Fungal fermentation for food protein production in upcycled agro-industrial side-streams.

Lübeck, Mette; Stephensen Lübeck, Peter

Publication date:
2023

ECFG16
INNSBRUCK | AUSTRIA
2023

www.ecfg16.org

Programme & Abstracts

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16th European Conference on Fungal Genetics

5-8 March 2023
Innsbruck, Austria

ECFG16
INNSBRUCK I AUSTRIA I 2023

PROGRAMME & ABSTRACTS BOOK

ECFG16
INNSBRUCK I AUSTRIA I 2023

CONTENT

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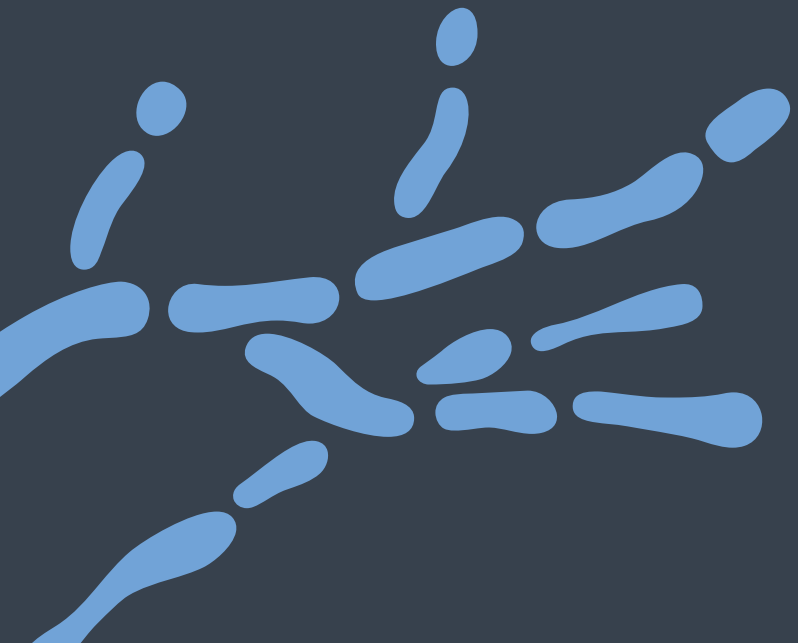

WELCOME

WELCOME

We warmly welcome you to the 16th European Conference on Fungal Genetics (ECFG16) in Innsbruck. After the challenging years of Covid-19, it is a great pleasure to host you in the Capital of the Alps and come together again in person to share data and ideas.
We expect ECFG16 to follow in the footsteps of previous ECFG conferences as a vibrant environment for interaction and exchange between scientists from all over the world who are interested in the field of fungal genetics. We are particularly happy to welcome students and young postdocs to the meeting, who make up roughly half of the over 850 participants. The scientific programme of ECFG16 includes three plenary sessions, three poster sessions, and 20 concurrent sessions on a diversity of topics ranging from fungal biodiversity to fungal biotechnology. Six satellite workshops focused on specific fungal genera complete the programme.
ECFG16 is hosted by Universität Innsbruck and Congress Innsbruck. We are extremely grateful to the Local Scientific Committee members who dedicated their energy and time to the organization of this meeting, to PCO Tyrol Congress, Universität Innsbruck and Medizinische Universität Innsbruck for their support of the local organizers, and to all sponsors and exhibitors for their cooperation.
We hope you will enjoy the scientific and social programme as well as the city of Innsbruck and its surrounding nature.

Susanne Zeilinger-Migsich & Hubertus Haas

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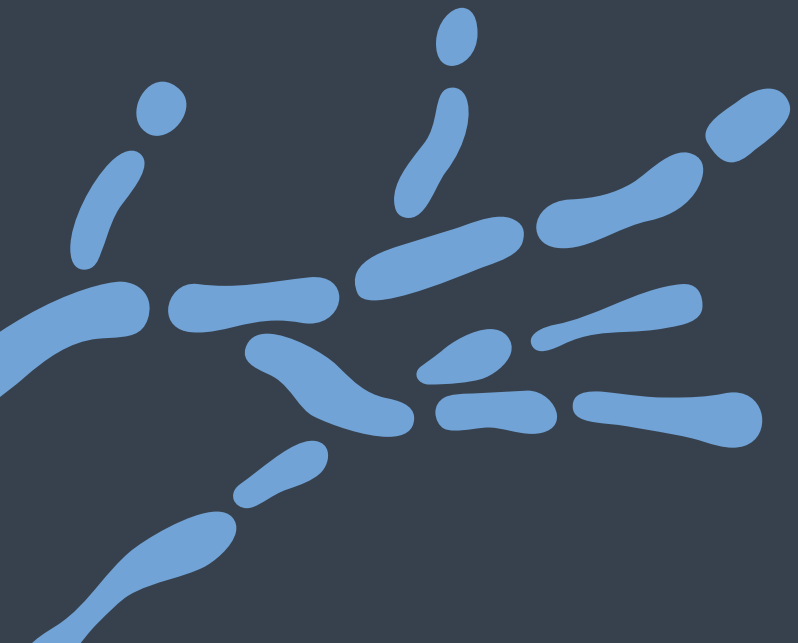

COMMITTEES

CONTACT & COMMITTEES

LOCAL ORGANIZING CHAIRS

Susanne Zeilinger-Migsich	Universität Innsbruck
Hubertus Haas	Medizinische Universität Innsbruck

LOCAL SCIENTIFIC COMMITTEE

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Lea Atanasova	BOKU-University of Natural Resources and Life Sciences, Vienna, Austria
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Monika Schmoll	University Of Vienna, Austria
Oded Yarden	Hebrew University of Jerusalem, Israel

CONGRESS ORGANISERS

PCO Tyrol Congress
Rennweg 3, 6020 Innsbruck
Tel.: +43 512 575600
ecfg23@cmi.at
www.cmi.at

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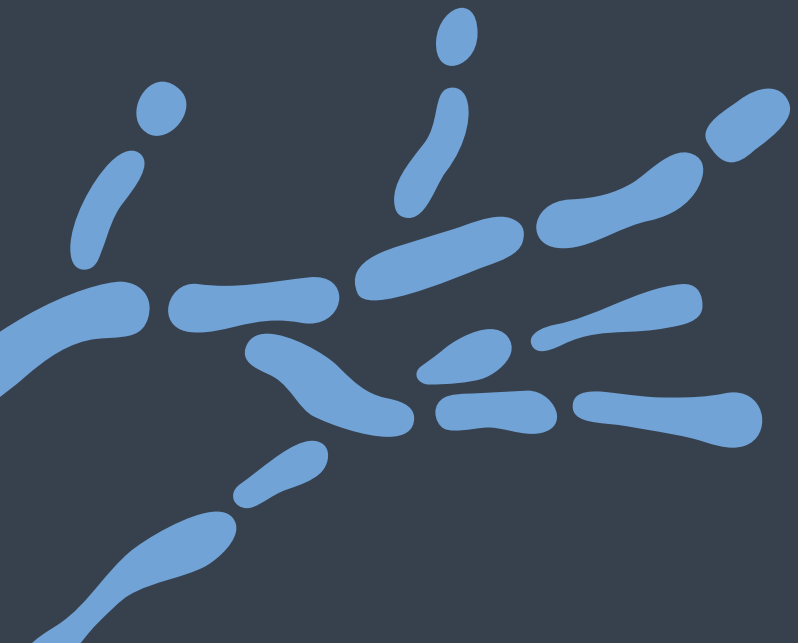

SPONSORS & EXHIBITORS

LIST OF SPONSORS

The ECFG16 would like to thank the following sponsors for their support.

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Medizinische Universität Innsbruck

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The ECFG16 would like to thank the following exhibitors for their support.

KF 01	FEMS
KF 02	Bisy
KF 03	Fungi DB
KF 04	Union Biometrica
KF 05	New England Biolabs
KF 06	Fungal Genetics and Biology (Elsevier)
KF 07	Microsynth Austria GmbH
KF 08	Inncellys GmbH
KF 09	Szabo Scandic
KF 10	Biosense Solutions
KF 11	Eppendorf
KF 12	Shimadzu
KF 13	Novogene
KF 14	sbi Scientific Bioprocessing
KF 15	Gilson
KF 16	Eurofins
GL	Tirol Werbung

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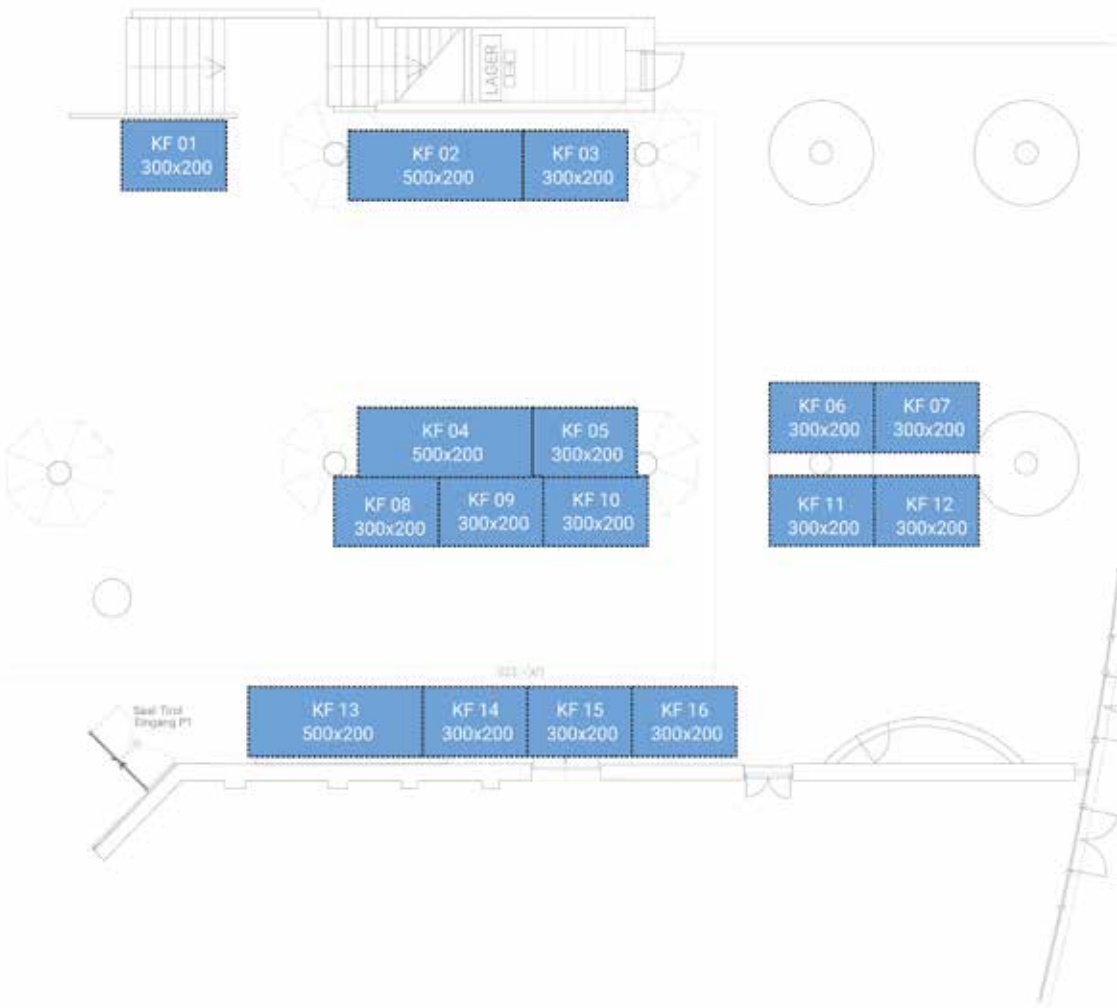
EXHIBITION FLOOR PLAN



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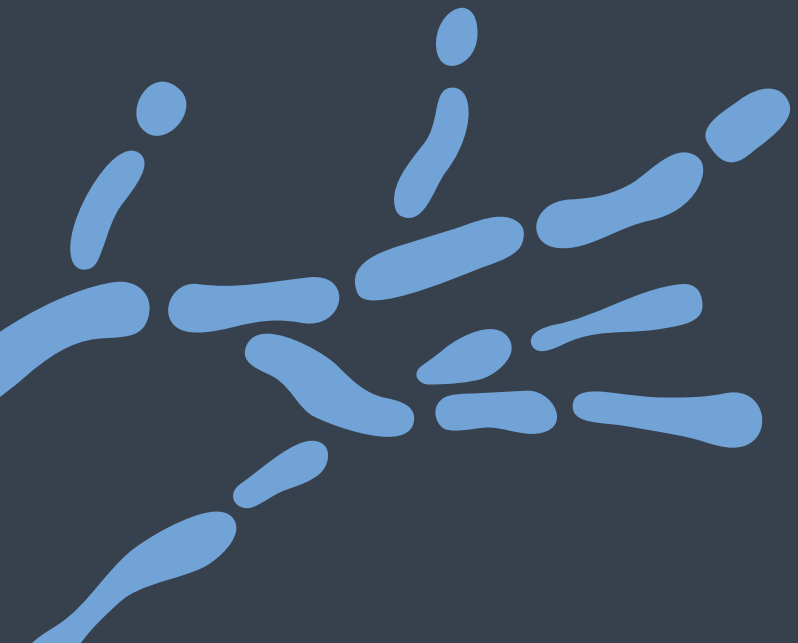

VENUE

VENUE

MAIN CONGRESS

ECFG16 will be held at Congress Innsbruck starting with the Opening Session in the evening of Sunday, March 5, 2023.

The venue Congress Innsbruck is situated right at the city centre making it possible for visitors of congresses to enjoy a car-free stay. The award-winning event venue offers a wide range of space and is geared to cater for individual requirements and wishes – from compact seminars to major congresses.

CONGRESS INNSBRUCK

Rennweg 3, 6020 Innsbruck
Austria
www.cmi.at

SATELLITE WORKSHOPS

Associated Satellite Meetings will take place on Saturday, March 4, 2023 (Asperfest) and / or Sunday, March 5, 2023 at SOWI Campus Universität Innsbruck. This university campus is within easy walking distance from Congress Innsbruck.

SOWI CAMPUS, UNIVERSITÄT INNSBRUCK

Universitätsstraße 15, 6020 Innsbruck
Austria
www.uibk.ac.at

ECFG16
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MAPS OF CONGRESS INNSBRUCK

3RD FLOOR:

Hall Freiburg
Hall Aalborg

1ST FLOOR:

Hall Tirol
(Plenary sessions)
Exhibition
Posters
Catering

GROUND FLOOR:

Registration desk
Cloakroom
Media check
Hall Brüssel
(Concurrent sessions)
Hall Strassburg
(Concurrent sessions)

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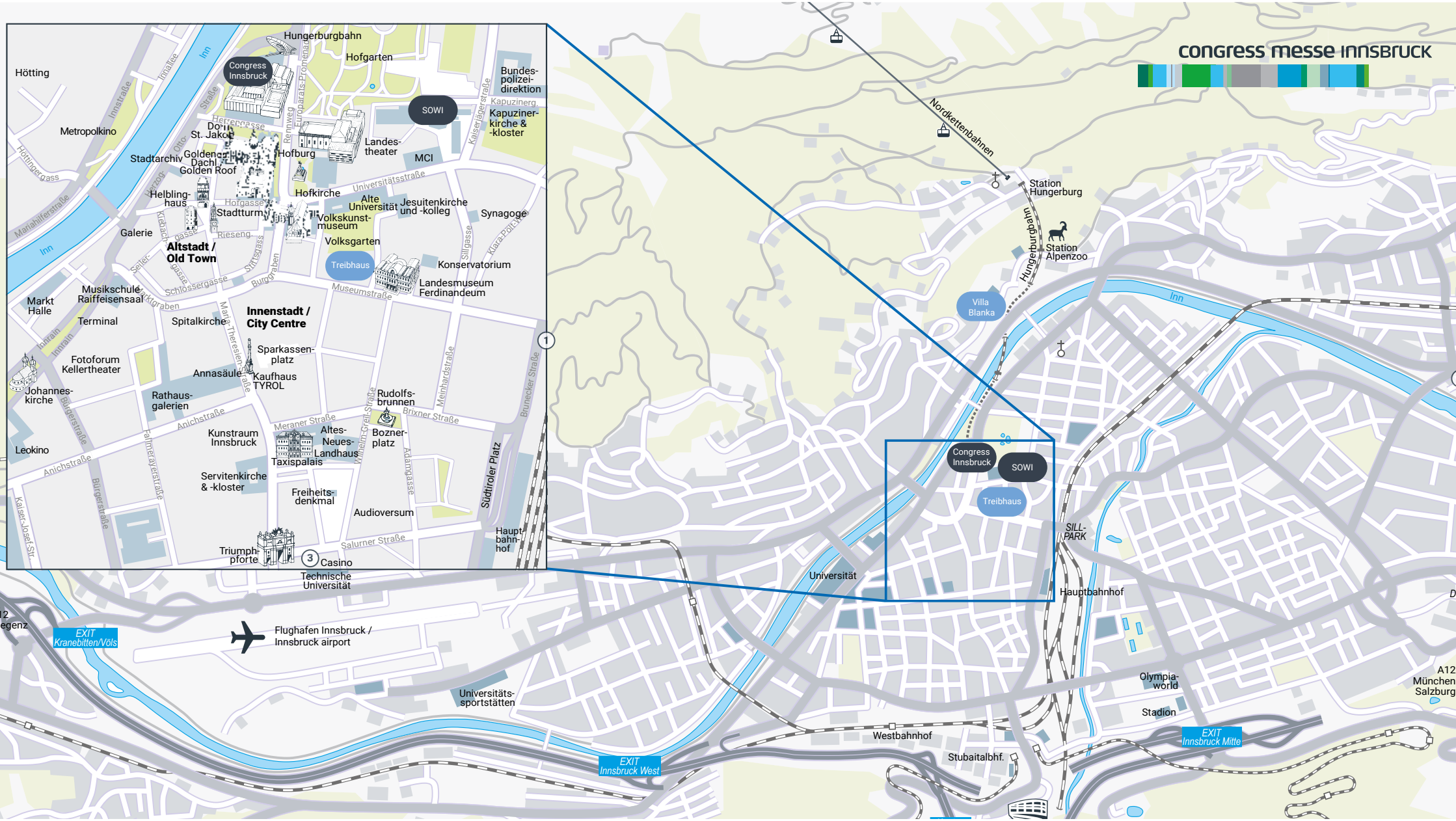

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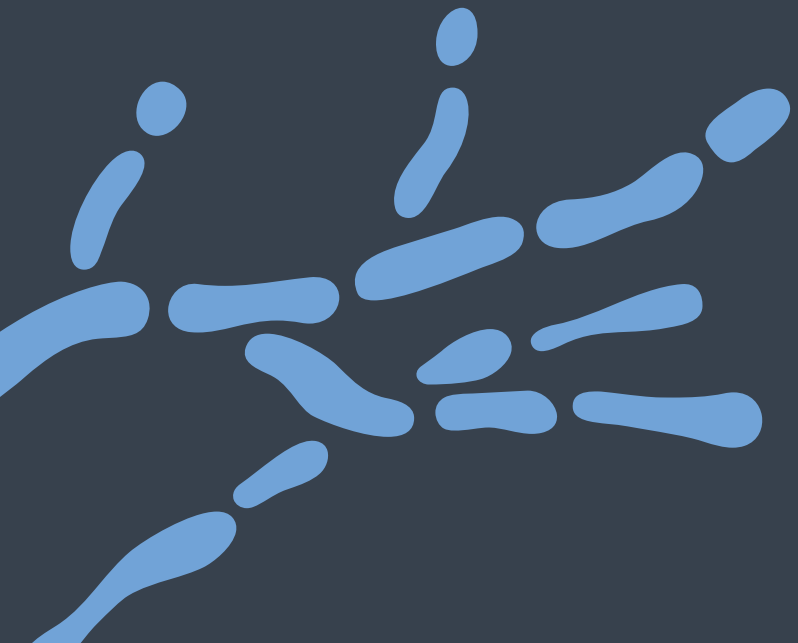

REGISTRATION INFORMATION

REGISTRATION INFORMATION

REGISTRATION OPENING HOURS
SOWI CAMPUS, UNIVERSITÄT INNSBRUCK

The ECFG16 registration desk for the Satellite Workshops is located close to the main entrance of the SOWI Campus, Universität Innsbruck. Opening hours are as follows:

Sunday, March 5 **08:15 – 11:00**

REGISTRATION OPENING HOURS
CONGRESS INNSBRUCK

The ECFG16 registration desk is located close to the main entrance of Congress Innsbruck at the ground level (Europa Foyer). Opening hours are as follows:

Sunday, March 5 **16:00 - 19:00**
Monday, March 6 **08:00 - 18:00**
Tuesday, March 7 **08:00 - 18:00**
Wednesday, March 8 **08:00 - 18:00**

CANCELLATION POLICY:

For detailed information regarding the ECFG16 registration cancellation policy, please refer to our website:
www.ecfg16.org (Registration – Registration Guidelines).

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FEES AND DEADLINES

	Early Fee Until Jan 2, 2023	Regular Fee Jan 3 - Feb 6, 2023	Late Fee Feb 7 - Mar 1, 2023	Virtual Registration
Participant	495 €	595 €	695 €	495 €
Student / Post Doc	295 €	395 €	495 €	295 €
Local Student	195 €	195 €	195 €	
Satellite Workshops	95 €	95 €	95 €	
Accompanying Person	195 €	195 €	195 €	

Conference registration fee includes:
- Participation in sessions
- Conference material
- Coffee breaks and lunches
- Welcome reception

Conference registration fee does not include:
- Conference Dinner *(Wednesday, March 8)*
- Satellite Workshops
- Accommodation

Satellite registration fee includes:
- Coffee breaks
- Light lunch

SATELLITE WORKSHOPS

Satellite workshops will be held at the SOWI Campus, Universität Innsbruck. The registration fee for each workshop is EUR 95,00. The fee includes coffee breaks and light lunch at the SOWI Campus, Universität Innsbruck.

PERSONAL DATA

The participant is entitled to revoke his/her consent to the specific data processing at any time by writing to the congress secretariat:
ecfg23@cmi.at.

LOST BADGE

Lost badge may be replaced onsite. A handling fee of EUR 50,00 will be charged.

FILMING AND PHOTOGRAPHY

We would like to inform you that there may be filming and photography during the meeting. A photo gallery with all photos taken during the congress may be available to registered participants on the ECFG16 website. In addition, photographs taken during the congress may be used for the organiser´s social media networks.

MODIFICATION OF THE PROGRAMME

The conference chairmen reserve the right to modify the conference programme, which is published as an indication only.

DATA PRIVACY

We take your privacy very seriously and in order to comply with GDPR consent requirements, your consent to our Privacy Policy is mandatory for a participation. For detailed GDPR guidelines please see:
www.ecfg16.org – Registration - Registration guidelines.

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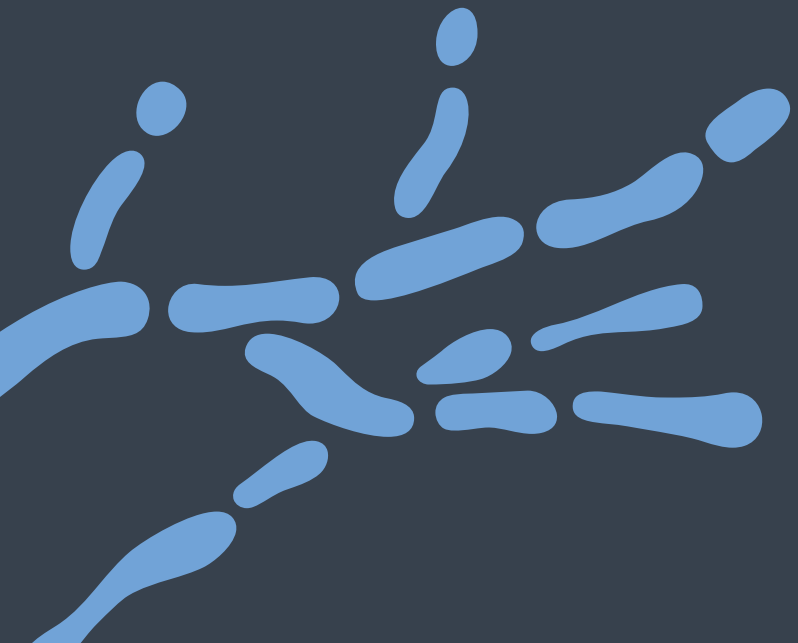

INFORMATION FROM A-Z

GENERAL INFORMATION FROM A-Z

ABOUT AUSTRIA

Austria has been a member of the European Union since 1995, the population is 8,5 million, the capital city being Vienna (Wien). Politically, Austria is a democratic republic with the prime minister as the head of the government and parliamentary elections every five years. The formal head of state is the president, who has more representative duties than political power. The country is divided into nine federal states, Innsbruck is the capital of the state of the Tyrol. The language spoken in Austria is German, but most Austrians speak English and many speak some French or Italian too and are happy to be of service to visitors.

ABOUT INNSBRUCK

Innsbruck, the capital of the Tyrol, is located in the Alpine region of Austria, in the valley of the river Inn, at 580 metres above sea level. It is surrounded by mountain ranges and numerous peaks which reach an altitude of approx. 2,700 metres above sea level. The city has 121,000 inhabitants and hosts one of the oldest universities in Europe, founded in the year 1669. Today, over 30,000 students attend the university in Innsbruck. Due to its location, Innsbruck has an excellent tourist infrastructure and is best known for its rich cultural heritage, as well as for its endless opportunities in sports and recreation. Innsbruck has been the host for Olympic Winter Games twice, in 1964 and 1976. In the town, some 160 restaurants, cafes and bars, most of them in walking distance to the convention centre, offer traditional Tyrolean and Austrian specialities as well as international dishes.

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ABSTRACTS

Abstracts selected for ECFG16 are presented in Concurrent Sessions and Poster Sessions. All accepted and confirmed abstracts are available via the abstract book online.
Please note, the additional abstract submission to the Satellite Workshops is separate from the ECFG16 submission. These abstracts are not included in the abstract book.

AM AND PM NETWORKING BREAKS

During the conference week, complimentary light snacks and refreshments will be available for registered delegates in the exhibition and poster area.

CAMERAS AND CELL PHONES

No unauthorised recording is allowed in any event during the ECFG16. As a courtesy to fellow attendees, please set your cell phones on silent mode during the session.

CERTIFICATE OF ATTENDANCE

A certificate of attendance will be sent to all delegates by email after the congress.

CITY TRANSPORTATION AND TAXI

There is a good public transport system in Innsbruck and its surroundings. Most busses and trams operate until midnight. Detailed information on bus schedules is available at your hotel. Tickets can be pre-purchased form ticket machines at the stops.
Taxis are usually available outside the conference centre's entrance. If you need support please contact the registration desk.

CONGRESS DOCUMENTS AND BADGES

Congress documents have to be collected on-site at the registration desk. Name badges must be worn visibly at all times during the conference, networking activities and in the exhibition and poster area.

COVID-19

By registering for the ECFG16, you agree to comply with the local (Innsbruck, Austria) COVID-19 measures in place at the time of the event. This includes any stipulations regarding being vaccinated, mask wearing and social distancing. The chairmen and organisers will accommodate all local regulations as part of the setup of the event.
For details on PCR testing, please see - PCR-testing possibility.

EMERGENCY

The emergency numbers can be called free of charge from any phone in Austria. In the event of traffic accidents, fire or other situations, emergency services should be contacted immediately on the European emergency number: 112. The police can be contacted by calling 133 and fire department by 122. For an ambulance service please call 144.

INFORMATION FOR SPEAKERS

Please bring your lecture on a USB stick and hand it in at the media check (located next to the registration desk on the ground floor of Congress Innsbruck). Please make sure to do so at least one hour before your session starts. You need not bring your own computer. The meeting rooms are equipped with PC and data projector.

LOST&FOUND

Lost and Found items should be returned/claimed at the registration desk.

OFFICIAL LANGUAGE

The official language of the ECFG16 is English. No simultaneous translation will be provided.

PARKING

There is an underground car park at the Congress Centre. Participants obtain tickets at reduced rates from the porter's desk on the ground floor of Congress Innsbruck. Please note that theses reduced fares only apply to the Congress garage (garage entry on the left side) and not the other parking facilities (garage entry on the right side). Please also note that street parking in the city is available but limited to 90 minutes.

PCR-TESTING POSSIBILITY

Medical Laboratory Priv.-Doz. Dr. Igor Theurl, PhD | Franz-Fischer-Str. 7b I 6020 Innsbruck
Opening hours: Monday - Frriday 07:00 - 15:00 without appointment (ATTENTION: After 15:00 the laboratory is closed, no more results will be given out!)
Testing takes place directly in the test tent after filling out a form.
After max. 5 hours the result is available. The result can be issued in English and is usually sent by mail.
ATTENTION: For China, a „medical certificate" is required in addition to the report, which is ONLY accepted if it is picked up locally at the laboratory. In this case, the medical certificate will also be given. Please keep an eye on the opening hours!
The PCR-Test costs EUR 60,00 per person and can be paid cash or by credit card.

RESTAURANTS & BARS

There are plenty of restaurants and bars in the inner-city of Innsbruck.
For dining options please refer to:
innsbruck.info/en/see-and-experience/food-and-drink/restaurant-search

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SMOKING

It is against the law to smoke in any indoor public place or worksite in Austria. Smoking is prohibited in the entire building of Congress Innsbruck. Please note that public transport, transit shelters, taxis and work vehicles are also smoke-free.

TRAIN STATION

Innsbruck main station is located in the centre of the city within walking distance to the conference venue. Taxis are also available outside the station's entrance.

WATER

The value of water is particularly evident in Innsbruck, because 100% of Innsbruck's water needs are met by spring water, the quality of which is constantly monitored.
The journey of Innsbruck's water begins at the very top of the Nordkette, where water from melted snow or precipitation seeps deep into the rock and then makes its long way down into the valley. This special geological situation determines the high quality of Innsbruck's drinking water.
For this reason, you will receive a drinking bottle at ECFG16 to keep your water needs topped up.

WI-FI CONNECTION

ECFG16 is providing free Wi-Fi in the conference area. To ensure a positive Wi-Fi experience for all users please do not use your own wireless hotspot device. The additional Wi-Fi devices create significant RF interference which can interfere with all Wi-Fi networks. Please turn these devices off and connect to the Wi-Fi network ISSW and open your web browser to connect to the internet.

LOG IN DETAILS:

Network name: congress
user name: ECFG
Password: ecfg16

SAVE THE DATE

Reconnect at the 10th Congress of European Microbiologists

9 - 13 July 2023 • Hamburg, Germany

FEMS 2023

Welcome to FEMS 2023 in Hamburg.

It is a great pleasure to announce the 10th Congress of European Microbiologists, FEMS 2023, which will be held 9-13 July 2023 in Hamburg, Germany. FEMS2023 will bring together leading scientists spanning different fields of microbiology to celebrate the best of microbiology.

This congress will showcase the most recent developments in microbiology to address some of the global challenges we face today, such as antimicrobial resistance, environmental pollution and the emergence of pathogenic disease.

We invite you to reconnect with us and be part of the FEMS2023 Congress. We hope to see you there!

KEYNOTE SPEAKERS

Kenneth Timmis, Switzerland
Jorge Galan, USA
Rita Colwell, USA
Julia Vorholt, Switzerland
Carmen Buchrieser, France
Paul Lehner, UK

REGISTER NOW!

www.fems2023.org

DISCLAIMER

The organizers have made every attempt to ensure that all information in this publication is correct. The organizers take no responsibility for changes to the program or any loss that may occur as a result of changes to the program. Some of the information provided in this publication has been provided by external sources. Although every effort has been made to ensure the accuracy, currency and reliability of the content, the organizers accept no responsibility in that regard.

LIABILITY AND INSURANCE

Neither the organizers nor CMI / PCO Tyrol Congress as their agency accept any liability for personal injuries, or loss of, or damage to property belonging to congress delegates or accompanying persons, either during or as a result of the conference or during any of the networking events. It is recommended that participant arrange for their own personal health, accident and travel insurance before they depart from their countries. Only written agreements shall be valid. The play of jurisdiction shall be Innsbruck.

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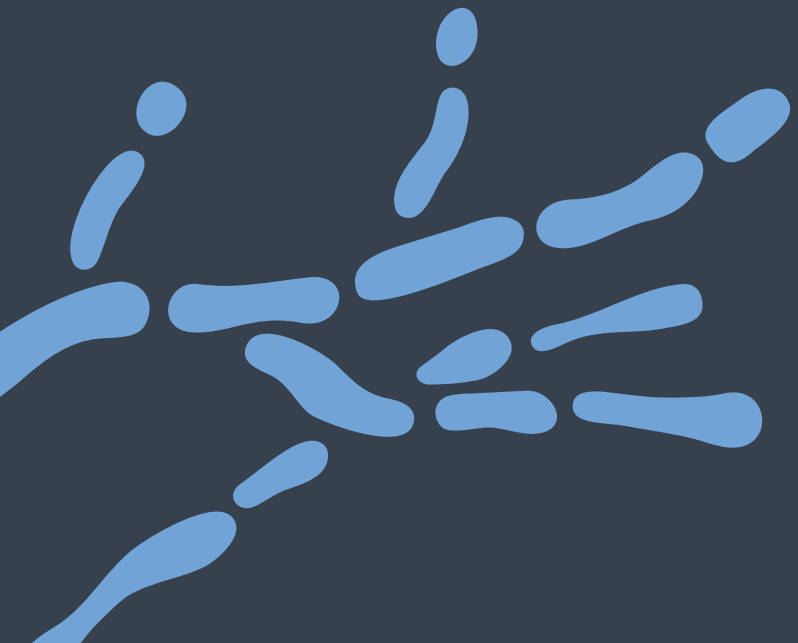

SOCIAL PROGRAMME

SOCIAL EVENTS

CONFERENCE DINNER

Wednesday, March 8, 2023 at 19:30 - 23:00

Ticket costs: EUR 120,00 per ticket, tickets are limited
Venue: Villa Blanka Eventcenter, Weiherburggasse 8, 6020 Innsbruck

ECFG16 PARTY @ TREIBHAUS

Wednesday, March 8, 2023 at 21:00 - 01:00

Address: Treibaus, Angerzellgasse 8, 6020 Innsbruck
Special Guest: Mais Uma

We would like to invite you to the ECFG16 party with live music and DJ.

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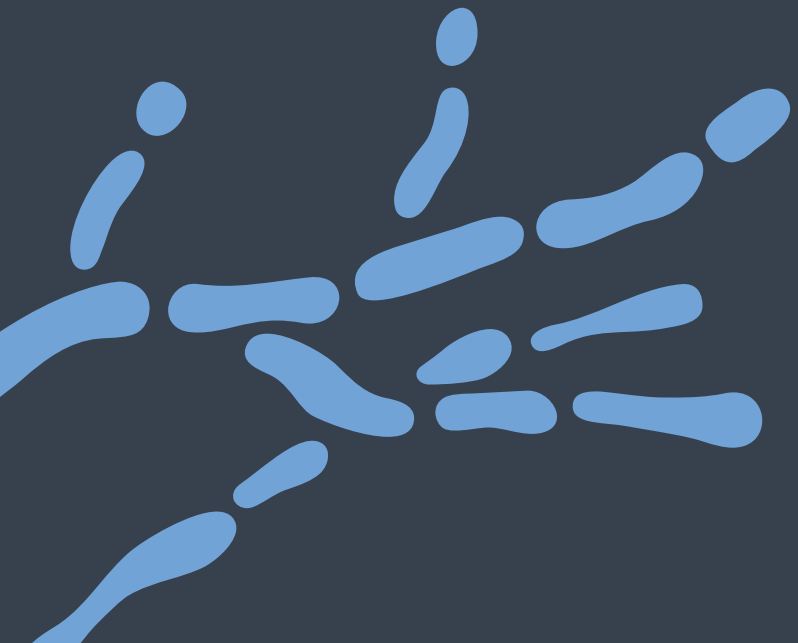

PROGRAMME
TIMETABLE

SUNDAY, MARCH 5

	SOWI Campus, Universität Innsbruck Meeting rooms	Congress Innsbruck Foyers	Congress Innsbruck Hall Tirol
08.15-09.00	Satellite Workshop Registration		
09.00-10.45	Satellite Workshops		
10:45-11.15	Coffee break		
11.15-13.00	Satellite Workshops		
13.00-14.00	Lunch break		
14.00-15.45	Satellite Workshops		
15.45-16.00	Coffee break		
16.00-16.15		ECFG16 Registration	
16.15-17.30	Satellite Workshops		
17.30-18.00			
18.00-20.00		Welcome Reception	
20.00-21.00			Opening Session

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MONDAY, MARCH 6

	Hall Tirol	Hall Brüssel	Hall Strassburg	Hall Freiburg	Foyer 1st floor
09.00-09.45	Keynote lecture 1				
09:45-10.45	Plenary session 1				
10.45-11.15	Coffee break, Exhibition and poster Foyer 1st floor				
11.15-12.45	Plenary session 1				
12.45-14.00	Lunch break, Exhibition and poster Foyer 1st floor				
14.00-16.00	CS1.1. Plant inter-actions	CS1.2. Bioactive metabolities, secondary metabolites	CS1.3 Genome function and epigenetics	CS1.4 Biocontrol and natural antagonists	
16.00-17.30					Poster session 1 Coffee break
17.30-19.30	CS2.1 Synthetic biology and biotechnology	CS2.2 De-velopment and morpho-genesis	CS2.3 Sensing and signaling	CS2.4 Molecular tools	

TUESDAY, MARCH 7

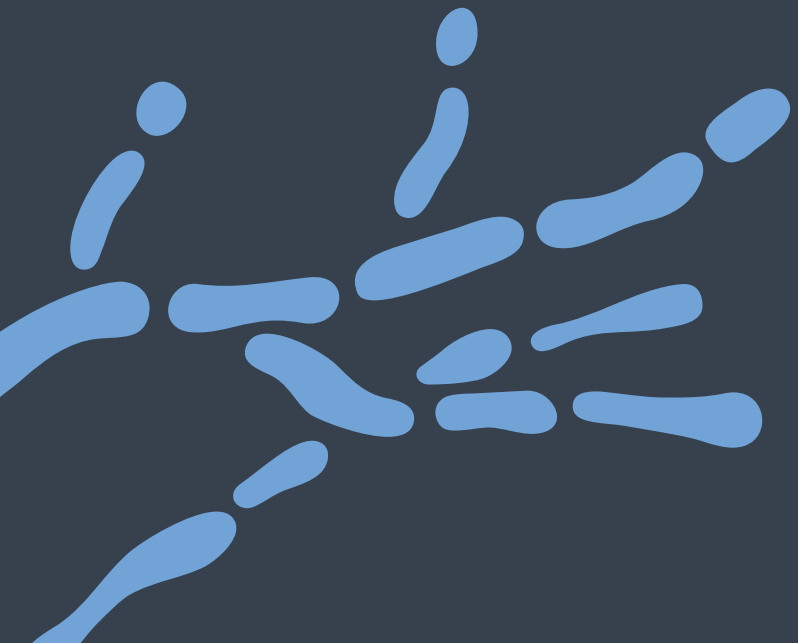
	Hall Tirol	Hall Brüssel	Hall Strassburg	Hall Freiburg	Foyer 1st floor
09.00-09.45	Keynote lecture 2				
09:45-10.45	Plenary session 2				
10.45-11.15	Coffee break, Exhibition and poster Foyer 1st floor				
11.15-12.45	Plenary session 2				
12.45-14.00	Early Career author workshop	Lunch break, Exhibition and poster Foyer 1st floor			
14.00-16.00	CS3.1 Evolution, biodiversity and taxonomy	CS3.2 Metabolism and physiology	CS3.3 Animal/human interactions	CS3.4 Symbionts and endophytes	
16.00-17.30					Poster session 2 Coffee break
17.30-18.30	CS4.1 Antifungals and resistance mechanisms	CS4.2 Fungal cell biology Concurrent session	CS4.4 Fungal epidemiology and diagnostics	CS4.3 RNA biology	
18.30-19.30			JGI Genomics Workshop		

WEDNESDAY, MARCH 8

	Hall Tirol	Hall Brüssel	Hall Strassburg	Hall Freiburg	Foyer 1st floor
09.00-09.45	Keynote lecture 3				
09:45-10.45	Plenary session 3				
10.45-11.15	Coffee break, Exhibition and poster Foyer 1st floor				
11.15-12.45	Plenary session 3				
12.45-14.00	Lunch break, Exhibition and poster Foyer 1st floor				ISC Meeting **Hall Aalborg**
14.00-16.00	CS5.1 Genomes and other – omics	CS5.2 Mycobiomes and microbial interactions	CS5.3 Stress and extreme environments	CS5.4 Regulatory networks	
16.00-17.30					Poster session 3 Coffee break
17.30-18.30	Closing ceremony, Poster prizes				
19.30-01.00	ECFG16 Conference Dinner @ Villa Blanka 19:30 - 23:00 ECFG16 party @ Treibhaus 21:00 - 01:00				

ECFG16

INNSBRUCK I AUSTRIA I 2023



SCIENTIFIC PROGRAMME

SUNDAY, MARCH 5

Satellite Workshops will take place at SOWI Campus, Universität Innsbruck.
For detailed programme see page 94.

16:00 – 19:00 REGISTRATION
Location: ***Congress Innsbruck,*** *ground floor*

18:00 – 20:00 WELCOME RECEPTION
Location: ***Congress Innsbruck,*** *first floor*

20:00 – 20:15 CONFERENCE OPENING
Location: ***Hall Tirol***
Susanne Zeilinger-Migsich
Universität Innsbruck, Austria
Hubertus Haas
Medizinische Universität Innsbruck, Austria
Marc-Henri Lebrun
President of ISC

20:15-21:00 OPENING SESSION
Location: ***Hall Tirol***

CHAIRS:
Miguel A. Peñalva
Consejo Superior De Investigaciones Científicas, Spain
Karl Kuchler
Medical University of Vienna, Campus Vienna Biocenter, Austria

From regulated cell death in yeast to anti-aging and anti-fungal treatments
Frank Madeo
Karl-Franzens Universität Graz, Austria

MONDAY, MARCH 6

09:00 – 12:45 PLENARY SESSION 1
Location: ***Hall Tirol***
Fungal interactions
supported by ORTNER

CHAIRS:
Hubertus Haas
Medizinische Universität Innsbruck, Austria
Gerhard Braus
Center for Molecular Biosciences, Georg-August-University Göttingen, Germany

09.00-09.05 WELCOMING REMARKS

Gregor Weihs
Vice Rector for Research, Universität Innsbruck, Austria

09:05-09:45 KEY NOTE 1
Location: ***Hall Tirol***
Root cap cell corpse clearance limits microbial colonization
Alga Zuccaro
University of Cologne, Institute for Plant Sciences, Germany

09:45-10:15 PS1.1 Regulated cell death in innate immunity of fungi
Teresa Pawlowska
School of Integrative Plant Science, Cornell University, USA

10:15-10:45 PS1.2 Lessons to learn from a gall-inducing fungus
Armin Djamei
Excellence University of Bonn, Germany

10:45-11:15	**Coffee break**
11:15-11:45	PS1.3 Fight on surfaces prior to the onset of fungal pathogenesis in insects **Chengshu Wang** Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, China
11:45-12:15	PS1.4 A master bZIP transcriptional regulator in the C. auris fungal pathogen links metabolism, tissue tropism, morphogenesis and antifungal resistance **Karl Kuchler** Medical University of Vienna, Campus Vienna Biocenter, Austria
12:15-12:45	PS1.5 Genomic and metabolic adaptation mechanisms of symbiotic fungi from insects **Christine Beemelmanns** Helmholtz-Institut für Pharmazeutische Forschung Saarland (HIPS), Germany
12:45-14:00	**Lunch break**

14:00-16:00 **CS1.1 PLANT INTERACTIONS**

Location: ***Hall Tirol***

CHAIRS:
Gerhard Adam
BOKU-University of Natural Resources and Life Sciences, Austria
Massimo Reverberi
Sapienza University, Italy

14:00-14:15	CS1.1.1The stress related transcription factor CRZ1 plays pivotal functions in host perception and fumonisin biosynthesis during Fusarium verticillioides-Zea mays interactions **Andrea Cacciotti,** Sapienza Università di Roma, Italy
14:15-14:30	CS1.1.2 Natural variation in Avr3D1 from Zymoseptoria sp. contributes to quantitative gene-for-gene resistance and to host specificity **Andrea Sánchez-Vallet,** Universidad Politecnica de Madrid, Spain
14:30-14:45	CS1.1.3 ZymoSoups : A forward genetics method for rapid identification of effector genes in Zymoseptoria tritici **Graeme Kettles,** University of Birmingham, United Kingdom
14:45-15:00	CS1.1.4 Towards identification of virulence factors contributing to the necrotrophic phase of Colletotrichum orbiculare **Katsuma Yonehara,** RIKEN, Tokyo University, Japan
15:00-15:15	CS1.1.5 Fusarium graminearum: does the trichothecene chemotype matter? **Gerhard Adam,** BOKU-University of Natural Resources and Life Sciences, Austria

15:15-15:30	CS1.1.6 Addressing redundant roles of phytotoxic proteins for necrotrophic infection of B. cinerea by multi-k.o. mutagenesis **Matthias Hahn,** University Kaiserslautern, Germany
15:30-15:45	CS1.1.7 Giant transposons facilitate horizontal gene transfer of the necrotrophic effector ToxA in fungal wheat pathogens **Megan McDonald,** University of Birmingham, United Kingdom
15:45-16:00	CS1.1.8 PWL2 modulates PAMP-triggered immunity through interaction with a host isoprenylated HMA **Vincent Were,** Norwich Research Park, United Kingdom

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14:00 – 16:00 CS1.2 BIOACTIVE METABOLITES, SECONDARY METABOLITES

Location: ***Hall Brüssel***

CHAIRS:
Lena Studt-Reinhold
BOKU-University of Natural Resources and Life Sciences, Austria
Jens Laurids Sørensen
Aalborg University, Denmark

14:00-14:15	CS1.2.1 Ubiquitous bacterial polyketides mediate cross-kingdom microbial interactions **Axel Brakhage,** Leibniz Institute for Natural Product Research and Infection Biology (HKI), Germany
14:15-14:30	CS1.2.2 Evolutionary Histories of Type I Fatty Acid Synthases in Fungi **Yanfang Guo,** Westerdijk Fungal Biodiversity Institute, The Netherlands
14:30-14:45	CS1.2.3 An HMM approach expands the landscape of sesquiterpene cyclases across the kingdom Fungi **Marie-Noelle Rosso,** INRAE, France
14:45-15:00	CS1.2.4 Novel Insights into Fungal Specialized Metabolite Biosynthesis **Uffe Mortensen,** Technical University of Denmark
15:00-15:15	CS1.2.5 Fungal Raincoats **Teis Sondergaard,** Aalborg University, Denmark

15:15-15:30 CS1.2.6 Genome analysis and elucidation of the biosynthetic pathway for the cRAS inhibitor rasfonin in Cephalotrichum gorgonifer
Andreas Schüller, BOKU-University of Natural Resources and Life Sciences, Austria

15:30-15:45 CS1.2.7 Heterologous production of ribosomal backbone N-methylated macrocyclic peptides
Lukas Sonderegger, ETH Zürich, Switzerland

15:45-16:00 CS1.2.8 Sustainable Conversion of Polyethylene Waste Plastics into Fungal Secondary Metabolites
Clay Wang, University of Southern California, USA

14:00 – 16:00 **CS1.3 GENOME FUNCTION AND EPIGENETICS**

Location: ***Hall Strassburg***

CHAIRS:
Ingo Bauer
Medizinische Universität Innsbruck, Austria
Özgür Bayram
Maynooth University, Ireland

14:00-14:15 CS1.3.1 Chromatin-wired nuclear compartments in Fusarium graminearum
Nadia Ponts, INRAE, France

14:15-14:30 CS1.3.2 Histone deacetylase 1 (HDA-1) activity regulates facultative heterochromatin formation in the model system Neurospora crassa
Felicia Ebot Ojong, The University of Georgia, USA

14:30-14:45 CS1.3.3 The DNA N6-Adenine Methyltransferase Complex of Mucorales and its role on gene expression and chromatin structure
Carlos Lax, University of Murcia, Spain

14:45-15:00 CS1.3.4 A comprehensive genomic atlas of chromatin remodelling activity in Candida albicans uncovers new regulatory circuits of fungal fitness
Adnane Sellam, University Of Montreal, Canada

15:00-15:15 CS1.3.5 Chromosome dynamics in the highly plastic genome of the fungal pathogen Fusarium oxysporum
Lucía Gómez Gil, University of Córdoba, Spain

15:15-15:30 CS1.3.6 Sirtuin E is involved in cell wall integrity, growth, secondary metabolism production, and virulence in Aspergillus fumigatus
André Damasio, University of Campinas, Brazil

15:30-15:45 CS1.3.7 The effects of phase separation on chromatin modifications, transcriptional regulation and virulence in the human fungal pathogen Candida albicans
Qing Lan, University of Macau, China

15:45-16:00 CS1.3.8 The role of histone modifications in morphological plasticity of Aureobasidium pullulans
Zainab Abdul Qayyum, Technical University Vienna, Austria

14:00 – 16:00 **CS1.4 BIOCONTROL AND NATURAL ANTAGONISTS**
supported by MyPilz GmbH
Location: ***Hall Freiburg***

CHAIRS:
Lea Atanasova
BOKU-University of Natural Resources and Life Sciences, Austria
Magnus Karlsson
Swedish University of Agricultural Sciences, Sweden

14:00-14:15 CS1.4.1 Genes for an extended phenotype: Fungal biosynthesis of volatiles in zombie flies entice male flies to mate with female cadavers
Henrik De Fine Licht, University of Copenhagen, Denmark

14:15-14:30 CS1.4.2 One Health approaches to Biocontrol: Breeding for Biologicals and Microbiome Resilience
Laura Grenville-Briggs Didymus, Swedish University of Agricultural Sciences, Sweden

14:30-14:45 CS1.4.3 Sensing and regulation of mycoparasitism-related processes in Trichoderma atroviride
Lea Atanasova, BOKU-University of Natural Resources and Life Sciences, Austria

14:45-15:00 CS1.4.4 Harnessing microbiota functions to combat Fusarium diseases in wheats
Yun Chen, Zhejiang University, China

15:00-15:15 CS1.4.5 Utilizing bacterial phyllosphere dynamics of Cercospora Leaf Spot-infected sugar beet to hunt for bacterial isolates involved in fungal antagonism
Lorena Rangel, United States Department of Agriculture, USA

15:15-15:30 CS1.4.6 Endophytic fungi as biocontrol agents of cranberry plant pathogens
FEMS grant
Bhagya C. Thimmappa, University de Montreal, Canada

15:30-15:45 CS1.4.7 Transcriptomic approach and functional genetics to unveil the interaction between a biocontrol yeast and a fungal pathogen on the host
Giuseppe Ianiri, University of Molise, Italy

15:45-16:00 CS1.4.8 MicroRNA profiling of the Metarhizium brunneum – Galleria mellonella pathosystem
Daniel Eastwood, Swansea University, United Kingdom

16:00-17:30 **Poster Session & Coffee break**

POSTER SESSION 1

CS1.1.9 – CS1.1.59
Plant interactions

CS1.2.9 – CS1.2.41
Bioactive metabolites, secondary metabolites

CS1.3.9 – CS1.3.30
Genome function and epigenetics

CS1.4.9 – CS1.4.25
Biocontrol and natural antagonists

CS2.3.9 – CS2.3.23
Sensing and signaling

CS2.4.9 – CS2.4.22
Molecular tools

17:30-19:30 **CS2.1 SYNTHETIC BIOLOGY AND BIOTECHNOLOGY**
supported by IFF
Location: ***Hall Tirol***

CHAIRS:
Matthias Steiger
Technical University Vienna, Austria
Arthur F.J. Ram
Leiden University, The Netherlands

17:30-17:45 CS2.1.1 A versatile high–throughput friendly system for construction and validation of fungal cell factories
Katherina Garcia Vanegas, Technical University of Denmark, Denmark

17:45-18:00 CS2.1.2 Synthetic Biology tools for genome mining of fungi for novel bioactive metabolites
Yit-Heng Chooi, University of Western Australia, Australia

18:00-18:15 CS2.1.3 Characterization of a GH5_7 β-mannanase by activity-based protein profiling in secretomes of A. niger
Massimo Tedeschi, Leiden University, The Netherlands

18:15-18:30 CS2.1.4 Controlling macromorphologies of Aspergillus niger during high and low shear stress bioreactor cultivation
Karin Engelbert, Technische Universität Berlin, Germany

18:30-18:45 CS2.1.5 The Emericellopsis genus, a comparison of its marine and terrestrial strains
FEMS grant
Raquel Ledo Doval, Westerdijk Fungal Biodiversity Institute, The Netherlands

18:55-19:00	CS2.1.6 Enhanced production of heterologous natural products in Aspergillus oryzae by large-scale metabolic engineering based on genome editing **Naoya Saito,** The University of Tokyo, Japan
19:00-19:15	CS2.1.7 Functional characterization of a highly specific L-arabinose transporter from Trichoderma reesei **Christopher Landowski,** Onego Bio Ltd, Finland
19:15-19:30	CS2.1.8 Bioconversion of lignocellulosic feedstocks to 3-hydroxypropionic acid using acidophilic fungi **Kyle Pomraning,** Pacific Northwest National Laboratory, USA

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17:30-19:30 CS2.2 DEVELOPMENT AND MORPHOGENESIS *Location:* ***Hall Brüssel***

CHAIRS:
Florentine Marx
Medizinische Universität Innsbruck, Austria
Fabienne Malagnac
Paris Saclay University, France

17:30-17:45	CS2.2.1 A novel secreted protein Stt1 adsorbed on the tip of sterigma mediates basidiosporogenesis **Hiroshi Yoshida,** Iwate Biotechnology Research Center, Japan
17:45-18:00	CS2.2.2 The important role of protein kinases in basidiomycete sporulation **Peter Jan Vonk,** Utrecht University, The Netherlands
18:00-18:15	CS2.2.3 Effects of NWD2 genes on the fruiting process of Coprinopsis cinerea **Shanta Subba,** Georg-August-Universität Göttingen, Germany
18:15-18:30	CS2.2.4 Global Analysis of Circuitry Governing Candida albicans Morphogenesis within Host Immune Cells **Nicola Case,** University of Toronto, Canada
18:30-18:45	CS2.2.5 A novel spore-specific transcription factor is essential for conidial maturation and dormancy in Aspergillus species **Ye-Eun Son,** Kyungpook National University, South Korea

18:55-19:00 CS2.2.6 Identification and functional characterization of the transcriptional cyclin-kinase CTDK-1 complex of Aspergillus nidulans as a regulator of growth and development
Oier Etxebeste, University of The Basque Country, Spain

19:00-19:15 CS2.2.7 F-box receptor mediated control of substrate stability and subcellular location organizes cellular development of Aspergillus nidulans
Özlem Sarikaya Bayram, Maynooth University, Ireland

19:15-19:30 CS2.2.8 Rapid and frequent loss of female fertility under culture condition in the rice blast fungus
Kohtetsu Kita, Tokyo University of Science, Japan

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MONDAY, MARCH 6
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17:30 – 19:30 **CS2.3 SENSING AND SIGNALING**

Location: ***Hall Strassburg***

CHAIRS:
Monika Schmoll
University of Vienna, Austria
Luis M. Corrochano
Universidad de Sevilla, Spain

17:30-17:45 CS2.3.1 Light dependent impact of methionine on metabolism of Trichoderma reesei and signal transmission by the GPCR GPR2
Monika Schmoll, University of Vienna, Austria

17:45-18:00 CS2.3.2 Mutagenesis approach to unravel the dual role of the Candida albicans Gpr1 receptor in methionine-induced morphogenesis and lactate-induced ß-glucan masking
Patrick Van Dijck, KU Leuven, Belgium

18:00-18:15 CS2.3.3 In vitro competitive fitness profiling reveals specific protein kinases and secreted proteases promote Aspergillus fumigatus adaptation to cystic fibrosis host
Kayleigh Earle, University of Manchester, United Kingdom

18:15-18:30 CS2.3.4 Role of the plasma membrane H+-ATPase Pma1 in development and virulence of the fungal pathogen Fusarium oxysporum
Melani Mariscal, University of Cordoba, Spain

18:30-18:45 CS2.3.5 Regulation of extracellular cellulase production by heterotrimeric G protein signaling in Neurospora
Katherine Borkovich, University of California Riverside, USA

18:55-19:00	CS2.3.6 Identification of F-Box proteins involved in the switch to cellulolytic metabolism **Philipp Benz,** Technical University of Munich, Germany
19:00-19:15	CS2.3.7 A light-sensing system in the common ancestor of the fungi **Luis Javier Galindo,** University of Oxford, United Kingdom
19:15-19:30	CS2.3.8 Local calcium signal transmission in mycelial network exhibits decentralized stress responses **Norio Takeshita,** University of Tsukuba, Japan

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MONDAY, MARCH 6

17:30 – 19:30 **CS2.4 MOLECULAR TOOLS**

Location: ***Hall Freiburg***

CHAIRS:
Fabio Gsaller
Medizinische Universität Innsbruck, Austria
Uffe Mortensen
Technical University of Denmark, Denmark

17:30-17:45	CS2.4.1 Locus-specific chromatin composition analysis by dCas9-driven proximity labelling **Thomas Svoboda,** BOKU-University of Natural Resources and Life Sciences, Austria
17:45-18:00	CS2.4.2 Utilization of CRISPR/Cas9-based methodology for genetic manipulation of the basidiomycete white-rot fungus Dichomitus squalens FEMS grant **Victor Manuel Gonzalez Ramos,** University of Helsinki, Finland
18:00-18:15	CS2.4.3 Non-Homologous End-Joining (NHEJ)-deficient filamentous fungal strains mitigate the impact of off-target mutations after the application of CRISPR/Cas9 **Sandra Garrigues,** Westerdijk Fungal Biodiversity Institute, The Netherlands
18:15-18:30	CS2.4.4 Modular Inducible Multigene Expression System for Filamentous Fungi **Clara Baldin,** Medizinische Universität Innsbruck, Austria
18:30-18:45	CS2.4.5 Online biomass monitoring enables characterization of the growth pattern of Aspergillus fumigatus in liquid shake conditions **Ingo Bauer,** Medizinische Universität Innsbruck, Austria

18:55-19:00	CS2.4.6 Genome-wide in vitro competitive fitness profiling reveals novel interconnected networks of genes associated with adaptation of Aspergillus fumigatus to antifungals **Can Zhao,** University of Manchester, United Kingdom
19:00-19:15	CS2.4.7 Building a redox flow battery to store renewable energy based on the fungal synthesized quinone phoenicin **Jens Laurids Sørensen,** Aalborg University, Denmark
19:15-19:30	CS2.4.8 Mass spectrometry toolbox for deciphering molecular heterogeneity of proteins **Mowei Zhou,** Pacific Northwest National Laboratory, USA

TUESDAY, MARCH 7

09:00 – 12:45 PLENARY SESSION 2

Location: ***Hall Tirol***

Evolution and biodiversity
supported by BISY GmbH

CHAIRS:
Irina Druzhinina
Royal Botanic Gardens Kew, United Kingdom
Scott Baker
Pacific Northwest National Laboratory and DOE Joint BioEnergy Institute, USA

09:00-09:45	Key Note 2 Fungal interactions and co-adaptation in the plant phyllosphere **Eva Stukenbrock** Christian-Albrechts University Kiel, Germany
09:45- 10:15	PS2.1 The global emergence of antifungal resistance: the calm before the storm? **Matthew Fisher** Imperial College School of Public Health, United Kingdom
10:15-10:45	PS2.2 Genomic and post-genomic diversity in fungal plant biomass utilization **Ronald de Vries** Westerdijk Fungal Biodiversity Institute, The Netherlands
10:45-11:15	**Coffee break**
11:15-11:45	PS2.3 Repeat Induced Point mutations, variations on a theme **Fabienne Malagnac** Université Paris Saclay, France

11:45-12:15	PS2.4 Crossroads in drug resistance and host adaptation in Candida opportunistic pathogens **Toni Gabaldón** Barcelona Supercomputing Centre and Institute for Research in Biomedicine, Spain	
12:15-12:45	PS2.5 Fungi in Glacial and Hypersaline Environments - what can we learn from their genomes? **Nina Gunde-Cimerman** Biotechnical Faculty, University of Ljubljana, Slovenia	
12:45-14:00	**Lunch break**	
12:45-13:25	EARLY CAREER AUTHOR WORKSHOP What to consider when publishing your research **Mark Gannon** Elsevier	*Location:* ***Hall Tirol***

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TUESDAY, MARCH 7

14:00-16:00	CS3.1 EVOLUTION, BIODIVERSITY AND TAXONOMY CHAIRS: **Martin Grube** University of Graz, Austria **Nina Gunde-Cimerman** University Of Ljubljana, Slovenia	*Location:* ***Hall Tirol***
14:00-14:15	CS3.1.1 Nuclear interactions in heterokaryons of the filamentous ascomycete Neurospora tetrasperma **Hanna Johannesson,** Stockholm University, Sweden	
14:15-14:30	CS3.1.2 Genomic consequences of hybridisation in a fungal syngameon **Fernando Fernandez-Mendoza,** Karl-Franzens-Universität Graz, Austria	
14:30-14:45	CS3.1.10 The pan-genome behind the multifunctional root symbiotic insect pathogenic fungus Metarhizium brunneum **Carolina Nogueira,** University of Copenhagen, Denmark	
14:45-15:00	CS3.1.4 From populations to pan-genomes: identifying patterns of global genomic evolution in the porcini mushroom, Boletus edulis **Keaton Tremble,** University of Utah, USA	
15:00-15:15	CS3.1.5 Massive transposons as the crucible of evolution in fungi **Aaron Vogan,** Uppsala University, Sweden	

15:15-15:30 CS3.1.6 Genome analysis of Candida orthopsilosis marine isolates unveils missing parental lineage and suggests environmental origin of hybrids with pathogenic potential
Valentina del Olmo Toledo, Barcelona Supercomputing Center, Spain

15:30-15:45 CS3.1.7 Lifestyle transitions in basidiomycetous fungi are reflected by tRNA composition and translation efficiency
Marco Alexandre Guerreiro, Max Planck Institute for Evolutionary Biology, Germany

15:45-16:00 CS3.1.8 Resolving species boundaries in the Diaporthe eres species complex
FEMS grant
Sandra Hilário, University of Aveiro, Portugal

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TUESDAY, MARCH 7

14:00-16:00 **CS3.2 METABOLISM AND PHYSIOLOGY**
Location: ***Hall Brüssel***

CHAIRS:
Bernhard Seiboth
Technical University Vienna, Austria
Levente Karaffa
University of Debrecen, Hungary

14:00-14:15 CS3.2.1 Revitalizing Post-Consumer Plastic Waste: Trash to Treasure
Benjamin Miller, University Of Southern California, USA

14:15-14:30 CS3.2.2 The “manganese effect” during Aspergillus niger citric acid fermentation is dependent on the cultivation stage
Levente Karaffa, University of Debrecen, Hungary

14:30-14:45 CS3.2.3 Manganese and its regulatory role on the citrate transporter CexA – exploring the citric acid production mechanism of Aspergillus niger
Aline Reinfurt, Technical University Vienna, Austria

14:45-15:00 CS3.2.4 Plant biomass conversion is differently organized in basidiomycetes compared to ascomycetes
Miia Mäkelä, University of Helsinki, Finland

15:00-15:15 CS3.2.5 The paralogous transcription factors LeuR and LeuB regulate leucine biosynthesis, nitrogen assimilation, and iron metabolic pathways in Aspergillus nidulans
Richard Todd, Kansas State University, USA

15:15-15:30 CS3.2.6 Mannitol metabolism and osmotic stress answer: a comparative study on Trichoderma reesei and Aureobasidium pullulans
Audrey Masi, Technical University Vienna, Austria

15:30-15:45 CS3.2.7 The Siderophore Ferricrocin Mediates Iron Acquisition during Germination in Aspergillus fumigatus
Isidor Happacher, Medizinische Universität Innsbruck, Austria

15:45-16:00 CS3.2.8 Organelle-dependent synthesis of nitric oxide in fungi
David Canovas, University of Sevilla, Spain

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14:00 – 16:00 CS3.3 ANIMAL/HUMAN INTERACTIONS

Location: ***Hall Strassburg***

CHAIRS:
Ulrike Binder
Medizinische Universität Innsbruck, Austria
Ilse Jacobsen
Leibniz Institute For Natural Product Research And Infection Biology (HKI), Germany

14:00-14:15 CS3.3.1 The impact of colonization on infection - systemic candidiasis in mice
Ilse Jacobsen, Leibniz Institute For Natural Product Research and Infection Biology (HKI), Germany

14:15-14:30 CS3.3.2 Roles of candidalysin of Candida albicans in the gut permeability and brain pathology
Courtney Smith, UTSA Texas, USA

14:30-14:45 CS3.3.3 Airway epithelial cells as a novel intracellular host reservoir for Cryptococcus spores
FEMS grant
Sébastien C. Ortiz, University of Manchester, United Kingdom

14:45-15:00 CS3.3.4 Do extracellular RNAs released upon infection with Aspergillus fumigatus contribute to antifungal defense?
Alexander Bruch, Leibniz Institute For Natural Product Research and Infection Biology (HKI), Germany

15:00-15:15 CS3.3.5 Host brain environment triggers MAPK RNAi-based epimutation in the human pathogen Mucor circinelloides
Maribel Navarro-Mendoza, Duke University School of Medicine, USA

15:15-15:30	CS3.3.6 Dual RNA-Seq reveals expression signatures beneficial for iron uptake and intracellular long-term interaction of Lichtheimia corymbifera (Mucorales) with macrophages **Kerstin Voigt,** University of Jena, Germany
15:30-15:45	CS3.3.7 Functional studies of Coccidioides CPS1, and creation of a live attenuated vaccine against Coccidioidomycosis **Marc Orbach,** University of Arizona, USA
15:45-16:00	CS3.3.8 Unraveling the biology of Nematophagy during a Fungal-Nematode Predator-Prey Interaction Using Time-Course Transcriptomic analysis **Hung-Che Lin,** Academia Sinica Taipei, Taiwan

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14:00 – 16:00 **CS3.4 SYMBIONTS AND ENDOPHYTES**

Location: ***Hall Freiburg***

CHAIRS:
Benjamin Horwitz
Technion - Israel Institute Of Technology, Israel
Ursula Peintner
Universität Innsbruck, Austria

14:00-14:15	CS3.4.1 Local Endoreduplication of the host is a conserved process during Phytomyxea-host interaction **Michaela Hittorf,** Universität Innsbruck, Austria
14:15-14:30	CS3.4.2 Genomic features of endophytism and host adaptation in the Arabidopsis thaliana root mycobiome **Fantin Mesny,** Max Planck Institute for Plant Breeding Research / University of Cologne, Germany
14:30-14:45	CS3.4.3 Mycoheterotrophic orchids and their symbionts: A metatranscriptomic approach **Gregor Langen,** University of Cologne, Germany
14:45-15:00	CS3.4.4 Role of the SP7-like effectors in the arbuscular mycorrhizal symbiosis **David Figueira-Galán,** Karlsruhe Institute of Technology, Germany
15:00-15:15	CS3.4.5 Biocontrol potential of a tomato fungal endophyte against P. syringae **Luisa Liu-Xu,** Universitat Jaume I, Spain
15:15-15:30	CS3.4.6 Are all the same? An evolutionary story of lichen symbioses **David Díaz Escandón,** University of Alberta, Canada

15:30-15:45 CS3.4.7 Synthetic mutualism in plant-fungus interactions
Marcel Bucher, University of Cologne, Germany

15:45-16:00 CS3.4.8 Reducing fungal endophytes in wheat and its effects on plant fitness
Or Sharon, Institute for Cereal Crops Research, Tel Aviv University, Israel

16:00-17:30 **Poster Session & Coffee break**

Poster Session 2

CS2.1.9 – CS2.9.55
Synthetic biology and biotechnology

CS2.2.9 – CS2.4.31
Development and morphogenesis

CS5.1.10 – CS5.1.69
Genomes and other -omics

CS5.2.9 – CS5.2.29
Mycobiomes and microbial interactions

CS5.3.9 – CS5.3.21
Stress and extreme environments

CS5.4.9 – CS5.4.20
Regulatory Networks

17:30-19:30 **CS4.1 ANTIFUNGALS AND RESISTANCE MECHANISMS** *Location:* ***Hall Tirol***

CHAIRS:
Michaela Lackner
Medizinische Universität Innsbruck, Austria
Gabriel Scalliet
Syngenta Crop Protection AG, Switzerland

17:30-17:45 CS4.1.1 Systematic Discovery of Antibacterial and Antifungal Bacterial Toxins
FEMS grant
Marina Campos Rocha, The Hebrew University of Jerusalem, Israel

17:45-18:00 CS4.1.3 Systematic characterization of anti-fungal resistance mutations and resistance function trade-offs using genome editing
Philippe Després, Université Laval, Canada

18:00-18:15 CS4.1.4 Mutator phenotypes in Aspergillus fumigatus drive the rapid evolution of antifungal resistance
Michael Bottery, The University of Manchester, United Kingdom

18:15-18:30 CS4.1.5 Remodelling the anti-oomycetes efficacy screenings: exploring new frontiers and refining the existing
Demetrio Marcianò, University of Milan, Italy

18:30-18:45 CS4.1.6 The complex genetic landscape of fungicide resistance evolution in Zymoseptoria tritici
Guido Puccetti, Université de Neuchâtel, Switzerland

18:55-19:00 CS4.1.7 The use of targeted antifungal liposomes against Rhizopus delemar
Quanita Choudhury, University of Georgia, USA

19:00-19:15 CS4.1.8 Analysis of the Aspergillus fumigatus proteomic response to amphotericin B (AmB) reveals involvement of a putative flippase in resistance
Olaf Kniemeyer, Leibniz Institute For Natural Product Research and Infection Biology (HKI), Germany

19:15-19:30 CS4.1.15 Resistance complex III inhibitors in the wheat pathogen Zymoseptoria tritici
Gabriel Scalliet, Syngenta Crop Protection, Switzerland

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TUESDAY, MARCH 7

17:30 – 19:30 **CS4.2 FUNGAL CELL BIOLOGY**
Location: ***Hall Brüssel***

CHAIRS:
Alexander Lichius
Universität Innsbruck, Austria
Miguel A. Peñalva
Consejo Superior De Investigaciones Científicas, Spain

17:30-17:45 CS4.2.1 The HUM complex is a myosin-5 adaptor to secretory vesicles
Miguel A. Peñalva, Consejo Superior De Investigaciones Científicas, Spain

17:45-18:00 CS4.2.2 Structural and molecular investigation of secondary metabolite compartmentalization in fungal vesicles
Fabio Gherlone, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Germany

18:00-18:15 CS4.2.3 The vacuolar morphology protein VAC14 plays an important role in sexual development of Sordaria macrospora
Stefanie Pöggeler, Georg-August University Göttingen, Germany

18:15-18:30 CS4.2.4 Exploring septation-dependent and -independent roles of the Aspergillus fumigatus Septation Initiation Network
FEMS grant
Xabier Guruceaga, University of Tennessee Health Science Center, USA

18:30-18:45 CS4.2.5 Septins, sterols, sphingolipids, and cell wall integrity
Michelle Momany
University of Georgia, USA

18:55-19:00 CS4.2.6 SIP-1 is essential for germling fusion of Neurospora crassa, probably by mediating the initiation of cell-cell communication
Anne Oostlander, TU Braunschweig, Germany

19:00-19:15 CS4.2.7 Cytoplasmic sequestering of the Cochliobolus heterostrophus stress-activated MAPK in response to a host plant phenolic acid
Benjamin Horwitz, Technion - Israel Institute of Technology, Israel

19:15-19:30 CS4.2.8 Filament branching in the human fungal pathogen Candida albicans
FEMS grant
Antonio Serrano Salces, Université Côte d'Azur, CNRS, INSERM, Institute of Biology Valrose (iBV), France

17:30 – 19:30 **CS4.3 RNA BIOLOGY**

Location: ***Hall Freiburg***

CHAIRS:
Astrid Mach-Aigner
Technical University Vienna, Austria
Erzsébet Fekete
University of Debrecen, Hungary

17:30-17:45 CS4.3.1 Uncovering the sequence and structural determinants guiding m6A evolution via inter and intra-species hybrids
Ran Shachar, Weizmann Institute of Science, Israel

17:45-18:00 CS4.3.2 Small RNAs from the plant pathogen Sclerotinia sclerotiorum associate with host quantitative disease resistance genes
Mark Derbyshire, Curtin University, Australia

18:00-18:15 CS4.3.3 RNAi spray-mediated silencing of Alternaria alternata AGO and DCL gene transcripts enhanced resistance to Alternaria black spot disease
Conrad Chibunna Achilonu, University of the Free State, South Africa

18:15-18:30 CS4.3.4 New Propagation Mechanism for Co-Existing Stwintrons and Derived Canonical Introns
Erzsébet Fekete, University of Debrecen, Hungary

18:30-18:45 CS4.3.5 The Dicer/R3B2 complex: a novel interaction in the center of the RNAi-related mechanisms of Mucor lusitanicus
José Tomás Cánovas-Márquez, University of Murcia, Spain

18:55-19:00	CS4.3.6 Pathogenic oomycetes with diverse hosts contain different RNA silencing proteins but similar small RNA profiles FEMS grant **Edoardo Piombo,** Swedish University of Agricultural Sciences, Sweden
19:00-19:15	CS4.3.7 Artificial nanovesicles for dsRNA delivery in Spray induced gene silencing (SIGS) for crop protection **Jonatan Niño Sánchez,** University of Valladolid, Spain
19:15-19:30	CS4.3.8 Long non-coding RNAs in the interaction between Mucorales causing mucormycosis and host defense cells **Ghizlane Tahiri,** Murcia University, Spain

17:30 – 18:30 **CS4.4A FUNGAL EPIDEMIOLOGY AND DIAGNOSTICS**

Location: ***Hall Strassburg***

CHAIRS:
Sigrid Neuhauser
Universität Innsbruck, Austria
Oliver Kurzai
University of Würzburg, Germany

17:30-17:45	CS4.4a.1 The importance of spore-dispersal to initiate new Armillaria root rot infections in gardens **Jassy Drakulic**, Royal Horticultural Society, United Kingdom
17:45-18:00	CS4.4a.2 Novel short tandem repeat typing and whole genome sequencing analysis on Sporothrix brasiliensis isolates reveal independent outbreaks in Brazil **Bram Spruijtenburg,** Center of Expertise in Mycology, Radboud University Medical Center/ Canisius-Wilhelmina Hospital, The Netherlands
18:00-18:15	CS4.4a.3 Population genomics links clinical Aspergillus fumigatus triazole-resistant isolates to environmental hotspots and uncovers an agricultural fungicide exposure history **Eveline Snelders,** Wageningen University & Research, The Netherlands
18:15-18:30	CS4.4a.4 #EUROBLAST: Disease surveillance of cultivated crop plants and their wild relatives for detection of emerging epidemics **Thorsten Langner,** The Sainsbury Laboratory, University of East Anglia, Norwich Research Park, United Kingdom

18:30 – 19:30	**JGI Genomics Workshop** CHAIRS: **Igor Grigoriev** Joint Genome Institute, USA **László Nagy** Biological Research Centre, Hungary	*Location:* ***Hall Strassburg***
18:30-18:45	JGI multi-omics resources for fungal biology **Igor Grigoriev,** Joint Genome Institute, USA	
18:45-19:00	CS5.1.69 Understanding the evolution and functioning of symbiosis: intra-genus and intra-species molecular diversity in the ectomycorrhizal genus Pisolithus **Annegret Kohler,** INRAE Grand-Est Nancy, France	
19:00-19:15	CS5.1.13 Transcriptomic analyses of white and brown rot basidiomycetes, with emphasis in Pleurotus ostreatus, reveal new enzymes involved in lignocellulose degradation **Idoia Jimenez,** Public University of Navarre, Spain	
19:15-19:30	Beyond primary coding sequence in fungal genomes: high-throughput functional assays for decoding fungal gene regulation. **László Nagy,** Biological Research Centre, Hungary	

WEDNESDAY, MARCH 8

09:00 – 12:45	PLENARY SESSION 3 **Metabolism and development** supported by Fungal Biology and Biotechnology (Springer Nature) CHAIRS: **Joseph Strauss** BOKU-University of Natural Resources and Life Sciences, Austria **David Canovas** Universidad de Sevilla, Spain	*Location:* ***Hall Tirol***
09:00-09:45	KEY NOTE 3 EMBO Lecture Dissecting the mechanism of function of a fungal transporter: a long persistent trip from genetics to structure **George Diallinas** Department of Biology, National and Kapodistrian University of Athens, Greece	
09:45-10:15	PS3.1 Chromatin-controlled regulation of fungal development and mycotoxin biosynthesis **Özgür Bayram** Maynooth University, Ireland	
10:15-10:45	PS3.2 Fungal metabolism that contributes to a new socio-economic development? Anything goes! **Vera Meyer** Technische Universität Berlin, Germany	
10:45-11:15	**Coffee break**	

11:15-11:45	PS3.3 RNAi-dependent epimutations evoke transient antifungal drug resistance **Joseph Heitman** Duke University, USA	
11:45-12:15	PS3.4 Cellular sorting of chitin synthases in Neurospora crassa **Meritxell Riquelme** Centro de Investigación Científica y de Educación Superior de Ensenada, Mexico	
12:15-12:45	PS3.5 Conidial transcription before dormancy matters **Chris Koon Ho Wong** University of Macau, China	
12:45-14:00	**Lunch break**	
12:45-14:00	ISC MEETING	*Location:* ***Hall Aalborg***

14:00-16:00	**CS5.1 GENOMES AND OTHER –OMICS** CHAIRS: **Igor Grigoriev** Joint Genome Institute, USA **László Nagy** Biological Research Centre, Hungary	*Location:* ***Hall Tirol***
14:00-14:15	CS5.1.1 Giant Starship elements are engines of adaptive variation in fungal pathogens **Emile Gluck-Thaler,** Université de Neuchatel, Switzerland	
14:15-14:30	CS5.1.2 Genomic Innovations and Horizontal Gene Transfer sculpt the lifestyle of Armillaria species FEMS grant **Neha Sahu,** Biological Research Center, Hungary	
14:30-14:45	CS5.1.57 Lessons from genomic and transcriptomic analysis of five marine-derived fungi **Frank Kempken,** Kiel University, Germany	
14:45-15:00	CS5.1.4 Chromosome-level assemblies from diverse clades reveal limited structural and gene content variation in the genome of Candida glabrata **Marina Marcet-Houben,** Barcelona Supercomputing Center, Spain	
15:00-15:15	CS5.1.5 Machine learning prediction of novel pectinolytic enzymes in Aspergillus niger through integrating heterogeneous (post-) genomics data **Mao Peng,** Westerdijk Fungal Biodiversity Institute, KNAW, The Netherlands	

15:15-15:30 CS5.1.6 Pan-genomics uncover mechanisms behind the rapid evolution of spinach downy mildew
Petros Skiadas, Utrecht University, The Netherlands

15:30-15:45 CS5.1.7 Phylogenetic analysis and investigation of RiPP cluster in 50 Trichoderma genomes
Matthias Schmal, Technical University Vienna, Austria

15:45-16:00 CS5.1.8 Exploration of transporter gene family evolution in ectomycorrhizal fungi in relation to mineral weathering capabilities
Katharine King, Swedish University of Agricultural Sciences, Sweden

14:00-16:00 **CS5.2 MYCOBIOMES AND MICROBIAL INTERACTIONS**
Location: ***Hall Brüssel***

CHAIRS:
Christoph Schüller
BOKU-University of Natural Resources and Life Sciences, Austria
Claire Stanley
Imperial College London, United Kingdom

14:00-14:15 CS5.2.1 Effect of fungal hyphae on dispersal and growth of anaerobic bacteria
Lukas Yvo Wick, Helmholtz Centre for Environmental Research, UFZ, Germany

14:15-14:30 CS5.2.2 The human fungal pathogen Aspergillus fumigatus holds a core bacteriome
Cristina Silva Pereira, ITQB Nova, Portugal

14:30-14:45 CS5.2.3 Fungal-bacterial interactions in glacier forefields: to the Alps and beyond
Edoardo Mandolini, Universität Innsbruck, Austria

14:45-15:00 CS5.2.4 Chemotactic signals regulating the interaction of Sclerotinia sclerotiorum and two soil oxalotrophic bacteria
Pilar Junier, University of Neuchatel, Switzerland

15:00-15:15 CS5.2.5 Antagonist-specific defence responses of the coprophile mushroom Coprinopsis cinerea against bacteria and fungivorous nematodes
Markus Künzler, ETH Zürich, Switzerland

15:15-15:30 CS5.2.6 Hyphosphere and microbial communities: can fungi also change their environment?
Irina Druzhinina, Royal Botanic Gardens Kew, United Kingdom

15:30-15:45	CS5.2.7 Polaramycin B, and not physical interaction, is the signal that rewires fungal metabolism in the Streptomyces-Aspergillus interaction **Harald Berger,** BOKU-University of Natural Resources and Life Sciences, Austria
15:45-16:00	CS5.2.8 A global survey of host, aquatic, and soil microbiomes reveals ecological properties shared between bacterial and fungal generalsists **Amelia Barber,** Friedrich Schiller University, Germany

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14:00-16:00 **CS5.3 STRESS AND EXTREME ENVIRONMENTS** *Location: **Hall Strassburg***

CHAIRS:
Drauzio Eduardo Naretto Rangel
Universidade Tecnológica Federal do Paraná, Brazil
Laura Selbmann
University Of Tuscia, Italy

14:00-14:15	CS5.3.1 Recombination, clonality and hybridization in fungi from extreme environments **Cene Gostincar,** University Of Ljubljana, Biotechnical Faculty, Slovenia
14:15-14:30	CS5.3.2 New insights into the mechanisms involved in resisting copper toxicity in the pathogenic fungus Aspergillus fumigatus **Nir Osherov,** Tel Aviv University, Israel
14:30-14:45	CS5.3.3 The roles DHN melanin and the stress-activated MAP kinase in the rock inhabitant Knufia petricola **Julia Schumacher,** Bundesanstalt für Materialforschung und –prüfung (BAM), Germany
14:45-15:00	CS5.3.4 Specific genomic traints drive diverse ecologies throughout the extremes in stress-tolerant black fungi **Claudia Coleine,** University of Tuscia, Italy
15:00-15:15	CS5.3.5 Phase separation as a potential mechanism for cold-stress tolerance in polar fungi **Steven Hanes,** SUNY Upstate Medical University, USA

15:15-15:30 CS5.3.6 Aspects of metal stress response of the ectomycorrhizal basidiomycete Tricholoma vaccinum
Manuela Östreicher, Friedrich Schiller University Jena, Germany

15:30-15:45 CS5.3.7 Increased protein solubility contributes to heat priming of the plant pathogenic fungus Botrytis cinerea
Mingzhe Zhang, Tel Aviv University, Israel

15:45-16:00 CS5.3.8 Potential and underlying mechanisms of Schizophyllum commune to remediate the Chernobyl exclusion zone
Lea Traxler, Friedrich Schiller University Jena, Germany

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14:00-16:00 **CS5.4 REGULATORY NETWORKS**

Location: ***Hall Freiburg***

CHAIRS:
Christian Zimmermann
Technical University Vienna, Austria
Manuel Sánchez López-Berges
Universidad de Córdoba, Spain

14:00-14:15 CS5.4.1 Light perception in Aspergillus nidulans, A. fumigatus and Alternaria alternata
Reinhard Fischer, Karlsruhe Institute of Technology (KIT), Germany

14:15-14:30 CS5.4.2 Apoplastic space of two cultivars provides highly different environments for the pathogen colonization: insights from proteome and microbiome profiling
Carolina Sardinha Francisco, Christian-Albrechts University of Kiel, Germany

14:30-14:45 CS5.4.3 A transcription profiling approach to study the Aspergillus nidulans kinome
Zhiqiang Dong, University of Macau, China

14:45-15:00 CS5.4.4 The influence of epigenetic modifications on effector gene expression and pathogenicity in Fusarium oxysporum
Slavica Janevska, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Germany

15:00-15:15 CS5.4.5 Role of the transcription factor MacA in Fusarium oxysporum pathogenicity
FEMS grant
Rafael Palos Fernández, Universidad de Córdoba

15:15-15:30	CS5.4.6 Investigation of gene regulatory networks underlying pattern formation in Coprinopsis cinerea **Hongli Wu,** Biological Research Centre, Hungary
15:30-15:45	CS5.4.7 Transcriptome meta-analysis unveils link between transcription and splicing networks in fungi **João Neves da Rocha,** University Of São Paulo, FMRP, Brazil
15:45-16:00	CS5.4.8 Identifying global regulators of effector gene expression in the rice blast fungus **Camilla Molinari,** The Sainsbury Laboratory, United Kingdom

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16:00-17:30	**Poster Session & Coffee break** **Poster Session 3** CS3.1.9 – CS3.1.53 Evolution, biodiversity and taxonomy CS3.2.9 – CS3.2.35 Metabolism and physiology CS3.3.9 – CS3.3.15 Animal/human interactions CS3.4.9 – CS3.4.17 Symbionts and endophytes CS4.1.9 – CS4.1.46 Antifungals and resistance mechanisms CS4.2.9 – CS4.2.46 Fungal cell biology CS4.3.9 – CS4.3.14 RNA biology CS4.4a.5 – CS4.4a.12 Fungal epidemiology and diagnostics

17:30-18:30 CLOSING CEREMONY

Location: ***Hall Tirol***

Announcement of the next meeting
Özgür Bayram
Maynooth University, Ireland

Concluding remarks
Susanne Zeilinger-Migsich
Universität Innsbruck, Austria
Hubertus Haas
Medizinische Universität Innsbruck, Austria

Presentation of the Poster Prizes

CHAIRS:
Monika Schmoll
University of Vienna, Austria
Oded Yarden
Hebrew University of Jerusalem, Israel
Massimo Reverberi
Sapienza University, Italy
Lene Lange
LI-bioeconomy, Denmark

19:30-22:30 CONFERENCE DINNER

Location: ***Villa Blanka***

bus transfer: departure 19:00 – 19:15
return shuttle: 22:30 – 23:30

21:00-01:00 ECFG16 PARTY AT TREIBHAUS

Location: ***Treibhaus***

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NOTES

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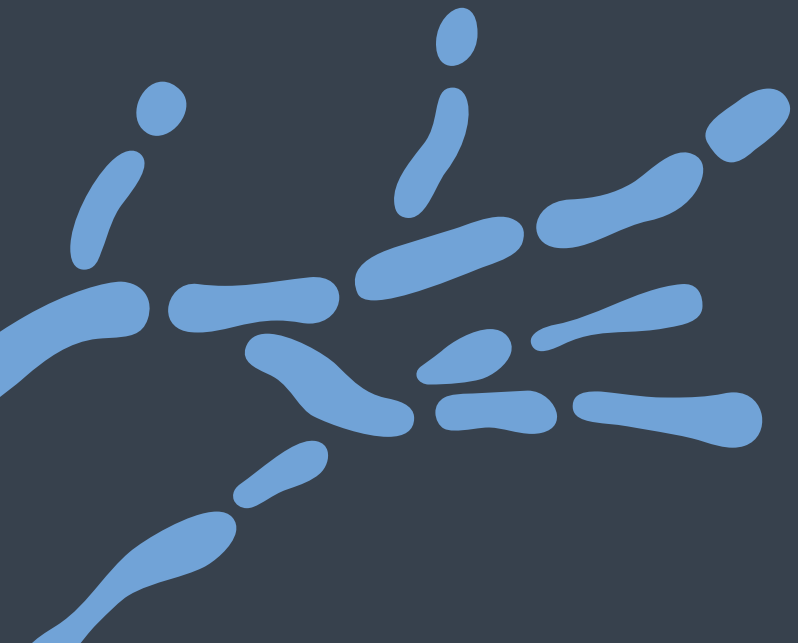

ABSTRACTS KEYNOTE AND PLENARY TALKS

OPENENING LECTURE

FROM REGULATED CELL DEATH IN YEAST TO ANTI-AGING AND ANTI-FUNGAL TREATMENTS

Frank Madeo[1]
[1]Karl-Franzens Universität Graz, Graz, Austria

Yeast can undergo programmed cell death displaying diagnostic features of apoptosis and necrosis. Physiologically, yeast cell death can be triggered by chronological aging. We used chronological aged yeast cells to discover the polyamine spermidine as a natural autophagy inducer. Subsequently, we found Spermidine supplementation to be geroprotective in higher eukaryotes.
Further, we screened for substances inducing yeast programmed cell death and identified novel natural metabolites that confer broad anti-fungal activity.

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PLENARY SESSION 1 - FUNGAL INTERACTIONS

KEY NOTE 1: ROOT CAP CELL CORPSE CLEARANCE LIMITS MICROBIAL COLONIZATION

Alga Zuccaro[1]
[1]University of Cologne, Cologne, Germany

Programmed cell death (PCD) in plants is a fundamental cellular process during development but can also be triggered upon biotic and abiotic stresses. In Arabidopsis thaliana, PCD is an integral part of the differentiation process of the root cap, a specialized organ that surrounds meristematic stem cells. The acquisition of cell death competence in lateral root cap (LRC) cells depends on the root cap-specific transcription factor ANAC033/SOMBRERO (SMB). Cell death is followed by a rapid cell-autonomous corpse clearing process on the root surface, involving the senescence-associated nuclease BFN1 downstream of SMB. Since the beneficial root endophyte Serendipita indica downregulates BFN1 during colonization, we investigated the roles of SMB and BFN1 in fungal accommodation. We find that roots of smb3 mutants, which are deficient in root cap PCD and corpse clearance, are entirely covered by undegraded LRC cell corpses loaded with protein aggregates. The accumulation of uncleared cell corpses promotes intra- and extra-radical colonization by S. indica, which in turn is sufficient to clear the cell corpses from the surface of smb3 roots. Compared to smb3, the bfn1-1 knockout mutant exhibits an attenuated corpse clearance phenotype, but still enhances intra-radical colonization by S. indica. These results highlight the importance of root cap differentiation in plant-microbe interactions and show that the constant production and clearance of LRC cells represents a sophisticated physical defense mechanism to prevent microbial colonization in close proximity to meristematic stem cells. Furthermore, we propose a mechanism by which S. indica manipulates PCD in Arabidopsis roots by downregulating BFN1 to promote fungal colonization through reduced clearance of dead LRC cells as a potential sources of nutrients.

PS1.1 REGULATED CELL DEATH IN INNATE IMMUNITY OF FUNGI

Teresa Pawlowska[1]
[1]School of Integrative Plant Science, Cornell University, USA

Innate immunity is an ancient cell-autonomous property of eukaryotes that allows them to regulate interactions with antagonistic microbes. Due to convergent evolution, animal and plant immune systems share remarkable functional similarities of the surveillance, signal transduction, and response modules. Among others, these similarities include regulated death of infected cells (RCD) as an ultimate mechanism for eliminating microbial intruders. While fungi react to antagonistic bacteria with behaviors resembling innate immunity responses of animals and plants, the role of RCD in fungal ability to restrict the proliferative niche of infective prokaryotes has not been explored in depth. Our experiments confronting asymbiotic isolates of Rhizopus microsporus and Mucor lusitanicus with Mycetohabitans sp. bacterial antagonists indicate that these fungi react to bacteria with production of reactive oxygen species and lipid peroxidation, leading to membrane damage and cell death. These findings suggest that fungi, like animals and plants, rely on immune RCD to protect their cellular integrity against microbial invasions.

PS1.2 LESSONS TO LEARN FROM A GALL-INDUCING FUNGUS

Armin Djamei[1]
[1]Excellence University of Bonn, Bonn, Germany

Smut fungi form a large group among the basidiomycetes and are biotrophic specialists in infecting a diverse set of mainly grasses, among them important crops like sorghum, millet, barley and maize. The maize smut fungus Ustilago maydis serves as an important model for smuts fungi and induces prominent galls on all aerial parts of its host, reflecting a metabolic and developmental reprogramming of the plant. This massive manipulation of the host is achieved with the help of fungal secreted molecules, so called effectors. In a systematic approach we screened in the past decade hundreds of putative effector proteins to identify their specific place of action and their functions on the plant side. Here I will present our current molecular understanding of the fungal effectome and the biotrophic interaction between the fungus and its host plant maize. Main focus will be given to a group of effectors suppressing the central negative regulator Topless in plants, thereby explaining various central aspects of biotrophy and revealing the surprising redirection of a conserved plant developmental program by U. maydis.

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PS1.3 FIGHT ON SURFACES PRIOR TO THE ONSET OF FUNGAL PATHOGENESIS IN INSECTS

Chengshu Wang[1]
[1]Shanghai Institute of Plant Physiology And Ecology, Chinese Academy of Sciences, Shanghai, China

Insect pathogenic fungi infect host through spore adhesion and penetration of cuticles. In addition to innate immunity in physiological context, insects evolved behavioral defenses against fungal parasite attacks. By using Drosophila melanogaster as an infection model, we found that the spore surface CFEM (common in fungal extracellular membrane) protein Mcdc9 of Metarhizium robertsii could be detected by fly through a chemosensory protein to activate the hygienic grooming to wipe off spores from body surfaces. The deletion of this gene could thus substantially increase fungal virulence against the flies. Similar to the barrier function of human skin microbiotas, emerging evidence has indicated that insect cuticles are inhabited by diverse bacteria to form ecto-microbiomes that can combat fungal parasite infections. We found that bacterial loads quickly increased on fly surfaces along with Drosophila age increase, which could facilitate insects to defend against fungal infections. Cross-inhibition assays indicated that the dominant bacteria isolated from the Drosophila surfaces could inhibit the germination of Beauveria bassiana and Metarhizium robertsii spores and therefore the penetration and infection of insects by these fungi. To outcompete insect cuticular microbiotas, Metarhizium species can produce and store the potent antibiotic of triterpene helvolic acid in conidial spores. Intriguingly, the closely-related Beauveria species do not have the biosynthetic gene cluster for the production of helvolic acid. Instead, the defensin-like antimicrobial gene is expressed by B. bassiana to suppress the insect cuticular microbiomes and therefore facilitate fungal penetration of cuticles and colonization of insects. Our data provide the previously-overlooked insights into the complex interactions prior to fungal colonization of insect hosts.

PS1.4 A MASTER BZIP TRANSCRIPTIONAL REGULATOR IN THE C. AURIS FUNGAL PATHOGEN LINKS METABOLISM, TISSUE TROPISM, MORPHOGENESIS AND ANTIFUNGAL RESISTANCE

Karl Kuchler[1]
[1]Medical University of Vienna, Campus Vienna Biocenter, Vienna, Austria

Candida auris is a newly emerging opportunistic human fungal pathogen, causing global hospital outbreaks of fungal infections of high mortalities in immunocompromised individuals. The pronounced skin tropism, its (pan-antifungal) multidrug resistance (MDR) traits, and the ease of transmission in clinical settings prompted the WHO to declare C. auris as a top priority fungal pathogen for drug development. Azoles cannot be employed for treatment, and even ~30% of patient isolates are AmB- and ~5-8% are echinocandin-resistant, thus severely threatening successful clinical therapy. Importantly, the precise mechanisms contributing to complex and dynamic MDR traits in C. auris remain ill-posed. We have thus employed integrative omics approches, including the integration of data to identify and dissect possible mechanisms underlying the pathophysiology and MDR development in C. auris. Comparative proteomics and extensive RNA-seq approaches enabled the discovery of C. auris genes controling pathogenicity, including those implicated in cellular detoxification, mitochondrial metabolism, cell wall function, adhesion and/or drug transport, as well as chromatin modifications. We discover a highly conserved bZip-family master regulator that controls bicarbonate metabolism and morphogenetic signaling, as well as fitness and host immune evasion. In my talk, I will discuss molecular principles of Candida auris pathophysiology, pan-antifungal MDR and host immune surveillance. Finally, I shall discuss whether potential Candida auris genes suitable for antifungal discovery may also serve as feasible drug targets in other medically relevant human fungal pathogens

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PS1.5 GENOMIC AND METABOLIC ADAPTATION MECHANISMS OF SYMBIOTIC FUNGI FROM INSECTS

Christine Beemelmanns[1]
[1]Helmholtz-Institut für Pharmazeutische Forschung Saarland (HIPS), Saarbrücken, Germany

The rapid development of metabolomics and genomic sequencing technologies has enabled a renaissance of natural product research and allowed the chemical analysis of complex symbiotic systems at unprecedented levels and depths. Many of the identified microbial metabolites were found to have be important regulators of these interactions and pharmacological important activities, which making them promising pharmaceutical drug candidates. One of our studies within the field of ecology and genome-driven natural product chemistry focused on the fungus-growing termite symbiosis, in which the termites cultivate a symbiotic fungus Termitomyces for nutrition. Our recent studies uncovered that the fungal mutualist likely regulates its intertwined life style via the emission of specific volatile blends, which are spiked with different sets of sesquiterpenes. Transcriptomic coupled with genomic studies supported these findings.
We also investigated members of the fungal subgeneric taxon Pseudoxylaria, which only emerge when the colony is weakened. Comparative genomic studies uncovered a certain degree of adaptation to fungus comb as substrate and the colony as host. Metabolomic analyses of Pseudoxylaria isolates revealed specialized metabolites that could weaken growth of the fungal mutualist, and by that allow Pseudoxylaria to siphon nutrients from vegetative mycelium without instantaneously killing the fungus.

PLENARY SESSION 2 - GENOMICS, EVOLUTION & BIODIVERSITY

KEY NOTE 2: FUNGAL INTERACTIONS AND CO-ADAPTATION IN THE PLANT PHYLLOSPHERE

Eva Stukenbrock[1]
[1]Kiel University, Kiel, Germany

Plants are associated with a variety of microorganisms. Some microbes are highly specialized to a plant-associated lifestyle and play a detrimental role in plant health by promoting growth or conferring disease. In spite of their fundamental importance, we know surprisingly little about the ecology of these microbial species. We use the fungal grass pathogen Zymoseptoria spp as a model system to study fungal evolution in the context of molecular interactions with the host and the plant-associated microbiota. Species of Zymoseptoria infects the phyllosphere of a variety of different grass hosts. The species Z. tritici has co-evolved with wheat during domestication and provides an excellent model system to study fungal adaptation. Genome and transcriptome analyses have elucidated how host specialization of this pathogen has involved the acquisition of adaptive substitutions in genes encoding secreted proteins as well as changes in gene expression. So far, we have considered the host as a main driver of adaptive evolution in the pathogen, however in the phyllosphere the pathogen not only interacts with host-produced molecules, but also with the host-associated microbiota. To understand the contribution of plant microbiota in adaptation of pathogens, we have set out to study the interactions of Zymoseptoria pathogens with the microbiota of different grass hosts. We have used amplicon sequencing to characterize the impact of pathogen invasion on microbial composition in susceptible and resistant hosts. Moreover, we have generated a collection of endophytic bacteria and fungi from different wheat cultivars and species to test hypotheses concerning pathogen-microbiota interactions. We find evidence that Z. tritici actively suppresses antagonistic bacteria during colonization of susceptible hosts, however a resistant wheat cultivar hosts bacteria with strong pathogen suppressing effects, possibly contributing to the defense response. Intriguingly, we show genetic variation in microbial interactions among Z. tritici isolates possibly reflecting adaptation to different host microbiota.

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PS2.1 THE GLOBAL EMERGENCE OF ANTIFUNGAL RESISTANCE: THE CALM BEFORE THE STORM?

Matthew Fisher[1]
[1]Imperial College School of Public Health, London, United Kingdom

The Kingdom Fungi is a biodiverse and essential component of our habitable Planet. However, recent decades have seen an increase in the number of pathogenic fungi infecting natural populations and managed landscapes; fungi are increasingly recognized as presenting a worldwide threat to food security and the healthy functioning of ecosystems. In parallel, clinicians and biomedical scientists are fighting emerging fungal pathogens that infect millions of people every year and there are signs that fungi are become increasingly adapted to resist frontline antifungal therapies. Traditional approaches to studying the biology of fungal infections are currently being transformed by the growing number of high-quality assembled genomes, by world-wide surveys of fungi and by new technological and informatic strategies. This talk will discuss current challenges in analysing emerging fungal diseases in order to identify weaknesses in our armamentarium against fungal infections. Rapid progress is being made in our understanding of how to manage fungal disease in clinical, agricultural and natural settings, however mass-deployment of antifungal drugs, the development of monocultures and massive international trade has brought new risks to health, biodiversity and biosecurity. This talk will discuss how modern genomic toolkits are generating insights into how we can understand and tackle the emerging fungal threat.

PS2.2 GENOMIC AND POST-GENOMIC DIVERSITY IN FUNGAL PLANT BIOMASS UTILIZATION

Ronald de Vries[1]
[1]Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands

The rapidly increasing number of fungal genomes and post-genomic datasets has altered the way we do research dramatically and provided unprecedented insights into the diversity of fungi in topics. In our group a main focus of research is the utilization of plant biomass by fungi, covering all aspects of this process: extracellular degradation of plant biomass polymers, uptake of the resulting monomers and small oligomers, metabolic conversion of these compounds and the regulatory systems involved in this process. These studies have revealed differences in the approach fungi use for utilizing the same substrate at the genomic and post-genomic level, even between closely related species. Classifying these differences enables us to get a much better understanding of the evolution of plant biomass utilization in the fungal kingdom and the molecular basis of this process.
In addition, these insight provide near-unlimited opportunities for biotechnology by implementing a much larger part of this biodiversity into application with respect to enzyme discovery, design of fungal cell factories and the range of products that fungi can synthesize. However, none of this can be achieved without detailed understanding of gene function, expression and diversity, which often requires in-depth biochemical or genetic analysis.
In this talk I will address both the potential and limitations of our current (post-) genomic driven research on plant biomass utilization by fungi, illustrated by recent insights and results.

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PS2.3 REPEAT INDUCED POINT MUTATIONS, VARIATIONS ON A THEME

Fabienne Malagnac[1]
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Eukaryotic genomes are constantly under the threat of being invaded by replicating selfish DNA elements such as transposons. To prevent these elements from proliferating, several defense mechanisms exist. In human, plants, ciliates and yeasts, repeated DNA is usually silenced transcriptionally via RNA-direct DNA methylation, heterochromatin formation or DNA rearrangements and post-transcriptionally through various RNAi pathways. Filamentous fungi display an additional genome defense mechanism, independent of RNAi, occurring during sexual reproduction called RIP (Repeat-Induced Point mutations). First described in Neurospora crassa, RIP targets repeats, introducing C to T mutations, methylating some of the remaining cytosines and triggering heterochromatin assembly. RIPed sequences are heavily mutated leading to AT-rich regions that are silenced, making RIP a unique mechanism with both genetic and epigenetic consequences. Since its first description, signatures of RIP were detected in most Dikarya indicating that RIP is a conserved genome defense mechanism whose consequences have a major impact on fungal genome evolution. Indeed, fungi with a highly efficient RIP displayed only relics of mutated and silenced transposons in their genome.

Active mutagenic processes are considered antagonistic to genome stability. However, RIP challenges this paradigm, since its high mutation rate protects genome integrity. To study RIP, we use a powerful experimental system, the filamentous fungus Podospora anserina. We discovered that a RIP key gene (PaRid) encoding a DNA methyltransferase-like protein is also required for sexual reproduction during zygote formation. To decipher the potential links between RIP and sexual development, we developed a readout tool to assay RIP efficiency in various mutant backgrounds. Our findings further link RIP and chromatin modification during sexual developement.

PS2.4 CROSSROADS IN DRUG RESISTANCE AND HOST ADAPTATION IN CANDIDA OPPORTUNISTIC PATHOGENS

Toni Gabaldón[1]
[1]Barcelona Supercomputing Centre and Institute for Research in Biomedicine, Barcelona, Spain

Invasive fungal diseases such as candidiasis, caused by Candida species, are a major public health problem. They are difficult to diagnose and have high mortality rates. Moreover therapeutic options are limited, and resistance to multiple antifungal drugs is increasingly reported, particularly in emerging species such as Candida glabrata, Candida parapsilosis, and Candida auris. Despite their common genus name, Candida pathogens, are evolutionarily diverse and belong to different lineages where the ability to infect humans has emerged independently. Over the last years we have used in vitro evolution, and comparative genomicsapproaches to understand how the different pathogenic lineages adapted to humans and how they become resistant to the drugs we use to fight their infections. Here I will discuss recent findings revealing complex trade-offs in drug and host adaptation.

PS2.5 FUNGI IN GLACIAL AND HYPERSALINE ENVIRONMENTS - WHAT CAN WE LEARN FROM THEIR GENOMES?

Nina Gunde - Cimerman[1]
[1]Biotechnical Faculty, University Of Ljubljana, Ljubljana, Slovenia

Hypersaline waters and glacial ice are inhospitable environments, linked by low water activity and high concentrations of osmolytes. They are inhabited by diverse microbial communities, of which extremotolerant and extremophilic fungi are an essential component. Some fungi are specialised in only one of these environments and can thrive in conditions that are lethal to most other life forms. Others are generalists, highly adaptable species that occur in both environments and tolerate a wide range of different extremes. Sequencing of genomes of model organisms isolated from both types of low water activity environments helped to reveal how both groups efficiently balance cellular osmotic pressure and ion concentration, stabilise cell membranes, remodel cell walls and neutralise increased oxidative stress. Some species use unusual reproductive strategies, as evidenced by population genomics studies. These studies will allow us to understand and predict the role of these fascinating organisms in a rapidly changing environment and to explain how they survive some of the most extreme conditions on our planet.

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PLENARY SESSION 3 - METABOLISM & DEVELOPMENT

THE EMBO KEYNOTE LECTURE: DISSECTING THE MECHANISM OF FUNCTION OF A FUNGAL TRANSPORTER: A LONG PERSISTENT TRIP FROM GENETICS TO STRUCTURE

George Diallinas[1,2]
[1]Department of Biology, National and Kapodistrian University of Athens, Athens, Greece
[2] Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology, Heraklion Crete, Greece

Transporters are transmembrane proteins mediating the selective uptake or efflux of solutes, ions, metabolites and drugs across cellular membranes. Despite their immense biological importance in cell nutrition, communication, signaling and homeostasis, their study remains technically difficult mostly due to their lipid-embedded nature. The study of eukaryotic transporters presents additional complexity due to multiple subcellular control mechanisms that operate to ensure proper membrane traffic, membrane localization and regulated turnover. Here, I will highlight how Aspergillus nidulans has developed to be an excellent genetic system to study several aspects of transporter biology. In particular, I will present a collection of findings concerning structure-function relationships in the UapA purine transporter, a paradigmatic case of elevator-type transporters present in all organisms, which shed light on how specific substrate recognition and transport is determined and how this knowledge can be applied to genetically modify transporters. I will also very briefly present how studies on UapA membrane trafficking has unraveled a novel route of translocation to the plasma membrane via Golgi-bypass.

PS3.1 CHROMATIN-CONTROLLED REGULATION OF FUNGAL DEVELOPMENT AND MYCOTOXIN BIOSYNTHESIS

Özgür Bayram[1]
[1]Biology Department, Maynooth University, Maynooth, Ireland

Chromatin complexes control a vast number of epigenetic developmental processes in eukaryotes. Filamentous fungi present an important clade of microbes with poor understanding of underlying epigenetic mechanisms. Here, we describe a chromatin binding complex in the fungus Aspergillus nidulans composing of a H3K4 histone demethylase KdmB, a cohesin acetyltransferase (EcoA), a histone deacetylase (RpdA) and a histone reader/E3 ligase protein (SntB). In vitro and in vivo evidence demonstrate that this KERS complex is assembled from two the EcoA-KdmB and SntB-RpdA, heterodimers. KdmB and SntB play opposing roles in regulating the cellular levels and stability of EcoA, as KdmB prevents SntB-mediated degradation of EcoA via proteasomes. The KERS complex is recruited to transcription initiation start sites at active core promoters exerting promoter-specific transcriptional effects. It mainly controls regulatory genes for morphogenesis and secondary metabolism. Interestingly, deletion of any one of the KERS subunits results in a common negative effect on morphogenesis and production of secondary metabolites, molecules important for niche securement in filamentous fungi. Consequently, the entire mycotoxin sterigmatocystin gene cluster is downregulated and asexual development is reduced in the four KERS mutants. The elucidation of the recruitment of epigenetic regulators to chromatin via the KERS complex provides the first mechanistic, chromatin-based understanding of how development is connected with small molecule synthesis in fungi.

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PS3.2 FUNGAL METABOLISM THAT CONTRIBUTES TO A NEW SOCIO-ECONOMIC DEVELOPMENT? ANYTHING GOES!

Vera Meyer[1]
[1]Technische Universität Berlin, Berlin, Germany

What will the world of tomorrow look like? We don't know. What we do know is that our current economy, including its fossil-based manufacturing processes, have contributed to climate change, and that humanity is called upon to stop such practices if we want to ensure survival of our civilization. This requires that we develop an alternative economy that both respects planetary boundaries and is based on renewable resources. Research on socio-economic transformation asks what such a future economy could look like, and how this can respect environmental limits within which humanity can safely operate while also serving social well-being. Fungal biotechnology can start playing a key role in this transformation, as fungal metabolism converts organic and especially plant-based renewable biomass into virtually any product for everyday life. In my talk, I will use two fungal species as examples – the industrial cell factory Aspergillus niger and the medicinal polypore Fomes fomentarius – to highlight the impressive variety of products and productivities of fungal metabolic processes. Additionally, I will discuss how the synthesis of systems biology thinking, modelling approaches and synthetic biology can expand this range of products even further and provides new leads for genetic, metabolic and morphology engineering. I will also explain how the integration of artistic research and citizen science into ongoing research projects can contribute to making fungal biotechnology more open and socially relevant, and thus potentially more sustainable.

PS3.3 RNAI-DEPENDENT EPIMUTATIONS EVOKE TRANSIENT ANTIFUNGAL DRUG RESISTANCE

Joseph Heitman[1]
[1]Duke University, Durham, USA

Microbes evolve antimicrobial resistance through stable and unstable genetic mechanisms, such as aneuploidy underlying azole resistance in Candida and Cryptococcus. We discovered a new mechanism conferring FK506 antifungal drug resistance in the human fungal pathogen Mucor circinelloides. The natural product FK506 binds to the peptidyl-prolyl isomerase FKBP12 forming a complex that inhibits the protein phosphatase calcineurin. Calcineurin inhibition by FK506 blocks M. circinelloides dimorphic transition to hyphae enforcing yeast growth. In some FK506-resistant isolates, mutations in the genes encoding FKBP12 or calcineurin A or B confer resistance, restoring hyphal growth. In other resistant isolates, RNAi has silenced the FKBP12 gene, yielding drug-resistant epimutants that revert to sensitivity in the absence of FK506. Epimutation is accompanied by generation of abundant small RNAs targeting the FKBP12 gene and requires some known RNAi pathway components whereas others are dispensable. Surprisingly, epimutants occur at a higher frequency and are more stable in mutants lacking RNA-dependent RNA polymerase 1 or 3 or the RNaseIII-like protein R3B2, revealing some RNAi components inhibit epimutation. FKBP12 silencing appears to involve generation of a double-stranded RNA intermediate using the fkbA mature mRNA as template to produce antisense fkbA RNA. These findings have been generalized showing epimutations occur in three Mucor species and identifying epimutations in the pyrF/pyrG genes conferring 5-fluoroorotic acid resistance. Studies in murine models reveal infection-related stress can induce epimutation and drug resistance, and that epimutants are stable during infection. Epimutations are inheritable following sexual reproduction and heterochromatic marks are not associated with RNAi-dependent epimutations. These studies uncover a novel, reversible epigenetic RNAi-based epimutation mechanism controlling phenotypic plasticity, with implications for antimicrobial drug resistance, mechanisms of pathogenesis, and RNAi-regulatory mechanisms in fungi and other eukaryotes. The full impact of epimutations in this and other systems may have eluded discovery given their unstable nature.

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PS3.4 CELLULAR SORTING OF CHITIN SYNTHASES IN NEUROSPORA CRASSA

Meritxell Riquelme[1]
[1]Centro de Investigación Científica y de Educación Superior de Ensenada, Ensenada, Mexico

In Neurospora crassa hyphae the localization of all seven polytopic membrane protein chitin synthases (CHS) has been well analyzed. The different CHS are transported along hyphae in chitosomal microvesicles until reaching the core of the Spitzenkörper (SPK) and developing septa. From the SPK, the CHS are delivered to the apical PM, where they catalyze chitin synthesis. Hitherto, the mechanisms involved in CHS biogenesis and sorting remain largely unknown. We have previously identified CSE-7 (NCU05720), the N. crassa orthologue of S. cerevisiae Chs7, as the putative endoplasmic reticulum (ER) receptor for CHS-4 (orthologue of ScChs3). In a N. crassa Δcse-7 mutant, CHS-4-GFP fluorescence was retained in hyphal subapical regions in an extensive network of endomembranes, suggesting that CSE-7 is required for the exit of CHS-4 from the ER and its arrival of to the SPK and septa. Recently, we have studied CSE-8 (NCU01814), a second orthologue of Chs7. CSE-7 and -8 display a very similar localization pattern and evidenced the complexity of the ER in N. crassa. CSE-8 appears to participate in CHS-3 (class 1) trafficking. In the Δcse-8 mutant strain, CHS-3-GFP is retained in subapical areas of the hyphae and does not reach SPK and septa. Both CSE-7 and -8 are highly conserved within the fungal kingdom. Interestingly, yeast and dimorphic Ascomycota show only one copy of CSE, whereas filamentous Ascomycota have two copies. These recent findings support a conserved activity of CSE-7 and -8 in the transport of CHS-4 and -3 to sites of new cell wall construction during polarized growth of N. crassa.

PS3.5 CONIDIAL TRANSCRIPTION BEFORE DORMANCY MATTERS

Chris Koon Ho Wong[1]
[1]University of Macau, Taipa, Macau, China

Filamentous fungi produce large quantities of clonal but phenotypically heterogeneous conidia that can stay dormant for a long time until favorable conditions are encountered. How fungal conidia achieve phenotypic heterogeneity and prepare for dormancy is not known. Studies have shown that conidia contain abundant stable messenger RNAs (mRNAs); however, their origin and purpose remain unclear. Our work showed that the so-called dormant conidia of three filamentous fungal species (Aspergillus nidulans, Aspergillus fumigatus and Talaromyces marneffei) have robust transcription activities to synthesize their own mRNAs. Conidia remain transcriptionally active and responsive to the changing environment until they leave the developmental structure or become dehydrated. These environment-specific transcriptional responses can influence conidial content, expedite gene expression during germination, and affect subsequent fitness and capabilities of the fungal cells including drug and stress resistance, mycotoxin and secondary metabolite production, and virulence. Our findings uncover a mechanism for how genetically identical conidia achieve phenotypic variation and suggest that conidia prepare for the future by synthesizing and storing transcripts according to their experience before entering dormancy

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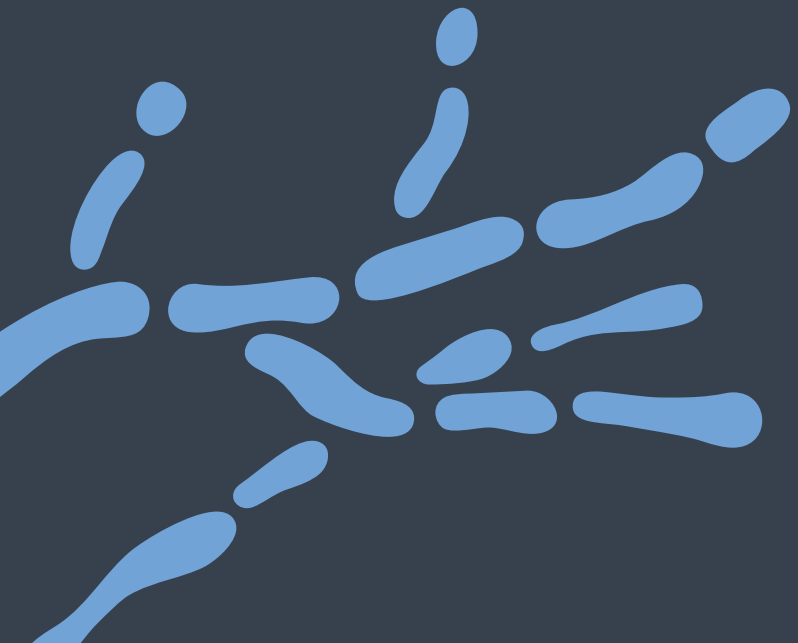

ABSTRACTS CONCURRENT SESSIONS

CONCURRENT SESSION 1.1
PLANT INTERACTIONS

MONDAY, MARCH 6
14:00 – 16:00
Location: Hall Tirol (Congress Innsbruck)

CHAIRS:
Gerhard Adam, Massimo Reverberi

CS1.1.1

THE STRESS RELATED TRANSCRIPTION FACTOR CRZ1 PLAYS PIVOTAL FUNCTIONS IN HOST PERCEPTION AND FUMONISIN BIOSYNTHESIS DURING FUSARIUM VERTICILLIOIDES-ZEA MAYS INTERACTIONS

Andrea Cacciotti[1], Marzia Beccaccioli[1], Luigi Faino[1], Stefania Vitale[2], Antonio di Pietro[3], David Turrà[4], Valeria Scala[5], Massimo Reverberi[1]

[1]Department of Enviromental Biology, University of Rome "Sapienza", Roma, Italy, [2]National Research Council, Institute for Sustainable Plant Protection (IPP-CNR), Portici (Naples), Italy, [3]Department of Genetics, Campus de Excelencia Internacional Agroalimentario ceiA3, Universidad de Córdoba, Cordoba, Spain, [4]Department of Agricultural Sciences, University of Naples "Federico II", Portici (Naples), Italy, [5]CREA-DC, Roma, Italy

Synchronization of the different metabolic pathways plays a key role in the onset of host-pathogen interactions. Here we investigated the biological role of the fungal protein Crz1, a downstream transcription factor of the calmodulin/calcineurin (CaM/CaN) pathway, in the fumonisin (FUM)-producing fungus Fusarium verticillioides. Previous studies have shown that fatty acids (FAs) and oxylipins (e.g., oxidized FAs) trigger FUM biosynthesis in Fusarium-infected maize plants, by acting as intra-/extra-cellular signals exchanged between the pathogen and its host.
In this study, we suggest that Crz1 indirectly controls this lipid-based communication via the action of phospholipase A2, thus modulating both oxylipin production and FUM biosynthesis that normally occurs in F. verticillioides-Zea mays interactions. To investigate the possible role of this transcription factor during the infection, we found, by using mass spectrometry and transcriptomic approaches, that crz1 deletion is consistently associated with an overall reduction in fatty acids, oxylipins and fumonisins contents in infected kernels. Notably, transcriptional data showed that the phospholipase pathway is one of the most enriched/affected by the deletion of crz1. We thus postulate that Crz1 can control the secretion of PLA2 during the interaction with the host that, in turn, can trigger the oxylipin pathway in maize. Under this scenario plant oxylipins (e.g. 9-HPODE) would provide feedback to the fungus to switch on the biosynthesis of FUM.

CS1.1.2

NATURAL VARIATION IN AVR3D1 FROM ZYMOSEPTORIA SP. CONTRIBUTES TO QUANTITATIVE GENE-FOR-GENE RESISTANCE AND TO HOST SPECIFICITY

Lukas Meile[1], Coraline Praz[1], María Garrido-Arandia[1], Zoe Bernasconi[3], Jules Peter[2], Alissa Schneller[2], Marta Suarez-Fernandez[1], Alessio Bernasconi[2], Julien Alassimone[2], Bruce A McDonald[2], **Andrea Sánchez-Vallet**[1,2]

[1]Universidad Politecnica de Madrid, Pozuelo De Alarcón-Madrid, Spain, [2]ETH, Zurich, Switzerland, [3]Zurich University, Zurich, Switzerland

Successful host colonization by plant pathogens requires the circumvention of host defence responses, frequently through sequence modifications in secreted pathogen proteins known as avirulence factors (Avrs). Although Avr sequences are often polymorphic, the contribution of these polymorphisms to virulence diversity in natural pathogen populations remains largely unexplored. We determined how natural sequence polymorphisms of the avirulence factor Avr3D1 in the wheat pathogen Zymoseptoria tritici contributed to adaptive changes in virulence and showed that there is a continuous distribution in the magnitude of resistance triggered by different Avr3D1 isoforms. These results demonstrate that natural variation in an Avr gene can lead to a quantitative resistance phenotype. We further showed that homologs of Avr3D1 in two non-pathogenic sister species of Z. tritici are recognized by some wheat cultivars, suggesting that Avr-R gene-for-gene interactions can contribute to nonhost resistance. We suggest that the mechanisms underlying host range, qualitative resistance and quantitative resistance are not exclusive.

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CS1.1.3

ZYMOSOUPS : A FORWARD GENETICS METHOD FOR RAPID IDENTIFICATION OF EFFECTOR GENES IN ZYMOSEPTORIA TRITICI

Haider Ali[1], Megan McDonald[1], **Graeme Kettles**[1]

[1]University of Birmingham, Birmingham, United Kingdom

The dothideomycete fungus Zymoseptoria tritici causes Septoria tritici blotch disease, a major threat to wheat productivity globally. Z. tritici has proven difficult to control due to the emergence of strains that that can overcome host genetic resistance provided by disease resistance (R) genes. How this fungus evolves to evade R gene recognition is not well understood, partly due to the unknown identity of most secreted effector proteins that are recognised by host R proteins to trigger immune responses. To date, identification of Z. tritici effector genes has been done through a combination of GWAS and QTL mapping. Whilst effective, this process is laborious and requires considerable resources. We therefore sought to develop a faster method for effector gene identification through UV mutagenesis and in planta forward genetic screens. Using the known gene-for-gene interaction between the R gene Stb6 and the effector AvrStb6, we found that inoculations of mixtures (soups) of virulent and avirulent spores led to observable disease symptoms even when virulent spores were greatly outnumbered. We then developed a UV mutagenesis and in planta screening protocol that allowed us to screen >100,000 UV mutants for gain-of-virulence (GoV) on wheat containing Stb6. We recovered five mutants that gained virulence on the Stb6-containing wheat cultivar Cadenza. Following confirmation of GoV by single isolate inoculations, whole genome sequencing was used to identify SNPs and other mutations in comparison to the IPO323 parental strain. All five GoV mutants contained either SNPs in AvrStb6, or larger chromosomal deletions involving the AvrStb6 locus. These results would have allowed rapid identification of the AvrStb6 gene as the virulence determinant on Stb6-containing wheat. This method could be useful for identifying currently unknown effector genes in this pathogen.

CS1.1.4

TOWARDS IDENTIFICATION OF VIRULENCE FACTORS CONTRIBUTING TO THE NECROTROPHIC PHASE OF COLLETOTRICHUM ORBICULARE

Katsuma Yonehara[1,2], Naoyoshi Kumakura[1], Pamela Gan[1], Ken Shirasu[1,2]

[1]RIKEN, Yokohama, Japan, [2]The University of Tokyo, Bunkyo-ku, Japan

Colletotrichum orbiculare, a causal agent of anthracnose disease, infects Cucurbitaceae plants as well as a model plant Nicotiana benthamiana. This infection is characterized by an early symptomless biotrophic phase and a later destructive necrotrophic phase. To complete these two phases, C. orbiculare is assumed to utilize different subsets of molecules including secreted proteins and secondary metabolites. We found 127 genes encoding secreted proteins and 2 genes encoding secondary metabolite biosynthesis enzymes specifically expressed at the necrotrophic phase, designated SPNs and SMNs, respectively. To characterize these genes, we utilized a multiplex gene disrupting method. So far, we have simultaneously disrupted 9 SPNs and 2 SMNs in the same strain. In addition, we tested whether these genes were able to induce host cell death via a transient gene expression system in N. benthamiana. As a result, 2 SPNs induced host cell death only when they contained signal peptides, implying that they functioned in the apoplast. These data raised two potential hypotheses: (i) they are recognized by receptors on the host plasma membrane to induce hypersensitive responses; (ii) they have toxicity and kill host cells. We are currently analyzing their functions in more detail.

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CS1.1.5

FUSARIUM GRAMINEARUM: DOES THE TRICHOTHECENE CHEMOTYPE MATTER?

Gerhard Adam[1], Gerlinde Wiesenberger[1], Krisztian Twaruschek[1], Herbert Michlmayr[1], Elisabeth Varga[2], Franz Berthiller[2], Alfia Khairullina[3], Christian Hametner[4]

[1]Univ. of Natural Resources and Life Sciences, Vienna (BOKU), Inst. of Microbial Genetics, Tulln, Austria, [2]Univ. of Natural Resources and Life Sciences, Vienna (BOKU), Institute of Bioanalytics and Agro-Metabolomics, Tulln, Austria, [3]Lund University, Lund, Sweden, [4]University of Technology, Vienna, Inst. of Synthetic Chemistry, Vienna, Austria

F. graminearum isolates produce different trichothecenes in culture. Most strains produce deoxynivalenol (DON) and have a loss of function allele of the cytochrome P450 encoding gene TRI13, while nivalenol (NIV) producers can introduce a hydroxy-group at C4. DON producers can be subdivided into 15-acetyl-DON or 3-acetyl-DON producers, which is caused by different alleles of the TRI8 esterase gene. A fraction of North American strains produces NX-3, which compared to DON lacks the keto group at C8. This structural difference could provide an advantage against glutathione-mediated inactivation by Michael adduct formation (DON-10-GSH). Interestingly, all natural NX-isolates are genotyped as 3-acetyl-DON producers and produce NX-2 in vitro. The causal gene for NX-production is TRI1, which is unlinked to the TRI-cluster – so it is surprising that during outcrossing with the dominant 15-acetyl-DON producers in the field no strains producing NX-4 (=15-acetyl-DON lacking C8 keto) accumulate, indicating that they might have a disadvantage. We have investigated plant UDP-glycosyltransferases and found that the switch from NIV to DON production reduces detoxification by the plant. We have also studied whether 3- or 15-ADON production has an effect on virulence, by changing the TRI8 allele in the PH-1 background. Although 3-ADON is far less toxic at the ribosomal target, surprisingly no significant virulence difference was found in wheat and oat, which is probably due to the rapid deacetylation of both ADONs to DON by plant carboxylesterases. Introduction of the NX-TRI1 allele into the 15-ADON producer PH-1 led to production of NX-4 and lower virulence. We found that NX-4 is less inhibitory at the ribosomal level, and in addition the NX-toxins come with another

fitness price, they have compromised thermal stability. Seemingly, the different chemotypes are maintained by balancing selection on different host plants with differing detoxification properties in the natural ecosystem.

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CS1.1.6

ADRESSING REDUNDANT ROLES OF PHYTOTOXIC PROTEINS FOR NECROTROPHIC INFECTION OF B. CINEREA BY MULTI-K.O. MUTAGENESIS

Matthias Hahn[1], Nassim Safari[1], Patrick Pattar[1], Thomas Leisen[1], Frederik Sommer[1], Michael Schroda[1], David Scheuring[1], Sophie Eisele[1]

[1]*University of Kaiserlautern, Kaiserslautern, Germany*

Botrytis cinerea is a necrotroph infecting a wide range of host plants. During invasion, it quickly kills host cells and colonizes dead tissue. Factors that contribute to this lifestyle include secretion of CWDE, cell death inducing proteins (CDIPs) and phytotoxic metabolites, tissue acidification and induction of host defence. However, it is still unknown how the fungus induces host cell death. An efficient CRISPR/Cas9 protocol was established to generate multiple mutants lacking phytotoxic metabolites and CDIPs of B. cinerea (Leisen et al., 2020, PLoS Pathogens; doi.org/10.1371/journal.ppat.1008326; Leisen et al., 2022, PLoS Pathogens; doi.org/10.1371/journal.ppat.1010367). By now, a series of up to 21-fold multiple mutants were constructed, lacking all currently known CDIPs. The mutants showed normal growth and in vitro differentiation, but decreased virulence with increasing numbers of deleted genes. The effects of the deletions were dependent on the origin of the infected tissue, indicating host-specific roles of some CIDPs. The 21x mutant showed strongly impaired lesion formation on leaves, and was virtually nonpathogenic on fruits. Since the secretome of the mutant still showed residual phytotoxic activity, we are continuing to search for and delete the remaining CDIPs, to generate finally a non-necrotrophic B. cinerea mutant. Our data document one of the first systematic approaches to address functional redundancy of virulence factors in a pathogenic fungus.
B. cinerea triggers the plant hypersensitive response (HR) as an infection strategy, and it has been suggested that the CDIPs contribute to HR induction by activating plant immune receptors and pattern-triggered immunity (PTI). However, infections of plant mutants or silenced tissues of Arabidopsis or tobacco lacking the coreceptors of pattern recognition receptor proteins (PRRs), BAK1 and SOBIR1, didn't reveal

differences to wildtype plants in susceptibility against B. cinerea. Further mutants are being tested to identify plant cell death pathways that are activated by the invading fungus.

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CS1.1.7

GIANT TRANSPOSONS FACILITATE HORIZONTAL GENE TRANSFER OF THE NECROTROPHIC EFFECTOR TOXA IN FUNGAL WHEAT PATHOGENS

Hannah Wilson[2], Ryan Gourlie[3], Michael Choi[1], Reem Aboukhaddour[3], Peter Solomon[2], **Megan McDonald**[1]

[1]University of Birmingham, School of Biosciences, Birmingham, United Kingdom, [2]The Australian National University, Division of Plant Scences, Canberra, ACT, Australia, [3]Agriculture and Agri-food Canada, Lethbridge, Alberta, Canada

Horizontal gene transfer (HGT) is a tool that many organisms use to rapidly adapt to novel hosts or environments. One well-known example of HGT is the movement of the necrotrophic effector ToxA between three fungal wheat pathogens, Parastagonospora nodorum, Pyrenophora tritici-repentis and Bipolaris sorokiniana. Defining the extent of horizontally transferred DNA is also important because it can provide clues to the mechanisms that facilitate HGT. Our previous analysis of ToxA and its surrounding 14 kb showed that this region was a type II DNA transposon we named ToxhAT due to the “hAT-like” transposase gene neighbour to ToxA. Importantly, there was some evidence that this transposon may remain active and mobile in B. sorokiniana. Long-read genome sequencing of eight ToxhAT carrying B. sorokiniana isolates confirmed that ToxhAT is an active transposon with a two base-pair “TA” target site duplication. This feature of the Class II transposon in combination with the long terminal inverted repeats suggests that it should be re-classified as a member of the Tc1/Mariner transposon superfamily. In addition to confirming the active movement of ToxhAT, these genome assemblies also revealed that this transposon was a passenger within a much larger 170-196kb mobile genetic element. This element, termed Sanctuary I, has been classified as a member of the giant “Starship” transposons a new wide-spread transposon family found in fungi.

CS1.1.8

PWL2 MODULATES PAMP-TRIGGERED IMMUNITY THROUGH INTERACTION WITH A HOST ISOPRENYLATED HMA

Vincent Were[1], Xia Yan[1], Jan Sklenar[1], Andrew Foster[1], Diana Gomez[1], Thorsten Lagner[1], Matthew Smoker[1], Darren Soanes[2], Dan MacLean[1], Matthew Muscou[1], Sophien Kamoun[1], Frank Menke[1], Nicholas Talbot[1]

[1]Sainsbury Laboratory, Norwich, United Kingdom, [2]The University of Exeter, Exeter, United Kingdom

The rice blast fungus, Magnaporthe oryzae, causes the most serious disease of cultivated rice and secretes a battery of effector proteins during infection. The ability of Magnaporthe isolates to infect weeping lovegrass (Eragrostis curvula) - a forage grass for livestock, was reported to be controlled by a single gene, PWL2. Here, we show that PWL2 belongs to a gene family and unlike other members in the family, PWL2 is present in all limited host forms and outside M. oryzae species. PWL2 has undergone genome expansion and occurs as multiple copies in the rice blast genomes. We used CRISPR/Cas9 genome editing to generate Dpwl2 mutants in M. oryzae strain Guy11, which has three copies of the gene, resulting in gain of virulence towards weeping lovegrass. Additionally, constitutively expressing PWL2 in transgenic rice and barley lead to suppressed production of reactive oxygen species (ROS) suggesting that Pwl2 is involved in modulating PAMP-triggered immunity. To further investigate the role of Pwl2 in suppression of plant immunity, we have performed co-IP/Mass spectrometry from stable transgenic barley plants expressing Pwl2-YFP to reveal a total of 46 proteins in the Hordeum vulgare (barley) proteome database that associated with Pwl2. One of the putative interactors was identified as an isoprenylated heavy metal binding domain containing protein called Hv-HIPP2. We have generated transgenic barley lines expressing Hv-HIPP2 and shown that they are attenuated in response to PTI like transgenic lines expressing Pwl2. We have subsequently carried out co-IP/Mass spectrometry that led us to identify multiple targets for HIPP2. Our study provides evidence that Pwl2 belongs to the MAX-fold group of conserved M. oryzae effectors and plays an important role during the biotrophic phase of infection by suppressing ROS burst and thereby suppressing plant immunity.

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CONCURRENT SESSION 1.2 BIOACTIVE METABOLITES, SECONDARY METABOLITES

MONDAY, MARCH 6
14:00 – 16:00
Location: ***Hall Brüssel (Congress Innsbruck)***

CHAIRS:
Lena Studt-Reinhold, Jens Laurids Sørensen

CS1.2.1

UBIQUITOUS BACTERIAL POLYKETIDES MEDIATE CROSS-KINGDOM MICROBIAL INTERACTIONS

Axel Brakhage[1], Mario Krespach[1], Maria Stroe[1,2], Lukas Zehner[1], Maira Rosin[1], Simone Edenhart[1], Sophie Tröger[1], Moemi Kawashima[1], Volker Schroeckh[1]

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Although the interaction of prokaryotic and eukaryotic microorganisms is critical for the functioning of ecosystems, knowledge of the processes driving microbial interactions between organisms of different kingdoms is in its infancy. We have shown that a key role for the communication is played by natural products (NPs). We have discovered an unprecedented tripartite interkingdom microbial consortium consisting of the bacterium Streptomyces rapamycinicus (or S. iranensis), the fungus Aspergillus nidulans and the green alga Chlamydomonas reinhardtii involving NPs. The streptomycete produces the arginine-derived polyketide azalomycin F that triggers the expression of the otherwise silent ors gene cluster of A. nidulans resulting in the production of orsellinic acid and derivatives. This way, the bacterium re-programs the epigenetic machinery of the fungus. Key fungal response genes are the histone acetyl transferase GcnE that specifically acetylates histones located in the ors gene promoters, and the transcription factor BasR. Presence of BasR and ors genes in fungal species allows

forecasting the inducibility of the fungal ors cluster by S. rapamycincus. Azalomycin F is also released in presence of C. reinhardtii. As a response, the alga swims to the mycelia of the fungus and is thereby protected from the toxic activity of azalomycin F. Furthermore, sublethal concentrations of azalomycin F trigger the formation of a protective multicellular structure by C. reinhardtii, which we named gloeocapsoid. Thus, the algae survive lethal NPs through formation of a multicellular structure and of an alliance with a fungus. Furthermore, arginine-derived polyketide-producing bacteria occur world-wide. The producer bacteria and the fungi decoding and responding to this signal can be co-isolated from the same soil sample. Arginine-derived polyketides impact surrounding microorganisms both directly as well as indirectly, by inducing the production of fungal NPs that further influence the composition of microbial consortia.
PNAS 2011; eLife 2018; ISME J 2020; PNAS 2021

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CS1.2.2

EVOLUTIONARY HISTORIES OF TYPE I FATTY ACID SYNTHASES IN FUNGI

Yanfang Guo[1,2], Jérôme Collemare[1]

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The biosynthesis of fungal secondary metabolites involves core enzymes that produce from primary metabolism precursors to the first stable intermediates, and tailoring enzymes that modify the chemical structure to yield final compounds. While the various catalytic activities of tailoring enzymes expand the chemical diversity in fungi, different precursor selectivity of core enzymes also contributes to this diversity. Mutations in the core gene may lead to selecting different precursors, however, in certain cases a specific precursor must be produced by other enzymes which are encoded with the biosynthetic gene cluster. One major example is the polyketide synthase (PKS) involved in the production of aflatoxin (and related compounds like dothistromin), which specifically uses hexanoyl-CoA synthesized by fatty acid synthases (FASs) instead of acetyl-CoA from primary metabolism. FASs have been reported to provide precursors in few secondary metabolite pathways, including HC-toxin. Type I FASs play a key role in primary metabolism, and how such enzymes have been recruited in secondary metabolite biosynthetic gene clusters is not known. In this study, we retrieved FASs from Pezizomycotina, including characterized ones, and performed phylogenetic and comparative genomics analyses to determine the evolutionary relationships between FASs involved in primary and secondary metabolism, and how they co-evolved with PKS enzymes.

CS1.2.3

AN HMM APPROACH EXPANDS THE LANDSCAPE OF SESQUITERPENE CYCLASES ACROSS THE KINGDOM FUNGI

Marie-Noëlle Rosso[1], Hayat Hage[1], Julie Couillaud[2], Asaf Salamov[3], Margot Loussouarn-Yvon[1], Fabien Durbesson[4], Elena Ormeño[5], Sacha Grisel[2], Katia Duquesne[2], Renaud Vincentelli[4], Igor Grigoriev[6], Gilles Iacazio[2]

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Fungi are among the most prolific producers of terpenoid compounds. Among them, C15 terpenes (sesquiterpenes) are thought to play a role in the chemical communication between fungi and other microbes, and thereby impact the establishment of microbial communities. Moreover, fungal sesquiterpenes include notorious bioactive mycotoxins, cytotoxins and precursors of anticancer molecules, of high biotechnological and medicinal interest. However, our knowledge on fungal sesquiterpenes is limited by the lack of an efficient tool for the identification of the genes coding for sesquiterpene cyclases from genomics, transcriptomics, and meta-omic data.

Our objective was to provide a tool for the reliable identification of sesquiterpene cyclase genes from sequence data. We also tested the possibility to predict the type of sesquiterpene compound the sesquiterpene cyclases produce, depending on the primary cyclization of the linear farnesyl diphosphate.
Using new HMM models on 656 published fungal genomes, we doubled the numbers of sesquiterpene cyclase genes identified as compared to previously existing tools. We identified more than 6,202 genes for which we could predict the type of primary cyclization catalyzed by the enzymes.
With the rising numbers of data being generated from genomics, metagenomics, transcriptomics and metatranscriptomics studies, we think this tool will be of relevance to a large community of scientists interested in understanding the roles of fungal sesquiterpenes in natural environments, or in data mining for the discovery enzymes that catalyze the production of new bioactive compounds.

CS1.2.4

NOVEL INSIGHTS INTO FUNGAL SPECIALIZED METABOLITE BIOSYNTHESIS

Uffe Mortensen[1], Xinhui Wang[1], Yaojie Guo[1], Jaqueline Gerhardt[1], Malgorzata Futyma[1], Jakob Hoof[1], Thomas Larsen[1]

[1]*Technical University Of Denmark, Lyngby, Denmark*

Fungi produce a vast amounts of specialized metabolites, SMs, of which only the tip of the iceberg has been characterized and many compounds and exciting biochemistry await discovery. To uncover new SM gene functions and products. We typically use a strategy where we delete the relevant genes in the native SM producer species, which may be a model or non-model fungus; as well as we express the relevant gene(s) in a well-characterized fungal cell factory. In both cases, new insights are gained by subsequent chemical metabolome analyses including feeding with isotope labeled precursors. Using this strategy, we have recently investigated SM pathways in Aspergillius homomorphus and A. californicus, which had never previously been genetically engineered, by CRISPR technology and discovered and characterized pathways with unusual fungal SM biochemistry. Hence, in the case of A. homomorphus we discovered a pathway for production of a hybrid SM that combines a precursor derived from the shikimate pathway with a polyketide to produce a pigment, representing a type of pyrones, which is often found in plants, but not in fungi. In the case of A. californicus, dissection of an SM pathway identified a novel ring-closure mechanism for production of 2-pyridones. Lastly, we revisited yanuthone production in A. niger and determined that a fraction of these compunds are derived from a precursor originating from the shikimate pathway. Previously, we have described that the initially described yanuthones in A. niger are derived from the polykeide 6-MSA. Hence, our new observation shows that the yanuthone SM pathway is more complex as precursors from two different sources of the primary metabolism are able to enter the same biosynthetic pathway to expand the repertoire of products that this pathway can produce.

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CS1.2.5

FUNGAL RAINCOATS

Trine Aalborg[1], Marie Overgaard[1], Klaus Westfall[1], Reinhard Wimmer[1], Henriette Giese[1], Jens Sørensen[2], **Teis Sondergaard**[1]

[1]*Aalborg University, Aalborg, Denmark,* [2]*Aalborg University, Esbjerg, Denmark*

We have identified a new type of compound that might be used by fungi to seal their cell wall making it impermeable to water. Small hydrophobic molecules may play a very important role cementing together proteins. The fungal cell wall is a highly plastic skeleton build of a robust network of different organic molecules to protect the fungi from crucial environmental challenges like UV light, hydration, dehydration, chemical substances, mechanical stress, and predators. It has been known for a long time that fungi produce hydrophobic cell wall components to survive different humidity conditions. Presently, the hydrophobic properties of the fungal cell walls have been attributed to hydrophobins, but we aim to demonstrate that cell wall components are glued together using small secondary metabolites. The commercial application and value for protein-based water insoluble nanomaterials is significant, and it would enhance our understanding of fungal biology. We have investigated these substances in two fungal genera, Apiospora and Fusarium, but propose that this mechanism is present throughout the Ascomycota branch of the fungal kingdom.

CS1.2.6

GENOME ANALYSIS AND ELUCIDATION OF THE BIOSYNTHETIC PATHWAY FOR THE CRAS INHIBITOR RASFONIN IN CEPHALOTRICHUM GORGONIFER

Andreas Schüller[1], Lena Studt-Reinhold[1], Harald Berger[1], Lucia Silvestrini[1], Roman Labuda[2,3], Ulrich Güldener[4], Markus Gorfer[5], Markus Bacher[1,2], Maria Doppler[1], Erika Gasparotto[1,2], Arianna Gattesco[1,2], Michael Sulyok[1], Joseph Strauss[1,2]

[1]Universität für Bodenkultur, Wien, Tulln an der Donau, Austria, [2]Research Platform Bioactive Microbial Metabolites (BiMM), Tulln an der Donau, Austria, [3]University of Veterinary Medicine Vienna, Vienna, Austria, [4]Technical University of Munich, Munich, Germany, [5]AIT Austrian Institute of Technology GmbH, Tulln an der Donau, Austria

Background: Fungi are important sources for bioactive compounds that find their applications in many important sectors like in the pharma-, food- or agricultural industries. In an environmental monitoring project for fungi involved in soil nitrogen cycling we also isolated Cephalotrichum gorgonifer (strain NG_p51). In the course of strain characterization work we found that this strain is able to naturally produce high amounts of rasfonin, a polyketide inducing autophagy, apoptosis, necroptosis in human cell lines and shows anti-tumor activity in RAS-dependent cancer cells.
Results: In order to elucidate the biosynthetic pathway of rasfonin, the strain was genome sequenced, annotated, submitted to transcriptome analysis and genetic transformation was established. Biosynthetic gene cluster (BGC) prediction revealed the existence of 22 BGCs of which the majority was not expressed under our experimental conditions. In silico prediction revealed two BGCs with a suite of enzymes possibly involved in rasfonin biosynthesis. Experimental verification by gene-knock out of the key enzyme genes showed that one of the predicted BGCs is indeed responsible for rasfonin biosynthesis.
Conclusions: The results of this study lay the ground for molecular biology focused research in Cephalotrichum gorgonifer. Furthermore, strain engineering and heterologous expression of the rasfonin BGC is now possible which allow both the construction of rasfonin high producing strains and biosynthesis of rasfonin derivates for diverse applications.

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CS1.2.7

HETEROLOGOUS PRODUCTION OF RIBOSOMAL BACKBONE N-METHYLATED MACROCYCLIC PEPTIDES

Lukas Sonderegger[1], Emmanuel Matabaro[1], Olivia Bossert[1], Markus Künzler[1]

[1]ETH Zürich, Institute of Microbiology, Zürich, Switzerland

Peptide backbone N-methylations and macrocyclization are desired properties for the development of peptide therapeutics, as these modifications can improve cell permeability, target selectivity and proteolytic stability. One famous example of a macrocyclic, backbone N-methylated peptide is the immunosuppressive agent cyclosporin A, a fungal non-ribosomal peptide natural product. Omphalotin A on the other hand is a ribosomally produced and post-translationally modified peptide (RiPP) that consists of a 12-amino acid macrocycle with 9 backbone N-methylations. This compound, produced by the mushroom Omphalotus olearius, exhibits strong toxicity against nematodes by an unknown mechanism. Also, the biosynthesis of omphalotin A is still not understood completely. Its precursor protein contains a SAM-dependent α-N-methyltransferase domain, which iteratively methylates the core peptide located at the C-terminus. After complete methylation, the core peptide is cleaved off by an unidentified protease and subsequently macrocyclized by a prolyl oligopeptidase. Heterologous production of omphalotin A by expression of the precursor protein and prolyl oligopeptidase has so far only been successful in yeast, but not in E. coli, suggesting that E. coli lacks the protease responsible for the initial cleavage of the precursor protein. We aim at establishing the production of omphalotin A or related backbone N-methylated macrocyclic peptides in E. coli by engineering the precursor protein via introduction of specific amino acid sequences that are recognized and cleaved by co-expressed proteases. This heterologous production platform will enable the biosynthesis of diverse novel backbone N-methylated macrocyclic peptides with potentially promising pharmacological properties. In addition, efficient production of omphalotin A in E. coli will allow us to screen for the still unknown molecular target of omphalotin A.

CS1.2.8

SUSTAINABLE CONVERSION OF POLYETHYLENE WASTE PLASTICS INTO FUNGAL SECONDARY METABOLITES

Clay Wang[1], Chris Rabot[1], Swati Bijlani[1], Yi-Ming Chiang[1], Yuhao Chen[2], Elizabeth Oakley[3], Berl Oakley[3], Travis Williams[2]

[1]Department of Pharmacology and Pharmaceutical Sciences, University Of Southern California , Los Angeles, United States, [2]Donald P. and Katherine B. Loker Hydrocarbon Institute and Department of Chemistry, Los Angeles, United States, [3]Department of Molecular Biosciences, Lawrence, United States of America

Waste plastics represent major environmental and economic burdens due to their ubiquity, slow breakdown rates, and inadequacy of current recycling routes. Polyethylenes are particularly problematic, because they lack robust recycling approaches despite being the most abundant plastics in use today. We report a novel chemical and biological approach for the rapid conversion of polyethylenes into structurally complex and pharmacologically active compounds. We present conditions for aerobic, catalytic digestion of polyethylenes collected from post-consumer and oceanic waste streams, creating carboxylic diacids that can then be used as a carbon source by the fungus Aspergillus nidulans. As a proof of principle, we have engineered strains of A. nidulans to synthesize the fungal secondary metabolites asperbenzaldehyde, citreoviridin, and mutilin when grown on these digestion products. This hybrid approach considerably expands the catalog of products to which polyethylenes can be upcycled.

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CONCURRENT SESSION 1.3
GENOME FUNCTION AND EPIGENETICS

MONDAY, MARCH 6
14:00 – 16:00
Location: ***Hall Strassburg (Congress Innsbruck)***

CHAIRS:
Ingo Bauer, Özgür Bayram

CS1.3.1

CHROMATIN-WIRED NUCLEAR COMPARTMENTS IN FUSARIUM GRAMINEARUM

Aurelie Etier[1], Fabien Dumetz[1], Nathalie Prunier-Leterme[2], Fabrice Legeai[2], Gael Le Trionnaire[2], **Nadia Ponts**[1]

[1]INRAE UR1264-MycSA, VILLENAVE D ORNON, France, [2]INRAE UMR IGEPP, Le Rheu, France

Fusarium graminearum is an efficient plant pathogen found in crops worldwide. Major causal agent of Fusarium head blight on wheat in Europe, this filamentous fungus can also produce toxic mycotoxins and a variety of secondary metabolites in developing kernels. These secondary metabolites are produced as the result of a cascade of coordinated regulations of an arsenal of genes dispersed accross the genome. Previous study highlighted the roles of the histone marks H3K27me3 and H3K4me3 in regulating the metabolism of F. graminearum. Here, we applied HiC in combination to histone profiling and transcriptomics to unravel the 3D organization of the four chromosomes in the nucleus. We show that the orchestration of genome regulation is mediated by histone-dependant highly topologically structured chromatin that organizes chromosomes in territories to potentiate coordinated genome regulation.

CS1.3.2

HISTONE DEACETYLASE 1 (HDA-1) ACTIVITY REGULATES FACULTATIVE HETEROCHROMATIN FORMATION IN THE MODEL SYSTEM NEUROSPORA CRASSA

Felicia Ebot Ojong[1], Zachary Lewis[1], Ailleen Ferraro[1]

[1]*The University Of Georgia, Athens, United States*

Neurospora crassa is a filamentous fungus with a rich history in epigenetics research. Several conserved epigenetic pathways operate in N. crassa to silence gene expression, including RNAi, DNA methylation, H3K9 methylation, and H3K27me3, which is absent in yeast models. Like higher eukaryotes, H3K27me3 is catalyzed by a conserved Polycomb Repressive Complex 2 (PRC2) comprised of three core subunits, SET-7/KMT6, EED, and SUZ12. The presence of a conserved Polycomb system in N. crassa presents an opportunity to study Polycomb-mediated repression using powerful fungal genetics approaches. We carried out a genetic screen to identify genes required for Polycomb repression in N. crassa. We found that histone deacetylase-1 (hda-1) is required for repression of PRC2-targeted genes in N. crassa. In the absence of HDA-1, H3K27me3 is lost from typical PRC2-targeted domains and instead accumulates aberrantly at constitutive heterochromatin domains marked by H3K9me3. Next, we used various molecular approaches to determine how HDA-1 is regulating H3K27me3 localization. Together, our results show that H3K9me3 recruits HDA-1 to prevent aberrant recruitment of PRC2 to constitutive heterochromatin domains.

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CS1.3.3

THE DNA N6-ADENINE METHYLTRANSFERASE COMPLEX OF MUCORALES AND ITS ROLE ON GENE EXPRESSION AND CHROMATIN STRUCTURE

Carlos Lax[1], José Francisco Martínez-Hernández[1], José Antonio Pérez-Ruiz[1], Stephen J. Mondo[2], Igor V. Grigoriev[2], Teresa E. Pawlowska[3], Eusebio Navarro[1], Francisco Esteban Nicolás[1], Victoriano Garre[1]

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Genetic mechanisms involved in processes such as gene expression regulation and chromatin structure have been traditionally overlooked in Mucorales and, by extension, in all the early-diverging fungi (EDF). Nevertheless, the next-generation sequencing (NGS) techniques combined with the recent development of state-of-the-art molecular tools for these fungi have allowed us to deepen the implications of epigenetic modifications in EDF. Interestingly, and sharing this characteristic with ciliates and green algae, EDF use N6-methyladenine (6mA) as their main DNA epigenetic mark, differentiating them from the rest of the eukaryotic organisms. In the case of Rhizopus microsporus, 1.5% of adenines are methylated. Remarkably, this modification is not randomly distributed across the genome but concentrated in specific locations and mostly on ApT dinucleotides. We found that 6mA preferentially localizes around the transcription start sites and is strongly associated with actively expressed genes transcribed by RNA pol II. In addition, 6mA appears predominantly in linker regions and rarely in nucleosome-bound DNA, suggesting its association with chromatin configuration. 6mA-rich regions display more strongly positioned nucleosomes than regions where 6mA is scarce. Conserved in ciliates, green algae, and EDF, a 6mA methylation complex formed by two DNA methyltransferase proteins (Mta1 and Mta9) and one DNA binding protein (P1), has proven to have an essential role in R. microsporus because mutation of its components is lethal. At the same time, a drastic reduction of its expression, enabled by the development of a regulable

expression system, causes severe effects on the growth of this fungus. Given the scarcity of 6mA and the absence of this complex in higher eukaryotic organisms, the study of this mechanism in Mucorales represents a new source of promising targets for developing new mucormycosis treatments.

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CS1.3.15

A COMPREHENSIVE GENOMIC ATLAS OF CHROMATIN REMODELLING ACTIVITY IN CANDIDA ALBICANS UNCOVERS NEW REGULATORY CIRCUITS OF FUNGAL FITNESS

Faiza Tebbji[3], Manon Henry[1], Antony Vincent[2], **Adnane Sellam**[1]

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Study of transcriptional circuits that modulate fungal fitness is an attracting research subject that can help fighting fungal pathogens by targeting specific circuits as a whole instead of individual effectors of virulence. Precise nucleosome organization at promoters, that is mediated by multiple chromatin remodeler (CR) enzymes, is critical for transcription initiation and gene expression control. CRs are multi-protein complexes that use the energy of ATP hydrolysis to mobilize nucleosomes and consequently define the chromatin states. Our recent works showed that the CR SWI/SNF of Candida albicans, is key modulator of fungal virulence by acting as a nexus integrating oxygen status to the fungal metabolic machinery. To build a comprehensive understanding of the role of all CRs in C. albicans, we used a multiple genome-scale measurements (ChEC-seq, MNase-seq and RNAPII occupancy) to assess the activity of the eight C. albicans CRs (SWI/SNF, ISW1a/b, ISW2, INO80, RSC, SWR and CHD) at their direct target promoters. This work provided a comprehensive atlas of CR activity and uncovered important contribution of some remodelers to the control of antifungal resistance and adaptation to the host environments. For instance, we uncovered that the Ino80 complex play a key role in modulating the growth of C. albicans under hypoxia, the predominant condition inside the host, in conjunction with the TOR (Target Of Rapamycin) pathway. We will provide a detailed mechanistic analysis of this new fungal regulatory axis and describe its crosstalk with different histone marks. Importantly, the C. albicans CR networks exhibited an extensive plasticity as compared to that of the saprophytic yeast Saccharomyces cerevisiae suggesting that chromatin remodelling is a driving evolutionary force that might contribute to pathogenicity.

CS1.3.5

CHROMOSOME DYNAMICS IN THE HIGHLY PLASTIC GENOME OF THE FUNGAL PATHOGEN FUSARIUM OXYSPORUM

Lucía Gómez Gil[1], Cristina López Díaz[1], Dilay Hazal Ayhan[2], Li-Jun Ma[2], Antonio Di Pietro[1]

[1]University Of Córdoba, Córdoba, Spain, [2]University of Massachusetts Amherst, Massachusetts, United States

Plant pathogenic fungi represent a major risk to human health and food security. The ascomycete Fusarium oxysporum causes vascular wilt disease on more than 150 different crops. A single isolate of Fusarium oxysporum f.sp. lycopersici, Fol4287, is capable of killing tomato plants, immunodepressed mice and the model insect host Galleria mellonella. Similar to other fungal pathogens, Fol4287 exhibits a remarkable genomic plasticity that implies changes in chromosome structure and organization. An experimental evolution approach involving serial passaging of a clonal isolate of Fol4287 through tomato plants or plates with complete or minimal medium revealed recurrent large-scale copy number variations (CNVs) in accessory or lineage specific regions of the genome, including chromosome duplications and losses. Karyotype analysis by Contour-Clamped Homogeneous Electric Field (CHEF) electrophoresis and Southern blot revealed the presence of previously unrecognized duplicated genomic regions of Fol4287 that are shared between different accessory chromosomes. Furthermore, we developed methodologies for monitoring chromosomal dynamics and quantitatively measuring the frequency of chromosome loss, both in the wild type strain and in mutants affected in histone methylation or DNA repair. Our results indicate that the frequency of spontaneous mitotic loss of an accessory mini-chromosome in Fol4287 can be as high as 1% in the near-absence of selection and that the epigenetic mark H3k9me3 may contribute to increase the stability of this accessory region.

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CS1.3.6

SIRTUIN E IS INVOLVED IN CELL WALL INTEGRITY, GROWTH, SECONDARY METABOLISM PRODUCTION, AND VIRULENCE IN ASPERGILLUS FUMIGATUS

Natália Wassano[1], Jaqueline Gerhardt[1], Everton Antoniel[1], Gabriela Silva[2], Daniel Akiyama[3], Leandro Neves[4], Bianca Oliveira[5], Adriana Leme[4], Fausto Almeida[5], Taicia Fill[3], Nilmar Moretti[2], **André Damasio**[1]

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Aspergillus fumigatus is a filamentous fungus and the main etiologic agent of Invasive Pulmonary Aspergillosis (IPA) with high mortality rates in immunocompromised individuals. A. fumigatus represents a public health problem due to the rise of immunosuppressive therapies; the increasing number of reports on COVID-19-associated IPA and antifungal-resistant isolates. Lysine acetylation is a posttranslational modification that orchestrates various biological processes such as fungal pathogenesis and targeting the enzymes that regulate acetylation status has been proposed as a potential strategy to treat fungal infections. Sirtuins are NAD+-dependent lysine deacetylases that participate in the regulation of the acetylation status of many proteins, however, the role of sirtuins in A. fumigatus virulence has not been reported to date. Here, we first describe the potential roles of sirE in A. fumigatus. Our findings indicate that SirE participates in growth, maintenance of cell wall integrity, secondary metabolite production, and virulence. The profile of acetylated proteins of the A. fumigatus sirE knockout strain (ΔAfSirE) demonstrates a hyperacetylation on histones, which influences the transcription of different genes. Gene Ortholog Enrichment analysis of differentially expressed genes indicated that upregulated genes are associated with secondary metabolites production and the downregulated genes are associated with the N-acetylglucosamine metabolic process, glucosamine catabolic

proess, and amino sugar catabolic process. Further in vivo studies will be necessary to assess the therapeutic potential of sirtuin inhibitors on the most common fungal pathogens among immunocompromised individuals. In that way, understanding the biology of these enzymes in A. fumigatus and their inhibitors may open perspectives for the development of new molecules targeting protein acetylation balance.

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CS1.3.7

THE EFFECTS OF PHASE SEPARATION ON CHROMATIN MODIFICATIONS, TRANSCRIPTIONAL REGULATION AND VIRULENCE IN THE HUMAN FUNGAL PATHOGEN CANDIDA ALBICANS

Qing Lan[1], Zhengqiang Miao[1], Songlin Wu[1], Koon Ho Wong[1]

[1]*University Of Macau, Macao, China*

Candida albicans is an opportunistic pathogen that can live in the human body as a commensal and cause deadly infection when the immune system is compromised. C. albicans can colonize different niches and survive the wide range of stresses elicited from the host during infection. These abilities are mediated by rapid and dynamic transcriptional responses, which are tightly controlled at transcription levels (e.g. transcriptional activation, elongation, and termination) and chromatin modification. The phenomenon of liquid-liquid phase separation can rapidly trigger the formation and dissociation of cellular compartments for biomacromolecules like proteins and nucleic acids without physical barriers. The process is reversible and tunable by factors relevant to the infection process, such as pH, temperature, and salinity. Phase separation has been shown to play crucial roles in controlling various biological processes and pathways in many organisms. We hypothesize that phase separation is important for the transcriptional responses of pathogens during adaption to diverse environmental conditions and infection. This study sets out to investigate the role of phase separation on the transcription and pathogenicity processes in C. albicans.

CS1.3.8

THE ROLE OF HISTONE MODIFICATIONS IN MORPHOLOGICAL PLASTICITY OF AUREOBASIDIUM PULLULANS

Zainab Abdul Qayyum, Eva Tenschert, Robert L. Mach, Astrid R. Mach-Aigner, Christian Zimmermann

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"Pleomorphic fungi" have the ability to alter their morphology following environmental cues i.e., switch between two or more growth forms. Aureobasidium pullulans is a pleomorphic, black, yeast-like fungus, also known as a significant producer of pullulan. In A. pullulans, the regulatory network responsible for switching between multicellular hyphae, unicellular yeast and other growth forms is explored very little. In the phenotypic plasticity context, fungal cells partake unique epigenetic programs. The study of epigenetic influence along with the deletion of the cell signalling regulatory factors has been a fruitful tactic in previous studies. In A. pullulans, histone modification acts as a significant regulatory factor, indicating epigenetic imprints for specific gene expression. Screening assays indicate Histone deacetylase inhibitors and histone acetyltransferase (HAT) inhibitors to have an impact on morphological switching. Our work aims to investigate the regulatory webbing and putative factors involved in this phenomenon. Constructing strains with locked phenotypes, followed by phenotype characterization, omics analyses and bioinformatics will help identify the regulatory network for morphological plasticity. This research will undoubtedly assist in finding unbiased correlations and contribute to a more satisfactory understanding of morphological plasticity in A. pullulans and fungi in general.

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CONCURRENT SESSION 1.4
BIOCONTROL AND NATURAL ANTAGONISTS

MONDAY, MARCH 6
14:00 – 16:00
Location: ***Hall Freiburg (Congress Innsbruck)***

CHAIRS:
Lea Atanasova, Magnus Karlsson

CS1.4.1

GENES FOR AN EXTENDED PHENOTYPE: FUNGAL BIOSYNTHESIS OF VOLATILES IN ZOMBIE FLIES ENTICE MALE FLIES TO MATE WITH FEMALE CADAVERS

Andreas Naundrup[1], Björn Bohman[2], Charles A. Kwadha[2], Annette B. Jensen[1], Paul G. Becher[2], **Henrik H. De Fine Licht**[1]

[1]*University Of Copenhagen, Frederiksberg C, Denmark,* [2]*Swedish University of Agricultural Sciences, Alnarp, Sweden*

To ensure dispersal, many parasites and pathogens behaviourally manipulate infected hosts. In the fungal kingdom, famous examples include so-called zombie ants and zombie flies. Here, we show that the host-specific and behaviourally manipulating pathogenic fungus, Entomophthora muscae, generates a chemical blend of volatile sesquiterpenes and alters the level of natural host cuticular hydrocarbons in dead infected female house fly (Musca domestica) cadavers. We used a combination of behavioural, chemical (GC-MS), and physiological (GC-EAD) analyses to identify the chemical cues eliciting male mating attraction and how the male antennae respond. We show that healthy male house flies respond to the fungal compounds and are enticed into mating with dead female cadavers. This is advantageous for the fungus as close proximity between host individuals leads to an increased probability of infection. We further use transcriptional profiling (RNAseq) of expressed genes in volatile chemical biosynthesis pathways to verify the fungus E. muscae as source of the behaviourally active volatile compounds in fungus-killed cadavers. It is unusual for

pathogens to rely on both behavioural host manipulation and sexual mimicry, and the E. muscae-emitted volatiles represent the evolution of an extended phenotypic trait that exploit male flies' willingness to mate and benefit the fungus by altering the behavioural phenotype of uninfected healthy male host flies.

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CS1.4.2

ONE HEALTH APPROACHES TO BIOCONTROL: BREEDING FOR BIOLOGICALS AND MICROBIOME RESILIENCE

Christian B Andersen[1], Bradley Dotson[1], Simon B Lassen[4], Kristin A Kadish[1], Kenneth Fredlund[3], Per Snell[3], Åsa Lankinen[1], Allan Rasmusson[2], **Laura Grenville-Briggs Didymus**[1]

[1]Swedish University Of Agricultural Sciences, Alnarp, Sweden, [2]Lund University, Lund, Sweden, [3]DLF Beet Seed, Landskrona, Sweden, [4]University of Copenhagen, Copenhagen, Denmark

In a one health context where we consider the plant as a holobiont, traits encoded for by symbiotic microbes and the microbiome are important contributors to overall plant health. We are studying the impact of fungal (Trichoderma afroharzianum T22) and oomycete (Pythium oligandrum) biocontrol agents on sugar beet and potato health. Studies on crop plant rhizosphere microbiomes have focused mainly on bacterial and fungal spatio-and-temporal dynamics between plant growth stages, genotypes and cropping systems. Very few studies have investigated manipulation of the plant rhizosphere microbiome with the amendment of a Biological Control Agent (BCA), which is of importance for our understanding of the function of BCAs in the environment and their overall impact on soil and plant health. We therefore conducted a series of field trials to investigate whether amendment of P. oligandrum induces growth promotion in potato, and further induces dynamic and temporal changes of the rhizosphere microbiome of the starch potato CV. Kuras, whilst at the same time offering protection from potato early blight. P. oligandrum induced significant changes in the fungal and bacterial diversity in the rhizosphere microbiome in a transient manner, indicating that potato rhizosphere microbial communities are highly resilient. P. oligandrum also had a biostimulatory effect in potato in a cultivar-dependent manner. In sugar beet we have also observed significant variation in the biocontrol of root rot and in growth promoting effects of T22 within sugar beet elite breeding lines leading us to hypothesize that host plant genetic factors may be important for the success of symbiotic microbes as plant symbionts, biocontrol agents and for biostimulation. Future work will focus on the identification of these plant genetic factors and their incorporation into crop breeding programs along with microbiome engineering approaches to produce crop plants with better interactions with symbionts.

CS1.4.3

SENSING AND REGULATION OF MYCOPARASITISM-RELATED PROCESSES IN TRICHODERMA ATROVIRIDE

Lea Atanasova[1], Martina Marchetti-Deschmann[2], Albert Nemes[2], Bianca Bruckner[2], Pavel Rehulka[2], Nancy Stralis-Pavese[3], Paweł P. Łabaj[3], David P. Kreil[3], Susanne Zeilinger-Migsich[4]

[1]University Of Natural Resources And Life Sciences, Vienna (BOKU), Department of Food Science and Technology, Vienna, Austria, [2]TU Wien, Institute of Chemical Technologies and Analytics , Vienna, Austria, [3]University of Natural Resources and Life Sciences, Vienna (BOKU), Chair of Bioinformatics, Vienna, Austria, [4]University of Innsbruck, Department of Microbiology, Innsbruck, Austria

Trichoderma atroviride is a successful mycoparasite applied for the protection of plants against fungal pathogens. The direct antagonism of plant pathogenic fungi by T. atroviride involves several stages and includes the host-triggered activation of molecular targets. The receptors and signaling pathways involved in sensing and responding to a fungal host are of great importance for the activation of the mycoparasitic response. We showed that G-coupled protein receptor Gpr1 and mitogen-activated protein kinase (MAPK) Tmk1 have mycoparasitism-associated functions in T. atroviride. Transcriptome and proteome analyses, moreover, revealed a set of host-responsive Tmk1 targets and their differential gene regulation further indicated a stimulating regulatory function of this MAP kinase in T. atroviride. Based on a data intersection analysis Tmk1 is strongly involved in molecular functions with GTPase and oxidoreductase activity, indicating that during the R. solani antagonism, Tmk1 mainly governs processes regulating cell responses to extracellular signals and those involved in putative redox reactions and oxidative stress.

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CS1.4.4

HARNESSING MICROBIOTA FUNCTIONS TO COMBAT FUSARIUM DISEASES IN WHEATS

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Fungal diseases of wheat caused by Fusarium spp. have caused severe yield loss, such as Fusarium head blight and Fusarium crown rot. Moreover, Fusarium spp. contaminate grains with various mycotoxins during infection. Application of biological control agents from crop associated microbiome is an attractive alternative strategy for control diseases. In our studies, a few biocontrol bacteria are pick up from wheat associated microbiome, subsequently their mode of action to suppress wheat Fusarium diseases are investigated. We found that Pseudomonas chlororaphis ZJU60 secreted antimicrobial substrate phenazine-1-carboxamide to inhibit fungal growth and virulence by altering histone acetylation. Streptomyces hygroscopicus S89 suppressed Fusarium diseases by disrupting the fungal autophagy homeostasis. More recently, we reported that Pantoea agglomerans ZJU23 isolated from fruiting bodies of F. graminearum showed potent efficient in reducing fungal growth and infectivity. Herbicolin A was identified as the key antifungal compound secreted by ZJU23. Genetic and chemical approaches led to the discovery of its biosynthetic gene cluster. Herbicolin A exerted a fungus-specific mode of action by directly binding and disrupting ergosterol-containing lipid rafts. Synthetic microbial communities based on above biocontrol agents and others are designing for enhancing wheat resiliency against Fusarium diseases.

CS1.4.5

UTILIZING BACTERIAL PHYLLOSPHERE DYNAMICS OF CERCOSPORA LEAF SPOT-INFECTED SUGAR BEET TO HUNT FOR BACTERIAL ISOLATES INVOLVED IN FUNGAL ANTAGONISM.

Lorena Rangel[1], Nathan Wyatt[1], Mari Natwick[2], Madison Christenson[2], Peter Hakk[2], Mohamed Khan[2], Melvin Bolton[1]

[1]U.S. Department of Agriculture - Northern Crop Science Laboratory, Fargo, United States, [2]North Dakota State University, Fargo, United States

Cercospora leaf spot (CLS), caused by the fungal pathogen Cercospora beticola, is the most destructive foliar pathogen of sugar beet (Beta vulgaris). Leaves of CLS-resistant and -susceptible cultivars grown in a single field were sampled to monitor changes in the phyllosphere microbiome over the growing season. CLS-resistant and -susceptible sugar beet varieties were allowed to naturally acquire CLS. Leaves from both genotypes were harvested every three weeks beginning at symptom development of the CLS-susceptible variety and ended during harvest. The full 16S rDNA gene was deep-sequenced for each sample to characterize the leaf bacterial microbiome. Bacterial communities were profiled, and composition and predicted functions were compared between sugar beet genotypes. Additionally, a culture collection was created by isolating phenotypically diverse bacteria from each genotype at each time point. A total of 453 isolates were taxonomically characterized by 16S sequencing and assayed for antagonism towards C. beticola. Future efforts will entail molecularly characterizing bacterial molecules and underlying biosynthetic genes responsible for this antagonism. By monitoring communities for taxa associated with the presence or absence of CLS as well as microbes with antagonistic tendencies towards the pathogen, we can develop advanced strategies that can inform better management of CLS in a dynamic environment.

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CS1.4.6

ENDOPHYTIC FUNGI AS BIOCONTROL AGENTS OF CRANBERRY PLANT PATHOGENS

Bhagya C. Thimmappa[1], Lila Naouelle Salhi[1], Lise Forget[1], Matt Sarrasin[1], Peniel Bustamante Villalobos[1], Marcel Turcotte[2], Franz B. Lang[1], Gertraud Burger[1]

[1]Department of Biochemistry and Robert-Cedergren Centre for Bioinformatics and Genomics, Université de Montréal, Montreal, Quebec, Canada, [2]School of Electrical Engineering and Computer Science, University of Ottawa, Ottawa, Ontario, Canada

Endophytes influence the health and growth of their host plant. Some endophytes play a significant role in controlling plant pathogens and are therefore used as biocontrol agents in agriculture. Only little is known about endophytes with biocontrol ability in Ericaceae. In the current study, we focus on one of the fungal endosymbionts of Vaccinium macrocarpon (Cranberry), provisionally called Endophytic Champignon 4 (EC4), which colonizes cranberry plant roots. 28S rRNA phylogeny places EC4 as Codinaeella sp, a poorly explored group of fungi (Chaetosphaeriaceae, Sordariomycetes). We show that EC4 can control the growth of a broad range of pathogens infecting cranberry and other plants by tests on plate and in-plantae. Pathogens induce the production of antimicrobial compounds in EC4. Genomic and transcriptomic analyses show that pathogens induce the production of secondary metabolites such as NonRibosomal Peptide Synthetases, PolyKetide Synthases, and fungal cell wall degrading enzymes by EC4, which most likely cause the suppression of the pathogens.
Many secondary metabolite gene clusters of EC4 have not been described in any other organism, and thus open a window on new antagonistic compounds. In addition, EC4 has the potential to be employed as a biocontrol agent in sustainable agriculture.

CS1.4.7

TRANSCRIPTOMIC APPROACH AND FUNCTIONAL GENETICS TO UNVEIL THE INTERACTION BETWEEN A BIOCONTROL YEAST AND A FUNGAL PATHOGEN ON THE HOST

Giuseppe Ianiri[1], Giuseppe Barone[1], Davide Palmieri[1], Filippo De Curtis[1], Giuseppe Lima[1], Raffaello Castoria[1]

[1]University of Molise, Campobasso, Italy

Biocontrol strategies are a promising alternative for food safety and food security. This study aims to decipher the molecular interactions involving the biocontrol agent (BCA) yeast Papiliotrema terrestris LS28, the pathogen Penicillium expansum, and Malus domestica. RNAseq analysis was performed during both their dual and tritrophic interactions to identify their differentially expressed genes. Analysis of transcriptome changes in the BCA revealed that nutrient transports and oxidative stress response are critical for the BCA to rapidly colonize the ecological niche and outcompete the pathogen. In the absence of P. expansum, BCA genes involved in metabolism and transport of carbohydrates were highly represented, suggesting a different nutritional requirement of P. terrestris when it is not competing with the pathogen. To confirm transcriptomic data, several P. terrestris mutants generated displayed reduced biocontrol activity against P. expansum. For transcriptomic analysis of P. expansum, genes involved in transcription, oxidation reduction process, and transmembrane transport were the most represented GO categories, regardless of the presence of the BCA. While in the absence of the BCA there was enrichment of oxidation reduction process, in the presence of the BCA metabolic processes of polysaccharides, aminoglycan and glucosamine-containing compounds were strongly enriched, suggesting a substantial nutritional rewiring of the pathogen to directly outcompete the BCA. Analysis of the M. domestica transcriptome revealed overexpression of genes involved in host defense signaling pathways both in the presence of the BCA and the pathogen, and a prevalence of PTI and ETI host genes overexpressed only during interaction with P. expansum. This comprehensive analysis contributes to advance the knowledge on the molecular mechanisms that underlie biocontrol activity and the tritrophic interaction with the pathogen and the host.

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CS1.4.8

MICRORNA PROFILING OF THE METARHIZIUM BRUNNEUM – GALLERIA MELLONELLA PATHOSYSTEM

Muneefah A. Alenezi[2], Christopher Coates[3], Bethany Greenfield[1], Tariq Butt[1], Vassili Kovelisn[4], Alexandra Kortsi[4], Marios Andrikopoulos[4], **Daniel Eastwood**[1]

[1]Swansea University, Swansea, United Kingdom, [2]University of Tabuk, Tabuk, Saudi Arabia, [3]University of Galway, Galway, Republic of Ireland, [4]University of Athens, Athens, Greece

The infection of invertebrate hosts by entomopathogenic fungi, such as Metarhizium spp., involves a series of regulated developmental changes from germinating spores, appressorial penetration, blastospore-driven colonisation and reversion to hyphal form during emergence from the host to sporulate. In addition, the fungus interacts with host physiology and immune defences in order to proliferate and establish in the liquid and solid tissues. Genetic and biochemical analyses have revealed key molecular mechanisms driving the infection process. However, the role of pathogen-derived microRNAs during the infection cycle has received much less attention, despite being implicated in morphological development in other systems. In this study, we isolated and sequenced microRNAs at different stages of Metarhizium brunneum infection of Galleria mellonella. MicroRNAs were mapped across pathogen and host genomes to identify putative gene targets. Finer scale effects on gene expression during infection were measured by qPCR across three different hosts, G. mellonella, Tenebrio molitor and Schistorcerca gregaria. The study also correlated host enzymatic defence measurements, including detoxification, to disease stage and transcript expression levels. The potential roles of microRNAs in Metarhizium-insect antibiosis are discussed.

CONCURRENT SESSION 2.1
SYNTHETIC BIOLOGY AND BIOTECHNOLOGY

MONDAY, MARCH 6
17:30 – 19:30
Location: ***Hall Tirol (Congress Innsbruck)***

CHAIRS:
Matthias Steiger, Arthur F.J. Ram

CS2.1.1

A VERSATILE HIGH–THROUGHPUT FRIENDLY SYSTEM FOR CONSTRUCTION AND VALIDATION OF FUNGAL CELL FACTORIES.

Katherina Garcia Vanegas[1], Kyle Richard Rothschild-Mancinelli[2], Martzel Antsotegi[1], Martí Morera Gómez[1], Tomas Strucko[1], Andreas Møllerhøj Vestergaard[1], Fabiano Jares Contesini[1], Uffe Hasbro Mortensen[1]

[1]Technical University of Denmark, Kongens Lyngby, Denmark, [2]Novozymes A/S, Bagsværd, Denmark

Filamentous fungi are industrially important because of their large production of metabolites and enzymes. However, compared to cell factories based on Escherichia coli and yeasts where high-throughput strain construction tools are highly developed, most strain construction work on filamentous fungi is still mostly done manually and, hence, in low throughput. One reason for this technology gap is a lack of fungal genetic tools that are compatible with an automated setup. However, recent developments in in vivo DNA assembly and CRISPR based gene-editing techniques are setting the stage for implementing high throughput fungal cell factory engineering. To this end, we have established a flexible platform, DIVERSIFY, for multi-species heterologous gene expression using methods that do not involve E. coli cloning steps. Specifically, relevant gene-expression cassettes compatible with the platform can quickly be assembled from libraries of parts, directly into the fungal strain by in vivo recombination. All individual parts for gene-expression cassette construction are made by PCR that are co-transformed into a given host. Importantly, the key parts of our expression cassettes are compatible with a string of DIVERSIFY strains that have all been pre-engineered to harbor a common landing platform, which is designed to accept the common gene-targeting expression cassette. Together the system makes it possible to produce a large number of gene-expression cassettes that can be easily introduced and tested for production efficiency in a number of different production hosts in parallel to increase the experimental success rate. We are currently adapting these technologies to fit into an automated workflow that will allow us to construct and validate 100-1000 in defined strains for heterologous protein production in a time frame of two weeks.

CS2.1.2

SYNTHETIC BIOLOGY TOOLS FOR GENOME MINING OF FUNGI FOR NOVEL BIOACTIVE METABOLITES

Yit-Heng Chooi[1], Indra Roux[1], Clara Woodcraft[1], Jinyu Hu[1]

[1]*University Of Western Australia, Crawley, Western Australia, Australia*

Fungi are prolific producers of bioactive molecules, called secondary metabolites (SMs), which serve as a valuable source of drugs and agrichemicals. With the increased availability of fungal genome sequences, improved knowledge of SM biosynthesis and development of knowledge-based computational tools, we now have the ability to rapidly identify biosynthetic gene clusters (BGCs) within fungal genomes that encode SMs and can predict the structural classes of these compounds. Significantly, these advances have also revealed that most fungi possess immense biosynthetic potential that far surpasses the diversity of SMs typically observed from classical fermentation and natural product isolation studies. To tap into this "biosynthetic dark matter" to fuel bioactive discovery, efficient synthetic biology tools are needed to translate these cryptic BGCs into novel molecules. To this end, we have developed a set of episomal yeast-Aspergillus-E. coli shuttle expression vectors for efficient refactoring of gene clusters up to twelve genes for heterologous expression in Aspergillus hosts, which has led to characterisation of a number of BGC products. To assist with whole gene cluster genome integration when it is more desirable, we have developed a cre-lox-based recombination system, in which we demonstrated efficient targeted integration of a 21 kb DNA fragment in a single step. Finally, we have developed the first CRISPR-mediated transcriptional activation based on dCas12a-VPR activator, which has the ability for multiplexing. We were able to successfully activate the production of the metabolite dehydromicroperfuranone. More recent development in this technology in our lab would be discussed.

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CS2.1.3

CHARACTERIZATION OF A GH5_7 B-MANNANASE BY ACTIVITY-BASED PROTEIN PROFILING IN SECRETOMES OF A. NIGER

Massimo Tedeschi[1], Vincent A. J. Lit[2], Prajeesh Kooloth Valappil[1], Mark Arentshorst[1], Hermen S. Overkleeft[2], Arthur F. J. Ram[1]

[1]*Leiden University, Institute of Biology Leiden, Microbial Sciences, Fungal Genetics and Biotechnology, Sylviusweg 72, 2333 BE, Leiden, The Netherlands,* [2]*Leiden University, Leiden Institute of Chemistry, Department of Bio-organic Synthesis, Einsteinweg 55, 2333 CC, Leiden, The Netherlands*

Activity-based protein profiling (ABPP) is an effective tool to characterize glycosyl-hydrolases in complex mixtures. ABPP relies on a simple yet powerful concept in which activity-based probes (ABPs) irreversibly inhibit an enzyme by covalently binding to its active site. ABPs are endowed with a reporter entity (either a fluorophore like Cy5 or a capture agent such as biotin) which allows detection and purification of the enzyme bound to the ABP. ABPP works on crude protein mixtures as long as the ABP is selective for the desired enzymes and is an ideal tool to screen fungal secretomes for interesting activities. In the context of plant biomass polysaccharide processing, several ABPs have been developed to target e.g. cellulases, xylanases and alpha-glucosidases. In this study we developed ABPs for β-mannanases.
The synthesized β-mannanases ABPs were evaluated on A. niger secretomes obtained from cultivations on various mannan containing substrates including Guar gum, Locust bean flour and Konjac flour. Furthermore, mannanase GH5_7 from A. niger was overexpressed in A. niger. The GH5_7 gene was cloned between the glucoamylase promoter and terminator and transformed into a genetically modified A. niger strain. This A. niger strain contains multiple integration sites for targeted integration of the gene of interest in the genome. Such a strategy allows high production of the protein of interest.
Culturing the developed strain in the presence of glucose results in the specific production of the recombinant mannanase. Selectivity of the ABPs for GH5_7 was confirmed by labelling the recombinant mannanase in the secretome of the engineered strain. Further experiments are in progress to characterize pH and temperature stability of GH5_7 and binding potency of the newly developed ABPs.

CS2.1.4

CONTROLLING MACROMORPHOLOGIES OF ASPERGILLUS NIGER DURING HIGH AND LOW SHEAR STRESS BIOREACTOR CULTIVATION

Karin Engelbert[1], Tolue Kheirkhah[1], Henri Müller[2], Charlotte Deffur[2], Stefan Junne[1,3], Heiko Briesen[2], Peter Neubauer[1], Vera Meyer[1]

[1]Technische Universität Berlin, Berlin, Germany, [2]Technical University of Munich, Munich, Germany, [3]Aalborg University, Aalborg, Denmark

Submersed cultivation of filamentous fungi is widely used in fungal biotechnology. The formation of different macromorphologies, however, range from dispersed mycelia over loose clumps to dense pellets, and thus limits productivity with shear stress as one of the main influencing parameters.
In this study, seed cultures with defined macromorphologies of the cell factory Aspergillus niger were exposed to high shear stress in stirred-tank (STR) and low shear stress in rocking-motion bioreactors (RMB). Talcum microparticles at 1 and 10 g L-1 were added to the seed cultures to achieve pellet populations with controlled diameter sizes. Physiological and morphological data were comprehensively investigated with high-throughput 2D image analysis and 3D synchrotron radiation based micro-computed tomography. This approach allowed us to determine the distribution of spore agglomerates, pellets and dispersed mycelia as well as hyphal densities and total hyphal lengths. Our data show that high shear stress in STR leads to breakage of pellets right after the stirrer was switched on. The mechanical stress from stirring also hindered pellets from surpassing a certain diameter during cultivation. In contrast, pellet size increased constantly until glucose was limited during RMB cultivations with largest macromorphological changes during the exponential growth phase.
This work will allow us to estimate hyphal growth rates and pellet breakage as a function of shear stress for the first time and will furthermore pave the way for better understanding of cell-bioreactor interactions, and thus morphology-optimised cultivation processes.

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CS2.1.5

THE EMERICELLOPSIS GENUS, A COMPARISON OF ITS MARINE AND TERRESTRIAL STRAINS

Raquel Ledo Doval[1], Bo Briggeman[1], Ronald de Vries[1]

[1]Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands

The diversity, phylogeny and ecological interactions of marine fungi remain poorly explored despite being pivotal elements of the marine ecosystem. They perform essential roles in the nutrient cycles and in maintaining the ecosystems' balance by stablishing ecological interactions with other marine organisms like algae. The metabolic pathways of terrestrial fungi have been the focus of research in the last years due to their potentiality for ecological and industrial applications. Many carbohydrate-active enzymes (CAZymes) have been identified and characterized as optimal biological tools for plant biomass degradation. When compared to terrestrial biomass, marine substrates widely vary in their sugar composition which most certainly tailored the enzymatic capacities of the marine fungi towards the production of novel degrading enzymes. Numerous Emericellopsis species has been putatively described as one of the most prevalent ones within marine habitats, being especially found in the decaying seaweed matter. In this study we focus on the comparative analysis of the enzymatic activities of three selected Emericellopsis species and their terrestrial counterparts. Thus, we aim to not only broadening our understanding on the underlying phylogenetic relationships of the Emerocellopsis genus but to explore novel enzymatic activities that can be used in the biorefinery of diverse algal substrates. Ultimately, this can provide more sustainable and efficient ways of extracting the valuable bioactive compounds found in most algal species and whose demand has drastically increase in recent years within the food, medical and bio-based economy fields.

CS2.1.6

ENHANCED PRODUCTION OF HETEROLOGOUS NATURAL PRODUCTS IN ASPERGILLUS ORYZAE BY LARGE-SCALE METABOLIC ENGINEERING BASED ON GENOME EDITING

Naoya Saito[1], Takuya Katayama[1], Atsushi Minami[2], HIdeaki Oikawa[2], Jun-ichi Maruyama[1]

[1]The University of Tokyo, Tokyo/113-8657 Bunkyo-ku/1-1-1 Yayoi, Japan, [2]Hokkaido University, Hokkaido/060-0810 Sapporo/Kita-ku, Japan

Aspergillus oryzae has recently been used as a useful host in heterologous production of natural products. To improve the productivity, optimal engineering based on the metabolic feature of A. oryzae is needed. However, due to limitations in the number of transformation marker genes available, it was difficult to modify multiple metabolic genes in addition to integration of whole biosynthetic genes for heterologous production. We developed the CRISPR/Cas9 system as a genome editing technique for A. oryzae(1). Along with recycling technique of genome editing plasmid, it enabled efficient multiple gene modifications for large-scale metabolic engineering in addition to heterologous production. Therefore, we aimed to develop a host for high-level production of heterologous natural products by enhancement of the metabolic pathway fluxes based on multiple gene modifications using genome editing.

As a model of heterologous production, we used pleuromutilin, an antibacterial diterpene produced by a basidiomycete fungus. First, transcriptomic and metabolomic analyses were performed to obtain the information on day-course variations regarding metabolic genes and metabolites. Based on the information, we designed for a higher productivity of the terpene by inhibition of ethanol fermentation, increase of acetyl-CoA supply via the TCA cycle, and enhancement of the entire mevalonate pathway. We modified the relevant genes of metabolic pathways and analyzed the productivity of pleuromutilin by GC/MS. As a result, each pathway modification caused metabolic change and increased the pleuromutilin production. Finally, combination of the three pathways modifications with 13 genes provided the highest productivity of pleuromutilin (161.6 mg/L, 8.4-fold higher than WT). The effect of large-scale metabolic engineering on production of other natural products will be presented.

(1) Katayama et al. (2019) Appl. Environ. Microbiol. 85:e01896-18.

CS2.1.7

FUNCTIONAL CHARACTERIZATION OF A HIGHLY SPECIFIC L-ARABINOSE TRANSPORTER FROM TRICHODERMA REESEI

Sami Havukainen[2], Jonai Pujol-Giménez[3], Mari Valkonen[2], Matthias Hediger[3], **Christopher Landowski**[1,2]

[1]Onego Bio Ltd., Helsinki, Finland, [2]VTT Technical Research Centre of Finland, Espoo, Finland, [3]Department of Biomedical Research, Inselspital, University of Bern, Bern, Switzerland

Background: Lignocellulose biomass has been investigated as a feedstock for second generation biofuels and other value-added products. Some of the processes for biofuel production utilize cellulases and hemicellulases to convert the lignocellulosic biomass into a range of soluble sugars before fermentation with microorganisms such as the yeast Saccharomyces cerevisiae. One of these sugars is L-arabinose, which cannot be utilized naturally by yeast. The first step in L-arabinose catabolism is its transport into the cells, and yeast lacks a specific transporter, which could perform this task.
Results: We identified Trire2_104072 of Trichoderma reesei as a potential L-arabinose transporter based on its expression profile. This transporter was described previously as D-xylose transporter XLT1. Electrophysiology experiments with Xenopus laevis oocytes and heterologous expression in yeast revealed that Trire2_104072 is a high-affinity L-arabinose symporter with a Km value in the range of 0.1-0.2 mM. It can also transport D-xylose, but with low affinity (Km = 9 mM). In yeast, L-arabinose transport was inhibited slightly by D-xylose but not by D-glucose in an assay with five-fold excess of the inhibiting sugar. Comparison with known L-arabinose transporters revealed that the expression of Trire2_104072 enabled yeast to uptake L-arabinose at the highest rate in conditions with low extracellular L-arabinose concentration. Despite the high specificity of Trire2_104072 for L-arabinose, the growth the T. reesei deletion mutant was only affected at low L-arabinose concentrations.
Conclusions: Due to its high affinity for L-arabinose and low inhibition by D-glucose or D-xylose, Trire2_104072 could serve as a good candidate for improving the existing pentose-utilizing yeast strains. The discovery of a highly specific L-arabinose transporter also adds to our knowledge of the primary metabolism of T. reesei. The phenotype of the deletion strain suggests the involvement of other transporters in L-arabinose transport in this species.

CS2.1.8

BIOCONVERSION OF LIGNOCELLULOSIC FEEDSTOCKS TO 3-HYDROXYPROPIONIC ACID USING ACIDOPHILIC FUNGI

Kyle Pomraning[1]

[1]*Pacific Northwest National Laboratory, Richland, United States*

Biological engineering of microorganisms to produce commodity chemicals is a promising route to sustainable manufacturing. 3-hydroxypropionic acid (3-HP) can be used directly to produce biodegradable polymers or modified to produce 1,3-propanediol, acrylic acid, methyl acrylate, acrylamide, and acrylonitrile as drop-in replacements for petroleum derived chemicals in existing industrial processes. Filamentous fungi are notable for their ability to convert a variety of complex feedstocks and produce organic acids at low pH making them attractive as a host for bioproduction of 3-HP. Here we engineered acidophilic fungi from the genus Aspergillus for the conversion of hexose and pentose sugars to 3-HP using the resources available via the DOE Agile BioFoundry. A synthetic carbon-neutral pathway was initially constructed to establish 3-HP production in Aspergillus pseudoterreus and then was transferred into Aspergillus niger, followed by two multi-omics enabled design, build, test, learn (DBTL) cycles, as well as pathway stability and scale-up assessment in small scale bioreactors. Overexpression of pyruvate carboxylase, a 3-HP plasma membrane transporter, and deletion of a malonate semialdehyde dehydrogenase in addition to over-expression of heterologous pathway genes improved yield to 0.31 C-mol 3-HP C-mol−1 glucose while optimization of culture conditions for production of 3-HP from deacetylated and mechanical refined corn stover hydrolysate improved yield to 0.48 C-mol 3-HP C-mol−1 sugar monomers and resulted in a final titer of 36.0 g/L 3-HP. This work establishes Aspergillus species as a platform for commercial production of renewable 3-HP.

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CONCURRENT SESSION 2.2 DEVELOPMENT AND MORPHOGENESIS

MONDAY, MARCH 6
17:30 – 19:30
Location: ***Hall Brüssel (Congress Innsbruck)***

CHAIRS:
Florentine Marx, Fabienne Malagnac

CS2.2.1

A NOVEL SECRETED PROTEIN STT1 ADSORBED ON THE TIP OF STERIGMA MEDIATES BASIDIOSPOROGENESIS

Hiroshi Yoshida[1], Yuichi Sakamoto[1]

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Coprinopsis cinerea Stt1 is a putative small secreted protein of 253 aa containing an N-terminal signal peptide of 20 aa but lacking any known functional domains. Stt1 homologues are found only in basidiomycetes fungi, whose function has not been reported previously. Recently it was shown that Stt1 was transcriptionally upregulated in basidiocarp pileus (cap) tissue at the sporogenesis stage, implicating it in sexual development. The objective of this study was to investigate the role of Stt1 in the life cycle of basidiomycetes fungi. First of all, we obtained STT1-knockout (Δstt1) strains to examine their growth and development. They showed normal growth and basidiocarp development but were completely devoid of basidiospore formation. The sterile basidia of Δstt1 contained four nuclei and formed sterigmata without any spores or prespores produced, indicating meiosis completion and postmeiotic arrest in Δstt1. We hypothesized that Stt1 might participate in prespore generation at the apex of sterigma and that Stt1 would be localized on the surface of the sterigma tip or around the swelling prespore since Stt1 was predicted to be a secreted protein. To examine the localization of Stt1, we constructed strains expressing mCherry-tagged Stt1 (Stt1-mCherry). Introduction of the gene coding for Stt1-mCherry into the Δstt1 restored basidiospore formation.

By fluorescence microscopy, Stt1-mCherry was detected only at the tips of sterigmata. Before prespore formation, Stt1-mCherry distributed around the apical part of elongating sterigma; it formed cap-like structure on the sterigma tip. After the initiation of prespore formation, Stt1-mCherry distributed around the narrowest part between the strigma and the swelling prespore but not around the prespore; it formed ring- or cylinder-like structure. Stt1-mCherry fluorescence was then attenuated following basidiospore maturation. These results demonstrate that Stt1 is produced at the apex of sterigma and adsorbed on the sterigma tip surface to perform essential functions in basidiosporogenesis.

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CS2.2.2

THE IMPORTANT ROLE OF PROTEIN KINASES IN BASIDIOMYCETE SPORULATION

Peter Jan Vonk[1], Julie Grosse-Sommer[1], Emmeline van Roosmalen[1], Brandon Simon[1], Natalia Escobar[1], Robin A. Ohm[1]

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Many basidiomycetes form mushrooms to produce basidiospores for sexual propagation. In the cultivation of edible mushrooms however, sporulation is an unwanted trait that is associated with allergic reactions. Because mushroom development and basidiospore formation are part of a single, highly complex developmental program, strains deficient in sporulation often also have defects in mushroom development. We identified four highly conserved protein kinases that are developmentally regulated and essential for basidiospore formation, but not mushroom development. Deletion strains of all four kinases resulted in strains deficient in basidiospore formation. Using fluorescent microscopy, we could localize one of these kinases to the basidia, which are involved in spore formation. Potential downstream targets of this kinase were identified using phosphoproteomics, which were then verified with a yeast-2-hybrid system.

CS2.2.3

EFFECTS OF NWD2 GENES ON THE FRUITING PROCESS OF COPRINOPSIS CINEREA

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Fruiting body development in the dung fungus Coprinopsis cinerea is strictly regulated by environmental conditions including light, temperature and aeration. It takes place at 25° C and follows a conserved scheme defined by day and night phases, with well predictable distinct stages over the time. The differentiation process begins with the formation of primary hyphal knots (Pks) in the dark, which, when exposed to light, transform into compact aggregates, secondary hyphal knots (Sks) in which stipe and cap tissues differentiate. Further light signals control differentiation of tissues within the growing primordia. Primordium development (P1 to P5) takes five days to culminate on day 6 of development in light-induced karyogamy (K) followed by meiosis (M) within the basidia and basidiospore production which parallels fruiting body maturation (stipe elongation and cap expansion). Mature fruiting bodies autolyze on day 7 to release the spores in liquid droplets. Little is so far understood about the physiological and genetic backgrounds of the complex differentiation processes in C. cinerea. A self-compatible mutant strain AmutBmut with mutations in both mating type loci was used to generate a collection of mutants in fruiting. In the collection of about 1500 different strains, mutations did not evenly distribute over the complete pathway. High numbers of mutants are available from the early development stages up to stage P1, comparably few in subsequent steps up to P3. Larger sets of mutants exist for P4 and P5 when karyogamy and meiosis have to occur and fruiting body maturation has to be initiated. Mutant Proto159 has a defect in formation of Pks. An NWD2 gene was found in transformation to be a suppressor gene to overcome the defect. NWD2 genes are unique to few Agaricomycetes. They encode small NTPases and occur in C. cinerea in seven gene families analyzed for their influences on fruiting.

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CS2.2.4

GLOBAL ANALYSIS OF CIRCUITRY GOVERNING CANDIDA ALBICANS MORPHOGENESIS WITHIN HOST IMMUNE CELLS

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The evasion of killing by immune cells is crucial for fungal survival in the host. For the human fungal pathogen Candida albicans, the morphogenetic transition from yeast to filament upon internalization by macrophages is a key intracellular survival strategy that occurs through mechanisms that remain largely enigmatic. To identify the C. albicans genes that orchestrate filamentation in macrophages, we performed a functional genomic screen of conditional expression mutants covering ~50% of the C. albicans genome and identified 127 genes important for filamentation upon phagocytosis. Notably, twenty-six of the genes were dispensable for filamentation in host-relevant culture conditions (RPMI with 3% serum, 37°C, 5% CO2), demonstrating specificity in the program governing morphogenesis within macrophages. Gene ontology enrichment of the genes selectively required for morphogenesis in macrophages revealed a key role for the mitochondrial ribosome, aerobic respiration, and gluconeogenesis, suggesting that C. albicans heavily relies on respiration to enable intraphagosomal growth. In line with this hypothesis, we determined that expression of genes encoding subunits of the mitochondrial ribosome and electron transport chain are needed for C. albicans to escape from and kill macrophages. Further, we explored filament-inducing stimuli within or produced by the macrophage and determined that macrophage lysate is sufficient to induce morphogenesis. Bioactivity-guided fractionation coupled to mass spectrometry identified the immune modulator, prothymosin alpha (PTMA), as a potential macrophage-derived trigger of filamentation. Immunoneutralization of PTMA within macrophage lysate abolished its ability to stimulate C. albicans filamentation, strongly supporting PTMA as a filament-inducing component of macrophage

lysate. Overall, this work is the first to implicate a specific host protein as a trigger of filamentation and highlights respiration as an Achilles' heel of C. albicans intraphagosomal growth and escape from macrophages.

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CS2.2.5

A NOVEL SPORE-SPECIFIC TRANSCRIPTION FACTOR IS ESSENTIAL FOR CONIDIAL MATURATION AND DORMANCY IN ASPERGILLUS SPECIES

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Aspergillus, a filamentous fungus that makes up the majority of airborne fungi, reproduces primarily by forming asexual spores called conidia. The process of making conidia is regulated by various transcription factors (TFs). Although previous studies have shown that some TFs, such as VosA, VelB, and WetA, mediate conidia formation and maturation, there are still unexplored TFs for conidiogenesis. Therefore, we studied putative spore-specific TFs based on transcriptome analysis of conidia and hyphae in three representatives Aspergillus. As results, we identified twenty-two spore-specific TFs and each deletion mutant was phenotypically analyzed in A. nidulans. Among them, we characterized one of the spore-specific-C2H2 zinc finger A SscA in A. nidulans. The ΔsscA mutant showed defective conidiation, conidial viability, and reduced stress tolerance in A. nidulans. And the amount of trehalose in the ΔsscA mutant was decreased compared to that of the WT. On the other hand, deletion of sscA caused induced germ tube formation with or without glucose and increased the amount of β-glucan in ΔsscA mutant conidia compared to wild-type conidia. Absence of sscA led to increase the amount of sterigmatocystin in conidia. Furthermore, transcriptome data suggested that SscA affected the mRNA expression of various genes in A. nidulans conidia. Interestingly, deletion of sscA resulted in alterations of gene expression involved in the response of conidia to stimuli and stress. The mRNA levels of β-glucan biosynthesis gene and sterigmatocystin gene cluster were upregulated in sscA mutant conidia. In addition, we confirmed that the roles of SscA in conidia were conserved in A. flavus and A. fumgiatus. Overall, these results suggest that SscA is a spore-specific transcription factor, essential for proper asexual development, conidia maturation,

conidial dormancy, and secondary metabolites in A. nidulans. And the functions of SscA in conidia are conserved in three representative Aspergillus spp.

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CS2.2.6

IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF THE TRANSCRIPTIONAL CYCLIN-KINASE CTDK-1 COMPLEX OF ASPERGILLUS NIDULANS AS A REGULATOR OF GROWTH AND DEVELOPMENT

Ziortza Agirrezabala[1], Xabier Guruceaga[2], Adela Martín-Vicente[2], Ainara Otamendi[1], Ane Fagoaga[1], Jarrod R. Fortwendel[2], Eduardo A. Espeso[3], **Oier Etxebeste**[1]

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The genus Aspergillus includes industrially, medically and agriculturally important species. All of them, as do fungi in general, disperse to new niches principally by means of asexual spores. When it comes to the genetic/molecular control of asexual development, Aspergillus nidulans is the main reference. Two pathways control in A. nidulans the production of conidiophores, the structures bearing asexual spores (conidia). The UDA pathway transduces environmental signals, determining whether the CDP pathway and thus the required morphological changes are induced. The transcriptional regulator BrlA links both pathways while loss-of-function mutations in flb (UDA) genes block brlA transcription and, consequently, conidiation. However, the aconidial phenotype of specific flb mutants is reverted under salt-stress conditions. Previously, we generated a library of ΔflbB mutants unable to conidiate on culture medium supplemented with NaH2PO4 (0.65M). Here, we identified a Gly347Stop mutation within flpA as responsible for the mutant phenotype FLIP57. The putative cyclin FlpA and the remaining putative components of the C-terminal domain kinase-1 (CTDK) complex are necessary in the transition from metulae to phialides during conidiophore development. We show that the three orthologs of A. fumigatus are also necessary for proper growth and developmental patterns. Cellular localization, functional dependencies and their importance in virulence are also assessed. Overall, this work links control of RNA polymerase II activity through the CTDK-1 complex with growth, development and virulence in aspergilli.

CS2.2.7

F-BOX RECEPTOR MEDIATED CONTROL OF SUBSTRATE STABILITY AND SUBCELLULAR LOCATION ORGANIZES CELLULAR DEVELOPMENT OF ASPERGILLUS NIDULANS

Özlem Sarikaya Bayram[1], Özgür Bayram[1], Betim Karahoda[1], Cindy Meister[2], Anna M Köhler[2], Nadia Elramli[1], Dean Frawley[1], Jamie McGowan[1], David A Fitzpatrick[1], Kerstin Schmitt[2], Leandro Jose de Assis[3], Oliver Valerius[2], Gustavo H. Goldman[3], Gerhard Braus[2]

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Fungal growth and development are coordinated with specific secondary metabolism. This coordination requires 8 of 74 F-box proteins of the filamentous fungus Aspergillus nidulans. F-box proteins recognize primed substrates for ubiquitination by Skp1-Cul1-Fbx (SCF) E3 ubiquitin RING ligases and degradation by the 26S proteasome. 24 F-box proteins are found in the nuclear fraction as part of SCFs during vegetative growth. 43 F-box proteins interact with SCF proteins during growth, development or stress. 45 F-box proteins are associated with more than 700 proteins that have mainly regulatory roles. This corroborates that accurate surveillance of protein stability is prerequisite for organizing multicellular fungal development. Fbx23 combines subcellular location and protein stability control, illustrating the complexity of F-box mediated regulation during fungal development. Fbx23 interacts with epigenetic methyltransferase VipC which interacts with fungal NF-κB-like velvet domain regulator VeA that coordinates fungal development with secondary metabolism. Fbx23 prevents nuclear accumulation of methyltransferase VipC during early development. These results suggest that in addition to their role in protein degradation, F-box proteins also control subcellular accumulations of key regulatory proteins for fungal development.

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CS2.2.8

RAPID AND FREQUENT LOSS OF FEMALE FERTILITY UNDER CULTURE CONDITION IN THE RICE BLAST FUNGUS

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The rice blast fungus Pyricularia oryzae (Syn. Magnaporthe oryzae) is a heterothallic ascomycete that causes most destructive disease in cultivated rice worldwide. The infection cycle of this fungus consists of asexual reproduction, and most field isolates have lost female fertility. Meanwhile, several isolates collected from the region of origin, including Yunnan, China, have retained female fertility, suggesting that female fertility was lost during geographic spread of the fungus from the region of origin. We found that partial morphological changes of mycelia in the female-fertile isolate CH598 from Yunnan occur during long-term culture (2–4 weeks) on the oatmeal agar (OMA) medium. Interestingly, the isolated lines from the changed mycelia showed loss of female fertility. The morphology of CH598 continued to change during culture, but there were no observable changes to that of isolated lines (female-sterile lines), and the morphology and female sterility thereof was irreversible at the culture level. Compared with the parental isolate CH598, the growth rate in female-sterile lines was decreased on OMA, potato dextrose, yeast glucose and rice flour agar media. Additionally, conidiation in female-sterile lines was increased on the OMA medium but that was decreased on the rice flour agar medium. Recently we have identified the mutation of gene responsible for loss of female fertility in Japanese field isolates (Uchida, unpublished data), the same mutation was not detected in these female-sterile line genomes. Crossing of the female-fertile isolate with two female-sterile-lines tended to produce a segregation ratio of female-fertile and female-sterile progenies of 1:1 to 2:1. These results suggest that female fertility in P. oryzae may be rapidly lost in certain conditions due to genetic mutations and/or epigenetic regulation.

CONCURRENT SESSION 2.3
SENSING AND SIGNALING

MONDAY, MARCH 6
17:30 – 19:30
Location: ***Hall Strassburg (Congress Innsbruck)***

CHAIRS:
Monika Schmoll, Luis M. Corrochano

CS2.3.1

LIGHT DEPENDENT IMPACT OF METHIONINE ON METABOLISM OF TRICHODERMA REESEI AND SIGNAL TRANSMISSION BY THE GPCR GPR2

Miriam Schalamun[2], Sabrina Beier[2], Wolfgang Hinterdobler[2,3], **Monika Schmoll**[1]

[1]University Of Vienna, Wien, Austria, [2]AIT Austrian Institute of Technology GmbH, Tulln, Austria, [3]MyPilz GmbH, Wien, Austria

The natural habitat of the ascomycete Trichoderma reesei is a tropical forest. There, it efficiently degrades dead plant biomass by production of highly prolific cellulases and hemicellulases. Their production is considerably influenced by environmental cues such as carbon source, light or pH. Depending on the condition, available resources are distributed to balance growth with development or efforts for competition and defense.
Here we investigated the impact of the amino acid methionine, which can act as a carbon-, nitrogen- or sulphur source. Previously we showed that methionine influences sulphate uptake in a light- and carbon source dependent manner. Our transcriptome study now revealed a strong and light dependent impact on gene regulation in T. reesei, with more than thousand genes regulated in light and darkness on cellulose. Cellulase formation is shut down and considerable rearrangements in metabolic pathways as well as protein sythesis are initiated along with up-regulation of protease genes.
Additionally, we analzyed the contribution of a G-protein coupled receptor putatively involved in methionine sensing. Lack of GPR2 positively affects cellulase production, but does not rescue the phenotype seen upon addition of methionine. Transcriptome analysis showed that GPR2 positively regulates two genes involved in sulphur metabolism and three protease genes in light. Accordingly, gene regulatory network inferrence revealed GPR2 as a regulator in light. BIOLOG analysis showed that GPR2 is required for efficient growth on storage carbohydrates and intermediates of the galactose and xylose catabolism in darkness, but decreases growth on some substrates such as xylitol or alanine in light. Furthermore, GPR2 is required for full sexual fertility, but does not alter chemical communication.
We conclude that methionine serves as an important and light dependent metabolic signal in T. reesei and that one of the predicted sensors of methionine, GPR2, influences carbon source utilization, sexual development and sulphur metabolism.

CS2.3.2

MUTAGENESIS APPROACH TO UNRAVEL THE DUAL ROLE OF THE CANDIDA ALBICANS GPR1 RECEPTOR IN METHIONINE-INDUCED MORPHOGENESIS AND LACTATE-INDUCED SS-GLUCAN MASKING

Wouter Van Genechten[1], Stefanie Wijnants[1], Jolien Vreys[1], **Patrick Van Dijck**[1]

[1]*Laboratory of Molecular Cell Biology, KU Leuven, Leuven, Belgium*

In our previous work we showed that the G-protein coupled receptor Gpr1 senses the presence of methionine, resulting in morphogenesis via activation of the cAMP-PKA pathway (Maidan et al. Biochem Soc Trans 33: 291-293, 2005). Recently it was shown that Gpr1 is also sensing lactate, which then triggers β-glucan masking in the cell wall, which according to recent work is also mediated by the PKA pathway and most likely acts through trimming β-glucan exposed regions by exo- and endoglucanases (Ballou et al. Nat Microbiol. 2: 16238, 2016, José de Assis et al. mBio, e0260522, 2022).
We further characterized the different roles of Gpr1 through a mutagenic approach. Specific point mutants were affected in methionine-induced morphogenesis and masking, whereas one other mutant only showed a defect in β-glucan masking. We confirmed (and therefore contradict the Ballou et al. hypothesis) that there is a single Gα protein, Gpa2, associated with Gpr1 and that the Gpr1-Gpa2 module activates morphogenesis as well as lactate-induced β-glucan masking. Whereas the former phenotype is mediated solely by the cAMP-PKA pathway, the latter phenotype has both PKA dependent (Schrevens et al. Mol Microbiol. 108: 258-275, 2018) and independent components. Indeed, β-glucan masking is partly regulated by the Tpk isomers but this is independent of sensing lactate, based on data obtained with our point mutants. Furthermore, we evaluated these Gpr1 point mutants and their respective phenotypic defects upon interaction with macrophages. We conclude that neither immune evasion nor methionine-induced filamentation determines the outcome and fitness of C. albicans in a macrophage survival assay, indicating that another downstream phenotypic effect of Gpr1 plays a major role in macrophage survival. We are currently determining the role of a putative Gβ protein for this additional downstream effect of Gpr1.

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CS2.3.3

IN VITRO COMPETITIVE FITNESS PROFILING REVEALS SPECIFIC PROTEIN KINASES AND SECRETED PROTEASES PROMOTE ASPERGILLUS FUMIGATUS ADAPTATION TO CYSTIC FIBROSIS HOST

Kayleigh Earle[1], Clara Valero[1], Michael Bromley[1], Paul Bowyer[1], Sara Gago[1]

[1]*Manchester Fungal Infection Group, University Of Manchester, Manchester, United Kingdom*

Aspergillus fumigatus is a frequent coloniser of the cystic fibrosis (CF) lung and is responsible for the development of several diseases including allergic bronchopulmonary aspergillosis (ABPA) and aspergillosis bronchitis (AB). Our understanding of the factors that drive A. fumigatus pathogenicity in CF is limited. Previous work has implicated several protein kinases and secreted proteases in the promotion of A. fumigatus virulence in the host environment. Therefore, in this study we aimed to provide functional genomic analysis to describe the role of protein kinases and secreted proteases in promoting A. fumigatus pathogenesis in the CF lung. Using a library of 109 genetically barcoded protein kinase null mutants and 35 secreted protease null mutants, we carried out competitive fitness profiling and assessed the relative fitness of each mutant under different growth conditions. Comparison of the null mutants identified several protein kinases from known signalling pathways, as well as other clusters, which showed increased fitness in a range of conditions including mucin-rich media. These mutants will be selected for further analysis including screening with our CFTR KO epithelial cell line to further elucidate their role in the promotion of pathogenesis in CF.

CS2.3.4

ROLE OF THE PLASMA MEMBRANE H+-ATPASE PMA1 IN DEVELOPMENT AND VIRULENCE OF THE FUNGAL PATHOGEN FUSARIUM OXYSPORUM

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Ambient pH regulates fundamental processes in fungi, including cell growth, development and virulence. We previously found that changes in ambient pH induce rapid and transitory fluctuations in cytosolic pH (pHc), both in Saccharomyces cerevisiae and in the vascular wilt pathogen Fusarium oxysporum. Moreover, our results demonstrated that pHc acts as a signal for the regulation of conserved mitogen-activated protein kinase (MAPK) cascades. How pHc control is linked to MAPK signaling is currently unknown. The essential plasma membrane H+-ATPase Pma1 is the major regulator of pHc homeostasis in fungi. Pharmacological inhibition of Pma1 with Diethylstilbestrol (DES) triggers a rapid and sustained decrease of pHc. Here we found that a F. oxysporum mutant carrying a temperature-sensitive pma1 allele (pma1-ts) showed a significant decrease in Pma1 activity within 60 minutes after the shift from the permissive (24ºC) to the restrictive temperature (37ºC). Inhibition of Pma1 resulted in a dramatic decrease of hyphal growth and conidiation. On the other hand, targeted deletion of the casein kinase 1 (Ck1), a negative regulator of Pma1, caused a significant increase in Pma1 activity, accompanied by extracellular acidification and increased resistance to acetic acid-triggered cell death. Furthermore, the ck1Δ mutants were impaired in hyphal chemotropism towards acidic pH, invasive growth through cellophane membranes and pathogenicity on tomato plants. Our results represent a step forward towards understanding how pHc regulates cell signaling and virulence in fungal pathogens.

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CS2.3.5

REGULATION OF EXTRACELLULAR CELLULASE PRODUCTION BY HETEROTRIMERIC G PROTEIN SIGNALING IN NEUROSPORA

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Heterotrimeric G-protein signaling pathways regulate environmental sensing in eukaryotes. GDP/GTP exchange on the Galpha subunit leads to its activation, while GTP hydrolysis (inactivation) is accelerated by Regulator of G protein Signaling (RGS) proteins. We investigated a role for G-protein signaling in cellulose degradation through analysis of 6 G protein subunits, 7 RGS genes and adenylyl cyclase, a downstream effector of G protein signaling in N. crassa. We compared strains with either knockout mutations or expressing predicted constitutively activated, GTPase-deficient alleles (denoted *) for each of the three Galpha subunit genes. Our results showed that deletion of the Galpha subunit genes gna-1 and gna-3, the Gbeta subunit genes gnb-1 and cpc-2, the Ggamma gene gng-1, or adenylyl cyclase (cr-1) resulted in loss of detectable cellulase activity. This defect was also observed in the gna-3* strain; deletion of gnb-1 partially restored cellulase activity in this background, suggesting that GNB-1 is a negative regulator of GNA-3. We observed reduction of GNA-1 protein levels in some backgrounds that likely contributes to their low cellulase activity. Expression patterns for five cellulase genes were consistent with transcriptional regulation for 5/6 G protein subunits. cAMP fully remediated cellulase activity defects in Δgna-3 mutants, but only partially in Δgna-1 and Δgnb-1 mutants, suggesting that GNA-1 and GNB-1 regulate additional cAMP-independent pathways that control cellulase activity. Our data revealed that four of the seven RGS mutants had significantly different extracellular cellulase levels relative to wild type. Of interest, the Δrgs-2 mutant had no detectable activity, similar to the gna-3* strain. In contrast, the Δrgs-1 and Δrgs-4 mutants and gna-1* and gna-2* strains exhibited significantly higher cellulase activity than wild type. Our results suggest that RGS-2 interacts with GNA-3, while RGS-1 and RGS-4 regulate GNA-1 and/or GNA-2 during regulation of cellulase activity in N. crassa.

CS2.3.6

IDENTIFICATION OF F-BOX PROTEINS INVOLVED IN THE SWITCH TO CELLULOLYTIC METABOLISM

Philipp Benz[1], Lisa T. Meyer[1], Kerstin Schmitt[3],
Maria Augusta Crivelente Horta[2], Gerhard Braus[3], Gustavo H. Goldman[2]

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The fungal ability to secrete enzymes for the deconstruction of lignocellulosic material is of particular interest for the circular bioeconomy using renewable plant biomass. However, natural "brakes" exist, preventing the fungi to waste energy. One of these is carbon catabolite repression (CCR), a highly conserved and multi-faceted signaling process leading to the repression of lignocellulose utilization in presence of preferred carbon sources like glucose. Therefore, CCR is a disadvantage for industrial enzyme production.
The proteins involved in CCR are regulated, among other things, by targeted degradation, an important regulatory process to switch between metabolic states. Key factors here are F-Box proteins, which destine proteins for proteasomal degradation. F-box proteins are conserved in all eukaryotes and known to be involved e.g. in carbohydrate sensing and sulfur assimilation. Nevertheless, the specific function of the majority of F-Box proteins present in filamentous fungi remains enigmatic.
To identify F-Box proteins involved in the switch between CCR and lignocellulose utilization, we employed the genetics reference organism Neurospora crassa. N. crassa deletion strains of 40 genes with F-Box domains were screened to identify aberrant phenotypes related to CCR. By combination of two screens, several deletion strains were found to display significant CCR-repressed or de-repressed phenotypes. Four candidate F-Box proteins with a strong potential for regulatory importance in lignocellulose signaling pathways were subjected to GFP pull-down experiments to elucidate potential interaction partners. In parallel, transcriptomic changes in the corresponding fbx gene deletion strains during the switch from repressed to de-repressed states (and vice-versa) were assayed by RNAseq to identify their regulatory influence on carbon utilization.
The results of these experiments allow for a better understanding of the function of F-Box proteins during carbohydrate metabolic switches in general and will be essential to allow rational strain modifications, leading to improved enzyme-producing strains of high interest for the industry.

CS2.3.7

A LIGHT-SENSING SYSTEM IN THE COMMON ANCESTOR OF THE FUNGI

Luis Javier Galindo[1], David S. Milner[1], Suely Lopes Gomes[2], Thomas A. Richards[1]

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Diverse light-sensing organs (i.e., eyes) have evolved across animals, with several subcellular analogs found in eukaryotic microbes. All of these systems have a common "recipe": a light occluding or refractory surface juxtaposed to a membrane-layer enriched in type I rhodopsins. In the fungi, several lineages have been shown to detect light using a diversity of non-homologous photo-responsive proteins. However, these systems are not associated with an eyespot-like organelle with one exception found in the zoosporic fungus Blastocladiella emersonii (Be). Be possesses both elements of this recipe: an eyespot composed of lipid-filled structures (often called the side-body complex [SBC]), co-localized with a membrane enriched with a gene-fusion protein composed of a type I (microbial) rhodopsin and guanylyl cyclase enzyme domain (CyclOp-fusion protein). Here, we identify homologous pathway components in four Chytridiomycota orders (Chytridiales, Synchytriales, Rhizophydiales, and Monoblepharidiales) adding to those identified within the Blastocladiomycota and within the recently described phylum Sanchytriomycota. To further explore the architecture of the fungal zoospore and its lipid organelles, we reviewed electron microscopy data and performed fluorescence-microscopy imaging of four CyclOp-carrying zoosporic fungal species, showing the presence of a variety of candidate eyespot-cytoskeletal ultrastructure systems. We then assessed the presence of canonical photoreceptors across the fungi and inferred that the last common fungal ancestor was able to sense light across a range of wavelengths using a variety of systems, including blue-green-light detection. Our data imply, independently of how the fungal tree of life is rooted, that the apparatus for a CyclOp-organelle light perception system was an ancestral feature of the fungi.

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CS2.3.8

LOCAL CALCIUM SIGNAL TRANSMISSION IN MYCELIAL NETWORK EXHIBITS DECENTRALIZED STRESS RESPONSES

Norio Takeshita[1]

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Many fungi live as mycelia, which are networks of hyphae. Mycelial networks are suited for the widespread distribution of nutrients and water. The logistical capabilities are critical for the extension of fungal survival areas, nutrient cycling in ecosystems, mycorrhizal symbioses, and virulence. In addition, signal transduction in mycelial networks is predicted to be vital for mycelial function and robustness. A lot of cell biological studies have elucidated protein and membrane trafficking and signal transduction in fungal hyphae, however, there are no reports visualizing signal transduction in mycelia. This paper, by using the fluorescent Ca2+ biosensor, visualized for the first time how calcium signaling is conducted inside the mycelial network in response to localized stimuli in the model fungus Aspergillus nidulans. The wavy propagation of the calcium signal inside the mycelium or the signal blinking in the hyphae varies depending on the type of stress and proximity to the stress. The signals, however, only extended around 1000 μm, suggesting that the mycelium has a localized response. The mycelium showed growth delay only in the stressed areas. Local stress caused arrest and resumption of mycelial growth through reorganization of the actin cytoskeleton and membrane trafficking. To elucidate the downstream of calcium signaling, calmodulin and calmodulin-dependent protein kinases, the principal intracellular Ca2+ receptors, were immunoprecipitated and their downstream targets were identified by mass spectrometry analyses. Our data provide evidence that the mycelial network, which lacks a brain or nervous system, exhibits decentralized response through locally activated calcium signaling in response to local stress.

CONCURRENT SESSION 2.4
MOLECULAR TOOL

MONDAY, MARCH 6
17:30 – 19:30
Location: ***Hall Freiburg (Congress Innsbruck)***

CHAIRS:
Fabio Gsaller, Uffe Mortensen

CS2.4.1

LOCUS-SPECIFIC CHROMATIN COMPOSITION ANALYSIS BY DCAS9-DRIVEN PROXIMITY LABELLING

Thomas Svoboda[1], Andreas Schüller[1], Dominik Loibl[1], Joseph Strauss[1]

[1]*Institute of Microbial Genetics, Department of Applied Genetics and Cell Biology, BOKU University of Natural Resources and Life Science Vienna, Campus Tulln/Donau, Tulln an der Donau, Austria*

Proximity labelling by biotinylation is a powerful tool to study the interaction of proteins in living cells. In this study, we aim to establish a system that allows to directly analyze a genomic locus of choice for the locally positioned proteome and thereby define the chromatin environment of this locus. Special interest is cast on biosynthetic gene clusters (BGCs) that are characterized by a strong dependence on the recently identified KERS complex (Karahoda et al., 2022), facultative heterochromatic marks and by the lack of H3K4 methylation marks despite active gene transcription (Gacek-Matthews et al., 2016).
Here we describe the application of the TurboID-dCas9 fusion protein that can be directed to any desired genomic locus with the goal of biotin labeling chromatin-associated proteins in close proximity to the locus of interest. As a proof of concept, we targeted the well-described niiA-niaD bidirectional promoter region in Aspergillus nidulans that controls nitrate assimilation genes with several known transcription factors such as AreA and NirA (Berger et al., 2008). Chromatin immunoprecipitation (ChIP) revealed that both TurboID-dCas9 and AreA were present at the AreA binding locus under nitrate-inducing, but not under repressing conditions. In order to detect whether the fusion protein was positioned correctly and working as expected, biotinylation of AreA was verified using a western blot with both anti-HA-antibody and streptactin-HRP. To confirm the correct working mode of the system and to identify other DNA-associated proteins within the proximity of the TurboID-dCas9 annealing site within the niiA niaD promoter, tryptic digest of the enriched biotinylated proteins, followed by LC-MS analysis was carried out. This work presents the concept and first results of locus-specific chromatin composition analysis by proximity biotinylation in A. nidulans.

CS2.4.2

UTILIZATION OF CRISPR/CAS9-BASED METHODOLOGY FOR GENETIC MANIPULATION OF THE BASIDIOMYCETE WHITE-ROT FUNGUS DICHOMITUS SQUALENS

Victor Manuel Gonzalez Ramos[1], Joanna E. Kowalczyk[1], Shreya Saha[1], Astrid Müller[1], Miia Mäkelä[1]

[1]*University of Helsinki, Helsinki, Finland*

The basidiomycete white-rot fungus Dichomitus squalens is an efficient wood-degrading species which produces a highly adjusted enzymatic response to various types of plant biomass. Detailed bioinformatic analyses of recent (post-)genomic studies in this fungus have revealed candidate regulators of the enzymes involved in the lignocellulose breakdown. However, functional characterization of genes encoding the lignocellulose degrading enzymes and their regulators, and engineering of enhanced lignocellulolytic strains require efficient and reliable genetic manipulation tools. At the moment, the genetic manipulation of basidiomycete white-rot fungi is still hampered by extremely low frequencies of homology directed recombination (HDR) and limited number of available selection markers.

Different CRISPR/Cas9-based genome editing methodologies have recently been developed for genetic manipulation of a few basidiomycete fungi, such as for the species from the genera Ganoderma, Pleurotus, Ustilago and Schizophyllum. The current strategies vary in Cas9 expression system, single guide RNA (sgRNA) synthesis, and HDR template type. Additionally, the disruption of the non-homologous end-joining (NHEJ) machinery is used for increased efficiency of HDR. The established approaches show promise on deletion, insertion, substitution, and disruption of single or multiplexed genes for their in vivo functional characterization.

In D. squalens, we have established a CRISPR/Cas9-based genome editing approach that utilizes Cas9-sgRNA ribonucleoproteins (RNP) with single-stranded oligodeoxynucleotides (ssODNs) as donors for HDR. Through the targeted introduction of STOP codons, we have successfully disrupted genes encoding candidate transcriptional regulators and lignocellulose degrading enzymes. We will showcase the development of the targeted CRISPR/Cas9 approach in D. squalens and its application in the analysis of gene functions, and discuss the challenges in genetic manipulation of basidiomycete (wood-degrading) fungi.

CS2.4.3

NON-HOMOLOGOUS END-JOINING (NHEJ)-DEFICIENT FILAMENTOUS FUNGAL STRAINS MITIGATE THE IMPACT OF OFF-TARGET MUTATIONS AFTER THE APPLICATION OF CRISPR/CAS9

Sandra Garrigues[1], Mao Peng[1], Roland S. Kun[1], Ronald P. de Vries[1]

[1]*Fungal Physiology, Westerdijk Fungal Biodiversity Institute, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands*

Filamentous fungi are widely used as cell factories for the production of industrially relevant compounds such as organic acids, proteins, enzymes, and secondary metabolites. One fundamental aspect for the future of fungal biotechnology is the improvement of production strains at the molecular level. The increased precision currently possible by CRISPR/Cas9-mediated genome editing represents a big change from conventional approaches, which largely rely on random chemical- or radiation-induced mutagenesis, or untargetted insertions of genes into the genomes through genetic engineering. Despite the high level of accuracy reported for CRISPR/Cas9, guide RNA (gRNA) binding to nucleotides that are similar to the target site can result in potential off-target effects that restrict its application. However, the searching for off-target effects is far from routine during the experimental procedures, sometimes leading to unexpected phenotypes misattributed to the originally intended mutation. In this study, we generated a large set of CRISPR/Cas9-derived fungal mutant strains and analyzed them to determine whether CRISPR/Cas9 induces off-target mutations, and to which extent genome stability may play a role in the accumulation of these mutations. As a test case, we deleted the (hemi-) cellulolytic regulator-encoding gene xlnR in in two different strains of the industrial workhorse Aspergillus niger: a wild-type (wt) strain and a NHEJ-deficient strain ΔkusA. Phenotypic analysis of the mutants already suggested a high prevalence of off-target effects in the wt strain. Nevertheless, whole genome sequencing of the generated mutants confirmed the much higher prevalence of off-target mutations in the wt strain compared to ΔkusA. Taking all results together, we determine that in ΔkusA strains Cas9 does not cause additional off-target mutations, concluding that CRISPR/Cas9 is a safe genome-editing system when a NHEJ-deficient strain is used.

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CS2.4.4

MODULAR INDUCIBLE MULTIGENE EXPRESSION SYSTEM FOR FILAMENTOUS FUNGI

Clara Baldin[1], Alexander Kühbacher[1], Petra Merschak[1], Johannes Wagener[2], Fabio Gsaller[1]

[1]*Medical University of Innsbruck, Innsbruck, Austria,* [2]*Trinity College Dublin, University of Dublin, Dublin, Ireland*

Efficient genetic manipulation of filamentous fungi is becoming more and more relevant to either optimize industrial strains or characterize clinically relevant species. Regulatory elements are extremely important for successful strain engineering and the possibility to finely regulate expression on demand, for example with the utilization of inducible promoters, offers several advantages. A major challenge remains the identification of promoters that display an appropriate activation in presence of the inducer as well as reduced leakiness in absence of the inducer or in presence of a repressor. Despite the list of available inducible systems is slowly increasing, the possibility of using more than one promoter for the simultaneous overexpression of different genes has been poorly explored. In this paper, we provide a pioneer study in a human pathogenic fungus, Aspergillus fumigatus, where we investigated various available inducible promoters, compared them, and proved for the first time that up to three systems could be used simultaneously without interfering with each other. The proof of concept was obtained by conditionally expressing three antifungal drug targets within the ergosterol biosynthetic pathway under the control of the xylose-inducible PxylP, the tetracycline-dependent Tet-ON and the thiamine-repressible PthiA.

CS2.4.5

ONLINE BIOMASS MONITORING ENABLES CHARACTERIZATION OF THE GROWTH PATTERN OF ASPERGILLUS FUMIGATUS IN LIQUID SHAKE CONDITIONS

Ingo Bauer[1], Beate Abt[1], Annie Yap[1], Bernd Leuchtle[2], Hubertus Haas[1]

[1]Medical University of Innsbruck – Biocenter, Innsbruck, Austria, [2]SBI Scientific Bioprocessing, Pittsburgh, USA

Numerous filamentous fungal species are extensively studied due to their role as model organisms, workhorses in biotechnology, or as pathogens for plants, animals, and humans. Growth studies are mainly carried out on solid media. However, studies concerning gene expression, biochemistry, or metabolism are carried out usually in liquid shake conditions, which do not correspond to the growth pattern on solid media. The reason for this practice is the problem of on-line growth monitoring of filamentous fungal species, which usually form pellets in liquid shake cultures. Here, we compared the time-consuming and tedious process of dry-weight determination of the mold Aspergillus fumigatus with online monitoring of biomass in liquid shake culture by the parallelizable CGQ ("cell growth quantifier"), which implements dynamic biomass determination by backscattered light measurement. The results revealed a strong correlation of CGQ-mediated growth monitoring and classical biomass measurement of A. fumigatus grown over a time course. Moreover, CGQ-mediated growth monitoring displayed the difference in growth of A. fumigatus in response to the limitation of iron or nitrogen as well as the growth defects of previously reported mutant strains (ΔhapX, ΔsrbA). Furthermore, the frequently used wild-type strain Af293 showed largely decreased and delayed growth in liquid shake cultures compared to other strains (AfS77, A1160p+, AfS35). Taken together, the CGQ allows for robust, automated biomass monitoring of A. fumigatus during liquid shake conditions, which largely facilitates the characterization of the growth pattern of filamentous fungal species.

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CS2.4.6

GENOME-WIDE IN VITRO COMPETITIVE FITNESS PROFILING REVEALS NOVEL INTERCONNECTED NETWORKS OF GENES ASSOCIATED WITH ADAPTATION OF ASPERGILLUS FUMIGATUS TO ANTIFUNGALS

Can Zhao[1], Marcin Fraczek[1], Lauren Dineen[1], Isabelle Storer[1], Ressa Lebedinec[1], Thorsten Heinekamp[2], Danielle Weaver[1], Takanori Furukawa[1], Norman Van Rhijn[1], Juliane Macheleidt[2], Hajer Alshammri[1], Narjes Alfuraiji[1], Daniela Delneri[1], Axel Brakhage[2], Paul Bowyer[1], Michael Bromley[1]

[1]University Of Manchester, Manchester, United Kingdom, [2]Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany

More than 10 million people globally suffer from lung diseases caused by the pathogenic fungus Aspergillus fumigatus, there are few antifungals available, and resistance is rising. In-depth knowledge of factors that contribute to drug resistance in pathogenic fungi is required to facilitate the development of diagnostics to rapidly detect drug resistance isolates and for the development of synergistic combination therapies. Functional genetic analysis has been used in several organisms to great effect and has provided useful insights to understand the factors governing drug adaptation. However, a complete picture of the chemogenetic networks of A. fumigatus remains elusive.
As part of the A. fumigatus genome-wide knockout program (COFUN), we have now completed the generation of a library that consists of over 6000 genetically dual-barcoded null mutants. Using a competitive-fitness profiling approach known as Bar-Seq, we performed a genome-wide assessment of fitness for each mutant in the library when exposed to the clinical therapeutics Voriconazole, Itraconazole and AmphotericinB as well as the novel antifungal Olorofim which is in late-stage clinical trials. We identified clear and near complete correlation in fitness profiles in the voriconazole and itraconazole challenge experiments. Strains with mutations in genes previously linked to azole adaptation, including the drug transporter Cdr1B and the target of the azoles Cyp51A were amongst those with the biggest fitness defects. In addition, isolates with defects in plasma membrane biosynthesis and the vesicle transport network were also enriched in the cohorts

with significant reductions in competitive fitness when under azole challenge. Comparison of the fitness datasets across all drugs has revealed a small number of genes that are linked to adaptation to multiple drugs.
This study showed that the competitive-fitness profiles can provide useful sensors to assess how fungi perceive challenges, and also provides a framework for future genome-wide functional genomic studies in A. fumigatus.

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CS2.4.7

BUILDING A REDOX FLOW BATTERY TO STORE RENEWABLE ENERGY BASED ON THE FUNGAL SYNTHESIZED QUINONE PHOENICIN

Charlotte Overgaard Wilhelmsen[1], Sebastian Birkedal Kristensen[1], Oliver Nolte[2], Ivan Volodin[2], Johan Vormsborg Christiansen[3], Thomas Isbrandt[3], Celine Petersen[4], Trine Sørensen[4], Thomas Ostenfeld Larsen[3], Jens Christian Frisvad[3], Martin Hager[2], Ulrich S. Schubert[2], Kåre Lehmann Nielsen[4], Teis Esben Sondergaard[4], Jens Muff[1], **Jens Laurids Sørensen**[1]

[1]Aalborg University, Esbjerg, Denmark, [2]Friedrich-Schiller-University, Jena, Germany, [3]Technical University of Denmark, Kgs. Lyngby, Denmark, [4]Aalborg University, Aalborg, Denmark

The fungal kingdom is full of colorful pigments with a quinone structure, which are used as protective agents against oxidative stress and competing microorganisms. The biosynthetic pathways for these quinones are initiated by non-reducing polyketide synthases (NR-PKSs) followed by various modifications (e.g., oxidation, methylation, ammonia incorporation and dimerization) resulting in huge structural variation. Besides their natural biological role, quinones are gaining increased interest as promising electrolytes in organic redox flow batteries (RFBs) that can be used to store energy from solar and wind power plants. However, the current quinones used in RFBs have been chemically synthesized from crude oil, which is not aligned with the sustainable thinking behind renewable energy.
Here we will present our work in developing a RFB based on the bibenzoquinone phoenicin, which is produced by several Penicillium species. The responsible gene cluster was identified by sequencing by tapping into our database of nearly 100 Penicillium genome sequences. The wildtype P. atrosanguineum strain produced approximately 3 g/L phoenicin in a week, which was further elevated by overexpressing the internal transcription regulator using a purpose generated CRISPR-Cas9 system. The extracted phoenicin was then used to generate a RFB with a cell voltage of 0.86 V and an initial capacity of 11.75 Ah/L. The electrochemical properties of phoenicin are similar to the published petro-quinones, which demonstrates that fungal biosynthesized quinones provide a sustainable solution for energy storage.

CS2.4.8

MASS SPECTROMETRY TOOLBOX FOR DECIPHERING MOLECULAR HETEROGENEITY OF PROTEINS

Mowei Zhou[1]

[1]*Pacific Northwest National Laboratory, Richland, United States*

Proteins are essential players in biology. In addition to their amino acid sequences, post-translational modifications (PTMs) and higher-order structures give rise to the molecular heterogeneity that are functionally important but not directly captured by genetic and transcriptomics analysis. Mass spectrometry (MS) methods have contributed significantly to the profiling and quantitation of proteins in complex mixtures. Most established methods follow a "bottom-up" approach, where proteins are indirectly detected via inference from proteolytically cleaved low-mass peptide fragments that are easy for MS detection. In contrast, the "top-down" approach characterizes intact proteins, offering unique information on the functional forms of proteins including stoichiometry of PTMs. When performed under nondenaturing conditions, noncovalent interactions and complexes can be studied to gain structural insights, which can be particularly useful for annotating proteins with unknown functions.
Herein, I will highlight several examples of top-down MS of microbial proteins for PTM analysis, structural elucidation, and spatial mapping to decipher the heterogeneity of proteins at different levels. I will focus on a top-down MS method coupled to hydrophilic interaction liquid chromatography to characterize highly heterogenous PTM patterns on glycoproteins. Glycosylation is a family of diverse PTMs that are vital for protein stability and molecular recognition. The role of glycosylation in immunity has long been recognized in human health research. Glycosylation is also correlated to the efficacy of important fungal enzymes such as cellulases. I will demonstrate the workflow using the recombinant glycoprotein SARS-CoV-2 spike receptor binding domain, and discuss recent work applying top-down MS method for fungal cellulase research. Comprehensive characterization of these heterogenous PTMs will provide critical attributes for precision engineering of fungal systems as efficient producers of renewable energy.

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CONCURRENT SESSION 3.1
EVOLUTION, BIODIVERSITY AND TAXONOMY

TUESDAY, MARCH 7
14:00 – 16:00
Location: ***Hall Tirol (Congress Innsbruck)***

CHAIRS:
Martin Grube, Nina Gunde-Cimerman

CS3.1.1

NUCLEAR INTERACTIONS IN HETEROKARYONS OF THE FILAMENTOUS ASCOMYCETE NEUROSPORA TETRASPERMA

Hanna Johannesson[1], Johan Reimegård[2], Judith Mank[3], Iulia Darolti[3], Cecile Meunier[4]

[1]*Stockholm University/ The Royal Swedish Academy of Sciences, Stockholm, Sweden,* [2]*Department of Cell and Molecular Biology, National Bioinformatics Infrastructure Sweden, Science for Life Laboratory, Uppsala University, Uppsala, Sweden,* [3]*Department of Zoology and Biodiversity Research Centre, University of British Columbia, Vancouver, Canada,* [4]*Department ECOBIO, UMR CNRS 6553, Université Rennes 1, Rennes, France*

Heterokaryosis is a system in which genetically distinct nuclei coexist within the same cytoplasm. While heterokaryosis dominates the life cycle of many fungal species, the transcriptomic changes associated with the transition from homokaryosis to heterokaryosis is not well understood. Here, I present a study in which we analyze gene expression profiles of homokaryons and heterokaryons from three phylogenetically and reproductively isolated lineages of the filamentous ascomycete Neurospora tetrasperma. We show that heterokaryons are transcriptionally distinct from homokaryons in the sexual stage of development, but not in the vegetative stage, suggesting that the phenotypic switch to fertility in heterokaryons is associated with major changes in gene expression. Heterokaryon expression is predominantly defined by additive effects of its two nuclear components. Furthermore, allele-specific expression analysis of heterokaryons with varying nuclear ratios show patterns of expression ratios strongly dependent on nuclear ra-

tios in the vegetative stage. In contrast, in the sexual stage, strong deviations of expression ratios indicate a co-regulation of nuclear gene expression in all three lineages. Taken together, our results show two levels of expression control: additive effects suggest a nuclear level of expression, whereas co-regulation of gene expression indicate a heterokaryon level of control.

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CS3.1.2

GENOMIC CONSEQUENCES OF HYBRIDISATION IN A FUNGAL SYNGAMEON

Fernando Fernandez-Mendoza[1], Eva Andrea Strasser[1], Ester Gaya[2], Martin Grube[1]

[1]*Institute of Biology, Karl-Franzens-Universität Graz, Graz, Austria,* [2]*Royal Botanic Gardens, Kew, Richmond, UK*

During the last decade, identifying cryptic lineages has become a common ground in fungal biodiversity surveys. While using phylogenetic concepts was instrumental in unveiling the “true” extent of fungal diversity, it implied interpreting all genetic variation as a result of macroevolutionary processes. Overlooking the evolutionary and selective processes acting at any level of organization but species strongly biases our understanding of evolutionary events and the adaptive potential of fungal lineages.

The lichen genus Pyrenodesmia s.s. (Teloschistaceae) provides a good example of the limitations of the phylogenetic paradigm, and of the need to consider evolution as a multilevel process. The taxonomic treatment of the genus is incomplete and difficult, despite the high morphological and genetic diversity it encompasses. With the aim to understand the source of its hyperdiversity, we surveyed a collection of Western European specimens using phylogenetic and phylogenomic methods.

Beyond the high nucleotide diversity found on each of the sequenced loci, we observed that the genus evolves as a syngameon, a supraspecific unit in which multiple species are partially interconnected through gene-flow. We identified widespread interspecific mating, i.e. low prezygotic isolation, using network analyses of MAT-loci cooccurrence in dikaryotic samples. Despite interbreeding, network modularity reflects significant regional and specific boundaries to gene-flow, as do population genetic analyses. The imbalance observed in gene-flow and mating networks suggests that reduced endolithic species introgress other species, in a fashion analogous to sexual parasitism.

The genomic study of P. erodens provides further evidence of the hybrid history of the genus. Contrary to diploid organisms, in which polyploidization is frequent, we identified multiple evidences of genome concertation in an aneuploid hybrid. This is mainly driven by genome defence mechanisms which modulate gene content, generate areas of suppressed recombination, and likely drive the reduced morphology and loss of reproductive specificity through gene loss.

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CS3.1.10

THE PAN-GENOME BEHIND THE MULTIFUNCTIONAL ROOT SYMBIOTIC INSECT PATHOGENIC FUNGUS METARHIZIUM BRUNNEUM

Carolina Nogueira[1], Dinah Parker[1], Jinglin Zhao[1], Michael Habig[2], Nicolai Meyling[1], Tue Nielsen[3], Knud Nielsen[3], Lars Hansen[3], Henrik De Fine Licht[1]

[1]Section for Organismal Biology, University of Copenhagen, Copenhagen, Denmark, [2]Environmental Genomics, Christian-Albrechts University, Kiel, Germany, [3]Microbial Ecology and Biotechnology, University of Copenhagen, Copenhagen, Denmark

The fungal genus Metahrizium belongs to the ascomycete's and presents a multifunctional lifestyle. Though being able to survive as a saprophyte, insect pathogen, and plant-root symbiont, the ability to grow in each of these lifestyles varies between and within species. The species M. brunneum has a cosmopolitan distribution and is able to grow well as all three lifestyles. The species is a generalist insect pathogen that infect insects from at least seven different orders. In addition, this species can also establish symbiotic relationships with plant-roots involving exchange of nutrients and the induction of plant resistance against pathogens. Despite having a variable and versatile lifestyle, the genome-wide genetic diversity within the species is not known. In order to increase the understanding of M. brunneum genetic diversity we are generating a reference pan-genome based on six isolates sampled across different habitats and three continents. These isolates exhibit different phenotypic traits such as growth rate, virulence against Tenebrio molitor larvae, and ability to colonize wheat roots. Our preliminary data gives a hint on the high genetic diversity within the species. Unlike the two reference genomes of M. brunneum previously sequenced, which do not differ in genome size, our genomes vary up to 9 Mb in length. The species pan-genome comprises 11,835 different orthogroups. The core orthogroups represent ± 67% of the pan-genome. A phylogeny with all available M. brunneum genomes based on the core genes shows a very consistent and defined division within the species into three clades. We are currently investigating variation in gene functional groups between these three groups of M. brunneum and looking for correlations with phenotypic characteristics with an aim to better understand local adaptation in this species.

CS3.1.4

FROM POPULATIONS TO PAN-GENOMES: IDENTIFYING PATTERNS OF GLOBAL GENOMIC EVOLUTION IN THE PORCINI MUSHROOM, BOLETUS EDULIS.

Keaton Tremble[1,2], Bryn Dentinger[1,2]

[1]*School of Bioscence, University Of Utah, SALT LAKE CITY, United States,*
[2]*Natural History Museum of Utah, Salt Lake City, United States*

During the process of speciation, the genome undergoes a dramatic array of genetic and structural evolution. These genomic changes often do not occur simultaneously; some changes only occur after the speciation process is nearly complete and reproductive isolation is present, while other changes may occur early in the process and be a primary cause of isolation. For most species, particularly the under-represented macro-fungi, it is currently unknown under which demographic scenarios genomic changes accrue, and whether any one genomic alteration is necessary for divergence. In this work, we take a pan-genomic approach to investigate patterns of gene-family and genomic structural evolution in the globally distribution ectomycorrhizal porcini mushroom “Boletus edulis”. B. edulis is a species actively in the process of speciation, with patterns of strong geographic structure yet ongoing gene flow between widely dispersed groups (Tremble et al. 2022, New Phytologist). Specifically, we use 350 genomes to identify the pan-genome of B. edulis and characterize gene-family composition on a global scale. Then, we use 21 high-quality reference genomes to identify unique patterns of gene family, transposable element, and secretome evolution that are associated with previously characterized patterns of population divergence and gene flow. Lastly, we assess whether changes in genomic synteny are necessary for population divergence to arise. This work provides a novel insight into the dynamic relationship between population divergence and genomic evolution during the process of speciation in a globally prized edible mushroom.

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CS3.1.5

MASSIVE TRANSPOSONS AS THE CRUCIBLE OF EVOLUTION IN FUNGI

Aaron Vogan[1], Emile Gluck-Thaler

[1]*Uppsala University, Uppsala, Sweden*

Fungi are famous for their ability to quickly adapt to novel environments and fill new niches. This has been to both the detriment and benefit of humans, exemplified by devastating plant pathogens like Fusarium or industrial workhorses such as Aspergillus. Over the past decade, investigations into various fungal genomes have uncovered a number of large genomic regions that appear to have been horizontally transferred among strains or species, and which carry genes important for adaptation. These include regions for cheese processing in Penicillium, toxin genes in various dothideomycetes, genes for resistance to heavy metals in Penicillium and relatives, and meiotic drive genes in the model species Podospora anseirna. Here I will present evidence that these apparently disparate phenomena are united by the action of a novel group of massive mobile genetic elements, named Starships. These elements are widespread across Pezizomycotina, suggesting an ancient origin, and can also be found in select basidiomycetes as apart of clear cases of recent horizontal transfer or as members of a cryptic lineage. Transposition is enacted by a single gene coding for a tyrosine recombinase, but four additional genes are often associated with the elements. The putative functions of these genes suggest that in addition to being mobile within a given genome, Starships may also be capable of active transfer between individuals via conjugation through hyphal fusion as a direct analog of the method that some bacteria use to exchange genetic material. In addition to these conserved genes, a vast array of “cargo” genes can also be found within the Starships. These include putative biosynthetic gene clusters and genes with predicted functions relating to heat stress, virulence, and metal resistance. Together the data suggests that the Starships alone may be the secret of success among fungi and challenge entrenched ideas regarding the evolution of eukaroyotes.

CS3.1.6

GENOME ANALYSIS OF CANDIDA ORTHOPSILOSIS MARINE ISOLATES UNVEILS MISSING PARENTAL LINEAGE AND SUGGESTS ENVIRONMENTAL ORIGIN OF HYBRIDS WITH PATHOGENIC POTENTIAL

Valentina del Olmo Toledo[1,2], Verónica Mixão[1,2], Ester Saus[1,2], Ewa Księżopolska[1,2], Juan Carlos Núñez-Rodriguez[1,2], Toni Gabaldón[1,2]

[1]Barcelona Supercompunting Center, Barcelona, Spain, [2]Institute for Research in Biomedicine, Barcelona, Spain

Hybridisation, i.e. the crossing of two lineages to form a hybrid with an admixed genome, is a common event in yeasts often leading to genomic variability and adaptation. Candida orthopsilosis is a human-associated opportunistic pathogen belonging to the Candida parapsilosis species complex. Most clinical isolates from this species are hybrids resulting from at least four independent crosses between two parental lineages of which only one has been identified. The rare presence or total absence of parentals amongst clinical isolates has been hypothesised to be a consequence of a reduced pathogenicity with respect to their hybrids. Here, we analyse the genomes of the first sequenced environmental C. orthopsilosis strains, which were isolated from warm marine ecosystems. We found a majority of hybrid strains among environmental isolates, and we determined they are phylogenetically closely related to hybrid clinical isolates. Furthermore we identified the long-sought missing parental lineage, thus providing a complete overview of the genomic evolution of this species. Our results suggest a marine origin of C. orthopsilosis and pave the way to identify the pre-existing environmental adaptations that rendered hybrids more prone to colonise and infect the mammalian host.

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CS3.1.7

LIFESTYLE TRANSITIONS IN BASIDIOMYCETOUS FUNGI ARE REFLECTED BY TRNA COMPOSITION AND TRANSLATION EFFICIENCY

Marco Alexandre Guerreiro[1,2], Minou Nowrousian[3], Andrey Yurkov[4], Eva Stukenbrock[1,2]

[1]Max Planck Institute For Evolutionary Biology, Plön, Germany, [2]Christian-Albrechts University of Kiel, Kiel, Germany, [3]Ruhr University Bochum, Bochum, Germany, [4]Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

Fungi are ubiquitous and inhabit every known terrestrial habitat. Pathogenic fungi are highly diverse, and recent years have seen an uprise in the emergence of new pathogens on crops, animals and humans. The order Trichosporonales (Tremellomycetes, Agaricomycotina, Basidiomycota) harbours saprobic and a few opportunistic human pathogenic species. These emerging pathogens cause superficial skin irritations, as well as invasive life-threatening infections. Yet, little is known about their evolution, ecology, virulence mechanisms and transition to pathogenic lifestyles. In this study we aimed to determine genomic signatures associated with lifestyle transitions, virulence and host/substrate specialization among 30 Trichosporonales species from a total of 41 genome sequences. We used comparative analyses of genome content, including gene functional categories, repetitive element content and tRNA composition among saprotrophic and reported opportunistic human pathogens. A genome-scale phylogenetic reconstruction revealed that even though the different genera are monophyletic, opportunistic pathogenic species are present in distantly-related clades. Statistical analyses showed that differences in genome structure among species did not correlate with predicted lifestyles. Intriguingly, we found that tRNA content varied widely across species (from 51 to 1455 manually curated tRNA genes). The expansion was independent from the phylogenetic structure. Opportunistic pathogenic species showed an overall increased efficiency in the translation of genes associated with host colonization (i.e. lipid metabolism), while exclusively saprotrophic species showed an increase translation efficiency for genes associated with a saprotrophic lifestyle (i.e. carbohydrate

metabolism). This pattern was consistent among distantly-related saprotrophic and pathogenic Cryptococcus species. In conclusion, our analyses link genomic information with ecology and fungal lifestyles across an entire order. We find evidence for an evolutionary scenario where distinct habitats select for an optimized translation of genes involved in successful proliferation in the respective habitat. We predict that lifestyles are not strictly defined by gene repertoires, but also by expression profiles in fungal pathogens.

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CS3.1.8

RESOLVING SPECIES BOUNDARIES IN THE DIAPORTHE ERES SPECIES COMPLEX

Sandra Hilário[1], Micael Gonçalves MF[1], Artur Alves[1]

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Diaporthe is a species-rich genus that encompasses endophytes and plant pathogens. Accurate detection and identification of these pathogens are crucial for a correct diagnosis of plant diseases. Therefore, we aimed to clarify the species limits in the D. eres species complex, which comprises several plant pathogens causing disease on economically important crops. Several criteria were implemented such as single and multilocus phylogenetic analyses, the Genealogical Phylogenetic Species Recognition principle (GCPSR), coalescent-based model Poisson Tree Processes (PTPs), phylogenetic networks, and recognition of genetic recombination events. The absence of reproductive isolation and barriers to gene flow as well as the high haplotype and low nucleotide diversity indices within the species complex suggest that D. eres constitutes a population rather than different lineages. Of relevance is the synonymization of D. vaccinii with D. eres. Diaporthe vaccinii was regarded as a common and important pathogen of crops from the genus Vaccinium and was listed for many years as a quarantine organism in Europe. The results provide evidence that D. vaccinii cannot be regarded as a distinct species from D. eres, representing an impact on the plant pathology community. The methodology used and validated in this study represents an important contribution to the systematics of the genus Diaporthe. Cohesive approaches comprising genealogical concordance criteria, coalescent models, and methods to detect recombination should be implemented in the genus Diaporthe. This will provide stronger support to infer the phylogenetic relationships between cryptic species.

CONCURRENT SESSION 3.2
METABOLISM AND PHYSIOLOGY

TUESDAY, MARCH 7
14:00 – 16:00
Location: ***Hall Brüssel (Congress Innsbruck)***

CHAIRS:
Bernhard Seiboth, Levente Karaffa

CS3.2.1

REVITALIZING POST-CONSUMER PLASTIC WASTE: TRASH TO TREASURE

Benjamin Miller[1], Chris Rabot[1], Yuhao Chen[1], Shu-Yi Lin[1], Maria Tangalos[1], Megan Fisk[1], Katie Macfee[1], Salma Durra[1], Yi-Ming Chiang[1], C. Elizabeth Oakley[2], Berl Oakley[2], Clay Wang[1], Travis Williams[1]

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Post-consumer plastic waste represents one of the most imminent environmental threats today. The same physical properties that confer their beneficial characteristics also lead to major challenges regarding their decomposition and valorization. Here, we leverage chemical and biological approaches to reclaim value embedded in post-consumer polystyrene (PS). First, we develop and optimize an oxidative catalytic process tolerant of post-consumer PS. This reaction enables the rapid conversion of PS to benzoic acid in high yield. We then utilize genetically engineered strains of Aspergillus nidulans to channel this benzoic acid into secondary metabolic pathways to generate structurally diverse and pharmacologically relevant secondary metabolites in high yield. Further, we generate large quantities of spores of the EPA-approved biocontrol agent A. flavus Af36 directly from this PS-derived benzoic acid. Current efforts are focused on expanding the catalog of fungal fermentation products that can be generated from these plastic-derived substrates. In addition, we are investigating the fungal metabolism of the PS substrate to induce high yields of essential organic acids. Taken together, we present a novel, hybrid chemical-biological approach to transform one of the world's most harmful pollutants into high-value fungal products.

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CS3.2.2

THE "MANGANESE EFFECT" DURING ASPERGILLUS NIGER CITRIC ACID FERMENTATION IS DEPENDENT ON THE CULTIVATION STAGE

Levente Karaffa[1], Vivien Bíró[1], Alexandra Márton[1], István Bakondi-Kovács[1], Katica Kramcsák[1], Andrea Kun[1], Erzsébet Fekete[1], Christian Peter Kubicek[2], Adrian Tsang[3]

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High-yield citric acid overflow requires a combination of unusual culture conditions of which the deficiency of manganese(II) ions in the growth medium is particularly critical: concentrations >5 µg/L (= 5 ppb) reduces final citric acid yield by some 25%. In this study we demonstrated that under high-yield citric acid producing conditions even a brand-new bioreactor releases more manganese(II) ions into the culture broth than the threshold level. The leaching of manganese(II) ions from metal surfaces does not limit citric acid accumulation as long as it occurs in the late stages of the fermentation. On the other hand, manganese deficiency in the first 48 hours of the cultivation appears critical for citric acid overflow. The results imply that once A. niger citric acid overflow commences due to the special cultivation conditions, it continues irrespective of the changing environment in the bioreactor. We thus hypothetized that the genes involved in the response to manganese(II) ion deficiency are expressed at an early stage of the cultivation. To test this hypothesis we analyzed the transcriptome of three parallel A. niger citric acid fermentations at 24, 48 and 72 hours and identified genes which display an at least 2-fold up- or downregulation in dependence on the availability of manganese(II) ions. The expression profile of cexA, encoding a citrate exporter that secretes citric acid from the cytosol into the culture broth, suggests that it plays a major role in the mechanism of the manganese effect.

CS3.2.3

MANGANESE AND ITS REGULATORY ROLE ON THE CITRATE TRANSPORTER CEXA – EXPLORING THE CITRIC ACID PRODUCTION MECHANISM OF ASPERGILLUS NIGER

Aline Reinfurt[1,2], Vivien Bíró[3], Alexandra Márton[3], Valeria Ellena[1,2], Erzsébet Fekete[3], Levente Karaffa[3], Matthias Steiger[1,2]

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Aspergillus niger is an important filamentous fungus used for the industrial production of citric acid. One of the main contributors to high citric acid accumulation by the fungus is the citrate transporter CexA. It belongs to the major faciliatory superfamily subclass DHA1 which act as drug-H+ antiporters[1]. Since cexA and its regulators are essential within the citric acid production process, it is crucial to study their regulatory mechanism, which is the focus of this work. The amount of manganese present in the culture broth has a major impact on the production process. Observations showed that the fungus develops a certain pellet-like morphology under manganese limitation conditions and that this limitation is decisive for high citric acid accumulation by A. niger[2 3]. However, the exact mode of action of manganese in the cell was not clear until now. Thus, in this work, we show the regulatory role of manganese on the citrate transporter CexA.

1. Steiger, M. G., Rassinger, A., Mattanovich, D. & Sauer, M. Engineering of the citrate exporter protein enables high citric acid production in Aspergillus niger. Metab. Eng. 52, 224–231 (2019).
2. Fejes, B. et al. The effects of external Mn2+concentration on hyphal morphology and citric acid production are mediated primarily by the NRAMP-family transporter DmtA in Aspergillus niger. Microb. Cell Fact. 19, 1–11 (2020).
3. Karaffa, L. & Kubicek, C. P. Aspergillus niger citric acid accumulation: Do we understand this well working black box? Appl. Microbiol. Biotechnol. 61, 189–196 (2003).

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CS3.2.4

PLANT BIOMASS CONVERSION IS DIFFERENTLY ORGANIZED IN BASIDIOMYCETES COMPARED TO ASCOMYCETES

Miia Mäkelä[1]

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Fungal species from the phyla Basidiomycota and Ascomycota are the most efficient organisms for plant biomass conversion and degradation. Several (post-)genomic studies have shown that ascomycetes and basidiomycetes produce highly similar sets of extracellular carbohydrate active enzymes (CAZymes) for the degradation of plant biomass polymers. However, the other components of the plant biomass conversion process seem to be distinctively different between asco- and basidiomycete fungi. This is exemplified by the regulatory system that controls the production of the extracellular CAZymes, since most of the identified ascomycete regulators do not have orthologs in basidiomycetes. The differences also extent to the compounds that induce the production of the CAZymes and sugar metabolic enzymes. While in ascomycetes plant biomass derived monomers or their metabolic products often act as inducers of the genes encoding these enzymes, our recent studies suggest that basidiomycetes use at least in part disaccharides as the inducing compounds. The disaccharide-based induction is also supported by an enrichment of putative disaccharide transporters in genomes of basidiomycetes compared to ascomycetes. The combined data will be presented to highlight the characteristic features in the organization of plant biomass conversion in basidiomycete fungi.

CS3.2.5

THE PARALOGOUS TRANSCRIPTION FACTORS LEUR AND LEUB REGULATE LEUCINE BIOSYNTHESIS, NITROGEN ASSIMILATION, AND IRON METABOLIC PATHWAYS IN ASPERGILLUS NIDULANS

Joel Steyer[1], Damien Downes[1], Cameron Hunter[1], **Richard Todd**[1]

[1]Kansas State University, Manhattan, United States

Many fungi can synthesize branched-chain amino acids (BCAAs), while animals cannot. Proper regulation of BCAA metabolism is important for protein synthesis, growth, secondary metabolite production and virulence. In Aspergillus nidulans, the Zn(II)2Cys6 transcription factor LeuB activates leucine biosynthesis pathway genes. LeuB also regulates expression of gdhA, which encodes the key nitrogen assimilation enzyme NADP-glutamate dehydrogenase. Using phylogenetic analysis and sequence comparisons, we have identified a paralog of LeuB named LeuR and examined the intersection of LeuB and LeuR in regulating leucine biosynthesis, gdhA, and iron metabolism. The leuBΔ mutant is a leaky leucine auxotroph. We deleted leuR and found the leuRΔ mutant to be a prototroph. However, the leuBΔ leuRΔ double mutant is a tight leucine auxotroph, indicating a redundant role for LeuR in regulating leucine biosynthesis. Using a gdhA-lacZ translational fusion reporter gene and exogenous leucine, we show that LeuR also regulates gdhA expression. By altering the levels of the leucine biosynthetic intermediate α-isopropyl malate (α-IPM) using leucine biosynthesis loss-of-function mutants, we show that α-IPM is an inducer of LeuB but not LeuR activity. Previously, we used a series of promoter deletions in gdhA-lacZ to identify two sites of action for LeuB in the gdhA promoter. We have now used these promoter deletions to identify the site of action for LeuR. Orthologues of LeuB in pathogenic fungi has been shown to regulate iron metabolism. We show that LeuB and LeuR play a role during iron starvation while LeuB is involved during iron excess conditions. Additionally, we performed RNA-Seq with the wild type, leuBΔ, leuRΔ, and leuBΔ leuRΔ mutants to determine the genome-wide direct and indirect targets and overall physiological roles of LeuB and LeuR. Our experiments show that the transcription factors LeuB and LeuR overlap in regulating leucine biosynthesis, nitrogen assimilation, and iron metabolism.

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CS3.2.6

MANNITOL METABOLISM AND OSMOTIC STRESS ANSWER: A COMPARATIVE STUDY ON TRICHODERMA REESEI AND AUREOBASIDIUM PULLULANS

Audrey Masi[1,2], Klara Wögerbauer[1], Georg Stark[1], Johanna Pfnier[1], Robert L Mach[2], Astrid R Mach-Aigner[1,2]

[1]Christian Doppler laboratory for optimized expression of carbohydrate-active enzymes, Institute of Chemical, Environmental and Bioscience Engineering, TU Wien, Vienna, Austria, [2]Institute of Chemical, Environmental and Bioscience Engineering, TU Wien, Vienna, Austria

Mannitol's role and metabolism in fungi have been widely debated. It has been proposed to serve as carbohydrate storage, neutralize reactive oxygen species, support germination, and increase resistance to stress conditions such as osmotic stress. But some publications demonstrate that mannitol is less critical for osmotic stress resistance than glycerol. We hypothesized that the predominance of mannitol or glycerol in the osmotic stress answer might depend on the environmental conditions. We decided to couple this investigation with a clarification of mannitol metabolism.
Mannitol metabolism was described as a cycle by Hult and Gatenbeck in 1978. Their proposed cycle, in Alternaria alternata, comprises four enzymes: a mannitol-1-phosphate dehydrogenase, a mannitol-1-phosphatase, a mannitol dehydrogenase, and a hexokinase. Since then, these enzymes have been identified in many fungi, opening the possibility to generate deletion strains for the "mannitol cycle" enzymes to challenge this model. Such tests performed in Aspergillus niger, A. alternata, or Botrytis cinerea demonstrated that the mannitol cycle model could not fully explain mannitol metabolism. Still, no new model could be built from these results. The tests performed vary from one strain to another, making comparison impossible.
To clarify the debate, we decided to explore mannitol metabolism by performing the same tests on two fungi: Trichoderma reesei and Aureobasidium pullulans. We aim to generate single and double deletion strains for mannitol-1-phosphate dehydrogenase and mannitol dehydrogenase. Once developed, the strains are cultivated with different carbon sources with or without osmotic stress. During cultivation, polyols and carbon source amounts are monitored. Biomass is measured

at the end of the experiment. Our observations will then be confronted with existing results in other strains to contribute to a new model for mannitol metabolism. These tests will also characterize the osmotic stress answer through polyol production.

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CS3.2.7

THE SIDEROPHORE FERRICROCIN MEDIATES IRON ACQUISITION DURING GERMINATION IN ASPERGILLUS FUMIGATUS

Isidor Happacher[1], Mario Aguiar[1], Beate Abt[1], Mostafa Alilou[2], Tim J. H. Baltussen[3], Gerald Brosch[1], Willem J. G. Melchers[3], Hubertus Haas[1]

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The opportunistic fungal pathogen Aspergillus fumigatus utilizes two high-affinity iron uptake mechanisms, termed reductive iron assimilation (RIA) and siderophore-mediated iron acquisition (SIA). The latter has been shown to be crucial for virulence of this fungus and is a target for development of novel strategies for diagnosis and treatment of fungal infections. So far, research on SIA in this mold focused mainly on the hyphal stage revealing the importance of extracellular fusarinine-type siderophores in iron acquisition as well as of the siderophore ferricrocin in intracellular iron handling.
The goal of the current study was the characterization of iron acquisition during germination. Expression of genes involved in siderophore metabolism, particularly in biosynthesis and uptake of ferricrocin mediated by the siderophore transporter Sit1, were found to be increased during germination independent of iron availability. This suggested a role of ferricrocin in iron acquisition during germination. In agreement (i) bioassays indicated secretion of ferricrocin during growth on solid media independent of iron availability, (ii) ferricrocin was identified in the supernatant of conidia germinating in liquid media independent of iron availability, (iii) in contrast to mutants completely lacking siderophores, mutants synthesizing ferricrocin but lacking fusarinine-type siderophores were able to grow under iron limitation in the absence of RIA, and (iv) genetic inactivation of Sit1 decreased germination in the absence of RIA.
Taken together, this study revealed that ferricrocin has not only an intracellular role but also functions as an extracellular siderophore in A. fumigatus, at least during germination. Furthermore, RIA is likely to assist germination. The iron-independent induction of Sit1 during germination indicates developmental rather than iron regulation.

CS3.2.8

ORGANELLE-DEPENDENT SYNTHESIS OF NITRIC OXIDE IN FUNGI

David Canovas[1], Reinhard Beyer[2], Francesca Cervellini[1], Joseph Strauss[2], Christoph Schüller[2,3]

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The role of nitric oxide (NO) in signalling and its synthesis pathways have been extensively studied in mammals. However, little is known about the role of NO and how it is synthesized in fungi. NO has been reported to contribute to the regulation of nitrogen metabolism, development and pathogenesis. We previously reported that NO production in A. nidulans requires a functional nitrate reductase gene (niaD) that is upregulated during conidiation even in the presence of the repressing nitrogen source ammonium. In addition, we also demonstrated that an arginine-dependent route of NO synthesis is operational in this fungus. However, based on biochemical data it is clear that additional NO biosynthesis routes must exist. To explore them, we screened the yeast genome-wide knock-out collection for mutants defective in NO synthesis. We identified 116 mutant strains with reduced levels of NO synthesis. Analysis of enriched GO terms suggested that mutations in the respiratory chain of mitochondria and in the import system for peroxisomal proteins affect NO synthesis. Inhibition of the respiratory chain with cyanide further demonstrated that nitrite is reduced to NO in the mitochondria in S. cerevisiae and in A. nidulans. We also found that A. nidulans cultures of pexC and pexG mutants as well as their conidia showed reduced production of NO similar to the pex3 and pex7 mutants of S. cerevisiae. These findings suggest connections between the peroxisome and the developmental regulation of NO synthesis. In summary, we identified two additional pathways for the biosynthesis of NO in fungi and both are related to mitochondrial and peroxisomal functions.

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CONCURRENT SESSION 3.3 ANIMAL/HUMAN INTERACTIONS

TUESDAY, MARCH 7
14:00 – 16:00
Location: ***Hall Strassburg (Congress Innsbruck)***

CHAIRS:
Ulrike Binder, Ilse Jacobsen

CS3.3.1

THE IMPACT OF COLONIZATION ON INFECTION - SYSTEMIC CANDIDIASIS IN MICE

Ilse Jacobsen[1]

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Systemic candidiasis in humans usually originates from strains that have long-term colonized mucosal surfaces – especially the gut – prior to the onset of infection. Yet, mice naïve to C. albicans are commonly used for systemic infection experiments. It has recently been shown that by inducing Th17 and antibody responses, respectively, colonization partially protects mice from systemic candidiasis.
To further elucidate the impact of microbiome composition and fungal colonization on systemic candidiasis, we used C57Bl/6 mice from different breeding colonies with differences in microbiome composition and germ free mice. We found microbiome composition affects the immune-phenotype of mice at baseline, and their susceptibility to systemic candidiasis in C. albicans naïve mice. Colonization, in contrast, was protective independent of microbiome composition and as effective in germ free animals as in SPF mice, even though the immunological response to colonization differed. Using two different C. albicans strains we observed limited protection against heterologous systemic challenge, and we are currently aiming the questions whether this is due to limited cross-protection or an intrinsic property of the non-protective strain. Finally, we show that antibiotics commonly used to facil-

itate stable gut colonization at high levels in mice affect susceptibility to systemic infection in naïve mice and the immune response to systemic challenge in colonized mice.
In summary, our work shows that colonization has a significant impact on systemic candidiasis in mice, which is affected by microbiome composition, antibiotic treatment, and the C. albicans strain.

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CS3.3.2

ROLES OF CANDIDALYSIN OF CANDIDA ALBICANS IN THE GUT PERMEABILITY AND BRAIN PATHOLOGY

Courtney Smith[1], Eun Young Huh[1,2], Emily Perez[1], Jenny Hsieh[3,4], Soo Chan Lee[1,4]

[1]South Texas Center for Emerging Infectious Diseases (STCEID), Department of Molecular Microbiology and Immunology, UTSA, San Antonio, United States, [2]Naval Research Unit (NAMRU-SA), Maxillofacial Injury and Disease Department, San Antonio, United States, [3]Brain Health Consortium UTSA, San Antonio, United States, [4]Department of Neuroscience, Developmental and Regenerative Biology, UTSA, San Antonio, United States

Alzheimer's disease (AD) is currently ranked as the seventh leading cause of death in the United Sates and is the most common cause of dementia. In the United States, AD affects at least two-thirds of dementia patients. AD is characterized as a neurodegenerative disorder with symptoms including memory loss, cognitive impairments, and behavioral changes; pathological features include extracellular deposits of amyloid-β (Aβ) plaques, intracellular neurofibrillary tangles of phosphorylated tau protein, and neuronal death and loss. There are large genetic components that are related to AD progression. Despite this knowledge, genetic risk factors fail to explain the systemic neuroinflammation associated with AD patients. Another line of thought suggests that AD may have a microbial origin, which relies on the model of the gut-brain axis. Recent studies have suggested that the gut is one of the major reservoirs for Candida in which it can translocate through mucosal barriers, enter the bloodstream, and disseminate. In fact, differences in the gut microbiome have been associated with AD. There is mounting evidence that suggest that the bacterial gut microbiota plays a pathogenic role but fungal mycobiome is largely ignored. In further support of this idea, 90% of postmortem brain biopsies were found to have colonization by Candida albicans. Despite this knowledge, it is not yet known how C. albicans enters the brain and what fungal factor(s) exacerbates common AD pathology. Our data for both in vivo mouse and in vitro cell line models suggest that a toxin secreted by C. albicans, called candidalysin, increases gut permeability of the epithelial barrier permitting the fungus to migrate from the gut to the brain.

These results strongly suggest that candidalysin plays a key role in the migration of C. albicans. Currently we are testing how candidalysin affects brain pathology and endothelial cell permeability.

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CS3.3.3

AIRWAY EPITHELIAL CELLS AS A NOVEL INTRACELLULAR HOST RESERVOIR FOR CRYPTOCOCCUS SPORES

Sébastien C. Ortiz[1], Rachael Fortune-Grant[1], Robin C. May[2], Rebecca A. Drummond[3], Margherita Bertuzzi[1]

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The inhaled human fungal pathogen Cryptococcus neoformans causes over 200,000 deaths a year. Cryptococcal disease occurs by dissemination of the pathogen from the lung into the brain and importantly, can ensue years after exposure; however, the mechanisms of Cryptococcus dissemination and intracellular latency are still unclear. The inhalation of Cryptococcus spores initiate infections and recent evidence in vivo has shown that spores display unique host-pathogen interactions and are able to escape the lung and disseminate to the brain more readily than yeast. Due to the difficulties associated working with these basidiospores, limited is known about how they interact with host cells. Airway Epithelial Cells (AECs), which cover the entire alveolar surface and comprise 24% of all cells in the human lung parenchyma, likely have instant and extensive contact with inhaled spores. A growing body of evidence has emerged demonstrating a role of AECs in host defence against inhaled pathogens, but in certain cases including diseased states, may be exploited by pathogens as a potential intracellular safe haven. Using state-of-the-art single-cell technologies, we demonstrated that Cryptococcus spores (14.6%) are more readily taken up by AECs, than yeast (0%). Furthermore, using live-cell microscopy, we demonstrated that while a fraction of internalized spores fail to germinate at all (~15%), internalised spores can germinate and replicate. On occasion, Cryptococcus can even escape AECs in a non-lytic manner, leading us to hypothesise that AECs may be a novel intracellular host reservoir that contribute to both dissemination and latency. To this end our work centres around characterizing the ability of spores,

unlike yeast, to invade, escape, and persist in AECs, both in tissue culture and in murine models of disease. Understanding this novel intracellular host reservoir could elucidate both the mechanisms of how fungi disseminate out of, and remain latent in, host lungs.

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CS3.3.4

DO EXTRACELLULAR RNAS RELEASED UPON INFECTION WITH ASPERGILLUS FUMIGATUS CONTRIBUTE TO ANTIFUNGAL DEFENSE?

Alexander Bruch[1], Matthew Blango[1]

[1]*Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany*

Severe fungal infections have greatly increased over the past few decades and still occur with case-fatality rates of up to 90% in some populations. Roughly 1.7 million deaths per year worldwide make fungi an important medical challenge of the current century. Wide use of immunosuppression is facilitating fungal infections caused by opportunistic pathogens like Aspergillus fumigatus. These infections are particularly difficult to treat and exacerbated by the lack of early diagnosis and emerging antifungal resistance. Thus, the search for new diagnostic and antifungal treatment strategies demands new innovative approaches. The quickly expanding field of extracellular RNAs (exRNAs) is one potential option to advance these goals. ExRNAs consist of a variety of RNA species and can be released by diverse cell types including immune cells in response to infections in association with RNA binding proteins (RBPs) or extracellular vesicles (EVs). In a recent study, EVs derived from polymorphonuclear leukocytes (PMNs) infected with the filamentous fungus A. fumigatus displayed antifungal capacity consistent with other reports of exRNAs in host-pathogen interactions. Exploring the possible involvement of exRNA in antifungal defense, we set up an isolation pipeline to extract RNA from PMNs, EVs, and extracellular supernatants. First analysis indicated an enrichment for 20-200 nt small RNAs (sRNA) together with RBPs like ANXA1 and ANXA11 in the EV fractions. Furthermore, sRNA-sequencing of the exRNA population indicated a high abundance of miRNAs, where specific, conserved candidates are already known to bear antimicrobial properties. Thus, subsequent investigations aim to further characterize the identified miRNAs and define their role in A. fumigatus defense. With this better foundation, we hope to build on our understanding of exRNAs in the host-pathogen interaction to facilitate development of new diagnostics and therapeutics against this important human pathogen in the future.

CS3.3.5

HOST BRAIN ENVIRONMENT TRIGGERS MAPK RNAI-BASED EPIMUTATION IN THE HUMAN PATHOGEN MUCOR CIRCINELLOIDES

Maribel Navarro-Mendoza[1], Carlos Perez-Arques[1], Zanetta Chang[1], Joseph Heitman[1]

[1]*Duke University School of Medicine, Durham, NC, United States*

Mucorales are understudied basal fungi that cause lethal human infections known as mucormycoses. Immunocompromised and COVID-19 patients are highly susceptible and mortality rates reach 90% in disseminated infections. These poor prognoses are associated with limited therapies further complicated by antifungal-drug resistance. Mucor species can develop transient drug resistances via RNAi-based epimutations, adapting to different stresses through a reversible epigenetic mechanism. Epimutations persist during infection and are induced after host passage, particularly during central nervous system (CNS) infections. In this work, we explore epimutations induced by different host microenvironments during infection. We found seven loci that were actively silenced following CNS infection in mice, named bep for brain epimutations. Six encode hypothetical proteins (bepB to bepG) and one, bepA, encodes a possible ortholog to the stress-activated protein kinase Mpk1. bepA showed decreased transcript levels following macrophage phagocytosis and small RNA accumulation during CNS infection. We developed a method to transform M. circinelloides and generated bepA deletion mutants. bepA mutants exhibit resistance to calcineurin inhibitors, FK506 and Cyclosporine A, manifested as mycelial growth. These results suggest that bepA is a MAPK involved in the yeast-hyphae transition transduction pathway, promoting yeast growth, and counteracting the protein phosphatase calcineurin or its targets that induce the transition to mycelium. To further characterize the role of BepA in host adaptation, we have taken two approaches. First, we evaluated the ability of Mucor to adhere to brain endothelial cells and then cross the blood-brain barrier (BBB), mimicking the CNS environment that triggered bepA silencing. Second, we studied the impact of bepA deletion in whole-animal models, monitoring infection of immunosuppressed mice. Our preliminary results suggest that bepA mutants damage the integrity of the tight junctions connecting the endothelial BBB cells, and further characterization will elucidate why silencing of bepA promotes adaptation to the CNS environment.

CS3.3.6

DUAL RNA-SEQ REVEALS EXPRESSION SIGNATURES BENEFICIAL FOR IRON UPTAKE AND INTRACELLULAR LONG-TERM INTERACTION OF LICHTHEIMIA CORYMBIFERA (MUCORALES) WITH MACROPHAGES

Kerstin Voigt[1,2], Felicia Adelina Stanford[1,2], Patricia Sieber[1], Hea-Reung Park[1,2], Philipp Kämmer[2], Mohamed Ismail Abdelwahab Hassan[1,2], Hans-Martin Dahse[2], Sascha Brunke[2], Stefan Schuster[1], Christine Skerka[2], Hortense Slevogt[3],
Jörg Linde[2], Ulrike Binder[4], Cornelia Lass-Flörl[4], Bernhard Hube[2]

[1]University of Jena, Jena, Germany, [2]Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany, [3]University Hospital Jena, Jena, Germany, [4]Medical University of Innsbruck, Innsbruck, Austria

Mucormycosis is a life-threatening fungal infection caused by pathogenic mucoralean species which predominantly belong to the genera Lichtheimia, Rhizopus, and Mucor. Among a multitude of predisposing factors ranges the increased serum iron levels in immunocompromised individuals. This, in-turn, impairs the immune system leading to the growth and dissemination of L. corymbifera. Here we provide a detailed dual- RNASeq-based analysis of the transcriptomic environment of intracellularly persisting L. corymbifera sporangiospores in murine alveolar macrophages. Using a total of de novo generated 70 million reads were screened from expression signatures which are specific for L. corymbifera and unique for mucormycosis by combination with publicly available RNASeq data from other pathogenic mucoralean fungi. Out of a total of 21 genes encoding for enzymes and transporters in the high-affinity iron-uptake systems, 12 genes have orthologs in S. cerevisiae and Candida spp. and 2 in Aspergillus spp. On the host side, the gene encoding the inducible nitric oxide synthase (iNOS) was identified to be highly upregulated under intracellular persistence after phagocytosis by macrophages. qRT-PCR-based validation of these differentially expressed genes confirmed their differential regulation in real interaction scenarios by human macrophages with causative agents of mucormycosis most reported to be the cause of fatal disease in immunocompromised patients. A total of 13 host genes were identified which are differentially regulated, 4 of which are directly involved in the host's proinflammatory response. Our host and pathogen transcriptomics data, iron starvation, supplementation studies, and quantitative validation indicate that these iron assimilation genes are essential for intracellular persistence, and most importantly, may contribute to virulence.

CS3.3.7

FUNCTIONAL STUDIES OF COCCIDIOIDES CPS1, AND CREATION OF A LIVE ATTENUATED VACCINE AGAINST COCCIDIOIDOMYCOSIS

Alejandra Mandel[1], Devin Seka[1], Christine Butkiewicz[1], Nitesh Khandelwal[1], Hien Trinh[1], Daniel Powell[1], Jeffrey Frelinger[1], Lisa Shubitz[1], John Galgiani[1], Thomas Tomasiak[1], **Marc Orbach**[1]

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A live-attenuated canine coccidioidomycosis vaccine has been developed by deletion of the CPS1 gene in Coccidioides posadasii. The Δcps1 mutant initiates the parasitic spherule phase but fails to produce mature spherules and is avirulent in all mouse strains tested, including severely immunodeficient NOD scid mice indicating it's safety. Recently we have shown that vaccination provides a high level of protection in dogs. To understand why the CPS1 deletion is so debilitating in Coccidioides, structural studies of the Cps1 protein and functional and expression studies of CPS1 are being used to define the role of CPS1 in normal Coccidioides growth and spherulation. Cps1 domain deletion derivatives of C. posadasii strain Silveira were created and screened for virulence in a mouse infection model and for in vitro spherulation. The three CPS1 domain deletion constructs each lacked a single conserved domain, the N-terminal DMAP1b domain proposed to be a protein-protein interactions domain, or one of the two adenylate-forming domains, AMP1 or AMP2. Gene constructs were tagged at the C-terminus for validation of protein expression. Deletion of either of the two Cps1 adenylate-forming domains, AMP1 or AMP2, resulted in strains that reiterated the Δcps1 phenotype; they were avirulent and failed to persist in mice, while deletion of the Cps1 DMAP1b domain produced strains that were fully virulent in mice. The Cps1 protein was previously predicted to be a transmembrane protein however AlphaFold2.1 predicts that Cps1 lacks transmembrane domains and is a globular peripheral membrane protein. For structural characterization of Cps1, tagged cDNA copies were expressed in Saccharomyces cerevisiae, and the protein was purified, supporting the peripheral membrane model. The catalytic activity of Cps1 is being defined using ATP hydrolysis studies. Understanding the molecular and biochemical role of CPS1 in spherulation is critical to support the safety of this live-attenuated fungal vaccine.

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CS3.3.8

UNRAVELING THE BIOLOGY OF NEMATOPHAGY DURING A FUNGAL-NEMATODE PREDATOR-PREY INTERACTION USING TIME-COURSE TRANSCRIPTOMIC ANALYSIS

Hung-Che Lin[1], Guillermo Vidal-Diez de Ulzurrun[1], Sheng-An Chen[1], Ching-Ting Yang[1], Pedro Gonçalves[1], Chih-Yen Kuo[1], Tsung-Yu Huang[1], Erich Schwarz[2], Yen-Ping Hsueh[1]

[1]Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan, [2]Department of Molecular Biology and Genetics, Cornell University, NY, USA

Nutritional deprivation triggers a saprotrophic to predatory lifestyle switch in soil dwelling nematode-trapping fungi (NTF). In particular, Arthrobotrys oligospora has evolved to secrete food and sex cues to lure their nematode prey into an adhesive network of traps, specialized structures that originate from the vegetative mycelium. Upon capture, the nematodes are invaded and digested by the fungus, thus serving as a food source. We employed RNA-sequencing to examine the response of A. oligospora upon exposure to the model nematode Caenorhabditis elegans. A dynamic transcriptomic reaction that indicated a strong reliance on protein secretion was observed. Two-thirds of the predicted secretome of A. oligospora are upregulated in the presence of C. elegans at all tested time points. We found a large number of genes related to ribosome biogenesis induced at an early time point, suggesting that the TOR signaling pathway might be critical. Moreover, a plasma membrane t-SNARE protein, SSO2, involved in membrane fusion of secretory vesicles, plays a major role in nematode-adhesion. We subsequently predicted the putative effectors of A. oligospora and found that they represent approximately 19% of the secretome. Specifically, we found that genes of the Egh16 family were highly upregulated upon nematode exposure and highly expanded in the genomes of several NTF, suggesting a role for the evolution of the predatory lifestyle in Ascomycetes. In situ hybridization and GFP fusion protein reveals the accumulation of the highly expressed Egh16 in the traps cell. Thus, we named these gene family as Trap Enriched Protein (TEP). Gene deletion of the highest expressed gene TEP1 impairs the function of traps. Lastly, upregulation of the serine and metalloproteases were observed during the nematode digestion process. Protease inhibitors thwart fungal infection after penetration, demonstrating that proteases facilitate hyphal growth and prey digestion.

CONCURRENT SESSION 3.4
SYMBIONTS AND ENDOPHYTES

TUESDAY, MARCH 7
14:00 – 16:00
Location: ***Hall Freiburg (Congress Innsbruck)***

CHAIRS:
Benjamin Horwitz, Ursula Peintner

CS3.4.1

LOCAL ENDOREDUPLICATION OF THE HOST IS A CONSERVED PROCESS DURING PHYTOMYXEA-HOST INTERACTION

Michaela Hittorf[1], Andrea Garvetto[1], Marianne Magauer[2], Martin Kirchmair[1], Willibald Salvenmoser[3], Stefan Ciaghi[1], Sigrid Neuhauser[1]

[1]Institute Of Microbiology, University Of Innsbruck, Innsbruck, Austria, [2]Institute of Botany, University Of Innsbruck, Innsbruck, Austria, [3]Institute of Zoology, University Of Innsbruck, Innsbruck, Austria

Endoreduplication is a modified cell cycle where cells duplicate their DNA without subsequent mitosis. This process is quite common in plants and can also be found in other organisms like algae and animals. Biotrophic plant pathogens have been shown to induce endoreduplication in their host to gain space and/or nutrients. Phytomyxea are obligate biotrophic parasites of plants, diatoms, brown algae and oomycetes. We demonstrate that Plasmodiophora brassicae (Brassica hosts) and Maullinia ectocarpii (brown algal hosts, E. siliculosus) induce local endoreduplication in the hosts. Spatiotemporal, microscopic analysis reveal, that endoreduplication is a conserved process of plant and brown algal hosts during phytomyxid infections. By combining fluorescent in situ hybridisation (FISH) coupled with nuclear area measurements and flow cytometry we confirm that endoreduplication is induced in infected plants and are able to demonstrate this process in combination with a biotrophic parasite in brown algae. Molecular signatures of this process can be identified in RNA-seq datasets of P. brassicae-infected Brassica oleraceae and M. ectocarpii-infected E. siliculosus. The expression pattern of gatekeeper genes for endoreduplication change in infected plants and brown algae accordingly: Cell cycle switch proteins (CCS52A1 and B in plants and CCS52 in algae) as well as the protein kinase WEE1 (in plants) were identified as genes potentially important for the phytomyxean-induced switch from the mitotic cell cycle to the endocycle. In this study we expand the knowledge on phytomyxea-host interactions by showing that induced endoreduplication in the host is a conserved feature in phytomyxid infections. The induction of this cellular mechanism by phytomyxid parasites in phylogenetically distant hosts further points at a fundamental importance of endoreduplication in these biotrophic interactions.

CS3.4.2

GENOMIC FEATURES OF ENDOPHYTISM AND HOST ADAPTATION IN THE ARABIDOPSIS THALIANA ROOT MYCOBIOME

Fantin Mesny[1,2], Thorsten Thiergart[1], Shingo Miyauchi[1,3], Bruno Hüttel[4], Annegret Kohler[3], Igor Grigoriev[5], Francis Martin[3], Stéphane Hacquard[1]

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The non-mycorrhizal model plant Arabidopsis thaliana hosts in its roots diverse fungal communities, that were demonstrated to negatively impact its health in absence of protection from bacterial commensals and innate immune responses. While multiple pieces of evidence pointed to the endophytism of A. thaliana mycobiota members, predicted evolutionary histories revealed that most of these fungi derived from pathogenic ancestors. Re-colonization experiments with individual isolates highlighted diverse fungal effects on plant performance, spanning along the mutualist-pathogenic continuum. This gradient of effects was correlated to fungal root colonization efficiency, revealing that fungi with detrimental effects dominate in natural root samples. We showed that pectin-degrading enzymes from family PL1_7 contribute in the aggressiveness of endophytic colonization. While further genomic and transcriptomic analyses corroborated the major role of carbohydrate-active enzymes in root endophytic colonization, intra-species comparative genomics of the highly prevalent mycobiota member Plectosphaerella cucumerina revealed a genomic architecture favoring the fast evolution of effector-encoding genes. We notably identified in this species a candidate genomic region predicted to be involved in fungal adaptation to A. thaliana. Taken together, these results offer a better understanding of the fine line between endophytism and parasitism in the root mycobiota. They show that fungi robustly colonizing A. thaliana roots in nature rely on carbohydrate-active enzymes to degrade host cell walls, and likely on effectors to overcome innate immune responses.

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CS3.4.3

MYCOHETEROTROPHIC ORCHIDS AND THEIR SYMBIONTS: A METATRANSCRIPTOMIC APPROACH

Guillermo Chumaceiro[1,2,3], Marc-André Selosse[4,5], Alga Zuccaro[1,2], **Gregor Langen**[1]

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Fungi belonging to the order Sebacinales have highly diverse interactions with plants and have a broad host range. Neottia nidus-avis is a mycoheterotrophic orchid that lost the ability to photosynthesize and relies on fungal partners for mineral and organic nutrients. Sebacinales fungi are commonly found in its roots based on environmental analyses of ITS sequences. The family Sebacinaceae has been recently established from rRNA sequence analysis, but no transcriptome or genome is available for any member of this group. Here, we have identified transcripts from orchid mycorrhizal fungi colonizing the roots of the host N. nidus-avis from three independent environmental root samples. ITS sequences and de novo assembly of their transcriptomes revealed the presence of two different Sebacinales taxa colonizing the roots of this orchid with a BUSCO completeness of 81% and 62%. A phylogenetic tree of the ITS region positions these two fungi closest to Sebacina flagelliformis and Sebacina epigaea. We have functionally annotated these transcriptomes to understand what genes are expressed in this symbiotic relationship. The effector and Carbohydrate-active enzyme (CAZyme) repertoires of these orchid mycorrhizal fungi give hints on their lifestyle and how they are able to colonize their hosts.

CS3.4.4

ROLE OF THE SP7-LIKE EFFECTORS IN THE ARBUSCULAR MYCORRHIZAL SYMBIOSIS

David Figueira-Galán[1], Sven Heidt, Ruben Betz[1], Natalia Requena[1]

[1]*Karlsruhe Institute Of Technology, Karlsruhe, Germany*

The vast majority of land plants are able to establish a symbiotic relationship with fungi from the subphylum Glomeromycotina called arbuscular mycorrhiza. Through specialized fungal structures named arbuscules, the nutrient exchange takes place. The fungus receives photosynthetically fixed carbon in the form of sugars and fatty acids from the plant, while it supplies with the plant with different mineral nutrients as well as water in exchange. Among these nutrients, phosphorous is the most decisive for the establishment and maintenance of the symbiosis. Our model mycorrhizal fungus, Rhizophagus irregularis, secretes different effectors of the SP7-like protein family in order to alter the host plant physiology to facilitate the colonization and prolong this interaction. Our research has shown that most of these effectors localize to the nucleus of the arbuscule-containing root cells, where they interact with several RNA-binding proteins of the plant. To analyze the effect of these fungal proteins in planta we have generated Solanum tuberosum transgenic plants that ectopically express RiSP7 and they show clear developmental changes when compared to control plants. Through the analysis of the root transcriptome of these lines we have been able to show that this fungal protein leads to changes in the plant's alternative splicing activity as well as in the expression levels of several plant genes, which will help us elucidate the function and molecular mechanism of this family of effectors.

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CS3.4.5

BIOCONTROL POTENTIAL OF A TOMATO FUNGAL ENDOPHYTE AGAINST P. SYRINGAE

Luisa Liu-Xu[1], Loredana Scalschi[1], Emma Fernandez-Crespo[1], Atefeh Farvardin[1], Lorena Sanchez-Gimenez[1], Gemma Camañes[1], Begonya Vicedo[1], Pilar Garcia-Agustin[1], Eugenio Llorens[1]

[1]*Universitat Jaume I, Castellon De La Plana, Spain*

Plant endophytes have been shown to improve crop's performance and resistance against environmental stress factors. In this work, we assessed the potential of an endophytic fungal strain isolated from traditional tomato (S. lycopersicum) as biocontrol agent against pathogenic bacteria Pseudomonas syringae pv. Tomato (Pst). First, in-vitro effect of fungal exudates was determined using culture filtrates (CF). This showed that the addition of CF in media significantly reduced Pst growth. To confirm this role of the endophytic fungi in host plant, we set up experiments with tomato var. Ailsa Craig. 4-week-old plants, with distinction between seed inoculated plants and plants treated with CF, were infected with Pst. At 72hpi, phenotype analysis of infected leaves and bacterial colony counting was performed. A significant reduction of symptoms and infection rate was seen in both seed inoculated and CF-treated plants. Transcriptomic analysis targeting stress related gene regions showed that gene expression of CF treated plants was not significantly different from control, while inoculated plants had a significantly lower expression of PR5, LOX and OPR3 genes post-infection. Thus, though both seed inoculation and application of CF help the plant fight Pst infection, the involved mechanisms are different. In addition, since CF application was effective, metabolomic analysis was performed to detect compounds with potential impact in the pathogenic system.

CS3.4.6

ARE ALL THE SAME? AN EVOLUTIONARY STORY OF LICHEN SYMBIOSES

David Díaz Escandón[1], Gulnara Tagirdzhanova[1], Philipp Resl[2], Toby Spribille[1]

[1]*Unviersity of Alberta, Edmonton, Canada,* [2]*University of Graz, Graz, Austria*

Almost two third of all fungi belong to the phylum Ascomycota, including some of the most studied organisms worldwide. However, some of the major lifestyles in the phylum have been
overlooked due to lack of economic importance or human pathogenicity. Lichen symbioses account for 25% of all ascomycotan diversity but only 1% of all the current ascomycotan genomic research. Lichens have been traditionally described as a mutualistic symbiosis between a fungal and a photosynthetic partner, and after 160 years, this concept has grown to include additional symbionts in the symbioses, but in terms of goods and services of the symbiosis little has changed. This symbiotic relationship is thought to be a nutrient-driven relationship—whereas the photobiont release photosynthates to the fungal symbiont—that has emerged multiple independent times across ascomycotan fungi evolution. In this study we compared genomes from lichens across all filamentous Ascomycota, representing 6 of the 10 currently circumscribed classes of Ascomycota. We collected all available genomes of fungal lichen symbionts in databases together with in-house sequenced genomes and functionally annotate them. We select Carbohydrate active enzymes as metabolic proxy and predicted metabolic flexibility based on substrate activity. Our results suggest that lichen symbioses have different metabolic enzymatic machinery despite the symbiotic lifestyle, suggesting that the independent origins are not converging in nutritional nature, but other services might be under selective pressures.

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CS3.4.7

SYNTHETIC MUTUALISM IN PLANT-FUNGUS INTERACTIONS

Johannes Gärtner[1], Svenja Hermanns[1], Hang Lu[1], **Marcel Bucher**[1]

[1]*University of Cologne, Cologne, Germany*

The best-studied mutualistic plant-fungal interaction is the arbuscular mycorrhizal (AM) symbiosis between terrestrial plants and soil-living fungi of the subphylum Glomeromycotina within the Mucoromycota. AM fungi colonise at least 80 % of land plant species, promote plant growth and plant diversity. The bidirectional transfer of phosphorus and organic carbon between the interacting AM partners as well as the obligate biotrophic lifestyle of AM fungi have stabilised this symbiosis over 500 million years.
In the project, we construct a synthetic mycorrhiza-like interaction with the Brassicaceae model plant Arabidopsis thaliana, which cannot form AM symbiosis, and its natural fungal endophyte Colletotrichum tofieldiae (C.t.). C.t. is genetically engineered to resemble AM fungi, which are fatty acid auxotrophs that transport nutrients into host roots.
The mechanistic study of the processes underlying the symbiotic traits of AM fungi is difficult because they have proven recalcitrant to conventional genetic transformation, because they are aseptate and protoplasts cannot be released, and because they have multinucleate cytoplasms.
Therefore, the synthetic symbiotic partnership created in this project will improve the growth and fitness of the Arabidopsis host and allow the identification of new fungal and plant traits underlying mutualism in mycorrhizal symbioses.
https://doi.org/10.1073/pnas.1812275115
https://doi.org/10.1126/science.abg0929

CS3.4.8

REDUCING FUNGAL ENDOPHYTES IN WHEAT AND ITS EFFECTS ON PLANT FITNESS

Or Sharon[1], Naomi Kagan Trushina[1], Amir Sharon[1]

[1]*Institute for Cereal Crops Research, Tel Aviv University, Tel Aviv, Israel*

All plant species contain fungal endophytes communities (FECs) that are part of their microbiome. Certain fungal endophytes have proven beneficial effects on their hosts; however, plants contain high number of uncharacterized fungi, which complicates the evaluation of the contribution of specific taxa. Production of endophytes free plants can help advance understanding of the true impact of specific fungal members and of the entire FECs on plants.
In this study we attempted to produce fungal endophyte free wheat (Triticum aestivum) plants using several methods: treatment of seeds with fungicides, production of plants from rescued embryos, and regeneration of plants from callus tissue. Analysis of the resulting plants and seeds using Next generation sequencing and digital droplet PCR showed that the different treatments reduced the fungal biomass but did not completely eliminate fungal endophytes. Germination and development of the endophytes-reduced plants was damaged and delayed compared to control plants, indicating that the FEC is necessary for proper plant development.
In conclusion, we showed that the biomass of fungal endophytes can be significantly reduced, however plants can't be completely cured from fungal endophytes and certain members of the FECs are transmitted in embryos and even in undifferentiated callus tissue. Still, reduction of endophyte content leads to changes in FEC composition and plant performance.

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CONCURRENT SESSION 4.1 ANTIFUNGALS AND RESISTANCE MECHANISMS

TUESDAY, MARCH 7
17:30 – 19:30
Location: ***Hall Tirol (Congress Innsbruck)***

CHAIRS:
Michaela Lackner, Gabriel Scalliet

CS4.1.1

SYSTEMATIC DISCOVERY OF ANTIBACTERIAL AND ANTIFUNGAL BACTERIAL TOXINS

Marina Campos Rocha[1], Nimrod Nachmias[2], Noam Dotan[2], Rina Fraenkel[3], Maor Shalom[2], Arbel Rivitz[2], Noam Deouell[3], Inbar Cahana[3], Netanel Tzarum[3], Naama Shamash-Halevy[2], Yaara Oppenheimer-Shaanan[2], Asaf Levy[2], Neta Shlezinger[1]

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Microbes employ toxins to kill competing microbes or eukaryotic host cells. Polymorphic toxins are proteins that encode C-terminal toxin domains. Here, we developed a computational approach to discover novel toxin domains of polymorphic toxins within 105,438 microbial genomes. We validated nine short novel toxins ("PTs") that cause bacterial or yeast cell death. The novel PTs are encoded by ~2.2% of the sequenced bacteria, including numerous pathogens. We also identified five cognate immunity genes ("PIMs") that neutralize the toxin activities. Intriguingly, we observed an antifungal effect of the PTs against various pathogenic fungi. Lysates of different PT-expressing cells were toxic at different levels to the six species of pathogenic fun-

gi we tested, including species of Aspergillus nidulans, Aspergillus fumigatus, Candida albicans, Candida auris, Cryptococcus neoformans, and Fusarium oxysporum. The toxicity was abolished or decreased when the fungal cells were grown with lysates of cells expressing PT mutant, PT-PIM. Each of the toxins we tested demonstrated antifungal activity against at least one fungal strain. Lysates of cells expressing PT1, PT7, PT8, and PT9 reduced hyphal length by at least 50% and PT7, PT8, and PT9 reduced germination by over 67% in comparison to Ctr. We followed the toxins under the microscope and found that the toxins clearly penetrated the cells in up to 30 minutes. The toxins likely act as enzymes that cause severe damage to cell shape, cell wall, membrane, and DNA. Finally, we solved the 3D structure of two PTs in complex with their PIMs, and showed that they function as novel DNAses. The new potent toxins likely play key roles in inter-microbial competition and can be utilized in various clinical and biotechnological applications.

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CS4.1.3

SYSTEMATIC CHARACTERIZATION OF ANTIFUNGAL RESISTANCE MUTATIONS AND RESISTANCE FUNCTION TRADE-OFFS USING GENOME EDITING

Philippe Després[1,3,4,5], Angel F. Cisneros[1,3,4,5], Emilie MM Alexander, Ria Sonigara[1,3,4,5], Cynthia Gagné-Thivierge[1,2,3,4,5], Alexandre K Dubé[1,2,3,4,5], Christian R Landry[1,2,3,4,5]

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Antifungal resistance is an emerging threat for public health and agriculture. The pool of resistance mutations remains largely uncharacterized, as do the mechanisms leading to resistance. In particular, we know that the success of resistance mutations depends on the trade-off between the benefits and costs they incur. These trade-offs are largely unknown for antifungal drug targets. Here, we use genome editing and high throughput sequencing to systematically measure the effect of all amino acid substitutions in the yeast cytosine deaminase Fcy1, the target of the antifungal 5-fluorocytosine (5-FC, flucytosine). We identify over 900 missense mutations granting resistance to 5-FC, a large fraction of which appear to act through destabilization of the protein. By comparing mutational effects on 5-FC resistance and the canonical function of Fcy1, we then systematically examine the resistance-function trade-off. We find the relationship between 5-FC resistance and growth sustained by cytosine deamination is characterized by a sharp trade-off, such that small gains in resistance universally lead to large losses in canonical enzyme function. This steep relationship can be explained by differences in the dose-response functions of 5-FC and cytosine. Finally, we observe the same trade-off shape for the orthologue of FCY1 in Cryptoccocus neoformans, a human pathogen. Our results provide a powerful resource and platform for systematically cataloging and interpreting drug target variants in pathogenic fungi as well as unprecedented insights into resistance-function trade-offs.

CS4.1.4

MUTATOR PHENOTYPES IN ASPERGILLUS FUMIGATUS DRIVE THE RAPID EVOLUTION OF ANTIFUNGAL RESISTANCE

Michael Bottery[1], Chris Knight[1], Michael Bromley[1]

[1]*The University of Manchester, Manchester, United Kingdom*

The occurrence of antifungal resistance in the fungal pathogen Aspergillus fumigatus is increasing globally, creating a barrier to the successful treatment of life-threating fungal infections. Of particular concern is the evolution of resistance to triazoles, the only class of antifungals suitable for long-term treatment. However, the cellular mechanisms that promote the emergence of resistance to both currently used and novel antifungal compounds are unclear. Here we demonstrate how mutator phenotypes caused by variants in DNA mismatch repair (MMR) genes, essential for maintaining genomic integrity during DNA replication, cause significantly elevated mutation rates, resulting in the rapid emergence of antifungal resistance. Loss of function of MMR genes MSH2 or PMS1 result up to 190-fold increase in the rate of triazoles resistance acquisition, and increases the rate of olorofim resistance (a novel antifungal in stage-3 clinical trials) by up to 100-fold. A screen of 218 clinical and environmental A. fumigatus isolates identified a high degree of genetic variation in MMR genes with up to 30% of isolates containing non-synonymous variants in MSH2, PMS1 and MSH6. Genetic variation in MMR genes within clinical and environmental A. fumigatus isolates strongly associate with clade A multi-azole resistance isolates and are shown to significantly increase the rates of resistance evolution in vitro. The mutation rate, and thus the propensity to evolve drug resistance, within A. fumigatus is variable and dependent upon genotype. The co-occurrence of highly adaptable mutator phenotypes and azole resistance means that novel, next generation antifungal resistance is more likely to arise within isolates already resistant to currently used drugs – resulting in multi-drug resistant pathogens. We anticipate this will have an important role in predicting the likelihood of treatment failure due to drug resistance and in the effective stewardship of antifungal drug treatments.

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CS4.1.5

REMODELLING THE ANTI-OOMYCETES EFFICACY SCREENINGS: EXPLORING NEW FRONTIERS AND REFINING THE EXISTING

Demetrio Marcianò[1], Silvia Laura Toffolatti[1]

[1]*Università degli Studi di Milano, Dipartimento di Scienze Agrarie e Ambientali (DISAA), Via Celoria 2, 20133, Milan, Italy*

Oomycetes borne diseases represent a serious issue for agriculture sustainability. Increasing concerns about fungicide use and the spreading resistance among the pathogen populations make the stakeholders in demand of new, specific and low-environmental impact active compounds. Despite these needs, poor descriptions of high-throughput methodologies for fungicide efficacy screening are reported for this kind of organisms. Our study aimed at developing high-throughput protocols designed for multiwell plates, using Phythopthora infestans (tomato and potato late blight agent) and Pythium ultimum (soil-borne pathogen for multiple crops) as model species. The developed protocols summarized the knowledge derived from previous studies, providing comparisons with the gold standard (agar amended medium) and validation of the outputs using known inhibitor molecules. Moreover, new insights and a careful description of procedures for statistical analyses suitable for the analysis of data arising from efficacy screenings (e.g. beta regression and growth curves analyses) were proposed. Finally, a solution for efficacy assays was also proposed for a non-cultivable parasite. The biotrophic adaptation of several oomycetes species, in fact, greatly hampers pathogen handling thus limiting the study of their biology and the efficacy screenings for new antifungals. To solve this issue, a protocol combining flow cytometry (FCM) and fluorescence-activated cell sorting (FACS) on sporangia of Plasmopara viticola (grapevine downy mildew agent) was developed with interesting results. Using FCM, non-germinated sporangia were individuated among debris, degenerated sporangia and zoospores that are present within the heterogeneous inoculum obtained from infected leaves. Then, non-germinated sporangia were individually inoculated with FACS on grapevine leaves. This analytical approach offers unmatched resolution, precision and accuracy compared with the traditional tech-

niques and further implementations could offer brighter opportunities also in the study of related phythopatogenic oomycetes with a significant and positive contribution to the field.

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CS4.1.6

THE COMPLEX GENETIC LANDSCAPE OF FUNGICIDE RESISTANCE EVOLUTION IN ZYMOSEPTORIA TRITICI

Guido Puccetti[2]

[1]*Université de Neuchâtel, Neuchatel, Switzerland,* [2]*Syngenta, Stein säckingen, Switzerland*

The evolution of fungicide resistance in fungal pathogens poses a threat to the health of animal and plant species. The repeated emergence of pathogens with reduced sensitivity to specific fungicides illustrates the rapid process of resistance evolution. The widespread application of target-site fungicides in European agricultural fields over the last three decades makes the continent an excellent geographic range to identify the rise of specific resistance mutations in space and time. Target-site fungicides such as demethylation inhibitors (DMIs) target the enzyme CYP51 involved in essential cellular processes. The most common mechanism leading to insensitivity DMIs is structural changes in the target protein. There is strong evidence that additional loci are also contributing to overall resistance. Here, we aimed to unravel the evolutionary trajectories driving the genetic architecture of fungicide resistance in Europe for the major wheat pathogen, Zymoseptoria tritici, towards five widely used DMIs. Using a panel of 1420 whole-genome sequenced isolates spanning 27 European countries and a period of 15 years, we performed genome-wide association mapping for resistance using multiple phenotyping and genotyping methods. We identified dozens of previously unknown loci contributing to DMI resistance in addition to mutations in the gene encoding the target of DMIs. Even though DMIs invariably target the same protein, we found significant divergence in the genetic architecture of resistance. Our study highlights the power of microbial genome-wide association studies to retrace rapid evolutionary processes.

CS4.1.7

THE USE OF TARGETED ANTIFUNGAL LIPOSOMES AGAINST RHIZOPUS DELEMAR

Quanita Choudhury[1], Suresh Ambati[1], Zachary Lewis[1], Richard Meagher[1]

[1]*University of Georgia, Athens, United States*

Our research group has designed DectiSomes as a novel therapeutic treatment against human fungal infections. DectiSomes are antifungal-loaded liposomes that are coated with C-type lectin receptors such as the Dectins. Dectins recognize specific components of the fungal cell wall and are involved in the immune response against a variety of fungi. Coating antifungal-loaded liposomes with Dectins should increase the local drug concentration on fungal cells and lower the required dose of antifungal drug. Our research group has previously published results showing that DectiSomes loaded with amphotericin B (AmB) have improved targeting efficacy over untargeted liposomes against Aspergillus fumigatus, Candida albicans, and Cryptococcus neoformans in vitro and in mouse models of aspergillosis and candidiasis. We recently evaluated the targeting efficacy of DectiSomes against Rhizopus delemar, the primary causative agent of mucormycosis. Although mucormycosis is primarily an opportunistic infection, mortality rates range between 50-96% depending on the severity of the infection and the patient's underlying condition. There has been a notable increase in cases of mucormycosis during the COVID-19 pandemic. Treatment usually involves a combination of liposomal AmB and surgery to remove the infected tissue. Since Dectin-1 is known to be involved in the immune response against mucormycosis infections, we hypothesized that Dectin-1-targeted DectiSomes would exhibit improved efficacy against R. delemar. Our data show that DectiSomes bind more efficiently to R. delemar germlings and hyphae compared to untargeted liposomes. DectiSomes loaded with AmB also inhibit and/or kill R. delemar in vitro more efficiently than untargeted liposomes. In the near future, we will begin to test the efficacy of DectiSomes in mouse models of mucormycosis.

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CS4.1.8

ANALYSIS OF THE ASPERGILLUS FUMIGATUS PROTEOMIC RESPONSE TO AMPHOTERICIN B (AMB) REVEALS INVOLVEMENT OF A PUTATIVE FLIPPASE IN RESISTANCE

Annica Pschibul[1], Ammar Abou-Kandil[2], Sophie Tröger[1], Franziska Schmidt[1], Maira Rosin[1], Yana Shadkchan[2], Thomas Krüger[1], Nir Osherov[2], Axel A. Brakhage[1], **Olaf Kniemeyer**[1]

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The filamentous fungus Aspergillus fumigatus is an opportunistic human pathogen, which can cause mycoses and allergies in susceptible individuals. With regard to therapy, the polyene drug amphotericin B (AmB) is still frequently used to treat Aspergillus fumigatus infections due to the fact that it remains effective against azole-resistant Aspergillus strains. AmB binds to ergosterol in the fungal membrane, but its mode of action and the fungal resistance mechanisms involved have not been fully understood yet. To get further insights into the mode of action of AmB, by LC-MS/MS analysis we investigated the proteomic profile of A. fumigatus in response to AmB and its liposomal formulation. A significant increase (≥ 2-fold) in abundance of 202 different proteins in response to AmB and 193 in case of liposomal AmB was observed, while the level of 70 and 83 proteins decreased, respectively. In particular, the level of proteins anchored to the membrane, involved in catabolic processes, aromatic acid degradation, or secondary metabolism prominently increased. It is worth mentioning that some of the most significantly AMB-upregulated proteins included enzymes involved in the biosynthesis of secondary metabolites such as the prenylated polyphenol fumicycline as well as xanthocillin and hexadehydro-astechrome, which both form complexes with transition metals. Of particular note was the more than 300-fold increase of an RTA1 domain-containing protein upon AmB treatment. Fungal RTA-like proteins represent lipid-translocating exporters, which are characterized by multiple transmembrane regions and often confer resistance to toxic chemicals. The deletion of the corresponding gene in A. fumigatus led to an increased susceptibility to AmB and other antifungal polyenes such as nystatin.

CS4.1.15

RESISTANCE TO COMPLEX III INHIBITORS IN THE WHEAT PATHOGEN ZYMOSEPTORIA TRITICI

Gabriel Scalliet[1], Dirk Balmer[1], Melanie Appertet[1], Urvashi Thacker[2], Dominique Edel[1], Yvonne Grether-Buehler[1], Stefano Rendine[1]

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The use of antifungals targeting the respiratory chain is widespread in agriculture. One of the main targets is the coenzyme Q: cytochrome c – oxidoreductase, the Complex III of the respiratory chain. Complex III inhibitors bind to the mitochondrially-encoded Cytochrome b subunit of complex III, either at the Quinone outside (Qo, quinol oxidation) site, or at the Quinone inside (Qi, quinone reduction) site. Novel chemical Qo and Qi inhibitors were discovered which do not display cross-resistance to the widespread G143A and F129L (Qo site) Strobilurin-fungicides resistance mutations. The prediction of Cytochrome b resistance mutations ahead of their development in the field is crucial to design next generation Complex III inhibitors not affected by current and future Qo and Qi target-site resistance. We will present our approach to the generation of single and double mutants of Zymoseptoria tritici cytochrome b, their preliminary fitness assessments, cross resistance profiles and their rationalization through molecular modelling.

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CONCURRENT SESSION 4.2 FUNGAL CELL BIOLOGY

TUESDAY, MARCH 7
17:30 – 19:30
Location: ***Hall Brüssel (Congress Innsbruck)***

CHAIRS:
Alexander Lichius, Miguel A. Peñalva

CS4.2.1

THE HUM COMPLEX IS A MYOSIN-5 ADAPTOR TO SECRETORY VESICLES.

Miguel A. Peñalva[1], Ana M. Alonso[1], Vivian de los Ríos[1], Ignacio Bravo-Plaza[1], Álvaro de La Gándara[1], Antonio Galindo[2], Ernesto Arias-Palomo[1], Mario Pinar[1]

[1]Centro de Investigaciones Biológicas Margarita Salas, CSIC, Madrid, Spain, [2]LMB Laboratory of Molecular Biology, Cambridge, UK

The biogenesis of secretory vesicles and their transport to the vesicle supply center (VSC) have been intensively studied in Aspergillus nidulans. The oligomeric complex TRAPPII, which acts as GEF for RAB11 —formerly denoted RabE (Pinar and Peñalva, 2021)— is recruited to trans-Golgi network (TGN) cisternae at late stages of their maturation. TRAPPII recruits RAB11, and when a sufficient amount of the GTPase accumulates on any given TGN cisterna, its identity shifts from 'Golgi' to 'post-Golgi', engages molecular motors and tears off into secretory vesicles that are swiftly transported to the VSC. F-actin dependent myosin-5 focuses secretory vesicles at the VSC. Myosin-5 contains a motor domain, a coiled-coil domain mediating dimerization, and a C-terminal globular head domain mediating cargo recognition. Using shotgun proteomics combined with bottom-up reconstitution approaches, we have characterized the HUM complex, consisting of UDS1 (upregulated during septation), HMSV (hook of myosin to secretory vesicles) and the dimeric globular head domain of myosin-5 (Pinar et al., 2022). By interaction of RAB11 with both the GTD and UDS1, the HUM complex acts as adaptor of RAB11 secretory vesicles to the motor. The phe-

notype of HUM ablation resembles that of a partial deficiency of myosin-5, and SVs do not concentrate in the SPK although they arrive to the apical dome by MT-dependent transport. As expected from the deficient SV focusing resulting from disabling HUM, uds1Δ and hmsVΔ affect hyphal morphogenesis.

Pinar, M., A. Alonso, V. de los Ríos, I. Bravo-Plaza, Á. de la Gándara, A. Galindo, E. Arias-Palomo, and M.Á. Peñalva. 2022. The type V myosin-containing complex HUM is a RAB11 effector powering movement of secretory vesicles. iScience. 25.
Pinar, M., and M.A. Peñalva. 2021. The fungal RABOME: RAB GTPases acting in the endocytic and exocytic pathways of Aspergillus nidulans (with excursions to other filamentous fungi). Mol Microbiol. 116:53-70.

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CS4.2.2

STRUCTURAL AND MOLECULAR INVESTIGATION OF SECONDARY METABOLITE COMPARTMENTALIZATION IN FUNGAL VESICLES

Fabio Gherlone[1], Vito Valiante[1], Katarina Jojić[1]

[1]*Leibniz Institue for Natural Product Research and Infection Biology (HKI), Jena, Germany*

Fungal secondary metabolites (SMs), are a rich source of active compounds. The discovery of the chemical repertoire and biosynthesis of fungal SMs has increased over the past years, such as their chemical variability, the regulation and the organization of biosynthetic gene clusters (BGCs), as well as the comprehension of the producing organisms. Genome mining prediction and pathway biochemical elucidation are crucial in moving forward compound discovery. However, to have a full picture of biosynthetic pathways, the knowledge on enzymes and their activity needs to be coupled with the understanding of their spatial and temporal organization within the cell.
Many subcellular compartments of SM-associated proteins have been already described, and they include peroxisomes, the cytosol, and ER-derived endosomes such as vesicles, vacuoles, and even more specialized organelles, such as melanosomes. The fungal vesicular transport system is essential in the formation of those compartments and the sorting of biosynthetic enzymes. We therefore started by identifying putative vesicle sorting signal peptides (SPs) of known compartmentalized BGCs. Batch alignment of gene orthologues in fumonisin (Fusarium verticillioides) and sphingofungin (Aspergillus fumigatus) clusters indicated that N-terminal regions are conserved. We created a mutant library of the first 66 amino acids of the putative SP of the sphingofungin biosynthetic enzyme SphH (a P450 monooxygenase). Each sequence was tagged to a GFP and fluorescent protein localization was analyzed using a confocal microscope, first in Saccharomyces cerevisiae and then in Aspergillus fumigatus. 21 amino acids were found to be sufficient for successful localization in perinuclear ER and ER-derived vesicles. Since very little is known about the mechanism of SP recognition, cleavage, and targeting in filamentous fungi, these mutant phenotypes gave us the first insights into the SP putative secondary structure and membrane topology.

CS4.2.3

THE VACUOLAR MORPHOLOGY PROTEIN VAC14 PLAYS AN IMPORTANT ROLE IN SEXUAL DEVELOPMENT OF SORDARIA MACROSPORA

Anika Groth[1], Svenja Ahlmann[1], Antonia Werner[1], **Stefanie Pöggeler**[1]

[1]*Georg-Agust University Göttingen, Göttingen, Germany*

The multiprotein Fab1p/PIKfyve-complex regulating the abundance of the phospholipid phosphatidylinositol 3,5-bisphosphate (PtdIns(3,5)P2) is highly conserved among eukaryotes. In yeast/mammals it is composed of the phosphatidylinositol 3-phosphate 5-kinase Fab1p/PIKfyve, the PtdIns(3,5)P2 phosphatase Fig4p/Sac3 and the scaffolding subunit Vac14p/ArPIKfyve. The complex is located to vacuolar membranes in yeast and to endosomal membranes in mammals, where it controls the synthesis and turnover of PtdIns(3,5)P2. In this study, we analyzed the role and function of the Fab1p/PIKfyve-complex scaffold protein SmVAC14 in the filamentous ascomycete Sordaria macrospora (Sm). We generated the Smvac14 deletion strain Δvac14 and performed phenotypic analysis of the mutant. Furthermore, we conducted fluorescence-microscopic localization studies of fluorescently labeled SmVAC14 with vacuolar and late endosomal marker proteins. Our results revealed that SmVAC14 is important for maintaining vacuolar size and appearance as well as proper sexual development in S. macrospora. In addition, SmVAC14 plays an important role in starvation stress response. Accordingly, our results propose that the turnover of PtdIns(3,5)P2 is of great significance for developmental processes in filamentous fungi.

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CS4.2.4

EXPLORING SEPTATION-DEPENDENT AND -INDEPENDENT ROLES OF THE ASPERGILLUS FUMIGATUS SEPTATION INITIATION NETWORK

Xabier Guruceaga[1], Adela Martin-Vicente[1], Ashley Nywening[1], Harrison Thorn[1], Jinhong Xie[1], Wenbo Ge[1], Jarrod R. Fortwendel[1]

[1]*Department of Clinical Pharmacy and Translational Science, Pharmacy College, UTHSC, Memphis, United States*

Previous work in our laboratory identified the Septation Initiation Network (SIN) kinases of Aspergillus fumigatus, SepH, SepL, and SidB, as essential for echinocandin fungistatic activity and virulence-associated tissue invasive growth. Although the best characterized role for SIN function is initiation of septum construction, downstream elements of this pathway involve signaling to cell wall biosynthetic components that may not be directly involved in septation. Therefore, SIN kinase deletion phenotypes could be driven by septum-dependent or -independent mechanisms. In the current study, we sought to delineate the extent to which the SIN kinase-deletion echinocandin hypersusceptibility phenotype is septum- or cell wall-dependent. To first examine possible interactions between the SIN and cell wall biosynthesis / integrity pathways, we examined the mutant strains for alteration of cell wall content and for expression of cell wall-associated genes. Hyphal staining revealed altered distribution of chitin cell wall deposition and reduced β-glucan levels in the SIN kinase mutants compared with the wild type control. In contrast, loss of SIN kinase genes did not alter baseline expression of the major cell wall integrity MAPK gene, mpkA, or the glucan synthase encoding gene, fksA. These data implied that mere absence of septation, or loss of SIN pathway activity, likely does not induce cell wall stress signaling. To attempt to restore septation in the absence of SIN pathway activity, we next overexpressed the small GTPase, rho4, in wild type and SIN deletion mutants. Surprisingly, although Rho4 is essential for septation in A. fumigatus and is a putative SIN pathway effector, overexpression resulted in minimal recovery of septation in only ΔsidB. Although septation was partially recovered, echinocandin activity remained fungicidal. Ongoing work will seek to

better separate cell wall- and septum-dependent phenotypes of the SIN pathway. However, our current findings suggest a non-linear SIN pathway branching at the SepL-SidB signaling node.

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CS4.2.5

SEPTINS, STEROLS, SPHINGOLIPIDS, AND CELL WALL INTEGRITY

Michelle Momany[1]

[1]*University Of Georgia, Athens, Georgia, United States*

Septins are best known as cytoskeletal elements that shape cells, especially at division planes. Septins fall into five major groups based on sequence conservation and are found in all fungi and animals, some algae, and some protists. Aspergillus nidulans has five septins, four “core septins” (AspA/Cdc11, AspB/Cdc3, AspC/Cdc12, AspD/Cdc10) and a single noncore septin (AspE). A. nidulans core septins form heterooctamers that contain all core septins and heterohexamers that lack AspDCdc10. Core septin deletion mutants challenged with cell wall-disturbing agents grew more slowly or not at all, while the noncore septin deletion mutant showed very little change. Cell wall polymer localization was modified in core septin deletion mutants, as was the localization of the chitin synthase cell wall biosynthetic enzyme. Similarly core septin mutants challenged with ergosterol-perturbing agents showed distinct sensitivities relative to the noncore septin mutant. Septin core hexamer mutants challenged with sphingolipid-disrupting agents were more sensitive than the other septins. Core hexamer septin localization was disrupted when sphingolipids were disrupted. Taken together our results suggest that septins are important for monitoring and modulating membrane composition and cell wall integrity with hexamers and octamers playing distinct roles.

CS4.2.6

SIP-1 IS ESSENTIAL FOR GERMLING FUSION OF NEUROSPORA CRASSA, PROBABLY BY MEDIATING THE INITIATION OF CELL-CELL COMMUNICATION

Anne Oostlander[1], Marcel Schumann[1], Ulrike Brandt[1], André Fleißner

[1]*Technische Universität Braunschweig, Braunschweig, Germany*

Cell fusion plays a central role in the development and proliferation of eukaryotic organisms. However, the molecular basis for this cellular process has not yet been fully elucidated. In the ascomycete fungus Neurospora crassa, germinating spores undergo chemotropic interaction and fusion to establish an interconnected colony. The proteins SO and MAK-2 are essential during this process. Their coordinated alternating recruitment to the cell tips of interacting germlings suggests that cells alternate between the physiological state of signal sending and signal receiving in the manner of a “cell dialog” to avoid auto-excitation. The MAK-2 MAP kinase module is associated with signal-receiving, while SO is recruited to the cell tip during signal-sending. The onset of this dialogue-like communication occurs remains unexplained.
Using co-immunoprecipitation and mass spectrometry, we identified SIP-1 as a new interaction partner of the SO protein. A deletion of the sip-1 gene results in inability to undergo chemotropic interactions and subsequent fusion. Live cell imaging revealed that SIP-1 is recruited to the cell tip of interacting germlings in an oscillating manner, coinciding with the SO recruitment. However, in contrast to all other known factors, SIP-1 already undergoes dynamic membrane recruitment in individual germlings prior to interaction. Based on these observations, we hypothesize that SIP-1 plays a role in the initiation of interaction or fusion competence. Predictions of the SIP-1 protein structure recently identified potential divalent ion binding sites as well as a homomer forming-domain. The functional role of these potential domains in cell-cell communication and its initiation is currently tested.

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CS4.2.7

CYTOPLASMIC SEQUESTERING OF THE COCHLIOBOLUS HETEROSTROPHUS STRESS-ACTIVATED MAPK IN RESPONSE TO A HOST PLANT PHENOLIC ACID

Benjamin Horwitz[1], Rina Zuchman[2], Ofri Levi[1], Tamar Ziv[2], Ilana Navon[2]

[1]*Faculty of Biology, Technion - Israel Institute of Technology, Haifa, Israel,* [2]*Smoler Protein Center and Faculty of Biology, Technion - Israel Institute of Technology, Hafia, Israel*

MAPK phosphorylation cascades are universal in cell signaling, and protein phosphorylation in response to stress is highly conserved. Ferulic acid (FA), an abundant phenolic found in plant host cell walls, promotes rapid cell death of Cochliobolus heterostrophus, a Dothideomycete causing Southern corn leaf blight. Stress-activated MAPK Hog1 has a basal level of dual phosphorylation which FA decreases, contrary to what might be expected. Gene deletion experiments showed that least two serine/threonine phosphatases: PtcB (PP2C family) and CDC14 (dual-specificity) are required for FA-induced dephosphorylation. To follow localization of Hog1 in response to stress, we established a functional Hog1:Gfp fusion. In response to osmotic stress, Hog1 partitioned to the nucleus as expected. Surprisingly, upon exposure to FA, Hog1 accumulated in cytoplasmic foci. The cytoplasmic granules where Hog1:Gfp accumulates following exposure to FA resemble stress granules. Proteomic study of Hog1 co-enrichment in a subcellular fraction from FA-treated germinating conidia compared to controls implicates protein networks belonging to a number of subcellular compartments. These include stress granules, P-bodies and peroxisomes among others. We aim to identify the type of granules, their protein composition, and their co-localization with Hog1 in the cell upon FA treatment. We propose that the combination of MAPK sequestering and dephosphorylation act to mitigate stress and cell death caused by exposure to defense compounds like FA deployed by the plant host.

CS4.2.8

FILAMENT BRANCHING IN THE HUMAN FUNGAL PATHOGEN CANDIDA ALBICANS

Antonio Serrano Salces[1], Charles Puerner[1], Martine Bassilana[1], Robert Arkowitz[1]

[1]*Université Côte d'Azur, CNRS, INSERM, Institute of Biology Valrose (iBV), Nice, France*

Candida albicans can cause superficial, as well as systemic infections in immunocompromised individuals. Formation of invasive hyphal filaments is associated with virulence and a single cell has the capacity to generate multiple growth sites, in a defined and coordinated process known as filament branching. Interestingly, hyphal branching is observed in murine tissues infected with C. albicans, with an increase in branching frequency in necrotic zones (wherein there is nutrient limitation), suggesting that it may be important for fungal virulence. Additionally, in some fungi, branching appears to be activated in response to host immune responses [1].

In order to investigate C. albicans branching and analyze morphological and molecular changes associated with this transition, we have been using live-cell microscopy and quantitating growth parameters of filament branching, compared to hyphal and germ tube extension. Our results indicate that filament branching is a distinct growth state based upon morphological and molecular characteristics. Specifically, we observed differences between branch and main filament morphologies, as well as differences in the distribution/levels of reporters for the secretory vesicles, lipids, and endocytosis during these two growth modes. In contrast, both the distribution and level of the small GTPase Cdc42, a key regulator of polarized growth, are similar during branch and main filament growth. Additionally, by investigating the cytoplasmic biophysical characteristics, using a micro-rheological probe [2], we have detected a striking difference with respect to cytoplasmic crowding/viscosity, between these growth modes. In summary, our results reveal that filament branching is a distinct growth mode compared to germ tube formation and filament extension, suggesting that these growth states are differentially regulated. To further investigate filament branching, a small-scale screen to identify candidate genes specifically important for filament branching will be carried out.

[1] Ellett et al. 2017, PLoS Pathog. 13: e1006154.
[2] Delarue et al. 2018, Cell, 174, 338.

CONCURRENT SESSION 4.3 RNA BIOLOGY

TUESDAY, MARCH 7
17:30 – 19:30
Location: ***Hall Freiburg (Congress Innsbruck)***

CHAIRS:
Astrid Mach-Aigner, Erzsébet Fekete

CS4.3.1

UNCOVERING THE SEQUENCE AND STRUCTURAL DETERMINANTS GUIDING M6A EVOLUTION VIA INTER AND INTRA-SPECIES HYBRIDS

Ran Shachar[1], Schraga Schwartz[1]

[1]*Weizmann Institute of Science, Molecular genetics department, Rehovot, Israel*

Similar to DNA and proteins, chemical modifications may be formed on diverse types of RNA molecules, co- or post-transcriptionally. The most widespread chemical modification on mRNA is N6-methyladenosine (m6A). Yet, the determinants governing the substantial differences in transcriptome-wide maps of this modification between and within species remain only partially understood. This is largely due to the pleiotropic and indirect effects typically associated with the disruption of components of the methylation machinery and the difficulty of precisely disrupting individual methylation sites and connecting them with functional readouts.
Here we leverage the power of different inter- and intra-species hybrids in yeast and mammals, which allows interrogating outputs from two distinguishable alleles in a shared environment to dissect the determinants leading to different RNA methylation patterns between species and individuals. We found that differences in methylation between species evolve almost exclusively due to evolution in cis-elements. We discovered that those differences in methylation are to a large extent driven by (1) disruptions of the m6A consensus motif and (2) by disruption of local secondary structure at the target site. Both on yeast (S. cerevisiae - S. paradoxus) and mammalian (human-mouse) interspecies hybrids, changes leading to local relaxation of mRNA secondary structure at m6A consensus motifs correlate positively with m6A accumulation. In addition, by using advanced sequence perturbation systems in both yeast and human cells, we demonstrate that secondary structure is causal and reveal that the gain of structure is sufficient to abolish methylation, whereas the loss of it is sufficient to gain m6A. Collectively, our findings establish substantial rewiring of m6A between species, driven primarily by local changes in sequence and secondary structure.

CS4.3.2

SMALL RNAS FROM THE PLANT PATHOGEN SCLEROTINIA SCLEROTIORUM ASSOCIATE WITH HOST QUANTITATIVE DISEASE RESISTANCE GENES

Mark Derbyshire[1]

[1]*Curtin University, Bentley, Australia*

The host generalist fungus Sclerotinia sclerotiorum infects hundreds of plant species. Little is known about how it interacts with so many different hosts at the molecular level. Several studies have shown that plant pathogenic fungi can secrete short non-coding RNAs called 'small RNAs' into host cells. These small RNAs can silence host gene expression by binding to complementary mRNAs in a process known as RNA interference. Our research uses targeted 'omics experiments to identify small RNAs from S. sclerotiorum that might interact with host mRNAs. Experimental follow-up has confirmed the roles of some of the targeted host genes in response to the pathogen. An increased association with disease resistance among alleles within host genes targeted by S. sclerotiorum small RNAs suggests a broad role in suppression of host quantitative disease resistance. Future research will aim to elucidate this association further.

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CS4.3.3

RNAI SPRAY-MEDIATED SILENCING OF ALTERNARIA ALTERNATA AGO AND DCL GENE TRANSCRIPTS ENHANCED RESISTANCE TO ALTERNARIA BLACK SPOT DISEASE

Conrad Chibunna Achilonu[1], Gert Marais[1]

[1]*Department of Plant Sciences | Division of Plant Pathology, Faculty of Natural and Agricultural Sciences, University of the Free State, Bloemfontein, Free STate, South Africa*

The novel application of RNA interference (RNAi) silencing has emerged as a potential alternative to target specific and environmentally friendly disease management strategies against phytopathogenic and human pathogenic fungi. However, targeted gene silencing mediated by plant expression of non-coding inhibitory double stranded RNAs (dsRNAs) can enhance plant resistance to various diseases with unparalleled efficiency. By generating RNAi silencing signals, plants can be protected from pathogens by spray-applied RNAi-based fungicides. Notwithstanding the remarkable efficiency of RNAi-based technologies, the molecular mechanisms underlying the spray-induced gene silencing (SIGS) approaches remain almost unsolved, a prerequisite for successful future application in the field. In this study, the SIGS strategy was employed to silence and counteract the infection of Alternaria alternata pathogen, which causes Alternaria black spot (ABS) disease in pecans, using the in vitro and in vivo designed dsRNA constructs targeting the Argonaute (AGO) and Dicer (DCL) gene transcripts of A. alternata. Our results indicated that the in vivo-design of dsRNAs resulted in higher gene-silencing efficiency compared to the in vitro designed dsRNA construct and thus, targeting core constituents of the fungal RNAi mechanism by SIGS could protect pecans from A. alternata-induced ABS disease. Furthermore, our results demonstrate the prospect of RNA silencing in the A. alternata-pecan interaction, a situation where sRNAs act as effector molecules to induce gene silencing between different fungal species in plant-pathogen interactions.

Keywords: Alternaria alternata, Alternaria black spot, RNA spraying, RNAi spray-induced gene silencing, AGO and DCL

CS4.3.4

NEW PROPAGATION MECHANISM FOR CO-EXISTING STWINTRONS AND DERIVED CANONICAL INTRONS

Erzsébet Fekete[1], Fruzsina Pénzes[1], Viktória Ág-Rácz[1], Norbert Ág[1], Claudio Scazzocchio[2], Michel Flipphi[1], Levente Karaffa[1]

[1]Department of Biochemical Engineering, Faculty of Science & Technology, University of Debrecen, Debrecen, Hungary, [2]Institute for Integrative Biology of the Cell (I2BC), Université Paris-Saclay, CEA & CNRS Unité Mixte de Recherche, Gif-sur-Yvette, France

Originally, introns were described as non-coding sequences interspersed with exons in primary transcripts. Spliceosomal U2 introns are ubiquitous in nuclear transcriptomes. Alternative splicing can increase proteome diversity and provide means for (post)transcriptional regulation. Some intron RNAs accumulate to perform crucial functions in response to, e.g., nutrient exhaustion. U2 intron excision requires a ribonucleoprotein complex, the U2 spliceosome, and involves highly coordinated RNA-RNA interactions with the intron's 5'-donor- and 3'-acceptor G's at opposite splice sites, and the branch-point A. However, continual generation of new U2 introns is a vexing mystery. Stwintrons consist of nested U2 introns excised by consecutive splicing reactions. In a [D1,2] stwintron, an internal intron is integrated in the 5'-donor of an external intron between the first and second nucleotide. Using the principle of nested U2 introns, we have identified many dozens of genuine [D1,2] stwintrons in Hypoxylon sp. CO27-5 and EC38. The large majority of them were sequence-unique but located at gene positions also occupied by [D1,2]'s in other Hypoxylaceae, suggesting a common ancestry. Conversely, 25 [D1,2]'s were sequence-similar and unique to Hypoxylon CO27-5/EC38. A striking distinction between these groups of stwintrons involves the density of a terminal symmetry sequence (40–50 nt), which is considerably higher in most sequence-similar "sister" stwintrons. Moreover, we found canonical introns which are sequence-similar to the termini of sister stwintrons but lack the latter's central spacer separating the terminal inverted repeats (TIR). Consequently, these new canonical introns only consist of TIRs, predicted to form a relatively stable hairpin secondary structure bringing the donor- and acceptor G's in very close proximity. Sister stwintrons would duplicate as [D1,2] stwintrons but occasionally give rise to highly symmetrical introns, which propagate as canonical introns. The TIR seems key to the inferred (stw)intron propagation.

CS4.3.5

THE DICER/R3B2 COMPLEX: A NOVEL INTERACTION IN THE CENTER OF THE RNAI-RELATED MECHANISMS OF MUCOR LUSITANICUS

José Tomás Cánovas-Márquez[1], Álvaro Sánchez-Ferrer[2], Francisco E. Nicolás[1], Eusebio Navarro[1], Victoriano Garre[1]

[1]Department of Genetics and Microbiology (Associated Unit to IQFR-CSIC), Faculty of Biology, University of Murcia, 30100 Murcia, Spain, [2]Department of Biochemistry and Molecular Biology-A, Faculty of Biology, University of Murcia, 30100 Murcia, Spain

In the opportunistic fungal pathogen Mucor lusitanicus two RNAi related pathways display a refined control of endogenous genes related to relevant biological processes as growth, stress resistance and virulence. The canonical RNAi mechanism, driven by Dicer enzymes, synthetizes the ex-siRNAs from dsRNA that guide the silencing of the endogenous transcripts. In contrast, the Non-Canonical RNAi pathway (NCRIP) directly degrades the target transcripts through an unusual RNase III, called R3B2, that has evolved to cut ssRNA. Both pathways exhibit a complex crosstalk were the functioning of the NCRIP negatively regulates the canonical RNAi, but at the same time the last mechanism requires R3B2 for the production of the majority of the ex-siRNAs.

Here we show that R3B2 interacts with Dicer enzymes and guided by the last advances in protein structure prediction (Alfaphold2) we dissected the interaction with Dicer-1 enzyme. We found that the dsRBD1 of R3B2 interacts with the helicase corner of Dicer-1, while a novel interaction region in R3B2, called PIR, interacts with the platform-PAZ domains suggesting a steric hindrance to the entrance of dsRNA in the catalytic center of Dicer-1. The analysis of the sRNAs profiles during vegetative growth and the stationary phase in the r3b2Δ strain revealed that while R3B2 is necessary for RNAi initiation, it becomes dispensable during silencing amplification. These results suggest that R3B2 produces adequate dsRNA ends for dicer processing of RdRP-1 products, while the dsRNAs produced by RdRP-2 during silencing amplification does not requires this end-trimming activity.

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These findings prompt us to propose a novel model for RNAi-related mechanism that explains the contradictory roles of R3B2 in RNAi silencing of the endogenous genes, the generation of transitory antifungal resistant strains (epimutants) and the control of transposable elements described in the last years.

CS4.3.6

PATHOGENIC OOMYCETES WITH DIVERSE HOSTS CONTAIN DIFFERENT RNA SILENCING PROTEINS BUT SIMILAR SMALL RNA PROFILES

Edoardo Piombo[1], Bekele Gelena Kelbessa[2], Poorva Sundararajan[2], Stephen C. Whisson[3], Ramesh Raju Vetukuri[2], Mukesh Dubey[1]

[1]Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden, [2]Department of Plant Breeding, Horticum, Swedish University of Agricultural Sciences, Lomma, Sweden, [3]Cell and Molecular Sciences, The James Hutton Institute, Invergowrie, UK

Oomycetes cause damaging diseases of plants, and RNA interference (RNAi) is increasingly being shown to play a role in the pathogenicity of Phytophthora species. Some oomycetes, however, also infect animals, fungi, and other oomycota. To gain a wider understanding of RNAi in oomycete species with diverse hosts, we analysed RNA silencing proteins in Phytophthora plurivora, Ph. cactorum, Ph. colocasiae, Pythium oligandrum, Py. periplocum, and Lagenidium giganteum. Each species possessed one Dicer-like protein, but a variable number and type of Argonautes and RNA-dependent RNA polymerases. Sequencing small RNAs (sRNAs) from the mycelium of each species revealed prevalence of 21nt, 25nt, and 26nt sRNA sizes in all species, but the abundance and 5' base preference of these classes differed markedly between genera. Most sRNAs mapped to mobile elements and other repeats, signifying that the major role for RNAi in oomycetes is to limit the expansion of these elements. We also found that sRNAs may act to regulate the expression of duplicated genes. Other sRNAs mapped to several gene families, and this number was higher in Pythium spp., suggesting a role for RNAi in regulating gene expression. Genes encoding most effector classes were the origin of diverse sRNA sizes, but in some cases precise gene families showed a preference for specific classes of sRNAs, such as RxLR effectors which originated predominantly 25/26 sRNAs in Phytophthora species. Novel microRNAs were discovered from all species, and two were predicted to act on transcripts of RxLR effectors in Ph. plurivora and Ph. cactorum, suggesting a putative role in regulating the induction of disease. Moreover, microRNAs from the biocontrol Pythium species had putative targets in Phytophthora infestans, and L. giganteum miRNAs matched candidate genes in the mosquito, Aedes aegypti. This suggests the possibility that trans-boundary RNAi may have a role in the biocontrol action of these oomycetes.

CS4.3.7

ARTIFICIAL NANOVESICLES FOR DSRNA DELIVERY IN SPRAY INDUCED GENE SILENCING (SIGS) FOR CROP PROTECTION

Jonatan Niño Sánchez[2], Lulu Qiao[1], Rachael Hamby[1], Luca Capriotti[3], Angela Chen[1], Bruno Mezzetti[3], Hailing Jin[1]

[1]University of California - Riverside, Riverside, US, [2]University of Valladolid, Palencia, Spain, [3]Universitá Politecnica delle Marche, Ancona, Italy

Fungal plant pathogens are a threat to global food security, and current management practices rely mainly on chemical control, which can be harmful to human health and the environment. Innovative, environmentally-friendly alternatives to chemical fungicides are thus needed for the sustainable management of fungal pathogens. Pioneering research by our group showed that Spray-Induced Gene Silencing (SIGS) can effectively inhibit fungal plant diseases, as pathogenic fungi can take up RNA from the environment through topical application of gene-targeting RNAs. These antifungal RNAs can be versatilely designed to be species-specific and to target multiple genes simultaneously. Despite the promise of SIGS, one of its major disadvantages is the instability of RNA in the environment, which is quickly degraded when exposed to rainfall, high humidity, or UV light. Our earlier studies showed that plants use extracellular vesicles to deliver small RNAs into fungal cells to suppress fungal virulence. We therefore explored the use in SIGS of three artificial nanovesicle formulations, which were designed to shield double-stranded RNAs targeting pathogen genes from degradation for topical application, to control the fungal pathogen Botrytis cinerea. We first tested the ability of B. cinerea to uptake dsRNA docked into the artificial nanovesicles, then we tested the SIGS treatments on tomato, grape, lettuce and rose petals, and we assessed dsRNA stability under a range of environmental conditions. All three formulations enhanced RNA delivery, improved the plant protection effect and significantly extended the duration of RNA-mediated protection.

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CS4.3.8

LONG NON-CODING RNAS IN THE INTERACTION BETWEEN MUCORALES CAUSING MUCORMYCOSIS AND HOST DEFENSE CELLS

Ghizlane Tahiri[1], Hrant Hovhannisyan[2], Francisco Esteban Nicolás Molina[1], Eusebio Navarro Ros[1], Toni Gabaldón[2], Victoriano Garre Mula[1]

[1]Murcia University, Murcia, Spain, [2]Barcelona Supercomputing Center, Barcelona, Spain

Long non-coding RNAs (lncRNAs) have been widely studied in human diseases. Nevertheless, little is known about their biological implications in fungi. Here, we performed a deep screening of lncRNAs in two of the main causal agents of the infectious disease mucormycosis, Rhizopus microsporus and Mucor lusitanicus. Our analysis has identified hundreds of intergenic lncRNAs based on a genome-guided transcriptome assembly using large RNA-seq datasets. We found that these RNA transcripts are shorter than protein-coding genes, have lower GC content and lower expression levels, which is in agreement with lncRNAs from other organisms. The differential expression analysis shows that some of the predicted lncRNAs may be involved in the interaction with macrophages in vitro and in vivo during mice infection, suggesting regulation of these non-coding transcripts by the immune host cells. Mouse RNA-seq data analysis reveals that host lncRNAs are also involved in the response to fungal infection. Additionally, based on the sequencing data of small RNAs, we could infer that some of these lncRNAs, similarly to protein-coding genes, are being regulated by RNA interference, indicating that they are transported to the cytoplasm where they might be playing important roles in the post-transcriptional processes. Their possible involvement in the virulence of Mucorales will be demonstrated by generating knockout mutants based on the CRISPR-Cas technology in those upregulated lncRNAs involved in the response to infection. Their low conservation among eukaryotes, even among related species, makes Mucorales lncRNAs promising therapeutic targets for drug development against Mucormycosis.

CONCURRENT SESSION 4.4A FUNGAL EPIDEMIOLOGY AND DIAGNOSTICS

TUESDAY, MARCH 7
17:30 – 18:30
Location: ***Hall Strassburg (Congress Innsbruck)***

CHAIRS:
Sigrid Neuhauser, Oliver Kurzai

CS4.4a.1

THE IMPORTANCE OF SPORE-DISPERSAL TO INITIATE NEW ARMILLARIA ROOT ROT INFECTIONS IN GARDENS

Jassy Drakulic[1], Matthew Cromey[1], Nicola Dennison[1], Liz Beal[1], Renaud Travadon[2], Kendra Baumgartner[2]

[1]Royal Horticultural Society, Woking, United Kingdom, [2]University of California, Davis, Davis, United States

Armillaria root rot (ARR) is a fungal disease that kills trees, vines, shrubs and herbaceous plants in forestry, agriculture, and horticultural settings. The Royal Horticultural Society is a gardening charity with over 600k members, and every year ARR is the most frequent disease diagnosed from UK home gardens through the members' advisory service. Armillaria mellea causes the majority of garden infections and we sought to clarify the population structure and reproductive strategy this species. Inherited knowledge from the forestry sector states that spores are unimportant in initiating new infection sites and that belowground vegetative spread is the most important dispersal route, which if true would imply low variability in pathogen populations. We analysed 76 UK isolates sourced from RHS Garden Wisley and the surrounding area, using microsatellite genotyping and somatic incompatibility testing. Of 13 microsatellite loci designed for North American A. mellea populations, 11 loci successfully amplified in the UK population. Seven loci were sufficiently variable in the UK population to find eight multilocus genotypes (MLGs) accounting for 20 isolates. Of 23 isolates sourced from RHS Wisley, eight were accounted for in MLGs. The observed heterozygosity (0.78 ±0.05) was much higher than was expected (0.55 ±0.03). Isolates in the MLGs and a further 19 isolates were tested in somatic incompatibility pairings in 231 combinations. We detected even greater population variability in the pairing assays; just five pairs of isolates were somatically compatible. The low number of somatically compatible pairs, high microsatellite heterozygosity and low count of MLGs indicates that spore dispersal and sexual reproduction is highly successful in initiating new A. mellea infections in UK gardens. As a result, the RHS now advises gardeners to remove mushrooms when they first start to pin, to reduce the incidence of new infections in gardens and neighbourhoods.

CS4.4a.2

NOVEL SHORT TANDEM REPEAT TYPING AND WHOLE GENOME SEQUENCING ANALYSIS ON SPOROTHRIX BRASILIENSIS ISOLATES REVEAL INDEPENDENT OUTBREAKS IN BRAZIL

Bram Spruijtenburg[1,2], Amanda Bombassaro[1,3], Maria Eduarda Grisolia[4,5], Vania Aparecida Vicente[3,4,5], Flavio de Queiroz-Telles[5], Eelco F.J. Meijer[1,2], Jacques F. Meis[1,2,4], Theun de Groot[1,2]

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Sporothrix brasiliensis causes the human and animal disease sporotrichosis and is highly prevalent in Brazil and neighboring countries. The increase of this infection is foremost driven by infected cats and treatment can be challenging due to limited diagnostic capabilities in South American countries and emerging resistance against antifungals. S. brasiliensis oubreaks were first identified in Rio de Janeiro and subsequently in adjacent states and countries, possibly involving clonal spread. Here, we applied a novel short tandem repeat (STR) genotyping scheme to 173 clinical S. brasiliensis isolates from Brazil, isolated from humans and cats. This resulted in the identification of 62 different genotypes and ten clusters comprised of more than two isolates. STR typing results were compared to whole genome sequencing (WGS) single nucleotide polymorphism (SNP) analysis for 21 isolates and were found to correlate well. Some isolates were differentiated by more than 200,000 SNPs, while other isolates differed less than 100 SNPs, despite some originating from different cities. The observed genetic diversity suggests independent introduction in the human and feral population while clonal spread is prevalent in Rio de Janeiro and Curitiba. Mating type loci were determined by WGS data. The MAT1-1 locus was found in isolates from Brasilía, São Paulo, Belo Horizonte and Curitiba, while the MAT1-2 locus was found in isolates from Rio de Janeiro and Curitiba as well. Altogether, we describe a rapid, reliable, and cost-effective method to study the hitherto unknown high genetic diversity of S. brasiliensis. We show that there are multiple independent outbreak events of S. brasiliensis in Brazil, with multiple introductions in feral and human populations and clonal spread across vast distances. Our findings challenge the current view that S. brasiliensis was initially identified in Rio de Janeiro and subsequently spread to adjacent states and countries.

CS4.4a.3

POPULATION GENOMICS LINKS CLINICAL ASPERGILLUS FUMIGATUS TRIAZOLE-RESISTANT ISOLATES TO ENVIRONMENTAL HOTSPOTS AND UNCOVERS AN AGRICULTURAL FUNGICIDE EXPOSURE HISTORY

Eveline Snelders[1], Brandi Nicole Celia-Sanchez[2], Li Wang[2], Ymke Nederlof[1], Jianhua Zhang[1], Hylke Kortenbosch[1], Karin Van Dijk[3], Marin Talbot Brewer[2], Michelle Momany[2], Bas Zwaan[1], Ben Auxier[1], Paul Verweij[4]

[1]Wageningen University & Research, Wageningen, Netherlands, [2]University of Georgia, Athens, USA, [3]AmsterdamUMC, Amsterdam, Netherlands, [4]RadboudUMC, Nijmegen, Netherlands

Aspergillus fumigatus is a ubiquitous fungus that causes a range of diseases in animals and humans. The most lethal manifestation is invasive aspergillosis for which treatment relies on triazole compounds. The efficacy of such triazoles is threatened by the emergence of resistance in A. fumigatus and exposure to triazole fungicides in the environment has proven to be the major route of resistance selection. Triazole resistant A. fumigatus is recovered from decaying plant material or so-called hotspots that contain residues of azole fungicides. Although observations support a strong link between resistance genotypes from environmental origin and clinical samples, a direct link between environmental hotspots and azole-resistant invasive aspergillosis has not been demonstrated. Genomic epidemiology has the potential to expose transmission routes and we therefore whole-genome sequenced 170 Dutch A. fumigatus isolates, obtained from three well-characterized hotspots, potable water samples, and two hospitals between 2016 and 2019, and included all publicly available global A. fumigatus genomes. In the Dutch dataset, A. fumigatus isolates from six patients showed near-identical genomes compared to environmental isolates. One hotspot isolate matched with three proven/probable cases of azole-resistant invasive aspergillosis, including one fatal case. Patient isolates were recovered up to 34 months after matching isolates had been cultured from the hotspots. In the whole dataset, the genomic population structure and single nucleotide polymorphisms (SNPs) associated with resistance to non-azole fungicides, such as benzimidazole (MBC) and quinone outside inhibitor (QoI) classes showed a strong indication of an agricultural fungicide exposure history. Our data shows that hotspots represent highly selective habitats for multiple fungicide-resistant A. fumigatus, which can be directly linked to clinical disease. As the emergence of multi-fungicide-resistant A. fumigatus isolates severely limits the usefulness of single-site fungicides it is of great relevance to understand the extent of this phenomena.

CS4.4a.4

#EUROBLAST: DISEASE SURVEILLANCE OF CULTIVATED CROP PLANTS AND THEIR WILD RELATIVES FOR DETECTION OF EMERGING EPIDEMICS

Thorsten Langner[1], A. Cristina Barragan[1], Sergio M. Latorre[2], Paul G. Mock[1], Adeline Harant[1], Joe Win[1], Angus Malmgren[1], Hernán A. Burbano[2], Sophien Kamoun[1]

[1]The Sainsbury Laboratory, University of East Anglia, Norwich Research Park, Norwich, United Kingdom, [2]Centre for Life's Origins and EvolutionDepartment of Genetics, Evolution and Environment, University College London, London, United Kingdom

Surveillance and early detection of emerging diseases are critical to control pathogen spread. Like zoonotic diseases, plant pathogens that cause destructive crop diseases often originate from wild hosts. However, surveys of major plant pathogens tend to be skewed towards crops and often neglect their wild host plants. We recently reported an emerging disease threat caused by the blast fungus Magnaporthe (Syn. Pyricularia) spp. in central Europe. We found that this notoriously devastating plant pathogen infects wild grasses in Germany, a region previously deemed unfavourable for blast disease. Using phenotypic characterization and genomic analyses, we determined that the observed disease symptoms are associated with the foxtail millet-infecting lineage of M. oryzae and its sister species M. grisea. We showed that M. oryzae isolates can infect barley and wheat, thus highlighting the risk of pathogen spread to crops. In addition, M. oryzae isolates which co-occur in natural populations display compatible mating types and variable candidate effector gene content, which may enhance the pathogen's adaptive potential. Our findings stress the risk of blast fungus infections expanding into the central European cereal belt through migration and host jumps, underlining the importance for pathogen surveillance in both, crops and wild grasses.

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WORKSHOP: JGI GENOMICS WORKSHOP

TUESDAY, MARCH 7
18:30 – 19:30
Location: ***Hall Strassburg (Congress Innsbruck)***

CHAIRS:
Igor Grigoriev, Laszlo Nagy

CS5.1.69

UNDERSTANDING THE EVOLUTION AND FUNCTIONING OF SYMBIOSIS: INTRA-GENUS AND INTRA-SPECIES MOLECULAR DIVERSITY IN THE ECTOMYCORRHIZAL GENUS PISOLITHUS

Annegret Kohler[1], Shingo Miyauchi[2], Krista Plett[3], Maira de Freitas Pereira[1], Benjamin Dauphin[4], Emmanuelle Morin[1], Vasanth Singan[5], Vivian Ng[5], Alan Kuo[5], Maurício Dutra Costa[6], Martina Peter[4], Ian Anderson[3], Igor V Grigoriev[5], Jonathan M Plett[3], Francis Martin[1]

[1]Université de Lorraine, INRAE, Interactions Arbres/Microorganismes, INRAE Grand Est - Nancy, Champenoux, France, [2]Evolutionary and Synthetic Biology, Okinawa Institute of Science and Technology , Okinawa, Japan, [3]Hawkesbury Institute for the Environment, Western Sydney University, Richmond NSW, Australia, [4]Swiss Federal Research Institute WSL, Birmensdorf, Switzerland, [5]US Department of Energy Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, California, USA, [6]Department of Microbiology, Universidade Federal de Vicosa, Vicosa, MG, Brazil

The growing number of sequenced fungal genomes allowed us in the last years to advance in our understanding of the evolutionary history of mycorrhizal symbiosis.
For all interactions studied so far, we could show that both conserved genes, like genes coding for transporter or CAZymes as well as clade-specific genes like mycorrhiza-induced small secreted proteins (MiSSPs) are necessary to establish a fully functioning mycorrhiza. The majority of the conserved ectomycorrhiza-induced genes predated the evolution of ectomycorrhizal symbiosis suggesting that these genes have been co-opted for ectomycorrhiza development during evolution from saprotrophic ancestors. In addition to a likewise unique set of novel genes, that evolved after the origins of ectomycorrhizal

symbiosis and most of which have functions yet to elucidate.
We recently analyzed the genomes of nine Pisolithus species, an ectomycorrhizal genus within the Boletales, both globally dominant but with species-specific gymnosperm and angiosperm hosts, giving us the opportunity to explore what pathways have developed to support host colonization.
We could highlight an unexpected level of variation between closely related species, in terms of genome size and content, transcriptional regulation and metabolic potential. Divergent CAZyme profiles include enzymes associated to symbiotic sugar processing, although metabolomic analysis suggests that neither copy number nor expression of these genes is sufficient to predict capture of sugar from a host plant.
Comparison of the Pisolithus mycorrhizal transcriptomes revealed a set of small-secreted proteins induced in interaction with their hosts that are conserved between genomes but expanded in gene copy number compared to other genomes.
Further, a Pisolithus microcarpus monocaryotic strain collection isolated from the same fruiting body showed striking differences in the ability to form mycorrhiza. Genome resequencing and gene expression analyses during mycorrhiza formation allowed further insights into genetic differences within this Pisolithus microcarpus progeny.

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CS5.1.13

TRANSCRIPTOMIC ANALYSES OF WHITE AND BROWN ROT BASIDIOMYCETES, WITH EMPHASIS IN PLEUROTUS OSTREATUS, REVEAL NEW ENZYMES INVOLVED IN LIGNOCELLULOSE DEGRADATION

Idoia Jimenez[1], Manuel Alfaro[1], Antonio G. Pisabarro[1], Igor Grigoriev[2], Lucia Ramirez[1]

[1]Public University of Navarre, Pamplona, Spain, [2]DOE Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, California, United States of America

Lignocellulose, the most abundant biopolymer on Earth, is composed of cellulose, hemicellulose, and lignin polymers. While different prokaryotic and eukaryotic microorganisms can use cellulose to produce second-generation biofuels, lignin is a resistant polymer degraded by a few basidiomycete fungi. White Rot (WR) fungi are lignin degraders, which make cellulose accessible to other microorganisms and Brown Rots (BR) enzymatically attack the cellulose, minimally modifying the lignin moiety.

In this study, the gene expression of four WR (Fomitiporia mediterranea, Heterobasidion annosum, Phanerochaete chrysosporium, and Pleurotus ostreatus) and three BR (Fomitopsis pinicola, Serpula lacrymans and Postia placenta) were studied. Fungi were grown in submerged cultures with two different carbon sources: glucose as control and poplar wood chips to compare strategies used by the different saprophytic basidiomycetes to understand the mechanisms of wood degradation for its application in other biotechnological fields, such as the development of new biofuels.

We have analyzed the expression of all carbohydrate-active enzymes (CAZy) to set differences in using these protein families considering fungal lifestyles to achieve this goal. Although the gene expression was analyzed in both WR and BR, we focused on WR, which overexpressed LPMO (Lythic Polysaccharide Monooxigenases) and CDH (Cellobiose Dehydrogenase) when they were grown on poplar chips.

The high abundance (31) of LPMO genes in P. ostreatus moved us to study their genome organization and expression in both culture media. We found two genes with high aminoacid similarity and proximity over-expressed in different culture media.

Clustering analysis of P. ostreatus LPMO genes showed that genes coding for highly amino acid-similar proteins did not group in the same cluster.

Gypsy fingerprints in flanking and coding sequences of some LPMO genes suggest that the LPMO gene family could have arisen by the mobilization of transposons.

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CONCURRENT SESSION 5.1
GENOMES AND OTHER –OMICS

WEDNESDAY, MARCH 8
14:00 – 16:00
Location: ***Hall Tirol (Congress Innsbruck)***

CHAIRS:
Igor Grigoriev, Laszlo Nagy

CS5.1.1

GIANT STARSHIP ELEMENTS ARE ENGINES OF ADAPTIVE VARIATION IN FUNGAL PATHOGENS

Emile Gluck-Thaler[1], Daniel Croll[1]

[1]*Université de Neuchatel, Neuchatel, Switzerland*

Accessory genes are variably present among members of a species and are a reservoir of adaptive functions. In fungal pathogens, accessory genes contributing to pathogenicity represent significant fractions of genome content and differences in accessory genes between individuals accumulate rapidly. However, we often lack a mechanistic understanding of how genes become accessory and why variation in accessory genes exists. Here, we demonstrate that differences in accessory gene content in many fungal pathogens is attributable to Starships, a newly described group of giant mobile elements that have evolved mechanisms to transpose fungal genes as genetic cargo. By systematically annotating Starships and classifying them into families, we found that individual fungal species harbour complex communities of distinct elements ranging from 60-600kb in length and differing in their activity. Active Starships represent between 4-8% of any given genome and carry diverse genes implicated in host- and environment-adaptation, including metabolic gene clusters and candidate virulence factors. Starship-associated genes typically make up around 25% of the overall accessory genome, implicating Starship activity as a direct mechanism generating variation in accessory gene content. However, we found that most Starship insertions are under strong purifying se-

lection, suggesting there are intrinsic costs to maintaining Starships despite any benefits their cargo may confer. Our results shed light on the origins of accessory variation in fungi, and reveal a novel mechanism for pathogen adaptation.

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CS5.1.2

GENOMIC INNOVATIONS AND HORIZONTAL GENE TRANSFER SCULPT THE LIFESTYLE OF ARMILLARIA SPECIES

Neha Sahu[1], Boris Indic[2], Johanna Wong-Bajracharya[3,4], Zsolt Merényi[1], Sandor Kocsube[5,6], Krista L Plett[3], Jason Slot[7], György Sipos[2], Jonathan Plett[3], László G. Nagy[1]

[1]Biological Research Center, Synthetic and Systems Biology Unit, Szeged, Hungary, [2]Functional Genomics and Bioinformatics Group, Faculty of Forestry, Institute of Forest and Natural Resource Management, University of Sopron, Sopron, Hungary, [3]Hawkesbury Institute for the Environment, Western Sydney University, Richmond, New South Wales, Australia, [4]New South Wales Department of Primary Industries, Elizabeth Macarthur Agricultural Institute, Menangle, Australia, [5]Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary, [6]ELKH-SZTE Fungal Pathogenicity Mechanisms Research Group, University of Szeged, Szeged, Hungary, [7]Department of Plant Pathology, The Ohio State University, Columbus, Ohio, United States

The genus Armillaria (Basidiomycete, Agaricales) includes some of the most devastating fungal pathogens of temperate forests worldwide, as well as saprotrophs that efficiently degrade the plant biomass. Despite their global importance, the evolutionary routes of how pathogenicity-related traits got incorporated into Armillaria genomes remain elusive. In this study, we combined new genomes and expression data from in-planta and in vitro pathosystems, to understand the evolution of pathogenicity and saprotrophic lifestyle of these fungi. Our findings show that apart from extensive gene duplications, and genus-specific innovations, Armillaria species also have also acquired at least 775 genes via 101 horizontal gene transfer (HGT) events, primarily from Ascomycota. Gene expression profiling in wood-decay, stem-invasion and root-infection experiments also suggested that an influx of horizontally transferred genes from Ascomycota might have impacted two major aspects of Armillaria biology - plant biomass-degradation and pathogenicity. Consistent with previous studies reporting Armillaria species exhibit a soft-rot type decay, a lifestyle restricted to Ascomycota, our genome analyses revealed that Armillaria species were distinct from white-rot species based on their CAZy gene content. Alien

CAZy genes acquired through HGT from Ascomycota might explain the evolution of unusual plant biomass-degrading systems in the Basidiomycota. Overall, our study provides insights into how evolution shaped pivotal traits like pathogenicity and plant biomass degradation in one of the most influential pathogens of temperate forest ecosystems.

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CS5.1.57

LESSONS FROM GENOMIC AND TRANSCRIPTOMIC ANALYSIS OF FIVE MARINE-DERIVED FUNGI

Abhishek Kumar[1], Pradeep Phule[1], **Frank Kempken**[1]

[1]*Kiel University, Kiel, Germany*

Marine Fungi are potent secondary metabolite producers. However, limited genetic information are available their biosynthetic gene clusters (BGCs) and their biotechnological applications. To overcome this lack of information, herein, we used next-generation sequencing methods for genome sequencing of two marine fungi (1), isolated from the German Wadden Sea, namely Calcarisporium sp. KF525 and Pestalotiopsis sp. KF079. In addition, we sequenced a strain of Scopulariopsis brevicaulis isolated from a sponge (2,3), a Fusarium isolate from the Baltic Sea, and finally a strain of Rhodotorula isolated from the seafloor of the Mid-Atlantic Ridge (4). Many novel secondary metabolite gene (SMG) clusters of Calcarisporium sp. and Pestalotiopsis sp., were detected, with the vast majority of all SMGs being unique for these two marine fungi. Only few of the SMGs were found to be expressed under laboratory conditions by RNA-seq analysis, but employing a marine strain of Fusarium sp. we also analyzed the effect of co-cultivation with marine bacterial populations from Baltic Sea, which led to activation of some SMGs. In addition, we observed specific activation of transporter proteins and certain transcription factors upon co-cultivation. These data include important clues for future genomic analyses of marine fungi.

(1) KUMAR A, ..., KEMPKEN F (2018) Sci Rep 8:10187 doi:10.1038/s41598-018-28473-z
(2) KUMAR A, ..., KEMPKEN F (2015) PLoS One 10:e0140398. doi:10.1371/journal.pone.0140398 Abstract
(3) LUKASSEN MB, ... KEMPKEN F, WIEBE MG, SORENSEN JL (2015) Mar Drugs 13: 4331-4343 doi:10.3390/md13074331
(4) BUEDENBENDER L, ... KEMPKEN F, TASDEMIR D (2021) 50-3-19/20B. Mar Drugs 2021, 19: 14

CS5.1.4

CHROMOSOME-LEVEL ASSEMBLIES FROM DIVERSE CLADES REVEAL LIMITED STRUCTURAL AND GENE CONTENT VARIATION IN THE GENOME OF CANDIDA GLABRATA

Marina Marcet-Houben[1,2], María Alvarado[3], Ewa Ksiezopolska[1,2], Ester Saus[1,2], Piet W. J. de Groot[3,4], Toni Gabaldón[1,2,5,6]

[1]Barcelona Supercomputing Center (BSC-CNS), Barcelona, Spain, [2]Institute for Research in Biomedicine (IRB Barcelona), Barcelona, Spain, [3]Regional Center for Biomedical Research, University of Castilla-La Mancha, Albacete, Spain, [4]Castilla-La Mancha Science & Technology Park, Albacete, Spain, [5]Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain, [6]Centro Investigación Biomédica En Red de Enfermedades Infecciosas, Barcelona, Spain

Candida glabrata is an opportunistic yeast pathogen displaying a large genetic and phenotypic diversity and a highly plastic genome. However, the lack of chromosome-level genome assemblies representing this diversity limits our ability to accurately establish how chromosomal structure and gene content vary across strains. Here, we expanded publicly available assemblies by using long-read sequencing technologies in twelve diverse strains, obtaining a final set of twenty-one chromosome-level genomes spanning the known C. glabrata diversity. Using comparative approaches, we inferred variation in chromosome structure and determined the pan-genome, including an analysis of the adhesin gene repertoire. Contrary to our expectation, C. glabrata has a largely stable pan-genome except for a highly variable subset of genes encoding cell-wall associated functions. Our analysis uncovered four new adhesin orthogroups, and inferred an ancestor encoding a rich adhesion repertoire, which was subsequently shaped through a still ongoing process of gene loss, gene duplication, and gene conversion.

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CS5.1.5

MACHINE LEARNING PREDICTION OF NOVEL PECTINOLYTIC ENZYMES IN ASPERGILLUS NIGER THROUGH INTEGRATING HETEROGENEOUS (POST-) GENOMICS DATA

Mao Peng[1], Ronald de Vries[1]

[1]Westerdijk Fungal Biodiversity Institute, KNAW, Utrecht, Netherlands

Pectinolytic enzymes are a variety of enzymes involved in breaking down pectin, a complex and abundant plant cell-wall polysaccharide. In nature, pectinolytic enzymes play an essential role in allowing bacteria and fungi to depolymerize and utilize pectin. In addition, pectinases have been widely applied in various industries, such as the food, wine, textile, paper and pulp industries. Due to their important biological function and increasing industrial potential, discovery of novel pectinolytic enzymes has received global interest. However, traditional enzyme characterization relies heavily on biochemical experiments, which are time consuming, laborious and expensive. To accelerate identification of novel pectinolytic enzymes, an automatic approach is needed. We developed a machine learning (ML) approach for predicting pectinases in the industrial workhorse fungus, Aspergillus niger. The prediction integrated a diverse range of features, including evolutionary profile, gene expression, transcriptional regulation and biochemical characteristics. Results on both the training and the independent testing dataset showed that our method achieved over 90 % accuracy, and recalled over 60 % of pectinolytic genes. Application of the ML model on the A. niger genome led to the identification of 83 pectinases, covering both previously described pectinases and novel pectinases that do not belong to any known pectinolytic enzyme family. Our study demonstrated the tremendous potential of ML in discovery of new industrial enzymes through integrating heterogeneous (post-) genomimcs data.

CS5.1.6

PAN-GENOMICS UNCOVER MECHANISMS BEHIND THE RAPID EVOLUTION OF SPINACH DOWNY MILDEW

Petros Skiadas[1], Sofía Riera Vidal[1], Melanie Mendel[1], Joyce Elberse[1], Ronnie de Jonge[1], Guido Van den Ackerveken[1], Michael F. Seidl[1]

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The extensive deployment of genetic disease resistances in crops exerts strong selective pressure on pathogens, which can rapidly break resistances. This rapid evolution is driven by the diversification of effectors, secreted proteins that are employed by plant pathogens to establish host colonisation. In contrast to the hundreds of predicted effector proteins very few have been functionally characterised, and consequently their function, genetic diversity, and the evolutionary processes that contributes to their diversification are largely unknown. Here, we generated complete and fully phased diploid genome assemblies of six races of Peronospora effusa, an obligate biotrophic oomycete that causes downy mildew on spinach, which is the economically most important disease in cultivated spinach worldwide. Each P. effusa race encodes almost 400 predicted host-translocated effectors belonging to the RXLR and Crinklier families. Using Alphafold2 for computational structural genomics, we uncovered conserved structural folds in the N-termini of Crinkler and in the C-termini of Crinkler and RXLRs effectors. We furthermore described the genomic organisation of P. effusa effectors and observed effector genes that cluster in few distinct genomic regions. These physically clustered effectors also cluster based on protein sequence and fold. A pan-genomic analysis of the six races revealed a highly conserved chromosome structure with few highly variable regions enriched in repetitive elements and clustered effector genes. The diversification of these regions is driven by retroduplication, which results in a high number of pseudogenes. Summarizing, we here demonstrate how pan-genomics complemented with computational structural genomics can uncover the evolution of P. effusa and provide the framework for further research into the molecular mechanisms underlying the interactions between P. effusa and spinach.

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CS5.1.7

PHYLOGENETIC ANALYSIS AND INVESTIGATION OF RIPP CLUSTER IN 50 TRICHODERMA GENOMES

Matthias Schmal[1], Gabriel Vignolle[2], Robert L. Mach[1], Astrid R. Mach-Aigner[1], Christian Zimmermann[1]

[1]Technical University of Vienna, Vienna, Austria, [2]Austrian Institute of Technology, Vienna, Austria

Many Trichoderma species have applications in biotechnology, agriculture and human healthcare. Often, secondary metabolites play an important role in the biological activity of the fungus. Ribosomally synthesized and post-translationally modified peptides (RiPPs) are a class of secondary metabolites that are genetically encoded of biosynthetic gene clusters (BGCs). These clusters contain modifying enzymes, which allow for a wide spectrum of potential compounds and give the possibility to alter the final product for different purposes. Using antiSMASH we mined the genomes of 50 different Trichoderma strains across multiple species, looking especially into potential RiPP cluster. The analysis of the Trichoderma genomes predicted nine RiPP BGCs as well as a polyketide-RiPP hybrid cluster in 10 different species. A subsequent analysis using BiGSCAPE revealed that 7 of these clusters are closely related to each other and the already welldescribed ustiloxin-B cluster from Aspergillus flavus. These ustiloxin-like clusters are found exclusively in species belonging to the „Harzianum clade“ and show a very conserved cluster structure. The three unique cluster show no resemblance to the ustiloxin-like cluster and might represent a new class of RiPPs. This is further supported by a multiple sequence alignment between homologous genes in all 10 clusters. One representative of the ustiloxin-like cluster, as well as the three unique clusters were chosen for further investigation. Using RT-qPCR we determined which genes are co-transcribed under different conditions. In order to complement the transcript analysis a classic proteomics experiment was conducted to confirm the expression of the linked genes. These results underscore the diverse biotechnological potential of the genus Trichoderma.

CS5.1.8

EXPLORATION OF TRANSPORTER GENE FAMILY EVOLUTION IN ECTOMYCORRHIZAL FUNGI IN RELATION TO MINERAL WEATHERING CAPABILITIES

Katharine King[1], Marisol Sanchez-Garcia[1], Petra Fransson[1], Roger Finlay[1]

[1]*Swedish University Of Agricultural Sciences, Uppsala, Sweden*

The role of ectomycorrhizal (ECM) fungi in biological weathering is increasingly recognised, although the quantitative significance of microbially mediated mineral dissolution for plant growth is debated. ECM fungi can weather minerals and mobilise essential nutrients such as base cations by physical force, extrusion of low molecular weight organic acids, enzymes, free radicals and chelators. Suillus species in particular, are found to preferentially inhabit mineral soils and are frequently reported to possess weathering capabilities. Though studies growing ECM fungi with minerals have shown heightened nutrient content in mycelia compared to growth without minerals, the mechanistic understanding of nutrient mobilisation associated with weathering remains largely unknown.

Here we focus on characterising the diversity and phylogenomic distribution of transporters in ECM genomes. We hypothesise that species with extensive mineral weathering capabilities will have higher copy-numbers of transporter genes associated with the transport of base cations to enable rapid uptake and transfer of recently mobilised nutrients. We examined transporter family gene copy-numbers of 110 Agaricomycetes species, and analysed evolutionary expansions and contractions of transporter gene families across the phylogeny.

Preliminary results show large variations in copy-numbers in all transporter gene families across all species. Overall, 70 transporter gene families are highlighted as rapidly evolving across the whole phylogeny, and there are rapid expansions of cation transporters at the Russulales and Suillus nodes. Genome analyses will be complemented by pure culture studies comparing base cation mobilisation and uptake by Suillus spp. to that by other species with varying copy-number and ecology. Fungal cultures will be grown with and without mineral additions (granite and gabbro) and mycelial base cation uptake and biomass will be quantified. These studies will aid in identification of isolates with high mineral weathering potential and provide a basis for future transcriptomic studies.

CONCURRENT SESSION 5.2 MYCOBIOMES AND MICROBIAL INTERACTIONS

WEDNESDAY, MARCH 8
14:00 – 16:00
Location: ***Hall Brüssel (Congress Innsbruck)***

CHAIRS:
Christoph Schüller, Claire Stanley

CS5.2.1

EFFECT OF FUNGAL HYPHAE ON DISPERSAL AND GROWTH OF OBLIGATE ANAEROBIC BACTERIA

Bijing Xiong[1], Sabine Kleinsteuber[1], Heike Sträuber[1], Christian Dusny[1], Hauke Harms[1], **Lukas Yvo Wick**[1]

[1]*Helmholtz Centre For Environmental Research UFZ, Leipzig, Germany*

Creating unique and dynamic microenvironments, hyphal surfaces and their surrounding often allow for spatially distinct microbial interactions and functions. The hyphosphere also is a favourable habitat for bacterial colonization and dispersal. Here, we analyzed whether and to which degree hyphae of Coprinopsis cinerea thriving in oxic habitats enable the germination, growth, and dispersal of obligate anaerobic soil bacterium Clostridium acetobutylicum. Time-resolved optical oxygen mapping, microscopy and metabolite analysis revealed the formation and persistence of anoxic circum hyphal niches allowing for spore germination, growth and fermentative activity of the obligate anaerobe in an otherwise inhabitable environment. Hypoxic liquid films containing ca. 80 % of atmospheric oxygen saturation around single air-exposed hyphae thereby allowed for efficient clostridial dispersal amid spatially separated (>0.5 cm) anoxic sites. Hyphae hence may serve as good networks for the activity and spatial organization of obligate anaerobic bacteria in oxygenated environments such as soil with its physico-chemically distinct zones, habitat patchiness, and hotspots of microbial activity.

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CS5.2.2

THE HUMAN FUNGAL PATHOGEN ASPERGILLUS FUMIGATUS HOLDS A CORE BACTERIOME

Cristina Silva Pereira[1], Daryna Piontkivska[1], Gustavo H. Goldman[1,2], Dalila Mil-Homens[3,4]

[1]*Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa (ITQB NOVA), Oeiras, Portugal,* [2]*Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, Brazil,* [3]*Institute for Bioengineering and Biosciences (iBB) and Institute for Health and Bioeconomy (i4HB), Instituto Superior Técnico, University of Lisbon, Lisboa, Portugal,* [4]*Department of Bioengineering, Instituto Superior Técnico, University of Lisbon, Lisboa, Portugal*

Fungi are globally ubiquitous and kill more than 2 million people annually worldwide. In particular, invasive aspergillosis comprises a diversity of diseases caused by Aspergillus spp. with clinical outcomes ranging from colonization (e.g. lungs) to systemic infections. The most vulnerable population, immunocompromised patients of all ages, continues to grow. Other groups at risk include those suffering from chronic obstructive pulmonary disease, asthma and cystic fibrosis. The burden associated with fungal diseases on healthcare systems is predicted to rise in the near future.

There are many examples of partnerships of fungi with other organisms with the most emblematic being with algae to form lichens. Recently, a diverse array of bacteria has been found to associate with various fungal hosts otherwise axenic. A. fumigatus usually exhibit extensive genomic and phenotypic heterogeneity in their virulence and drug-resistance profiles, questioning if the bacteriome influences the observed phenotypic heterogeneity.

In this study, we focus our attention on the bacteriome of A. fumigatus clinical isolates to dissect how the bacterial partners influence fungal growth, infection capacity and drug susceptibility. A. fumigatus strains grown in media containing an antibiotic lost some of the most abundant bacterial partners. To eliminate additional ephemeral bacteria partners (able to survive the antibiotic treatment) and reduce

inter-spore variability, the spores were submitted to a high-temperature shock, and then single-conidium cultures were generated. These strains showed great genotypic and phenotypic variability (colony growth rate, antifungal-susceptibility profiles, and in vivo infection capacity in Galleria mellonella). The bacteriome (full-length 16S) of strains having distinct phenotypic characteristics was analysed. The acquired data clearly show that the analysed A. fumigatus cultures, otherwise axenic, harbour a complex core bacteriome but each has specific bacterial partners. Ongoing analyses are extrapolating correlations of how the assembly of a fungal-bacteria partnership influences the potential for virulence of A. fumigatus.

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CS5.2.3

FUNGAL – BACTERIAL INTERACTIONS IN GLACIER FOREFIELDS: TO THE ALPS AND BEYOND

Edoardo Mandolini[1], Maraike Probst[1], Anusha Telagathoti[1], Luis Miguel Rodriguez-Rojas[1], Ursula Peintner[1]

[1]*University Of Innsbruck, Innsbruck, Austria*

Bacterial-fungal interactions in recently deglaciated ecosystems promote biogeochemical cycles, mineral soil fertility, and pioneer plant growth, but the diversity of keystone microbes and the quality of their interactions remain largely unexplored. Here, we investigated the diversity of both fungal and bacterial communities to predict core microbial networks and to estimate conserved interactions across comparable deglaciated systems.
We studied the soil fungal and bacterial communities at the early stages of soil development (0-25 years) in four receding calcareous glaciers of the Alps (>200 samples). High-resolution marker-gene (16S and ITS) analysis were performed alongside detailed soil geochemical analysis. Furthermore, we included 13 datasets from publicly available projects on forefields of receding glaciers (world-wide) whose sequencing libraries resembled the criteria of our own dataset. Network analysis (SpiecEasi) was performed for each dataset. Then, core microbial interactions were identified across glacier forefields.
Bacterial and fungal communities differed in a location-specific manner, sharing remarkably few common taxa. We found extremely dense networks in all locations, with fungi clearly dominating the keystone nodes for all major interactive clusters. We speculate that conserved interactions across glacier forefields are rather based on trophic preferences than on phylogenetic diversity.
Our data emphasize (i) the unique diversity of soil microbial communities in glacier forefields likely depending on stochastic processes of dispersion, but provide (ii) evidence for common ecological roles based on conserved microbial interactions.

CS5.2.4

CHEMOTACTIC SIGNALS REGULATING THE INTERACTION OF SCLEROTINIA SCLEROTIORUM AND TWO SOIL OXALOTROPHIC BACTERIA

Pilar Junier[1], Aislinn Estoppey[1], Saskia Bindschedler[1], Thierry Kuhn[1], Armelle Vallat-Michel[1], Xiang-Yi Li[1]

[1]*University Of Neuchatel, Neuchatel, Switzerland*

Microorganisms exhibit different behaviours depending of their environment. Different chemical stimuli are known to affect the overall directionality of the movement of motile organisms. This is also possible in the soil matrix by a combination of attractive and repulsive stimuli. The production of these chemical signals might affect not only the behaviour of individual organism, but also of multiple interacting species. In this study the interaction between Sclerotinia sclerotiorum, a soil-borne phytopathogenic fungus, and two soil oxalotrophic bacteria, Cupriavidus necator and Cupriavidus oxalaticus was investigated. In confrontation assays on diluted malt agar, C. necator and C. oxalaticus were repulsed by S. sclerotiorum, while on Reasonner's 2 agar, the bacteria were attracted to the fungus. Chemotaxis experiments with the spent medium from pure cultures or after confrontation experiments showed that the molecule responsible for the chemotactic signal was not produced constitutively. Only the spent medium from confrontations experiments triggered the attraction or repulsion behaviour previously seen in the confrontation assays. In order to determine the molecules responsible for the repulsion behaviour the spent medium was fractionated in poly-lysin containing columns. These fractions will be subjected to further chemical characterization to identify the chemotactic signal. Interkingdom communication via chemical signalling is a key component of bacterial-fungal interactions (BFI) and identifying these signalling molecules and how do they regulate complex microbial behaviours such as competition/cooperation and communication is essential to advance in the field.

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CS5.2.5

ANTAGONIST-SPECIFIC DEFENSE RESPONSES OF THE COPROPHILE MUSHROOM COPRINOPSIS CINEREA AGAINST BACTERIA AND FUNGIVOROUS NEMATODES

Markus Künzler[1]

[1]*ETH Zürich, Zürich, Switzerland*

Fungi are engaged in antagonistic interactions with other organisms including competitors and micropredators. The main survival strategy of fungi against such antagonists is chemical defense. As a matter of fact, fungi are well known to produce a plethora of defense effectors, including secondary metabolites, peptides and proteins, that are able to negatively affect the fitness of other organisms. The regulation of the biosynthesis of these chemicals, however, is often poorly understood. In our laboratory, we take a reductionist and experimental approach aiming at the identification and characterization of novel fungal defense effectors as well as a molecular dissection of the regulatory mechanisms governing the biosynthesis of these chemicals. Our studies focus on the coprophile model mushroom Coprinopsis cinerea and its antagonistic interaction with bacteria and fungivorous nematodes and involve a variety of experimental tools. For example, we used an experimental setup involving glassbeads immersed in liquid culture medium to confront the mycelium of the mushroom with bacteria. Using this setup in combination with RNAseq, we demonstrated that the fungus is able to mount a specific defense response against bacteria. Similar results were obtained with fungivorous nematodes, where the confrontation between the C. cinerea mycelium and the predators was performed in a microfluidics device allowing a spatial control of predation. This device, in combination with a fluorescent reporter strain of C. cinerea, also enabled us to follow the propagation of the antinematode defense response within the fungal mycelium at single hyphal resolution. The results revealed a novel type of hyphal differentiation and mechanism of intrahyphal signal propagation/nutrient transport. We are currently trying to identify the signals responsible for the induction and propagation of the antagonist-specific defense responses of C. cinerea. Results of recent FluidFM experiments suggest that the antinematode response may be triggered by a sudden drop in hyphal turgor pressure.

CS5.2.6

HYPHOSPHERE AND MICROBIAL COMMUNITIES: CAN FUNGI ALSO CHANGE THEIR ENVIRONMENT?

Irina Druzhinina[1]

[1]*Royal Botanic Gardens Kew, Richmond, United Kingdom*

Absorptive nutrition, i.e., the uptake of pre-digested small molecules from the environment, is the only rational way of feeding for heterotrophic organisms covered by a rigid cell wall, such as fungi or fungi-like protozoans. This strategy also determines either spheroid or filamentous bodies, as this geometry maximizes the surface-to-volume ratio and explains the requirement for moisture. Thus, fungi frequently live inside their food or on its surface. The feeding hyphae secrete a cocktail of hydrolytic enzymes capable of digesting a vast diversity of substrates ranging from sugars to resilient polymers. Furthermore, they release antimicrobial secondary metabolites, which defend their foraging grounds from competitors. However, recent studies showed that feeding hyphae also massively secreted surface-active proteins, which inevitably modified the physicochemical properties of the adjacent area - the hyphosphere. Interestingly, fungal structures involved in the aerial dispersal of spores - conidiophores, fruiting bodies, and others, secrete a drastically different profile of surface-active proteins, reflecting different functional demands for their hyphosphere.
In this report, we will define the hyphosphere concept considering different stages of the fungal life cycle and explore the role of surface-active proteins in solving specific tasks required to complete the fungal life cycle. We will use the model filamentous fungi from the genus Trichoderma (Hypocreales, Ascomycota) and show how the hydrophilicity of feeding hyphae is maintained through the activity of cerato-platanins, hydrophobicity of aerial hyphae and spores is provided by hydrophobins, and what might be the putative function of the other families of surface-active small secreted cysteine-rich proteins. Furthermore, we will discuss the impact of surface-modulating substances secreted by fungi into their environment on other members of the microbial community, the mobility of enzymes, small molecules, and water. Thus, we will address the selfish ability of fungi to modify their habitat, which makes them similar to humans.

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CS5.2.7

POLARAMYCIN B, AND NOT PHYSICAL INTERACTION, IS THE SIGNAL THAT REWIRES FUNGAL METABOLISM IN THE STREPTOMYCES-ASPERGILLUS INTERACTION

Harald Berger[1], Markus Bacher[2], Roman Labuda[2], Isabel Eppel[1], Florentina Bayer[1], Michael Sulyok[1], Erika Gasparotto[1], Franz Zehetbauer[1], Maria Doppler[1], Hannes Gratzl[1], Joseph Strauss[1]

[1]*University of Natural Resources and Life Sciences, Tulln, Österreich,* [2]*Research Platform Bioactive Microbial Metabolites (BiMM), Tulln, Österreich*

Co-culturing the bacterium Streptomyces rapamycinicus and the ascomycete Aspergillus nidulans has previously been shown to trigger the production of orsellinic acid (ORS) and its derivates in the fungal cells. Based on these studies it was assumed that direct physical contact is a prerequisite for the metabolic reaction that involves a fungal amino acid starvation response and activating chromatin modifications at the biosynthetic gene cluster (BGC). Here we show that not physical contact, but a guanidine containing macrolide, named polaramycin B, triggers the response. The substance is produced constitutively by the bacterium and above a certain concentration, provokes the production of ORS. In addition, several other secondary metabolites were induced by polaramycin B. Our genome-wide transcriptome analysis showed that polaramycin B treatment causes downregulation of fungal genes necessary for membrane stability, general metabolism and growth. A compensatory genetic response can be observed in the fungus that included upregulation of BGCs and genes necessary for ribosome biogenesis, translation and membrane stability. Our work discovered a novel chemical communication, in which the antifungal bacterial metabolite polaramycin B leads to the production of antibacterial defence chemicals and to the upregulation of genes necessary to compensate for the cellular damage caused by polaramycin B.

CS5.2.8

A GLOBAL SURVEY OF HOST, AQUATIC, AND SOIL MICROBIOMES REVEALS ECOLOGICAL PROPERTIES SHARED BETWEEN BACTERIAL AND FUNGAL GENERALISTS

Daniel Loos[2], Ailton Pereira[1], **Amelia Barber**[1], Gianni Panagiotou[2,3]

[1]Friedrich Schiller University, Jena, Germany, [2]Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany, [3]University of Hong Kong, Hong Kong, China

Microbiome engineering is a fast-evolving area with relevance for human health, agriculture, and climate management solutions. Despite significant efforts to repair dysbiotic communities, new microbes often fail to establish and/or alter ecosystem function. To identify bacterial and fungal genera with the desired ability to alter microbial communities, we retrieved paired 16S and ITS rRNA amplicon sequence data from 1,580 host, soil, and aquatic samples and explored the ecological patterns of the 2,977 bacteria and 1,740 fungal genera detected across all samples. Through this large-scale analysis, we revealed that a small number of bacterial and fungal generalists with high prevalence across all environments positively contribute to the taxonomic diversity of their respective kingdom and explain a large percentage of the variation in the cross-kingdom community structure. We also observed that bacterial and fungal generalists have a significantly higher abundance compared to specialists, or genera whose prevalence was strongly associated with a single habitat - possibly due to their ability to stimulate positive associations with other highly prevalent genera. These findings can streamline existing strategies to identify bacterial and fungal inoculants with a higher probability to establish in recipient ecosystems and confer noticeable changes in their structure and function.

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CONCURRENT SESSION 5.3
STRESS AND EXTREME ENVIRONMENTS

WEDNESDAY, MARCH 8
14:00 – 16:00
Location: ***Hall Strassburg (Congress Innsbruck)***

CHAIRS:
Drauzio Eduardo Naretto Rangel, Laura Selbmann

CS5.3.1

RECOMBINATION, CLONALITY AND HYBRIDIZATION IN FUNGI FROM EXTREME ENVIRONMENTS

Cene Gostinčar[1], Nina Gunde-Cimerman[1]

[1]University Of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia

Clonality has long been the presumed reproductive strategy of many extremophilic and extremotolerant fungi. The absence of sexual reproduction was long considered the best option for inhabitants of rare and fragmented extreme environments, where energy sources are scarce and recombination load would compromise efficient genomic configurations of well-adapted strains. Only population genomics has shown that most of these species recombine, some with surprising frequency. Although the complete absence of recombination is much rarer than previously thought, strict clonality has been reported for some species, including two extremotolerant black yeasts from Dothideomycetes: Hortaea werneckii and Aureobasidium melanogenum. However, genome sequencing of over 100 strains of these two species unexpectedly showed that they occasionally form diploid intraspecific hybrids, which account for about two thirds of all wild isolates of each species. The mechanism of these hybridization events, which are not followed by meiosis or haploidisation, remains unknown. The resulting hybrids are highly heterozygous and stable enough to spread over large geographical distances. The origin of the diploid strains collected worldwide can thus be traced back to only a handful of hybridization events. The unusual reproductive strategy observed in Hortaea werneckii and

Aureobasidium melanogenum raises several questions. How common is hybridisation in otherwise clonal species? Does it play a role in the extremotolerance of H. werneckii and A. pullulans? How should the resulting hybrid lineages be treated in taxonomy? Possible answers to these questions will be discussed together with the opportunities and challenges of working with clonal hybrids in extremotolerant fungi.

Gostinčar C, Sun X, Černoša A, Fang C, Gunde-Cimerman N, Song Z. Clonality, inbreeding, and hybridization in two extremotolerant black yeasts. Gigascience. 2022. 6 (11). doi: 10.1093/gigascience/giac095. PMID: 36200832; PMCID: PMC9535773.

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CS5.3.2

NEW INSIGHTS INTO THE MECHANISMS INVOLVED IN RESISTING COPPER TOXICITY IN THE PATHOGENIC FUNGUS ASPERGILLUS FUMIGATUS

Nir Osherov[1], Zohar Meir[1], Mariana Handelman[1], Ammar Abou Kandil[1], Hila Werner[1], Orin Amano[1], Yana Shadkchan[1]

[1]*Tel Aviv University, Tel Aviv, Israel*

High levels of copper (Cu), generated by phagocytes of the immune system, are one of the mechanisms used to kill invading fungi. Fungi have evolved intricate mechanisms to delicately balance between acquiring Cu and defending against its toxicity. Aspergillus fumigatus is the most common human mold pathogen in humans, causing both chronic and life threatening diseases. In the presence of excess Cu, the A. fumigatus transcription factor AceA increases expression of the Cu transporter CrpA to eliminate excess toxic levels of Cu. We show that CrpA is expressed in response to Cu and localizes to the ER and plasma membrane. We constructed in silico a model structure of CrpA and identified two regions that are unique to fungi: the N-terminal region (amino acids 1-211) and an intracellular loop (amino acids 542-556). Using deletion and replacement mutants, we discovered that the intracellular loop is important for CrpA function and localization to the plasma membrane, while the N-terminal region seems to bind Cu. To identify novel genes involved in Cu resistance, we performed an evolutionary experiment on gradually increasing Cu concentrations. Whole genome sequencing of Cu-resistant A. fumigatus mutants identified two genes that confer resistance following mutation: Pma1, a plasma membrane H+-ATPase, and Gcs1, a putative glutamate-cysteine ligase. Both genes elevated Cu resistance in an additive manner. Surprisingly, no AceA or CrpA mutations were found in the evolved strains. Our research sheds light not only on the known and studied mechanisms of copper homeostasis, but also suggests that there are additional unrelated pathways involved in the process.

CS5.3.3

THE ROLES OF DHN MELANIN AND THE STRESS-ACTIVATED MAP KINASE IN THE ROCK INHABITANT KNUFIA PETRICOLA

Antonia K.M. Brandhorst[1,2], Sarah Nitsche[1,2], Eileen A. Erdmann[1,2], Ruben Gerrits[1], Anna A. Gorbushina[1,2], **Julia Schumacher[1,2]**

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Black fungi/yeasts exhibit high stress tolerance, yeastlike or meristematic growth, and constitutive 1,8-dihydroxynaphthalene (DHN) melanin formation. Due to their slow growth, robust cell walls and the lack of sexual cycles and genetic tools, the underlying mechanisms of their phenotypic traits have remained largely unexplored. Using recently developed genetic tools, it is now possible to manipulate the genome of the rock-inhabiting model fungus Knufia petricola. Thus, gene functions and the cell biology of black fungi can be studied using CRISPR/Cas9-based genome editing and live-cell imaging with genetically encoded fluorescent proteins. Here, we are addressing the question to which extent constitutive pigment formation (melanin and carotenoids) and responses mediated by the stress-activated mitogen-activated protein (MAP) kinase contribute to the observed extremotolerance of K. petricola. The mutations of pks1, phs1 and both genes result in melanin-free (pink), carotenoid-free (black) and pigment-free (white) strains, respectively. The other putative melanogenic genes were identified in the genome, deleted to confirm their involvement in DHN melanogenesis and co-expressed in Saccharomyces cerevisiae for reconstruction of the synthesis pathway. Sak1 encoding the stress-activated MAP kinase was deleted in the wild-type and different pigment-deficient backgrounds. Growth of the obtained single, double and triple deletion mutants was tested by droplet tests on media supplemented with different stress-inducing agents. The Δsak1 mutants show slightly reduced growth rates even without environmental pressure and are hypersensitive to different stresses: e.g. osmotic, oxidative, membrane, pH and heat stress. Melanin-free Δsak1 mutants are more sensitive than black Δsak1 mutants to some but not all stress conditions, suggesting that melanin and the SAK1 pathway have complementary roles in protecting K. petricola from stress.

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CS5.3.4

SPECIFIC GENOMIC TRAITS DRIVE DIVERSE ECOLOGIES THROUGHOUT THE EXTREMES IN STRESS-TOLERANT BLACK FUNGI

Claudia Coleine[1], Giulia Calia[2], Tani Kurbessoian[3], Manuel Delgado-Baquerizo[4], Alessandro Cestaro[2], Nicola Segata[5], Claudio Donati[2], Jason Stajich[3], Laura Selbmann[1]

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Black fungi form a polyphyletic group within Ascomycota, mainly in the classes Eurotiomycetes and Dothideomycetes. They are among the most successful extreme-tolerant organisms on Earth, encompassing an astonishing capability to perpetuate in all type of extreme environments, both natural and anthropogenic, where colonization by most life-forms is hampered, with a recurrent ability to colonize lithic niches. Partly because of their dark, melanin-based pigmentation, black fungi are resistant to stresses including UV- and ionizing-radiation, heat and desiccation, toxic metals, and organic pollutants. Nevertheless, the genomic traits that govern their success in the extremes and their ecologies are still largely unknown. To fill this knowledge gap, we present a comparative genomic analysis of over 100 strains dothideomycetous and eurotiomycetous black fungi, selected to represent diverse ecologies and global distribution for a comprehensive study of adaptations of these fungi. Here, the link between the considered ecologies and diverse competences is shown in a class-wide phylogenetic analysis as a statistically significant difference in incidence and abundance of specific genomic traits. In fact, the genomes of Dothideomycetes, dominating mostly extreme natural habitats (e.g. hot and cold deserts), were significantly enriched in cold temperature and UV radiation resistance and in genes that encode for DNA damage repair, while no such signatures were observed in Eurotiomycetes. The latter, instead, were particularly enriched in genes related to hot temperature tolerance and hydrocarbons degradation and were significantly correlated with human influence, revealing that Eurotiomycetes are more prone to dominate polluted and anthropogenic environments. This study rep-

resents the basis to fully unearth the ecologies, genetic and metabolic competences of these enigmatic microorganisms towards advancing the current understanding of life on our planet and its adaptive potential.

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CS5.3.5

PHASE SEPARATION AS A POTENTIAL MECHANISM FOR COLD-STRESS TOLERANCE IN POLAR FUNGI

Steven Hanes[1], Ryan Palumbo[1], Nathan McKean[1], Erinn Leatherman[1], Cene Gostinčar[2], Nina Gunde-Cimerman[2], Laurie Connell[3], Kevin Namitz[4], Alaji Bah[1]

[1]SUNY Upstate Medical University, Syracuse, New York, United States, [2]Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia, [3]2 School of Marine Sciences, Sciences & Department of Molecular and Biomedical Sciences, University of Maine, Orono, Maine, United States, [4]Department of Chemistry, Penn State University, University Park, Pennsylvania, United States

Most of the world's biodiversity lives in cold (-2°C to 4°C) and hypersaline environments. To understand how cells adapt to such stressful conditions, we focused on the RNA polymerase II (RNAPII) transcription machinery from fungal species that live in extreme cold and high salt environments. We cloned the Ess1 prolyl isomerase and its target, the carboxy-terminal domain (CTD) of the catalytic subunit of RNAPII from five cold (and salt) -tolerant species; three of which can be found in the Arctic, (Aureobasidium pullulans, Hortaea werneckii, Wallemia ichthyophaga), and two of which have only been isolated in Antarctica (Dioszegia cryoxerica, Naganishia vishniacii).
The cold-tolerant Ess1 enzymes are highly conserved and fully functional in the mesophilic model host, Saccharomyces cerevisiae, and show key amino acid substitutions consistent with predictions of increased flexibility and solvation. By contrast, the CTD, which in S. cerevisiae is composed of a consensus heptad repeat (YSPTSPS)26 that is intrinsically disordered and known to promote phase separation in vitro and nuclear clustering of RNAPII in vivo, is highly divergent in the cold-adapted fungi. The divergent CTDs did not complement well in S. cerevisiae, nor did they direct efficient nuclear localization. We found that the CTDs from cold-adapted fungi possess very unusual phase separation properties in vitro. Based on these and other results, we propose that environmentally-tuned phase separation by the CTD and other intrinsically disordered regions in proteins plays an adaptive role in cold tolerance. One way in which phase separation could increase cold-stress adaptation is to concentrate enzymes and substrates to overcome energetic barriers to metabolic activity in the cold.

CS5.3.6

ASPECTS OF METAL STRESS RESPONSE OF THE ECTOMYCORRHIZAL BASIDIOMYCETE TRICHOLOMA VACCINUM

Manuela Östreicher[1], Nina Carl[1], Thomas Krüger[2], Judith Hoffmann[1], Katrin Krause[1], Erika Kothe[1]

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Tricholoma vaccinum was chosen as model organism to view various aspects of the metal stress response of an ectomycorrhizal basidiomycete with usual tolerance ranges in axenic culture or co-culture with its compatible symbiont, the coniferous tree Picea abies.
T. vaccinum showed different expanses of the concentration ranges with decreasing growth performance between a series of metal chloride species while starting at highly different minimal inhibitory concentrations. In co-culture with P. abies, similar growth patterns were observed. Concomitant monitoring of the plant arose the opportunity to establish a white- and a blacklist for valuable morphological and developmental parameters for preparatory studies.
The molecular basis of the fungal heavy metal stress response and the impact of the underlying lifestyle were analyzed on transcriptional level with an RNA-Seq approach using Cd, Cu and Ni. The regulation patterns revealed a high lifestyle dependency and metal species-specificity with a low count of genes with overlap between two or three metal treatments. In the comparisons pure vs. co-culture, each metal treatment background was correlated with rather a smaller upregulation of metabolism pathways and a smaller downregulation of genetic information processing pathways.
The adaptation to heavy metals in the laboratory was exemplarily studied in detail for Cd adaptation. The adapted lineage showed improved growth and normalized hyphal morphology due to Cd treatment. Putatively involved genes and pathways were analyzed via a proteomics approach. Cd adaptation clearly influenced the amount of more proteins than the Cd treatment did.
For both above mentioned contexts, candidates putatively involved in heavy metal stress associated detoxification processes showed their involvement but with clear differences between treatments, lineages or lifestyles - considering transporters, antioxidant enzymes, the glutathione metabolism and in detail glutathione S-transferases (GST). Especially GSTs within the classes GSTFuA, Phi, Ure2p and Xi showed a regulation as consequence of heavy metal circumstances.

CS5.3.7

INCREASED PROTEIN SOLUBILITY CONTRIBUTES TO HEAT PRIMING OF THE PLANT PATHOGENIC FUNGUS BOTRYTIS CINEREA

Mingzhe Zhang[1], Naomi Kagan Trushina[1], Tabea Lang[2], Metsada Pasmanik Chor[1], Amir Sharon[1]

[1]Tel Aviv University, Tel Aviv, Israel, [2]Technical University of Kaiserslautern, Kaiserslautern, Germany

Botrytis cinerea causes grey mold disease in leading crop plants. The disease develops only at cool temperatures, but the fungus remains viable in warm climates and can survive periods of extreme heat. We discovered a strong heat priming effect in which the exposure of B. cinerea to moderately high temperatures greatly improves its ability to cope with subsequent, potentially lethal temperature conditions. We showed that priming promotes protein solubility during heat stress and discovered a group of priming-induced serine-type peptidases. Several lines of evidence, including transcriptomics, proteomics, pharmacology and mutagenesis data, link these peptidases to the B. cinerea priming response, highlighting their important roles in regulating priming-mediated heat adaptation. By imposing a series of sub-lethal temperature pulses that subverted the priming effect, we managed to eliminate the fungus and prevent disease development, demonstrating the potential for developing temperature-based plant protection methods by targeting the fungal heat priming response.

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CS5.3.8

POTENTIAL AND UNDERLYING MECHANISMS OF SCHIZOPYLLUM COMMUNE TO REMEDIATE THE CHERNOBYL EXCLUSION ZONE

Lea Traxler[1], Katrin Krause[1], Martin Richter[1], Thorsten Schäfer[1], Erika Kothe[1]

[1]Friedrich Schiller University Jena, Jena, Germany

The pollution of the environment with metals is an omnipresent and growing problem. One possible approach to make such contaminated soils usable is remediation by fungi. This process is called mycoremediation.
In order to investigate the mycoremediation abilities of the wood-digesting S. commune, the fungus was inoculated in a test field near the nuclear power plant in Chernobyl, which was damaged 35 years ago. Using DNA isolation from soil samples and quantitative PCR with species-specific primers, it was possible to demonstrate that S. commune not only survived in the test field but also even spread at a rate of around 8 mm / day.
To understand the mechanism behind this tolerance, an mRNA sequencing of S. commune, grown on two different intensely contaminated soils and minimal medium with and without the addition of metal, was carried out. This showed that the growth substrate has a higher impact on transcription than the different metal contents. The analysis revealed that genes belonging to transporters mostly seem to be downregulated under metal stress, whereas genes associated with secretion tend to be upregulated. Furthermore, a connection between metal stress and inositol signaling could be found in S. commune.
Additionally, the metal tolerance mechanism of S. commune was investigated using a Sr adapted strain. Both the wildtype and the adapted strain were able to transport Cs and Sr along their hyphae. These results indicate that S. commune is a promising candidate for mycoremediation.

CONCURRENT SESSION 5.4 REGULATORY NETWORKS

WEDNESDAY, MARCH 8
14:00 – 16:00
Location: ***Hall Freiburg (Congress Innsbruck)***

CHAIRS:
Christian Zimmermann, Manuel Sánchez López-Berges

CS5.4.1

LIGHT PERCEPTION IN ASPERGILLUS NIDULANS, A. FUMIGATUS AND ALTERNARIA ALTERNATA

Reinhard Fischer[1], Michael Pitz, Kai Leister, Lars Schuhmacher, Alexander Landmark

[1]*Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany*

Many fungi contain several photoreceptor proteins which enable them to sense and respond to different wavelengths. Light controls developmental processes as well the physiology and the production of secondary metabolites (1). Phytochrome is a biliverdin-containing red-light receptor, whereas the flavin-containing white collar proteins perceive blue and opsins green light. Phytochrome controls a large proportion of the genome of A. nidulans whereas the white-collar protein LreA appears to control less genes (2). Interestingly, A. fumigatus contains two phytochromes, one of which however does not bind a chromophore and appears to play different functions in the cell. A. alteranta is an attractive model for light sensing because it contains phytochrome, a blue-light receptor and two functional opsins. We found that one opsin resides in the vacuolar and one in the cytoplasmic membrane. Both together cause an acidification of the cytoplasm in the presence of green light. This could lead to a dormant state which in turn could control germination and polar growth.

(1) Yu, Z. and R. Fischer, 2019. Light sensing and responses in fungi. Nat. Rev. Microbiol., 17(1): 25-36.

(2) Yu, Z., C. Streng, R. Seibeld, O.A. Igbalajobi, K. Leister, J. Ingelfinger and R. Fischer, 2021. Genome-wide analyses of light-regulated genes in Aspergillus nidulans reveal a complex interplay between different photoreceptors and novel photoreceptor functions. PLoS Genet, 17(10): e1009845.

CS5.4.2

APOPLASTIC SPACE OF TWO CULTIVARS PROVIDES HIGHLY DIFFERENT ENVIRONMENTS FOR PATHOGEN COLONIZATION: INSIGHTS FROM PROTEOME AND MICROBIOME PROFILING

Carolina Sardinha Francisco[1], Mohammad Abuhhalaf[2], Marco Alexandre Guerreiro[1], Liam Cassidy[2], Susanne Braun[1], Andreas Tholey[2], Eva Holtgrewe Stukenbrock[1]

[1]Environmental Genomics group, Christian-Albrechts University of Kiel, Kiel, Germany, [2]Systematic Proteomics and Bioanalytics group, Christian-Albrechts University of Kiel, Kiel, Germany

The apoplast comprises the intercellular space between cell membranes. During plant colonization by microbes, the apoplast is a space where the intruder lives for most of its lifetime within host. Therefore, crucial biological and regulatory processes occur in the apoplast, including microbial components triggering apoplastic immunity. The role of the plant immune system in detecting and controlling pathogenic microorganisms has been well described; however, its effect on plant-associated endophytes is still poorly understood. Z. tritici is a hemibiotrophic fungal pathogen that colonized the apoplastic space of wheat plants. Wheat resistance and susceptibility can be mediated via gene-specific interactions between wheat and Z. tritici. Here, we used Z. tritici as a model system to unveil 1) cultivar-specific microbiota and protein profiling changing during pathogen infection; and 2) plant-associated microbiota members engaging in direct and indirect interactions with Z. tritici. We developed a novel method for microbial isolation, in which we validated the apoplastic fluids as a proxy to understand the warfare between plants and microbes. We use proteomic analysis to show that Z. tritici diminishes the photosynthetic functionality in the susceptible Obelisk cultivar, while the resistant Chinese Spring is significantly enriched in defense response-related proteins. We observed that this difference in plant immune response causes a drastic shift in apoplastic microbial composition. Next, we experimentally determined the tolerance of apoplastic microbes to plant-produced immune-related antimicrobial compounds. We also screened them for antagonistic fungal-bacterial interactions. We found several bacterial isolates showing susceptibility to antimicrobial compounds and negatively affecting the growth of Z. tritici. Finally, we analyzed the genome of antagonistic bacterial strains and found that secondary metabolite biosynthetic gene clusters associated with fungal growth inhibition are under positive selection. Overall, our findings highlight the potential of a multi-omics approach targeting the outcomes of complex plant-defense-associated and microbial-microbial interactions in the apoplastic fluids.

CS5.4.3

A TRANSCRIPTION PROFILING APPROACH TO STUDY THE ASPERGILLUS NIDULANS KINOME

Zhiqiang Dong[1], Niranjan Shirgaonkar[1], Zhengqiang Miao[1], Kaeling Tan[1], Koon Ho Wong[1]

[1]*University Of Macau, Macao, China*

Fungi can adapt and grow in diverse environments, the ability of which is mediated by complex sensing, signalling, and regulatory mechanisms. As a quick reversible covalent modification, protein phosphorylation catalyzed by protein kinases plays essential roles in signal transduction and regulation of cellular processes. Despite their importance, the function of many kinases is still unknown even in well-studied model organisms such as A. nidulans, which has more than half of the non-essential kinases uncharacterized. In this work, we performed transcription profiling analysis on ninety-nine non-essential kinase mutants of A. nidulans grown in preferred carbon (e.g., glucose) and nitrogen (e.g., ammonium) conditions. The overall results reveal the transcriptional effects of each kinase and portray a function map of the A. nidulans kinome during active growth.

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CS5.4.4

THE INFLUENCE OF EPIGENETIC MODIFICATIONS ON EFFECTOR GENE EXPRESSION AND PATHOGENICITY IN FUSARIUM OXYSPORUM

Slavica Janevska[1], Lena Studt-Reinhold[2], Tongta Sae-Ong[3], Gianni Panagiotou[3], Vito Valiante[1], Martijn Rep[4]

[1]*Biobricks of Microbial Natural Product Syntheses, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany,* [2]*Applied Genetics and Cell Biology, BOKU Vienna, Tulln, Austria,* [3]*Systems Biology and Bioinformatics, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Jena, Germany,* [4]*Molecular Plant Pathology, University of Amsterdam, Amsterdam, Netherlands*

Fusarium oxysporum strains infect a wide range of different agronomically important crops (e.g., tomato, banana, cucurbits, cotton, oil palms), and moreover, represent a threat as opportunistic human pathogens. Each host-specific form (forma specialis, f. sp.) has a narrow host range, which relies on the set of small secreted proteins. These effector genes are specifically upregulated in planta and are physically co-localized in small accessory chromosomes. Due to these characteristics, evidence suggests that they are co-regulated by epigenetic modifications, and are mobile between strains. It has been demonstrated that transfer of the 'pathogenicity' chromosome 14 of F. oxysporum f. sp. lycopersici (Fol) to a non-virulent endophyte confers pathogenicity on tomato. Little is known about regulation mechanisms governing pathogenicity-related gene expression in these regions.
In the present work, we show that under non-inducing conditions, effector genes are silenced by facultative heterochromatin, histone 3 lysine 36 (H3K36) and H3K27 methylation, in tomato-infecting Fol 4287. Therefore, we elucidated the two histone methyltransferases responsible for these modifications, i.e., H3K36-specific Ash1 and H3K27-specific Kmt6. Deletion of ASH1 or KMT6 released repressive histone marks, and upregulated effector gene expression under laboratory conditions, as shown by RNA- and ChIP (chromatin immunoprecipitation)-sequencing. Extraordinarily, multiple H3K27-specific methyltransferases (Kmt6a-c) were identified in F. oxysporum, while a single homolog is conserved in other fungi. Cross-complementation

with Fusarium graminearum revealed that all three Fol Kmt6 homologs are functional H3K27 methyltransferases, and ChIP data suggest their differential targeting to the Fol pathogenicity chromosome. Finally, deletion of ASH1 or KMT6A strongly reduced or abolished pathogenicity on tomato, respectively.

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CS5.4.5

ROLE OF THE TRANSCRIPTION FACTOR MACA IN FUSARIUM OXYSPORUM PATHOGENICITY

Rafael Palos Fernández[1], Harald Berger[2], Lena Studt-Reinhold[2], Joseph Strauss[2], Antonio Di Pietro[1], Manuel Sánchez López-Berges[1]

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The transcription factor MacA is a key regulator of copper homeostasis, governing the transcriptional response to copper limiting conditions in fungi. It has been described as a virulence factor in human pathogens such as Candida albicans and Aspergillus fumigatus but, surprisingly, its role in plant pathogenesis is poorly understood.
Here, we have characterized MacA in the phytopathogenic fungus Fusarium oxysporum, causal agent of vascular wilt disease on more than one hundred plant species. Predictably, under copper limitation (-Cu), targeted deletion of macA (macAΔ) caused deactivation of genes required for copper acquisition, resulting in the inability to grow both on -Cu solid and liquid cultures. In addition, RNA-seq analysis performed in -Cu conditions revealed 605 genes downregulated in macAΔ compared with the wild-type (wt). However, ChIP-seq analysis detected the presence of MacA on the promotor of only 14 of these genes.
Inactivation of MacA leads to the inability of F. oxysporum to kill tomato plants, even when the irrigation water is supplemented with copper. Surprisingly, RNA-seq analysis under plant infection conditions showed low transcript levels of genes responding to -Cu in the wt. Furthermore, several genes that are induced in the wt during plant infection (and not in -Cu) are downregulated in macAΔ. These results suggest that the role of MacA in virulence is not linked to copper limitation.
RNA-seq and ChIP-seq revealed that the superoxide dismutase 3 (Sod3), which employs manganese as cofactor, is transcriptionally regulated by MacA. On the other hand, two additional superoxide dismutases, Sod1 and Sod5, use copper as cofactor. Our results show that MacA is required for normal superoxide dismutase activity, how-

ever, preliminary pathogenicity assays suggest that only Sod5 plays a role in virulence. Further studies are needed to elucidate the complex role of MacA in F. oxysporum pathogenicity.

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CS5.4.6

INVESTIGATION OF GENE REGULATORY NETWORKS UNDERLYING PATTERN FORMATION IN COPRINOPSIS CINEREA

Hongli Wu[1], Xiao-Bin Liu[1], Torda Varga[1], Máté Virágh[1], Zhihao Hou[1], Zsolt Merényi[1], Botond Hegedüs[1], László Nagy[1]

[1]*Biological Research Centre, Szeged, Hungary*

Complex multicellular organisms are subject to intense research, and they are dominating the visible world. The development of morphological patterns of multicellular organisms has been well-elucidated in plants and animals while less effort has been put into morphologically diverse fungi. Cap development in mushrooms is a spectacular and widely known pattern formation mechanism. Here, we identified a conserved transcription factor cpl1 which we show is a key regulator of cap development in Coprinopsis cinerea. The cpl1 gene is highly expressed in the cap region in C. cinerea. Knocking out cpl1 in C. cinerea caused an abolishment of cap development. Preliminary phenotyping of mutant ectopically expressing the cpl1 gene under a stipe-specific gene promoter yielded several fruiting bodies growing from one stipe. Transcriptomic analysis revealed that some cap-enhanced genes identified by tissue-specific transcriptomics in C. cinerea were significantly down-regulated in the cpl1 mutant. Cpl1 orthologs are found in many mushroom-forming fungi and similar expression patterns were observed in six species. Our results suggest that cpl1 plays a vital role in cap development in C. cinerea. This result could lead us to unveil the genetic mechanisms of pattern formation in fungi.

CS5.4.7

TRANSCRIPTOME META-ANALYSIS UNVEILS LINK BETWEEN TRANSCRIPTION AND SPLICING NETWORKS IN FUNGI

João Neves da Rocha[1], Pablo Rodrigo Sanches[1], Marcos Eduardo Ramos Lopes[1], Antonio Rossi[1], Nilce Martinez-Rossi[1]

[1]*Department of Genetics, Ribeirão Preto Medical School – University of São Paulo, Ribeirão Preto, SP, Brazil*

The regulation of gene expression in eukaryotes is knowingly responsive to stimuli through a network of competing signaling pathways. This constitutes a central system associated with transistor functions, tuning physiological responses and transcription rates to environmental conditions. Using a meta-transcriptomic workflow, we searched to determine the extent of downstream regulatory networks by alternative splicing (AS) for the control of gene expression in fungi. Our results support the occurrence of AS in a large subset of genes in RNA-Seq data with different viewpoints of the biology of the dermatophyte model species Trichophyton rubrum: growth in glucose- or keratin-supplemented medium (1,340 genes), exposure to the antifungal Undecanoic Acid (UDA) (654 genes), and knock-out mutations in the transcription factors (TF) genes ap1 (1,432 genes) and stuA (1,940 genes). In the set of 17 libraries, 5,449 AS events by Intron Retention (IR) were detected in 2,763 genes. Notably, this number represents 31.71% of the 8,713 genes found in T. rubrum. Most importantly, we highlight AS as a stress-responsive system. In fact, the number of splicing-regulated was equivalent to differentially expressed genes in two experiments: ap1 mutation and UDA exposure. Furthermore, we ran an analysis to search for AS specific to TF. Libraries from all experiments were enriched with IR events in TF. A total of 123 events were detected in 67 TF genes. The results revealed a clear overlap between splicing factors and TF responses. Gene Ontology classification of AS events showed that libraries were also enriched with regulatory functions such as translation, regulation of transcription, RNA binding, and mRNA processing. Altogether, these results show that compensatory mechanisms involving the transcription and splicing machineries must act in a concerted manner. We propose that overlaps between these systems play an adaptive function during fungal metabolic reprogramming, providing fine-tuning of gene expression in response to stimuli.

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CS5.4.8

IDENTIFYING GLOBAL REGULATORS OF EFFECTOR GENE EXPRESSION IN THE RICE BLAST FUNGUS

Camilla Molinari[1], Xia Yan[1], Bozeng Tang[1], Lauren Ryder[1], Nicholas Talbot[1]

[1]*The Sainsbury Laboratory, Norwich, United Kingdom*

Global rice production is threatened by rice blast a devastating disease caused by the filamentous fungus Magnaporthe oryzae. To invade its host the fungus secretes a battery of effectors which are differentially expressed during infection with distinct functions and sub-cellular targets. Little is known, however, regarding the specific mechanisms by which M. oryzae regulates effector gene expression. Typically, effector-encoding genes are not expressed when the fungus is not growing in a plant and show enhanced expression during host infection. Based on this simple concept, we have designed a series of forward genetic screens to discover novel transcriptional regulators that control effector expression in the rice blast fungus. We have selected representative effectors which peak in expression at 48h of M. oryzae infection-MEP3 and MEP4. Then we constructed fusions of each effector to GFP and expressed these in M. oryzae. Later we selected mutants showing constitutive GFP expression, which must result from an alteration that leads to up-regulated effector gene expression. Using a combination of bioinformatic analysis and gene mapping experiments we are identifying the corresponding regulators. In parallel, using a transcriptomic dataset of M. oryzae-infected rice tissue I have identified putative transcriptional regulators of effector-encoding genes, which are being functionally characterised. I will present our complementary strategies and progress in identifying novel transcriptional regulators in the rice blast fungus.

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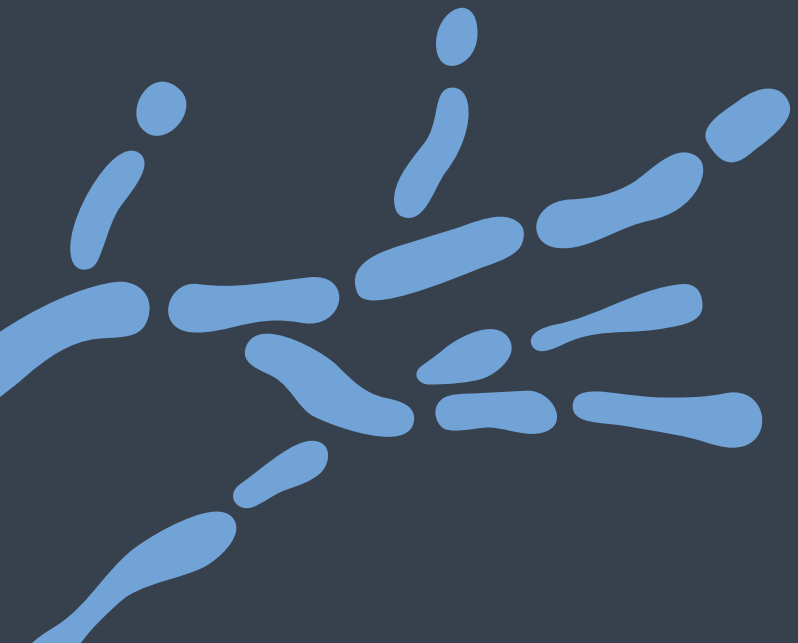

ABSTRACTS POSTER SESSIONS

POSTER SESSION I

MONDAY, MARCH 6
16:00 – 17:30
Location: ***Tirol Foyer (Congress Innsbruck)***

CS1.1 PLANT INTERACTIONS

CS1.1.9

EXPERIMENTAL EVIDENCE THAT LIGNIN-MODIFYING ENZYMES ARE ESSENTIAL FOR DEGRADING PLANT CELL WALL LIGNIN BY PLEUROTUS OSTREATUS USING CRISPR/CAS9

Takehito Nakazawa[1], Iori Yamaguchi, Yufan Zhang, Chinami Saka, Hongli Wu, Keita Kayama, Moriyuki Kawauchi, Masahiro Sakamoto, Yoichi Honda

[1]*Kyoto University, Kyoto, Japan*

Lignin-modifying enzymes (LMEs), which include laccases (Lacs), manganese peroxidases (MnPs), versatile peroxidases (VPs), and lignin peroxidases (LiPs), have been considered key factors in lignin degradation by white-rot fungi because they oxidize lignin model compounds and depolymerize synthetic lignin in vitro. However, it remains unclear how much these enzymes (or each enzyme) contribute to the actual degradation of natural lignin in plant cell walls. Therefore, we examined the lignin-degrading abilities of multiple mnp/vp/lac mutants of Pleurotus ostreatus. One vp2/vp3/mnp3/mnp6 quadruple-gene mutant was generated from a monokaryotic wild-type strain PC9 using plasmid-based CRISPR/Cas9. Also, two vp2/vp3/mnp2/mnp3/mnp6, two vp2/vp3/mnp3/mnp6/lac2 quintuple-gene mutants, and two vp2/vp3/mnp2/mnp3/mnp6/lac2 sextuple-gene mutants were generated. The lignin-degrading abilities of the sextuple and vp2/vp3/mnp2/mnp3/mnp6 quintuple-gene mutants on the beech wood sawdust medium (BWS) reduced drastically, but not so much for those of the vp2/vp3/mnp3/mnp6/lac2 mutants and the quadruple mutant strain. The sextuple-gene mutants also barely degraded lignin in Japanese cedar sawdust and milled rice straw. Thus, this study presented evidence that the LMEs, especially MnPs and VPs, play a crucial role in the degradation of natural lignin by P. ostreatus, and demonstrated their redundant but dispensable role for the first time.

CS1.1.10

THE TRANSCRIPTIONAL LANDSCAPE OF PLANT INFECTION BY THE RICE BLAST FUNGUS MAGNAPORTHE ORYZAE REVEALS TEMPORALLY CO-REGULATED AND STRUCTURALLY CONSERVED EFFECTORS

Xia Yan[1], Bozeng Tang[1], Lauren Ryder[1], Dan MacLean[1], Vincent Were[1], Alice Eseola[1], Neftaly Cruz-Mireles[1], Weibin Ma[1], Andrew Foster[1], Miriam Osés-Ruiz[1], Nicholas Talbot[1]

[1]*The Sainsbury Laboratory, Norwich, United Kingdom*

The blast fungus Magnaporthe oryzae causes a devastating disease which threatens global food security. The biology of plant tissue invasion during blast disease remains poorly understood. It is therefore important to investigate the interaction between the blast fungus and its host plant. Fungi secrete effector proteins into the apoplast and the plant cells, to facilitate invasive hyphal growth. Here we report a high resolution, transcriptional profiling study of the entire plant-associated development of the blast fungus. Our analysis revealed major temporal changes in fungal gene expression during plant infection. The rice blast gene expression could be classified into 10 modules of temporally co-expressed genes, providing evidence of induction of pronounced shifts in primary and secondary metabolism, cell signalling and transcriptional regulation. A set of 863 genes encoding secreted proteins are differentially expressed at specific stages of infection, and 546 were predicted to be effectors and named MEP (Magnaporthe effector protein) genes. Computational prediction of structurally-related MEPs, including the MAX effector family, revealed their temporal co-regulation in the same co-expression modules. We functionally characterised a group of MEP genes and demonstrate that Mep effectors are predominantly targeted to the cytoplasm of rice cells via the biotrophic interfacial complex (BIC), and use a common unconventional secretory pathway. Taken together, our study reveals major changes in gene expression associated with blast disease development and identifies a diverse repertoire of effectors critical to successful plant infection.

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CS1.1.11

THE GPI-ANCHORED PROTEIN HAM-7 REGULATES MYCELIAL NETWORK FORMATION AND ROOT ADHESION IN FUSARIUM OXYSPORUM

Valentino Maria Guastaferro[1], Stefania Vitale[2], Emanuela Russo[1], David Turrà[1]

[1]*Department of Agricultural Sciences, University of Naples "Federico II", 80055, Portici (Na), Italy,* [2]*Institute for Sustainable Plant Protection, National Research Council, 80055, Portici (Na), Italy*

The fungal cell wall is a dynamic structure protecting cells from environmental stresses, providing cell type-specific morphology and functioning as a physio-chemical rheostat for the transmission of extracellular signals through a large set of cell-wall-anchored proteins. HAM-7, a highly conserved protein present in all filamentous ascomycetes, is characterized by an N-terminal signal peptide (SP), a fungal lytic polysaccharide monooxygenase domain (LPMO) and a C-terminal glycosylphosphatidylinositol (GPI)-anchor. In the saprophytic ascomycete Neurospora crassa, HAM-7 is hypothesized to form a sensor complex at the cell wall/plasma membrane interface for the activation of the MAK-1 cell wall integrity (CWI) mitogen-activated protein kinase (MAPK) pathway required for cell-to-cell fusion and sexual development. The biological role of the CWI MAPK pathway upstream component HAM-7 in fungal plant pathogens is largely unknown. Here, we identified a single 233-amino-acid long orthologue (64.19% identity) in the Fusarium oxysporum f. sp. lycopersici (Fol) genome by using BlastP homology searches and the N. crassa OR74A HAM-7 protein as a bait. We further used a reverse genetic approach to dissect the contribution of the Fol ham-7 gene in the regulation of stress response, vegetative hyphal fusion, hyphal agglutination, plant root adhesion and virulence. Similarly to N. crassa ham-7Δ mutants, gene knock-out in Fol resulted in fungal cells that were severely impaired in vegetative hyphal fusion, but not in vegetative growth under control or stress conditions (i.e. cell wall, hyperosmotic and heat stress). Additionally, Fol ham-7Δ mutants were unable to undergo hyphal agglutination in liquid media and to adhere on host plant root tissues. We further show that absence of HAM-7 leads to a minor however significant reduction in

plant virulence. Collectively, our results reveal a new role for HAM-7 in mycelial network formation, host surface attachment and virulence in the soil plant pathogen Fusarium oxysporum.

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CS1.1.12

THE USTILAGO MAYDIS EFFECTOR PROTEIN STS2 IS A TRANSCRIPTIONAL ACTIVATOR THAT REGULATES TUMOR FORMATION IN MAIZE

Weiliang Zuo[1], Jasper Depotter[1], Sara Christina Stolze[2], Hirofumi Nakagami[2], Gunther Doehlemann[1]

[1]Institute for Plant Sciences and Cluster of Excellence on Plant Sciences (CEPLAS), University of Cologne, Cologne, Germany, [2]Basic Immune System of Plants, Max Planck Institute for Plant Breeding Research, Cologne, Germany

Ustilago maydis is a biotrophic fungus causing common smut in maize. One of its exclusive symptoms is the localized tumor induction on the infected maize organs. In leaves, U. maydis infection results in two types of tumor cells, the hypertrophic cells that consist of enlarged mesophyll, and the hyperplasic cells which result from de nova cell division of bundle sheath. However, little is known about fungal effectors orchestrate these processes. In a previous study we conducted a cross-species transcriptome analysis between U. maydis and Sporisorium reilianum, which is the phylogenetically closest relative of U. maydis that infects maize without inducing leaf tumors. This approach identified a U. maydis effector (Sts2; small tumor on seedlings 2) with two homologs in S. reilianum, which were differentially regulated and likely evolved diversified virulence functions in these two pathogen species.
Here, we show that U. maydis Sts2 is specifically required for the formation of hyperplasia tumor cells upon U. maydis infection. Sts2 is translocated from the fungal hypha into the maize cell nucleolus and acts as a transcriptional activator in the host and this function is crucial for its virulence in U. maydis. Sts2 interacts with ZmNECAP1 (adaptin-ear-binding coat-associated protein 1), a yet unknown plant transcriptional activator. RNA-seq analysis revealed that Sts2 is required to activate several maize key regulators being involved in leaf meristem maintenance and development, which corresponds with the induction of the hyperplasic tumor cells. Consequently, introduction of a suppressive SRDX motif into Sts2 causes a dominant negative effect on tumor formation. To the best of our knowledge, we here provide the first evidence of a fungal effector protein that acts as a transcriptional activator to reprogram the host's developmental process.

CS1.1.13

IDENTIFICATION AND INITIAL CHARACTERIZATION OF EFFECTOR CANDIDATE GENES IN THE WHEAT FUNGUS ZYMOSEPTORIA TRITICI EXPRESSED DURING INFECTION

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Wheat is the second-most important cereal crop. It is affected by Zymoseptoria tritici, the cause of Septoria tritici blotch. This disease has an asymptomatic stage for 1–13 days, followed by a rapid transition to necrotrophy. The lifestyle of Z. tritici makes this fungal pathogen an attractive model to investigate infection phase-specific gene expression. Differential gene expression was determined in Z. tritici during a compatible interaction with the susceptible cultivar Taichung29, two incompatible interactions with the resistant cultivars Veranopolis and Israel493, and one non-host interaction with barley. Differential gene expression was calculated at 1, 3, 6, 10, 17 and 23 days after inoculation (DAI). We found 978 up-regulated genes at 1 DAI and 2,317 up-regulated genes at 3 DAI in the compatible compared to the non-host interaction, which suggests the early activation of effector genes. We found that Z. tritici activates over 1,300 genes at 10 DAI in the compatible interaction compared to the incompatible interactions which correlates with the initiation of the necrotrophic lifestyle. The enriched KEGG pathways at 1 and 3 DAI were oxidative phosphorylation, proteasome, peroxisome, glycerophospholipid metabolism and autophagy. Biosynthesis of secondary metabolites and biosynthesis of antibiotics were significantly enriched at 17 and 23 DAI. We found that Z. tritici activates 32 putative effector genes as early as 1 DAI in the compatible interaction. Many of the candidate effectors are not predicted to target specific compartments within the cell. However, ZtCE109991 and ZtCE102792 contain predicted nuclear localization signals (NLS). ZtCE3106456 contains a chloroplast transit peptide (cTP), and ZtC394290 contains a mitochondrial targeting sequence and possible cTP and NLS. Two putative effectors are Hce2 domain-containing proteins, a conserved protein family within Dothiodemycetes that induce plant necrosis. The predicted effectors are being evaluated for cell localization, immunity suppression and necrosis induction using a Nicotiana benthamiana-based heterologous expression system.

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CS1.1.14

THE INFECTION CUSHION, A KEY ORGAN OF VIRULENCE FOR BOTRYTIS CINEREA

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A collection of Botrytis cinerea ATMT-strains revealed that random mutants exhibiting total loss of virulence toward different host plants share a common profile including impaired secretion of hydrolytic enzymes and severe deficiency in mature infection cushion (IC). IC is a multicompound appressorium differentiated by an epiphytic mycelium to penetrate a plant host. Transcriptomic and proteomic analysis of the mature IC highlighted high secretion of ROS and proteins involved in virulence, e.g. phytotoxins, proteases, plant cell wall degrading enzymes and plant cell-death inducing proteins. These results support a role for the IC in stimulating plant immunity and inducing necrotrophy by the pathogen. But surprisingly, effectors suppressing the plant chitin-triggered immunity were also induced in the IC. Chitin deacetylases genes (cda) are up-regulated and the conversion of chitin into chitosan was confirmed by differential staining of the IC cell wall. Chitosan is a poor substrate for plant chitinases. Cda mutants show a reduced pathogenicity compared to the wild-type strain and stimulate plant immunity. A LysM effector accumulated by the IC can bind the chitin in the fungus cell wall and protects hyphae against degradation by external chitinases. It is also able to sequester chitooligosaccharides and to prevent them from inducing ROS production in A. thaliana. Deletion strains of the LysM gene show a delay in infection initiation and a default in adhesion to bean leaf surfaces. It is hypothesized that the infection cushion must play a major role during the early phase of Botrytis cinerea infection, hiding from the plant during the asymptomatic phase and then inducing the necrotrophic phase and the appearance of symptoms.

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CS1.1.15

LEV MYCOVIRUS STRAINS AFFECT PHENOL OXIDASE ACTIVITY AND FRUITING BODY YIELD IN THE EDIBLE MUSHROOM LENTINULA EDODES

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In this study, we investigated the biological functions of LeV mycovirus strains in the edible mushroom Lentinula edodes. Following mycelial fragmentation of virus-infected fungi, samples were subjected to serial dilution and spread on plates to obtain virus-cured colonies. We confirmed the absence of mycoviruses known to infect L. edodes worldwide in the virus-cured strains using RNA sequencing and reverse-transcription polymerase chain reaction (RT-PCR) analyses. Then, we tested several LeV mycovirus strains in virus-infected and -cured L. edodes colonies for the occurrence of multiple viral infections in L. edodes using quantitative RT-PCR. Once cured, all fungal cultures remained virus-free for 2 years. Next, we investigated viral effects of LeV on mycelial growth and phenol oxidase activity in isogenic virus-infected and -cured strains of L. edodes. Although no discernable phenotypic changes in colony morphology were observed, the isogenic virus-cured L. edodes strain LeV-C2 had a slightly higher growth rate and phenol oxidase activity, and produced more fruiting bodies, than the virus-infected strain. These results indicate that LeV infection affects growth and fruiting body formation in L. edodes through decreased phenol oxidase activity.

CS1.1.16

GUANOSINE-SPECIFIC SINGLE-STRANDED RIBONUCLEASE EFFECTORS OF A PHYTOPATHOGENIC FUNGUS POTENTIATE HOST IMMUNE RESPONSES

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Plants activate immunity upon recognition of pathogen-associated molecular patterns. Although phytopathogens have evolved a set of effector proteins to counteract plant immunity, some effectors are perceived by hosts and induce immune responses. Here, we show that two secreted ribonuclease effectors, SRN1 and SRN2, encoded in a phytopathogenic fungus, Colletotrichum orbiculare, induce cell death in a signal peptide- and catalytic residue-dependent manner, when transiently expressed in Nicotiana benthamiana. Using a transient gene expression system in cucumber, an original host of C. orbiculare, we show that SRN1 and SRN2 potentiate chitin-triggered immunity. Consistent with this, C. orbiculare SRN1 and SRN2 deletion mutants exhibited increased virulence on the host. Importantly, the potentiation of C. sativus responses by SRN1 and SRN2 depends on the signal peptide and ribonuclease catalytic residues, suggesting that catalytic residues of SRNs or cleaved RNAs are detected by the host. We propose that the pathogen-derived apoplastic guanosine-specific single-stranded endoribonucleases lead to immunity potentiation in plants.

CS1.1.17

VOCS ANALYSIS OF CRYPHONECTRIA PARASITICA AND CHANGES ACCORDING TO CHV1

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The chestnut blight fungus, Cryphonectria parasitica, its hypovirus comprise useful model system to the mechanisms of hypovirus infection. Infection by hypovirus, Cryphonectria hypovirus 1 (CHV1), results in attenuation of virulence, known as hypovirulence, in C. parasitica, as well as other related symptoms such as altered metabolism, retarded development, and reduced sporulation. Moreover, CHV1 can be naturally transferred to other fungal hosts during hyphal fusion, converting virus-free virulent strains into virus-infected hypovirulent strains, resulting in the protection of chestnut trees from detrimental blight disease. This virus-fungus-plant interaction is a successful example of a naturally occurring biological control. VOC (Volatile Organic Compound) is known as one of means of communication among ecosystem members. Recently, studies on VOCs emitted by fungi under various conditions such as life-cycle and growth condition are conducted. Thus, we analyzed the changes in VOCs depending on the virus infection. A total of 45 VOCs were identified when virus-free EP155/2 was cultured on standard PDAmb medium. In case of virus-infected UEP1, 44 VOCs were identified. Among these VOCs, 23 VOCs were found in both cases. Of the 23 common VOCs, 21 VOCs showed difference depending on CHV1 infection, i.e., 19 VOCs were decreased and two VOCs were increased by CHV1 infection. Two VOCs showed no changes in their amounts due to CHV1 infection. On the other side, among 24 EP155/2-specific VOCs, cis-Thujopsene was strongly detected in all tested samples and among 21 UEP1-specific VOCs, three VOCs such as cis,cis-2,7-Nonadiene, (3E,5E)-2,6-Dimethylocta-3,5,7-trien-2-ol, and Bicyclo[5.3.0]decane, 2-methylene-5-(1-methylvinyl)-8-methyl- as UEP1 specific VOCs were consistently detected. We have identified major VOCs in C. parasitica and demonstrated that VOCs production can be affected by CHV1 infection. Further studies on VOCs function will be conducted.

CS1.1.18

CHARACTERIZATION AND FUNCTIONAL ANALYSIS OF CO-INFECTED MYCOVIRUSES FROM TRICHODERMA POLYSPORUM

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Mycoviruses are widespread in most fungal groups. Here we report co-infection of two unrelated mycoviruses, Trichoderma polysporum fusagravirus 1 (TpFV1) with a dsRNA genome and Trichoderma polysporum partitivirus 1 (TpPV1) with a bipartite dsRNA genome, in a Trichoderma polysporum strain NFCF205. The genome of TpFV1 showed that the complete genome sequence was 9,502 bp in size and contained two large open reading frames. The deduced amino acid sequence of ORF1 and ORF2 showed highest similarity to the hypothetical protein and RNA-dependent RNA polymerase (RdRp) of Trichoederma atroviride mycovirus (TaMV1). Phylogenetic analysis using RdRp domain sequences revealed that TpFV1 is related to the previously reported fusagraviruses. The genome of TpPV1 consists of two separated dsRNA segments with sizes pf 2,001 bp and 2,048 bp, each of which encoded a single ORF showing highest similarity to the RdRp and capsid protein of known members of Partitiviridae. Northern blot analysis validated that the sequence was derived from the corresponding segments. Following single-spored progeny of parental mycoviruses-containing T. polysporum, we have obtained a virus-free strain and strains with each of fusagravirus or partitivirus. Regardless of mycovirus infections, strains did not show any significant difference in conidia production and β-1,3-glucanase activity. However, the increased mycelial growth was observed in fusagravirus and partitivirus co-infected strain. In addition, the lower growth inhibition in co-cultured Rhizoctonia solani by T. polysporum was observed in co-infected strain. This study will yield new insights on the virus taxonomy and the interactions between the mycoviruses and the plant-pathogenic fungal hosts.

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CS1.1.19

INTO THE WILD BLUE YONDER: FORWARD AND REVERSE GENETICS APPROACHES UNCOVER NOVEL VIRULENCE-RELATED LOCI IN PENICILLIUM EXPANSUM

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Blue mold, an economically impactful post-harvest disease of pome fruits, is caused by the mycotoxigenic fungus Penicillium expansum. In addition to the economic losses caused by P. expansum, food safety is also compromised as this pathogen produces patulin, a mycotoxin regulated by many government agencies worldwide. Although there have been many advances in understanding the molecular biology of P. expansum, there is a need to unravel this pathogen's disease strategies to develop additional management tools. In this study, forward and reverse genetic approaches were used to identify genes involved in blue mold infection biology in apple fruit. For this, P. expansum R19 strain was transformed with Agrobacterium tumefaciens to generate a random T-DNA insertional mutant library. A total of 448 transformants were generated, single-spore propagated, and screened for reduced lesion size on inoculated apple fruits. The mutants ranged in reduced diseased severity from WT levels up to a 20% reduction in lesion diameter. Of these mutants, 6 (T-193, T-275, T-434, T-588, T-625, T-711) were selected and 5 unique genes were identified of interest via TAIL-PCR. Southern Blot hybridization revealed single insertional events where the T-DNA integrated into non-coding regions except in T-625. Gene expression levels of the WT strain confirmed that the identified genes were transcribed both in vitro and in vivo. To further characterize these genes and their role in blue mold decay, 2 deletion mutants (Δt625 and Δt588) and a knock-down strain (t-434KD) were generated for 3 loci. Three loci (T-193, T-275, T-711) were recalcitrant to either deletion or knock-down approaches. Preliminary data suggests that

the obtained deletion mutants phenocopy the T-DNA insertion strains, have virulence penalties during apple fruit decay, and represent previously unknown members of signaling networks and genetic factors that regulate fungal virulence.

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CS1.1.20

IDENTIFICATION OF NOVEL CANDIDATE VIRULENCE GENES OF FUSARIUM GRAMINEARUM USING NETWORK ANALYSIS

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Fusarium graminearum which infects wheat and other cereals is the causative agent of the highly destructive fungal disease Fusarium Head Blight (FHB). FHB causes devastating crop losses by dramatically decreasing grain quality before harvest. Furthermore, the pathogen produces harmful toxins which deem grains unfit for human or animal consumption. Faced with a growing population, climate change, environmental pressures, and fungicide resistance, the ability to control fungal plant pathogens has become a global concern requiring urgent solutions. With F. graminearum being one of the most economically important plant pathogenic fungi globally, increasing our understanding of its ability to infect and inflict disease is paramount.
Recent advances in fungal genomics and NextGen sequencing technologies enabled a substantial number of RNA-sequencing (RNA-seq) studies investigating the genetic interaction between F. graminearum and its cereal hosts during infection. This includes stage and tissue specific investigation of the biphasic F. graminearum infection process (1). Using this dataset, we performed a weighted gene co-expression network analysis (WGCNA) (2) to generate the first fungal pathogen/crop dual co-expression networks in wheat. Virulence specific modules were identified and by studying these modules, we have discovered previously uncharacterised candidate virulence genes in F. graminearum. This includes a novel secondary metabolite cluster and a hub gene encoding for a cell wall protein (FgCWP). Deletion of FgCWP resulted in a defective growth phenotype in vitro and loss of pathogenicity in planta. Overall, this project and its results demonstrate the utility of an integrated network-level analytical approach to provide new knowledge on wheat fungal infection.
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tors promote Fusarium head blight on wheat. PLoS Pathogens, 15(4), p.e1007666.
(2) Langfelder, P. and Horvath, S., 2008. WGCNA: an R package for weighted correlation network analysis. BMC bioinformatics, 9(1), pp.1-13.

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CS1.1.21

D-PROTEIN SPECIFICITY IN FUSARIUM OXYSPORUM FF.SPP. INTERACTIONS

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Effector molecules are used by microbes to suppress immunity and colonize the host. Usually, a specific effector is common to a few strains within the same species and rarely is shared between species. An exception are effectors of Verticillium dahliae (Vd) and Fusarium oxysporum (Fo). In fact, functional studies characterized few effector proteins that are shared by these two plant pathogens and play a role in pathogen-host interaction. One of them is the d gene isolated in Vd (d-Vd) strains belonging to the defoliating pathotype. Vd defoliating pathotypes are strains that cause defoliation on cotton upon infection. Homology analysis showed that many Fo strains has a homolog gene to the d-Vd located the dispensable chromosome. In order to test the functionality of the Fo d-homolog proteins, we produced and purified different version of the protein from Fo f.sp. vasinfectum (Fov), Fo f.sp. radices-cucumerinum (Forc) and non-defoliating Vd strains. In planta assays with the protein showed that all the protein variants can induce wilting on cotton suggesting a conserved functionality. Additionally, we generate a deletion mutant for the d-Forc homolog to test whether the d-Forc plays a role in Forc-Cucurbits interaction. Interestingly, deletion mutants of d-Forc showed a reduction of virulence on cucumber and watermelon after infection while no difference on melon plant.
Homology search showed no functional homology to other predicted protein therefore, the d-Fov homolog was sent for crystal structure analysis. The 3D model was used as a query in structural homology search and the results showed that the d-Fov has a toxin like structure. Currently, we are performing localization experiments via Agrobacterium transient expression in both cotton and Bentha and we are expressing the d-Forc in different Cucurbits species to perform pull-down assays.

CS1.1.22

AN ARABIDOPSIS/CERCOSPORA PATHOSYSTEM TO STUDY THE MOLECULAR BASIS OF CERCOSPORA DISEASES OF SOYBEAN

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Cercospora leaf blight (CLB) is an important disease of soybean worldwide. Historically, Cercospora kikuchii was considered to be the causal agent of CLB, but other Cercospora spp. have recently been associated with the disease. In particular, Cercospora cf. flagellaris has displaced C. kikuchii as the primary causal agent of CLB in the U.S. Although C. cf. flagellaris is associated with a broad range of hosts, little is known about the molecular basis of pathogenesis. A key bottleneck is that CLB symptoms are difficult to reproduce in greenhouse and growth chamber conditions. The goal of this study was to develop a C. cf. flagellaris - Arabidopsis thaliana pathosystem as a proxy for CLB of soybean. We determined that C. cf. flagellaris wild type strain ARCK7—first isolated from soybean leaves affected by CLB—readily infected A. thaliana. Symptoms on A. thaliana consistently appeared six to seven days after inoculation, consistent with reports of latent infection in soybean. Symptoms included necrotic lesions and premature leaf abscission, although foliar bronzing/purpling was not observed. Approximately ten days after inoculation, ARCK7 produced conidia abundantly from mature lesions on leaves of A. thaliana. To determine if the ability to infect A. thaliana was strain-specific, eighteen additional strains of C. cf. flagellaris isolated from soybean were evaluated and confirmed to infect A. thaliana. Experimental conditions including spore concentration, inoculation technique, temperature, light, and humidity were optimized for consistent, reliable infection. The ability of C. cf. flagellaris strains associated with CLB to infect A. thaliana suggests a broad host range for the pathogen, which is an important consideration for disease epidemiology and control. Additionally, the utilization of A. thaliana as a proxy for soybean has numerous advantages for discovering/engineering novel resistance genes and advancing the fundamental understanding of molecular mechanisms underpinning CLB.

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CS1.1.23

TOMATO XYLEM SAP HYDROPHOBINS VDH4 AND VDH5 ARE IMPORTANT FOR LATE STAGES OF VERTICILLIUM DAHLIAE PLANT INFECTION

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Verticillium dahliae causes economic losses to a wide range of crops as a vascular fungal pathogen. This filamentous ascomycete spends long periods of its life cycle in the plant xylem, a unique environment that requires adaptive processes. Specifically, fungal proteins produced in the xylem sap of the plant host may play important roles in colonizing the plant vasculature and in inducing disease symptoms. RNA sequencing revealed over 1,500 fungal transcripts that are significantly more abundant in cells grown in tomato xylem sap compared with pectin-rich medium. Of the 85 genes that are strongly induced in the natural plant environment, four genes encode the hydrophobic proteins Vdh1, Vdh2, Vdh4 and Vdh5. Vdh4 and Vhd5 are structurally distinct from each other and from the three other hydrophobins (Vdh1-3) annotated in V. dahliae JR2. Their functions in the life cycle and virulence of V. dahliae were explored using genetics, cell biology and plant infection experiments. Our data revealed that Vdh4 and Vdh5 are dispensable for V. dahliae development and stress response, while both contribute to full disease development in tomato plants by acting at later colonization stages. We conclude that Vdh4 and Vdh5 are functionally specialized fungal hydrophobins that support pathogenicity against plants.

CS1.1.24

FUSARIUM GRAMINEARUM CHEMOTYPE DIFFERENCES AND VIRULENCE

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We have previously identified F. graminearum strains producing a novel type A-trichothecene. These strains seem to be still confined to North America, but are potentially increasing there in frequency. They produce NX-2 in vitro, the equivalent of 3-acetyl-deoxynivalenol lacking the C-8 keto-group. Interestingly, all natural NX producing isolates were genotyped as 3-ADON producers. Allelic differences at TRI8 are responsible for preferential production of 3-acetyl- or 15-acetyl-deoxynivalenol, while different TRI1 genes determine if the C8 is hydroxylated (and subsequently converted to C8-keto) or not. Since the TRI1 gene is unlinked to the core TRI cluster containing TRI8, it would be expected to freely combine during outcrossing of NX-producers with the 15-ADON chemotype dominating in the field. Apparently, strains combining the 15-ADON version of TRI8 with the NX-version of TRI1 have a selective disadvantage. Comparing the virulence of natural isolates with different chemotypes is usually inconclusive due to abounding differences in the genomic background. We have therefore developed positive-negative selectable transformation markers, which allow construction of near isogenic strains containing swapped alleles. Introduction of the NX-TRI1 allele into the 15-ADON background caused production of NX-4 (equivalent of 15-ADON lacking C8-keto) in vitro and to reduced virulence on wheat. An even stronger reduction of virulence was found in mutants lacking TRI1, which accumulate in calonectrin (lacking both C7 and C8 hydroxylation) in vitro. Interestingly, we found that both, NX-4 and calonectrin, have lower inhibitory activity for wheat ribosomes, which could explain the reduced virulence on wheat. However, the deacetylated compound NX-3 has very similar inhibitory activity to DON. It is unclear whether differences in deacetylation and glycosylation of NX-2 and NX-4 exist in planta, which may also affect the potential of the toxins as virulence factors.

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CS1.1.25

CHARACTERIZATION OF P23, CPCO23, OF CRYPHONECTRIA PARASITICA; FROM THE START OF SPORES TO THE END OF MYCELIAL GROWTH AND PATHOGENICITY.

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Co-chaperon p23 of a heat shock protein 90 (Hsp90) upregulated by Cryphonectria hypovirus 1 (CHV1) and / or tannic acid (TA) treatment, was confirmed by proteomic analysis. The amino acid of CpCo23 showed the highest sequence homology with Neurospora crassa Hsp90 co-chaperone p23 and was found to be Saccharomyces cerevisiae Sba1 ortholog. Transcription of CpCo23 peaked at 24 h with CHV1 and/or TA supplementation or both conditions, and the accumulation of CpCo23 transcripts decreased dramatically with the passage of time in response to CHV1 and TA supplementation. For the functional analysis of CpCo23, a CpCo23-null mutant in which the CpCo23 gene was deleted was obtained and verified by Southern blot analysis. The sporulation rate of ΔCpCo23 was similar to that of the wild-type strain (EP155/2). It was confirmed through single-sporing and microscopic observation that showed very low germination rate. Comparing ΔCpCo23 with the wild-type strain on PDAmb, retarded growth with less aerial mycelia and strong pigmentation was observed. In addition, it was observed that the number of branching of ΔCpCo23 mycelium was decreased. The CHV1-infected ΔCpCo23 with restored mycelial branching and growth except the pigmentation was obtained through mycelial fusion by co-culturing CHV1-infected wild-type strain UEP1 and ΔCpCo23 on the same plate. C. parasitica, a strong plant pathogen, confirmed that ΔCpCo23 was associated with pathogenicity by observing smaller lesions than wild-type in the ΔCpCo23. Therefore, it was confirmed that CpCo23 is related to spore germination rate, mycelial branching, and pathogenicity.

CS1.1.26

HOW FUNGAL NUDIX HYDROLASE EFFECTORS PROMOTE PLANT DISEASE

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Plant pathogens secrete proteins, known as effectors, that often function to modulate plant immune responses. Effectors with predicted Nudix (nucleoside diphosphate linked to moiety-X) hydrolase domains are secreted by diverse plant pathogens, but how they function to promote infection is poorly understood. To understand the roles of Nudix hydrolases effectors in plant disease we have employed structural biology, protein biophysics, biochemistry and in planta studies. Here, I will present our recent findings demonstrating that Nudix hydrolases secreted by pathogenic fungi interfere with plant immune function, remove the protective 5' cap from mRNA transcripts, and hydrolyse inositol pyrophosphates to activate plant phosphate-stress responses.

CS1.1.27

HISTONE ACETYLTRANSFERASES REGULATE EFFECTOR GENE EXPRESSION IN THE WHEAT PATHOGEN ZYMOSEPTORIA TRITICI

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Zymoseptoria tritici is a major pathogen of wheat that, similar to other plant pathogenic fungi, harbours effectors to colonize its host. Although effector genes are frequently located in heterochromatic regions of the genome and are typically silenced in the absence of the host, they are highly expressed at specific stages of the infection. This indicates that modifications in the chromatin structure should be involved in effector gene activation. Accordingly, induction of effectors -including the two well-characterized effector genes, Avr3D1 and AvrStb6- is associated with a reduction in the levels of H3K27me3 and H3K9me3. We have now shown by ChIP-qPCR in planta that the acetylation levels in H3K9 and H3K14 increase in AvrStb6 locus during infection. Therefore, we propose that histone acetylation is involved in effector gene induction during host colonization. In this work, we have functionally characterized 5 Histone Acetyltransferases (KATs) from Z. tritici (Sas2, Sas3, Ngs1, Gcn5 and Elp3). We determined that KATs have distinct roles in growth, development, and infection. For example, ΔSas2 showed enhanced hyphal growth, whereas ΔGcn5 and ΔSas3 produced smaller colonies than the controls. Knock-out mutants led to altered effector gene expression patterns during host infection. Particularly, we demonstrated that ΔSas3 is involved in the activation of effector genes at the cellular level thanks to a reporter line to monitor AvrStb6 expression during the penetration through the stomata. In accordance, ΔSas3 did not show the characteristic necrotic lesions of the wild-type controls and caused a yellowing phenotype on the leaves. Importantly, ΔGcn5 and ΔSas3 mutants displayed few and no pycnidia -respectively- on infected leaves. Overall, the results suggest that his-

tone acetylation mediated by Gcn5 and Sas3 is involved in regulating infection and asexual reproduction. These analyses provide us with new insights into the mechanisms involved in chromatin-mediated regulation of the infection machinery of plant pathogens.

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CS1.1.28

MOLECULAR BASIS OF PLANT CELLULAR UPTAKE OF EXTRACELLULAR VESICLES SECRETED BY BIOTROPHIC PATHOGEN USTILAGO MAYDIS.

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Extracellular vesicles (EVs) are nanoparticles composed of lipid bilayer and released from cells including fungi. EVs contain proteins and nucleic acids, and are considered to deliver such molecules to another cells. However, the molecular basis related to EVs-mediated delivery remains to be unclear particularly in host-pathogen interaction. Here we examined the unknown function of transmembrane protein ILP1 identified by proteome analysis of EVs from biotrophic plant pathogen Ustilago maydis. The deletion mutant of ilp1 showed reduced virulence to host maize plants. Immunocytochemistry revealed that ILP1 proteins are detectable as speckles mainly inside biotrophic filamentous cells, suggesting vesicular localization of ILP1. Structural prediction suggested that ILP1 would have a single-pass transmembrane domain at C-terminus and N-terminal region forms beta-propeller structure, which would mediate protein-protein interaction. Dot blot analysis using EVs from U. maydis strains expressing HA-ILP1 or ILP-HA found that N-terminal region of ILP1 would be exposed to the outside of EVs. When EVs labeled with membrane-anchoring fluorophore were incubated with maize protoplast, plant cell membrane could be labeled with fluorescence, suggesting the occurrence of membrane fusion between EVs and plant cells. In contrast, we could detect a certain number of plant cells that do not show membrane labeling, when EVs lacking ILP1 were applied to plant cells. This suggests that ILP1 would play a role in the intermediation of membrane fusion between EVs and plant cells, possibly through the interaction with a cognate receptor protein localized on plant plasma membrane.

CS1.1.29

CYTOLOGY OF THE PATHOGENIC INTERACTION OF BOTRYTIS CINEREA AND ITS HOST PLANTS

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Botrytis cinerea is a plant pathogen with a wide host range. During invasion, the fungus quickly kills host cells and colonizes dead tissue. Factors shown to contribute to infection are plant cell wall degrading enzymes, cell death inducing proteins (CDIPs), phytotoxic metabolites and induction of plant defence responses such as the hypersensitive response (HR). However, the relative contributions of these factors, and the sequence of events that lead to successful necrotrophic infection are poorly understood. By performing infections with fluorescently labeled B. cinerea wild type and mutant strains, we are investigating the infection process microscopically, to evaluate the role of virulence factors during the different stages of penetration, primary lesion formation and lesion expansion. To evaluate the contribution of plant defence, we are analysing the oxidative burst and the mechanisms of pathogen-induced HR, by using Arabidopsis and tobacco mutants or silenced tissues deficient or suppressed in signaling components related to oxidative burst, PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI).
The virulence of B. cinerea was found to be positively correlated with activation of host oxidative burst and induction of defence gene PR1, indicating that these responses are ineffective against B. cinerea. The loss of multiple CDIPs and two major polygalacturonases resulted in a slight delay in fungal penetration and primary lesion formation, but to significant reduction in lesion expansion. The effects of multiple CDIP deletions were host dependent, indicating that the redundancy of virulence factors contribute to the wide host range of B. cinerea. Several CDIPs have been shown to activate pattern recognition receptors (PRRs), followed by activation of PTI. Nevertheless, Arabidopsis or tobacco mutants lacking the PRR coreceptors BAK1 and SOBIR1 showed similar susceptibility to B. cinerea as wild type plants. Further mutants are tested to identify plant defence pathways that are important for fungal infection of plant resistance.

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CS1.1.30

INTRODUCING DIDYMELLA CARI AND HETEROSPHAERIA SP.: TWO UNIQUE PATHOGENS OF CORIANDER AND CARAWAY BLOSSOMS IN WESTERN CANADA

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Blossom blight diseases significantly impact the stability and sustainability of coriander and biennial caraway production in western Canada, particularly when cool, wet conditions occur during flowering. As niche crops, research into these disease issues has limited history and scope. In our region, the pathogens causing the most damage to caraway, Didymella cari, and to coriander, an unnamed Heterosphaeria species, are new to science. Efforts to determine the disease and life cycle related to these organisms have included field-based epidemiology trials, spore-trapping and commercial crop monitoring, as well as lab-based teleomorph induction experiments. Initial results show that limited dispersal of stubble-borne and secondary inoculum can occur under less than conducive weather conditions. Laboratory studies identified two pathogenic anamorph stages of the unnamed Heterosphaeria species, and revealed QoI fungicide insensitivity among D. cari isolates. Greenhouse studies indicated that Heterosphaeria sp. can also infect cumin, chervil and fennel.

CS1.1.31

QUANTITATIVE PHOSPHOPROTEOMIC ANALYSIS OF APPRESSORIUM DEVELOPMENT BY THE RICE BLAST FUNGUS MAGNAPORTHE ORYZAE IDENTIFIES NOVEL PMK1 TARGETS

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Rice blast is one of the most devastating plant diseases and a major risk to global food security. To infect rice, the blast fungus Magnaporthe oryzae develops a specialised dome-shaped infection structure called an appressorium. Previous work has shown that the Pmk1 mitogen activated protein kinase is essential for development of appressoria and invasive growth in planta. Despite being discovered more than two decades ago, very little is known about how Pmk1 precisely controls infection-related development. We used quantitative phosphoproteomics aproaches to quantify a set of over 2000 phospho-proteins in germinating conidia during appressorium development. We quantified more than 6800 phosphorylation sites using label free quantification, which provides a valuable resource for understanding the regulation of appressorium development. We selected a subset of 440 phospho-peptides for in depth analysis by parallel reaction monitoring (PRM) to accurately quantify changes in phosphorylation, comparing delta-pmk1 mutants with the wild type Guy11. During a time series between 0-6 h post germination, we identified 33 proteins as potential direct Pmk1 targets. This subset includes previously reported members of the Pmk1 pathway such as the transcription factors Hox7 and Mst12 that are required for appressorium development and function, respectively, as well as novel proteins required for plant infection. To verify whether these are direct Pmk1 targets, we used an analogue-sensitive mutant of Pmk1 (pmk1AS) to selectively inhibit Pmk1-dependent phosphorylation from 1h after conidial germination. Phosphorylation of MAPK motifs in several targets were nearly completely abolished in the presence of the ATP-analogue NA-PP1 at 2h, 3h and 4h post germination, confirming these sites as Pmk1-dependent. Our work lays the basis for a step-change in understanding of the regulation of appressorium development and shows the power of quantitative phosphoproteomics to identify new regulators of appressorium development in an important plant fungal pathogen.

CS1.1.32

PLANT CELL DEATH INDUCING PROTEINS OF THE NECROTROPHIC FUNGAL PATHOGENS BOTRYTIS ELLIPTICA AND BOTRYTIS SQUAMOSA

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Botrytis fungi are necrotrophic phytopathogenic Ascomycetes that require cell death to establish disease and colonize the host plant. Cell death is achieved by mean of secreted necrotrophic effector proteins. In this study we aim to identify necrotrophic effectors in the sister species Botrytis elliptica and Botrytis squamosa, host specific in lily and onion, respectively. Despite their occurrence in different host plants, genomic and transcriptomic data indicate that both species share necrotrophic effector genes which are highly expressed during infection in their respective hosts. To study the cell death inducing activity of secreted proteins we grew each Botrytis species in liquid culture and collected all secreted proteins in culture filtrate samples. Infiltration of lily, onion and tobacco leaves with the culture filtrate samples caused a necrotic response. Mass spectrometry analysis revealed the presence of common necrotrophic effectors in both culture filtrates. Since the sample derived from Botrytis elliptica caused the most severe response in all tested plants, this was further fractionated via ion exchange chromatography. Lily infiltration with the different protein fractions showed differences in necrotic response and we found an incremental necrotizing activity when fractions were combined and simultaneously infiltrated. Mass spectrometry analysis of protein fractions identified one particularly abundant protein in the most active fractions. The corresponding gene was subsequently cloned in yeast for heterologous expression. The purified protein was tested alone in different lily genotypes where it caused a necrotic response. Functional analysis of the necrotrophic effector gene and biochemical characterization of the protein are ongoing to assess its contribution to fungal virulence.

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CS1.1.33

ZYMOSEPTORIA PASSERINII, A NEW FUNGAL PATHOSYSTEM MODEL FOR SEPTORIASIS IN WILD AND DOMESTICATED BARLEY

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Zymoseptoria passerinii causes Septoria Speckled Leaf Blotch (SSLB) disease in wild and domesticated barley (Hordeum vulgare) and is a sister species of the prominent wheat pathogen Zymoseptoria tritici responsible for the disease Septoria tritici blotch (STB). The broader host range of Z. passerinii and the diploid nature of barley make it an excellent model for studying host specialization and septoriasis in cereals. Here, we introduce the Z. passerinii in Hordeum pathosystem; we evaluated the ability of ten isolates (six from domesticated- and four from wild-host) to infect three Hordeum species. We observe a similar disease development as the one described for STB in wheat. Z. passerinii strains from domesticated host infected four domesticated barley cultivars and the crop wild relative Hordeum spontaneum. However, only one isolate from a wild host led to the establishment of SSLB on the wild barley species H. murinum. To survey the demographic history of this pathogen, we analyzed whole-genome SNPs of 40 haploid strains: nine from domesticated barley from North America and the remaining from wild grasses from northern Iran. We observe a high extent of clonality among Z. passerinii isolates and identify four populations that reflect the distinct host species from which the isolates were obtained. The phylogenetic clustering suggested a monophyletic origin of Z. passerinii on domesticated hosts, with extensive genome divergence to the isolates from wild grasses (FST = 0.38±0.06). Populations from wild hosts showed a higher extent of genetic variation (π) compared to those coming from domesticated barley. The persistent ability of this pathogen to infect wild grasses suggests that Z. passerinii expanded its host range during barley domestication, despite the observed loss of variation. Moreover, the similarities that we observed in disease progression make this pathosystem a useful tool for identifying effectors of septoriasis in cereal crops.

CS1.1.34

ADAPTATION OF LEPTOSPHAERIA MACULANS TO A NONHOST SPECIES: WHICH GENES ARE INVOLVED?

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Leptosphaeria maculans is a plant-pathogenic fungus that infects Brassica species, including Brassica napus (rapeseed). Breeding for rapeseed varieties with genetic resistance is efficient to control the disease, nevertheless, L. maculans can adapt and evolve to bypass these resistances. Deciphering the mechanisms that enable L. maculans to adapt is essential to manage emergence of new and better adapted isolates. Brassica carinata (Ethiopian mustard, closely related to B. napus) is considered a nonhost species of L. maculans as this fungus cannot infect this plant species. Despite the extreme resistance of Ethiopian mustard, one natural isolate of L. maculans has been identified as causing moderate and atypical symptoms on B. carinata, but unable to infect B. napus. A cross was performed between this isolate (the "carinata" isolate) and an isolate adapted to B. napus (the "napus" isolate). A progeny of a hundred individuals was obtained. With this "carinata" isolate and the progeny, the aim is to identify genes involved in the adaptation of L. maculans toward B. carinata and B. napus. A genetic approach (QTL analysis), a transcriptomic and a genomic analysis will be combined, using the "carinata" and the "napus" isolates, in order to identify L. maculans candidate genes potentially involved in its adaptation to the two Brassica species. Functional analyses on candidate genes identified through these approaches will be performed to confirm their implication in adaptation of L. maculans to its hosts. Here, we focus on the genomic regions identified through the QTL analyses and on functional analyses of a few candidate genes. These first results will provide opportunities to study L. maculans adaptive capacities as well as data to initiate analysis of the extreme resistance of B. carinata.

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CS1.1.35

NEW TOOLS FOR INFECTION STUDIES AND GENETIC TRANSFORMATION OF THE FOREST PATHOGEN DIPLODIA SAPINEA

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Diplodia sapinea is a globally distributed endophyte but also pathogen of conifers, damaging forests worldwide. Common symptoms of D. sapinea infections are shoot tip dieback, tree canker, discoloration, root disease, and tree death. The spread and impact of D. sapinea on forest health is expected to increase in the context of climate change. A better understanding of the biology of D. sapinea and its infection mechanisms is essential for the development of diagnosis and control strategies. Therefore, we developed methods for cultivation and genetic manipulation of D. sapinea in the laboratory. First, an efficient, standardized protocol for the production and storage of highly viable vegetative spores was developed. The spores were then used to infect healthy pine seedlings, comparing different inoculation methods. Most efficient symptom development was achieved when spores were inoculated on small wounds. The application of spores on non-wounded plants led to high rates of asymptomatic infection, suggesting endophytic fungal development. This inoculation method allows highly reproducible large-scale spore infection and virulence assays and promotes the use of D. sapinea as a model for studying the switch from endophytic to pathogenic life styles of forest pathogens.
Second, a protocol for the Agrobacterium-mediated transformation of D. sapinea was developed, allowing molecular genetic studies for the first time. Our method is easy to implement and leads to a high rate of homologous integration. This will allow the creation of gene knockout mutants and the expression of fluorescently labelled proteins for live cell imaging, in order to identify and characterize factors controlling endophytic and pathogenic development. This new tool set will enable experimental verification of hypotheses obtained by previous studies, leading to a better understanding of the opportunistic pathogen and its interaction with the environment and the hosts.

CS1.1.36

GENE REGULATORY NETWORKS IN SOLANUM LYCOPERSICUM DEFENSE RESPONSE AGAINST BOTRYTIS CINEREA MODULATED BY PLANT NITROGEN AVAILABILITY

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Nitrogen (N) is a limiting nutrient for plant growth and crop yield. Despite its role as a nutrient, nitrate, the primary N source in agricultural soils, can act as a signaling molecule that modulates gene expression networks of a wide range of plant processes. Plant N availability influences their capacity to respond effectively when challenged by different pathogens. However, the molecular mechanisms involved in the N-modulation of plant susceptibility to pathogens are poorly characterized. In this work, we show that Solanum lycopersicum defense response to the necrotrophic fungus Botrytis cinerea is affected by plant N availability, with higher susceptibility in nitrate-limiting conditions. In addition, we performed a dual RNA-Seq time-course of infected leaves of plants grown with contrasting nitrate concentrations. Genome-wide expression responses reveal that plant and fungal transcriptomes showed different profiles during the infection. Indeed, only a limited set of plant genes were differentially expressed at early time post-infection (8 hours), compared with a later time (72 hours). In contrast, fungal genes were mostly affected at early time points after inoculation (8-24 hours). Using a systems biology approach, we identified gene regulatory networks implicated in plant-fungus infection under contrasting nitrate conditions. Interestingly, several transcription factors in this network are known key genes involved in hormone plant defense: ethylene (ET) and jasmonic acid (JA). Our results provide insights into potential crosstalk mechanisms between necrotrophic defense response and N status in plants.

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CS1.1.37

VALIDATION AND CHARACTERIZATION OF PYRENOPHORA TERES F. TERES EFFECTOR GENES VR1 AND VR2 CONFERRING VIRULENCE ON RIKA BARLEY

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The fungal pathogen Pyrenophora teres f. teres causes net form net blotch of barley. Previously, a population developed from a cross of P. teres f. teres isolates 15A and 6A was used to identify two loci (VR1 and VR2), conferring virulence on Rika barley. Here, we identified candidate genes for VR1 and VR2, giving priority to genes encoding small, secreted proteins. Five and three genes at the VR1 and VR2 loci, respectively, were identified as the top candidates. CRISPR-Cas9-based gene disruption was used to knockout each of the genes. Disruption of a predicted carboxypeptidase at the VR1 locus and disruption of a gene encoding a hypothetical small, secreted protein at the VR2 locus changed virulent isolates to avirulent. Subsequently, we conducted CRISPR-Cas9-based gene editing (allele swaps) in an avirulent isolate, and the VR1 andVR2 edited strains became virulent on Rika. Collectively, these results validated VR1 and VR2, the genes conferring virulence on Rika barley. VR1 encodes a relatively large secreted effector (64.4 kDa) with a serine carboxypeptidase protease domain and homologous proteins were found in various plant pathogens. In contrast, VR2 (47.6 kDa) lacks any predicted functional domains and homologues only appear in the Pyrenophora genus. Inoculation of the VR1 and VR2 edited strains onto the Rika × Kombar host mapping population showed that both VR1 and VR2 were targeting the same 6H susceptibility locus in Rika barley, however, it is not clear if these proteins are targeting the same host gene or if there are multiple host genes being targeted at the same locus. VR1 expression gradually increased from 4 hours post inoculation (hpi) until peaking at 36 hpi, while VR2 showed maximum expression at 4 hpi and rapidly decreased with the progression of the disease, indicating that VR1 and VR2 may have different functions during infection.

CS1.1.39

THE SECRETED VLPLA2 PHOSPHOLIPASE FROM VERTICILLIUM LONGISPORUM IS A PATHOGENICITY FACTOR TARGETS HOST NUCLEUS AND MODULATES PLANT IMMUNITY

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Phospholipases are lipolytic enzymes that hydrolyze phospholipids. They are involved in several biological processes including signal transduction, cytoskeletal dynamics, protein secretion and microbial virulence. However, their precise role in fungus-plant interactions remain understudied. In the present study, the role of a secreted phospholipase A2 (VIPLA2) are characterized in the phytopathogenic fungus Verticillium longisporum that infects plants in Brassicaceae family and causes severe annual yield losses worldwide. VlPLA2 gene was highly induced in V. longisporum upon B. napus infection and encodes an active phospholipase A2. Transient expression of VlPLA2 in Nicotiana benthamiana plants, resulted in increased production of certain phospholipids compared to the plants where the enzymatically inactive VlPLA2 was expressed (VlPLA2mut). Further, VlPLA2 was able to suppress chitin-induced ROS burst, and hypersensitive response (HR) triggered by the Cf4/Avr4 complex, but not by Pseudomonas syringae pv. tomato DC3000 (PsPto). Verticillium longisporum VlsPLA2 overexpression strains showed increased virulence to Arabidopsis thaliana and B. napus plants, and induction of fungal genes with a confirmed role in pathogenicity. Confocal microscopy showed that VlPLA2 is initially localized to the host nucleus, while is translocated to the chloroplasts at the later time points. In addition, our result showed that VIPLA2 can bind to plant vesicle associated membrane proteins A (VAMP-A), and transported to the nuclear membrane, while the VlPLA-2mut version failed to bind to these vesicles and no nuclear localization was observed. In nucleus, VlPLA2 causes major alterations in gene expression known for their role in plant immunity. In conclusion, the VlPLA2 phospholipase, is a virulence factor, co-regulating induction of pathogenicity factors in fungal cells. It hijacks the host VAMP-A proteins to facilitate entry to the nucleus, where it hydrolyzes phospholipids from the nuclear membrane. This action might act as a signaling cascade, suppressing basal plant immunity responses, such as the PTI-induced HR.

CS1.1.40

FUSARIUM OXYSPORUM RESISTANCE MEDIATED BY FOUR DIFFERENT TOMATO R-GENES CORRELATES WITH ACCUMULATION OF A SHARED SET OF XYLEM SAP "GUARDIANS"

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The fungus Fusarium oxysporum (Fo) is a destructive pathogen causing wilt disease in many crops thereby contributing to food insecurity. Pathogenic strains extensively colonize the host xylem tissues, resulting in wilting, stunting and plant death. Four resistance (R) genes have been introgressed in cultivated tomatoes to protect the plant against Fo f.sp. lycopersici (Fol). These R-genes encode structurally different immune receptors that recognize distinct fungal effector proteins. R-gene-mediated resistance halts the fungus in the xylem preventing disease development. It is unknown which factors restrict the fungus in the vasculature, and whether these molecules are similar for the different R-genes. Using grafting experiments, we show that a resistant rootstock prevents disease development in a susceptible scion, notwithstanding the presence of the fungus in the latter tissues. This implies that xylem sap transported from a resistant rootstock contains compounds that limit Fol proliferation in the foliage. To determine the nature of these compounds, xylem sap proteomes of Fol-inoculated resistant plants were compared to that of mock-treated plants using label-free quantitative LC-MS/MS. As expected, fungal effectors were identified confirming the presence of the pathogen in the xylem vessels of a resistant plant. The abundance of a relatively small fraction (4-12%) of plant-derived proteins changed one-week post inoculation with an avirulent Fol race. Notably, all four R-proteins consistently induced accumulation of the same set of five proteins, making these prime candidates to be causal for resistance. These candidates include pathogenesis-related proteins (PR2, PR4 and PR5) and a leucine-rich repeat protein-like. To assess the involvement of these proteins in Fol resistance, transgenic plants have been generated in which these genes are either knocked out or overexpressed. Preliminary data indicate that these proteins are key players in Fol resistance.

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CS1.1.42

MYCORRHIZAL FUNGI DRIVE PLANT UPTAKE OF THE ANTIOXIDANT ERGOTHIONEINE FROM SOIL AND CONTRIBUTE TO CROP NUTRITIONAL CONTENT

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The amino acid ergothioneine (ERGO) has recently gained attention as an important antioxidant for human health, which has been shown to prevent chronic diseases of the heart and brain. ERGO is known to be produced only by fungi and certain types of bacteria. Humans acquire ERGO exclusively through their diet, and express a transporter for tissue-specific localization, though no specific transporters are known outside of animal systems. Aside from mushrooms, plant-based foods are major sources of ERGO; however, plants don't synthesize ERGO and the mechanism by which they acquire it from microbial sources in the soil is unknown. Arbuscular Mycorrhizal Fungi (AMF) have been shown to affect plant uptake of organic nitrogen compounds from the soil, including proteinaceous amino acids, which was the basis of our hypothesis that AMF contribute to ergothioneine uptake as well. To test this out, we used controlled inoculation trials of asparagus, black beans, oats, wheat, and potatoes, and found that AMF inoculation enhanced the ERGO content in all crops evaluated, and that the level of AMF colonization, which varied among inoculation treatments, positively correlated with ERGO content. Additional work to investigate the mechanism of AMF-mediated transport of ERGO is ongoing. Our results demonstrate a novel role for AMF in their contribution to the nutritional quality of crops by mediating the uptake of a specific bioactive molecule.

CS1.1.43

EXPLORING THE ROLE OF ON HOST HORMONAL MANIPULATION OF GENE CANDIDATES OF A FUNGAL PATHOGEN OF WHEAT

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Manipulation of the plant hormonal network is a mechanism that plant pathogens can exploit to promote virulence and disease. To do so, pathogens secrete molecules, such as proteins or metabolites (also known as effectors); although, their mode of action on hormonal signaling components is not fully understood. Our research focuses on the fungus Zymoseptoria tritici, a devastating pathogen in wheat, responsible for Septoria tritici blotch disease (STB), which has been largely associated with severe economic losses. We investigate whether Z. tritici manipulates hormone-regulated defense pathways by producing its own hormone metabolites or protein effectors that interfere with hormone signaling. Based on phylogenetic analyses and gene expression profiles during in planta infection, we selected genes putatively related to cytokinin production (Ztipt, Ztlog1, Ztfmo), and interference with salicylic acid biosynthesis (ZtIsc1) for functional validation. Using Agrobacterium-mediated transient gene overexpression in the model plant, Nicotiana benthamiana, the cytokinin biosynthetic gene Ztipt was found to induce a cell death phenotype upon infiltration, while the ZtIsc gene promotes immune suppression. These preliminary results suggest a relevance of cytokinin manipulation and salicylic acid signaling for Z. tritici virulence. Ongoing research involves the generation of mutant fungal strains, lacking these candidate genes, to test whether these strains are impaired in virulence during infection in wheat.

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CS1.1.44

EXPLORING THE FUNCTION OF TWO PARALOGOUS F. GRAMINEARUM EFFECTORS REVEALS AN ALTERNATIVE GENETIC PATHWAY REQUIRED FOR VIRULENCE ON WHEAT SPIKES

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The effector repertoire of plant pathogens is a key determinant of the success of pathogen-host interactions. The ascomycete fungus Fusarium graminearum is the causal agent of fusarium head blight (FHB), one of the most destructive diseases threatening wheat production worldwide. Despite the publication of the refined F. graminearum secretome in 2012, prioritising candidates for functional studies from a pool of almost 300 secreted proteins with unknown functions remains problematic (Brown et al., 2012). We have adopted in silico bioinformatic pipelines encompassing a multifaceted approach to effector discovery, incorporating transcriptional analysis (RNA-seq and microarray), proteomics, taxonomic distribution and genomic location of candidates. We identified a paralogous pair of candidate effectors that are expressed during the early symptomless (latent) stage of F. graminearum infection. These effectors, FgSSP34 and FgSSP53 are adjacent to each other on the F. graminearum chromosome and divergently orientated. Both effectors, and their orientation, are highly conserved within the wider F. graminearum species complex (FGSC) and also in some distantly related grass infecting ascomycetes. Of the pair, FgSSP53 induces cell death in the non-host Nicotiana benthamiana, independent of the cell-surface co-receptors NbBAK1 and NbSOBIR1, whereas FgSSP34 fails to induce a detectable response in tobacco. In wheat coleoptile infections ΔFgSSP53, ΔFgSSP34 and ΔFgSSP34ΔFgSSP53 fungal deletion strains produced smaller lesions compared to the wildtype, indicating a reduction in virulence. Whereas, in wheat spike inoculations, both the single ΔFgSSP34 and ΔFgSSP53 strains have reduced virulence, however the double ΔFgSSP34ΔFgSSP53 mutants unexpectedly restored wildtype pathogenicity. These results reveal that the simultaneous loss of two crucial effector paralogs leads to

the abandonment of the preferred primary genetic pathway controlling infection and the adoption of an alternative genetic pathway to ensure successful wheat spike colonisation.

Brown (2012) The predicted secretome of the plant pathogenic fungus Fusarium graminearum: a refined comparative analysis. PLoS One, 7, e33731

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CS1.1.45

NOVEL ECTOMYCORRHIZAL INTERACTIONS IN MAYAN FOREST: MOLECULAR IDENTIFICATION OF DIFFERENT GENERA OF FUNGI ASSOCIATED TO ROOTS OF GYMNOPODIUM FLORIBUNDUM

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Climate change and human activity is causing several disturbances in different ecosystems around the globe. Forest biodiversity and crop management in Latin America are drastically affected by droughts, severe floods and soil contamination. Fungal interactions with plants in rhizosphere contribute to adequate adaptations against biotic and abiotic stress factors and maintains productivity in relevant crops for human consumption. In the case of Mayan area several species of plants in forests have supported microbial biodiversity required to sustain land management. Gymnopodium floribundum (tzitzilche in Mayan language) has a relevant role in absorption of water, mainly in severe floods, and it has noteworthy resistance to hurricanes and droughts. G. floribundum nectar is also highly nutritional for pollinators and thus, most of high-value honey obtained in Mayan area is produced in places where this plant grows. However, most of fungal interactions with this species remains unknown and these relationships are critical in climate change scenario. Based on this available knowledge, this work determined for the first time the interactions of fungal species in external and internal cells of G. floribundum roots. DNA extraction protocols were established for roots and fungal species associated to this tissue. Using molecular markers as ITS region we identified fungal species with interactions in the rhizosphere of G. floribundum which belong to Trichoderma, Agaricus and Tremelloscypha genera. Moreover, microscopic studies also showed for the first time internal colonization of hyphae in G. floribundum roots. This works contributes to identify remarkable species that may be crucial to resilience of biodiversity and land management in Mayan area that are highly affected by climate change. The results could have an impact on food security, biodiversity and could contribute to the discovery of new species of beneficial fungi.

CS1.1.46

INDICATIONS FOR A CONSERVED PATHWAY FOR THE SENSING OF DIVERSE PLANT-DERIVED SIGNALS

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In the last years, a molecular pathway responsible for the chemotropic growth to class III peroxidases was described for different tomato-interacting fungi. The core module of this pathway consists of the cell wall integrity (CWI) MAPK pathway and the upstream pheromone receptors Ste2 and St3. Additionally, NADPH oxidase complex 2 (Nox2) and extracellular superoxide dismutase (SOD) provide reactive oxygen species essential for peroxidase signal amplification.
The hemibiotrophic fungus Colletotrichum graminicola is a maize pathogen infecting several plant tissues like leaves, stems, and roots. We have shown recently that C. graminicola generates two distinct asexual spore types, oval and falcate conidia, adapted for efficient root or leaf infection, respectively. Basis for root infection by oval conidia is the recognition of host-secreted diterpenoids, inducing a chemotropic growth response of oval conidia-derived germlings to maize roots. To get further insights into the molecular mechanism, we have tested the role of the CWI MAPK scaffold CgSo in diterpenoid sensing. Intriguingly, the corresponding mutant was unable to grow towards diterpenoids. Further, we investigated peroxidases as probable chemoattractant for C. graminicola, examining the probable host-specificity of the overall process. Surprisingly, comparable concentrations of peroxidases are eliciting strong directed growth responses in C. graminicola as described for tomato-interacting fungi. Further, deletion mutants in Cgnox2 and Cgso are unable to sense peroxidases, indicating a conserved peroxidase-sensing mechanism in fungi, independent of its relevance for host recognition. Together, these findings raise the questions whether 1) a single conserved pathway exists for the molecular translation of different plant-derived signals and 2) how the sensing of the different signals is achieved. To answer these questions, we are currently investigating additional core pathway components for their role in diterpenoid and peroxidase sensing and test the ability of further root-interacting fungi to respond to both plant derived signals.

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CS1.1.47

VERTICILLIUM DAHLIAE VTA3 PROMOTES ELV1 VIRULENCE FACTOR EXPRESSION IN XYLEM SAP, BUT TAMES MTF1-MEDIATED LATE PLANT COLONIZATION AND MICROSCLEROTIA FORMATION

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Verticillium transcription activator of adhesion 3 (Vta3) is required for plant root colonization and pathogenicity of the soil-borne vascular fungus Verticillium dahliae. RNA sequencing identified Vta3-dependent genetic networks required for growth in tomato xylem sap. Vta3 affects the expression of more than 1,000 transcripts, including candidates with predicted functions in virulence and morphogenesis such as Egh16-like virulence factor 1 (Elv1) and Master transcription factor 1 (Mtf1). The genes encoding Elv1 and Mtf1 were deleted and their functions in V. dahliae growth and virulence on tomato (Solanum lycopersicum) plants were investigated using genetics, plant infection experiments, gene expression studies and phytohormone analyses. Vta3 contributes to virulence by promoting ELV1 expression, which is dispensable for vegetative growth and conidiation. Vta3 decreases Mtf1-mediated infection of tomato plants by V. dahliae at advanced stages, which induces the expression of fungal effector genes and tomato pathogenesis-related protein genes. The levels of pipecolic and salicylic acids functioning in tomato defense signaling against (hemi-) biotrophic pathogens depend on the presence of MTF1, which promotes the formation of resting structures at the end of the infection cycle. In summary, the presence of VTA3 alters gene expression of virulence factors and tames the Mtf1 genetic subnetwork for the late stage of plant colonization and subsequent survival of the fungus in the soil.

CS1.1.48

RECOGNITION OF AVIRULENCE FACTORS BY RESISTANCE PROTEINS CONTRIBUTE TO NON-HOST RESISTANCE IN WHEAT

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Zymoseptoria tritici is a devastating pathogen that is highly specialized on wheat and not pathogenic on other grasses. In contrast, species related to Z.tritici, such as Z.pseudotritici and Z.ardabiliae infect wild grasses species but are incapable of infecting wheat. Resistance of one host genotype to specific genotypes of an adapted pathogen is frequently based on recognition of specific avirulence factors (Avr) of a pathogen by the corresponding host resistance proteins (R). In contrast, resistance of an entire host species (non-host plant) to all the genotypes of a pathogen species (non-adapted pathogen), known as non-host resistance (NHR), is postulated to be based on distinct and mostly unknown molecular mechanisms. In this study, we used Zymoseptoria species to investigate the contribution of Avr-R interactions in NHR.
We first demonstrated that Z. tritici avirulence factors were conserved and expressed in Z.ardabiliae and Z.pseudotritici. We then showed that three of these Avr homolog genes in the related species were recognized by wheat and triggered an immune response. We finally demonstrated that the Avrs are induced during wheat colonization and restrict the infection of the non-adapted Z.ardabiliae. In fact, disruption of a single Avr homolog in Z.ardabiliae leads to the production of pycnidia (asexual spores) in wheat, which was never observed in wheat plants infected with the wild type Z.ardabiliae. In other words, we demonstrated that a non-adapted pathogen can gain virulence towards a non-host plant by losing only one Avr. These results suggest that race-specific resistance and NHR share similar mechanisms involving recognition of Avrs by R proteins. We propose that an accumulation of Avrs in the closely related Zymoseptoria species and their recognition by specific host resistance genes in wheat are responsible for their non-ability to infect wheat.

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CS1.1.49

HOW DO MINI CHROMOSOMES OF THE PLANT PATHOGEN COLLETOTRICHUM HIGGINSIANUM CONTRIBUTE TO ITS HOST RANGE?

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Colletotrichum higginsianum is a hemibiotrophic plant pathogen infecting different Brassicaceae species. Since C. higginsianum is able to infect the model plant Arabidopsis thaliana, this interaction serves as a model system for the analysis of plant pathogen relations. The C. higginsianum mini chromosome 11 is mandatory for this infection process because mutants lacking this chromosome (Δchr11) are not able to switch into the necrotrophic infection phase.
To investigate the role of different genes on mini chromosome 11 during the infection process, we produced deletion mutants lacking different regions of this chromosome. With this approach we could show that genes located on the left arm of the mini chromosome 11 play an important role during the infection process.
We analyzed the importance of mini chromosomes for the host range of C. higginsianum by protoplast fusion experiments with a Colletotrichum destructivum strain that is not virulent on A. thaliana. In these we forced the two strains to exchange and segregate their genetic material. By analyzing the genetic contribution of C. higginsianum to such segregants and their host ranges we investigated genomic regions important for virulence on A. thaliana.
We also report the identification of A. thaliana mutants that become hosts to the disarmed Δchr11 strain. Common genetic features of these A. thaliana lines are examined.

CS1.1.50

FROM RED QUEEN HYPOTHESIS TO FUNCTIONAL REVERSE CHEMICAL ECOLOGY

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Interactions between plants and their microbiota play a central role in the functioning of ecosystems. These interactions are governed by chemical dialogues involving the production of a large number of specialized metabolites of various chemical types. Indeed, organisms, and in particular pathogenic microorganisms, impose very strong selection pressure on plants, forcing them to develop increasingly diversified phytochemical defense systems. In this context of the "evolutionary arms race" formalized by the "Red Queen" hypothesis, herbivory organisms have also developed detoxification systems that enable them to resist these toxic molecules.
From that, we have proposed that the study of enzymes, such as Glutathione transferases (GSTs), involved in detoxification pathways should allow a better understanding of the chemical environment encountered by these organisms. This proposed approach has been successfully tested using GSTs from the white rot Trametes versicolor. A high-throughput method has been indeed developed allowing to quantify the interactions between GSTs from this fungus and more than 300 extracts from different wood species. The obtained data coupled to structural analysis of these isoforms revealed a specific pattern of interactions between this library of extracts and each isoform allowing to unravel the structural and biochemical evolution of ligand binding sites within GSTs. Furthermore, using the same set of data, a positive correlation has been shown between the antifungal properties of these extracts and their interactions with these GSTs. Targeted fractionation based on this method also made it possible to identify antifungal molecules within complex mixtures. In addition, this approach has been used to model the natural sustainability of tropical tree species. Overall, these data strongly support the hypothesis that detoxification systems, and in particular GSTs, can be used as targets in "functional reverse chemical ecology" approaches to characterize functionally and chemically an ecological niche.

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CS1.1.51

UNTANGLING THE EFFECTOR-HOST DOMINANT SUSCEPTIBILITY 'GORDIAN KNOT' INTERACTIONS IN SEPTORIA NODORUM BLOTCH OF WHEAT

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Breeding for durable resistance to fungal diseases is a continual challenge for crop breeders as fungal pathogens have developed many ways to overcome host resistance. Association mapping (AM) of wheat populations infected with pathogen mixtures, is frequently used to seek out novel sources of genetic resistance. However, QTL linked to disease resistance detected through AM are often minor or inconsistent. This is a particular problem with septoria nodorum blotch (SNB) of wheat caused by Parastagonospora nodorum. P. nodorum uses a suite of proteinaceous necrotrophic effectors (NEs) to cause necrosis on wheat carrying a matching dominant susceptibility gene (Tsn/Snn). Interactions between these NEs are complex during infection. Once thought to quantitatively contribute to SNB, we now know that NEs suppresses each other's contribution to disease through complex epistatic interactions. Alluding to the title, the Gordian Knot is used as metaphoric representation of a difficult problem that can be solved using an unconventional approach. We liken the complexity of effector epistasis to the Gordian knot of SNB that impedes progress in resistance breeding. In our study, a genetic element called PE401 was discovered in the promoter of the Tox1 NE gene. PE401 functions as a repressor of Tox1 and suppresses the contribution of Tox8-Snn8 interaction in SNB. P. nodorum isolates in Australia generally lacked PE401 and favour the Tox1-Snn1 interaction in SNB, as opposed to most other wheat-growing regions of the world where P. nodorum isolates predominantly harbour PE401. In the context of crop protection, constant surveillance of the pathogen population for the PE401 frequency will

enable agronomists to provide the best advice to growers on which wheat varieties can be tailored to provide optimal SNB resistance to regional pathogen populations. We advocate for the removal of Snn1 and Snn8 to further minimise the impact of SNB.

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CS1.1.53

UNMASKING A CEREAL KILLER: WHAT TRIGGERS ZT3LYSM EFFECTOR EXPRESSION IN THE FUNGAL PHYTOPATHOGEN ZYMOSEPTORIA TRITICI?

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The fungal phytopathogen Zymoseptoria tritici causes Septoria Leaf Blotch disease on wheat (Triticum aestivum). This devastating disease is associated with high fungicide use and yield losses. Current management strategies do not provide full control of Z. tritici.

An effector protein essential for Z. tritici virulence on wheat, Zt3LysM, sequesters fungal chitin fragments, preventing their recognition by wheat immune receptors and 'masking' the pathogen. It also protects Z. tritici from host chitinases. This effector is strongly upregulated during the initial, symptomless phase of infection, but little is known about how this expression regulation occurs.

Experiments were conducted in planta and in vitro using Zt3LysM promoter::GFP Z. tritici strains. Early findings indicated GFP expression was affected by nutrient source and changed temporally during wheat leaf infection. Future work will verify these observations.

To investigate the consequences and dynamics of Zt3LysM immune suppression, dual RNAseq was performed on Z. tritici WT and mutant strains across a wheat leaf infection time course. The experiment also enables us to address the question of how the pathogen compensates for a lack of Zt3LysM, and survives enhanced wheat defence responses during attempted infection.

Understanding more about Zt3LysM could enable the development of new disease management strategies, blocking the secretion of the effector. This would allow wheat to mount a full immune response to Z. tritici, preventing disease and associated yield losses

CS1.1.54

CHARACTERIZATION AND ANTIFUNGAL ACTIVITY ANALYSIS OF NOVEL DSRNA MYCOVIRUS OF TRICHODERMA HARZIANUM NFCF305

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We reported the 15 agarose gel band patterns of double-stranded RNA (dsRNA) from Trichoderma spp.. Here, we describe that a band pattern XV in Trichoderma harzianum NFCF305, which showed four dsRNA bands at 3.5, 2.6, 2.3 and 1.8 kbp, represents a new member of the proposed family "Alternaviridae". Among these dsRNAs, dsRNA1 at 3.5 kbp encodes RNA-dependent RNA polymerase (RdRp). dsRNA2, dsRNA3, and dsRNA4 at 2.6, 2.3, and 1.8 kbp, respectively, encode hypothetical proteins. BLAST search and evolutionary analysis of the amino acid sequences of RdRp and hypothetical proteins indicated that the dsRNAs represent segments of the genome of a novel alternavirus, designated as Trichoderma harzianum alternavirus 1 (ThAV1). Through repetitive single-spore isolation, we were able to cure mycovirus, which resulted in a virus-free strain. Virus-cured strain did not show any significant difference in growth rate, colony morphology, and conidia production from the virus-infected strain. However, the β-1,3-glucanase activity was increased in the ThAV1-infected strain. This study is the first report of an alternavirus of the proposed family "Alternaviridae" from T. harzianum and revealed the mycovirus-induced fungal enzyme activity.

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CS1.1.55

GENOMIC DETERMINANTS OF ECTOMYCORRHIZA FORMATION AND TRANSCRIPTOMIC FINGERPRINTING OF SYMBIOSIS GENES IN A BASIDIOMYCETE WITH POPLAR

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Forest ecosystems are made up of a wide diversity of organisms that interact at all levels of biological organisation, from genes to communities. In resource-limited environments, trees have associated with mycorrhizal fungi to facilitate the uptake and exchange of nutrients and water as well as carbon elements produced by photosynthetic activities. As such, the emergence of these symbiotic interactions was a major evolutionary innovation for plants and fungi. However, although many fungal genomes have recently been sequenced, the molecular mechanisms underlying ectomycorrhizal traits remain poorly understood. Here, we investigate the basidiomycete Pisolithus microcarpus, an ectomycorrhizal fungus that can form such relationships with eucalyptus trees as well as non-host trees under experimental conditions. Using a unique collection of 41 monokaryons, the parental dikaryon and five dikaryons formed by spontaneous crosses between monokaryons, six ectomycorrhizal traits were measured including mycorrhization rate, which revealed a wide phenotypic variation in the proportion of poplar roots colonised by the different monokaryons, ranging from incompatible to fully compatible strains. Although originating from the same fruitbody, ectomycorrhizal traits of poplar clones inoculated with these strains exhibited contrasting responses, suggesting a genetic role underlying the signalling pathway of these symbiotic organs. Hence, we conducted a quantitative trait locus analysis and found key gene variants in P. microcarpus that are involved in ecto-

mycorrhizal traits. Our results also revealed genetic recombination among monokaryon progeny and random allele sorting at four of the eleven mating type loci known to date. In parallel, we performed gene expression analysis to compare functional responses between compatible and incompatible strains with poplar and found lifestyle-specific transcriptomic profiles for ectomycorrhizal and free-living mycelium tissues. Our study provides a better understanding of the genetic basis underlying ectomycorrhizal symbioses and thus the functioning of forest ecosystems.

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CS1.1.56

NEONECTRIA DITISSIMA TRANSCRIPTOMIC PROFILE REVEALS POTENTIAL EFFECTORS WITH STRUCTURAL SIMILARITY TO OTHER PLANT NECROTROPH VIRULENCE PROTEINS

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European canker, caused by the necrotrophic fungal pathogen Neonectria ditissima, is one of the most damaging apple diseases in New Zealand and north-western European countries. During plant colonization, N. ditissima secretes virulence factors, also known as effectors that can be recognised by plant susceptibility proteins to activate a hypersensitive response (HR). The HR-generated dead tissue serves as an ideal nutrition source for this necrotrophic pathogen and allows for extensive plant colonization. Understanding the molecular basis of N. ditissima virulence may ultimately lead to the formulation of novel control strategies against this necrotroph pathogen. This study reports the first RNA-seq transcriptome of N. ditissima during colonization of apple fruit and twigs. Analysis of this transcriptome revealed temporal waves and host-specific gene clusters during early and late stages of infection. The most upregulated genes in planta were selected for their effector features using bioinformatics tools such as SignalP 5.0, SecretomeP 2.0, WoLFPSORT and EffectorP – fungi 3.0. To further gain insights into their function, the tertiary structures of the candidate effectors (CEs) were predicted using AlphaFold2 and then investigated for similar protein structures using the Dali server. Unique predicted functions were identified for three CEs (g4542, g5809 and g7123) with similarity to virulence proteins in other plant necrotroph fungi. Similarly, these CEs were knocked out of N. ditissima, through CRISPR-Cas9 gene editing, and a reduction of the virulence phenotype in apple fruit and twigs was observed for g4542 KO and g5809 KO. g7123 KO only showed a reduction of virulence in apple twigs which aligns with g7123 transcriptomic profile of being highly upregulated during twig infection but low expressed during fruit infection. Our study reveals

novel effectors in the necrotroph pathogen N. ditissima, their predicted function and the potential interplay of their virulence role in different host tissues.

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CS1.1.57

FUNGAL PLANT-PATHOGEN EFFECTOR MIMIC OF PLANT IMMUNE RECEPTORS

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Fungal plant-pathogens use small-secreted proteins (effectors) to manipulate host immunity and metabolism. The focus of our research is to study effectors from Zymoseptoria tritici, an economically significant pathogen of wheat that is responsible for the disease, Septoria tritici blotch (STB). During infection, Z. tritici undergoes an extended latent growth phase where the pathogen has to evade host defences, before finally inducing necrotic symptoms (approximately two weeks post initial infection). Very little is known about how this pathogen is able to evade host defences, and which of its effectors play important roles as immune suppressors during this phase.

We have identified that Z. tritici expresses a secreted leucine rich repeat (sLRR) during the latent growth phase of infection. Homologues of the Z. tritici sLRR homologue are shared among a limited number of plant pathogens; however, beyond these fungal sequences, the sequences with closest homology are plant immune receptors. Subsequent structural prediction of the Zt-sLRR showed that these effectors share a strong similarity with the ectodomain of BAK1-dependent plant-immune receptors. Therefore, we hypothesized that the Zt-sLRR is being used as a molecular mimic of these plant proteins in order to interfere with plant immune signalling. We have successfully demonstrated that Zt-sLRR is able to suppress BAK1-dependent cell-death when transiently expressed in Nicotiana benthamiana. Similarly, we also showed that the Zt-sLRR is able to suppress BAK1-dependent ROS burst (Flg22-triggered) in N. benthamiana leaf discs, but not BAK1-independent ROS burst (laminarin-triggered). We are currently studying how the Zt-sLRR inhibits these immune responses (i.e., what are its interaction partners?). We have also identified additional effectors that can suppress similar immune responses, suggesting effector redundancy during Z. tritici's infection of wheat.

CS1.1.58

EXPLOITATION OF THE LEPTOSPHAERIA MACULANS LATE EFFECTOR REPERTOIRE FOR DIVERSIFICATION OF RESISTANCES TO BLACKLEG IN BRASSICA NAPUS

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Leptosphaeria maculans is a phytopathogenic fungus being responsible for a damaging disease of oilseed rape (Brassica napus): stem canker. The disease is mainly controlled by plant genetic resistance: single-gene specific resistance or quantitative, adult-stage resistance. During its particularly complex and long infectious cycle, L. maculans colonizes asymptomatically the stems of oilseed rape, producing late effectors specific to this colonization stage. In the context of a strong need to identify new sources of disease resistance, we exploited the repertoire of 'late' effectors to identify genes in the plant that could contribute to quantitative disease resistance. Our hypothesis was that quantitative resistance partly rely on gene-for-gene interactions, with fungal effectors produced during stem infection being recognized by resistance proteins. Using an innovative strategy of early expression of late effector genes, we validated that the interaction between the late effector LmSTEE98 and the resistance RlmSTEE98 obeys a typical gene-for-gene interaction, occurring during the colonization of oilseed rape stems by L. maculans, that contributes partly to quantitative resistance, in controlled conditions. We then used the same strategy to search for new sources of resistance after having established criteria to select the most relevant late effectors, and chosen ten of these for screening. Our screening approach of 130 diversified genotypes representative of the available diversity of B. napus, allowed us to identify new sources of resistance, displaying diversified interaction phenotypes. The next steps of this project now are further validation of the efficacy of the new sources of resistance in the field and of the validity of the quantitative resistance markers. However, as it stands, our results demonstrate the existence of unsuspected sources of resistance that are potentially more durable than the classic major genes expressed early after penetration in plant tissues.

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CS1.1.59

A LEAP INTO THE UNKNOWN: UNDERSTANDING HOST-JUMPING BY FUSARIUM OXYSPORUM IN CUCURBITS

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Fusarium wilt disease, caused by the fungus Fusarium oxysporum (Fo), affects over one hundred plant species, resulting in significant crop losses globally. Pathogenic Fo strains are often host specific, only able to infect one or a few related plant species. Fo. f. sp melonis (Fom) and Fo f. sp. cucumerinum (Foc) are host-specific Fusarium strains that cause disease in melon and in cucumber, respectively. In contrast, Fo f. sp. radicis-cucumerinum (Forc) can infect three different hosts within the cucurbits: cucumber, melon and watermelon. In previous research, the first 'non-host' avirulence gene was found in Fom. Transferring this gene, which encodes a small secreted protein, into a Forc strain compromised its ability to infect cucumber. Based on these findings, we aim to determine plant responses that control compatibility with cucurbit-infecting Fo isolates and to uncover how Fo evolved compatibility towards different cucurbit species. To identify the role of the aforementioned avirulence gene and its homologs in host compatibility, knock-out mutant strains of Fom and Forc were generated using a CRISPR/Cas9-mediated genome editing approach. The knock-out mutants are currently being tested in bioassays for altered virulence on cucurbits.

CS1.2 BIOACTIVE METABOLITES, SECONDARY METABOLITES

CS1.2.9

FORMATION OF LACTYL- AND PROPIONYL-DON

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The use of lactic acid bacteria is increasingly proposed for stabilization of Fusarium damaged and deoxynivalenol (DON) contaminated grain. The compound 3-lactyl-DON was reported in 1982 as the toxic principle “responsible for vomiting in humans and swine” in naturally contaminated barley in China. Theoretically, 3-lactyl-DON could be produced by a) DON challenged lactic acid bacteria, b) by plant cells (in the presence of DON and lactic acid), or c) by lactic acid stressed Fusarium. We found that the FgTri101 acetyltransferase can utilize lactyl-CoA instead of acetyl-CoA to acylate the C3-OH of DON. Lactate is utilized via lactate dehydrogenase and pyruvate-dehydrogenase, so normally no lactyl-CoA is formed. Yet, Escherichia coli possesses prpE encoding propionyl-CoA synthase that can also act also as lactyl-CoA synthase (ATP + CoA + Lactate = Lactyl-CoA + AMP + PPi). Combining recombinant affinity-purified prpE and FgTri101 proteins in vitro with these substrates allowed enzymatic synthesis of both 3-lactyl-DON and 3-propionyl-DON in good yield, which were purified by preparative HPLC. The structures were confirmed by NMR. F. graminearum possesses a functional propionyl-CoA synthase (FGSG_10126) which can fully replace prpE. Interestingly, both lactyl-DON and propionyl-DON can be hydrolyzed back to DON by the 15-ADON chemotype Tri8 esterase, and also by human cells. The enzymatically generated standards are currently used to investigate under which conditions the “new” (potentially re-emerging) modified mycotoxins are formed, and to characterize their toxicological properties.

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CS1.2.10

SIMILAR PHENOTYPES OF WCOA AND WCOB MUTANTS REVEAL REGULATORY FUNCTIONS AS A COMPLEX IN FUSARIUM FUJIKUROI

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The fungus Fusarium fujikuroi has a rich secondary metabolism, subject to the regulation of different environmental signals. Among them, light has received special attention. In other fungi, the main light regulation system is mediated by the White-Collar complex, formed by a flavoprotein of type WC-1 and its partner WC-22. In F. fujikuroi, special attention has been paid to photoinduction of carotenogenesis as a model of regulation by light, and mutants of the orthologous WC-1 gene, wcoA, show a strong decrease in transcript levels of carotenoids biosynthesis structural genes in both light and dark, although they retain a photoresponse and maintain the production of carotenoids. Interestingly, the wcoA mutation in F. fujikuroi also affects other pathways of secondary metabolism, both in light and in dark, indicating that WcoA is a key regulator of many metabolic processes.

In this work, we have proposed to find out if the diversity of phenotypic effects of the wcoA mutation is also shown in mutants lacking its partner ortholog WC-2 gene, wcoB. Complete phenotypic screening of both types of mutants has been carried out. This includes the kinetics of photoinduction of carotenoid biosynthesis and the effects on the expression of cryD and opsA photoreceptors, as well as the production and regulation of other secondary metabolism pathways, such as those for bikaverins and gibberellins. As a result, almost all regulatory alterations in the wcoA mutants were observed in the wcoB mutants, either in light or in darkness, supporting the joint regulatory action of WcoA and WcoB forming a complex. As the only exception, a strong decrease in conidiation was observed in wcoA mutants that was not seen in wcoB mutants, indicating a regulatory action of WcoA by itself in this important developmental process.

CS1.2.11

DETANGLING THE MYSTERY OF THE INTRIGUING ODOR OF DERMATOPHYTES

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Dermatophytes, filamentous fungi from the Arthrodermataceae family, the most common fungal skin pathogens of mammals produce a whole variety of secondary metabolites. A specific group of secondary metabolites is called volatile organic compounds (VOCs), some of them are compounds forming the specific odour, produced during growth of fungi. VOCs were previously described as a taxonomy tool. In addition to that, the role of VOCs in the ecology of different fungi was hypothesized. 14 taxonomical groups of dermatophytes were analysed in this study. The SPME fibre was used to sorb the compounds directly from the headspace of dermatophytes cultivated on sheep wool. Not only the odour creating VOCs were characterised, also their role in ecology of dermatophytes was inspected. From the 80 features found by the GC-MS analysis, some may be used as potential markers for the diagnosis of general dermatophyte infection. We also tested whether the GC-MS spectra of VOCs correspond with the phylogenetic analysis of the species. The clustering analysis on the whole GC-MS spectra was performed, however, the result did not correspond with the phylogenetic analysis. The bioassays of characterised compounds, which were also verified by standards, suggest the antimicrobial potential and therefore a role in the ecology of the dermatophytes.

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CS1.2.12

ALTERATION OF THE CHROMATIN LANDSCAPE VIA CCLA DELETION MODULATES SECONDARY METABOLISM PRODUCTION IN ASPERGILLUS TERREUS

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Secondary metabolites (SMs) are complex, structurally heterogenous organic compounds that can be produced by fungi, bacteria, and plants. SMs are of profound interest in the scientific and medical communities because of their potent bioactivities. Functional analyses of fungal genomes suggest that these organisms have the ability to biosynthesize a large number of SMs. A significant problem in SM discovery is that the majority of putative biosynthetic gene clusters are inactive under standard laboratory conditions. For this reason, it would be greatly beneficial to employ methodologies to activate these silent biosynthetic gene clusters, facilitating discovery of novel SMs. One gene common to several Aspergillus species, cclA, has been identified as a modulator of secondary metabolite production. The cclA gene product works to restrict SM biosynthesis through histone 3 lysine 4 methylation, a process which tightly wraps DNA around histone proteins, thereby suppressing SM biosynthetic gene clusters (1). Thus, deletion of cclA would effectively alter the chromatin landscape, permitting transcription of genes involved in SM biosynthesis. The goal of our project is to delete cclA from Aspergillus terreus ATCC20516 in an effort to stimulate production of SMs. Preliminary data suggest that cclA deletion successfully altered SM production relative to the wild-type strain, with 25 unique compounds being upregulated by several orders of magnitude. Thus, cclA deletion stands as a viable way to significantly increase the yield of desired SMs under otherwise standard conditions.

(1) PeerJ: Loss of CclA, required for histone 3 lysine 4 methylation, decreases growth but increases secondary metabolite production in Aspergillus fumigatus 2013; Palmer et Al.; Page 2

CS1.2.13

FUNGAL MELANIN BIOSYNTHESIS PATHWAY AS SOURCE FOR FUNGAL TOXINS

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The black mold Alternaria alternata is one of the most widespread contaminants of food and feed, and a weak plant pathogen. It produces a large diversity of secondary metabolites with alternariol and its derivatives as prominent examples. Other important phyto- and mycotoxins are perylene quinones (PQs), some of which exhibiting some anticancer activity. We discovered recently that the PQ altertoxin (ATX) biosynthesis shares most enzymes with the DHN-melanin pathway1. However, melanin is formed in aerial hyphae and spores, but ATXs are synthesized in substrate hyphae. Furthermore, we proved that 1,8-DHN is the last common intermediate required for DHN-melanin and ATXs formation. However, the enzyme dimerizing 1,8-DHN to ATXs remained unknown. To identify the dimerization enzyme encoding gene, we performed genome-wide expression analyses with different mutant strains producing much more or much less ATXs as compared to the wild type. A small gene cluster was discovered where the expression in the different mutants correlated well with the amount of ATXs formed. The cluster contains six genes, namely the transcription factor encoding gene atbA, the dehydrogenase encoding gene atbB, the anthrone oxygenase encoding gene atbC, the oxidoreductase encoding gene atbD, the cytochrome P450 encoding gene atbE and the major facilitator superfamily transporter encoding gene atbF. Deletion of atbA resulted in a strain unable to produce ATXs, suggesting a key role of this gene cluster in ATXs biosynthesis. Hence, ATXs are an example for secondary metabolites which require two gene clusters plus several genes scattered in the genome. One of the other genes, atbB-E, should code for the dimerizing enzyme. Deletion experiments to solve this question are on the way.

Reference

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CS1.2.14

FUNCTIONAL ANALYSIS OF THE PROTOCATECHUATE BRANCH OF THE B-KETOADIPATE PATHWAY IN ASPERGILLUS NIGER

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Plants produce an abundant supply of aromatic compounds which many bacterial and fungal species are able to catabolize by funneling into one of seven central intermediates prior to ring fission. Two of the most common of these intermediates are protocatechuic acid and catechol, which converge on β-ketoadipate before further conversion to TCA cycle intermediates. While the β-ketoadipate pathways are well understood in bacteria, these pathways in fungi have not been fully characterized and knowledge of the genes involved is incomplete. In this study we have used functional annotation and data from transcriptome sequencing in the presence of protocatechuic acid to predict the genes possibly encoding each of the enzymes involved in the protocatechuic acid branch of the β-ketoadipate pathway in the filamentous fungus Aspergillus niger. For each candidate gene, single deletion mutants were generated and grown on protocatechuic acid to observe the growth phenotype and detect the accumulation of metabolites by mass spectrometry. Enzyme assays were also performed using recombinant proteins encoded by each of the candidate genes. Based on the results of these experiments we have assigned genes for each of the five enzymes in the protocatechuic acid branch of the pathway: protocatechuate 3,4-dioxygenase is encoded by NRRL3_01405 (prcA); carboxy-cis,cis-muconate cyclase is encoded by NRRL3_02586 (cmcA); 3-carboxymuconolactone hydrolase/decarboxylase is encoded by NRRL3_01409 (chdA); β-ketoadipate:succinyl-CoA transferase is encoded by NRRL3_01886 (kstA); and β-ketoadipyl-CoA thiolase is encoded by NRRL3_01526 (kctA). A sixth gene, NRRL3_00837, was essential for growth using protocatechuic acid as the sole carbon source, however its function is currently unknown.

CS1.2.15

LIBERATION OF PHENOLICS FROM OAT HULL SAMPLES BY ZYGOMYCETES FUNGAL ENZYMES

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Phenolic compounds are important secondary metabolites in plants. In addition, these molecules have beneficial effects on the human health through antioxidative, antimicrobial, anti-diabetic, and anti-inflammatory properties. Majority of phenolics can be found in carbohydrate-ester and carbohydrate-glycoside forms in plants decreasing their bioavailability. However, treatment with cellulase and lipase enzymes can be an ecofriendly strategy to liberate these bound phenolics. Oat has gained attention in the recent year, due to its high content of health-beneficial compounds. The hull part (a by-product of oat processing) has been characterized to have higher soluble and bound phenolic content than the groat part. Although many zygomycetes are great extracellular enzyme-producers, their ability to enrich phenolics from oat hull samples is rarely investigated.
An enzyme-assisted approach using a cellulolytic and lipolytic cocktail from Rhizomucor miehei was applied to enrich phenolics from two different oat hull samples.
The enzyme cocktail was produced in a wheat-bran based solid state fermentation system and was partially purified by gel filtration before use. During enzyme treatments, a mass of 1 g grounded hull was treated with 10 mL of enzyme cocktail, and the mixtures were incubated for 7 hours at 50 °C. Samples were taken at predefined intervals, then, total phenolic content (TPC) and antioxidant activity measurements were carried out.
For both hull samples, the TPC increased markedly until the 3rd hour of incubation, that was followed by an increase in antioxidant capacity as well. Hull sample from the colored variety had higher bound phenolic content and exerted higher antioxidant activity. In conclusion, the cellulase/lipase treatment with enzymatic cocktail of R. miehei had a positive effect on the release of antioxidative phenolics from oat hull.
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CS1.2.16

IS THE ABC TRANSPORTER FUM19 INVOLVED IN FUMONISINB1 PRODUCTION IN FUSARIUM SPP?

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Fumonisins are mainly produced by several Fusarium species, such as F. verticillioides and F. proliferatum on maize. An FWF project aiming to study the formation of modified fumonisins and their bioavailability in animals we set out to optimize the production of FB1 to find suitable growth conditions for the later production of 13C- and 14C-labelled FB1. These labelled forms of FB1 will subsequently be used for treatment of fast flowering mini maize (FFMM) and a maize cell culture (Black Mexican Sweet) to investigate the formation of modified fumonisins in planta. A total of 51 Fusarium strains from the BOKU collections were screened on different complex and synthetic media under several growth conditions. The strain performing best on synthetic liquid media containing 3% glucose as sole carbon source had been isolated in the Tulln area in 2015 from maize; it produces close to 1 g/L FB1. In 2020 Janevska et al reported that the proposed fumonisin transporter Fum19 acts as a repressor of FB1 production in the Fusarium verticillioides strain M3125. The authors claimed that deletion of the gene encoding this ABC (ATP binding cassette) transporter leads to an approx. 25-fold increase in FB1 production, while overexpression causes reduction of FB1 formation. This prompted us to investigate if deletion of FUM19 might even further improve the FB1 yield in our isolates. We deleted the FUM19 gene in seven of our best fumonisin producers and compared the FB1 production by the deletion strains to that in untransformed and ectopically transformed control strains. Surprisingly, six of our fum19 strains produced less FB1 than the control strains on various synthetic media, while we observed no significant change in FB1 production in the fum19 mutant of one F. verticillioides. Further investigation on the role of Fum19 as possible repressor of FB1 production is warranted.

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CS1.2.17

CHARACTERIZATION OF LAEA AND ITS IMPACT ON SECONDARY METABOLISM IN PODOSPORA ANSERINA

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Secondary metabolites (SMs) are structurally heterogeneous low-molecular-mass molecules bioactive natural products. The model ascomycete Podospora anserina, a saprophyte fungus developing in a highly competitive environment, constitutes an unexploited reservoir of natural products. More than 40 putative biosynthetic gene clusters (BGCs) have been found in the genome. There are several levels of regulation that control the expression of BGCs, including epigenetic control and environmental signal stimuli. LaeA (loss of aflR expression A) is a global transcription factor, belonging to the velvet complex, which coordinates development and a large number of SM biosynthesis production in response to variation in light levels in many filamentous fungi.
Here, we focus on the functional characterization of LaeA and its involvement in the regulation of the expression of secondary metabolites in P. anserina. For this purpose, the deleted, complemented and overexpressed strains for LaeA gene (ΔPaLaeA, PaLaeAComp and OE-PaLaeA, respectively) were constructed. A large phenotypical analysis was done. In particular, we showed that the loss of the function of LaeA affect the vegetative growth and the sexual reproduction. Accumulation of a pink pigment in liquid culture, and whose identification is in progress, was observed in the ΔPaLaeA strain. LaeA is also involved in the adaptation to osmotic and oxidative stresses. The implication of LaeA in interspecific confrontations was also tested. Finally, by using HPLC-UV-MS approach, three metabolites, Sterigmatocystin, Secosterigmatocystin and an unknown compound (whose identification is also in progress), were specifically found in ΔPaLaeA, when compared to WT.

CS1.2.18

TOWARDS IDENTIFICATION OF THE GENETIC BASIS OF 6-PENTYL-ALPHA-PYRONE BIOSYNTHESIS IN TRICHODERMA ATROVIRIDE

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The filamentous fungus Trichoderma atroviride is commonly used as a biocontrol agent against crop diseases caused by other fungi due to its high antagonistic activity that includes mycoparasitism and the production of antifungal and antibiotic secondary metabolites (SM). One of these bioactive and mycoparasitism associated SMs is 6-pentyl--pyrone (6-PP). This volatile organic compound is produced by some Trichoderma species like Trichoderma atroviride and Trichoderma harzianum but not Trichoderma reseei or Trichoderma virens. We previously showed that 6-PP production in T. atroviride reaches its maximum when the fungus is cultivated in complete darkness but is largely inhibited by white light. Although a recent study has evidenced a polyketide nature of 6-PP, its exact biosynthesis pathway has not yet been elucidated and the genes responsible for 6-PP production are still unknown.
The aim of this study was to identify candidates with a putative role in 6-PP biosynthesis among the 22 PKS genes encoded in T. atroviride. Comparative analyses of PKS gene transcription in the T. atroviride wild type, cultivated in the presence of light or in complete darkness, as well as in mutants with altered 6-PP production resulted in the identification of the most promising candidates. This combination of transcriptomic and metabolomic analyses provided evidence that pks4 is involved in the biosynthesis of 6-PP. Moreover, quantification of 6-PP levels produced by histone deacetylase-deficient mutants missing the hda1 gene revealed a regulatory role of Hda1 on the genes responsible for 6-PP production. Finally, a similar time course of production of 6-PP and 2-pentylfuran was observed which could indicate similarities in the biosynthetic origin and/or physiological role of the two substances.

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CS1.2.19

SECONDARY METABOLITES FROM EARLY DIVERGING FUNGI: PHARMACEUTICAL AND MEDICAL APPLICATIONS

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Fungi represent a prolific resource of bioactive natural products such as antibiotics, cholesterol-lowering drugs and immunomodulating agents. In the past 100 years, natural product research primarily has focused on higher fungi (Dikarya). In contrast, the secondary metabolism of early diverging fungi (EDF) has been scarcely investigated. Moreover, EDF (divisions Mucoromycota and Zoopagomycota) have long time been considered not to produce natural products. Here, we showcase the evolutionary origin, the biosynthesis and the pharmaceutical potential of metabolites of EDF by a combination of genomic, phylogenetic, and expression analyses along with LC/MS-based techniques and bioassays.
Mortierella alpina (Mucoromycota) turned out as a model organism to study biosynthetic routes of EDF and produces preferably leucine-containing peptide families: The malpinins are hexapeptides that are biosynthesized by a highly promiscuous heptamodular nonribosomal peptide synthetase (NRPS) MalA whose terminal module remains inactive. Fluorophore-coupling by click-chemistry revealed that malpinins are carrier peptides to specifically target human macrophages. While the malpinin synthetase gene malA is constitutively expressed, transcription of the NRPS genes mpbA and mpcA strictly rely on fructose in the culture medium. Both NRPS genes are responsible for the biosynthesis of the cyclopentapeptides malpibaldins and the antibiotic malpicyclins. Interestingly, all investigated NRPS genes are not related to counterparts from Dikarya, but may originate from (endo)bacterial NRPS genes. Using a strain screening approach and a heterologous expression system for long NRPS genes (>20 kb), we give evidence that M. alpina additionally produces insecticidal and anti-mycobacte-

rial compounds.
For the first time, we show that a species of the family Kickxellomycotina (Zoopagomycota) produces secondary metabolites. We observed a glucose-dependent production of polyenic compounds. In addition, some compounds are active against plant pathogenic oomycete . Biosynthesis of these compounds is currently under investigation.
Hence, EDF are an underestimated resource of compounds with potential applications in medicine, pharmacy and agriculture.

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CS1.2.21

INTERDISCIPLINARY APPROACH FOR THE CHARACTERIZATION OF ANNULARINS IN THE FILAMENTOUS FUNGUS PODOSPORA ANSERINA

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Podospora anserina has been considered as an efficient laboratory model ascomycete as well as a huge reservoir of specialized metabolites, as others coprophilous fungi which evolved in the complex ecosystem of herbivore dungs (Bills et al., 2013). Comprehensive research has conducted on the morphology, cytology, senescence hyphal interference, while fewer on chemical investigations of this specie, leaving the contribution of secondary metabolites during the life cycle of P. anserina unclear. With the whole genome sequence analyzed and current mutation protoplasts for transformation well-established, we functionally characterized the involvement and interplay of the genes PaStcA PaVvd and PaNsdD (Shen et al., 2019, 2022) in the biosynthesis pathway of the sterigmagtocystin in P. anserina, during which, a small molecule annularin F, was isolated recently. This specified metabolite, bearing an α-pyrone core structure and biosynthesis gene cluster remaining unknown, has motivated us to decipher the code between molecular genetics biology and natural products chemistry, leading to the illustration of biosynthesis pathway of annularins by interdisciplinary approach. Considering the 3-substituted methoxy characterized in their chemical structures, we narrowed the candidates into 3 potential gene clusters assembled with putative methyltransferase, 3 inactivation mutants of targeted PKS genes were constructed respectively. LC-MS analysis of the extracts from those mutants were compared with that from the wild type and revealed that there was no annularin F traced from the culture of one mutant ΔPa_4_3840, indicating that the cluster 19 could be the potential one involved in the biosynthesis of annularins in P. anserina. Mutants of overexpression and complementation are supposed to be constructed to verify and further stand

for our point. Meanwhile, phenotype characterization of those strains could facilitate a better understanding of physiological functions of this gene during the life cycle.

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CS1.2.22

STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF SIDF, A POSSIBLE SECOND ASPERGILLUS FUMIGATUS N5-ACETYL-N5-HYDROXY-L-ORNITHINE TRANSACETYLASE

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Siderophores, microbe-produced iron-sequestering chelators, play an important role in the storage and acquisition of iron and are consequently crucial for pathogens during host infection. The mold Aspergillus fumigatus excretes fusarinine C (FsC) and triacetylfusarinine C (TAFC) for iron uptake. Moreover, this fungal specie produces ferricrocin (FC) and hydroxyferricrocin (HFC) for intracellular iron handling in hyphae and conidia. The transacylases SidF and SidL, which are classified into the same protein family but are not closely related, were previously reported to produce the siderophore pathway intermediates N5-anhydromevalonyl-N5-hydroxy-L-ornithine and N5-acetyl-N5-hydroxy-L-ornithine for the synthesis of the extracellular and intracellular siderophores, respectively. In contrast to SidF, the expression of SidL was shown to be not regulated by iron availability.
Here we report for the first time the SidF structure at a resolution of 1.87 Å using X-ray crystallography. We discovered that SidF comprises two domains, with the N-terminal largely resembling the C-terminal domain. The conserved motif characterizing Gcn5-related N-acetyltransferases (GNATS), however, is found only in the C-terminal domain. In line, Ellman's assays indicated that the N-terminal domain is enzymatically inactive. SidF is observed as a unique tetramer in crystal structure and in solution, which was confirmed by Small Angle X-ray Scattering (SAXS). Surprisingly, a computed structure model of SidL revealed a similar structure to SidF. Previous studies indicated that SidF uses anhydromevalonyl-CoA as a donor and N5-hydroxy-L-ornithine as an acceptor. Remarkably, Ellman's assays indicated that SidF can accept acetyl-CoA as a donor, as previously proposed for SidL, and has some

selectivity to the N5-hydroxy-L-ornithine acceptor. Previous studies indicated that A. fumigatus possesses an unidentified N5-acetyl-N5-hydroxy-L-ornithine transacetylase that complements SidL activity mainly during iron limitation. Therefore, we propose that SidF could be the second N5-acetyl-N5-hydroxy-L-ornithine transacetylase.
Taken together, our results elucidated the SidF protein structure and suggested a novel function for SidF in siderophore biosynthesis.

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CS1.2.24

ACTIVATION OF AN UNUSUAL BIOSYNTHETIC GENE CLUSTER IN TRICHODERMA REESEI USING SYNTHETIC TRANSCRIPTION FACTORS

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Most biosynthetic gene clusters of the industrial working horse Trichoderma reesei remain silent under standard laboratory conditions. Therefore, the full repertoire of potentially bioactive secondary metabolites formed by this fungus has yet to be exploited. One biosynthetic gene cluster in T. reesei seems to have evolved by the fusion of two distinct clusters. We successfully activated both parts of this fusion gene cluster by introducing synthetic transcription factors. Activation was verified by a strong upregulation of the core biosynthetic genes in comparison to the parent strain. Expression analysis sheds more light on the regulation of this complex gene cluster. Culture supernatants of the activated cluster strain displayed antifungal properties against Alternaria alternata, Botrytis cinera, Fusarium oxysporum, and Rhizoctonia solani and antibacterial properties against Escherichia coli. Deletion of the core biosynthetic gene prevented antimicrobial properties of the culture supernatants. Deletion of cluster genes, expression analysis, and chemical and bioactive analysis of cluster products will contribute to a better understanding of this silent fusion cluster.

CS1.2.25

BIOLOGICALLY ACTIVE ISOINDOLINONES FROM S. CHARTARUM OBTAINED BY MICROSCALE SEMISYNTHETIC APPROACH

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Stachybotrys chartarum is a fungus of the genus Stachybotrys whose occurrence is described in dead plant materials and water-damaged buildings. These fungi produce a diverse group of secondary metabolites including macrocyclic trichothecenes, atranones, and phenylspirodrimanes (PSDs). Some known PSD representatives possess highly reactive odialdehyde groups at their aryl fragment. These phenylspirodrimanes including stachybotrydial, stachybotrydial acetate and acetoxystachybotrydial acetate were used as a starting material for the synthesis of their corresponding isoindolinones. To access the library of the PSDs derivatives and screen them against physiologically relevant serine proteases, a microscale semisynthetic approach was developed. Generated library of 35 lactams was tested for the inhibitory activity toward thrombin (FIIa), FXIIa, FXa, and trypsin. Among them, the agmatine-derived lactam showed the highest inhibitory activity. Subsequently, the lead compound was shown to demonstrate the anticoagulant properties in two plasma coagulation tests. The stability test and metabolism study of the agmatine derivative were performed by using human liver microsomes. Moreover, it has been demonstrated that semi-synthetic isoindolinones were significantly less toxic compared to their parental natural PSDs. Additional efforts were undertaken to investigate the water-mediated lactonization of isolated PSDs utilizing quantum chemical DFT calculations corroborated by HPLC-MS and NMR experiments.

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CS1.2.26

MINING SECONDARY METABOLITES IN APIOSPORA

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The fungus Apiospora Arundinis has a huge potential for the discovery of novel secondary metabolites i.e. Non- ribosomal peptides (NRP) and polyketides (PKS), because it harbours a great amount of predicted biosynthetic gene clusters, but only few have been linked to their respective secondary metabolites. This project aims to uncover secondary metabolites in Apiospora Arundinis which are not detectable at normal conditions and link some of the known 264 secondary metabolites found in Apiospora Sp. to their respective gene clusters. We are going to overexpress local transcription factors found in clusters of PKS synthetases, NRP synthetases and hybrids thereof by insertion of constitutive promoters. The mutants will be verified by whole genome sequenced (nanopore) and the secondary metabolites will be extracted and then elucidated on HPLC-MS.

CS1.2.27

EARLY DIVERGING FUNGI AS PRODUCERS OF INSECTICIDAL COMPOUNDS

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The fungal realm represents a source for a plethora of bioactive secondary metabolites. Nevertheless, early diverging fungi (EDF) such as Mortierella alpina (division Mucoromycota) have not been in the focus of natural product researchers for many decades. Here, we showcase the production of insecticidal cyclodepsitetrapeptides, called cycloacetamides A – F, by M. alpina. Screening 23 different strains of this species along with precursor-directed biosynthesis facilitated the isolation of six novel threonine-linked compounds. They possess selective insecticidal activity against fruit fly larvae while showing no cytotoxic effects against human cells or microbes. Structure elucidation was performed using 2D-NMR and LC-MS/MS analyses, and the absolute configuration was determined with Marfey's analysis and total synthesis. Therefore a liquid phase peptide synthesis approach with a final macrolactamisation step was successfully implemented. While EDF can harbour endobacteria with the potential to produce nonribsomal peptides, we were able to verify the fungal origin of the cycloacetamides. After iterative antibiotic treatment of the fungus, metabolite production was not affected. Additionally, a subsequent PCR amplification of bacterial 16S rDNA from mycelium of M. alpina failed. Investigation of the responsible nonribosomal peptide synthetase gene is currently under investigation by sequencing the producer strain. This work highlights M. alpina as a model organism to study secondary metabolite biosynthesis of EDF and, moreover, discloses EDF as a fruitful, but yet underrepresented resource of bioactive molecules.

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CS1.2.30

TRANSCRIPTIONAL CHANGES AND RELATED PHYSIOLOGICAL EFFECT CAUSED BY TYROSOL EXPOSURE IN CANDIDA AURIS

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Candida-derived quorum sensing molecules are key regulators in fungal physiology especially in fungal morphogenesis and biofilm development. Under physiological conditions, tyrosol induces the yeast-to-hypha transition in Candida albicans; however, its effect is unknown in the case of the majority of non-albicans species such as Candida auris. In this study, we examined the effect of tyrosol on growth, redox homeostasis, antioxidant enzyme activities, and intracellular metal content. Furthermore, to gain detailed insights into the abovementioned effects, we also determined genome-wide gene expression changes using transcriptome sequencing (RNA-Seq). Fifteen mM tyrosol exposure caused a significant inhibition in growth within 2 hours incubation. Tyrosol increased the production of reactive oxygen species (2',7'-dichlorofluorescein (DCF) [nmol DCF (OD640)-1]: 7.1±1.9 and 16.8±3.9 for untreated and treated culture, respectively), which were associated with the increased superoxide dismutase, catalase, and glutathione peroxidase activity. Tyrosol treatment significantly decreased the intracellular iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) contents compared to untreated control cells (mg/kg) (Fe: 114.3±16.2 vs. 152.2±21.1; Mn: 42.6±1.14 vs. 67.9±5.1; Zn: 516.5±22.12 vs. 787.8±22.; Cu: 123.5±9.8 vs. 274.6±15.7). Regarding the total transcriptome, tyrosol exposure resulted in 142 and 108 differentially expressed genes with at least a 1.5-fold increase or decrease in transcription. Genes involved in virulence (ALS4, ALS9, IFF4, LIP10), oxidative stress (CAT1, CIP1, YCP4) showed up-regulation; while genes associated with biofilm development (NRG1, BCR1) fatty-acid metabolism (FAS1, FAS2), and iron metabolism (CFL4, FRE9, FRE10, FTR1, HMX1, RBT5) were down-regulated. Our data showed that tyrosol exposure remarkably influences the physiology and gene expression of Candida auris planktonic cells especially in terms of oxidative stress and iron metabolism,

which contribute the better understanding of tyrosol-related effect in Candida auris.

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CS1.2.32

SECONDARY METABOLITES OF BISCOGNIAUXIA PETRENSIS: AS PRIMARY TARGET AGAINST CANCER

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The pharmaceutical industry has found a wealth of unique secondary metabolites in marine environments, some of which are being tested as potential anticancer therapeutic leads. Numerous findings have shown that marine endophytic fungi are a significant source of these pharmacological leads.
In the present work, we introduce Biscogniauxia petrensis, a marine algae-associated endophytic fungus, isolated from Halimeda macroloba, collected from the Bay of Bengal. Biscogniauxia petrensis showed significant cytotoxicity with CC50 (cytotoxic concentration) values of 18.04 and 24.85 µg/ml against HeLa and A431 cells, respectively. Furthermore, the growth media and extraction solvent optimization revealed the highest cytotoxic compounds in ethyl acetate extracts from the potato dextrose yeast extract broth medium. The bioactive guided purification led us to reach two fractions (C2 and M4) from culture filtrate and mycelia extract with CC50 values of 31.18 and 25.52 g/ml, respectively against HeLa, and neither compound was toxic to non-cancerous cells (HEK) at the same time. C2 and M4 were identified using MetFrag as 2-(1,3-benzothiazol-2-ylsulfanyl) ethanol and 2,2-bis(azidomethyl)butan-1-ol respectively. Both compounds induced up to 60% cell death in HeLa, in a dose-dependent manner and arrested the cell cycle at the Sub-G1 phase to inhibit cell proliferation. The apoptotic cell death was confirmed by Annexin V FITC/PI, MMP loss, DNA fragmentation and Caspase 3/7 activation assays. Adding B. petrensis as a notable apoptotic agent can be employed in pharmaceutical applications, this work is the first to detail the cytotoxic characteristics of an endophytic fungus from the Rameswaram coastline region of Tamil Nadu, India.
Keywords- Marine algae, endophytic fungi, secondary metabolites, anticancer compounds, apoptosis.

CS1.2.34

ACTIVATION OF SECONDARY METABOLITE BIOSYNTHESIS BY TRANSCRIPTION FACTOR OVEREXPRESSION IN ASPERGILLUS TERREUS

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Filamentous fungi, such as Aspergillus terreus, are extensive producers of secondary metabolites that exhibit a diverse range of biological activities. Enzymes involved in secondary metabolite biosynthesis are frequently encoded by genes that are organized within biosynthetic gene clusters. Although several well-known compounds, including lovastatin, are produced by A. terreus, the secondary metabolic capacity of this fungus has remained largely unexplored. To further uncover the secondary metabolome of A. terreus, we replaced, en masse, the promoters of multiple putative $Zn(II)_2Cys_6$ transcription factors with the constitutive gpdA promoter. These transcription factors were selected due to their colocalization within biosynthetic gene clusters. Metabolic profiling of the transcription factor-overexpressing strains revealed the production of several secondary metabolites. Current work is focusing on structural elucidation of these compounds. The activation of secondary metabolite production by Aspergillus terreus supports the immense potential for discovery and production of pharmacologically and industrially relevant secondary metabolites.

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CS1.2.35

INDUCTION OF CRYPTIC GENE CLUSTERS IN ASPERGILLUS NIGER FOR NATURAL PRODUCT DISCOVERY

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Microbial interactions have been recognized as the driver force for chemical diversity. Microbial secondary metabolites (SM) are produced by micro-organism to ensure their formidable adaptive capacity for variable environments. The genome sequence of Aspergillus niger has revealed the presence of over 75 gene clusters potentially interesting SM. However, only a part of the SM encoding genes in Aspergillus niger are transcribed under routine laboratory conditions. To screen for conditions that activate particular gene clusters, reporter strains were constructed by fusing the promoter of the core biosynthetic gene from that cluster (encoding a PKS or NRPS) directly upstream of the luciferase gene. The reporter constructs were targeted for integration into the genome by double cross-over at nicB locus. Several reporter strain were constructed and screened for conditions in a 96-wells setting that induce luciferase expression. A particular gene cluster was identified that was induced by pectin and in co-cultivation with Trichoderma reesei or Penicillium chrysogenum in a synergistic manner. Ongoing gene knock outs experiments in combination with LC-MS-MS analyses are carried out to identify the compound(s) produced by this gene cluster. This study provides a new reporter strategy to screen for conditions that activate cryptic SM gene cluster and emphasize the effectiveness of co-cultivation and exploiting the hidden SM treasure.

CS1.2.36

COMPARATIVE EVALUATION OF THE SECONDARY METABOLOME OF TRICHODERMA ATROVIRIDE UNDER THREE COMMON LIGHT CONDITIONS

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Many studies aim at maximizing the production of fungal secondary metabolites. Although it is known that their formation is affected by light, its influence has hardly been systematically studied so far.
Here, we combined an untargeted isotope-assisted liquid chromatography - high resolution mass spectrometric metabolomics approach with standardized cultivation of Trichoderma atroviride on minimal medium to compare the number and relative amount of secondary metabolites produced under three light regimes: complete darkness, minimal light exposure and 12/12 h light/dark cycle. For two of them we investigated the secretion and mobility of the metabolites in the medium, by cutting the culture medium into 5 parts before analysis.
The unambiguous assignment of the global metabolome resulted in more than 900 metabolites of fungal origin including the known 6-pentyl-pyrone (6-PP) and peptaibols. For each of them, the abundance in each part of the slide was determined and the metabolites were clustered according to their spatial distribution patterns. The majority of metabolites, including 6-PP, was significantly less abundant under 12h light/dark cycle conditions compared to minimal light- or dark conditions. Interestingly, while the latter two conditions resulted in similar numbers and overall abundance of metabolites, a significant difference remained when comparing the abundances preceding the hyphal growth front.
Our study reveals that cultivation of T.a. under minimal light conditions helps to maximize the production of secondary metabolites.

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CS1.2.37

ACTIVATION AND CHARACTERIZATION OF THE DIAPORTINIC ACID GENE CLUSTER IN TRICHODERMA REESEI

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The industrially important ascomycete Trichoderma reesei possesses a relatively small number of biosynthetic gene clusters in comparison to other Trichoderma species. Further, most of these gene clusters are not active under laboratory conditions. Consequently, little is known about the secondary metabolism of this fungus. We successfully activated a biosynthetic gene cluster by overexpressing the transcription factor contained in the cluster. The core gene, encoding for a polyketide synthase, and other genes were strongly expressed in comparison to a wild-type strain. The overexpression strain secretes diaportinic acid and other related isocoumarins. The culture supernatant exhibits antibacterial and antifungal activities in assays against Escherichia coli, Bacillus subtilis, Rhizoctonia solani, Alternaria alternata, and Botrytis cinerea, respectively. Deletion of the polyketide gene in the overexpression strain abolished the diaportinic acid secretion and the observed antibiotic effects. Deletion of the other genes in the gene cluster followed by transcript analyses, antibiotic assays, and chemical analysis contribute to a detailed characterization of the diaportinic acid gene cluster.

CS1.2.39

SUSTAINABLE PRODUCTION OF INDUSTRIALLY-RELEVANT BIOACTIVE COMPOUNDS BY CONTROLLED FERMENTATION WITH ASCOMYCETE FUNGAL SPECIES

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Among fungi, ascomycetes are known to naturally produce an abundance of bioactive secondary metabolites with a broad spectrum of possible applications in industry, medicine and agriculture. Examples for those highly valued compounds are enzymes, terpenes and volatile aroma compounds. In an effort to establish sustainable fungal production platforms we propose a circular economy, based on the use of agricultural waste streams, such as wheat bran, by natural and efficient hydrolysis. To that end, a highly active, specifically tailored mixture of enzymes was applied to hydrolyze wheat bran. These enzymes were obtained from different ascomycete species cultivated on media supplemented with wheat bran. The resulting sugar-rich hydrolysate was then added to different media formulations for the cultivation of other fungal species producing various industrially-relevant secondary compounds.

For a better understanding of metabolic pathways resulting in the production of these relevant enzymes and compounds, different omics methodologies were deployed. These include whole genome sequencing (PacBIO), transcriptomics and proteomic approaches, differentiating between secretome and intracellular proteins. Knowledge of these pathways may enable the optimization of production processes by specific media optimization, supplementing favorable precursors and nutrients.

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CS1.2.40

STUDY ON THE CONNECTION BETWEEN MYCOTOXINS AND KASHIN-BECK DISEASE IN CHINA

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Kashin-Beck disease (KBD) is a chronic, disabling disease characterized by multiple deformed bones and joints, mainly affecting remote, rural Asian populations. The disease is developing in childhood. Microscopically, the degenerative changes in the KBD cartilage are characterized by deep zone chondrocyte necrosis, middle zone with adjacent necroptosis, and apoptosis. However, the etiology of KBD remains unknown. Selenium deficiency and fungal contamination in the last decade, especially Fusarium mycotoxins, are considered multifactorial environmental risk factors. From 1995-2015, an extension of a Belgian physical therapy program “Doctors Without Borders (MSF)” was carried out in the TAR (Tibetan Autonomous Region) to investigate the role of cereal fungal contamination, mainly Alternaria species, in KBD. We hypothesized that unknown mycotoxins from the Tibetan endemic region may be additional risk factors that cause KBD. We took advantage of the Endemic-Area (EA) and Non-Endemic-Area (NEA) cereal collection to identify compounds specifically present or more abundantly present in the KBD-related regions, especially in Tibet. Metabolomics analysis of the data obtained by liquid chromatography coupled to a high-resolution tandem mass spectrometer (LC-HRMS(/MS)) was performed to analyze compound profiles between the endemic and non-endemic regions areas. Molecular network analyses of the LC-HRMS/MS data further allowed us to identify a new mycotoxin as a specific compound present in the samples from KBD endemic regions. Further analysis with cytotoxicity in vitro and genes involved in Fusarium from cereal samples need to be completed to enable the role of mycotoxin and KBD.

CS1.2.41

MANIPULATION OF MCRA UPREGULATES SECONDARY METABOLITE PRODUCTION IN ASPERGILLUS WENTII USING CRISPR-CAS9 WITH IN VITRO ASSEMBLED RIBONUCLEOPROTEINS

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Most secondary metabolite biosynthetic gene clusters (BGCs) are silent under normal laboratory growth conditions. To activate these BGCs, we deleted the negative transcriptional regulator mcrA by an in vitro CRISPR-Cas9 system in Aspergillus wentii. The deletion of mcrA (mcrAΔ) resulted in differential production of a total 17 secondary metabolites (SMs). Nine out of the initial fifteen SMs purified were fully characterized as emodin (1), physcion (2), sulochrin (3), physcion bianthrone (4), 14-O-demethylsulochrin (5), (trans/cis)-emodin bianthrone (6 and 7), and (trans/cis)-emodin physcion bianthrone (8 and 9). These compounds were all found to be produced by the same polyketide synthase (PKS) BGC. We then performed a secondary knockout targeting this PKS cluster in the mcrAΔ background. The dual-knockout strain revealed two additional SMs that were not previously detected in the mcrAΔ parent strain – aspergillus acid B (16) and a structurally related but previously unidentified compound (17). For the first time, this work offers a simple genetic system capable of targeted gene editing in A. wentii, illustrating the utility of performing a dual knockout to exclude major metabolic products and enable additional SM discovery.

CS1.2.42

FUNCTIONAL ANALYSIS OF TWO TRANSCRIPTION FACTOR-ENCODING GENES WITHIN SECONDARY METABOLITE BIOSYNTHETIC CLUSTERS IN PARASTAGONOSPORA NODORUM

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The necrotrophic fungus Parastagonospora nodorum, the causal agent of glume blotch on wheat is responsible for major economic losses worldwide. Genome mining of P. nodorum revealed that it harbors 44 secondary metabolite biosynthetic gene clusters (BGCs), some of which contain a predicted transcription factor (TF) gene that might regulate the corresponding BGC expression. Here, we assessed the role of two BGCs in the pathogenicity of P. nodorum by deleting the TF genes they contain. The selected BGCs share similarities with BGCs linked to choline (synthesized by a class V adenylate forming reductase) and duclauxin (synthesized by a non-reducing polyketide synthase) production, respectively, in other fungi. Expression profiling of both TF genes showed they are induced under carbon and nitrogen starvation and the choline BGC TF is highly up-regulated at early stages of infection compared to the late stages. Gene deletion mutants of both TFs exhibit sporulation defect. While deletion of the TF in the duclauxin-like BGC led to mutants with pigmentation defect but no alteration on virulence, deletion mutants of the TF in the choline BGC are non-pathogenic, consistent with the gene expression profile. These results point the choline metabolism as important for the pathogenicity of P. nodorum, while duclauxin-like compounds contribute to the pigmentation of P. nodorum and might have a protection role outside of the host plant.
Keywords: Transcription factor, Gene deletion, Secondary metabolite, Pathogenicity

CS1.3 GENOME FUNCTION AND EPIGENETICS

CS1.3.9

ROLE OF PENICILLIUM EXPANSUM HISTONE DEACETYLASES HOSA AND HOSB ON GROWTH, CONIDIATION, PATULIN PRODUCTION AND VIRULENCE

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Penicillium expansum is one of the most common foodborne fungi on pome fruits, causing the 'blue mold rot'. P. expansum is of particular concern in fruit products because of the production of patulin. This polyketide-derived mycotoxin has a wide range of toxic effects, including cytotoxicity or genotoxicity. This fact has generated great concern in society, and therefore different international authorities have established maximum patulin levels in different food products.
Recent studies have shown that the secondary metabolism of filamentous fungi is controlled by a complicated regulatory network, which is influenced not only by various transcription factors but also by epigenetic regulators. Histone posttranslational modifications such as methylation, acetylation, and phosphorylation, among others, are key epigenetic mechanisms for gene regulation in response to environmental stimuli. In particular, histone acetylation is an important modification for the regulation of chromatin accessibility and is controlled by two kinds of histone-modifying enzymes: histone acetyltransferases (HATs) and histone deacetylases (HDACs). In filamentous fungi, HATs and HDACs are involved in mycelial growth, stress response, pathogenicity and secondary metabolite production.
In this study, to understand the role of HDACs in the regulation of patulin biosynthesis in P. expansum, hosA and hosB – genes encoding two of the classical family HDACs – were deleted. While the ΔhosB mutant maintained a phenotype similar to the wild-type strain, the ΔhosA mutant showed a characteristic fluffy phenotype, a drastic reduction in conidiation, and a marked decrease in patulin production. The complemented strains recovered the wild type phenotype. In addition, the ΔhosA mutant showed a slight reduction in virulence, which may be attributed to delayed growth and impaired conidiation.

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CS1.3.10

CHARACTERISATION OF SEXUAL REPRODUCTION MECHANISMS OF PYRICULARIA ORYZAE TO DETERMINE THE GENETIC BASES OF BOTH MALE AND FEMALE FERTILITY.

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The reproductive system of an organism affects the emergence and evolution of adaptive variants in response to selective constraints. Comprehension of the mode of reproduction in pathogens helps to understand their life history. Mechanisms and genes involved in sexual reproduction in Ascomycetes, are relatively well described in several model organisms such as Neurospora crassa or Podospora anserina (Brun et al., 2021). However, in Pyricularia oryzae, the fungal pathogen responsible for blast diseases on several species of Poaceae, the biology of sexual reproduction remains poorly documented and phenotyping of fertility is based on the result of sexual reproduction, ie the formation of perithecia (Saleh et al., 2012). Previous studies had reported microconidia in P. oryzae (Chuma et al., 2009) that were recently demonstrated to be the male gametes (Lassagne et al., 2022).
The identification of microconidia as male gametes in P. oryzae allowed a precise phenotyping of male fertility by counting microconidia. Following this new method, we measured the male fertility of strains from a recombinant population isolated from a rice field. We also sequenced the genome of the same strains. Genome Wild Association Study (GWAS) analysis based on these phenotypic and genotypic data permitted to identify 3 genomic regions involved in male fertility.
To identify genes involved in female fertility, a GWAS analysis was also performed on the number of perithecia formed by strains of the P. oryzae population mentioned above. A complementary approach based on spontaneous mutants was also used to identify genes involved in female fertility. Bulk Segregant Analysis was performed on pools of female fertile and female sterile progenies of crosses between female fertile wild-type strains and female sterile mutants (Saleh et al., 2012).

CS1.3.11

ROLE OF HMBC, A PROTEIN OF THE HMG-BOX FAMILY, IN FUSARIUM FUJIKUROI

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The phytopathegenic fungus Fusarium fujikuroi is a study model for secondary metabolite production that includes giberellins, bikaverin, fusarins, or carotenoids. The car genes responsible for the biosynthetic steps of carotenogenesis pathway in this fungus, carB, carX, carRA, are clustered with the rhodopsin gene carO and regulated by light and by CarS repressor [1]
To identify new potential regulators of these genes, a biotin-mediated pulldown of proteins that bind to car promoters was carried out with wild type and carS mutant extracts. In this screening, among the proteins more frequently bound to the carB and carX-carRA promoters, two proteins of the HMG-box family stood out because of their frequency and the differences between both extracts. Knock out mutants of the hmbC gene were obtained in F. fujikuroi IMI58289 to study its role in carotenogenesis. The mutants exhibit a more intense orange pigmentation in light or dark conditions that was confirmed by carotenoid analyses, suggesting a possible role of this protein in the carotenogenesis pathway like a possible negative regulator of car genes. To check if the regulation is carried out at transcription level, RT-PCR analysis of car genes will be performed. On the other hand, no protoplasts could be obtained from the hmbC mutants, and therefore gene complementation could not be carried out. Moreover, morphological differences were observed between wild type and hmbC mutants under different osmotic stress conditions as 1.2 M sorbitol. Taken together, the results suggest a role of the HmbC on cell wall formation, possibly independent of its role on the regulation of carotenogenesis. Other possible phenotypic effects of the hmbC mutation are under study.

Reference:
[1] Avalos, et al. (2017b). Carotenoid Biosynthesis in Fusarium. J. Fungi 3, 39.

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CS1.3.12

CONSTRUCTION OF A NEW REPORTER STRAIN TO DISSECT ESTABLISHMENT AND EPIGENETIC INHERITANCE OF FACULTATIVE HETEROCHROMATIN

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Neurospora crassa is a filamentous fungus with a rich history in epigenetics research. Like higher eukaryotes, Neurospora has heritable phenotypes that persist through mitosis or meiosis and are independent of the DNA sequence. The higher-order assembly and maintenance of chromatin controls many biological processes and is largely regulated by post translational modifications such as histone modifications. How these histone modifications are established and maintained through mitosis or meiosis remains unknown. We have successfully developed a new reporter strain that will enable us to dissect the establishment and maintenance of facultative heterochromatin in Neurospora crassa. To accomplish this, we took advantage of the bacterial DNA-binding protein tetR and its DNA-binding site, the tet operator sequence (tetO). We obtained a N. crassa strain harboring a large array of tet operator repeats (tetO array; a gift from Dr. Eugene Gladyshev). Three different transgenes encoding individual PRC2 components were fused to a tetROFFGFP module and introduced into the strain containing the tetO array. Hence, recruitment of PRC2 to the tetO array is expected to induce an artificial facultative heterochromatin domain and addition of tetracycline to the growth medium should trigger the disassociation of the tetROFF fusion. Epigenetic maintenance of H3K27me3 should be maintained by the native PRC2 complex in the absence of the tethered PRC2 complex. We have fully validated the strain and next, we will delete individual components of the Polycomb Repression Network in this reporter strain background to probe their roles in establishment and/or maintenance of H3K27me3-marked facultative heterochromatin. We expect that this innovative system will yield important new insights into the assembly of facultative heterochromatin.

CS1.3.13

INHERITABLE CRISPR BASED EPIGENETIC MODIFICATION IN A FUNGUS

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The CRISPRoff system was recently introduced as a programmable epigenetic memory writer that can be used to silence genes in human cells. The system makes use of a dead Cas9 protein (dCas9) that is fused with the ZNF10 KRAB, Dnmt3A, and Dnmt3L protein domains. The DNA methylation resulting from the CRISPRoff system can be removed by the CRISPRon system that consists of dCas9 fused to the catalytic domain of Tet1. Here, the CRISPRoff and CRISPRon systems were applied for the first time in a fungus. The CRISPRoff system resulted in an inactivation up to 100% of the target genes flbA and EGFP in Aspergillus niger. Phenotypes correlated with the degree of silencing in the transformants. Silencing was stably maintained after removing the CRISPRoff plasmid from the flbA silenced strain. On the other hand, introducing the CRISPRon system in a strain in which the CRISPRoff plasmid was removed fully reactivated flbA showing a phenotype similar to that of the wildtype. Together, the CRISPRoff and CRISPRon sys-tems can be used to study gene function in A. niger.

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CS1.3.14

HETEROCHROMATIN MARKS AFFECT THE MITOTIC STABILITY OF ACCESSORY CHROMOSOMES DIFFERENTLY BETWEEN SPECIES OF ONE GENUS OF PLANT PATHOGENIC FUNGI

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Heterochromatin marks have been shown to have variable effects on distinct biological processes among different genera of fungi. To investigate the applicability of this observation to chromosome segregation integrity, fungi harbouring accessory chromosomes – non-essential chromosomes present in only some individuals of a species – provide excellent model systems. The genome of the economically important wheat pathogen Zymoseptoria tritici contains up to eight accessory chromosomes, the mitotic transmission fidelity of which is affected by the heterochromatic histone modifications H3K9me3 and H3K27me3. We herein analysed the impact of H3K9me3 and H3K27me3 on the mitotic stability of accessory chromosomes in the closely related species Zymoseptoria ardabiliae to test whether the roles of these two histone modifications in accessory chromosome transmission are conserved within the genus. We abolished H3K9me3 and H3K27me3 in Z. ardabiliae by deleting the responsible lysine methyl-transferases Kmt1 and Kmt6, respectively. We propagated the obtained deletion strains in an evolution experiment with limited selection over a course of four weeks (≈ 80 mitotic divisions) and assessed the frequency of accessory chromosome losses. Preliminary data indicate that abolishment of H3K9me3 decreases accessory chromosome loss and abolishment of H3K27me3 increases accessory chromosome loss in Z. ardabiliae, which is inverse to the pattern previously described in Z. tritici. Our data therefore suggest that the same histone modification can have opposing roles in mitotic chromosome stability even within closely related species.

CS1.3.16

ROLE OF SPOK GENES IN CHROMOSOME DYNAMICS AND STABILITY IN THE CLONALLY EVOLVING PATHOGEN FUSARIUM OXYSPORUM

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The ascomycete Fusarium oxysporum causes vascular wilt disease in more than a hundred crops and opportunistic infections in immunocompromised humans. Its ability to adapt to a wide variety of environments is thought to be related to its highly dynamic genome structure and chromosomal plasticity, including partial duplication or complete loss of chromosomes. The mechanisms underlying the dynamics of dispensable chromosomes in F. oxysporum are largely unknown. Here we study the role of Spoks (Spore Killers), a type of genetic elements that act as meiotic drivers by actively killing sexual spores that lack the element. Because the F. oxysporum genome contains a considerable number of Spok-like sequences, we set out to examine their role in chromosome dynamics by using two complementary experimental approaches: 1) removing a single Spok gene from a chromosome and 2) introducing a single Spok gene into a chromosome that lacks it. Quantitative monitoring of chromosome loss in the generated strain will provide new insights into the mechanisms determining chromosome stability in this clonally evolving fungal pathogen.

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CS1.3.17

COPY NUMBER VARIATION DRIVEN BY A MASSIVE MOBILE ELEMENT MAY UNDERLIE CLIMATE ADAPTION IN A FUNGAL WHEAT PATHOGEN

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Copy number variation (CNV) can drive rapid evolution of numerous traits including fungicide resistance and pathogen virulence. Yet, the contribution of CNV to environmental adaptation of fungal populations remains poorly understood. Here, we systematically investigate CNVs in the largest to-date genome sequencing dataset of a fungal pathogen to assess the contribution of CNVs to trait architecture and climatic adaptation. We analyzed a worldwide collection of 1109 Zymoseptoria tritici isolates sampled in 42 countries. The chromosome complements of this destructive wheat pathogen are highly plastic with accessory chromosome variation and core chromosome duplications. We found that most gene CNVs were very rare in the species (i.e. singletons) with only 3% of gene CNVs segregating at high frequency across populations. We found that secondary metabolism functions were the main targets of gene CNV events and important factors of population differentiation. We used global environmental datasets to associate CNVs with climatic gradients across wheat production areas. We found multiple gene CNVs significantly associated with environmental gradients, including a gene of the NAD-dependent Sirtuin family, paramount for metabolism regulation and chromatin silencing in eukaryotes. The CNV locus is part of a larger region of ~90kb encoding proteins highly expressed during infection. Furthermore, the structural variation of the locus was dominated by a Starship mobile element unique to the species and with the capacity to mobilize gene content in the genome. Taken together, CNVs are likely a major factor driving climatic and metabolic adaptation of the species. The presence of a massive mobile element governing metabolic capacity and climate adaptation opens new avenues to understanding the rapid evolution of fungal plant pathogens.

CS1.3.18

FUNCTIONAL ANALYSIS OF THE CONSERVED HISTONE CHAPERONE ASF1 IN THE ASCOMYCETE SORDARIA MACROSPORA

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The highly conserved histone chaperone ASF1 is involved in the assembly and disassembly of nucleosomes during transcription, replication and DNA repair. S. macrospora is one of the few multicellular organisms where asf1 deletions are viable, which makes it exceptionally useful for in vivo analysis. Deletion of asf1 leads to a reduction of DNA methylation and upregulation of genes that are usually weakly expressed in the wild type. Here, we focused on the functions of ASF1's highly conserved core and divergent C-terminal tail, studied the effects of ASF1 on histone modifications and tested its relevance for genomic stability. By Co-IP and complementation analysis we showed that substitutions of V94 or truncations of the C-terminal tail abolish histone binding and lead to strains resembling a deletion mutant. Δasf1 is sensitive to the DNA damaging agent MMS, while complementation strains, even those with non histone-binding variants, regain wild type-like resistance. By using HI-C we detected a tandem duplication of around 600 kb on chromosome 2 in the mutant. Further testing revealed that the viability of strains with ASF1 variants that are at least able to participate in DNA damage protection does not depend on the presence of this duplication. We speculate that the occurrence of this duplication is the reason for viability of asf1 mutants in Sordaria compared to almost all other multicellular eucaryotes. Crossing experiments might clarify the relationship between asf1 deletion and the duplication on chromosome 2. ChIP-seq analysis revealed a significant increase of the heterochromatic mark H3K27Me3 in Δasf1, contradicting the slightly upscaled expression patterns in the mutant. We suspect a compensation mechanism, possibly related to the viability of Sordaria asf1 deletion mutants. High levels of H3K27Me3 in genes like rad53, known to be lethal when overexpressed in S. cerevisiae Δasf1, underscore this hypothesis.

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CS1.3.19

PHENOTYPIC PROFILING OF EPIGENETIC FACTOR KNOCKOUT MUTANTS IN THE HUMAN FUNGAL PATHOGEN ASPERGILLUS FUMIGATUS

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Aspergillus fumigatus is one of the most important mould pathogens and allergens of humans. Estimates suggest that over 3 million people have invasive or chronic aspergillosis that leads to in excess of 600,000 deaths every year. Resistance to the very limited available set of antifungal drugs is rapidly developing and mortality due to therapeutic failure is on the rise. However, a lack of vital molecular insight precludes a mechanistic understanding of the pathogenicity and development of antifungal resistance in A. fumigatus. Beyond transcriptional control mediated by transcription factors, a growing body of evidence has suggested the important roles of epigenetic mechanisms in diverse aspects of fungal infection biology including pathogenesis, antifungal resistance, and genome evolution. However, the epigenetic regulatory landscape largely remains unexplored in A. fumigatus.
In this study, we carried out a systematic phenotyping of epigenetic factor knockout mutants to gain insight into the epigenetic systems in A. fumigatus. Through bioinformatic exploration of the A. fumigatus genome, we have identified a cohort of putative epigenetic factor-encoding genes that are potentially involved in epigenetic processes including DNA methylation and histone modification. Transcriptional profiling of the identified factors revealed that A. fumigatus dynamically modulate the expression of the epigenetic factors during growth and after exposure to antifungal compounds. To identify the key epigenetic factors, we constructed a collection of gene deletion mutants and characterised their general growth fitness, antifungal drug susceptibility, and pathogenicity in a Galleria mellonella infection model. Our phenotypic profiling identified a cohort of epigenetic factors that play an important role in A. fumigatus and provide an important basis to further understand the epigenetic regulatory landscape in A. fumigatus.

CS1.3.20

THE HOMEOBOX TRANSCRIPTION FACTOR HBXA INFLUENCES THE EXPRESSION OF OVER A THOUSAND GENES IN THE FUNGUS ASPERGILLUS NIDULANS

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Fungi present conserved homeobox-domain (HD) proteins known to that act as transcriptional regulators governing asexual and sexual development. In some Aspergillus species, several HD transcription factor genes have been characterized, among them, HbxA/Hbx1. For instance, in the opportunistic human pathogen Aspegillus fumigatus, HbxA is involved in development, secondary metabolism and virulence. In A. flavus, disruption of hbx1 results in fluffy colonies unable to produce sclerotia. Transcriptome analysis of A. flavus hbx1 showed that it regulates expression of more than five thousand genes, including those involved in mycotoxin production. In the model fungus A. nidulans, deletion of hbxA also results in aconidial colonies with reduced sterigmatocystin production, however, the extent or conservation of its regulatory scope is unknown. Our present study of the A. nidulans hbxA-dependent transcriptome, revealed that more than one thousand genes are differentially expressed when this regulator was not transcribed at wild-type levels. Among them numerous transcription factors, including those involved in development as well as in regulation of secondary metabolism. Furthermore, our study revealed that several secondary metabolite gene clusters, and concomitant metabolite production, are under the control of hbxA in A. nidulans.

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CS1.3.21

INVESTIGATING THE IMPACT OF FLOCCULATION UPON GENE REGULATION BY THE TUP1-CYC8 COMPLEX IN SACCHAROMYCES CEREVISIAE USING A CYC8 CONDITIONAL MUTANT

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The Tup1-Cyc8 (Ssn6) co-repressor complex is a regulator of gene transcription in the yeast Saccharomyces cerevisiae. It has been shown that the complex brings about a repressive chromatin structure at regulatory regions of target genes or prevents the recruitment of the factors needed for activation of transcription. Despite this knowledge, the activity of the Tup1-Cyc8 complex is not fully understood. The Anchor Away (AA) technique allows for a target nuclear protein to be conditionally sequestered to the cytoplasm. I compared changes in transcription in a Cyc8-AA strain after the removal of Cyc8p from the nucleus, to a cyc8 deletion mutant compared to wt. Using an AA strain to study this complex provides many advantages over a gene deletion mutant strain. For instance, the FLO family of genes are repressed by the Tup1-Cyc8 complex. These genes encode the proteins required for flocculation, a stress response where the cells aggregate to protect cells within the floc. Cells with a CYC8 deletion display a striking flocculation phenotype. However, the Cyc8-AA strain only develops a flocculation phenotype after the removal of Cyc8p from the nucleus.
An important aspect of my project was to investigate if flocculation influences global gene expression. To test this, I designed an experiment whereby global transcription was compared in a flocculant and non-flocculant Cyc8-AA strain. The results showed that flocculation played a role in repression of 249 genes, whereas it increased the expression of 95 genes. Additionally, the experiment revealed 477 genes that showed changes in gene expression that were not affected by the presence or absence of a flocculation phenotype. These results identify the cohort of genes that are directly regulated by the Tup1-Cyc8 complex, and those genes that are indirectly influenced by the flocculation phenotype.

CS1.3.22

DIFFERENTIAL ROLES OF RID1 AND DIM2 CYTOSINE METHYLASES IN TRICHODERMA REESEI MEIOSIS

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Meiotic recombination in most eukaryotes is initiated by Spo11-induced DNA double-strand breaks (DSBs), which are repaired by Rad51 and/or Dmc1 recombinases. The industrial workhorse fungus Trichoderma reesei loses dmc1 and its spo11 is dispensable for meiosis. We mapped genome-wide single-strand DNA (ssDNA)-associated DSBs that accumulate in processing-capable, repair-defective rad51Δ and spo11Δrad51Δ mutants, respectively. T. reesei has two fungus-specific DNA cytosine methyltransferase genes, dim2 and rid1. Our genetic and genomic results reveal that dim2 and rid1 differentially regulate homologous recombination and genome-wide hypermutation in Trichoderma reesei meiosis.

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CS1.3.23

UNBIASED MASS SPECTROMETRY REVEALS THE HISTONE CODE IN THE FUNGAL GENUS ASPERGILLUS

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In eukaryotes, chromatin is a dynamic and multi-fold nucleoprotein structure that regulates access to genetic information via modifications like methylation and acetylation on histone proteins H2A, H2B, H3, and H4. The combination of different histone modifications – the histone code – at a single genomic locus can impact chromatin dynamics, i.e., the transition between the compact and transcriptionally silent state and the loose and transcriptionally active state. Chromatin dynamics is crucial for organisms to adapt gene expression patterns in response to a variety of developmental and environmental clues. The fungal genus Aspergillus comprises at least 350 species, including human and crop pathogens, food contaminants, as well as important cell factories for industrial and medical applications. Most catalytic subunits of well-characterized eukaryotic chromatin modifiers (16 complexes with 86 subunits) that catalyse the deposition or removal of histone modification are evolutionarily conserved in Aspergilli. However, we still lack an experimental and unbiased overview of the occurrence of histone modifications and their relative abundance of histone modification in Aspergilli. Here, we employed unbiased mass spectrometry to measure histone modifications in three Aspergillus species (A. niger, A. nidulans (two strains), and A. fumigatus). We detected 18 histone modifications, including mono-, di-, or tri-methylation and acetylation on both the tail and core domains of histone proteins in all four strains. While they all carry the same modifications, we uncovered significant differences in the relative abundance of H3K4me3, H3K9me3, H3K14ac, H3K36me1, and H3K79me1 in a strain-specific manner. Based

on these data, we are currently performing the ChIP-seq experiment to reveal the genes associated with quantitatively different histone modifications, and we foresee that this will provide valuable clues about the complexity of the histone code and its functional implications on genome architecture and gene regulation in fungi.

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CS1.3.24

FREQUENT HORIZONTAL TRANSFER OF WHOLE CHROMOSOMES ENHANCES EVOLUTIONARY FITNESS OF A FUNGAL PATHOGEN

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Horizontal transfer of whole chromosomes was postulated to influence fitness and host specificity of several pathogenic fungi. Population genomic data indeed indicate that such a horizontal chromosome transfer can occur occasionally. To date, however, experimental evidence for such transfers is limited and these transfers seem to occur at low frequency. Here, we report three independent horizontal transfer events of the same accessory chromosome between two strains of the asexual entomopathogenic fungus Metahizium robertsii during experimental evolution with infected insect hosts. Intriguingly, only a single accessory chromosome but no other donor chromosome was transferred to the recipient strain. The recipient strain with the horizontally acquired accessory chromosome seemed to increase its fitness and out-competed the donor strain during insect infection. Importantly, using genomic data, we demonstrate that the same accessory chromosome was transferred in the field between M. roberstsii and another insect pathogen M. guizhouense, thereby revealing a unique horizontal whole-chromosome transfer between distinct fungal species. The transferred accessory chromosome shows high TE content and relatively low gene density. It contains genes encoding two putative histones, several chitinases, as well as homologs to spore killer proteins which could account for the fitness increase and/or its transmission. In summary, our study demonstrates that the horizontal transfer of a single accessory chromosome seems to enhance evolutionary fitness of the recipient fungus and occurs frequently between different Metarhizium species under both experimental and field conditions.

CS1.3.25

CAPTURING TRANSPOSON DYNAMICS IN THE FUNGAL PATHOGEN FUSARIUM OXYSPORUM

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Transposable elements (TEs) represent an important source of genetic variation and are thought to act as major drivers of genome evolution in eukaryotic organisms. The ascomycete Fusarium oxysporum causes vascular wilt disease in more than 150 different crops and opportunistic infections in humans. The ability to infect organisms from different kingdoms makes this fungus an ideal model to study the genetic mechanisms underlying adaptation to different host environments. Genome re-sequencing of experimentally evolved lines of a clonal F. oxysporum isolate submitted to serial passages through different experimental conditions revealed an important role of TEs in adaptive evolution. Hormin, a non-autonomous miniature element of the the hAT-type TE Hornet, accounted for almost a quarter of all mutations detected in the passaged lines. To understand the dynamics of TE movement in F. oxysporum at a genome-wide scale, we are using an NGS strategy based on Insertion Sequencing to capture the TE excision/insertion events under different environmental conditions. Furthermore, we are studying the expression of the Hornet transposase during development of this fungal pathogen. Taken together, our results suggest that TEs act as drivers of genetic variation and play key roles in adaptation to diverse environments. These experimental approaches will advance our understanding on the mechanisms of TE dynamics and its role in adaptive evolution of F. oxysporum.

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CS1.3.26

DECIPHERING THE ROLES OF JUMONJI DOMAIN CONTAINING PROTEINS IN PODOSPORA ANSERINA

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In eukaryotic organisms, histone proteins are associated with DNA inside the nucleus. This structure called chromatin is associated with the epigenetic component of gene regulation. Indeed, epigenetic histone post-translational modifications or histone marks are set up by specialized enzymes, which lead to either an open conformation of chromatin, namely euchromatin, in which transcription occurs; or a close conformation of chromatin, namely heterochromatin, blocking transcription. However, the chromatin conformation needs to be dynamics to allow a fine regulation. Thus, these histone marks have to be periodically removed for other marks to be deposited. The methylation of histone lysine is performed by histone methyltransferases (HMTs), whereas the removal of methyl group, or demethylation, is performed by histone demethylases (HDMs). HDMs containing a JmjC domain are widely conserved among Eukaryotes. In the model fungus Podospora anserina, it has been shown that the deletion of HMT coding genes caused defects in many aspects of the life cycle such as growth, differentiation, gamete production, sexual development, etc. (Carlier et al., 2021). However, compared to HMTs, there are only few studies on HDMs in fungi. We searched P. anserina's genome for genes predicted to encode a JmjC domain containing proteins. We detected 12 candidates for which knock-out mutants have been constructed. I present here the analysis of this gene family and describe the phenotypes of the deletion mutants.

CS1.3.27

RECOVERY FROM UV IRRADIATION

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Fusarium oxysporum f. sp. lycopersici (Fol) is a soilborne fungal pathogen that is the causal agent of tomato vascular wilt disease. This phytopathogen can remain in the soil as dormant spores and can actively withstand UV-exposure utilising three DNA-repair mechanisms, namely Nucleotide Excision Repair (NER), Photolyase dependent photoreactivation (Phr) and an alternate excision repair pathway centred around the UV-damage endonuclease (UVDE). Our study investigates how damages in the fungal genome due to the exposure to UV and subsequent DNA repair can affect the normal development of Fol and in turn its ability to infect tomatoes. A time scale analysis of the growth of Fol starting from single celled conidia to mature multicellular hyphae revealed that Fol conidia exposed to UV-C showed a significant delay in germination compared to the control condition. This has been further supported by a sharp increase in the expression of FoSir5 two hours after exposure to UV-C which is known to act as a negative regulator for conidial germination. The recovery even after 48 hours post irradiation was found to be incomplete as observed from the reduced conidiation in the irradiated fungal population. An initial screening of the major players involved in selected DNA repair pathways showed a significant induction of NER related genes in the irradiated conidia 2 to 4 hours post irradiation. These results were also reflected in the development of Fol in its host. Microscopic observation of the roots of Rehovot-13 tomato seedlings infected with UV-C exposed Fol conidia showed fungal conidia adhering to the root surface 24 hours post infection whereas in the control condition, filamentation and hyphal networking were mostly observed. Further analysis of the involvement of specific DNA repair pathways in Fol at different time points during fungal development after UV exposure is currently ongoing.

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CS1.3.28

THE HISTONE DEACETYLASE HDA1 IS INVOLVED IN MYCOPARASITISM, STRESS RESPONSE AND METABOLITE BIOSYNTHESIS IN THE MYCOPARASITIC FUNGUS TRICHODERMA ATROVIRIDE

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An outstanding feature of filamentous fungi is their ability to produce a wide variety of bioactive secondary metabolites (SMs) that contribute to their survival, fitness and pathogenicity. The genes for SM biosynthesis are arranged in a contiguous fashion as clusters on the fungal chromosomes that commonly remain silent under standard laboratory conditions because of a repressive chromatin architecture. Consequently, a wide arsenal of yet unknown fungal metabolites is waiting to be discovered. Here, we describe the effects of deletion of hda1, one of the four classical histone deacetylase (HDAC)-encoding genes in the mycoparasitic fungus Trichoderma atroviride. We show that Hda1 acts as a major regulator of SM biosynthesis in T. atroviride, affecting the production of several soluble SMs and volatile organic compounds (VOCs). In contrast to its orthologues in other fungi, hda1 deletion did not lead to major alterations in the pigmentation of mycelia or the supernatant of T. atroviride grown in liquid culture but resulted in a reduced progress of the mycoparasitic attack and alterations of the phenotype in direct confrontation with host fungi. Moreover, phenotypic analysis of the Δhda1 deletion mutants revealed significantly reduced growth under conditions of oxidative as well as osmotic stress. Finally, the expression of several genes encoding members of important protein categories such as enzymes of the phospholipid metabolism or proteins involved in antagonism, root colonization and induction of plant defence responses emerged as being affected by the loss of Hda1.

CS1.3.29

COMPOSITION AND ACTIVITY OF RPDA COMPLEXES IN THE OPPORTUNISTIC MOLD PATHOGEN ASPERGILLUS FUMIGATUS

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The class 1 lysine deacetylase (KDAC) RpdA is an essential protein and a critical virulence determinant of the most common airborne opportunistic mold pathogen Aspergillus fumigatus. Class 1 KDACs are highly conserved in all eukaryotic organisms up to humans and have shown to be important regulators of chromatin function as components of diverse multi-protein complexes. Thus, to exploit the pathogen-relevant character of RpdA for treatment and prophylaxis of disease, mainly two possibilities are conceivable: (i) interference with its enzymatic function by the application of KDAC inhibitors, some of which have already been approved by the FDA due to their impact as anticancer drugs, and (ii) disturbance of the formation of critical (fungal-specific) RpdA complex(es). The latter requires a precise knowledge of number and composition of RpdA complexes. Recently, the existence of at least four of those complexes, RpdA-L, RpdA-S, RcLS2F and KERS, has been demonstrated in the filamentous fungal model organism Aspergillus nidulans. Here we present the characterization of corresponding RpdA complexes in A. fumigatus, which might finally aid in the development of efficient novel antifungal therapies.

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CS1.3.30

DNA-METHYLATION DYNAMICS IN COLLETOTRICHUM HIGGINSIANUM

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DNA methylation occurs in all domains of life. Different DNA methylation patterns in different tissues, alteration of these patterns in cancer cells and association with biological age are some examples of the importance of DNA methylation in genome regulation. Maintenance of DNA methylation and epigenetic reprogramming during embryo development are well understood. In contrast, how and where DNA methylation is established de novo and its dynamics are largely unknown.
Throughout the kingdom of fungi, we find different sets of DNA methyltransferases and different methylation patterns. Paired with their small genome sizes, fungi are an excellent eukaryotic model system to study DNA methylation in an accessible and economic way. We hypothesise that DNA methylation is context dependent, and that specific sequence features determine how cells prioritize DNA methylation.
To test this hypothesis, we will demethylate the genome of Colletotrichum higginsianum by growing it in presence of the DNA methylation inhibitor 5-azacytidine, followed by monitoring re-methylation over time in the absence of the inhibitor. We are determining DNA methylation by direct sequencing using Oxford Nanopore Technologies (ONT), where single strands of DNA pass through nanopores that are embedded in a membrane. When a DNA strand passes through, it causes changes in the electric current that is applied across the membrane. These voltage changes are characteristic for each base and their modifications, which enables measuring DNA methylation directly.
Learning about DNA methylation dynamics after a demethylation event will let us draw conclusions about genome regulation. For example, transposable elements (TEs) must be silenced to avoid their activity, as TEs are essentially mutators. We expect to be able to draw conclusions about how a cell recognises a TE that jumped and in which genomic contexts TEs become methylated.

CS1.3.31

TRICHODERMA PHYTOHORMONE PRODUCTION: COMPARATIVE ANALYSIS OF ETHYLENE BIOSYNTHESIS-RELATED GENES

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Trichoderma is a fungal genus with a high biotechnological value with several species currently used as biological control agents. In addition, Trichoderma spp. can induce plant systemic responses, promote plant growth and abiotic stress tolerance. It has been suggested that the beneficial interaction of Trichoderma spp. with plants is associated to the ability of the genus to modulate the plant's phytohormone network. Trichoderma spp. can produce phytohormones (including auxins, abscisic acid, gibberellins and cytokinins) with the phytohormone production profile depending on the strain. However, little is known about the pathways and genes involved in phytohormone production in Trichoderma spp. In this work, we have performed a comparative analysis to identify genes involved in ethylene biosynthesis in 37 selected Trichoderma spp. Based on protein sequence of five genes putatively involved in ethylene biosynthesis in T. atroviride, we have identified possible orthologs of these genes within the proteomes of several selected Trichoderma spp. using the OrthoFinder software. In addition, we have performed phylogenetic analyses for each of these orthogroups as well as searching for conserved protein domains and evidence of local positive selection events. Results showed that the analysed Trichoderma spp. were predicted to be able to synthesise ethylene by two different pathways: through 1-aminocyclopropane-1-carboxylic acid (ACC) or by 2-oxoglutarate (2-OG) conversion. In addition, most of the species included in the study have a single copy of each of the analysed genes, except for ACC synthase gene, which interestingly was present in two copies. Also, protein domains in all the analysed orthogroups were highly conserved. These results indicate that ethylene biosynthesis-related genes are highly conserved within the Trichoderma genus, suggesting a potential role of ethylene in fungal development or plant interaction.

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CS1.4 BIOCONTROL AND NATURAL ANTAGONISTS

CS1.4.9

TOWARDS THE DISCOVERY OF ANTIMICROBIAL NATURAL PRODUCTS FROM TRICHODERMA KONINGIOPSIS AND STUDY OF THEIR BIOSYNTHESIS

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Erwinia mallotivora has been identified as the causal agent of papaya dieback, a disease that has caused important yield losses to Malaysia and other East Asian countries [1,2]. Biocontrol is the most promising strategy to explore as others such as crop management, chemical treatment or the use of resistant plants are either not manageable or not available. Three strains of Trichoderma koningiopsis have been identified as being active against E. mallotivora [3]. This project aims to characterise secondary metabolites from those three Trichoderma strains showing bioactivity against E. mallotivora. So far, results show that crude extracts of all three strains have antibacterial activity with one showing additional antifungal activity. Citric acid was identified as one of the antibacterial compounds active against Gram negative bacteria and E. mallotivora specifically. Other antimicrobial compounds have been isolated using different cultivation strategies (carbon sources, solid/liquid cultivation methods, epigenetic manipulation) and their structures are being elucidated using NMR spectroscopy. In addition, the genomes of the three Trichoderma strains have been sequenced and PKS and NRPS biosynthetic gene clusters (BGC) have been identified through AntiSMASH. A transformation method for the environmental strains of T. koningiopsis is being developed and CRISPR/Cas9 knockouts will be created to investigate the BGCs producing the anti-

microbial compounds.

1 Mat Amin et al., (2010), Int J Mol Sci 12, 39–45
2 Sekeli et al., (2018), Frontiers in Plant Science 9, 1380
3 Tamizi et al., (2022), Journal of Fungi 8, 246

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CS1.4.10

CHARACTERIZATION OF THE GATA TRANSCRIPTION FACTOR ARE1 IN TRICHODERMA ATROVIRIDE

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The ascomycete Trichoderma atroviride is a mycoparasitic fungus proliferating mostly in soil and on wood, but it can adapt to different ecological conditions. Due to its antagonistic and parasitic activity against various fungal pathogens, T. atroviride is used as pest control agent alternatively to synthetic fungicides. The ability to sense nutrient availability in the surrounding environment and adjust accordingly the metabolism for optimal growth, development and reproduction is an essential trait for the fungus adaptability, being as well of relevance for its mycoparasitic activity. Several secondary metabolites, that weaken the fungal host and support attack, are produced during mycoparasitism. As it has been previously shown that the quality and quantity of nitrogen can impact the biosynthesis of many known secondary metabolites in fungi, we decided to characterize Are1 in T. atroviride. Are1 is the orthologue of Aspergillus nidulans AreA , which is supposed to be one of the main positive-acting GATA transcription factors in the activation of genes subjected to nitrogen catabolite repression (NCR). We could show that the growth of T. atroviride Δare1 mutants was impaired on different primary and secondary nitrogen sources, with the only exception of glutamine, which allowed growth comparable to the wt. Deletion of are1 also led to an enhanced resistance against sorbitol-mediated osmotic stress and a reduced sensitivity to the TOR kinase inhibitor rapamycin. Most importantly, the Δare1 mutant was found completely avirulent and could not attack and overgrow Rhizoctonia solani, indicating a pivotal role of Are1 in the activation of mycoparasitism-related gene expression.

CS1.4.11

EXTRACELLULAR ACIDIFICATION IS NOT ESSENTIAL FOR BEAUVERIA BASSIANA BIOCONTROL ACTIVITY AGAINST FUSARIUM OXYSPORUM

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Entomopathogenic fungi belonging to Beauveria bassiana (Bb), apart from being widely used in agriculture for the control of insect pests, they also play different roles in natural agroecosystems, including endophytism, plant growth promotion and disease control. Rhizosphere pH alkalinization represents a renowned pathogenicity mechanism of several fungal pathogens including the soil-borne ascomycete F. oxysporum f. sp. lycopersici (Fol), the causal agent of vascular wilt disease on tomato plants. Here, to understand if Bb biocontrol activity against fungal pathogens might be related to its ability to modify rhizosphere pH, we characterized the pH modulating and biocontrol activity in vitro and in vivo of ten Bb isolates. In vitro Bb acidifying activity was evaluated by using the pH indicator bromophenol blue and an acidification index was calculated for each of the tested isolates. Nine isolates out of ten were able to acidify the culture medium and six of them produced a more intense acidification halo around the colony. However, when biocontrol activity was tested in vitro only three highly-acidifying and one non-acidifying isolate greatly inhibited Fol growth. Unexpectedly, when Bb biocontrol activity was evaluated in vivo against Fol, all isolates similarly protected tomato plants from wilting, thus suggesting that rhizosphere acidification might be only one of the biocontrol mechanisms used by Bb in vivo. Metabolomic analysis of Bb exo-metabolites through LC-MS qTOF and GC-MS approaches is currently being performed to identify potential molecules involved in Bb biocontrol activity against F. oxysporum.

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CS1.4.12

PHYTOHORMONES: PUTATIVE SIGNALLING MOLECULES IN MYCOPARASITIC TRICHODERMA SPECIES

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The fungal genus Trichoderma contains a vast array of species that are well known for their high opportunistic potential and adaptability to various ecological niches. The ability of many Trichoderma species to both colonize the rhizosphere and antagonize plant pathogenic fungi has led to their use in biological pest control for several decades. Beside their ability to ward off fungal phytopathogens, these biological control agents were shown to further protect their plant host by activating plant defence genes and promoting plant growth.
Phytohormones, which are signalling molecules known for controlling various aspects of plant growth and development, are produced by both plants and microorganisms, including fungi that interact with plants, both in a beneficial and harmful manner.
In the present study, we characterized and compared the phytohormone production profiles of three Trichoderma species through UHPLC MS/MS analysis. To this end, Trichoderma atroviride, Trichoderma virens and Trichoderma asperellum were cultivated on solid medium in the presence or absence of a plant host and the production of secreted phytohormones was assessed. All three Trichoderma species produced two auxins, indole-3-acetic acid (IAA) and oxidised indole-3-acetic acid (oxIAA), as well as salicylic acid. Notably, the presence of the plant host only had minor effects on the quantity of phytohormones produced by the Trichoderma spp. These data indicate that the phytohormones produced by Trichoderma spp. could play a role in the physiology of the fungus, regardless of their role during plant interactions. The analysis of phytohormones in these Trichoderma spp. is the basis for currently ongoing experiments that aim at further elucidating the functions of phytohormones in the physiology of filamentous fungi.

CS1.4.13

COMPARATIVE EXO-PROTEOMIC ANALYSIS OF TRICHODERMA ANTAGONISTS INDUCED BY OOMYCETE MYCELIUM HIGHLIGHTS DIVERSE ANTAGONISM STRATEGIES

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Plant-beneficial fungi from the genus Trichoderma (Hypocreales, Ascomycota) can control oomyceteous plant-pathogenic Pythium myriotylum (Peronosporales, Oomycota) and thus serve as bioeffectors for the eco-friendly products of crop protection. However, the underlying mechanisms of microbe-microbe interactions have yet to be fully understood. In this study, we focused on the role of the Trichoderma secretome induced by P. myriotylum mycelia. For this purpose, we selected strains showing strong (T. asperellum, T. atroviride, T. virens), moderate (T. cf. guizhouense, T. harzianum, T. reesei), and weak (T. parepimyces) activities, respectively, and cultured with the sterilized P. myriotylum mycelia. Secreted proteins were analyzed using label-free LC-MS/MS, bioinformatic localization prediction, gene ontology (GO) annotation, and ortholog analysis. The exoproteomic analysis quantified the range from 132 up to 811 proteins in the seven Trichoderma spp., suggesting unequal antagonistic mechanisms among the strong and weak strains, respectively, with different proportions of putative cellulases, proteases, redox enzymes, and extracellular proteins of unknown function. Proteins from 47 conserved orthogroups (i.e., at least one protein in all species) composed about half of the total secretome and were mainly related to carbohydrate metabolism. Notably, proteolysis-related proteins ranged between 4% and 50% of the total abundance, while the abundant proteases tended not to be conserved across the species (i.e., non-orthologous). Putative cellobiohydrolases were detected abundantly in all Trichoderma species except for the weak antagonist T. parepimyces, even though its genome encodes for these proteins (JGI MycoCosm unpublished). Notably, secretomes of the most potent anti-Pythium bioeffectors tended to have higher endo-cellulase activity. Cellulose and other glucans are major components of the oomycete cell wall, which was partly reflected in the cellulases produced by the Trichoderma species. The varying abundances of orthologous proteins suggested the evolution of differing transcription regulation mechanisms across the Trichoderma genus in response to the ubiquitous presence of Oomycota.

CS1.4.14

CLONOSTACHYS ROSEA AS A BIOCONTROL AGENT AGAINST FUSARIUM GRAMINEARUM AND ITS MYCOTOXIN IN OAT

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While oats (Avena sativa) is affected by Fusarium head blight (FHB) to a similar degree as other cereals, biological control of FHB in oats has been neglected. Previously, a mycoparasitic fungus Clonostachys rosea was found to control Fusarium infection in wheat and maize. In the present work, we studied the ability of C. rosea IK726 to control FHB and reduce mycotoxin content in oat and investigated effect of C. rosea on mycotoxin detoxification. Deoxynivalenol (DON), produced by F. graminearum is known to act as a virulence factor during the infection. An increased detoxification of DON with the help of UGT-glycosyltransferases (UGTs) has been directly linked to increased resistance to FHB in cereals.
We found that C. rosea-treatment of oat spikelets at anthesis 3 days prior to F. graminearum inoculation substantially reduced both Fusarium biomass (79%) and DON level (80%) in mature oat kernels. In addition, the percentage of DON-3-Glc over the total level of DON, 3ADON and DON-3-Glc was higher in C. rosea-treated spikelets compared to mock treated ones, which indicates higher activity of DON-glucosylation enzymes. Application of DON to C. rosea pre-inoculated oat spikelets led to powerful enhancement of expression of two oat UGT genes (AsUGT1 and AsUGT2) , especially at the earliest time points. These genes were previously characterised and found to be highly inducible by DON-treatment and F. graminearum infection. We found that conjugation of DON into DON-3-Glc occurred at a much higher level in C. rosea-treated spikelets compared to mock-treated ones. We conclude that C.rosea IK726 has good potential to be used as biocontrol agent against FHB in oat as it helps the plant not only to hamper the F.graminearum infection effectively, but also to detoxify mycotoxin produced by the pathogen more rapidly.

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CS1.4.15

ANTAGONISTIC EFFECT OF VOLATILE ORGANIC COMPOUNDS FROM OLIVE ROOTS ENDOPHYTIC BACTERIA AGAINST THE TOMATO VASCULAR WILT PATHOGEN FUSARIUM OXYSPORUM

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Fusarium oxysporum (Fo) is one of the major vascular wilt fungi attacking hundreds of different crops worldwide. Environmentally friendly and cost-effective approaches such as biocontrol with bacterial or fungal agents (biocontrol agents, BCAs) have long been regarded as the most promising alternative to fungicides.

Increasing evidence suggests that volatile organic compounds (VOCs) produced by plant roots and rhizosphere microorganisms play important roles in signaling and biocontrol. However, our knowledge on the chemical diversity of VOCs produced by wilt pathogens and BCAs and their role in fungal-bacterial-plant interactions remains fragmented. VOCs from Fo were shown to enhance plant growth by affecting auxin signaling. The antagonistic effect of a non-pathogenic Fusarium isolate against Verticillium dahliae is partially due to the production of VOCs and several metabolic pathways of Fusarium are altered upon contact with V. dahliae volatiles. Our starting hypothesis is that VOCs emitted by fungal pathogens, bacterial BCAs and plant roots mediate ecological interactions that crucially impact the infection process.
Preliminary experiments performed in our lab detected VOCs in root samples induced specifically after inoculation with Fo f.sp lycopersici (Fol), demonstrating that the GC-IMS technique can be successfully applied to the analysis of the Fusarium-tomato volatilome.
In this study we have analyzed the antagonistic capacity of several olive-roots endophytic bacterial BACs (Paenibacillus and Pseudomonas) against Fol. A sandwiched plate assay was used to analyze the phenotypic effect of VOCs from different BCAs on Fusarium growth. Under the in vitro conditions tested, both Pseudomonas sp. PICF6 and

P. simiae PICF7 induced a drastic reduction on microconidia production and germination, suggesting that volatiles emitted by these bacterial BACs may be involved in the in vitro antagonism against the tomato pathogen Fol. Progress on the identification of the specific bacterial VOCs showing antagonistic activity against Fol will be presented.

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CS1.4.17

REDUCING AFLATOXIN CONTAMINATION BY USING ASPERGILLUS ORYZAE STRAIN ISOLATED FROM THE TRADITIONAL FERMENTED SOYBEAN BRICK, MEJU

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In Korea, a traditional soybean paste, Doenjang, is very important for Korean cuisine. The soybean brick, Meju, is a starting material of the soybean paste and is sometimes vulnerable for mycotoxin contamination due to the inaccurate control of fungal fermentation. To secure the safety against mycotoxin contamination of traditional meju produced by natural fermentation, a metagenomic analysis of meju was performed, and filamentous fungal strains were isolated from meju to confirm their characteristics. A total of 30 species filamentous fungi in 7 genera, including Aspergilli, Penicilli, Cladosporia, and Mucor, were isolated and identified, and the production of mycotoxins was confirmed. The results of metagenome analysis showed that the distribution and abundance of fungi were very diverse according to the difference in production regions and fermentation conditions of meju. The activity of peptidase, amylase, and lipase was investigated in nonaflatoxigenic Aspergillus flavus/oryzae among strains isolated from meju, and this activity was compared with commercially available yellow-koji strains for meju production. Nonaflatoxigenic A. oryzae strain AO3222 showed more suitable enzyme activity for fermentation of meju than conventional yellow-koji. Also, result of whole genome analysis, it was found that all yellow-koji strains were clustered in very similar close relationships, thus they had almost identical genomic characteristics. However, strain AO3222 was clustered into A. oryzae group different from yellow-koji. To analyze the possibility of using the AO3222 strain as a new koji for the production of soybean meju, the AO3222 strain was inoculated and fermented simultaneously with strains such as A. flavus, Mucor racemosus, and Rhizopus arrhizus. Through this, the ability to reduce the production of aflatoxin in AO3222 strains was analyzed, and the distribution pattern of each strain inoculated in meju was analyzed using the metagenomic method.

CS1.4.18

ASPERGILLUS CARBONARIUS MUTANTS DEFECTIVE IN OCHRATOXIN A PRODUCTION AS POSSIBLE BIOCONTROL AGENTS

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Aspergillus carbonarius is one of the most common species responsible for toxin contamination of grapes and their derivatives, such as wine, coffee, and cocoa. Ochratoxin A (OTA), the main mycotoxin produced by A. carbonarius, is a secondary metabolite that has been categorized as a potential human carcinogen due to its significant nephrotoxicity and immunosuppressive effects. The first enzyme in the OTA biosynthetic pathway is encoded by a polyketide synthase (pks) gene. Furthermore, secondary metabolism in filamentous fungi is regulated by both pathway-specific and global regulatory factors. The VELVET family of regulatory proteins (VeA, VelB, LaeA, among others) works to coordinate fungal growth and secondary metabolites production.
In the present study, we investigated the competitive exclusion of A. carbonarius ΔveA- and Δpks-gene deletion mutants from the wild-type strain, as well as their effect on OTA production. During both in vitro culture and grape infection, the knockout mutants displaced the wild-type strain. Consequently, the establishment of the non-mycotoxigenic producer reduced the amount of OTA. Although a non-mycotoxigenic strain can still infect the fruits, the risk of mycotoxin contamination of the fruits is lowered, and the final product is safer for human and animal consumption. This preliminary study suggests that non-mycotoxigenic strains of A. carbonarius could be used as biocontrol agents.

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CS1.4.20

TRICHOTHECENE PRODUCTION BY TRICHODERMA ARUNDINACEUM ISOLATES RECOVERED FROM BEAN (PHASEOLUS VULGARIS) FIELDS AFFECTS DEFENSE RELATED GENES EXPRESSION IN BEAN PLANTS

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The filamentous fungus Trichoderma arundinaceum has been studied for biological control applications in crop protection against plant pathogens. Since most studies have focused on the reference IBT 40837 strain, this work was based on the isolation of Trichoderma strains from bean fields, where the fungus is well adapted to the environmental conditions. The common bean, Phaseolus vulgaris, is a legume crop distributed worldwide. In the last years, its production has experienced increasing difficulties due to relatively low yields. Most of these economic losses are caused by fungal pathogens such as Rhizoctonia solani and Sclerotinia sclerotiorum. Among the 55 Trichoderma spp. isolates recovered from bean field soils located at the Protected Geographical Indication “Alubia La Bañeza-León” (Spain), three correspond to the Trichoderma arundinaceum species, and showed production of the trichothecene harzianum A (HA) and trichodermol, an intermediate in the HA biosynthesis. HA production by these isolates correlated with significant in vitro antifungal activity against R. solani and S. sclerotiorum. Furthermore, the soil isolates stimulated germination of bean seeds and the growth of above ground parts of the plants. Transcriptomic analysis of RNAs extracted from leaves of bean plants inoculated with these T. arundinaceum bean-field soil isolates and an HA non-producing strain, used as a control, showed that

HA production significantly modified the expression level of plant defense-related genes, such as chitinase encoding genes. Taken together, these results underscore the interest in searching for Trichoderma species capable of producing nontoxic trichothecenes to trigger plant defense responses without negatively affecting their germination and development.

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CS1.4.21

INHIBITION OF GROWTH AND AFLATOXIN B1 PRODUCTION IN ASPERGILLUS FLAVUS BY PSEUDOMONAS STRAINS

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Fungal pathogens can cause a great number of plant diseases leading to significant crop losses in the field and during postharvest processes. Rhizosphere bacterial species such as Bacillus, Streptomyces and Pseudomonas have been proved to be efficient biocontrol agents to combat plant diseases.
In the present study, antagonistic behaviour of fifty-eight corn rhizosphere Pseudomonas strains was investigated against Aspergillus flavus. 16S rRNA gene sequence analyses together with rpoD housekeeping gene sequencing demonstrated that the bacterial isolates belong to five groups: P. fluorescens, P. putida, P. chlororaphis, P. jessenii, and P. koreensis.
The isolated Pseudomonas strains were screened for their biocontrol activities by co-culturing of the bacteria isolates with A. flavus both in liquid and on agar media. The vast majority of Pseudomonas strains completely inhibited the growth of A. flavus in liquid co-cultures, while some other Pseudomonas strains allowed fungal growth. In most of these co-cultures the aflatoxin B1 level was reduced. The aflatoxin B1 degradation ability of the bacterial strains were evaluated by supplementing the liquid bacterial cultures with aflatoxin B1 followed by analytical detection of the toxin level.
High-performance liquid chromatography-hybrid quadrupole-orbitrap mass spectrometry method was applied to explore the metabolomic profile of the co-cultured bacterial and fungal strains (using pure cultures as control) and to identify the degradation products of aflatoxin B1. According to our results, aflatoxin B1 is transformed isolate-dependently. Among known aflatoxin-derived compounds we detected several, yet undescribed products too.
This research has been supported by NKFI K139312 project and National Talent Programme NTP-NFTÖ-22-B-0096.

CS1.4.22

THE ROLE OF CIRCADIAN CLOCK COMPONENTS IN THE TRICHODERMA ATROVIRIDE - BOTRYTIS CINEREA PHYSIOLOGICAL AND METABOLIC INTERACTIONS.

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Circadian clocks are important for an individual's fitness, and recent studies have underlined their role in the outcome of biological interactions. However, the relevance of circadian clocks in fungal-fungal interactions remains largely unexplored. We sought to characterize a functional clock in the biocontrol agent Trichoderma atroviride to assess its importance in the mycoparasitic interaction against the phytopathogen Botrytis cinerea. Thus, we confirmed the existence of circadian rhythms in T. atroviride, which are temperature-compensated and modulated by environmental cues such as light and temperature. Nevertheless, the presence of such molecular rhythms appears to be highly dependent on the nutritional composition of the media. Complementation of a clock null (Δfrq) Neurospora crassa strain with the T. atro-viride-negative clock component (tafrq) restored core clock function confirming the role of tafrq as a bona fide core clock component. Confrontation assays between wild-type and clock mutant strains of T. atroviride and B. cinerea, in constant light or darkness, revealed an inhibitory effect of light on T. atroviride's mycoparasitic capabilities. Interestingly, when confrontation assays were performed under light/dark cycles, T. atroviride's overgrowth capacity was enhanced when inoculations were at dawn compared to dusk. Deleting the core-clock negative element FRQ in B. cinerea, but not in T. atroviride, was vital for the daily differential phenotype, suggesting that the B. cinerea clock has a more significant influence on the result of this interaction.
Additionally, we observed that T. atroviride clock components modulate development and secondary metabolism in this fungus, affecting the production of several molecules. Notably, we detected the rhythmic production of distinct T. atroviride volatile organic compounds (VOCs), which depended on its circadian clock. Thus, this study provides evidence on how clock components impact diverse aspects of T. atroviride lifestyle and how daily changes modulate fungal interactions and dynamics.

CS1.4.23

CHARACTERIZATION OF AN ENVIRONMENTALLY ISOLATED PSEUDOMONAS SPECIES AS A PROMISING BIOCONTROL AGAINST ASPERGILLUS FLAVUS

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Aspergillus flavus is an opportunistic plant pathogen that colonizes crops of agricultural importance, contaminating them with potent mycotoxins. This results in a devastating health and economic impact. Among A. flavus mycotoxins is Aflatoxin B1, the most carcinogenic natural compound known. In an effort to reduce A. flavus plant colonization, we search for effective biocontrol methods. A new isolate from Pseudomonas fluorescens, 20EI1, was obtained from an aquatic environment. P. fluorescens 20EI1 has shown promising results inhibiting fungal growth. Through our investigation of this novel strain, we hope to gain insight into the mechanism of action responsible for the antifungal activity against A. flavus. P. fluorescens 20EI1's application has high potential to prevent fungal colonization, dissemination, as well as mycotoxin production, and could be the basis for a control strategy, reducing crop losses as well as mitigating negative health outcomes caused by A. flavus, and possibly those caused by other fungi.

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CS1.4.24

GENOME-WIDE ASSOCIATION STUDY OF CLONOSTACHYS ROSEA MEDIATED BIOCONTROL OF ZYMOSEPTORIA TRITICI IN WHEAT GERMPLASM

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Beneficial microorganisms can act as biocontrol agents by directly targeting pathogens or indirectly by enhancing the plant's defense mechanisms against pathogens. However, efficiencies with which plants benefit from biocontrol agents vary, potentially because of genetic variation in plants for plant-biocontrol agent compatibility. The aim of this study was to explore the genetic variation in winter wheat for Clonostachys rosea mediated biocontrol of septoria tritici blotch caused by Zymoseptoria tritici. In total, more than 200 winter wheat genotypes comprising of landraces and old cultivars cultivated between the 1900s and 2000s in Scandinavian countries were tested under controlled conditions in a greenhouse. Foliar spray application of the pathogen and the biocontrol agent in two treatments, i.e., Z. tritici (Zt) only and Z. tritici along with C. rosea (ZtCr) was used to assess the disease progress over time. The absence and presence of C. rosea in Zt and ZtCr, respectively, allowed to dissect variation for plant disease resistance and biocontrol efficacy for disease control. The study showed significant phenotypic variation among plant genotypes for disease progress in Zt and ZtCr treatments. Moreover, disease progress for individual plant genotypes differed significantly between the two treatments, reflecting the plant genotype dependent variation in biocontrol efficacy. For the phenotypic variation in disease progress and biocontrol efficacy, Genome-Wide Association Study (GWAS) using a 20K single-nucleotide polymorphism (SNP) marker array was also performed. In total, five genetic regions were found to be associated with disease resistance and C. rosea biocontrol efficacy. This work will serve as a basis for future studies to further characterize the loci associated with plant-biocontrol agent interactions, which ultimately can improve the efficacy of biocontrol applications.

CS1.4.25

BIOCONTROL ABILITY OF RATIONALLY DESIGNED PEPTIDE DERIVATIVES OF A NOVEL SOLANUM LYCOPERSICUM L. ANTIFUNGAL DEFENSIN

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Recently, the control of fungal pathogens represents a challenge for the agriculture due to the fast resistance spreading, and limited number of EU approved fungicides. Therefore, there is a substantial demand to develop fundamentally new and safely applicable plant protection strategies. According to our recent investigations rationally designed synthetic antifungal peptides derivatives spanning the evolutionarily conserved γ-core motifs (GXC-X[3-9]-C) of antifungal plant defensins are promising candidates. In the present work we investigated the biocontrol ability of rationally designed synthetic γ-core peptide derivatives of a novel Solanum lycopersicum L. defensin, K4CBP6. In vitro antifungal susceptibility testing against different plant pathogenic fungi pointed out that the efficacy of a K4CBP6 peptide derivative highly depends on the number of hydrophilic and positively charged amino acid residues. The biocontrol ability of K4CBP6 peptide derivatives was tested in a tomato plant-Botrytis cinerea model system. None of these peptides showed cell disruption effect on intact tomato plant leaves. They were able to inhibit the B. cinerea infection development on detached tomato plant leaves, or mitigated the symptoms depending on the in vitro antifungal efficacy. These proof-of-concept results suggest that γ-core peptide derivatives of K4CBP6 are promising biocontrol agents; however, several other tests (e.g. ecotoxicity testing) are needed to prove their safe environmental application in agricultural fields.

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CS1.4.26

ZOMBIE-FLIES: BEHAVIOURAL MANIPULATION BY AN INSECT-DESTROYING FUNGUS

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Certain insect pathogens can manipulate the behaviour of their hosts to increase their chance of transmission. The specialist fungal pathogen Entomophthora muscae turns their housefly hosts into so-called 'zombie-flies', where it spectacularly manipulates the behaviour of its host before killing it at sunset six days after initial infection. There are three stereotypical and sequentially observed behaviours, (1) summiting, whereby the fly climbs to an elevated position, (2) proboscis affixation, where the fly 'glues' itself to the substrate surface, and (3) wing raising, where the wings raise to not obstruct the fungal spores that will be shot out of the abdomen. Following behavioural observations for these behaviours, specific time points were determined and pooled-head samples collected for RNAseq analysis. We are using comparative transcriptomics of gene expression differences to assess what this pernicious hijacker is doing to provoke these striking moribund displays and how the host responds. Preliminary analyses have uncovered a handful of candidate genes, including several secreted effector proteins. With this work, we hope to gain an insight into the genetic underpinnings of the co-evolutionary processes leading to the extended phenotypic response of zombie-flies.

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CS2.3 SENSING AND SIGNALING

CS2.3.9

GREEN-LIGHT PERCEPTION IN FUNGI - ATTEMPTS TO SOLVE AN ONGOING CONUNDRUM

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For fungi, photoreceptors are of superior importance as light controls many substantial processes in the fungal life cycle such as reproduction and pathogenicity. Among the arsenal of different fungal light sensors reacting to a broad plethora of wavelengths, fungal rhodopsins are responsible for the perception of green light. Fungal rhodopsin are representatives of type I (microbial) rhodopsins and as such consist of seven transmembrane helices that form an interior pocket around the chromophore all-trans retinal. The retinal is covalently bound to the protein via a protonated Schiff-base and undergoes conformational changes upon light-activation that provide protein function. Fungal rhodopsins act either as proton pumps or as sensory proteins, however, detailed knowledge about their physiological function and biological role is still marginal. Considering our recent analyses of different fungal rhodopsins from the ascomycetes Fusarium fujikuroi and Aureobasidium pullulans and the basidiomycete Ustilago maydis, we will summarize the to-date available information about fungal rhodopsins. We will highlight the role of these green light sensors in the biology of fungi while taking into account the occurrence of rhodopsins in dependence of the inhabited niche, especially regarding the green-light-enriched phyllosphere. Besides that, we will compare the biophysical characteristics of different rhodopsins and report on the augmentation of proton-pump activity induced by the plant auxin indol acetic acid observed in certain fungal rhodopsins.

CS2.3.10

MOLECULAR EVOLUTION OF NOD-LIKE RECEPTORS IN SORDARIALES FUNGI

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Fungi have homologs of cytoplasmic immune receptors of the NLR family found in plants and animals. Fungal NLRs were identified in studies of conspecific non-self recognition and the associated programmed cell death process. Fungal NLRs are characterized by extensive diversity of domain architectures. However, a large fraction of fungal NLR domains remain unannotated, the variability of NLR repertoires within species is largely unknown, and we therefore lack a clear understanding of their origins and evolutionary trajectories. We performed detailed bioinformatic analyses of NLR genes found in 84 assembled genomes of Sordariales, the fungal order that includes Neurospora and Podospora. We discovered that the number of NLRs varied extensively across the order, ranging from 10 to 162 NLR loci per species. We also found a previously undescribed Nucleotide-Binding (NB) domain, that presents features of both NACHT and NB-ARC - the two NB domains classically described in NLRs. Using population genomic and phylogenomic analyses on resequencing data for 12 species, we show that some Sordariales NLRs have been under long-term balancing selection, consistent with their possible role in heterospecific non-self recognition. Our evolutionary studies firmly establish the remarkable variability of fungal NLR repertoires and highlight their original evolutionary signatures as impetus for their functional characterization in Fungi.

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CS2.3.11

CONSERVED SIGNALING FACTORS MEDIATE INTERSPECIES INTERACTIONS BETWEEN NEUROSPORA CRASSA AND TRICHODERMA ATROVIRIDE

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Cell fusion is a crucial process for the development of eukaryotic organisms, including filamentous fungi such as Neurospora crassa. In many species, germinating spores fuse with each other to form a supracellular network, which develops into the mycelial colony. In N. crassa, the interacting partner cells coordinate their behavior and switch between signal sending and receiving. This unique behavior is reflected in the alternating recruitment of the MAP kinase MAK-2 and the scaffold protein SO to the plasma membrane. We recently found that this cell dialog-like mechanism is conserved in the distantly related grey mold Botrytis cinerea, in which the MAK-2 homologue, BMP1, and the SO homologue, BcPro40, undergo the same subcellular dynamics. In mixed populations of B. cinerea and N. crassa spores, interspecies interactions are frequently observed albeit without evidence for fusion across the species barrier after physical contact. Interspecies communication is also mediated by the cell-dialog mechanism. Based on these observations, we hypothesized that this conserved signaling mechanism might also be relevant for other types of fungal interspecies interactions, including mycoparasitism. In the mycoparasitic fungus Trichoderma atroviride, knockout mutants of the mak-2 and so homologous genes tmk1 and tso are fully deficient in germling interactions. When wild-type T. atroviride and N. crassa spores were mixed, interspecies interactions between the two species were frequently observed. During these interactions, the typical dynamic subcellular localization of MAK-2-GFP occurred in N. crassa, whereas SO-GFP was also recruited to the plasma membrane but mislocalized compared to the intraspecies interaction. Strikingly, we obtained first evidence for cytoplasmic exchange between N. crassa and T. atroviride germlings after physical contact, which was followed by rapid cell death in both species. In conclusion, our data suggest that interspecies interactions are common in filamentous fungi and that they are mediated by a conserved signaling mechanism.

CS2.3.12

SCREENING A. NIDULANS MUTANTS FOR THE ABILITY TO GERMINATE IN WATER

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Filamentous fungi produce large amounts of stress-resistant conidia that can stay dormant for a long time. Conidial germination is the first critical step to initiate growth and is triggered by favourable environmental conditions such as water availability and nutrients (e.g. carbon sources). How dormancy is maintained and regulated is not clear. We attempt to identify the regulators involved in controlling dormancy and germination. Considering the importance of protein kinases in environmental sensing and signal transduction, we screened the Aspergillus nidulans kinase deletion library for mutants capable of germinating in pure water (i.e. the absence of nutrients) and identified the yakAΔ mutant. Further characterizations of the mutant show that the YakA kinase is important for germination behaviour, conidial development, conidial stress resistance and carbon regulation.

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CS2.3.13

A BAR1-INDEPENDENT DESENSITIZATION MECHANISM CONTROLS A-STE2 SIGNALLING IN FUSARIUM OXYSPORUM

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Fusarium oxysporum (Fo) is one of the most destructive pathogens affecting a wide variety of plant crops used for both human food and animal feed worldwide. Its growth and development are regulated by the interaction between peptide pheromones, A and α, and their cognate receptors, Ste3 and Ste2, that modulate different aspects of fungal physiology and pathology, including germination, chemotropism and quorum sensing. To retain cell responsiveness in molecularly overcrowded environments fine-tuning of pheromone-receptor desensitization is critically required to localize and discriminate among different signalling molecules.
In the model fungus Saccharomyces cerevisiae, the secreted aspartyl protease Bar1 acts as a "barrier" and antagonist of α-Ste2 signalling. Here we performed an HPLC-Q-TOF-MS analysis of synthetic Fo α-pheromone (WCTWRGQPCW) either pre-incubated with wild-type or ΔBar1 fungal germlings or their exudates. Notably, α-pheromone treatment with either wt or ΔBar1 fungal exudates generated two complementary peptides generated by cleavage between the Cys² and Thr³ residues. On the contrary, direct incubation of α-pheromone with wt but not ΔBar1 fungal germling produced two complementary sub-products deriving from pheromone cleavage between Thr³ and Trp⁴ residues, suggesting that Bar1 activity is associated with fungal cells in Fo. In line with this, bioinformatic analysis identified a C-terminal localized transmembrane domain in Fo BAR1 protein, differently from other fungal counterparts (i.e. Candida albicans and S. cerevisae). Altogether our results suggest the existence of a yet unknown Bar1-independent α-Ste2 desensitization mechanism in Fo.

CS2.3.14

CHROMATIN-IMMUNOPRECIPITATION REVEALS THE PNPF2 TRANSCRIPTIONAL NETWORK CONTROLLING EFFECTOR-MEDIATED VIRULENCE IN A FUNGAL PATHOGEN OF WHEAT

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The regulation of virulence in plant-pathogenic fungi has emerged as a key area of importance underlying host infections. Pf2 is a member of the Zn_2-Cys_6 family of fungal transcription factors (TFs), where orthologues regulate the expression of genes linked to parasitism in several plant-pathogen lineages. These include PnPf2 which controls the expression of necrotrophic effector (NE) genes in Parastagonospora nodorum, the causal agent of septoria nodorum blotch of wheat. As a result, regulation exerted by PnPf2 dictates the outcome of effector-triggered susceptibility on wheat, wheat. However, direct genomic targets and whether other are regulators involved in NE gene regulation, remain unknown. We used chromatin immunoprecipitation (ChIP-seq) and a mutagenesis analysis to investigate these components. Two distinct binding motifs connected to positive gene-regulation were characterised and genes directly targeted by PnPf2 were identified. These included genes encoding major effectors and other components associated with the P. nodorum pathogenic lifestyle, such as carbohydrate-active enzymes and nutrient assimilators. This supports a direct involvement of PnPf2 in coordinating virulence on wheat. Other TFs were also prominent PnPf2 targets, suggesting it also operates within a transcriptional network. Several TFs were therefore functionally investigated in connection to fungal virulence. Distinct metabolic and developmental roles were evident for the newly characterised Pro1, Ada1, Ebr1 and the carbon-catabolite repressor CreA. Overall, the results uphold PnPf2 as the central transcriptional regulator orchestrating genes that contribute to virulence on wheat and provide mechanistic insight into how this occurs.

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CS2.3.15

VEGETATIVE INCOMPATIBILITY IN A. BISPORUS

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Fungal colonies encountering each other are either vegetatively compatible and fuse to one larger colony or incompatible, usually resulting in cell death in the interaction zone. This phenomenon has mainly been studied in ascomycetes. In this group, direct interaction between gene products of specific loci of incompatible partners occurs induce cell death. Self/non-self recognition in ascomycetes mostly occurs between homokaryons. In contrast, in basidiomycetes VI has generally been described to occur between heterokaryons. However, in Agaricus bisporus we observed that VI also occurs between homokaryons and that this was independent of the mating type genes. Furthermore, interactions between heterokaryons with one set of chromosomes different and the other shared (e.g. heterokaryon AB with AD) showed VI. These observations suggest a different, more complex mechanism of VI and suggest that in basidiomycetes, a direct interaction between gene products is probably not occurring. Instead, we recently proposed a reader-writer (R/W) system modifying and recognizing proteins, as found in ascomycetes. A crucial prediction of this model is that a cytoplasm with one nucleus can be incompatible with a cytoplasm with a different nucleus, while these two nuclei can coexist in a single cytoplasm. Evidence for this model using experiments with homokaryons and heterokaryons of A. bisporus will be discussed. A population of homokaryotic single spore isolates (SSI's) from a cross between two homokaryons derived from incompatible strains of A. bisporus was produced. The obtained 150 SSI's were tested for VI against the two parent homokaryons, when both the tester and parent homokaryon were combined with a common nucleus. The VI screen used a newly developed method using Evans blue . SNP analysis indicated the involvement of four quantitative trait loci (QTL) on four chromosomes. The identified chromosomes involved in VI were the same as involved in an assay using chromosome substitution lines.

CS2.3.16

DISTINCT INTRA- AND EXTRACELLULAR N-ACETYLGLUCOSAMINE SIGNALING CASCADES IN A FILAMENTOUS SOIL FUNGUS

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Communication is essential within the soil microbiome for the survival in a fast-changing environment. Environmental triggers and signals such as small molecules and volatile organic compounds (VOCs) have been identified for the interaction of the biotic community. N-acetylglucosamine (GlcNAc) is a ubiquitous structural component of the fungal and bacterial cell wall, biofilms, and animal glycosaminoglycans of the extracellular matrix. Due to its abundance, the amide derivative sugar serves as carbon and nitrogen source for many microorganisms. Importantly, it also acts as signaling molecule and stimulates virulence in human pathogenic fungi leading to a switch to filamentous growth in dimorphic yeasts.
Previously, we identified a gene cluster in soil dwelling, filamentous model fungi Trichoderma spp. and Neursopora crassa, which coordinates enzymes for GlcNAc catabolism through the NDT80-like transcription factor RON1. In our recent study we show that the presence of GlcNAc additionally induces differential expression of a plethora of genes from 22 functional groups, including cell wall differentiation, virulence and secondary metabolism. In dimorphic yeasts, such as Candida albicans, deletion mutants of the transporter gene ngt1 are defective in inducing filamentous growth, indicating GlcNAc has to enter the cell to serve as a signaling molecule. In T. reesei we identified NGT1 as the only specific GlcNAc transporter. However, at high concentrations of GlcNAc, the molecule can also enter the cell via unspecific transporters. In contrast to the data from dimorphic yeasts, the pathways for e.g. defense and virulence seem to be induced already by external GlcNAc in T. reesei, whereas GlcNAc has to enter into the cell for activating the catabolic pathway.
These observations suggest the presence of distinct GlcNAc signaling cascades in Trichoderma reesei and possibly other filamentous soil fungi, which are activated differentially via intra- or extracellular GlcNAc.

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CS2.3.17

FUNCTIONAL ORGANIZATION OF THE STRIPAK COMPLEX IN CRYPTOCOCCUS NEOFORMANS

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The eukaryotic serine/threonine protein phosphatase PP2A is a heterotrimeric enzyme composed of a scaffold A subunit, a regulatory B subunit, and a catalytic C subunit. Of the four known B subunits, the B‴ subunit (known as striatin) interacts with the multi-protein striatin-interacting phosphatase and kinase (STRIPAK) complex. Functional studies on the STRIPAK complex in Cryptococcus neoformans have not been reported. Using protein sequences of STRIPAK components from other species, protein orthologs were identified for STRIPAK complex subunits in C. neoformans, namely the A subunit, the C subunit, the B‴ subunit striatin, the striatin-interacting protein STRIP, the tail-anchor domain protein SLMAP, and the striatin-associated protein Mob3. Distinct protein domains that mediate the structure and/or function of STRIPAK complex subunits in other species were also found to be conserved in the protein orthologs in C. neoformans. To characterize the organization of the STRIPAK complex, we are taking two complementary approaches. First, the genes are being deleted or modified by transformation and homologous recombination to recover gene deletion mutants or conditional alleles for genetic analysis. If the genes prove to be essential, we will construct and characterize inducible-repressible alleles for phenotypic analysis. Second, yeast two-hybrid analyses are being performed. Plasmid constructs encoding GBD (Gal4 DNA binding domain) and GAD (Gal4 transcriptional activation domain) fused to each STRIPAK complex subunit are being generated and transformed into yeast two-hybrid reporter strains. Pair-wise interactions among STRIPAK components will be analyzed by assaying for Gal4-dependent expression of reporter genes ADE2, HIS3, and lacZ. Previous studies on the fungal STRIPAK complex have shown that organization of STRIPAK subcomplexes partially mediate its functions in connecting multiple cellular processes, such as TORC2 signaling, mitophagy, and cell wall integrity pathways. The conserved nature of these genes may hint at similar roles for the STRIPAK complex in development of C. neoformans.

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CS2.3.18

DENSITY-DEPENDENT BEHAVIOUR OF ASPERGILLUS NIGER CONIDIA

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Quorum sensing (QS) is a density-dependent stimulus-response system mediated by secreted signalling molecules (QSM). The concept has broadened throughout the decades: it has been proven not only being a bacterial characteristic. Within the fungal kingdom, besides dimorphic species whose density-dependent behaviour is well-understood, mycelial QS of some filamentous species has also been investigated. However, the existence of this behaviour has not been examined before in the case of conidia. We show that conidia of the industrially significant Aspergillus niger demonstrate quorum sensing that can be observed in their swelling pattern. The behaviour was transferable by the supernatant. With a supernatant transfer experiment coupled with organic extraction we managed to prove that the density-dependence is likely caused by an apolar compound. The assessment of the chemical composition of the supernatant to identify the QSM candidates and to find out whether conidial QS is independent from the mycelial QS in this regard is in progress. We anticipate that in the long run our results will be beneficial for both medical science and industrial biotechnology. The better understanding of fungal quorum sensing is expected to result in the emergence of new antifungal therapies, as well as the more efficiently controlled usage of fungi by the industry.

CS2.3.19

EFFECT OF THE TRANSCRIPTION FACTOR ASFLBC DELETION ON ENZYME PRODUCTION IN SOLID STATE CULTURE IN ASPERGILLUS SOJAE

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In Japan, Aspergillus fungi are widely used in traditional Japanese foods, seasonings, and beverage fermentation. One of them, Aspergillus sojae is exclusively used for production of soy sauce and miso (soybean paste), which are traditional Japanese seasonings. A. sojae is generally cultivated on soybeans and wheat, known as solid state culture, during soy sauce manufacturing. In another industrially important fungus, Aspergillus oryzae, it is known that solid state culture induced the secretion of a large amount of hydrolases, and some hydrolases are rarely expressed in submerged culture. Previously, we found that the transcription factor FlbC regulates the glucoamylase gene glaB and the secretory acid protease gene pepA, which are specifically expressed in solid state culture in A. oryzae (1). A. sojae has a gene highly homologous (96.4%, hereafter referred to as AsflbC) to A. oryzae flbC. In this study, we performed disruption of the AsflbC gene and examined the involvement of AsFlbC in the hydrolases production in solid state culture. The AsflbC disruption strains were generated using the CRISPR/Cpf1 system, tuned for genome editing of A. oryzae and A. sojae (2). The AsflbC disruptant showed that glucoamylase production is remarkably decreased than the wild-type (WT) strain in solid state culture using wheat bran as a substrate. In addition to glucoamylase, acid protease activity in solid state culture was also decreased by AsflbC disruption. In this conference, we will also provide the results of the expression levels of amylolytic and proteolytic (acid, neutral, and alkaline protease) genes in WT and the AsflbC disruptant.

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CS2.3.20

BIG1 CONTROLS ARF2 ACTIVATION DURING MUCOR LUSITANICUS YEAST DEVELOPMENT THROUGH THE PKA PATHWAY

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Mucor lusitanicus (previously M. circinelloides f. lusitanicus) is a dimorphic mucoral fungus. It is a biological model to understand cell differentiation and the opportunistic fungal infection known as mucormycosis. The Arf family proteins are monomeric G proteins involved in the regulation of vesicular trafficking in eukaryotes, and they depend on ArfGEF-type proteins (guanine nucleotide exchange factor) for their activation by exchanging GDP for GTP. The central role of Arf-ArfGEF proteins is the regulation of vesicle transport between the endoplasmic reticulum, the Golgi apparatus and the plasma membrane. Previously, we reported the role of the Arf2 protein in the yeast development of M. lusitanicus. arf2 transcript accumulates mainly in the yeast stage and the Δarf2 mutant showed a decreased in yeast germination compared to the wild-type strain. In this work, we identified that M. lusitanicus possesses six genes in its genome that encode ArfGEF proteins. Expression analysis of arfGEF genes showed that the transcript of big1 (arfGEF2), arfGEF3 and arfGEF4 accumulates mainly during yeast development, suggesting these proteins could be activators of Arf2. Through a genetic, molecular, and physiological approaches, we demonstrate that Big1 physically interacts with Arf2. Interestingly the Δbig1(+)(-) mutant showed a similar phenotype as Δarf2, and the mutation of the codon that encodes threonine 871 (phosphorylation site by PKA) of Big1 abolishes its activity as a guanine nucleotide exchanger, demonstrating the role of Big1 and the PKA pathway in the control of yeast development. By other hand, we determine that the yeast development of M. lusitanicus is through the participation of Ras2, Cyr1,

Pde2b, PkaR1, Big1 and Arf2. With these results, it is possible to propose new molecular targets for the control of yeast growth which is considered the avirulent morphology in M. lusitanicus, as well as this knowledge could be suitable for the optimization of fermentation process

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CS2.3.21

LIPID RAFTS IN SCHIZOPHYLLUM COMMUNE – INSIGHTS IN LOCALIZATION AND COMPOSITION

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Lipid rafts are small microdomains of the plasma membrane. Their unique composition of lipids and proteins and their role in several cell mechanisms like cell signaling and cell polarity makes them highly interesting. Due to the size of ~10-200 nm it is difficult to investigate the formation, composition and localization of lipid raft microdomains.
We are investigating the lipid rafts of the basidiomycete Schizophyllum commune, a polarized growing white rot fungus. Its high competitive ability is based on the recognition of other fungi and bacteria, the production of specific extracellular metabolites and a strategy of fast growth. Therefore, fast transport processes within the hyphae and an efficient cytoskeleton are essential. Lipid rafts are involved in these transport processes, but their role in polarized growth is still not fully understood. We used microscopy with LSM 780 to locate rafts at the plasma membrane of hyphal tips by staining with the fluorescent dye filipin that binds to the high amount of sterols in this microdomains. Further we labelled the raft associated protein called stomatin with the fluorescence protein dTomato. We also investigated the co-localization of the actin cytoskeleton and the raft protein stomatin by mating of S. commune Lifeact-EGFP and S. commune Sto-dTom.
The results showed that sterol rich domains are mainly located at the tip as expected. The raft associated protein stomatin however shifts its location over time. Fluorescence microscopy also showed a co-localization of actin and stomatin, in particular at clamp sites of the Schizophyllum commune dikaryon. In addition, an interesting phenotype of hyphal growth could be observed.
Investigations about localization of special membrane domains in S. commune showed similarities to lipid rafts, whereas insights into formation and protein association revealed differences to lipid rafts of e.g. humans or ascomycetes.

CS2.3.22

CONVERGENT EVOLUTION OF G-PROTEIN COUPLED RECEPTORS OF ARTHROBOTRYS FLAGRANS AND CAENORHABDITIS ELEGANS FOR SENSING ASCAROSIDES

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Nematode-trapping fungi form three-dimensional trapping-networks to capture nematodes when the environment is deprived of nutrients and in the presence of nematodes. The predacious fungus Arthrobotrys flagrans emits attractive olfactory substances to lure the prey Caenorhabditis elegans into the traps. Meanwhile C. elegans secretes ascarosides as pheromones which control many developmental processes in C. elegans (Park et al., 2019). However, they are recognized by A. flagrans and induce trap formation by downregulation of the trap-inhibiting arthrosporol biosynthesis pathway (Yu et al., 2021). The question arises of how the fungus senses nematode-deriving ascarosides and how the external signals are transmitted to down-regulate the arthrosporol biosynthesis. G-protein dependent signaling is likely to fulfill this task.
To find ascaroside-sensing receptors in A. flagrans, the reported ascaroside-sensing receptors of C. elegans were analyzed, and two conserved motifs were found in SRBC-64, SRBC-66, and one of them in Daf-37 and Daf-38. Both motifs were also present in the receptor GprC of A. flagrans. The expression of gprC was induced by nematodes. Gene-deletion revealed its involvement in the regulation of trap production. Normal trap morphogenesis in a gprC-deletion strain was restored by introducing GprC or a chimeric protein composed of the N-terminal half of SRBC-64, SRBC-66 or Daf-38 and the A. flagrans-derived C-terminal half of GprC. Hence, ascaroside binding is conserved in C. elegans and A. flagrans. Three-dimensional modeling and docking predictions suggested arginine174 to be crucial for ascaroside binding in GprC. This was proven by site-directed mutagenesis. Since there is very little overall sequence conservation, our results suggest convergent evolution of the ascaroside receptors.

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CS2.3.23

INVESTIGATING THE H2O2 SENSITIVITY OF LBAP1, A YAP1-LIKE HOMOLOG IN THE ECTOMYCORRHIZAL FUNGUS LACCARIA BICOLOR.

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Ectomycorrhizal fungi (ECM) are an important group of organisms that ensure that trees can flourish in all types of environmental conditions. By providing nutrients, water and protection against stress conditions in exchange for sugars, they allow their host trees to grow. The stress response of plants has already been studied in detail. However, stress response signalling remains poorly understood in ECM fungi. In this study, we assess the potential of H2O2 to regulate a putative transcription factor, LbAP1, using a bio-informatic approach, and localisation of heterologously expressed eGFP-fused LbAP1 upon H2O2 treatment. LbAP1 is a homolog of YAP1, a key regulator of ROS responses in Saccharomyces cerevisiae. Oxidation of two cysteines in YAP1, located in the N- and C-terminal CRD, results in nuclear accumulation through masking of the NES by disulfide bond formation and transcriptional activation of target genes. In contrast to YAP1, results obtained by fluorescence microscopy showed that an LbAP1-eGFP fusion protein did not accumulate in the nucleus upon treatment of transformed Saccharomyces cerevisiae BY4741 with 0.4 mM H2O2. As C-terminal eGFP could be interfering with disulfide bond formation, the H2O2 sensitivity of eGFP-LbAP1 was examined and found to also be unresponsive to H2O2. Upon analysis of both protein sequence and structure of YAP1 and LbAP1, it was revealed that LbAP1 contains 2 Cys less than YAP1. Furthermore, the Cys responsible for nuclear localisation upon oxidation in YAP1 are not conserved in LbAP1. The NLS and NES, however, are partially conserved. Structural predictions of LbAP1 also showed that the helix structures containing these important cysteines are not conserved, possibly indicating that LbAP1 nuclear localisation is not H2O2 dependent. From these results, we can conclude that LbAP1 regulation might be H2O2 independent, and that the cellular mechanisms of ECM fungi should be investigated separately to other fungi.

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CS2.4 MOLECULAR TOOLS

CS2.4.9

ESTABLISHMENT OF IN VIVO PROTEIN-PROXIMITY LABELING WITH BIOTIN IN SORDARIA MACROSPORA

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Recently our group established the Biotin Identification (BioID) technique in the filamentous fungus Sordaria macrospora (1). This method relies on the in vivo labeling of proximal proteins through a promiscuous biotin ligase which is fused to the protein of interest. Biotinylated proteins are enriched through biotin affinity capture and subsequently identified by liquid chromatography coupled to mass spectrometry (LC-MS). Since proteins in the microenvironment of the bait are covalently labeled with biotin, protein-protein binding does not have to stay intact during cell lysis, allowing the identification of proteins that weakly or transiently co-localize with the bait.

For the establishment of BioID in Sordaria macrospora, we used the well-characterized striatin-interacting phosphatase and kinase (SmSTRIPAK) complex as an example. This multiprotein complex is evolutionary highly conserved from yeast to man and regulates cellular pathways by phosphorylation/dephosphorylation. Deletion of SmSTRIPAK subunits causes sterility and developmental defects in hyphal fusion and vegetative growth. For the proof of concept, one component of the complex, the SmSTRIPAK interactor 1 (SCI1), was fused to a codon-optimized TurboID biotin ligase. Expression of this SCI1-TurboID fusion construct controlled by the native sci1 promoter in the Δsci1 deletion strain complemented the deletion strain phenotype and fertility was restored. In the following SCI1-BioID experiment the already known SmSTRIPAK components PRO11, SmMOB3, PRO22, and SmPP2Ac1 were captured, thus demonstrating the successful application of BioID in S. macrospora. By the fusion of TurboID with other subunits, we aim to achieve a multi-perspective proximity view on the SmSTRIPAK complex in vivo. We hope that the BioID proximity labeling approach will

provide a powerful proteomics tool for other fungal biologists.

1. Hollstein, L. S., Schmitt, K., Valerius, O., Stahlhut, G., & Pöggeler, S. (2022). Establishment of in vivo proximity labeling with biotin using TurboID in the filamentous fungus Sordaria macrospora. Scientific Reports, 12(1), 17727. https://doi.org/10.1038/s41598-022-22545-x

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CS2.4.11

SIMPLY CUT OUT – COMBINING CRISPR/CAS RNPS AND TRANSIENTLY SELECTED TELOMERE VECTORS FOR MARKER FREE-GENE DELETION IN TRICHODERMA ATROVIRIDE

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Trichoderma atroviride is a well-known mycoparasite used to protect plants against fungal pathogens. To facilitate genetic manipulation in this species, such as multiple gene deletions and retransformations, expanding the genetic toolbox is essential. Based on previous studies in Botrytis cinerea and Magnaporthe oryzae, a robust strategy that combines Cas9-gRNA ribonucleoprotein complex (RNP) delivery with co-transformation of transiently stable vectors containing telomeres was applied to T. atroviride. With this approach, we deleted the pex5 gene coding for the peroxisomal targeting signal type-1 (PTS1) receptor, a cytosolic cycling adaptor protein required for protein import into the matrix of peroxisomes. During co-transformation, two DNA double-strand breaks were induced with the CRISPR/Cas9 system and subsequently repaired by the cell's own non-homologous end joining (NHEJ) repair mechanism, which resulted in loss of the ORF of pex5. Transformant selection was accomplished by a transiently stable telomere vector carrying the hygromycin-resistance gene hph, that was rapidly lost after cultivation on non-selective media. For comparison, the marker gene pyr4, being part of a homology repair donor, was used to replace pex5. Δpex5 deletion mutants resulting from both approaches were confirmed by locus-specific genotyping PCR and sequencing and exhibited biotin auxotrophy, consistent with mutants of other fungi whose PTS1 import is impaired. By simply cutting our target DNA using the new approach, we were able to bypass the tedious generation of knock-out cassettes and benefit from NHEJ instead of the less efficient homology-directed repair in the cell. We demonstrate that combining CRISPR/Cas9 RNP delivery with transiently stable telomere vectors allows marker-free gene deletion and vector recycling in T. atroviride.

CS2.4.12

BOSCALID AS A NEW SELECTABLE MARKER FOR ASCOCHYTA LENTIS AND ASCOCHYTA RABIEI

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Fungal pathogens use an array of infection strategies to survive. The host-specific pathogens Ascochyta lentis and Ascochyta rabiei infect lentil and chickpea, respectively. These pathogens secrete small molecules known as effectors to colonise their hosts. Tools to characterise effectors using genetic manipulation based on Agrobacterium tumefaciens mediated transformation (ATMT) of TDNA exist for both pathogens. Recently, a robust method to perform genetic manipulation in A. lentis which enabled the targeted disruption of a gene involved in melanin production, AlSCD1, and the deletion of AlAvr1 genes using ATMT has been reported. However, gene manipulation has been limited to performing a single gene manipulation at a time due to the lack of a second selectable antibiotic marker suitable for use in A. lentis and A. rabiei.
The fungicide boscalid is a known succinate dehydrogenase inhibitor (SDHI) and single nucleotide changes within the succinate dehydrogenase (Sdh) genes have been found to lead to boscalid resistance. Here we describe the use of mutated versions of the A. rabiei and A. lentis Sdh homologues as selectable markers for random and targeted integration of transgenes, gene knockout and gene complementation in both pathogens. As a proof of concept, we have successfully deleted AlSCD1 in the AlKewell transformant where AlAvr1-2 has been disrupted using the hygromycin B cassette as well as complemented AlSCD1 in the knockout in a random and targeted way to reenable melanin production. Using the same strategy, we also show double knockout and complementation in A. rabiei. This study will enable us to characterise further the role of effectors and gain better understanding of the pathogen's mechanism of infection.

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CS2.4.13

TRANS-NUCLEI CRISPR/CAS9 FOR SAFE GENOME EDITING TECHNOLOGY IN PLEUROTUS OSTREATUS

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CRISPR/Cas9 is a promising system for genome editing in many organisms including edible fungi, such as mushrooms. It is a strong tool for reverse genetic to see the function of gene of interest for basic science and can also be applied for breeding of new strains with desirable functions in industry. However, for especially edible use, a safe technology to avoid restriction for genetically modified organisms (GMO) is to be developed.
We have successfully introduced CRISPR/Cas9 system in an edible mushroom, Pleurotus ostreatus by introducing a recombinant plasmid expressing Cas9 protein and single guide RNA (sgRNA) into monokaryotic strains (Boontawon et al., AMB Express, 2021). We used this technique to isolated disruptant of single gene or multiple genes using multiple sgRNA expressing cassettes (Xu et al., FEMS Microbiol. Lett., 2022). And the same technology was applied to a dikaryotic strain to isolate strains with gene knock-out in both of the nuclei. And less spore dikaryotic mutants with deficiency in msh4 or mer3 were successfully isolated in one transformation experiment (Yamasaki et al., FEMS Microbiol. Lett., 2022). We have also developed a marker-less genome editing system using transient transformants screening protocol (Koshi et al., J. Wood Sci., 2022) However, integration of the introduced plasmid was frequently observed that hamper safe and efficient non-GM genome editing using plasmid DNA. Furthermore, we established a DNA-free, RNP-dependent CRISPR/Cas9 in P. ostreatus (Boontawon et al., FEMS Microbiol. Lett., 2021), but this technique is dependent on the screening by the introduced mutation in the target gene, pyrG, and not applicable for general target genes.
Here a new protocol for safe genome editing is reported by applying a cross of a donor monokaryon that harbors Cas9/sgRNA expressing cassette in the genome with a recipient monokaryon to cause a genome editing in a recipient nucleus in trans.

CS2.4.14

ESTABLISHMENT OF GENETIC TRANSFORMATION SYSTEMS FOR FUNCTIONAL ANALYSIS OF POLYKETIDE SYNTHASE GENE CLUSTERS IN THE INDOOR FUNGUS STACHYBOTRYS CHARTARUM

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Stachybotrys chartarum is an indoor fungus that has health relevance due to the production of mycotoxins. The most toxicologically relevant group of mycotoxins from Stachybotrys are the satratoxins. Quantitatively, the phenylspirodrimanes represent the most important group and exhibit a high structural diversity, depending on the individual strains and cultivation conditions. To date, however, little is known about the genetic background of Stachybotrys secondary metabolites and their biosynthesis. Moreover, to functionally study gene clusters, a method for the introduction of exogenous DNA into the fungus should be developed.
To enable the identification of new secondary metabolites and to functionally characterize targeted gene clusters, particularly polyketide synthase (PKS), we develop an efficient method for the genetic manipulation of Stachybotrys chartarum. The phylogenetic analyses of Stachybotrys and its comparison with other filamentous fungi, revealed a number of interesting PKS gene clusters, which offer the potential for previously undiscovered secondary metabolites. With Agrobacterium-mediated and protoplast-mediated transformations, methods for the introduction of exogenous DNA into Stachybotrys have been established. These have already been applied successfully in the generation of gfp, overexpression and deletion mutants. These methods offer the starting point for the investigation of a large number of Stachybotrys gene clusters.

CS2.4.15

TOWARDS THE ESTABLISHMENT OF A CRISPR/CAS9 AND A TET-ON SYSTEM IN NEUROSPORA CRASSA

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The ability to genetically modify an organism of interest is important for understanding gene functions and corresponding molecular mechanisms. In filamentous fungi one of the standard methods is the homologous recombination (HR) for generating knock-outs. But HR is inefficient and the number of selection markers available is restricted (1). The RNA-guided CRISPR/Cas9 system on the other hand is highly efficient, easy to handle and allows a precisely targeted mutagenesis. It has already been successfully applied and adapted in some filamentous fungi (2). In Neurospora, non-replicating plasmids carrying the cas9 and the gRNA sequence, respectively, were successfully applied (3). But so far this method is not implemented as a standard method for Neurospora. Here, we intend to establish a different version of the CRISPR/Cas9 system by integrating the cas9 sequence into the N. crassa genome and comparing the efficiency of a constitutive vs an inducible promotor. Another inducible promotor system which is already established in Aspergillus niger (4) is the so-called Tet-On system, deriving from regulatory elements of the bacterial Tetracycline Operon (tetO). It consists of a reverse tetracycline-dependent transactivator (rtTA) and the tetO array in combination with a minimal Promotor (Pmin). In a next step we now want to establish the Tet-On system in N. crassa, by using a strain with an ectopically integrated rtTA sequence. In combination with a tetO-Pmin construct integrated in the promotor region of the gene of interest this system will allow an inducible gene expression by Doxycycline.
References:
1. Wang, S. et al. Applied Microbiology and Biotechnology (2017).
2. Schuster, M. & Kahmann, R. Fungal Genetics and Biology (2019).
3. Matsu-ura, T., Baek, M., Kwon, J. & Hong, C. Fungal Biol Biotechnol (2015).
4. Meyer, V. et al. Appl Environ Microbiol (2011).

CS2.4.16

APPLICATION OF RECYCLABLE, MARKER-FREE CRISPR/CAS9 TOOLS FOR TARGETED GENOME EDITING IN THE POSTHARVEST PATHOGENIC FUNGI PENICILLIUM DIGITATUM AND PENICILLIUM EXPANSUM

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Penicillium digitatum and Penicillium expansum are plant pathogenic fungi that cause the green and blue mold diseases, respectively, leading to serious postharvest economic losses worldwide. Moreover, P. expansum can produce mycotoxins, which are hazardous compounds to human and animal health. The development of tools that allow multiple and precise genetic manipulation of these species is crucial for the functional characterization of their genes. In this context, CRISPR/Cas9 represents an excellent opportunity for genome editing due to its efficiency, accuracy and versatility. In this study, we developed protoplast generation and transformation protocols and applied them to implement the CRISPR/Cas9 technology in both species for the first time. For this, we used a self-replicative, recyclable AMA1-based plasmid which allows unlimited number of genomic modifications without the limitation of integrative selection markers. As a test case, we successfully targeted the wetA gene, which encodes a regulator of conidiophore development. Finally, CRISPR/Cas9-derived ΔwetA strains were analyzed. Mutants showed reduced axenic growth, differential pathogenicity and altered conidiogenesis and germination. Additionally, P. digitatum and P. expansum ΔwetA mutants showed distinct sensitivity to fungal antifungal proteins (AFPs), which are small, cationic, cysteine-rich proteins that have become interesting antifungals to be applied in agriculture, medicine and in the food industry. With this work, we demonstrate the feasibility of the CRISPR/Cas9 system, expanding the repertoire of genetic engineering tools available for these two important postharvest pathogens and open up the possibility to adapt them to other economically relevant phytopathogenic fungi, for which toolkits for genetic modifications are often limited.

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CS2.4.17

UPREGULATION OF SECONDARY METABOLITE PRODUCTION IN ASPERGILLUS MELLEUS USING IN VITRO CRISPR

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[1]University of Southern California, Los Angeles, United States, [2]University of California, Riverside, Riverside, United States

Fungal secondary metabolites are organic compounds produced by biological gene clusters (BGCs) in the fungal genome. These compounds are not vital to the survival of the organism, but are often associated with medicinal and industrial properties. Our strain of focus, Aspergillus melleus, produces several known secondary metabolites with antibiotic, anti-parasitic, insecticide, and nematicide potential. However, additional compounds may still be discovered from this fungal strain as antismash shows that there are multiple gene clusters. A negative global regulator of fungal secondary metabolite production, mcrA (multicluster regulator A), aids in the inactivation of many BGCs in the genome. Targeting mcrA will allow for the upregulation of known secondary metabolites and the possibility for discovery of new natural products.
Gene editing is a promising technology that can be used for genome manipulation. Using the CRISPR Cas9 genome editing system, we designed primers to target the regions surrounding mcrA to knockout the gene and replaced it with an antibiotic selection marker. Successful transformation of the strain was determined by innoculating the mutant strain on agar plates containing our selection marker to confirm marker uptake by resistance to antibiotic. This confirmation was further verified by sequencing primers comparing the wild type and mutant strain.
LC-MS traces of the wild type and mutant strains were grown in different conditions and compared. Our successful deletion of mcrA allowed for activation of new pathways, demonstrated by the emergence of new compounds and the upregulation of others. Structural characterization using tools such as cryo electron microscopy (cryoEM) confirm several compounds.

CS2.4.19

DEVELOPMENT OF A SYNTHETIC BIOLOGY TOOLKIT FOR THE HETEROLOGOUS GENE EXPRESSION IN AGARICUS BISPORUS

Panward Prasongpholchai[1], Tiantian Fu[1], Fabrizio Alberti[1]

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Basidiomycota fungi are prolific producers of high-value metabolites including terpenes, terpenoids, alkaloids, and sugar derivatives. Although many fungal-derived natural products are known today, the production of these compounds in the native producers are limited primarily due to the low/cryptic expression level of biosynthetic genes under the laboratory conditions. In recent years, new genetic and cultivation-based strategies for engineering of Basidiomycota fungi are becoming more available, yet these are often species-specific and genetic engineering techniques require a sophisticated knowledge of the biology of the producing organisms. Heterologous expression of Basidiomycota biosynthetic pathways in Ascomycota fungi such as Aspergillus spp. is feasible, however, more often they are unable to process numerous introns found within Basidiomycota genes. As such, we aim to develop a robust synthetic biology toolkit for the heterologous expression of Basidiomycota genes in a tractable Basidiomycete Agaricus bisporus (white button mushroom), for which several protocols for introducing exogenous DNA as well as genetic manipulation have been established. We hope that the toolkit would benefit both synthetic biology community as well as fungal natural products researchers in order to exploit Basidiomycota genomes as the source for the production of high-value compounds and in other applications.

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CS2.4.20

A VERSATILE SELECTION FREE CRISPR-CAS9 TRANSFORMATION SYSTEM FOR ASPERGILLUS FUMIGATUS

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Aspergillus fumigatus is a saprophytic fungus that is the cause of more than 300,000 life-threatening infections annually. Unlike the model yeast Saccharomyces cerevisiae, targeted allele replacement in A. fumigatus is complicated by low rates of homologous recombination and the fact that replacement cassettes require long homology arms of c. 1kb. CRISPR-Cas9 mediated transformation has been widely using as a genome editing tool to overcome some of these issues. However, successful editing normally relies on time consuming multi-step cloning procedures paired with the use of selection markers, which can result in a metabolic burden for the host and unintended transcriptional modifications at the site of integration. Recently we published data showing that an in vitro CRISPR-Cas9 assembly methodology could be used to perform genome editing without the need for selectable markers. We have introduced a functional GFP-epitope tag to the N- and C-terminus of the PacC and SrbA transcriptional regulators. In addition, we have generated targeted point mutations within genes by using a single-strand oligonucleotide as a repair template. Overall, our selection free method decreases the time required for complex construct synthesis and can potentially be translated to other fungi.

CS2.4.21

OPTIMIZING GENETIC TRANSFORMATION OF MYCELIOPHTHORA THERMOPHILA

Lisa Wasserer[1], Anton Glieder[1]

[1]*Bisy GmbH, Hofstätten an der Raab, Österreich*

The filamentous fungus Myceliophthora thermophila ATCC 42464 is a promising production host for both homologous and heterologous proteins. Its capability to secrete high amounts of proteins enables the hydrolysis of all major polysaccharides found in biomass and therefore provides an efficient cellulose degradation system. This thermophilic organism can be used for a high yield production of thermostable enzymes, which makes it a great candidate for biotechnological applications. There are different available transformation protocols. In order to optimize known techniques a protoplast mediated transformation protocol was investigated and optimized for the M. thermophila ATCC42464 strain, for an efficient DNA uptake. As a first step towards using this strain as an efficient protein production host, new expression cassettes were constructed and used for transformation with a reporter to analyse various constitutive regulated promoters. The choice of the promoter of the selection marker gene influenced the transformation efficiency.

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CS2.4.22

INSERTIONAL MUTAGENESIS USING THE TC1-MARINER TRANSPOSON IMPALA IN THE WHEAT FUNGAL PATHOGEN ZYMOSEPTORIA TRITICI

Marc-Henri Lebrun[1], Yohann Petit[1], Anais Pitarch[1], Camille Delude[2], Gabriel Scalliet[2]

[1]*Inrae, Palaiseau, France,* [2]*Syngenta Crop Protection, Stein, Switzerland*

Zymoseptoria tritici is a fungal pathogen of wheat. To decipher molecular interactions between this fungus and its host plant, we developed a strategy for transposon-based insertional mutagenesis. impala is a fungal DNA transposon of the TC1-mariner family moving in host genome by a cut-and-paste mechanism. This transposon is composed of two Terminal Inverted Repeats and a transposase coding gene necessary to excise and reinsert the transposon at a TA site. impala was originally identified in the fungus Fusarium oxysporum, but it transposed successfully in other fungal species. Excision vectors containing an autonomous copy of impala inserted in Aspergillus nidulans nitrate reductase gene were used to select impala excision events in Z. tritici. impala had high rates of excision (85%) and re-insertion (90%) in this fungal species. impala also displayed a positive bias toward genes (90%), preferentially close to transcription starting sites (50%). This pattern was not described before. Overall, impala re-insert randomly in genes from core chromosomes, but seemed to avoid accessory chromosomes poor in expressed genes. We added a strong constitutive promotor (pGdpA) in impala for activation tagging. The chimeric impala:pGpdA transposon was able to excise and re-insert in Z. tritici genome with the same pattern as native impala. We also developed vectors for a double component transposition in which a defective impala carrying pGdpA was trans-activated by an impala transposase encoding gene. These tools provided new methods for mutagenesis in Z. tritici that will be used to identify genes involved in infection. These vectors will be also used to identify mutants of impala transposase with an increased transposition frequency.

POSTER SESSION II

TUESDAY, MARCH 7
16:00 – 17:30
Location: ***Tirol Foyer (Congress Innsbruck)***

CS2.1 SYNTHETIC BIOLOGY AND BIOTECHNOLOGY

CS2.1.9

MODIFICATION OF TRANSCRIPTIONAL FACTOR ACE3 ENHANCES PROTEIN PRODUCTION IN TRICHODERMA REESEI IN THE ABSENCE OF CELLULASE GENE INDUCER

Yun Luo[1], Mari Valkonen[2], Raymond Jackson[3], Jon Palmer[1], Aditya Bhalla[1], Igor Nikolaev[4], Markku Saloheimo[2], Michael Ward[1], **Robin Sorg[4]**

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Background: Trichoderma reesei is one of the best-known cellulolytic organisms, producing large quantities of a complete set of extracellular cellulases and hemicellulases for the degradation of lignocellulosic substances. Hence, T. reesei is a biotechnically important host and it is used commercially in enzyme production, of both native and foreign origin. Many strategies for producing enzymes in T. reesei rely on the cbh1 and other cellulase gene promoters for high-level expression and these promoters require induction by sophorose, lactose or other inducers for high productivity during manufacturing.
Results: We described an approach for producing high levels of secreted proteins by overexpression of a transcription factor ACE3 in T. reesei. We refined the ace3 gene structure and identified specific ACE3 variants that enable production of secreted cellulases and hemicellulases on glucose as a sole carbon source (i.e., in the absence of an inducer). These specific ACE3 variants contain a full-length Zn2Cys6 binuclear cluster domain at the N-terminus and a defined length of truncations at the C-terminus. When expressed at a moderate level in the fungal cells, the ACE3 variants can induce high-level expression of cellulases and hemicellulases on glucose (i.e., in the absence of an inducer), and further improve expression on lactose or glucose/sophorose (i.e., in the presence of an inducer). Finally, we demonstrated that this method is applicable to industrial strains and fermentation conditions, improving protein production both in the absence and in the presence of an inducer.
Conclusions: This study demonstrates that overexpression of ACE3 variants enables a high level of protein production in the absence of an inducer, and boosts protein production in the presence of an inducer. It is an efficient approach to increase protein productivity and to reduce manufacturing costs.

CS2.1.10

RESTRAINING ASPERGILLUS NIGER PELLET SIZE IN A CONTROLLED BIOREACTOR SYSTEM

Tolue Kheirkhahhasanzadehfoumani[1], Peter Neubauer[1], Stefan Junne[1,2]

[1]*Technische Universität Berlin / Department of Biotechnology, Berlin, Germany,*
[2]*Aalborg University, Esbjerg, Denmark*

Filamentous fungi develop various types of macromorphology in a submerged cultivation depending on the growth environment. The relation of growth, supply deficiencies inside the pellet, product synthesis and secretion are not yet completely understood.
As high shear stress regimes in stirred tank reactors lead to the breakage of pellets, a 2-D rocking motion bioreactor (RMB) was applied in this study. It became then possible to avoid the appearance of high mechanical stress and provide sufficient mass transfer to avoid oxygen limitation. The effect of adding different concentrations of talcum microparticles on the pellet size and process performance was studied. A. niger grew into pellets of up to 2 mm in diameter when cultivated without microparticle in the RMB. Image analysis showed that the macromorphology shifted to a combination of dispersed hyphae and loose clumps at a talcum concentration between 2.5 and 10 g L-1. A concentration between 0.1 and 2.5 g L-1 resulted in the formation of pellets with various sizes depending on the microparticle concentration. This proved the suitability of talcum addition to achieving a certain pellet size in RMB cultivation. By adjusting the talcum concentration between 0.5 and 1 g L-1, the diameter of interest was finally achievable. At 1 g L-1, the population size distribution (mean pellet diameter of 450 µm) was similar to what is obtained in industrial scale STR cultivations with fluctuating shear stresses. This suggests that the presented workflow can be used for further investigation of how a certain macromorphology can affect the productivity and overall process performance of A.niger while decoupling the relation of mechanical shear stress and oxygen limitation as it exists in many reactors. This concept is currently used to investigate the attachment of spores to microparticles and the effect of different macromorphologies on co-cultures of filamentous organisms.

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CS2.1.11

HARMONIZED ASPERGILLUS ORYZAE PLATFORM STRAIN FOR THE PRODUCTION OF DIFFICULT-TO-EXPRESS PROTEINS

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[1]*Novozymes, Denmark*

The filamentous fungi Aspergillus oryzae (A. oryzae) has been extensively used to produce soy sauce, liquors, other traditional brewed products and, thanks to its GRAS status and high secreting capacity it is also widely used in industry to produce proteins and metabolites.
However, in comparison to other laboratory workhorses, such as S. cerevisiae, the limited availability of characterized parts and integration sites makes strain construction more difficult and time consuming. Additionally, the expression and secretion of heterologous proteins in A. oryzae can be hindered by the presence of bottlenecks at various production stages, from translation and protein folding to secretion.
To tackle these issues and expand the available toolkit for A. oryzae engineering, we performed a detailed investigation of the OMICS profile of A. oryzae under industrially relevant conditions. By means of mRNA sequencing, proteomics, secretomics and metabolomics analysis at multiple time points we aim to identify new and stable integration sites, promoters for high expression under requires conditions, improved signal peptides and a blueprint of active metabolic pathways that could be used to redirect carbon consumption towards the production of valuable molecules.
The learnings obtained through the OMICS analysis will then be used within the scope of the Harmonize project to construct a platform strain to produce difficult-to-express proteins based on the combinatorial screening of newly identified elements, targeted deletions and auxiliary genes such as chaperones, transporters and maintenance genes.

CS2.1.12

HIGH THROUGHPUT OVEREXPRESSION OF REGULATORY PROTEINS TO BOOST PERFORMANCE OF THE INDUSTRIAL FUNGUS TRICHODERMA REESEI

Barbara Kozak[1], Nuria Barrajon Simancas[1], Jon Palmer[2], Igor Nikolaev[1]

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Due to its natural capacity to express high levels of secreted proteins the filamentous fungus Trichoderma reesei is one of the most efficient cell factories for enzyme production used in food, feed, grain and other industrial applications. Despite increased understanding of gene expression and cellular metabolism in industrially relevant fungi, there is an ongoing need for strains with improved heterologous protein expression, product stability, suitable morphology and fermentation robustness at industrial scale. Using the T. reesei genome annotation we identified more than 700 putative regulatory factors (e.g. transcription factors) and aimed to characterize their effect on fungal cell properties relevant for industrial protein production. This was accomplished by measuring cell productivity, strain morphology, product degradation and other parameters in a T. reesei dual heterologous reporter strain expressing a fungal alpha-amylase and a bacterial phytase. We generated 691 overexpression mutants that were subsequently screened for improved protein production under various conditions. These data identified several regulatory factors whose overexpression led to enhanced production under inducible and non-inducible conditions, at higher temperature and / or with higher production speed. The best performing strain overexpressing regulatory factor Trire2_62386 showed up to 66% increase of productivity under standard fermentation conditions. Moreover, the top 12 regulatory factors were selected to construct a combinatorial library yielding an additional 134 strains, each of them overexpressing two regulatory factors. Certain combinations of two regulatory factors resulted in further increase of protein secretion up to 107%. Finally, we performed RNA-Seq analysis of the best performing strains to identify transcriptional changes that led to the enhanced strain performance.

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CS2.1.13

A GLYCOSIDE HYDROLASE FAMILY 31 CARBOHYDRASE WITH ALPHA-GLUCOSIDASE ACTIVITY FROM LICHTHEIMIA RAMOSA

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Mucoromycota fungi are excellent producers of various carbohydrases to be used for industrial purposes. Glycosidases, which are capable of both hydrolyzing and synthesizing oligosaccharide molecules, are particularly interesting among enzymes of practical importance. The alpha-glucosidase activity hydrolyzes alkyl- and aryl-alpha-glycosides as well as di- and oligosaccharides. In our recent studies, a Lichtheimia ramosa isolate proved to be an excellent glycosidase producer, and beta- and alpha-glycosidase activities secreted by the fungus have been purified and characterized. During the glycosidase purifications, an enzyme protein belonging to the Glycoside Hydrolase Family 31 (GH 31) was identified by mass spectrometry analysis; members of this group typically catalyze the hydrolysis of alpha-glycosidic bonds.
In silico analysis showed two putative GH 31 coding elements in the Lichtheimia genome. Gene expression was studied on medium containing wheat bran or wheat bran and lactose, since these environments proved to be best conditions for the enzyme production. The two genes showed different expression in the fermentation tests. Namely, high expression level was identified for GH 31A on the first and second day of cultivation, whereas GH 31B showed a relatively stable expression on these media.
An attempt was also made for the production of GH 31A enzyme protein in heterologous expression system. With this context, cDNA was generated and the his-tagged protein was expressed in Pichia pastoris. Most enzyme proteins were produced on the fifth day during the seven-day incubation period. The enzyme protein was purified using affinity chromatography, and the analysis of biochemical properties of

alpha-glucosidase activity identified has been started. In addition, vector systems have also been constructed for GH 31A overexpression or gene disruption experiments. These assays may provide more knowledge on the production and function of these enzymes in L. ramosa.
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CS2.1.14

HETEROCHROMATIN PROTEIN 1 (HP1) KNOCK-OUT MUTANTS EXHIBIT CELLULOTIC ENZYME COCKTAIL ALTERATIONS IN TRICHODERMA REESEI

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The filamentous fungus Trichoderma reesei secretes high amounts of cellulolytic enzymes which are used to produce fermentable sugars from lignocellulosic biomass. In fungi, various biological processes involve epigenetic control mechanisms via chromatin status changes. The acquisition of knowledge on epigenetic regulation is thus crucial to design strategies aiming at efficiently controlling fungal productions.
Recently, Zhang et al. (2016) investigated the contribution of Heterochromatin Protein 1 (HP1) to the regulation of cellulolytic enzymes in the filamentous fungus Penicillium oxalicum. The hp1 gene encodes the H3K9me3 reader protein and is responsible for the recruitment of the DIM2 protein, which affixes methylation to DNA cytosines (5mC). The authors observed a down-regulation of the main cellulases genes in the mutant strain whereas hp1 overexpression results in an overall up regulation of gene expression.
To investigate the role of HP1 in cellulase production in T. reesei, hp1 was deleted in two strains: the wild type QM6a and the hyperproducer RutC30. In lactose fed batch culture, a reduced protein production was measured for RutC30Δhp1 but not for QM6aΔhp1.
A transcriptomic study was performed in both mutants compared to their respective reference strains. A down-regulation of cellulase genes is observed in both backgrounds, even though no phenotype of decreased cellulase secretion is observed in QM6aΔhp1. Apart from cellulase genes, the low overlap of differentially expressed genes (DEG) between the two transcriptomic dataset suggests that the mutations present in Rut-C30 genetic background interfere with hp1 knock-out to generate additional consequences.

HP1 has been shown to be tightly associated with the boundaries of heterochromatin region in AT-rich region. A thorough examination of the localization of DEG revealed an enrichment in mutants of down regulated genes close to AT-rich regions.
Zhang X, Qu Y, Qin Y. (2016). Biotechnol Biofuels. 9:206.

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CS2.1.15

THE N-TERMINAL REGION OF HYDROPHOBIN ROLA OF ASPERGILLUS ORYZAE REGULATES SELF-ASSEMBLY OF ROLA

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Hydrophobin is an amphipathic low molecular weight protein conserved in filamentous fungi and is localized on the fungal cell wall. Hydrophobin RolA of Aspergillus oryzae interacts with polyesterase CutL1 via ionic interaction to promote polymer degradation by CutL1. Positively charged residues in the N-terminal region of RolA are important for the interaction. At the air-water interface, RolA self-assembles to form β-amyloid–like structure called rodlet under acidic conditions but does not form rodlet under alkaline conditions. It is suggested that the surface potential of RolA affects the rodlet formation via ionic interaction. In this study, we focused on the elongation of rodlets and analyzed the factors involved in the regulation of rodlet elongation.

The droplets of RolA solution were incubated for different time in a wet environment. Rodlets formed on the droplet surface were transferred to the surface of SiO2 substrate and observed by an atomic force microscope. At first, the elongation of rodlets under various conditions (pH and ionic strength) was analyzed over time. The result showed that the elongation rate of rodlets becomes higher under conditions where the electrostatic repulsion among RolA molecule is reduced (acidic-pH and high ionic strength). Therefore, it is suggested that the surface potential of RolA affected the elongation of rodlets via ionic interaction. Next, we focused on N-terminal region of RolA, where positively charged residues are clustered. We analyzed the effect of N-terminal region on the elongation of rodlets by using mutants in which multiple positively charged residues were substituted to serine. The elongation rate of rodlets of RolA mutants were higher than that of wild-type RolA,

suggesting that the N-terminal region of wild-type RolA suppresses rodlet elongation. It is also suggested that N-terminal region of RolA is the bifunctional region involved in both the interaction with CutL1 and self-assembly of RolA.

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CS2.1.16

A CRISPR/CAS9 BASED MULTICOPY INTEGRATION SYSTEM FOR PROTEIN PRODUCTION IN ASPERGILLUS NIGER

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The filamentous fungus Aspergillus niger is well known for its high protein secretion capacity and is therefore a preferred host for the production of homologous and heterologous proteins. Glucoamylase is one of the highest expressed genes in A. niger and the promoter and terminator regions of glucoamylase (PglaA and TglaA) are often used to drive the expression of (heterologous) genes of interest. Moreover, the introduction of multiple copies of such gene constructs is known to further boost product yields. Therefore, we have designed a CRISPR/CAS9 based gene targeting method to integrate multiple copies of a gene of interest to predetermined sites in the genome. Genes encoding extracellular enzymes that could interfere with the purification of the protein of interest such as alpha-amylase and alpha-glucosidases or protease encoding genes that could interfere with the stability of the protein of interest, were deleted and replaced by a so-called Glucoamylase Landing Site (Gla_LS). Each Gla_LS consists of a 410 bp long glaA promoter region and a 428 bp long glaA terminator region. In between the glaA promoter and glaA terminator regions one or more unique DNA sequences were introduced for which unique CAS9 compatible guide RNAs were designed. In total five different DNA sequences (dubbed KORE sites) were designed. A strain lineage in a non-homologous end joining mutant background was made in which up to ten Gla_LS were introduced with different KORE sequences. Using CRISPR/CAS9 mediated genome editing in combination with specific guide RNAs, various copy numbers of genes of interest can be integrated at these landing sites. In our hands, three to four integrations could be accomplished in a single transformation. As an illustration of its potential, we successfully used the expression platform to generate strains producing the Penicillium expansum PatE protein extracellularly in A. niger.

CS2.1.17

MARINE PENICILLIA VS. THEIR TERRESTRIAL COUNTERPARTS IN THEIR ABILITY FOR MARINE AND PLANT BIOMASS DEGRADATION

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A global transition towards more sustainable and biobased food production systems is urgently needed. Macroalgae has been lately spotted by the biotechnological industry as promising renewable sources of functional proteins and other bioactive compounds used for the development of alternative plant-based food and feed products. Extracting valuable compounds from macroalgae is challenging due to the absence of suitable enzyme cocktails able to process this substrate. To develop such enzyme cocktails, nature can provide key cues when looking at those organisms that already possess the natural capacity to degrade macroalgae. One such group of organisms is marine fungi, which despite being largely unexplored in marine habitats, form a diverse and ecologically essential group of species. In a recent project we sampled many locations across the North Sea and Atlantic Ocean (including the Caribbean), resulting in approximately 200 marine fungal isolates. Interestingly, many of these belonged to the genus Penicillium, which is also an abundant genus in many terrestrial environments. We compared a subset of marine isolates of this genus to their closest terrestrial relatives with respect to their ability to degrade and utilize terrestrial and marine biomass. For that, a multidisciplinary approach was used by combining various genomics, phenotypic and enzymatic assays. The highlights of this study will be presented within the framework of a larger project aimed at the establishment of a sustainable and renewable protein production system for macroalgal biomass valorization.

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CS2.1.18

REDUCING PROTEOLYTIC ACTIVITY OF THE FILAMENTOUS FUNGUS THERMOTHELOMYCES HETEROTHALLICA C1

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Thermothelomyces heterothallica C1 is a well-known industrial enzyme production host able to reach enzyme titers over 120 g/l in a 6-7-day process. We have further developed the C1 Technology and are exploiting its excellent protein production efficiency for low-cost manufacturing of different types of therapeutic and vaccine proteins.

One of the major bottle necks in recombinant protein production is the proteolytic activity by host cell proteases. Therefore, one of our main goals in C1 strain development is reducing proteolytic activity to achieve higher production levels and better quality of the recombinant proteins. The work was initiated by characterization of C1 extracellular proteases using transcriptomics, proteomics, and biochemical methods such as inhibitor affinity chromatography and zymogram analysis and by expressing C1 proteases in Pichia pastoris. Based on characterization results, a set of proteases was selected and sequentially deleted with a marker recycling transformation procedure. In addition to gene deletions, a promoter swapping technique was used. The strains generated were tested using a total protease assay and spiking experiments with selected target proteins. As a result, multiple protease deletion strains, depleted up to fifteen proteases, were generated.

By sequential deletion of proteases, the total extracellular protease activity was reduced in each deletion step reaching over 50-fold reduction. Simultaneously, we observed increasing stability of several target proteins. Many difficult-to-produce proteins, which could not be produced in early protease deletion strains, showed step-wise improved production as more proteases were deleted, and were successfully produced in the most advanced deletion strains. We also identified

two main proteases responsible for degradation of monoclonal antibodies (mAbs) in C1 and by deleting those proteases were able to produce stable mAbs in quantities higher than 20g/l. To summarize, our work in reducing proteolytic activity has successfully generated a set of C1 production strains for efficient production of therapeutic proteins.

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CS2.1.19

CHARACTERIZATION AND ENGINEERING OF THE XYLOSE-INDUCIBLE XYLP PROMOTER FOR USE IN MOLD FUNGAL SPECIES

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Conditional promoters allowing both induction and silencing of gene expression are indispensable for basic and applied research. The xylP promoter (pxylP) from Penicillium chrysogenum was demonstrated to function in various mold species including Aspergillus fumigatus. pxylP allows high induction by xylan or its degradation product xylose with low basal activity in the absence of an inducer. Here we structurally characterized and engineered pxylP in A. fumigatus to optimize its application. Mutational analysis demonstrated the importance of the putative TATA-box and a pyrimidine-rich region in the core promoter, both copies of a largely duplicated 91-bp sequence (91bpDS), as well as putative binding sites for the transcription factor XlnR and a GATA motif within the 91bpDS. In agreement, pxylP activity was found to depend on XlnR, while glucose repression appeared to be indirect. Truncation of the originally used 1643-bp promoter fragment to 725 bp largely preserved the promoter activity and the regulatory pattern. Integration of a third 91bpDS significantly increased promoter activity particularly under low inducer concentrations. Truncation of pxylP to 199 bp demonstrated that the upstream region including the 91bpDSs mediates not only inducer-dependent activation but also repression in the absence of inducer. Remarkably, the 1579-bp pxylP was found to act bi-bidirectionally with a similar regulatory pattern by driving expression of the upstream-located arabinofuranosidase gene. The latter opens the possibility of dual bidirectional use of pxylP. Comparison with a doxycycline-inducible TetOn system revealed a significantly higher dynamic range of pxylP. Taken together, this study identified functional elements of pxylP and opened new methodological opportunities for its application.

CS2.1.20

MICROPOLLUTANT DEGRADATION BY WASTED SPENT MUSHROOM SUBSTRATE

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An increase in toxic levels of micropollutants in water bodies is the topic of political documents worldwide. It is clear that these levels should be reduced. In this research a waste stream from the commercial production of the white button mushroom, Agaricus bisporus, is used to degrade and sorb a wide range of micropollutants. The global white button mushroom industry produces 15,000,000 ton of waste, called spent mushroom substrate. We show that a gram spent mushroom sustrate can remove overnight around 25% of several different micropollutants (pesticides, pharmaceutials, and dyes). Over two weeks, this removal can go to 90%. The removal rate depends on the compound. In addition, around 10% of the micropollutants is sorbed, however, this fraction can also be degraded by re-incubation with an enzyme mix made of spent mushroom substrate. Key players of the substrate and this enzyme mix are the lignin modifying enzymes. For this, the mechanism behind the removal and degradation pathways is explored by transforming manganese peroxidase and laccase genes of A. bisporus into Schyzophyllum commune. By exploring the use of spent mushroom substrate and the mechanism, a promising new way to clean our waters from organic micropollutants is arising.

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CS2.1.21

THE DEVELOPMENT OF PELLET POPULATIONS DURING SUBMERGED CULTIVATIONS OF ASPERGILLUS NIGER

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Macromorphological entities of filamentous fungi during submerged growth include dispersed mycelia, loose clumps and pellets. However, their evolution is unpredictable and limits productivity during biotechnological processes.
We have therefore cultivated the cell factory Aspergillus niger under 48 different submerged conditions to comprehensively analyse the formation of different macromorphologies. The parameters that varied in these cultivations included spore inoculum, the presence of talcum microparticles and stirrer speed. Notably, a wide range of different macromorphologies could be generated that were analysed for particle sizes using image analyses. Next to dispersed mycelia and loose clumps, pellet populations with diameters ranging from a few 100 µm to several millimetres were observed. With increasing talcum concentration, however, pellet sizes decreased. Likewise, a higher spore titre used for inoculation led to reduced pellet diameters. These and further data will be presented and discussed on how such analyses contribute to a better understanding of population dynamics and the formation of heterogeneous cultures during filamentous growth.

CS2.1.22

RATIONALLY DESIGNED ANTIFUNGAL PROTEIN CHIMERAS REVEAL NEW INSIGHTS INTO STRUCTURE-ACTIVITY RELATIONSHIP

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Antifungal proteins (AFPs) are promising antimicrobial compounds that represent a feasible alternative to fungicides. Penicillium expansum encodes three phylogenetically distinct AFPs (PeAfpA, PeAfpB and PeAfpC) which show different antifungal profiles and fruit protection effects. To gain knowledge about the structural determinants governing their activity, we solved the crystal structure of PeAfpB and rationally designed five PeAfpA::PeAfpB chimeras (chPeAFPV1-V5). Chimeras showed significant differences in their antifungal activity. chPeAFPV1 and chPeAFPV2 improved the parental PeAfpB potency, and it was very similar to that of PeAfpA. chPeAFPV4 and chPeAFPV5 showed an intermediate profile of activity compared to the parental proteins while chPeAFPV3 was inactive towards most of the fungi tested. Structural analysis of the chimeras evidenced an identical scaffold to PeAfpB, suggesting that the differences in activity are due to the contributions of specific residues and not to induced conformational changes or structural rearrangements. Results suggest that mannoproteins determine protein interaction with the cell wall and its antifungal activity while there is not a direct correlation between binding to membrane phospholipids and activity. This work provides new insights about the relevance of sequence motifs and the feasibility of modifying protein specificity, opening the door to the rational design of chimeras with biotechnological applicability.

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CS2.1.23

PRODUCTION OF BOVINE BETA-LACTOGLOBULIN AND HEN EGG OVALBUMIN BY TRICHODERMA REESEI USING PRECISION FERMENTATION TECHNOLOGY

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To satisfy the increasing food and nutrient supply requirements for our growing future human population, fundamental changes in food protein production methods are needed. Providing sufficient amount and nutritionally balanced protein diet for an increasing population will be a great challenge that needs to be solved in a manner that rather alleviates pressure to our planet than increases it even more. Cellular agriculture is a field in bio-based economy that focuses on the production of agriculture products, proteins, fats and meat tissue from cell cultures using molecular biology tools and biotechnology. Cellular agriculture allows us to produce animal proteins through microbial precision fermentation.
In this work we have studied the use of Trichoderma reesei as production host for hen ovalbumin (OVA) and bovine beta lactoglobulin (BLG). These food proteins were expressed using more common cellulase (cbh1) promoter and synthetic expression system (SES). Production of BLG and OVA was studied in 24-well plate- and bioreactor-scale to compare the different expression systems. Both proteins were successfully produced by T. reesei. Purified proteins were characterized and used for functional studies on gelation and foaming.

CS2.1.25

EXPLORING HIGHLY REDUCING FUNGAL POLYKETIDE SYNTHASES FOR NOVEL BIOFUEL PRODUCTION IN YEASTS

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Climate change is affecting all corners of the world and has become one of the biggest global challenges. This acute problem has prompted efforts to replace fossil fuels with biofuels produced by cell factories, aiming to reduce greenhouse gas emissions and decelerate the pace of climate change. The search for new biofuels has led us to the underexploited chemical diversity of fungal polyketide natural products, which is a fascinating group of hydrocarbons with chemical features desired in novel alternative biofuels. These molecules are produced by highly reducing iterative polyketide synthases (HR-PKS).
The goal of my research is to develop a heterologous expression platform for screening biofuel production systems based on HR-PKS. I am using phylogenomic methods to identify HR-PKSs, that can make molecules rich in isomeric carbons, and identify thioesterase candidates needed to release the products. For proof of concept, I selected the HR-PKS from the azanigerone biosynthetic pathway as a model synthase (AzaB) and 10 HR-PKS-related thioesterase candidates for further analysis. I am co-expressing AzaB with the thioesterase candidates in two host organisms, ascomycetes, and basidiomycetes.
My overall aim is to develop a cell-factory platform that efficiently produces polyketides for detecting and testing biofuel candidates. I will present the overall strategy of the project as well as bioinformatics and experimental results.

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CS2.1.26

SUCCESSFUL PRODUCTION OF VACCINE CANDIDATE MOLECULES INCLUDING SARS-COV-2 ANTIGENS IN THE FILAMENTOUS FUNGUS THERMOTHELOMYCES HETEROTHALLICA C1

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Thermothelomyces heterothallica C1 is a well-known industrial enzyme production host able to reach 120 g/l enzyme levels in a 6-7-day process. We have developed the C1 Technology for low cost manufacturing of therapeutic and vaccine proteins. We are exploiting the excellent protein productivity of this system for animal and human vaccine candidates and monoclonal and bispecific antibodies, Fab fragments, Fc fusion proteins, enzymes and other therapeutic proteins.
One of the major hurdles in fungal recombinant protein production is the abundance of host proteases. Our protease characterization and deletion work has lead to production strains with 14-15 proteases deleted or modified, allowing efficient production of vaccine antigens. These strains have been used to express vaccine candidates of different types including virus-like particles (VLPs), nanoparticles and individual antigens. Production levels ranging from several hundreds of mg/l to 10 g/l of secreted protein have been obtained in 5-7 day fermentations.
Our work for developing a SARS-Cov-2 vaccine started soon after the outbreak of the coronavirus pandemic. To date, we have expressed the receptor binding domain (RBD) of the spike protein from the original variant and six emerging variants of concern including Omicrom BA.5. We have also expressed RBDs for several variants in one C1 strain to make multivalent vaccines. Excellent production levels of up to 2-3 g/l have been obtained for the RBDs, and their good immunogenicity and protection potential have been shown in animal tests in mice, hamsters and rabits by numerous collaborators. A toxicology study in New Zealand White rabbits showed no adverse effects in the rabbits

injected with the RBD. A permit for Phase 1 clinical trials in humans has been obtained, and these studies will be in progress during the conference. To our knowledge this is the first protein produced in any filamentous fungus to enter human clinical trials.

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CS2.1.27

AP2 AND ERF TRANSCRIPTIONAL FACTORS PROMOTING TAXOL BIOSYNTHESIS FROM ENDOPHYTIC FUNGI

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Abstract:
Taxol® is an anticancer drug and Taxol biosynthetic genes are described in much detail in Taxus plant-associated endophytic fungi. Transcription factors (TFs) such as WRKY, ERF, AP2, bHLH, MYB, NAC etc., are involved in Taxol biosynthesis (TB) upon elicitor stress response signalling in Taxus plants. However, have not yet been reported from Taxol producing endophytic fungi. Hence, in this study, the fungal Apetala2 (AP2) and Ethylene Response (ERF) TF genes were isolated from Didymella glomerata and Pestalotiopsis microspora cultures. The full-length cDNA of PmERF and DgAP2 are 528 bp, and 807 bp respectively, including the expected open reading frames. Homology analysis indicated that the deduced DgAP2 and PmERF and proteins are highly homologous to the conserved domain of AP2 and ERF proteins from Taxus plants followed by other plants and microbes. Phylogenetic analysis displayed that fungal ERF and AP2 belong to their respective groups of TF families in plants and microbes. In addition, the role of fungal ERF and AP2 in signal transduction of elicitors such as methyl jasmonate (MeJA), salicylic acid (SA) and abscisic acid (ABA) to the TB of ggpps and tasy genes was proven by RT-PCR analysis. The respective gene promoters binding studies by computational docking have been documented. Moreover, high-performance liquid chromatography (HPLC) analysis showed an increase in the production of Taxol at various elicitor treated fungal cultures. Our study suggests that fungal ERF and AP2 TFs may be involved in Taxol biosynthesis and promote the production of Taxol in endophytic fungi. This research encourages further studies on regulatory mechanisms of TB in endophytic fungi.
Keywords: Taxol Biosynthesis, Secondary metabolites Elicitors, Transcription factors, endophytic fungi

CS2.1.28

A DUAL REPORTER STRAIN TO MONITOR THE INDUCTION MECHANISM OF THE GLUCOAMYLASE GENE IN A. NIGER

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The potential of filamentous fungi to breakdown plant polysaccharides has been well investigated. Starch is the major storage polysaccharide in plants and amylolytic enzymes such as alpha-amylases, glucoamylases, and alpha-glucosidases are produced to hydrolyse starch. Glucoamylase is one of the highly expressed genes in Aspergillus niger in the presence of starch, maltodextrins or maltose as inducing sugars. The genetics of the induction mechanism of starch degrading enzymes (SDEs) including glucoamylase (GlaA) is quite well studied in Aspergillus niger as well as in other Aspergilli. However, detailed knowledge on how AmyR is activated at the molecular level is lacking.

To fill this gap, we have developed a dual reporter strain to study the regulation of the glaA gene. The dual reporter strain monitors both quantitative and qualitative aspects of glucoamylase induction. As reporters, the acetamidase gene (amdS) and the luciferase gene (lux613) were expressed from the glucoamylase promoter.

The PglaA-lux613 reporter gene was used to quantitatively monitor glaA expression in germinating spores on a variety of inducing and non-inducing compounds. In agreement with previous data, the glaA gene of A. niger was induced by maltose and glucose in a concentration-dependent way. The addition of an alpha-glucosidase inhibitor (castanospermine) reduced the luciferase expression indicating that inducer formation requires alpha-glucosidase activity.

The amdS reporter gene was used to quantitatively monitor glaA expression by growing the reporter strain on acetamide or acrylamide plates under inducing and non-inducing conditions. The growth phenotype of the reporter strain on acrylamide showed again specific induction of the glaA gene on maltose and glucose. The dual reporter strain was also used to isolate constitutive mutants expressing the glaA-based reporters by selecting mutants on non-inducing acrylamide plates. The trans activity of the mutations were monitored using the PglaA-lux613 based reporter. Initial characterization of the obtained mutants will be presented.

CS2.1.29

TRANSCRIPTOME ANALYSIS OF PENICILLIUM SUBRUBESCENS XLNR AND ARAR MUTANTS

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Penicillium subrubescens is a promising candidate for industrial application as its plant cell wall-degrading enzyme production levels and saccharification abilities are similar to that of the industrial species A. niger. It has an expanded repertoire of specific types of hemicellulases and inulinases in its genome, that may enable a more targeted degradation of the corresponding polysaccharides.
The transcriptional factor XlnR (Xyr1/XLR-1) is essential for the expression of xylanolytic genes and is commonly found in genomes of filamentous ascomycete fungi. The transcriptional factor AraR (a homolog of XlnR), controls the arabinanolytic system as well as L-arabinose catabolism. Transcriptomic data from P. subrubescens revealed that most xylanolytic genes had a higher expression level on L-arabinose than on D-xylose, suggesting a different organization of the regulatory system in P. subrubescens. In this study, we characterized strains in which xlnR or araR were deleted, to better understand the regulatory system in P. subrubescens. Results of this study will be presented.

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CS2.1.30

KOMATAGAELLA PHAFFII: A MODERN CELL FACTORY FOR PRODUCING CYSTEINE-RICH ANTIMICROBIAL FUNGAL PROTEINS AND AI-PREDICTED CYSTEINE RICH PEPTIDES

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Antimicrobial resistance (AMR) is a serious threat to human health worldwide. The COVID-19 era has once again highlighted the need for novel, improved, and cost-effective antimicrobials due to the continual emergence of pathogenic and/or resistant organisms, the unbalanced use of antimicrobials, and the nature of resistance evolution. Due to their abundance in nature, antimicrobial peptides (AMPs) and antimicrobial proteins may provide novel alternatives to existing antimicrobials. Komagataella phaffii (aka Pichia pastoris) is a methylotrophic yeast capable of secreted protein expression in high titers along with PTMs required for eukaryotic proteins.
In this study, we used K. phaffii microbial cells due to their superior properties as modern cell factories to produce two families of antimicrobials: Cysteine-Rich Peptides(CRPs) and fungal defensins. CRPs are small AMPs of 10-40 aminoacids, contain several cysteine residues with ability to form “Cysteine stabilized alpha-helical” structure. Defensins are small, secreted and cysteine-rich proteins with antimicrobial activity. Both are products of the innate immunity in a pleuthera of organisms from mammalians to plants and fungi, and are potent therapeutic agents against pathogenic bacteria and fungi.
In an attempt to find novel antimicrobials, we trained several deep learning architectures (CNN, LSTM, CNN-biLSTM etc.) on a diverse set of AMPs/non-AMPs curated from databases and datasets. Nov-

el AMPs were predicted from a library of 20 aminoacid long protein fragments compiled from Uniprot. Alternatively, a homology-based methodology was used to screen for eurocin-like fungal defensins. We formed a selection strategy based on the structural, physico-chemical properties. Rationally selected five novel defensins and three CRPs were expressed in K. phaffi using the AOX promoter and α-factor secretion signal. A high-throughput screening strategy was employed for the selection of high-producers. Purification and antimicrobial screening of the CRPs and defensins indeed verified K. phaffii as a suitable host for high yield, cost-effective production of active antimicrobials.

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LEARNING CHEMISTRY FROM FUNGI TO MAKE SUSTAINABLE CHEMICALS.

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Transportation, manufacturing, and agriculture depend on petroleum and produce large amounts of greenhouse gas emissions which are accelerating climate change. We need sustainable alternatives to make the products that we use and power our activities. Nature's chemistry is the result of millions of years of natural selection, as a result it covers a huge chemical space beyond the reach of organic chemistry. We are cataloging the fungal natural products chemical and genetic space. We are using this information to program fungal hosts to make new molecules using sustainable and inexpensive feedstocks.
We believe that many products currently derived from petroleum can be made using Polyketide synthases (PKSs) for C-C chemistry and non-ribosomal peptide synthases (NRPSs) for C-N chemistry. Our goal is to develop platforms for heterologous production of chemicals using fungal PSKs and NRPSs in fungal hosts. So far, we have developed a bioinformatics toolkit and mined thousands of fungal genomes for biosynthetic gene clusters (BGCs). We have catalogued tens of thousands of PKSs and NRPSs. We use tandem mass spectrometry to assess the chemical repertoire deployed by the strains in our collection. And we have found the BGCs for new and known molecules. We have obtained a genetic and chemical catalog of bioparts, and we are using it for design and assembly of biosynthetic pathways to produce new chemicals.
In this opportunity I will present our workflow: from genomes to heterologous products, and examples of the application of our approach to make fuels, materials and pesticides

CS2.1.32

HARNESSING COEXPRESSION NETWORK DATA AND SYNTHETIC BIOLOGY TO METABOLICALLY ENGINEER ASPERGILLUS NIGER

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Fungal secondary metabolites are bioactive, small molecular weight molecules which have saved millions of human lives as antibiotics, immunosuppressants, cholesterol lowering agents, and other therapeutics. A wealth of genome and transcriptional data confirms that the vast majority of fungal secondary metabolites are not produced during laboratory growth, presumably requiring unknown cue(s) encountered in the natural niche for transcriptional activation. Consequently, fungal secondary metabolites are drastically underexplored in drug discovery pipelines. In this study, we used the microbial cell factory Aspergillus niger to demonstrate that coexpression networks can be used for accurate predictions of transcription factors which regulate multiple secondary metabolite biosynthetic loci. By elevating expression of these transcription factor targets using a titratable synthetic Tet-on driven gene switch, we could activate secondary metabolite biosynthetic gene clusters at transcriptional and metabolite levels. This approach also enabled us to re-engineer other aspects of A. niger biology, including organic acid synthesis for industrial applications. We finally compared coexpression networks from RNA-seq and microarray platforms, revealing good concordance and confirming that meaningful networks can be derived from only 32 transcriptional experiments. The combination of systems and synthetic biology in this study can generate new high-priority genetic leads for functional analysis, and enable rapidly re-engineering of fungi for drug discovery and many other purposes.

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CS2.1.33

DESIGNER SACCHAROMYCES 'BOULARDII' PROBIOTIC YEASTS: HETEROLOGOUS GENE EXPRESSION FOR AN IN-HOST VIRULENCE ASSAY AND ANTIBACTERIAL THERAPY

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The yeast Saccharomyces 'boulardii' is a worldwide marketed probiotic in more than 80 countries. However, infants, the elderly, immunocompromised patients, and patients who have undergone prolonged hospitalization are at increased risk of probiotic yeast-derived fungemia. Thus, research on probiotic yeast has to focus on reducing the incidence of such cases.
Genomic and phenotypic properties might be present in the case of S. 'boulardii' that allow it to survive in the mammalian bloodstream. Thus, our probiotic isolate collection was subjected to genomic analysis to reveal the characteristics that enable the probiotic yeast to persist in the bloodstream. We identified copy number variations and various single nucleotide polymorphisms and compared these among commercial, mycosis-causing, and non-mycosis human isolates. However, blood isolates did not have unique, distinguishing mutations.
Since bloodstream survival could not be linked to well-defined genomic adaptations, we designed a competitive in-host assay to study probiotic yeast pathogenicity. A probiotic control strain was developed, constitutively expressing a visually detectable color marker. The marker coding gene was inserted in multiple copies into the yeast genome. This strain can be injected into laboratory test animals simultaneously with tested isolates. The competitive assay relies on identifying colonies on an isolate level visually. This enables the identification of relative survival/growth rates in the bloodstream and in various organs, and this data may subsequently be correlated with phenotypic differences, to link phenotypic characteristics with pathogenic potential.
In the same manner, the integration of the gene encoding for the leucocine C (lecC) antilisterial peptide was also designed. In this way, this probiotic yeast strain is not only able to produce lecC, but this phenotype can be inherited. Our work demonstrates that S. 'boulardii' is not only a probiotic, but a probiotic that can be further developed and utilized in various aspects of research and medicine.

CS2.1.34

FUNGAL FERMENTATION FOR FOOD PROTEIN PRODUCTION IN UPCYCLED AGRO-INDUSTRIAL SIDE-STREAMS.

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There is an increasing demand for proteins due to the human population rise and increase in living standards. At the same time, animal production has a huge negative climate impact and demand for agricultural land for feed production. This calls for a shift in the dietary pattern towards more sustainable food sources with better utilization of the arable land to sustain feeding the population without further burdening the environment. Due to their efficient biomass degradation apparatus, filamentous fungi are excellent organisms to retrieve nutrients from complex material. Thereby they have the capacity to upcycle agro-industrial side-streams into food proteins. The production of food proteins can be carried out using native strains or engineered strains to produce multiple proteins or to produce specific secreted proteins in highly specialized fungal hosts. Production of proteins is carried out in Solid State Fermentation (SSF) and Submerged Fermentation (SmF) where SmF is based on easily fermentable sugar streams. These sugars can come from 1. generation processes or pre-treated and hydrolyzed 2. generation biomasses, whereas SSF can utilize substrates without pre-treatment. The fermentation substrates are often considered among the most important components in the cost of the fermentation products, which usually can account for almost 50% of the whole production process. Thus, to lower the costs of production for lower value products that need to be produced in high amounts such as food proteins, the search for cheaper sources of fermentation substrate has high priority for the industry. We are working on upcycling of low-cost side-streams from the food- and agro-industry and adapting these streams to specific fungal production hosts as alternative cheap and sustainable fermentation substrates in the production of alternative proteins for the food industry in SSF and SmF.

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CS2.1.35

UNDERSTANDING THE INTERPLAY OF FUNGAL MORPHOLOGY DEVELOPMENT AND OXYGEN SUPPLY: A MULTISCALE MODEL APPROACH

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Filamentous fungi cultivated as bio-pellets are well established in the biotechnology industries. Thereby, hyphal growth and fungal morphology affect product titers and the choice of process conditions. Inside the pellet, mass transfer, substrate consumption and biomass formation strongly depend on local hyphal density and pellet size. Commonly, a pellet is divided into an active outer layer and an inactive, substrate-limited pellet core. Models used to predict biomass concentration, substrate uptake, and product formation often idealize pellets as being spherical, porous particles with constant density. In such case, reaction kinetics are averaged over the entire pellet population. In reality, the pellets show different density distributions across the pellet radius, are therefore differently supplied with oxygen, and contribute individually to the overall productivity. In this study, the population development of pellet morphology was investigated while considering individual oxygen-dependent growth rates. For this purpose, a large number of pellet structures are simulated with a stochastic single-pellet-model by varying growth parameters and hyphal abrasion rates at the pellet border. By knowing the effective diffusion into the pellet and oxygen uptake rates, quasi-stationary oxygen concentration profiles can be assigned to the simulated pellet structures. This allows to determine oxygen-dependent growth rates (change in pellet radius and change in hyphal density). Furthermore, pellets are described in a population balance model with a limited number of characterizing variables, which allows appropriate mapping to the previously generated hyphal distributions, oxygen profiles and calculated growth rates. These variables are the volume of the convex hull that encloses all hyphal elements, the sum of all hyphal volumes in the pellet and a param-

eter representing the shape of the hyphal distribution in the pellet. With this model, the evolution of pellet size distribution and hyphal density can be predicted more precisely compared to existing simplified models.

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ASPERGILLUS ORYZAE STRAIN ENGINEERING FOR ANTICANCER L-ASPARAGINASE PRODUCTION

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Aspergillus oryzae is an important industrial workhorse used for the production of different enzymes due to its efficient protein secretory pathway and low or no levels of mycotoxin production. Interestingly, A. oryzae is a natural producer of L-asparaginases. Those enzymes, mainly from bacteria produced in Escherichia coli are commercialized as anticancer enzymes against acute lymphoblastic leukemia (ALL) and for the best of our knowledge it has not been studied at the genetic level in A. oryzae. This study aimed to engineer an A. oryzae strain for recombinant production of L-asparaginase, characterize the enzyme and assess its anticancer properties in vitro. Initially, six potential L-asparaginase genes were identified and individually deleted in A. oryzae using CRISPR/Cas9 technology. The knockout of a candidate gene, here named asp5, resulted in no L-asparaginase activity in plate assays and this mutant was selected for the reintegration of the six different L-asparaginase genes. This process was facilitated as the strain contains a target expression site that harbors an uidA marker gene resulting in an easy identification of the correct mutants via white or blue color formation in the colonies. A total of 10 mutants constructed with different signal peptides resulted in one L-asparaginase, named here Asp2, with molecular mass of approximately 75 kDa and 40 kDa before and after N-glycans removal, respectively. A native gel showed that the enzyme is at least dimeric as the molecular mass was substantially higher compared to denaturation conditions. Asp2 showed optimal activity at pH 7-8 and 37°C, which is compatible with human body conditions. When applied to several cancer cell lines, Asp2 has been found to be highly efficient against ALL. The results show that the L-asparaginase, Asp2, from A. oryzae has the potential to be further studied as an anticancer drug.

CS2.1.37

ANALYSIS OF THE MOLECULAR BASIS FOR THE ABERRANT PHENOTYPE OF ASPERGILLUS VADENSIS

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In the last decades, much research has been applied for the development of Aspergillus, mainly A. niger and A. oryzae, as hosts for recombinant protein production. A. vadensis, a close relative of A. niger, has been suggested as a suitable and more favorable alternative for recombinant protein production as it does not acidify the culture medium and produces very low levels of extracellular proteases. Growth profiling of this species revealed further phenotypical differences, with A. vadensis being unable to degrade starch and protein. Preliminary data suggested that A. vadensis phenotype is likely caused by the very low expression of the transcription factor-encoding genes amyR and prtT rather than amino acid mutations of these regulators. We confirmed this hypothesis after comparing the prtT and amyR genes between our reference strain, A. vadensis CBS113226, and DTO 422-H3, which is second recently isolated A. vadensis strain that can naturally utilize protein and starch. Sequence alignment indicated no difference in PrtT and AmyR amino acid sequences between these two strains. Nevertheless, the cause of non-amylolytic and non-proteolytic phenotype of A. vadensis CBS113226 remained unclear.
In this study we further addressed this question by expressing A. niger amyR and prtT genes with their own promoter and terminator sequences in A. vadensis to see whether this fungus could recover amylolytic and proteolytic activities. Results of this study will be presented.

CS2.1.38

BIOCHEMICAL INVESTIGATION OF THE NON-LINEAR COPROGEN NON-RIBOSOMAL PEPTIDE SYNTHETASE

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In fungi, the majority of siderophore compounds produced belong to the hydroxamate class. This functionality originates from L-ornithine, which is N5-hydroxylated and subsequently N5-acylated to yield a bidentate ligand. Whilst the physiological function of hydroxamate siderophores in fungi is well established, the molecular details of their biosynthesis remain poorly characterised. Genes encoding for large non-ribosomal peptide synthetase (NRPS) enzymes are known to be responsible for the assembly of peptidyl siderophores. Recent work on the SidD NRPS from Aspergillus nidulans, responsible for the biosynthesis of the siderophore fusarinine C, revealed a highly unusual non-linear behaviour to construct the depsipeptide siderophore structure.
The work on SidD also provides useful insights into the biosynthesis into the coprogen-type siderophores. The coprogen NRPS, PssA, from Penicillium rubens shares several similarities with the fusarinine NRPS, SidD. Firstly, they adopt near-identical domain architectures and secondly, they utilize very similar building blocks (SidD activates cis-anhydromevalonate-hydroxyorthinine (AMHO) while PssA activates trans-AMHO). Despite the similarities, they make distinct products: coprogen is a partially linear depsipeptide whilst fusarinine C is a macrocycle.
The work aims to characterise the PssA NRPS and determine how structural differences in the product arise, as well as comparing the biosynthetic routes to the monomer units, cis- and trans-AMHO. This will be achieved through molecular cloning of the biosynthetic genes from Penicillium rubens, utilising heterologous expression and in vitro biochemical assays.

CS2.1.39

RECOMBINANT PRODUCTION AND ANTIMICROBIAL ACTIVITY SCREENING OF NOVEL FUNGAL DEFENSINS IN KOMAGATAELLA PHAFFII

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Antimicrobial resistance originating from misuse of antibiotics occurs when bacteria, fungi, and viruses are unresponsive to the antimicrobial drugs, making the infections harder to treat. This results in inability to treat common infections and the spread of multi-resistant species globally. Current, single target approach of antibiotics is contributing to this phenomenon; however antimicrobial peptides such as defensins provide a new alternative to antibiotics by acting on multiple targets on the plasma membrane as well as the intracellular targets.

Defensins are antimicrobial cationic peptides composed of disulfide-linked cysteines, rich in beta-sheets. Fungal defensins like the cysteine-rich CSab defensin, Eurocin, are produced by fungi, exhibit antimicrobial activity, and are promising candidates as novel antimicrobials. Due to their structures, they can tolerate high pH, extreme temperatures, and proteolytic hydrolysis. They are mainly found in mammalian epithelial cells, scattered throughout the body, and contribute as a host defense mechanism of innate immunity.

In the framework of this study, previously uncharacterized fungal defensin-like proteins with high sequence homology to eurocin were mined from the NCBI BLASTp database. Two candidates from Aspergillus udagawae and Hyaloscypha hepaticicola were selected based on structural-physicochemical properties. Codon-optimized defensins are recombinantly produced in the methylotrophic yeast Komagataella phaffi, aka Pichia pastoris under the AOX promoter using the α-factor secretion signal. The pPICZαA plasmids carrying the candidate defensin-like proteins were subcloned into competent E. coli strain DH5α. Following PCR verification of the target genes, plasmids are isolated, linearized, and transformed into K. phaffii strain by electroporation. Colony PCR-verified transformants were further selected in a high-throughput setup for submerged fermentation. Candidate proteins secreted into the medium are collected and purified by Ni-affinity purification. Biochemical characterization was performed through BCA assay, Tris-Tricine SDS-PAGE, and Western blotting. Finally, antimicrobial activity was screened against bacterial and fungal strains via broth microdilution assay.

CS2.1.40

INFLUENCE OF TRANSCRIPTION FACTORS ON THE SECRETION OF A RECOMBINANT A-L-ARABINOFURANOSIDASE IN ASPERGILLUS NIDULANS

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Filamentous fungi can secrete significant amounts of proteins and are used as a platform for the production of industrially important enzymes. However, in terms of recombinant protein production, there is still potential for improvement. In this regard, many strategies have been explored to increase the capacity of fungal cell factories, and as consumer demand grows, identifying new genetic targets to increase enzyme secretion becomes increasingly critical. Therefore, our aim was to assess the secretion of enzymes in A. nidulans strains overexpressing a heterologous enzyme in which target transcription factors had been genetically manipulated. To identify these transcription factors, we used RNA-seq data from strains overexpressing recombinant enzymes and performed differential expression, GO enrichment, and comparative genomic analysis. Next, using mainly CRISPR-Cas9, we deleted or overexpressed these transcription factors in A. nidulans overexpressing a heterologous α-L-arabinofuranosidase (AbfA) and analyzed their phenotype to determine if they have any effect on enzyme activity. These analyses allowed us to identify transcription factor candidates with predicted functions in vesicle transport, protein folding, and cellular heat response. We found that the mutants ΔorsD and ΔAN9373 had a slight reduction in AbfA activity, and the strain OE::msnA had a 90% increase. However, the mutants ΔAN0094, ΔzipB, ΔAN3420, and ΔAN7592 did not exhibit any differences in enzymatic activity (U/mg/dry weight). This finding might suggest that the transcription factors orsD, AN9373, and msnA influence protein secretion and that overexpressing orsD and AN9373 might be an effective way to boost the production of recombinant proteins. We concluded that transcriptomic data could be used to identify transcription factors that might be involved in the protein secretion of A. nidulans, and we intend to continue our research by carrying out additional experiments to ascertain the role of these transcription factors in the secretion of biotechnologically important recombinant enzymes.

CS2.1.41

RELEVANCE OF THE COP9 SIGNALOSOME TO PLANT CELL WALL DEGRADATION IN TRICHODERMA REESEI

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Trichoderma reesei is one of the main producers of lignocellulolytic enzymes, especially cellulases, for the conversion of plant biomass to sustainable fuels and chemicals. The transcriptional regulatory machinery triggering CAZyme production is well known in this fungus, and therefore it has been the main target for strain improvement. In recent years the dogma of cellulase regulation exclusively at the level of transcription in T. reesei was challenged by data showing discrepancies between transcript levels and enzyme production, indicating that posttranscriptional mechanisms might be involved.
The COP9 signalosome represents a mechanism which might contribute to this second regulation step after transcriptional activation of cellulases and their regulators. The COP9 signalosome (CSN), is a multiprotein complex conserved in all eukaryotic organisms, involved in regulation of development, cellular signaling and influences protein stability via its connection to the 26S proteasome. Therefore, we investigated the role of the subunits CSN1, CSN4 and CSN5 for their role in cellulase and CAZyme production.
Deletion strains (Δcsn1, Δcsn4 and Δcsn5) in T. reesei QM6a were investigated by enzyme activities, growth performance assays and transcriptomics (RNA-seq). Cellulolytic and xylanolytic activities were massively reduced in all three mutans, but not their morphology. Also their transcript levels were affected as well as the expression of several transcription factors (TFs) including the main (hemi-)cellulolytic regulator xyr1. Interestingly, our transcriptome analyses showed a severe reshaping in gene expression encoding not only CAZymes, but including transcriptional regulation (TFs), signaling pathways, sugar transport, secondary metabolism or RNA interference.
Our data suggests that not only cellulases, but also other CAZymes and their regulators are subject to regulation by the COP9 signalosome, highlighting the multilevel complexity of the regulatory system related to plant biomass degradation in T. reesei, yet to be fully understood. Furthermore, this dataset provides leads for strain improvement in industrial biotechnology.

CS2.1.42

BIOPROSPECTING TRICHODERMA: A PARAGON RESOURCE OF STRUCTURALLY DIVERSE PHARMACEUTICALS AND BIOPESTICIDES

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Abstract
Fungi represents a rich repository of taxonomically restricted, yet chemically diverse, secondary metabolites that are synthesised via specific metabolic pathways. Enzyme's specificity and biosynthetic gene clustering are the bottleneck of secondary metabolite evolution. Trichoderma spp. produce many pharmaceutically important molecules; however, their specific biosynthetic pathways remain inaccessible. Our genomic-based analysis of this species, reveal the biosynthetic diversity of its specialised secondary metabolites, were over 50 BGCs were predicted, most of which were characterised as polyketide like compounds associated clusters. Gene annotation of the biosynthetic candidate genes predicted theproduction of many medically\ industrially important compounds including Enniatin, squalestatin, clavaric acid, ascochlorin, ankafalvin, naphthopyrone. Our genome mining and putative functional analysis of the biosynthetic genes annotated in this species, indicated the evolutionary processes that have shaped its current genetic structure and the structural diversity
of their chemical compositions. Revealing the biogenetic background of these natural molecules is a step forward towards the expansion of their chemical diversification via engineering their biosynthetic genes heterologously, and the identification of their role in the interaction between this fungus and its biotic\abiotic conditions as well as its role as bio-fungicide.

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KOMAGATAELLA PHAFFII (AKA PICHIA PASTORIS) AS A MICROBIAL CELL FACTORY FOR THE PRODUCTION OF NOVEL FUNGAL DEFENSINS

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As a result of the discovery of antibiotics and antifungals, life quality and treatment options have significantly improved. Despite this, a few years after the discovery, microorganisms have proven to be resistant to the treatments, which has resulted in antimicrobial resistance becoming one of the world's most serious health problems, causing an estimated 700.000 deaths every year worldwide. Recent studies have reported secondary fungal infections in patients on ventilators and with poor prognoses following Covid-19's worldwide spread. In turn, secondary fungal infections such as candidiasis and Aspergillosis were found to contribute to some of the fatalities due to Covid-19.
Defensins are small peptides that are secreted by the innate immune system of mammal, fungus, plant, and bacteria in order to eliminate microorganisms or foreign molecules. Defensins secreted by fungi constitute the fungal defensin subtype and novel antimicrobial drugs may be derived from them.
The methylotrophic yeast Komagataella phaffii uses methanol as a carbon source and an inducer via the AOX promoter. K. phaffii as a microbial factory, has an efficient expression system with its high capability to secrete recombinant proteins. This host's ability to perform post-translational modifications makes it a suitable host for the expression of fungal proteins.
During the course of this study, we recombinantly produced codon optimized defensins from Coprinopsis cinerea, Trichophyton rubrum, Trichophyton equinum in the K. phaffii KM71H strain in an attempt to characterize their antimicrobial activity. In order to select higher-yield colonies, a high-throughput selection strategy was applied following the colony PCR verification. Defensins were produced using submerged fermentation in BMM medium and then characterized with

BCA assays, Tris-Tricine SDS-PAGE, and Western Blotting. As a final test, broth microdilution was used to detect antimicrobial activity against S. aureus and C. albicans.

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CS2.1.44

CARBON METABOLISM RELATED DEHYDROGENASES AND REDUCTASES FORM DISTINCT SUBGROUPS WITHIN THEIR PFAM FAMILIES

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Sugar reductases and polyol dehydrogenases are common enzymes in fungal central metabolism. They catalyze steps in various metabolic pathways, such as the pentose catabolic pathway (PCP), the oxido-reductive D-galactose pathway and the D-galacturonic acid pathway. Most of these enzymes belong to PFAM family 248, 107 or 106. We downloaded all the members of these PFAM families from the Aspergillus niger genome and performed phylogenetic analysis for each PFAM family to explore the genetic diversity of dehydrogenases and reductases of ascomycete Aspergillus niger. This analysis showed that sugar-related aldo/keto reductases of PFAM family 248 and of polyol dehydrogenases of PFAM family 107 form distinct subclades in their respective PFAM families. These clades also contain so far uncharacterized members, which we hypothesize are also involved in sugar metabolism. In contract, the PFAM 106 sugar reductases were found in different branches of its phylogenetic tree. The difference in phylogenetic distribution of these enzymes could be a clue to the evolutionary diversity of sugar metabolic pathways and guide us towards novel enzymes for synthetic biology.

CS2.1.45

"A FIGHT WITHIN" – SECRETORY STRESS IN TRICHODERMA REESEI, STALLING ITS BRIMFUL CAPACITY TO PRODUCE HYDROLYTIC ENZYMES

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A microorganism isolated from Solomon island (namely Trichoderma reesei) is capable of magnificent protein expression and secretion, and has established its importance in various industries including biorefineries and bioprocessing. It is likely that massive enzyme secretion goes along with high accumulation of proteins and is also accompanied by high amounts of unfolded proteins in the endoplasmic reticulum (ER). This probably results in induction of the unfolded protein response (UPR) and another mechanism that was described for T. reesei: repression by secretion stress (RESS). While UPR results in halting translation and activating chaperone expression, RESS responds specifically at the transcript level. Hence, to break new scientific ground and enable knowledge-based strain design, we aim to elucidate the molecular mechanism of RESS. We will first verify the occurrence of RESS in T. reesei and if it is present, we will further determine whether it occurs at initiation of transcription or it is the result of mRNA instability. To answer these two questions, the T. reesei wild-type strain will be cultivated in the presence and absence of transcription and translation inhibitors, respectively. If RESS occurs, and the reason for reduced transcript levels is not mRNA degradation, a recombinant strain, which has RESS-target promoters (e.g. cbh1 and egl1) replaced with non-RESS target promoters (e.g. sar1 and bgl2), will be subjected to artificial secretion stress by chemical treatment. This will suggest whether promoter engineering might be useful for preventing RESS. Additionally, whole transcriptome data of conditions under UPR will be mined for further targets and possible mediators.

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CS2.1.46

ANALYSIS, IMAGING AND SORTING OF GERMINATED FUNGAL SPORES ON THE COPAS VISION LARGE PARTICLE FLOW CYTOMETER

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Large particle flow cytometers from Union Biometrica provide automation for the analysis and dispensing of a wide range of samples like large fragile cells, cell clusters, small model organisms, encapsulated cells, seeds and germinated fungal spores.
A flow cytometry approach to analyze and dispense germinated fungal spores, without compromising their morphology and viability will make possible developing high-throughput spore germination assays or fungitoxicity studies.
Here, we were able to analyze, select and sort germinated fungal spores using a large object imaging flow cytometer, the COPAS VISION. As proof-of-concept models, we used germinated spores from 3 important plant pathogens. We tested Phakopsora pachyrhizi which is a pathogen that causes Asian soybean rust. We also tested Botrytis cinerea which is a necrotrophic fungus that affects many plant species like wine grapes and finally Zymoseptoria tritici was evaluated which is a species of filamentous fungus and is a known wheat plant pathogen. We were able to discriminate between germinated and ungerminated spores, determine the length of the mycelia and dispense into wells of multiwell plates or directly onto plant leaves.

CS2.1.47

EVALUATION OF THE POTENTIAL OF PFAS DEGRADATION BY FUNGI

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PFAS has become one of the most addressed pollutants in many environments after the realization that PFAS can cause many health issues in humans and other higher eukaryotes. Its persistence in many environments adds to the problem, as it is not feasible to remove all contaminated soil or water to a processing plant for PFAS removal. It is therefore crucial to develop in situ bioremediation approaches for the removal of PFAS.
In this project, we evaluated the possibility of PFAS degradation by fungi. Literature so far is non-consistent with respect to the ability of fungi to degrade PFAS and no concrete data is available on the mechanism through which this could occur.
Initial testing identified several fungi that appear to benefit from the presence of PFAS in their medium, suggesting that they are in fact able to convert these compounds. To validate this, we have performed a large-scale experiment in which seven fungal species were grown on four carbon sources in the absence or presence of PFOA or PFOS, two of the main PFAS compounds. Samples were taken over time and analyzed for PFAS degradation, transcriptomics, substrate utilization and enzyme activities, to determine if these fungi can degrade PFAS and if so, by which approach. Highlights of this study will be presented.

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CS2.1.48

HETEROLOGOUS EXPRESSION OF TYPE I FUNGAL POLYKETIDE SYNTHASES IN YARROWIA LIPOLYTICA

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Fungal polyketides are a large group of secondary metabolites, valuable due to their diverse spectrum of pharmacological activities. Polyketide production in fungi is associated with several challenges: small yield and low-purity titers. To tackle these aspects, we switched from fungi to the yeast Yarrowia lipolytica, an easily cultivable heterologous host.
As an oleaginous yeast, Yarrowia lipolytica displays a high flux of acetyl- and malonyl-CoA precursors used in fatty acid biosynthesis. Likewise, acetyl- and malonyl-CoA are the building blocks of fungal polyketides, and we explored the possibility of redirecting this flux toward polyketide production. Despite its promising prospect, Y. lipolytica has so far only been used for heterologous expression of simple type III polyketide synthases (PKSs) from plants. We, therefore, decided to examine the potential for more complex polyketide production in Y. lipolytica by targeting fungal polyketides derived from type I PKSs. We employed a CRISPR-Cas9-mediated genome editing method first to investigate the production of the simple polyketide 6-MSA and achieved a titer of 100 mg/L in the initial trials. Subsequently, we used the same markerless gene integration system to express in Y. lipolytica the genes fsr1, fsr2, and fsr3, together with the activating co-enzyme phosphopantetheinyl transferase, FsPPT1, all being responsible for bostrycoidin biosynthesis in Fusarium solani. We obtained a 9 mg/mL titer in the initial unoptimized shake flask cultures and are currently working on a more promising fermentation set-up in bioreactors.
The work demonstrates the potential of Yarrowia lipolytica as a platform for the heterologous production of complex fungal polyketides. In our
ongoing research, we are exploring polyketide yield optimization by over-expressing participants in the β-oxidation pathway, which should favor precursor recruitment for polyketide biosynthesis.

CS2.1.49

GENOME EDITING USING A VERSATILE VECTOR-BASED CRISPR/CAS9 SYSTEM IN FUSARIUM SPECIES

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Fusarium species include important filamentous fungal pathogens that can infect plants, animals, and humans. Meanwhile, some nonpathogenic Fusarium species are promising biocontrol agents against plant pathogens. We developed a vector-based CRISPR/Cas9 system for the tomato wilt fungus Fusarium oxysporum f. sp. lycopersici (Fol). Thes system harbors an endogeneous U6 small nuclear RNA promoter for the expression of single-guide-RNA and an endogenous H2B nuclear localization signal for the localization of Cas9. This optimized system enabled efficient targeted gene knock-out, knock-in, and base editing, including in the accessory chromosomal regions of Fol. This system was also applicable for genome editing in F. oxysporum ff.spp. spinaciae, conglutinans, pisi, and raphani, and F. commune, a nonpathogenic biological agent for F. fujikuroi, without any modifications to the vector. We further demonstrated that large chromosomal regions (approximately up to 750 kb) could be removed by simultaneously cutting of two sites in the genome of Fol. This developed system and genome editing strategy will allow for a broad range of functional genomics in Fusarium species.

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CS2.1.50

DEVELOPMENT OF NOVEL COMPOSITE MATERIALS FOR THE CONSTRUCTION INDUSTRY USING THE BASIDIOMYCETE FOMES FOMENTARIUS

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The construction sector contributes decisively to the overall emission of greenhouse gases. In Germany, for example, the contribution from the construction, modernization and operation of buildings is estimated to account for 40 % of the total CO2 equivalents emitted. In addition, a growing human population requires increasing building activities worldwide, although many mineral resources become scarce. To meet these challenges, the construction sector must be transformed towards sustainability and filamentous cell factories can make an important contribution here.
Composite materials based on basidiomycetous mycelium have a particular potential, since the basis for these materials - lignocellulosic raw and residual materials from agriculture and forestry - is available in large quantities worldwide. In the present work, we provide data for composite materials that were generated using the tinder fungus Fomes fomentarius whilst growing on different agricultural residues. We show that the fungus feeds on different plant substrate particles that allow us to produce composite materials that can either be used as insulating material or, when compressed, as construction panels. They therefore provide a sustainable alternative to insulating materials such as expanded polystyrene or gypsum board and particle board, respectively.

CS2.1.51

AN INVESTIGATION ON "BURNOUT" IN INDUSTRIAL FUNGI AND HOW TO CURE IT

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"Burnout," or spontaneous degeneration, is frequently observed in various microorganisms and could be generalized as the loss of an essential ability of the organism during extensive usage. This can range from the loss of virulence in a pathogenic microorganism during lab research to the depletion of the productivity of an industrial strain. Although this depicts a severe problem, especially for the biotechnology industry, the underlying mechanisms are yet poorly studied. Recently, this phenomenon has been reported in the filamentous fungus Trichoderma reesei, which is applied in the biotechnology industry to produce cellulase on a large scale. In general, cellulases are amongst the most abundant enzymes in industries, such as pulp and paper, food, textile, and agricultural sector, thus of high importance. According to the report published, cellulase productivity is spontaneously lost during the production process in an industrial T. reesei lineage and its publicly available ancestors. However, any other T. reesei lineage has not yet been investigated for strain degeneration. Our research aims to determine whether this phenomenon occurs in another lineage and to which degree of severity. Therefore, a standardized induced strain degeneration workflow is used to artificially degenerate T. reesei strains while monitoring cellulase production and activity changes. A percentage of the non-productive population is determined from the still cellulase-producing population by a cellulase agar plate screening assay. These parameters are compared to the already investigated lineage and will be presented and discussed. The outcome of this study will help to improve the understanding of mechanisms underlying burnout in industrial fungi and how to prevent it to ensure cellulase production in T. reesei.

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CS2.1.52

SYNCHROTRON RADIATION BASED X-RAY MICROTOMOGRAPHY FOR THREE-DIMENSIONAL GROWTH ANALYSIS OF ASPERGILLUS NIGER PELLETS

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Filamentous fungi are an indispensable part of industrial biotechnology. Submerged cultivated in bioreactors with several 100 m³ capacity, these cell factories produce relevant biotechnological compounds. The close relationship between fungal morphology and productivity has led to many high-throughput methods to quantify their macromorphology [1]. Nevertheless, only micro-computed tomography (µCT) is the method of choice to study the three-dimensional micromorphology of fungal pellets during submerged cultivation [2]. However, as morphological heterogeneity of pellets makes it necessary to measure hundreds of pellets per sampling time, there is a need for high-throughput µCT approaches.
To meet this challenge, we applied synchrotron radiation based X-ray microtomography at the P05 beamline (Helmholtz-Zentrum hereon) of PETRAIII (Deutsches Elektronen Synchrotron - DESY) and extended our developed method [2], to generate and analyze 3D images of ~20,000 single fungal pellets. We revealed micromorphological properties such as number and density of spores, tips, branches, and hyphae from 26 sampling points during 48-hour Aspergillus niger cultivations. The computed data allowed us to follow the growth of submerged cultivated fungal pellets in highly resolved 3D for the first time.
With our previously developed methods for diffusion computations and growth simulations of filamentous fungal pellets [3][4], the generated morphological database from synchrotron measurements can be used to understand, describe, and model the growth and substrate supply of fungal cultivations.

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[3] Schmideder, Barthel, Müller et al., Biotechnol. Bioeng., 2019, doi: 10.1002/bit.27166
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CS2.1.53

USING NON-SACCHAROMYCES YEAST FOR THE FERMENTATION OF DAIRY BY-PRODUCTS

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Dairy products processing leads to the accumulation of by-products that result in large amounts of food waste. For example, for every 10 pounds of cheese, 9 pounds of liquid whey is created. Sweet whey is the crude liquid whey that results after cheddar cheese production while acid whey results after yogurt and cream cheese production. The main compositional difference between sweet and acid whey is pH and protein content. Non-Saccharomyces yeasts that encode functional LAC genes can hydrolyze lactose in crude liquid whey to produce ethanol. Here, we used Kluveromyces spp. to test the feasibility of converting lactose in sweet and acid whey into ethanol. Proximate analysis for protein content and pH was conducted to understand the differences between sweet and acid whey. Spoilage microorganisms were also identified in the sweet and acid whey collected. Micro-fermentations were conducted comparing filtered crude sweet whey from cheddar, acid liquid whey from cream cheese and yogurt, and lactose broth representing the pH of sweet and acid whey (positive control). The whey by-product will be inoculated with Kluveryomyces marxianus, 4 different strains of K. lactis, and Saccharomyces cerevisiae (negative control). PH, ethanol, and lactose content were measured at 0 and 72 hours. Optical density (OD) was measured every hour over 72 hours. There were no significant differences observed in pH over time in the acid whey, while in sweet whey the pH was lowered (statistical significance, p-value < 0.05). Our data suggests that non-Saccharomyces strains can grow in liquid whey, hydrolyze lactose, and produce up to 10% ethanol supporting use of fungi in a circular and sustainable bioeconomy.

CS2.1.55

ENGINEERING FUNGAL ITERATIVE POLYKETIDE SYNTHASES TO MAKE SUSTAINABLE CHEMICALS

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Fungi are natural chemists with the capability to produce a diverse range of specialized metabolites, also known as natural products (NP). Among them, polyketides like the cholesterol lowering-drugs Lovastatin and the agricultural fungicide Strobilurin are outstanding for their conserved biosynthetic mechanism which involves the condensation of acyl-coA units, followed by their total or partial reduction. These reactions are catalysed by enzymatic complexes called polyketide synthases (PKS) which can be (i) multimodular: where each module condenses a specific extender unit and potentially reduces the growing product or (ii) iterative (iPKS): where the same module catalyses multiple condensations and reductions cycles. Most PKS in fungi are iterative.

Fungal iPKS products can be converted into alpha amino acids via three-step mechanism. These building blocks can then be processed by non-ribosomal peptide synthetases to produce complex molecules. In this study, we chose the iPKS involved in the biosynthesis of the fungal non-ribosomal peptide, cyclosporin to be produced in yeast. We are using phylogenomics to identify the genetic space to create diversity in the amino acid pool by engineering the PKS. We hope that this research helps defining the mechanistic principles behind the chemical diversity of PKSs and will help in the production of useful polyketides that may be used as drugs or bioproducts.

Here I will present the experimental methods that were used to produce the polyketide-derived alpha amino acid in yeast and the use of phylogenetics based rationale to engineer iPKS to create the diversity in the amino acid pool.

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CS2.2 DEVELOPMENT AND MORPHOGENESIS

CS2.2.9

VE-1 REGULATION OF MAPK SIGNALLING CONTROLS SEXUAL DEVELOPMENT IN NEUROSPORA CRASSA

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The velvet complex is a fungal-specific protein complex that participates in the regulation of gene expression during development, pathogenesis, and secondary metabolism in response to environmental signals such as light. In Neurospora crassa the velvet complex is composed of VE-1, VE-2, and LAE-1. Strains with deletions in ve-1 or ve-2 have increased conidiation, and a delayed and reduced sexual development. Alterations in the development of female structures (protoperithecia) in the ve-1 and ve-2 mutants suggested that a protein complex composed of VE-1/VE-2 regulates transcription during sexual development. The transcriptome of wild-type and ve-1 mutant strains was characterized in a time course experiment during sexual development in both dark and light. We have identified 2,117 genes with different transcriptional profiles between the wild-type and the mutant strain in cultures kept in the dark, and 4,364 genes when cultures were kept in the light with an overlap of 1,648 genes. Among the misregulated genes, we detected genes that are known for their regulatory roles in sexual development, including genes in the mitogen-activated protein kinase (MAPK) signaling pathways, cell-cell fusion genes (ham genes) and transcription factor genes involved in fruiting body development. We have detected in vitro binding of VE-1 and VE-2 to the promoter sequences of mak-1, mak-2, mek-2 and os-4, suggesting that VE-1/VE-2 plays a direct regulatory role in the transcription of MAPK genes. Furthermore, we detected transcription of ve-1, ve-2, and lae-1 during all stages of sexual development, but the three proteins were not detected in the later stages of development (96 and 144 hours after fertilization). Our results suggest that the absence of VE-1 results in transcriptional changes that disrupt the signal transduction cascade regulating sexual development in N. crassa.

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CS2.2.10

SYSTEMS AND 3D IMAGING APPROACHES TO UNDERSTAND THE ASPERGILLUS NIGER CHITIN SYNTHASE GENE REPERTOIRE

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The filamentous fungus Aspergillus niger is broadly applied as a cell factory for the production of proteins, enzymes and organic acids. In all filamentous fungi, hyphal growth and (protein) secretion are strictly coupled processes. One organelle affecting both processes is the fungal cell wall with chitin as one of its main components. In this project, we aimed to understand the role of nine predicted chitin synthase genes on the growth, morphology and productivity of Aspergillus niger. We therefore constructed a chitin synthase knock-out library and compared the phenotypes of all mutant strains during solid and submerged cultivations with its parental wild-type strain. Corresponding data will be shown including transcriptomic data, microscopic and macroscopic data based on 2D and 3D synchrotron X-ray microtomography analyses as well as data addressing the cell wall stress responsiveness of all strains.

CS2.2.11

SYSTEMATIC KNOCKOUT EFFORTS HIGHLIGHT CONSERVED TRANSCRIPTION FACTORS REGULATING THE INITIATION OF MUSHROOM FORMATION IN COPRINOPSIS CINEREA

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Mushroom-forming fungi produce fascinating fruiting bodies, the development of which represents a transition from simple to complex multicellularity within a few days. Mushroom development starts with localized hyphal branching, giving rise to primary and secondary hyphal knots. However, the gene regulatory networks orchestrating this process remain largely elusive. Here, we identified 35 conserved transcription factors (TFs) in Coprinopsis cinerea through a genome comparison of 137 Basidiomycota species. Based on the high-resolution time series developmental RNA-seq data from C. cinerea, and a re-analysis of developmental transcriptome data for eight Agaricales species, we revealed most of the TFs are up-regulated early in fruiting body development in most of the species. Moreover, some of the TFs are orthologs or close homologs of the morphogenetic TFs in yeast/pathogenic fungi. Using CRISPR/Cas9, we generated 35 TFs mutants from the C. cinerea AmutBmut #326 strain, which showed phenotypes ranging from the lack of hyphal knot formation to arrest at the primary/secondary hyphal knot or later developmental stages. Combined with four phenotyping approaches, we identified eight TFs that regulate early developmental events (Vegetative mycelium -> Hyphal knot -> Primordium1). Intriguingly, we identified two TFs, which showed no reduction in vegetative mycelium growth but were defective in hyphal knot formation. This suggests that these two TFs probably play an important role in mushroom initiation. RNA-seq analysis of mutant and wild-type strains provides a preliminary glimpse into the transcriptional network and a set of potential genes which function in mushroom initiation. Our study paves the way to identifying key regulators of mushroom formation in Agaricomycetes.
Keywords: Agaricomycetes, gene editing, regulatory network, transcriptome, fruiting body development

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CS2.2.12

THE ASPERGILLUS FUMIGATUS SEPTATION INITIATION NETWORK REGULATOR, MOBA, IS ESSENTIAL FOR SEPTATION, SURVIVAL UNDER ECHINOCANDIN STRESS, AND VIRULENCE

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Aspergillus fumigatus is a major invasive mold pathogen and is the most frequent etiologic agent of invasive aspergillosis. The currently available treatment options for invasive aspergillosis are limited in both number and efficacy. Recent work in our lab showed that the β-glucan synthase inhibitors, the echinocandins, are fungicidal against strains of Aspergillus fumigatus with defects in Septation Initiation Network (SIN) kinase activity, whereas they are fungistatic against strains with normal septation. Surprisingly, SIN kinase deletion strains also failed to invade lung tissue, and were therefore significantly reduced in virulence capacity, in immunosuppressed mouse models of invasive aspergillosis. Inhibiting septation to unlock hyphal compartments of filamentous fungi is therefore an exciting clinical prospect that could both reduce virulence and improve current antifungal therapy. However, the SIN remains woefully understudied in many species. To begin to address this knowledge gap, we aimed to characterize the putative activators of the A. fumigatus SIN kinases SepL and SidB: SepM and MobA, respectively. Our data suggest that deletion of sepM (ΔsepM) or mobA (ΔmobA) results in phenotypes similar to deletion of their SIN kinase binding partners. Loss of either sepM or mobA resulted in hypersusceptibility to cell wall stressors, including echinocandins, and caused reduced invasion of murine lung tissue. Cell wall staining revealed that septum formation was absent in ΔmobA yet was detectable, though greatly diminished, in ΔsepM. Correspondingly, the echinocandins were fungicidal against ΔmobA and remained fungistatic against ΔsepM. Strikingly, microcolonies of ΔsepM that formed under caspofungin stress were found to be hyperseptate, suggesting septation is a cell wall stress-responsive process in A. fumigatus. Our work underscores the scarcely breached complexity of the A. fumigatus SIN and highlights the need for a deeper understanding of SIN regulation to find where in the pathway druggable targets may lie.

CS2.2.13

DISTINCT FUNCTIONS OF VMH3, ONE OF THE MAJOR HYDROPHOBINS IN THE WHITE-ROT FUNGUS PLEUROTUS OSTREATUS

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Hydrophobins, which are small-secreted proteins with both hydrophobic and hydrophilic parts, can self-assemble into an amphiphilic film at air-water interface helping the fungus to form aerial hyphae which was first reported in Schizophyllum commune. In white-rot fungus Pleurotus ostreatus, three hydrophobins 'Vegetative Mycelium Hydrophobin (Vmh1, Vmh2, Vmh3)' have been identified 20 years ago. In our previous study, vmh2 and vmh3 has been reported with predominant expression level in wild-type strain. The roles of Vmh2 and Vmh3 have not yet been elucidated, we attempted to find their physiological function in this study. The single deletion mutants (Δvmh2 and Δvmh3) and a double deletion mutant (Δvmh2Δvmh3) were obtained by gene targeting with homologous recombination. The Δvmh3 and Δvmh2Δvmh3 strains exhibited relatively slower aerial mycelia formation on liquid medium despite no significant alternation was found on agar medium. When the hyphae were observed by Transmission Electron Microscope (TEM), deletion of Vmh2 or Vmh3 both resulted in loss of the layer at the interface between cell wall and outer environment. All mutants showed the same reduced level of hydrophobicity when 0.2% SDS was dripped onto the mycelial surface. Δvmh3 and Δvmh2Δvmh3 strains grew slower than WT under oxidative stress on agar plate. The Δvmh3 and Δvmh2Δvmh3 strains also showed delay in lignin degradation compared with WT on beech wood sawdust medium. These results suggested that hydrophobins Vmh2 and Vmh3 were both important in mycelial hydrophobization, but Vmh3 took on major roles in development, stress resistance and white rot compared with Vmh2. Although there are more than 20 hydrophobins in P. ostreatus, our study indicated that while there are overlapping functions, such as the cell surface hydrophobicity seen in vmh2 and vmh3, there are also unique physiological functions, as shown in vmh3.

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CS2.2.14

STUDYING SEXUAL REPRODUCTION IN LEPTOSPHAERIA MACULANS, THE CAUSAL AGENT OF BLACKLEG DISEASE OF RAPESEED

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Plant-associated fungi have an important role in our ecosystems, either through beneficial, neutral or harmful interactions. In particular, phytopathogenic fungi represent a threat to global food security. Rapeseed (Brassica napus), the world's second-largest oilseed crop, is mainly threatened by the ascomycete Leptosphaeria maculans. The latter displays a complex life cycle switching from an asymptomatic colonization (biotrophic stage) followed by a necrotrophic phase, and eventually a saprotrophic step. During the saprotrophic stage, sexual reproduction take place on stems debris. This step is paramount in its life and pathogenic cycle since sexual reproduction (i) favors genetic recombination and appearance of new pathogenicity traits and (ii) produces ascospores that are the main inoculum throughout the plant life. Sexual reproduction in L. maculans is controlled in laboratories, but this stage has never been detailed. Our first aim of this project is to describe sexual reproduction per se. This involves identifying male and female gametes, and characterizing the different steps between fertilization and production of ascospores. Under laboratory conditions, fruiting bodies (pseudothecia) are mature after 3-4 weeks of culture. Nevertheless, the precise dynamics of pseudothecia production is not yet known. Besides, the development of fruiting bodies depends on environmental conditions that are not yet fully understood. At a molecular level, we aim to determine key genes involved in the successive stages. The second aim linked to sexual reproduction consists in studying the epigenetic mechanism RIP (Repeat-Induced Point mutation) occurring before karyogamy. This genome defense mechanism detects duplicated sequences and induces C-to-T mutations and methylation in repeats. The mechanism of RIP and its ability to recognize duplications throughout the genome are not well understood. This specific process can result in the emergence of new pathogenicity-related alleles, and thus new virulent strains. The first results of this project will be presented.

CS2.2.16

DEVELOPMENTALLY EXPRESSED UNANNOTATED GENES REGULATE FRUITING BODY MORPHOGENESIS OF THE BASIDIOMYCOTA MODEL ORGANISM COPRINOPSIS CINEREAC

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Fruiting body morphogenesis is a complex process in fungal development determined by a genetically encoded program, which reached the highest complexity level in the Agaricomycetes. According to the literature, thousands of genes are involved in this process in the Basidiomycota model organism, Coprinopsis cinerea. While previous studies described numerous novel morphogenesis-related conserved gene families, recent reports pointed out the existence of conserved developmentally expressed gene groups, which encode proteins without any known conserved domain signatures and may form novel gene families. A part of these genes is especially interesting, because they are significantly upregulated during the primordium initiation or sporogenesis. Therefore, in the present work we set out to investigate the function of selected unannotated genes, using reverse genetics.
To select the most relevant genes for disruption, we combined previously published gene expression data sets of C. cinerea fruiting body development with a recently conducted RNA-Seq analysis of sporogenesis. We selected six genes which are upregulated in the primordium and later stages of fruiting body development compared with the vegetative mycelia, or solely show a high expression level in the gills during spore formation. We used the CRISPR/Cas9 system for gene deletion. According to our preliminary results, the observed phenotypes of the knock-out mutants showed various developmental changes, ranging from minor defects (weak sporulation) to more serious deformities (lack of the lamellae, snowball-like primordia with incomplete cap and thickened stipe). These results demonstrate that several conserved developmentally expressed unannotated genes are involved in morphogenesis and their investigation can highlight new exciting patterns in mushroom development.

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CS2.2.17

THE FUNCTION OF A CONIDIA SPECIFIC TRANSCRIPTION FACTOR CSGA IN ASPERGILLUS NIDULANS

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Aspergillus spp. mainly reproduce through asexual reproduction, producing the asexual spore called conidia. The process of conidia formation (conidiation) is controlled by various transcription factors. Among them, BrlA, AbaA, and WetA have been defined as the central regulators which regulate gene expressions related to conidiation. Our previous transcriptomic analysis identified twenty novel conidia-specific transcription factors. In this study, we characterized one of the conidia-specific transcription factors CsgA, the Zn2Cys6 transcription factor containing the GAL4-like zinc-finger domain. The roles of CsgA were investigated in two Aspergillus species, the model organism Aspergillus nidulans and the aflatoxin producer Aspergillus flavus. In A. nidulans, the ΔcsgA strain showed an increase in conidiation and fungal growth. The expression levels of brlA in the ΔcsgA strain increased in the early stage of conidiation. Deletion of csgA exhibited a defect in sexual growth. Overexpression of csgA resulted in decreased conidiation and increased sexual development, suggesting that CsgA plays a role in maintaining the balance between asexual and sexual development in A. nidulans. In conidia, deletion of csgA resulted in increased trehalose content and higher tolerance to thermal, oxidative, and UV stresses. In ascospore (sexual spore), the absence of csgA showed higher trehalose content and stress tolerance compared to control. Germination ability of ascospore was lower in the ΔcsgA strain. The production of sterigmatocystin increased in the ΔcsgA conidia and ascospore. In A. flavus, deletion of csgA showed a decrease in fungal growth but an increase in conidiation. The ΔcsgA strain exhibited abnormal sexual development. Deletion of csgA resulted in increased trehalose content and higher tolerance in thermal and oxidative stresses. The aflatoxin B1 production was lower in the ΔcsgA conidia compared to control. Overall, these results suggest that CsgA plays a crucial role in proper fungal development and mycotoxin production in A. nidulans and A. flavus.

CS2.2.18

SENSING IN THE DATING GAME: ASSUMING THE MALE OR FEMALE PART IN A CROSS IS INFLUENCED BY THE GPCR GPR8

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Fungal reproduction in an environment with scarce resources needs precise timing, securing of nutrient supply and preparation of defence chemicals. For heterothallic species, such as Trichoderma reesei, the 'dating game' begins when two strains of the opposite mating type get in contact. Pheromones and corresponding receptors ensure mate recognition. Successful reproduction further involves chemical communication incorporating sensing and signalling via several other G-protein coupled receptors (GPCRs) and secondary metabolites.
We identified 14 GPCRs to be involved in the regulation of secondary metabolite production upon partner interaction. Of them, gpr8 is located in close vicinity to the most prominent secondary metabolite cluster of T. reesei responsible for the yellow pigmentation of in vitro cultures – the SOR cluster. We previously showed that GPR8 influences secondary metabolite production with a strong overlap in gene regulation with the main transcription factor of the SOR cluster, YPR2. In addition, gpr8 is regulated by the carbon catabolite repressor CRE1 in a light dependent manner. Therefore, we asked whether these characteristics might be relevant for its function in partner interaction as well.
Crossings of strains in fertile background revealed that the absence of the receptor GPR8 leads to mating type dependent sterility: MAT1-1 Δgpr8 strains are male sterile, while MAT1-2 Δgpr8 strains are female sterile. Consequently, the signal received by GPR8 may trigger the decision whether a strain will act as a male or female partner in a cross. Transcriptome data indicates a strong influence of GPR8 on cellular transport and genes coding for secondary metabolite synthases. Female sterility of gpr8 deficient strains is linked to altered regulation of genes assigned to RNA synthesis, processing, and modification.
Consequently, sexual reproduction from partner sensing to ascospore production includes a wide array of GPCR-mediated sensing and signalling as well as signals from nutrient availability and the abiotic environment.

CS2.2.19

THE EFFECT OF THE DUPLICATION OF A SINGLE SEPTIN ON THE DEVELOPMENT OF AGARICALES FRUITING BODIES

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Carpogenesis in Agaricomycetes is a varied process leading to a high number of different cell-types, tissue-types and fruiting body forms. The genetic background of this high diversity is poorly known. Here we report that the duplication of a single septin might underlie some order-specific developmental processes within the largest group of Agaricomycetes, the order Agaricales.
Septins are conserved cytoskeletal proteins that can form higher-order structures, such as filaments, gauzes or rings. They can scaffold other cytoskeletal proteins or function as physical barriers and play important roles in diverse cellular processes such as cell division or cellular differentiation. The building blocks of the septin higher-order structures are septin heteropolimers consisting of different septin monomers in a well defined order. The terminal position of the heteropolymers are occupied by Cdc11.

We have found that Cdc11 septin has been duplicated in the Agaricales after the split of the family Pleurotaceae. The newly gained paralog (termed cdc11b) acquired a limited number of conserved substitutions and deletions and is developmentally regulated during fruiting body formation. We created knockout mutants of cdc11a and cdc11b in Coprinopsis cinerea and found that the Δcdc11a strain produced fruiting bodies identical to that of the wild type, though delayed in time and less in number. In contrast, the development of the Δcdc11b strain aborted in the early primordial stage and produced an abnormally shaped primordia, with a bowl-like nodulus.
We hypothesize that Cdc11B is needed for the proper differentiation of a subpopulation of cells within the nodulus that later gives rise to the fruiting body initial.

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CS2.2.20

NSDD-DEPENDENT SEXUAL DEVELOPMENT AND THE DOWNSTREAM REGULATOR NDRC IN ASPERGILLUS NIDULANS

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Aspergillus nidulans is a filamentous model fungus that has both of asexual and sexual life cycles, which are regulated by genetic and environmental factors such as nutritional conditions and stresses. The nsdD gene is a well-known GATA type transcription factor responsible for the regulation of sexual and asexual development in A. nidulans. Previous report identified an interaction partner protein IndD as a physical regulator of the NsdD protein. In this study, we revealed that the IndD protein interact with NsdD in cytosol in vivo. Also, we identified a gene, named ndrC (nsdD-dependent regulation) by using RNA-seq experiment followed by DEG analysis. The NdrC protein is conserved in some Aspergillus species but not in other organisms and it has no known domain except DUF4267 regarded as a hypothetical protein. To characterize the function of the gene, deletion mutants were generated, and the phenotypes under the various developmental conditions were observed. The colony size of the mutant was similar to the host strains and the control strains, but more conidia were produced compared to the control strains, suggesting that the gene is negatively regulate asexual development or conidia production. Microscopic observations showed that there was no cleistothecium or hüll cell formed after incubation of sexual induction condition. In wilt-type strain the ndrC gene expression was not detected in cleistothecia whereas the gene was expressed in conidia. Taken together, the ndrC gene is responsible for the sexual development and repression of asexual development in A. nidulans.

CS2.2.21

CHITOSAN METABOLISM: A WAY TO MODULATE MAGNAPORTHE ORYZAE PATHOGENICITY

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Chitosan is a partially deacetylated linear polysaccharide composed of β-1,4-linked units of d-glucosamine and N-acetyl glucosamine. As well as a structural component of fungal cell walls, chitosan is a potent antifungal agent. However, the mode of action of chitosan is poorly understood. We reported that chitosan is effective for control of rice blast disease. Chitosan application impairs growth of the blast fungus Magnaporthe oryzae and has a pronounced effect on appressorium-mediated plant infection. Chitosan inhibits septin-mediated F-actin remodelling at the appressorium pore, thereby preventing repolarization of the infection cell. Chitosan causes plasma membrane permeabilization of M. oryzae and affects NADPH oxidase-dependent synthesis of reactive oxygen species, essential for septin ring formation and fungal pathogenicity. We further show that toxicity of chitosan to M. oryzae requires the protein kinase C-dependent cell wall integrity pathway, the Mps1 mitogen-activated protein kinase and the Nox1 NADPH oxidase. A conditionally lethal, analogue (PP1)-sensitive mutant of Pkc1 is partially remediated for growth in the presence of chitosan, while Δnox1 mutants increase their glucan: chitin cell wall ratio, rendering them resistant to chitosan. Taken together, our data show that chitosan is a potent fungicide which requires the cell integrity pathway, disrupts plasma membrane function and inhibits septin-mediated plant infection.

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CS2.2.22

UNIQUE CELL WALL STRUCTURE OF BASIDIOMYCETE FOCUSED ON A-1,3-GLUCAN IN PLEUROTUS OSTREATUS

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The α-1,3-glucan (α-glucan), a notable cell wall component of the ascomycetes, has been reported to have distinctive functions in mycelial aggregation and escape from the immune system of the host. Despite these characteristic functions being reported in the ascomycetes, no functional analysis has been conducted in the basidiomycetes. In addition, the structure and biosynthesis of the basidiomycete cell wall has not been well elucidated. In this study, we analyzed cell wall constitution and a gene for α-glucan synthesis in Pleurotus ostreatus. Firstly, fractionation of the cell wall based on the alkali solubility of its components, quantification of sugars, revealed the amount of constituent sugars in each fraction is much different from that of the ascomycetes. Notably, content of α-glucan is much lower while β-glucan content is higher in P. ostreatus. The cell wall structure was visualized by confocal microscopy using α-glucan and β-glucan specific fluorescent probes for P. ostreatus and ascomycete Aspergillus oryzae. Imaging analysis revealed that the outermost layer of the cell wall is covered by β-glucan with occasional small α-glucan particles in P. ostreatus, whereas in A. oryzae it is covered with α-glucan. In P. ostreatus, the α-glucan layer was observed after β-glucanase treatment. These results suggest that basidiomycetes have a unique cell wall structure, and that α-glucan may play roles different from those proposed within the ascomycetes. A gene for α-glucan synthase ags1 was identified and disrupted in P. ostreatus. No α-glucan was observed in both imaging and sugar measurement in the Δags1 strain. The Δags1 strain had thinner cell walls and was more sensitive to cell wall synthesis inhibitors than the control strain. Moreover, the deletion of ags1 did not affect mycelial aggregation, suggesting that basidiomycetes have a specific aggregation factor that differs from ascomycete. Further phenotypes of Δags1 strain are being investigated.

CS2.2.23

INTRODUCED AND NATURAL GENETIC VARIATION ENABLE UNISEXUAL REPRODUCTION IN CRYPTOCOCCUS NEOFORMANS

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Cryptococcus is a global fungal pathogen of humans that comprises a group of closely related species including C. neoformans, C. deneoformans, and C. gattii. While all three species from this complex can undergo canonical bisexual reproduction between cells of opposite mating types, unisexual reproduction (i.e. selfing), where sex can be initiated and completed from a single cell or between cells of the same mating type, has been observed and characterized in C. deneoformans and C. gattii, but not thus far in C. neoformans under laboratory conditions. We recently found that in C. deneoformans, RIC8 (encoding a guanine-exchange factor) is a negative regulator that represses hyphal development and unisexual reproduction. By deleting the RIC8 gene in C. neoformans, we showed for the first time that C. neoformans can also undergo unisexual reproduction and produce basidiospores that are genetically identical to the parent. Based on genetic epistasis analysis, the genes encoding the Galpha subunit Gpa3 or the pheromone receptor Ste3 are not required, whereas the gene encoding the pheromone-activated Ste7 MAP kinase kinase is required for unisexual reproduction in ric8 mutant C. neoformans. F1 progeny from unilateral crosses between ric8 deletion lab strain and wild-type natural isolates exhibit transgressive enhanced self-filamentation and unisexual reproduction, all of which inherited the ric8 mutation from the lab strain, affirming the central role of Ric8 in governing unisexual development and revealing that natural genetic variation can promote selfing. Self-filamentation and unisexual reproduction of the ric8 mutant strains are affected by media supplementation with exogenous cAMP, providing evidence that Ric8 influences selfing at least in part via cAMP-PKA signaling. In summary, our study uncovers a key gene controlling unisexual development in Cryptococcus through highly conserved cellular signaling pathways, with implications for the cell biology, ecology, population genetics, and evolutionary dynamics of Cryptococcus in nature.

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CS2.2.24

RHYTHMIC PATTERNS: EVIDENCES TOWARDS A BI-SINUSOIDAL DYNAMIC OF APICAL SECRETION IN BOTRYTIS CINEREA

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The development and morphology of filamentous fungi rely on the polar delivery of secretory vesicles to growing hyphal apexes. Those vesicles bring lipids required for the extension of the plasma membrane, and parietal enzymes involved in the synthesis of the cell wall. While extensively studied in plant pollen tubes that also exhibit polar growth, the temporal dynamic of vesicles at fungal apexes has been understudied. Here, the chitin synthase CHSIIIa of the phytopathogenic fungus Botrytis cinerea, known to be transported by secretory vesicles, has been fused with eGFP. Confocal microscopy has been used to study the temporal dynamics of labelled vesicles. First, pulses of vesicles were observed in actively growing hyphae that were neither observed in non-growing hyphae nor in differentiated penetrative structures. Second, the kinetic measurement and analysis of the fluorescence signals collected from growing hyphae highlighted a rhythmic behavior characterized by a bi-sinusoidal pattern. To our knowledge, this is the first description of a bi-sinusoidal dynamic of vesicle secretion in filamentous fungi. Together, these results shed new light on the current models of fungal cell elongation.

CS2.2.26

THE C2H2 TRANSCRIPTION FACTOR SLTA IS REQUIRED FOR GERMINATION AND FUNGAL HYPHAL DEVELOPMENT IN ASPERGILLUS FUMIGATUS

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Germination of inhaled Aspergillus fumigatus conidia is essential to establish an infection in the host. Germination of conidia includes breaking of dormancy which is accompanied by an increase of the cellular perimeter dubbed isotropic growth. This swelling phase is followed by polarized growth, which results in the formation of a germ tube. The multinucleate tubular cells start to elongate at the hyphal tip after which lateral branches emerge to form a mycelial network. Transcription factors (TFs) and other genes governing conidial germination remain largely unclear. In this study we identified a novel role for TF SltA in directing normal germination and hyphal development. The ΔsltA conidia started to swell earlier and subsequently switched to polarized growth faster. Additionally, hyphal development was distorted as hyphae were hyper-branching, were wider, and showed splitting of the apical tip. Exposing ΔsltA conidia to antifungal drugs resulted in a growth benefit. A time series transcriptomic analysis was carried out to assess the effect of SltA loss. We identified highly down regulated genes during all analyzed growth phases, of which we obtained five knock out strains (ΔAFUB_035430, ΔAFUB_071880, ΔAFUB_085360, ΔAFUB_099730, ΔAFUB_084520). We monitored the germination showing three of these had rapid germ tube emergence. Different from ΔsltA, these conidia swelled at the same time and at a similar rate as seen in the WT strain, but switched to polarized growth faster. Here we identify the regulatory mode of action of SltA upon polarized growth and characterize several genes involved in this process.

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CS2.2.27

TRANSCRIPTION FACTOR CHIP-SEQ REVEALS REGULATORY NETWORK OF MUSHROOM DEVELOPMENT IN SCHIZOPHYLLUM COMMUNE.

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Basidiomycete fruiting is among the most complex multicellular developmental programs in fungi. Previously, a number of transcription factors have been identified that play an important role in mushroom development in Schizophyllum commune, identified by clear defects in fruiting of deletion strains. These transcription factors include Wc2 and Hom2, which are essential for fruiting. Wc2 is involved in blue-light sensing and Hom2 has been proposed to play a role in CO2 sensing. More recently we reported that Fst1 and Zfc4 are two transcription factors that are required for fruiting body maturation. However, the exact role of these transcription factors has not been identified. We used ChIP-Seq to identify the direct targets of multiple transcription factors that are involved in fruiting body development. With this we can, for the first time, identify the regulatory network of several stages of mushroom development.

CS2.2.28

A GENE INVOLVED IN THE DEVELOPMENT OF ASPERGILLUS FUMIGATUS COULD BE THE MISSING FUNGAL GRANULIN

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The Afu6g07200 gene encodes a hypothetical protein with a granulin domain homologous in its 3D structure to human Granulin A at 95.4% confidence. This growth factor is involved in proliferation and migration and is believed to be extinct in the fungal kingdom. The aim of this study is to determine the role of the Afu6g07200 gene in fungal biology and as a possible growth factor. Using the A. fumigatus ΔakuBku80 (Wt) strain as genetic background, a deletion (Δ72), its complemented, a GFP-tagged, and a truncated strain were generated using a CRISPR-Cas9 technique. The absence of the granulin domain caused a decrease in proliferation, swollen areas along the hyphae and tips, cell vacuolization, and death. In addition, the mitotic rate was mismatched with septation and branching. Septation in the Δ72 strain started earlier than in the Wt, with some septa very close even without nuclei between them, and its hyphae showed more branches. Furthermore, the cell wall of the deletion strain presented poor organization, including a thinner outer layer and wall-to-membrane spaces. These changes could be related to the increase in sensitivity to Calcofluor White and Congo Red. Fluorescence microscopy observations suggest that this protein could be secreted following the secretory pathway since the protein was localized in vesicles along the cytoplasm. This study, on the one hand, shows that the Afu6g07200 gene (that seems to codify for a fungal Granulin) is involved in hyphal polarization and proliferation, cell cycle, septation, colony morphogenesis, and cell wall construction; on the other hand, brings to light the need to study the A. fumigatus hypothetical proteins to discover new genes/proteins important for this pathogenic mold, that could be promising therapeutic targets.

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CS2.2.29

MYCOPARASITES INFECTING MUSHROOMS IN NATURE

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Fungicolous mycoparasites causing distinct diseases in mushroom farms are well investigated due to economic interests. In contrast, mycoparasites in nature tend to be much ignored although they are potential sources for infestations in commercial mushroom cultures. The majority of infestations on fruiting bodies in nature seem to come from the Ascomycota. Certain mycopathogenic species exhibit board host ranges, while others show adaptation and are restricted to an order, family or fungal genus. Mycoparasites can be biotrophs and obtain nutrition from living host cells or necrotrophs that kill the host cells consumed by them. The most destructive necrotrophic mycoparasites on mushrooms of Agaricomycetes belong to certain conidiogenous ascomycete genera which spread rapidly via masses of asexual spores and infect and completely colonize their fungal hosts in a few hours to days. Humid weather in nature with repeated rainfalls at comfortable lower temperatures is of advantage for both, mushroom formation and mushroom infestations. Mycopathogens of e.g. the ascomycetous genera Cladobotryum and Mycogone were variably detected on polypores and agarics. Deformations of primordia and carpophores by cobweb and wet bubble disease were observed on different mushroom species. Mycopathogens tended to grow from the ground over the stipe up to the pores or the lamellae with the hymenia and spores. We harvested infected mushrooms and analyzed the infections on the mushrooms. Strains of the Hypomyces oderatus were isolated from Agaricus macrosporus and identified by conidiophore morphology, sclerotia formation, production of pigment aurofusarin being yellow to red in color dependent on the pH, and ITS sequencing. The strains infested carpophores of different Agaricus species while Pleurotus mushroom caps were resistant. Agaricus and Pleurotus mycelium was also resistant to the strains unlike mycelium of the dung fungus Coprinopsis cinerea.

CS2.2.30

HOMEODOMAIN TRANSCRIPTION FACTORS IN THE WHITE-ROT FUNGUS GANODERMA LUCIDUM

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Ganoderma lucidum is a white rot filamentous fungus that belongs to the Polyporales. Besides a use in traditional Asian medicine, the species has gained enormous interest for applications in the field of sustainable mycelium materials these past years. Homeodomain (Hox) transcription factors play critical roles in the regulation of development and morphogenesis processes and although this importance, not much is known about their functioning and physiological role in G. lucidum. Previously, deletion of Hox transcription factors in the model fungus Schizophyllum commune was shown to lead to an altered vegetative mycelium growth and fruiting body development. In this project, we aim to investigate putative Hox transcription factors in a G. lucidum strain used for myco-material production, using a combined in silico and experimental approach. The genome of this strain was sequenced and bioinformatic analyses enabled to predict 12 genes encoding putative Hox transcription factor-encoding genes. This included homologs of transcription factors that were previously characterized in S. commune, such as Hom2 but also Fst4. A cDNA library was prepared and a selection of 5 genes encoding homeodomain TFs were amplified indicating expression. Followed cloning of the cDNA transcripts in pET24a for heterologous expression of proteins in E. coli. Protein purification was performed using IMAC allowing further biophysical characterization. Additionally, purified proteins were used as antigen for antibody generation. Currently, a chromatin immunoprecipitation protocol is being developed for G. lucidum that enables the mapping of genome-wide binding profile of the transcription factors as a first step towards insights into the regulon and physiological functioning.

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CS2.2.31

ADDING PIECES TO THE PUZZLE OF ASPERGILLUS FUMIGATUS SEXUALITY: TOMA IS REQUIRED FOR CLEISTOTHECIA FORMATION AND THE A-FACTOR-LIKE PHEROMONE EXISTS

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The filamentous ascomycete Aspergillus fumigatus is the major cause of a variety of fungal infections commonly termed aspergillosis. Besides vegetative growth and asexual spore formation, this mold is capable of fruiting body formation accompanied by ascosporogenesis under specific environmental conditions. Knowledge about the sexual phase of its lifestyle and regulatory determinants is, however, incomplete. A. fumigatus sexuality is governed by a bi-polar mating-type system comprising an idiomorphic region that contains one of the master regulator-encoding genes MAT1-1-1 or MAT1-2-1. Transcriptional profiling data from strains overexpressing the mating-type regulators turned out to be a rich source shedding light on a variety of fungal traits, such as secondary metabolism, antimicrobial defense, or cleistothecia formation. For the latter, an annotated gene encoding a conserved hypothetical protein and assigned as tomA (target of MAT1-1-1) was investigated. Transcription of tomA strictly depends on the MAT1-encoded master regulators and putative MAT1-1-1 binding sites are present within the corresponding upstream regulatory region. Preliminary inspection of strains expressing GFP-tagged TomA indicates its nucleolar localization, supporting functionality of a deduced nuclear localization sequence in the tomA coding region. To explore any cellular function of the tomA gene product, deletion strains in either mating-type background were generated to reveal that the absence of TomA in the heterokaryon results in sterility, indicating that tomA is crucial for A. fumigatus sexual reproduction. Moreover, we made use of the MAT1 overexpressing strains to produce A. fumigatus pheromones. Using sensitive yeast strains as chassis to express the respective pheromone receptor, existence of the so far elusive a-factor-like peptide could be demonstrated; current efforts are focused on its identification and functional characterization.

CS5.1 GENOMES AND OTHER -OMICS

CS5.1.10

THE TRANSCRIPTOME OF TUBER BORCHII UNDER DIFFERENT IN VITRO CULTURE CONDITIONS

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Truffles (Tuber spp.) are ascomycete fungi that grow associated with the roots of Pinus, Quercus (holm oaks, oaks, and Kermes oaks), and Corylus (hazel). They are known for their peculiar aromas and flavors, wich are especially appreciated in gastronomy. Their cultivation is slow, and obtaining fruiting bodies can take years. For this reason, there is a great interest in optimizing the culture of mycelium in vitro for biotechnological applications. The most appreciated truffle species are T. melanosporum, T. magnatum, T. aestivum, and T. borchii.
Intending to study the genetic factors responsible for the growth characteristics of Tuber, a strain characterized morphologically as T. borchii obtanied from a fruiting body collected in the community of Castilla y León was isolated and maintained in culture using Potato Dextrose Agar (PDA) at 22±1°C in the dark. The taxonomic classification of the isolate was carried out by studying the ITS (internal transcribed spacer) regions using specific primers for ascomycetes and fungi. The isolated species was classified as T. borchii strain SP1. The transcriptome of the mycelium of this strain, cultivated in submerged fermentation under different conditions, was then studied using the genomes of T. melanosporum and T. borchii available in the Mycocosm database as references. 91% of the genes present in the reference genome are expressed in different media. The analysis of genomic variants verified the novelty of this strain of T. borchii. This comparative transcriptomic study allows us to visualize the transcriptomic profiles of the central metabolic pathways and regulation of gene expression in this species as a preliminary step for the study of the factors involved in symbiotic association and production of secondary metabolites of interest.

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CS5.1.11

EVOLUTION OF ECTOMYCORRHIZAL SYMBIOSIS IN INOCYBACEAE LINEAGE OF FUNGI

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Ectomycorrhizal (ECM) fungi play a key role in recycling of nutrients in the ecosystems by facilitating the nutrient uptake of plants. They have evolved multiple times in several lineages from saprotrophic ancestors. Genomic signatures of ECM include contractions in ancestral carbohydrate degrading proteins, expansion of repeat elements and expansion of mycorrhiza specific small secreted proteins in ectomycorrhizal genomes. Whether these ECM related changes are happening abruptly at the shift to ECM habit from saprotroph or there has been gradual change along the course of evolution in ECM lineages is not well known yet. We sampled 20 species from the ECM lineage Inocybaceae and performed comparative analyses with 7 saprotrophic species, including its sister lineage Crepidotaceae. This is the first extensive genomic study of Inocybaceae and the most densely sampled from a single ECM lineage to date. The results show that most of the previously observed ECM related changes e.g contractions in Carbohydrate Active enZymes (CAZymes), Secondary Metabolites, serine peptidases and sc3 hydrophobins etc and expansions in transposons related genes are happening significantly at a higher rate at the transition point to ECM, though changes in these groups are also happening before and after the transition. Most of the overall gene family contractions in CAZymes happen at the transition point but contractions in peptidases show a slow and general trend of evolution after transition to ECM. This study sheds a new light on the evolutionary events happening around the origin of ECM and this may help in understanding of the evolution of ECM symbiosis better.

CS5.1.12

TRANSCRIPTOMIC LANDSCAPE OF XYLANASE REGULATOR 1 (XYR1) MUTATION IN TRICHODERMA REESEI

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Trichoderma reesei is known for its protein secretion abilities and is one of the most important industrially used filamentous fungi. Xyr1 as the master regulator is responsible for activation of cellulase gene expression, normally during inducing conditions. It has been reported that mutations in certain areas of xyr1 bypass the carbon catabolite repression and cells are able to produce cellulases also when growing on glucose (1). These mutations also change the pattern of produced proteins, shifting it more towards xylanase production, and increase the protein production in inducing conditions.

The aim of the study was to explore the changes in the cells that enable increased protein production both in repressing and inducing conditions and better understand the role of xyr1 in protein production. Xyr1 mutant and strain with a wild type xyr1 were cultivated in 250 ml bioreactors on lactose or glucose using minimal media and multiple parameters were measured online to monitor and control the cultivations. The amount of produced proteins along with differences in the patterns of produced proteins were measured and mRNA-sequencing was conducted to evaluate the changes in gene expression.

Based on the cultivation results, xyr1 mutant strains produced more protein compared to the wild type both with glucose and lactose as a carbon source. On glucose, strains had similar biomass dry weights, whereas on lactose the mutant had higher biomass dry weight. RNA-seq experiment was conducted to achieve a transcriptome-wide image of the effects of the mutation. In cultures with lactose as the carbon source the comparison of the xyr1 mutant and wild type strain revealed a higher number of differentially expressed genes compared to cultures grown on glucose.

(1) Derntl C. et al. (2013) Biotechnology for Biofuels 6:62

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CS5.1.15

THE FUSARIUM OXYSPORUM PANGENOME: MIX AND MATCH OF ACCESSORY CHROMOSOMES

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Fusarium oxysporum (Fo) is a notorious pathogen that causes wilting and rotting symptoms in a large number of host plants. Individual strains often have a narrow host range under greenhouse conditions and are grouped into formae speciales according to their host-preference. Research into the pan-genome of Fo has revealed a wealth of transposon-rich accessory chromosomes and chromosomal regions. Some of these are specific to strains with the same host-preference, and horizontal transfer of these chromosomes to a non-pathogenic strain can result in gain of pathogenicity. To study the role of horizontal transfer in the emergence of new diseases we use comparative genomics to identify signatures of past horizontal transfer events and study crosstalk between core and accessory chromosomes. We find that some formae speciales share the same host-associated accessory material, while others mix-and-match different putative pathogenicity chromosomes.

In addition to comparative genomics of natural isolates we also performed a mutation accumulation study in which we iteratively inoculated tomato-plant with strains that received a chromosome through horizontal transfer in the lab. Nanopore sequencing of the progeny allows us to identify and compare genomic rearrangements and transposon activity after horizontal chromosome transfer.

CS5.1.16

EVOLUTIONARY DYNAMICS OF THE ADAPTIVE GENOME IN FUSARIUM SPECIES AFFECTING GLOBAL BANANA PRODUCTION

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Plant pathogens engage in a constant evolutionary arms race to overcome the host immune system. Many fungal plant pathogens have compartmentalized genomes with conserved core regions and variable adaptative regions, containing effector genes involved in host colonization. The evolvability of adaptive regions enables rapid diversification of effectors supporting host adaptation. The Fusarium oxysporum species complex (FOSC) contains major fungal plant pathogens. FOSC collectively affects many economically important crops while individual isolates are mostly host-specific. The F. oxysporum genome contains adaptive regions, some of which span entire chromosomes. Essential effector genes are located in these regions, and the transfer of an adaptative chromosome can establish pathogenicity in non-pathogenic isolates. Although adaptive regions play important roles in host pathogenicity, how these regions emerge and evolve, and how variable these regions are between host-specific strains remains largely unknown. Here we provide insights into the dynamics that shape the adaptative genome of Fusarium species infecting banana, the most popular fruit and a major staple crop worldwide. We applied pangenome approaches to study 69 banana-infecting Fusarium strains that have been isolated from banana-growing regions worldwide and that differ in pathogenicity towards a panel of banana varieties. Identified adaptative regions range from sub-telomeric areas to multiple complete chromosomes. Notably, extensive variation between adaptive regions of isolates was observed, even when infecting the same banana varieties. On gene level, the genomes vary considerably, especially in the presence and absence of potential effector genes. Interestingly, 25% of all genes located on adaptative regions are duplicated, and we speculate that duplication events play an important role in shaping these regions. Our study provides novel insights into the origin and evolution of the adaptive genome in banana infecting Fusarium species, which might provide a framework to better understand how pathogenicity and host-specificity in this pathogen evolves.

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CS5.1.17

PANGENOMIC ANALYSIS OF ASPERGILLUS FUMIGATUS REVEALS PUTATIVE HETEROKARYON INCOMPATIBILITY LOCI

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Allorecognition plays an important role in preventing the transmission and invasion of parasitic genetic elements, such as transposons. This process is tightly governed by heterokaryon incompatibility loci. Formation of a heterokaryon is clinically and environmentally important as it can increase the genetic diversity of an organism and as a result spread antifungal resistant alleles. Only a few heterokaryon (het) loci have been identified in Aspergillus fumigatus, in comparison over 30 het loci have been characterized in Neurospora crassa. Het loci are distinguishable by domain architecture, e.g. HET, NACHT, WD-domains and Ankyrin repeats, and high nucleotide divergence. Using nucleotide and amino acid divergence metrics, analysis of conserved gene neighbourhoods and variant calling, we have been able to scan the results from a previously published pangenome to identify several putative het loci, uncovering novel forms of allelic difference in Aspergillus fumigatus.

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CS5.1.18

LONG-DISTANCE MIGRATION OF THE MAIZE ANTHRACNOSE PATHOGEN ALLOWS GENETIC RECOMBINATION BETWEEN ISOLATED LINEAGES

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The fungus Colletotrichum graminicola, causal agent of maize anthracnose, is an important plant pathogen worldwide. Understanding mechanisms underlying genetic variation in emerging fungal diseases is crucial to the development of effective control strategies. The population structure of a plant pathogen is the result of evolutionary interactions with hosts and local environments, migration at different scales, as well as human-mediated long-distance dispersal events. The reproductive biology of plant pathogens also has marked effects on the population structure. Fungal reproductive biology is conducive to genetic exchange, and genetic recombination (i.e., genetic exchanges within a lineage) could result from both sexual reproduction or parasexual events via hyphal anastomosis. In addition, genetic introgression (genetic exchanges between lineages) could be important in the context of adaptive evolution since admixture could promote adaptation by rapidly creating novel allelic combinations. We employed a population genomics approach to investigate the genetic diversity and reproductive biology of C. graminicola isolates infecting maize. We sequenced 197 isolates of C. graminicola collected in 14 countries using restriction site-associated DNA sequencing (RAD-Seq) and whole-genome sequencing (WGS). Clustering analyses based on single-nucleotide polymorphisms showed populational differentiation at a global scale, with three genetic groups delimited by continental origin (South America, Europe, and North America). Intra and inter-continental migration were predicted between Europe and South America, likely associated with the movement of contaminated maize germplasm were discovered. Our population genomics data reveal signatures of genetic exchanges. Investigation of the recombination history reveals the

presence of recombinant blocks within and between lineages, which differ largely in age (in terms of generation numbers), suggesting relatively recent hybridization events. The long-distance migration of the pathogen, likely mediated by human activities (global trade of infected plant material, and/or seeds), can allow hybridization between previously isolates lineages.

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CS5.1.19

COMPARATIVE GENOMICS OF PUBLIC SEQUENCES ILLUMINATE THE SIGNATURES OF RECENT SELECTION AND DRUG RESISTANCE IN CANDIDA PATHOGENS

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Infections caused by Candida species are a major threat for immunocompromised patients. Understanding how recent selection shaped virulence and drug resistance within each species is key to improve current diagnostic and therapeutic options. Recent studies have clarified some aspects about such recent evolution, including the importance of genomic plasticity and the drivers of in vitro-evolved drug resistance. However, the genome-wide signatures of recent selection in clinical isolates, which underlie host adaptation and drug resistance, remain elusive. In addition, the similarities in recent selection between species are unclear (since most studies are species-specific), which is a key question to develop personalized therapies.
To address these gaps we generated and analyzed variant calls (small, copy-number and structural variants) for a collection of ~2,000 (mostly) clinical isolates with available genomes from six major Candida species (glabrata, auris, albicans, tropicalis, parapsilosis and orthopsilosis). We find hundreds of genes with signs of selection (with an excess of recent, functional variants), suggesting the role of many cellular functions in clinical adaptation. Most of the related pathways are species-specific, but some processes (such as cell adhesion or pseudohyphal growth) are convergently affected in several organisms. These common functions may be at the core of recent adaptation. To find the drivers of clinical drug resistance we used our collection to do a Genome Wide Association Study (GWAS) on eight antifungals (azoles, echinocandins and polyenes) in C. albicans, C. glabrata and C. auris. We find significant associations around the expected FKS, ERG11 and PDR1 genes, but also on hundreds of novel genes and pathways. Some of these novel players are significant across multiple species and drugs, underscoring their importance. In summary, our work improves our understanding about recent, clinically-relevant, selection in Candida pathogens.

CS5.1.20

JLOH: EXTRACTING LOSS OF HETEROZYGOSITY BLOCKS FROM SHORT-READ SEQUENCING DATA

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Genome evolution occurs through the accumulation of genetic changes that range from single-nucleotide polymorphisms (SNPs) to large structural variations (SVs) and copy-number variations (CNVs). In heterozygous genomes, loss of heterozygosity (LOH) through chromosomal recombination is an important factor that is often neglected due to a lack of appropriate tools. Heterozygous sites are a platform for natural selection, especially when the organism is under stress, as they offer two alleles to select upon rather than one. In hybrid organisms heterozygosity is high, as their subgenomes derive from separate species. LOH happens when a heterozygous site (or haplotype) loses one of the alleles in favour of the other. It has been shown experimentally that LOH is capable of driving adaptation in hybrid yeasts from the Saccharomyces and the Candida genera. Its impact on genome evolution in other phylogenetic groups is also being studied. However, despite the research interest, the tools to extract LOH are very limited. Hence, we developed JLOH, a toolkit to extract, filter, and analyse LOH blocks. JLOH can work with normal and hybrid genomes, and is capable of extracting LOH blocks starting from short sequencing reads and a reference genome. Blocks are characterized by the lack of heterozygous SNPs and, often, by a different coverage profile than the rest of the genome. When analysing hybrids, JLOH identifies the LOH blocks in both parental reference genomes, allowing the assignment of each LOH block to either parent. The results produced by JLOH can be easily manipulated to be used in common pipelines. We tested JLOH in five wild strains from a S. cerevisiae x S. paradoxus hybrid. We could observe extensive LOH over multiple chromosomes, and one potential case of mislabelling. All in all, we believe that JLOH will substantially facilitate the study of LOH in genomics.

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CS5.1.21

TRANSPOSON ACTIVITY SHAPES SHORT-TERM EVOLUTION OF A FUNGAL PATHOGEN

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Structural variation is a common source of intraspecific genetic variation. Human diseases, crop improvement traits and pesticide resistance have all been associated with such structural rearrangements. Understanding the mechanisms promoting genomic instability is therefore essential for the study of rapid evolution. In this work, we show that transposable elements are major drivers of the pangenome architecture of the fungal wheat pathogen Zymoseptoria tritici. We find that specific transposon families and chromosomal sequence characteristics are tightly correlated with the occurrence of specific genomic rearrangements. Using machine-learning, we were able to predict the emergence of spontaneous insertion-deletion variants produced during meiosis in a four-generations pedigree. Some of the most expansive structural variants generated in the pedigree were tied to a single highly active transposon. Retracing the activity of the element in the Zymoseptoria genus, we identified multiple independent reactivation events generating a complex set of transposon copies. Within the focal species Z. tritici, we identified recent reactivation of the transposon with a geographically localized rapid expansion in copy numbers. Our results retrace the recent origin of a transposon that successfully evaded host control to promote major structural rearrangements within a species.

CS5.1.22

A NEW CHROMOSOME IS DESCRIBED IN FUNGAL SPECIES INFECTING WILD GRASSES

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Fungal plant pathogens have dynamic genomic architectures that can contribute to rapid evolution and adaptation to new niches. Zymoseptoria tritici, an important fungal pathogen of wheat, has a diverse, highly adaptive and compartmentalized genome. In the reference Z. tritici isolate IPO323, 8 of the 21 chromosomes are accessory, comprising around 12% of its haploid genome. In spite of the profound impact on genome organization, the origin of accessory chromosomes in Z. tritici is still poorly understood. We explored genomic variation in new populations of Z. tritici infecting wild wheat species from the genus Aegilops. Using a multi-omics approach (genomics, transcriptomics and epigenomics), we discovered a new chromosome in these Z. tritici isolates. The newly identified chromosome present similar characteristics to known accessory chromosomes in Zymoseptoria species, including presence-absence variation among Aegilops-infecting isolates, low gene expression in vitro and in planta, and enrichment with heterochromatic histone methylation marks (H3K27me3). Interestingly, we found an orthologous chromosome sharing almost 50% of the genes in Zymoseptoria ardabiliae, a closely related fungal species also infecting wild grasses. This ortholog chromosome also presents accessory chromosomes characteristics, but lacks the enrichment of heterochomatin methylation marks. Transcriptomic analyses revealed that the orthologous chromosome in Z. ardabiliae presents a high expression of transposable elements (TE) coupled with lower signatures of host-genome defense mechanisms against TE expansion and spread (RIP), indicating that this chromosome is a reservoir of active transposons. We suggest that the chromosome has been exchanged between Z. tritici and Z. ardabiliae by introgressive hybridization events and propose that hybridization may play an important role in the evolution of new accessory chromosomes.

CS5.1.23

GENOME ARCHITECTURE AND STRUCTURAL VARIATION SURROUNDING EFFECTOR LOCI IN CERCOSPORA JANSEANA

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Cercospora janseana is a hemibiotrophic fungal pathogen of rice and causes the emerging disease narrow brown leaf spot. The effector repertoire of this pathogen is completely uncharacterized and this system lacks any substantial genomics resources which hinders efforts towards functional validation of genes involved in host-pathogen interactions. To overcome these obstacles, we generated the foundational genomic resources to characterize the diversity and evolution of effectors in C. janseana. Nanopore sequencing enabled the assembly of a nearly complete telomere-to-telomere reference genome consisting of 15 chromosomes. RNAseq-mediated gene annotation identified 10,833 genes, including 370 predicted effectors. Genes encoding predicted effectors are localized significantly closer to transposable elements (TEs) and significantly more distant from neighboring genes. Microsynteny with other agronomically important Cercospora spp. was evaluated to further characterize the genome architecture of effector loci. Synteny was highly conserved surrounding the cercosporin biosynthetic gene cluster, but was disrupted by a proliferation of transposable elements at various species-specific effector loci. Effector diversity was further explored by sequencing a natural population of 130 C. janseana isolates collected from Louisiana and Texas. A total of 747,119 polymorphisms were identified, among which were 113,472 nonsynonymous single nucleotide polymorphisms. Substantial nucleotide diversity within the coding region of effectors, as well as presence/absence variation, was found and observed to vary among populations. Coverage-based analyses and comparison to a Nanopore assembly of C. janseana isolate RL485 identified large segmental duplications and deletions involving predicted effector gene loci. These results shed light on the effector and genome dynamics of this important emerging rice pathogen and will facilitate future work on their functional validation.

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CS5.1.24

GENOMICS ANALYSIS OF THE FRESHWATER FUNGUS FILOSPORELLA FISTUCELLA (HELOTIALES) INDICATES POTENTIAL FOR PLANT LITTER DEGRADATION IN COLD TEMPERATURES

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Freshwater fungi are key players in the decomposition of organic matter such as leaf litter in streams. Furthermore, they are equipped with strategies to survive colder temperatures. This includes the production of antifreeze proteins (AFPs) and cold-active enzymes. The Filosporella fistucella genomic DNA was extracted from 14 grams of cell biomass after cultivation in Potato Dextrose liquid medium and sent to Macrogen, Netherlands for Hifi sequencing. The genome assembly was performed with Flye v2.9. and was compared whit the phylogenetic close organism Tricladium varicosporioides (syn. Hymenoscyphus varicosporioides; GenBank accession: GCA_021365295.1). The gene prediction was performed using the ab initio tool Funannotate v1.8.9 and annotated at the dbcan2 online database using the hmmer tool to identify Carbohydrate-Active enzymes (Cazy). The results were filtered for enzymes associated with leaf litter degradation (eight in total). The CryoProtect web tool was used to predict if the identified plant-litter degradation sequences could have antifreeze properties. The F. fistucella genome assembly (45.7 Mbp) has a smaller size than T. varicosporioides (48.2 Mbp). The Ab initio gene prediction identified more protein sequences in F. fistucella (13.507) than in T. varicosporioides (12.628). We also identified more sequences associated with plant-litter degradation in F. fistucella than in T. varicosporioides, 855 and 719 proteins, respectively. The F. fistucella also shows more AFPs than T. varicosporioides, 75 and 66, respectively. The F. fistucella has been shown to have a more complex enzymatic system to act in plant and wood cell wall degradation, as well as sugar molecules. Our results suggest that F. fistucella has a higher potential for growth in the water, more adaptation to freshwater environments, and higher resistance to low temperatures than T. varicosporioides.

CS5.1.25

GENETIC DIVERSITY AND EVOLUTION OF RHIZOCTONIA SOLANI AG-1 ISOLATES ASSOCIATED WITH DIFFERENT PLANT HOSTS

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Rhizoctonia solani is a species complex classified into anastomosis groups (AGs) based on hyphal fusion. AG-1 is an important group divided into further intraspecific groups based on differences in host range and molecular diversity including AG1-IA, AG1-IB, AG1-IC, AG1-ID, AG1-IE and AG1-IF. Although all subgroups are phytopathogenic, Rhizoctonia solani AG1-IA is one of the most relevant subgroups for being the causal agent of sheath blight in rice and aerial blight in soybean, two devastating diseases in economically important crops. In some areas, these crops are often used in rotation and increase inoculum in the field each season. Currently, there is limited resistance to sheath blight in rice and no soybean cultivars known to be resistant to aerial blight. Monitoring genetic variability and addressing host-pathogen dynamics in R. solani AG-1 is necessary to develop new resistant cultivars. We sequenced 119 R. solani isolates representing five intraspecific groups of AG-1 obtained from different hosts and regions over multiple years and determined the genetic diversity of this group based on high-quality single nucleotide polymorphisms (SNPs) and their phylogenetic relationships using the ITS gene. Illumina reads were mapped to the genome of R. solani isolate B2 (AG1-IA); SNPs were identified and genotyped in AG1-IA isolates to infer genomic differences among this subgroup based on a genetic distance matrix. The low sequence homology between subgroups further confirms the genetic divergence and evolutionary distances between AG-1 intraspecific groups. The results will help improve our understanding of the genome-wide genetic variability of R. solani AG-1 isolates and their associations with different hosts and allowed us to establish a better understanding of the diversity of this group both at the inter- and intraspecific level.

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CS5.1.26

PHYLOGENOMICS OF ADMIXED SACCHAROMYCES CEREVISIAE STRAINS AND METAGENOME-DERIVED SAMPLES WITH SHORT- AND LONG-READ SEQUENCING

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The history of the yeast S. cerevisiae is deeply interwoven with that of human migrations, developments in agriculture and food technology, and trade. Its use in fermentation and the consequent domestication process had made it one of the most important and beneficial microorganisms. The species is a minor and probably transient member of the human microbiome, while it may also cause opportunistic infections.
An ever-increasing number of whole S. cerevisiae genomes have been sequenced and analyzed. Regarding the species' phylogeny, there is a consensus about the main clades, but the high proportion of mosaic genomes poses a significant methodological challenge.
In this work, we aimed to test and optimize methods for the phylogenomic evaluation of polyploid admixed yeasts. We sequenced and assembled local baker's, wine, and probiotic yeast isolates with high coverage Illumina and ONT technology and compared two reference-based strategies, namely mapping to the species' S288C reference genome and mapping to multiple de novo assembled references. Subsequent allele calling and filtering practices were compared for these. Maximum Likelihood-based and phylogenomic network methods were tested on the final dataset, and whole-genome phylogenies were compared with results obtained for individual chromosomes or chromosomal regions. The phylogenies created involved hundreds of strains representing all known clades, along with several yeast genomes recovered from metagenomic samples from human stool.
We show that in the case of admixed and highly heterozygous, polyploid genomes, specifying chromosome-region copy number upon calling and the correct choice of representing phylogenomic relationships are especially important. We found the SNP-network-based representation of smaller chromosome regions to be the most informative in understanding the origins of such yeasts that are common in domesticated environments and/or reside in the human gastrointestinal tract.

CS5.1.27

PROVIDING HUMAN AND MACHINE READABLE FAIR DATA RESOURCES TO EXPLORE AND INTERCOMPARE DIVERSE PLANT, ANIMAL AND HUMAN INFECTING PATHOGENS

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Complex phenotypes, such as virulence, pathogenicity and resistance are important observable traits controlled by the genomes of interacting organisms and individual virulence/avirulence genes. PHI-base, www.phi-base.org, is a gold-standard manually curated phenotype database storing molecular information on genes implicated in virulence (1) and aims to help researchers to uncover new fundamental insights and to find novel intervention targets for disease control. Our Nov 2022 release provides information on 283 pathogens tested on 234 hosts, covering 19,881 interactions and 8,993 genes curated from >4,800 peer reviewed articles. PHI-base also provides information on the target sites of commercial and experimental anti-infective chemistries and the first host targets of pathogen effector genes. PHI-base's mission is to be the primary information source for researchers studying plant, animal, and/or human pathogens, and to make pathogen phenotype data computable. Species neutral high-level phenotypes are implemented to allow comparative plant-pathogen analysis using our newly developed Pathogen-Host Interaction Phenotype Ontology (PHIPO) registered at the OBO Foundry. In addition, we recently developed a web-based community annotation tool, called PHI-Canto, canto.phi-base.org, to permit authors to capture wild-type and mutant phenotype data once their original research articles are published through peer review. This new curation tool will be rolled out for global community use in 2022/23. PHI-base phenotype data and ontology terms are exported to Ensembl, NCBI, UniProtKB, FungiDB and the KnetMiner knowledge graphs. This allows linking of phenotypes to genomes and evolutionary trees, and enables enhanced computational analysis utilising variant and transcriptomic data, comparative genomics, gene network analysis and mapping to biochemical pathways. A new gene-centric version of PHI-base, which hosts all published data for each gene on a single page, will be described as well as how the yearly rollout of the new author curation tool for community use will be implemented.

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CS5.1.28

A GENOME-BASED STUDY ON THE BIOSYNTHETIC GENE CLUSTERS OF SECONDARY METABOLITES IN PENICILLIUM SPECIES OF SECTION RAMOSUM

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Within Penicillium subgenus Penicillium, the section Ramosum consists of 14 species clustered to 5 series. However, the biosynthetic gene clusters (BGCs) of secondary metabolites (SMs) within this section are largely unexplored. Here, we performed a study of SM BGCs in genomes of P. simile and P. raistrickii of series Raistrickiorum, P. swiecickii of series Lanosa and, for comparative analysis, Penicillium species of other sections. A total of 42 SM BGCs were identified in the genome (29.8 Mb, GC 48%) of P. simile, 52 in P. raistrickii (31.4 Mb, GC 46.62%) and 66 in P. swiecickii (34.1 Mb, GC 46.62%), with core enzymes mainly belonging to PKS, NRPS, PKS-NRPS and terpenes. Some BGCs were found in all Penicillium genomes and are most likely related to the production of non-specific SMs. For example, the BGCs for nidulanin A (NRPS) and naphthopyrone (T1PKS), which were widely conserved within the section Ramosum and followed the Penicillium phylogeny. However, even the closely related P. simile and P. raistrickii shared few SM BGCs. Among these clusters, the BGC (NRPS) for asperphenamate. The analysis of the homologous BGC from P. brevicompactum, Penicillium sp. MUT6482 of section Brevicompacta and P. arizonense, P. antarcticum of section Canescentia indicated an evolutionary scenario of the cluster largely congruent with the phylogeny of these sections and a loss-based distribution pattern (e.g., P. swiecickii). Interestingly, some SM BGCs most likely have not co-evolved with a given Penicillium species and were possibly acquired by horizontal gene transfer (HGT). For example, in P. swiecickii a relatively recent HGT was predicted for the mpa cluster (probably from P. roqueforti, section Roquefortorum) that is responsible for the production of mycophenolic acid, which may be relevant in competing environments. Finally, some cluster-specific transcription factors and global regulatory genes were identified, as a basis for future transcriptome analysis.

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CS5.1.29

GENOMICS OF ASPERGILLUS SP. SPH2, AN ENDOPHYTE ISOLATED FROM THE CANARY ISLANDS ENDEMISM BETHENCOURTIA PALMENSIS

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Some endophytic fungi (eg. arbuscular mycorrhiza) have long been known to play important roles in plant nutrition. Lately, the ability of many endophytic fungi to synthesize bioactive secondary metabolites is gaining attention, in view of their biocontrol potential. Bethencourtia palmensis (Nees) Choisy is an endemic plant from the Tenerife and La Palma islands that has been extensively studied for its ability to produce bioactive metabolites. Our group has isolated a number of endophytic fungi from B. palmensis plants and studied their ability to produce bioactive metabolites. One such isolate, SPH2, has been found to secrete compounds with strong fungicidal and ixodicidal effects [1]. Characterization of SPH2 suggested that it is an Aspergillus sp. within the Circumdati section, and closely related to the saprophytic, ochratoxin A (OTA) producing species A. ochraceous and, especially, A. westerdijkiae. However, no OTA was detected in SPH2 extracts [1]. The genome of SPH2 was sequenced and assembled with the Oxford Nanopore technology and MinION instruments, and the base calling accuracy was improved by using Illumina NextSeq reads. Genomic analyses showed that SPH2 is a member of a new Aspergillus Circumdati genomospecies. Gene clusters potentially involved in the production of the previously isolated bioactive metabolites were identified. A complete, highly conserved OTA-encoding cluster was also identified, even though SPH2 does not produce OTA. Given that the food toxin OTA can have deleterious effects on the plant host, we hypothesize that B. palmensis has selected an endophytic variant that no longer produces OTA. We are investigating the molecular bases for this selection and trying to identify new SPH2-like isolates in order to properly define the new species.

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[1] Morales-Sánchez et al. (2021). Bioactive Metabolites from the Endophytic Fungus Aspergillus sp. SPH2. J. Fungi 7(2), 109. https://doi.org/10.3390/jof7020109

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DIVERGENT CARBOHYDRATE-ACTIVE ENZYME (CAZYME) GENE EXPRESSION PATTERNS RELATED TO PLANT BIOMASS DEGRADATION IN SIX EVOLUTIONARILY DIVERSE FUNGI

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Plant biomass is one of the most abundant renewable carbon sources, which holds great potential for replacing current fossil-based production of fuels and chemicals. In nature, fungi can efficiently degrade plant polysaccharides through secreting a broad range of carbohydrate-active enzymes (CAZymes), such as cellulases, hemicellulases and pectinases. Due to the crucial role of plant biomass degrading (PBD) CAZymes in fungal growth and various biotechnologies applications, investigation of their genomic diversity and transcriptional dynamics has raised increasing attention.
In this project, we compared the CAZome content related to plant biomass degradation of six taxonomic distant species: Aspergillus niger, Aspergillus nidulans, Penicillium subrubescens, Trichoderma reesei, Phanerochaete chrysosporium, and Dichomitus squalens. We also determined their transcriptomic profiles during growth on nine monosaccharides. A considerable genomic variation and remarkable transcriptome changes of CAZyme-encoding genes were identified, which are suggested to be linked with their preferred carbon source and different transcriptional regulation. In addition, fungal growth profiling on different sugars only partially correlated with the corresponding carbon utilization ability inferred from genomics and transcriptome profiles. Our study provides new insights for a better understanding of fungal adaptation to different habitats and for developing new strategies for related industrial applications.

CS5.1.31

UNRAVELING THE REGULATORY NETWORK MEDIATED BY THE TRANSCRIPTION FACTOR STUA IN TRICHOPHYTON RUBRUM: FROM METABOLISM REPROGRAMMING TO PATHOGENICITY REGULATION

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Trichophyton rubrum is a fungal species of worldwide concern because of its ability to cause infections in keratinized tissues. This condition, known as dermatophytosis, is often persistent and hard to treat, affecting skin, hair, and nails. The severity related to dermatophytosis concerns immunocompromised individuals more dramatically. However, drug resistance has been reported worldwide in isolated strains from immunocompetent people. This fungus' virulent character relies on many mechanisms that depend on the balance between signaling processes and transcriptional regulation. Transcription factors (TFs) play a pivotal role in controlling the levels of target transcripts in cells. They are embedded into complex regulatory networks that mutually interact, promoting the coordination of processes such as RNA transcription, alternative splicing, and metabolism control. In previous RNA-sequencing (RNA-seq) analysis, we addressed the role of the TF StuA (a member of the fungal-conserved APSES family) in T. rubrum. As a follow-up study, our research was carried out through a complete mapping of StuA-target TFs, as they highlight the role of StuA in directly or indirectly modulating the transcript levels of other TFs, playing a regulation that could elicit significant responses in T. rubrum. In silico research showed that the TFs-StuA targets are somehow responsible for the transcription of genes related to nutrient assimilation, physiological responses, and pH maintenance. The differential expression of relevant TFs validated by RT-qPCR using a ΔstuA strain showed that the target genes' positive or negative regulation corroborated previous RNA-seq findings. We also observed that StuA could mediate a direct regulation over the promoter regions of TF genes, as we found putative StuA-binding sites in their 5' untranslated regions. Our results showed that the StuA-mediated regulatory network is involved in distinct biological processes in T. rubrum. Remarkably, our study shed light on the search for possible new antifungal therapies for controlling dermatophytosis worldwide.

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CS5.1.32

COMPARATIVE GENOMICS AS THE FIRST STEP TOWARDS DECIPHERING MULTIPLE ASPECTS OF ENDOPHYTISM EXHIBITED BY ENTOMOPATHOGENIC FUNGI

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Entomopathogenic fungi (EPF) are widely known for their use as biological control agents against a wide range of pests. Alongside this function, they possess the ability to affect plant growth and confer plant protection characteristics as both endophytes and rhizosphere colonisers, rendering them promising candidates for the development of a whole range of novel biocontrol products and biofertilizers. This, however, is hindered by limited knowledge related to the mechanisms behind these abilities and the ways that these fungi evolved for this dual mode of living. In this work, an attempt is made to highlight the significance of the deployment of genomics in revealing the underlying molecular mechanisms of endophytism by entomopathogenic fungi.
The whole genome sequencing of Metarhizium brunneum strains ARSEF 4556 and V275, alongside Beauveria bassiana ATHUM 4946, confirmed that entomopathogenic fungal genomes are versatile in respect to their gene content and structure. Comparative genomic analyses revealed that all strains employ a broad metabolic repertoire, with a high capacity for multiple modes of life. Furthermore, they were found to share orthologue genes with other endophytic and entomopathogenic strains, as well as several singleton genes. Synteny analysis revealed major syntenic clusters among other endophytic and entomopathogenic strains. Phylogenetic trees constructed by using these genes as matrices reveal taxonomic relationships among these fungi.
Therefore, insights acquired via this comparative genomic approach offer the needed knowledge of the molecular mechanisms and the metabolic pathways implicated in endophytism and insect pathogenicity by EPF. It is the cornerstone for other omic approaches needed to pave the way for the commercialization of these fungi as safe biofertilizers, through their endophytic mode of life.

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TRANSCRIPTOME ANALYSIS OF NEOFUSICOCCUM LUTEUM DURING AVOCADO BRANCH AND FRUIT INFECTION

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The most important aerial diseases affecting avocado orchards on the Andalusian coast (Spain) are caused by species of fungi belonging to the Botryosphaeriaceae family, one of the most common being Neofusicoccum luteum. The symptoms produced by this fungus are branch dieback and fruit rot. Currently, it is essential to develop strategies to control this disease. In this line, knowledge of the infection mechanisms of the pathogen is considered an essential objective. Therefore, a transcriptomic study was carried out by RNAseq of N. luteum growing on avocado branch and fruit in comparison with its in vitro growth in PDA medium. Transcriptome analysis revealed a total of 903 and 1271 genes significantly downregulated (- 2 > fold change > 2) during growth on branch and fruit compared to growth on PDA, respectively. Among the genes overexpressed in the N. luteum/branch/fruit interaction, genes related to mycotoxin production, wall degradation, detoxification of harmful compounds, protein degradation and candidate effector proteins were identified, three of which showed 100% probability (Effector P3) and apoplastic localization. The analysis of N. luteum transcriptome during the infection process will be a very useful tool to better understand the biology and virulence of this emerging pathogen and will help in the development of strategies for its control

CS5.1.34

A GENOMIC PERSPECTIVE ON FILAMENTOUS FUNGAL HUMAN PATHOGENS: THE SCEDOSPORIUM CASE.

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Fungi are mostly investigated for their impact on agriculture, food and technology, however, these organisms are neglected, compared with bacteria, viruses and other eukaryotes. Moreover, new fungal pathogens are expected to emerge with climate change and environmental pollution.
Most human fungal pathogens are opportunistic: their ecological success is not dependent on host infection and their growth in the human body is occasional. However, the connection between extremophilic and pathogenic lifestyles is largely unexplored. Here, we focused on the genus Scedosporium (Microascaceae), a group of life-threatening fungi with outstanding degradative skills. Since the genomic data is fragmentary for the genus, we sequenced the genomes of S. aurantiacum MUT6114 and S. minutisporum MUT6113, isolated from tannery wastewater and a Polyciclic Aromatic Hydrocarbons (PAH)-contaminated soil, respectively. We characterized the publicly available Microascaceae genomes, most of which lacked annotation, and provided the first comparative framework for this family. By comparing Microascaceae with other pathogenic and non-pathogenic ascomycetes, we found that quantitative traits are not sufficient to outline a common pathogenic genomic asset and that, from a phylogenomics perspective, there is a lack of convergence of putative pathogenicity traits. We investigated the role of point mutations and transposons insertions in pathogenicity and resistance to drugs, and found that these roles might have both ancient and recent roots. We also used RNA-seq to describe the response of the two isolates to the antifungal voriconazole, confirming the involvement of previously identified genomic traits. Our results highlight the strengths and limitations of genomics applied to opportunistic fungal human pathogens and the multifactoriality of pathogenicity and resistance to drugs. Finally, we discuss an evolutionary scenario where pressures other than anthropic ones shaped pathogenic lifestyle in filamentous fungi.

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EYE SEE YOU: GENOMIC AND TRANSCRIPTOMIC FEATURES OF FUSARIUM SOLANI KERATITIS ISOLATES

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The Fusarium solani species complex (FSSC) are important and ubiquitous plant pathogens. They are also increasingly recognized as causes of human disease, particularly causing eye infections or keratitis that are difficult to treat and can lead to blindness or require enuculation. To understand the features in this species complex that underpin their success as both plant and human pathogens, we generated highly contiguous genome assemblies of three isolates of Fusarium keratoplasticum and three isolates of Fusarium petrophilum from keratitis patients in Germany. When analyzed jointly with two additional genomes from aquatic environments, the F. keratoplasticum genomes had a mean length of 50.7 Mb (range 49.5-53.5 Mb) with an average of 6.5% repeat content and 15,364 genes (range 14,050-15,777). F. keratoplasticum showed a pronounced level of lineage specific genes and only 81% of the total genes detected were shared between five genomes. The F. petrophilum genomes had a mean length of 49.3 Mb (range 47.9-51.2 Mb) with an average of 5.3% repetitive elements and predicted 15,313 genes (range 15,041-15,570). Compared to F. keratoplasticum, the genomes of F. petrophilum showed fewer lineage-specific genes and 92% of genes were shared between all genomes examined. To identify shared and distinct responses between isolates and species, RNAseq was performed on conidia and mycelia under variable nutritional conditions, including carbon and nitrogen starvation. Altogether this work builds the tools needed to understand the virulence potential of the Fusarium solani species complex and the features underpinning their success as pathogens of both plants and humans.

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CS5.1.36

IMPROVED GENOME ANNOTATION OF THE FUNGAL WHEAT PATHOGEN ZYMOSEPTORIA TRITICI USING ISO-SEQ AND RNA-SEQ DATA

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The genome of the fungal wheat pathogen Zymoseptoria tritici IPO323 strain was sequenced and annotated in 2011. Since, additional IPO323 genome annotations were released using different ab initio software and RNA-Seq evidences. These annotations displayed many discrepancies, and only a few CDS have identical structures (n: 3918, 30%). Iso-Seq long-read sequencing delivers full-length transcripts, facilitating gene model prediction. Iso-Seq transcriptomic data, corresponding to 11 biological conditions, were obtained for IPO323. This dataset was used with other evidence (RNA-Seq data and fungal protein sequences from public databases) to generate new ab initio annotations of IPO323 genome sequence. They were compared to previous annotations to select the best gene models according to transcriptomic and protein evidence using ingeannot, a suite of bioinformatics tools for the annotation, selection, and validation of gene models, and their comparison. The new annotation corrected many errors (2047) found in previous annotations (CDS fusions, false introns, and missing exons), and added 671 new genes, leading to 13,414 Re-annotated Gene Models (RGMs). Iso-Seq and RNA-Seq data were used to define 5' and 3'UTRs for 73% of the genes. 13% of RGMs displayed alternative transcripts, mostly corresponding to intron retention (75%). However, 353 genes displayed alternative transcripts with new combination of existing exons or new exons. Long non-coding transcripts (51 lncRNAs) were also identified, as well as DsRNA from two fungal viruses. Most lncRNAs corresponded to antisense transcripts of genes (52%). lncRNAs up- or down-regulated during infection (17) were enriched in antisens transcripts (70%) suggesting their involvement in the control of gene expression during infection. Overall, Iso-seq data were very effective for the improvement of Z. tritici genome annotation. It also provides new insights in its transcriptional landscape.

CS5.1.37

COMPLETE GENOME OF THE FUNGICOLOUS SPECIES CLADOBOTRYUM MYCOPHILUM ATHUM6906, A DAMAGING SPECIES TO THE EDIBLE MUSHROOM AGARICUS BISPORUS

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Fungicolous species which belong to the order Hypocreales have been verified as the main reason for the destruction of edible mushroom cultivations of the species Agaricus bisporus. Cobweb disease, caused by Cladobotryum spp., reduces the quantity and quality of the produced mushrooms which are aimed to reach the market, resulting to a great damage in the production. Genome analyses of these important mycophilic species will set the background to decipher the interactions between Cladobotryum spp. and their mushroom host. In this study, the complete genome of the Greek strain Cladobotryum mycophilum ATHUM 6906 is presented. High-quality DNA sequencing was performed in-house by MinION sequencer (Oxford Nanopore Technologies). The raw sequences were assembled to obtain a 40.67Mb genome. It was organized into 16 contigs (N50 was 5Mb) including the 76.5 kb mitochondrial genome and one 11.7 kb mitochondrial circular plasmid. Telomeric sequences were found in several contigs and thus, chromosomes were determined. 273 tRNA genes, 56 rRNA genes and 12,282 protein coding genes were identified. The structural organization and functional annotation of the protein coding genes were achieved using BlastP against SwissProt, RCSB Protein Data Bank (PDB), Cluster of Orthologous Groups (COG), Pathogen-Host Interaction-PHI (PHI) and CAZyme databases. BUSCO analysis was additionally performed. Genes which derived from Horizontal Gene Transfer (HGT) events were also identified. 106 Biosynthetic Gene Clusters (BGC) for the biosynthesis of plethora of secondary metabolites were found, distributed in most contigs. Comparative genomic analyses with other mycophilic species whose genomes are publicly available, were also performed. This is the first report on the complete genome of Cladobotryum mycophilum providing genomic data for the study of the mycophilic mode of life.

CS5.1.38

VIRUS AND VIRUS-LIKE ELEMENTS IN A COLLECTION OF TRICHODERMA ISOLATES FROM NATURAL ENVIRONMENTS IN SARDINIA

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Trichoderma species are free-living soilborne fungi playing a crucial role in disease suppression. A collection of Trichoderma isolates from Sardinia (a biodiversity hotspot) had been previously characterized genetically and morphologically, suggesting that the population of Trichoderma from Sardinia is not significantly different from those commonly found in Eurasia. Here we started a characterization of the viral and viroid-like components associated to 110 selected Trichoderma isolates, representatives of twelve species. We carried out NGS sequencing of total RNA after ribosome depletion following a bioinformatic pipeline that detects virus RNA-dependent RNA polymerases (RdRP) and other conserved virus protein sequences, and a specific pipeline developed to detect ORFAN sequences. Each virus, firstly characterized in silico, is then associated to a specific fungal isolate through qRT-PCR and RT-PCR. The viral pipeline detected 16 viral RdRPs. Two of them correspond to virus isolates already detected in other regions of the world. The remaining 14 represent isolates of new virus species: surprisingly, 8 of them are from new negative stranded RNA viruses, which for the first time are reported in the genus Trichoderma. Among them a cogu-like virus, very closely related to plant-infecting viruses, is being further characterized in its putative movement protein. Among the positive strand viruses, it is noticeable the presence of an ormycovirus, a recently characterized group of bi-segmented ss-

RNA genome viruses with still uncertain phylogenetic assignment. It is noteworthy the absence of members of the lenarviricota (mitovirus, ourmiavirus and narnaviruses) which is the most represented phylum in Ascomycetes. Finally, one of the ORFAN segments detected both in silico and in vivo belongs to a new group of genetic/infectious agents of circular RNA in fungi, that carry a self-cleaving ribozyme. We are currently attempting different approaches to obtain virus-infected and corresponding virus-free isogenic strains, in order to compare molecular and phenotypic differences.

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BURSTS, DEATHS, AND SURVIVAL OF A RETROTRANSPOSON IN THE PODOSPORA SPECIES-COMPLEX WHILE FACING REPEAT INDUCED POINT MUTATIONS (RIP)

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The filamentous fungus Podospora anserina is a part of a species-complex with six other species. P. anserina is known to harbor a small abundance of repeats, and also possess the host defence mechanism known as repeat induced point-mutations (RIP). RIP induces C-to-T mutations in repetitive regions of the genome, and hence can introduce nonsense mutations into transposable elements (TEs). Typical classification relies on a number of assumptions, such as constant rates of evolution, which are violated by RIP due to its stochastic nature. Thus, classifying TEs in many fungal species is a complex and imprecise process. In this study, we utilized a combination of a sequence similarity network (SSN) approach with typical alignment-based classification to explore the evolution of an LTR retrotransposon family, Crapaud, across the Podospora species-complex. LTR-retrotransposons rely on an RNA-mediated transposition mechanism and contain protein domains for reverse transcription and integration and have structurally important long terminal repeat sequences that provide sequence promotors and transcription termination sites. Crapaud is the most abundant TE in the species complex and initial results revealed variations in the terminal repeats, where half has diverged in multiple variations. We identified 16 new subfamilies of the Crapaud LTR family, based on their terminal repeats. While full-length copies of subfamilies are present only in some species, fragmented versions of all subfamilies are present in most or all of the seven species. This indicates that the diversification of Crapaud occurred in the ancestor of the species-complex. Differences in repeat composition between the genomes can thus be explained through sorting and later bursts of subfamilies. This study highlights the utility of adding SSNs to the typical methods of studying TE evolution in genomes with RIP. These results also provide key insights into the evolution of LTR retrotransposons and how they manage to evade the host defence.

CS5.1.40

EXPRESSION PROFILING OF GENES RELATED TO 4'-PHOSPHOPANTETHEINYL TRANSFERASE (PPTASE) IN ASPERGILLUS NIDULANS

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Polyketide synthases and/or nonribosimal peptide synthetases are central components of secondary metabolism in bacteria, plants, and fungi. Aspergillus nidulans is a model filamentous fungus widely used to study fungal development and metabolism. In this study, we investigated genes related to the npgA gene, which encodes 4'-phosphopantetheinyl transferase (PPTase), in wild-type (WT) and mutant strains of A. nidulans using multi-omics analyses. A total of 9,968 unigenes were annotated and analyzed using the Gene Ontology, EuKaryotic Orthologous Groups, and Kyoto Encyclopedia of Genes genome databases, among others. Gene expression analysis revealed 477 differentially expressed genes, among which 299 and 178 were upregulated in the WT and mutant strains, respectively. These transcriptomes will be useful for elucidating the molecular mechanisms of secondary metabolite biosynthesis, and aid exploration of metabolite biosynthesis-related genes in A. nidulans. To further investigate gene expression regulation, we analyzed protein expression changes caused by PPTase. We selected protein spots on two-dimensional gel electrophoresis maps and successfully identified them via matrix-assisted laser desorption/ionization tandem time-of-flight mass spectrometry (MALDI–TOF/TOF/MS). We also generated a high-quality cDNA library and performed a yeast two-hybrid screen using NpgP as bait, finally identifying 24 candidate-interacting proteins. The results of our comparative proteomic analyses provide valuable insights and highlight the need for further functional genomic studies of the effects of genes encoding PPTase in WT and mutant strains of filamentous fungi.

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FUNGICIDE RESISTANCE AND POPULATION STRUCTURE OF BOTRYTIS CINEREA FROM MICHIGAN GREENHOUSES

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Floriculture crops in the United States are worth an estimated $4.8 billion annually. Michigan is the third largest producer in the country. The temperate and high humidity greenhouse environment favors development of foliar and flower blighting incited by the fungal pathogen, Botrytis cinerea. Geranium (Pelargonium x hortorum), petunia (Petunia x atkinsiana), and poinsettia (Euphorbia pulcherrima) are highly susceptible. Fungicides are an important tool in integrated disease management, but pathogen resistance may develop. To assess fungicide resistance among B. cinerea populations, isolates (96) were cultured from symptomatic geranium, petunia, and poinsettia plants obtained from eleven greenhouses across three major production regions in Michigan from 2018-2021. A germination-based assay was used to screen B. cinerea isolates against site-specific fungicides representing seven classes including thiophanate-methyl, pyraclostrobin, boscalid, fluopyram, iprodione, cyprodinil, fenhexamid, and fludioxonil. The majority of the isolates screened (63%) were resistant to four or more chemical classes, while only six isolates were sensitive to all fungicides. All isolates were whole genome resequenced using the Illumina NovaSeq 6000 platform. This resulted in an average of 5.2M reads per sample with 23.8X coverage across the genome. Filtered reads were aligned to the reference genome (Botrytis cinerea B05.10) and variants were called using GATK HaplotypeCaller. Following quality filtering, 1.7M variants were identified. Phylogenetic analysis using G2PDH, HSP60, RPB2, and Mrr1 genes indicated that the collection consisted of B. cinerea and B. cinerea Group S, except for one isolate. Mutations known to confer resistance to common fungicides were located and compared to phenotypic data. Population structure was analyzed to evaluate population clusters among greenhouses and crops. Results indicate that B. cinerea populations in Michigan greenhouses have high levels of genetic diversity and relying on fungicides for disease control is not sustainable.

CS5.1.42

COMPARATIVE SECRETOME ANALYSIS OF ZYMOSEPTORIA TRITICI ISOLATES AND DOTHIDEOMYCETE SPECIES TO IDENTIFY CONSERVED SECRETED EFFECTOR PROTEINS

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Zymoseptoria tritici (Zt) is the cause of Septoria tritici blotch in wheat crops. This disease has an asymptomatic stage for 1–13 days, followed by a rapid transition to necrotrophy. Pathogens secrete effectors that modulate innate immunity of the plant and can overcome pattern-triggered immunity mechanisms to facilitate infection. Effector molecules from fungal pathogens are small, secreted proteins (SSPs). They typically are fewer than 300 amino acids in length, are cysteine-rich, contain signal peptides at the N-terminus, and lack transmembrane domains. Proteins of five Zt isolates and seven Dothideomycete species with representative lifestyles within the class were retrieved from JGI Mycocosm and NCBI databases. Functional domain annotations were conducted using BLAST+ 2.12.0., Pfam v.35.0, dbCAN, and the MEROPs protease database. Secreted proteins were identified by the presence of signal peptides and the absence of transmembrane domains detected by Phobius v1.01, TargetP v.2.0. and SignalP v4.1. EffectorP v.3.0, ApoplastP and LOCALIZER were applied to identify effector features and predict the localization of the effectors within the plant cell. The four European isolates of Zt showed the highest number of secreted proteins among all the organisms. The Zt IPO323 strain has similar numbers of SSPs and predicted effector proteins as Cladosporium fulvum and Parastagonospora nodorum. A clustering analysis was conducted to identify conserved predicted effectors among the Dothideomycete species, and at least two clusters that contain effectors with significant sequence similarity were identified among all the species. We identified shared effectors in 11 species including non-pathogens Baudoinea compniacensis and Cryomyces antarcticus.

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CS5.1.43

PROFILING OF FUNGAL CELL TYPES WITH AN OPTIMIZED SINGLE-CELL RNA SEQUENCING PROTOCOL

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As complex multicellular fungi, mushroom-forming fungi (Agaricomycetes) have evolved numerous cell types. However, how many cell types we can estimate in fungi is still elusive. Single-cell RNA sequencing (scRNA-seq) is a cutting-edge technology which has been widely used in animals and plants for exploring cell type diversity at the finest level, yet to our knowledge has not been applied to complex multicellular fungi. Here we present an optimized protocol of scRNA-seq in the model mushroom-forming fungus Coprinopsis cinerea, and demonstrate a diversity of specific cell types in preliminary experiments. We optimized the enzyme cocktail, homogenization and purification during the protoplastation of fruiting bodies. Our protocol results in >10^6 protoplasts/ml with >80% cell viability in the fruiting body samples after 1.5h digestion, which has rarely been attempted before. The Pearson-correlation analysis of gene expression between protoplasted and un-protoplasted samples showed a high correlation (r=0.87), with most of the differential expressed genes related to the environmental response, suggesting that protoplastation induced moderate, and controllable gene expression changes to the cells. Using the 10x Genomics technology, we profiled >7,000 cells from the mycelium sample and >3,000 cells from fruiting body samples. We clustered the cells based on their expression profile and identified several distinct

clusters. Compared to bulk RNA-seq data that reveals the cell identify for several of these, including a distinct subcluster corresponding to asexual spores (oidia) of C. cinerea was identified in the mycelium sample. Some cluster-specific genes in fruiting body samples had a high correlation with the tissue-specific expression dataset. Taken together, our study demonstrates a feasible protocol of scRNA-seq in C. cinerea and provides a first-generation of cell type landscape of complex multicellular fungi at single-cell resolution.

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CS5.1.44

DEFINING THE PAN-GENOME OF ASPERGILLUS FLAVUS

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The saprotrophic and pathogenic fungus Aspergillus flavus is a clinically and agriculturally important species responsible for devastating human disease and crop contamination. In the United States, populations of A. flavus have been shown to have distinct genetic structure and characteristics, such as the ability to produce certain secondary metabolites, but studies of global genetic diversity are lacking. Pan-genomic analyses provide many additional metrics of genetic diversity and capture enormous amounts of relevant data, including functional annotations and rare genes. To define the pan-genome of A. flavus, we assembled a collection of 250 isolates from 9 countries (95 clinical and 155 environmental isolates), including 70 newly sequenced clinical isolates. We annotated or re-annotated the genomes of all isolates and identified over 19,000 gene families that included 99.7% of all predicted genes. Genome size and predicted genes did not differ between clinical and environmental isolates. We found 4,230 of the 19,000 gene families were single copy orthologs present in all studied strains. We also predicted biosynthetic gene clusters for each genome to examine gene presence or absence within clusters of interest. The number of biosynthetic gene clusters was similar for both clinical and environmental isolates. We will next examine the distribution of functional annotations within shared and accessory genes, including identifying shared and rare biosynthetic gene clusters within our dataset and exploring presence or absence of 50 A. flavus virulence-associated genes. Using a set of single copy orthologous proteins present in all isolates, we will also construct a maximum likelihood phylogeny to examine whether isolate origin source (clinical or environmental) or geographic location correlate with phylogenetic relationships.

CS5.1.45

COMPARATIVE GENOME ANALYSIS OF TWO ASCOCHYTA LENTIS ISOLATES SHOWING OPPOSITE PATHOGENICITY PATTERNS ON DIFFERENT LENTIL CULTIVARS TO IDENTIFY PUTATIVE EFFECTORS

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Ascochyta blight, caused by the hemibiotrophic fungal pathogen Ascochyta lentis is one of the most damaging fungal diseases of lentil, responsible for up to 40% crop loss in severely infected fields. This lentil-specific pathogen infects all above ground parts of the plant and can severely reduce grain quality and yield. An avirulence effector protein, AlAvr1, has been identified from the biparental mapping population between two A. lentis isolates, AlKewell and P94-24. Currently, there are two forms of AlAvr1 that mediate specificity in two Australian lentil cultivars, Nipper and PBA Hurricane XT. The avirulence form, AlAvr1-1 identified from P94-24, elicits a hypersensitive response from Hurricane while AlAvr1-2 from AlKewell does not. However, fungal pathogens use a suite of effector molecules, thus we investigated further the genomes of AlKewell and P94-24 in order to identify putative effectors that contribute to pathogenesis. In this study we generated telomere to telomere genome assemblies from long and short sequencing reads and improved gene annotations with in vitro and in planta RNAseq data. The assemblies of AlKewell and P94-24 are near complete i.e., 22 gapless chromosomes from telomere to telomere, 1 chromosome with 1 telomere and rRNA repeats on the other end and complete mitogenomes. Curated annotations were used to rank effector candidates in both isolates and compare gene presence/absence and mutations to shortlist candidates for agroinfiltration and knockout experiments. We found several homologues to effector genes of other fungal pathogens and small structural variations, especially in highly repetitive regions. One of the candidates present in both isolates elicits necrosis after infiltration into Nicotiana benthamiana. These improved assemblies of AlKewell and P94-24 offer a great resource to mine for candidate effectors and increase our competency to identify effectors to be used as a valuable tool for effector-guided breeding of AB resistance responses in Australian lentil varieties.

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CS5.1.46

EXPRESSION PROFILE EVOLUTION OF METABOLISM IN MONOKARYONS OF PLEUROTUS OSTREATUS AND ITS HETEROKARYON ALONG THE TIME IN SUBMERGED LIQUID CULTURE

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Pleurotus ostreatus (Oyster mushroom) is a tetrapolar, edible, lignin-degrading basidiomycete and an active producer of bioactive compounds with important biotechnological properties. P. ostreatus can grow as a monokaryotic or a dikaryotic mycelium during its vegetative phase. In monokaryons, the mycelial cells contain a single haploid nucleus (n), whereas in dikaryons (heterokaryons) the cells contain two haploid nuclei (n+n) coming from the fusion of two compatible monokaryotic mycelia. This particularity of basidiomycetes' life cycle permits studying the evolution of the transcriptomic profile of monokaryons and dikaryons throughout their submerged culture in a maltose medium. Here, we show the analysis of the comparative evolution of the transcriptomes, the sugar-reducing-ends concentration, and the pH of the cultures of the mkPC9 and mkPC15 protoclones and the dikaryon dkN001 containing their two nuclei. The comparative kinetics of sugar consumption revealed complex patterns that suggest a different use of sugars of low (glucose, maltose, and maltotriose) and high molecular weight (maltodextrins) present in the culture medium. Moreover, these patterns were different in mkPC15 (slow growth) compared to mkPC9 (fast growth) and dkN001 (fast growth). Complementarily, the co-expression analysis of the transcriptomes showed the occurrence of eight different groups of co-expression genes. permitted obtaining eight different groups. Three of them deserve particular interest due to clustered genes appeared with differential co-expression profiles in dkN001-mkPC9 vs. mkPC15 (group M1), dkN001-mkPC15 vs. mkPC9 (group M2), and mkPC9-mkPC5 vs. dkN001 (group M6). The most relevant KOG categories in the co-expressed genes were carbohydrate transport and metabolism, with differences associated with the pecu-

liarities of sugar consumption by each monokaryon. In contrast, in the case of the M6 group, the genes associated with mating, heterokaryon, and chromatin-modifying genes appear, revealing peculiarities of the monoaryotic vs dikaryotic life style.

CS5.1.47

ADAPTATION OF ASPERGILLUS FUMIGATUS TO LOW GLUCOSE AVAILABILITY

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Glucose is a widely used carbon source in laboratory routine when stress responses of fungi are studied. The availability of glucose is rather limited in the human body and adaptation to it may alter the physiology of the fungus, including the stress tolerance attributes or the activity of other virulence determining processes. Here, we used a transcriptomical approach to elucidate the physiological differences between Aspergillus fumigatus Af293 cultures incubated on glucose, glucose and peptone, peptone (carbon limitation), or without any carbon source (carbon starvation).
Autolytic cell wall degradation processes were upregulated by carbon starvation and, unexpectedly, by carbon limitation as well. The importance of autolytic cell wall degradation in adaptation to the absence of glucose was also supported by that approximately 12.4% of the A. fumigatus genomes have duplication of N-acetyl glucosamine utilization genes. Absence of glucose altered the transcription of antioxidative enzyme genes which was accompanied with increased redox imbalance and oxidative stress tolerance. Glucose withdrawal downregulated iron acquisition processes, but it upregulated heme protein genes and increased the tolerance against the iron chelator deferiprone. Transcriptional activity of the Gliotoxin cluster was low in all experiments, while the Fumagillin cluster showed substantial activity both on glucose and under carbon starvation but not in the presence of peptone, and the Hexadehydro-astechrome cluster only on glucose. We concluded that glucose withdrawal substantially modified the physiology of A. fumigatus, including processes that contribute to virulence. This may explain why it is challenging to predicting the in vivo behavior of A. fumigatus merely based on data gained from glucose rich cultures.
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CS5.1.48

GENOME-WIDE PREDICTION AND EXPRESSIONAL ANALYSIS OF SUGAR TRANSPORTERS IN FOUR ASCOMYCETE SPECIES

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The degradation and conversion of plant biomass by fungi holds great potential for the production of biofuel, biochemicals and many other bioproducts. Sugar transporters (STs) play an important role in mediating the uptake of small sugars generated from fungal decomposition of plant polysaccharides. A systematical comparison of the STs encoded in different fungal genomes and their dynamic transcriptome changes in response to different sugars can provide a better understanding the diversity and biological role of specific STs, as well as improve related industrial applications.
In this study, we investigated the genome distribution and transcriptome dynamics of four commonly studied filamentous fungi: Aspergillus niger, Aspergillus nidulans, Penicillium subrubescens and Trichoderma reesei. Through computational search of the conserved sugar transport domain and manual inspection of predicted sequences, we predicted 90, 83, 117, and 52 STs for A. niger, A. nidulans, P. subrubescens and T. reesei, respectively. Putative STs were further classified into nine subfamilies and their sugar specificities were predicted according to phylogenetic analysis and literature mining. In addition, comparative analysis of transcriptome of fungi grown on nine different sugars revealed complex expression profiles of STs across different species. Many ST genes were closely co-expressed with other genes involved in sugar utilization, such as CAZymes and sugar catabolic genes. Our study provides new insights into the diversity of STs across different fungi at both genomics and transcriptomic levels, which will facilitate further biochemical characterization and metabolic engineering of these genes.

CS5.1.50

DRAFT GENOME SEQUENCE OF THE BIOSURFACTANT-PRODUCING MINIMEDUSA POLYSPORA FBL 503 (BASIDIOMYCOTA; AGARICOMYCOTINA)

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The soil microfungi that release biosurfactants and tolerate recalcitrant organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) may be of outstanding importance for mycoremediation of polluted sites, in a cost-efficient and environmentally friendly manner. Minimedusa polyspora FBL 503 (Agaricomycetes, Cantharellales) showed characteristics with a high biotech potential. The strain FBL 503 tolerated naphthalene, phenanthrene, pyrene, each PAH at 20 mg/L, and a mix of them (1:1:1) at 60 mg/L as well as exhibited biosurfactant activity. In particular, the production of biosurfactants was carried out in yeast extract broth (10g/L) with the addition of 40g/L of olive oil. Fourteen days after inoculation, the culture medium was recovered and the production of biosurfactants was evaluated by means of the oil dispersion test. The activity of the biosurfactants found in the culture filtrate was evaluated on olive oil, diesel oil, and exhausted motor oils from a scooter and a freeze-dryer vacuum pump. The biosurfactants from FBL 503 resulted efficient, to different extent, in displacing all the tested oils.
In this context, we obtained the draft genome sequence of the strain FBL 503 with Illumina platform. The genome was assembled denovo and was used to functionally annotate M. polyspora FBL 503. We calculated the predicted genes, including genes encoding secreted CAZymes, proteases, and lipases present in the genome. Moreover, mining the genome for genes involved in secondary metabolism biosynthesis resulted in the identification of biosynthetic gene clusters. The genes encoding enzymes for the enhanced solubility of PAHs such as biosurfactants and lipases, which could be included in the PAH degradation pathway, were found in the M. polyspora FBL 503 genome. Our genomic data on FBL 503 are a basis for transcriptomic approaches under exposure to organic pollutants.
The INAIL-DIT (PAR 2019-2021) financially supported the genome sequencing of M. polyspora FBL 503.

CS5.1.51

THE CARBOHYDRATE-ACTIVE ENZYME DATABASE: LITERATURE, FUNCTIONS AND SUBFAMILIES

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Thirty years have elapsed since the emergence of the classification of carbohydrate-active enzymes in sequence-based families that became the CAZy database over 20 years ago. CAZy is updated monthly and freely available at www.cazy.org.
In the era of large scale sequencing and high-throughput Biology, it is important to examine the position of this specialist database that is deeply rooted in human curation. The three primary tasks of the CAZy curators are (i) to maintain and update the family classification of these enzymes, (ii) to classify sequences newly released and (iii) to capture and present functional information for each family.
In our recent publication [1], we summarized the increase in novel families and annotations during the last eight years. We also presented important changes developed to facilitate taxonomic navigation and the download of the entirety of CAZy annotations.
We also highlighted the considerable amount of work that accompanies the capture of biochemical data from the literature. We notably now invited authors to contact CAZy for every missing or novel publications of a biochemically characterized CAZyme. In return, the links to the publications now appear on CAZy website, providing an increased visibility for the biochemists who performed the work and facilitating bibliography survey.
We hope that with the newly displayed information and recently added features, the CAZy database will continue to be a useful resource to the community in the years to come.

Additionally, since 2010, CAZy invested important efforts in the curation of fungal genomes released by the JGI program 1KFG. Each year, more than 200 genomes are semi-manually annotated and displayed by the JGI website. This collaboration highlights the incredible diversity of CAZyme repertoires across fungal genomes. These annotations are uploaded by the Mycocosm project (https://mycocosm.jgi.doe.gov).

[1] Drula E.; Garron M.-L.;...;Lombard V.; Henrissat B.; Terrapon N. Nucleic Acid Res. 2022

CS5.1.53

USING MACHINE LEARNING TO PREDICT PROTEIN-PROTEIN INTERACTIONS BETWEEN A ZOMBIE ANT FUNGUS AND ITS CARPENTER ANT HOST

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Parasitic fungi produce proteins that modulate virulence, alter host physiology, and trigger host responses. These proteins, classified as a type of "effector," often act via protein-protein interactions (PPIs). The fungal parasite Ophiocordyceps camponoti-floridani (zombie ant fungus) manipulates Camponotus floridanus (carpenter ant) behavior to promote transmission. The most striking aspect of this behavioral change is a summit disease phenotype where the infected host ascends and attaches to an elevated position. Plausibly, cross-species PPIs drive aspects of Ophiocordyceps infection and parasitic behavioral manipulation. Machine learning PPI predictions offer high-throughput methods to produce mechanistic hypotheses on how this behavioral manipulation occurs. Using D-SCRIPT to predict host-parasite PPIs, we found ca. 6,000 interactions involving 2,083 host proteins and 129 parasite proteins, which are encoded by genes upregulated during manipulated behavior. Our analyses identified multiple overrepresentations of functional annotations among these proteins. In the fungal parasite, we found enrichment of Ophiocordyceps proteases and frequent involvement of unannotated small secreted proteins. The strongest signals in the host highlighted that neuromodulatory G-protein coupled receptors could be targeted by Ophiocordyceps. We also detected Camponotus structural and oxidation-reduction related proteins as overrepresented targets of putative fungal effectors. From these in silico protein interactome data, we refine behavioral manipulation hypotheses generated from existing genomic, transcriptomic, and metabolomic data.

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CS5.1.54

FUNGIDB: FREE ONLINE INFORMATICS RESOURCE FOR EXPLORATION OF FUNGAL AND OOMYCETE OMICS SCALE DATA

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FungiDB (https://fungidb.org) is a component of the Eukaryotic Pathogen, Vector and Host Informatics Resource (VEuPathDB.org) that enables browsing, querying and mining of genomic-scale datasets across diverse groups of organisms including hosts (HostDB.org), invertebrate vectors of human pathogens, pathogenic and non-pathogenic species, and also environmental and epidemiological studies (ClinEpiDB.org). As of Release 60, FungiDB hosts 277 genomes and over 290 datasets. With FungiDB, you can access transcriptomic (RNA-Seq, microarrays, etc.), proteomic (peptides, quantitative and post-translational modifications), genetic variation (polymorphisms and copy number variation), epigenetic, phenotypes, and other types of data via a user-friendly web interface. The embedded bioinformatics tools support in silico experiments via the search strategy system that enables users to combine results from diverse data types and across species. Data can also be analyzed in the VEuPathDB Galaxy and then exported privately into the FungiDB "My Workspace" for further analysis. With FungiDB, you can also browse genomes and the integrated data in the genome browser JBrowse, peruse gene record pages, and improve gene models in Apollo, a real time collaborative genome annotation and curation platform. Expert knowledge about genes, knockout phenotypes and much more can be recorded via the User Comments system. Have questions, want to nominate a dataset for integration, or invite us for a demo on Zoom? - Click on the Contact Us tab at the top of the site or email directly to help@fungidb.org.

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* EB presenting on behalf of the entire VEuPathDB Bioinformatics Resource Center

CS5.1.55

HYBRIDIZATION DRIVES MITOCHONDRIAL DNA DEGENERATION AND METABOLIC SHIFT IN A SPECIES WITH BIPARENTAL MITOCHONDRIAL INHERITANCE

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Mitochondrial DNA (mtDNA) is a cytoplasmic genome that is essential for respiratory metabolism. While uniparental mtDNA inheritance is most common in animals and plants, distinct mtDNA haplotypes can coexist in a state of heteroplasmy, either because of paternal leakage or de novo mutations. mtDNA integrity and the resolution of heteroplasmy have important implications, notably for mitochondrial genetic disorders, speciation, and genome evolution in hybrids. However, the impact of genetic variation on the transition to homoplasmy from initially heteroplasmic backgrounds remains largely unknown. Here, we use Saccharomyces yeasts, fungi with constitutive biparental mtDNA inheritance, to investigate the resolution of mtDNA heteroplasmy in a variety of hybrid genotypes. We previously designed 11 crosses along a gradient of parental evolutionary divergence using undomesticated isolates of Saccharomyces paradoxus and Saccharomyces cerevisiae. Each cross was independently replicated 48 to 96 times, and the resulting 864 hybrids were evolved under relaxed selection for mitochondrial function. Genome sequencing of 446 MA lines revealed extensive mtDNA recombination, but recombination rate was not predicted by parental divergence level. We found a strong positive relationship between parental divergence and the rate of large-scale mtDNA deletions, which led to the loss of respiratory metabolism. We also uncovered associations between mtDNA recombination, mtDNA deletion, and genome instability that were genotype-specific. Our results show that hybridization in yeast induces mtDNA degeneration through large-scale deletion and loss of function, with deep consequences for mtDNA evolution, metabolism and the emergence of reproductive isolation.

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CS5.1.56

GENETIC FACTORS INVOLVED IN THE REGULATION OF ENZYME SECRETION IN THE HYPERPRODUCING STRAIN TRICHODERMA REESEI RUT-C30

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Second generation biofuels are a promising alternative to fossil fuels. Therefore, efficient and low-cost hydrolytic enzymes are needed for the breakdown of lignocellulose into glucose which is then fermented into ethanol. The fungus Trichoderma reesei is one of the most commonly used industrial cellulase producers due to its high capacity of protein secretion with a titer reaching 100 g/L. Strains of T. reesei with enhanced protein secretion capacity, such as RUT-C30, have been obtained after several rounds of random mutagenesis. But the gaps in our knowledge of the genes responsible for the protein hypersecretion phenotype prevent us from further enhancing this capacity by genetic engineering.

A transcriptomic study was performed on RUT-C30 strain after exposing it to two secretion stress conditions: induction of protein secretion and addition of chemicals affecting the secretion pathway. As expected, many genes of the secretion pathway were upregulated in both secretion stress conditions. To unravel regulation mechanisms, genes encoding transcription factors co-regulated with secretion stress genes were selected as targets for deletion. Further targets were chosen by comparing results published in the literature obtained with other fungi under different secretion stress conditions. In total, 10 RUT-C30 deletion strains were constructed. Some of the mutants show an altered phenotype, such as reduced growth or lowered protein secretion. One of the deletion strains, lacking the gene coding for the transcription factor Res2, was also analyzed by transcriptomics and the results will be presented.

CS5.1.58

EXPLORING THE ECTOMYCORRHIZAL PAN-GENOME AND TRANSCRIPTOME TO PROVIDE CLUES ON ADAPTIVE EVOLUTION AT LOCAL SCALE UNDER A CLIMATE CHANGE SCENARIO

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Ectomycorrhizal fungi are able to form symbiosis with the roots of several plant species and play pivotal roles in forest ecosystem functions, including carbon cycling and dynamics. These fungi show a broad ecological distribution and are considered enhancers of forest health and growth. To identify genomic signatures of local adaptation, the genomes of ectomycorrhizal fungi showing different phenotypes adapted to diverse habitats, can be compared. Despite their ecological impact, little is known about the genetic and functional diversity of these fungi in nature. To fill this knowledge gap, we, in collaboration with the Joint Genome Institute, have launched a project aimed at deciphering ectomycorrhizal genetic diversity in different microclimatic areas. Our goal is to broaden our understanding on how adaptive evolution in various habitats shaped their genomes, focusing on Carbohydrate-Active enZymes (CAZyme) genes. The project is focused on the whole genome re-sequencing of 36 isolates belonging to widespread ectomycorrhizal fungal species, including Pisolithus tinctorius, Tuber borchii, and Suillus collinitus, collected from a plethora of different habitats. Additionally, RNA-seq of selected isolates of S. collinitus and T. borchii with differing degree of heat tolerance will be performed. Gene expression will be monitored during in vitro growth under different temperature regimens, mirroring the future expected scenario due to the climate change. RNA-seq profiling will provide insights into the divergent expression of CAZyme encoding alleles, to identify their putative role in carbon cycling under heat stress. Integration of population genomics and transcriptomic data will reveal insights on pan-genomes of widespread ectomycorrhizal fungi sharing the same habitat, as well as their local adaptive evolution at the genomic level.

CS5.1.59

NEW SECRETS IN AN OLD MODEL: NEUROSPORA ORPHAN GENES SHED LIGHT ON THE EVOLUTION OF DE NOVO ELEMENTS

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Orphan genes have long been postulated to play roles in the establishment of genetic barriers to intercrossing and speciation. In Neurospora they have received little attention, due to a deficit of recent gene duplications attributable to repeat-induced point mutation and other genome-defense mechanisms. Accordingly, there is little understanding of the origin, evolution, and function of these lineage-specific elements. Nevertheless, we have been able to identify 670 orphan genes aggregated adjacent to telomeres. We (1) investigated their genome organization, origin, and evolution, and (2) assessed their functions in Neurospora biology and ecology. To distinguish Neurospora orphan genes that likely evolved by gene duplication followed by fast divergence, horizontal gene transfer, non-coding sequences abundant with long repeats, and by coding capture of “junk” non-coding sequences, we performed comparative genomic sequence conservation analyses among closely related species. 63% of these orphan genes compose clusters with 61% of the het-like genes that regulate self-recognition and confer hyphal fusion incompatibility. To explore functional roles, we assembled 68 transcriptomic data sets and reconstructed co-regulatory networks. Orphan genes were sparsely distributed and did not form regulatory hubs, indicating their marginalized roles in the biology of the species. However, among the 342 orphan genes that were dynamically expressed during both asexual and sexual phases, 64% were detectably expressed on the unusual carbon sources furfural and HMF—wildfire-produced chemicals that strongly induce sexual development. This expression implies a functional role in reproduction, the asexual-sexual switch, and sensitivity to light and temperature. Furthermore, orphan genes and clustered het-like genes were expressed similarly in mutants of hyphal communication transcription factors adv-1 and pp-1, and more than one quarter were affected by a mating locus mutant. Orphan genes appear to work in concert with the vegetative compatibility processes that drive reproduction isolation and that likely play a role in speciation in Neurospora.

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CS5.1.60

ON THE INTEREST OF QUANTITATIVE GENETICS APPROACH TO DECIPHER FUNGAL BIOLOGY IN THE OMIC ERA

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In fungi, numerous traits exhibit a quantitative variation within natural populations, reflecting a complex genetic architecture : yield in mushroom, aggressiveness in pathogen, or life history traits in filamentous fungi ... A deeper understanding of the genetic control of these traits is essential for numerous issues such as breeding, evolutionary genetics, or fundamental biology. Indeed, several questions could be addressed : How many factors are involved in the genetic control of those traits? Where are they located on the genome? What are their relative importance and their possible interaction? All these questions are addressed through quantitative genetic approaches based on association between genotype and phenotype variation, measured in recombinant population. Two major strategies have been developed: classical quantitative trait locus (QTL) mapping based on experimental population derived from crosses and genome wide association studies (GWAS) using natural populations. Both strategies have received less interest in non-model fungi than in plants or animals. However, their relevancies and their uses are increasing, especially thanks to the contribution of next-generation sequencing. We will draw an overview of some basics of quantitative genetics, illustrated by examples from the recent literature. We will particularly emphasize on the added value of the genomics in the renewal of such classical genetic approaches. Further challenges offered by the development of other omics will also be proposed.

CS5.1.61

RAPID ADAPTATION OF A MAJOR WHEAT PATHOGEN TO FLUCTUATING ENVIRONMENTS

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The constantly fluctuating environments of human-managed agroecosystems, often characterized by the deployment of new hosts and pesticides, are likely to accelerate the evolution of the associated pathogen populations, but little is known of how pathogen populations evolve in the field during a single growing season. We sampled, phenotyped, and sequenced nearly a thousand Zymoseptoria tritici (cause of septoria tritici blotch on wheat) strains from twelve wheat cultivars growing at the same time in the same field to identify short-term evolutionary responses to host genotypes and potentially identify new virulence loci. First, we investigated the impact of selection imposed by the local host and pesticide environment using genome-scans for selective sweeps. Five outlier loci were identified, including one in a Major Facilitator Superfamily (MFS) transporter, potentially encoding a generalist adaptation to fungicides. Next, we explored host-associated genomic determinants using a genome-wide association study (GWAS) for each wheat genotype based on pairwise comparisons. We found one locus shared among eight pairwise host associations, encompassing potential host-associated genetic determinants important for the infection of different wheat genotypes. Finally, we examined the genetic basis for i) growth responses associated with “host-like factors” including acidic pH, two phytohormones, and oxidative stress, as well as ii) growth responses associated with “survival outside the host factors” including cold and warm temperatures, and osmotic stress. Among the diverse loci identified using GWAS, we located a shared genomic region associated with response to oxidative stress and sensitivity to one of the phytohormones. Our study highlights the power of combining high-throughput genomic and phenotyping approaches to increase understanding of the processes affecting pathogen adaptation.

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GENOMIC PATTERNS OF ADAPTATION FOLLOWING SERIAL INFECTION OF A SPECIALIST FUNGAL PATHOGEN IN A NOVEL HOST

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The genus Metarhizium comprises a set of diverse insect-pathogenic fungi that exhibit a wide spectrum of host ranges. Within this genus, M. acridum is a specialist pathogen that infects orthopteran insects and is currently used in environmentally friendly biological control of locust pests. Although this species exhibits a global distribution across tropical and sub-tropical regions, much of the current genotypic and phenotypic characterization is based on only two isolates. To increase our understanding of how evolutionary factors and genomic diversity drive host colonization, we first expand current knowledge of intra-specific genomic diversity by establishing a reference-quality pangenome of M. acridum based on six assembled genomes of isolates from four continents. We find that 7,242 of the 10,177 gene clusters (71%) are shared among all isolates (core genome), and use enrichment analysis to determine the functional differences between the core and accessory regions. Using this foundational genomic dataset, we then selected three isolates displaying divergent genotypic and virulence profiles for a serial passage experiment to investigate how standing genetic variation in a pathogen contributes or constrains the ability to colonize novel hosts. We passaged M. acridum through three host environments for five generations: Locusta migratoria grasshoppers, representing the natural host; Tenebrio molitor beetles, representing a novel host; and sabouraud dextrose agar media representing an experimental control. We observe clear changes in virulence and gene expression following serial passaging, and use our studies to reveal how standing genetic variation and the pan-genomic structure of a specialist pathogen influence genomic patterns of early adaptation.

CS5.1.63

POPULATION GENOMICS OF FUSARIUM ASIATICUM: DRIFT AND FUNCTIONAL DIVERSITY

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Fusarium head blight (FHB) is an economically important disease on wheat caused by Fusarium. FHB can result in yield losses and infection reduces the quality of grains. In addition, infected grains can accumulate trichothecenes, such as DON, 3ADON, 15ADON and NIV. Contaminated kernels pose a serious threat to human and livestock health. The Fusarium graminearum species complex (FGSC) represents the foremost etiological agent of FHB of wheat. F. asiaticum is one of the major species, and dominant in Eastern Asian regions, such as in southern China. In this study, we performed comparative genomics and phylogenetic studies on F. asiaticum mitochondrial and nuclear genomes. Over 2000 strains were collected from southern China to understand the drivers and the gene functions responsible for the diversity within F. asiaticum populations found in our previous studies. Based on the mitochondrial genome analysis, we can conclude that: 1) F. asiaticum mitogenome proves extremely conserved; 2) intron patterns can be used as genetic markers for monitoring population dynamics; 3) recombination in mitogenomes was identified in F. asiaticum. The more extensive nuclear genome analysis generated that: 1) a high-quality reference genome assembled at chromosome level; 2) 245 F. asiaticum genomes assembled with high quality and completeness; 3) a graphically based pan-genome of F. asiaticum that can represent the F. asiaticum population in China; 4) 3ADON strains in Hunan and ML-RYR were shown to belong to the same population indicating recent spread potentially caused by the human activity; 5) core genomic regions show relatively low genetic diversity, in contrast the accessory/unique regions drive evolution; 6) recombinations were identified in the phylogeny network analysis, and a detailed analyses also showed a recombination event in a biosynthetic gene cluster responsible for the trichothecene production and its flanking regions.

CS5.1.64

3D MODELING OF HIC CONTACT MAPS: SEEING THE SPATIAL ORGANIZATION OF CHROMOSOMES AND VISUAL INTEGRATION OF OMICS DATA

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In recent years, significant technical advances have been made to better observe the structure and the organization of chromatin. Many Hi-C data are now available in public databases, at different accuracy and resolution and for various species. Generally, the final results of Hi-C data analyses are summarized with the representation of “contact maps”. Using a color scale, these maps (heat-maps) show the frequency of contacts observed between different portions of a genome, both at short-range and long-range distances.
Even if they are widely used, our observation is that the biological interpretation of contact maps is not trivial. It requires training and significant experience to be useful to validate or invalidate hypotheses concerning the overall organization of the studied genome. In this context, alternative tools have been developed. Some of them produce 3D models of contact networks, as a substitute for visualization of Hi-C outputs. By 3D models, we mean representations in a 3D space of a genome, in which euclidean distances are derived from the contact frequency matrices. The information contained in the Hi-C data is thus directly translated into distances in a 3D space. Although care must be taken in interpreting these models (they are not “pictures” of the interior of a cell), we observed they are very helpful to highlight specific structural patterns in chromatin organization, originally hidden in contact maps.
Developing new strategies to visualize and integrate large scale multi-omics data is the major objective of Thibault Poinsignon’s thesis (ANR MinOmics). These led us to start building 3D models of yeasts and filamentous fungi genomes, from Hi-C raw sequencing data. We present here our evaluations of the biological significance and the interest to use these models for projecting heterogeneous epigenomics data and hence better support their biological interpretations.

CS5.1.65

EXPLORING THE ROLE OF MINI-CHROMOSOMES IN BOTRYTIS ELLIPTICA

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Genomes of microbial plant pathogens are highly dynamic and typically exhibit an architecture that facilitates rapid adaptation to their hosts. One of the extreme cases is the presence of isolate-specific, supernumerary chromosomes (also called accessory-, conditionally dispensable- or mini-chromosomes). In some fungal species, the mini-chromosomes are directly associated to the emergence of new virulent traits, underlying the importance of understanding their role in evolution and pathogen adaptation. However, the diversity of mini-chromosomes across plant pathogens and their contribution to the gene plasticity are poorly understood. The fungal genus Botrytis contains well known necrotrophic plant pathogens. A few of them have a broad host range such as B. cinerea. The majority of Botrytis spp. is considered host-specific because they occur only on one host plant species. The complete genome assembly of B. cinerea strain B05.10 revealed two mini-chromosomes with sizes of 247 and 209 kb, which encode uncharacterized proteins. So far, no association of mini-chromosomes with virulence has been described for this pathogen. Interestingly, a recent well assembled genome from a lily infecting species, Botrytis elliptica, contains at least two mini-chromosomes, whereas these mini-chromosomes are completely absent in another well assembled Botrytis elliptica strain. Although these mini-chromosomes contain few (predicted) genes, they contain orthologs of genes that are also present on the mini-chromosomes of B. cinerea. In this study we aim to identify the presence and absence of mini-chromosomes in a small Botrytis elliptica population, which consists of genomes of three strains assembled with long reads and eight strains with short reads. By mapping the short reads to the well assembled genome as references and to other Botrytis spp., we obtain an overview of the variation within the small B. elliptica population and hope to obtain clues to the origin of these mini-chromosomes.

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A THOUSAND-GENOME PANEL RETRACES THE GLOBAL SPREAD AND CLIMATIC ADAPTATION OF A MAJOR CROP PATHOGEN

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Human activity impacts the evolutionary trajectories of many species worldwide. Global trade of agricultural goods contributes to the dispersal of pathogens reshaping their genetic makeup and providing opportunities for virulence gains. Understanding how pathogens surmount control strategies and cope with new climates is crucial to predicting the future impact of crop pathogens. Here, we address this by assembling a global thousand-genome panel of Zymoseptoria tritici, a major fungal pathogen of wheat reported in all production areas worldwide. We identify the global invasion routes and ongoing genetic exchange of the pathogen among wheat-growing regions. We find that the global expansion was accompanied by increased activity of transposable elements and weakened genomic defenses. Finally, we find significant standing variation for adaptation to new climates encountered during the global spread. Our work shows how large population genomic panels enable deep insights into the evolutionary trajectory of a major crop pathogen.

CS5.1.67

PANGENOME SEQUENCING AND ANALYSIS OF THE PENICILLUM GENUS

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Penicillia are known to produce a wide range of bioactive natural compounds, some with devastating outcome for the agricultural industry and others with unexploited application potential. However, a large-scale overview of the biosynthetic potential of the genus has been lacking. In this study, we sequenced 93 Penicillium isolates using long read sequencing and assembled them into high quality near chromosome-level models. Together with eleven published genomes that hold similar assembly characteristics, we established species phylogeny, as well as defining the Penicillium pangenome. A total of 5,612 genes were shared between ≥98 isolates corresponding to approximately half of the average number of genes a Penicillium genome holds. We further identified 15 lateral gene transfer events that have occurred in this collection of Penicillium isolates, which might have played an important role, such as niche adaption, in the evolution of these fungi. Finally, we have analyzed the Penicillium pangenome for statistical association with the presence of 37 known mycotoxins and penicillin and found significant and credible associations between specific genes and compounds. This comprehensive characterization of the genomic diversity in the Penicillium genus capture a much larger proportion of the genome diversity and provides a deeper understanding of the biosynthetic potential and evolution of the genus.

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CS5.1.68

COMPARATIVE GENOMICS REVEALS ACCESSORY CHROMOSOMES AND DIFFERENTIAL EFFECTOR CATALOGUES IN FUSARIUM SPECIES CAUSING WILT DISEASE OF BANANAS IN CUBA

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Fusarium wilt of bananas (FWB) is a destructive plant disease that causes devastating economic losses to banana production worldwide. In Cuba, FWB is one of the major concerns for banana cultivation. The disease is caused by the soilborne fungus Fusarium oxysporum f. sp. cubense (Foc), which is classified in pathogenic races based on their capacity to infect a subset of banana varieties. Recently, Foc was subdivided in a suite of genetically distinct Fusarium species, based on genotyping analyses. The present study aimed to unravel the genomic diversity and the repertoire of putative effector genes of Fusarium spp. that infect bananas in Cuba. Whole genome sequencing was performed on 22 Cuban pathogenic strains of F. purpurascens, F. phialophorum and F. tardichlamydosporum and compared with the genome of reference strains of a global Fusarium panel. The presence of previously identified Secreted In Xylem (SIX) genes was determined, and putative effector genes were identified by selecting genes encoding small secreted proteins (0-600 amino acids) in proximity to a miniature impala transposable element. Whole-genome comparisons revealed that Cuban strains of F. tardichlamydosporum race 2 share two accessory chromosomes that could not be identified in the genome assemblies of the other specie representatives included in the study. Fusarium phialophorum and F. purpurascens genomes contain one and two accessory chromosomes, respectively; with regions that are

highly rearranged between all the species. A hierarchical cluster analysis, based exclusively on SIX genes presence/absence, showed that Fusarium species cannot be distinguished from each other. However, the overall predicted effector profile correlated well with the species of the studied strains but not with the race classification, which indicates that effector profiles in Fusarium spp. infecting bananas are more diverse than hitherto assumed. Thus, we here provide the first report on the genomic structure of banana infecting Fusarium species across Cuba.

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CS5.2 MYCOBIOMES AND MICROBIAL INTERACTIONS

CS5.2.9

CANDIDA ALBICANS AND ENTEROCOCCUS FAECALIS FORM A COMPLEX NUTRITIONAL NETWORK DURING IN VITRO COINFECTION AFFECTING HOST CELL DAMAGE

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Disseminated candidiasis a severe life-threatening condition is commonly caused by endogenous strains colonizing mucosal surfaces. As a member of the microbiota, Candida has to interact with bacteria on the mucosae and positive associations between C. albicans and E. faecalis were observed during colonization in murine models and mixed bloodstream infections in human patients. If these interactions promote or prevent host damage is, however, unclear.
By comparing different E. faecalis isolates during mono- and coinfection of enterocytes with C. albicans we found that cytolysin-positive E. faecalis strains lead to increased host cell damage during coinfection. These findings could be translated into a murine model of oral pharyngeal candidiasis where cytolysin-positive E. faecalis strains resulted in enhanced tissue damage and fungal invasion during coinfection.
Since synergistic damage was only partially dependent on physical contact between bacteria and fungi in vitro, we hypothesized that the metabolic activity of C. albicans could impact bacterial virulence by changing nutrient availability. Therefore, we analyzed the metabolome of mono- and coinfection supernatants and identified several metabolites with altered abundance in the presence of fungi that might contribute to bacterial virulence. Supplementation of cell culture media with selected metabolites during infection, revealed that addition of spermine or malate significantly increased damage caused by E. fae-

calis. Other metabolites tested had no effect or led to reduced damage. In summary, our results suggest that host cells, fungi, and bacteria form a complex nutritional network. The balance of different metabolites appears to influence host cell damage caused by E. faecalis. We are currently the mechanisms by which metabolites mediate a protective or detrimental effect.

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CS5.2.10

WHAT DOES YOUR GUT TELL YOU? INFLUENCES OF ZOMBIE-MAKERS AND GENERALIST FUNGAL ENTOMOPATHOGENS ON CARPENTER ANT MICROBIOTA

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'Zombie-making' fungi of the species complex Ophiocordyceps unilateralis infect ants from the genus Camponotus. These fungi hijack the behavior of ants in a matter of weeks, causing them to leave their nests, climb to an elevated position, and execute a 'death grip' bite. This unique behavior is adaptive to Ophiocordyceps to create the ideal conditions for the parasite's successful transmission. In contrast, generalist fungal kill their hosts within a few days, without obvious induction of fungus-adaptive behaviors. There is significant evidence that the microbiome impacts many facets of a host including health, nutrition, reproduction, and pathogen defense. The bacterial microbiome of the host C. floridanus has been well characterized across different regions of the body and maturation level. However, the fungal communities and potential effects from disease on the gut microbiome have yet to be assessed. Moreover, the strategies of Ophiocordyceps fungi to manipulate their hosts currently remain elusive. Investigation of the microbiome could provide valuable insight into potential infection and manipulation strategies exploited by fungal entomopathogens. To add context to our limited understanding of fungal entomopathogens and infection, we performed a time course study of C. floridanus infected with Ophiocordyceps camponoti-floridani and Beauveria bassiana to characterize the gut microbiome. By comparing a specialist fungal entomopathogen against a generalist our aim is to 1) describe the dynamics of the myco- and microbiome of C. floridanus, 2) determine the effects of infection on gut microbiota, and 3) establish if results are distinctive to behavioral manipulation or a general characteristic of fungal infection.

CS5.2.11

THE EPIDEMIOLOGY OF FUNGAL PATHOGENS OF APPLES UNVEILED BY FRUIT MICROBIOME: THE CASE OF DRY LENTICEL ROT AND WHITE HAZE

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Different methods, including cultural and molecular ones, can be used to study the epidemiology of postharvest pathogens. In recent years, metabarcoding is emerging as a flexible tool to elucidate the biology and epidemiology of pathogens. With the introduction of new apple varieties, emerging diseases emerged including dry lenticel rot and white haze. Dry lenticel rot symptoms are brown-to-black spots occurring on the fruit peel, caused by Ramularia mali. White haze is a waxy, opaque white film forming on the cuticle, associated with yeast-like basidiomycetes of Entylomatales and Golubeviales. The epidemiology of these pathogens remains unclear. In the present study, we used metabarcoding to characterize both epiphytic and endophytic microbial communities of two apple cultivars, 'Opal' and 'Ambrosia', across six time points from early fruit development up to the end of shelf life. Moreover, we measured fruit disease incidence and quality parameters. R. mali first developed in both cultivars as an endophyte at second fruit fall phenological phase, then it appeared as an epiphyte from fruit ripe for picking onward, when symptoms became visible. This was confirmed in endophytic samples through qPCR specific for R. mali: a higher amount of the pathogen was detected in June, lower in July and August, and the pathogen was not anymore detectable later. Among the genera associated to white haze, Golubevia was the most abundant epiphyte from beginning of ripening to the end of shelf life. Alpha and beta diversity analyses showed significant difference both in richness and composition among different tissue, time points and cultivars. The study helps to explain the epidemiology of white haze and dry lenticel rot, and to design a targeted crop protection strategy, reinforcing the hypothesis that fruit metabarcoding could be a valuable tool for assessment and prediction of postharvest diseases, before symptoms occurrence in fruit.

CS5.2.12

THE INTERACTION BETWEEN RESIDENT BACTERIA AND A FUNGAL PATHOGEN IN AN INSECT PEST

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Fungi are predominant natural enemies of insects and are a promising strategy for environmentally-safe pest control. Many insects also harbor bacterial associates that can have a major influence on the biology of their host, either as specialized symbionts or as members of the gut microbiota. While interactions between insects and insect pathogenic fungi or insects and their bacterial symbionts have been studied independently, little is known about the tripartite interaction between these players. Using Western Flower Thrips (Frankliniella occidentalis), a globally invasive insect pest, we are investigating if and how symbiotic bacteria affect infections by the generalist entomopathogen Beauveria bassiana. In vitro assays show that metabolites produced by the bacterial gut symbiont BFo1 (Erwiniaceae) hinder the germination of B. bassiana conidia. Also, preliminary in vivo assays indicate that the presence of the gut bacteria delays infection by B. bassiana in thrips larvae. We are interested in the mechanisms by which bacteria and fungi interact in the insect, and what factors determine the role of the resident microbiome in the progression of fungal infections. Fungal gene expression profiles upon infection of symbiotic and symbiont free insect hosts in this system are providing insights on the direct and indirect effects that insect symbiotic bacteria can have on a fungal entomopathogen.

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CS5.2.13

FIRST IN VIVO DETECTION IN A FUNGUS, TULASNELLA SP., OF TWO NEW PUTATIVE VIROIDS OR VIROID-LIKE RNAS

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Fungi host a number of biological entities able to self replicate, from bacteria to viruses, while viroids (i.e. infectious agents, with a small, single-stranded circular and non-coding RNA genome -circRNA-, endowed with autonomous replication and sometimes containing ribozymes) and viroid-like RNAs (including satellite RNAs, retroviroids, retrozymes and Ribozyviruses) have not been detected yet. However, the list of putative viroids and viroid-like RNAs, has been recently expanded by high-throughput analysis of metatranscriptomic data from plant, animals and/or environmental sources. A specific pipeline to detect putative circular RNAs carrying ribozymes (the ribozycirculome) identified 20,364 novel species-like operational taxonomic units (sOTU), suggesting that circRNAs containing ribozymes are more widespread than expected in ecological niches where fungi thrive.
The same pipeline was applied to RNAseq data previously used to characterize the virome from a collection of orchid mycorrhizal fungi of the genus Tulasnella and Ceratobasidium, allowing the identification of a number of members of the ribozycirculome. We focused on two contigs (contig_11108 and contig_6686) whose presence in Tulasnella sp. and circularity have been confirmed by RT-PCR. Contig_11108 and contig_6686 are 1212 and 1575 nt long, respectively, and both contain two different ribozymes (hammerhead ribozyme, HHRz, in the plus polarity and hairpin ribozyme, HPRz, in the minus polarity), and a region with high percentage of sequence identity with a virus infecting the same isolate, Tulasnella Ambivirus 4 and Tulasnella Ambivirus 1, respectively.

Next experiments will aim to verify: i) the autocatalytic activity of the ribozymes in vitro and in vivo and the presence of circular RNA of both polarity strands to investigate the replication mechanism; and ii) the possible dependency on the coinfecting virus/es for their replication. These results will help to assess whether these two new circRNAs can be considered bona fide viroids or satellite RNAs of fungi.

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CS5.2.14

UNCOVERING THE IMPACT OF THE PHYLLOSPHERE MYCOBIOME ON THE RESISTANCE OF STRAWBERRY AGAINST BOTRYTIS CINEREA

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Infections by phytopathogenic fungi are responsible for a significant amount of crop loss each year, causing a huge economic burden for agriculture. The broad application of fungicidal chemicals is still the most common strategy to manage crop plant diseases, but this practice is accompanied by environmental pollution and shifts in biodiversity. Therefore, the development of novel and environmentally friendly forms of fungal control is essential for organic farming.
Recent studies have suggested that the necrotrophic plant pathogen Botrytis cinerea, which causes grey mold disease in a variety of crops, is forced into developmental reprogramming in the presence of the saprophytic fungus, Neurospora crassa. As a consequence, B. cinerea undergoes germling fusion and builds a hyphal network on the plant surfaces, instead of forming infection structures. Since B. cinerea and N. crassa occupy vastly different ecological niches, we are now asking the question if these observations are also relevant for interspecies interactions in natural fungal communities that include B. cinerea.
For that purpose, we will characterize the interaction between cultivatable members of the strawberry phyllosphere mycobiome on a macroscopic, microscopic, and metabolomic level. This project aims to identify species that can manipulate B. cinerea development in a way similar to N. crassa. With this approach, we also set out to elucidate whether the presence of non-pathogenic fungi in the phyllosphere contributes to the initial defense response of plants towards fungal pathogens. Moreover, we aim to uncover the variety and dynamics of the cultivatable phyllosphere mycobiome of strawberry plants at different growth stages and sampling sites in addition to exploring the metabolic changes of the occupying fungi during developmental reprogramming. Taken together, we anticipate that this project will broaden our understanding of the role of the phyllosphere mycobiome in plant defense against pathogenic fungi.

CS5.2.15

THE ROLE OF PENICILLIUM EXPANSUM SECONDARY METABOLITES IN THE MOLDY APPLE MICROBIOME

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Fungal secondary metabolites (SMs) have long been of interest due to their antibiotic activity and potential applications in medicine and agriculture. However, the role of SMs for the producer and the members of their natural microbial community is largely unknown. To investigate the role of SMs in the development and structure of a microbial community, we apply moldy windfall apples as a model system due to its defined spatial structure and relatively simple composition of potent SM producers. Specifically, Penicillium expansum, the causal agent of blue mold rot, is a dominant SM producer with more than 20 known compounds and 69 predicted Biosynthetic Gene Clusters (BGCs). We have established a marker-free CRISPR/Cas9-based genetic engineering toolbox for manipulating natural isolates of P. expansum, allowing for the construction of a library of individual BGC deletion mutants. The impact of losing the ability to produce individual SMs on the fitness and competitiveness of the producer was tested via in vitro mono-cultures and co-cultures with another dominant apple pathogenic fungi, Monilinia fructigena. Analysis of the metabolic profiles in co-cultures showed that the presence of P. expansum stimulated the SM production. Furthermore, the role of the individual SMs produced by P. expansum for the outcome of interactions with a broader variety of members of the natural community (Tricothecium roseum, Mucor spp., Metschnikowia pulcherrima etc.) is currently being investigated by automated high-throughput screening of the P. expansum mutant library in co-cultures.

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CIRCULAR RNA MOBILE GENETIC ELEMENTS IN FUNGI: NEW PLAYERS IN DEFINING FUNGAL HOLOBIONTS

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Phenotypic variability of isogenic fungal strains can be often associated to mycovirus infections. High throughput sequencing (HTS) approaches have allowed the untargeted detection of virus-like elements. In specific, a bioinformatic pipeline aimed at detecting orphans-encoding RNA segments has revealed new virus-like elements (e.g., ambiviruses), which encode a putative RNA-dependent RNA polymerase (RdRp) and a protein of unknown function. A different bioinformatic pipeline aimed at detecting RNA circularity associated to ribozymes (catalytic RNAs) has revealed, in metatranscriptomes from various ecological niches, thousands of elements combining these two features (circularity and ribozymes). The hosts of these circular RNAs remain mostly unknown, but the largest ones coincided with ambiviruses. Here, we summarize our in-silico findings and describe in vivo detection and characterization of these new mobile RNA genetic elements in diverse fungi, namely isolates of the plant pathogen Rhizoctonia solani, isolates of the orchids endomycorrhizal symbiont Tulasnella spp. and, in Cryphonectria parasitica, the model system for virus-caused hypovirulence. In these three systems, we reveal the presence of ambiviruses and prove i) their circular genomic nature and ii) the ribozyme-mediated cleavage of the circular RNAs at specific sites. These features are the hallmark of a symmetric rolling circle replication mechanism previously proposed for several ribozyme-encoding infectious RNAs (i.e., viroids, some satellite RNAs and members of the realm Ribozyviria), which however do not code for an RdRp. Therefore, our results

identify ambiviruses as the first example of self-cleaving circular RNAs coding for their own RNA polymerase, thus providing evidence that they are extant hybrids of RdRp-carrying mobile genetic elements and ribozyme-encoding circular RNAs. Additional classes of small circular RNAs with ribozymes were also found in these fungal isolates. Our biological characterization confirms that these new elements can provide specific phenotypes, and therefore need to be included in future assessment of fungal holobionts.

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CS5.2.17

ANTIMICROBIAL DEFENSES IN MODEL MUSHROOM COPRINOPSIS CINEREA

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Lactone rings are common structural features of natural products. In bacteria and fungi, lactone-based natural products, or lactones, can serve as signaling mediators of intra-species communication, e.g. quorum sensing in Gram-negative bacteria, but also as antimicrobial agents and virulence-promoting toxins. Lactones can be inactivated by enzymes that hydrolyze the ester bond of the lactone ring. These enzymes are commonly referred to as lactonases. The saprophytic mushroom Coprinopsis cinerea dwells in herbivore dung and competes with several bacterial and fungal species for this ecological niche. Hence, C. cinerea is a model system to study fungal antagonism and defense. Coprinopsis cinerea possesses two intracellular lactonases, which are known to be active against the lactones of Gram-negative bacteria. In this study, we aim to establish whether the same enzymes are relevant to the interactions of C. cinerea with other microbes, such as Gram-positive bacteria and fungal competitors. To address this question, we will test the activity of C. cinerea lactonases against lactones with antifungal activity of different origins. These will include the antifungal agent rapamycin, produced by the Gram-positive bacterium Streptomyces hygroscopicus, and the mycotoxins patulin and zearalenone produced by plant-pathogenic fungi. The model yeast Saccharomyces cerevisiae will be used for assaying the activity of the C. cinerea lactonases against the selected lactones. Firstly, we will determine the working concentrations of these compounds against S. cerevisiae. Secondly, S. cerevisiae will be transformed to express C. cinerea lactonases and tested for growth at inhibitory lactone concentrations. Lactonase activity will be revealed by the ability of transformants to grow.

CS5.2.18

TWO KEY REGULATORS OF DEFENSE OF THE MUSHROOM-FORMING FUNGUS SCHIZOPHYLLUM COMMUNE AGAINST ITS ANTAGONISTS

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Mushrooms are a valuable food source for a growing world population. However, commercially interesting species suffer from pests and diseases, which can lead to significant crop losses. Mushroom-forming fungi have evolved defense strategies to resist attacks, such as production of secondary metabolites and effector proteins. However, the regulatory mechanisms of this immune system are still unknown. Therefore, we investigated interactions between the mushroom-forming fungus Schizophyllum commune and multiple antagonists: the fungi Trichoderma harzianum, Trichoderma aggressivum and Purpureocilium lilacinum, and the bacterium Serratia quinivorans.

Gene expression in S. commune during interaction with these antagonists was assessed by RNA-sequencing. We identified a small but clear systemic effect, suggesting that a signal travels from the interaction zone to non-interacting parts of the colony. More pronounced is the large transcriptomic response in the interaction zone, including an antagonist-specific expression profile. This indicates that S. commune responds differently to each antagonist and possibly has a tailored response for each of them.

Eleven transcription factors were up-regulated during interaction with multiple antagonists, suggesting they regulate aspects of the defense response. Gene deletion of the regulators TF21 and TF22 rendered S. commune more susceptible to certain antagonists. The vegetative mycelium and mushrooms were overgrown, and less pigment was produced in the interaction zone. Complementation of the missing gene restored the wild-type phenotype. Next, we are performing ChIP-Seq and RNA-Seq on knockout strains to find target genes of these important regulators of defense.

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CS5.2.19

A GFPS-NANOLUCIFERASE SYSTEM TO MONITOR THE INDUCTION OF SILENT FUNGAL NATURAL PRODUCT GENE CLUSTERS IN THE ENVIRONMENT

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In all habitats on earth microorganisms form consortia with many different species closely living together in the soil. The interspecies communications in these communities are decisive for function of microbial communities and further lead to the induction of otherwise silent natural product biosynthesis gene clusters. One prominent example is the interaction of the bacterium Streptomyces rapamycinicus with the fungus Aspergillus nidulans. Upon co-cultivation, the streptomycete is able to activate the otherwise silent ors biosynthesis gene cluster in A. nidulans (Schroeckh et al. 2009). Recently, we discovered that the compound family of arginine-derived polyketides including azalomycin F produced by S. iranensis (and S. rapamycinicus) serve as the long sought-after bacterial signals for this induction (Krespach, Stroe et al. submitted). To measure the induction of silent gene clusters, we employed a reporter system encoding the gene for the green fluorescence protein (GFP) coupled with the nanoluciferase gene. It allows to measure qualitatively and quantitatively the transcriptional activation of genes. This construct was translationally fused to the orsA gene of the orsellinic acid biosynthesis gene cluster of A. nidulans that is silent when the fungus is cultivated in monoculture. Transformants of A. nidulans with the reporter system showed fluorescence and luciferase activity upon addition of S. iranensis or of azalomycin F to the culture. The sensitivity of the reporter system allowed detection of azalomycin F concentrations as low as 10 nanomolar. In line, the reporter was induced even when soil extract was added to the culture medium indicating that arginine-derived polyketides are indeed present in the soil. Further, with this reporter we were able to identify several bacterial strains that induce green fluorescence in the fungus.

CS5.2.20

MICROBIOME DYNAMICS AND INTERACTIONS DURING THE BIOTROPHIC GROWTH OF ZYMOSEPTORIA SPP. IN WILD GRASSES

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Plants harbor diverse microbiomes important for plant health. Zymoseptoria spp. are fungal hemibiotrophic leaf pathogens with both biotrophic and necrotrophic infection stages. Closely related species have specialized to distinct grass hosts making this a powerful model system to identify plant traits relevant for fungal host specificity. Host-driven specialization can arise from traits related to invasion, manipulation of plant defenses, nutrient uptake, and reproduction. Evidence shows that the microbiome can intervene in those processes (e.g., antibiosis, interaction via effectors) and potentially play a role in influencing host specificity. In the wheat - Zymoseptoria tritici pathosystem, we previously showed that the microbiome assembly is determined by fungal infection, and the host's development and genotype. These changes were attributed to the host's metabolic and immune status induced by the pathogen. Nevertheless, different Zymoseptoria species infect wild grasses and represent promising models for microbiome studies because domesticated crops assemble more stochastic microbial communities than wild relatives. If different Zymoseptoria species have encountered differential microbial assemblies during their evolution within their host, we hypothesize that they have specialized to coexist and compete with the specific microbiota of their given host. In this study, we aim to determine the leaf microbiome dynamics of wild grasses and their molecular interactions with four host-specific lineages of Zymoseptoria. First, we have characterized and compared the leaf microbiome of wild grass species (Hordeum, Aegilops cylindrica) during the biotrophic invasion of specialized (virulent) and non-specialized (avirulent) Zymoseptoria isolates by amplicon sequencing and isolation of microbial partners. We have analyzed amplicon data to characterize the microbial dynamics and predicted microbial interactions induced by fungal colonization. Finally, we are testing the causality of such interactions using synthetic microbial communities and characterize their molecular basis. This study will provide new insights into the co-adaptation and interaction of fungal plant pathogens with their host microbiomes.

CS5.2.21

FRUITING BODY SPECIFIC SC4 HYDROPHOBIN GENE PLAYS A ROLE IN SCHIZOPHYLLUM COMMUNE HYPHAL ATTACHMENT TO STRUCTURED GLASS SURFACES

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Genes encoding hydrophobins play distinct roles at different stages of the life cycle of fungi, and they foster hyphal attachment to surfaces. The hydrophobin Sc4 is known to provide a hydrophobic membrane lining of the gas channels within Schizophyllum commune fruiting bodies. Here, we cultivated non-fruiting, monokaryotic S. commune 12-43 on glass surfaces that could be verified by micrography. Differential gene expression profiling of nine hydrophobin genes and the hydrophobin-like sc15 gene by quantitative PCR showed significant up-regulation of sc4 when S. commune was attaching to glass surfaces, confirmed also with RNA-Seq data analysis. Another silicate, namely quartz sand, was investigated, and induction of sc4 was seen as well. The up-regulation of the hydrophobin gene sc4 may indicate involvement in S. commune hyphal attachment to glass as well as quartz surfaces. We propose that the covering of hyphae by Sc4 allows for direct interaction with the hydrophobic surfaces of silicates, and that differential functions of specific hydrophobin genes is depending on the surface interface involved. This study could help with the clarification of the biological functions of hydrophobins in natural surroundings, including hydrophobic surface attachment. Therefore, the analysis of growth on glass serves as a basis for understanding S. commune interaction with glass surfaces while providing the possibility to visualize the interaction microscopically.

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CS5.2.22

CRYPTIC DIVERSITY AND MICRO-NICHE SPECIALIZATION OF MICROBACTERIUM SPP. ASSOCIATED TO SERPULA LACRYMANS

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The dry rot fungus Serpula lacrymans, that causes significant damage in construction timber by decomposing cellulose and hemicellulose, offers a diverse set of microbial niches and is known to be accompanied by bacteria, although exact mechanisms of this association are largely elusive. We aimed to analyse the taxonomy and physiology of Microbacterium spp., which have been isolated from S. lacrymans fruiting bodies, mycelia and rhizomorphs, and to study synergistic or detrimental interactions between Microbacterium spp. and S. lacrymans. The genus Microbacterium contains about 300 species isolated from a variety of environments. All Microbacterium spp. isolates from S. lacrymans were characterised in their (i) ability to use carbohydrates, specifically those associated with wood-decay, (ii) growth adaptation in stressful environmental conditions, (iii) interaction with S. lacrymans (co-culture- or wood-degradation microcosm assays). Here we present 29 Microbacterium genomes, showing that 16S rRNA gene analysis fails to capture the diversity in the genus Microbacterium, and introduce 16 new Microbacterium species. We show that bacteria are equipped with a broad range of carbohydrate active enzymes and therefore should be able to use the wood resources becoming available during fungus-induced decay. Moreover, co-cultivation indicated that S. lacrymans reacts with a rather unspecialized chemical response to biotic stressors. Exploring cryptic species, i.e. two or more distinct species that are mistakenly classified as a single species due to morphological similarity, is especially important for bacteria, as they cannot be distinguished solely by morphology. Our results provide an overview on the diversity and ecology of fungus associated Microbacterium spp..

CS5.2.23

IDENTIFICATION OF EFFECTOR PROTEINS DURING INTERACTION OF MUSHROOM-FORMING FUNGI WITH THEIR COMPETITORS

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Mushroom-forming fungi are prone to pests and diseases, which can lead to devastating crop losses. To counteract predation, fungi have evolved several methods to defend themselves, including the secretion of effector proteins. However, few secreted proteins involved in defence have been identified in mushroom-forming fungi.
To elucidate the secreted arsenal of mushroom-forming fungi against their competitors, we determined gene expression during interactions between vegetative mycelium of the mushroom-forming fungus Schizophyllum commune and four fungal or bacterial competitors. Additionally, RNA-seq was performed on the mushroom-forming fungus Pleurotus ostreatus against a fungal competitor to study conserved defence responses.
Upregulation of transcripts encoding thaumatins and glycosyl hydrolases were observed as the main annotated groups for putative secreted proteins in S. commune, whereas redox-related proteins were predominantly present during interaction in P. ostreatus. However, most drastic changes in expression were observed in unannotated genes, which lack known domains. Moreover, there was a conserved response, since 31 and 21 orthologous secreted proteins of S. commune and P. ostreatus, respectively, showed a similar expression profile during interaction. These orthologues are all widely conserved among fungi.
The gene encoding the small secreted protein Tip1 was strongly upregulated during interaction, and we studied this gene in more detail. A reporter strain was generated by placing the red fluorescent dTomato gene under the control of the tip1 promotor. No fluorescence was observed during mono-cultivation, whereas strong fluorescence was only found during interaction. Furthermore, Tip1 was heterologously expressed and purified to analyse its function. Tip1 inhibited growth of fungal competitors, although its exact function is still unknown. Further investigation of function and regulatory network will contribute to the understandings of the defence system of mushroom-forming fungi.

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CS5.2.24

EXPLORING THE DIVERGENCE OF FUNCTIONAL AND EVOLUTIONAL INTERACTIONS BETWEEN FUNGI AND BACTERIA

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Fungi and bacteria comprise a large fraction of biomass in the soil and bacterial-fungal interactions are crucial for understanding the microbial ecosystem which is closely related to agriculture, medicine and the environment. The interactions between microbes promote the activation of cryptic biosynthetic pathways that produces secondary metabolites and other bioactive compounds that drive interactive dynamics.

Our recent study described the distinctive nature of the mutualistic relationship between the filamentous fungus Aspergillus nidulans and gram-positive bacterium Bacillus subtilis depicting evidence to show their spatial and metabolic interactions that facilitates the communication in between species to explore untraversed environmental niches and obtain nutrients. Confronting this interactive nature, the current study was comprised of co-culturing of 36 environmental fungal species and 22 bacterial species to further investigate the interaction dynamics in the co-cultures. Parameters such as the effect on the fungal growth, the affinity of the bacterial cells to the fungal hyphae, bacterial cell dispersal distance and the velocity of movement of bacteria were analyzed to define the phenotypic interaction specificity. Depending on the nature of interactions, the combinations were then classified into genres of positive, negative, and neutral. In depth analysis of the diversity of interaction specificity even among the species of Aspergillus species with Bacillus subtilis revealed that specific antagonism demonstrated in Aspergillus niger was directed primarily through Pyranonigrin A. Subsequently, selected combinations were subjected to LCMS/MS analysis and transcriptomic analysis to visualize their genomic potential and expression in coexistence compared to their monoculture state which would assist in defining the factors that help establish and maintain the relationships of the microbes in the microbial network setting.

This study intended to impart insights to the ecological context of interactions of the environmental microbiota and utilization of the metabolic capacity of the chemically prolific microorganisms.

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CS5.2.25

A CARNIVOROUS MUSHROOM PARALYZES AND KILLS NEMATODES VIA A VOLATILE KETONE

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Fungal predatory behavior on nematodes has evolved independently in several lineages. Pleurotus ostreatus, the basidiomycete oyster mushroom, is a nematophagous fungus that preys on nematodes when limited nutrients are available. Our previous study showed that when Caenorhabditis elegans contacted the mycelium of P. ostreatus, the toxins could enter through cilia, causing head muscle hyper-contraction and calcium influx in pharyngeal and body wall muscles, finally resulting in the necrosis process. However, the molecular identity of the nematode-paralyzing toxins produced by P. ostreatus remained unclear. To study this question, we conducted random mutagenesis forward genetic screens in P. ostreatus to isolate mutants that were unable to paralyze C. elegans. We generated ~12,000 UV and EMS-mutagenized clones and identified 22 P. ostreatus loss-of-toxicity (lot) mutants that lacked nematicidal activity toward C. elegans. Viewing the morphology of the lot mutants revealed that they all lack the spherical structures, toxocysts, on the hyphae. Toxocysts are fragile and lose nematicidal activity easily. Therefore, we hypothesized that toxins inside toxocysts are volatile. We conducted GC-MS and identified 3-octanone as a promising candidate for the nematicidal compound. 3-octanone treatment recapitulated the phenotypes of rapid calcium influx and neuronal cell death by targeting the cell membrane and disrupting membrane integrity in vivo and in vitro to trigger rapid cell death in C. elegans. Our work reveals that P. ostreatus develops a specific structure to concentrate a volatile ketone to disrupt the membrane integrity of C. elegans, resulting in rapid organismal cell death.

CS5.2.26

IMPACT OF ANAEROBIC DIGESTION RESIDUES (ESP. DIGESTATE) ON THE BIODIVERSITY AND PHYSICO-CHEMICAL COMPOSITION OF CULTIVATED SOILS

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Agricultural methanization is now booming. The residues of this process (digestate) can be used to replace all or part of the chemical fertilizers and also to valorize what was considered as an ultimate waste. However, no study has yet been conducted on the effect of digestate application on soil biodiversity, both fungal and bacterial. The objective of our research, conducted jointly with chemists (1) and biologists (2), is to highlight the effects of the application of digestate from anaerobic digestion on the microbial biodiversity and the metabolic capacities of soil microorganisms.
For this purpose, we will compare the results obtained on 2 agricultural soil test plots, one amended, the other not, which have been fertilized with digestates more or less recently, with controls from the same plots without treatment.
The samples were taken at two different depths (surface and sub-surface). Three approaches were implemented for the microbiological analysis: i macroscopic approach by cultivating microorganisms; ii a metabolic approach using BIOLOG® microplates; iii a global metagenomic approach.
In parallel, soil pH, heavy metals, inorganic and organic carbon and nitrogen composition are also analyzed.
Statistical analyses of the results show an impact on biodiversity as well as on the metabolism of bacterial communities. A taxonomic analysis has been performed and the detailed analysis of the sequences is still in progress. Particular attention will be paid to the fungal distribution which is characteristic of the good health of the soils studied.
(1) Shen L., et al. (2022). https://doi.org/10.1128/aem.02378-21.

(2) Dicko M., Ferrari R., et al. (2020) https://doi.org/10.3390/jof6040278

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CS5.2.27

DOES A BRASSICA NAPUS RESISTANCE GENE TO LEPTOSPHAERIA MACULANS INFLUENCE THE MYCOBIOTA STRUCTURE? A 2-YEAR CASE STUDY

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One of the main rapeseed pathogens, Leptosphaeria maculans, is mainly controlled by using cultivars with quantitative and/or specific resistance genes against the pathogen. The influence of resistant genotypes on the targeted pathogen is expected. However, this work considers the pathogen in a broader environment, interacting with the whole microbiota locally present. With new sequencing technologies, it is now possible to describe the microbiota associated with a pathogen and analyze how it can be modified by the plant genetic background and over time.

Here, we used metabarcoding sequencing of two barcode genes in duplex for fungal identification: the Internal Transcribed Spacer (ITS) region and the single-copy Actin gene . The generated datasets were used to describe the fungal mycobiota present on different rapeseed tissues sampled in fields (leaves, stems and residues) at key time points (sampling date) in the L. maculans life cycle over two cropping seasons. This work was done on isogenic rapeseed cultivars carrying or not the Rlm11 resistance gene.

The whole data set first demonstrated the complementarity of ITS and Actin barcodes to identity and quantify, respectively, fungal species, including the main rapeseed fungal pathogens. The dynamics of pathogens over each cropping season fits our knowledge about their epidemiology. The main factors structuring fungal community were the sampling date (SD), and the cropping season (CS).The plant genotype (PG) explained only a small percentage of fungal diversity variation. These two last effects (CS and PG) increased when the dataset anal-

ysed was divided per SD or CS. An alternation of species between the different ecological niches (leaves, stems, residues) was visible, with variations among the two sampling years.

In conclusion, the introgression of a resistance gene to a fungal pathogen in Brassica napus does not seem to be the main driver of the mycobiome structure whatever the ecological niche.

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CS5.2.28

NEW CLADES OF MYCOVIRUSES INFECTING THE OBLIGATORY BIOTROPH BREMIA LACTUCAE REPRESENTING DISTINCT EVOLUTIONARY TRAJECTORY FOR VIRUSES INFECTING OOMYCETES

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Recent advances on NGS approaches allowed a broad exploration of viromes from different fungal hosts, unveiling a great diversity of mycoviruses with interesting evolutionary aspects. Moreover, the majority of virome studies are focused on mycoviruses infecting true fungi, with less mycoviruses found and characterized in oomycetes, particularly in the obligatory biotrophs. Aim of our work was to characterize the virome associated to the lettuce downy mildew Bremia lactucae, which is an important biotrophic pathogen for lettuce and that was studied in details for the molecular aspects of the plant-pathogen interactions. Metatranscriptomic analysis and homology approaches allowed the identification of 13 new RNA viruses. Among the 13 viruses, we could identify 2 new negative sense ssRNA viruses related to the yueviruses; one example of a bipartite toti-like dsRNA virus which accumulates more negative strand RNA and could be a link to the origin of negative strand RNA from dsRNA; a new splipalmivirus; a positive sense ssRNA virus called Bremia lactucae associated ssRNA virus 1 which only shares homology with some flavi-like viruses recently detected in metatranscriptomic studies. Interestingly, a virus that we called Bremia lactucae associated ssRNA virus 2 shows an ORF encoding for a putative protein that doesn't show any hit against the NCBI database, but could find a hit against permutotetraviruses RdRP when investigating the folded structure prediction. Investigation of the ORFan contigs from the metatransciptome allowed the identification of a virus whose RdRP shows distant homology to a mycovirus previously characterized in Phytophthora infestans and two viral contigs encoding for putative proteins that can not be associated to a viral genome. The results obtained show a great diversity of viruses previously unreported for oomycetes and set up the basis to study tripartite interactions between plants, oomycetes pathogens and viruses using Bremia lactucae -lettuce as a model.

CS5.2.29

CATCHING ALTERED RELATIONSHIPS BETWEEN MYCOVIRUS AND ITS HOST FUNGI DURING SYMBIOSIS

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Mycoviruses are virus detected from fungi. They are clearly different from cell-disruptive viruses infecting human, plants or bacteria, because they have no infection machinery. Thus, mycoviruses are thought to persistently exist inside the host cells as symbionts. However, it is unclear whether specific interaction is established between the host and virus through co-evolution. To gain an insight into this, we used two Aspergillus flavus strains, IFM65241 and IFM65242 (designated as A and B, respectively), which have been isolated from a single patient 1 months apart. Based on genomic sequence, lineage of the strains is identical. Both are infected by partitivirus (named AflPVa or AflPVb), whose sequences are compared, and 3 nucleotides are altered. To compare the virus effects on the host physiology, we generated the virus-free strains from A and B by eliminating the viruses. Regarding colony growth, morphology, stress tolerance, and secondary metabolism, there were no significant differences between virus-infected and -free strains in both sets. However, transcriptome analysis revealed that no more than 500 genes are differentially expressed between them in both sets.
Next we reintroduced the viruses, AflPVa and AflPVb, into the parental host fungi as well as the counter host. First, introduction of the viruses showed no alterations in the host phenotypes in any combinations. However, gene expression was changed in all virus-transmitted strains compared with each virus free strain. Interestingly, the larger number of genes were differentially expressed in host B than A, when either of AflPVa or AflPVb was reintroduced. Transcriptome data also indicated the larger number of genes were differentially expressed in strains harboring AflaPVa than AflaPVb. These results suggested that host fungi B became more responsible mode than fungi A and AflaPVb became more stealth mode than AflaPVa.

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CS5.3 STRESS AND EXTREME ENVIRONMENTS

CS5.3.9

DIVERSITY OF FILAMENTOUS MICROBES IN SEDIMENTS OF BASQUE ESTUARIES: ASSESSMENT OF THE POTENTIAL TO PROCESS COMPLEX CARBOHYDRATES AND PRODUCE SECONDARY-METABOLITES

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Marine environments are home to a vast diversity of microorganisms. Fungi and bacteria within marine ecosystems contribute to ecological balance by playing critical roles in nutrient cycles and by shaping food webs. In this context, marine microbes developed genomic resources to adapt to stress conditions such as, e.g., high salt concentrations and nutrient scarcity, or to degrade complex polymeric substrates. These features make marine microorganisms a valuable source for the development of new biotechnological tools. Research on marine microorganisms mainly focused on bacteria, with a couple of hundreds of fungal species retrieved from marine environments, despite the fact that the kingdom fungi is composed of millions of species. Here, we focused on the isolation of filamentous microbes, using sediment samples collected in estuaries of the Basque Country, Bay of Biscay. Their phenotypic characterization led to the identification of strains potentially able to process complex polysaccharides or to produce secondary metabolites. Two isolates belong to the order of Hypocreales (Marquandomyces marquandii and Albophoma yamanashiensis), while a third one is a Streptomyces strain. Analysis and comparison of their CAZYme and secondary metabolite gene-cluster repertoires suggest that these isolates could be used as a source of novel enzymatic activities and secondary metabolites.

CS5.3.10

POLYMYCOVIRUS REGULATES STRESS RESPONSE AND VIRULENCE OF ASPERGILLUS FUMIGATUS

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Mycoviruses – are viruses that use fungal cells for proliferation. While some mycoviral infections are benign there are examples of mycoviruses having both beneficial and detrimental effects on their fungal host, such as alteration of fungal virulence and adaptation to challenging environments. Yet the molecular mechanisms driving mycoviral replication and their impact on fungal pathogens are poorly understood – especially the interplay between mycoviruses of human pathogenic fungi, the fungal host, and the mammalian host. Here, we study the biology of Aspergillus fumigatus polymycovirus-1M (AfuPmV-1M) – which is a natural habitant of Aspergillus fumigatus Af293, and the viral impact on the fungal stress response and cell fate during infection. To investigate the impact of AfuPmV-1M on its fungal host, we generated a congenic virus-cured strain and a re-infected strain.
We found that AfuPmV-1M enhances the pigmentation of the colonies and conidial viability over time. Moreover, AfuPmV-1M confers a survival benefit under oxidative stress and in the murine lungs leading to increased fungal virulence in a murine model of invasive aspergillosis. In agreement with these findings, mycoviral replication was elevated under oxidative, high/low/ pH, temperature stress, and during infection. Our results suggest that AfuPmV-1M may have a beneficial impact on fungal survival and adaptation under stress conditions. We propose that AfuPmV-1M hijacks fungal survival and stress pathways in order to survive and maintain reproduction, and thereby modify fungal virulence.

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CS5.3.11

QTL MAPPING FOR TEMPERATURE TOLERANCE IN THE WHEAT PATHOGEN ZYMOSEPTORIA TRITICI

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Global warming is expected to have adverse impacts on global agriculture, as it influences plant disease occurrence and severity. Understanding the genetic basis of adaptation to temperature in fungal plant pathogens is crucial to predict how pathogen populations will respond to warming climates and how they may impact agricultural systems in the future. Temperature can influence fungal fitness in multiple ways, by directly influencing their growth, virulence and reproduction, and also indirectly by influencing plant immune responses. In this study we used QTL mapping to identify large effect loci associated with tolerance to temperature stress in Z. tritici. The traits of colony size and colony melanization were measured in vitro across three temperatures (10, 18, 27oC) at 8 and 12 days post inoculation in 259 and 265 offspring from two separate crosses. We found a single QTL peak specific to adaptation to high temperature that contained several promising candidate genes, including a heat shock protein (Hsp90) that explained 21% of the variance associated with growth at high temperatures.

CS5.3.12

LANTHANIDE/ACTINIDE DECONTAMINATION BY FUNGI ACCUMULATION AND LOCALIZATION OF EUROPIUM IN THE FILAMENTOUS FUNGUS PODOSPORA ANSERINA

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In the environment, fungi play a major role due to their ability to degrade and absorb various compounds. Because their wide geographical distribution, abundance, capacity to accumulate heavy metals (i.e. cadmium and arsenic) and variety, they are considered as good bioindicators of environmental pollution. Fungi are also able to accumulate natural and anthropogenic radionuclides. These compounds are found in all compartments of the biosphere. Among these radionuclides, actinides are a major concern for modern nuclear societies. The complexity of their chemistry, in particular that of the lanthanides/actinides, leads to a poorly understood behavior in the environment.

Most of the few studies on radionuclide decontamination by fungi have focused on the determination of actinide concentrations in different fungal species and the speciation of actinides in fungi remains largely unknown. Moreover, as many fungal components are likely to interact with radionuclides, the molecular mechanisms involved in their accumulation and impact on fungal development have never been investigated.

Using radiochemical, biochemical and cytological approaches, we have undertaken the study of the speciation of actinides at different oxidation levels during different fungal developmental stages in order to understand the mechanisms of transfer and accumulation. The study focus on the accumulation of europium (Eu), an analogue of trivalent lanthanides (the most reactive rare earth elements) in the filamentous fungus Podospora anserina, used as a model system. We will present some promising results on Eu accumulation, localization and speciation in P. anserina. In particular, by a fluorescence microscopy approach, we are now able to follow the accumulation of Eu in different cellular compartments, and showed an accumulation in asci during their maturation.

One of the strategies developed by fungi to survive in environments polluted by heavy metals is to accumulate the excess toxic compound in the spores, thus protecting the rest of the fungal colony.

CS5.3.13

IMPORTANCE OF THE ERGOSTEROL BIOSYNTHETIC PATHWAY INTEGRITY FOR THE CANDIDA GLABRATA TOLERANCE TO ENVIRONMENTAL STRESSES

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Candida glabrata is an opportunistic fungal pathogen associated with life-threatening systemic infections in immunocompromised individuals. C. glabrata is outstanding in its capacity to rapidly develop resistance to antifungal drugs and its tolerance to various environmental stresses. In this work we studied the impact of ergosterol biosynthesis disruption on C. glabrata response to various stresses. We disrupted the CgERG6 gene and prepared the deletion mutant appointed as Cgerg6Δ. The CgERG6 gene deletion was accompanied by accumulation of specific sterol precursors. The absence of sterol transmethylation reaction affects C. glabrata response to various stresses: oxidative, cell wall, osmotic and weak acid stress. Cgerg6Δ deletion strain exhibits increased sensitivity to hydrogen peroxide and diamide compared with its parental strain. qRT-PCR analysis showed increased expression of CgCTA1 and CgMSN4 genes together with decreased expression of CgYAP1 gene in the Cgerg6Δ mutant. Deletion of CgERG6 leads to increased susceptibility of C. glabrata to compounds interfering with cell wall (caspofungin, congo red, caffeine). Decreased expression of CgFKS1, CgFKS2 and CgCRZ1 genes can explain the susceptibility of Cgerg6Δ strain to cell wall inhibitors. The absence of the CgERG6 gene also affects the capacity of mutant cells to grow in the presence of osmotic and weak acid stresses. qRT-PCR analysis showed the inability of weak acid to induce the expression of CgPDR12 gene in Cgerg6Δ mutant. Our observations point to changes in the plasma membrane properties or in the high osmolarity glycerol signaling pathway in Cgerg6Δ strain. Taken together, our results indicate that the absence of CgERG6 gene and concomitant accumulation of sterol intermediates significantly affects C. glabrata tolerance to various stresses.

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CS5.3.14

THE ROLE OF REACTIVE OXYGEN SPECIES SCAVENGING ENZYMES IN COLD TOLERANCE OF POSTHARVEST PHYTOPATHOGENIC FUNGI

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Postharvest fungal pathogens are a significant threat causing loss of fresh fruits and vegetables estimated at approximately 30 percent of total crop yield worldwide. Low-temperature (LT) storage is an efficient practice to prolong the postharvest performance of perishable crops with a minimal negative impact on human health and the environment. LT reduces cellular respiration and metabolic activities, which delay fruit ripening and senescence and arrest the growth of pathogenic microorganisms. However, some phytopathogenic fungi are highly tolerant to LT conditions and can develop during cold storage, causing fruit deterioration. To better understand the effect of LT conditions on fungal physiology and pathogenicity, two economically important postharvest pathogens were studied: the psychrotolerant Botrytis cinerea, and the mesophilic Colletotrichum gloeosporioides. To analyze the molecular basis of fungal tolerance to LT, we characterized both fungi's morphological and physiological responses to a downshift in temperature. Samples were collected before and during 72 hours of a time-course cold shift experiment (22□C to 5□C). These samples were subjected to morphological characterization, RNAseq, and enzymatic activity assays, which all indicated a rapid response to the cold stress (within 1-2 hours). In both fungi, LT exposure generated oxidative stress resulting in elevated levels of reactive oxygen species (ROS) and specifically H2O2. This ROS burst was accompanied by rapid induction of antioxidant enzymatic defense activity in B. cinerea (especially Catalases), whereas C. gloeosporioides showed a delayed response. These results suggest that while both fungi have the metabolic capability to handle ROS stress, variation in regulation may account for the differences in growth and pathogenicity between the two fungi during cold storage. These findings demonstrate how environmental factors like LT can influence fungal morphology, ROS production, recognition, and detoxification of ROS, potentially affecting pathogen survival and the epidemiology of postharvest diseases.

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CS5.3.15

MAGNETIC AND ELECTRIC FIELDS INDUCE PRIMING OF METARHIZIUM ROBERTSII TO STRESS

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Little is known about the phenotypic effects caused by magnetic and electric fields on fungal mycelial growth and its priming responsiveness on conidial tolerance to different stress conditions. In this study, conidia of the insect-pathogenic fungus Metarhizium robertsii were produced on 1) potato dextrose agar medium (PDA = control), 2) under nutritional stress on minimal medium (MM), and on PDA medium under 3) magnetic field (MF) and 4) electric field (EF). All four treatments were incubated in the dark. The tolerances of conidia produced on these conditions were evaluated in relation to oxidative and osmotic stress, heat, and UV-B radiation. The cultures of the fungus grown on the PDA medium under magnetic and electric fields were similar to the fungus grown on the control PDA medium; however, the three treatments produced more conidia than the fungus produced on the minimal medium. M. robertsii conidia produced under magnetic and electric fields were more tolerant to oxidative and osmotic stress, heat, and UV-B radiation. Both treatments – MF and EF – produced conidia with similar tolerances to all stress conditions, except for osmotic stress, where conidia from MF were more tolerant than conidia from EF. On the other hand, conidia produced under magnetic and electric fields were less tolerant than conidia produced on minimal medium, which causes nutritional stress. In conclusion both magnetic and electric fields induced priming on Metarhizium robertsii but not at the same level as nutritive stress.

CS5.3.16

UNDERSTANDING THE ROLE OF DHN MELANIN IN CRYOMYCES ANTARCTICUS

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Cryomyces antarcticus – a cryptoendolithic black fungus endemic to Antarctica – is taxonomically classified in phylum Ascomycota, class Dothideomycetes incertae sedis. C. antarcticus has shown high capability to survive extreme environmental conditions like those found in space (ionizing radiation, vacuum, microgravity), thus fueling fundamental astrobiological questions like "searching for life beyond Earth" (Onofri et al. 2020, Extremophiles Astrobiol Model). Its extraordinary resilience has been attributed to the presence of thick, highly melanized cell walls, which may contain both DHN and DOPA melanins (Pacelli et al. 2020, Appl Microbiol Biotechnol). To better understand the contribution of DHN melanin to the overall resilience of C. antarcticus, we initially adopted chemicals e.g., tricyclazole to inhibit the DHN melanin synthetic pathway; however, these studies gave inconclusive results. Eventually, we decided to generate melanin-deficient mutants by genetic engineering. Using the genetic toolkit developed for the black fungus Knufia petricola (Voigt et al. 2020, Sci Rep; Erdmann et al. 2022, Front Fungal Biol), we designed a strategy for mutating the key enzyme (polyketide synthase)-encoding gene capks1 by transient delivery of Cas9 and capks1-specific sgRNA from AMA-containing plasmids and PCR-generated donor DNA i.e., resistance cassettes flanked by ~75-bp-long sequences homologous to capks1. For this, the melanin-PKS encoding ortholog was identified in the C. antarcticus CBS 116301 genome (mycocosm.jgi.doe.gov) and used to design primers for re-sequencing of the capks1 locus in the strain CCFEE 515. Transformation of C. antarcticus is challenging because of its very slow growth; we expect that 4-6 months are needed from obtaining enough biomass for cell wall lysis until transferring putatively resistant transformants for genotyping. Important parameters were evaluated: protoplasts can be generated, and they survive the transformation procedure, and suitable concentrations of selective agents have been identified. Nowadays, we are waiting for the first C. antarcticus mutants considered to be deficient in DHN-melanogenesis.

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CS5.3.17

SYSTEMATIC COMPARATIVE PHENOTYPIC SCREENING OF CLINICAL ISOLATES OF HUMAN PATHOGENIC CANDIDA SPECIES

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Candida species are associated with humans and animals. Host persistence requires phenotypic adaptation and flexibility. We used a collection of 1366 clinical isolates to explore the inter- and intra- phenotypic variation of 13 species of human associated Candida. We performed a high-throughput quantitative analysis of fitness under environmental stress conditions, antifungal drugs and biofilm formation. Three major phenotypic clusters emerged consisting of Heat-resistant Fast Growers, Osmo-Sensitives and Slow Growers which could be further divided into species enriched sub-clusters. Comparative correlation analysis detected general and species-specific phenotypic properties. Among other insights, we found a considerable phenotypic variation among the genetically uniform C. parapsilosis isolates. Another striking example was the diverse phenotypic response to environmental stresses of the genetically closely related C. dubliniensis and C. albicans isolates. Our quantitative phenotypic analysis method proved suitable to establish the shape of the phenotypic landscape of the Candida clade.

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CS5.3.18

CENOCOCCUM GEOPHILUM, AN EMERGING MODEL FOR STUDYING HOST-SYMBIONT INTERACTIONS AND ADAPTATION TO DROUGHT IN ECTOMYCORRHIZAL FUNGI

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Recent extreme climatic events, such as extended drought periods, impose serious threats to the functioning of forest ecosystems. These may be overcome at one or multiple levels of biological organisation, all of which ultimately contribute to the resilience of forest ecosystems to climate change. To date, the role of symbiotic plant–fungus interactions in response to drought is poorly understood and its assessment requires an integrative framework linking genotypic, phenotypic, and environmental data from natural populations as well as experimental validation of in situ observations. One of the most abundant ectomycorrhizal fungi in forest ecosystems throughout the world is Cenococcum geophilum, a generalist ascomycete that associates with most tree species in various soil types, enhancing nutritional status of its host trees, and tolerating adverse environmental conditions such as water scarcity or high temperature. Here, we highlight the potential role of C. geophilum in water and nutrient supply of host trees and its resilience to such environmental extremes. We then discuss whether natural C. geophilum populations are locally adapted and how different genotypes affect the resistance and resilience of trees facing climate change. Predicting maladapted fungal populations under future climates is critical to help establish tomorrow's forests.

CS5.3.19

FUNCTIONAL CHARACTERIZATION OF LBCDF-A AND LBCDF-B, TWO METAL TRANSPORTERS OF THE ECTOMYCORRHIZAL FUNGUS LACCARIA BICOLOR

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Heavy metal contaminated soils have a negative impact on plant growth and production. Through ectomycorrhiza (ECM), a mutualistic symbiosis between soil-borne fungi and the plant roots, both partners can survive and thrive in these polluted environments. Organisms are obliged to take up specific heavy metals, such as the essential micronutrient Zn, for its structural and enzymatic role in the cell. However, a high concentration will become toxic. Therefore, the availability of cytoplasmic Zn should be tightly regulated. Transmembrane transport plays a crucial role in this process. The molecular mechanisms underlying Zn homeostasis in the model organism Laccaria bicolor are poorly understood. We hypothesized that L. bicolor CDF proteins transport Zn across cellular membranes. In this study we aimed to determine the metal specificity and subcellular localization of two of these transporters. First a phylogenetic tree of CDF transporters was constructed to predict the function of the putative transporters and guide wet lab experiments. Almost all of the putative transporter sequences clustered together with a previously characterized CDF transporters from Saccharomyces cerevisiae, except for the proteins LbCDF-A and LbCDF-B. These were targeted for further investigation by heterologous expression in S. cerevisiae and its mutants. Drop-out assays were performed and translational fusion proteins with GFP were visualized using microscopy. Besides, expression levels in response to Zn availability were assessed in L. bicolor by qPCR and illustrated their specific function in Zn homeostasis. All together, these results provide insights into how the environment and in particular Zn pollution is impacting on ECM fungi and open pathways for the development of applications in the bioremediation of waste land.

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CS5.3.20

COMPREHENSIVE FUNCTIONAL ANALYSES OF THE BZIP TRANSCIPTION FACTORS ATFA AND ATFB IN ASPERGILLUS NIDULANS

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The bZIP transcription factors AtfA and AtfB regulate secondary metabolism, sexual development and stress response pathways in Aspergilli.
To examine the physiological functions of atfA and atfB we have generated the deletion (Δ) and overexpression (OE) mutants in all combinations and investigated their phenotypes in Aspergillus nidulans.
Based on stress sensitivity studies with agar plate assays, the ΔatfAatfBOE, ΔatfAΔatfB, atfAOEatfBOE mutants showed increased sensitivity to the oxidative stress elicited by diamide. The ΔatfA mutant was sensitive to MSB, whose sensitivity could be alleviated by atfBOE. The atfAOE, atfBOE, and atfAOEatfBOE mutants showed increased tolerance to tBOOH, whereas the ΔatfA and ΔatfAΔatfB mutants were sensitive to tBOOH. The atfAOEatfBOE mutant showed increased tolerance to NaCl. Hyphal growth of the ΔatfB mutant was significantly reduced in the presence of NaCl, yet it was the most tolerant to sorbitol. Upon heavy metal stress, vegetative growth of the ΔatfAatfBOE mutant was slightly reduced, and the atfBOE, atfAOEatfBOE mutants showed sensitivity to CdCl2. The cell wall stress inducing CongoRed affected only the ΔatfA mutant, where moderate tolerance was observed.

Production of the mycotoxin sterigmatocystin (ST) was decreased in the ΔatfAatfBOE, ΔatfBatfAOE, and atfAOEatfBOE mutants. The absence of atfA resulted in the loss of ST production, whereas, intriguingly, the ΔatfAΔatfB mutant was able to produce ST. Notably, the atfBOE mutant produced larger asexual spores. The conidiospores of ΔatfAatfBOE and ΔatfBatfAOE mutants showed increased viability, whereas the ΔatfB spores exhibited reduced viability under 50oC thermal stress. The number of conidiospores for ΔatfA, ΔatfAΔatfB, atfBOE, ΔatfAatfBOE mutants reduced, while for the atfAOE and ΔatfBatfAOE mutants increased. The ΔatfA, ΔatfAΔatfB mutants were unable to produce sexual fruiting bodies, whereas the ΔatfB and ΔatfBatfAOE mutants produced cleistothecia in larger quantities.
To test the hypothesis of AtfA and AtfB heterodimer formation in vivo, the bi-molecular fluorescence complementation experiment will be performed.

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CS5.3.21

BIOREMEDIATION USING NATIVE MICROBIAL COMMUNITIES - FUNGAL AND BACTERIAL OIL DEGRADATION FOR BIOREMEDIATION PURPOSES AT ACTIVE MINES IN NORTHERN SWEDEN

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When oil spills occur, remediation methods are employed to mitigate the negative effects, for example soil excavation or the usage of dispersants, which are very resource demanding. Newer methods are looking into bioremediation, using fungi and/or bacteria, to remove oil pollutants. New studies have looked at using certain microbes for direct bioremediation methods and enzyme production, highlighting industrial applications. Research shows that the most applicable and useful microbes for bioremediation purposes are the ones adapted to the environmental conditions of the site of the spill, but further research is needed to fully understand and describe the potential of the individual communities. Several strains of fungal species have been shown to be able to degrade petroleum products and oils, for instance Aspergillus niger and certain strains of Fusarium oxysporum. A number of bacteria are also capable of degrading and/or facilitate the degradation of oils. The Kiruna mine is the world's largest underground iron ore mine. The mining industry relies on the use of oil and oil products. By introducing oils into an underground abiotic environment, the proportion of carbon available in the oils is high in comparison other carbon sources. In addition to this, the mine also has high concentrations of metals in the industrial process water. We hypothesize that, having adapted to the extremes of oil pollutants and high heavy metal concentrations, the exposure to pollutants and oils in an industrial and abiotic environment has led the microbial community in the Kiruna mine to adapt to degrading and using the nutrients in the otherwise harmful pollutants. With this project, using a multi-omics approach, comparative genomics and growth experiments, we aim to describe the microbial community in the mine, the activity and rate of degradation of the microbial community, and to create an in-situ bioremediation method for the mining industry.

CS5.4 REGULATORY NETWORKS

CS5.4.9

TRANSCRIPTION FACTOR RAP1 REGULATES CELL WALL INTEGRITY AND HOST-PATHOGEN INTERACTION IN CANDIDA ALBICANS

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Candida albicans is an important fungal pathogen. The cell wall of C. albicans plays a crucial role in maintenance of cellular integrity as well as interaction with the host cells. Repressor activator protein 1 (Rap1) is a multifunctional protein involved in different cellular processes in Saccharomyces cerevisiae and mammals, including telomeric and nontelomeric functions. However, the role of Rap1 in C. albicans are poorly characterized. To explore nontelomeric functions of C. albicans Rap1, the rap1Δ/Δ mutant and RAP1-reintegrated strain were constructed. We found that the deletion of the RAP1 gene altered cell wall properties, composition, and gene expression. Moreover, the deletion of RAP1 affected biofilm formation and host-C. albicans interactions. Finally, the rap1Δ/Δ mutant attenuated C. albicans virulence in a Galleria mellonella infection model. Collectively, this study provides insights into functions of Rap1 in cell wall maintenance and virulence.

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CS5.4.10

RIMO (SRRB) IS REQUIRED FOR CARBON STARVATION SIGNALING AND PRODUCTION OF SECONDARY METABOLITES IN ASPERGILLUS NIDULANS

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Depending on the prevailing environmental, developmental and nutritional conditions, fungi activate biosynthetic gene clusters (BGCs) to produce condition-specific secondary metabolites (SMs). For activation, global chromatin-based de-repression must be integrated with pathway-specific induction signals. Here we describe a new global regulator needed to activate starvation-induced SMs. In our transcriptome dataset, we found locus AN7572 strongly transcribed solely under conditions of starvation-induced SM production. The predicted AN7572 protein is most similar to the stress and nutritional regulator Rim15 of Saccharomyces cerevisiae, and to STK-12 of Neurospora crassa. Based on this similarity and on stress and nutritional response phenotypes of A. nidulans knock-out and overexpression strains, AN7572 is designated rimO. In relation to SM production, we found that RimO is required for the activation of starvation-induced BGCs, including the sterigmatocystin (ST) gene cluster. Here, RimO regulates the pathway-specific transcription factor AflR both at the transcriptional and post-translational level. At the transcriptional level, RimO mediates aflR induction following carbon starvation and at the post-translational level, RimO is required for nuclear accumulation of the AflR protein. Genome-wide transcriptional profiling showed that cells lacking rimO fail to adapt to carbon starvation that, in the wild type, leads to down-regulation of genes involved in basic metabolism, membrane biogenesis and growth. Consistently, strains overexpressing rimO are more resistant to oxidative and osmotic stress, largely insensitive to glucose

repression and strongly overproduce several SMs. Our data indicate that RimO is a positive regulator within the SM and stress response network, but this requires nutrient depletion that triggers both, rimO gene transcription and activation of the RimO protein.

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CS5.4.11

REGULATION OF EFFECTOR-GENE EXPRESSION AS CONCERTED WAVES IN LEPTOSPHAERIA MACULANS: A TWO-PLAYERS GAME

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Leptosphaeria maculans is a phytopathogenic fungus causing stem canker on oilseed rape (Brassica napus), on which it displays a complex lifecycle. As for other host-interacting fungi, effector-genes (encoding sets of molecules involved in manipulation of the host immune defense system allowing for spreading into the host) are specifically expressed during infection. So far, all effector-genes cloned in L. maculans are located within repeat-rich regions of its genome. We have previously shown that i) effector-genes are associated with the repressive histone modification H3K9me3 during axenic culture, ii) a local removal of H3K9me3 was a pre-requisite for their specific induction in planta, yet iii) removal of H3K9me3, through inactivation of the lysine methyltransferase KMT1, does not induce expression of effector-genes at the same level as observed during infection of oilseed rape. Our hypothesis is that, although the control of H3K9me3 localization is key to regulate expression of effector-genes, a second layer of regulation, such as action of a transcription factor (TF), is involved in the tight coordination of effector-gene expression. We investigated the involvement of Pf2, a TF belonging to the fungal specific Zn2Cys6-family, in the control of effector-gene expression, and showed its concerted role in the regulation of pathogenicity, together with KMT1. Notably, transcriptomic analyses revealed an enhanced effect of the over-expression of LmPf2 on effector-gene expression in a Δkmt1 background. Thus, LmPf2 and KMT1 would have an antagonistic role: KMT1 inhibiting and LmPf2 activating the expression of effector-genes. Our work unraveled a dual layer control of pathogenesis and effector-gene expression, involving a chromatin-based control and a TF-regulation.

CS5.4.12

NEW REGULATORS INVOLVED IN CAROTENOID BIOSYNTHESIS IN FUSARIUM FUJIKUROI

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Fusarium fujikuroi is a model organism in the regulation of secondary metabolism, including the biosynthesis of neurosporaxanthin, a xanthophyl with antioxidant properties. Carotenoid biosynthesis is induced by environmental signals that include light and abiotic stresses. Three structural genes of the pathway (carRA, carB and carX) are linked and coregulated with carO rhodopsin gene. The white-collar complex is a positive regulator of these genes, with a global effect on the transcriptome, and the ubiquitin ligase CarS is a negative regulator whose mutation provokes carotenoid overproduction and pleiotropic effects. This communication summarizes our recent progress in the study of the genes that control the activity of this biosynthetic pathway. Upstream of the carS locus, a long non-coding RNA, carP, was found. Knock-out mutants of carP are albino. This lncRNA may be acting on cis, as the complemented strains recover the ability to produce carotenoids only when the gene is inserted in its native locus but not when is ectopically integrated. In a screening for transcription factors bound to to carB and carRA promoters, proteins belonging to the HMG-box family were detected. Deletion of one of them, hmgC, confirmed a regulatory role in carotenogenesis. Finally, the role of a gene for a zinc finger transcription factor, carZ, adjacent to the carX gene, was investigated. According to available RNAseq data, carZ mRNA levels are affected by signals that also regulate carotenogenesis, such as nitrogen starvation and light, suggesting its participation in the regulation of the car cluster. Knock-out mutants and carZ overexpression strains show alterations in the accumulation of carotenoids, indicating the participation of CarZ in their regulation. Although the changes were not drastic enough to attribute a central role to CarZ on its regulation, the data support that this transcription factor is an additional element participating in the complex regulation of carotenoid biosynthesis.

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CS5.4.13

THE ROLE OF OSAA GENE IN ASPERGILLUS FUMIGATUS DEVELOPMENT AND VIRULENCE

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Aspergillus fumigatus is ubiquitous, saprophytic, and an important opportunistic pathogen. It is the leading cause of aspergillosis, affecting particularly immunocompromised individuals. In search for novel genetic targets against aspergillosis infections, we studied the WOPR transcription factor OsaA in A. fumigatus. We found OsaA to be conserved in Ascomycota. In the model fungus A. nidulans, the OsaA homolog was shown to orchestrate both sexual and asexual development, repressing formation of fruiting bodies and activating conidiation. Our present study revealed that A. fumigatus OsaA is necessary for proper growth and also for conidiation, as in the case of A. nidulans. Interestingly, deletion or overexpression of the osaA gene resulted in alterations in secondary metabolism, including changes in gliotoxin production. Furthermore, absence of OsaA in A. fumigatus decreases mortality rate when using Galleria mellonella as animal model system.

CS5.4.14

A ROLE OF THE TRANSCRIPTION FACTORS ACUK AND ACUM IN SIDEROPHORE BIOSYNTHESIS OF ASPERGILLUS FUMIGATUS.

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Iron is essential for the mold Aspergillus fumigatus, which employs two high-affinity uptake systems: reductive iron assimilation (RIA) and siderophore mediated iron acquisition (SIA), the latter of which has been shown to be crucial for virulence in diverse infection models. Previously, the Aspergillus fumigatus transcription factors required for activation of gluconeogenesis, AcuK and AcuM, have been implicated in iron regulation in A. fumigatus Af293, but the underlying rationale remains unclear.
To further investigate the potential role of AcuK und AcuM in iron regulation we analyzed growth, siderophore production and expression of iron-regulated genes in respective gene deletion mutant strains in A. fumigatus A1160+ using minimal medium with glucose as carbon source and either glutamine or ammonium as nitrogen source.
Expression of the investigated iron-regulated genes involved in RIA and SIA was largely unaffected by lack of AcuK or AcuM, which contrasts previous studies. However, lack of either AcuK or AcuM decreased extracellular siderophore production during iron starvation and decreased biomass formation in liquid culture under both iron sufficiency and to a lower degree under iron starvation. In agreement with a role of gluconeogenesis in adaptation to iron starvation, transcript levels of malate synthase-encoding acuE, which depends on activation by AcuK and AcuM, was found to be upregulated by iron starvation.
Taken together, this study suggests that neither AcuK nor AcuM is directly involved in transcriptional regulation of genes involved in RIA or SIA in A. fumigatus A1160+. Therefore, the previously published findings might indicate strain-specific effects. Similar to the the reduced biomass formation in liquid culture, reduction of siderophore production caused by lack of AcuK or AcuM appear to be caused indirectly by dysfunctional metabolic pathways such as gluconeogenesis, TCA cycle, synthesis of or production of pyruvate or acetyl-CoA

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CS5.4.15

REGULATION OF XYLANASE GENE EXPRESSION IN ASPERGILLUS NIGER

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The expression of xylanases in Aspergillus niger is tightly controlled by the Zn(II)2Cys6 transcription factor XlnR. One of the target genes of XlnR is xlnB, encoding a beta-xylanase. Furthermore, the expression of xylanases is under carbon catabolite repression mediated by the transcriptional repressor protein CreA.
In this study, the xlnB promoter region was used to create an acetamidase (AmdS)-based reporter strain to study the transcriptional activation of xlnB. Growth analysis of the reporter strain containing the PxlnB-amdS construct on acetamide containing medium confirmed that the amdS gene was specifically expressed in the presence of xylan and xylose in an XlnR dependent way. In addition, the creA gene was deleted in the reporter strain to further examine the role of carbon catabolite repression on the expression of xlnB.
Both reporter strains were UV-mutagenized to obtain mutants showing constitutive expression of xylanases in the absence of an inducer. Five and four mutants were isolated in both backgrounds, respectively, and the constitutive expression of xylanase genes was confirmed by AZCL-xylan plate assays. Sequencing of the xlnR gene in these mutants revealed that only one of the four mutants obtained in the ΔcreA mutant background contains a mutation in the xlnR gene. Interestingly, the mutation is at a position in the xlnR gene which results in a valine to phenylalanine mutation at position 756 in the XlnR protein. Constitutive mutations at this position were also obtained recently in our lab (see abstract of Rahnama et al.) and by Hasper et al. 2004.
For one of the constitutive xylanase mutants obtained in the CreA+ background, we show that the constitutive expression of xylanases is independent of the XlnR transcription factor via AZCL-xylan plate assays and gene expression studies.

CS5.4.16

NOVEL INSIGHTS INTO THE ROLE OF THE HISTONE VARIANT H2A.Z IN THE PLANT PATHOGEN FUSARIUM FUJIKUROI

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Fusarium fujikuroi is a pathogen of rice and as such well known for inducing hyperelongation of internodes (so-called bakanae) that is tightly associated with the production of the phytohormones gibberellins by the fungus. Although, recently, another pathotype displaying a distinct chemotype (no gibberellins) was described that resulted in severe 'stunting' of the infected crop plants. Noteworthy, while both pathotypes largely harbor the same set of biosynthetic gene clusters involved in the production of these small molecular-weight compounds, expression levels thereof differ significantly. In this regard, gene regulation by chromatin structure is plausible.
To alter gene expression the chromatin structure must be dynamic, and different mechanisms to expose or silence underlying genes are known. These include histone post-translational modifications, ATP-dependent chromatin-modifying complexes, and the so far only poorly understood histone variants.
Here, we functionally characterized the highly conserved histone variant H2A.Z in a member of the economically relevant Fusarium fujikuroi species complex. By a combination of RNA- and ChIP-sequencing, and the assessment of the nucleosome position (MNase-seq), we give novel insights on the genome-wide distribution of this prominent variant and its correlation with gene expression as well as with relevant histone marks (i.e., H3K4me3 and H3K27me3). We have previously shown that deletion of H2A.Z is lethal in F. fujikuroi. By generating an inducible knock-down strain, we were now able to functionally characterize H2A.Z. Reduced levels of H2A.Z resulted in a severe phenotype, including significant changes in the transcriptome.

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CS5.4.17

CONSTITUTIVE EXPRESSION OF XYLANASES IN ASPERGILLUS NIGER

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The xlnR gene of Aspergillus niger encodes a Zn(II)2Cys6 transcription factor required for the induction of genes encoding xylanases and xylosidase. One of the target genes of XlnR is xlnD, encoding a beta-xylosidase. Previous RNA expression studies have shown that the xlnD gene is specifically induced by xylan or xylose in a XlnR dependent way.
In this study we have used the xlnD promoter region to create a reporter strain to isolate mutants with constitutive expression of xylanases in A. niger. To obtain these mutants the xlnD promoter region was fused to the acetamidase gene (amdS) and the reporter construct was transformed to A. niger. Growth analysis of the reporter strain on acetamide containing medium confirmed that the amdS gene was specifically induced by xylan and xylose in a XlnR dependent way. Spores of the PxlnD-amdS reporter strain were UV mutagenized and constitutive mutants were selected based on their ability to grow on acetamide (as nitrogen source) and fructose (as non-inducing carbon source). In total five independently obtained constitutive mutants were isolated and the constitutive expression of xylanases in those five mutants was confirmed by AZCL-xylan assays. Sequencing of the xlnR gene in those five mutants revealed that all five mutants have exactly the same mutation in the xlnR gene resulting in a valine to phenylalanine mutation at position 756 in the XlnR protein. This mutation has also been earlier identified in a screen for constitutive XlnR mutants (Hasper et al., 2002) and suggests that this amino acid position is highly preferred as a position leading to constitutive activated XlnR.
Using CRISPR/CAS9 mediated genome editing we confirmed that reintroduction of the XlnRV756F mutation results in a constitutive phenotype. Experiments to evaluate whether also other amino acid changes at the 756 position result in a constitutive phenotype are currently conducted.

CS5.4.18

NOVEL APPROACH FOR DISCOVERING TRANSCRIPTION FACTORS CONTROLLING FUNGAL PLANT BIOMASS CONVERSION

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In nature, many fungi rely on plant biomass as a carbon source. Fungal plant biomass conversion (FPBC) plays a key role in the global carbon cycle and industrial production of food, enzyme, biofuel and biochemicals. Typically, an efficient FPBC system consists of four crucial aspects: extracellular enzymatical degradation of plant polymers, transport of the small sugars mediated by transporters, intracellular enzymes for metabolizing these sugars, and transcription factors (TFs) for activating or repressing the expression of related genes. The extracellular degrading enzymes, sugar transporters and metabolic enzymes can be reliably predicted by searching for homologous genes or conserved domains. However, the identification of TFs is more challenging due to their large sequence and functional diversity across fungi kingdom. So far, only around 20 Ascomycete and two Basidiomycete TFs related to FPBC have been experimentally characterized. Therefore, a novel approach that can accurately and efficiently predict the FPC related TFs without relying on sequence homology is urgently needed.

To bridge this knowledge gap, in this project we have developed a novel approach for screening of FPBC related TFs based on reconstructing gene regulatory networks (GRN) and enrichment analysis of crucial FPBC genes in GRN. Applying this method on big transcriptome data of the model Ascomycetes fungus Aspergillus niger, we identified 15 FPBC related TFs, including seven previously known ones out of 558 TF encoding genes in the A. niger genome. Another proof of concept study was performed on transcriptome data of the Basidiomycete Dichomitus squalens, which predicted 48 PBC related TFs. The orthologs of the known Basidiomycete TFs related to FPBC, Roc1 and PacC, were successfully predicted. Our study provides a bioinformatics framework for predicting novel TFs through computational analysis of GRN on large transcriptome data, and demonstrates its tremendous potential in prioritizing candidate genes for further experimental characterization.

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CS5.4.19

EXPERIMENTAL VALIDATION OF GENE REGULATORY NETWORKS IN BOTRYTIS CINEREA DURING CONFRONTATION ASSAYS WITH TRICHODERMA ATROVIRIDE

Nicolas Arias[1], Consuelo Olivares-Yañez[1], Gabriel Pérez-Lara[1], **Paulo Canessa**[1]

[1]*Universidad Andres Bello, Santiago, Chile*

Recent advances in genomics technologies have resulted in rich datasets containing global gene expression information for different organisms of interest under diverse conditions. When integrated with pure bioinformatics approaches, researchers can generate dependable hypotheses that can be later experimentally validated. With this idea in mind, we recently developed a reference Gene Regulatory Network (GRN) for Botrytis cinerea and Trichoderma atroviride that serves that purpose for both fungal communities: it predicts regulatory interactions between transcription factors (TF) and their regulated target genes. When fed in with context-specific differentially expressed genes (DEGs), e.g., from the “contact zone” of a dual RNA-seq of a Botrytis-Trichoderma confrontation assay, the network predicted several TFs commanding the expression of genes whose biological function appears relevant in the context of a mycoparasitic interaction. In particular, in the phytopathogen’s network, 4 clusters of genes regulated by the same number of TF appear as key regulators that trigger and/or command defense mechanisms against Trichoderma. To evaluate the predictive value of the network, we generated a knockout mutant for each TF. For example, the loss-of-function mutant of the Zinc-finger TF Bcin07g06800, the most connected TF of the former GRN, displays more susceptibility to Trichoderma, as revealed by in plate confrontation assays after seven days of co-cultivation. RNA-seq experiments are underway to determine the transcriptional impact of the TF mentioned above, which will also allow us to better understand the predictive value of the generated GRN.

CS5.4.20

DNA-BINDING SEQUENCES OF THE TRANSCRIPTION FACTOR FLBC INVOLVED IN THE REGULATION OF ASPERGILLUS ORYZAE GENES SPECIFICALLY EXPRESSED IN SOLID-STATE CULTURE

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Aspergillus oryzae, a koji mold used in traditional fermentation industries in Japan, produces a large amount of amylolytic and proteolytic enzymes required for the production of sake, shoyu, and miso. Among these enzyme genes, the glucoamylase gene glaB, the acid protease gene pepA, and the neutral protease gene nptB are specifically expressed in solid-state culture with rice or wheat bran as the substrate. We have previously shown that the transcription factor FlbC is involved in the transcriptional regulation of those genes specifically expressed under solid-state culture condition in A. oryzae (1). In this study, we aimed to identify the DNA-binding sequences of FlbC in those promoter regions. MEME analysis using those promoter sequences has suggested the region consisting of 7 nucleotides located between Region III and TATA-box of the glaB promoter is a DNA-binding sequence of FlbC. Then, electrophoretic mobility shift assay (EMSA) using the glaB promoter confirmed that FlbC can bind to the DNA fragment containing the predicted cis-element candidate. In addition to the sequence, there are two highly similar sequences located distally in the glaB promoter, and FlbC was able to bind, albeit to a lesser extent, to one of these sequences. Furthermore, FlbC was found to bind to conserved sequences also present in the pepA and nptB promoters. EMSA analyses of the glaB, pepA, and nptB promoters altogether showed that the essential sequence of the cis-elements for FlbC is 4 nucleotides of 5'-GATC-3'.
(1) Tanaka et al., Appl. Microbiol. Biotechnol., 100, 5859–5868 (2016).

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POSTER SESSION III

WEDNESDAY, MARCH 8
16:00 – 17:30
Location: ***Tirol Foyer (Congress Innsbruck)***

CS3.1 EVOLUTION, BIODIVERSITY AND TAXANOMY

CS3.1.9

GENETIC MOSAICISM AND INHERITANCE OF SOMATIC MUTATIONS IN THE FAIRY-RING MUSHROOM MARASMIUS OREADES

Markus Hiltunen Thorén[1,2,3], Boel Olsson[3], Martin Ryberg[3], Hanna Johannesson[1,2]

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Heritable genetic variation forms the basis for adaptive evolution. In unitary organisms, the germline is defined early during development, and only germline mutations have evolutionary impact, since somatic mutations cannot be inherited to the next generation. In modular organisms, on the other hand, the germline is often assumed to be defined much later, meaning that mutations that have accumulated over growth have a larger potential for inheritance. Here we investigated somatic mutation accumulation and inheritance in a mushroom-forming fungus. By making use of the fairy-ring system of Marasmius oreades, we traced the accumulation of somatic mutations over growth, and their distribution within and between fruiting bodies. From one fairy ring of the fungus, i.e. one genetic individual, we collected fruiting bodies and spore prints. Different tissues were dissected from the fruiting bodies, including stipe, cap and hymenium. By using whole-genome sequencing, we scored somatic mutations as genetic differences between fruiting body tissues. We found many mutations in vegetative tissue types of the fruiting bodies, such as stipe and cap, and much fewer mutations in the hymenium and spore prints. Furthermore, the

frequency of each mutation in sequencing reads was lower in vegetative tissues than in the hymenium, meaning that vegetative tissues likely were a mosaic of cell lineages carrying different somatic mutations. In the hymenium and spore prints, on the other hand, mutations were very close to a 1:2 ratio, supporting the presence of only two haploid genotypes. Together, our results suggest a tightly controlled partitioning of genotypes in fungal fruiting bodies, where certain cell lineages are destined for spore production, and others relegated to vegetative tissues. Our findings shed light on the timing of germline definition in filamentous fungi, and have large implications for how genetic variation is accumulated and spread in fungal populations.

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CS3.1.11

AEGEROLYSINS – WHAT DO WE ALREADY KNOW ABOUT THEM?

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Aegerolysins are remarkable proteins. They are distributed throughout the tree of life and are relatively abundant in fungi and bacteria, and also present in a few insects, plants, protozoa, and viruses. Despite their occurrence in cells of certain developmental stages and their presence in secretomes, few aegerolysins have been studied in detail. Above all, their function is intriguing.
We summarize the evidence published to date on the distribution, molecular interactions, and function of these versatile proteins. The machine learning approach of the AlphaFold algorithm, complemented by additional genomic support, provides us with new insights into the aegerolysins and their pore-forming partners.
Aegerolysins have very different protein sequences but a common fold. They are involved in various interactions by recognizing a molecular receptor in the target organism. The formation of pores in combination with larger, non-aegerolysin-like protein partners is one of the possible responses of the aegerolysin-producing organism in competitive exclusion of other organisms from the ecological niche.
Despite the limited knowledge of aegerolysin function, several potential applications are already emerging. Most commonly, some fungal aegerolysins serve as probes for the detection, labeling, and imaging of specific membrane lipids, lipid rafts, cancer cells, invertebrates, or parasites. At high concentrations, they can induce both artificial lipid vesicles and living cells to bend and bud. Their genes and expression, or antibodies produced against aegerolysins, can serve as biomarkers or immunodiagnostic tools for the progression of fruiting body differentiation, fungal pathogens exposure, or infectious disease progression. Strong promoters regulating aegerolysin genes can promote secretion of heterologous proteins. For some aegerolysins, a role in combating obesity and related metabolic disorders has been recog-

nized. In combination with larger protein partners, some of them are able to form pore-forming complexes that can be used to selectively eliminate insect pests or to treat certain types of cancer cells.

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CS3.1.12

ONGOING AND RAPID SPECIATION IN THE WIDESPREAD PURPLEPORE BRACKET FUNGUS LEADS TO POROUS INTERSTERILITY BARRIERS

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Reproductive barriers within morphospecies in Basidiomycota tend to be stronger between sympatric lineages, but the genetic mechanisms maintaining these barriers and how they arise are largely unknown. In this study we apply population genomic analyses together with in vitro crosses and wood decay experiments to understand the evolution of reproductive barriers within the purplepore bracket fungus Trichaptum abietinum. We have whole genome sequenced ~350 T. abietinum samples from Asia, Europe and North America, to cover most of the range of this widespread morphospecies. Our phylogeographic analyses show that T. abietinum is delimited into six major lineages, one in Asia, two in Europe, and three in North America. We found the two lineages present in Europe to be interfertile and admixed, whereas the North American lineages were reproductively isolated. In Asia a more complex pattern appears, with partial intersterility between multiple sub-lineages that likely originated independently and more recently than the pre-mating barriers in North America. Using genome scans and association studies we identified candidate genes including metacaspases, suggesting that programmed cell death may be involved in intersterility in basidiomyetes, similar to what has been found in acomycetes. We found reduced wood decay for in vitro hybrids to be strongly correlated with the genomic divergence between parents, indicating Bateson-Dobzhansky-Muller incompatibilities. On the other hand, pre-mating barriers were moderately correlated with genomic divergence, suggesting that other mechanims have been involved in generating these barriers in North America and Asia. Our demographic modelling and phyloge-

netic network analyses support that these pre-mating barriers in T. abietinum were reinforced upon secondary contact between lineages that diverged in allopatry during the pleistocene glacial cycles.

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CS3.1.13

ESTIMATING THE SPONTANEOUS MUTATION RATE IN CANDIDA SPECIES

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Invasive fungal diseases have become a significant source of concern in public health in recent times since they result in high morbidity and mortality. According to various estimates, about 70–80 percent of all mycoses are caused by fungi from the Candida genus. Even though the most prevalent and invasive species is Candida albicans, over the past two decades, non-albicans Candida species have become one of the emerging causes of candidiasis. To understand evolutional processes occurring in the most prevalent Candida species, we are going to assess the mutation rate variation across these species and analyze the molecular spectrum of mutations using an in vitro evolutionary approach combined with next-generation sequencing. The approach consists of random mutation-accumulation experiments with different Candida parental species. We used 15 replicates for each of studied species that were passed through the sharp bottleneck for 40 passages (only one colony was randomly selected for each passage). After reaching approximately 1000 generations, high-throughput sequencing was used to obtain the unique set of mutations within each individual line. The final set of mutations includes all possible base substitution mutations, small indels, as well as large segmental changes such as duplications, deletions, and chromosomal rearrangements. The results will help us to identify mutational hotspots in Candida genomes and obtain an estimation of neutral mutation rates per cellular division that will be useful in epidemiological studies. To confirm the validity of our mutation rate estimates, we will contrast our data with that of publicly available genomes of dated serial isolates from hospital outbreaks. Thus, acquired information will be of broad relevance

and interest, especially for quantitatively understanding and describing stochastic evolutionary processes and their possible role in the different adaptation mechanisms in Candida species.

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CS3.1.14

A MINIMAL FANCONI ANEMIA COMPLEX PRESENT IN EARLY DIVERGING FUNGI

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Fanconi Anemia (FA) is a DNA repair pathway that recognises damage caused by DNA interstrand crosslinks (ICL). ICL is one of the lethal forms of DNA damage which prevents separation of two strands of duplex DNA and blocks major cellular processes of transcription and replication. A typical FA pathway consists of an endonuclease complex, a core complex, activation complex and a repair complex coordinating together upon stimulation of damage signals. This pathway was initially thought to be restricted to vertebrates, but was later confirmed in other metazoans. The identification of FA homologs of FANCM, FANCL, FANCP and FANCJ proteins in yeasts opened the discussion about a rudimentary form of FA DNA repair system that lacks a majority of FA core proteins. In this work, we attempt to expand the search for FA components in the fungal kingdom using an in-silico approach. We identified homologs of five proteins belonging to the core complex, five from the endonuclease complex and four from both FA activation and repair complexes respectively. We noted the lack of several key components of the pathway in all fungal taxa including FANCC, FANCF and FANCG responsible for post-replication repair and chromosome stability. The observed distribution can be attributed to a gradual loss of FA components from the early diverging fungi to Dikarya. While FANCD2 and FANCI are conserved in EDFs, they are absent from Dikarya. On the other hand, protein FANCM is conserved across the fungal tree of life with a loss in Glomeromycotina. Interestingly, FANCA can be found in a handful of Mucoromycotina and Zoopagomycotina representatives. Moreover, we found that some FA components are overexpressed during stress according to publicly available transcriptomic datasets. Our results might be an indicator of the presence of a minimal Fanconi Anemia pathway in EDF, with gradual loss of their components towards Dikarya.

CS3.1.16

NONSELF RECOGNITION MODULES IN ASPERGILLUS FUMIGATUS ARE SHARED ACROSS ASPERGILLI

Ben Auxier[1]

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The distinction between self and nonself is a fundamental requirement for multicellular life. In fungi, this distinction is provided by het genes, genes where allelic differences lead to cell death when brought together in a common cytoplasm. Recently, in Aspergillus fumigatus we identified 5 loci whose alleles trigger nonself recognition, hetA-E. One of these, hetE showed a phenotype of delayed, but not blocked, heterokaryon formation. This phenotype was strikingly similar to a previous description of “partial” het genes from A. nidulans. Here, we show that the hetE region of A. fumigatus is in fact homologous to hetA of A. nidulans. This complex locus is composed of three proteins; a NACHT+Ankyrin type NLR, a homolog of a yeast vesicle transporter boi1, and rosA, a repressor of sexuality. All three of these genes are under balancing selection, with divergent alleles found within populations. As these two Aspergilli are estimated to have diverged ~87 million years ago, the sharing of this complex locus is clearly unexpected. While the evolutionary pressures maintaining variation at the NLR and boi1-homolog may be similar to other het genes – the maintenance of the individual – the selective force maintaining variation for rosA is unclear. As rosA is known to regulatethe sexual cycle, an alternative explanation for balancing selection is heterozygote advantage, where matings between isolates with different alleles are either more likely, more productive, or offspring have higher fitness. We will present ongoing work detailing investigations of the effect of heterozygosity at rosA, and whether allelic differences are associated with higher fecundity or fertility.

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CS3.1.17

REVISED TAXONOMY OF CORTINARIUS SUBGENUS DERMOCYBE FROM THE CENTRAL EUROPEAN ALPINE RANGE

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Cortinarius is one of the most species-rich genera in Basidiomycota. Cortinarius subgenus Dermocybe was defined for including small to medium-sized, brightly coloured Cortinarii with anthraquinone pigments. The chemotaxonomic approach has always been an important method for defining species within the subgenus along with classical morphological and ecological characters. However, taxonomic confusion was still very high. We therefore decided to address this topic based on a combined phylogenetic, morphological, and pigment-chemical approach. The HPLC-MS pigment profiles and spore size distribution were combined in order to enable a better resolution of taxa. This was done based on recent collections and the comparative study of type material. More than 135 collections were found in the coniferous forests of Tyrol and surrounding areas over a period of 3 years. A total of 73 new rDNA ITS sequences were produced in this study, 96 sequences were retrieved from databases. The examined species clustered into 17 different phylogenetic clades, and 5 pigment groups. The phylogenetic analysis showed that the diversity of dermocyboid Cortinarius spp. is generally very high, with 31 European species intermixed with at least 25 species from North America or the Southern Hemisphere. In combination with morphological and pigment profiles, we were able to clearly delimit 14 Dermocybe species occurring in coniferous habitats. Furthermore, type material from three species was analysed here for the first time. This analysis of Dermocybe species occurring in a restricted habitat confirmed the hypothesis that species diversity is much larger than currently assumed. The real diversity is blurred by too wide and wrong species concepts of several classical species like C. croceus and C. cinnamomeus. Molecular and chemotaxonomical studies carried out in parallel with phenotypical analyses resulted in a good differentiation of species.

CS3.1.18

MOLECULAR BIODIVERSITY IN FUSARIUM SUBGLUTINANS AND F. TEMPERATUM: A VALUABLE TOOL TO DISTINGUISH TWO SISTER SPECIES AND DETERMINE BEAUVERICIN CHEMOTYPE

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Fusarium subglutinans and Fusarium temperatum are common maize pathogens that produce mycotoxins and cause plant disease. F. subglutinans sensu lato in 2011 has been divided into Fusarium temperatum sp. nov. and F. subglutinans sensu stricto, showing different phylogeny and beauvericin production, even though the presence of bea gene in both species.
Genetic differences in bea gene cluster could be related to different abibity to produce beauvericin by these two closely related species, and related genetic interspecies variabilty could provide insights into the evolutionary processes involved in their separation into different species.
In a previous work, has been highlighted that Bea1, which encodes the NRPS22, the nonribosomal peptide synthase responsible for synthesizing the beauvericin backbone, in F. subglutinans, compared to F. temperatum, is affected by polymorphisms, presumibly related to its inhability to produce beauvericin, in despite of the presence of related 4 biosynthetic genes.
In this work, genetic differences in TEF1a has been exploited to design species-specific PCR primers useful for correct species identification; 3 polymorphisms in bea1 gene were searched in a set of strains (48 F. subglutinans and 43 F. temperatum), collected from maize worldwide (Argentina, Austria, Belgium, Germany, Iowa, Italy, Poland, Serbia, Slovakia, Switzerland, Netherland). The most frequent polymorphism detected was SNP528. Considering that SNP frequency is proportional to the recombination frequency, the high frequence of SNP528, leads to suppose that mutations occurred after speciation divergence between F. subglutinans and F. temperatum.

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CS3.1.20

INTERSPECIFIC HYBRIDIZATION AMONG PATHOGENS CAUSING CERCOSPORA LEAF BLIGHT IN SOYBEAN

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Cercospora leaf blight (CLB) is one of the most prevalent foliar diseases of soybean in the U.S. and South America. Although the causal agent of CLB was originally described as Cercospora kikuchii, two other pathogens are now associated with CLB: Cercospora cf. flagellaris, and Cercospora cf. sigesbeckiae. Recent surveys of pathogens associated with CLB across the U.S. failed to detect C. kikuchii. This led us to question whether C. kikuchii fell victim to interspecific competition from C. cf. flagellaris and C. cf. sigesbeckiae, or if other mechanisms were responsible. We sequenced the genome of a historical isolate of C. kikuchii (isolated in the 1990s), and two isolates of C. cf. sigesbeckiae (isolated in Louisiana in 2012, and Arkansas in 2017). Surprisingly, the majority of the genomes (>70%) of all three strains were identical, which indicated close taxonomic relatedness. The genomic regions differing most between C. kikuchii and C. cf. sigesbeckiae were clustered into distinct regions spread broadly across the assembly scaffolds. The regions distinguishing C. kikuchii from C. cf. sigesbeckiae were highly conserved in the two C. cf. sigesbeckiae strains despite the time and distance distinguishing their collections. Together, these factors suggest that the genomic polymorphisms distinguishing C. kikuchii and C. cf. sigesbeckiae are introgressions resulting from interspecific hybridization. The high level of genomic identity among the three strains suggests that the hybridization event was relatively recent. Furthermore, the high level of genomic identity between the two C. cf. sigesbeckiae strains implies that sexual reproduction is uncommon or nonexistent in this species. In sum, the finding that C. cf. sigesbeckiae is likely a hybrid derived from C. kikuchii and another as-yet unknown Cercospora species indicates that species barriers among Cercospora spp. are semi-permeable to the exchange of genetic information, an important consideration for breeding strategies to control Cercospora diseases.

CS3.1.21

THE EFFECTOR GENE TOXB IS PRESENT ON A PUTATIVE STARSHIP TRANSPOSON IN PYRENOPHORA TRITICI-REPENTIS (TAN SPOT OF WHEAT)

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The role of transposable elements in the evolution of virulence has been well detailed for ToxA (the necrosis coding gene) and its carrying wheat pathogens. Here, we provide evidence for the first time for the potential mobility of ToxB (the chlorosis coding gene) via a large and novel transposable element in the tan spot pathogen (Pyrenophora tritici-repentis). In this study, the genomes of two P. tritici-repentis (Ptr) isolates, one carrying ToxB and another carrying its non-functional homolog toxb, were sequenced with PacBio RS II and assembled with Flye+Pilon. Comparison with reference isolates lacking the ToxB/toxb genes showed a large 294 to 340 kb region with no co-linearity to reference chromosomes. Edge analysis revealed terminal-inverted repeats which indicated a possible transposon activity. Gene annotations within the region confirmed the presence of known “Starship” cargo genes and DDE transposases in the ‘captain’ position. DDE transposases are known to be associated with some Class II transposons (e.g. Mutator, hAT, etc.) and are known captains of bacterial ICE transposons. Searching the available fungal genomes revealed that the distribution of ToxB-like protein sequences extends beyond the genus Pyrenophora into other genera of the class Dothidiomycetes. Additionally, the putative ToxB protein homologs are also present in Sordariomycetes and Leotiomycetes classes. These findings together suggest that ToxB could have been mobile at certain evolutionary times contrary to the previous assumption of its vertical inheritance.

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CS3.1.24

THE ORIGIN, EVOLUTION, AND CHEMICAL ECOLOGY OF AN EMERGING FUNGAL PATHOGEN OF SOYBEAN, XYLARIA NECROPHORA

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Xylaria necrophora has likely caused impacts to soybean production for more than a decade, but it was long mistaken for other ailments or diseases. The description of the disease, taproot decline, and a preliminary identification of the pathogen was published in 2017. Since that time, we have compiled a suite of phylogenetic, phylogenomic, population genomic, and secondary metabolite data to understand the origin, evolution, and chemical ecology of X. necrophora. We incorporated sequence and distribution data from historical specimens of species in the X. arbuscula species complex to determine that X. necrophora likely transitioned from forested to agricultural habitats. Population genomic data from more than 160 whole genome sequences suggests that the pathogen likely underwent a population bottleneck during its emergence in agricultural habitats and lost its ability to reproduce sexually. It currently consists of atleast two clonal lineages distributed throughout the southern United States with little evidence for geographical population structure. Secondary metabolites produced by X. necrophora are important mediators of its ecological interactions and may determine its pathogenicity and virulence. Culture filtrates of X. necrophora are phytotoxic to soybean and inhibit the growth of a diverse suite of fungi. Ten compounds, including cytochalasins, pyrone derivates, and diterpenoids were identified by bioactivity-guided fractionation to be antimicrobial, phytotoxic, or both. An analysis of secondary metabolite clusters (SMC) in the genome of X. necrophora shows a high proportion of SMCs are NRPS-like, type 1 PKS, or terpenoid gene clusters, consistent with our expectations from the chemical analyses. The multidisciplinary approach to characterizing X. necrophora has laid the groundwork for the management of this pathogen as well as understanding the ecology of other Xylariales.

CS3.1.25

VARIATION IN ADAPTIVE PROTEIN EVOLUTION WITHIN GENOMES AND ACROSS FUNGAL SPECIES

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Disentangling the effects of natural selection and genetic drift in species represents a step forward in understanding the molecular basis of adaptation. In fungi, the lack of a broad multi-species overview accounting for confounding factors stemming from different types of selection is hindering our understanding of the adaptive process in nature. Here, we use a comprehensive panel of 20 fungal species to unveil the drivers and mechanisms of adaptive evolution. We sought to understand the impact of gene function, population size, gene presence/absence variation (PAV), and recombination on the rates of adaptation at the protein level. To this end, we analyze single nucleotides segregating as polymorphism and substitutions across sequence alignments of 109,438 protein-coding genes extracted from the genome of 2,461 individuals. Employing state-of-the-art statistical methods accounting for confounding effects from demography and modeling the distribution of fitness effects of mutations, we determined the proportion of non-synonymous substitutions that are attributed to be adaptive (α). We found that higher α across species is driven by higher rates of adaptation (ωA) together with lower rates of non-adaptive substitutions (ωNA). Considering gene categories, genes predicted to encode effector proteins have in general the highest ωA, followed by genes encoding other secreted proteins and genes encoding non-secreted proteins. When comparing genes present in all individuals and genes showing patterns of PAV, genes in PAV have a higher ωA in most species analyzed. Finally, our results suggest that a higher effective population and higher recombination rates are also correlated with lower levels of non-adaptive substitutions, supporting the idea of more efficient purging of deleterious mutations in species with larger populations and with frequent sexual recombination. Overall, our findings highlight the potential of disentangling and quantifying the outcomes of adaptive evolution at the molecular level and unveils factors influencing the adaptive substitution load across species.

CS3.1.27

GENOMICS-ASSISTED DIRECTED EVOLUTION FOR THE DEVELOPMENT OF BIOCONTROL STRAINS OF THE VASCULAR WILT PATHOGEN FUSARIUM OXYSPORUM

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Root infecting vascular wilt fungi attack hundreds of crops worldwide and are extremely difficult to control by chemical fungicides. Fusarium oxysporum comprises a species complex that causes vascular wilt disease in more than 150 different crops, provoking devastating losses in global agriculture. The evolutionary mechanisms underlying host adaptation and host range dynamics in this pathogen remain poorly understood. In an experimental evolution experiment, we obtained multiple independently evolved lines from a tomato pathogenic clone of F. oxysporum by performing serial passage through growth media plates. Interestingly, the plate-passaged lines displayed striking phenotypic differences compared to the initial clone, including asignificant increase in colony growth speed and conidiation and a dramatic reduction in pathogenicity. Furthermore, some of these lines strongly outcompeted the ancestral pathogenic isolate when co-inoculated on tomato roots and effectively protected plants from vascular wilt disease. Re-sequencing of these lines revealed that the trajectories of mutational events leading to plate adaptation and concomitant loss of pathogenicity are highly reproducible and thus predictable. Currently, re-evolution experiments through tomato plants are carried out with the previously evolved lines to test the potential reversibility of the attenuated virulence phenotype upon successive passages under selective pressure by the main plant host. Our findings suggest that genomics-assisted directed evolution of fungal root pathogens could be used to generate new biocontrol strains that offer a promising alternative to chemical funcicides for protection of crops against vascular wilt disease.

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CS3.1.28

MEIOTIC DRIVE IS ASSOCIATED WITH SEXUAL INCOMPATIBILITY IN NEUROSPORA

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Evolution of Bateson-Dobzhansky-Muller (BDM) incompatibilities is thought to represent a key step in the formation of separate species. They are incompatible alleles that have evolved in separate populations and are exposed in hybrid offspring as hybrid sterility or lethality. In this study, we reveal a previously unconsidered mechanism promoting the formation of BDM incompatibilities, meiotic drive. Theoretical studies have evaluated the role that meiotic drive, the phenomenon whereby selfish elements bias their transmission to progeny at ratios above 50:50, plays in speciation, and have mostly concluded that drive could not result in speciation on its own. Using the model fungus Neurospora, we demonstrate that the large meiotic drive haplotypes, Sk-2 and Sk-3, contain putative sexual incompatibilities. Our experiments revealed that although crosses between Neurospora intermedia and Neurospora metzenbergii produce viable progeny at appreciable rates, when strains of N. intermedia carry Sk-2 or Sk-3 the proportion of viable progeny drops substantially. Additionally, it appears that Sk-2 and Sk-3 have accumulated different incompatibility phenotypes, consistent with their independent evolutionary history. This research illustrates how meiotic drive can contribute to reproductive isolation between populations, and thereby speciation.

CS3.1.29

GENETIC DIVERSITY AND POPULATION STRUCTURE OF PENICILLIUM ROQUEFORTI ISOLATES FROM TURKISH BLUE CHEESES

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Turkiye has different types of blue cheeses locally produced using spontaneous mold-ripening. Penicillium roqueforti is the principal mold giving the characteristic blue-green color to these cheeses. In this study, it was aimed to determine the genetic diversity and the population structure of P. roqueforti isolates obtained from Turkish blue cheeses. For this purpose, 148 molds were isolated form 61 cheese samples. Among these isolates, 120 (81%) were molecularly identified as P. roqueforti. Fingerprinting analyses conducted using (GTG)5 and M13 primers were very succesful in differentiating different fungal species; however, these analyses provided limited information on intraspecies diversity. All P. roqueforti isolates were shown to harbor Wallaby and CheesyTer, which are the horizontal gene transfer regions frequently found in cheese fungi, using PCR-screening. The mating type (MAT) locus PCR showed that the MAT distribution of the isolates was skewed in favor of MAT1-2 (95%). On the other hand, three cheese samples contained P. roqueforti isolates of both mating type together, indicating possible sexual reproduction on cheese. The population structure of P. roqueforti isolates was analyzed using three microsatellite loci (Proq01_3, Proq02_2 and Proq16). As a result, 36 sequence types were found and the most common one harbored 42 isolates and comprised 35% of all isolates (n=120). In addition, at least thirty one cheese samples contained isolates of different sequence types, showing that different strains might grow and multiple on cheeses during spontaneous mold ripening. The analysis of microsatellite loci together with mating genotypes using Splitstree, resulted in a diagram showing a generally clonal population with restricted recombination. Phylogenetic analysis of polymorphic loci (cmd, benA, proq235, proq631 and proq845) indicated that Turkish P. roqueforti isolates were separated into different clades, one clade close to P. roqueforti isolates of European blue cheeses, as well as clades related to but distinct from non-cheese isolates.

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CS3.1.30

DIVERSITY OF MARINE ALGICOLOUS ENDOPHYTIC FUNGI: EXPLORING THEIR CYTOTOXIC SECONDARY METABOLITES

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Marine organisms comprise approximately half of the total biodiversity on earth. Among marine organisms, fungi are a group of biotechnologically valuable and remarkable cradle for bioactive secondary metabolites. The stress tolerance response is triggered by secondary metabolites that fungi produce in order to live in challenging marine environments. Several of these distinctive secondary metabolites also have exceptional therapeutic efficacy.
31 endophytic fungi that were dwelling inside red, green, and brown marine algae were isolated and identified in the current study. The phylogenetic relationship among the fungi and their species richness were revealed by the maximum likelihood analysis and diversity indices. It was observed that Aspergillus was the dominant genus of endophytic fungi among 17 genera, representing seven species (highest) out of 27 different species belonging to all classes of fungi such as Ascomycetes (13), Basidiomycetes (1), Hyphomycetes (14), and Coelomycetes (3). Cladosporium spp was observed to be the second dominant with 3 species followed by Periconia and Aschotricha. The Aspergillus colonies were isolated from all three groups (green, brown, and red) of algae. Further, the cytotoxic potential of each endophytic fungal extract was evaluated against HeLa (cervical cancer) A431 (epidermoid carcinoma) and non-cancerous cells (HEK). In the preliminary screening of ethyl acetate extracts, nine fungi showed notable cytotoxicity with cell death above 60%, and 22 fungal total extracts exhibited 30–50% while showing negligible toxicity towards HEK. We may be able to purify novel bioactive compounds from potent fungal extracts and understand the mechanism underlying the anticancer property by using various work models.

Keywords: Biodiversity, marine macro-algae, endophytic fungi, Aspergillus, secondary metabolites

CS3.1.31

DIVERSITY OF THE FUNGAL PATHOGEN ALTERNARIA SPP. ON WILD TOMATO PLANTS

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The wild relatives of modern tomato crops are native to South America. These plants occur in habitats as different as the Andes and the Atacama Desert and are to some degree all susceptible to fungal pathogens of the genus Alternaria. Alternaria is a large genus. On tomato, several species cause early blight, leaf spot, and other diseases.
We collected Alternaria-like infection lesions from the leaves of eight wild tomato species from Chile and Peru. Using molecular barcoding markers, we characterized the pathogens. The infection lesions were caused predominantly by small-spored species of Alternaria of the section Alternaria, like A. alternata, but also by Stemphylium spp., Alternaria spp. from the section Ulocladioides, and other related species. Morphological observations and an infection assay confirmed this. Comparative genetic diversity analyses show a larger diversity in this wild system than in studies of cultivated Solanum species. Furthermore, we sequenced the whole genomes of 20 small-spored isolates to elucidate their evolutionary history.
As A. alternata has been reported to be an increasing problem on cultivated tomato, investigating the evolutionary potential of this pathogen is not only interesting to scientists studying wild plant-pathosystems. It could also inform crop protection and breeding programs to be aware of potential epidemics caused by species still confined to South America.

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CS3.1.32

HISTORICAL HERBARIUM GENOMES REVEALS CENTURY-LONG GENETIC CONTINUITY OF A CLONAL LINEAGE OF THE RICE BLAST FUNGUS IN EUROPE

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Many crop disease outbreaks are characterized by clonal expansions of single pathogenic lineages that in some instances reach pandemic proportions. Understanding the timescales of clonal lineage expansions is important for evolutionary studies and the implementation of informed disease response programs. We have previously determined that the population structure of the rice blast fungus Magnaporthe (Syn. Pyricularia) oryzae is characterized by three pandemic clonal lineages that are globally distributed and expanded in the last 300 years. However, the time frames of a particular clonal lineage in a given geographical region remain to be investigated. To answer this question we have sequenced historical genomes of European M. oryzae strains derived from herbarium specimens. By analyzing these century-old genomes in conjunction with a set of hundreds of modern genomes, we determined the genetic continuity of the same clonal lineage in Europe for more than 100 years. To identify genetic changes that have permitted the persistence of the same clonal lineage in spite of radical changes in agricultural practices across several decades, we ascertained absolute difference in single nucleotide polymorphisms (SNPs) and presence/absence of genes variants (PAVs) between historical and present-day genomes. Moreover, we used a set of mini-Chromosomes (mChr) ascertained in present-day European isolates as a reference dataset to determine the mChr content of historical genomes. Our analysis quantified and timed the emergence of major drivers of phenotypic diversity (SNPs, PAVs and mChr) and generate a set of candidate genomic regions that might have played a role in the adaptation and evolutionary success of a clonal lineage of M. oryzae in Europe.

CS3.1.33

ELUCIDATION OF SPECIES-SPECIFIC FUNGAL SIDEROPHORE RECOGNITION

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Iron is an essential cofactor for several cellular processes. Despite its high abundance in the earth crust, the bioavailability of this metal is very low due to oxidation by atmospheric oxygen. Consequently, fungi evolved high-affinity iron uptake mechanisms including siderophore-mediated iron acquisition. Ascomycota and Basidiomycota produce exclusively hydroxamate-type siderophores that are subclassified into fusarinines, coprogens, ferrichromes, and rhodotorulic acid, whereby siderophore-class production is highly species-specific. Additionally, most fungal species are able to utilize xenosiderophores, i.e, not self-produced siderophore classes. For example, the mold Aspergillus fumigatus is able to utilize ferrioxamines, which are bacterial hydroxamate-class siderophores.
Recent studies elucidated the substrate specificities of four siderophore-iron transporters (SITs) of A. fumigatus, which, as shown here, facilitates prediction of the substrate specificity of fungal orthologs. Phylogenetic analysis of SITs from diverse representatives of Ascomycota, Basidiomycota and Mucoromycota revealed insights into the evolutionary conservation of SITs as well as the diversity of siderophore utilization by different species. For example, in contrast to A. fumigatus, Aspergillus nidulans is shown to be able to utilize the bacterial catecholate-type siderophore enterobactin via the SIT MirA supported by the genomic clustered enterobactin-hydrolysing enzyme EstA. Moreover, the so far uncharacterized SIT SitC was found to improve coprogen uptake in coprogen-producers such as Aspergillus terreus in comparison to the non-coprogen producer A. fumigatus. As siderophores might improve or decrease iron availability of other niche habitants dependent on their siderophore utilization spectrum, the production of a certain siderophore functions as either a cooperative or a competitive trait. Therefore, the elucidated diversity in siderophore-class utilization most likely reflects ecological niche adaptation mirroring differences in cooperative and competitive interactions.

CS3.1.34

CONCERNING THE CURATION OF FUNGAL METABARCODING DATA SETS

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Accurate species identification is a requirement to answer the questions of function, resilience, interaction and diversity effects in ecological studies in all kinds of habitats. The cultivation bias introduced with isolation strategy and culture media limit the information about the fungal diversity at the time point of sampling.
For more than a decade, fungal diversity has been estimated using DNA metabarcoding applying the partial ITS rDNA operon as the most widely used genetic marker.
We conducted several metabarcoding studies from different habitats. It became evident that fungal metabarcoding has the advantages of overcoming the cultivation bias, increasing the number of possible samples by high-throughput sequencing, and realizing the detection of yet uncultivated fungal taxa. However, the limitations, in particular the lack of taxonomic accuracy when using automated classification, became apparent, too:
Many widespread taxa are difficult to identify because the phylogenetic resolution of the ITS region is not sufficient for species delimitation, which in turn can lead to underestimates, but multiple heterogeneous ribosomal operons lead to overestimation of fungal diversity.
During curation of the retrieved dataset, quality of classification i.e. phylogenetic rank and current nomenclature were checked. The manual curation step showed that 20 % of the OTUs represented either chimeric or very short (> 80 bp) uninformative sequences despite standard quality filtering and chimera-tests. Re-blasting OTU resulted in taxonomic correction (including nomenclature) of another 30 % of OTU. About 60% of the insufficient identifications can be attributed to database shortcomings, i. e. as yet unidentified species or missing reference sequences for taxa not yet sequenced.
Parameters that can be adjusted to improve the classification of species in metabarcoding studies include changing the cut-off threshold for sequence similarity, updating and refining of databases, and aligning and collapsing of OTU.

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CS3.1.35

GENOMIC DIVERSITY OF THE QUARANTINE ORGANISM PHYLLOSTICTA CITRICARPA AND RELATED SPECIES

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Members of the genus Phyllosticta can colonize a variety of plant hosts, including several Citrus species such as Citrus sinensis (orange), Citrus limon (lemon), and Citrus maxima (pomelo). Some species may cause serious disease, such as citrus black spot caused by Phyllosticta citricarpa, resulting in necrotic lesions on fruit and in severe cases early fruit drop. P. citricarpa is of quarantine status in Europe and import of citrus fruits showing black spot symptoms is strictly prohibited. P. citricarpa has a global distribution but is currently absent in Europe's citrus growing regions, though occasional reports of P. citricarpa in Mediterranean countries have been made. Several years ago, a closely related new species from Europe called Phyllosticta paracitricarpa was described. Very little information is available on this species and distinguishing it from P. citricarpa has proved to be challenging due to high genomic similarity of the two species. Species boundaries have always been difficult to define in fungi but are especially important when quarantine organisms are involved. In this study, we used a bioinformatics approach to assess the genomic variation within several citrus-colonizing Phyllosticta species, including P. capitalensis, P. citribraziliensis, P. citrichinaensis, P. citriasiana, P. citricarpa and P. paracitricarpa. We assessed several genomic parameters, such as overall whole-genome similarities, mitochondrial assemblies, and repeat landscape. By mapping the diversity within well-defined species, we aim to conclude on species boundaries within the genus Phyllosticta and with that on the relation between P. citricarpa and P. paracitricarpa.

CS3.1.36

GENOME EVOLUTION IN THE FUNGAL ORDER SORDARIALES

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The order Sordariales (Ascomycota) is of large biochemical and ecological importance. It is taxonomically diverse, with closely related species inhabiting a wide variety of natural habitats. For example, the Chaetomiaceae family contains the widest known variety of thermophilic fungi. Thermophily in this group is a polyphyletic trait with many biotechnological applications. The order thus provides a unique opportunity to study the evolution and correlation with genomic properties.
To study the connections between trait variation and genomic properties, a robust framework of phylogenetic relationships within the order is essential. Previous molecular phylogenetic analysis of Sordariales has relied on a few genes-many taxa approach. In our project, we used whole genome data from 106 genomes to infer a robust genome-wide phylogenetic framework of the order.
We have used the phylogeny as a basis for the comparisons and correlations of genomic properties and inference of the direction of evolutionary change. Analysis amongst the three largest families (Podosporaceae, Sordariaceae and Chaetomiaceae) in our dataset indicate that the Chaetomiaceae show the highest average GC content, and a low average genome size. The Sordariaceae have the highest average genome size and repeat percentages, while having the lowest average GC content and the smallest number of genes. Local autocorrelation tests show furthermore that Chaetomiaceae, and to a slightly lesser extent Sordariaceae, species are the main drivers of phylogenetic signal of the genomic properties investigated in this study.
Together, the results provide insights into the determinants of fungal genome evolution and provide a basis for our ongoing studies on the influence of thermophily on genomic properties and the rate of molecular evolution. Additionally, the phylogeny and order-wide overview of genomic properties will be helpful for researchers studying other biochemical, ecological, genetic and evolutionary questions in this group of fungi.

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CS3.1.37

PHYLOGENY AND SEXUAL REPRODUCTION OF KWONIELLA HEVEANENSIS-RELATED YEASTS, SAPROBIC RELATIVES OF PATHOGENIC CRYPTOCOCCUS SPECIES

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Kwoniella heveanensis is a yeast closely related to the pathogenic Cryptococcus species complex. A sexual cycle involving dikaryotic hyphae with fused clamp connections, basidia, and basidiospores has been defined for K. heveanensis. The species has a multi-allelic homeodomain locus and a bi-allelic pheromone-receptor locus consistent with a tetrapolar mating system. To resolve the taxonomic status of K. heveanensis and related isolates and species, we analyzed 46 isolates from seven different countries. Whole genome phylogenetic analyses revealed eight clades. Seventeen Thailand isolates clustered with the type strain CBS569 isolated from Indonesia (clade 1) whereas 20 Brazilian isolates formed a separate cluster (clade 2) closely related to but distinct from the Asian clade. The average nucleotide identity (ANI) between clades 1 and 2 is ~92.8%, whereas their interspecific ANI values are 98.8% and 97.7%, respectively. Two additional Brazilian isolates clustered together with a more distantly related strain CBS6097, isolated in South Africa (clade 3). A few other isolates from the USA, China, and Spain formed additional separate clades populated by only one or two isolates (clades 4-8). The majority of isolates were determined to be haploid by fluorescence-activated cell sorting with the exception two diploid isolates, one in clade 2 and the other in clade 5. The diploid isolates harbored two different versions of the homeodomain and pheromone receptor alleles, indicating possible cell fusion between haploid strains of different mating types. While sexual reproduction with basidia and basidiospores could be observed for fertile isolates in clade 1, mating structures were not observed in the other clades. Fu-

ture work will include 1) mating assays with mutant strains within and between each clade to stimulate mating and 2) analysis of F1 progeny to determine if reproductive barriers exist between the different clades/species.

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CS3.1.39

ON THE ORIGIN OF BLAST FUNGUS MINI-CHROMOSOMES

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The blast fungus Magnaporthe oryzae is a highly devastating plant pathogen that infects a variety of cereal crops and wild grasses. A key feature promoting pathogen evolution and facilitating adaptation to its host, is genomic variation. In addition to its essential core chromosomes, the blast fungus, like many plant pathogenic fungi, carries small (500kb - 3Mb) supernumerary mini-chromosomes (mChr). These mChr sometimes have an adaptive value and can impact the pathogen's virulence to its host. While some mChr are conserved across blast fungus isolates, others show high presence/absence variation. In addition, specific mChr are not always limited to a single host-specialized blast fungus lineage, but can be present across strains belonging to different lineages. Which factors ultimately result in the variable mChr distribution patterns we observe across blast fungus isolates remains unclear. We hypothesize that these could be the result of migration events followed by differential mChr loss, of recombination events between isolates from the same or from different blast fungus lineages, of horizontal mChr transfer between isolates, or of a combination of these events. We propose that mChr acquisition and loss may be an important determinant of adaptation in the blast fungus. This may be especially relevant in asexual lineages where sexual recombination does not occur, and where genetic variation in the core genome is limited. Studying how clonal lineages of the blast fungus adapt to their host is of great interest since these have been responsible for recent blast pandemics in crops.

CS3.1.40

SINGLE CELL BASED BIODIVERSITY SCREENING OF AQUATIC FUNGI

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Aquatic fungi are key destruents and parasites in aquatic ecosystems. They perform important functions in the transfer of energy to higher trophic levels and complement the microbial cycle. In the fungal tree of life, aquatic fungi are widely distributed across several lineages, mainly located within the Chytrdiomycota and Ascomycota. However, most of their biodiversity is unknown to science. Our goal is to explore the genetic diversity of aquatic fungi using single cells from environmental samples to circumvent bottlenecks in cultivation and reference data. To this end, we developed a single cell protocol that can be used for targeted dissection of individual cells and whole genome amplification. In total, we examined 357 single cells with a success rate of 59% for the fungal short ITS region. In addition, we were able to generate long 5-kb ribosomal amplicons in 24% of the cells, indicating high genome integrity and suitability for whole genome sequencing. Subsequent sequencing confirmed the difference in quality between the short and long amplicons. From the barcodes, we identified ten new lineages for which there is no reference in the database. Future work using this protocol will search for functional genes for aquatic adaptations, carbon turnover, and parasitic traits at the genome level.

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CS3.1.41

GENETIC DIVERSITY AMONG NUCLEI IN ARBUSCULAR MYCORRHIZAL FUNGI: THE ROLE OF GENETIC DRIFT AND SELECTION

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Arbuscular mycorrhizal fungi (AMF) form obligate symbioses with the roots of the majority of land plants and are found in all terrestrial ecosystems. The source and structure of intra-organismal genetic variation in AMF has been a long-standing topic of debate due to difficulties in the axenic cultivation and generation of high-quality genome assemblies from most species of AMF. Furthermore, how the fungus survives long-term without a single nuclear stage is puzzling, and there are hypotheses on selection at the nuclear level which functions to purge deleterious mutations.

In this study, we aimed to characterize inter and intra-organismal genetic variation in AMF by analyzing genomic information from individual nuclei of three strains in the genus Claroideoglomus. We analyzed unique and shared variance across strains and species, and assessed signatures of selection among nuclei.

In line with earlier studies, we confirmed nuclei to be haploid and identified one mating type allele in each of the three strains. We also observed an overall low level of genetic variation within the strains and a

low number of fixed differences between the strains and between the species. Strain-specific variation is mostly by rare, presumably young variants that appear to be under strong purifying selection. Noteworthy, we observed variants that were polymorphic in different strains and across the species. We propose that it represents variation that predates speciation and is maintained in strains as a result of drift in a large population of nuclei.

Altogether, these results affirm our conceptual understanding that the strains function as populations of asexually reproducing nuclei. New deleterious mutations are purged by selection while some mutations that rise to higher frequency can be maintained across speciation events. Further, we infer that there is selection acting on different levels within the individual, and on nuclei within the nuclei population of a strain.

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CS3.1.43

THE STRUCTURE OF BIOCHEMICAL FUNCTIONAL TRAITS: THEIR ROLE IN FUNGAL ECOLOGY AND EVOLUTION

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The nutritional requirements of Basidiomycete fungi are key components to the functioning of temperate forest ecosystems, because of their role in the breakdown of lignocellulose and their subsequent effects on carbon and nitrogen dynamics, as well as primary productivity. Fungal nutrition as well as their life history strategies rely on the expression of enzymes; among which Carbohydrate Active Enzymes (CAZymes) are an integral part of the fungal enzymatic arsenal. Functional traits are traits related to organism fitness and can be used to discover and examine trait covariation patterns spatially and temporally, helping us understand underlying ecological mechanisms and the history behind ecological strategies. This study focuses on examining genetic proxies that encode for these important biomolecules using Phylogenetic Comparative Methods. This approach has aimed to identify new sets of functional traits and the hidden lifestyles of mycorrhizal and saprotrophic Basidiomycete fungi, by uncovering latent traits and proposing new evolutionary trajectories of trait syndromes.

CS3.1.44

BIODIVERSITY OF BLACK YEASTS INVOLVED IN THE CARTON NESTS OF LASIUS FULIGINOSUS (HYMENOPTERA: FORMICIDAE)

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Fungi are an integral, stabilising part of the carton nests of the jet black ant L. fuliginosus, however their biodiversity is still not understood in detail. Here we defined the fungal core mycobiome of Lasius fuliginosus carton nests. Carton nests were sampled at three locations and fungi were isolated using a dilute to extinction approach. Overall, 355 fungi were isolated, which were then determined. All isolated fungi belong to phylum Ascomycota. The largest number of isolates belonged to Chaetothyriales (Eurotiomycetes), also known as black yeasts, containing fungi from the genera Cladophialophora (114 isolates), Exophiala (91 isolates), Phialophora (9 isolates) and Cyphellophora (5 isolates). Phylogenetic placement showed, that the majority of the Chaetothyriales isolates clustered with or in near distance to fungi known from other carton nests. Also 83 isolates belonging to the genus Scolecobasidium (Venturiales) were regularly found. Fungi present in low abundance and not in all samples belonged to the genera Lophiostoma and Nigrograna (Pleosporales) and Rhizodiscina (Aulographales). Species found in all nests in relatively high abundances allowed the definition of a core mycobiome consisting of a hitherto undescribed Cladophialophora species and two undescribed Scolecobasidium species. Beyond the core mycobiome, carton nests of the jet black ant are a highly diverse habitat hosting a vast number of hitherto undescribed fungal species.

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CS3.1.45

CHARACTERIZATION OF A COLLECTION OF ISOLATES OF FUSARIUM CHLAMYDOSPORUM SPECIES COMPLEX FROM BRAZIL: SPECIES DIVERSITY, COMPARATIVE GENOMICS, AND TOXIGENIC POTENTIAL

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The Fusarium chlamydosporum species complex (FCSC) includes nine distinct phylogenetic species, commonly found in association with soils and plants, but also induces opportunistic mycoses in humans and animals. Although members of FCSC have been reported to produce diverse mycotoxins, including beauvericin, butanolide, chlamydosporol, and moniliformin, the ability of each species of causing mycotoxin contamination has not been settled yet. The objective of this study was to investigate the mycotoxin potential in FCSC by determining both the chemotypes and genotypes for strains from this species complex from Brazil. Seventy-seven strains collected from soil and plants in Brazil were identified by phylogenetic analysis and were resolved in four previously described phylogenetic species: F. atrovinosum, F. chlamydosporum, F. nelsonii, and F. spinosum. Furthermore, screening of toxin production by high performance liquid chromatography showed that none of the strains examined produced fumonisins and trichothecenes, while production of beauvericin was confirmed for most of the strains. We examined genomes of three species within the FCSC (F. nelsonii, F. chlamydosporum, and F. spinosum), to determine the distribution of and variation in genes and gene clusters responsible for the synthesis of mycotoxins and other secondary metabolites (SMs). Overall, our results indicate that variation exists in the genetic potential of FCSC members to produce SMs, including mycotoxins.

CS3.1.46

MEIOTIC DRIVER HOMOLOGS IN ASEXUAL FUSARIUM SPECIES

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According to Mendel's law of segregation, in a diploid organism the two copies of a gene are equally likely to be passed on to the next generation. Exceptions to this are, however, common. Recent work into one such exception, namely the spore killing genes (Spoks) of Podospora anserina, has begun to unravel how these genes are able to kill any offspring cell that does not carry them. By genetically transforming Podospora with either full or partial copies of the Spoks, the researchers have been able to deconstruct the protein product into a toxin and an anti-toxin domain. Not only do several variants of Spoks exist in P. anserina, but homologs of these genes in unrelated fungal species abound. Here, I will present my research into the functionality of diverged homologs of the Spok genes within the fungal genus Fusarium, using yeast genetics. I will show evidence of the conserved function of the toxin and anti-toxin domains, and the apparent separation of these domains in certain Fusarium species. The functional conservation of these genes across fungal orders suggests that they play an important role in fungal evolution, and I will propose one mechanism by which they may influence genome evolution in the Fusarium genus.

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CS3.1.48

THE EVOLUTION OF ARBUSCULAR MYCORRHIZAL FUNGI IS MARKED BY EXPANSIONS OF SIGNATURE PHOSPHORUS TRANSPORTERS

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Arbuscular mycorrhizal (AM) fungi have been instrumental to plant growth since the emergence of terrestrial plants. Enhanced phosphorus (P) uptake is one of the key plant benefits of this association (Bonfante & Genre 2019). Upon mycorrhizal colonization, the plant pathway for P uptake is downregulated in favor of the mycorrhizal pathway and a number of different P transporters are involved in the fungal pathway for uptake of P from soil to plants. Given the central role that P uptake occupies in our understanding of the AM symbiosis, we wanted to explore if the evolutionary history of these obligate plant symbionts is associated with a diversification of P transporter genes. We distinguished five sub-groups of P transporters in a comprehensive phylogeny including previously identified fungal P transporter genes and predicted proteins from 955 published fungal genome assemblies. We identified functional domains specific to each sub-group and used these to estimate the number of P transporters in each sub-group across Glomeromycota, that include all AM fungi, and their sister phyla Mucoromycota and Morteriellomycota. The four sub-groups of inorganic P transporters were detected across AM fungi, with the high affinity proton symporter Pho84 by far being the most abundantly detected sub-group marking an possible expansion specific to Glomeromycota. The potential organic P transporter Git on the other hand was not detected in Glomeromycota supporting our understanding of AM fungi as specialists of inorganic P uptake.

CS3.1.49

GENOME BIOLOGY AND EVOLUTION OF MATING TYPE LOCI IN RUST FUNGI

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In basidiomycetes, two distinct gene loci confer mate compatibility. These loci encode for homeodomain (HD) transcription factors and pheromone receptor (PR)-ligand pairs. In some fungi these two loci are physically linked and give rise to large evolutionary strata with extensive recombination suppression. Genome level mating type (MAT) loci analysis is lacking for obligate biotrophic basidiomycetes, including the order Pucciniales. These important plant pathogens have long-term binuclear life-stages in which matched nuclei must carry compatible mating type loci. The binuclear state can arise via plasmogamy in the sexual cycle or via somatic hybridization during asexual reproduction and mating type loci define compatibility in either case. Therefore, it is important to understand genetic architecture of MAT loci in these fungi.
We focus on four Puccinia species that infect wheat and oat. Our analysis of available haplotype-phased chromosome scale assemblies provides novel insight into MAT loci evolution. We identify the MAT locations on two separate chromosomes which supports previous suggestions of a tetrapolar mating system in this fungal order. Transcription factors encoded at the HD locus genealogically group by species and showed no trans-specific polymorphisms between species. At a population-level HD locus appears highly multi-allelic within each species. In contrast, we were able to identify only a very limited number of alleles for PR encoding genes. Regions surrounding the PR loci displayed extensive genomic degeneration and up to megabases long tracks of recombination suppression, which are linked to centromeres in some species. Expression analysis suggests that both mating type loci are involved in appropriate nuclear pairing during spore formation in the asexual cycle. Together, our study provides insights into the evolution of mating type loci in key pathogenic Puccinia species. This detailed understanding is important to predict the possibility of novel epidemic lineages to arise via nuclear paring that combine advantages effector alleles.

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CS3.1.50

NEW TAXA OF ANTARCTIC CRYPTOENDOLITHIC BLACK FUNGI BY MULTI LOCUS PHYLOGENY

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At the fringe for life sustainability in the ice-free areas of Continental Antarctica, including the Mars-like environment of the McMurdo Dry Valleys, cryptoendolithic microbial communities represent the dominating life-forms, exploiting the airspaces of porous rocks as ultimate refuge to find more buffered conditions. These communities are composed of highly adapted prokaryotic and eukaryotic organisms living altogether in spatial association. Black meristematic fungi, also known as Rock-inhabiting black fungi (RIF), among the most stress-resistant organisms known to date, are recurrent components of these communities playing an irreplaceable role in protecting algae and other microbes from intense and harmful solar exposition. The geographic and genetic isolation and strongest environmental pressure promoted adaptive radiation, making these ecosystems a boundless reservoir of still unknown fungal diversity.
In the present study, we describe a relevant number of new RIF genera and species, defined by a multilocus phylogeny in rock samples collected along an altitudinal transect (1,000 to 3,300 m asl) in Victoria Land (Continental Antarctica) in the frame of Italian Antarctic Campaigns.
All new taxa were included in Dothideomycetes; in particular, 2 new genera and 4 new species were sitting in the Teratosphaeriaceae, 3 new species were discerned in the genus Friedmanniomyces (Teratosphaeriaceae), 1 new species in the genus Rachicladosporium (Cladosporiaceae), recently formally described, and 1 in the genus Cryomyces (incertae sedis). Representatives of these guilds have been selected for class-wide genome comparisons in the frame of the “Shed LighT in the daRk lineagES of the Fungal tree of life” project (US JGI), which aims to sequence nearly 100 species of black fungi from extreme environments as reference species.

CS3.1.52

ANCESTRAL ENZYME "RESURRECTION" PROVIDES SEVERAL CLUES ON EVOLUTION OF THE LIGNINOLYTIC CAPABILITY BY WOOD-ROTTING FUNGI

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Ancestral enzyme "resurrection" allows studying evolutionary hypotheses otherwise impossible to be tested. Here, we target fungal peroxidases playing a key role in lignin degradation, a central process for carbon recycling in land ecosystems. Ligninolytic peroxidases are secreted by wood-rotting fungi (order Polyporales), whose origin was established in the Carboniferous period associated to the appearance of these enzymes [1]. The lignin peroxidase (LiP) family includes the most efficient extant ligninolytic peroxidases. To reconstruct their evolution from the common ancestor of Polyporales peroxidases (CaPo), sequences in representative fungal genomes were curated, their phylogeny established by RAxML, and ancestral nodes reconstructed by PAML and heterologously expressed in Escherichia coli [2]. First, we found that the high reduction-potential that characterizes LiPs today was not inherited from CaPo but progressively increased through evolution. Secondly, CaPo, and its immediate progeny, were recognized as manganese peroxidases, a second peroxidase family acting on phenolic lignin via Mn(III) chelates. Interestingly, we found that the production of more recalcitrant lignin by angiosperms correlated with the appearance of a solvent-exposed catalytic tryptophan in ancestral peroxidases resulting in a shift from softwood to hardwood lignin preference, as revealed by kinetic and 2D-NMR analyses [3]. Moreover, this lignin-oxidizing tryptophan is a convergent trait appearing not only in LiP ancestors but also in the evolutionary line leading to versatile peroxidases [2] a minor third peroxidase family in Polyporales.

1. Floudas D; Binder M; Riley R; Barry K; Blanchette RA; Henrissat B; Martínez AT et al. (2012) The Paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. Science 336, 1715-9
2. Ayuso-Fernández I; Ruiz-Dueñas FJ; Martínez AT (2018) Evolutionary convergence in lignin degrading enzymes. PNAS 115, 6428-33
3. Ayuso-Fernández I; Rencoret J; Gutiérrez A; Ruiz-Dueñas FJ; Martínez AT (2019) Peroxidase evolution in white-rot fungi follows wood lignin evolution in plants. PNAS 116, 17900-5

CS3.1.53

USING DIGESTIVE SECRETOME RELATEDNESS FOR ELUCIDATING FUNGAL EVOLUTION AND SPECIATION

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A new analysis of relatedness of digestive secretome composition, among species within the genus Fusarium will be presented. For this we will use CUPP, a peptide-based functional annotation method, enabling robust (in both precision and sensitivity) prediction of function directly from sequence (available as online web-based functional annotation). Combined with Enzyme Profile Relatedness, EPR, based on CUPP, for comparative analysis of secretome composition across taxonomies. The EPR analyze, annotating the digestive CAZyme secretome to integrated "Function;Family" observations, are calculated to give equal weight to the "F;F" observations, the fungal species share to have, and the "F;F" observations they share NOT having. EPR analysis has been used to test the hypothesis, that the digestive CAZyme secretome is an integrated part of fungal evolutionary speciation. The hypothesis was first tested on all species of Aspergillus and Penicillium with the following result: With stunning congruence, between the organismal phylogenetic tree and the EPR based dendrogram, we found that the clades of the CAZyme EPR dendrogram was congruent with the clustering of these two fungal genera into phylogenetic Sections. Here we will report on the outcome of using this EPR analysis method on all genome-sequenced species of the genus Fusarium, to see how their secretomes cluster within the Fungal kingdom and identifying occurrence of major clades within the Fusarium EPR dendrogram; and to compare these findings with the ecophysiology and substrate affinities found in the different clades. The objective of this analysis is to provide an additional approach to shedding light on the complexity of the genus Fusarium.

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CS3.2 METABOLISM AND PHYSIOLOGY

CS3.2.9

CHARACTERIZATION OF MULTIPLE TRICHODERMA REESEI SUGAR TRANSPORTERS WITH ELECTROPHYSIOLOGICAL TWO-ELECTRODE VOLTAGE CLAMP TECHNIQUE

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Trichoderma reesei is an established production host for industrial enzymes. It natively produces high amounts of enzymes which degrade lignocellulose biomass, an abundantly available feedstock for the production of renewable fuels and chemicals. Although the biomass degradation system of T. reesei has been studied intensively, there are aspects of T. reesei physiology which have received less attention. It is known that different sugars present in the environment of T. reesei affect the production of the biomass degrading enzymes. However, only few proteins involved in the transport of sugars into the cell have been characterized, although the T. reesei genome has been predicted to contain about 50–100 genes coding for such proteins.

To gain more knowledge of T. reesei sugar transporters, we have characterized a group of them with electrophysiology [1]. Transporter genes were expressed in Xenopus laevis oocytes and studied with two-electrode voltage clamp (TEVC). This technique can be used to study electrogenic transporters, e.g. sugar proton symporters, although its use for studying fungal sugar transporters has been rare. We characterized eight sugar transporters with TEVC. Additionally, we did yeast uptake experiments with four T. reesei transporters, of which some were passive facilitators and thus could not be studied with TEVC. The studied proteins included transporters of hexoses, β-linked disaccharides and uronic acids. Transport function was shown for the first time for 3 transporters. Besides providing new information about the studied

transporters, the results also demonstrate the usefulness of TEVC for the characterization of fungal sugar transporters.

[1] Havukainen, S., Pujol-Giménez, J., Valkonen, M., Westerholm-Parvinen, A., Hediger, M. A., & Landowski, C. P. (2021). Electrophysiological characterization of a diverse group of sugar transporters from Trichoderma reesei. Scientific Reports 11:14678.

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CS3.2.10

NICOTINIC ACID CATABOLISM IN ASPERGILLUS NIDULANS: AN EXAMPLE OF CONVERGENT EVOLUTION

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Nicotinic acid (niacin) is a precursor of NAD and NADP and thus an essential metabolite. Its degradation has been previously described only in bacteria.
We describe, for the first time, a complete eukaryotic pathway of nicotinic acid (NA) catabolism. The genes are organised in three co-regulated gene clusters, which encode eight enzymes, two transporters and the pathway specific transcription factor. The pathway is conserved; and its variable organisation in the Pezizomycotina illustrates cluster evolution and horizontal gene transfer.
Reverse genetics was coupled with state-of-the-art chemical characterisation of each intermediate. The first step, the conversion of NA to 6-hydroxynicotinic acid (6-NA) is common to prokaryotic and eukaryotic pathways, even if catalysed by independently evolved enzymes. While two downstream metabolites, 2,5-dihydroxypyridine and 2,3,6-trihydroxypyridine, are common to various prokaryotic routes, other steps and metabolites are unprecedented: 3-hydroxypiperidine-2,6-dione and 5,6-dihydroxypiperidine-2-one intermediate metabolites have not been identified previously in any organism, the latter being a completely novel chemical compound. Furthermore, the hydrolytic N-C ring opening results in α-hydroxyglutaramate, a compound not detected in analogous prokaryotic pathways. Remarkably, the physiological inducer of the whole pathway is a near-terminal intermediate metabolite: 5,6-dihydroxypiperidine-2-one.
While most steps are cytosolic, two steps take place in the peroxisomes. 6-NA monooxygenase (HxnX) enters peroxisomes through a canonical PTS-1 signal, while HxnW, a polyol dehydrogenase, is co-transported by piggybacking HxnX.

The genomic organisation and phylogeny of the pathway cognate genes and proteins, showed that this catabolic pathway is of fungal (Ascomycota) origin and thus it exemplifies convergent evolution of catabolic pathways between fungi and bacteria, where at least four different pathways occur.
This work was supported by NKFI-K16 119516.

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CS3.2.11

AN UNEXPECTED ROLE FOR THE CARBON CATABOLITE REPRESSOR PROTEIN CREA AS AN ACTIVATOR FOR GLUCONIC ACID PRODUCTION IN ASPERGILLUS NIGER

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Aspergillus niger is one of the main producers of gluconic acid in industry. The conversion reaction involves three enzymes including glucose oxidase (GoxC), catalase (CatR), and lactonase (LctA). It has previously been shown that genes encoding these enzymes are co-regulated and induced by high levels of glucose and by hydrogen peroxide. To identify a possible transcriptional activator required for the induction of glucose oxidase, we screened our transcription factor knock out library of 239 individually deleted knockouts. We discovered that the mutant in which the transcription factor CreA was deleted was unable to produce glucose oxidase.
To study the role of CreA in more detail, glucose oxidase activity assays and gene expression studies were preformed of wild-type and ΔcreA deletion strains under a variety of gluconic acid producing conditions which showed that both the glucose oxidase activity and the induction of goxC, lctA and catR were CreA dependent.
Furthermore, the effect of deleting genes that control the stability of the CreA protein via the process of ubiquitination and deubiquitination was addressed by creating ΔcreB, ΔcreC, and ΔcreD mutants and by overexpression of creA. CreB and CreC are proteins involved in deubiquitination of CreA and deletion of creB and creC is expected to result in more ubiquitinated CreA which is targeted for degradation by the proteasome. Indeed, the deletion of creB and creC resulted in a similar phenotype as deletion of CreA. Deletion of the gene encoding the ubiquitination protein CreD is expected to result in more stable CreA as no ubiquitination of CreA occurs in the ΔcreD mutant. Indeed, deletion of creD resulted in higher and constitutive glucose oxidase expression which was also achieved by overexpression of CreA. We conclude that CreA has an activator role in the regulation of gluconic acid production.

CS3.2.12

THE CERAMIDE SYNTHASE CER1 PLAYS A ROLE IN SELF-PROTECTION AGAINST FB1

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Fumonisins are categorized as sphinganine-analogue mycotoxins (SAMs) due to their structural similarity to sphinganine. They are inhibitors of eukaryotic sphingolipid biosynthesis, by specifically targeting ceramide synthases of plants and animals. They are produced by Fusarium verticillioides, F. proliferatum and other species, with fumonisin B1 (FB1) representing the most relevant metabolite of this class. Our successful construction of a FB1 sensitive baker's yeast strain led to interesting observations on the self-protection mechanism in F. verticillioides. The fumonisin cluster genes FUM17 and FUM18 encode candidate ceramide synthases implicated in self-protection against the toxin alongside FUM19, encoding an ATP-binding cassette transporter. After transformation of the sensitive yeast strain with these genes, only the strain expressing FUM18 was able to grow at concentrations of 5 – 10 µM of FB1. Yet, our screening tests implicate another ceramide synthase located outside the FUM cluster, FvCER1, in self resistance. It conferred resistance to more than 25 µM FB1 when expressed in the sensitive yeast strain. The fumonisin producing Aspergillus niger, which lacks a fumonisin cluster associated ceramide synthase, has higher FB1 resistance than e.g. A. nidulans. The tomato pathotype Alternaria alternata f. sp. lycopersici, which produces the fumonisin analog AAL toxin, has a cluster associated ceramide synthase, but this gene has been previously shown to be dispensable for AAL toxin resistance. AAL toxin producing Alternaria alternata from tomato has much higher resistance to FB1 than other A. alternaria isolates from apple or potato. We therefore set out to investigate the possible role of FvCER1 and its homologs in self-resistance to sphinganine-analogue mycotoxins.

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CS3.2.13

JANUS-FACED FUCOSE AS A NUTRIENT LIGAND FOR DIKARYA AND A BUILDING BLOCK OF EARLY DIVERGING LINEAGES

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Fucose is a deoxyhexose sugar present and studied in mammals. The process of fucosylation has been the primary focus in studies relating to fucose in animals due to the presence of fucose in Lewis antigens. Very few studies have reported its presence in Fungi, mostly in Mucoromycotina. The constitution of 25% and 12% of this sugar in the carbohydrates of the cell wall in the respective Umbelopsis and Mucorales strains boosts the need to bridge the gap of knowledge on fucose metabolism across the fungal tree of life. In the absence of a network map involving fucose proteins, we carried out an in-silico approach to construct the fucose metabolic map in Fungi. We analyzed 85 protein families against the fungal proteome to predict the presence of fucose proteins in Fungi including diverse early diverging fungal lineages. The presence of these predicted proteins was validated with the help of whole transcriptome studies conducted in several species of early diverging fungi. We found proteins involved in several metabolic activities apart from fucosylation such as synthesis, transport and binding. Most of the identified protein families are shared with Metazoa suggesting an ancestral origin in Opisthokonta. However, the overall complexity of fucose metabolism is greater in Metazoa than in Fungi. Massive gene loss has shaped the evolutionary history of these metabolic pathways leading to a complete deletion in most yeast-forming lineages. Our results point to a distinctive mode of utilization of fucose among fungi belonging to Dikarya and the early diverging lineages. While Dikarya used fucose as a source of nutrients for metabolism, the early diverging group of fungi depended on fucose as a building block and signalling compound.

CS3.2.14

INFLUENCE OF CA2+ AND CL- ON AMYLASE ACTIVITY AND GROWTH BEHAVIOR OF SELECTED AGARICOMYCETES

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Amylases catalyze the hydrolysis of glycosidic bonds in amylolytic carbohydrates such as starch which is composed of D glucose residues in linear amylose and branched amylopectin. Three classes of amylases are distinguished. The metalloenzyme α amylase from the GH13 family cleaves α 1,4 endo-glycosidic bonds in amylose yielding maltotriose and maltose, and in amylopectin yielding maltose and glucose. Its function strictly depends on the cofactor calcium and is allosterically regulated by chloride. β-Amylase as exoamylase breaks the second α 1,4 glycosidic bond from the terminal ends of starch. GH15 family γ-amylase primarily cleaves α-1,4-bonds from non-reducing ends of amylose and amylopectin, and α-1,6-linkages from amylopectin, yielding glucose. A genomic search of five basidiomycetous fungi Ganoderma resinaceum, Pleurotus ostreatus, Schizophyllum commune, Trametes hirsuta and Trametes versicolor detected between 7 and 13 genes for α-amylases and 3 to 4 genes for γ-amylases but no genes for β-amylases. Effects of CaCl2 as a stimulant of α-amylases and alternatively of MgCl2 and CaCO3 on starch degradation in the five fungi were tested. All fungi showed an increase in α-amylase activity upon addition of CaCl2, albeit with individual optimum CaCl2 concentrations. The fungi in liquid starch medium responded with an overall higher growth rate in presence of CaCl2. CaCl2 increased hyphal branching frequencies in G. resinaceum cultures and caused more uniform pellet-sizes in liquid cultures of all fungi, with exception of S. commune. Addition of MgCl2 or CaCO3 as alternative Ca2+ and Cl- sources did not lead to a higher amylase activity in liquid cultures and fungal morphologies were also not influenced.

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CS3.2.15

TRYPTOPHAN BIOSYNTHESIS IN THE MUSHROOM COPRINOPSIS CINEREA

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Trp is an amino acid underrepresented in protein sequences and often confined to catalytic centers of enzymes. This non-polar aromatic residue is the most costly amino acid in terms of its biosynthesis. Basal lines to fungi (Cryptomycota, Microsporidia) seem not possess trp biosynthesis genes, but younger fungal lines obtained the ability of Trp biosynthesis, probably by horizontal gene transfer (HGT) from photosynthetic Chloroflexi. A common bacterial operon structure is trpEGDFCBA, which follows roughly the order of the enzymatic actions in the Trp biosynthesis pathway. In the fungi, different genes in the biosynthesis pathway have been fused. Trp biosynthesis in Coprinopsis cinerea is covered by four genes, trp1+ to trp4+. Anthranilate synthase subunit Trp3 in combination with the anthranilate synthase subdomain of the trifunctional Trp2 undertakes the first step in the biosynthesis to transform chorismate from the shikimate pathway into anthranilate. Next, phosphoribosyl transferase Trp4 produces N-(5´-phosphoribosyl)-anthranilic acid (PRA) as substrate for Trp2 which in two further steps gives rise to 1-(2-carboxyphenylamino)-1-deoxy-D-ribulose 5-phosphate (CDRP) by phosphoribosyl anthranilate isomerase activity and then indole-3-glycerol-phosphate (InGP) by indole-3-glycerol phosphate synthase activity. InGP is substrate for the bifunctional Trp1 which with its N-terminal TrpA half converts InGP into indole and with the C-terminal TrpB half the indole with serine into tryptophan. Trp biosynthesis underlies strict genetic controls in order to avoid toxic effects by intermediates, the Trp end-product and degradation products. Mutations had been generated in the 1970ties for all four Trp biosynthesis genes. Of these, mutations in trp1 and trp3 are still available in laboratory strains and used as selection markers in DNA transformation. Mutations in trp3 and in the separable trpA and trpB halves of trp1 are analyzed to define effects and regulation in the Trp biosynthesis, aromatic amino acid cross pathway control, and on growth and developmental fungal processes.

CS3.2.17

ON THE INTERACTION OF SREBP SAH2 AND THE PLEIOTROPIC REGULATOR XPP1 IN TRICHODERMA REESEI

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Sterol regulatory element binding proteins (SREBPs) are transcription factors that are conserved from yeast to humans. In mammals they regulate cholesterol synthesis, in fungi they are additionally involved in hypoxic response (Sre1 in Schizosaccharomyces pombe), resistance to azole compounds (Sre1 in Cryptococcus neoformans), and pathogenesis (SrbA in Aspergillus fumigatus), among others. More recent studies also reveal the role of SREBP Sah2 in protein secretion in Neurospora crassa.
Typically, the N-terminus of SREBPs harbors the transcription factor domain, which is a basic-helix-loop-helix leucine zipper (bHLH-LZ) structure. Thereby, the leucine zipper allows for dimerization. Structure analysis of Sah2 of Trichoderma ressei revealed, that its bHLH-LZ is highly similar to the C-terminal bHLH-LZ of transcription factor Xpp1.Our recent work elucidated the critical role of Xpp1 in the metabolism of T. reesei. When Xpp1 is deleted, growth is impaired and the expression of sorbicillinoids and low weight molecules is increased. Those findings support the conclusions from RNA-Seq results, that Xpp1 activates primary metabolism, however, it represses secondary metabolism.
In this work, we investigated the interaction between Sah2 and Xpp1 in order to regulate metabolic processes in T. reesei. Different imaging techniques were used to observe the co-localisation of the fluorescence-tagged molecules inside the cell. Additionally, we analyzed different metabolic processes and their dependencies from the presence of either transcription factor.

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CS3.2.18

IRON UPTAKE BY THE ROCK-INHABITING FUNGUS KNUFIA PETRICOLA: CHELATION SHORTCOMINGS OFFER ECOLOGICAL INSIGHTS AND MITIGATION STRATEGY

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Iron is arguably the most essential metal in living organisms. For rock-inhabiting fungi its acquisition might be unconventional as they (1) tend to inhabit iron-deficient, oxygen-rich surfaces like marble monuments and solar panels and (2) produce the black, iron-adsorbing pigment melanin. We used a range of analytical methods, ongoing mineral dissolution experiments and gene deletion mutants of the model rock-inhabiting fungus Knufia petricola to figure out the mechanisms and substrate deteriorating effects of iron uptake by these organisms. To study both siderophore-mediated and reductive iron assimilation (RIA), genes like sidC, encoding a putative siderophore synthetase and ftr1 and fet3 encoding the subunits of an iron permease-oxidase were deleted.
At iron deficient conditions, growth of the wild type (WT) and ΔsidC mutant was similar, whereas growth of the Δftr1-fet3 mutant and the double mutant ΔsidC/Δftr1-fet3 was diminished and absent, respectively. We were not able to detect the siderophore of K. petricola and the WT and mutants were not able to grow at low concentrations of strong iron chelators. However, in a cross-feeding experiment, an overexpression strain of sidC allowed more growth of ΔsidC/Δftr1-fet3 on iron deficient medium than the WT, whereas the ΔsidC mutant could not do so at all. Compared to the WT, the sidC overexpression strain also withstood oxidative stress better and had a shorter lag time and higher growth rate. Combined, these results indicate that K. petricola relies more on RIA than siderophore-mediated uptake as it likely excretes low quantities of a primarily intracellular siderophore. Interestingly, Δftr1-fet3 had a higher iron content than the WT at iron deficient conditions. This difference disappeared upon deletion of melanin synthesis (Δpks1 vs. Δpks1/Δftr1-fet3): melanin-bound iron can likely not be used without RIA. K. petricola's chelation incapacity implies a habitat free of competition for iron while offering us a mitigation strategy.

CS3.2.19

CHARACTERIZATION OF HIGH-AFFINITY ARABINOSE TRANSPORTER HINTS TOWARDS THE INVOLVEMENT OF MULTIPLE TRANSPORTERS IN ARABINOSE TRANSPORT BY TRICHODERMA REESEI

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Lignocellulose biomass has been considered as a promising feedstock for biofuels and other value-added products. The sugars in lignocellulose biomass can be released enzymatically and converted to the desired products by organisms such as yeast Saccharomyces cerevisiae. However, some of these sugars cannot be utilized naturally by S. cerevisiae. One of these sugars is arabinose, for which yeast lacks both a metabolic pathway and a specific transporter. Saprophytic fungi like Trichoderma reesei produce mixtures of enzymes that allow them to degrade cellulose, hemicellulose, and pectin, and hence to efficiently utilize lignocellulose biomass. Their genomes possess numerous genes coding for sugar transporters which are needed to internalize the biomass-derived sugars, including arabinose. Therefore, fungal sugar transporters have been investigated for improving yeast arabinose utilization.

We identified a known xylose transporter from T. reesei as a candidate arabinose transporter based on its expression profile [1]. We confirmed the arabinose transport activity by heterologous expression in Xenopus laevis oocytes and yeast. Electrophysiological experiments performed with oocytes showed that sugar transporter Trire2_104072 can transport arabinose, xylose and glucose. Arabinose was transported with high affinity and xylose with low affinity. With the yeast system, we observed relatively low inhibition of arabinose transport activity by xylose and glucose. Deletion of trire2_104072 gene in T. reesei affected growth only at low arabinose concentrations.

Due to its high specificity for arabinose, we hypothesize that Trire2_104072 could serve as a good candidate for improving yeast arabinose utilization. The obtained results also hint towards the presence of more than one arabinose transporters in T. reesei.

[1] Havukainen, S., Pujol-Giménez, J., Valkonen, M., Hediger, M. A., & Landowski, C. P. (2021). Functional characterization of a highly specific L-arabinose transporter from Trichoderma reesei. Microbial cell factories 20:177.

CS3.2.20

WHY DOES CANDIDA GLABRATA NEED FIVE SUGAR KINASES?

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Candida glabrata is a commensal of the human body. However, when conditions become virulence-favouring it can become pathogenic leading to infections. As C. glabrata is intrinsically resistant to the frequently used azoles, its treatment is challenging and over the years, there is a slight increase in the frequency of C. glabrata infections, which can be lethal. To infect the human host, C. glabrata possesses several virulence factors, but over the past decades it became clear that central metabolism is of equal importance for fungi to establish infections. In this project, we focus on the sugar metabolism of C. glabrata. As this fungus is only able to use glucose and trehalose, a glucose-disaccharide, as fermentable carbon sources, this sugar metabolism is odd compared to related fungi which can grow on a variety of sugars. Because of this inability to use other sugars, it was surprising to see that C. glabrata encodes five putative sugar kinases, converting sugars into sugar-6-phosphate, as they only have one putative substrate, glucose. Moreover, five sugar kinases is more than observed in related fungi. We aim to investigate the role of these sugar kinases and we hypothesise that they may have gained additional regulatory functions. Therefore, we are generating single and multiple deletion strains of these putative sugar kinases. We will determine the contribution of each of the sugar kinases to sugar phosphorylation. Besides, we will investigate whether they can translocate from the cytoplasm to the nucleus and influence the expression of the other sugar kinases. Later on, as several virulence-related pathways require sugar phosphorylation, we will look into the role of the sugar kinases in virulence. This project will contribute to the further elucidation of the sugar metabolism, -sensing and -signalling in C. glabrata possibly leading to the identification of novel antifungal drug targets.

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CS3.2.21

FUNCTIONAL CHARACTERIZATION OF L-ARABITOL TRANSPORTER LATA OF ASPERGILLUS NIGER IN SACCHAROMYCES CEREVISIAE

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Plant biomass degrading fungi are essential players in the sustainable solutions for bio-based economy. L-arabinose is an abundant pentose sugar present in plant biomass and it can be converted by many fungi through the pentose catabolic pathway (PCP). Recently, a transporter LatA for one of the PCP intermediates, L-arabitol, was identified in the filamentous fungus Aspergillus niger[1]. Despite the extensive in silico identification of sugar transporters (STs)[2] of A. niger, most transporters have remained uncharacterized both in vivo and in vitro. In vitro uncharacterized STs include polyol transporters such as LatA.
To describe the in vitro functional properties of the A. niger LatA transporter, we chose it to be characterized in the yeast Saccharomyces cerevisiae. Since S. cerevisiae lacks pentose pathways metabolizing L-arabinose or L-arabitol, we engineered S. cerevisiae strains to be able to metabolize these sugars using CRISPR/Cas9 technology. For this, we used a S. cerevisiae platform strain that is devoid of all hexose transporters, disaccharide transporters and disaccharide hydrolases[3]. The set of the metabolic platform strains of S. cerevisiae is an effective strategy to study the biochemical properties of the STs that mediate the uptake of polyols originating from fungal plant biomass conversion. Biochemical characterization of transporters enables us to also engineer tools for physiological in vivo characterization of the STs, by deleting multiple polyol transporter encoding genes of A. niger. Our ultimate aim is to connect the sugar transport from exogeneous environment to the endogenous processes to get a comprehensive view of plant biomass conversion by A. niger.

[1]Meng J. et al. Submitted
[2]Peng M. et al. Front. Microbiol. 9: 1 – 10 (2018)
[3]de Valk S.C. et al. Biotechnol. Biofuels 15: 47 (2022)

CS3.2.22

TRACE METAL IONS IN FUNGAL ORGANIC ACID FERMENTATIONS

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Organic acid accumulation is probably the best-known example of primary metabolic overflow. Both bacteria and fungi are capable of producing various organic acids in large amounts under certain conditions, but in terms of productivity – and consequently, of commercial importance – fungal platforms are unparalleled. For high product yield, chemical composition of the growth medium is crucial in providing the necessary conditions, of which the concentrations of four of the first-row transition metal elements, manganese (Mn2+), iron (Fe2+), copper (Cu2+) and zinc (Zn2+) stand out. Three of them – Mn, Fe, Cu – provide the necessary redox and catalytic activity for many important biological process-es. They possess a stable +2 oxidation state and can generate many additional stable states, which allows them to change their oxidation states in biological reactions. Manganese concentrations > 5 µg/L decrease the final yield of citric acid in A. niger and itaconic acid in A. terreus, respectively. Various methods have therefore been patented or published to remove the surplus manganese ions from the growth medium, but a more convenient strategy is to counteract their effect. Both for A. niger citric- and A. terreus itaconic acid fermentations, low product yield in the presence of high Mn-concentrations can be counteracted by increasing the copper concentration in the culture broth. We recently described that the ratio of copper to manganese – rather than their respective absolute concentration – modulates itaconic acid production yield on D-glucose and D-fructose. In this study we demonstrate that the high-affinity Mn2+ transport in A. niger is inhibited – in addition to copper – also by Zn2+ roughly to the same extent.

CS3.2.23

SUBSTRATE SPECIFICITY OF SIDEROPHORE UPTAKE BY ASPERGILLUS FUMIGATUS

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The opportunistic human pathogen Aspergillus fumigatus employs two high-affinity uptake systems for the essential micronutrient iron: reductive iron assimilation (RIA) and siderophore-mediated iron acquisition. This mold produces two fusarinine-type (fusarinine C and triacetylfusarinine C (TAFC)) and two ferrichrome-type siderophores (ferricrocin and hydroxyferricrocin) for acquisition and storage of iron. Siderophores have been shown to play a crucial role in virulence of several fungal pathogens and to have high potential as biomarker as well as for imaging of fungal infections. Moreover, the siderophore transporter (SIT) Sit1 was found to mediate uptake of the novel antifungal drug VL-2397. However, siderophore uptake in filamentous fungi is poorly characterized.
To enable characterization of siderophore uptake in A. fumigatus by growth studies, the genes encoding the five potential SITs (Sit1, Sit2, MirB, MirD and MirC) were inactivated individually or in combination in an A. fumigatus background lacking siderophore biosynthesis (ΔsidA) and RIA (ΔftrA). These studies demonstrated that (i) Sit1 and Sit2 have overlapping as well as unique substrate specificities with respect to different ferrichrome-type siderophores, e.g., utilization of ferrirhodin and ferrirubin depends exclusively on Sit2, use of ferrichrome A depends mainly on Sit1, and utilization of ferrichrome, ferricrocin, and ferrichrysin is mediated by both transporters; (ii) both Sit1 and Sit2 mediate weak use of the coprogen-type siderophores; (iii) Sit1 transports the bacterial ferrioxamine-type xenosiderophores; (iv) MirB transports TAFC; (v) MirD transports fusarinine C; (vi) MirB but not MirD is crucial for virulence in a murine aspergillosis model; vii) lack of MirC causes a growth defect under iron limitation that cannot be cured by siderophore supplementation, which questions a role in siderophore metabolism

in line with MirC localization to the vacuolar membrane. Furthermore, A. fumigatus was found to lack utilization of the xenosiderophores schizokinen, basidiochrome, rhizoferrin, ornibactin, rhodotorulic acid and enterobactin.

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CS3.2.24

REGULATION OF HIGH-AFFINITY IRON ACQUISITION IN ASPERGILLUS FUMIGATUS IS COORDINATED BY ATRR, SRBA AND SREA

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Iron acquisition is crucial for the virulence of the human pathogen Aspergillus fumigatus. Previous studies indicated that this mold regulates iron uptake via both siderophores and reductive iron assimilation by the negatively-acting and iron-sensing GATA-factor SreA and the positively-acting SREBP-type regulator SrbA. Here, characterization of loss-of-function as well as hyperactive alleles revealed that transcriptional activation of iron uptake depends additionally on the Zn_2-Cys_6-regulator AtrR, most likely via cooperation with SrbA. Mutational analysis of the promoter of the gene encoding an iron transporter involved in RIA, FtrA, iron permease-encoding ftrA gene identified a 210-bp sequence, which is both essential and sufficient to impart iron regulation. Further studies located functional sequences, densely packed within 75 bp, that largely resemble binding motifs for SrbA, SreA and AtrR. The latter, confirmed by ChIP analysis, is the first one not fully matching the 5´-CGG(N)12CCG-3´ consensus sequence. The here presented results emphasize for the first time the direct involvement of SrbA, AtrR and SreA in iron regulation. The essential role of both AtrR and SrbA in activation of iron acquisition underlines the coordination of iron homeostasis with biosynthesis of ergosterol and heme as well as adaptation to hypoxia. The rationale is most likely the iron-dependence of these pathways along with the enzymatic link of biosynthesis of ergosterol and siderophores.

CS3.2.25

THE BOLA FAMILY PROTEIN BOL3 IS DUAL LOCALISED BY ALTERNATIVE TRANSLATION INITIATION IN A. FUMIGATUS

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Numerous enzymes involved in various metabolic pathways depend on iron-sulphur clusters (FeS) as co-factors. The biosynthesis of FeS requires complex biosynthetic machineries: the mitochondrial core FeS assembly machinery generates [2Fe-2S] clusters, representing precursors of mitochondrial and cytosolic [4Fe-4S] clusters. Trafficking of [2Fe-2S] has been shown to involve BolA family proteins in cooperation with glutaredoxins (Grx). In the mold Aspergillus fumigatus, mitochondrial [2Fe-2S] biosynthesis and the cytosolic/nuclear glutaredoxin GrxD have been shown to be essential for iron sensing. Most eukaryotes possess genes encoding BolA homologs with and without mitochondrial targeting sequences (MTS). In contrast, both A. fumigatus homologs possess putative MTS, which suggests the lack of cytosolic/nuclear versions. However, closer inspection of the Bol3 protein sequence revealed a methionine residue, located downstream of the MTS and highly conserved in various Aspergillus species. Proteomic analyses identified a Bol3 peptide that indicates that this methionine is derived from alternative translational initiation. Generation and phenotyping of different bol3 mutant strains that lack the Bol3-encoding gene (Δbol3), the putative cytosolic/nuclear Bol3 (bol3M41L) or the mitochondrial Bol3 (bol3M1L, bol3Δ38) revealed different phenotypes, supporting a dual-localisation of Bol3. The most pronounced phenotype – a growth defect under iron limitation – was caused by the loss of the cytosolic Bol3. Fluorescence microscopy confirmed dual localisation of Venus-tagged Bol3 protein versions in mitochondria and the cytosol/nucleus. Purification of C-terminally Venus-tagged Bol3 proteins followed by nLC-MS/MS analysis revealed peptides, confirming different Bol3 proteins derived from alternative translational initiation, followed by proteolytic processing. Interestingly, this analysis indicated that mitochondria and the cytosol/nucleus contain the very same Bol3 protein discriminated only by N-terminal acetylation of the cytosolic/nuclear form. Mutation of the initial Kozak sequence demonstrated that increased translation initiation at the first AUG decreases translational initiation at the downstream AUG.

CS3.2.27

THE ACYLTRANSFERASE SIDF IS INVOLVED IN BIOSYNTHESIS OF FUSARININE-TYPE AND FERRICHROME-TYPE SIDEROPHORES IN A. FUMIGATUS

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The opportunistic human pathogen Aspergillus fumigatus employs two high-affinity uptake mechanisms for iron: reductive iron assimilation (RIA) and siderophore mediated iron acquisition (SIA), which has been shown to be crucial for its virulence in diverse infection models. This mold species produces fusarinine-type siderophores such as triacetylfusarinine C (TAFC) and ferrichrome-type siderophores such as ferricrocin (FC). The first committed enzymatic step for all siderophores is hydroxylation of ornithine catalyzed by SidA. Subsequently, the pathways for synthesis of fusarinine- and ferrichrome-type siderophores split. For fusarinine-type siderophores an anhydromevalonyl group is linked to hydroxyornithine mediated exclusively by the transacylase SidF, while for ferrichrome-type siderophores an acetyl group is linked by the transacylase SidL and a yet unknown enzyme. Both SidF and SidL belong to the GNAT (Gcn5-related N-acetyltransferases) protein family but are only distantly related, showing similarity only in the C-terminal half. SidF is localized in peroxisomes and the encoding gene is induced by iron starvation, while SidL is a cytosolic enzyme and expression of the encoding gene is largely iron-independent. Here we found that simultaneous inactivation of both SidF and SidL abrogates biosynthesis of both fusarinine- and ferrichrome-type siderophores. In line, the ΔsidFΔsidL double mutant phenocopies the ΔsidA mutant, e.g., growth inhibition by the iron-specific chelator bathophenanthroline disulfonate. Our studies also revealed an interdependence of fusarinine- and ferrichrome-type siderophores as inactivation of SidF blocked biosynthesis of TAFC but increased production of FC. Moreover, we demonstrate that truncation of either the GNAT-motif containing C-terminal half showing similarity to SidL or the N-terminal half blocks all SidF functions.
Taken together, this study suggests that SidF is the so far unknown enzyme catalyzing acetylation of hydroxyornithine, i.e., SidF is a bifunctional enzyme accepting acetyl-CoA and anhydromevalonyl-CoA as substrates for acylation of hydroxyornithine for biosynthesis of both fusarinine- and ferrichrome-type siderophores.

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CS3.2.28

ACCESSORY ORGANIC NITROGEN BOOSTS VEGETATIVE AND REPRODUCTIVE BIOMASS BUILD-UP IN A WHITE ROT FUNGUS WITH A FLEXIBLE LIFE STYLE

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The Black Poplar Mushroom Cyclocybe aegerita (syn. Agrocybe aegerita) is a saprotrophic white-rot fungus with a flexible life style. Its fruiting bodies form on tree trunks, declining trees, buried wood and wood chips of deciduous trees. Its substrate versatility may go hand in hand with a respective carbohydrate-active enzyme repertoire that is intermediate between plant litter decomposers and prototypical white-rot fungi. Since wood is poor in nitrogen, nitrogen mobilization from surrounding litter is one way to ensure nitrogen acquisition for cellular homeostasis and reproduction in wood-destroying fungi. To date, the impact of accessory nitrogen for vegetative and reproductive biomass build-up has not yet been investigated applying a uniform minimalistic laboratory setup. For C. aegerita, such a growth and fruiting setup is available. Here, we have grown this species with and without an organic nitrogen source which is present in plant litter. In contrast to the control treatment, elevated nitrogen levels were positively correlated with aerial mycelium weight, reproductive biomass and primordium formation. This implies that C. aegerita applies additional organic nitrogen resources to vegetative and reproductive biomass augmentation simultaneously. Growth on substrates like buried wood, which is near the plant litter layer, may, accordingly, confer an evolutionary fitness advantage. Anthropogenically altered global nitrogen dynamics may affect mycelia-driven processes as well as fruit body-driven food webs.

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CS3.2.29

UNRAVELLING THE DIVERSITY OF SUGAR RELATED REDUCTASES IN ASPERGILLUS NIGER

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Metabolic engineering of filamentous fungi gained more attention in recent years. Especially in the context of creating better fungal industrial cell factories to produce a wide range of enzymes and valuable metabolites from plant biomass. Recent studies into the pentose catabolic pathway (PCP) in Aspergillus niger, revealed functional redundancy in most of the pathway steps. Interestingly, this redundancy appears to be even larger when the fungus is grown on plant biomass compared to pure pentose sugars. In this study, we have explored this redundancy by identifying the function of paralogous genes of known pathway genes, to unravel the metabolic diversity of A. niger and related fungi. Phylogenetic analysis of the PFAM family that contains the currently known pentose reductases identified five additional genes in A. niger with high similarity to the already characterized genes. Genome editing by CRISPR/Cas9 technology and biochemical characterization were used to elucidate the function of theses putative reductases.

CS3.2.30

REVISITING ASPERGILLUS NIGER MST SUGAR TRANSPORTERS

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Aspergillus niger is one of the most studied plant-biomass-degrading filamentous fungus. In silico study reported an extensive set of putatively diverse sugar transporters (STs) in A. niger, which are predicted to play key roles in plant biomass conversion[1]. However, the comprehensive understanding of STs diversity in A. niger is largely limited as only a few of them have been characterized. It is hypothesized that A. niger STs also have different affinities and overlapping specificities.
We selected seven Mst transporter candidates from A. niger NRRL3, some of which have previously been shown or predicted to participate in glucose transport[2] [3] [4]. To characterize them functionally, we used engineered Saccharomyces cerevisiae strain deficient in hexose transporters, disaccharide transporters and disaccharide hydrolases[5]. Physiological characterization was performed through multiple A. niger Δmst strains engineered by CRISPR/Cas9 method.
Expression of Green Fluorescent Protein-tagged Mst transporters showed that these transporters were localized to yeast plasma membrane. The preliminary growth analysis of the recombinant S. cerevisiae strains indicated that MstA, MstC, MstE, MstG and MstH are capable of transporting glucose and fructose, whereas MstD and MstF are not involved in the transport of these sugars. It also showed that MstE is an effective glucose ST with the previously characterized MstA, MstG and MstH[2] [4]. MstC has been classified as glucose ST based on expression[3], which is supported by our results. The multiple Δmst strains will reveal physiological contribution of these STs in A. niger. This study will shed light on the role of the Mst transporters and increase the possibilities to find novel glucose transporters in A. niger.

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CS3.2.31

LACCASE EXPRESSION IN THE DUNG FUNGUS COPRINOPSIS CINEREA WITH 17 NATURAL LACCASE GENES

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Laccases are phenoloxidases that can oxidize phenolic and aromatic compounds and occur in bacteria, fungi, insects, and plants. Among fungi, wood-rotting Basidiomycetes are considered to be main producers of laccase in nature that under different environmental conditions secrete various forms of this enzyme, being either laccase isoforms encoded by the same gene, isoenzymes encoded by different laccase genes or allozymes encoded by different alleles of a gene. The ink-cap mushroom Coprinopsis cinerea for example has 17 different laccase genes divided into 2 subfamilies, which are differentially expressed during growth on distinct media and different temperature regimes, during fungal differentiation and as defense in confrontation with other microbes. Different monokaryotic strains of the fungus can have inactivated copies of some of these genes. Most often, laccase gene lcc15 is affected from gene inactivation. Monokaryotic strains usually express Lcc1 and Lcc5 as main laccases, under stress at lower temperature of 25-28 °C much higher than at 37 °C as the best growth temperature. Lcc9 can also be expressed in traces while full expression is encountered as response in presence of competitors. Laccases of C. cinerea are of interest for biotechnological applications, which requires good production rates of properly glycosylated enzymes. Enzymes can be overexpressed in C. cinerea upon cloning their genes behind highly active promoters, transformation of constructs into suitable monokaryotic strains and cultivation of transformants under favorable environmental conditions, with yields up to 30 U/ml culture supernatant depending on the gene used for cloning. Crossing of transformants can further enhance laccase yields with dikaryons expressing a single enzyme or mixtures of laccases when transformants of different laccase genes were mated.

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CS3.2.32

FUNCTIONAL ANALYSIS OF ACID PHOSPHATASE OF ASPERGILLUS ORYZAE

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Miso, fermented soy bean paste, is a traditional Japanese seasoning. It is made from soybeans, salt, water, and koji (solid-state culture of A. oryzae on rice, soybean, or barley). During the koji preparation, A. oryzae secretes variety of enzymes essential for efficient maceration and degradation of solid materials. Among these secreted enzymes, acid phosphatase may hydrolysis the ribonucleotide umami components yielding insipid ribonucleosides and phosphoric acid. Therefore, heat treatment is required in the manufacturing process of umami added miso to inactivate the acid phosphatase of A. oryzae. However, heat treatment needs energy and special equipment, and it reduces the quality of miso.
Through the screening of 503 practical A. oryzae strains stocked in Bio'c Co., we found the strain with greatly reduced acid phosphatase activity while maintaining protease and amylase activities sufficient for miso fermentation. In the A. oryzae genome, 13 putative extracellular acid phosphatase genes (aphA-M) were identified and there is no information which acid phosphatases are mainly involved in the hydrolysis of ribonucleotide umami components.
In this study, we investigate the cause of the reduced acid phosphatase activity in screened strain (KBN-p) to obtain better strains. The genome sequence of KBN-p was compared with 4 other A. oryzae strains normally used for miso fermentation, and 5 acid phosphatases have special amino acid substitution in KBN-p strain. Furthermore, we checked the transcriptional pattern of aph genes in response to the addition of phosphate. As a result, among the 13 acid phosphatases, AphC may be the cause of low acid phosphatase activity in KBN-p. Now we purified AphC from several strains and confirm its activity and stability to determine the actual cause of reduced acid phosphatase activity in KBN-p strain.

CS3.2.33

MANGANESE HOMEOSTASIS SHAPES FUNGAL BIOLOGY AND VIRULENCE OF CANDIDA ALBICANS

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Trace metals including iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn) are essential micronutrients that are sequestered by the host to limit microbial growth, a process known as “nutritional immunity”. For a human fungal pathogen like Candida albicans, the acquisition of these metals is essential for its survival and also for its ability to infect the host as many virulence factors such as the superoxide dismutases require these metals for their activity. While seminal contributions were made to understand how fungal pathogens acquire Fe, Cu and Zn, processes required for Mn internalization and keeping this central metal at homeostatic level remain completely unexplored. To understand the contribution of Mn homeostasis in fungal virulence, we performed RNA-seq transcriptional profiling in the opportunistic yeast C. albicans under both Mn limitation and excess. Our data emphasized a significant impact of Mn fluctuations on different biological and virulence processes. For instance, Mn deprivation promote the expression of ECE1 a gene encoding the lytic cytotoxin Candidalysin required for mucosal invasion. In accordance with this observation, we found that C. albicans cells pre-grown in Mn-depleted medium caused more damage to human enterocytes as compared to cells grown under Mn sufficiency. We also uncovered that the transcriptional level of different transporters including members of the Nramp (Natural resistance-associated macrophage proteins) Mn transporters were modulated by Mn (SMF12, SMF11, SMF2 and SMF3). Genetic inactivation of Smf transporters uncovered their role in Mn homeostasis, antifungal tolerance, unfolded protein response and host invasion. To our knowledge, this work represents the first assessment of the genetic determinism of Mn homeostasis and its contribution to fungal virulence.

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CS3.2.34

DISTINCT EFFECTS OF ANAEROBE AND AEROBE FUNGAL ACTIVITY ON LIGNOCELLULOSE COMPOSITION AND STRUCTURE DURING ITS DEGRADATION

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Understanding effective biodegradation of lignocellulose is essential to advancing renewables-based biotechnology. Similarly, in livestock farming, lignocellulose degradation by the rumen microbiome is essential for feed digestion, and therefore critically impacts ruminant farming. As producers of effective carbohydrate active enzymes, fungi are highly important in both areas. Our insight in the fungal physiology and metabolism relating to lignocellulose degradation is ac-celerating. However, to fully understand fungal degradative mechanisms, we now need to combine this with knowledge of the effects that fungi and their enzymes have on the composi-tion and structure of the actual complex lignocellulose substrates.
We previously investigated how industrial work-horse Aspergillus niger regulates gene expression and enzyme secretion in response to lignocellulose (PMID 32313551, 28184248). We then investigated how lignocellulose composition and structure changed after exposure to this fun-gus, to create a full picture of its degradative mechanism. We are now comparing the degrada-tive activity of Aspergillus niger with that of three species of anaerobe rumen fungi, phylum Neocallimastigomycota, as both fungi display stark differences in physiology (aerobe vs anaerobe) and degradative mechanism (soluble enzymes vs enzyme complexes). We employed complementary techniques to assess changes caused in the lignocellulose matrix, including fiber analysis and glycoprofiling to assess changes in polysaccharides, and mass spectrometry-based imaging to identify surface exposure of lignin and polysaccharides. We identified differences in lignocellulose degradation efficiency between fungal species as well as distinct patterns in deg-radation of hemicelluloses and cellulose. We will contrast the effects of the degradative activity of the different fungi, and highlight how these may be exploited.

CS3.2.35

PEPTIDE-BASED FUNCTIONAL ANNOTATION (BY CUPP ANALYSIS OF ALL GENOME-SEQUENCED FUNGI) LED TO DISCOVERY OF UNCHARACTERIZED AA3 OXIDOREDUCTASES, PROMINENTLY PRESENT ACROSS FUNGAL KINGDOM

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Fungal digestive enzyme secretome blend, (enzyme composition; substrate, affinity and function), can be characterized most efficiently by annotating to “Function;Family” observations (“F;F”). This is achievable by annotation to CAZyme family (and subfamily), integrated with a peptide-based functional prediction (by CUPP, Conserved Unique Peptide Pattern analysis; available online). Initially, “F;F” enzyme blend analysis was done (of the enzymatic biomass conversion capacity of the secretome) of all genome sequenced species of Aspergillus. This resulted in identifying several “AA3;Unknown function” observations. This result inspired to further investigation, across the Fungal Kingdom, of the occurrence of such most likely novel/uncharacterized AA3 enzymes with unknown function. By annotating all genome sequenced fungal species to “Function;Family” observations, we found four types of AA3 observations, “AA3;1.2.3.10”, “AA3;1.1.2.13”, “AA3; 1.1.3.4” and “AA3;1.1.99.18”. Furthermore, a surprisingly prominent and widespread presence, of AA3 with unknown function was found; and such “AA3;Unknown function” enzymes were found to be associated in degradation of both lignin and cellulose. The 35 fungal species, found to have the highest redundancy level of AA3 enzymes with unknown functions, were analyzed with-regard-to their eco-physiological life-form specialization and Fungal Kingdom taxonomic position. Interestingly, a very broad diversity of eco-physiological specializations were found among the “AA3;Unknown function”-top scoring species, e.g., saprotrophic mushrooms and soil-inhabiting saprotrophic ascomycetes; ascomycetous and basidiomycetous plant pathogens; wood degrading fungi (both Asco- and Basidiomycota); molds, e.g. Aspergillus and Penicillium species; and several more. The presentation will also include a preliminary (bioinformatic) characterization of the “AA3;Unknown function” enzymes identified, to be found so widespread among highly diverse types of fungal life forms and taxonomies.

CS3.3 ANIMAL/HUMAN INTERACTIONS

CS3.3.9

MORPHOTYPE-SPECIFIC FUNGAL FACTORS DRIVE UPTAKE AND CLEARANCE OF ASPERGILLUS FUMIGATUS BY AIRWAY EPITHELIAL CELL

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The fungal pathogen Aspergillus fumigatus (Af) affects over 3,000,000 individuals annually, with invasive aspergillosis having mortality rates of over 50%. Airway Epithelial Cells (AECs), which cover the entire alveolar surface and comprise 24% of all cells in the human lung parenchyma, have instant, extensive, and likely prolonged contact with Af conidia upon inhalation. We previously demonstrated that AECs provide a potent means of antifungal defense against Af in vivo, and that dysfunctional epithelial antifungal activity in at-risk patients may provide an opportunity for Af to exploit AECs as a safe haven to reside intracellularly. However, relatively little is known about the fungal factors controlling Af uptake and clearance by AECs and the dependency of these processes on the morphotype-specific changes associated with fungal germination. To this end, using fluorescent auxotrophic pyrG- strains, we locked Af at specific morphological stages, and determined that swollen conidia locked at 3 and 6 hours of germination are 2-fold more readily internalized than conidia locked at 0 hours. Internalization rates decrease as the germ tube extends and the fungus becomes larger (from 9 hours onwards). Probing with fluorescent lectins, we identified mannose and N-acetylglucosamine as two key surface carbohydrates that show a morphotype-specific increase during germination, making them likely surface epitopes involved in Af-AEC interactions. Supporting this, mannose and the mannose-binding lectin Concanavalin A were able to respectively reduce (by 88%) and abolish (100%) Af internalization. Using a combination of Af cell wall mutants, and AEC receptor mutants, we are systematically evaluating morphotype-specific factors on Af surface and characterizing key host receptors for their role in mediating fungal uptake and clearance both healthy and diseased AECs. Understanding how AECs contribute to antifungal clearance by recognizing morphotype-specific fungal factors is of mayor clinical importance as it could inform the development of much needed novel antifungal therapeutics.

CS3.3.10

TRANSCRIPTIONAL RESPONSES DURING TRICHOPHYTON RUBRUM AND HUMAN KERATINOCYTES INTERACTION: AN UPDATE ON RECOGNITION PATTERNS, SIGNALING AND CELL WALL REMODELING

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Superficial infections of keratinized tissues, called dermatophytosis, are the most prevalent mycoses worldwide, being Trichophyton rubrum as the most frequent etiological agent. Despite being restricted to cornified layers of the epidermis, these infections could be severe in people with diabetes or immunocompromised patients, dramatically decreasing the quality of life.
Keratinocytes play a pivotal role in the innate immune response during a dermatophyte-host interaction. For a suitable immunological response, pathogens-associated molecular patterns (PAMPs) need to be recognized by host-specific pattern recognition receptors (PRRs). Ligand recognition of fungal chitin, glucans, and mannans triggers the activation of distinct signaling pathways, such as the nuclear factor NF-kβ, stimulating an immunomodulatory response. Furthermore, the recognition of fungal β-glucan by immune cells can lead to metabolic reprogramming increasing aerobic glycolysis, also known as the Warburg effect. This metabolic change is signalized by the recruitment of the intracellular sensor mTOR that mediates the increased expression of the transcription factor HIF-1α, favoring aerobic glycolysis. As a result, high glucose levels are utilized to generate lactate, and oxidative phosphorylation by mitochondria is prevented.
Considering that molecular mechanisms that trigger the host recognition, signaling, and cell wall remodeling during T. rubrum- host interaction is still scarce, the present work evaluated by RT-qPCR the transcriptional response of several human PRRs, fungal PAMPs, and genes involved in the Warburg effect during a co-culture with T. rubrum and human keratinocytes for 3, 24 and 48h. Our results showed the upregulation mainly in 3h and 24 h of several human PRRs, such as toll-like receptors and Nod-like receptors, as well as the NF-kβ transcription factor. Genes of fungal β-glucans and chitins showed distinct transcriptional response patterns after 24h and 48 h. Furthermore, upregulation of mTOR, HIF-1α, and lactate dehydrogenase genes was observed, suggesting that keratinocytes could lead to metabolic reprogramming during infection.

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CS3.3.11

STOMATIN IS REQUIRED FOR RECRUITMENT OF DECTIN-1 TO THE PHAGOSOMAL MEMBRANE AND FOR FULL ACTIVATION OF MACROPHAGES AGAINST ASPERGILLUS FUMIGATUS

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Alveolar macrophages are part of the first line of defense against inhaled conidia of the human-pathogenic fungus Aspergillus fumigatus. In the alveoli, they contribute to phagocytosis and elimination of the conidia. As a defense measure, the conidia possess a grey-green pigment that enables them to survive for some time in phagosomes of macrophages. Previously, we had shown that this pigment interferes with the formation of flotillin-dependent lipid-raft microdomains in the phagosomal membrane, thereby preventing the formation of functional phagolysosomes.
Besides flotillins, stomatin is a major component of lipid rafts and can be targeted to the membrane. However, little information is available on stomatin, especially on its role in defense against pathogens. To determine the function of this integral membrane protein, a stomatin-deficient macrophage cell line was generated by CRISPR/Cas9 gene editing. Immunofluorescence microscopy and flow cytometry revealed that stomatin contributes to phagocytosis of conidia and is important for the recruitment of the β-glucan receptor dectin-1 to both the cytoplasmic and phagosomal membranes. In stomatin knockout cells, phagosome-lysosome fusion and vATPase recruitment to phagosomes were reduced when infected with pigmentless conidia. The data presented here provide new insights into the important role of stomatin in the immune response against human pathogenic fungi.

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CS3.3.12

MICROSCOPIC ANALYSIS OF HOST-PATHOGEN INTERACTIONS USING MICROPATTERNED HUMAN ALVEOLAR A549 CELLS INFECTED WITH ASPERGILLUS CONIDIA

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Attachment, internalization and processing of fungal conidia by airway epithelial cells orchestrate conidia clearance mechanisms as well as early immune signalling. Investigating these processes in spatially defined cellular compartments is yet a technical challenge. In vitro models of airway cell lines suggest that accurate assessment of conidia-cell interactions can only be determined using multi-plane microscopy. Moreover, spatial organization of compact polarized epithelial monolayers precludes resolving the compartmentalization of cellular events due to the complexity of multicellular systems.
Previous studies indicated that changes in the extracellular microenvironment that control fundamental parameters of cellular state, like confinement, adhesion, and contractility, can be defined using micropatterned substrates. Micropatterns coated with extracellular matrix proteins impose adhesive and non-adhesive areas to individual cells and thus, allow controlling internal cell organization. For example, studies using fibronectin-coated micropatterns have illustrated that cell polarity and mechano-sensitivity respond to the geometry of cellular adhesion cues. Furthermore, micropatterns of larger sizes enable the formation of defined/synchronized multi-cellular islands.
Here, we have used multi-plane live cell imaging and high-resolution fluorescent microscopy to analyse the processing of Aspergillus conidia by micropatterned human alveolar A549 cells stably expressing lysosomal/late endosomal protein LAMP1-NeonGreen. To gain insights into the molecular mechanisms involved in the conidial fate, we have used conidia of different Aspergillus strains including A.fumigatus CBS 144.89, ΔPksp, ΔUgm1, ΔGt4bc as well as patient isolates of A.fumigatus and A.terreus. Our preliminary data show that A549 cells seeded onto fibronectin-coated micropatterns adhere and grow to the patterned areas until a certain cell confluence, forming an

island. Upon addition of dormant conidia, the pathogen specifically adheres and becomes immobilized to the cell islands with some conidia being internalized. This ongoing work constitutes a novel model to study host-pathogen interactions allowing high spatial resolution of cellular events to extend our understanding of conidia processing by non-phagocytic cells.

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CS3.3.13

VISUALIZATION OF CD226 IN THE NK-CELL-FUNGUS INTERACTION

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Natural killer (NK) cells play an important role in the clearance of fungal infections including invasive pulmonary aspergillosis (IPA) that causes severe mortality in immunocompromised patients. Deciphering the mechanism of interactions of NK cells with Aspergillus fumigatus- the causative agent of IPA - is an ongoing and puzzling topic.
Surface-associated proteins are significant mediators that function in the interaction of immune cells with their target. Among them, the membrane associated DNAX accessory molecule 1 (DNAM-1; CD226) plays an important role in the regulation of NK-cell activity. Malfunction of CD226 is an assignable cause of different diseases ranging from autoimmune diseases to cancer and viral infections.Here we investigate by means of fluorescence microscopic techniques if and in how far CD226 is involved in the fungal clearance by NK cells. Purified, fluorescently tagged CD226 protein stained the cell wall of A. fumigatus hyphae suggesting direct interaction between CD226 and the fungal surface. Single molecule analysis (dSTORM) of CD226 moieties in naïve NK cells or cells exposed to fungal hyphae revealed no clear relocalization of CD226 toward the immunological synapse as observed for the pattern recognition receptor CD56 [1]. However, bioinformatic analysis suggested the cell-wall located proteases opsB in A. fumigatus and Sap10 in C. albicans as potential target proteins for CD226. Indeed, NK cells treated with purified surface-associated protease (Sap10) from C. albicans showed increased protein and chemokine secretion highlighting the role of Sap10 in NK-cell activation. Furthermore, binding of fluorescent Sap10 obviously requires the presence of CD226 as Sap10 signal was only present in primary NK cells unlike in ΔCD226 cells.
In conclusion, our data suggest binding of CD226 to A. fumigatus and activation of NK cells via CD226 by fungal cell-wall located proteases.

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CS3.3.14

A PUTATIVE RAB GTPASE, CNYPT7, INFLUENCES GROWTH AT ELEVATED TEMPERATURE, HEME USE AND VIRULENCE IN CRYPTOCOCCUS NEOFORMANS.

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In fungi, one of the Rab GTPases, Ypt7, plays critical roles in late endosome trafficking, and is required for homotypic fusion events in vacuole biogenesis. Previously, we described the involvement of the HOPS (homotypic fusion and vacuole protein sorting) tethering complex proteins, Vam6 and Vps41, in iron/heme utilization and virulence in Cryptococcus neoformans, a fungal pathogen causing life threatening meningoencephalitis in immunocompromised individuals. In this study, we identified a putative YPT7 homolog in C. neoformans, CnYPT7, and characterized its functions. Deletion of CnYPT7 caused abnormal vacuolar morphology, increased sensitivity to trafficking inhibitors, defective endocytic trafficking, failure to undergo autophagy, and mis-localization of Aph1, a secreted vacuolar acid phosphatase. These results suggest that CnYpt7 possesses conserved functions in vacuole biogenesis, intracellular membrane trafficking, and autophagy. CnYpt7 localized to the vacuolar membrane. Interestingly, the absence of CnYpt7 resulted in defective growth at the elevated temperature of 39ºC, and reduced capsule formation, compared to the wildtype strain, suggesting a role for CnYpt7 in the virulence of this pathogenic fungus. Most likely, CnYpt7 influences thermotolerance via the calcineurin signaling pathway because ypt7 mutants displayed increased susceptibility to calcineurin-specific inhibitors, FK506 and cyclosporin A, and impaired growth in either limiting or high levels of calcium. Furthermore, CnYPT7 deletion mutants showed defective growth in minimal medium supplemented with heme as the only iron source, but normal growth with inorganic iron sources. Deletion of CnYpt7 also caused the mis-localization of GFP-Rim101, further supporting the hypothesis that CnYpt7 plays a role in acquisition of iron from heme in C. neoformans. Finally, CnYpt7 is required for survival during interactions with macrophages, and the mutants exhibited attenuated virulence in a mouse inhalation model of cryptococcosis. Therefore, CnYpt7 contributes to virulence in C. neoformans likely through pleiotropic functions in membrane fusion, virulence factor elaboration, and the use of iron from heme.

CS3.3.15

THE ROLE OF RHIZOFERRIN IN GROWTH AND VIRULENCE OF RHIZOPUS MICROSPORUS

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Among the wide variety of fungal infections, those caused by mucormycetes are tremendously life threatening, characterized by fulminant progression of disease, difficulties in diagnosis and high resistance to antifungal treatment. Recently, exploding numbers of mucormycete-infections have been reported in association with severe COVID-19 infections especially in India. This highlights that understanding virulence traits of mucoralean fungi is of major importance to speed up identification of novel drug targets or optimize drug structures for better efficacy.
One essential element for all living organisms is iron. Fungi secrete siderophores to enable chelation and uptake of ferric iron. For clinically relevant mucormycetes it has been shown that a polycarboxylate siderophore, identified as rhizoferrin, is secreted. To elucidate the role of rhizoferrin in virulence, we achieved disruption of rfs gene in Rhizopus microsporus by applying plasmid free CRISPR/Cas9 system and HR-mediated DNA repair. Homokaryon formation was induced by repetitive plating and confirmed by PCR and Southern Blot analysis. First, the impact of rfs deletion on growth and germination in different media was studied, and if if growth inhibition could be reverted by addition of freely available iron. Rfs deletion resulted in significantly reduced virulence potential in the Galleria melonella infection model and currently investigations are carried out to determine if virulence can be restored in the presence of xenosiderophores.

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CS3.4 SYMBIONTS AND ENDOPHYTES

CS3.4.9

ABUNDANCE AND DISTRIBUTION OF ENDOPHYTIC FUNGI IN TOMATO PLANTS: TRADITIONAL VERSUS COMMERCIAL GENOTYPES

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It is currently known that plant genotype is reported to affect the microbiome composition and structure. We hereby study the endophytic communities of several tomato genotypes to understand their relation. Four traditional varieties from the Mediterranean area (ADX, TH-30, ISR, MO-10) were examined along with two commercial cultivars (Ailsa Craig, Moneymaker). Seeds were sown and grown under controlled conditions in growth chamber. 4-week-old plants were collected, and their stems sampled. DNA was extracted and used to perform amplicon sequencing targeting ITS region. The results of the following bioinformatics analyses showed that traditional tomato genotypes possessed significantly higher number of microbial taxa than their commercial counterparts, with preponderance of communities of the Sordariomycetes class. In addition, the composition of these fungal communities was more diverse and had a wider phylogenetic background. Thus, traditional tomato, which has been subject to lower pressure of manipulation than commercial cultivars, constitutes a richer source of novel fungal endophytes with potential to help crops endure stressful conditions.

cS3.4.10

CRANBERRY PLANT GROWTH PROMOTION: THE ROLE OF FUNGAL ENDOSYMBIONTS

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All plants in natural habitats are associated with microbial symbionts. Traditionally, plant-microbe associations have been studied from a crop-disease perspective, while we are interested in microbes that stimulate plant growth (biofertilization) and/or protect their host from pathogens (biocontrol).
Arbuscular Mycorrhizal Fungi (AMF; Glomerales), which colonize more than 90% of all land plant species, are arguably the best-investigated fungal symbionts living inside plant tissues (endophytes). In contrast, Ericaceae are among the few plant groups that harbour fungi other than AMF. The fact that Ericaceae readily grow in nutrient-poor, acidic soil, is probably facilitated by their endophytes. We examined the host-endosymbiont relationships in the poorly investigated ericacean plant Vaccinium macrocarpon (Cranberry). Among the isolated fungal strains with biofertilization and biocontrol abilities is a species provisionally designated 'Endophytic Champignon 4' (EC4). EC4 colonizes cranberry plant roots intracellularly, with hyphae reaching into the rhizosphere. In-plantae tests show that EC4 stimulates growth on water-soluble (potassium phosphate) and water-insoluble (tricalcium phosphate) phosphate sources. Analysis of the nuclear genome and transcriptome revealed that EC4 expresses numerous genes involved in mineral uptake, assimilation, and transport, which most likely give rise to the growth promotion of its host.
Molecular phylogeny based on 28S rRNA sequences places EC4 in the ascomycete genus Codinaeella (Chaetosphaeriales, Sordariomycetes). Earlier identified fungal endophytes of Ericaceae mainly belong to quite distant taxa, such as Helotiales (Leotiomycetes). Thus, EC4 is the first characterized Vaccinium endophyte and the first member of the Chaetosphaeriaceae family with a sequenced genome and transcriptome. Given its biofertilization potential, EC4 could be used for cranberry farming, by improving crop yield and decreasing the adverse impact of chemical fertilizers on the environment and human health.

CS3.4.11

MYCELIAL ARCHITECTURE AND TRANSCRIPTIONAL CHANGES OF FUNGAL PHOSPHATE TRANSPORTERS IN MYCORRHIZAL PLANTS OF DIFFERENT MAIZE INBRED LINES

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Phosphorus (P) is often limiting in agricultural soils, though it is required by plants for their growth and development. With the aim of reducing external P inputs, an array of microbial species able to live in association with plant roots, such as arbuscular mycorrhizal fungi (AMF), may be exploited to facilitate the optimization of phosphate use by plants in agroecosystems. To gain a better knowledge of fungal and plant traits possibly involved in P uptake and translocation, we studied the expression patterns of genes encoding phosphate transporters (PTs) in extraradical and intraradical mycelium (ERM and IRM), along with ERM extent and structure, plant growth and P uptake. Four maize inbred lines differing for their tolerance to low-P availability (Oh40B, Mo17, Oh43 and B73) were inoculated with Rhizoglomus irregulare and grown in a bidimensional experimental system in vivo. PT genes showed higher expression levels in ERM (and roots) of Mo17 (a low-P tolerant maize line) than in B73 (a low-P susceptible line), which also displayed a larger P increase in response to AMF colonization. Moreover, the ERM developing from roots of the B73 maize line was characterized by the highest hyphal density and interconnectedness. Interestingly, correlations were found between ERM structural traits, and both PT genes expression levels and plants mycorrhizal responsiveness. These results represent a sound basis for further studies aimed at unveiling the genetic mechanisms which regulate AMF symbiosis functioning, eventually allowing the design of “microbial management strategies” for a sustainable use of low-P tolerant crops.

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CS3.4.12

VARIATIONS IN HYPHAL FUSION OUTCOMES AMONG DIFFERENT STRAINS OF ARBUSCULAR MYCORRHIZAL FUNGI DURING THE ASYMBIOTIC, PRE-SYMBIOTIC AND SYMBIOTIC STAGES

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Arbuscular mycorrhizal fungi (AMF) are obligate root symbionts of most land plants, receiving carbon in exchange for mineral nutrients absorbed by large belowground networks of extraradical mycelium (ERM). The non-septate, multinucleated hyphae forming ERM are interconnected by hyphal fusions (anastomoses), functional to the maintenance of hyphal communication and to establish connections among different hosts. Data showing the occurrence and frequency of anastomoses in AMF and their potential role in nutrient and genetic flow are accumulating, although the knowledge on fungal determinants regulating compatibility/incompatibility in hyphal interactions is still limited. In a bi-dimensional system, devised for both asymbiotic and symbiotic stages, hyphal self-recognition ability of individual AMF has been confirmed on most Glomeracean isolates, where perfect anastomoses showing protoplasm continuity occurred. Variations in the rate of perfect anastomoses were recorded among isolates, both in asymbiotic and in pre-symbiotic mycelium, i.e. asymbiotic hyphae branching in response to host plants root exudates. Incompatibility, consisting of protoplasm withdrawal and septa formation either in fused hyphae or in the approaching hyphal tip before contact with the neighboring hypha, occurred in hyphal interactions between genetically different germlings and networks, belonging to co-specific isolates of Funneliformis mosseae and Rhizoglomus irregulare. Strains of R. irregulare show a homokaryotic/dikaryotic-like genetic pattern for putative mating-type (MAT) loci, that contains inversely transcribed homeodomains resembling those of Basidiomycota. Interestingly, when co-cultured in the two-dimensional system, some pairings of theoretically compati-

ble strains with distinct homokaryotic MAT-loci showed perfect anastomoses and/or post-fusion incompatible responses. Further work is needed to solve the genetic puzzle responsible of the outcomes of hyphal fusions in AMF, and to assess their impact on the occurrence of genetic segregation and variations among isolates, which may have important impacts on AMF ecology, evolution and symbiotic efficiency.

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CS3.4.13

INSIGHTS IN TRICHOMA VACCINUM ECTOMYCORRHIZOSPHERE

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Well-functioning forest ecosystems are of high importance, especially in times of climate change and forest dieback. In our research we focus on ectomycorrhiza-forming fungi like Tricholoma vaccinum known to stabilize the health of trees. We investigated the ectomycorrhizospheric habitat of T. vaccinum with its plant host spruce (Picea abies), characterized the soil and the community of co-occurring microorganisms, and identified mycorrhiza helper bacteria. We could show production of phytohormones and volatile compounds by the ectomycorrhizal fungus, plant host and co-occuring microorganisms, and investigated fungal phytohormone biosynthesis. RNASeq was carried out to identify transcriptomic changes in T. vaccinum while interacting with abiotic stressors like metal-containing seepage water axenically and while interacting with the plant host. Thus, we could not only show specific reactions in interactions occurring in pristine habitats, but can put ideas for helping to establish healthy forests even in anthropogenically contaminated areas like metal-rich environments present in former mining sites.
With our studies we were able to gain new insight into interactions and communication between organisms as well as with the non-living environment in the ectomycorrhizosphere, and we were able to develop and to adapt methods for these studies to build a powerful tool-set.

CS3.4.14

PHAGOCYTOSIS UNDERPINS THE BIOTROPHIC LIFESTYLE OF INTRACELLULAR PARASITES IN THE CLASS PHYTOMYXEA (RHIZARIA)

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Phytomyxea are intracellular biotrophic parasites infecting plants and stramenopiles and they include agriculturally impactful pathogens such as Plasmodiophora brassicae. Long thought to be fungi, recent molecular investigations assigned them to the clade Rhizaria, mainly composed of free-living amoeboflagellates where phagotrophy is the most widespread mode of nutrition. Phagocytosis is a complex multigene trait specific to eukaryotes, well documented in free-living unicellular eukaryotes and specialised cellular types of animals. Studies on phagocytosis in intracellular biotrophic parasites are scant, since the direct consumption of host organelles and cellular components is seemingly at odds with the biotrophic requirement of keeping the invaded cell alive. Here we provide evidence that phagotrophy is part of the nutritional strategy of phytomyxea, using morphological and genetic data (including a novel transcriptome of the brown algae parasite Maullinia ectocarpii). We document intracellular phagocytosis in P. brassicae and M. ectocarpii by transmission electron microscopy and fluorescent in situ hybridization. Our investigations confirm the presence of molecular signatures of phagocytosis in Phytomyxea and hint at a small specialised subset of genes used for intracellular phagocytosis. Microscopic evidence confirms the existence of intracellular phagocytosis, which in phytomyxea targets primarily host organelles. Phagocytosis seems to coexist with the manipulation of host physiology typical of "traditional" biotrophic interactions. Our findings resolve long debated questions on the feeding behaviour of Phytomyxea, suggesting an unrecognised role for phagocytosis in biotrophic interactions.

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CS3.4.16

28 MINUTES LATER: A PROOF OF CONCEPT FOR TESTING BEHAVIOR-MANIPULATING COMPOUNDS FROM ZOMBIE-MAKING FUNGI

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Manipulating fungi of the genus Ophiocordyceps have evolved to change the behavior of insects and arachnids. Some of the most conspicuous examples are found in infected carpenter ants, colloquially known as “zombie ants”, which climb and latch onto elevated positions, aiding fungal spore dispersion. The molecular driving force behind behavioral manipulation is unknown. Multi-omics approaches in the zombie ant fungus Ophiocordyceps camponoti-floridani have led to a wealth of new hypotheses regarding the bioactive compounds involved. To test these hypotheses, we established a methodology for systematically testing putative behavior-manipulating compounds. To pilot these protocols, we selected aflatrem since the production of a similar compound is highly upregulated during fungal manipulation of Camponotus floridanus. Previous studies linked aflatrem to neurological disorders in vertebrates (“staggers syndrome”) reminiscent of staggering behaviors observed in Camponotus floridanus ants during late-stages Ophiocordyceps infection. Furthermore, the availability of pre-synthesized aflatrem reduced the number of variables in our experimental setup, allowing for a greater focus on procedural design. To test if aflatrem-like compounds are responsible for staggering behavior in zombie ants, we injected healthy individuals with purified aflatrem and recorded their behavior for 30 minutes. Using both the machine learning tool MARGO and manual behavioral quantification, we found that aflatrem not only elicited staggering behavior in ants, but also reduced their overall activity and speed. Subsequent RNA-seq indicated 262 genes that were significantly dysregulated in aflatrem-injected ants. Comparison to previous transcriptomics data for Ophiocordyceps¬-infected ants showed that 108 of these genes were also dysregulated during behavioral manipulation, including several neuromuscular, olfactory sensing, and hormonal genes. Together, these protocols provide a framework for future studies examining the role of other putative behavioral effectors.

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CS3.4.17

TRANSMISSION OF RNAI SIGNALS BY A BENEFICIAL FUNGAL ENDOPHYTE TO NICOTIANA BENTHAMIANA LINES

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Fusarium solani strain K (FsK) is a non-pathogenic, endophytic fungus, previously isolated from the roots of tomato plants. It is a beneficial organism that confers resistance to biotic and abiotic stressors and also promotes plant growth. It can also colonize the roots of Nicotiana benthamiana and the whole body of Lotus japonicus. There is growing evidence that during interactions of plants with pathogenic fungi, there is bi-directional movement of small RNAs. However, the mechanisms of trans-kingdom RNAi during symbiotic relationships, are poorly understood. Previous work from our laboratory revealed that FsK encodes the core RNAi proteins (AGO1-2 and DCL1-2) and the machinery is functional. The goal of this experiment is to study small RNA transmission from a beneficial fungal endophyte to its host. To monitor if small RNA transmission takes place during the interaction of FsK with its host, we used gfp expressing Nicotiana benthamiana lines inoculated with an FsK transformant containing a transgene that targets host GFP. The effect of colonization levels of the root system by the endophyte was also tested. The efficiency of silencing mediated by FsK was monitored for a period of nine weeks, both visually under ultraviolet light as well as quantitatively by PCR.

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CS4.1 ANTIFUNGALS AND RESISTANCE MECHANISMS

CS4.1.9

DIRECTED EVOLUTION OF VORICONAZOLE RESISTANCE IN ASPERGILLUS FUMIGATUS IDENTIFIES NOVEL MUTATIONS RESPONSIBLE FOR TRIAZOLE RESISTANCE.

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Aspergillus fumigatus is the leading invasive mold pathogens in humans. The first line of treatment for invasive A. fumigatus infections are the triazole antifungals that inhibit Erg11/Cyp51 lanosterol demethylase activity, blocking ergosterol biosynthesis.
In recent years, triazole resistance of A. fumigatus has been increasingly reported, both as a result of widespread agricultural use of fungicidal triazoles and long-term treatment in patients with chronic aspergillosis. To date, the most common triazole resistance mechanisms in A. fumigatus are alterations in the erg11A/ cyp51A gene or promoter, followed by overexpression of efflux pumps and mutations in hmg1, encoding HMG-CoA reductase. To identify novel triazole resistance mechanisms, we passaged A. fumigatus wild type and cyp51A-null strains under increasing concentrations of voriconazole (0.25 µg/ml to 20 µg/ml) to generate resistant strains. Resistant isolates were whole-genome sequenced and compared to untreated controls. We identified known cyp51A and cyp51B mutations, and novel mutations in HMG1 and in previously uncharacterized genes in the ergosterol biosynthesis pathway as well as several efflux pumps. We identified the contribution of each mutation to the resistance phenotype by re-introduction, alone and in combination, into the susceptible parental strain, using a novel seamless CRISPR-Cas9-based system we will describe. Our study identified novel genes conferring triazole resistance and helps outline the complex stepwise evolutionary paths by which A. fumigatus develops resistance.

CS4.1.10

UNRAVELLING THE RESPONSE TO MEMBRANE DAMAGE IN THE ASCOMYCETE NEUROSPORA CRASSA

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The plasma membrane forms the barrier between the cytosol and the outer environment. Restoration of membrane integrity after injury is therefore essential for cell survival. Threats to membrane integrity include mechanically induced wounding or membrane-targeting chemicals. To counteract these events, cells have evolved membrane repair mechanisms (RMs), which involve Ca2+-signaling, cytoskeleton remodeling, and recruitment of different cell components to the wound site.
To study the molecular basis of fungal membrane repair, we are employing the ascomycete fungus Neurospora crassa. We identified the proteins PEF1 and ANX14 as part of RMs with different but overlapping functions. Subcellular localization revealed that PEF1 and ANX14 accumulate at wound sites during cell-cell fusion-induced lysis. Since membrane damage can also be induced by membrane-targeting chemicals, we tested the subcellular dynamics of these proteins in response to the anti-fungal drug nystatin and the plant defense compound α-tomatine. PEF1 and ANX14 are recruited to the membrane in response to α-tomatine, while only PEF1 is responding to nystatin. We recently gathered evidence that the cytoskeleton protein actin plays a role during membrane repair in N. crassa. We observed that actin is recruited to the site of injury in spore germlings and hyphae treated with nystatin, α-tomatine or lysing enzyme, a substance produced by Trichoderma species that targets the cell wall but will also lead to membrane damage. When actin polymerization is inhibited with latrunculin A, PEF1 is not recruited anymore in response to membrane damage. ANX14 recruitment, however, is not affected by the loss of actin cables. This strikingly different behavior of PEF1 and ANX14 reinforces the hypothesis that both proteins are part of different RMs. Future studies aim to unravel the interactions between different repair proteins and cytoskeletal structures during their task of mending the broken membrane.

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CS4.1.11

CBCYP51 MEDIATED DMI RESISTANCE IS MODULATED BY CODON BIAS

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Cercospora leaf spot (CLS) is the most damaging foliar disease of sugar beet globally. To combat CLS, multifaceted efforts are widely employed, including breeding for resistance, cultural practices, and the application of fungicides. However, populations of Cercospora beticola have become resistant to most fungicides used for CLS management, including those in the sterol demethylation inhibitor (DMI) class of fungicides. In this study, we sampled nearly 600 isolates of Cercospora beticola from MN and ND during the 2021 sugar beet growing season. For each isolate, EC50 values were determined for DMIs tetraconazole (Eminent), prothioconazole (Proline), difenoconazole (Inspire), and mefentrifluconazole (Revysol). Using the CYP51 gene sequence for each isolate, we determined that the synonymous E170 mutation and the synonymous/nonsynonymous L144(F) can be used to predict resistance to these four DMIs. The prevalence and accuracy of the six mutation combinations were calculated and specific combinations can predict resistance with greater than 90% accuracy. Interestingly, one prevalent mutation combination resulted identified cross-resistance to difenoconazole and mefentrifluconazole, but sensitivity to tetraconazole and prothioconazole. This data reveals the importance of codon bias in fungicide resistance and is the first demonstration of the use of synonymous mutations to predict cross-resistance.

CS4.1.12

CHARACTERIZATION OF PHANEROCHAETE CHRYSOSPORIUM MUTANTS RESISTANT TO BAGASSA GUIANENSIS WOOD EXTRACTIVES

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Wood decaying fungi possess an array of enzymes able to degrade lignocellulosic material. During wood degradation, they have to cope with toxic molecules released from wood and defined as “extractives”. Thousands of wood extractives molecules have been described but very little is known concerning their putative antifungal activity. To highlight the molecular targets of extractives in fungi, a collection of Phanerochaete chrysosporium mutants was generated using UV mutagenesis and screened for resistance to Bagassa guianensis wood extractives. B. guianensis is a tropical species exhibiting high amounts of extractives. Among the 34 isolated mutants, allelic series of AGR57_7124 mutations have been identified for which 13 mutants display the same allele. The AGR57_7124 gene codes for an orthologous to the human DENND6 protein. Our physiological studies show that the ectopic expression of the mutated allele in a wild type genetic background confers resistance to B. guianensis wood extractives. Moreover, the mutants resistant to B. guianensis wood extractives are able to mineralize B. guianensis wood while the WT cannot. The functional characterization of PcDENND6 is currently under investigation to better understand its role in resistance to extractives.

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CS4.1.13

THE ASPERGILLUS FUMIGATUS SPINDLE ASSEMBLY CHECKPOINT COMPONENTS, SLDA AND SLDB, PLAY ROLES IN MAINTENANCE OF TRIAZOLE SUSCEPTIBILITY

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Aspergillus fumigatus is the most common cause of invasive mold infections in susceptible human populations. Invasive aspergillosis is characterized by high mortality ranging from 30-90%. The recent rise of antifungal resistance in A. fumigatus is of increasing concern as infection with resistant isolates is associated with increased treatment failure. Much remains unknown concerning adaptation to antifungal stress and development of antifungal resistance, threatening the future use of triazole antifungals. Recent studies have linked genomic instability, specifically the presence of numerical or structural chromosomal abnormalities within the nuclear DNA, to acquisition of triazole resistance in several species of pathogenic fungi. Few studies have attempted to address the potential for genomic instabilities to promote antifungal tolerance in this pathogen. In this study, deletion of the gene putatively encoding the singular ortholog of Bub1/BubR1/Mad3 proteins in A. fumigatus, SldA, or that encoding the ortholog of Bub3, SldB, in a wild type background strain each resulted in 4-fold increased minimum inhibitory concentration (MIC) to voriconazole as determined by broth microdilution assay. Both strains exhibited increased MIC to other compounds targeting ergosterol biosynthesis but not to DNA damage or oxidative stressors. These mutant strains retained the hypersusceptibility to benzimidazole phenotype reported in other eukaryotic species where function of the SldA ortholog is lost, implying conserved roles in regulation of the SAC. We confirmed neither strain possessed aberrant number of nuclei per hyphal compartment nor were there abnormal chromosome configurations visible among stained nuclei, such as lagging chromosomes or chromosome

bridges which have been previously reported in SAC dysfunction. Loss of sldA did not potentiate voriconazole resistance acquisition through experimental adaptation. Future studies will focus on evaluating these strains for aneuploidy and delineating connections between SAC dysfunction and triazole susceptibility in A. fumigatus.

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CS4.1.14

AN ATYPICAL SUBUNIT OF THE PROTEIN TRANSLATION MACHINERY IS A PROMISING TARGET FOR THE DISCOVERY OF NEW ANTIFUNGAL COMPOUNDS

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Bio-based molecules have become one of the main alternatives to traditional chemicals for limiting the propagation of harmful fungi. However, a better understanding of the mode of action of these molecules is needed to evaluate the benefits/risks of using them as antifungal treatment. Protein translation is a relevant target for limiting cell proliferation. eEF1Bγ is one of the subunits of the elongation factor complex eEF1 of protein translation. This protein is atypical in that it possesses a glutathione transferase (GST) domain. GST are detoxifying enzymes being able to bind both glutathione and a large panel of molecules. The main described role of eEF1Bγ has been attributed to the stabilization of the elongation factor complex during translation, thanks to the GST domain that physically interacts with the other subunit eEF1Bα. However, we have shown that the binding of oxidized glutathione onto eEF1Bγ leads to structural modification of the protein and that small molecules could directly interact with eEF1Bγ as a ligand/substrate. Since the binding of eEF1Bα and small molecules occur at the same binding site, we hypothesize that some molecules can compete with eEF1Bα for interaction and affect the translation process.

CS4.1.16

WHOLE-GENOME SEQUENCING ELUCIDATES THE SPECIES-WIDE DIVERSITY AND EVOLUTION OF FUNGICIDE RESISTANCE IN THE EARLY BLIGHT PATHOGEN ALTERNARIA SOLANI

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Early blight of potato is caused by the fungal pathogen Alternaria solani and is an increasing problem worldwide. The primary strategy to control the disease is applying fungicides such as succinate dehydrogenase inhibitors (SDHI). SDHI-resistant strains, showing reduced sensitivity to treatments, appeared in Germany in 2013, shortly after the introduction of SDHIs. Two primary mutations in the SDH complex (SdhB-H278Y and SdhC-H134R) have been frequently found throughout Europe. How these resistances arose and spread, and whether they are linked to other genomic features, remains unknown. For this project, we performed whole-genome sequencing for 48 A. solani isolates from potato fields across Europe to better characterize the pathogen's genetic diversity in general and understand the development and spread of the genetic mutations that lead to SDHI resistance. The isolates can be grouped into seven genotypes. These genotypes do not show a geographical pattern but appear spread throughout Europe. We found clear evidence for recombination on the genome, and the observed admixtures might indicate a higher adaptive potential of the fungus than previously thought. Yet, we cannot link the observed recombination events to different Sdh mutations. The same Sdh mutations appear in different, non-admixed genetic backgrounds; therefore, we conclude they arose independently. Our research gives insights into the genetic diversity of A. solani on a genome level. The mixed occurrence of different genotypes, apparent admixture in the populations, and evidence for recombination indicate higher genomic complexity than anticipated. The conclusion that SDHI tolerance arose multiple times independently has important implications for future fungicide resistance management strategies. These should not solely focus on preventing the spread of isolates between locations but also on limiting population size and the selective pressure posed by fungicides in a given field to avoid the rise of new mutations in other genetic backgrounds

CS4.1.17

FROM PLANT-PATHOGEN INTERACTION TO DISEASE MANAGEMENT TOOLS: NEW PERSPECTIVES FOR THE CONTROL OF GRAPEVINE DOWNY MILDEW

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The management of grapevine downy mildew, a devastating disease of a major crop (Vitis vinifera), is constantly challenged by increasing restrictions on plant protection products and fungicide resistance. The development of innovative, effective, safe and target-specific disease management can take advantage from a deep knowledge on the plant, the pathogen and their interaction. The recent discovery of a downy mildew resistant cultivar (Mgaloblishvili), originated in the domestication center (Georgia, South Caucasus) of the V. vinifera species (traditionally susceptible to the causal agent Plasmopara viticola), led to the identification of genes and antimicrobial compounds involved in the infection process of potential interest for the pathogen control. On one hand, comparative transcriptome analysis and GWAS revealed new genes and loci involved in resistance to the pathogen that are currently exploited for the development of resistant grapevine varieties through marker assisted breeding (MAS). On the other hand, candidate susceptibility genes (i.e. plant genes that are necessary for pathogen infection) have been identified and one of this (VviLBDIf7, encoding for a LBD protein) was transiently silenced through RNAi triggered by exogenous double-stranded RNA (dsRNA). The dsRNA, delivered through spray application (spray-induced gene silencing, SIGS), caused a significant disease severity reduction. Moreover, the analysis of volatile compounds produced by infected plants led to the discovery of several terpenes (farnesene, nerolidol, ocimene and valencene) able to limit the disease in vitro. Regarding the pathogen, the identification of genes expressed during grapevine infection, and the heterologous expression and functional characterization of effector proteins, offer an interesting opportunity to shed light on the plant-pathogen interaction thus facilitating rational drug design and screening aiming at specifically inhibit selected P. viticola targets. These tools represent promising innovations for grapevine downy mildew control, however their integration in the disease management strategies requires further implementations at laboratory, field and industrial levels.

CS4.1.18

ANEUPLOIDY CONFERS AZOLE RESISTANCE IN THE FILAMENTOUS FUNGUS, ASPERGILLUS FLAVUS

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Aspergillus flavus is a mycotoxigenic fungal species that contaminates many important agricultural crops and produces aflatoxin B1, the most toxic and carcinogenic natural compound. The fungus is also the second leading cause of human invasive aspergillosis, after Aspergillus fumigatus, especially in individuals with impaired immune system. Azole antifungal drugs are considered the most effective chemical compounds to control Aspergillus infections both in the clinic and in agricultural field. Emergence of azole resistance in Aspergillus spp. have been mostly linked to point mutations in cyp51 genes encoding lanosterol 14α-demethylase, the target of azole antifungals in the ergosterol biosynthesis pathway. We hypothesized that alternative molecular mechanisms are also responsible for acquisition of azole resistance in filamentous fungi. Here, we found that exposure to voriconazole (VRC) at concentrations above the MIC resulted in adaptation of aflatoxin-producing A. flavus strain to this triazole antifungal agent through whole or segmental aneuploidy of specific chromosomes. The VRC-resistant clones returned to their original level of susceptibility following repeated transfers on drug-free media, highlighting the reversibility of this type of resistance in A. flavus. This study provides new insight into mechanism of azole resistance in filamentous fungi.

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CS4.1.19

NANOPARTICLES AND PATHOGENIC FUNGI: A NON-UPTAKE DELIVERY OF COMPOUNDS

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Newly discovered antifungal substances often display pharmacological problems, like low solubility or high toxicity. Several studies showed that nanoparticles (NPs) can be used to overcome these problems of antimicrobials. Liposomal Amphotericin B is an example for such a nanoformulated antifungal drug already being on the market. The mechanism, how polymeric NPs deliver encapsulated substances into pathogenic fungi, was suggested to be either via endocytosis or an endocytosis-independent uptake of the whole NP. However, the mechanism of uptake remains to be understood.
Therefore, we investigated the interaction of different NPs with several human pathogenic fungi to elucidate the uptake mechanism irrespective of the polymer or fungal species. NPs were prepared by utilizing 4 different polymers and were labelled with 2 different fluorescent dyes covalently attached and/or a fluorescent dye or antifungal drug encapsulated. The interaction of the fluorescently labelled NPs with the filamentous fungi Aspergillus fumigatus, A. nidulans, A. terreus, and A. oryzae, and the yeasts Cryptococcus neoformans and Candida albicans was investigated by confocal laser scanning microscopy. The efficacy of itraconazole-loaded NPs on these species was determined by MIC-testing following the respective EUCAST methodology.
Irrespective of the applied conditions, none of the used NPs reached

the fungal cytosol, but adhered to the fungal surface. Investigations on the exact localization of NPs revealed their appearance in the interspace between cell wall and membrane of the fungi. Nevertheless, encapsulation of a fluorescent dye or itraconazole led to an accumulation of the fluorescent dye in the fungal lumen or a lower MIC compared to the pristine drug, respectively.
In conclusion, polymeric NPs are not taken up by pathogenic fungi. Nevertheless, the delivery of hydrophobic substances like antifungals into these fungi with the help of NPs is possible and effective, making NPs a promising tool for antifungal treatment.

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CS4.1.20

THE CONTRIBUTION OF THE I.-III. PHASE DETOXIFICATION SYSTEM TO DECREASED SUSCEPTIBILITY OF FILAMENTOUS FUNGI

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Resistance of fungal cells to antifungal agents is attracting the attention of many research groups. Much is known about the mechanisms of fungal strains that help the fungus to overcome the toxicity of antifungal compounds. However, the knowledge about the contribution of the conserved detoxification system in eukaryotes is still limited. We focused our attention on the transcriptomic response of the (i) detoxification genes of the phase I coding for cytochrome P450 monooxygenases [CYP450s], (ii) genes of the phase II coding the conjugating enzyme glutathione-S-transferase (GST) and (iii) phase III genes coding for ABC efflux transporters when our model fungus Neurospora crassa was exposed to azole compounds. We have observed the increased expression of the cyp65_025 gene coding the CYP450 monooxygenase, especially when exposed to ravuconazole (11-fold higher gene expression compared to the control conditions). Considering the chemical structure of azole compounds, these could serve as a substrate for monooxygenase reaction, resulting in more soluble molecules that could be easier transported out of the cell. We observed slightly increased gst gene expression, but no increase in activity of GST on the enzymatic level when exposed to azoles, so we have concluded that azoles could not serve as the substrates for GST enzyme. The increased gene expression of the two ABC-C transporters located in the plasma membrane was detected after azole exposure. Finally, we can conclude that CYP450 monooxygenases and efflux pumps could contribute to the decreased susceptibility of fungal cells and the unique fungal CYP450 enzymes could serve as new antifungal targets or targets for synergy with known antifungals.
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CS4.1.21

ELUCIDATION OF INTRINSIC MICAFUNGIN DRUG RESISTANCE MECHANISMS IN MUCOR

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Mucormycosis is a life-threatening fungal infection especially to immunocompromised patients. Recently, there was a sharp increase of mucormycosis in patients with COVID-19 or post-COVID-19 patients. The causal fungi in Mucorales are resistant to most antifungal drugs. As a result, the mortality resulting from mucormycosis remains unacceptably high reaching up to 90-100% among disseminated infections. Echinocandins are the newest antifungal drug class that inhibits the enzyme β-(1,3)-D-glucan synthase. Mucorales however exhibit resistance to these drugs despite harboring the drug target fks genes. There is little or no knowledge regarding the mechanisms of the resistance. This study is to elucidate the genetic mechanisms underlying the intrinsic resistance of Mucorales to micafungin. We found that the model Mucorales species, Mucor circinelloides (denoted Mucor), carries three echinocandin drug target genes fksA, fksB, and fksC. A phylogenetic analysis with other fungi and Mucorales demonstrates that fksA and fksB were duplicated in an ancestral Mucorales lineage and fksC, on the other hand, has been diverged in a more ancestral point of Mucorales speciation. When Mucor was challenged with micafungin we found fksA and fksB were overexpressed. Our study further found that the serine/threonine phosphatase, calcineurin, regulates the expression of the fksA and fksB genes, in which deletion of calcineurin results in a decrease in expression of all three fks genes and a lower minimal effective concentration (MEC) to micafungin. Furthermore, we found that the fksA and fksB genes have their own essential role and we only obtained a heterokaryotic mutant of the fksA and fksB genes in Mucor. The heterokaryotic fksA or fksB mutants were more sensitive to micafungin compared to wildtype. Taken together, this study demonstrates that the fks gene duplication and calcineurin contribute to the intrinsic resistance to echinocandins in Mucor. We will also discuss how micafungin treatment alters host-recognitions in Mucor.

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CS4.1.22

KILLERS AND POTENTIATORS: POLYPHENOLS AS MULTIPURPOSE ANTIFUNGAL AGENTS

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Increasing resistance to common antifungal medications has become an emerging problem and strongly highlights the need for the development of new antifungal agents. In the past years, polyphenols, a group of secondary metabolites in plants, have gained increasing interest as potential antifungal agents (PAFAs). Thus, we tested a panel of different compounds for their antifungal activity against pathogenic fungi. We were able to identify a PAFA candidate that possesses antifungal activity against different Candida ssp. Intriguingly, the PAFA candidate was able to increase the antifungal capacity of commercially used antifungal medications when combined in lower (or non-toxic) doses in laboratory as well as clinically relevant and resistant strains of different Candida ssp. Taking advantage of the well-established Caenorhabditis elegans infection model we could show that the observed in vitro antifungal and potentiating activity also holds true in in vivo. Altogether, our data underline the potential of polyphenols as both discrete antifungal agents and direct or indirect potentiators of known antifungal drugs.

CS4.1.23

A HOST DEFENSE PEPTIDE MIMETIC, BRILACIDIN, POTENTIATES CASPOFUNGIN ANTIFUNGAL ACTIVITY AGAINST HUMAN PATHOGENIC FUNGI

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Fungal infections cause more than 1.5 million deaths a year. Due to emerging antifungal drug resistance, novel strategies are urgently needed to combat life-threatening fungal diseases. Here, we identified the host defense peptide mimetic, brilacidin (BRI) as a synergizer with caspofungin (CAS) against CAS-sensitive and CAS-resistant isolates of Aspergillus fumigatus, Candida albicans, C. auris, and CAS-intrinsically resistant Cryptococcus neoformans. BRI also potentiates azoles against A. fumigatus and several Mucorales fungi. BRI acts in A. fumigatus by disrupting cell membrane potential and impairing cell wall integrity pathway. BRI combined with CAS significantly clears A. fumigatus lung infection in an immunosuppressed murine model of invasive pulmonary aspergillosis. BRI alone also decreases A. fumigatus fungal burden and ablates disease development in a murine model of fungal keratitis. Our results indicate that combinations of BRI and antifungal drugs in clinical use are likely to improve the treatment outcome of aspergillosis and other fungal infections

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CS4.1.24

SAMPLING, SCREENING, AND IDENTIFICATION OF POTENTIALLY VORICONAZOLE RESISTANT ENVIRONMENTAL STRAINS IN THE BASQUE COUNTRY AIR

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Fungi constitute a ubiquitous group of microorganisms that play an important role in the environment, including opportunistic pathogen species. The treatment of the infections they cause consists mainly of antifungal agents such as azoles. However, lately, the resistance to these compounds has become an issue of major concern worldwide. The development of these resistances relies on two possible origins. On the one hand, the clinical route, due to prolonged treatments in chronic patients; on the other hand, the environmental route, due to extensive use of azoles in agriculture. Therefore, it is necessary to assess the prevalence of antifungal resistance in the environment. For this aim air samples were collected between November 2021 and July 2022 using the MAS-100 Eco Air Sampler in three different areas (hospital surrounding, rural, and urban areas) in each province of the Basque Country (Araba, Bizkaia, and Gipuzkoa). All the colonies able to grow on Sabouraud plates supplemented with 1 mg/L of voriconazole incubated at 37ºC were isolated. Afterwards, the identification of the species was performed by sequencing the region between the primers ITS1 (Internal Transcribed Spacer 1) and ITS4. Preliminary results showed the detection of at least 26 species distributed in 14 genera; among them, Talaromyces and Alternaria appear to be the most abundant. Furthermore, around 20 Aspergillus fumigatus isolates have been identified. In conclusion, this study sheds light to the importance of developing air samplings in order to detect fungal strains resistant to azoles and identify new resistance mechanisms in potentially pathogenic fungi for both humans and plants.

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CS4.1.25

FUSARIUM SPP. AND THE INTRINSIC RESISTANCE TO AZOLES

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Fusarium species are trans-kingdom pathogen and due to the increasing incidence of fungal infections have gained importance in human health. Intrinsic resistance to antifungal agents is characteristic, which impairs the Fusariosis treatment. A well-known mechanism of fungal azole resistance is the mutation of the cyp51A gene, which encodes 14α-demethylase, a central enzyme in the fungal ergosterol biosynthesis pathway. Our study aimed to evaluate the cyp51A gene sequence from Fusarium spp. clinical isolates from the State of São Paulo, Brazil. First, primers were designed for amplification and sequencing of cyp51A gene from F. solani, F. oxysporum, and F. fujikuroi species complex (FSSC, FOSC, and FFSC, respectively) clinical isolates. As a result, no Fusarium spp. CYP51A amino acid substitutions associated with high MICs of ITR and VOR were found. Thus, considering the conservative features of Cyp51A protein sequences among different species, we analysed the Fusarium spp. CYP51A peptide sequence concerning the well-known Cyp51A sequence of Aspergillus fumigatus. Different amino acid substitutions located in the peripheral Cyp51A protein and unrelated to the modulation of azoles MIC were determined. Interestingly, CYP51A sequences of FSSC (F. keratoplasticum, F. solani, F. petroliphilum, and F. falciforme), FOSC (F. oxysporum), and FFSC (F. proliferatum) clinical isolates presented a conserved leucine (L) in the equivalent position M220; and F. oxysporum (FOSC) clinical isolates have isoleucine (I) in a position equivalent to L98 of A. fumigatus Cyp51A which lead to azole resistance in this fungus. Thus, the conserved L220 and I98 residues of FSSC, FOSC, and FFSC CYP51A may explain the higher MIC (intrinsic resistance) of these fungal species to azole antifungals.

CS4.1.26

EXPLORATION OF DOWNSTREAM EFFECTORS OF TAC1B IN CANDIDA AURIS

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Background
Candida auris emerges as a multidrug-resistant pathogen. Rapid development of fluconazole resistance is a hallmark of C. auris. Tac1b, a transcription factor, is responsible for azole resistance. We previously identified that an amino-acid substitution S611P in Tac1b was associated with azole resistance. However, by real-time PCR, the expression of the transporter gene CDR1 was not significantly affected by the presence of Tac1b substitution. Therefore, we speculated that Tac1b-dependent and Cdr1-independent mechanisms could exist for azole resistance, and we planned to study the downstream effectors of Tac1b.

Methods
We effectuated RNA-sequencing by comparing a wild-type isolate IV.1 and its mutant IV.1Tac1bS611P. All the significantly up- or down-regulated genes (with ≥1.5 fold-change and p-value≤0.05) in the IV.1Tac1bS611P isolate were selected, and their functions were predicted by comparing their orthologs in Candida albicans. The candidate genes possibly responsible for azole resistance were studied by deleting them individually in both IV.1 and IV.1Tac1bS611P isolates using CRISPR-Cas9 system. The phenotype of these mutants were characterized by fluconazole susceptibility testing by CLSI protocol.

Results
By RNA-sequencing analysis, we explored a list of genes upregulated by the presence of Tac1b substitution: ADA2, ZCF22, QDR3, FCR1, FTR1. These genes were found to be responsible for azole resistance/tolerance or stress response in C. albicans. We also found that the transporter genes CDR1 and MDR1 were overexpressed by 1.93-time and 1.52-time, respectively. We then deleted these genes to confirm their function. However, deletion of these genes had no impact on fluconazole susceptibility, except the CDR1 mutant, in which we observed a significant decrease of fluconazole resistance.

Conclusion
These results actually confirm that Tac1b controls azole resistance via Cdr1 in C. auris, although the upregulation of CDR1 is relatively modest. The downstream effectors of Tac1b will be confirmed by studying the genomic occupancy of Tac1b in C. auris using CheC-seq.

CS4.1.27

WHOLE GENOME SEQUENCING ANALYSIS DEMONSTRATES THERAPY-INDUCED ECHINOCANDIN RESISTANCE DEVELOPMENT OF URINARY CANDIDA AURIS ISOLATES

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Candida auris is an emerging, multidrug-resistant yeast, causing outbreaks in healthcare facilities especially in intensive care units. Echinocandins are used as the antifungal drug of choice for treating invasive candidiasis. C. auris resistance to echinocandines is found in 1-8% of isolates. A downside of this treatment is the low penetration into the urine, where Candida species colonization may be present. Several patients from Kuwait with a C. auris infection were treated with echinocandins. Within weeks to months after initiation of treatment, echinocandin resistant isolates were found in urine samples of seven patients, while all obtained isolates were susceptible at treatment onset. To determine whether the echinocandin-resistant isolates were due to independent introductions or resulted from intra-patient resistance development, whole genome sequencing (WGS) was performed on susceptible (n=18) and echinocandin-resistant (n=9) isolates from seven patients. WGS analysis demonstrated that resistant isolates were nearly identical to previous susceptible isolates from the same patient. Between patients, isolates differed less than 120 SNPs. These findings demonstrate that echinocandin resistance may be acquired in the urinary tract during prolonged treatment. These echinocandin-resistant strains may cause long-term colonization or subsequent systemic disease, transfer to other patients and remain viable for longer time periods in the hospital environment.

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CS4.1.28

POTENTIAL FOR RESISTANCE DEVELOPMENT TO AN ANTIFUNGAL PROTEIN (NFAP2) IN CANDIDA ALBICANS IN COMPARISON WITH A CONVENTIONAL DRUG, FLUCONAZOLE

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As the consequence of the increasing number of antifungal drug-resistant strains, only few effective drugs are available to treat fungal infections. Therefore, there is a need to find new and safely applicable compounds with high antifungal efficacy and limited potential for resistance development. The features of NFAP2, a small, cysteine-rich antifungal protein secreted by Neosartorya (Aspergillus) fischeri NRRL 181 render it to be a promising candidate. In this study, we investigated the ability of Candida albicans CBS 5982 to develop resistance mechanisms to NFAP2 in comparison with fluconazole (FLC), a widely used antifungal agent. In a microevolution experiment, C. albicans was able to adapt only to the 1×MIC (minimum inhibitory concentration) of NFAP2, whereas this yeast survived even at 32×MIC of FLC. Whole genome sequencing revealed that the FLC-resistant strain carries single nucleotide variations (SNVs) and an insertion in the clathrin heavy chain, deletions in PTC2 (type 2C protein phosphatase), and a multiple nucleotide variation (MNV) in PHB1 (prohibitin 1) genes. The NFAP2-resistant strains carry an SNV in BNI4 (a scaffold protein that tethers chitin synthase III), an MNV in PGA58 (putative glycosylphosphatidylinositol-anchored protein) and eIF4G (eukaryotic translation initiation factor 4G), and deletions also in PTC2 genes. Resistance to 1×MIC of NFAP2 resulted in increased susceptibility to FLC; meanwhile the FLC-resistant strains showed decreased susceptibility to NFAP2. Metabolic adaptation experiments revealed that NFAP2 and FLC resistance developments have fitness cost depending on the composition

of the applied medium. The resistance development diminished the virulence of the NFAP2- and FLC-resistant isolates in a Galleria mellonella infection model. Summarizing our results, it seems to be possible that C. albicans has limited ability to develop potent resistance mechanisms to NFAP2 than FLC.

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CS4.1.29

OVERCOMING MELANIN TOLERANCE USING UV-C OPTICAL STRATEGIES

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Optical strategies to combat microorganisms provides an alternative to reliance on chemical control, particularly in food production. UV-C light (within the spectrum of 200 to 280nm) is well-known as an effective germicidal wavelength, widely used even during the pandemic. In greenhouses, UV-C can be deployed against a variety of microorganisms, including pathogenic fungi such as Botrytis cinerea (grey mould). Yet a balance must be struck between controlling infection and causing damage to the plants. Melanised fungi display particular tolerance to high light conditions, requiring more UV-C exposure to both plant and fungi. Unlike constitutively melanised fungi, melanogenesis in B. cinerea is regulated depending on the reproductive, survival and/or infection stage (Schumacher, 2015). The contribution of six melanin biosynthesis genes together with light-dependent cryptochrome/photolyase gene (CRY1) in germinating conidia and young mycelium can explain increased tolerance. Our preliminary findings show that when conidia were treated with non-toxic UV-C followed by dark incubation, cryptochrome exhibited a 3-fold increase in expression within the first 30 minutes post-UV-C exposure (PE). At four hours PE, corresponding to the appressorium developmental stage of B. cinerea, all melanin genes were downregulated. The fungus has, during this early germination period, likely established itself within the tissue and melanin tolerance is no longer required (Milo-Cochavi et al., 2019). Conversely, when treating 24-hour old colonies, melanin genes usually expressed during conidial development (ie. at 4 days) (Schumacher, 2015) are significantly upregulated at 4 hours PE, possibly as mechanism to trigger early conidial production due to UV-C stress. The unique regulation of melanin biosynthesis under UV-C treatment can better assist us in finding a targeted treatment within the developmental stages of this necrotrophic fungus.

CS4.1.30

EXPOSURE TO AGRICULTURAL DHODH INHIBITORS RESULT IN CROSS-RESISTANCE TO THE NOVEL ANTIFUNGAL OLOROFIM IN A. FUMIGATUS

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Pesticides, including fungicides, are extensively used in agricultural practice to protect plants from unwanted growth of weeds, plant pathogens and other pests. Dual use of antifungals in the environment and in the clinic, with similar mode of actions, has been shown to drive the development of resistance. Although not a plant pathogen, A. fumigatus is ubiquitous in the environment and therefore exposed to agricultural fungicides. Extensive use of triazoles in the environment has led to high rates of resistance found in clinical A. fumigatus isolates. The development of novel antifungals is paramount to be able to treat azole-resistant aspergillosis. Olorofim is a novel antifungal for clinical use, targeting the essential protein DHODH, for which resistance is rare. Recently, several agricultural DHODH inhibitors, including ipflufenoquin and tetflupyrolimet, have gone through the approval process. We show that these DHODH inhibitors are active against A. fumigatus, and have the same mode of action as olorofim. Spontaneous mutation analysis revealed we can select for ipflufenoquin resistant A. fumigatus isolates. These ipflufenoquin resistant mutants show cross-resistance to olorofim. Furthermore, other agricultural DHODH inhibitors recently approved as herbicide have the potential to result in cross-resistance to olorofim. Our results highlight the potential dangers of using DHODH inhibitors in agriculture and the future threat of resistance development to novel antifungals by selection in the environment.

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CS4.1.31

DUAL MODE OF ACTION OF NEOSARTORYA (ASPERGILLUS) FISCHERI ANTIFUNGAL PROTEIN 2 (NFAP2) ON CANDIDA ALBICANS

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The small, cysteine-rich, and cationic Neosartorya (Aspergillus) fischeri antifungal protein 2 (NFAP2) secreted by the isolate N. (A.) fischeri NRRL 181 is considered as a highly effective antifungal compound to treat drug-resistant superficial Candida albicans infections. One of the requirements of its therapeutic application is understanding the antifungal mode of action; therefore, our recent investigations focused on it. We observed that NFAP2 promptly disrupts the plasma membrane integrity of C. albicans cells when it is applied at minimum inhibitory concentration (MIC). Molecular dynamics simulation studies indicated that NFAP2 interacts primarily with ceramide-phosphoinositol, a representative lipid of the fungal cell membrane, and forms readily dimers in water. These features can be responsible for the plasma membrane disruption effect. Applying NFAP2 below the MIC, first it is localized in outer layers of C. albicans cells without disrupting the plasma membrane. Later NFAP2 is taken up into the cells and can be found in vacuoles, from where it is released resulting in cell death. Mass spectrometry coupled in vivo chemical crosslinking experiment applying SDAD (NHS-SS-Diazirine) (succinimidyl 2-((4,4'-azipentanamido)ethyl)-1,3'-dithiopropionate) revealed that NFAP2 interacts with transketolase and malate dehydrogenase, key enzymes of glycolysis. According to the above mentioned observations, we suppose that NFAP2 has a

concentration-dependent dual mode of action on C. albicans: 1) It influences the membrane integrity by a disruption mechanism; and/or 2) inhibits the glycolysis by interaction with key enzymes.
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CS4.1.33

DIFFERENTIAL GENE EXPRESSION OF MUCOR LUSITANICUS AFTER AZOLE TREATMENT

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Mucorales species have great biotechnological importance such as producers of extracellular enzymes, carotenoids or organic acids, but several species (e.g., Rhizopus oryzae, Lichtheimia corymbifera and Mucor circinelloides) can also act as opportunistic human pathogens causing frequently lethal systemic infection in immunocompromised patients, called as mucormycosis. In recent years, the number of mucormycosis cases has significantly increased.
Treatment of mucormycosis is challenging because these fungi are intrinsically resistant to most of the routinely used antifungal agents, such as echinocandins and short-tailed azoles. Usage of posaconazole is recommended as salvage therapy in the treatment of mucormycosis, while voriconazole and fluconazole have no in vitro or in vivo activity against Mucorales species. In this study, transcriptomic analysis was carried out after the treatment of Mucor lusitanicus with posaconazole, voriconazole, and fluconazole.
In total, 355, 177, and 232 unique genes were differentially expressed after posaconazole, fluconazole, and voriconazole treatment, respectively, while 145 common genes were observed after azole treatments. 66 genes were differentially expressed after fluconazole and voriconazole treatment, 30 genes after fluconazole and posaconazole, while after posaconazole and voriconazole treatment 205 common genes were expressed differentially compared to the untreated control. The RNA-Seq analysis was validated by performing real-time qRT-PCR on a selection of genes representing various affected treatments. These results confirmed the direction of regulation (up or down) for all genes studied. The different azoles affected the transcription of trans-

porters. Several transcription factor coding genes showed altered transcript level after azole treatment as well. Based on this data, a mutant library has been started to construct from M. lusitanicus including ABC and MFS coding genes.
This study was supported by the grants NKFIA K131796, ELKH 2001007, and ITM NKFIA TKP-2021-EGA-28."

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CS4.1.34

DRUG RESISTANCE ACQUISITION LEADS TO THE EMERGENCE OF FITNESS TRADE-OFFS IN CANDIDA GLABRATA

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Human fungal infections are a major global health problem of particular concern due to increasing drug resistance. Candida glabrata, which is the second most common fungal pathogen, has an remarkable ability to adapt to different environments and easily develop resistance to the most common antifungal drugs. The process of resistance acquisition and its consequences on fitness have not yet been studied in depth and do not always take into account the challenging diversity between clades. In this study we used an interdisciplinary approach integrating in vitro microevolution, whole genome sequencing and quantitative large-scale phenotyping. Strains from the 7 clades were evolved under different azole and echinocandin conditions and were subsequently sequenced. In this poster we explore the phenotypic consequences of evolution in antifungal drugs.
For this purpose we developed a high throughput quantitative phenotyping methodology on solid medium plates, where up to 1152 colonies can be analyzed at once. These strains are incubated in an in-house computer-controlled imaging system, and then analyzed automatically by a pipeline developed in our group called q-CAST. Strikingly, we found that not only the fitness is reduced, but also there is a general trend of loss in stress response. Our data indicate that resistance to different drugs influence the likelihood of being more susceptible to a given stress. They also show that genetic background may be a determining factor. Coupling phenotypes with genomic variants allow us to relate some genes with acquired stress sensitivity. These fitness trade-offs not only lead us to a better understanding of evolutionary processes but also could be a target for developing new approaches to treat drug-resistant strains.

CS4.1.35

CHARACTERIZATION OF THE QDR2 MULTIDRUG TRANSPORTER GENES OF MUCOR LUSITANICUS

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Mucor lusitanicus is an opportunistic human pathogenic fungus causing a frequently fatal infection, mucormycosis, in immunosuppressed patients. It is resistant to most clinical antifungals. Qdr2 is a plasma membrane transporter of the major facilitator superfamily that can efflux quinidine, barban, cisplatin and bleomycin in Saccharomyces cerevisiae. It has a role in cation homeostasis and the oxidative stress response in Candida glabrata.
Qdr2a, qdr2b, qdr2c and qdr2d single knock-out mutants of M. lusitanicus were created by using a plasmid free CRISPR-Cas9 system. The double-strand break caused by the Cas9 enzyme was repaired by homologous-directed repair.
Relative transcript level of qdr2 genes was analyzed after antifungal treatment (ravuconazole, isavuconazole, voriconazole, itraconazole, posaconazole, fluconazole, ketoconazole, and amphotericin B). Qdr2a, qdr2b and qdr2c were upregulated in response to all tested antifungals under aerobic condition. Transcript level of qdr2d increased significantly after voriconazole, itraconazole, posaconazole, fluconazole, ketoconazole, and amphotericin B under aerobic condition. Qdr2a and qdr2c were upregulated after ravuconazole, isavuconazole, itraconazole, posaconazole, fluconazole, ketoconazole, and amphotericin B treatment under anaerobic condition. Qdr2b and qdr2d showed increased transcript level in response to all tested antifungals under anaerobic condition.
Disruption of qdr2 genes has no effect on the antifungal susceptibility and growth in the presence of cell wall stressors. Spore production ability of the qdr2 deletion mutants was significantly lower than that of the control strain. In the in vivo Galleria mellonella model, qdr2a, qdr2b, qdr2c and qdr2d deletion mutants had significantly decreased virulence.
These results suggest that qdr2 genes may have a role in the pathogenicity of M. lusitanicus. The loss of qdr2a, qdr2b, qdr2c and qdr2d genes may be compensated by each other since they have overlapping expression patterns.
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CS4.1.36

CHARACTERIZING AZOLE DRUGS IN THE FUNGAL PATHOGEN CRYPTOCOCCUS NEOFORMANS

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Understanding mechanisms of anti-fungal drugs in the pathogenic fungus, Cryptococcus neoformans, is crucial for improving anticryptococcal therapy. We have previously determined that presence of anti-oxidants in growth medium can reverse growth inhibition of C. neoformans caused by the azole drug, fluconazole. We have also shown that fluconazole treatment leads to an increase of reactive oxygen species (ROS) in C. neoformans. To further explain the effects of anti-oxidants and fluconazole, we screened 8 chemically distinct azole drugs (voriconazole, propiconazole, cyproconazole, miconazole, isavuconazole, tebuconazole, myclobutanil, and ketoconazole) to determine the following: (1) ability to inhibit growth of C. neoformans (2) ability to induce ROS in C. neoformans, and (3) ability of the anti-oxidant vitamin C to reverse growth inhibition of C. neoformans in the presence of each of the drugs. We found that all azole drugs inhibit growth of C. neoformans. Isavuconazole, voriconazole, and propiconazole showed the largest effect against C. neoformans. However, growth inhibition in the presence of these drugs (voriconazole, propiconazole, and isavuconazole) was reversed by vitamin C. Other drugs that showed decreased growth inhibition in the presence of vitamin C were ketoconazole and cyproconazole. Inhibitory effects of miconazole, tebuconazole, and myclobutanil were not affected by vitamin C. We found that all the drugs whose inhibitory potential was lowered by vitamin C also induced higher ROS. We conclude that while all the tested azole drugs inhibit growth of C. neoformans, only five drugs (voriconazole, propiconazole, isavuconazole, ketoconazole, and cyproconazole) induce ROS and their inhibitory potential is reduced by addition of an antioxidant, vitamin C.

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CS4.1.37

DEVELOPING A TET-OFF SYSTEM FOR IN VIVO ANALYSIS OF ANTIFUNGAL RESISTANCE AND VIRULENCE OF MUCORALES

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The order Mucorales is a group of ancient fungi classified in the subphylum Mucoromycotina characterized for its high biodiversity, being isolated from forest soils, freshwater and river sediments (Nguyen et al., 2019). Several species belonging to this group of fungi are important agents of the concerning human infection known as mucormycosis. This disease has a severe effect in patients with impaired immune system, diabetes, or deep trauma. In addition, these fungi display a remarkably high resistance to most currently available antifungal drugs, including first-line therapies used to treat mucormycosis (Dannaoui et al., 2017), which supposes an added problem to the difficulty of finding specific treatments against fungal infections. Species belonging to the genus Rhizopus are among the most frequent casual agents. The recent development of genetic and molecular tools in Rhizopus microsporus has enhanced the possibilities in the understanding of mucormycosis and the biology of this fungus (Lax et al., 2021). Characteristically, we have demonstrated that Rhizopus presents a high level of 6-methyladenine (6mA) which predominates its genomic landscape and that it is a rare and almost non-existent modification in human DNA. We have also identified a 6mA methylation complex that is proposed to be involved in the deposition of this epigenetic mark. To test the importance of this epigenetic modification and the role of this methylation complex in the virulence and the antifungal resistance of R. microsporus we are developing a Tet-off system using the recent CRISPR/Cas9 assisted genetic modification tools available in R. microsporus (ATCC 11559). The controlled expression of one of the components of this complex will allow us to generate a useful system to evaluate the importance of 6mA in a murine invasive pulmonary mucormycosis model and its potential as a specific target for drug development against mucormycosis.

CS4.1.38

EXTRUSION OF 5-FLUOROCYTOSINE DERIVED FLUOROPYRIMIDINES DIMINISHES ITS ANTIFUNGAL ACTIVITY AND GENERATES A CYTOTOXIC ENVIRONMENT

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Invasive aspergillosis illustrates one of the deadliest fungal diseases and is predominantly linked to infections caused by the human mold pathogen Aspergillus fumigatus. Major treatment procedures involve the use of antifungal agents belonging to the azole, polyene and echinocandin drug classes. The prodrug 5-fluorocytosine (5FC), which is the only representative of a fourth class, the nucleobase-analogs, is barely used for the treatment of aspergillosis due to an apparent lack of activity in vitro. The main route of 5FC activation in A. fumigatus comprises its deamination into 5-fluorouracil (5FU) by FcyA, which is followed by Uprt-mediated 5FU phosphoribosylation into 5-fluorouridine monophosphate (5FUMP). In this study, we characterized and examined the role of a metabolic bypass that generates this nucleotide via 5-fluorouridine (5FUR) through uridine phosphorylase and uridine kinase activities. Resistance profiling of mutants lacking distinct pyrimidine salvage activities suggested a minor contribution of the alternative route in 5FUMP formation. We further analyzed the contribution of drug efflux in 5FC tolerance and found that A. fumigatus cells exposed to 5FC reduce intracellular fluoropyrimidine levels through their export into the environment. This release generates a toxic environment for cytosine deaminase lacking mutants as well as mammalian cells. Employing the broad-spectrum fungal efflux pump inhibitor clorgyline, we demonstrate synergistic properties of this compound in combination with 5FC, 5FU as well as 5FUR. Our results suggest that the inhibition of fungal efflux pumps during 5FC treatment may serve as a potential therapeutic strategy to bolster its antifungal activity and circumvent the generation of a toxic environment for the host cells.

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CS4.1.39

FUNCTIONAL CHARACTERIZATION OF THE GENE ENCODING THE ANTIFUNGAL PROTEIN PEAFPA FROM PENICILLIUM EXPANSUM

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Antifungal proteins (AFPs) from filamentous fungi are promising molecules to control fungal pathogens. The main postharvest pathogen of apple fruit, Penicillium expansum, encodes three AFPs (PeAfpA, PeAfpB and PeAfpC). PeAfpA shows the highest in vitro antifungal activity against phytopathogenic and mycotoxigenic fungi. However, its mode of action and potential biological function in the producer fungus remains unknown.

This work describes the functional characterization of the gene encoding the PeAfpA protein by generating null mutants in the parental strain CMP-1 of P. expansum. ΔafpA strains were phenotypically and pathogenically characterized. Deletion mutants did not show differences from the parental strain other than the absence of PeAfpA production. This gene disruption neither produced changes in PeAfpB and PeAfpC production. Finally, a transcriptomic study was conducted in order to determine gene expression changes in two independent ΔafpA mutants and the parental P. expansum strain at two culture times, one before and one after the start of PeAfpA detection in the parental supernatant. Transcriptomic profiling of wild-type P. expansum over time revealed 1819 differentially expressed genes (DEGs) when PeAfpA is detected, being 866 upregulated and 953 downregulated. Pairwise expression analysis of ΔafpA strains showed 235 common DEGs (2.2% of total annotated genes) compared to the parental strain, being 109 repressed and 126 overexpressed. A total of 216 DEGs have been identified as potentially relevant in PeAfpA biological function, as they present a reversal expression pattern in the wild-type analysis at two culture times compared to the ΔafpA versus parental strain exper-

iment supposedly to be reciprocal. 90 of these genes (41.6%) have an unknown function, while 32 (14.8%) are annotated as transmembrane transporters and 8 (3.7%) are related to fatty acid metabolism. Overall, this study provides a rich source of information to further advance on the characterization of PeAfpA.

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CS4.1.40

SCREEN OF POTENTIAL MULTI-DRUG RESISTANT ZYMOSEPTORIA TRITICI FIELD ISOLATES REVEALS GENOTYPIC AND PHENOTYPIC DIVERSITY, SUGGESTING MULTIPLE MECHANISMS INVOLVED IN MDR

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Septoria Leaf Blotch is an important wheat disease caused by Zymoseptoria tritici, mainly controlled by fungicides in the field. Over the last decades, target-site resistance has increased in Z. tritici populations. In parallel, resistance involving enhanced efflux of multiple classes of fungicides has also raised, named multidrug resistance (MDR). MDR in Z. tritici was shown to be driven by the overexpression of the membrane efflux pump encoding gene MFS1, due to the presence of various inserts in its promoter (Omrane et al. 2015, 2017). These inserts are classified type I, IIa/b, or III and their lengths and insertion sites vary.
In order to estimate if MDR in Z. tritici was driven by MFS1 promoter inserts solely, we performed a large scale screen of MDR isolates. 475 potential Z. tritici MDR strains were isolated from different regions of France and Europe in 2020 and 2021, through a screen using a spore germination test on media with medical fungicides, tolnaftate and terbinafine. We determined terbinafine resistance levels for all strains as well as MFS1 promoter alleles. Type I insert was found in 59% of isolates, type II in 20%, type III in 5%. Interestingly, 13% displayed the WT promoter allele. Furthermore, qualitative and quantitative phenotyping results of terbinafine resistance showed discrepancies between resistance level of isolates with the same MFS1 promoter allele. This indicates that new and unknown mutations are involved in the MDR phenotype in some isolates. Some of them have an impact on MFS1 expression, studied by RT-qPCR, while others cause an increase in MDR level, independent from MFS1 expression indicating the involvement of additional and undescribed mechanism(s) leading to MDR in Z.

tritici. Fungicide efflux studies and expression analysis of membrane transporter genes are underway to confirm the MDR phenotype and to identify the transporter(s) involved in increased resistance.

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CS4.1.41

GENETICALLY ENGINEERED MUTATOR LINES OF THE WHEAT PATHOGEN ZYMOSEPTORIA TRITICI FOR FUNGICIDE RESISTANCE RISK ASSESSMENTS

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The main resistance mechanisms to fungicides involve mutations in the molecular target, including its regulatory sequence, or mutations in detoxification genes such as efflux transporters and / or their regulators (regulatory sequences, transcription factors). In rarer cases, resistance can be caused by loss of function mutations in genes that abolish antifungal activity (suppressor phenotype). To predict resistance risk toward new fungicides in the laboratory, different mutagenesis techniques are applied which include forced selection or directed evolution, often combined with rounds of highly mutagenic treatments such as UV or EMS. To increase the frequency of resistant individuals and better assess the diversity of resistance mechanisms, an increased rate and diversified set of mutations is highly desired. We generated a collection of genetically engineered mutator and hypermutator lines of the plant pathogen Zymoseptoria tritici, enabling higher speed and higher-quality predictive fungicide resistance risk assessments. Our mutator collection showed resistance rates that are similar or superior by few orders of magnitude to classical UV treatment. We show how the improved frequency and diversity of mutations enabled by mutator phenotypes enables the early characterization of a diverse set of resistance mechanisms.

CS4.1.42

TRANSCRIPTOMIC PROFILE OF PENICILLIUM DIGITATUM REVEALS NOVEL ASPECTS ON THE MODE OF ACTION OF THE ANTIFUNGAL PROTEIN AFPB

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Antifungal proteins (AFPs) from filamentous fungi are promising molecules to control fungal pathogens. The understanding of their biological role and mode of action is essential for their future application. AfpB from the phytopathogen Penicillium digitatum is highly active against plant pathogens, including the native fungus. Our previous data showed that AfpB acts through a multitargeted three-stage process: interaction with the outer mannosylated cell wall, energy-dependent cell internalization and intracellular actions that result in cell death. Herein, we extend these findings by conducting a transcriptomic study to characterize the functional role of AfpB and its interaction with P. digitatum. For this, the transcriptome of P. digitatum wild type treated with AfpB, the null ΔafpB and an AfpB overproducing strain were compared. Results showed that treatments under strong aeration of the culture increase the antifungal potency of AfpB, demonstrating that growth conditions determine the apparent activity of this protein. Transcriptomic data indicates that afpB represses the expression of a gene putatively involved in the biosynthesis of indol alkaloids, which are well-known phytotoxins. Gene knockout mutants confirmed that genes coding for acetolactate (AL) synthase and AL decarboxylase from the acetoin biosynthetic pathway contribute to the inhibitory activity of AfpB. Moreover, a gene coding for a previously uncharacterized extracellular tandem repeat peptide (TRP) protein shows high induction in the presence of AfpB, while its putative TRP monomer enhances AfpB antifungal activity. Our study offers a rich source of information to further advance on the characterization of the multi-faceted mode of action of AFPs.

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CS4.1.43

MOLECULAR AND PHENOTYPIC CHARACTERIZATION OF CADOPHORA LUTEO-OLIVACEA ISOLATES FROM STORED APPLE FRUIT IN SOUTH TYROL (NORTHERN ITALY)

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Cadophora luteo-olivacea is known as a causal agent of trunk disease of grapevine and cordon dieback of kiwifruit, but has also been associated with postharvest diseases, such as skin pitting of kiwifruit or side rot of pear and apple. In South Tyrol (northern Italy), C. luteo-olivacea has been isolated from apple fruit with postharvest side rot symptoms on several occasions. Ten of these isolates were selected to be further assessed for their pathogenicity on different apple cultivars and their sensitivity to a selection of common fungicides used in integrated and organic apple production. The pathogenicity test was performed on detached fruit of four apple cultivars, Granny Smith, Cripps Pink, Golden Delicious and Fuji, which were artificially wounded and inoculated with a defined conidial suspension. Two different in vitro approaches were applied for the fungicide sensitivity testing. The inhibition of radial growth was studied on solid medium enriched with the recommended dose of five different fungicides (active substances: captan, boscalid, fludioxonil, orange oil and a mixture of eugenol, geraniol and thymol). The inhibitory effect of different concentrations of ten fungicides (active substances: captan, boscalid, fludioxonil, orange oil, a mixture of eugenol, geraniol and thymol, dithianon, fluazinam, dodine, copper sulfate, and cyprodinil) on spore germination was examined in a microtiter plate assay with liquid medium. Finally, ISSR fingerprinting with four primers was applied to assess the genetic diversity of C. luteo-olivacea isolates from South Tyrol and to associate genotypes to different fungicide sensitivity groups. Even though C. luteo-olivacea currently represents a minor post-harvest pathogen of apple, the knowledge of biological characteristics is a prerequisite to understand the epidemiology of the disease and to define disease control strategies.

CS4.1.44

INVESTIGATING THE MECHANISMS OF BOTRYTIS CINEREA MULTIDRUG RESISTANCE TO FUNGICIDES

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Botrytis cinerea is a notorious plant pathogen causing severe yield losses worldwide. The pathogen can infect more than 200 plant species at preharvest and postharvest stages and is mainly controlled by fungicide applications. Extensive fungicide use against B. cinerea has led to the development of multidrug resistance (MDR). MDR is caused largely by increased fungicide efflux, attributed to mutations in the transcription factors and promoters of transporter genes, resulting in non-lethal levels of fungicides in the cell. While MDR is well-studied in human pathogens, studies in plant pathogens are rather limited. Here we investigated the MDR mechanisms in B. cinerea by comparing the transcriptomes of B. cinerea MDR isolate Ap2 and the reference strain B05.10 during exposure to the fludioxonil fungicide at different time points. B. cinerea cultured without fungicide exposure were used as control treatments. RNA-seq analysis showed that 238 genes, putatively encoding transporter proteins, were up-regulated ($P \leq 0.05$) at 0h in the MDR isolate Ap2 as compared to B05.10, even in the absence of fungicide. However, at 8h the number of up-regulated transporter genes was considerably reduced, suggesting a rapid response by Ap2 against fludioxonil compared to the sensitive B05.10. Interestingly, a cluster of genes encoding putative polygalacturonases was also up-regulated in Ap2 as compared to B.05.10. In contrast, a cluster of genes putatively involved in DNA repair and homologous recombination processes, were down-regulated in the Ap2 resistant isolate compared to B05.10. In conclusion, these data show that certain membrane transporters are constitutively expressed at a high level in B. cinerea MDR isolate Ap2 and may contribute to fungicide efflux. The data from the current study will give insights to the phenomenon of MDR in fungal phytopathogens, contributing to a better management of fungicide resistance. This project has been funded by H.F.R.I.

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CS4.1.45

INTRINSIC AMINO ACID SUBSTITUTIONS IN THE SDM-F5 PARALOGUE OF MUCOR CIRCINELLOIDES ARE A MAJOR REASON IN SHORT-TAILED AZOLE RESISTANCE

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Mucor circinelloides is one of the most prevalent causative agent of mucormycosis in Europe. Intrinsic resistance to short-tailed azoles such as voriconazole limits treatment options for mucormycosis to amphotericin B, posaconazole, and isavuconazole. The amino acid substitutions (AA) Y129F, V293A within the ligand-binding pocket (LBP) of the Mucor circinelloides (Mc) sterol-14-α-demethylase (SDM) paralog F5 are suspected to cause this resistance due to in-silico work. Subsequently, we aim to experimentally proof the impact of the AA changes in the LBP on drug resistance, using a heterologous Saccharomyces cerevisiae model.

McSDM paralogues (SDM-F1 and SDM-F5) were overexpressed with and without their cognate cytochrome-P450-reductase (CPR) at PDR5 and PDR15 loci in a hypersensitive parental strain. To study the effect on drug binding within the LBP, AA changes were reverted in the SDM-F5 gene (F129Y, A293V, F129Y & A293V). The susceptibility profiles were assessed by EUCAST for all azoles. Strains were characterized using growth kinetics, SDS-PAGE, and western blots. The ergosterol pathway response to azole exposure was quantified using GC-MS.

Recombinant protein expression did not impact growth rates compared to the parental strain and gave comparable (SDM-F5: 98%) or lower levels (SDM-F1: 74%) than the control overexpressed ScERG11. According to resistance profiles, SDM-F1 and SDM-F5 mutants presented susceptible phenotypes for voriconazole (0.016-0.06 mg/L) and posaconazole (0.008-0.125 mg/L). SDM-F5 showed a comparable MIC for posaconazole (0.06 mg/L) but significantly higher values for voriconazole (4 mg/L). According ergosterol content, voriconazole treatment was more effective on SDM-F1.

The heterologous McSDM isoforms were functional and advantageous in the inhibition of azole efficacy on ergosterol biosynthesis, especially for SDM-F5. MIC profiles support these findings. Summarizing, the heterologous model identifies SDM-F5 as primary reason for intrinsic short-tailed azole in M. circinelloides.

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CS4.1.46

THE NON-HOMOLOGOUS END JOINING REPAIR PATHWAY IS IMPLICATED IN STABLE FLUCONAZOLE RESISTANCE IN CANDIDA GLABRATA

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Candida species are commensal yeasts that asymptomatically colonize healthy humans but act as opportunistic pathogens in immunocompromised patients. C. glabrata is the second most frequent Candida species in Candida infections and accounts for approximately 15–25% of invasive clinical cases. This yeast is associated with a high frequency of drug resistance resulting in high mortality rates. Particularly, C. glabrata shows a great ability to adapt to azole and echinocandin antifungal agents which are widely used for Candida treatment. Known mutations involved in azole and echinocandin resistance imply, respectively, points mutations in PDR1, which encodes a transcriptional regulator of drug efflux pumps, and in the FKS genes, implicated in the biosynthesis of a major structural component of fungal cell walls. However, mechanisms leading to drug adaptation are still poorly understood. Non-Homologous End-Joining (NHEJ) is a double-strand break repair pathway which can introduce mutations in the genome and, to our knowledge, its implication in drug resistance has never been studied. In this work, we used a laboratory micro-evolution experiment to better assess adaptation of C. glabrata to fluconazole (azole) and caspofungin (echinocandin) and to decipher the implication of NHEJ in drug resistance. WT and lig4Δ (NHEJ mutant) C. glabrata strains were exposed for prolonged periods to twice their MIC50 (Minimum Inhibitory Concentration required to inhibit the growth of 50% of cells) of fluconazole and caspofungin. Our results show that in C. glabrata, NHEJ is implicated in the stable fluconazole resistance but not in the caspofungin resistance. Both the wild-type and mutant strains evolved in flucozanole and in caspofungin show a higher resistance to osmotic stress and evolved a higher capacity to form biofilms. The mechanisms contributing to this adaptation remain to be unveiled and will tell us if the WT and lig4Δ strains' adaptation followed the same or different evolutionary pathways.

CS4.2 FUNGAL CELL BIOLOGY

CS4.2.9

A MODEL OF BEM46 MODE OF ACTION IN NEUROSPORA CRASSA

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Despite the ubiquitous presence of BEM46 homologs in eukaryotic organisms, the role of these proteins is still elusive. They belong to the α/β-hydrolase superfamily (1). The human homolog ABHD12 is a phosphatidylserine-lipase, localized in the ER and BAR-domain protein mediated AMPAR complex (2,3). Patients with abhd12 mutations develop the neurodegenerative disease PHARC (4). Our investigations focus on the Neurospora crassa BEM46 protein which is localized in the ER and BAR-domain mediated MCC/eisosome complex (5,6,7). Bioinformatical investigations show, that N. crassa BEM46 and human ABHD12 share the conserved catalytic domain and phosphorylation sites, making the N. crassa BEM46 an intriguing model for the human system.
Our investigations on bem46 overexpressing (OE), knock down and knock out (KO) fungal strains show, that in the asexual cycle, overexpression leads to significant delay of conidial germination and reduction of hyphal elongation. In the sexual development, bem46 overexpressing strains show strongly reduced ascospore germination with polarity defects. KO strains show only mediate alterations in all developmental stages (8). We showed, that BEM46 interacts with the anthranylate synthase component II and influences the auxin biosynthesis of the fungus (5). Whole transcriptome analyzes of OE and KO conidia and mycelia support our former data, however they also show the importance of further investigations on the lipidome level.
Here, we present a new model in which BEM46 and auxin interdependently influence plasma membrane subdomain structuring, thereby providing a flexible platform for signaling proteins.

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CS4.2.10

IDENTIFICATION OF ASPERGILLUS FUMIGATUS COTA EFFECTORS REGULATING HYPHAL MORPHOGENESIS AND GROWTH ON DIVERSE CARBON SOURCES

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Aspergillus fumigatus displays immense metabolic plasticity promoting growth in varied environments. The metabolic and signaling pathways that allow this pathogen to cause invasive disease in the host lung environment are not fully understood. We recently identified the conserved NDR-kinase, CotA, as essential for A. fumigatus hyphal development and virulence in a murine model of invasive aspergillosis. Our data suggest that CotA supports virulence through orchestration of growth in the presence of alternative carbon sources, such as fatty acids or aminoacids, in an isoform-specific manner. Although CotA is expressed as both a long and short protein isoform in wild type strains, a mutant expressing only the short isoform fails to grow on non-sugar carbon sources, suggesting a role for this kinase in carbon source sensing, signaling or utilization. To further explore the importance of CotA kinase activation, mutations of two conserved phospho-sites (S464 and T635) required for activation of NDR kinases were generated. Whereas the cotAS464A mutant only displayed a slight growth defect, the cotAT635A mutant revealed a striking reduction in colony diameter. Upon prolonged incubation, the cotAT635A mutant developed spontaneous suppressors with improved growth and conidiation and ten of these suppressors were isolated for differential gene expression studies. Only 16 genes (predicted to be involved in the degradation, metabolism and binding of extracellular polysaccharides, fungal development, metabolism of energy reserves and glycerol metabolic processes) were upregulated 2-fold or more in common among all suppressor mutants. Each of these 16 genes are also significantly downregulated in the cotAT635A mutant versus the parental strain. These findings suggest that CotA may regulate cell wall and glycerol metabolism to ensure hyphal morphogenesis. Genetic manipulation of these candidate genes will help in deciphering how CotA is able to orchestrate morphogenetic machinery in response to carbon source, and therefore its importance for A. fumigatus pathobiology.

CS4.2.11

THE LOCALIZATION OF THE VENUS-TAGGED ANTIMICROBIAL PROTEIN PAFB SHEDS LIGHT ON ITS ROLE IN GROWTH AND DEVELOPMENT OF PENICILLIUM CHRYSOGENUM

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The genome of the antibiotic producing filamentous fungus Penicillium chrysogenum harbors three genes that encode small, cysteine-rich and cationic antimicrobial proteins (AMPs), namely PAF, PAFB and PAFC. For these three AMPs other functions in addition to their well studied antifungal activity have been postulated. PAF is highly expressed in submerse culture under nutrient starvation during stationary growth, whereas PAFB is expressed under nutrient excess during logarithmic growth of P. chrysogenum. Furthermore, PAF was shown to support optimal conidiation and found in the exudate of aged surface cultures together with PAFB and PAFC.
In this study, we wanted to elucidate in more detail the intrinsic role of PAFB and studied its expression and localization in P. chrysogenum surface cultures, which better reflect the natural environment in which P. chrysogenum grows than the biotechnological relevant submerse cultivation conditions. To reach this aim, we analysed the expression pattern of pafB in P. chrysogenum Q176 (wt), and generated a pafB deletion mutant (P. chrysogenum delpafB) and a mutant (P. chrysogenum pafBvenus) that expresses PAFB that is tagged with the fluorescent protein Venus (PAFBVenus).
We report here on the characterization of the phenotype of the generated P. chrysogenum mutants using a multidisciplinary approach. The spatio-temporal expression of pafB and the distribution pattern of fluorescent PAFBVenus in surface cultures assigns PAFB an intrinsic function for the optimal growth and development of P. chrysogenum.

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CS4.2.12

THE INTRA-SPECIES DIVERSITY OF HYDROPHOBIN ROLA AND CUTINASE CUTL1 POSSIBLY AFFECT PHYSICOCHEMICAL PROPERTIES AND BIOLOGICAL FUNCTIONS OF THESE PROTEINS.

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Hydrophobins are amphipathic low molecular-proteins that are localized on the surface of fungal cell wall. Hydrophobins self-assemble at the air-water/solid-liquid interfaces and are involved in morphogenesis of filamentous fungi. A filamentous fungus Aspergillus oryzae produces hydrophobin RolA. RolA adsorbs to the solid surfaces, then recruits and condenses a cutinase (polyesterase) CutL1 on the solod surface to stimulate hydrolysis of solid substrates by CutL1[1]. The mechanism of solid polymer degradation via hydrophobin-enzyme interaction is unique and may widely conserved among filamentous fungi [2]. Recently, a genome-wide comparative analysis revealed that various A. oryzae strains has wide genomic diversity and are divided into many clades [3]. The fact suggests that and there is possibility of intra-species diversities in both RolA and CutL1, however, the diversities in these proteins have not been studied. It is also unknown whether the variation of amino acid sequences affect the physicochemical properties and biological functions of RolA and CutL1.

In this study, we compared the amino acid sequences of both RolA and CutL1 mainly in over 100 A. oryzae strains. The number of amino acid substitution in CutL1 was only one, however, the residue is known to be involved in RolA-CutL1 interaction [4]. In RolA, the substitution was found in 11 residues of 151 total amino acids. The substitutions were concentrated in particular regions that are important in self-assembly of hydrophobin RodA of Aspergillus fumigatus [5], a RolA orthologue. Therefore, it is suggested that there are diversities in both physicochemical properties and biological functions; RolA-CutL1 interaction and self-assembly of RolA.

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CS4.2.14

A RANDOM MUTAGENESIS APPROACH TO ELUCIDATE THE BIOLOGY OF EXTREMOTOLERANT BLACK FUNGI

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Microcolonial black fungi ubiquitously inhabit sun-exposed natural and man-made surfaces of our planet. To promote genetic studies, which are hindered by slow growth, lack of sexual cycles and transformation difficulties, CRISPR/Cas9-based genetic tools were implemented (Erdmann et al. 2022, Front Fungal Biol). Now efficient targeted mutagenesis of the rock inhabitant Knufia petricola (Eurotiomycetes/Chaetothyriales) - as a representative of the polyphyletic group of black fungi - enables the elucidation of extremotolerance, oligotrophism, unusual types of cell division, mineral weathering and symbiotic interactions. Still more progress on assigning functions to yet unknown genes can be expected if a forward genetics approach is available. We chose the two-component Activator/ Dissociation (Ac/Ds) transposon system from maize for generating a collection of insertional mutants by in-vivo mutagenesis of K. petricola. For the optimal use of this genetic tool, an inducible promoter for the expression of the Ac transposase (AcTPase) and by this the regulatable transposition of the resistance cassette-containing Ds transposon is desired. However, endogenous promoters for nitrate assimilation and galactose catabolism - often used in fungi for regulatable gene expression - are not inducible by their substrates in K. petricola suggesting that the regulatory networks for nutrient acquisition differ significantly in oligotrophic fungi. Therefore, the metabolism-independent Tet-on system was combined with the AcTPase coding sequence and subsequently transformed into Ds-carrying K. petricola strains. In total, four auxotrophic Ac|Ds starter strains containing the Ds transposon at different position of ade2 or ura3 were generated. The cultivation of these strains with doxycycline for induction of TET::Ac and subsequent selection of cells on ADE/ URA-lacking media resulted in prototrophic colonies (revertants) for

some but not all Ac|Ds strains. Currently, the transposition events in the obtained revertants are studied to validate the procedure. First amplicon sequencing of excision sites revealed footprint patterns, proving the transposon jumped.

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CS4.2.15

COTH GENES ARE NECESSARY FOR NORMAL SPORE FORMATION AND VIRULENCE IN MUCOR LUSITANICUSC

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Clarifying the pathomechanism of mucormycosis, understanding the interaction of Mucorales fungi with their hosts, and identifying potential virulence factors are essential. Thus, our research is focused on the extensive analysis of the CotH kinases and other surface proteins by tracking the phenotypic alterations of CRISPR-Cas9-derived mutants. Disruption of some of the cotH genes resulted in variances in the structure of the inner spore coat, differences in spore size distribution, fungal growth, and sporulation. The CWF fluorescence intensity was higher for the cotH4 mutant, which may be related to an increase in chitin content or to changes in cell wall structure that facilitate easier access of the dye to chitin. The CotH protein-mediated interaction proved to be crucial for the fungal invasion, as the number of the GRP78 molecules significantly increases in sinuses, lungs, and brain during DKA causing vulnerability towards the R. oryzae infection. IgG antibodies produced against the Rhizopus CotH3 protein protected mice with DKA from mucormycosis and anti-CotH3 antibodies were proposed promising for immunotherapy treatment of human mucormycosis. Viability studies

in DKA mice demonstrated that deletion of either cotH3 or cotH4 genes attenuates the pathogenicity of M. lusitanicus. Importantly, the cotH3 mutant did not show reduced virulence in a Galleria mellonella but did in a DKA mouse with elevated GRP78 receptor expression. However, the protein does not carry the characteristic motif determined earlier for R. delemar. Due to its sequence similarity to the Rhizopus CotH3 and the presence of the "CotH-motif", CotH4 could be a potential ligand for the GRP78 receptor, which requires further investigation.
Our results suggested that CotH proteins are involved not only in pathogenicity but also in the development of spore size and its structure in M. lusitanicus.
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CS4.2.16

OXYGEN MASS TRANSFER EFFECTS ON RECOMBINANT PROTEIN PRODUCTION BY THE HYPHAL DISPERSED ASPERGILLUS ORYZAE MUTANT IN THE LAB-SCALE FERMENTATION

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Aspergillus oryzae has been widely used in the industrial production of enzymes. In submerged fermentation, the mycelial form of culture exhibits high viscosity and limits oxygen mass transfer. Previously, we characterized the hyphal dispersed mutant of Aspergillus oryzae lacking both α-1,3-glucan and galactosaminogalactan (AGΔ-GAGΔ). Recombinant protein production of AGΔ-GAGΔ was significantly higher than that of wild-type strain (WT), and the viscosity of AGΔ-GAGΔ culture decreased significantly in a lab-scale bioreactor. In this work, we investigated the relationship between volumetric oxygen mass transfer coefficient (kLa) and the recombinant protein production of WT and AGΔ-GAGΔ over a wide range of agitation speed (400-1200 rpm). Protein production was correlated with kLa for both strains , as higher productivity under higher kLa conditions. At 500-1200 rpm, enzyme productivity and kLa of AGΔ-GAGΔ were higher than those of WT. In addition, computational fluid dynamics (CFD) simulation was performed to quantify the hydrodynamics in a culture vessel based on the measured physical properties. CFD model confirmed higher shear stress at higher stirring speed, whereas enzyme production continued to increase with speeds up to 1200 rpm. Simulated flow patterns and gas volume fractions are in good agreement with experimental data of dissolved oxygen and kLa at different agitation speeds. Furthermore, RNA-seq analysis was performed to identify the metabolic responses of the AGΔ-GAGΔ cells to different kLa values, resulting in

approximately 5000 differentially expressed genes between stirring at 400 and 800 rpm. Especially, the genes related to glyoxylate bypass and the GABA shunt were up-regulated at lower kLa conditions, suggesting a cellular response to reduce NADH formation from the TCA cycle when fewer oxygen supplied. These results indicate that AGΔ-GAGΔ displays marked shear tolerance and oxygen mass transfer has dynamic effects on fungal metabolism and recombinant protein production.

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CS4.2.17

ANALYSIS OF PHOSPHORYLATED COMPONENTS OF THE SEPTATION INITIATION NETWORK AS POTENTIAL TARGETS OF THE STRIPAK COMPLEX IN SORDARIA MACROSPORA

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The stratin-interacting phosphatases and kinases (STRIPAK) complex is a highly conserved signaling complex that performs a similar function in diverse eukaryotes, including humans and fungi; STRIPAK regulates protein functions through de-/phosphorylation, which is essential for their biological activities [1]. In fungi, STRIPAK is crucial for vegetative growth, hyphal fusion, and sexual development, and our aim is the identification of potential phosphorylation or dephosphorylation targets of STRIPAK. We recently found that STRIPAK communicates with conserved proteins, such as components of the septation initiation network (SIN), which is homologous to the Hippo signaling pathway from animals. Recent proteome and phosphoproteome studies using wild-type and mutant strains from S. macrospora have identified components of SIN (CDC7, SmKIN3, and DBF2) as putative targets of STRIPAK [2,3]. Based on our previous results, we propose that the deletion of STRIPAK subunits promotes higher phosphorylation of CDC7, which prevents SIN assembly. This results in a lower level of phosphorylated SmKIN3, which consequently cannot phosphorylate the downstream kinase DBF2. For functional analysis of SIN components, we constructed deletion and complementation strains. SIN deletion strains, for instance, are sexually sterile and show severe defects in septum formation. For a functional analysis, we have generated phosphomimetic and -deficient variants of conserved (de)phosphorylation residues in SIN subunits. The results of our study will gain deeper insight into the control of STRIPAK on cellular development in filamentous ascomycetes.

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CS4.2.18

GLYCOSYLPHOSPHATIDYLINOSITOL-ANCHORED A-AMYLASE REGULATES THE MOLECULAR WEIGHT OF CELL WALL A-1,3-GLUCAN IN ASPERGILLI

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Aspergillus fungi possess α-1,3-glucan with a low proportion of α-1,4-glucan as a major cell wall polysaccharide. Glycosylphosphatidylinositol (GPI)-anchored α-amylases are conserved in Aspergillus fungi. The GPI-anchored α-amylase AmyD in Aspergillus nidulans has been reported to directly suppress the biosynthesis of cell wall α-1,3-glucan but not to degrade it in vivo (1). However, the detailed mechanism of cell wall α-1,3-glucan biosynthesis regulation by AmyD remains unclear. We comparatively studied AoAgtA, which is encoded by the Aspergillus oryzae agtA gene, an ortholog of the A. nidulans amyD gene. Similar to findings in A. nidulans (2), agtA overexpression in A. oryzae grown in submerged culture decreased the amount of cell wall α-1,3-glucan and led to the formation of smaller hyphal pellets in comparison with the wild-type strain. We analyzed the enzymatic properties of recombinant (r)AoAgtA produced in Pichia pastoris and found that it degraded soluble starch, but not linear bacterial α-1,3-glucan. Furthermore, rAoAgtA cleaved 3-α-maltotetraosylglucose of which structure is similar to the predicted boundary structure between the α-1,3-glucan main chain and a short spacer composed of α-1,4-linked glucose residues in cell wall α-1,3-glucan. Interestingly, rAoAgtA randomly cleaved only the α-1,4-glycosidic bonds of 3-α-maltotetraosylglucose, indicating that AoAgtA may cleave the spacer in cell wall α-1,3-glucan. Consistent with this hypothesis, heterologous overexpression of agtA in A. nidulans decreased the molecular weight (MW) of cell wall α-1,3-glucan as well as homologous overexpression of amyD in A. nidulans (2). These in vitro and in vivo properties of AoAgtA suggest that GPI-anchored α-amylases of Aspergilli can degrade the spacer α-1,4-glycosidic linkages in cell wall α-1,3-glucan before its insolubilization, and this spacer cleavage decreases the MW of cell wall α-1,3-glucan in vivo.

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CS4.2.19

ROLE OF KINESIN-3 MOTOR PROTEINS IN PODOSPORA ANSERINA ORGANELLE DYNAMICS

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Kinesin-3 motors have been proposed as global regulators of intracellular trafficking during polarized growth of fungal hyphae and in neuronal axons. In the basidiomycete Ustilago maydis the only kinesin-3 motor protein mediates the endosomal-dependent hitchhiking of peroxisomes, endoplasmic reticulum (ER) and lipid droplets, but not of mitochondria. However, in Ascomycetes, which possess two kinesin-3 motor proteins, the panorama is not fully elucidated. UncA and NKIN2, the orthologues of metazoan Unc-104 in Aspergillus nidulans and Neurospora crassa, respectively, are required for peroxisome and early endosome transport. However, their involvement in mitochondrial dynamics remains ambiguous and their role in ER motility remains unknown. In this work we studied the role of the Podospora anserina class 3 kinesins –Kin2 and Kin3– in organelle dynamics. We deleted KIN2 and KIN3 and found that KIN2 (UncA and NKIN2 orthologue), but not KIN3, is required for optimal mycelial growth and proper hyphal ramification. We found that the genetic elimination of Kin2 affects Spitzenkörper behavior, reduces peroxisome abundance and motility, and disturbs apical ER dynamics. In addition, we discovered that loss of Kin2 altered the distribution of mitochondria by reducing their abundance at the growing hyphal tip, without impeding their motility. Finally, we found that Kin3 absence did not notably affect the dynamics of these organelles, and that the Kin2Kin3 double deletion recapitulated all ΔKin2 defects. Our research revealed a major role for the kinesin-3 motor Kin2 in the regulation of P. anserina organelle dynamics. This research was supported by grant CONACYT-DFG 277869 from CONACYT.

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CS4.2.20

THE ANTIMICROBIAL PEPTIDE ANAFP ACTS AS MEDIATOR OF AUTOPHAGY IN A. NIGER

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Antimicrobial peptides are used by various organisms to fight off predators, invaders, or microbial competitors and can be found virtually in all domains of life. Yet, recent insights into the expression profile of one of these, the peptide AnAFP from Aspergillus niger point towards a second, endogenous function.
For example, anafp is expressed in axenic cultures and only for a short period of time that coincides with carbon depletion where it parallels expression of genes predicted to function in autophagy, nutrient recycling and development. We therefore proposed earlier that AnAFP could act as a cannibal toxin that drives development and programmed cell death. It kills a subpopulation of A. niger cells to support the survival of the other subpopulation and thus survival of the species. To verify this hypothesis, we put anafp expression under control of the doxycycline-dependent Tet-On gene switch. Indeed, a reduced growth of A. niger as well as a reduces spore formation was measured during cultivation in shake flask or on solid agar when anafp expression was induced prematurely, i.e., before carbon depletion, with doxycycline. To determine whether the reduced growth is the result of enhanced autophagy, A. niger mycelia expressing anafp or anafp::gfp were monitored regarding autophagy and apoptosis and its subcellular localization using confocal fluorescence microscopy, qRT-PCR, and co-immunoprecipitation. Our results show a complex post-transcriptional, spatial and temporal regulation of AnAFP expression with clear impact on autophagy and degradation of fungal biomass in its host. Interestingly, AnAFP acts as a mediator in this process and does not appear as a final effector.
Key Words: #filamentous fungi #antimicrobial peptides #programmed cell death #autophagy #autolysis #confocal microscopy #live cell imaging #vacuole

CS4.2.21

KINASE ACTIVITY OF COT-1 IS ESSENTIAL FOR MAINTAINING STABLE CELL POLARITY AXES AND DIRECTED GROWTH IN NEUROSPORA CRASSA

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The Neurospora crassa strain cot-1 (Colonial Temperature sensitive 1) was among the first temperature-sensitive mutants found in an early forward genetics mutant screen of this fungus. When grown at restrictive temperatures, this mutant exhibits a hyperbranching, dendritic-spine like phenotype. Besides its severe polarity defect, the mutant also forms more septa and thicker cell walls compared to wild type. To investigate the subcellular dynamics of the kinase COT-1 via live cell imaging we used chemical genetics as a tool to inhibit the kinase activity on the protein level by adding an ATP-analog. The mutation of the ATP-binding pocket resulted in an analog sensitive kinase (COTas), which is readily inhibitable at different developmental stages. Inhibition of COT-1as in germinating conidial spores results in an increased number of germ tubes and a swollen spore body. The tips of these germ tubes show an unusual accumulation of various proteins at the plasma membrane, including proteins for polarization, cell communication and COT-1 itself. These substantially disturbed cells grow significantly slower than the wild type, while nuclei division appears not to be affected, resulting in cells with increased numbers of nuclei per cellular volume.
In wild type, germinating conidia interact to form a supracellular network via cell-cell interaction and fusion. When the COT-1 kinase activity was inhibited, cell interaction rates are highly reduced and cells communicate only over short distances. In these cells, communication proteins, such as SOFT and MAK-2, accumulate permanently at all cell tips of the hyperbranching germlings.
Taken together, these data indicate that the COT-1 kinase activity is essential for the proper subcellular dynamics of several critical signaling and polarity factors.

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CS4.2.22

A NOVEL HOLOTOMOGRAPHIC LIVE CELL IMAGING METHOD TO STUDY CELLULAR PROCESSES IN FUNGI

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Imaging of living cells allows a fascinating insight into morphological as well as subcellular changes of organisms. The inclusion of in vivo fluorescent microscopy has expanded the scope of application by visualizing fluorescent proteins to study localization, dynamics and interactions over time. Fungal development is studied with various super-resolution microscopy methods that require individual operation conditions.
Here, we want to extent this toolkit and propose live cell imaging of fungi, e.g. Aspergillus niger, with a technique based on refractive index measurements. This stain and marker free image acquisition allows spatiotemporal observation of fungal growth in real time with an acquisition speed up to 1.7 seconds per image. The focus of this work lies in the characterization of the RI-map of the fungus – the visualization of subcellular compartments including the mitochondrial network, liposomes, vacuoles and microtubuli based on their individual physical properties. This is followed by tracking the organization of structures during growth.
A non-invasive 3D tomography microscope, that can be operated with additional fluorescent channels, enables new insights into fungal morphology and development.

CS4.2.25

DISSECTING THE GENETIC MACHINERY OF ARYL HYDROCARBON RECEPTOR (AHR) AGONIST PRODUCTION IN THE SKIN COMMENSAL YEAST MALASSEZIA

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The lipophilic yeast Malassezia is by far the most abundant member of the skin mycobiome, representing over 90% of all commensal skin fungi. While being a commensal fungus, Malassezia has also been associated with some skin disorders like seborrheic dermatitis (SD), atopic dermatitis (AD) and pityriasis versicolor (PV). Recent studies also report a clear involvement of Malassezia species in Crohn's disease and progression of pancreatic cancer. Our understanding of the interaction between Malassezia and the host remains incomplete, both in the healthy and in the diseased skin. Previous studies found that Malassezia furfur converts tryptophan into brown-pigmented indoles that activate aryl hydrocarbon receptor (AhR) signaling in human keratinocytes. To elucidate the biochemical pathway responsible for AhR ligand production in M. furfur, we applied an insertional mutagenesis approach through Agrobacterium tumefaciens-mediated transformation. The screen allowed the isolation of several mutants impaired in the production of the characteristic brown indoles in media supplemented with tryptophan as the sole nitrogen source, and defective AhR activation in keratinocytes. A combination of omics approaches is in progress to characterize the active molecules and decipher the biochemical pathway of AhR agonist production in M. furfur. These genetics data will be integrated with available data from in vivo and in vitro models for fungus-host interaction to establish the mechanism by which Malassezia indoles affect antifungal immunity, with implications for therapeutical approaches targeting the tryptophan metabolic pathway in the fungus or AhR signaling in the host.

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CS4.2.26

FUNCTIONAL ANALYSES OF SUBSTANCES RELATED TO HYPHAL HYDROPHOBICITY IN BIPOLARIS MAYDIS

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The surface hydrophobicity of hyphae and conidia in plant pathogenic fungi is a critical factor in their adhesion and colonization to host plants. In general, the amphiphilic secreted protein hydrophobin is known to be strongly involved in the surface hydrophobicity of cells in filamentous fungi. Hydrophobins are mainly classified into Class I and II based on their hydrophobicity. The southern corn leaf bright fungus Bipolaris maydis retains a total of four putative hydrophobin genes in its genome: Class I (HYP1) and Class II (HYP2, HYP3, and HYP4). On the other hand, since the single disruption of any of the genes showed hyphal hydrophobicity comparable to that of the wild-type strain, it is inconclusive whether or not hydrophobins contribute to the hyphal surface characteristics of this fungus. In this study, we generated multiple deletion strains of the hydrophobin genes in B. maydis and attempted to analyze their phenotypes to clarify the function of hydrophobins. First, the hyp2 gene was disrupted using the Δhyp3 strain as the parental strain, followed by the disruption of the hyp4 gene. The resulting Class II hydrophobin-deficient strain (triple disruption strain) did not show obvious differences from the wild-type strain in vegetative growth and in hyphal hydrophobicity evaluated by dropping SDS solution on the colonies, but analyses of pathogenicity and conidial hydrophobicity are underway to understand their essential roles in this fungus. We are also in the process of attempting to disrupt all genes to clarify the function of the hydrophobins in B. maydis. Furthermore, since previous studies have shown that the NPS4 (non-ribosomal peptide synthetase 4) gene is strongly involved in the hyphal hydrophobicity of this fungus[1], we would like to discuss the function of the NPS4 gene, cell surface hydrophobicity, and its relationship with the hydrophobins. [1]Turgeon et al., Mycol. Res., 2008

CS4.2.27

THE ROLE OF SFP1 IN CANDIDA ALBICANS CELL WALL MAINTENANCE

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The cell wall is the outermost structure for Candida albicans to interact with the surrounding environment and the host cells. Therefore, cell wall maintenance is crucial for C. albicans survival and host-pathogen interaction. Here, we explored the role of the transcription factor Sfp1 in cell wall integrity (CWI). A deletion of the SFP1 gene not only caused changes in cell wall properties, cell wall composition and structure but also modulated expression of cell wall biosynthesis and remodeling genes. In addition, Cas5 is a known transcription regulator for C. albicans CWI. Interestingly, our results indicated that Sfp1 negatively controls the CAS5 gene expression by binding to its promoter element. Together, this study provides new insights into the regulation of C. albicans CWI and stress response.

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CS4.2.28

INSIGHTS INTO THE ASPERGILLUS FUMIGATUS AFU4G10610 GENE OVEREXPRESSED DURING IN VIVO AND IN VITRO INFECTIONS.

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Aspergillus fumigatus is the most pathogenic species among the fungi of the genus Aspergillus and has a high incidence and mortality in immunocompromised patients. Therefore, studying the virulence of the fungus and defining new diagnostic and therapeutic targets has become a priority. For this purpose, using the AWAFUGE (Agilent Whole A. fumigatus Genome Expression) microarray designed by our research group, we studied the whole A. fumigatus transcriptome during infection of in vitro cell cultures (murine RAW 264.7 macrophages and human A549 lung epithelial cells) and in vivo infections of immunosuppressed BALB/c mice. Comparison of these transcriptomes in the three conditions abovementioned allowed us to detect overexpression of the Afu4g10610 gene, that encodes a hypothetical protein of unknown function. The bioinformatic analysis of the sequence revealed homology to a dimeric A/B barrel domain possibly involved in the response to stress and indicated its location in the plasmatic membrane. To assess the role of this protein, we generated the deletion mutant strain Δ10610, using CRISPR-Cas9 gene-editing technique, and Af293 as a wild-type strain (WT). Phenotypic analysis showed increased sensitivity to Congo red cell wall stress agent and increased resistance to NaCl/KCl osmotic stress agents compared to the WT strain. On the other hand, the mutant strain showed the same response to caffeine than the WT, discarding the link of this protein with TOR signalling pathway and suggesting a possible link of this protein with the Cell Wall Integrity (CWI) pathway. Although the detailed role and interactions of this gene remain to be elucidated, this protein could be a new promising A. fumigatus sensor possibly involved in cell wall homeostasis.

CS4.2.29

A COMPUTATIONAL STRATEGY IDENTIFIES A PUTATIVE REPRESSOR OF ASPERGILLUS SPP. TO DIFFERENT CHEMICAL STRESSES

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Aspergillus spp. have been extensively studied due to their biotechnological and ecological relevance and pathogenic potential. Despite the impressive knowledge acquired in the last years about their genomes, many gene functions remain unknown. In this study, a Comprehensive CO-expression Analysis (COCOA) strategy that explores several well-established bioinformatics tools was performed. Under the goal to find a global stress regulator in aspergilli, five transcriptome-based datasets of aspergilli exposed to different organic compounds were selected and analysed using COCOA. Among the differently expressed genes, only one gene showed the same response in the five datasets (up-regulation), AN9181. This gene belongs to an orthogroup that also comprises the gene AN8970. The gene AN9181 encodes for a protein containing a NmrA-like domain and the gene AN8970 encodes a protein containing a NAD(P)-binding domain. We generated Aspergillus nidulans single- and double-deletion mutants of the two genes composing the AN9181 orthogroup and analysed their phenotypes in the presence of each organic compound of the five initial datasets. In addition, the phenotype of the mutants in the presence of a fluorescent dye binding the cell wall and in the presence of different antifungal drugs was also analysed. Our data showed that the deletion mutants are less susceptible to sodium salicylate, resveratrol and calcofluor-white. Different susceptibilities to different antifungal drugs were observed. These results suggest that AN9181 is involved in the regulation of A. nidulans to different chemical stresses. This study is a step forward to better understand the genome of this model organism and shows that COCOA is a valid strategy that can be applied to test the strength of a hypothesis before a complete experimental validation.

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CS4.2.30

ASSEMBLY OF THE NATIVE FUNGAL COP9 SIGNALOSOME

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The conserved eight-subunit COP9 signalosome (CSN complex) controls the exchange of E3 ubiquitin cullin RING ligase receptors and thus, the specificity of protein degradation in eukaryotic cells. CSN of the filamentous fungus Aspergillus nidulans assembles through a heptameric pre-CSN, which is activated by integration of the catalytic CsnE deneddylase. We addressed the assembly of the native fungal pre-CSN by combined genetic and biochemical approaches. Interactomes of functional GFP-Csn subunit fusions in pre-CSN deficient fungal strains were compared by affinity purifications and mass spectrometry. Two distinct cellular heterotrimeric CSN subcomplexes were identified as pre-CSN assembly intermediates. CsnA-C-H and CsnD-F-G form parallel and independently of CsnB, which connects the heterotrimers to a heptamer. CsnB is prerequisite for the association of CsnE to the pre-CSN. Surveillance mechanisms control accurate Csn subunit amounts and correct cellular localization for sequential assembly, because losses of Csn subunits change the abundance and location of remaining Csn subunits.

CS4.2.31

2-DEOXYGLUCOSE RESISTANCE AND SORBOSE RESISTANCE OF THE MUTANTS INVOLVED IN CARBON CATABOLITE REPRESSION OF NEUROSPORA CRASSA.

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Neurospora crassa shows linear growth on Vogel's nutrient (VN) medium containing 1.2 % sucrose, but forms compact colony on VN medium containing 1 % sorbose and 0.2 % sucrose. Several mutants which showed linear growth on sorbose medium have been isolated as sorbose resistant mutants. Among them, only sor-4 gene which encodes a glucose sensor had been identified. In this study, we found that the deletion mutant of col-26 which encodes AmyR like transcription factor also show the sorbose resistance. Both SOR-4 and COL-26 are factors involving in carbon catabolite repression (CCR). However, the mutants of other CCR factors such as hexokinase HXK-2, AMP-activated protein kinase PRK-10 and transcription factor CRE-1 did not show sorbose resistance. The sor-4 and col-26 mutants were also resistance to a glucose analog 2-deoxyglucose (2-DG). It is known that sor-4 gene are allelic to the 2-DG resistant dgr-3. Among dgr mutants, dgr-1, dgr-2 and dgr-3 mutants showed sorbose resistance, but dgr-4 did not. From genetical and physical mapping of dgr mutants, we identified the mutations in hxk-2 and col-26 genes in dgr-4 and dgr-1 mutants, respectively. Recently dgr-2 gene which encodes a F-box protein EXO-1 has been identified, and also single amino acid S11L substitution in exo-1 gene were identified in dgr-2 mutants. Interestingly, different from the exo-1 S11L mutants, deletion mutants of exo-1/dgr-2 did not show sorbose resistance. Double mutant analysis revealed that sorbose resistance in sor-4 and col-26 mutants did not affect by the deletion of hxk-2, prk-10, and cre-1 genes. Deletion mutant of prk-10 gene was hypersensitive to 2-DG, the double mutants, sor-4;prk-10 and col-26;prk-10, showed hypersensitivity to 2-DG, suggesting of independence sorbose resistance and 2DG resistance. These results indicate that SOR-4-EXO-1-COL-26 signaling confer sorbose resistance in CCR.

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CS4.2.32

SHINING THE LIGHT UPON THE TEMPORAL DYNAMICS OF MITOSIS IN ASPERGILLUS FUMIGATUS

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Nuclear division in the model organism Aspergillus nidulans has been well characterised. However, there has been less focus on the cell cycle of the human pathogen A. fumigatus. The cell cycle of A. fumigatus is of interest as it the primary filamentous fungal pathogen of humans and may have key differences to A. nidulans that currently are overlooked. Here we investigate the spatial and temporal differences in mitotic regulation in a range of commonly used A. fumigatus growth media. Furthermore, we investigate the parasynchrony of mitotic regulation and division along different cellular structures. We use live-cell imaging of an A. fumigatus-optimised green fluorescent NimX and a red fluorescent nuclear-reporter fusion protein to visualise both the G2/M transition and karyokinesis. We report on the mitotic activity of A. fumigatus to inform the choice of experimental growth media in laboratory assays.

CS4.2.33

FUNGI USE THEIR CELL WALLS TO STORE MICRONUTRIENTS

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The cell wall is key to functioning of fungi in nature. It provides mechanical strength and is a barrier for the diffusion of nutrients. Recently, we showed that proteins and B-(1,3)(1,6)-glucans in the cell wall of the mushroom forming fungus Schizophyllum commune bind Cu(II) ions. This suggests that the cell wall may be a storage organelle for micronutrients that are essential for growth. Indeed we here show that the cell wall of Schizophyllum commune can bind micro-nutrients, in particular at neutral and alkaline pH. Moreover, we show that bound micro-nutrients can be released at low pH. We also postulate how anions are bound to the fungal cell wall. Together, these results show that the fungal cell wall can store micro-nutrients at neutral and alkaline pH that can be released, and probably taken up by the fungus, when the fungus acidifies its medium.

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CS4.2.35

POLYKETIDE SYNTHASE PKS1 OF C. ORBICULARE FORMS GRANULAR-LIKE STRUCTURE DURING APPRESSORIUM FORMATION BY SELF-ASSEMBLY AND ESSENTIAL FOR MELANIN BIOSYNTHESIS

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Melanin biosynthesis in Colletotrichum and Magnaporthe species is essential for appressorial penetration. In this study, we analyzed the localization of Pks1, a melanin-synthesizing polyketide synthase, during appressorium formation in Colletotrichum orbiculare. We found that the Pks1: GFP fusion protein exhibited prominent granule-like appearance in the appressoria. Interestingly, this localization was not consistent with any cellular organelles, including peroxisome, mitochondria endosome, or vacuole, indicating that this was not an accumulation at specific cellular organelle. This characteristic localization was also confirmed by immunoelectron microscopy for a Pks1:GFP introduced strain with immunogold labelled GFP antibody. Interestingly, the granular formation of Pks1 in 15 single nucleotide polymorphisms (SNPs) mutants revealed that six mutants Pks1s, including mutations near the putative active center of ketosynthase and in the thioesterase domain, formed granular structure, while nine others did not. This suggests that the granular structure is formed by self-assembly and the specific molecular structure of the Pks1 protein is involved in granular structure formation. On the contrary, the downstream melanin biosynthetic enzymes, 1,3,8-trihydroxynaphthalene reductase and scytalone dehydratase showed no cellular localization during appressorium differentiation. The assembled structure formation of polyketide synthases is the first report in nature and we are interested in the significance of structural-functional relationship.

CS4.2.36

ROLE OF THE ADAPTER PROTEINS HOOK1 AND PXD1 IN PODOSPORA ANSERINA ORGANELLE DYNAMICS

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Microtubule-based organelle trafficking is crucial for eukaryotic cell functioning and development. In filamentous fungi the transport of a number of organelles, including peroxisomes, lipid droplets and the endoplasmic reticulum (ER), depends on early endosomes. These organelles establish physical interactions with early endosomes, which in turn are transported by cytoplasmic dynein and kinesin-3 motors along microtubules. Dynein-early endosome interaction in Aspergillus nidulans and in Ustilago maydis depends on the cargo adapter protein HookA/Hok1. In addition, U. maydis Hok1 has been implicated in kinesin-3 recruitment to early endosomes. On the other hand, early endosomes bind to peroxisomes by means of the adapter protein PxdA. Here, we analyzed the function of Hook1 and Pxd1 (PxdA orthologue) in Podospora anserina organelle dynamics. We found that the genetic elimination of Hook1 or Pxd1 affects hyphal growth and morphogenesis. In addition, we observed that the dynamics of endocytic compartments depends on Hook1, and that both adapters are required for peroxisome motility and distribution. Moreover, we discovered that loss of Hook1 affects mitochondrial dynamics and distribution, while that of Pxd1 alters the dynamics of the apical ER subcompartments. Our findings reveal that Hook1 and Pxd1 have an key role in the regulation of the dynamics of several organelles in P. anserina. This research was supported by CONACYT-DFG grant 277869 from CONACYT.

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CS4.2.37

NIEMANN-PICK TYPE C PROTEINS OF COLLETOTRICHUM ORBICULARE: STEROL TRANSPORT AND APPRESSORIUM-MEDIATED PLANT INFECTION

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The cucumber anthracnose fungus Colletotrichum orbiculare uses appressoria to directly penetrate the host plant surface. Differentiation of appressoria requires accurate cell cycle progression. Two-component GTPase-activating protein (GAP) CoBub2-CoBfa1 interacts with downstream GTPase CoTem1 and is required for G1/S progression to establish plant infection in C. orbiculare. To explore the mechanisms by which the CoTem1 cascade regulates plant infection, we identified a Niemann-Pick type C2 homolog (CoNpc2) as a novel physical interaction factor with CoTem1. Whereas Niemann-Pick type C proteins NPC1 and NPC2 are essential for sterol transport from lysosomes (vacuoles) in humans and yeasts, their functions in plant invasion by pathogenic fungi have remained unclear. In this study, we showed that CoNpc2 colocalized with CoNpc1 in late endosomes and vacuoles. Disruption of its gene resulted in aberrant sterol accumulation in vacuoles and loss of sterol membrane localization, suggesting that they have a critical role in sterol transport of C. orbiculare. For appressorium infection, appropriate sterol distribution mediated by CoNpc1 and CoNpc2 are required for membrane integrity and membrane curvature with actin assembly that leads to penetration peg emergence and the pathogenicity of C. orbiculare. Our findings suggest the importance of sterol distribution by NPC proteins in fungal morphogenesis during appressorium-mediated plant infection.

CS4.2.38

THE MONOTHIOL GLUTAREDOXIN GRX4 IS INVOLVED IN IRON SENSING, SECONDARY METABOLISM, FUNGAL CELL SURFACE FUNCTIONS AND VIRULENCE IN USTILAGO MAYDIS

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The basidiomycete Ustilago maydis, the causal agent of corn smut disease, is a model for biotrophic plant-fungal interactions and is also used to investigate fungal cellular processes. Monothiol glutaredoxins (GRX) are key regulators of fungal metabolism. GRXs are involved in iron and redox homeostasis via interactions with iron-responsive transcription factors such as Aft1 or Cir1 from yeast or Cryptococcus neoformans. Further, iron homeostasis or redox status is maintained via interactions of Grx4 with iron-sulfur clusters and glutathione. Here, we report the characterization of Grx4 in Ustilago maydis. We initially found that Grx4 is essential in U. maydis. Thus, we constructed a glucose-repressible and arabinose-inducible allele by promoter swapping of the wt with the Pcrg promoter in the FB1 and FB2 strain backgrounds. The EC50 value for Pcrg:grx4 promoter activation was 0.012% arabinose to allow a 50% growth rate in the presence of glucose. On arabinose no differences were seen for wt or Grx4-depleted strain. RNAseq of glucose repressed and arabinose induced Pcrg:grx4 strains was then conducted. Identified Grx4-controlled functions such as iron metabolism (sid1; sid2), organic acid uptake (jen2; jen20), cell surface changes (rep1), melanin (mtf1), mating (bE; bW) and virulence (ztf1; fox1) were further investigated. Repression of Pcrg:grx4 by glucose could partially be rescued by supplementation with iron, heme or glutathione. Furthermore, the biotrophy-mimicking media condition of glucose plus malate also increased the growth of Grx4-depleted strains. Siderophore secretion was not detectable and cell surface hydrophobicity during mating was reduced in Grx4-depleted strains. In contrast, melanin formation after extended cultivation could be observed in Grx4-depleted cultures. Finally, the regulated strains showed reduced mating and were unable to cause disease on maize seedlings thus indicating a requirement for Grx4 during biotrophic development. Taken together, we identified conserved and new functions of Grx4 in U. maydis compared to other fungi.

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CS4.2.40

THE IMPORTANCE OF PHOSPHATIDYLCHOLINE BIOSYNTHESIS IN CRYPTOCOCCUS NEOFORMANS DURING ADAPTATION TO THE HOST ENVIRONMENT

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The basidiomycete fungus Cryptococcus neoformans serves as a useful model for investigating mechanisms of fungal pathogenesis. This pathogen is the causative agent of cryptococcal meningitis in immunocompromised patients and is listed in the critical priority group of the World Health Organization fungal priority pathogens list. The plasma membrane of C. neoformans is primarily comprised of phosphatidylcholine (PC) (45%), phosphatidylethanolamine (PE) (20%), and phosphatidylserine (PS) (12%). In fungi, PC can be synthesized via the CDP-diacylglycerol de novo pathway, or via the Kennedy pathway which makes use of exogenous choline. An earlier precursor of PC, PS is a known essential component for cryptococcus viability. However, as the most abundant glycerolipid in the fungal lipid bilayer, phosphatidylcholine and its essentiality to virulence in cryptococcus has not been described.
In this work, we characterized the role of phosphatidylcholine on Cryptococcus neoformans viability, osmotic stress, capsule formation, and lipid homeostasis using a genetic knockout of a Methylene-fatty-acyl-phospholipid synthase called opi3. We show that opi3 is required for growth in nutrient limiting conditions and is rescuable with choline, phosphatidylcholine, and sorbitol. Using an opi3-GFP tagged strain, we confirm localization of opi3 to the endoplasmic reticulum. Importantly, we show that phosphatidylcholine synthesis influences capsule formation and that a lack of PC leads to an accumulation of neutral lipids shown as lipid droplets. We also provide evidence for PC's role in lipid homeostasis and how the synthesis of other major lipid classes (PA, PS, PE, PG) are altered when PC is depleted. Moreover, despite the choline auxotrophy of opi3 in vitro, survival assays with

alveolar macrophages and in vivo animal models suggest host choline is sufficient for C. neoformans infection. This work describes the fundamental contributions of phosphatidylcholine to C. neoformans's adaptation to the host environment and how Cryptococcus can utilize host choline for PC synthesis.

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CS4.2.41

THE INTERCONNECTION OF THE ADAPTIVE RESPONSE OF NEUROSPORA CRASSA TO AZOLES AND ECHINOCANDINS

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The response of filamentous fungi to stress caused by antifungal compounds is governed by evolutionary conserved mechanisms. It is believed that exposure to echinocandins induces these mechanisms to stimulate chitin synthesis. However, the interconnected nature of stress-response mechanisms suggests that even azoles may trigger the cell wall changes. Using Neurospora crassa as a model filamentous fungus, we aimed to prove this hypothesis. We analyzed the expression of genes encoding chitin synthases by real-time PCR under azole exposure and we verified the level of chitin by fluorescence microscopy (calcofluor white staining) and by analytical determining the N-acetylglucosamine after chitin hydrolysis. The azole exposure indeed caused the increase in both, the expression of genes encoding chitin synthases as well as the chitin content in the cell wall. The azole-affected hyphae shared, generally, features (increase in chitin, hyperbranching) with echinocandin-affected hyphae. Conversely, echinocandins stimulated the expression of genes encoding ergosterol biosynthesis enzymes, a characteristic of azole exposure. Taken together, our work demonstrates that our understanding of the response to stress caused by antifungal compounds may need revision, mainly what traits are compound-specific and what occurs more broadly than initially thought.

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CS4.2.43

DISCOVERING THE ROLE OF THE CASEIN KINASE 2 COMPLEX IN THE GROWTH, DIFFERENTIATION, STRESS RESPONSES, AND PATHOGENICITY OF CRYPTOCOCCUS NEOFORMANS

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The basidiomycete human fungal pathogen Cryptococcus neoformans causes fatal meningoencephalitis both in immunocompromised patients and immunocompetent individuals. However, the therapeutic options for treatment of cryptococcosis are currently highly limited. As a potential antifungal drug target, kinases have been considered to be good candidates as some of them play critical roles in cellular mechanisms and virulence of fungal pathogens. In our previous studies, we demonstrated that Cka1, which is a serine/threonine kinase and the catalytic subunit of the casein kinase 2 (CK2) complex, is involved in controlling the growth, morphology, and pathogenicity of C. neoformans. In this study, we aim to further characterize the functions and regulatory mechanism of the whole CK2 complex in C. neoformans. The cryptococcal CK2 complex consists of the catalytic subunit Cka1 and two regulatory subunits, Ckb1 and Ckb2. The ckb1Δ, ckb2Δ, and ckb1Δ ckb2Δ mutants exhibited increased susceptibility to antifungal drugs, oxidative stress, and DNA damaging agents, albeit to a lesser extent to the cka1Δ mutant, indicating that Ckb1 and Ckb2 play accessary roles for Cka1. Notably, however, the cka1Δ ckb1Δ ckb2Δ triple mutants showed more severe growth defects and greater stress susceptibility than the cka1Δ mutants, indicating that the two regulatory subunits may have Cka1-independent functions. Supporting this, we found that the CK2 complex is required for maintaining normal cell cycle and morphology. Coimmunoprecipitation assay revealed physical interactions between Cka1 and Ckb1, Cka1 and Ckb2, and Ckb1 and Ckb2, suggesting that the CK2 complex has a heterotetramer structure (Cka1-Ckb1-Ckb2-Cka1). Considering pleiotropic roles of the CK2 complex in C. neoformans, we elucidated its downstream effector genes and proteins through RNAseq-based transcriptomics and mass spectrometry-based proteomics analyses, respectively. In conclusion, this study provides a comprehensive insight into the function and regulatory mechanism of the fungal CK2 complex.

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CS4.2.44

UNRAVELING THE CRYPTIC FUNCTION OF MITOGEN-ACTIVATED PROTEIN KINASES CPK2 AND MPK2 IN CRYPTOCOCCUS NEOFORMANS

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In basidiomycetous human fungal pathogen, Cryptococcus neoformans, has five mitogen-activated protein kinases (MAPKs), of which Mpk1, Cpk1, and Hog1 play central roles in various physiological functions. Apart from these three major MAPKs, C. neoformans has Cpk2 (CNAG_02531) and Mpk2 (CNAG_04282), which are paralogs of Cpk1 and Mpk1, respectively, but their roles remain elusive. Our previous genome-wide functional analysis of cryptococcal kinases revealed that Cpk2 plays minor roles in osmotic and genotoxic stress response and melanin production but is dispensable for mating process unlike Cpk1. Deletion of CPK2 does not lead to defects in virulence and infectivity of C. neoformans. Similarly, unlike Mpk1, Mpk2 plays minor roles in cell membrane stress response, resistance to fludioxonil and fluconazole, and melanin as well as urease production. Mpk2 is essential for virulence in the murine infection model but not in the insect model unlike its paralog Mpk1, which is crucial for virulence in both models. In this study, we aimed to elucidate the functional connection of Cpk2 and Mpk2 to Cpk1- and Mpk1-dependent signaling pathways in C. neoformans. In support of their phylogenetic relationship, here we provide the following experimental evidence showing that Cpk2 and Mpk2 play redundant roles with Cpk1 and Mpk1, respectively. Overexpression of CPK2 could almost completely restore the mating defect of cpk1Δ, including mating pheromone production, filamentation, and sporulation. Cpk2 was shown to regulate the expression of Mat2, which is known as a downstream transcription factor of the Cpk1 mating pathway. Then, overexpression of MPK2 could also partially restore the growth defect of mpk1Δ under cell wall destabilizing conditions as well as restore the basal urease production level. Moreover, mpk1Δ mpk2Δ displayed more drastic defect in melanin production compared to mpk1Δ, which is a key virulence factor of the C. neoformans.

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CS4.2.45

COMPREHENSIVE INSIGHT INTO RAS1/CAMP/PKA SIGNALING PATHWAY IN CANDIDA AURIS

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Candida auris is an invasive human fungal pathogen which cause high fatality disease in immunocompromised patients. In addition, since C. auris has a multi-drug resistance, the importance of research on C. auris is increasing. Our recent study reported that adenylyl cyclase Cyr1 and protein kinase A (PKA) pathways play distinct and redundant roles in drug resistance and various pathobiological functions of C. auris, but its upstream and negative feedback regulatory mechanisms remain elusive. In this study, we focused on the upstream regulatory mechanisms of Ras/cAMP/PKA signaling pathway, which are believed to play an important role in the pathogenicity and drug resistance of pathogenic fungal species. Among the various genes related with the signaling pathway, we constructed knockout strains for the G-protein-coupled receptor Gpr1, G-protein alpha subunit Gpa2, RAS signal transduction GTPase Ras1, Guanyl-nucleotide exchange factor Cdc25, GTPase-activating protein Ira2, and cyclic nucleotide phosphodiesterase Pde1, Pde2. Then, we conducted a phenotypic analysis of each mutant to find out which genes are the main up-regulator of adenylyl cyclase Cyr1 in this pathway, and as a result, we found that Ras1 acts as the main upper regulator of Cyr1, not Gpr1 or Gpa2. The phenotypes of the Ras1 deletion strain and the Cyr1 deletion strain were generally similar, and the phenotypes were also very related to the Cdc25 deletion strain which regulates Ras1. We also confirmed that when the Ras/cAMP/PKA signaling pathway was inactivated, the drug resistance and growth rate was significantly reduced, and Sap activity involved in the pathogenicity of Candida species was remarkably decreased. Furthermore, we confirmed that the hyperactivation of Ras/cAMP/PKA signaling pathway can attenuate pathogenicity of C. auris.
Consequently, these results will indicate that targeting Ras/cAMP/PKA signaling pathway could serve as an effective alternative to antifungal therapy against emerging multidrug-resistant fungal pathogen C. auris.

CS4.2.46

DECIPHERING THE SIGNALING NETWORKS OF A PP2A-LIKE PHOSPHATASE SIT4 REQUIRED FOR BRAIN INFECTION OF CRYPTOCOCCUS NEOFORMANS

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Phosphatases play critical roles in regulating cellular signaling networks involved in the survival and virulence of fungal pathogens. Specifically, protein phosphatase 2A (PP2A) is a highly conserved and abundant serine-threonine phosphatase composed of catalytic, scaffold, and regulatory subunits. In this study, we aim to unravel the signaling networks of a PP2A-like phosphatase SIT4 in Cryptococcus neoformans, an opportunistic fungal pathogen that causes fatal meningoencephalitis accounting for 180,000 deaths annually. From a previous systematic analysis, we have identified SIT4 as a virulence-related phosphatase that promotes blood-brain barrier adhesion and crossing. To elucidate the factors involved in the regulation of SIT4, a red-fluorescent fusion protein was constructed for pull-down assay, through which one putative regulatory subunit, SAP190 (SIT4-associating protein 190), was identified. In Saccharomyces cerevisae, four copies of the SAP genes are present, while in C. neoformans, only one copy was identified through sequence alignment and confirmed through LC-MS/MS analysis. As SIT4 is downstream of the TOR (target of rapamycin) pathway, both sit4Δ and sap190Δ displayed increased susceptibility against rapamycin. The loss of the SAP190 gene also showed reduced BBB crossing but at a reduced severity compared to sit4Δ, while virulence was not affected in the insect virulence model. Additionally, both sit4Δ and sap190Δ formed abnormal capsule structures, which may be responsible for reduced BBB crossing. Moreover, because the TOR pathway regulates cell growth and metabolic status, the expression of SIT4 and SAP190 under glucose starvation condition was observed. As a result, the expression of both SIT4 and SAP190 increased in the wild type strain under glucose starvation. Surprisingly, in basal condition, SIT4 transcription increased in sap190Δ while SAP190 transcription increased in sit4Δ at a level higher than the wild type. From here, we aim to identify the signaling networks of SIT4 to uncover its role and mechanism in brain infection.

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CS4.2.47

UV INDUCE TRANSLATION IN A DEVELOPMENTAL DEPENDENT MANNER IN FUSARIUM SPECIES

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The hallmark of rapid growth in fungi and other organisms is ribosome biogenesis. Therefore, one of the outcomes of DNA damage exposure is reduction in ribosome biogenesis. We analyzed the transcriptomic response of Fusarium mangiferae to UV. Surprisingly, we observed induction of pyrimidine biosynthesis genes and ribosome biogenesis genes but this is only when fungi were irradiated 14 hours post inoculation and not 8. Moreover, we found that not only ribosome biogenesis is induced following UV but also translation capacity. Inhibition of rRNA synthesis blocked UV induced ribosome biogenesis and sensitized fungi to UV. The UV induced ribosome biogenesis was independent on TOR signaling. Taken together, we report here a novel concept of DNA damage induced general translation capacity that is regulated by the developmental stage of the fungus and involve non-canonical ribosome biogenesis mechanism.

CS4.3 RNA BIOLOGY

CS4.3.9

EXPLORING SIGS TECHNOLOGY FOR DISEASE TREATMENT IN MEDITERRANEAN FORESTS

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Plant diseases seriously compromise our life quality and global food security. In the Mediterranean region some forestry species are seriously threatened by pathogenic fungi and oomycetes that have been treated for a long time with chemicals, which have a high ecological impact on nature. Moreover, these methods are not entirely effective in controlling the diseases causing some pathogens to develop resistance. In addition, the use of such products is not allowed in forests. Thus, other sustainable alternatives are in the spotlight of plant pathology at the moment. One of these alternatives is spray-induced gene silencing (SIGS), which is a plant protection method based on the ability of eukaryotic organisms to uptake small RNAs from the environment that induce silencing of genes through RNA interference (RNAi). This strategy is environmentally friendly and could be a sustainable alternative to chemical disease control methods. We are studying the potential of SIGS in the treatment of forest diseases such as those caused by Fusarium circinatum and Phytophthora cinnamomi. Environmental RNAi approach is being used to silence genes involved in critical pathways of the pathogens, such as vesicle trafficking, signaling and the RNAi machinery. Silencing these genes would reduce growth and infectivity of the pathogens, offering a sustainable alternative for the treatment of these diseases. The results of preliminary trials with F. circinatum are promising and will help to explore the use of this technology especially in forestry where it is not widely developed.

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CS4.3.11

THE ROLE OF SMALL RNAS-MEDIATED GENE EXPRESSION REGULATION IN MYCOPARASITIC INTERACTIONS

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Small-RNAs (sRNAs), vital components of post-transcriptional gene regulation known as RNA interference (RNAi), are emerging as key players in pathogenic and mutualistic fungus-plant interactions, although their role in mycoparasitism remain understudied. We employed the necrotrophic mycoparasite Clonostachys rosea and plant pathogenic mycohosts Botrytis cinerea and Fusarium graminearum and investigated the role of sRNA-mediated RNAi in mycoparasitism. sRNA sequencing showed that the majority of differentially expressed sRNAs were down-regulated in C. rosea during the interactions with the mycohosts compared to a C. rosea self-interaction control, thus allowing de-suppression (up-regulation) of mycohost-responsive genes. Degradome tags sequencing led to the identification of 201 sRNA-mediated potential gene targets for 282 differentially expressed sRNAs. Deletion of dcl2 in C. rosea resulted in a mutant with reduced secondary metabolite (SM) production, antagonism towards B. cinerea, and reduced biocontrol of fusarium foot rot disease on wheat, caused by F. graminearum. Deletion of dcl1 affected instead the conidial production in C. rosea. Transcriptome sequencing of the in vitro interaction between the Δdcl strains and B. cinerea or F. graminearum identified differentially regulated genes coding for transcription factors, membrane transporters, hydrolytic enzymes and SM biosynthesis enzymes putatively involved in antagonistic interactions, in comparison with the C. rosea wild type interaction. Sixty-one putative novel microRNA-like RNAs (milRNAs) were identified in C. rosea, and 11 were downregulated in the Δdcl2 mutant. In addition to putative endogenous gene targets, these milRNAs were predicted to target B. cinerea and F. graminearum virulence factor genes, which showed an increased expression during interaction with the Δdcl2 mutant incapable of producing the targeting

milRNAs (cross-species RNAi). In summary, our work constitutes the first step in elucidating the role of sRNA-mediated RNAi in regulating mycoparasitism and poses the base for future studies focusing on the role of cross-species RNAi in interspecific fungal interactions.

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CS4.3.12

A SMALL RNA SIGNATURE CONFERS INHERITABLE, EPIGENETIC ANTIFUNGAL DRUG RESISTANCE IN THE HUMAN PATHOGEN MUCOR CIRCINELLOIDES

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Heritable, epigenetic modifications that alter gene expression are a widespread phenomenon in eukaryotic organisms. These are known as epimutations and may arise from RNAi, DNA methylation, and/or heterochromatin modifications, often resulting in gene silencing. Recently, epimutations were identified as a novel mechanism conferring antimicrobial drug resistance, one of the gravest threats to public health. Epimutations were discovered in the early-diverging fungus Mucor that result from sRNA silencing of the gene fkbA encoding the FK506 target, FKBP12. This silencing results in transient drug resistance that reverts to sensitivity after several mitotic growth cycles in the absence of FK506. Our work has discovered that epimutations conferring drug resistance in three Mucor species are RNAi-exclusive and post-transcriptional.
Mucor epimutations are sufficiently stable to be trans-generationally inherited following sexual reproduction and meiosis, despite lacking heterochromatin marks frequently associated with epigenetic inheritance. We identified epimutants in the pathogenic Mucor circinelloides phylogenetic species 15, that is capable of generating viable meiotic progeny after zygospore germination. Epimutational resistance was found to be inherited and epimutant progeny are resistant to FK506 and harbor sRNAs targeting fkbA. Intriguingly, the epimutant F1 meiotic progeny inherit the same sRNA signature as their parent epimutant, and are consistently distinct from other parent-progeny pairs. Following passage in the absence of FK506, the epimutant progeny revert to drug sensitivity and have lost the sRNA targeting FKBP12. Our findings demonstrate epimutations are broadly present across the Mucor species complex, acting exclusively through RNAi. Although epimutations are stable through mitosis and meiosis, their detection may pose a challenge to typical culture methods given that these involve growth in

the absence of drug selective pressure. Understanding how epimutations arise and the mechanisms via which they confer resistance may enable their detection in clinical settings and provide solutions to overcome the challenges posed by rising antimicrobial drug resistance.

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CS4.3.13

CONTINUAL PROPAGATION OF STWINTRONS IN DIVERGENT XYLARIALES

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Spliceosomal introns are ubiquitous in nuclear transcriptomes but generation of new U2 introns is a vexing mystery. The availability of complete genomes of more than a thousand fungi allows to study intron gain. Stwintrons consist of nested U2 introns excised by consecutive splicing reactions. In a [D1,2] stwintron, an internal intron interrupts the 5'-donor of an external intron between the first and second nucleotide. One can classify [D1,2] stwintrons in two groups. Uniquely occurring stwintrons are typically integrated at gene positions occupied by an apparently sequence-unrelated [D1,2] stwintron in a broad range of related fungal taxa (genus, family, order). On the other hand, sequence-similar "sister" stwintrons, cross-identified by blastn, occur at new intron positions in very narrow taxa, like Hypoxylon sp. CO27-5 and EC38. Four of the 25 sister stwintrons are unique to either CO27-5 or EC38, strongly suggesting recent proliferation events.When such blastn screens were performed in genomes of more remotely related Xylariales taxa, series of new sister stwintrons were identified. Those of Hypoxylon pulicicidum all localize in genes the orthologs of which do not harbor stwintrons in CO27-5/EC38, suggesting they arose after divergence of the taxa. We have identified some 230 sister stwintrons in published genomes of Hypoxylaceae and Xylariaceae species. Some species contain more than 50 sister stwintrons, others harbor less than ten. Some genomes investigated also specify a group of related, smaller canonical introns that derive from the fusion of the opposite terminal quarters of a sister stwintron, and propagate as canonical introns. We analyzed all genuine sister stwintrons, their internal structure, their respective integration sites and the bounding exonic sequences. In addition, the interrelations were estimated for the ensemble of collected [D1,2] sister stwintrons which may allow to reflect on the deeper origin(s) of the Xylariales sister stwintron(s).

CS4.3.14

THE RNA-BINDING PROTEIN JSN-1 IS REQUIRED FOR ASEXUAL REPRODUCTION IN NEUROSPORA CRASSA

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RNA-binding proteins (RBPs) play key roles in the polar growth of filamentous fungi. In Neurospora crassa, the RBP GUL-1 has been shown to have a regulatory function, binding over 2000 mRNA species, many of which encode genes involved in cell wall integrity. gul-1 is an extragenic suppressor of the colonial hyperbranched cot-1(ts) mutant. Inactivation of gul-1 results in minor morphological changes, suggesting that additional proteins may have overlapping functions with GUL-1. Protein co-immunoprecipitation (co-IP) was employed to identify additional components of the GUL -1 ribonucleoprotein complex (RNP). One of the proteins identified was JSN-1, a member of the Pumilio family RBPs. Inactivation of jsn-1 resulted in partial suppression of cot-1 (ts). The epistatic nature of the suppression of cot-1 by gul-1 and jsn-1 supports the possibility of functional overlap between the two RBPs Moreover, both RBPs were found to affect MAK-1 phosphorylation under stress conditions. JSN-1::GFP was observed in the cytoplasm with partial association with the perinuclear space. In addition, and in contrast to GUL-1, punctate accumulation of JSN-1 was observed in conidiophores. While inactivation of jsn-1 did not confer defects in radial growth, a marked defect in condition, as evident by the formation of short aerial hyphae and marked arrest of development prior to the formation of major constrictions in the conidial chains (12 hours after induction of conidiation) was observed in the mutant. Results of co-IP experiments showed that JSN-1 interacts with approximately 400 proteins, half of which were also associated with GUL-1. JSN-1-associated proteins included the products of the conidiation-specific genes con-6 and con-8. con-6 was also predicted to have a protein-RNA interaction with JSN-1, as predicted by the catRAPID server. We concluded that GUL-1 and JSN-1 are components of the same RNP and that while their functions partially overlap, only JSN-1 is required for asexual development.

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CS4.4A FUNGAL EPIDEMIOLOGY AND DIAGNOSTICS

CS4.4a.5

FROM LABORATORY TO THE FIELD: INVESTIGATING THE OOSPORE GERMINATION DYNAMICS TO OPTIMIZE THE GRAPEVINE DOWNY MILDEW MANAGEMENT

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Downy mildew, caused by the polycyclic, biotrophic and obligate parasite Plasmopara viticola, is one of the most economically impacting diseases of grapevine. The pathogen overwinters by differentiating resting structures, the oospores, originated by sexual reproduction, which produce the inoculum for primary infections. Each year, in the main grapevine growing area, the prediction of primary infection occurrence represents a challenge. It must be pointed out that, to preserve human health and the environment, the European regulations strictly points towards a reduction of the fungicide applications. A key point to improve the effectiveness of grapevine downy mildew control strategies consists in determining the right time window for fungicide schedule, avoiding the application of unnecessary treatments. A previous study demonstrated that the decrease in the number of days required by the oospores to germinate (t) decreases when grapevine reaches susceptibility to P. viticola. This study aimed at estimating the probability of infection occurrence in ten vineyards located in Franciacorta, an important Italian viticultural area, integrating the oospore germination data (t) with indications of the EPI disease forecasting model, in two growing seasons. Starting from grapevine sprouting (April) until bunch closure (July), weekly bulletins reporting the infection risk were provided to the farmers. The results obtained with the oospores and the model were compared with the real epidemics in field, by estimating disease incidence and severity on untreated plots. The results

showed that, in correspondence with the infection risk indicated by the EPI model, the oospores also showed a reduction in t. The a posteriori evaluation indicated that the model correctly predicted the occurrence of primary infections and the subsequent disease epidemic trend, at different disease pressure levels. Overall, the adoption of EPI model combined with real biological data, provided by oospore germination assays, contributed to the definition of a rational treatment strategy.

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CS4.4a.6

A SIMPLE AND EFFECTIVE AIR-SAMPLING APPROACH TO ASSESS AERIAL RESISTANCE FRACTIONS IN ASPERGILLUS FUMIGATUS.

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Triazole resistance in the airborne human fungal pathogen Aspergillus fumigatus is a significant health problem as the environmental use of triazoles selected for cross-resistance to life-saving clinical triazoles in medicine. Despite the environment being well established as a source of triazole resistant A. fumigatus, the aerial transmission routes of its spores from the environment to patients remains unclear. This is mainly due to the lack of an affordable, reliable, and simple-to-use method for wide-scale environmental air-sampling. Previous methods were ineffective in capturing sufficient A. fumigatus colony-forming units (CFUs) to allow the quantitative assessment of aerial triazole resistance fractions. Here we show that 14 days of exposure of sticky seals to the air along with selectively culturing colonies directly from the seals proved key for increasing CFUs per sample. We also tested the use of delta traps for passive outdoor spore capture and show that together with the sticky seals and selective culturing, they are a simple and effective tool for outdoor air sampling. We suggest the use of this cost-effective air-sampling technique for wide-scale outdoor sampling to map resistance fractions, assess health risks, and pinpoint environmental resistance hot- and coldspots. Doing so will close the knowledge gap between environmental triazole resistance selection and the transmission to patients.

CS4.4a.8

GENERATION AND EVALUATION OF ASPERGILLUS-SPECIFIC DNA APTAMERS TO IMPROVE DIAGNOSIS OF ASPERGILLOSIS

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The rapid technological, climatic and demographic changes characterizing the last few decades are exerting drastic effects on our planet. In this scenario, the occurrence of infectious diseases, including fungal infections, appears to increase steadily. Aspergillosis, caused by Aspergillus fungal species, poses a particular threat as it is estimated to affect millions of people every year. The high mortality rate of this infection is often associated with delayed diagnosis.
This project aims at the development and evaluation of DNA aptamers recognizing Aspergillus cells which can be implemented into a novel diagnostic tool for the detection of aspergillosis.
In order to select DNA aptamers which specifically recognize conidia and hyphae of Aspergillus species causing aspergillosis, a SELEX approach previously developed for bacteria [1] will be adapted for fungal cells (e.g. A. niger). The establishment of this SELEX pipeline includes setting up a robust PCR procedure to amplify ssDNA aptamers bound to fungal cells and an evaluation method to characterize the newly selected aptamers.
In order to establish a method to evaluate the aptamer binding capabilities, fluorescently labelled versions of previously developed aptamers [2] were used. Upon incubation with A. niger cells, the binding of the aptamers to the target cells was evaluated by means of fluorescence measurements and epifluorescence microscopy. The aptamer evaluation method established here will be applied to new aptamers obtained by the SELEX procedure.

[1] Kolm C. et al., Sci Rep., 2020, 10(1), 1–16.
[2] Seo J. W. et al., RSC advances, 2021, 11(5), 2608-2615

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CS4.4a.10

AIRBORNE FUNGI IN INDOOR ENVIRONMENTS: WHICH FUNGI ARE USEFUL INDICATORS FOR THE EVALUATION OF INDOOR AIR SAMPLES?

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Exposure to airborne fungal spores and fragments in damp indoor environments is linked to negative impacts on human health. For some taxa, most notoriously Stachybotrys chartarum, there is additional concern about the risks of exposure to mycotoxins via inhalation of fungal material. Detecting and evaluating putative indoor fungal growth (i.e. mold) is therefore an important part of building remediation and similar procedures. Culture-based active sampling of indoor air is a widespread method to assess fungal contamination, but useful interpretation of results can be difficult. We evaluated air samples from over 600 indoor sites in Austria, along with appropriate outdoor air references. 59.8% of indoor samples showed increased counts of colony-forming units (CFU) for one or more groups of fungi. From our results, we make the case for using elevated indoor counts of Penicillium spp., as well as the presence of Wallemia sebi and that of Aspergillus of the sections Versicolores and Restricti, as useful indicators for indoor fungal growth, and show that these fungi occur in distinct CFU ranges when found in increased concentrations in indoor air. We also argue that Cladosporium spp., despite being common and abundant in indoor air (present in 88.4% of samples), are less useful for routine evaluation because of their ubiquitous presence in outdoor air (present in 97.3% of samples), and being subject to strong seasonal influences. Finally, using own and previously published data, we show why assessing indoor growth of Stachybotrys chartarum via air sampling is not useful, and discuss possible implications for the risk of exposure to airborne mycotoxins to the residents of contaminated sites.

CS4.4a.11

POPULATION DYNAMICS OF CERCOSPORA BETICOLA FUNGICIDE RESISTANCE IN THE RED RIVER VALLEY OF MINNESOTA AND NORTH DAKOTA, USA.

Nathan Wyatt[1,2], Rebecca Spanner[2], Viviana Rivera[2], Gary Secor[2], Melvin Bolton[1,2]

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Cercospora beticola is an economically important fungal pathogen of sugar beets causing the foliar disease Cercospora Leaf Spot (CLS). Yield losses due to CLS primarily stem from reductions in sucrose content and lower sucrose purity, though total crop failure is possible under environmental conditions favorable to the pathogen. The Red River Valley (RRV) is a large sugar beet growing region spanning from the Canadian border to southern Minnesota and producing nearly 50% of the United States domestic sugar production. Management of CLS is typically accomplished through the timely application of fungicides. However, fungicide resistance to most chemistries has been identified and presents a challenge to CLS management, as evidenced by CLS epidemics in recent years. To better understand the population dynamics of C. beticola in the RRV, isolates were collected across the geographic range over multiple years, phenotyped for fungicide sensitivity, and whole genome sequenced. Preliminary population genetic results showed evidence of gene flow throughout the RRV, indicative of a singular panmictic population and evidence of sexual and clonal reproduction. A genome wide association study approach was used to identify genomic loci contributing to fungicide resistance for the most used fungicide chemistries in the RRV, including the triazoles, benzimidazoles, quinone outside inhibitors, and organotin compounds. Both novel and previously identified loci were identified, and the prevalence, distribution, and diversity of these fungicide resistance associated loci were examined over time across the RRV geographic distribution.

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INTERACTION OF FUSARIUM OXYSPORUM F. SP. CUBENSE TR4 WITH BANANA AND OTHER HOSTS, AND ORIGIN OF THE PATHOGEN IN ISRAEL

Marcel Maymon[1], Uri Shpatz[1], Navot Galpaz[2], Abraham Gamliel[3], Stanley Freeman[1]

[1]Dept. of Plant Pathology and Weed Research, ARO, The Volcani Institute, Rishon Lezion, Israel, [2]R&D, Kiryat Shmona, Israel, [3]Institute of Agricultural Engineering, ARO, The Volcani Institute, Rishon LeZion, Israel

Fusarium oxysporum f.sp. cubense tropical race 4 (TR4), the causal agent of wilting and plant mortality of Cavendish banana varieties, is considered one of the most devastating soilborne fungal pathogens of this crop worldwide. The pathogen was first detected in South East Asia in the 1990s, and has since spread to Australia, the greater Mekong sub region, reaching India, Pakistan, Oman, Turkey, Mozambique (Africa), and most recently Colombia, Ecuador and Peru (South America). TR4 was discovered in the Middle East, in Jordan and Lebanon in 2014, and in Israel in 2016. Typical disease symptoms include leaf-yellowing and wilting, accompanied by internal vascular discolorations of rhizomes and pseudostems and eventual plant mortality. In Israel, TR4 representative isolates were tested for pathogenicity, and identification from symptomatic plants was reconfirmed by PCR. According to resistance/susceptibility screening of banana germplasm, resistant germplasm included 6-7 genotypes, 6-8 tolerant and 15-16 susceptible genotypes. In inoculated plants, the pathogen was not detected within the vascular bundles of resistant germplasm while in susceptible wilted plants conidia and mycelia of the pathogen were observed within these tissues. TR4 colonization of roots and shoots of selected weed species, growing in diseased banana plantations, indicated that systemic infection of alternative hosts by the pathogen occurred without exhibiting wilt symptoms. Furthermore, artificial inoculation of citrus, mango, avocado and grapevine seedlings indicated that most of the alternative host seedling roots were colonized by TR4. The origin of isolates from Israel was determined by sequencing the genomes of 5 representative TR4 isolates (two from Israel, one from Jordan, the Philippines, and Indonesia each) and 11 additional worldwide isolates

by single nucleotide polymorphisms (SNPs) analysis. Phylogeographical and SNP analyses detected a close relatedness among the Middle Eastern isolates, indicating that the origin of TR4 in Israel is from Jordan.

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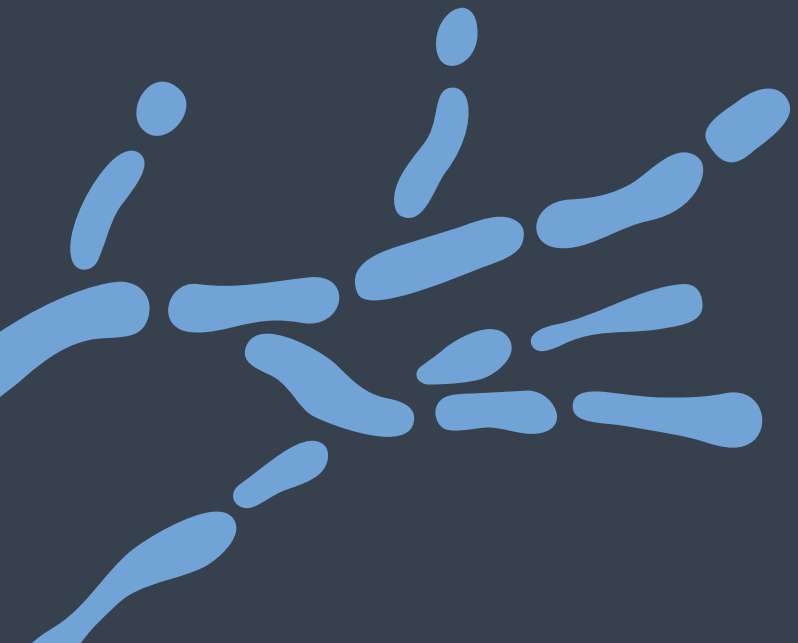

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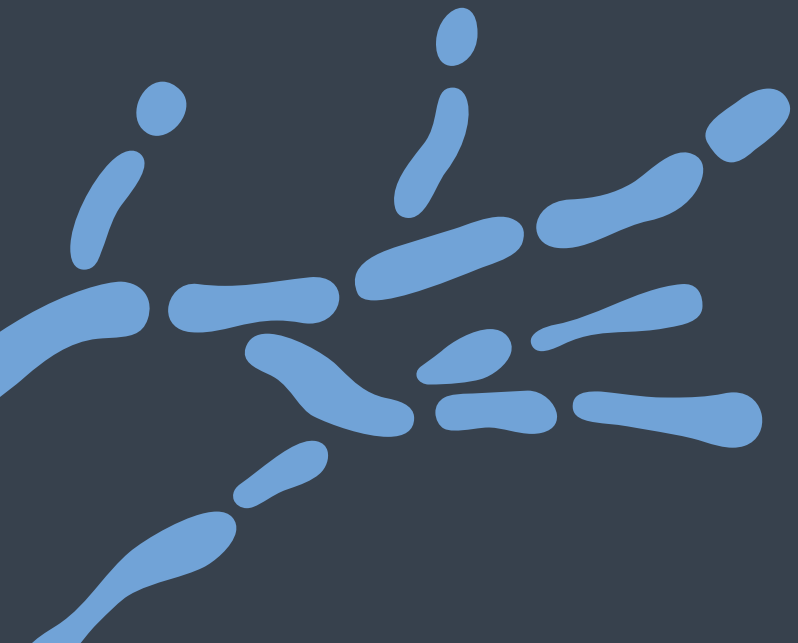

SATELLITE WORKSHOPS

SATURDAY, MARCH 4

ASPERFEST WORKSHOP

supported by Bartelt
supported by Novozymes
supported by Ginkgo Bioworks

Location: ***SOWI Campus, Universität Innsbruck, Aula***

CHAIRS:
Fabio Gsaller
Medizinische Universität Innsbruck, Austria
Richard Todd
Kansas State University, USA

17:00 – 20:00	**Registration and poster hang up, Poster and Welcome Reception (sponsored by Novozymes, A/S.)**
17:00 - 18:30	Odd-numbered poster presenters
18:30 - 20:00	Even-numbered poster presenters

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SUNDAY, MARCH 5

ASPERFEST WORKSHOP

supported by Bartelt
supported by Novozymes
supported by Ginkgo Bioworks

Location: ***SOWI Campus, Universität Innsbruck, Aula***

CHAIRS:
Fabio Gsaller
Medizinische Universität Innsbruck, Austria
Richard Todd
Kansas State University, USA

09:00 – 09:15	Welcome, introductions and announcements
09:15 – 10:45	SESSION I
	CHAIRS: **Richard Todd** Kansas State University, USA **David Canovas** University of Sevilla, Spain
	Aegerolysins – What Do We Already Know About Them? **Nada Kraševec**, National Institute of Chemistry, Slovenia
	The successful spread of azole-resistant Aspergillus fumigatus; sex, drugs and flying spores **Eveline Snelders**, Wageningen University and Research, The Netherlands
	A metabologenomic approach for secondary metabolite production **Isabelle Benoit Gelber,** Concordia University, Canada

The genomics of virulence and drug resistance in Aspergillus fumigatus
Amelia Barber, Friedrich Schiller University Jena, Germany

10:45 – 11:15 **Coffee break**

11:15 – 12:25 SESSION II

CHAIR:
Gerhard Braus
Center for Molecular Biosciences, Georg-August-University Göttingen, Germany

Antifungal potency of the airway epithelium in health and disease
Margherita Bertuzzi, University of Manchester, United Kingdom

Advances in genetic engineering to boost research applications in Aspergillus
Fabio Gsaller, Medizinische Universität Innsbruck, Austria

FLASHTALKS

CHAIRS:
Michelle Momany
University of Georgia, USA
Mike Bromley
University of Manchester, United Kingdom

Insights into the Aspergillus fumigatus Afu4g10610 gene overexpressed during in vivo and in vitro infections
Eduardo Pelegri-Martinez, University of the Basque Country, Spain

Engineering of a reporter tool to quantify carbon catabolite repression in filamentous fungi in real-time
Marcel Rüllke, Technical University of Munich, Germany

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Analysis of the Aspergillus fumigatus proteomic response to amphotericin B (AmB) reveals involvement of a putative flippase in resistance
Olaf Kniemeyer, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Germany

Global spatial and temporal analysis of Aspergillus fumigatus reveals insights into evolution of drug resistance
Johanna Rhodes, Radboudumc, The Netherlands

Pangenomic analysis of Aspergillus fumigatus reveals putative heterokaryon incompatibility loci
Harry Chown, University of Manchester, United Kingdom

NADPH oxidase-dependent antifungal activity of extracellular vesicles of macrophages against Aspergillus fumigatus
Thomas Orasch, Leibniz Institute for Natural Product Research and Infection Biology (HKI), Germany

Transcriptome analysis of Penicillium subrubescens xlnR and araR mutants
Dujuan Liu

Nonself recognition modules in Aspergillus fumigatus are shared across Aspergilli
Ben Auxier, Wageningen University, The Netherlands

The Aspergillus fumigatus Septation Initiation Network regulator, MobA, is essential for septation, survival under echinocandin stress, and virulence
Harrison Thorn, University of Tennessee Health Science Center, USA

12:25 – 12:45	FungiDB update Community directions discussion; Elections **Evelina Basenko**, FungiDB, University of Liverpool, United Kingdom CHAIR: **Richard Todd** Kansas State University, USA
12:45 – 14:00	**Lunch break**
14:00 – 15:45	SESSION III Talks from Abstracts CHAIRS: **Neta Shlezinger** School of Veterinary Medicine, Israel **Jarrod Fortwendel** University Of Tennessee Health Science Center, USA
	Generation and evaluation of Aspergillus-specific DNA aptamers to improve diagnosis of aspergillosis **Valeria Ellena**, Austrian Center of Industrial Biotechnology (ACIB) Gmbh/Technical University Vienna, Austria
	The antimicrobial peptide AnAFP acts as mediator of autophagy in A. niger **Stephan Starke**, Technische Universität Berlin, Germany
	Oxygen mass transfer effects on recombinant protein production by the hyphal dispersed Aspergillus oryzae mutant in the lab-scale fermentation **Shunya Susukida**, Tohoku University, Japan
	Screening A. nidulans mutants for the ability to germinate in water **Weng In Chong**, University of Macau, Macau SAR, China

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	A novel holotomographic live cell imaging method to study cellular processes in fungi **Susanne Fritsche**, Austrian Centre of Industrial Biotechnology (ACIB), Austria
	A gene involved in the development of Aspergillus fumigatus could be the missing fungal granulin **Uxue Perez-Cuesta**, University of the Basque Country, Spain
	Harnessing coexpression network data and synthetic biology to metabolically engineer Aspergillus niger **Timothy Cairns**, Technische Universität Berlin, Germany
15:45 – 16:15	**Coffee break**
16:15 – 17:00	PONTECORVO LECTURE „Omics-based Aspergillus stress biology“ sponsored by Ginkgo Bioworks CHAIR: **Richard Todd** Kansas State University, USA Omics-based Aspergillus stress biology **István Pócsi,** University of Debrecen, Hungary
17:00 – 17:30	Election results; Novozymes student poster prizes; other discussion items
17:30	Dismiss

LIST OF POSTERS

*Presenter indicated in underlined bold type, * denotes a student poster presenter*

1. Identification of fungal-specific effectors of regulated cell death (RCD) in *Aspergillus fumigatus
John Adeyemi Adeoye, Neta Shlezinger

2. Substrate Specificity Of Siderophore Uptake By *Aspergillus fumigatus*
Mario Aguiar, Thomas Orasch, Matthias Misslinger, Ana Maria Dietl, Fabio Gsaller, Clemens Decristoforo, Hubertus Haas

***3. In vivo transcriptomics for identification of drug targets in *Aspergillus fumigatus* and characterization of the candidates LaoA and SHMT**
Reem Alharthi, Monica Sueiro-Olivares, Jennifer Scott, Rachael Fortune-Grant, Andreas Beilhack, Gloria Lopez-Castejon, Amy Saunders, Jorge Amich

***4. Metabolic variability correlates with genotypic diversity in *Aspergillus fumigatus* isolates from patients with respiratory diseases**
Renad Aljohani, Andrew Scourfield, Matthew Fisher, Johanna Rhodes, Darius Armstrong-James

5. A CRISPR/CAS9 based multicopy integration system for protein production in *Aspergillus niger*
Mark Arentshorst, Prajeesh Kooloth Valappil, Selina Forrer, Gwen Tjallinks, Marco Fraaije, Sjoerd Seekles, Jaap Visser, Arthur Ram

6. Nonself recognition modules in *Aspergillus fumigatus* are shared across Aspergilli
Ben Auxier

7. Modular Inducible Multigene Expression System for Filamentous Fungi
Clara Baldin, Alexander Kühbacher, Petra Merschak, Johannes Wagener, Fabio Gsaller

8. The C2H2 transcription factor SltA is required for germination and fungal hyphal development in *Aspergillus fumigatus
Tim Baltussen, Norman van Rhijn, Jordy Coolen, Jan Dijksterhuis, Paul Verweij, Michael Bromley, Willem Melchers

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***9. Systems and 3D imaging approaches to understand the *Aspergillus niger* chitin synthase gene repertoire**
Lars Barthel, Sven Duda, Henri Müller, Heiko Briesen, Vera Meyer

10. FungiDB: Omics-scale data and bioinformatics tools for advanced data mining in support of your research
Evelina Basenko

11. Composition and activity of RpdA complexes in the opportunistic mold pathogen *Aspergillus fumigatus*
Ingo Bauer, Leopold Kremser, Bettina Sarg, Özgür Bayram, Stefan Graessle

12. The (NAD)-dependent histone deacetylase SIRT-1 has a role on redox signaling and aflatoxin production in *Aspergillus flavus*
Marco Zaccaria, **Marzia Beccaccioli**, Andrea Doddi, Rosita Silvana Fratini, Babak Momeni, Jeffrey Cary, Wei Qijian, Geromy Moore, Anna Adele Fabbri, Massimo Reverberi

13. Elucidating the pathway genes involved in the degradation of gentisate in *Aspergillus niger
Idowu Bello-Osagie, Patrick Semana, Marcos Di Falco, Ian D. Reid, Adrian Tsang, Justin Powlowski

14. Morphotype-specific fungal factors drive uptake and clearance of *Aspergillus fumigatus* by Airway Epithelial Cell
Sébastien C. Ortiz, Patrick J. Dancer, Rachael Fortune-Grant, **Margherita Bertuzzi**

15. Mutator phenotypes in *Aspergillus fumigatus* drive the rapid evolution of antifungal resistance
Michael Bottery, Chris Knight, Michael Bromley

16. Activation of Secondary Metabolite Biosynthesis by Transcription Factor Overexpression in *Aspergillus terreus
Zoey Bowers, Christian Rabot, Shu-Yi Lin, Michael De Guzman, Clay Wang

17. A versatile selection free CRISPR-Cas9 transformation system for *Aspergillus fumigatus*
Norman van Rhijn, Takanori Furukawa, Lauren Dineen, Michael Bottery, **Michael Bromley**

***18. A Role of the Transcription Factors AcuK and AcuM in Siderophore Biosynthesis of *Aspergillus fumigatus*.**
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20. Harnessing coexpression network data and synthetic biology to metabolically engineer *Aspergillus niger*
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21. Organelle-dependent synthesis of nitric oxide in fungi
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***22. Sampling, screening, and identification of potentially voriconazole resistant environmental strains in the Basque Country air**
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***23. Screening *A. nidulans* mutants for the ability to germinate in water.**
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***24. Pangenomic analysis of *Aspergillus fumigatus* reveals putative heterokaryon incompatibility loci**
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***25. Knockout of *cclA* gene activates cryptic biosynthetic gene clusters for secondary metabolite production in *Aspergillus terreus* ATCC 20516**
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***82. Manipulation of mcrA upregulates secondary metabolite production in *Aspergillus wentii* using CRISPR-Cas9 with in vitro assembled ribonucleoproteins**
Bo Yuan, **Justin M. Su**, Nancy P. Keller, Berl R. Oakley, Jason E. Stajich, Clay C. C. Wang

***83. Marine algae-associated endophytic fungi as sources of oxalic acid dihydrate, an inhibitor of lactate dehydrogenase (LDH) enzyme**
Subhadarsini Sahoo, S. Kamalraj, C. Jayabaskaran

***84. Oxygen mass transfer effects on recombinant protein production by the hyphal dispersed *Aspergillus oryzae* mutant in the lab-scale fermentation**
Shunya Susukida, Kiyoaki Muto, Hikaru Ichikawa, Ken Miyazawa, Akira Yoshimi, Yoshikazu Kato, Toshitaka Kumagai, Sachiyo Aburatani, Keietsu Abe

***85. The N-terminal region of hydrophobin RolA of *Aspergillus oryzae* regulates self-assembly of RolA**
Nao Takahashi, Yuki Terauchi, Takumi Tanaka, Akira Yoshimi, Hiroshi Yabu, Keietsu Abe

86. Characterization of a GH5_7 β-mannanase by activity-based protein profiling in secretomes of *A. niger
Massimo Tedeschi, Vincent A. J. Lit, Prajeesh Kooloth Valappil, Mark Arentshorst, Hermen S. Overkleeft, Arthur F. J. Ram

***87. The *Aspergillus fumigatus* Septation Initiation Network regulator, MobA, is essential for septation, survival under echinocandin stress, and virulence**
Harrison Thorn, Xabier Guruceaga, Adela Martin-Vicente, Wenbo Ge, Ashley Nywening, Jinhong Xie, Jarrod Fortwendel

88. The paralogous transcription factors LeuR and LeuB regulate leucine biosynthesis, nitrogen assimilation, and iron metabolic pathways in *Aspergillus nidulans*
Joel Steyer, Damien Downes, Cameron Hunter, **Richard Todd**

89. Exposure to agricultural DHODH inhibitors result in cross-resistance to the novel antifungal olorofim in *A. fumigatus*
Norman Van Rhijn, Michael Bottery, Mike Bromley

90. Sustainable Conversion of Polyethylene Waste Plastics into Fungal Secondary Metabolites
Clay Wang, Christian Rabot, Swati Bijlani, Yi ming Chiang, Yuhao Chen, Elizabeth Oakley, Berl Oakley, Travis Williams

***91. Characterization and engineering of the xylose-inducible xylP promoter for use in mold fungal species**
Annie Yap, Irene Glarcher, Matthias Misslinger, Hubertus Haas

***92. Regulation of high-affinity iron acquisition in *Aspergillus fumigatus* is coordinated by AtrR, SrbA and SreA**
Annie Yap, Ricarda Volz, Sanjoy Paul, Scott Moye-Rowley, Hubertus Haas

***93. Revisiting *Aspergillus niger* Mst sugar transporters**
Dongming Zhang, Christina Lyra, Jack T Pronk, Robert Mans, Miia R Mäkelä

94. Parasexual recombination in Aspergillus is mostly due to chromosomal shuffling
Ben Auxier, Eveline Snelders, Alfons Debets, **Jianhua Zhang**

95. Genome-wide *in vitro* competitive fitness profiling reveals novel inter-connected networks of genes associated with adaptation of *Aspergillus fumigatus* to antifungals
Can Zhao, Marcin Fraczek, Lauren Dineen, Isabelle Storer, Ressa Lebedinec, Thorsten Heinekamp, Juliane Macheleidt, Hajer Alshammri, Danielle Weaver, Narjes Alfuraiji, Takanori Furukawa, Norman Van Rhijn, Paul Bowyer, Axel Brakhage, Daniela Delneri, Michael Bromley

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INNSBRUCK I AUSTRIA I 2023

FUSARIUM WORKSHOP

Location: ***SOWI Campus, Universität Innsbruck, Hörsaal 1***

CHAIRS:
Lena Studt-Reinhold
BOKU-University of Natural Resources and Life Sciences, Austria
Manuel Sánchez López-Berges
Universidad de Córdoba, Spain

09:00 – 09:15	Welcome
09:15 – 10:45	SESSION I Secondary metabolism and mycotoxin production CHAIRS: **Lena Studt-Reinhold** BOKU-University of Natural Resources and Life Sciences, Vienna, Austria **Manuel Sánchez López-Berges** Universidad de Córdoba, Spain
09:15 - 09:30	New regulators involved in carotenoid biosynthesis in Fusarium fujikuroi **M. Carmen Limón**, University of Seville
09:30 - 09:45	The ceramide synthase CER1 plays a role in selfprotection against FB1 **Tamara Krska**, Universität für Bodenkultur Wien
09:45 - 10:00	The Fusarium PKS8 gene cluster facilitates biosynthesis of the dihydroisocoumarin derivates fusamarins **Anna K. Atanasoff-Kardjalieff**, Universität für Bodenkultur Wien
10:00 - 10:15	Filling out the gaps – identification of the product of the PKS2 cluster in Fusarium graminearum **Jens L. Sørensen**, Aalborg University

10:15 - 10:30	Role of HmbC, a protein of the HMG-box family, in Fusarium fujikuroi **Marta Franco-Losilla**, University of Seville
10:30 - 10:45	Fusarium graminearum chemotype differences and virulence **Gerlinde Wiesenberger**, Universität für Bodenkultur Wien
10:45 – 11:15	**Coffee break**
11:15 – 12:40	SESSION II Fusarium-host interaction CHAIRS: **Gerhard Adam** BOKU-University of Natural Resources and Life Sciences, Austria **Teis Esben Sondergaard** Aalborg University, Denmark
11:15 - 11:30	The structural repertoire of Fusarium oxysporum f. sp. lycopersici effectors revealed by experimental and computational studies **Simon Williams**, Australian National University
11:30 - 11:45	Identification and characterization of effector proteins from F. graminearum using Proximity-dependent biotin identification (BioID) **Gopal Subramaniam**, Agriculture and Agri-Food Canada
11:45 - 12:00	A leap into the unknown: understanding host-jumping by Fusarium oxysporum in cucurbits **Babette Vlieger**, University of Amsterdam
12:00 - 12:15	Eye see you: genomic and transcriptomic features of Fusarium solani keratitis isolates **Amelia Barber**, Friedrich Schiller University

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12:15 - 12:20*	A comprehensive comparison in virulence and immune response of plant and two human pathogenic isolates of Fusarium oxysporum **Marina Campos Rocha**, The Hebrew University of Jerusalem
12:20 - 12:25*	Comparative genomics reveals accessory chromosomes and differential effector catalogues in Fusarium species causing wilt disease of bananas in Cuba* **Einar Martinez de la Parte**, Wageningen University & Research
12:25 - 12:30*	Exploring the function of two paralogous F. graminearum effectors reveals an alternative genetic pathway required for virulence on wheat spikes* **Kim Hammond-Kosack**, Rothhamsted Research
12:30 - 12:35*	Fusarium oxysporum resistance mediated by four different tomato R-genes correlates with accumulation of a shared set of xylem sap “guardians”* **Margarita Šimkovicová**, University of Amsterdam
12:35 - 12:40*	The media composition affects Fusarium oxysporum infection of plants* **Clara Sánchez-Rodríguez**, ETH Zurich
12:40 - 12:45*	Virulence and host-specificity in Fusarium oxysporum ff.spp. interactions **Andrea Doddi,** University of Rome

*presentation will be given in a flash-talk format

12:45 – 13:45	**Lunch break**

13:45 – 15:45	SESSION III Evolution, taxonomy and genome dynamics CHAIRS: **Nadia Ponts** INRAE, France **Shay Cuovo** Hebrew University, Israel
13:45 - 14:00	Genomics-assisted directed evolution for the development of biocontrol strains of the vascular wilt pathogen Fusarium oxysporum **Antonio Di Pietro**, University of Cordoba
14:00 - 14:15	Pangenome analysis of Fusarium solani **Abbeah Mae Navasca**, North Dakota State University
14:15 - 14:30	The Fusarium oxysporum pangenome: mix and match of accessory chromosomes **Like Fokkens**, Wageningen University & Research
14:30 - 14:45	Meiotic driver homologs in asexual fusarium species **Linnea Sandell**, Uppsala University
14:45 - 15:00	Evolve and Resequence approach in the phytopathogenic fungus Fusarium graminearum : from concept to proof of concept? **Marie Foulongne-Oriol**, INRAE
15:00 - 15:15	Capturing transposon dynamics in the fungal pathogen Fusarium oxysporum **Ana Rodríguez López**, University of Cordoba
15:15 - 15:30	Using digestive secretome relatedness for elucidating fungal evolution and speciation **Lene Lange**, LL-BioEconomy, Denmark
15:30 - 15:45	FungiDB: Omics-scale data and bioinformatics tools for advanced data mining in support of your research **Dave Starns**, FungiDB

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15:45 – 16:15	**Coffee break**
16:15 – 17:15	SESSION IV Gene regulation and signaling CHAIRS: **M. Carmen Limón** University of Seville, Spain **Slavica Janevska** Leibniz Institute for Natural Product Research and Infection Biology (HKI), Germany
16:15 - 16:30	Phenotypic plasticity and adaptive potential under abiotic stresses in the phytopatogenic fungus Fusarium graminearum **Antoine Vajou**, INRAE
16:30 - 16:45	Modification of the mitogen-activation protein kinase kinase 1 (Mkk1) activation loop in Fusarium graminearum **Nora Foroud**, Agriculture and Agri-food Canada
16:45 - 17:00	Similar phenotypes of WcoA and WcoB mutants reveal regulatory functions as a complex in Fusarium fujikuroi **Julia Marente**, University of Seville
17:00 - 17:15	UV induce translation in a developmental dependent manner in Fusarium species **Quyen Hoang**, Hebrew University of Jerusalem
17:15 – 17:30	Discussion and closing remarks

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NEUROSPORA WORKSHOP

Location: ***SOWI Campus, Universität Innsbruck, Seminarraum 1***

CHAIRS:
Alexander Lichius
Universität Innsbruck, Innsbruck, Austria
Luis Corrochano
Universidad De Sevilla, Spain

08:00 – 08:40	Welcome
08:40 - 09:00	Analysis of Neurospora strains isolated from burnt Joshua trees after the Cima Dome fire in the Mojave Desert of California **Katherine Borkovich**, University Of California Riverside
09:00 - 09:20	Conserved gene methylation found throughout the genus Neurospora **Jesper Svedberg**, Stockholm University
09:20 - 09:40	Functional analysis of the conserved histone chaperone ASF1 in the ascomycete Sordaria macrospora **Jan Breuer**, Ruhr-University Bochum
09:40 - 10:00	Unexpected Polycomb silencing factors revealed through forward genetics mutant hunt **Colleen Mumford**, University of Oregon
10:00 - 10:20	Transcriptional Adaptation in Neurospora crassa,MPI for Heart and Lung Research **Hamzeh Hammadeh**, Bad Nauenheim
10:20 - 10:40	VE-1 regulation of MAPK signalling controls sexual development in Neurospora crassa **Luis M. Corrochano**, Universidad de Sevilla
10:45 – 11:15	**Coffee break**
11:20 - 11:40	The RNA-binding protein JSN-1 is required for asexual reproduction in Neurospora crassa **Anne Yenewodage**, Hebrew University of Jerusalem
11:40 - 12:00	Kinase activity of COT-1 is essential for maintaining stable cell polarity axes and directed growth in Neurospora crassa **Lucas Well**, TU Braunschweig
12:00 - 12:20	From a Cap to a Collar, ontogeny of the subapical endocytic collar in filamentous fungi **Rosa Mouriño-Pérez**, CICESE
12:20 - 12:40	A model of BEM46 mode of action in Neurospora crassa **Krisztina Kollath-Leiß**, CAU Kiel
12:45 – 14:00	**Lunch break**
14:00 - 14:20	Unravelling the response to membrane damage in the ascomycete Neurospora crassa **Linda Matz**, TU Braunschweig
14:20 - 14:40	Deorphanization of sugar transporters in Neurospora crassa **Elisabeth Tamayo**, Technical University of Munich
14:40 - 15:00	Identification of Cys redox regulated proteins in Neurospora crassa during biomass degradation **Lucia Bidondo**, INRAE/Aix-Marseille University
15:00 - 15:20	Identification of protein-protein interactions based on in vivo proximity labeling with biotin in Sordaria macrospora **Lucas Sebastian Hollstein**, Georg August University of Göttingen
15:20 - 15:40	Developing a temperature-inducible transcriptional rheostat in Neurospora crassa **Luis Larrondo**, IBio- P. Univ Catolica De Chile
15:50 – 16:15	**Coffee break**
16:15 – 17:30	open get together and discussion

SYMPOSIUM ON THE BASAL FUNGAL KINGDOM

supported by ÖGMM

Location: ***SOWI Campus, Universität Innsbruck, Hörsaal 2***

CHAIRS:
Ulrike Binder
Medizinische Universität Innsbruck, Austria
Joseph Heitman
Duke University, USA
Victoriano Garre
University of Murcia, Spain
Luis M. Corrochano
Universidad de Sevilla, Spain

09:00 - 10:45 SESSION
„Phylogeny and evolution“

CHAIRS:
Maribel Navarro-Mendoza
Duke University School of Medicine, USA
Victoriano Garre
University of Murcia, Spain

Molecular phylogeny and character evolution in Mucor and relatives
Grit Walther, Leibniz Institute for Natural Product Research And Infection Biology (HKI)

Basidiobolus: the herptile gut microbiome life-style of an enigmatic member of Basal Kingdom Fungi
Joey Spatafora, Oregon State University

Phylogenomics Supports the Monophyly of Aphelids and Fungi and Identifies New Molecular Synapomorphies
Luis Javier Galindo, University of Oxford

Expanding the Host Range for a Fungal Endosymbiont Through Implantation by FluidFM
Gabriel Giger, ETH Zürich

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Ecology and interkingdom relationships of Umbelopsis spp.
Alicja Okrasińska, University of Warsaw

10:45 – 11:15 Coffee break

11:15 - 12:45 SESSION
„Pathogenesis and antifungal resistance“

CHAIRS:
Teresa Pawlowska
School of Integrative Plant Science, Cornell University, USA
Sheng Sun
Duke University Medical Center, USA

Future directions in translational research and management of mucormycosis
Dimitrios Kontoyiannis, UT MD Anderson Cancer Center

Long non-coding RNAs in the interaction between Mucorales causing mucormycosis and host defense cells
Ghizlane Tahiri, Murcia University

Host brain environment triggers MAPK RNAi-based epimutation in the human pathogen Mucor circinelloides
Maribel Navarro-Mendoza, Duke University School of Medicine

Siderophore utilisation by Lichtheimia corymbifera (Mucorales), a causative agent of mucormycosis
Kerstin Voigt, University of Jena

Strain optimization of Mucor circinelloides reporter strains allows for monitoring of and drug efficacy testing against mucormycosis
Ulrike Binder, Medizinische Universität Innsbruck

12:45 – 14:00 Lunch break

14:00 - 15:30 SESSION
„Gene regulation, pathways and metabolism“

CHAIRS:
Nina Gunde-Cimerman
University of Ljubljana, Slovenia
Carlos Perez Arques
Duke University, USA

The DNA N6-Adenine Methyltransferase Complex of Mucorales and its role on gene expression and chromatin structure
Carlos Lax, University of Murcia

Big1 controls Arf2 activation during Mucor lusitanicus yeast development through the PKA pathway
José Alberto Patiño-Medina, Universidad Michoacana De San Nicolás De Hidalgo

The Dicer/R3B2 complex: a novel interaction in the center of the RNAi-related mechanisms of Mucor lusitanicus
José Tomás Cánovas-Márquez, University Of Murcia

Distinct effects of anaerobe and aerobe fungal activity on lignocellulose composition and structure during its degradation.
Jolanda van Munster, Scotland‘s Rural College

Distribution of lipid metabolism enzymes in Mucoromycota shows repeated loss of ergosterol synthesis genes in plant-associated fungi
Anna Muszewska, Institute Of Biochemistry And Biophysics, Polish Academy of Sciences

Regulation of the secondary metabolism in early diverging fungi: Mortierella alpina as a model organism
Markus Gressler, Friedrich-Schiller-University Jena

15:50 – 16:15 Coffee break

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16:15 - 17:30 SESSION
„pathogenesis and antifungal resistance“

CHAIRS:
Ulrike Binder
Medizinische Universität Innsbruck, Austria
Tamas Papp
University of Szeged, Hungary

A small RNA signature confers inheritable, epigenetic antifungal drug resistance in the human pathogen Mucor circinelloides
Carlos Perez-Arques, Duke University School of Medicine

Characterizing the sterol biosynthesis pathway and azole drug efflux transporters in mucormycetes to elucidate their role in intrinsic azole resistance
Michaela Lackner, Medizinische Universität Innsbruck

Elucidation of Intrinsic Micafungin Drug Resistance Mechanisms in Mucor
Soo Chan Lee, University Of Texas At San Antonio

Farewell / open discussion

COLLETOTRICHUM WORKSHOP

Location: ***SOWI Campus Universität Innsbruck, Seminarraum 2***

CHAIRS:
Elena Baraldi
University of Bologna, Italy
Henrik Hjarvard de Fine Licht
University of Copenhagen, Denmark

09:00 – 09:15	Arrival of participants and Welcome
09:15	Species diversity in Colletotrichum causing anthracnose on Lamiaceae and SYBR Green qPCR assay for the species-specific detection of C. ocimi **Ilaria Martino**, University of Torino, Italy
09:45	Occurrence of Colletotrichum species, causal agents of anthracnose on walnuts, in France by metabarcoding and culture-dependent approach during ripening **Flora Pensec**, University of Western Brittany, France
10:15	Colletotrichum gloeosporioides species complex as a destructive agent of apple orchards in Italy: from characterisation to genome analysis **Greice Amaral Carneiro**, University of Bologna, Italy
10:45 – 11:15	**Coffee break**
11:15	An updated genome sequence for Colletotrichum graminicola **Daniela Nordzieke**, University of Göttingen, Germany
11:45	Reconstruction of a mutualistic symbiosis between plant and fungus. **Johannes Gaertner**, University of Cologne, Germany
12:15	Unravelling the inter-kingdom host shifts of Colletotrichum nymphaeae – from plants to insects **Daniel Buchvaldt Amby**, University of Copenhagen, Denmark
12:45 – 13:45	**Lunch break**
14:00	Transcriptomics and lipidomics approach to characterize the response of Colletotrichum gloeosporioides to low temperatures during cold storage **Carmit Ziv**, The Volcani Center, Israel
14:30	How do mini chromosomes of the plant pathogen Colletotrichum higginsianum contribute to its host range? **Anna Henning**, University of Erlangen-Nuremberg, Germany
15:00	Plant surface signal recognition and infection-related morphogenesis of Colletotrichum orbiculare **Yasuyuki Kubo**, Setsunan University, Japan
15:50 – 16:15	**Coffee break**
16:15	Group photo, Social Event and Networking

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TRICHODERMA & CLONOSTACHYS WORKSHOP

Location: ***SOWI Campus, Universität Innsbruck, Hörsaal 3***

CHAIRS:
Bernhard Seiboth
Technical University Vienna, Austria
Magnus Karlsson
Swedish University of Agricultural Sciences, Sweden

09:00 – 10:40 SESSION I

CHAIRS:
Sabrina Sarrocco
University of Pisa, Italy
Magnus Karlsson
Swedish University of Agricultural Sciences, Sweden

09:00 - 09:05	Welcome address and opening of the workshop **Magnus Karlsson**, Swedish University of Agricultural Sciences, Sweden
09:05 - 09:30	Use of a Trichoderma gamsii beneficial isolate for the control of Fusarium Head Blight on wheat **Sabrina Sarrocco**, University of Pisa, Italy
09:30 - 09:5	Additional roles of Trichoderma in agriculture: indirect biocontrol and biostimulation **Enrique Monte**, University of Salamanca, Spain
09:55 - 10:10	Editing the LeEIX locus, which determines pathogen resistance in tomato, increases host receptivity to Trichoderma bio-control **Maya Bar**, ARO Volcani Institute, Israel
10:10 - 10:25	Metabolomic approach to select beneficial microorganisms and/or their metabolites for a new generation bio-formulates **Alessia Staropoli**, University of Naples Federico II, Italy
10:25 - 10:40	Two Trichoderma virens strains defined by gliotoxin production: comparative genomics and host interactions **Benjamin Horwitz**, Technion - Israel Institute of Technology, Israel
10:45 – 11:15	**Coffee break**

11:15 – 12:35 SESSION II

CHAIRS:
Lisa Kappel
Universität Innsbruck, Austria
Bernhard Seiboth
Technical University Vienna, Austria

11:15 - 11:40	40 years of taming Trichoderma reesei for industrial needs **Igor Nikolaev**, International Flavors & Fragrances, The Netherlands
11:40 - 12:05	Development of T. reesei strain and cellulase production process for hydrolysis of steam pretreated sawdust **Nina Aro**, VTT Technical Research Centre of Finland, Finland
12:05 - 12:20	Elucidating the sugar transport system in Trichoderma reesei during cellulase formation **Roberto Silva**, University of Sao Paulo, Brazil
12:20 - 12:35	Outbreeding sexual reproduction promote improvement of Trichoderma reesei hyper producer strain RutC-30 **Frédérique Bidard-Michelot**, IFP Energies Nouvelles, France
12:45 – 14:00	**Lunch break**
14:00 - 14:30	Trichoderma Taxonomy Meeting **Irina Druzhinina**, Kew Royal Gardens, United Kingdom

14:30 – 15:50	SESSION III
	CHAIRS: **Sabrina Sarrocco** University of Pisa, Italy **Magnus Karlsson** Swedish University of Agricultural Sciences, Sweden
14:30 - 14:55	A hidden chemical crosstalk shapes the mycoparasitic behavior of Trichoderma atroviride **Susanne Zeilinger-Migsich**, Universität Innsbruck, Austria
14:55 - 15:20	Small RNAs in mycoparasitic interactions **Mukesh Dubey**, Swedish University of Agricultural Sciences, Sweden
15:20 - 15:35	Revising Clonostachys and allied genera in Bionectriaceae **Lin Zhao**, Westerdijk Fungal Biodiversity Institute, The Netherlands
15:35 - 15:50	Trichoderma reesei Rad51 tolerates mismatches in hybrid meiosis with diverse genome sequences **Ting-Fang Wang**, Academia Sinica, Taiwan
15:50 – 16:15	**Coffee break**

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16:15 – 17:00	SESSION IV
	CHAIRS: **Lisa Kappel** Universität Innsbruck, Austria **Bernhard Seiboth** Technical University Vienna, Austria
16:15 - 16:40	Trichoderma reesei – the sensitive workhorse and its signaling highways **Monika Schmoll**, University of Vienna, Austria
16:40 - 17:05	Genomic footprints of fitness in Trichoderma suggest the widespread involvement of genes with unknown functions. **Irina Druzhinina**, Kew Royal Gardens, United Kingdom
17:05 - 17:20	Relevance of the COP9 signalosome to plant cell wall degradation in Trichoderma reesei. **Tiziano Benocci**, Austrian Institute of Technology, Austria
17:20 - 17:25	Closing remarks of the workshop **Bernhard Seiboth**, Technical University Vienna, Austria

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