X[th] International Fungal Biology Conference &
VIIIth Mexican Congress of Molecular and Cellular Biology of Fungi

Frontiers in Fungal Biology

Microbiology Department
Applied and Experimental Biology Division

December 6-10, 2009
Ensenada, B.C., Mexico

X International Fungal Biology Conference &
VIII Congreso Nacional de Biología Molecular
y Celular de Hongos

Frontiers in Fungal Biology

December 6-10
Ensenada, B. C.
Mexico

WELCOME

The Department of Microbiology of CICESE, the Center for Scientific Research and Higher Education of Ensenada, extends a warm welcome to fungal biologists of 22 different countries.

The International Fungal Biology Conferences and the National Congresses in Molecular and Cellular Biology of Fungi of the Mexican Biochemical Society have become long-standing, prestigious events. It was with great pride and enthusiasm that we accepted the responsibility of organizing the 10th International Conference together with the 8Th National Congress. This double event offers ample opportunities for fruitful exchanges among the >200 participating members of the international community of fungal biologists.

By joining the Mexican Congress with the International Conference, we are giving our guests from abroad the opportunity to learn more intimately about progress in, and commitment to fungal biology within Mexican Institutions and also valuable opportunities for mutual interaction with several enthusiastic groups of Mexican researchers and their students.

Faculty of the Microbiology Department
CICESE

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ACKNOWLEDGEMENTS

The X International Conference on Fungal Biology received generous support from CONACYT (the National Council for Science and Technology of Mexico), CICESE (the Center for Scientific Research and Higher Education of Ensenada) and UNAM (National Authonomus University of Mexico).

We also received valuable support from the exhibitors of scientific equipment listed on the back cover

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Program

PROGRAM

Chronogram

	6-Dec	7-Dec	8-Dec	9-Dec	10-Dec
	SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY
8:30		REGISTRATION			BUSINESS SESSION
9:00		OPENING CEREMONY	SYMPOSIUM POLARITY & CYTOSKELETON	SYMPOSIUM RHYTHMS & PHOTOBIOLOGY	SYMPOSIUM FRONTIERS IN FUNGAL BIOTECHNOLOGY
9:30		OPENING LECTURE			
10:00					
10:30		Coffee break			
11:00		SYMPOSIUM MORPHOGENESIS & CELL BIOLOGY	Coffee break		
11:30			SYMPOSIUM CHARLES BRACKER MICROSCOPY	SYMPOSIUM GENOME-WIDE APPROACHES TO STUDY NUCLEUS DYNAMICS	SYMPOSIUM FRONTIERS IN MEDICAL MYCOLOGY
12:00					
12:30					
13:00					
13:30		LUNCH	LUNCH	LUNCH	LUNCH
14:00					
14:30					
15:00		SYMPOSIUM SIGNAL TRANSDUCTION	FREE AFTERNOON TOURS EXCURSIONS	SYMPOSIUM FUNGUS – HOST INTERACTION	SYMPOSIUM FRONTIERS IN FUNGAL RESEARCH
15:30					
16:00					
16:30					
17:00	REGISTRATION				CLOSING CEREMONY
17:30					
18:00					
18:30		POSTER SESSION I *Posters 46-112* MEXICAN BUFFET - DRINKS - MUSIC		POSTER SESSION II *Posters 113-180* MEXICAN BUFFET DRINKS - MUSIC	
19:00					
19:30					
20:00	WELCOME PARTY				GALA DINNER & D A N C E
20:30					
21:00					
21:30					
22:00					

SUNDAY Dec 6, 2009

17:00 - 20:00 **REGISTRATION**
LOBBY – HOTEL CORAL

20:00 - 23:00 **WELCOME PARTY**
CASINO ROOM, CENTRO SOCIAL CIVICO Y CULTURAL RIVIERA

MONDAY Dec 7, 2009

ALL SYMPOSIUM LECTURES WILL BE HELD IN ENSENADA HALL OF HOTEL CORAL

9:00 **OPENING CEREMONY**
CHAIR: SALOMON BARTNICKI-GARCIA
Rosa Mouriño Perez, Alexei Licea Navarro, Jesús Aguirre, Kurt Mendgen, Federico Graef Ziehl

9:30 RECESS

9:40 **OPENING LECTURE**
Gordon Beakes, Newcastle University, UK,
Trivial pursuits - insights gained from a career studying unusual fungi

10:30 **C O F F E E   B R E A K**

11:00 ***SYMPOSIUM: MORPHOGENESIS & CELL BIOLOGY***
CHAIRS: MERITXELL RIQUELME & TERRY HILL

11:05 **Michelle Momany,** University of Georgia, USA,
Septins: cytoskeletal scaffolds that direct morphogenesis

11:35 **Oded Yarden,** Hebrew University Jerusalem, ISRAEL,
Cell elongation and branching are regulated by differential phosphorylation states of the NDR kinase COT1 in Neurospora crassa

12:05 **Vera Meyer,** Leiden University, NETHERLANDS,
Chasing regulatory networks involved in polar growth control of Aspergillus. Niger

12:35 **Barry Bowman,** University of California, Santa Cruz, USA,
Structure and distribution of organelles and the cellular location of calcium transporters in Neurospora crassa

C o n t i n u e d…

13:05	**Peter Phillipsen,** University of Basel**,** SWITZERLAND, *Control of polar growth and nuclear migration in fungal hyphae: lessons learned from Ashbya gossypii.*
13:35 – 15:00	**L U N C H**
15:00	***SYMPOSIUM: SIGNAL TRANSDUCTION*** CHAIRS: WILHELM HANSBERG & RICHARD TODD
15:05	**Kazuhiro Shiozaki,** University of California, Davis, USA, *Regulation of TOR complex 2 essential for stress survival in fission yeast*
15:35	**Jesus Aguirre,** IFC-UNAM, MEXICO, *Stress signaling and development in Aspergillus nidulans*
16:05	**Michael Bölker,** University of Marburg, GERMANY, *Regulation of Rac1 GTPase signalling during dimorphic switching of Ustilago maydis*
16:35	**Richard A. Wilson, University** of Nebraska. USA *Trehalose-6-phosphate synthase integrates metabolism and virulence in Magnaporthe oryzae via a novel NADP(H)-dependent genetic switch*
19:00 - 22:00	**POSTER SESSION I - MEXICAN BUFFET - DRINKS – MUSIC** THIRD FLOOR OF THE BIOLOGY DIVISION (DBEA) BUILDING **Posters 46-112**

TUESDAY Dec 8, 2009

9:00	***SYMPOSIUM: POLARITY & CYTOSKELETON***
	CHAIRS: ROSA R. MOURIÑO-PEREZ & MARJATTA RAUDASKOSKI
9:05	**Reinhard Fischer**, University Karlsruhe, GERMANY, *Microtubules in Aspergillus nidulans*
9:35	**Miguel Peñalva**, CSIC Madrid, SPAIN, *Membrane traffic in the Aspergillus nidulans hyphal tip*
10:05	**Peter Sudbery**, University of Sheffield, UK, *Hyphal growth in Candida albicans requires the phosphorylation of sec2 by the cdc28- hgc1 kinase*
10:35	**Brian Shaw**, Texas A&M University, USA *Endocytosis and development in Aspergillus nidulans*
11:05	**C O F F E E B R E A K**
11:30	***CHARLES BRACKER MICROSCOPY SYMPOSIUM***
	CHAIRS: ROBERT ROBERSON & SALOMON BARTNICKI-GARCIA
11:35	**Rosamaria Lopez-Franco**, ITESM Monterrey, MEXICO, *Charles E. Bracker Jr: The man, the teacher and the scientist*
12:05	**Martha Powell,** University of Alabama, USA, *Zoospores, microscopes and molecules*
12:35	**Adrienne Hardham,** Australian Natl. University, AUSTRALIA, *Molecular cytology of Phytophthora pathogenicity*
13:05	**Robert Roberson,** Arizona State University, USA, *Hyphal tip structure and diversity*
13:35 – 15:00	**L U N C H**

F R E E   A F T E R N O O N and E V E N I N G - TOURS - EXCURSIONS

WEDNESDAY Dec 9, 2009

9:00	***SYMPOSIUM: RHYTHMS & PHOTOBIOLOGY*** CHAIRS: STUART BRODY & ERNESTINA CASTRO-LONGORIA
9:05	**Alfredo Herrera-Estrella,** CINVESTAV Guanajuato, MEXICO, *A functional genomics analysis of light responses in Trichoderma*
9:35	**Deborah Bell-Pedersen**, Texas A&M University, USA, *How fungi keep time: Circadian oscillators and rhythmic outputs*
10:05	**Gerhard Braus**, University of Göttingen, GERMANY, *Light control of Aspergillus nidulans development*.
10:35	**Monika Schmoll,** Vienna University of Technology**.** AUSTRIA *Assessing signal relevance: How Hypocrea jecorina (Trichoderma reesei) adjusts the interplay between heterotrimeric G-protein signaling and light response*
11:00	**C O F F E E B R E A K**
11:30	***SYMPOSIUM: GENOME-WIDE APPROACHES TO STUDY NUCLEUS DYNAMICS*** CHAIRS: MICHAEL FREITAG & JORGE FOLCH-MALLOL
11:35	**Steve Osmani,** Ohio State University, USA, *Mitotic regulation of nuclear structure*
12:05	**Miriam Zolan,** Indiana University, USA, *Global analysis of meiotic gene expression and the roles of DNA repair proteins in meiotic chromosome structure and function*
12:35	**Kristina Smith,** Oregon State University, USA, *Centromeres of filamentous fungi*
13:05	**Judith Berman,** University of Minnesota**,** USA**,** *Genome instability in Candida albicans*
13:35 – 15:00	**L U N C H**

15:00	***SYMPOSIUM: FUNGUS – HOST INTERACTION*** CHAIRS: ALFREDO HERRERA-ESTRELLA & RUFINA HERNANDEZ-MARTINEZ
15:05	**Natalia Requena,** University of Karlsruhe, GERMANY, *Fungal signals and plant perception during the arbuscular mycorrhizal symbiosis*
15:35	**Kurt Mendgen,** University of Konstanz, GERMANY, *Specific communities of Oomycetes colonise live or dead leaves and roots of Phragmites australis in a changing environment*
16:05	**Raymond St. Leger,** University of Maryland, USA, *Metarhizium anisopliae as a model for studying host pathogen interactions*
16:35	**Ana Lila Martínez-Rocha,** University of Exeter, UK *Investigating the delivery of effector proteins by Magnaporthe oryzae*
16:50	**Sergio Casas Flores,** IPICYT, MEXICO *The plant growth-promoting fungus Aspergillus ustus alters root architecture through a hormone signaling mechanism and induces resistance against foliar pathogens in Arabidopsis thaliana*
19:00 - 22:00	**POSTER SESSION II - MEXICAN BUFFET - DRINKS - MUSIC** THIRD FLOOR OF THE BIOLOGY DIVISION (DBEA) BUILDING **Posters 113-179**

THURSDAY Dec 10, 2009

8:00	**BUSINESS SESSION -- INTERNATIONAL FUNGAL BIOLOGY CONFERENCES**
8:30	**SESION DE NEGOCIOS -- RAMA DE BIOLOGIA MOLECULAR Y CELULAR**

9:00 ***SYMPOSIUM: FRONTIERS IN FUNGAL BIOTECHNOLOGY***

CHAIRS: RAFAEL VAZQUEZ-DUHALT & CEES VAN DEN HONDEL

9:05 **Rafael Vazquez-Duhalt,** Institute of Biotechnology-UNAM, MEXICO,
Genetic design of fungal enzymes for environmental purposes

9:35 **Louise Glass,** University of California - Berkeley, USA,
Biofuels: Systems analysis of plant cell wall degradation by the model filamentous fungus Neurospora crassa

10:05 **Andrea Stierle,** University of Montana, USA,
Use of signal transduction enzyme assays to guide the isolation of anticancer compounds from acid mine waste extremophiles.

10:35 **Fabio Squina,** Center of Science & Technology of Bioethanol, Sao Paulo, BRAZIL
Gene library generation for biomass-conversion enzymes

10:50 **Rosa Quiroz Castaneda.** Institute of Biotechnology-UNAM, MEXICO
Expression in yeast of a new type of expansin-like protein of the Basidiomycete Bjerkandera adusta and quantification of expansin activity.

11:05	**C O F F E E B R E A K**

11:30 ***SYMPOSIUM: FRONTIERS IN MEDICAL MYCOLOGY***

CHAIRS: NEIL GOW & ALEJANDRO DE LAS PEÑAS

11:35 **James Kronstad,** University of British Columbia, CANADA,
Mechanisms of iron sensing and uptake in Cryptococcus neoformans

12:05 **Irene Castaño,** IPICYT, San Luis Potosí**,** MEXICO,
What do we know about silencing in Candida glabrata?

12:35 **Neil Gow**, University of Aberdeen, UK,
Fungal cell wall biosynthesis and immune regulation

C o n t i n u e d…

13:05 **Rafael Sentandreu,** University of Valencia, SPAIN,
Functional characterization of Pga13 and Pga31, two Candida albicans GPI-anchored cell wall proteins

13:20 **Alejandra Mandel,** University of Arizona, USA,
Role of chitin synthases (CHS) from classes V and VII in the mechanism of action of Nikkomycin Z in the infectious cycle of Coccidioides posadasii

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13:35 – 15:00 **L U N C H**

15:00 ***SYMPOSIUM: FRONTIERS IN FUNGAL RESEARCH***
CHAIR: SALOMON BARTNICKI-GARCIA

15:05 **José Ruiz-Herrera**, CINVESTAV Irapuato, MEXICO,
On how the cell wall structure and its synthesizing machinery evolved in the mycota kingdom

15:35 **Joan Bennett**, Rutgers Univ, USA,
Mycotoxins, sick buildings, and the molds of hurricane Katrina

16:05 **Nick Read,** University of Edinburgh, UK,
Cytoskeletal, nuclear and signalling dynamics during colony initiation in Neurospora crassa

16:35 **Nicholas Money,** Miami University, USA,
The fastest flights (and rotations) in nature: fungal spore discharge at one million frames per second

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17:05 **R E C E S S**

17:20 **CLOSING CEREMONY**
Stuart Brody, University of California, San Diego, USA**,**
The future of fungal biology

19:30 - ???? **GALA DINNER & D A N C E**
ENSENADA HALL OF HOTEL CORAL

Opening lecture

1. TRIVIAL PURSUITS - INSIGHTS GAINED FROM A CAREER STUDYING UNUSUAL FUNGI.

Beakes G.
School of Biology, Newcastle University, Newcastle upon Tyne, United Kingdom

This symposium series of meetings began with the Fungal Spore Symposium held in Bristol in 1966, during which a number of pioneering ultrastructural studies describing zoospore development in oomycetes (Gay and Greenwood) and chytrids (Fuller) were presented. The published volumes from first two meetings in this series greatly influenced both my undergraduate and post-graduate studies and helped stimulate my interest in zoosporic fungi. Over the years I appear to have been drawn to species that are of little economic importance and consequently these studies may be considered as trivial pursuits (and this certainly has been the view of most funding bodies!). I will cover such topics as the transitions that take place during encystment of oomycete zoospores and the maturation and germination of their oospores. After a brief detour into some aspects of chytrid biology, I will return to the oomycete fungi, with accounts of differentiation in two little known but extra-ordinary oomycete genera, Haptoglossa and Eurychasma, which infect nematodes and marine algae respectively. These organisms have shed light on the evolutionary origins of this important group of fungi (sensu lata) and revealed the central role that parasitism has always played in the biology of oomycetes. Along the journey I will throw in some trivia related to various happenings that have occurred during this immensely enjoyable and stimulating symposium series!

Symposium

MORPHOGENESIS & CELL BIOLOGY

2. SEPTINS: CYTOSKELETAL SCAFFOLDS THAT DIRECT MORPHOGENESIS

Momany, M.
Department of Plant Biology. University of Georgia, Athens, Georgia, USA. E-mail: momany@plantbio.uga.edu.

Septins are novel cytoskeletal proteins found in fungi, microsporidia and animals. They were first identified in *S. cerevisiae* through temperature-sensitive cell division cycle mutants that form elongated buds and fail to complete cytokinesis at restrictive temperature. In fungi and animals septins have been shown to localize to division planes where they appear to anchor several other proteins important for nuclear and cellular division. In *S. cerevisiae*, at least thirty proteins have been shown to localize to the mother/bud neck in a septin-dependent manner. In animals, septins have been shown to sometimes co-localize as filaments with actin and microtubules and appear to have roles not associated with cytokinesis.

We are investigating the roles of septins in multicellular organisms using *Aspergillus nidulans* as a model. We have constructed *A. nidulans* strains in which each of the five septins is fused to GFP as well as strains in which each septin has been deleted. Not surprisingly, septins localize to forming septa and emerging branches and germ tubes. They also localize as filaments or rods in conidia and hyphae, more reminiscent of animal septin localization patterns. The phenotypes of *A. nidulans* septin deletion mutants range from mild to severe, with disorganized conidiophores being common. Surprisingly, another common phenotype of septin deletion strains is increased emergence of germ tubes and branches suggesting that septins play a role in negatively regulating the emergence of new growth foci.

3. CELL ELONGATION AND BRANCHING ARE REGULATED BY DIFFERENTIAL PHOSPHORYLATION STATES OF THE NDR KINASE COT1 IN *Neurospora crassa*

Ziv, C.[1], Kra-Oz, G[1]., Gorovits, R.[1], Maertz, S.[2], Seiler, S.[2], Yarden, O.[1]
[1]Department of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Rehovot, Israel. [2]Institute of Microbiology and Genetics, Goettingen University, Goettingen, Germany. E-mail: Oded.Yarden@huji.ac.il.

Dysfunction of the *Neurospora crassa* nuclear Dbf2-related (NDR) kinase COT1 leads to cessation of tip extension and massive induction of new sites of growth. To determine the role phosphorylation plays in COT1 function, we mutated COT1 residues corresponding to positions of highly conserved NDR phosphorylation sites. Analyses of the point-mutation *cot-1* strains (mimicking non- and constitutively-phosphorylated states) indicate the involvement of COT1 phosphorylation in the regulation of hyphal elongation and branching as well as asexual development by altering cell wall integrity and actin organization. Phosphorylation of COT1's activation segment (at Ser417) is required for proper *in vitro* kinase activity, but has only a limited effect on hyphal growth. In marked contrast, even though phosphorylation of the C-terminal hydrophobic motif (at Thr589) is crucial for all COT1 functions *in vivo*, the lack of Thr589 phosphorylation did not significantly affect *in vitro* COT1 kinase activity. Nevertheless, its regulatory role has been made evident by the significant increase observed in COT1 kinase activity when this residue was substituted in a manner mimicking constitutive phosphorylation. We conclude that COT1 regulates elongation and branching in an independent manner, which is determined by its phosphorylation state.

4. CHASING REGULATORY NETWORKS INVOLVED IN POLAR GROWTH CONTROL OF *Aspergillus niger*

Meyer V., Arentshorst M., Kwon M. J., Nitsche B., van den Hondel C., Ram A.
Leiden University, Institute of Biology, Molecular Microbiology and Biotechnology, Kluyver Centre for Genomics of Industrial Fermentation, Leiden, The Netherlands: E-mail: v.meyer@biology.leidenuniv.nl.

Aspergillus niger is of considerable economic importance as cell factory for the production of proteins, bulk chemicals and pharmaceuticals. In terms of protein production, the morphology it adopts in a bioreactor is critical for the productivity of the process. Whereas the preferred morphology would consist of highly branched dispersed mycelia, *A. niger* either produces long hyphae with relative low branching frequencies or pellets which are also not desirable because of the high proportion of pellet biomass that does not contribute to product formation.
To understand in more detail the genetic basis of the morphogenetic program of *A. niger*, we have undertaken different genomic, transcriptomic and bioinformatic approaches and identified several signaling networks, including TORC2, phospholipid, calcium and cell wall integrity signalling, that likely regulate the morphogenetic program of *A. niger*. We have furthermore implemented different functional genomic approaches to query the function and biological role of predicted modulators of these signaling networks.

5. STRUCTURE AND DISTRIBUTION OF ORGANELLES AND THE CELLULAR LOCATION OF CALCIUM TRANSPORTERS IN *Neurospora crassa*.

Bowman, B. J., Bowman, E. J.
Department of Molecular, Cell and Developmental Biology, University of California, Santa Cruz, CA, USA. E-mail: bowman@biology.ucsc.edu

Calcium is an important signaling molecule in all cells and has been hypothesized to direct polar growth in filamentous fungi. We wished to determine which organelles sequester calcium in *Neurospora crassa*. However, a comprehensive description of the structure and distribution of organelles in filamentous fungi has not yet been reported. Using green and red fluorescent proteins (eGFP and tdimer (2)12) we tagged putative marker proteins for nuclei, ER, Golgi, vacuoles, and mitochondria. In the large, fast-growing hyphae of *N. crassa* we obtained excellent images and movies of these organelles. Red and green tagged proteins could be viewed in the same cells, allowing us to see which proteins were common to a single type of organelle. In *N. crassa* five putative calcium-transporting proteins have been identified. Three of these, encoded by the genes *nca-2*, *nca-3* and *cax*, localized to the vacuolar system; the NCA-2 and NCA-3 proteins also appeared in the plasma membrane in regions 1-2 mm behind the hyphal tip. The transporter encoded by *nca-1* appeared in the ER, which includes the nuclear envelope in *N. crassa*. The gene *pmr* encodes a calcium transporter in the Golgi. In the region 50–200 microns behind the hyphal tip we observed a novel organelle that was enriched with the proton-pumping vacuolar ATPase, and with CAX, a Ca^{2+}/H^{+} exchange protein.

6. CONTROL OF POLAR GROWTH AND NUCLEAR MIGRATION IN FUNGAL HYPHAE: LESSONS LEARNED FROM *Ashbya gossypii*

Philippsen P.[1], Köhli M.[1], Kaufmann A.[1], Birrer C.[1], Masai K.[1], Grava S.[1], Finlayson M.[1], Voegeli S.[1], Seeger S.[1], Molzahn L.[1], Rischatsch R.[1], Knechtle P.[1], Laissue P.[1], Helfer H.P.[1], Boudier K.[1], Jaspersen S.[2], Roberson R.[3]

[1] Biozentrum Basel, Switzerland. [2] Stowers Institute, Kansas, U.S.A. [3]Arizona State University, Tempe, U.S.A. E-mail: peter.philippsen@unibas.ch.

Over the past 20 years we have developed *Ashbya gossypii* as an experimental system to study two cell biological processes essential for fungal life style: Sustained polar surface expansion at tips of hyphae and controlled dynamics of multiple nuclei within hyphae. Our experimental approaches were influenced by many years of research experience in yeast genetics and our unexpected discovery that the genome of *A. gossypii* is highly syntenic with that of *Saccharomyces cerevisiae*.
After an introduction into *A. gossypii* biology, genomics and transcriptomics the presentation will focus on new data obtained by monitoring different GFP- or RFP-labeled proteins involved in polar growth or nuclear dynamics. The results can be summarized as follows.
Polar growth: In young, slowly growing hyphae all tested factors form a cortical layer at the tip front. These fluorescent caps comprise 40% to 50% of the total tip cortex area. During development to mature hyphae (growth speeds up to 3.5 micrometer/min) exocyst and polarisome components (except AgBud6) gradually accumulate in the tip as a spheroid. This so-called Spitzenkörper also accumulates secretory vesicles. Remarkably, during hyphal maturation (up to 30 fold acceleration in growth) the cortical tip zone for vesicle fusions only slightly enlarges. Thus, in fast growing hyphae, the Spitzenkörper is needed for increased vesicle fusion efficiency in a confined tip surface. This area overlaps with the distribution of the GTPase AgCdc42 and shows spatial fluctuations centred around the growth axis. The zone of surface growth is separated from sites of highly active endocytosis at the rim of hyphal tips. Mechanistic implications will be discussed.
Nuclear migration: We found three types of cytoplasmic MT-dependent nuclear mobility: oscillation, rotation and bypassing. These are superimposed on one MT-independent mechanism, cotransport with the cytoplasmic streaming. *A. gossypii* SPBs can nucleate short (perpendicular) and very long (tangential) cytoplasmic MTs. The long MTs are important for nuclear bypassing and the short MTs (most likely in combination with the long MTs) for oscillation as concluded from phenotypes of SPB deletion mutants. The differences to nuclear migration in other fungi will be discussed.

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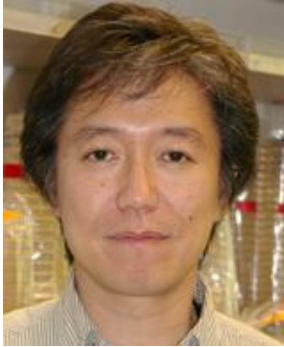
Symposium
SIGNAL TRANSDUCTION

7. REGULATION OF TOR (TARGET OF RAPAMYCIN) COMPLEX 2 ESSENTIAL FOR STRESS SURVIVAL IN FISSION YEAST

Shiozaki K.[1], Tatebe H.[1], Morigasaki S.[1,2], Zeng C.[1]
[1]Department of Microbiology, University of California, Davis, USA. [2]Graduate School of Biological Sciences, Nara Institute of Science and Technology, Japan. E-mail: kshiozaki@ucdavis.edu.



TOR protein kinases were first identified in budding yeast as cellular targets of rapamycin, an anti-proliferative drug used as immunosuppressant. From yeast to humans, TOR kinases form two distinct protein complexes referred to as TOR complex (TORC) 1 and 2 (TORC2), which play discrete roles in cellular signaling partly through activation of different AGC-family protein kinases. TORC1 is activated by the Ras-family Rheb GTPase, which is negatively regulated by the tumor suppressor Tsc1/2 complex. On the other hand, very little is known about the mechanisms that control TORC2 signaling, despite the crucial role of TORC2 in activating the AGC-family Akt kinase during cellular response to insulin and growth factors.

We set out to search for activators of TORC2 signaling, using the fission yeast *Schizosaccharomyces pombe* that has the TOR pathways highly homologous to those in higher eukaryotes. The subunit composition of TORC2 is well conserved in *S. pombe*, including the TOR kinase Tor1, Sin1, Ste20 and Wat1/Pop3. In addition, strains lacking functional TORC2 are defective in phosphorylating and activating an AGC-family kinase, Gad8. Thus, the *S. pombe* TORC2–Gad8 pathway appears to parallel the TORC2–Akt pathway in higher eukaryotes. We will report newly discovered regulatory factors of the TORC2–Gad8 pathway, which is essential for cellular survival of environmental stress.

8. STRESS SIGNALING AND DEVELOPMENT IN *Aspergillus nidulans*.

Lara-Rojas, F., Sánchez, O., Kawasaki, L. and Aguirre J.
Departamento de Biología Celular y Desarrollo, Instituto de Fisiología Celular UNAM. E-mail: jaguirre@ifc.unam.mx.

To sense and process different environmental signals, fungi use a phosphorelay system coupled to a MAP kinase module. *Aspergillus nidulans* response regulator SskA transmits osmotic and oxidative stress signals to the stress activated protein kinase (SAPK) SakA. We characterized the *atfA* gene, encoding a transcription factor of the ATF/CREB family, and analyzed its role as downstream component of SakA. Although AtfA is localized in the nucleus even in the absence of SakA, mutants lacking SakA (*DeltasakA*), AtfA (*DeltaatfA*) or both, show similar patterns of oxidative stress sensitivity, derepression of sexual development and decreased asexual spore (conidia) viability. AtfA is required for expression of several genes, and for conidial accumulation of the spore-specific catalase CatA and the MAPK SakA, linking SakA function and spore viability. Our results support a model in which AtfA interacts with SakA in the nucleus to regulate the expression of different stress response genes, and suggest that SAPK phosphorylation is a conserved mechanism to regulate spore dormancy and germination.

9. REGULATION OF RAC1 GTPASE SIGNALLING DURING DIMORPHIC SWITCHING OF *Ustilago maydis*

Frieser, S., Schink, K. O. and Bölker, M.
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The morphogenetic transition from yeast to filamentous growth is a characteristic feature of many pathogenic fungi. In the corn pathogen *Ustilago maydis*, dimorphic switching is part of its sexual life cycle and is controlled by the multiallelic *b* mating-type locus, which encodes a pair of homeodomain transcription factors. The molecular mechanisms by which the *b* mating-type proteins govern cell polarization remain poorly understood. We could previously show that filamentous growth of *U. maydis* requires the small GTP binding protein Rac1 and its downstream effector, the p21-activated kinase Cla4. We now present evidence that dimorphic switching involves *b* mating–type dependent stimulation of the Rac1-specific guanine nucleotide exchange factor (GEF) Cdc24. During polarized growth, the Rho-GEF Cdc24 recruits GTP bound Rac1 into a Bem1-scaffolded ternary complex, which is located at the hyphal tip and also contains Cdc24 and the Rac1 effector Cla4. Remarkably, ternary complex formation triggers degradation of Cdc24 most presumably by direct Cla4 dependent phosphorylation. This negative autoregulatory feedback loop requires that Rac1 has the ability to pass through its GDP bound state. We propose that degradation of Cdc24 is critical to restrict the dynamic localization of active Cla4 kinase to the apical growth zone.

10. TREHALOSE-6-PHOSPHATE SYNTHASE INTEGRATES METABOLISM AND VIRULENCE IN *Magnaporthe oryzae* VIA A NOVEL NADP(H)-DEPENDENT GENETIC SWITCH

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The most serious disease of cultivated rice, rice blast, results from infection by the filamentous fungus *Magnaporthe oryzae*. Plant infection is mediated by a specialized structure, called the appressorium, which develops from an asexual spore of *M. oryzae* adhered to the surface of the leaf. The mature appressorium generates enormous turgor through the accumulation of glycerol, and this pressure is translated into mechanical force acting on a penetration peg emerging at the base of the cell, causing it to breach the leaf cuticle and enter the plant. However, the physical nature of *M. oryzae* host entry belies the fact that once within the plant, the fungus forms an intimate association with the plant tissue and proliferates from cell to cell in a symptomless, biotrophic manner for the first 72 hours of infection. How the fungus monitors the transition from the surface to the interior of the leaf, and how it undergoes the rapid genetic reprogramming necessary to produce infectious hyphae from the slender penetration peg, acquire nutrients, and evade the plant host defense response, was not known. Previously, the role of *M. oryzae* trehalose-6-phosphate synthase (Tps1) in glucose-6-phosphate (G6P) sensing had been shown to be essential for virulence. Here, I show that the interaction of G6P and NADPH at the Tps1 substrate binding site affects the activity of an NADP-dependent transcription factor-inhibitor complex and finely regulates gene expression in response to the metabolic status of the cell. Consequently, this novel NADP(H)-dependent genetic switch, essential for virulence, provides a framework for understanding how *M. oryzae* senses the nutritious environment of the plant interior and establishes disease. Such an understanding is vital for the development of durable control strategies against this recalcitrant plant pathogen.

Symposium

POLARITY & CYTOSKELETON

<u>11.</u> MICROTUBULES IN *ASPERGILLUS NIDULANS*

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The interplay between the actin and the microtubule (MT) cytoskeleton in polarized growth of fungi has recently been revealed. In *Schizosaccharomyces pombe*, Tea1 is a key protein – a so-called cell end marker protein - in this process. Tea1 is transported to the plus ends of MTs by the kinesin Tea2, and is delivered to cell ends by hitchhiking with growing MTs. Mod5, which is posttranslationally modified by prenylation, anchors Tea1 at the cell ends, where Tea1 recruits formin (Snaith & Sawin, 2003). Formin initiates actin assembly and the establishment of cell polarity. We characterized recently homologues of several *S, pombe* cell end marker proteins in *Aspergillus nidulans* and showed that the functions are essentially conserved (Higashitsuji, et al., 2008; Konzack, et al., 2005; Takeshita et al., 2008). However, we found that the cell end marker complex is not only required for the polarization of the actin cytoskeleton but also for temporary attachment of the MTs to the complex. In addition, we discovered a novel role for TeaA in the control of MT polymerization. We showed that TeaA interacts with the MT plus-end localized MT polymerase AlpA (XMAP215) and triggers its enzymatic activity (Takeshita et al., unpublished).

In order to identify KipA (Tea2 homologue) interacting proteins, we performed a yeast-two-hybrid analysis using the tail of the motor as bait. We identified a novel protein, KatA, which interacts with KipA but also with TeaA. KatA displays some similarity to the kinetochore protein CENP-H. These results suggest partial conservation of MT attachment to chromosomes and to the cortex (Herrero de Vega et al., unpublished).

Recently, there is increasing evidence that endocytosis plays an important role for polarized growth. We characterized two Unc-104 related motor proteins and discovered that one of them, UncA, which is involved in endocytic vesicle transportation, preferentially moves along a detyrosinated MT. This is the first evidence for different MT populations in filamentous fungi (Zekert and Fischer, 2009).

<u>12.</u> MEMBRANE TRAFFIC IN THE *Aspergillus nidulans* HYPHAL TIP

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In coenocytic hyphal tip cells of the filamentous fungus *Aspergillus nidulans*, intracellular distances between apexes and basal regions and between the different nuclei within the same cytoplasm are notably large. Secretion predominates in the apexes and is spatially coupled to subapical compensatory endocytosis. Early endosomes (EEs) undergo long-distance movement riding on microtubules (MTs) and traffic anterogradely towards the dynein loading region at the tip where MTs are oriented with their plus-ends towards the apex before undergoing dynein-dependent retrograde movement. Dynein inactivation results in an exaggerated early endosomal compartment at the tip, denoted the NudA compartment. Evidence suggests that this trafficking of EEs facilitates recycling of endocytosed materials to the plasma membrane by polarised secretion. The *A. nidulans trans-* and *cis*-Golgi is formed by a dynamic network of rings, tubules and fenestrated structures that is strongly polarised and does not disassemble during mitosis. Unlike early endosomes, Golgi equivalents do not undergo rapid long-distance movements. Polarisation of the Golgi is possibly mediated, at least in part, by polarisation of the ERES and involves F-actin. Thus, the burning and as yet standing question is what determines polarisation of the

transitional ER. The organisation of both the *cis*- and the *trans*-Golgi is dramatically but reversibly affected by brefeldin A, to which *A. nidulans* is largely resistant, possibly acting at two different levels. Disorganisation of the Golgi arrests apical extension but growth is restored after washing out the drug, correlating with recovery of the normal Golgi organisation. Notably, whereas ERES and *cis*-Golgi elements reach the apical dome region, the *trans*-Golgi seems to be actively excluded from the tip and does not reach the dynein loading region. Our data underscore the remarkable and very poorly understood complexity of membrane traffic that takes place ahead of the leading nucleus.

13. HYPHAL GROWTH IN *Candida albicans* REQUIRES THE PHOSPHORYLATION OF SEC2 BY THE CDC28- HGC1 KINASE

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Candida albicans hyphae grow in a strongly polarized fashion from the tip driven by a Spitzenkörper, a subapical body rich in secretory vesicles. Cytological studies using protein fusions to GFP show that Mlc1, Sec4 and Sec2 localize to the Spitzenkörper, while exocyst and polarisome components localize to surface crescent that is spatially distinct from the Spitzenkörper. In the budding yeast Sec4 is a Rab GTPase which in its GTP-bound form is required for the transport of post-Golgi secretory vesicles along actin cables formed by the polarisome to sites of polarized growth, and their fusion with the plasmamembrane after docking with the exocyst. FRAP experiments show that Sec4 is much more dynamic than polarisome and exocyst components suggesting that the stream of secretory vesicles arriving at the tip is captured by the more static exocyst compenents. Sec2 is the guanine exchange factor mediates the conversion of Sec4 to its active, GTP-bound form. Mlc1 is the regulatory light chain of a class V myosin, Myo2, that provides the motive force for the transport of secretory vesicles. In order to understand the mechanisms that result in the accumulation of secretory vesicles in the Spitzenkörper, we have dissected the regulation of the Sec2. We present genetic and biochemical data showing that shortly after hyphal induction Sec2 is phosphorylated on residue S584 the Cdc28 kinase partnered by the hyphal-specific Hgc1 cyclin and that this phosphorylation is necessary for hyphal growth.

<u>14.</u> ENDOCYTOSIS AND DEVELOPMENT IN *Aspergillus nidulans*

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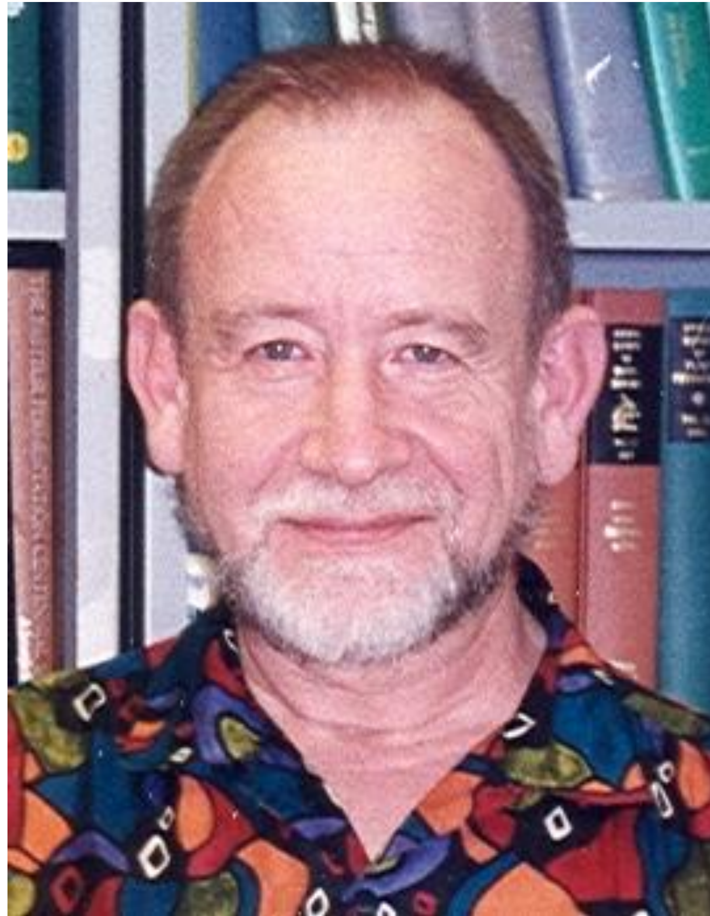
Cell shape is an important determinant of function. Filamentous fungi present an interesting paradigm to study this connection since their life cycle is dominated by cells growing exclusive in the hyphal form. Models explaining the hyphal shape have long been dominated by studies of exocytosis, the outward flow of new growth material to the cell apex. Recent studies have shown that endocytosis, the inward flow of membrane, at the hyphal apex is equally important for determining cell shape. Evidence will be presented that supports an 'Apical Recycling Model' that explains a need for endocytosis at the apex to determine cell shape. Endocytic sites correspond with actin patches that are found at the cell cortex in a sub-apical collar in growing hyphae. Hyphae that are not actively growing present actin patches within the cell apex. Mutants in which endocytosis is disrupted lose the hyphal shape during active growth. A proposed role for endocytosis in shaping conidia will be discussed.



We dedicate this Symposium to Prof. **Charles E. Bracker** to honor his life-long career devoted to the study of fungi and to celebrate his multiple contributions in elucidating the fine structure of fungal cells and his pioneering efforts in using transmission electron microscopy and video microscopy to study fundamental aspects of fungal biology.

CHARLES BRACKER MICROSCOPY
Symposium

15. CHARLES E. BRACKER JR: THE MAN, THE TEACHER AND THE SCIENTIST

Lopez-Franco R. M.

16. ZOOSPORES, MICROSCOPES, AND MOLECULES

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Typical of opisthokonts, the posteriorly uniflagellate zoospore is the calling card of chytrid fungi. The small size of zoospores, 3-7 micrometers, belies their ultrastructural complexity that electron microscopic studies have revealed. Differing arrangements and compositions of organelles accompany an abundance of unique features in the flagellar apparatus. The use of molecular sequence data to generate phylogenetic hypotheses now helps us postulate how organelles in lineages have evolved. So faithful is zoospore structure that specific zoospore ultrastructural types predict molecular-based phylogenetic relationships, and molecular phylogenetics predict zoospore ultrastructural types. Changes in the microbody lipid globule (MLC) cisterna and range of structures associated with the kinetosome are discussed in terms of habitat, a potential strong driver in the evolution of zoospore architecture.

17. MOLECULAR CYTOLOGY OF *Phytophthora* PATHOGENICITY

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Many of the more than 60 species of *Phytophthora* are aggressive plant pathogens that cause extensive losses in agricultural crops, horticultural plants and natural ecosystems. Some *Phytophthora* species infect only a small number of plant species while others have broad host ranges. *Phytophthora* and other members of the class Oomycetes form fungus-like hyphae and conidia-like asexual sporangia, but they are not fungi. The Oomycetes cluster with a range of other protists such as diatoms and coloured algae within the Stramenopiles, an assemblage whose taxon-defining characteristics include possession of tubular hairs on their flagella.

Species of *Phytophthora* produce motile, biflagellate zoospores that play a key role in the initiation of plant disease. Zoospores target suitable infection sites where they encyst

and attach. Cysts germinate and invade the underlying plant tissues. *Phytophthora* species may initially establish a biotrophic relationship with living host cells, obtaining nutrients through the development of haustoria within infected cells. In other cases, they grow necrotrophically, feeding on dead or dying cells. Like fungi, *Phytophthora* and other Oomycetes secrete effector proteins that are required for pathogenicity. Some effectors, such as cell wall degrading enzymes, function in the plant apoplast but others are transported across the plant plasma membrane into the host cytoplasm where, in susceptible plants, they are believed to suppress the plant defence response and orchestrate metabolic changes that favour pathogen growth. Successful colonisation of the plant allows sporulation to occur within a few days. During asexual sporulation, uninucleate zoospores are formed through subdivision of multinucleate sporangia that form at hyphal apices.

Early studies of the infection of plants by species of *Phytophthora* used light and electron microscopy to describe the major features of disease development. More recently, these traditional approaches have been extended by advanced light and electron microscopy techniques that use a variety of methods, such as immunocytochemical labelling, GFP-tagging and confocal microscopy, to mark and visualise a range of pathogen molecules and cell components. This molecular cytology not only facilitates identification of specific cell structures in fixed and sectioned material but also allows studies of living cells.

In this presentation, I will review our current understanding of cellular and molecular aspects of the infection of plants by *Phytophthora*. In so doing, I will highlight how modern molecular cytology can draw on genomic and transcriptomic sequence data and how it is revolutionising our ability to elucidate the roles of selected proteins and cell components in *Phytophthora* pathogenicity.

18. HYPHAL TIP STRUCTURE AND DIVERSITY

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Hyphal growth and morphogenesis are complex processes that have allowed the fungi to successfully utilize a wide range of ecological habitats and develop multiple lifestyles. Bioimaging studies, and more recently molecular studies, of hyphal tip biology have placed great emphasis on the Spitzenkörper. The Spitzenkörper appears to have evolved only in filamentous fungi where it is present in all members of the Basidiomycota and Ascomycota studied thus far. Spitzenkörper have been verified only in a few members of the 'lower fungi.' Though progress is being made towards better understanding the cellular and molecular biology of the hyphal tip growth, important details remain unclear regarding the structural diversity among the filamentous fungi. In this presentation, light and electron microscopy are used to address hyphal tip structure and diversity among fungal taxa.

Symposium
RHYTHMS & PHOTOBIOLOGY

<u>19.</u> FUNCTIONAL GENOMICS ANALYSIS OF LIGHT RESPONSES IN *Trichoderma atroviride*.

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Species belonging to the genus *Trichoderma* have been successfully used, several decades, for the control of phytopathogenic fungi. It is has been shown that *Trichoderma* mycoparasites the pathogens, produces antibiotics, and induces plant defense responses. In addition, *Trichoderma atroviride* has been used as a model for photo-morphogenesis. Light is an environmental signal that impacts on the behavior of almost all living forms, regulating growth, development, reproduction, pigmentation and circadian rhythms. In *T. atroviride*, light regulates asexual reproduction, growth and metabolism. The BLR1/BLR2 proteins, homologous to photoreceptor complex of *Neurospora crassa* WC1/WC2, regulate these responses to blue light. BLR1 has a LOV domain, which contains all amino acids necessary to bind FAD as chromophore and as described for WC1, light impacts the flavin activating the BLR complex, consequently, regulating many physiological responses to light.
Recently, we have applied pyrosequencing technology to the study of the *Trichoderma atroviride* transcriptome. We obtained RNA samples from the wild type strain grown in the dark or after exposure to a pulse of white light, as well as from a photoreceptor mutant (Δblr-1) exposed to light. Based on statistical tests and the relative abundance of a given transcript among all three samples we have been able to identify 331 light responsive genes and if their abundance depends on a functional *blr-1* gene. Among them we identified 12 transcription factors, DNA-repair enzymes, and a set chaperons, including heat shock proteins, suggesting that light is perceived as an stress signal by *Trichoderma*. In addition, our data suggest an important link between light and carbon metabolism. We have initiated the study of several of the light responsive genes through gene disruption of several of the TFs, and other key genes.

<u>20.</u> CIRCADIAN CLOCK OUTPUT PATHWAYS IN *Neurospora crassa*

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About 20% of *Neurospora* genes are under control of the circadian clock system at the level of transcript accumulation, and the bulk of the clock-controlled mRNAs have peak accumulation in the late night to early morning. These data suggested the existence of global mechanisms for rhythmic control of gene expression. Consistent with this idea, we found that the Neurospora OS pathway, a phosphorelay signal transduction pathway that responds to changes in osmotic stress, functions as an output pathway from the FRQ/WCC. ChIP/Solexa sequencing with known oscillator proteins revealed that phosophorelay/MAPK pathway components are direct targets of the White Colar Complex (WCC), providing a direct connection between the clock and the output pathway. Activation of the OS pathway by the FRQ/WCC oscillator culminates in rhythmic OS-2 MAPK activity, which through time-of-day-specific activation of downstream effector molecules, controls rhythms in several target clock-controlled genes. Hijacking conserved signaling pathways by the circadian clock provides a new paradigm for global rhythmic control of target genes of the pathway.

<u>21.</u> LIGHT CONTROL OF *Aspergillus nidulans* DEVELOPMENT

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The homothallic filamentous ascomycete *A. nidulans* is able to form fruit-bodies (cleistothecia) either by mating of two strains or by selfing in the absence of a partner. The three-dimensional *A. nidulans* cleistothecium is the most complicated structure this fungus is able to form. Differentiation and secondary metabolism are correlated processes in fungi that respond to various parameters including light, nutrients, aeration or pheromones. Our work on several proteins will be described, which are involved in the crosstalk between developmental regulation and secondary metabolism control in *Aspergillus nidulans.* They include the heterotrimeric *velvet* complex VelB/VeA/LaeA, where VeA bridges VelB to the nuclear master regulator of secondary metabolism LaeA, the eight subunit COP9 signalosome complex controlling protein turnover , and the MAP kinase-related protein kinase ImeB.

<u>22.</u> ASSESSING SIGNAL RELEVANCE: HOW *Hypocrea jecorina* (*Trichoderma reesei*) ADJUSTS THE INTERPLAY BETWEEN HETEROTRIMERIC G-PROTEIN SIGNALING AND LIGHT RESPONSE

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The biotechnological workhorse *Hypocrea jecorina* (anamorph *Trichoderma reesei*) is predominantly known for its efficiency in cellulase production. Previously we could show that heterotrimeric G-protein signaling in this fungus is impacted by light and that both GNA1 and GNA3, two G-protein alpha subunits, have a light dependent function in regulation of cellulase gene transcription. We were consequently interested how this interplay between environmental nutrient signals, light perception and cellulase regulation could work. As candidate factor for the missing link between these pathways we analysed the function of the class I phosducin like protein PhLP1, which is assumed to act as co-chaperone for G-protein beta-gamma folding and assembly. By analysis of deletion strains in *gna1*, *phlp1*, the genes encoding the G-protein beta and gamma subunits *gnb1* and *gng1*, the photoreceptors *blr1* and *blr2* and the light regulatory gene *env1*, we could draw a first draft of a model how the interaction between these signaling pathways could work.
Our data suggest, that the relevance of signals transmitted by G-alpha subunits in light and darkness is adjusted by an integration of the respective pathways. The regulatory circuit established by ENV1 and PhLP1 is likely to play a crucial role in connecting nutrient sensing with the light signaling pathway.

Symposium

GENOME-WIDE APPROACHES TO STUDY NUCLEUS DYNAMICS

<u>23.</u> MITOTIC REGULATION OF NUCLEAR STRUCTURE.

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Defying the textbook descriptions of mitosis as being either open or closed many organisms, including fungi, undergo variations between these two extreme modes of mitosis. For example, *Aspergillus nidulans* undergoes a partially open form of mitosis during which the mechanisms by which proteins locate to the nucleus are dramatically modified. During interphase nuclear location of proteins depends upon specific nuclear transport signals that target them to nuclei through nuclear pores in a highly regulated manner. However, during entry into mitosis active transport through nuclear pores is halted by the action of the mitotic NIMA kinase which promotes partial nuclear pore disassembly. This dramatically modifies the function of nuclear pores so that rather than acting as regulated gatekeepers between the nucleoplasm and cytoplasm, they act as unregulated conduits allowing equilibration of proteins across the nuclear envelope during mitosis. This provides a mechanism to allow proteins required for the rapid mitosis of *A. nidulans*, such as tubulin, immediate access to the nuclear interior in a regulated manner that does not rely on modulating their active nuclear transport. In addition to the reorganization of mitotic nuclear pores, other major changes to nuclear structures also occur during *A. nidulans* mitosis. The nucleolus is the most prominent sub-nuclear compartment occupying a significant portion of the nuclear volume in *A. nidulans*. During mitosis the nucleolar organizing regions of the genome are removed from the nucleolus at anaphase. In telophase double pinching of the nuclear envelope expels the nucleolus to the cytoplasm. At G1 the cytoplasmic nucleolar remnant undergoes sequential disassembly and the released proteins are imported to the daughter nuclei as active nuclear transport is reestablished. Thus the nucleolus undergoes a round of “cleansing” after each partially open mitotic nuclear division. These studies reveal previously unexpected, and surprisingly complex, modifications of nuclear structures during *A. nidulans* mitosis. These findings also pose some interesting questions regarding the evolution of mitosis and provide opportunities to understand mitotic regulation of nuclear structures beyond the more highly studied yeast closed mitoses.

<u>24.</u> GLOBAL ANALYSIS OF MEIOTIC GENE EXPRESSION AND THE ROLES OF DNA REPAIR PROTEINS IN MEIOTIC CHROMOSOME STRUCTURE AND FUNCTION

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We are studying meiosis in the basidiomycete *Coprinus cinereus* (also known as *Coprinopsis cinerea*), in which meiosis is naturally synchronous. Using classical genetics, we isolated mutants defective in DNA repair and meiosis, and molecular studies revealed that a subset of these strains contain mutations in the genes encoding Mre11, Rad50, and Nbs1, which form a complex (called MRN) required for DNA double-strand break repair and meiotic recombination. We are interested in the roles of the MRN complex and its individual members in meiotic chromosome structure and function.

Recent advances in *C. cinereus* genomics are allowing us to take global approaches to the analysis of MRN complex proteins and other genes essential for meiosis. We constructed a 70-mer oligonucleotide microarray for all predicted *C. cine-*

reus genes and used this microarray to examine a 15-hour meiotic time course, encompassing stages from before haploid nuclear fusion until tetrad formation. The prolonged prophase of *C. cinereus* allowed us to define nine clusters of sequential gene expression. Interestingly, *mre11* falls into a different cluster from that containing *rad50* and *nbs1*, perhaps reflecting independent roles for the encoded proteins in some aspects of meiosis. We also compared the *C. cinereus* meiotic program with those previously reported for *Saccharomyces cerevisiae* and *Schizosaccharomyce pombe*. As for these unicellular fungi, approximately 20% of *C. cinereus* genes exhibited altered expression during the meiotic time course. Comparative analysis of meiotic gene expression profiles showed that "meiotic process" genes, including *rad50* and *mre11*, were more highly correlated in their expression profiles than "non-meiotic process" genes. Our observations of within-species coordinate regulation and between species conserved regulation should enable the discovery of previously uncharacterized genes important for meiosis.
The *C. cinereus* genome sequence also facilitated the development of a whole-genome simple sequence repeat (SSR) map. We are using this map to examine meiotic crossover recombination distribution and frequency in wild-type strains and MRN complex mutants. We have found that a mutation in the gene encoding Nbs1, which confers a single amino acid substitution within the protein's FHA domain, leads to substantial map expansion, meaning that the number of crossovers is increased in this mutant strain. Our genome-wide approaches will facilitate mechanistic analyses of crossover control in *C. cinereus*.

25. CENTROMERES OF FILAMENTOUS FUNGI

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Centromeres serve as a platform for the assembly of kinetochores, the attachment points for spindle microtubules that transport chromosomes into daughter nuclei during nuclear division. Filamentous fungi are excellent models to study centromere function and evolution, as closely related fungi have highly divergent centromere components and centromere DNA composition. We analyzed fungal homologs of the centromere proteins CenpA (CenH3) and CenpC to identify motifs that are either under adaptive or purifying selection. CenH3 swapping experiments between different fungi are providing information about what is required for centromere function in these organisms. To learn about centromere assembly and maintenance, we subjected *Neurospora crassa* and *Fusarium graminearum* to ChIP-sequencing with tagged CenH3 and CenpC as well as antibodies against histone modifications thought to be required for centromere function. In Neurospora, we found colocalization of CenH3, CenpC and H3 K9me3 in a 100-300 kb region on each chromosome. H3 K4me2 was not enriched at Neurospora centromeres, in contrast to results from studies with plant, fission yeast, Drosophila and human core centromeric regions. DNA methylation, almost always tightly associated with H3 K9me3 in Neurospora, was enriched only at the centromere peripheries and overlapped little with Cen protein distribution. Mutation of *dim-5*, encoding an H3 K9 methyltransferase, resulted in loss of CenH3-GFP binding from the edges of the centromere regions. In the absence of HP-1, the chromo domain protein that binds H3 K9me3, CenH3-GFP was still mislocalized and restricted to the centromere core. We propose a model where CenH3 is maintained at the centromere core even in the absence of H3 K9me3 and HP-1, but both marks are required for normal spreading of CenH3 to the centromere peripheries.

26. GENOME DYNAMICS IN *Candida albicans*

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C. albicans does not undergo a normal meiosis, yet it generates a large amount of genome diversity, much of it due to changes in the copy number of whole chromosomes and/or chromosome segments. We are exploring the kinds of conditions that stimulate the generation of aneuploidy and the mechanisms by which aneuploidy arises. Conditions that affect genome stability include a range of stresses including the stress conditions used to introduce DNA into cells for transformations. A range of mechanisms including recombination, replication defects and mitotic defects that result in chromosome non-disjunction and the outcomes of those mechanisms will be discussed.

Symposium

FUNGUS – HOST INTERACTION

27. FUNGAL SIGNALS AND THEIR PERCEPTION BY THE PLANT IN THE ARBUSCULAR MYCORRHIZAL SYMBIOSIS

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Arbuscular mycorrhizal (AM) fungi form a mutualistic symbiosis with the root of most vascular plants. This mycorrhiza association is evolutionarily dated as one of the oldest fungal-plant symbiosis on earth reflecting the success of this interaction. Amazingly, it is perhaps one of the most obscure associations due to the genetic intractability of the fungal partner. Thus, while enormous advances on the knowledge about plant perception and accommodation of AM fungi has been achieved in the last years, not so much is known about the details governing the life cycle of the fungus. In our group we are interested in understanding how AM fungi talk to plants and persuade them of their good intentions. With a combination of several molecular approaches we are aiming to identify the chemical signals that trigger plant fungal recognition during the AM symbiosis. We have identified a family of putative effector proteins that are secreted and able to enter the plant and travel to the nucleus. Expression of these proteins *in planta* appears to increase the susceptibility to mycorrhiza formation, indicating that they might play a role in silencing the immune response of the plant. We are currently investigating how the plant perceives these and other fungal signals leading to the activation of the symbiotic program.

28. SPECIFIC COMMUNITIES OF OOMYCETES COLONISE LIVE OR DEAD LEAVES AND ROOTS OF *Phragmites australis* IN A CHANGING ENVIRONMENT

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We have used conventional baiting techniques and internal transcribed spacer clone libraries to characterise and quantify oomycete populations colonising live or dead leaves and roots of reed (*Phragmites australis*). In live reed leaves, the aggressive leaf pathogen *Pythium phragmitis* was among the dominant species. Field studies and microcosm experiments proved it to be promoted by increasing water temperatures and frequent flooding events. In addition, another, as-yet uncultured *Pythium* reed pathogen was particularly adapted to higher water temperatures during summer. In dead leaves, saprotrophic oomycetes displaced the pathogens, and the communities were less diverse and less sensitive to rising water temperatures. In roots and rhizomes we have detected a particularly virulent *Pythium* taxon, representing a newly emerged hybrid between *P. phragmitis* and a closely related, as-yet unknown species that might have been introduced from neighbouring agricultural crops.

Our results show that reed stands harbour diverse sets of substrate- and season-specific oomycete species with particular capabilities to infect and colonise live leaves, dead plant material, or roots and rhizomes. Furthermore, we could show that the different oomycete communities inhabiting natural ecosystems might be particularly sensitive to the proposed consequences of environmental change.

<u>29.</u> METARHIZIUM AS A MODEL FOR STUDYING INSECT-FUNGAL-PLANT INTERACTIONS

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Metarhizium anisopliae is an economically important insect pathogen and rhizosphere competent plant symbiont that provides a tractable experimental system with which to address fundamental questions of insect/fungal/plant interactions.
M. anisopliae is a radiating species, and contains both generalist and specialized lineages with broad and narrow insect host ranges. Strains can be selected representing evolutionary distances ranging from <1 to 21 MY and we have found examples where evolution has occurred by: 1) altered regulation of gene expression; 2) gene loss; 3) gene duplication and divergence, and 4) horizontal gene transfer. We have exploited easily identified marker genes and functional genomics to provide detailed knowledge of movement, persistence and short term modes of genetic change in *M. anisopliae*. Array based mutation accumulation assays demonstrated that three and a half years after release, field recovered *Metarhizium* isolates differed from the input strain by an average of 0.28% of the arrayed genes. However, hypotheticals, stress and cell wall genes were disproportionately altered in expression. Cell wall and stress tolerance genes are likely to have a direct impact on fitness of individual conidia and germtubes, and we have demonstrated that the accumulating mutations increased adaptation to the Maryland field site. Most genes for secreted enzymes and metabolic pathways were highly conserved which suggests that mutations are selected against over much of the genome.
Agrobacterium-mediated random mutagenesis studies and construction of deletion strains has identified genes required for rhizosphere competence and insect pathogenesis. Some of these genes encode regulators such as protein kinase A that have also been shown to be crucial in plant and animal pathogens as well as *M. anisopliae*. Other *M. anisopliae* genes with orthologs in plant and animal pathogens include an osmosensor that signals to penetrant hyphae that they have reached the haemocoel and a perilipin (the first characterized in a fungus) that regulates lipolysis, osmotic pressure and formation of infection structures. Some genes are highly adapted to the specific needs of *M. anisopliae* e.g., Mcl1 (immune evasion) with its collagen domain is so far unique to *M. anisopliae*. *M. anisopliae* has specialist genes for a bifunctional lifestyle including separate adhesins (Mad1 and Mad2) that allow it to stick to insect cuticle and plant epidermis, respectively. Field trials in plots co-inoculated with RFP-ΔMcl1 and GFP-ΔMad2 demonstrated that both rhizosphere competence and pathogenicity are required to maintain high population levels of *M. anisopliae*. Recent coverage of specialist and generalist genomes is further helping determine the identity, origin and evolution of traits needed for diverse lifestyles and host switching.
Finally, *M. anisopliae* is also a good model for studies on bioengineering. *M. anisopliae* has been used to express arthropod toxins and single chain antibodies directed against malaria. Current studies on genetic stability and genetic containment strategies will provide a model of general relevance to releases of transgenic fungi.

30. INVESTIGATING THE DELIVERY OF EFFECTOR PROTEINS BY *Magnaporthe oryzae*

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Magnaporthe oryzae is the causal agent of rice blast disease. To cause disease *M. oryzae* invade the plant, cell by cell, using invasive hyphae and delivering proteins directly into plant cells, but how the fungus delivers proteins during plant infection is currently unknown. Previous analysis has shown that the *MoAPT2* gene, which encodes an aminophospholipid translocase, is involved in secretion of proteins during plant infection and required for the rapid induction of host defence responses in an incompatible reaction. However, the loss of *MoAPT2* does not significantly affect hyphal growth, indicating that the establishment of rice blast disease involves the use of MoApt2-dependent exocytotic processes that operate during plant infection. One of the main objectives in this project is to determine whether those effectors are delivered to plant cells in a MoApt2-dependent manner. Fluorescent-tag gene fusion effectors have been created with native signal peptides and promoter sequences and a GFP at the C-terminus. *M. oryzae* effectors are expressed only during invasive growth in rice tissue. Our results showed that these effectors localize to discrete sub-apical aggregates at the periphery of hyphae, suggesting this localization is dependent upon the signal peptide. We have investigated the roles of cytoskeletal elements and the sites of exocytosis and endocytosis during vegetative hyphal growth, appressorium formation and invasive hyphae growth. Live imaging of MoSnc1, a synaptobrevin homologue and MoFim cytoskeletal component, reveals that vesicles accumulate in the Spitzenkorper (apical body) and fuse with the plasma membrane at the extreme apex of the hypha during vegetative hyphae growth in wild type strain Guy11 and the Δ*Moapt2* mutant. However the Δ*Moapt2* mutant exhibits a miss-localization of GFP-Snc1 during appressorium formation at 24h and GFP-Snc1 shows distinct distribution pattern in invasive hyphae compared to vegetative hyphae. We co-localize RFP-Snc1 with the effector AVR-Pia-SP-GFP and the expression was observed after 48hpi in rice plant tissue. The localization of RFP-Snc1 is at hyphal tip, while the localization of AVR-Pia-SP-GFP is sub-apical in aggregates at the periphery of hyphae. This preliminary analysis demonstrates MoSnc1 does not co-localise with AvrPia effector and this could indicate a new secretion system for deliver effectors which are produced and deliver only *in planta* where they may play, as yet uncharacterized roles in plant defence suppression and could also be important triggers for activating the immune response of the host plant.

31. THE PLANT GROWTH-PROMOTING FUNGUS *Aspergillus ustus* ALTERS ROOT ARCHITECTURE THROUGH A HORMONE SIGNALING MECHANISM AND INDUCES RESISTANCE AGAINST FOLIAR PATHOGENS IN *Arabidopsis thaliana*

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Due to health and environmental issues, there is a need to reduce the use of agrochemicals to enhance plant growth and control diseases in crops. This has raised the need to study alternatives, such as plant growth promoting microorganisms and biocontrol agents. We have identified an *Aspergillus ustus* isolate that promotes growth and induces developmental changes in *Arabidopsis thaliana* seedlings. *A. ustus* inoculation on *A. tahliana* roots induced an increase in shoot and root growth, lateral-root and root-hair number. In base to these results we hypothesized that this behavior could be due to the presence of auxin or ethylene-like molecules secreted by the fungus. Taken into account this result, we decide to test a mutant affected in the root hair initiation, whose phenotype is recued by the addition of exogenous Indole acetic acid (IAA) or by the ethylene precursor 1 -aminocyclopropane-1-carboxylic acid (ACC). Inoculation of *rhd6* mutant with *A. ustus* rescued the root hair initiation phenotype, which

support our hypothesis that an auxin-like mechanism is working during *A. ustus*-Arabidopsis interaction. Assays to measure reporter gene expression from auxin induced/repressed or cell cycle controlled genes (*DR5* and *CycB1* respectively) fused to *uidA* reporter gene, showed an enhanced GUS activity for *CycB1::uidA*, while not activity was observed in *DR5::uidA* lines when compared with mocked-inoculated seedlings. These results indicated that a molecule different to auxin is provoking the effect on Arabidopsis seedlings. In order to determine the contribution of phytohormone signaling pathways in the effect elicited by *A. ustus*, we evaluated the response of a collection of hormone mutants of *Arabidopsis* (*aux1-7*, *axr4-1*, *eir1-1*, *etr1-3*, *ahk2-2*, *ahk3-3*, and *abi4-1*), defective in auxin, ethylene, cytokinin or abscisic acid signaling respectively, to inoculation with this fungus. All mutant lines tested showed increased biomass production when inoculated with *A. ustus*. In addition, *A. ustus* induced the expression of defense genes in *Arabidopsis* Col-0 seedlings, which induced systemic resistance against *Botrytis cinerea* and *Pseudomonas syringae*. Taken together, our results suggest that the increase in plant growth and root architecture changes are induced by molecules produced by *A. ustus* different from those molecules that do not produce an effect on the *Arabidopsis* lines we used in this work. In addition, this fungus is able to protect plants against plant phytopathogens.

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32. FUNGAL ENZYMES FOR ENVIRONMENTAL PURPOSES; A MOLECULAR BIOLOGY CHALLENGE.

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In their capacity to transform xenobiotics and polluting compounds, fungal peroxidases and their use in the environmental field have a recognized and important potential. These peroxidases are able to oxidize a wide range of substrates with high redox potential, and they catalyze substrate oxidation by a mechanism involving free radicals, showing low substrate specificity. These ligninolytic enzymes are directly involved in the degradation of various xenobiotic compounds, including industrial dyes, polycyclic aromatic hydrocarbons, pesticides, dioxines, chlorophenols, explosives, and Kraft pulp bleaching.

However, both fundamental and practical issues, such as enzyme stability and availability, have delayed the development of industrial applications. Three main protein engineering challenges have been identified; i) Enhancement of operational stability, specifically hydrogen peroxide stability. ii) Increase of the enzyme redox potential in order to widen the substrate range. iii) Development of heterologous expression and industrial production.

All hemeproteins, including peroxidases, are inactivated in the presence of catalytic concentrations of hydrogen peroxide. Protein elements, such as the porphyrin ring or the protein backbone, act as simultaneous and competing electron sources even in the presence of exogenous reducing substrates, leading to a decline in activity. It is hypothetically possible to alter the intramolecular electron transfer pathways by direct replacement of low redox potential residues around the active site; as a consequence, the inactivation process would be delayed or even suppressed. To demonstrate this hypothesis, a redox-inspired strategy was implemented until an iso-1-cytochrome c variant fully stable at catalytic concentrations of hydrogen peroxide was obtained. The results presented here clearly demonstrate that redox-inspired protein engineering is a useful tool for the rational modulation of intramolecular electron transfer networks.

On the other hand, the range of substrates for peroxidases seems to have a thermodynamic limit. No substrate was oxidized over the enzyme redox threshold value. The redox potential of heme-peroxidases varies according to a combination of structural components within the active site and its vicinities. Here we describe a novel approach to estimate the redox potential of peroxidases, which directly depends on the catalytic performance of the activated enzyme. The results obtained with this catalytic approach correlate well with the oxidative capacity predicted by the redox potential of the Fe(III)/Fe(II) couple.

Thus, hydrogen peroxide stability, redox potential manipulation, and efficient heterologous production of ligninolytic enzymes in industrial microorganisms, represent three main problems for enzyme improvement and large-scale production. These issues could be addressed and may be solved by using recombinant DNA technology. In this presentation, the opportunities and challenges for the use of fungal enzymes in environmental applications are discussed.

33. BIOFUELS: SYSTEMS ANALYSIS OF PLANT CELL WALL DEGRADATION BY THE MODEL FILAMENTOUS FUNGUS, *Neurospora crassa*

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Plant biomass, primarily composed of lignocellulose, is widely viewed as a potential feedstock for the production of liquid fuels and other value-added materials. However, the principal barriers to production of liquid fuels from lignocellulose are the high costs of pretreatment and conversion of insoluble polysaccharides to fermentable sugars. Conversion of insoluble plant cell wall polysaccharides currently involves using hydrolytic enzymes produced by filamentous fungi. The model filamentous fungus *Neurospora crassa* is commonly found growing on dead plant material in nature, particularly grasses. Using functional genomics resources available for *N. crassa,* which include a near full genome deletion strain set and whole genome microarrays, we undertook a system-wide analysis of plant cell wall and cellulose degradation. We identified ~770 genes that showed expression differences when *N. crassa* was cultured on ground *Miscanthus* stems as a sole carbon source, which we then compared to expression analysis of *N. crassa* grown on pure cellulose. Functional annotation showed an enrichment for proteins predicted to be involved in plant cell wall degradation, but also many genes encoding proteins of unknown function. As a complement to expression data, the secretome associated with *N. crassa* growth on *Miscanthus* and cellulose was determined using a shotgun proteomics approach. Over 50 proteins were identified, including predicted *N. crassa* cellulases and hemicellulases. Strains containing deletions in genes encoding proteins detected by microarray and mass spectrometry experiments were analyzed for phenotypic changes during growth on crystalline cellulose and for cellulase activity. Growth of some deletion strains on cellulose was severely diminished, while other deletion strains produced higher levels of extracellular proteins that showed increased cellulase activity. The genomic tools available in *N. crassa* allow for a comprehensive system level understanding of plant cell wall degradation mechanisms used by a ubiquitous filamentous fungus.

34. USE OF SIGNAL TRANSDUCTION ENZYME ASSAYS TO GUIDE THE ISOLATION OF ANTICANCER COMPOUNDS FROM ACID MINE WASTE EXTREMOPHILES.

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Microbes isolated from an acid mine waste lake in southwestern Montana have proven a rich source of bioactive secondary metabolites. Berkeley Pit Lake, part of the largest Superfund site in North America, is an acid mine waste reservoir rich in toxic metals. Microbes isolated from the surface waters down to the basal sediment 800 feet below the surface have been grown using a series of physicochemical conditions. The organic and aqueous extracts of these microbial cultures have been tested for biological activity. These assays include assessment of antifungal potential against *Aspergillus* and *Candida* strains as well as *Pneumocystis carinii*, causative agent of PC pneumonia. Extracts are also evaluated as potential enzyme inhibitors of two different signal transduction enzymes that have been implicated in the development and/or metastasis of certain cancers and various autoimmune dis-

eases. Compounds isolated on the basis of their enzyme inhibitory potential have been sent to NIH for evaluation against human cancer cell lines. The isolation and characterization of two of these metabolites isolated from a deep water extremophilic fungus will be described.

35. GENE LIBRARY GENERATION FOR BIOMASS-CONVERSION ENZYMES

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Agricultural and forestry residues are abundant and low-cost sources of stored energy in the biosphere. Biomass conversion into feedstock sugars has moved towards the forefront of the biofuel industry. However, the saccharification of plant biomass is a complicated and lengthy process, mainly due to the inherent recalcitrance and the complex heterogeneity of the polymers comprising plant cell walls. The generation of a library of biomass conversion enzymes, made through heterologous expression and secretion, represents potential of finding best cocktail activities for lignocellulose degradation, thus fostering second-generation ethanol production. Herein we describe heterologous expression and secretion in *Aspergillus awamori* of a set of hemicellulotic enzymes isolated from *A. clavatus*. Based on genome mining data, the hemicellulases genes were targeted for cDNA amplification by polymerase chain reaction (PCR), including two endo-1,4-beta-xylanases, one beta-xylosidase, one arabinofuranosidase, one glucorunosidase, three ferulic acid esterases, one acetyl/xylan esterase, one xyloglucanase, five arabinanases and four laminarinases. All gene models were cloned into pFE2 expression/secretion vector for fungal expression and secretion and transformed into our *A. awamori* production strain. In order to figure out the biochemical action of these enzymes, a comprehensive functional analysis of the cloned enzymes was carried out. Natural polysaccharides were used to determine the enzyme specific activity on the majority of covalent linkage types. Additionally, the hydrolytic breakdown pattern was profiled by capillary zone electrophoresis of 8-aminopyrene-1, 3, 6-trisulfonic acid (APTS)-labeled oligosaccharides and mass spectra of natural polysaccharides digested with enzymes.

36. CHARACTERIZATION OF CELLULOLYTIC ACTIVITIES OF *Bjerkandera adusta* and *Pycnoporus sanguineus* ON SOLID WHEAT STRAW MEDIUM.

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Basidiomycete fungi have the ability to degrade all wood components since they secrete of a variety of enzymes that can oxidize lignin and hydrolyze cellulose and hemicellulose. Much attention has been focused on such activities due to their potential in the production of cellulosic bioethanol. Vegetal biomass is composed of three main polymers, lignin, cellulose and hemicelluloses. Cellulose is organized as glucose polymeric long lineal chains, arranged in different levels of fibrils that are associated by intramolecular interactions. Hemicelluloses, in contrast, are ramified polymers composed of different types of carbohydrates and phenolic compounds, among which xylan, glucan and arabinoglucan form the sugar backbone of the structure. Harsh physical-chemical pretreatments (such as steam explosion, with or without diluted sulfuric acid) are used to loosen lignin and release fibrils of cellulose and hemicellulose, as well as their component monomers. To complement, or even to avoid these costly treatments, cellulose and hemicellulose can be completely saccharified by a number of enzymes due to the synergism displayed by combinations of endo- and exo-glucanases and □-glucosidases. For this reason, it is important to establish the whole cellulolytic capabilities of wood-degrading fungi.

In this work we analyzed the cellulolytic properties of two white rot fungi, *Bjerkandera adusta* strain UAMH 8258 and *Pycnoporus sanguineus* CEIBMD01, originally isolated from two different geo-

graphical locations. *B. adusta*, a basidiomycete well known for its elevated ligninase activity, was isolated from temperate woods of the northern hemisphere (University of Alberta Herbarium, Canada). *P. sanguineus*, another basidiomycete able to grow at moderately high temperatures given its tropical origin was isolated from an oil-polluted environment in the south of the state of Veracruz, México (our laboratory).

Wheat straw solid medium was used to evaluate cellulolytic activities produced by both fungi. Optimum production for *B. adusta* was achieved after 6 days of culture at pH 5 and 28ºC. *P. sanguineus* achieved maximum cellulolytic activity also at pH 5 and 28ºC but at day 7. While *B. adusta* produced more overall cellulolytic activity, *P. sanguineus* cellulases were thermostable, withstanding up to 60 ºC for one hour without losing activity. Both cellulolytic extracts showed good activity in a broad range of pH (4 to 7). Using different substrates (avicel, carboxymethylcellulose, xylans) we could detect endo and exo glucanase activities as well as xylanolytic activities from both fungi.

A cDNA library from *P. sanguineus* was constructed and screened for cellulase sequences using a PCR approach with degenerate oligonucleotides. Partial cDNA clones for an endo glucanase and an exo glucanase were isolated which are currently being studied.

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37. MECHANISMS OF IRON SENSING AND UPTAKE IN *Cryptococcus neoformans.*

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Cryptococcus neoformans causes ~1 million cases of meningitis and ~650,000 deaths per year in AIDS patients. The major virulence factors of the fungus include the production of a polysaccharide capsule, deposition of melanin in the cell wall and the ability to grow at mammalian body temperature. Remarkably, the expression of all of these traits, as well as functions for signal transduction, cell wall biosynthesis and sterol production, are regulated by the GATA-type transcription factor Cir1. This protein also serves as the main sensor protein to regulate iron homeostasis. We found that the stability of the Cir1 protein is influenced by iron levels in *C. neoformans* and in the heterologous host *Saccharomyces cerevisiae*. The availability of iron is an important nutritional feature of the host environment and we found that iron plays a major role in the global regulation of gene expression via its influence on Cir1 stability. To develop a more detailed view of the iron regulatory circuitry, we examined the roles of iron permeases and ferroxidases that are controlled by Cir1 and that in turn regulate iron homeostasis by functioning in high affinity, reductive iron uptake. We found that this system is required for iron acquisition from transferrin and for full virulence. However, mutants with defects in the high affinity iron permease/ferroxidase system still cause disease suggesting that other iron sources such as heme may be utilized by *C. neoformans* during growth in mammalian hosts. We believe that Cir1 also regulates heme utilization via the expression of components of the HAP complex. Deletion mutants lacking components of the HAP complex (*hapX, hap3, hap5*) fail to grow with heme as the sole iron source and have altered expression of virulence factors. This observation reinforces the connection between iron acquisition and virulence. Finally, our transcriptional profiling for *C. neoformans* cells during pulmonary infection implicated glucose as an additional key regulator (with iron) of the metabolic remodeling that occurs in the host environment. Surprisingly, Cir1 also regulates at least part of the influence of glucose on gene expression.

38. WHAT DO WE KNOW ABOUT SILENCING IN *Candida glabrata*?

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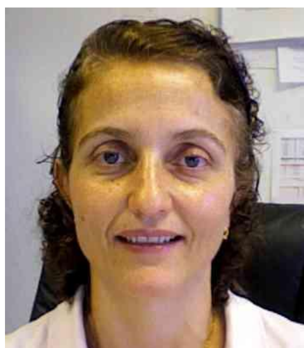

Candida glabrata is a common yeast pathogen of humans that accounts for around 15% of all *Candida* bloodstream infections worldwide. *C. glabrata* is naturally less susceptible to the antifungal drug fluconazole than other *Candida* species; additionally it is phylogenetically more closely related to *Saccharomyces cerevisiae* than to *Candida. albicans*. In *C. glabrata*, like in *S. cerevisiae*, several regions of the genome are subject to transcriptional silencing: the subtelomeric regions for example, are maintained transcriptionally repressed by chromatin based silencing that depends on Sir2-4, Rap1, Ku70/80 and Rif1 proteins; the *S. cerevisiae*, silent mating-type loci (*HML* and *HMR*) also are transcriptionally repressed very efficiently by the formation of a silent chromatin structure nucleated at discreet DNA elements flanking the two silent loci (distinct from the telomere), in a process that depends on the Sir proteins, Abf1, Orc and Rap1.

Subtelomeric silencing in *C. glabrata* plays a role in virulence by regulating the expression of a large family of genes encoding putative cell wall proteins (the *EPA* genes) the majority of which are located close to several telomeres. *C. glabrata* adheres tightly to cultured mammalian epithelial cells and this is mainly dependent on the expression of the adhesin Epa1. *EPA1* gene is located close to the right telomere of chromosome E and is transcriptionally repressed by chromatin-based silencing. Subtelomeric silencing however, varies between subtelomeric regions, and this probably depends on specific sequence elements present at these regions that can confer different requirements for the silencing proteins Sir2-4, Ku70/80 and Rif1. Mutations in any one of these proteins results in increased adherence of *C. glabrata* cells since several of the *EPA* genes are derepressed. In our collection of clinical isolates we found several that display increased adherence to epithelial cells and express different sets of adhesins.
C. glabrata, like *S. cerevisiae*, contains three mating-type like loci (*MTL*), and even though it also contains orthologues of the vast majority of genes involved in mating, it has no known sexual cycle, and strains containing both types of information at the *MTL1* locus are commonly found in clinical isolates. *MTL2* and *MTL3* are localized 29 and 10 Kb respectively from two telomeres on different chromosomes, and in sharp contrast to *S. cerevisiae*, only *MTL3* (and not *MTL2*) is subject to transcriptional silencing that is not completely efficient, resulting in cells that can express two types of mating-type information. The protein requirements for silencing at *MTL3* differ from the ones required for silencing of the orthologous locus *HML* in *S. cerevisiae*, resulting in a different pattern of expression of genes controlled by the transcription factors encoded in these loci.
Subtelomeric silencing is involved in the virulence of *C. glabrata* through regulation of the *EPA* genes, however whether chromatin-based silencing of the genes encoded at the *MTL* loci has an impact in virulence or persistence in the host, is still under investigation.

39. FUNGAL CELL WALL BIOSYNTHESIS AND IMMUNE REGULATION

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The core components of the cell wall are shared by most fungal species and consequently the immune system has evolved to try to detect these molecules to induce protective responses. In the human pathogen *Candida albicans* the cell wall and septal cross-walls are composed of a robust chitin-glucan inner skeleton to which an outer shield of a matrix of highly glycosylated mannoproteins at attached. Emerging evidence that has shown that all of the main components of the *C. albicans* cell wall skeleton and matrix may be recognised, both singly and in combination, by the immune system. However, while some components stimulate immune recognition others attenuate or block it. The composition of the cell wall is also far from fixed, and is modulated during cellular morphogenesis and in response to environmental changes. Therefore the dynamic nature of the cell wall makes it a moving target for the immune surveillance. This presentation will summarise how the fusion of fungal molecular genetics and immunology has led to the systematic dissection of the mechanisms by which cells of the innate immune system grapple and destroy fungal invaders and reciprocally how pathogenic fungi have adapted their cell wall structure create a defensive shield and camouflage jacket to protect themselves from the immunological sentinels of the body.

40. **FUNCTIONAL CHARACTERIZATION OF *Pga13* Y *Pga31*, TWO *Candida albicans* GPI-ANCHORED CELL WALL PROTEINS.**

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Transcription profiling carried out by our group (Castillo *et al.,* 2006) has unmasked a high number of genes that are directly related to the process of protoplasts regeneration. Two of the genes detected, *PGA13* and *PGA31* were found significantly over expressed. Both genes encode proteins that have been localized in the cell wall by immunological techniques and they are linked to the beta-1,6-glucans through a GPI anchor.
Independent homozygous mutants of *PGA13* and *PGA31* were constructed using the URA-blaster method. Both mutants show a higher sensitivity to compounds affecting the cell wall architecture such as the calcofluor white and the red Congo; in addition they present defects in their ability to regenerate a new cell wall. The absence of Pga13 or Pga31 induce an increase of the cell surface hydrophobicity, a better capacity to flocculate, increase of the biofilm formation and modification of the virulence of the cells in a mice system.
Transcription profiling of the mutants *PGA13* and *PGA31* suggest that they are important in the construction of *C. albicans* cell wall but also for other biological processes as lots of other genes are found differentially expressed.

41. **Role of chitin synthases (CHS) from classes V and VII in the mechanism of action of Nikkomycin Z in the infectious cycle of *Coccidioides posadasii***

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Coccidioides immitis and *C. posadasii* are the causative agents of coccidioidomycosis, commonly known as Valley Fever. These soilborne fungi infect healthy mammals when spores are inhaled and cause respiratory symptoms similar to pneumonia. If the host's immune system does not respond, the fungus may spread throughout the body and cause a variety of debilitating symptoms and in some cases death of the host. Valley Fever is endemic in the southwestern United States, along with parts of Mexico, Central and South America. There are an estimated 150,000 new infections per year in the US, of which 100,000 occur in Arizona.
The cell wall of *Coccidioides* spp. is rich in chitin, thus chitin synthases (CHSes) are likely to play an important role in producing morphogenetic changes during the transition from saprobic (soil) to parasitic (lung) growth. *Coccidioides* has seven single CHSes that belong to classes I to VII (Mandel et al, 2006). The role of CHS5 and CHS7 in the life cycle of *Coccidioides* was analyzed via generation of deletion mutants that lack each one of these CHSes, as well as a double mutant that lacks both genes. Preliminary results show that both ΔCHS5 and ΔCHS7 mutants produce aberrant morphologies during mycelial growth, with thickening of the cell wall, balloon-like structures and a reduced growth rate. The ΔCHS5 mutant produces very few conidia. Both mutants are non-infectious in our mouse model system, indicating their importance for the infectious phase. In *in vitro* experiments, both mutants are hypersensitive to Calcofluor White, Congo Red and SDS. Interestingly, both mutants are highly resistant to nikkomycin Z (nikZ), a chitin synthase inhibitor that *Coccidioides* is sensitive to and that is being developed as a new anti-fungal drug for coccidioidomycosis. Our hypothesis is that CHS5 and CHS7 are important for part of the integrity of the cell wall in *Coccidioides* and are also the targets of nikZ. We are exploring whether other CHSes compensate for CHS5 and CHS7 in the mutants to explain the nikZ phenotype.

Symposium
FRONTIERS IN FUNGAL RESEARCH

42. ON HOW THE CELL WALL STRUCTURE AND ITS SYNTHESIZING MACHINERY EVOLVED IN THE MYCOTA KINGDOM

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The most accepted model of the structure of the fungal cell wall has been elaborated based on studies made with ascomycete yeasts, mainly *Saccharomyces cerevisiae*. According to this model, the basic structure of the wall is considered to be made of a lattice constituted by repeating units of □ 1, 3 glucan microfibrils covalently bound to small amounts of chitin, □ 1,6 glucans and two classes of glycoproteins: Pir and GPI. This structure retains non-covalenly bound glycoproteins and other polysaccharides. Since our preliminary evidence on the structure of the wall from the basidiomycete *Ustilago maydis* did not agree with this model, we started an *in silico* study to analyze the phylogenetic relationships of the enzymes responsible for the synthesis of different wall polysaccharides, and the covalently bound proteins that make up the fungal cell wall in members of the different fungal *phyla*. The results obtained provided evidence that the model above described could be applied to ascomycetes only. Perhaps more interesting, they allowed envisaging a possible pathway to explain the steps occurring in the differentiation of the fungal cell wall, suggesting that its structure in the different fungal *phyla* constitutes a recapitulation of the evolutionary history of the compounds that make it up. According to our results, the following hypothetical series of events in fungal wall evolution may be suggested: *1*) Construction of an archaic wall made of the structural components chitin and chitosan, that formed a primitive girdle that retained non-covalently bound proteins in the periplasm and allowed cell growth in hypotonic media; *2*) duplication and differentiation of genes encoding the several polysaccharide synthases, *3*) horizontal transfer of some genes encoding synthesizing enzymes, *4)* development of systems for the selective regulation of specific synthases during the fungal life cycles, *4*) changes leading to modifications of the synthesized products, and *5*) acquisition of the capacity to associate different proteins through covalent linkages.

43. MYCOTOXINS, SICK BUILDINGS AND THE MOLDS OF HURRICANE KATRINA.

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Mycotoxins are a heterogeneous group of low molecular weight secondary metabolites, toxic to vertebrates in low concentrations, which constitute a global problem in agriculture. Most mycotoxins exposure occurs through the consumption of contaminated foods and feeds; dermal and inhalation exposure are less common. Sick building syndrome is a loosely defined condition thought to be associated with poor indoor air quality. Molds and their toxins are implicated in the etiology of sick building syndrome but the physiological basis of their involvement remains conjectural and in many cases mycotoxin exposure is not proven. The widespread flooding in New Orleans after Hurricane Katrina provided a living laboratory for studying indoor molds and their possible health effects. Over forty strains of fungi were isolated from a flooded home. Most isolates were species of *Aspergillus*, *Penicillium* and *Trichoderma.* Volatile organic compounds (VOCs) produced by selected Katrina molds grown in microcosms on common building materials were identified. Biological assays for mixtures and single standards of mold VOCs were developed using *Arabidopsis thaliana*, *Caenorhabditis elegans* and *Drosophila melanogaster*. At low concentrations, several major fungal VOCs displayed activity in all

three systems. Fungal VOCs bleached *A. thaliana* and caused behavioral and toxicity effects in both worms and flies. Using green fluorescent protein in a construct that drives the tyrosine hydroxylase gene in transgenic *D. melanogaster*, low concentrations 2, 5 dimethylfuran (DMF), trans-2-octenal, 2-octenone, and 1-octen-3-ol were associated with loss of expression of dopaminergic neurons. Different VOCs had different effects. These studies provide biologically plausible mechanisms for some of the wide range of symptoms associated with building related illness.

44. CYTOSKELETAL, NUCLEAR AND SIGNALLING DYNAMICS DURING COLONY INITIATION IN *Neurospora crassa.*

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Colony initiation in *Neurospora crassa* involves the formation of two types of hyphae: germ tubes that are involved in colony establishment, and conidial anastomosis tubes (CATs) that fuse to form interconnected networks of conidial germlings. These two hyphal types are morphologically and physiologically distinct and under separate genetic control. The interconnected state of conidial germlings allows the young fungal colony to operate as a coordinated individual and to regulate its overall homeostasis.

Using the anti-cytoskeletal drugs (benomyl, latrunculin A and latrunculin B), we have shown that germ tube formation is dependent on both microtubule and F-actin function but that CAT fusion only requires F-actin. Live-cell imaging of F-actin dynamics has been achieved using Lifeact fused to either GFP or TagRFP. Lifeact is a 17 amino acid peptide derived from the non-essential *Saccharomyces cerevisiae* actin binding protein Abp140p, and is an outstanding live-cell probe for F-actin. F-actin cables and patches localized to sites of active growth during the establishment and maintenance of polarized growth of germ tubes and CATs. Recurrent phases of formation and retrograde flow of complex arrays of actin cables were observed at growing germ tube and CAT tips. Following fusion, actin cables disappeared from the non-growing, connected CATs but actin patches were retained at sites of fusion suggesting that they play a role in endocytic recycling at this location.

Nuclear behaviour and mitosis were imaged in ungerminated macroconidia, germ tubes and during CAT fusion using H1 histone and microtubules labelled with GFP. Mitosis occurred more slowly in non-germinated macroconidia (1.0-1.5 h) than in germ tubes (15-20 min) at 25°C. The nucleoporin SON-1 was not released from the nuclear envelope during mitosis, suggesting that *N. crassa* exhibits a form of 'closed mitosis'. During CAT homing, nuclei did not enter CATs and mitosis was arrested. Benomyl treatment and the analysis of three *ropy* mutants showed that nuclear migration through fused CATs does not require microtubules, dynein/dynactin or mitotic division.

CAT fusion can be divided into a succession of events: CAT induction; CAT chemoattraction; and cell-cell adhesion, cell wall breakdown and membrane merger between CATs. Mutant screening has identified a number of signalling pathways involved in CAT induction, homing and fusion. A novel form of signaling involving the oscillatory recruitment of signaling proteins to CAT tips that are homing towards each other is observed. This 'ping pong' mechanism operates over a very short time scale and probably does not involve transcriptional regulation. It is proposed that this spatiotemporal coordination of signaling allows genetically iden-

tical cells to avoid self-stimulation and coordinate their behavior to achieve self-fusion. It is further suggested that this mechanism has evolved to increase the efficiency of fusion between genetically identical cells that are non-motile.

45. THE FASTEST FLIGHTS (AND ROTATIONS) IN NATURE: FUNGAL SPORE DISCHARGE AT ONE MILLION FRAMES PER SECOND

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Fungi are the evolutionary champions of fast movement and use a variety of microscopic devices to propel themselves into the air. These include pressurized cannons that squirt streams of spores at accelerations of up to 180,000 *g*, the explosive membrane that launches the artillery fungus, and a catapult powered by surface tension that discharges mushroom spores. In the last two years, technological advances in high-speed video microscopy have allowed unprecedented insights into the operation of these astonishingly beautiful mechanisms. This presentation will showcase these experiments, screen some wondrous videos, and explain their wider significance.

Abstracts of Poster Sessions

POSTER SESSIONS

Poster Session I- From 46 to 112
Poster Session II- From 113 to 180

SIGNAL TRANSDUCTION AND METABOLISM

46. CENTRAL METABOLISM OF *Ustilago maydis* HAPLOID CELLS.

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The 10 glycolytic enzymes activities, Piruvate Dehidrogenase and some TCA cycle enzymes were determined in crude extracts of *Utilago maydis* haploid cells. Enzyme activities in yeast like form grown in minimal medium and harvested in the stationary stage were two fold higher than those from yeast grown in rich medium. By the contrary, yeast harvested in exponential stage, the enzyme activities were higher in cells grown in rich media. The conventional enzymes which control the glycolytic flux were similar in all conditions. Phosphofructokinase activity was the lowest in the four growth conditions.*U. maydis* aldolase was found belongs to the class II type of metalo-aldolases and 3-Phosphoglycerate mutase (PGAM) activity was 2,3- bisphosphoglycerate cofactor independent, which contrasted with the cofactor dependency predicted by the amino acid sequence alignment analysis. About TCA cycle, our results shown that PDH, IDH, succinate DH and KGDH have the lower specific activities, contributing to the control of Krebs cycle. This is the first report of central metabolism in *U maydis* with the intention to evaluate the metabolic flux in this organism.

47. STUDY OF THE BIOCHEMICAL AND PHYSIOLOGICAL FUNCTION OF THE *Odc*1 GENE FROM THE ENTOMOPATHOGENIC FUNGUS *Metarhizium anisopliae* by HETEROLOGOUS COMPLEMENTATION IN *Yarrowia lipolytica*.

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Metarhizium anisopliae is an entomopathogenic fungus that invades its host directly through the exoskeleton using mechanical pressure and a battery of lytic enzymes. Conidia germinate on the surface of the insect to differentiate and form an adhesion structure called appressorium. The infection hypha penetrates the host cuticle to reach the haemocele, spreading and infecting the insect. The formation of the appressorium appears to be an important event in the process of differentiation and pathogenicity of the fungus to invade its host. In the formation of this structure, the ornithine decarboxylase activity (ODC) could have a significant role. ODC is a key enzyme of the synthesis of polyamines; these compounds have an important role in differentiation in a number of organisms like fungi, plant and mammalian. The yeast *Yarrowia lipolytica* has received increasing attention as a model to study dimorphic transition because of its ability to alternate between a unicellular yeast form and distinct filamentous forms (hyphae and pseudohyphae). In . *lipolytica,* a strain deleted in the Odc1 gene (strain FJOD) the dimorphic response is affected, this mutant grows only as yeast like cell, even in the presence of the

mycelia inductor N-acetyl glucosamine, unless polyamines are added to the culture medium. In our group, we are interested to study the physiological role of the Odc1 gene from *M. anisopliae* (*Ma*Odc1). For this purpose, the cDNA from the *Ma*Odc1 gene was cloned under the control of the promoter CUP1 and used to transform the Δodc . *lipolytica* mutant. The results showed that the phenotype mutant of Δodc . *lipolytica* was complemented by *Ma*Odc1 gene. The transformant was able to grow and form mycelium as the wild type strain, in contrast to the mutant strain, which was unable to grow and differentiate. Moreover, the *Ma*Odc1gene restored the ornithine decarboxylase activity. These results indicate that the *Ma*Odc1p is functional in . *lipolytica* complementing the proper functions of the *Yl*Odc1p: biochemical ornithine decarboxylase activity and participation in growth and dimorphism yeast-hypha process. These observations suggest that this gene (*Ma*Odc1) could be participating in differentiation processes in *M. anisopliae*.

48. DELETION OF THE *Aspergillus nidulans sumO* GENE INDIRECTLY PREVENTS AREA NUCLEAR ACCUMULATION IN RESPONSE TO NITROGEN STARVATION.

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In *Aspergillus nidulans,* nitrogen metabolic gene expression is activated by the GATA transcription factor AreA under conditions of nitrogen nutrient limitation and starvation. During nitrogen starvation, but not in the presence of nitrogen nutrients, AreA accumulates in the nucleus and AreA-dependent gene expression is elevated. We have previously demonstrated that the *sumO* gene encodes a small ubiquitin-like modifier peptide, which is covalently attached to a wide variety of proteins potentially modifying their localization and/or function. The AreA nuclear export signal (NES) overlaps a predicted sumoylation site and we investigated whether sumoylation of this site regulates AreA subcellular localization. In a *sumO* deletion mutant, AreA nuclear accumulation is not observed. However, mutation of the putative SUMO attachment residue does not affect AreA nuclear accumulation. Furthermore, sumoylation of AreA was not detectable. Therefore, it is unlikely that SumO directly affects AreA nuclear export. In *Saccharomyces cerevisiae,* it was shown that the AreA homolog Gln3p is imported into the nucleus by the alpha-importin Srp1p and the SUMO peptide Smt3p is required for Srp1p recycling to the cytoplasm. We have investigated whether overexpression of the Srp1p ortholog KapA suppresses the effect of *sumO* deletion on AreA nuclear accumulation. Our findings suggest that the effect of SumO on AreA nuclear accumulation is at the level of nuclear import via KapA.

49. THE ROLE OF GLYCEROL IN HALOTOLERANCE OF *Debaryomyces hansenii* WHEN GROWING AT ELEVATED BUT CONSTANT SALINITY.

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Survival to high salinity environments in tolerant species is accomplished by the appropriate accumulation of compatible solutes, that is, organic molecules acting as osmolytes, whose chemical nature has a minimal effect on cell function. In yeasts, glycerol plays such a role, behaving as an osmometer, as its concentration varies in direct proportion to the external osmolarity. Exposing yeast cells to elevated but constant salinity conditions, posses a fundamental question as to the need to synthesize glycerol for driving in such environments. In order to answer this question, the growth and glycerol accumulation were followed in halotolerant *Debaryomyces hansenii* yeast cells exposed to a constant salty environment for 10 generations. The presence of glucose, by signaling favorable conditions for growth, initiates the flux of carbon that is partly diverted to glycerol synthesis, in order to compensate the osmotic imbalance between the cell and its surroundings. The rate of conversion of glucose into glycerol seems to diminish as the salinity of the medium increases, which can impose a tolerance limit for the

cells. As the culture ages, a tendency for glycerol synthesis to increase can be appreciated, probably related to a lower capacity of the cells to accumulate other osmo-protective molecules. The results show the fundamental role played by glycerol in osmoregulation, being a necessary condition for *D. hansenii* to complete its cell cycle, even when growing at constant salinities.

50. THE GENE *Odc1* FROM *Metarhizium anisopliae* CODIFIES FOR A PROTEIN WITH ORNITHINE DECARBOXYLASE ACTIVITY

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Metarhizium anisopliae is a mitosporic entomopathogenic fungus used for biological control of agricultural pests. This fungus is one of the best-characterized enthomopathogens and parasitizes more than 300 species of insects, including vectors of human's diseases, such as *Anopheles gambiae* (Roberts and St Leger 2004, Scholte et al 2004). *M. anisopliae* infects its hosts by direct cuticle penetration. The process begins with conidia arrival to insect surface, followed by germination and differentiation to form a specialized adhesion structure called appressorium. From appressorium, emerges the infection hyphen, which penetrates through the insect cuticle to reach the hemocoel. In *Beauveria bassiana* and *M. anisopliae* the formation of the appressorium could be pivotal in establishing a pathogenic relationship with the host (Clarkson and Charnley 1996). In our group we are interested in the differentiation events taking place during the *M. anisopliae* invasion to the insects. In this sense, we isolated a sequence of 2630 bp from *M. anisopliae* strain CARO19, which codes a 447 amino acids protein with high homology to ornitinhe decarboxylases (ODC) mainly those from *Neurospora crassa* and *Oculimacula yallundae*. ODC is a key enzyme of the synthesis of polyamines; these compounds have an important role in differentiation in a number of organisms like fungi, plant and mammalian (Jimenez-Bremont *et al.* 2006). In this work, we manipulated the putative *Ma*odc1 gene for over-expression in a heterologous system for its characterization. The cDNA from the putative *Ma*odc1 gene was recovered by RT-PCR and cloned in the pRSETB vector (Invitrogen). This vector adds to the expressed protein six histidine residues, useful for its purification. The recombinant plasmid was introduced in *E. coli* BL21pLYS. The cells were induced with 1 mM IPTG for protein over-production. Over-expression of a 45-kDa protein was observed in a polyacrilamide gel electrophoresis. Then, the Odc-His tagged protein was purified using Ni-agarose columns. The purified Maodc1p protein was eluted using imidazole. With the purified protein, ODC activity was assayed and some properties of the protein obtained. The optimum parameters for ODC activity were: pH 8.5, temperature 37 °C, affinity constant (Km) to the substrate, ornithine, Km of 22 μM. These results demonstrated that the putative *Ma*odc1 gene codifies a protein with ornithine decarboxylase activity.

51. AN UNUSUAL STRUCTURE OF THE LOCUS CONTAINING THE TELOMERASE REVERSE TRANSCRIPTASE CATALYTIC DOMAIN IN *Ustilago maydis.*

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Telomere is a nucleoproteic complex consisting of telomeric-repeats and telomere specific proteins. This structure shelter the end-most terminal sequences of each chromosome from exonucleolytic degradation, recombination, and fusion. Telomeric DNA is dynamic by nature, it goes through rounds of shortening and lengthening as cell proceeds through several rounds of cell cycle. Telomerase is a enzyme responsible for telomere lengthening. It is a ribonucleoprotein whose essential core is composed by the telomerase reverse transcriptase catalytic subunit (TERT) and by the RNA template for telo-

mere synthesis. We found that in *Ustilagomaydis* the hypothetical TERT (*utert*) is encoded by the locus UM00761.1. Surprisingly, this locus has an unexpected annotation: it encodes one transcript that renders a single open reading frame 1931 aa residues long that includes a region containing the canonical TERT motifs fused to a fragment showing similarities to CDK1; utert is encoded in exon1 and CDK1 sequences in exon2 (we named *tck2* this second reading frame). The similarity to CDK1 is to several CDK1 from diverse fungi, and to CDK11 (PITSLRE) cyclin dependent kinases, a protein involved in microtubule stabilization and mitotic spindle assembly in mammals. Since we are pursuing the characterization of *utert* we were eager to verify the structure the UM00761.1.*In silico* analysis on the chromosomal regions surrounding TERT in several taxa revealed that the disposition of TERT fused to a putative CDK1 might be unique to*U. maydis*, since it is not present in any of the genomes that were analyzed. Transcript analysisby RT-PCR from wild type and 521^{-utert} disrupted mutants showed transcription spanning exons 1 and 2 in the wild type strain as well as transcripts corresponding to *tck2*in the 521^{-utert} disrupted mutant. Preliminary qRT-PCR assays on cDNA isolated from synchronous cultured cells shows differences in transcript abundances and expression timing during the cell cycle at sporidia. These results suggest that *utert* and *tck2* are contained in the UM00761.1 *locus* and that independent transcription for *utert* and *tck2* reading frames and/or that apost-transcriptional regulation is occurring at the *locus*.

52. FUNCTIONAL ANALYSIS OF *utert*, THE TELOMERASE REVERSE TRANSCRIPTASE CATALYTIC SUBUNITOF*Ustilago maydis*.

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We are using the basidiomycetous fungus *Ustilagomaydis*as a model to study telomere metabolism. The telomeric hexanucleotide repeated-motif of *U.maydis* $(TTAGGG)_{37-39}$ is identical to repeated motif present in vertebrates and several filamentous fungus, as in these organisms an autoradiography pattern of telomere termini consists of large smears of hybridization signals, indicating the existence of telomerase-synthesized telomeres. The hypothetical telomerase reverse catalytic subunit (TERT), named *utert*, is encoded the UM00761.1 locus. It harbors a 6528 bp gene composed by three exons of 4006, 1772, and 18 bp put aside by two introns of 712 and 18 pb. UM00761.1 encodes a hypothetical product of 1931 aa residues that combines the predicted product of the first exon, which share homology to all TERT motifs reported to date from several organisms, and that of the second exon, that harbors an open reading frame homologous to several CDK1 from diverse fungus. Gene-disrruption experiments on strains 520 and 521 using sequences from first exon (*utert*)*,* generated telomere shortening and strains delayed in growth at their early 180 rounds of replication. As seen first in *Saccharomyces-cerevisiae*, survivors rise promptly by an alternative-like mechanism no yet characterized in *U. maydis*. In order to assess infection phenotypes we compared wild type *U.maydis*strains 520 x 521with *utert* mutants 520^{-utert} x 521^{-utert}. Infections were carried out in five days old maize seedlings in green house, 14 hours of daylight at 28° to 30°C. Analysis of the infected seedlings indicates that symptoms appear likewise for both wild-type and mutants crosses at 5 –7 days after inoculation. Then, life cycle was accomplished at days 13 to 15 for wild-type strains, rendering mature galls, gray and full of black masses of teliospores. In contrast, mutant strains showed a slowdown of at least 15 days before some teliospores occasionally appear into the galls; in general, the galls were apparently devoid of teliospores. Phenotypes and telomere lengths determinations are currently assessed. The possible involvement of *utert* in teliospore maturation and completion of life cycle will be discussed.

53. ANAEROBIC ELEMEMTAL SULFUR REDUCTION BY *Aspergillus nidulans*

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Hypoxia (oxygen (O_2) depletion) imposes a challenge on most fungi since O_2 serves as the terminal electron acceptor, and induces various metabolic mechanisms. Reduction of elemental sulfur (S^0) to hydrogen sulfide (H_2S) by the fungus *Fusarium oxysporumi* is such a mechanism that we previously found. Here, we investigated hypoxic S^0 reduction mechanism by the fungus *Aspergillus nidulans*. When adding S^0 to the culture medium, the fungus accumulated sub-micromolar levels of H_2S in the culture medium, indicating that *A. nidulans* reduced S^0 to H_2S. Growth of the glutathione reductase (GR) gene disruptant was sensitive to various oxidants, indicating that GR constituted antioxidant system like other fungi. We also found that the gene disruptant produced lower levels of H_2S than wild type strain (WT) under the hypoxic S^0-reducing conditions. Recombinant GR (rGR) showed NADPH-GR activity. Furthermore, NADPH-S^0 reductase activity was re-constituted by using rGR and GSSG. These results indicated that GR contributed to the hypoxic S^0 reduction by *A. nidulans*.
Next we cultured the fungus in the presence of S^0 under normoxic and hypoxic conditions, resolved intracellular protein by two-dimensional electrophoresis, and identified 300 spots by using MALDI-TOF/MS. Proteins up-regulated over 2-fold under the hypoxic conditions included a hypothetical protein involved in thioredoxin reductase superfamily (TrrX, AN3963.3). Expression of *trrX* was induced in the hypoxic cells cultured with S^0. Absorption spectra of recombinant TrrX (rTrrX) indicated that it was a flavoprotein and was reduced by thiolate reagents such as dithiothreitol and GSH, while none of NADH and NADPH reduced rTrrX, which was consistent with lack of pyridine nucleotide-binding motif in the predicted TrrX sequence. We constructed the *trrX* gene disruptant and found that it produced less H_2S than WT, indicating that *trrX* was critical for reducing S^0. Growth of the gene disruptant was unaffected by oxidants. Our results indicated that both GR and TrrX constituted the fungal S^0-reducing mechanism under the hypoxic conditions.

54. DELETION OF THE UNIQUE GENE ENCODING THE *Yarrowia lipolytica* PKA CATALYTIC SUBUNIT REVEALS ITS REGULATORY ROLE IN DIMORPHISM AND MATING

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Fungal dimorphism, the capacity to alternate between yeast-like and mycelium forms, has been considered an attractive model to study cell differentiation. Besides, this phenomenon is a common trait observed in numerous fungal pathogens. This morphological switch is regulated by several environmental conditions including pH, temperature carbon or nitrogen source, as well as different compounds. The stimulus generated by these inducers is internalized into the cells mainly through the MAPK and PKA signaling pathways. In the dimorphic fungus *Yarrowia lipolytica* our previous results suggested opposite roles of these signaling cascades in the dimorphic transition. To further study the participation of PKA in this phenomenon, we conducted an *in silico* analysis to identify the gene(s) encoding the PKA catalytic subunit, demonstrating the existence of a single gene with the corresponding attributes, that we named *TPK1*. The gene was isolated and disrupted using the Pop in-Pop out technique. *TPK1* was not regulated at the transcriptional level, but PKA activity was found to increase during yeast-like growth. *tpk1* null mutants were viable and without growth defects, but were more sensitive to different stress conditions, and their mating capacity was reduced. Interestingly, mutants grew constitutively in the mycelium form. On the other hand, we had observed that mutants lacking the MAPKKK function (s*te11*) were unable to grow in the mycelium form. This same phenotype was

shown by the double mutants *ste11/tpk1*, indicating that this is the default growth pattern of the fungus. Our data confirm that the MAPK and PKA pathways operate in opposition during the dimorphic behavior of . *lipolytica*, but synergic in mating. These findings stress the hypothesis that in different fungi both signal transduction systems may operate distinctly, and be antagonist or synergic in the cell responses to different stimuli.

55. MOLECULAR STUDIES ON pH REGULATION IN CEPHALOSPORIN C PRODUCTION BY *Acremonium chrysogenum.*

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The pH plays an important role in determining transcription levels of many fungal genes, whose products work at the cell surface or in the environment; there is evidence that genes regulated by the environmental pH include those encoding proteins related with the β-lactam antibiotics biosynthesis. In filamentous fungi, the transcription factor PACC mediates gene expression in response to environmental pH. Besides, previous studies in our laboratory also indicate the need of keeping the pH of *A. chrysogenum* cultures in a narrow neutral to alkaline range to get higher cephalosporin C (CPC) yields.
To verify the effect of recombinant-DNA mutations in the *pacC* gene of *A. chrysogenum,* two constructions were used to trigger the effect of alkalinity-mimicking mutation in a middle-level CPC producer strain. The C10 strain and its transformants showed similar trends in pH kinetics, and although most of the transformants showed maximum yields lower than that of the control strain, some of them showed higher production levels. Specifically, the CP17 strain produced 55% more CPC and 118% more total antibiotics than the control strain in acid-pH liquid cultures. A steady and exponential increase of total antibiotics specific production by the transformant strain was observed, suggesting that the introduction of plasmid pLpacC1 generated the desired effect of alkalinity mimicry and might have led to the increased transcription of, at least, the genes involved in the first part of the antibiotic biosynthesis pathway. Therefore, based on the increased CPC yield shown by the alkalinity-mimicking *A. chrysogenum* mutant, a new genetic improvement strategy for this antibiotic production is proposed.

56. THE CCAAT-BINDING COMPLEX COORDINATES THE OXIDATIVE STRESS RESPONSE IN *Aspergillus nidulans*.

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The heterotrimeric CCAAT-binding complex is a global regulator in all eukaryotes, but the regulation of its activity is still poorly understood. The *Aspergillus nidulans* CCAAT-binding factor (AnCF) consists of the subunits HapB, HapC and HapE and senses the redox status of the cell via oxidative modification of thiol groups within the histone fold motif of HapC. Mutational and *in vitro* interaction analysis revealed that two of these cysteine residues are indispensable for stable HapC/HapE subcomplex formation, high affinity DNA-binding and proper nuclear import of AnCF. Oxidized HapC is unable to participate in AnCF assembly and localizes in the cytoplasm, but can be recycled by the thioredoxin system *in vitro* and *in vivo*. In this study, we demonstrate that the central transcription factor AnCF is regulated at the post-transcriptional level by interconnected feedback loops with the peroxide sensor NapA. AnCF represses full expression of *napA* and some NapA target genes. Oxidative stress inactivates AnCF via oxidation of HapC, which increases expression of *napA* and NapA target genes

directly (via release of AnCF repression) and indirectly (via NapA activation). This response includes the activation of the thioredoxin system, which represses NapA activity and reactivates AnCF. The coordinated activation and deactivation of antioxidative defense mechanisms, i.e., production of enzymes such as catalase, thioredoxin or peroxiredoxin, and maintenance of a distinct glutathione homeostasis very likely represents a evolutionary conserved regulatory feature of the CCAAT-binding complex in eukaryotes.

57. DETERMINATION OF EXPRESSION OF THE GENE FOR A MANNOSYLTRANSFERASE OF *Sclerotium cepivorum* Berk DURING GROWTH IN VARIOUS CULTURE MEDIA

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Mexico ranks among the ten top garlic (*Allium sativum*) producing nations worldwide, and the state of Guanajuato is one of the leading producers in Mexico. Production of garlic has been diminished by the disease called white rot, caused by the fungus *Sclerotium cepivorum* Berk. This fungus forms resistance structures called sclerotia, able to survive under unfavorable conditions. The prevalence of these structures creates a problem for disease control.
In order to control the disease, we must know more about the developmental biology of S. *cepivorum*. In this work we decided to determine the levels of a mannosyltransferase (MNT) expression in various growth conditions of this fungus, since it has been reported that the expression of some MNTs are involved in cell wall formation and virulence of some pathogenic fungi.
Primers were designed based on the Kre5 gene sequence corresponding to a mannosyltransferase of S. *sclerotiorum*. We amplify the full frame and an internal fragment of the gene, and confirmed their homology with the Kre5 gene. Internal primers of the Kre5 gene were used to determine expression level at different incubation time; mycelium samples were taken at 3, 5, 7 and 10 days of growth, finding maximum Kre5 gene expression at 3 days of growth.
Expression level of the Kre5 gene of *S. cepivorum* was also analyzed, when the fungus was growing in minimal medium with glucose or garlic as carbon source. We found that when *S. cepivorum* is grown in minimal medium, Kre5 gene transcript expression is greater, than when it was grown in rich medium. When garlic was used as a carbon source, expression levels were minimal as compared to growth with glucose.
These data suggest that we should carefully analyze the regulatory region of this gene, which it seems to be involved in mycelium growth and not in the formation of sclerotia of *S. cepivorum* Berk,
Financial support: CONACYT Ref. 83414, and DAIP, U. de Guanajuato.

58. EFFECT OF GENE SILENCING OF ORNITHINE DECARBOXYLASE (ODC) IN THE FORMATION AND DEVELOPMENT OF SCLEROTIA OF *Sclerotium cepivorum* Berk.

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Sclerotium cepivorum Berk is a pathogenic fungus of genus *Allium* plants and is the causal agent of garlic white rot, a disease that impacts negatively on the production of this vegetable, causing significant economic losses for producers in the Bajio region of central Mexico. The control of *S. cepivorum* is a major challenge, mainly due to the substantial ability of the fungus to differentiate, forming resistance structures called sclerotia, which can remain viable in soil for over 20 years.
The study of cell differentiation has demonstrated that ornithine decarboxylase (ODC), the initial enzyme of the biosynthetic pathway of polyamines, and responsible for the synthesis of putrescine, plays an important role in differentiating and development events in all organisms analyzed. Polyamine bio-

synthesis in bacteria, plants and mammals uses a complementary route, via agmatine. However, studies on fungal pathogens suggest that the only route of putrescine synthesis is mediated by ODC.
The objective of this study is to determine whether the expression of the ODC gene plays a role in the formation and development of sclerotia of *S. cepivorum*. To this end, we intend to use RNAi to reduce protein expression levels and determine whether there is any effect on sclerotia formation.
Primers were designed to amplify an internal fragment of the ODC gene in sense and antisense orientations; fragments were sent to be sequenced; determination was made of gene expression at different days of growth by RT-PCR, finding that the gene of interest is expressed during the mycelial growth phase. The number of gene copies was determined by Southern blot and, subsequently, using the aforementioned primers, internal fragments were cloned in sense and antisense orientations into the pSilent-1 vector.
The results of the transformation of *S. cepivorum* with the vector pSilent-1-ODC were presented using the protoplast formation technique, also presenting ODC gene expression levels in the transformants and ability to form sclerotia as a function of time.
The study was supported by grants DAIP, U. de Guanajuato & CONACYT-SAGARPA 2005-11919, Mexico.

59. MITOCHONDRIAL CONTROL OF LIGNIN PEROXIDASE PRODUCTION IN *Phanerochaete chrysosporium.*

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Phanerochaete chrysosporium is a fungal model able to degrade a wide range of toxic compounds and complex nutrients such as lignin. One feature of this fungus is the production of a wide array of extracellular enzymes such as lignin peroxidases (LIPs). The induction of these enzymes has been at least in part attributed to reactive oxygen species (ROS); however, the mechanism by which this induction occurs is still an open question. By focusing on the physiological properties of the fungus we show that LIPs are neither regulated by nitrogen starvation signalling inside the cell, nor by the nitrogen catabolic repression. We have observed a strong enhancement of the alternative respiratory pathways in ligninolytic conditions. The main complexes of the respiratory chain were not impaired and the alternative pathways are rather controlled by an integrative regulation system in response to a signal of stress, bypassing complex I and III, the main mitochondrial ROS producers. These major changes in mitochondrial respiration could be connected to metabolic signalling pathways, which also trigger nuclear gene expression responses. This mechanism has been called Mitochondrial Retrograde Regulation (MRR). In our study, both the enhancement of the alternative pathways and the maintenance of the glutamate pool in the fungus grown under ligninolytic condition are consistent with such a regulation in *P. chrysosporium*. We suggest that LIP induction could follow the MRR through ROS signalling.

60. Gln3p COULD REGULATE THE EXPRESSION OF ZINC TRANSPORTER GENES IN *Saccharomyces cerevisiae*

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Saccharomyces cerevisiae grows in a wide variety of nitrogen sources, but its growth rate differs due to the regulatory systems that keep some genes in their basal states when cells grow in a good nitrogen source. In glutamine, for example, some genes are regulated by a mechanism known as NCR (Nitrogen Catabolite Repression), and their expression is low under this condition. Instead, when cells are grown in proline, a poor nitrogen source, those genes that encode for permeases and catabolite enzymes for poor nitrogen sources are generally transcribed at high levels.
NCR gene transcription mainly depends on GATA factors, Gln3p and Gat1p that bind to promoters in their target genes. When cells are growing in a good nitrogen source Gln3p, is retained in cytoplasm by a protein called Ure2p. In proline, Gln3p enters into the nucleus and increases the transcription of their target genes.
Microarray experiments performed to analyze gene expression patterns of *ure2*Δ mutant versus a wild type strain grown in asparagine, a good nitrogen source, showed unexpectedly that some genes encoding for vacuolar zinc transporters were induced in the mutant strain. Furthermore, *ure2*Δ mutant grown in glutamine, glutamate, ammonia or proline is more resistant than the wild type strain to 15 mM of zinc sulphate. Nevertheless, to date the transcriptional regulation mechanism of zinc transport in response to different nitrogen sources is not known, nor the possible interaction between Gln3p and/or Ure2p regarding to cell zinc resistance.
The results of this work show that Ure2p is a negative regulator of zinc resistance in cells grown in all nitrogen sources analyzed. Gln3p seems to positively regulate the transcription of *ZRC1* even when the cells are grown in a good nitrogen source as glutamine. These data suggest that Gln3p, a nitrogen transcription factor, could regulate the expression of some zinc transporter genes.

61. PROTEOMIC ANALYSIS OF *Aspergillus nidulans* CULTURED UNDER HYPOXIC CONDITIONS

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The fungus *Aspergillus nidulans* reduces nitrate to ammonium and simultaneously oxidizes ethanol to acetate to generate ATP under hypoxic conditions in a mechanism called ammonia fermentation (Takasaki, K., *et al. J. Biol. Chem.,* 279, 12414-12420 (2004)). To elucidate the mechanism, the fungus was cultured under normoxic and hypoxic (ammonia fermenting) conditions, intracellular proteins were resolved by 2-DE, and 332 protein spots were identified using MALDI-TOF-MS after in-gel tryptic digestion. Alcohol and aldehyde dehydrogenases that play key roles in oxidizing ethanol, were produced at the basal level under hypoxic conditions but were obviously provoked by ethanol under normoxic conditions. Enzymes involved in gluconeogenesis, as well as the TCA and glyoxylate cycles, were down-regulated. These results indicate that the mechanism of fungal energy conservation is altered under hypoxic conditions. The results also showed that proteins in the pentose phosphate pathway as well as the metabolism of both nucleotide and thiamine were up-regulated under hypoxic conditions. Levels of intracellular xanthosine and other low molecular weight nucleotides were increased in hypoxic cells, indicating an association between hypoxia and pentose (nucleotide) metabolism. Furthermore, enzymes involved in the biosynthesis of branched-chain amino acids were up-regulated. We found that the fungus *A. nidulans* excretes branched-chain amino acids into the culture medium under the hypoxic conditions. These results strongly suggested that a *de novo* biosynthetic mechanism for branched-amino acid synthesis was activated, which is consistent with hypoxic accu-

mulation of the branched-chain amino acids. This study is the first proteomic comparison of the hypoxic responses of *A. nidulans*.

62. DETECTION AND FUNCTION OF THE HOG PATHWAY (HIGH-OSMOLARITY GLICEROL) IN *Ustilago maydis*

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Ustilago maydis is a basidiomycete fungi with dimorphic properties and like this organism there are other fungi with the same capacity of growing as yeast or mycelium, an example of this would be *Candida albicans*, in this organism the molecular mechanisms involved in the dimorphism are well known. The HOG pathway (high-osmolarity glicerol) is described as the mainly responsible of the morphogenesis when it means of osmotic stress adaptation, this route is composed by a MAP kinases signaling cascade that starts with a membrane receptor (Sho1), and with it stimulation triggers the respective phosphorylation pathway, the transcriptional factor Hog1 is inducible in presence of osmotic agents and it is repressed by feedback inhibition when it is not exposed to these conditions, when Hog1 is phosphorylated as the last step of the HOG pathway it leads to the activation of genes related to osmotic stability.
On this study we identify the HOG pathway genes Sho1 & Hog1 in *Ustilago maydis* by bioinformatics methods, first we designed an *in silico* probe to find sequences of the respective genes by alignments made on Sho1 and Hog1 homolog's reported in other fungi, with this, we were able to locate the exact position of these genes sequences, after this we designed specific primers to evaluate their expression levels meanwhile growing *U. maydis* on different media conditions like pH and nutritional variation by RT-PCR, the results show that there are notorious changes on the expression levels of the two genes when they are exposed to pH variations more than the nutritional ones, leading to the conclusion that regulation of the HOG pathway in *Ustilago maydis* has changes compare to another ascomicetes whose HOG regulation is closely related to osmotic stress. To our knowledge this is the first report of the presence and response of the Sho1 & Hog1 system in *Ustilago maydis* and it opens the opportunity to evaluate the HOG pathway relevance in this important basidioycete.

63. CpsA A PROTEIN WITH TWO ADENYLATION DOMAINS IS REQUIRED FOR *Aspergillus nidulans* ASEXUAL DEVELOPMENT

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The *cfwA/npgA* gene encodes a 4´-phosphopantetheinyl transferase (PPTase) that is required for the activation of all polyketide synthases (PKSs) and non-ribosomal peptide synthatases (NRPSs) in *Aspergillus nidulans*. Delta*cfwA* mutants are unable to produce PKS and NRPS derived secondary metabolites, cannot synthesize lysine and fail to produce chemicals signals that regulate asexual development (conidiation).
We searched the *A. nidulans* genome for putative CfwA substrates and deleted genes AN8504.2, AN9291.2, AN0842.2, AN8412.3 and AN0016.3, which encode putative NRPSs components. The inactivation of AN0842.2 produced a mutant that conidiates very poorly and accumulates a brown mycelial pigment. We have named CpsA to the protein encoded by AN0842.2 as it is orthologous to *Cochliobolus heterostrophus* Cps1, a protein involved in plant pathogenicity. A phylogenetic analysis indicates that Cps1 and CpsA belong to the superfamily of adenylate-forming enzymes and are more closely related to a group of acyl-CoA synthetases. Although these enzymes participate in diverse reactions, all of them involve the activation of a substrate (carboxylic acid), followed by its transfe-

rence to the reactive thiol group of CoA, or to the phosphopantheinyl prosthetic group of peptidyl carrier domains (PCP) present in NRPSs or acyl carrier domains (ACP) present in PKSs.
As part of the characterization of CpsA function, we generated delta*cpsA cfwA2* double mutants to determine its conidiation and if the pigment accumulated by delta-*cps*A mutants was produced by PKSs or NRPSs. The double mutants showed enhanced conidiation defects and produced the brown mycelial pigment, suggesting that CpsA and CfwA regulate asexual sporulation and/or secondary metabolism through different pathways.

64. NEGATIVE REGULATION OF HEAT SHOCK TRANSCRIPTION FACTOR (Hsf1) BY THE THREE CATALYTIC SUBUNITS OF PKA IN *Saccharomyces cerevisie*.

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Depending on the environmental and/or nutritional conditions, the yeast Saccharomyces cerevisiae regulates, in a coordinated manner, metabolism, growth, and the stress response. One of the pathways involved in remodeling these cellular processes is the cAMP-dependent protein kinase (PKA) pathway. Moreover, glucose-induced stimulation of PKA leads to a dramatic reconfiguration of the gene expression profile, of which the transcriptional repression of stress-activated genes is well characterized. PKA inhibits the stress response through the Msn2/4 transcription factors which are maintained in the cytoplasm after phosphorylation. Decrease of PKA activity results in their translocation to the nucleus.
The principal transcription factor that regulates the response to stress is Hsf1, a protein evolutionarily conserved in all eukaryotes. Previous studies had implicated the activity of PKA in the regulation of this transcription factor. Here, we used a reporter gene (HSE-lacZ) to analyze Heat-Shock Element (HSE)-dependent gene expression in PKA-pathway mutants. Mutants that weakly affected PKA-regulated processes showed no alterations in reporter gene expression, whereas the opposite was found for mutants that strongly affected the PKA-pathway. Also, deletion of the genes encoding the catalytic subunits (TPK1, TPK2 or TPK3) affected the expression of HSE-lacZ, maintaining levels similar to wild type only in the mutant Δtpk3, while Δtpk2 and Δtpk1 single mutants showed lower levels. The three double mutant combinations of PKA (Δtpk1 Δtpk3, Δtpk2 Δtp3 and Δtpk1 Δtpk2) strongly inhibited the expression of the HSE reporter gene. In contrast, the expression of STRE-dependent expression was increased.
These data support a model of inhibitory interactions between the three catalytic subunits of PKA. Homodimers of PKA strongly repressed Hsf1. Currently, we are studying the possible regulatory mechanisms by comparing, in the wild type, single and double TPK mutants: transcript and protein levels, catalytic activity, and intracellular localization of each Tpk subunit. Obtaining these data will help to elucidate the regulatory mechanism by which PKA inhibits Hsf1 activity.

65. VACUOLAR PROTEOLYTIC SYSTEM OF *Candida glabrata*

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Candida glabrata is the second or third most common cause of systemic candidiasis after *C. albicans*, however it's phylogenetically more related to *Sacharomyces cerevisiae*. Vacuolar proteases of *Candida glabrata* have been scarcely studied. Biochemical studies highlighted the presence of different intracellular proteolytic activities: acid aspartyl proteinase (PrA), which acts on substrates such as albumin and denatured acid hemoglobin; neutral serine protease (PrB) on Hide powder azure; metallo

aminopeptidase (APE), which acts on Lys-pNA; serine dipeptidyl-aminopeptidase, acting on Ala-Pro-pNA(DAP); and serine carboxypeptidase, acting on N-Benzoyl-Tyr-pNA (CP). *In silico* analysis of *C. glabrata* genome revealed ten genes encoding putative vacuolar proteases. Gene expression of six of these genes showed that they are differentially regulated, for example *PEP4Cg*, and *APE3Cg* genes by the source of nitrogen (ammonium); *PRB1Cg*, *APE1Cg*, and *APE3Cg* by nitrogen starvation; and *PRB1Cg* by carbon starvation were up-regulated. In all conditions tested the *PRC1Cg*, and *DAP2 Cg* genes don't showed significant change in its expression.

MORPHOGENESIS AND CELL BIOLOGY

66. FUNCTIONAL AND STRUCTURAL STUDY OF *Neurospora crassa* CATALASE-PEROXIDASE (CAT-2)

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Catalases, peroxidases and catalase/peroxidases (KatGs) are involved in defense mechanisms against reactive oxygen species. KatGs have a predominant catalase activity but, different to catalases, they exhibit substantial peroxidatic activity. KatGs are present only in bacteria and fungi; fungal enzymes probably arose by gene transfer from bacterial. Crystal structures of different bacterial KatGs revealed the presence of a characteristic Met-Tyr-Trp (Met264, Tyr238, Trp90 in *Neurospora crassa* numbering) covalent link that has been shown to be essential for the catalase activity. *N. crassa* CAT-2 has ~60% sequence identity with bacterial KatGs, but has an extra loop of 25-30 amino acid residues. CAT-2 is a cytosolic enzyme that is induced during late stationary growth, asexual spore formation, growth on poor carbon sources, and under heat shock. Using mass spectrometry, we have confirmed the presence of a similar Met-Tyr-Trp adduct in the purified CAT-2 that was expressed in *E. coli.* Highly purified CAT-2 is now been subjected to crystalization procedures and small crystals have been obtained.

Compared to Wt, mycelium of a Δ*cat-2* strain aggregated more during stationary growth of liquid cultures and these aggregates formed abundant conidia and showed increased carotene production. Aggregation and spore formation increased when strains were grown in a poor carbon source. Because of CAT-2 bi-functionality, it is necessary to elucidate of which of its two activity is related to these phenotypes.

Different point mutations in conserved amino acids cause loss of catalase activity in bacterial KatGs. We obtained a W90A substitution in CAT-2. A bacterial enzyme with the equivalent substitution only has peroxidase activity. Δ*cat-2* was transformed with the W90A mutated gene. *cat-2*(W90A) transcript was detected but enzyme activity was absent even when the transformant was grown on a poor carbon source. A 6His-tag W90A mutant was expressed in *E. coli* and purified by affinity chromatography. Compared to CAT-2, CAT-2(W90A) showed a different electrophoretic mobility in a SDS-PAGE and also had a shifted Soret band. Peroxidase activity in CAT-2(W90A) was detected, but no catalase activity. We are analyzing D120A, Y238A and R426 mutants of CAT-2 to obtain another mono-functional enzyme that will be introduced to *N. crassa*.

<u>67.</u> CHARACTERIZATION OF A NOVEL MEMBRANE PROTEIN THAT AFFECTS CELL WALL INTEGRITY IN *Aspergillus nidulans*

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This research describes a novel gene designated *SccA*, which affects cell wall integrity in the filamentous fungus *Aspergillus nidulans*. Plasmid-based, overexpression of *SccA* can suppress the *calC*2 mutation in the *A. nidulans* orthologue of protein kinase C (PkcA), which results in hypersensitivity to the chitin-binding agent Calcofluor White (CFW). In filamentous fungi, as in yeasts, hypersensitivity to CFW correlates with defects in cell wall integrity. The hypothetical translated product of *SccA* is an ST-rich 271-aa protein (42% S/T) of 27.4 kDa mass (unprocessed), with a cleavable N-terminal ER-targeting domain and a probable internal membrane anchor. No known homologies have been identified in the sequence databases. A SccA-GFP hybrid localizes to the plasma membrane of vegetative hyphae. When *SccA* is placed under the control of the regulatable *AlcA* promoter, vegetative growth is normal. When *SccA* is grown under low expression conditions (growth on glucose) we observed a sensitivity to CFW, indicating it plays an important role in cell wall integrity. When subjected to high expression conditions (growth on glycerol) asexual sporulation is suppressed. Taking into consideration the protein's cell surface location and its influence on the function of
PkcA, we hypothesize that SccA plays a role in signal transduction as part of a cell wall integrity pathway.

<u>68.</u> 3H8 ANTIGEN of *Candida albicans*: LINKAGES, TECNIQUES OF IDENTIFICATION AND LOCATION IN THE CELL WALLS.

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We obtained a few monoclonal antibodies against *C. albicans* cell wall mannoproteins and one of them, mAb 3H8, is the basic component of the BICHROLATEX ALBICANS kit used in the identification of *C. albicans* cells.
The antigen 3H8 is retained by the wall structure by two different types of linkages: i) disulphide bridges and ii) alkaline sensible linkages. During growth of *C. albicans* the antigen is partially released to the growth medium and during protoplast regeneration is initially released to the medium also but as the time goes by it is incorporated in specific spots in the nascent "cell wall".
The 3H8 antigen is a protein highly glycosilated and with an apparent MW higher that 300 KD as determined by molecular filtration. The antigen that is released by NaOH is more polydispersed than the one released beta-mercaptoethanol (beta-ME) and both of them lost the antigenic activity when treated with diluted acid or alkali solutions or following chemical deglycosilation by periodate treatment.
Purification of the antigen released from the walls of *C. albicans* CAI4, the mutant *pmt1* (cells with reduced glycosilation levels) and from the supernatant of regenerating protoplasts was carried out by covalently attaching the mAb 3H8 to a NHS-activated Sepharose 4 fast flow column. The antigen was eluted initially with 0.1 M glycine, pH 2.8 and later with 0.1 M ammonium hydroxide, pH 10.5. The antigen was eluted in higher amounts with the second reagent. Both eluted antigens were finally purified by SDS-PAGE and the antigen released from the walls by beta-ME was resolved in two bands whereas the antigen free by NaOH in a single one.
Localization of the antigen in the walls of growing cells and in the "cell walls" of regenerating protoplast was carried out in a confocal microscope.

<u>69.</u> cAMP LEVELS DURING THE FIRST TWO HYPEROXIDANT STATES OF *Neurospora crassa* CONIDIATION PROCESS

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Cyclic AMP (cAMP) levels play key roles in regulation of cellular processes such as virulence, chemotaxis, gene expression, cell division, growth, differentiation and death. The intracellular cAMP level is regulated by the activity of adenylate cyclase (AC) and phosphodiesterases. Protein kinase A is the principal cAMP target, but other targets have been reported. In *Neurospora crassa*, cAMP is involved in morphology of mycelia, formation of aerial hyphae and conidia development. Mutant strains without AC activity (crisp strain) do not form aerial hyphae, but conidiate profusely, indicating cAMP requirement for aerial hyphae growth, but not conidia formation. The *N. crassa* AC has a predicted *RAS* association domain, suggesting that *RAS* (or a *RAS* homologue) could interact with and regulate AC activity.
On the other hand, the band (*bd*) strain has a *ras-1* mutation that gives a T79I substitution in *RAS-1*. *ras-1*^{bd} strain exhibits cycles of growth and conidiation. The same behavior is observed in *sod-1* mutant strains. Cycle period in *ras-1*^{bd} is 22 h, but Paraquat shortens the period and N-acetyl-cysteine suppresses the rhythm. Thus mutations in *ras-1*, oxidants and antioxidants can alter the cell fate decision to grow or to conidiate. Compared to Wt, *ras-1*^{bd} strain displays increased reactive oxygen production during conidiation, measured as chemiluminescence, and also increased growth of aerial hyphae. Increased aerial growth could be related with increased cAMP levels in *ras-1*^{bd}.
Asexual differentiation in *N. crassa* involves three morphogenetic transitions: growing hyphae to adhered hyphae, adhered hyphae to aerial hyphae and aerial hyphae to conidia. A hyperoxidant-state develops at the start of each of these morphogenetic transitions. Here we report cAMP levels in Wt and *ras-1*^{bd} during the first two transitions. In Wt, as soon as the mycelial mat is exposed to the air, cAMP level descended quickly reaching a minimum at about 10 minutes air exposure, when maximal oxidation of glutathione and NAD(P)H occurs. cAMP levels recovered in the next 10 min and after an overshoot, initial levels were reached at 50 min after air exposure. Then cAMP levels remained constant but a second decrease related to the second hyperoxidant state was observed at 100 min of air exposure. As predicted, basal cAMP levels were higher in *ras-1*^{bd} mutant in which levels also decreased during the two hyperoxidant-states, but it did so faster and also recovered earlier compared to the Wt. Thus, we conclude that cAMP levels are regulated by *RAS-1* and the redox state of the cell.

<u>70.</u> EFFECT OF CARBON SOURCE IN THE MORPHOLOGICAL PATTERN OF *Mucor circinelloides* WILD-TYPE AND YR-1 STRAIN ISOLATED FROM PETROLEUM CONTAMINATED SOILS.

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Oil is a mixture of components, mainly aliphatic and aromatic hydrocarbons with small amounts of sulfur, oxygen, nitrogen and heavy metals such as iron, chromium, nickel and vanadium between others. It is a major source of energy and raw materials in the world but its improper management is one of the principal causes because the pollution problems have grown in recent times. There are many physical and chemical methods to disposal it from the environment, but these shows multiple disadvantages, meanwhile currently work with microorganisms that are capable of degrading petroleum components using it as sole carbon and energy source, could be a more suitable methodology.
Mucor circinelloides YR-1 was isolated from petroleum-contaminated soil, from a place near to the oil-refinery in Salamanca Guanajuato, México. The strain is capable to grow in a wide range of hydrocarbons as sole carbon source and energy. It also presents different morphologic patterns compared with the laboratory strain R7B, depending of the carbon source used for growth.

By these reasons, it is indispensable the study of the relation between morphology and growth conditions in the strain YR1 and compared it with the strain R7B.
A morphological analysis was performed for both strains growing in the following carbon sources: glucose, glycerol, ethanol, decanol, pentane, decane, hexadecane, and naphthalene. We also assessed the differences in the percentages of germination. The results show that YR1 strain was able to grow in all tested carbon sources, and presents marked morphological differences among each one. In the other hand, the strain R7B was unable to grow in most hydrocarbons and in some of them, its growth was smallest than YR-1 strain.

71. CDC-42 LOCALIZES AT THE CELLULAR APEX AND SEPTA OF MATURE HYPHAE OF *Neurospora crassa*

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Polarized growth in fungal cells starts at specific sites by the action of cortical markers. The subsequent apical growth is maintained by the assembly of multiple protein complexes that ensure that cellular components get incorporated into the plasma membrane and provide precursors and enzymes required for cell growth. CDC-42 is a highly conserved member of the family of Rho GTPases that presumably functions as a cortical marker in fungal cells. In *Saccharomyces cerevisae* Cdc42p is required for polarity establishment and maintenance and in *Aspergillus nidulans* Cdc42 is involved in polarity establishment and appears to have a more important role in hyphal morphogenesis. In this work, the *N. crassa* CDC-42 protein was labeled with the mCherry fluorescent protein (mChFP) or the green fluorescent protein (GFP) and its localization was determined by laser scanning confocal microscopy in growing cells. Preliminary results show that both CDC-42-mChFP and CDC-42-GFP are localized at the cellular apex and at sites of septum formation in mature hyphae. Fusion of the *N. crassa* CDC-42-mChFP strain with a *N. crassa* strain expressing SPA-2-GFP indicated only partial colocalization of the polarisome and CDC-42. Also, CDC-42 appears to be located at the core of the Spitzenkörper and not in the form of a crescent as in *A. nidulans*. Analysis of the *Δcdc-42* strain using FM4-64 revealed a severe defective growth pattern showing hyphae with an aberrant morphology and multiple septa accompanied by a very slow growth rate, indicating that CDC-42 in *N. crassa* has a key role in hyphal morphogenesis.

72. THE *fluf1* MUTATION DEFINES A NEW ALLELE OF THE *flbD* GENE, WHICH REGULATES CELL DIFFERENTIATION IN *Aspergillus nidulans*

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Asexual differentiation in *Aspergillus nidulans* is induced by environmental signals like exposure to air or nutrient starvation and depends on *brlA* gene activation. Developmental regulators acting upstream of *brlA* (*fluG* and *flbA-E* genes) are required for its proper expression, mutants in these genes show delayed asexual development (“fluffy” phenotype) and reduced expression of *brlA*. However, the exact function of these regulators remains unknown. We have found that the *fluF1* mutation, which also causes a “fluffy” phenotype, is a new allele of *flbD*. *brlA* expression in *fluF1* mutant is delayed, while *flbD* expression is not altered. A complete deletion of *flbD* results in a more severe mutant phenotype, suggesting that the protein FluF1 retains some function. The *fluF1* allele corresponds to a point mutation that results in the substitution R47P. As R47 and the contiguous cysteine (C46) are highly conserved residues within the Myb domain of FlbD, we generated mutant alleles in C46. The alleles that were expected to impair Myb DNA binding showed decreased conidiation, similar to the *fluF1* mutant. Moreover, inactivation of the *atfA* gene, which encodes a transcription factor needed for

a full antioxidant response, enhanced *fluF1* conidiation mutant phenotype. We propose that the redox properties of C46, which is critical for DNA binding of other Myb DNA *in vitro*, are modified by the presence of P, instead R, at position 47 of FlbD.

73. THE ROLE OF BEM-1 AS POSSIBLE REGULATOR OF NOX-1 IN *Neurospora crassa*

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Reactive oxygen species (ROS) play essential roles in cell differentiation in microbial eukaryotes (Lara-Ortíz *et al*., 2003, Aguirre *et al*., 2005, Cano-Domínguez *et al*., 2008). ROS are generated mainly during mitochondrial electron transport and by certain enzymes. The NADPH oxidases (NOX) catalyze the production of superoxide by transferring electrons from NADPH to O_2. In phagocytic cells, the NOX consists of the membrane-associated catalytic core $gp91^{phox}$ and $p22^{phox}$ subunits (cytochrome *b558*). The assembly of the cytosolic regulatory proteins $p47^{phox}$, $p67^{phox}$ and Rac1/Rac2 with the cytochrome *b558* results in NOX activation. *Neurospora crassa* contains two NADPH oxidase genes (*nox-1* and *nox-2*), which encode proteins that are homologous to phagocyte Nox2 ($gp91^{phox}$). We reported that deletion of *nox-1* results in mutants defective in differentiation of sexual fruiting bodies and show reduction of growth and asexual development. The inactivation of *nox-2* only affects the germination of the ascospores. *N. crassa* NOX activity requires other proteins like the $p67^{phox}$ ortologue NOR-1, and possibly other proteins like BEM-1 which could be the functional homologue of $p40^{phox}$ (Kawahara and Lambeth, 2007)). BEM-1 contains two amino-terminal Src homology 3 (SH3) domains and a carboxy-terminal Phox and PB1 domain. We characterized mutants lacking the only *N. crassa* BEM-1 (bud emergence protein or like Bem1p in *Saccharomyces cerevisiae*), and show that BEM-1 might be related to NOX-1 during asexual development but it is not required for development of sexual fruiting bodies, indicating that BEM-1 is not essential for NOX-1 activity.

74. *Candida albicans* PHOSPHOMANOSYLATION DEPENDS ON CARBON SOURCE AND CELL MORPHOLOGY

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The cell wall of the opportunistic pathogen *Candida albicans* is composed of chitin, beta-glucans and mannoproteins. These proteins have high-mannose oligosaccharides linked to asparagine (*N*-linked mannans) and/or serine/threonine (*O*-linked mannans) residues. *N*-linked mannans are highly branched oligosaccharides, while *O*-glycosylation produces shorter linear glycans with up to five alpha 1,2-mannose units. Both *O*-linked and *N*-linked mannans contain phosphomannans which are mannose residues linked to the glycans through a phosphodiester bond. Thus far, *C. albicans* Mnt3 and Mnt5 have been identified as phosphomannosyltransferases, and Mnn4 as a positive regulator of this enzyme activity. The loss of phosphomannans do not affect the *C. albicans* virulence; however, phosphomannans are responsible for cell wall surface negative charge, are required for normal phagocytosis by macrophages, and for the anti-*Candida* activity of cationic antimicrobial peptides similar to those secreted by macrophages.

In this work we determine the relationship between *C. albicans* phosphomannosylation and culture growth conditions. We found than *C. albicans* significantly increases cell wall phosphomannosylation when cells are grown in poor carbon sources, such as mannose and lactose. Next, we determined the effect of dimorphism on the phosphomannan content at the cell wall. In time-course experiments, growth in N-acetylglucosamine or RPMI 1640 medium induced cell filamentation but did not change the ability of the cell wall to bind the cationic Alcian blue dye, which is bound to the negative change

present in the phosphomannan. Microscopic analysis showed that Alcian blue dye was only bound by the yeast-like mother cells but not the filament cells. Therefore our results indicate that *C. albicans* is able to adapt their phosphomannan content to changes in the external environment, and that hyphal cell wall does not have mannosylphosphate residues or they are not exposed enough as in yeast cells. These observations have significant implications to the immune recognition of *C. albicans* cell walls.
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75. BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF THE DEVELOPMENT PROTEIN SC1 OF *Sclerotium cepivorum* Berk

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Sclerotium cepivorum Berk is a deuteromycete fungus that parasitizes plants of the *Allium* genus, such garlic and onion, is the etiological agent of known as "garlic white rot." disease. This fungus remains in a dormant state in a resistant structure with a melanized covering called sclerotia and is able to persist in the soil for years.
During sclerotium formation, a 34.4 kDa protein called Sc1 is synthesized, and reaches 70% of the total protein of the sclerotia and is not present in mycelial phase. It is possible that this protein plays a major role in sclerotia formation, maintenance and/or germination.
In order to determine the role of Sc1 in the formation and development of sclerotia of *S. cepivorum* Berk, some experimental strategies have been employed. Using two-dimensional electrophoresis, we found that Sc1 shows 3 isoforms with different isoelectric point, and by mass spectrometry, the sequence of one of them was obtained. The analysis shown sequence shows high homology with a protein present in sclerotia-forming fungi.
We are trying to affect the protein expression levels and determine it´s effect on the formation of sclerotia using few strategies. We have the cDNA belonging to *sc1* protein cloned into the plasmid pUE08 that is a constitutive expression, in order to generate antisense and overexpressing transformants. A replacement cassette was contructed by double join PCR, and we are working in the transformation of *S. cepivorum* in order to generate transformants and then analyze their phenotypes.
The study was supported by grants DAIP, U. de Guanajuato & CONACYT-SAGARPA 2005-11919, Mexico.

76. EXPLORATION OF COMPONENTS RELATED TO ENDOCYTOSIS IN THE FILAMENTOUS FUNGUS *Aspergillus oryzae*.

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According to the localization analysis of AoAbp1 and AoEnd4, which are thought to function in endocytosis, endocytosis occurs mostly at the tip region in the filamentous fungus *Aspergillus oryzae*. Endocytosis-deficient hyphae displayed the severe growth defect, suggesting that endocytosis and apical growth are highly linked. Based on these results, the mechanism of endocytosis which is specific to filamentous fungi seems to exist at the tip region. In this study, we explored novel components associated with endocytosis by the yeast two-hybrid (YTH) screening using AoAbp1, having two SH3 domains, which are related to endocytic protein-protein interaction, as bait. *A. oryzae* cDNA library was used as prey. Part of a gene which probably encodes AAA (ATPases associated with diverse cellular activities) type ATPase was obtained as prey by the YTH screening. The prey interacted with AoAbp1 SH3 domains in the YTH analysis. We designated the gene as *aipA* (AoAbp1 interacting protein). Motif analyses showed that AipA has one coiled-coil region at the N-terminus region and one AAA type ATPase domain at the C-terminus region. To investigate the localization of AipA, we generated a strain co-expressing EGFP-AipA and AoAbp1-mDsRed. EGFP-AipA and AoAbp1-mDsRed

were colocalized at the tip region, suggesting that AipA functions in endocytosis. We are studying *in vitro* and *in vivo* interaction between AipA and AoAbp1 and functional analyses of AipA.

<u>77.</u> THE *ugeA* GENE PRODUCT MEDIATES AN EARLY STAGE IN GENERATING THE GALACTOFURANOSE COMPONENT OF THE *Aspergillus nidulans* WALL.

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Aspergillus nidulans spore and hyphal walls contain a sugar, galactofuranose (Gal*f*) that is not found in metazoans. Gal*f* has five-membered-ring, is a pathogenicity determinant, and can be monitored in blood serum for tracking the progress of antifungal therapy. In *A. nidulans*, UDP-Gal*f* mutase, encoded by *ugmA* mediates an equilibrium between UDP-galactopyranose (Gal*p*, the six-membered ring form, which predominates) and UDP-Gal*f* prior to incorporation of Gal*f* into the wall. Deletion of *ugmA* from *A. nidulans* impairs hyphal growth and morphogenesis, but is not essential. Upstream of *ugmA* function, UDP-glucose-4-epimerase, encoded by *ugeA* mediates an equilibrium between UDP-glucose and UDP-galactose. Like *A. nidulans ugmA*, *ugeA* is not essential, however, its deletion causes a similar phenotype. A related sequence that we call *ugeB* lacks a significant deletion phenotype. Characterization of *ugeA* and *ugeB* deletion strains using immunofluorescence microscopy, electron microscopy, drug sensitivity studies, as well as standard molecular methods will be presented.

<u>78.</u> MpkC, A SECOND MAPK OF THE SAPK FAMILY IN *Aspergillus nidulans* REPRESS ASEXUAL DEVELPOMENT.

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Fungi utilize a phosphorelay system coupled to a MAP kinase module as a major mechanism for sensing and processing environmental signals. In *Aspergillus nidulans*, the response regulator SskA transmits osmotic and oxidative stress signals to the stress MAP Kinase (SAPK) SakA. All members of genus Aspergilli contain a second SAPK, which is also phosphorylated in SskA dependent manner. We characterized *A. nidulans* mutants lacking the SAPK MpkC (*DeltampkC*) and found that MpkC is not required for osmotic or oxidative stress resistance or the utilization of poor carbon or nitrogen sources. However, the *DeltampkC* mutant produced about 50% more spores that a WT strain, indicating a role for MpkC in repression of asexual development in this fungus.

<u>79.</u> *IN VITRO* PHENOTYPIC TRANSFORMATION OF THE FUNGUS *Ustilago maydis* FROM YEAST-LIKE CELLS TO BASIDIOCARPS

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Ustilago maydis, a phytopathogenic ustilaginomycete basidiomycete, is the causal agent of corn smut. During its life cycle *U. maydis* alternates a yeast-like, haploid nonpathogenic stage with a filamentous, dikaryotic pathogenic form that invades the plant and induces tumor formation. Same as the rest of ustilaginomycetes, *U. maydis* is unable to produce basidiocarps, instead it produces teliospores within the tumors. This characteristic may be due to either, that the *Ustilagynomycota* never acquired the capacity to make them, or during evolution they lost this faculty.
We have now established conditions allowing a completely different developmental program of *U. maydis:* when diploids or mating yeasts are incubated on dual cultures side by side to maize embryogenic tissue in the presence of plant growth regulators, the fungus suffers what might be well de-

scribed as a phenotypic "evolutionary jump" of complexity. Under these conditions *U. maydis* changes its quasi-obligate biotrophic pathogenic mode of life to that displayed by different saprophytic basidiomycetes, or plant growth-promoting mycorrhizal fungi (truffle-like), producing fruiting bodies. Morphology of these bodies mimics those displayed by gasteromycetes; they are highly organized, and develop skeletal and generative hyphae. Generative hyphae form clamp connections, holobasidia and basidiospores. This is evidence that *U. maydis* is able to produce tertiary mycelium similar to higher fungi, with morphological characteristics similar to those of exobasidiomycetes and Agaricomycotina species.
The results obtained suggest that the genetic information derived from its ancestors (Glomeromycota) or relatives to produce fruiting-bodies, may have remained silent in *U. maydis*, since when grown under appropriate conditions, like those here used, it was able to develop such structures. Phylogenetic data suggest that the fungus shares genetic information not only with plant pathogens, but also with mycorrhizal fungi (fruiting-body forming species). It is therefore tempting to suggest that the silent information existing in *U. maydis* may well be a strategy to survive under changing environmental conditions, mainly in the absence of its specific host

80. HYPHAL TIP STRUCTURE OF *Basidiobolus ranarum* (ZYGOMYCOTA)

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Fungal hyphae contain many of the organelles and sub-cellular inclusions found in other heterotrophic eukaryotic organisms. However, because of their mode of growth and diverse interactions with the ecosystem, hyphae have certain cytoplasmic features that are unique to the fungi. Some of these features show significant structural plasticity or are present in only certain fungal groups. Such features (e.g., Spitzenkörper, cytoskeletal order, septal structure, nuclear division) are of particular importance in understanding aspects of hyphal behavior and are used as indicators of evolutionary relationships. A thorough characterization of hyphal tip structure in members of the Zygomycota has not been performed. To date, among the zygomycetes that have been examined, a Spitzenkörper has yet to be reported. In this presentation, the cytoplasmic order of hyphal tip cells of *Basidiobolus ranarum* will be illustrated using a combination of light microscopy and transmission electron microscopy methods. Results show that Spitzenkörper are present in growing tip cells of *B. ranarum*. To our knowledge this is the first report of a *bona fide* Spitzenkörper in a member of the Zygomycota.

81. AoSO PROTEIN ACCUMULATES AT THE SEPTAL PORE IN RESPONSE TO VARIOUS STRESSES IN *Aspergillus oryzae*.

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Filamentous ascomycetes form hyphal networks that are compartmentalized by septa. These septa contain pores allowing the flow of the cytoplasm and organelles between hyphal compartments. This characteristic is shared with higher eukaryotic organisms such as gap junctions in animal cells and plasmodesmata in plant cells. The septal pore can be plugged by a wound-healing organelle, Woronin body, upon hyphal lysis to prevent excessive loss of the cytoplasm. To keep homeostasis as multicellular organisms, the filamentous fungi would have an acute system regulating intercellular communication in response to sudden environmental changes. However, proteins plugging the septal pore in a stress-dependent manner had not been extensively studied in the filamentous fungi. In this study we focused on *Aspergillus oryzae* protein (AoSO) homologous to the *Neurospora crassa* SO protein, which was reported to accumulate at the septal pore in aging (Fleissner and Glass, 2007). The Δ*Aoso* strain showed excessive loss of the cytoplasm upon hyphal lysis like the Woronin body-deficient strain

(Δ*Aohex1*). Cellular localization studies using EGFP showed that the AoSO protein accumulated at the septal pore adjacent to the lysed compartment, while it was found in the cytoplasm in normal growth condition. These indicate that the AoSO protein contributes to preventing excessive cytoplasmic loss upon hyphal lysis. Furthermore, the AoSO protein accumulated at the septal pore under various stress conditions (low/high temperature, extreme acidic/alkaline pH, nitrogen/carbon starvation). It also dissociated from the septal pore after relieved from a stress condition, low temperature. These data give a hypothesis that the AoSO protein may participate in regulation of intercellular communication via the septal pore in response to environmental stresses.

82. SEXUAL REPRODUCTION IN *Aspergillus flavus* AND *A. parasiticus.*

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The fungal phylum Ascomycota comprises a large proportion of species with no known sexual stage, despite high genetic variability in field populations. Two such asexual species, *Aspergillus flavus* and *A. parasiticus* are potent producers of carcinogenic and hepatotoxic aflatoxins, polyketide-derived secondary metabolites that contaminate a wide variety of agricultural crops. Population genetics data lead us to show that both species are heterothallic, with individuals containing either a *MAT1-1* or *MAT1-2* mating-type gene. In this study, individuals of *A. flavus* and *A. parasiticus* from populations showing evolutionary histories of recombination were examined for sexual reproduction. Strains with opposite mating-type genes *MAT1-1* and *MAT1-2* within each species were crossed in an attempt to induce sexual reproduction. Lengthy incubation resulted in the development of multiple, indehiscent ascocarps containing asci and ascospores within the pseudoparenchymatous matrix of stromata, which places the teleomorphs in the genus *Petromyces*. Sexually compatible strains in both species belonged to different vegetative compatibility groups. Recombination through the independent assortment of *A. parasiticus* chromosomes 3 and 6 was detected using loci for mating type, aflatoxin gene cluster, and a protein-encoding gene. The teleomorph of *Petromyces flavus* could not be distinguished morphologically from that of *P. parasiticus*. The two *Petromyces* species can be separated by anamorph morphology, mycotoxin profile, and molecular characters.

83. ROLE AND DISTRIBUTION OF CHITIN SYNTHASES WITH MYOSIN-LIKE MOTOR DOMAIN (CHS-5-GFP AND CHS-7-GFP) DURING HYPHAL GROWTH OF *Neurospora crassa*

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The fungal cell wall is composed primarily of chitin and glucan. Chitin synthases (CHS) catalyze the addition of N-acetilglucosamine subunits to a growing chain of chitin. *N. crassa* has a representative of each of the reported seven classes of CHS. Two of them, CHS-5 and CHS-7, belonging to classes V and VII, respectively, have a myosin-like motor domain (MMD) at their amino terminus. Previous studies in *Ustilago maydis* have reported that MMD shows a 22% sequence identity with the motor domain of Myo5, a class V myosin. Previously, other CHS belonging to classes I, III and VI from *N. crassa* were tagged with GFP. These CHS (CHS-1, CHS-3 and CHS-6) accumulated at the core of the Spitzenkörper (Spk) and forming septa and appeared to follow an unconventional secretory route. In this work, using a split marker approach, we have endogenously tagged with *gfp* the CHS encoding genes *chs-5* (NCU04352) and *chs-7* (NCU04350) from *N. crassa*, and studied their expression in living hyphae of *N. crassa.*

We observed that both, CHS-5-GFP and CHS-7-GFP were localized in the developing septa, but only CHS-5-GFP was observed in the core of the Spitzenkörper. Although no CHS-7-GFP was observed at the apex, a *Δchs-7* strain (FGSC#11996) showed reduced growth. To discern whether the MMD of these CHS behaves as a class V myosin, we tagged with *gfp* the *myo-4* gene (NCU11354). MYO-4-GFP was found at the core of the Spitzenkörper but not at septa. CHS-5 colocalizes with CHS-1, CHS-3 and CHS-6 at the core of the Spitzenkörper. These results suggest that MYO-4 is probably the actin-associated motor involved in the transport of CHS-containing vesicles (chitosomes) to the Spk, but it is not involved in the transport of these vesicles to septa.

84. ROLE AND DYNAMICS OF THE SEPTIN HYP -1 IN *Neurospora crassa*

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Septins constitute an evolutionary conserved family of GTPases that participate as organizational cues or scaffolds in various cellular processes. In yeast, they participate in septum formation, mitosis and cytokinesis. In *Aspergillus nidulans,* the septin AspB is essential for the formation of septa and to synchronize mitotic events and morphogenesis. In mammals Sept7, the orthologue of AspB, is part of the kinetochore and participates in mitosis, particularly in chromosome segregation. By searching the genome of *Neurospora crassa* seven likely septin-encoding genes were identified.
We analyzed the role and dynamics of the septin HYP-1 in *N. crassa* during germination, branching, septation, tip growth and mitosis. Using a split marker approach and fusion PCR, we tagged the *hyp-1* (NCU08297.3), a homologue of *aspB* (group II septin) with *gfp* by homologous recombination. Analysis by Laser Scanning Confocal Microscopy showed that HYP-1-GFP starts accumulating at septation sites in the lateral membrane of the hyphae. Gradually, fluorescence moves towards the center, temporarily occupying the central region of the septum corresponding to the pore, and subsequently it irreversibly disappears. The overall process lasts about 7 to 10 minutes (n=20). Cells expressing HYP-1-GFP were co-labeled with the dye FM4-64. During septum formation, GFP fluorescence accumulated prior to FM4-64 fluorescence. After septum completion, and HYP-1-GFP disappearance, FM4-64 fluorescence remained all along the septum membrane. At the hyphal apex, there was an accumulation of fluorescence, whose intensity decreased at the subapex and completely disappeared in distal regions. In damaged hyphae, HYP-1-GFP was observed at the septa that separated the damaged apical compartment from the closest compartment without any apparent damage. In some of these hyphae, Woronin bodies were found in close proximity to the HYP-1-GFP labeled septa. In conidia, HYP-1-GFP was observed as dispersed granules. Upon germination, these granules became clouds of fluorescence gathered close to nuclei along the germlings.These preliminary observations indicate that HYP-1 may act as positional cue for septum formation. We are currently analyzing the distribution of other proteins known to participate in septum formation (i. e. chitin synthase 1 and actin) relative to presence of HYP-1. By analyzing the expression of the centromeric protein CenH3 labeled with GFP in an *N. crassa* Δ*hyp-1* strain we will also study the participation of HYP-1 during mitosis in germlings of *N. crassa.*

85. THE RAB GTPASA SEC-4 CONTRIBUTES TO HYPHAL MORPHOGENESIS IN *Neurospora crassa.*

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The apex of *Neurospora crassa* hyphae presents an accumulation of secretory vesicles that form the Spitzenkörper, whose function is to direct growth, maintain hyphal morphology and regulate the flow of vesicles before they fuse with the plasma membrane (PM). In yeast, vesicle fusion with the PM at growth sites depends on the function of Sec4p, a Rab (*Ras-related protein in brain*) GTPase, which associates with vesicles generated at Golgi, and that controls the assembly of the exocyst complex. Mutagenesis studies of Sec4p in yeast demonstrated that the absence of this protein results in a lethal phenotype and causes an accumulation of vesicles in the cytoplasm.
The aim of this study was to better understand the role of SEC-4 in the regulation of vesicle traffic and exocytosis in the filamentous fungus *N. crassa.* We obtained a strain of *N. crassa* with the *sec-4* gene (NCU06404.3) deleted. Contrary to what was observed in yeast, the absence of *sec-4* in *N. crassa* was not lethal. The mutant showed significant morphological changes during vegetative growth and a lower growth rate (3.0 µm min^{-1}) compared to that of the control host strain (FGSC#9718; 23.0 µm min^{-1}). At the macroscopic level the mutant colonies showed a white cottony appearance. At the microscopic level the mutant presented hyphae with swollen and amorphous regions and with narrow regions, an indication presumably of an increase or decrease in diameter in the apical region of the hypha during growth. By phase-contrast microscopy we observed that the Spitzenkörper presented abrupt movements, which caused sudden changes of growth direction and permanent deformations in the apical region of hyphae. Furthermore, we observed unusual branching, formation of abortive branches close to each other, an increase in the presence of septa close to successful branches and an accumulation of vacuoles in the distal regions of the hyphae.
The morphological changes observed during hyphal growth indicate that SEC-4 is involved in hyphal morphogenesis as well as other events such as branching and septation.

86. LOCALIZATION OF THE GENERAL AMINO ACID PERMEASE IN *Neurospora crassa.*

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Fungi are capable of concentrating solutes from the surrounding milieu into the cell. These solutes enter the cell via permeases and channels, which are embedded in the cell membrane. Among all the solutes, amino acids are one of the most actively transported through the cell membrane in eukaryotes. In fungi transport of amino acids is mediated by two types of permeases: specific systems, which are capable of introducing only chemically related amino acids, and the general systems, that have affinity for the majority of the amino acids. In the yeast *Saccharomyces cerevisiae* the general amino acid permease (Gap1p) has been previously characterized using green fluorescent protein (GFP) as a tag. It was observed that its intracellular sorting was nitrogen source dependent. When cells were grown on an amino acid rich medium, Gap1p was sorted to the vacuole. Conversely, during growth on urea or ammonia, Gap1p was sorted to the plasma membrane. In both *S. cerevisiae* and *N. crassa* the general system is involved in nitrogen metabolism, and internalizes amino acids as source of nitrogen in low nitrogen conditions. However in *N. crassa* the general system has not been localized. We analyzed whether a similar sorting process to the one reported in yeast for the general amino acid permease system occurs in *N. crassa* as a plausible way to analyze the secretory pathway in this filamentous fungus.
We fused GFP to the *N. crassa* closest homolog of Gap1p (NCU10262.3), which we named *ngap-1l* (for Neurospora gap-1 like) and expressed it in *N. crassa*. Using laser scanning confocal microscopy we observed that the GFP labeled putative NGAP-1L was localized in the cell in a nitrogen dependent

fashion, although it was never seen in the plasma membrane. In poor nitrogen source conditions (urea or potassium nitrate) the labeled protein was observed in tubular vacuoles; whereas in optimal nitrogen medium (ammonium nitrate) the protein was only seen in distal spherical vacuoles. These findings suggest that NGAP-1L could be a vacuolar permease involved in the nitrogen metabolism.
We identified 2 other putative candidates (NCU05830.3 and NCU03509.3) with homology to Gap1p. We are currently labeling with *gfp* the gene *naap-1* (NCU03509.3).

HOST-FUNGUS INTERACTION

87. EFFECT OF TRICHODERMA ISOLATES ON COMMON BEAN PLANTS

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Trichoderma spp. are free-living fungi in root and soil environments. They could be opportunistic, avirulent plant symbionts, or parasites of other fungi. *Trichoderma* species have been recognized as agents for the control of plant disease and for their ability to promote plant growth and development. Root rots caused by fungi (*Fusarium* sp. and *Macrophomina phaseolina* among others) reduce common bean (*Phaseolus vulgaris* L.) production and grain quality at northern Mexico. Fungal genetic and pathogenic diversity analyses are important for disease control in wide production areas of Mexico. Previously we identificated *F. oxysporum*, *F. solani* and *M. phaseolina* as the main causal agents of root rots in common bean in Durango, Mexico. Seeds of common bean cultivar Pinto Saltillo were treated with several *Trichoderma* species (*T. virens, T. asperellum, T. harzianum and T. gamsii*) and their abilities to promote plant growth and control of plant pathogenic fungi (*F. oxysporum, F. solani and M. phaseolina*) were tested. Experiments were carried out greenhouse and effects on plants were evaluated in flowering stage. The results showed that *T. asperellum* is able to reduce the damage in plant caused by those pathogens, whereas *T. virens* was efficient to control *F. solani*. *T. asperellum* showed substantial positive effects on growth (dry weight root, leaf area and plant height, in the presence or absence of pathogens.

88. CLONING AND NUCLEOTIDE SEQUENCE OF THE HISTIDINE KINASE *NIK1* GENE FROM *Micosphaerella fijiensis*, A FUNGAL PATHOGEN OF *Musa* sp.

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Histidine kinases, also known as two component systems play key roles in perception and signal transduction of extracellular stimuli. This protein kinase system (HKS) is used mainly by prokaryotic organisms, although eukaryotes as plants and fungi use it in lesser extent. The histidine kinases are involved in the regulation of important functions such as the nutrient intake, osmotic adjustment, mating, toxin production and virulence.
In fungi the HKS are relatively well studied and currently almost 50 genes encoding HKS have been described. One of them is the *NIK1* gene, which in non pathogenic fungi *Neurospora crassa* (*NcNIK-1/OS-1*) and *Lentinula edodes* (*Le.Nick*) seems to be involved in the osmotic adjustment, hyphal development and resistance against fungicide. In the opportunistic fungus *Candida albicans* the *CaNik1* gene is involved in the development of pathogenic process; *C. albicans* strains lacking of the expres-

sion of *Nik1* do not developed disease. In the case of phytopathogenic fungus *Cochliobolus heterostrophus* the *NIK1* system (*ChDIC*) seems to be involved in fungicide resistance and osmoregulation. *Mycosphaerella fijiensis* an hemibiotrophic pathogenic fungus and the causal agent of black Sigatoka disease of banana and plantain. The disease produces decrease in photosynthesis and important fruit losses. To face the economic impact, the producers apply combination of fungicides to control *M. fijiensis*. Unfortunately, the pathogen has breaking down the effect of some fungicides and parallelaly the virulence on *Musa* sp also increases dramatically.
It is expected that HKs and particularly the *NIK1* be fundamental for virulence or resistance against fungicides like in other phytopathogenic fungi. We searched for *NIK1* homologs into the genome of *M. fijiensis* and in the present work using long distance PCR and specific primers flanking the start and ending codon from a putative *NIK1* gene, we amplified a 5114 pb DNA fragment. This fragment was cloned into pGem-T-easy vector and then sequenced it. Comparison by Blastx with the NCBI database retrieved accessions described like *NIKI* genes in other fungi. Southern blot using a 814 pb probe showed that NIK1 in *M. fijiensis* is unicopy.
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<u>89.</u> THE GLYOXYLATE CYCLE IN *Metarhizium anisopliae*.

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Metarhizium anisopliae is an entomopathogenic fungus, currently with an increasingly important role in the control of agricultural insect pests, including vectors for human diseases. Several factors promote the virulence of *M. anisopliae*. Different proteins have a particular and specific function during the invasion process. Adhesins are involved in the adherence to host cuticle. Secreted proteases, chitinases and toxins have significant role in the penetration, with other proteins allowing immune evasion. Together, these factors provide the mechanism for successful dissemination and invasion of this fungus to the host.
We reasoned that these factors of virulence are programmed in parallel with adjustment of the metabolism for assimilation of nutrients that allow growth and development of the *M. anisopliae* into the host, as already demonstrated in other pathogens. One of these metabolic pathways is the glyoxylate cycle, a modified form of the tricarboxylic acid cycle, which permits the utilization of C2 compounds. One example is the fatty acids from lipids commonly used as an energy source via degradation by beta-oxidation into acetate molecules. The key enzyme for the glyoxylate cycle is the isocitrate lyase (ICL), the interruption of this gene has been associated with decrease in the pathogenicity and virulence in the mammalian pathogen fungus *Candida albicans* and plant pathogenic fungi *Magnaporthe grisea*, *Leptosphaeria maculans* and *Gibberella zeae*.
In this work we describe the isolation of the *M. anisopliae* genes for citrate synthase (CIT1 and CIT2), aconitase (ACO1) related to the tricarboxylic acid (TCA) cycle and the glyoxylate cycle, and the genes for isocitrate lyase (ICL1) and malate synthase (MLS1) limited to the glyoxylate cycle. We analyzed their expression under *in vitro* conditions of induction of the glyoxylate cycle as well as in pathogenicity and saprophytic conditions. The genes CIT2, ICL1 and MLS1 showed induction, suggesting a putative role of this glyoxylate cycle in the life cycle of *M. anisopliae*. In order to investigate this role in *Metarhizium anisopliae* we are also working on the characterization of the gene for isocitrate lyase, the key enzyme of the glyoxylate cycle.

<u>90.</u> MORFOLOGICAL CHANGES AND NITRIC OXIDE PRODUCTION BY HUMAN OSTEOBLASTS DURING *C. albicans* AND *C. glabrata* INFECTION

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C. albicans is the most common fungal opportunist pathogen associated to infections of immunocompromised individuals. However, infections by other yeast species are becoming increasingly frequent, as infections caused by *C. glabrata.* Some of the most extensively investigated virulence factors of Candida genus include its ability to attach to host tissue and then penetrate them. Some adhesins of Candida and their host co-receptor on phagocytic cells are well recognized, however Candida interaction with osteoblasts and their cellular responses to the infection are not known in details. In this work, we investigated the adherence and internalization of C. albicans and C. glabrata with human osteoblasts and nitric oxide production induced by these yeasts. Adherence and internalization were evaluated by optical and scanning electron microscopy; nitric oxide production was quantified by a fluorometric method using DAF as indicator. For all assays, yeast-complement-opsonized against yeast-non-opsonized were compared. For both yeasts, the internalization was low (10-20%) however, adhesion was very high (90%). Opsonization did not have any influence on adhesion and internalization events. Scanning electron microscopy assays showed that C. albicans infection induced membrane ruffling and C. glabrata stimulate the formation of large membrane projections similar to networks or nets. In addition, nitric oxide production induced by non-opsonized-yeast was higher than those induced by opsonized-yeast. In conclusion, our results suggest that interaction of C. albicans and C. glabrata with osteoblasts induce different cytoskeleton rearrangements and differential nitric oxide production, most probably due to different signaling pathways trigger by each yeast.

<u>91.</u> OVER-EXPRESSION OF *SM-1* IN *Trichoderma atroviridae* ENHANCES BIOMASS PRODUCTION AND INDUCES PLANT DEFENSE RESPONSE IN PEPPER PLANTS

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Crop production is often impaired due to attack by pests and diseases. Plants have evolved sophisticated mechanisms that recognize biotic and/or abiotic stresses through signals that are translated to metabolic and biochemical reactions that provoke an altered physiological status in plants. Salicylic Acid (SA) plays important roles in plant defense and it is involved in the activation of the defense response against biotrophic and hemi-biotrophic pathogen. An endogenous increase in SA results in the induction of pathogenesis related (PR) genes and establish the Systemic Acquired Resistance (SAR). On the other hand, induced systemic resistance (ISR) is mediated by Jasmonic Acid (JA) and Ethylene (ET) and usually is associated with defense against necrotrophic pathogens. Recently it was described that SM-1 (small protein), from *T. virens* induced plant defense response. Induced protection by SM-1 has been associated with the accumulation of phenolic compounds, reactive oxygen species, as well as the accumulation of plant defense genes mediated by SA, and JA/ET. It was suggested that during *Trichoderma* -plant interaction SM-1 plays an important role in signaling. Data from our lab showed that SM-1 from *T. virens* and *T. atroviride* induce systemic plant defense against *Botrytis cinerea, Alternaria solani* and *Pseudomonas syringae* pv *tomato*. In addition, we also demonstrated that SM-1 induces plant growth in tomato. In base to this, we decided to evaluate *sm*-1 deletion and overexpressing strains (OE) during their interaction with pepper (*Capsicum annuum*) plants. We determined that inoculated plant with overexpression strains of *Trichoderma* ssp. enhanced protection levels against *Botritys cinerea* and *Alternaria solani* compared with plants that were inoculated with WT, knockout (KO) strains or mocked plants. For instance the *T. atroviride* strain 1.1 in pepper plants protected 30 %

against *B. cinerea* compared with mocked plant, similar result was obtained in pepper plants inoculated with *A. solani.* Furthermore, we determined the level expression of some genes related to systemic plant defense after 72 h *Trichoderma* spp-pepper interaction, finding that some genes involved in SAR, SIR and hypersensitive response (HR) were upregulated. In addition, OE strains enhanced growth and biomass to pepper plants when compared with WT, KO or mocked plants, in some cases was up to 40 %.

92. ECTOMYCORRHIZAL FUNGI ASSOCIATED WITH A RELICT *Fagus grandifolia* var. *mexicana* POPULATION IN THE MONTANE CLOUD FOREST FROM VERACRUZ, MÉXICO.

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Members of the genera *Carpinus* and *Quercus* are common trees of montane cloud forest (MCF) in Mexico. On the contrary, trees of the genus *Fagus* persist only as relict populations. From nearly ten relict *Fagus* populations known to exist in Mexico (Williams *et al.* 2003), just two fragments dominated by *F. grandifolia* var. *mexicana* are present in the State of Veracruz, practically surviving as natural refuges in the MCF of the Sierra Madre Oriental and perhaps marking the southernmost range of *Fagus* distribution in the continent (Montoya *et al.* 2009). One of these sites, the Acatlán Volcano, was selected as our study site. *Fagus* trees in the Volcano occur naturally at 1840-1900 m above sea level and covering ca. 4.7 *ha*. Two of the authors (Montoya and Bandala) have elaborated a research program considering that ectomycorrhizal (EM) fungi related with *Fagus grandifolia* var. *mexicana* populations in Mexico are of special interest: *i)* due to the important ecological role that their symbiotic associations represent for the distribution and maintenance of this tree in this part of the continent, *ii)* the restricted range of *Fagus grandifolia* var. *mexicana* in Mexico, currently showing a rare presence in fragmented habitats, *iii)* its potential vulnerability and *iv*) the scarce documentation of macrofungi in the aforementioned natural refuges. The present contribution is part of that project and reports the advances after studying aboveground fruit bodies and underground EM root tips collected in the Acatlán relict forest during September-November 2008 and February-August 2009. Fruit bodies gathered during random visits in the forest were processed macro- and microscopically for taxonomic identification according to standards for different taxa. EM root systems were separated from soil samples and the morphotypes were characterized macroscopically. Species identification of fruit bodies and mycobiont (in individual EM tips) were identified by PCR amplification and sequencing the ITS region of rDNA as described by Haug *et al.* (2005). Sequence similarities were determined by comparing with those provided by GenBank (NCBI), employing the BLAST sequence similarity search tool. Based on the aboveground fruitbodies collected in Acatlán Volcano we have identified different ectomycorrhizal members of the genera: *Amanita, Boletellus, Boletus, Cantharellus, Cortinarius, Elaphomyces, Gyroporus, Hebeloma, Hydnum, Inocybe, Lactarius, Lycoperdon, Phellodon, Pulveroboletus, Ramaria, Russula, Scleroderma, Strobilomyces, Tricholoma*, and *Tylopilus.* Underground EM fungal genera detected to date are represented by *Cenococcum, Lactarius, Russula,* and *Tomentella*. Relevant ecological data obtained during the monitoring field work, taxonomic study and macro- and microscopic analyses are discussed.

93. MOLECULAR CLONING, IN VITRO, IN PLANTA AND HETEROLOGOUS EXPRESSION OF ENDOPOLYGALACTURONASE FROM *Ustilago maydis*

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The plant cell wall is one of the major barriers that protect plants against pathogens. It provides plants shape, support, regulates physiological processes (including defense responses) and offers protection against pathogen invasion. Many plant pathogens produce an enzyme array capable of degrading plant cell wall components. Among the different cell wall-degrading enzymes (CWDEs) produced by pathogens, most attention has been focused on pectin depolymerizing enzymes. Pectin-degrading enzymes are among the first enzymes produced by plant fungal pathogens during the early stages of penetration and enzymatic degradation of this polymer in plant cell walls is considered an important aspect in plant infection by pathogenic fungus. Polygalacturonases (PGs) are a pectinases class, which participate in plant cell wall degradation by catalyzing homogalacturonan skeleton hydrolysis.
Several PG genes have been isolated from plant pathogenic fungi, due to their importance in the infection process and virulence. In several cases there is conclusive evidence for the role of PGs in the infection process and/or pathogenesis. Whereas had been demonstrated that PGs gene knockout do not affect fungal pathogenicity. *Ustilago maydis* is a biotrophic pathogenic fungus that infects maize, which is the causal agent of the corn smut, a disease with a worldwide distribution, which under some conditions may cause severe economical losses. Biochemical analysis of infection process in *U. maydis*, had demonstrated that PG secretion occurs during fungal colonization and development in maize plant. In a global genome analysis lead to the conclusion that *U. maydis* is poorly equipped with CWDEs and has been demonstrated that PG gene deletion did not result in any discernible effect on the pathogenicity of *U. maydis* to *Zea mays*. However, in *U. maydis* little is known about regulation of PG genes expression.
In this job we are reporting the molecular cloning of a PG coding gene, *Pgum*, from *U. maydis*. The coding sequence of *Pgum* predicts a 37.9 kDa product of 364 amino acid residues. The *U. maydis Pgum* gene is expressed when the fungus is grown in liquid culture media containing different carbon sources. In glucose, *Pgum* expression level decreased after 6 h of growth, but polygalucturonic acid (PGA) induction was constitutive and on plant tissue the expression increased as a function of incubation time. These results suggest that *Pgum* gene expression has a complex regulated path by carbon source. On the other hand, *Pgum* gene expression was detected during plant maize infection around 10 days post-infection with *U. maydis* FB-D12 strain, which coincide with teliospore formation. Finally, expression and secretion of active recombinant polygalacturonase (PGUM) was successfully produced using expression vector pPICZαB and the yeast *P. pastoris* X33 (Mut^+).

94. STANDARDIZATION OF A PROTOCOL FOR THE IDENTIFICATION OF ARBUSCULAR MYCORRHIZAL FUNGI ASSOCIATED WITH ROOTS OF AVOCADO (*Persea Americana* MILL)

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Mycorrhizae are mutualistic symbiotic associations between a group of soil fungi, belonging to the class Glomeromycetes, and roots of most land plants. These associations promote the uptake of water and nutrients from plants, which is particularly important in those plants with small number of root hairs such as avocado. A high diversity of arbuscular mycorrhizal fungi (AMF) spores has been reported in the soil of avocado orchards in the state of Michoacan, Mexico; however, neither the species of AMF directly involved in the mycorrhization of avocado or the role they may have within the pro-

duction system have been identified. This is mainly because there are no evident morphological differences between AMF species once the symbiotic association is established. Thus, the AMF species composition within roots may only be analyzed by molecular markers. To the best of our knowledge, the specificity of AMF colonizing avocado roots has not been reported before. One limiting factor to the use of molecular markers in this symbiosis is that the DNA extraction from such woody plant roots with high phenolics content is complicated. Herein we evaluated five different protocols to isolate DNA from avocado roots. This DNA was used in nested PCR assays with specific primers to identify AMF. The commercial Illustra TM kit (Tissue & cell genomic DNA, GE Healthcare) render the best results and DNA from Glomeraceae, Acaulosporaceae and Gigasporaceae families were amplified.

95. ROLE OF ETHYLENE IN REGULATING LATERAL ROOT DEVELOPMENT AND PLANT GROWTH STIMULATION BY *Trichoderma* FUNGI in *Arabidopis thaliana*

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Plants display considerably plasticity in response to endogenous and environmental signals. Inoculation of *Arabidopsis* seedlings with plant beneficial *Trichoderma* fungi stimulates pericycle cells within elongating primary roots to produce new lateral roots, and this effect is related to an increased biomass production. *Trichoderma virens* has been found to produce auxins, including indole-3-acetic acid (IAA) and other related indolic compounds, which play a role in root system architecture remodeling and plant growth promotion. Crosstalk between auxin and ethylene in root elongation and root hair formation has been demonstrated, but interactions between these phytohormones in plant responses to rhizospheric fungus are not well characterized. In this study, we investigated the role of ethylene in root developmental responses to *Trichoderma* fungi. We found that enhanced ethylene synthesis, resulting from the application of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) in uninoculated WT (Col-0) plants, or ethylene overproduction in Arabidopsis ethylene overproducer (*eto1*) mutants strongly induce root hair formation, an effect reminiscent of *T. virens* inoculation. In these plants, however, both primary root growth and lateral root development were inhibited by ethylene. Mutations that block ethylene signaling, *etr1*, *ein2* and *ein3* increase both primary root growth and lateral root initiation. Inoculation of ethylene insensitive mutants with *Trichoderma virens* or *Trichoderma atroviride*, caused a further increase in primary root growth, lateral root initiation, lateral root elongation and plant biomass production, indicating that ethylene plays a negative role in plant responses to these fungi. Taken together, our data show that auxin and ethylene play complimentary roles in root hair formation, but opposite roles in primary and lateral root development in response to *Trichoderma* fungi.

96. MULTILOCUS ANALYSIS OF *Histoplasma capsulatum* ISOLATES ASSOCIATED WITH CAVE-DWELLING BATS

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Histoplasma capsulatum, Darling 1906, is a saprobe-geophilic fungus belonging to the Ascomycota phylum, class Eurotiomycetes, order Onygenales, family Ajellomycetaceae. *H. capsulatum* has a teleomorphic state (sexual) represented by the genus *Ajellomyces*, which has only a "*capsulatus"* species. The anamorphic state (asexual) includes three taxonomic varieties identified by their micromorphology, geographical distribution, and association with the host as well as the clinical forms of the disease. The three varieties, *H. c.* var. *capsulatum, H. c.* var. *duboisii*, and *H. c.* var. *farciminosum* are dimorphic and causative agents of histoplasmosis capsulati, histoplasmosis duboisii, and histoplasmo-

sis farciminosi diseases, respectively. *H. capsulatum* is an intracellular preferential pathogen of the phagocytic mononuclear system of mammals. *H. capsulatum* grows favorably in accumulated bird and bat guano with different feeding behaviors: insectivorous, frugivorous, pollinivorous, nectarivorous, hematophagous, etc., indicating that the diet of chiropters does not influence the nutrimental conditions of this fungus.

We studied several *H. capsulatum* isolates from naturally infected bats, captured randomly in different caves of Mexico, using a phylogenetic multilocus analysis of five molecular markers [arf, H-anti, ole1, tub1, and (GA)n] evaluated in a concatenated way, considering both neirghbor-joining (NJ) and maximum parsimony (MP) algorithm. The NJ and MP trees of the five concatenated markers defined two large groups of Mexican *H. capsulatum* isolates in relation to the outgroup from US. The first group was formed by all the isolates from the migratory bat *Tadarida brasiliensis mexicana* together with one isolate from *Mormoops megalophylla* (previously considered as a lineage). The second group clumped all the isolates from the non-migratory bats. These results support the proposal that the group containing *H. capsulatum* isolates from *T. brasiliensis mexicana* and *M. megalophylla* bats should be considered as a new *H. capsulatum* genetic population (clade). It is likely that the *T. brasiliensis mexicana* species, which has very particular migratory characteristics, has shared the ecological niche of the fungus during a considerable lapse of time along the evolution time, modulating the environmental parameters of the habitat and favoring changes that allowed for a better adaptation and survival of both organisms. This postulation is based on the notion that the interaction between the *T. brasiliensis* bat and the *H. capsulatum* fungus could have started since the Pleistocene. Certainly a common evolutionary history can be proposed for both, *T. brasiliensis* (wild host) and *H. capsulatum* (pathogen) whose outcome is still unknown.

97. THE SECRETOMES ON THE INTERFACE OF *Magnaporthe grisea*-RICE LEAF INTERACTION

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Magnaporthe grisea, a fungal plant pathogen, is a major threat to rice production globally. In response to various growth conditions it secretes extracellular proteins, which play indispensable roles in host-pathogen interactions. Plant pathologists have concentrated on elucidating the relation between environmental factors and field development of the disease. However, molecular basis of the resistance mechanisms in rice plants against *M. grisea* or of host specificity for *M. grisea* has not been clearly elucidated. A catalog of secreted proteins would therefore represent a valuable resource to elucidate processes such as pathogenesis and disease resistance. The aim of this study is to gain new insights into the dynamics and complexity of the interaction of secreted proteins by *M. grisea* during rice infection.

To obtain the extracellular protein samples, rice seedlings were inoculated with *M. grisea* conidia for three days. The infected leaves were gently agitated in a bath of water. The extracellular proteins (ECPs) were concentrated and fractionated according to their isoelectric points (pI) using a liquid-phase isoelectric focusing system (rotofor). Then, samples were trypsin digested. A portion of each rotofor fraction was subjected to ConA affinity chromatography to separate N-linked glycoproteins. The glycproteins were trysin digested, followed by a second ConA column to extract N-glycopeptides from the nonglycosylated peptides. Samples were analyzed by LCMSMS on a linear ion trap mass spectrometer. Data was searched using Mascot algorithm and statistically evaluated using ProValT algorithm. Preliminary data by non-gel based fractionation method, 97 proteins have been identified. Seventy-three of these were identified as fungal proteins and twenty-four were identified as rice proteins. The functional categories of secreted proteins from *M. grisea* were identified as protein degradation, cell wall catabolism, secondary metabolism, pathogenicity, and unknown. Proteins from *Oryza*

sativa L were classified into two categories: defense and unknown. Currently work is being performed on the analysis of glycopeptides. To the best of our knowledge, this is the first proteomic and glycomic study performed on *M. grisea*-rice leaf interaction. Details of invasion strategies of fungal plant pathogens and the interplay between host and these pathogens are largely unknown. By studying secreted proteins of the interaction of *M. grisea* during rice leaf infection, we expect to gain vital information for deciphering the biological events happening in both plant and pathogen perspectives during the invasion process.

98. RECOMBINANT EXPRESSION OF MfAtr4, AN ABC TRANSPORTER FROM *Mycosphaerella fijiensis* USING *Pichia pastoris*

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ATP-binding cassette (ABC) transporters comprise the largest family of membrane transport proteins and are ubiquitous in all living kingdoms. ABC transporters use the energy derived from ATP hydrolysis to drive the transport of endogenous metabolites and exogenous toxic compounds over biological membranes. In pathogenic fungi ABC transporter can protect fungi against fungicides, plant defense compounds, or they may also play a role in pathogenesis by secretion of pathogenicity factors. So far, only in *Giberella pulicaris* (ABC1), *Magnaphorte grisea* (ABC1), *Botrytis cinerea* (*Bcatr*B) and *Mycosphaerella graminicola* (*MgAtr*4) it has been shown that the ABC transporters have a direct function in the pathogenesis on the host.

Mycosphaerella fijiensis is the most important threaten for bananas and a serious agronomic problem worldwide. Knowledge about its molecular pathogenic battery is largely unknown, but it is probable that some of its ABC transporters being involved. In the phylogenetic related fungus *M. graminicola* it has been identifies the *MgAtr4* as the ABC transporter member involved in pathogenesis, although its specific role has not been established. We speculate that the largest homologous Mf ABC transporter may play an equivalent role in *M. fijiensis*. We call this gene *MfAtr4* and our goal is to determine its function during the *M. fijiensis*-Musa spp interaction. One challenge to characterize the function of MFATR4 protein is the little amount that is being expressed by *M. fijiensis* and the difficulties to purify this kind of membrane proteins. To facilitate the structure/function analysis, we are cloning its CDS in the methylotrophic yeast, *Pichia pastoris*. In the present work we described the isolation and cloning of the full CDS encoding the putative *MfAtr4* (4977 pb). The nucleotide blastX analysis showed that putative *MfAtr4* gene has the firts hist with *MgAtr4* gene, with an identity of 69.3%. Searching into the *M. graminicola* sequence genome it was retrieved ABC transporter sequences, and *MgAtr*4 (the first described virulence factor in this genus) shows the largest identity with the query. And viceversa, using *MgAtr4* as the query and searching in the *M. fijiensis* sequence genome the first hit was with the gene we cloned. Currently we have subcloned from pGEM-Teasy vector into pPICZA for the heterologous expression in the *P. pastoris* system.

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99. IMPACT OF ENVIRONMENT(AL STRESS) ON MYCORRHIZA

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Mycorrhizal fungi have a deep impact on nutrition and health of plants, especially under stress conditions as heavy metals. Field experiments show that mycorrhizal profile depends on the bioavailability of heavy metals.
First investigations of gene expression indicate a specific expression in lab experiments with microarray assay too. Different basidiomycetes *Tricholoma vaccinum*, *Paxillus involutus*, *Amanita muscaria*, and for control the wood rotting fungus *Schizophyllum commune* were used for comparison of expression pattern after growth on heavy metal and fungicide containing media.
Using an *in vitro* model system, differential gene expression in *Tricholoma vaccinum* spruce ectomycorrhiza was investigated and genes expressed mycorrhiza specific but also in adding toxic substances in pure cultures of the fungus were investigated.

100. *Trichoderma* FUNGI PROMOTES GROWTH AND REGULATES ROOT SYSTEM ARCHITECTURE IN *Arabidopis thaliana* VIA THE PRODUCTION OF AUXIN SIGNALS

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Trichoderma spp. belong to a class of free-living fungi beneficial to plants that are common in the rhizosphere. We investigated the role of auxin in regulating the growth and development of *Arabidopsis thaliana* seedlings in response to inoculation with *Trichoderma virens* and *Trichoderma atroviride* by developing a plant-fungus interacting system. WT Arabidopsis seedlings inoculated with either *T. virens* or *T. atroviride* showed characteristic auxin-related phenotypes including increased biomass production and stimulated lateral root development. Mutations in genes involved in auxin transport or signaling *AUX1*, *BIG*, *EIR1* and *AXR1* were found to reduce the growth-promoting and root developmental effects of *T. virens* inoculation. When grown under axenic conditions, *T. virens* produced the auxin-related compounds indole-3-acetic acid, indole-3-acetaldehyde and indole-3-ethanol. A comparative analysis of all three indolic compounds provided detailed information about the structure-activity relationship based on their efficacy at modulating root system architecture, activation of auxin-regulated gene expression and rescue of the root hair-defective phenotype of the *rhd6* auxin response Arabidopsis mutant. Auxin determinations in plant tissues by gas-chromatography/mass spectrometry (GC-MS) revealed increased IAA accumulation in plants inoculated with either *T. virens* or *T. atroviride*. Our results highlight the important role of auxin signaling for plant growth promotion by *Trichoderma* fungi.

101. IDENTIFICATION OF *Ustilago maydis* PATHOGENICITY- AND PLANT DEFENSE-RELATED GENES USING THE *U. maydis-Arabidopsis thaliana* PATHOSYSTEM

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We used *Arabidopsis thaliana* L. inoculation with *Ustilago maydis* (DC.) Cda. haploid strains as an experimental model for the analysis of genes that become up- or down-regulated during infection of the plant with *U. maydis*, in both, the host and the pathogen. For these analyses we used two approaches: construction of substractive cDNA libraries, and microarrays. Six-seven dais-old *A. thaliana* plantlets, were inoculated with strain FB1 (F. Banuett) haploid *U. maydis* cells. At different times

post-inoculation total RNA was isolated from the plants. Controls included un-inoculated plants and fungal cells obtained after 20 h of incubation in complex medium.
Substractive libraries corresponding to RNA isolated at 24, 72 or 96 h post-infection were obtained using the Kit of PCR cDNA substractive system from CLONTECH to obtain *U. maydis* and *A. thaliana* gene fragments. A total of 267 clones of cDNA were obtained and subjected to sequencing. All of the sequences corresponded to either the *A. thaliana* or *U. maydis* genomes. In total, 21 genes from *U. maydis* and 37 genes from Arabidopsis were identified.
Up- or down-regulated genes from the host were also identified with microarrays, using the system of 29,000 element Arabidopsis Oligonucleotide Microarrays from the Quiagen-Operon Arabidopsis Genome Array Ready Oligo Set (AROS) version 3.0, available from the University of Arizona. By this mean we identified 700 genes, using values of 2.0/0.5 (ANOVA 0.05 p value) for up or down-regulated genes respectively. Values of 93/47, 132/28, and 63/21 genes were identified in RNA samples obtained 24, 72 or 144 h post- infection, respectively. Using the taxonomy from GENE ontology we found that the up- or down-regulated genes belonged to the following categories: 229 to biological process unknown, 26 to defense response, 13 to defense response to fungi plus a few to other different categories. These results were validated by measuring differential expression of some selected genes by RT-PCR.
It must be stressed that the expression of the different genes followed distinct kinetics, and in particular we must emphasize the significant number of genes whose expression responded to fungal infection (ten), some other ones whose expression was regulated by fungal infection, and others known to be involved in plant defense in general.
We conclude that these results exemplify the advantages of using this pathosystem for the analysis of the interactions of *U. maydis* with its host during the infection process.
This work was partially supported by PROMEP, CONACyT and CONCYTEG (México).

102. YEAST INFECTION OF PRIMARY MURINE FIBROBLASTS: INFECTION AND IMMUNE RESPONSE FEATURES.

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The main and well characterized fibroblasts' function is tissue repairing. Now, their participation in the immune responses is known. However, fibroblasts' interactions with pathogens and the innate immune response triggering by these cells have not been analyzed in detail. In this study, the response of several types of fibroblasts was analyzed; among them were mouse primary culture of corneal and skin fibroblasts as well as the mouse lung fibroblast cell line MLg. These cells were challenged with two opportunist yeasts, C. albicans and C. glabrata and to the non-pathogenic yeast *S. cerevisiae* (heat killed). Endocytosis assays with opsonized and non opsonized yeasts were performed; cellular responses, as production of reactive oxygen species (ROS), and nitric oxide (NO) production were evaluated; finally, complement receptors engagement in adherence and yeast uptake were also assayed. Ours results showed that, the three types of fibroblasts had low uptake ability for S. cerevisiae; however this feature was improved when the yeasts were opsonized. With alive opportunist yeast (C. albicans and C. glabrata), uptake rates were much higher than those for non-pathogenic yeast; the highest endocytosis rate was observed with corneal fibroblasts and opsonized C. albicans interaction. Yeast internalization trigger a high ROS production only with opsonized S. cerevisiae, while both opportunist yeasts trigger only a low ROS production. In comparison, nitric oxide production was induced mainly when the three types of fibroblasts interacted with opsonized S. cerevisiae, and C. albicans; the highest NO production was trigger by opsonized S. cerevisiae followed by C. albicans, the lower production was observed with C. glabrata. In general, fibroblasts from MLg cell line were the highest NO producers; NO production induced by S. cerevisiae in the three types of fibroblasts increased in a time dependent-fashion, situation that was not observed with C. albicans in which an early NO production

was observed and then a sudden fall. Finally, CR3 blockade inhibited significantly only the pathogenic yeast uptake by skin fibroblasts; accordingly by immunostaining skin fibroblasts showed the highest distribution of CR3 receptors on their cellular surface. All these results showed that fibroblasts had endocytic capabilities; are able to display an innate immune response against yeasts and they had a differential CR3 distribution on their cell membrane.

103. IDENTIFICATION OF SUCEPTIBLE AND RESISTANT MAIZE CULTIVARS TO *Ustilago maydis*.

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Ustilago maydis (DC) Corda is a basidiomycete, dimorphic, biotrophic and pathogenic fungus specific for teozintle and maize (*Zea mays* L.). In maize *U. maydis* cause the disease know as common smut or "huitlacoche" that is world-wide distributed. Typical symptom of disease is the flesh, edible galls on corn ears. Huitlacoche (syn. cuitlacoche) is named "maize mushroom", "Mexican truffle" or "caviar azteca", and since prehispanic culture represent a culinary delicacy in Mexico, but recently has been accepted by North American and Europe public, who view it as a gourmet food item. Common smut cause large economic losses in susceptible corn cultivars. On the other hand, 'huitlacoche' means money incomes because represent an economic activity by his great quote in the market. Teliospores disperse, apply teliospores on stigmatas and artificial inoculation of susceptible maize varieties, are some farmer's efforts in order to increase incidence of disease and yield of huitlacoche. Exist several corn cultivars that show differences in susceptibilitiy or resistance to the fungus. However host resistance is the only practical control method of disease. By means techniques of artificial inoculation on maize plantlets in greenhouse, using *U. maydis* natives strains we analized 30 maize cultivars (hybrids and experimental lines) of the Northeastern of México, in order to identify resistance and susceptibility to the fungus, for huitlacoche production and increase the yielding of the maize grain.

104. GENES OF THE MULTIDRUG AND TOXIC COMPOUND EXTRUSION (MATE) FAMILY IN FUNGI

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All over the world fungi can be found in different habitats and in interaction with a multiplicity of organisms. These widespread distribution and the contact to other organisms have a lot of advantages but also bear the risk of having contact to antagonistic defense mechanisms including toxic compounds. To prevent their cells from these substances a lot of facilities are given. One possibility is the extrusion via multidrug transporters. These proteins can transport toxic substances out of the cell and save the cells from damages. Because of the high number of transporters in the membrane only a part of these proteins is well investigated yet. A new familiy of multidrug transporters are the proteins from the multidrug and toxic compound extrusion (MATE) family. For some orthologes in human, bacteria and plants their role in detoxification is understood. They can transport e.g. chemotherapeutics, antibiotics and secondary plant metabolites. In fungi only ERC1 (ethionine conferring resistance) from *S. cerevisiae* is described as being responsible for accumulation of ethionine when it is overexpressed in the cell. Like most fungi yeast has two MATE paraloges. In drop test experiments both MATE gene knock-out mutant strains were tested on heavy metal containing media. Not delta*erc*1 but the second MATE gene knock-out mutant strain shows reduced growth in contact to some metals. Both strains were used for heterologues expression experiments with a MATE gene from the ectomycorrhizal fun-

gus *Tricholoma vaccinum TvMMF*1. The *T. vaccinum* MATE sequence shares approx. 60 % aa similarity to other basidiomycetes MATE proteins and has the characteristic 12 transmembrane domain structure. Interestingly, this gene is higher expressed in the symbiosis with its host *Picea abies*. The investigation of the role of *TvMMF*1 in ectomycorrhizal interaction can give a first insight into MATE transporter function in basidiomycetes.

105. ANALYSIS OF THE EXPRESSION OF HOMOLOGOUS GENES ASSOCIATED WITH VIRULENCE FACTORS IN *Mycosphaerella fijiensis,* THE CAUSAL AGENT OF BLACK SIGATOKA DISEASE OF BANANA

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Mycosphaerella fijiensis is the causal agent of black Sigatoka disease, the most devastating disease of banana and plantain at the worldwide (FAO, 2006). Currently, this disease is present in all the productive regions of the tropics.
One of objectives of research efforts to control this disease has been focused on the search for virulence factors whose roles may be preponderant during the host-pathogen interaction and which are expressed exclusively or differentially during the host-pathogen interaction are preferred. The known virulence factors include proteins with enzymatic activities (lipases, esterases, proteases, etc.) and elicitor avirulence proteins (Avrs). Until recently it was believed that there was no conservation between these genes. However in 2008 Bolton *et al*. reported the identification of virulence genes in *Cladosporium fulvum* Ecp6 which had homologues in other fungal species.
During the *in silico* screening of the *M. fijiensis* genome, we found genes which had homologues *C. fulvum* Avrs factors; this fungus belongs to the same order of as *M. fijiensis*. The identified homologues were for *Avr4, Ecp2 Ecp6* genes. The same genes were also identified *in silico* by Dr. DeWit's work group.
In this report our goal is being to explore if these genes are expressed during the infective process, the presence of theirs transcripts was evaluated by RT-PCR on field banana plants infected with black Sigatoka disease. All *M. fijiensis´s Avr4, Ecp2 Ecp6* homologues are expressed in the samples, in some cases over-expressed, which could support a hypothesis that they have in *M. fijiensis* similar function to their *C. fulvum* homologues.
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106. THE TYPE IV DIPEPTIDYL AMINOPEPTIDASE FROM *Ustilago maydis*

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Dipeptidyl aminopeptidases (DPP) are proteases which remove dipeptides from the N-terminus of peptides. These enzymes are classified according to the dipeptide released. Type IV dipeptidyl aminopeptidase (DPP IV) removes N-terminal X-Pro and X-Ala dipeptides. Many biologically active peptides are protected from general proteolytic degradation by evolutionary conserved prolines (Pro), due to conformational constraints imposed by the Pro residue.
U. maydis, a pathogenic fungus that causes the corn smut, produce an intracellular typo IV dipeptidyl aminopeptidase (DPP IV) encoded by *dap2* gene. This gene was expressed in *P. pastoris,* the recom-

binant protein removes dipeptides from the N-terminal of Ala-Pro-pNA and was specifically inhibited by PMSF and Pefabloc indicating that this is a serine protease.
From variety yeasts these proteases are involved in the general amino acids metabolism and protein processing. However the role of the DPPIV in the fungus life-cycle is unknown so, the construction of null mutants of this gene could allow to Known this.

107. IDENTIFICATION OF *Botryosphaeriaceae* ASSOCIATED WITH GRAPEVINE DECLINE IN BAJA CALIFORNIA

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In Baja California, Mexico, grapevine (*Vitis vinifera* L.) is the most economically important fruit crop. Production is used mainly for the wine industry. Around 95% of Mexican wine originates from this State. Trunk diseases produced by fungi are a very serious threat for the grapevine industry. Pathogens invade through pruning wounds created in the woody parts of the plants; colonize the xylem interrupting its conductivity, and cause slow decline and dieback of the vine. In recent years an increasing number of anamorphic Botryosphaeriaceae had been found in all growing regions worldwide. Grapevine decline in Mexico had been associated with *Eutypa lata* in Aguascalientes and Durango and with *Lasiodiplodia theobromae* and *Diplodia seriata* in Baja California. Since a total of 14 species has been reported in others parts of the world, is highly probable that there are more *Botryosphaeria* spp in Baja California. With the aim of identify the species of *Botryosphaeria* associated with grapevine decline and dead arms, a survey was conducted from 2007-2009 in Ensenada, Baja California. A total of 17 putative *Botryosphaeria* spp were isolated from trunks and cordons. Isolates were identified based on their morphological characteristics along with the phylogenetic analysis of the internal transcribed spacer region (ITS-5.8S-ITS2) and the partial sequence of the ß-tubulin gene. Data analysis showed that at least four *Botryosphaeria* spp occur in Baja California: *Diplodia seriata*, *Neofusicoccum vitifusiforme*, *Diplodia corticola*, and *Neofusicoccum australe*. *D. seriata* was the most common species found in the vineyards, and it was mainly isolated from wedge-shaped sectors in the wood and dead arms, while *N. vitifusiforme*, *D. corticola*, and *N. australe* were found mainly associated with black streaks and brown-red wood. A pathogenicity test done using green grapevine shoots of the *Vitis vinifera* cv. Merlot, showed that *D. seriata*, *N. vitifusiforme*, and *N. australe* were able to produce symptomatic infections; however lesions were smaller than those produced by *L. theobromae* used as positive control. *D. corticola* seems to be a weak pathogen since it did not produce disease symptoms under the same conditions. These findings confirm the presence of three new species of fungi in Baja California: *N. vitifusiforme*, *D. corticola*, and *N. australe*.

108. BIOLOGICAL CONTROL OF TRUNK DISEASE FUNGI USING *Trichoderma spp.*

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Grapevine is one of the most important fruit crops in Baja California; production is mainly used for wine making. This state alone generates 95% of the wine produced in Mexico. Grapevine trunk diseases, caused mainly by fungi, are among the main factors limiting vineyard longevity and productivity. Recent studies in the Ensenada'coast of Baja California had found several fungi associated with trunk diseases, including several Botryospharaceaus spp., *Cylindrocarpon* sp., *Phaeoacremonium aleophium* and *Phaeomoniella chlamydospora*. Due to the importance of these pathogens worldwide,

finding diseases control is a priority. *Trichoderma* spp. are good competitors in soil, and producers of volatile and nonvolatile antibiotics that suppress plant pathogens. Here, we evaluated six *Trichoderma* spp isolated from field-grown grapevines in their potential to control fungi associated with trunk diseases and compare them with a well characterized isolate of *T. atroviride* and a commercial strain of *T. harzianum*. Pathogens tested were: *Lasiodiplodia theobromae*, *Diplodia seriata*, *Neofusicoccum vitifusiforme*, *Diplodia corticola*, *Neofusicoccum australe, Cylindrocarpon* spp., *P. aleophium* and *P. chlamydospora.* Results showed that two isolates named CCMT01-1 and SACH26-1, showed higher percent of inhibition than the commercial strain and *T. atroviride* against all the fungi tested. Isolate CCMT01-1 inhibited all the pathogens but *P. chlamydospora* while SACH26-1 inhibited all of them. Both CCMT01-1 and SACH26-1 produce volatile and nonvolatile compounds. Isolates were identified based on their morphological characteristics and the analysis of their internal transcribed spacer region (ITS-5.8S-ITS2) as members of the species *T. gamsii*. These results open the possibility of using these isolates to control fungi affecting grapevine in the Ensenada'coast.

109. INITIAL STUDIES OF *Aspergillus* POPULATIONS FROM SOILS IN JALISCO, MEXICO

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Aflatoxins are carcinogenic secondary metabolites produced by *Aspergillus flavus* and related fungi, that contaminate feed and food. In Mexico, specifically on the state of Jalisco, not much research has been conducted on the etiology of this group of fungi. There are 3 strains of *A. flavus* (S, T, and L), and it is not known in which proportion they occur in maize field soils or grains of corn from Jalisco state. During 2008-2009, seventeen samples of soil from corn fields and recently harvested grains were collected in Jalisco state and plated on two different media. We recovered 188 strains each on CU medium and on Si10, but the CU medium was more useful to get only *Aspergillus flavus* and *A. parasiticus* strains. Use of AFPA medium favored aspergillic acid production by various *Aspergillus* strains obtained from Si10 medium. In general, for isolates from soils 15% of *Aspergillus* strains originated in Zacoalco de Torres, 14% in Tizapán El Alto, and 15% for Atotonilco. S strains of *A. flavus* were only reported in Puerto Vallarta region. Several other isolates produced high concentrations of B1, B2, and G2 aflatoxins. This is the first report on populations of aflatoxigenic fungi from soils of corn fields in Jalisco region.

GENOMICS

110. TRANSCRIPTIONAL PROFILING OF *Yarrowia lipolytica* CELLS DURING THE YEAST TO HYPHA TRANSITION (DIMORPHISM)

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The yeast *Yarrowia lipolytica* has received increasing attention as a model to study dimorphic transition because of its ability to alternate between a unicellular yeast form and distinct filamentous forms (hyphae and pseudohyphae). This fact, combined with the availability of specific molecular and genetic tools, has provided . *lipolytica* with a number of advantages over *Saccharomyces cerevisiae* and *Candida albicans* for investigation the molecular mechanisms underlying dimorphic transition. Like *C. albicans*, . *lipolytica* displays complete yeast to hypha transition that can be induced by different

environmental factors: carbon source, pH, nitrogen source, N-acetylglucosamine, serum, etc. In our previous work we have isolated the transcription factor *YlHOY*1*,* involved in the yeast-hypha transition. This gene codifies a 509 amino-acid protein with a homeo domain and nuclear location, without a clear orthologs in other yeast species. Deletion of *HOY*1 in . *lipolytica* prevents the yeast-hypha transition.
Microarray technology is one of the resources created by the genome projects and other sequencing efforts to answer the question: what genes are expressed in a particular cell type of an organism, at a particular time, under particular conditions? Using 70 mer DNA microarrays from the *Yarrowia* consortium built by Eurogentec representing 6404 ORF from . *lipolytica* CLIB122 (98 % of total annotated ORFs), we have analyzed and compared the transcription profiles between wild type cells and a *hoy*1 deleted strain, during the yeast to hypha transition induced by N-acetylglucosamine at different times (15, 60 and 180 minutes). We have analyzed every spot in each microarray to calculate signal and background intensities. Signals were log transformed and per-chip normalized by an intensity-dependent method (LOWESS). The first filter applied to the normalized data was based on an arbitrary fold change of 1.5 (decrease or increase) between *hoy*1 versus wild type, on at least 1 ratio. Next, to identify genes regulated by the yeast-hypha transition, a non-parametric test of Wilcoxon-Mann-Withney, with a Benjamini and Hochberg multiple test correction (P-value < 0.01) was applied to the list of genes. All the data were analyzed with the GeneSpring program. The gene annotations of Génolevures (http://www.genolevures.org/) were used. Ten genes up and down regulated were confirmed by RT-PCR. The results obtained in this work constitute the first evidence of transcriptional profiles of the yeast . *lipolytica* using microarrays technology.

111. THE PHOSPHORELAY SYSTEM Sln1p-Ypd1p-Ssk1p IS NOT ESSENTIAL FOR THE OSMOTIC STREES RESPONSE IN THE YEAST *Kluyveromyces lactis*

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All cells respond to stresses generated by the environment in which they develop. Concerning to osmotic adaptation, yeast cells contain at least two branches to cope with high osmolarity conditions, the Sho1p branch and the Sln1p branch (phosphorelay system). The phosphorelay pathway is similar to prokaryotic two component systems. The two component system in bacteria refers to phosphate transfer between two proteins: a protein domain that contains a sensor histidine kinase and an acceptor domain containing an aspartate residue. In the active state the sensor protein autophosphorylates and then it donates the phosphate group to the aceptor protein. Phosphorelay systems have been described in organisms such as *Saccharomyces cerevisiae*, *Arabidopsis thaliana*, *Candida albicans*, and *Dictyostelium discoideum*. In *S. cerevisiae* the phosphorelay is carried out by the osmosensor Sln1p, which phosphorylates the transfer protein Ypd1p and this phosphorylates the response regulator Ssk1p. In normal growing conditions the phosphorelay system is on, but when the cell is exposed to high osmolarity the system turns off, activating the HOG pathway (High Osmolarity Glycerol). Under osmotic stress, the response regulator Ssk1p remains unphosphorylated, allowing the MAPKKK Ssk2p to activate the MAPKK Pbs2p, which activates the MAPK Hog1p, which finally activates transcription of genes required for adaptation to high osmotic conditions. *Δsln1* and *Δypd1* mutants are lethal because of constitutive activation of Hog1p, while *Δssk1* mutants are viable and sensitive to high osmotic stress.
In the budding yeast *Kluyveromyces lactis*, the phosphorelay system operates differently from that of *S. cerevisiae*. Disruption of the genes *KlSLN1*, *KlYPD1* and *KlSSK1* generates viable mutants that show moderate sensibility to osmotic stress. These phenotypes displayed by the *K. lactis* mutants are unique among fungi and suggest that in this species, the phosphorelay system is not essential for the osmotic response and that it regulates positively the HOG pathway. Currently we are studying the

phosphorylation state of Hog1p in the *K. lactis* mutants, and we are investigating the impact that the two branches, Sln1p and Sho1p have in the adaptation to high osmotic conditions in this yeast.
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112. RESISTANCE MARKERS DETECTION IN *Candida albicans* ISOLATES WITH DIFFERENT FLUCONAZOLE SUSCEPTIBILITY USING ARMS PRIMERS

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Candida albicans is the fungal pathogen most frequently isolated from skin and mucoses. *C. albicans* represents 70 to 80% of the isolates obtained from infected patients. Thus, antifungal therapies with azoles have been employed which lead to an increase in resistance incidence and occasionally to the treatment failure. Nowadays in an effort to understand the molecular mechanisms implicated in the apparition of resistance and susceptibility in *C. albicans* gene expression analyses have been employed. In the present work the prescence-abscense of molecular markers for fluconazole resistance are identified in clinical isolates. To characterize the antifungal susceptibility of the isolates, M27-A2 protocol was used. Initially, fluconazole resistance of 40 isolates from vulvovaginitis patients from the department of ginecology and obstetrics from Hospital Universitario were analyzed. The results obtained from antifungal susceptibility testing were compared with those obtained with E-Test and agar dilution, which allowed us to analyze the efficiency of the 3 methods and from this results 8 strains were selected according to their characteristics and in order to proceed with the presence searching of the markers *ERG3* and *TAC1*, employing the ARMS oligonucleotide technology for mutation detection. Results obtained using ARMS oligonucleotides indicated differences in the DNA sequences for isolates 09 and 11 that didn't allow amplification with the oligonucleotides that were designed. The rest of the isolates are presented without mutation, with the exception of strain 36 which shows a pattern that is different for both the wild type pattern and the T57A/G mutation. For *TAC1*, only strain 34 shows the mutation pattern for N977D. Strains 36 and 40 resulted in no amplification of *ERG11* while susceptible strain 21 showed an evidently reduced expression of this gene. Results of this work indicate the relationship between expression of resistance factors and the type of growth to which the organisms is submitted to and ARMS technology is presented as an effective and relatively cheap method to search for fluconazole resistance markers in clinical isolates of *Candida albicans.*

113. IDENTIFICATION OF GENES INVOLVED IN THE SYNTHESIS OF BETA - (1,3)-D-GLUCANS IN *Ganoderma lucidum*

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Ganoderma lucidum is a macromycete economically and culturally important in China, Korea and Japan because of its medicinal properties. This species contain different bioactive compounds, including polysaccharides that have demonstrated immunomodulatory, anticancer and radioprotective effects. Polysaccharides from *G. lucidum* such as beta-D-glucans and heteropolysaccharides have been isolated and characterized. However the genes involved in the synthesis of beta-(1,3)-D-glucans have not been identified.
Previous works have identified genes involved in this pathway for other organisms, based on this background the enzymes involved in the formation of beta - (1,3)-D-glucans from sucrose are the alpha glucosidase, beta fructofuranosidase, hexokinase, glucose-6-phosphate isomerase, phosphoglucomutase, UDP-glucose pyrophosphorylase and 1,3-beta-glucan synthase.

The aim of this study was to identify the genes involved in the synthesis of beta-(1,3)-D-glucans in *G. lucidum*. First, specific and degenerate primers were designed for each of the enzymes using the previous reported genes as templates. Subsequently PCR reactions were performed using the DNA extracted from *G. lucidum* and *Ustilago maydis* as positive control. Correct weight bands were cloned and are ready for the sequencing process. This will be the first report of the genes involved in metabolic pathway of the synthesis of beta-(1,3)-D-glucans in *G. lucidum.*

114. ANALYSIS OF THE GENETIC INSTABILITY AND HOMOLOGOUS RECOMBINATION PATHWAYS OF *rad51*-ΔΔ MUTANTS OF *Candida albicans*

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We have characterized the *RAD51* orthologue of the pathogenic fungus *Candida albicans*. *rad51*-ΔΔ mutants were constructed by sequential deletion of both alleles in the CAI4 background. Null *rad51* strains retained the ability to integrate linear DNA with long flanking homology in the genome. However, both the transformation efficiency of the disruption cassette a non-essential gene of chromosome 2 (*SHE9*) into *rad51*-ΔΔ strains as well as the frequency of correct targeting were significantly decreased as compared to wt. By comparison, *rad52* mutants did not showed any transformant. *rad51*-ΔΔ strains heterozygous for *SHE9* (*SHE9/she9::hisG-URA3-hisG*) generated 5-FOA^R segregants at a rate similar to wt. About 67% of these segregants (79% for wt) were characterized as *SHE9/she9::hisG*, indicating that Rad51 is dispensable for single strand annealing (SSA). The rest (33%) carried exclusively the *SHE9* allele (18% for wt) and at least 50% of them carried both Chr2 homologues, as deduced from the heterozygosity of selected SNPs makers indicating that gene conversion can also occur in the absence of Rad51. The other 50% was homozygous for all the markers, suggesting chromosome loss. Null *rad51* strains, which are heterozygous for *HIS4* (*HIS4/his4*), produced His$^-$ auxotrophs at a frequency of 10^{-3} per viable cell, which is similar to that yielded by *rad52* mutants, and the number of auxotrophs increased significantly after UV treatment. Most of the *rad51* auxotrophs (90%) have lost Chr4b and only 10% carried a truncated version of that homologue. These values were 50% each for those auxotrophs derived from *rad52*. Our results indicate that *rad51* strains of *C. albicans* are as genetically unstable as their *rad52* counterparts, but the later are more deficient in homologous recombination.

115. A MORE ROBUST CHITIN SYNTHASE PHYLOGENY AND EXAMINATION OF CHITIN SYNTHASE EXPRESSION IN *Chaetomium globosum*

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Fungi are able to tolerate an extreme range of environmental conditions through the presence of a cell wall, that in the majority of true fungi, consists of chitin, a linear polymer of N-acetyl-glucosamine. Chitin synthesis is dependent upon the regulation of distinct chitin synthase isoenzymes that have been divided into seven distinct classes on the basis of their structure in conserved regions. Previous studies of fungi indicate that chitin synthase (CS) gene number varies in the fungi; from one copy in the genome of *Schizosaccharomyces pombe* to seven in filamentous fungi like *Aspergillus fumigatus*. A more robust phylogenetic tree has been constructed using new CS protein sequence data in hopes of further elucidating the taxonomic relationships between fungi and to determine if there are specific CSs characteristic of pathogenic fungi versus non-pathogens.
Several CS genes from *A. fumigatus*, *Wangiella (Exophiala) dermatitidis*, and *Ustilago maydis* are known contributors to fungal virulence. We are characterizing this association in detail in the ascomy-

cete *Chaetomium globosum* by studying CS gene expression at both room temperature (25^oC) and temperature of infection (37^oC). *C. globosum* is normally a saprophyte, but has been increasingly observed on interior building materials following water leaks and is associated with respiratory problems. Six putative CS genes have been identified in the recently sequenced genome of *C. globosum* and we are monitoring CS gene expression using qRT-PCR. Cultures transitioning from 25^oC to 37^oC and those grown at 37^oC allow for the detection of quantitative changes in CS gene activity, which may indicate which *C. globosum* CS genes play a role in respiratory infection.

116. ROLE OF KU70 AT TELOMERES OF *Candida albicans*

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We have characterized the function of Ku70 in the diploid fungal pathogen *Candida albicans*. Ku70 binds Ku80 to form a heterodimer, the Ku complex, which is conserved from bacteria to man. The Ku complex participates in non-homologous end joining (NHEJ), a recombination pathway that can rejoin the two ends of a double strand break (DSB) by simple ligation after little or no nucleolytic processing of the ends. In addition to its role in NHEJ, the Ku proteins have been implicated in a variety of functions at telomeres. Telomeres are nucleoprotein structures located at the ends of chromosomes that are crucial for maintaining chromosome stability. Telomeres in most organisms consist of G-rich repeats that terminate with a single-stranded 3' overhang. Conventional DNA polymerases cannot fully replicate the very ends of linear DNA molecules. Thus, without a specific compensatory mechanism, the ends of chromosomes shorten during each cell division. Many eukaryotes utilize telomerase, a specialized reverse transcriptase consisting of a catalytic protein subunit (Tert), an RNA template (Ter), and several accessory proteins for telomere addition. Previous studies from yeast to mammals have implicated the Ku complex in performing a multitude of functions at telomeres. In the budding yeast *S. cerevisiae*, the Yku70/Yku80 heterodimer has been shown to protect chromosomal ends from nucleolytic degradation. In addition, the *S. cerevisiae* Ku complex promotes telomere elongation by recruiting the telomerase complex through an interaction between Yku80 and telomerase RNA. Furthermore, binding of Ku to telomeres facilitates the recruitment of Sir3 and Sir4 to the subtelomeric regions to enhance telomeric silencing.
We have cloned the *C. albicans KU70* homologue and constructed deletions mutants. Interestingly, *ku70* heterozygous and homozygous deletion mutants are sensitive to both high temperature and the DNA-damaging agent MMS. Disruption of one copy of *KU70* resulted in telomeres that were shorter than those of wild type cells and this effect was independent of the allele present in the heterozygotes. By contrast, homozygous *C. albicans ku70* deletion strains exhibit long and heterogeneous telomeres. Analysis of *tert-/- ku70-/-* double mutants suggests that the telomere elongation in the *ku70* null mutant is due mostly to unregulated telomerase action. In addition, both *Candida* Ku70 and Ku80 in cell extracts associate specifically with telomerase RNA. Thus, like the *S. cerevisiae* Ku complex, the *C. albicans* complex appears to mediate a positive recruitment function for telomerase. Several abnormal phenotypes observed in our *C. albicans KU70* mutants, including thermosensitivity, susceptibility to MMS, and elongated and heterogeneous telomeres, could not be fully reverted by reintegrating the missing allele(s) into the heterozygous or the null homozygous strain. This suggests the existence of epigenetic changes in the *KU70* mutants that cannot be reversed upon reintroduction of the gene.

117. DEVELOPMENT OF AN EFFECTIVE GENE TARGETING SYSTEM IN *Schizophyllum commune* USING *ku70* KNOCK-OUT

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Fungi are involved in decomposing organic matter, nutrient cycling and nutrient exchange. *Schizophyllum commune* a wood rotting homobasidiomycete is considered a model organism because it is manageable, passing the entire life cycle within 14 days and it is a well known subject for studying mating types, sexual development, gene regulation and signalling systems.
In this study, *S. commune* is used for investigating genes involved in the degradation of organic material. By creating a knock-out strain with increased gene targeting efficiencies, the targeted construction of different gene deletions is going to be facilitated. This is achieved by reducing the heterologous recombination which, in homobasidiomycetes, greatly exceeds homologous recombination (HR). The homologous- recombination enhanced strain is created by impairing one of the major DNA repair mechanisms, the non-homologous end joining pathway (NHEJ), by knocking out the *ku70* gene which codes to one of the initiating proteins in this pathway. In molecular biology, gene manipulation is often achieved by integration of extra chromosomal DNA into a target site through homologous recombination. But fungi and higher eukaryotes favour NHEJ rather than HR resulting in low gene deletions efficiencies.
Current work is dealing with the construction of a gene targeting vector for *ku70* knock-out. This can be achieved by cloning the amplified flanking regions of *ku70* in to the proposed gene targeting vector. The *ku70* gene is replaced by the marker gene *ura1* which is involved in the pyrimidine biosynthesis. Knock-out strains were selected by culturing in minimal media and confirmed by PCR. The knock-out strain is characterised with respect to HR versus NHEJ frequencies and subsequently used in gene deletion approaches with higher gene targeting for genes with environmental impact like laccases or hydrophobins.

118. GENETIC AND FUNCTIONAL ANALYSIS OF THE GLUCOSIDASE II FROM *Sporothrix schenckii.*

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Sporothrix schenckii is a pathogenic dimorphic fungus. Its cell wall consists, among others, of *N*-glycosylated proteins that are involved in the adhesion of the fungus to host cells. *N*-glycosylation is a posttranslational modification that is initiated in the endoplasmic reticulum (ER), where $Glc_3Man_9GlcNAc_2$ oligosaccharide is processed by alpha-glucosidase I . Upon trimming by this enzyme, alpha-glucosidase II (GII) removes the two alpha1,3-glucose residues from this oligosaccharide, followed by alpha1,2-mannosidase to generate $Man_8GlcNAc_2$, which is further modified by Golgi glycosyl hydrolases and transferases. In most of the eukaryotic systems characterized thus far, the GII is conformed for a catalytic-subunit -member of the glycosyl hydrolase family 31 (GH31), and a beta-subunit responsible of its retention within the ER lumen. We have previously demonstrated that *S. schenckii* also has GII activity, which is associated with a 70 kDa polypeptide. In the present work degenerate primers were designed to amplify a 1500 bp DNA fragment by PCR. Bioinformatic analysis showed that this DNA fragment contains part of an ORF with homology to GII from the GH31.
A Southern blot assay demonstrated the presence of only one copy of this ORF within the *S. schenckii* genome. In order to isolate the full length of this ORF, a partial genomic library was constructed cloning ≈7.9 Kpb genomic DNA fragments in pBlueScript. The library is currently under screening and so far, two colonies containing the GII ORF have been identified.
This work is supported by CONACYT-México Ref. 83414 and University of Guanajuato.

119. IDENTIFICATION OF GENES REGULATED BY RIM101 IN *Ustilago maydis*

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Rim101 (PACC) is a zinc finger transcriptional factor, whose expression is alkaline pH-dependent. Its importance relies on functions it regulates, some of them have been reported in *Ascomycetes* and *Deuteromycetes*, includes: proteases, cell wall proteins, Ferredoxins, Flocculation Genes, ATPases, transcription factors, and virulence regulation. Previous studies found (for the first time in a *Basidiomicete*), the presence of this gene (Rim101) in *Ustilago maydis*, the null mutant for this gene (BMA), resulted affected in ionic stress response, cell wall stability and morphology. In order to study the direct regulation of this transcriptional factor in several genes, we select 5 ones previously reported genes regulated by Rim101 in other fungy: Ena1 (sodium pump), Lcc3 (acid Laccase) Pbr1 (Vacuolar Protase), SMP1 (Transcription factor responsive to meiosis, invasive growth, sporulation) and NRG1 (Transcription factor responsive to alkaline growth and ionic stress).
We proceed to identify these genes in two ways: 1) *In silico*. We search the homologous genes of the selected ones in a database, and created probes used to identify them in the *Ustilago maydis* genome. We founded genes in a not annotated region of the genome of *U. maydis*, where sequences were not scored. Once we identified the genes in the *U. maydis* genome, we designed primers for each one 2) *In vitro*: The primers were used to analyze the expression of the selected genes at different culture conditions, varying the pH and the Carbon Source, between Glucose and Glycerol. Our results show a not well known regulating function of Rim101, and constitute evidence that other factors influence the expression of these pH response genes.

FRONTIERS IN FUNGAL BIOTECHNOLOGY

120. EVALUATION OF DIFFERENT LIGNOCELLULOSIC SUBSTRATES FOR THE PRODUCTION OF CELLULOLYTIC ACTIVITIES OF *Bjerkandera adusta* and *Pycnoporus sanguineus.*

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White rot fungi have the capacity to exploit all wood components due to the secretion of a variety of lignocellulolytic enzymes. Much attention has been focused on finding such hydrolytic activities since their potential in the production of cellulosic bioethanol. Plant wastes (bagasse, stubble, sawdust, etc.) are composed mainly of three polymers: lignin, cellulose and hemicelluloses. Cellulose is composed of long lineal chains of glucose, arranged in different levels of fibrils that are associated by intramolecular interactions. Hemicelluloses, in contrast, are ramified polymers composed of different types of carbohydrates and phenolic compounds, among which xylan, glucan and arabinoglucan form the sugar backbone of the structure. Several different physical-chemical pretreatments (such as steam explosion or different treatments with sulfuric acid) are used to loosen lignin and release cellulose, hemicellulose and their monomers. Alternatively, cellulose and hemicellulose can be completely saccharified by a number of enzymes due to the synergism displayed by combinations of endo- and exo-glucanases and □-glucosidases. For this reason, it is important to establish the whole cellulolytic capabilities of wood-degrading fungi.
In this work we evaluated different lignocellulosic substrates (mainly agricultural or forestry wastes) for the production of cellulolytic activities of two white rot fungi, *Bjerkandera adusta* and *Pycnoporus sanguineus*, originally isolated from two different geographical locations. *B. adusta* strain UAMH 8258, a basidiomycete well known for its elevated ligninase activity, was isolated from temperate

woods of the northern hemisphere (University of Alberta Herbarium, Canada). *P. sanguineus*, another basidiomycete able to grow at moderately high temperatures given its tropical origin was isolated from an oil-polluted environment in the south of the state of Veracruz, México (our laboratory).
Wheat straw, maize stubble, cedar and oak sawdust, rice husks, *Jathropha curcas* leaves and *Ricinus comunis* stubble were evaluated as substrates for the production of cellulases and xylanases in both fungi. Our results show that growth was optimum in wheat straw and rice husks for both fungi, although maximum cellulolytic activities were found when grown in cedar and oak sawdust. These data may indicate that sawdust has probably less free sugars (or other alternative carbon sources) than other substrates so the fungi depend entirely on the production of lignocellulolytic activities for growth. Experiments are under way to test this hypothesis. Xylanolyitc activities were also better produced in oak sawdust for both fungi.
For *P. sanguineus*, zimogram analysis of the extracts indicated that different sets of proteins with cellulolytic activities were produced in different substrates. Data concerning zimogram analysis for *B. adusta* and xylanolytic activities for both fungi will be presented.

121. DECOLORIZATION OF INDUSTRIAL TEXTIL DYES BY FUNGI IN SOLID AND LIQUID MEDIA

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Seven fungal strains were isolated and selected based on their capacity to decolorize azo, indigo and sulfonic dyes. Six strains were identified as *Mucor sp*., and only one as *Fonsecaea compacta*. The fungal decolorization efficiency on the dyes dianix black, dianix yellow, dianix red, remazol yellow, remazol red, remazol blue and indigo was determined on plate with and without an extra carbon source and in liquid fermentation with a limited carbon source. The strain identified as *Mucor sp* (MAP03) was able to grow and generate dye decolorization halos on blue and red remazol in plate without an extra carbon source. All of the isolated strains were able to co-metabolize the studied dyes in the presence of glucose as an additional carbon source, growing and giving rise to dye decolorization. In liquid fermentation the decolorization percentages varied in accordance with the strain and dye. Two of the remazol dyes, red and yellow**,** were less susceptible to decolorization for all the strains. On the other hand, dianix black and indigo were the most susceptible, showing decolorization percentages above 70% for strains MAP02, MAP04 and MAP07. Strains MAP02 and MAP03 were found to be the most efficient in terms of decolorization percentages and number of dyes they were able to decolorize. Plate-taste screening for lignocellulolytic enzymes shown that five out of six strains were positive for peroxidese and only three strains (MAP03, MAP4 and MAP5), were also positive for laccase and tyrosinase.

122. ISOLATION AND MOLECULAR CHARACTERIZATION OF *Trichoderma spp.*

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Thichoderma is a kind of anaerobic-facultative fungi which is found in natural status within important number of soils and other sort of media, this research aimed to analyze the variability of this fungi throughout the coast (Guayas, Los Rios, Esmeraldas) and highland (Riobamba) in Ecuadorian soils. A morphological identification was made from isolated Trichoderma with two types of growth media at 28ºC, then, we proceeded to measure the radial growth or the growth rateo of Trichoderma isolations, we complete the measurements with observations at the microscope of the different fungi structures which contributes to the differentiation of the Trichoderma species, this includes the characteristics of

the isolation or colony, conidia production, pigments or color, smell, ramification of the conidiosphoros, philiades, Clamidosphoros and others. Therefore a research using molecular techniques was made, this included the analysis of DNA ribosolmal (rDNA) showing some relevant differences in ITS1 (transcribed spacer internal) region between the sections of Trichoderma. We used the ITS x, ITSy indicators and the PCR`s product was cut with Mbol enzyme revealed distinct banding patterns that made it possible to distinguish sections, we obteining two groups which are within the 5 sections of the fungi, at the same time we made the universal PCR`s technique (UP-PCR) using the L45, L15/ AS19 AS15 inv. We found differences in isolation analyzed.

123. PRODUCTION OF LIGNOCELLULOSIC BIOMASS DEGRADING ENZYMES

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Plant cell wall polysaccharides are the most abundant carbon-based organic compounds found in nature. They make up 90% of the plant cell wall and can be divided in to three groups: cellulose, hemicelluloses and pectin. The degradation of cell walls by enzymes is a complicated and lengthy process, taking a multitude of recalcitrant hydrolytic activities. Much of the recalcitrance is due to intrinsic structural heterogeneity (e.g., crystalline cellulose fibers), which confers strength to plant cell walls. Thus, because of functional and structural heterogeneity, degradation of plant cell walls requires a suite of synergistic enzymes. As a result of this complexity, microorganisms have acquired a large set of enzymes that attack these polymers. In this work we describe an *Aspergillus nidulans* protein production system, which results in high yield expression and secretion of heterologous commercial proteins. Here we develop the expression and secretion of a complete set of 15 enzymes needed for complete hemicellulose breakdown. Our expression/secretion system, results in high yield enzyme production recovered in a clean culture filtrate as compared to other expression systems such as *E. coli* or *P. pastoris*.

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124. TRANSFORMATION OF ENDOCRINE DISRUPTIVE CHEMICALS BY LACCASE FROM *Coriolopsis gallica*

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Endocrine disruptive chemicals (EDCs) are exogenous substances that are structurally similar to hormones and can interact with the endocrine receptors of animals. These compounds can disturb the normal functionality of the endocrine system causing adverse health effects in the organisms or their progeny. The list of EDCs is long and constantly increasing. They can mainly be found in domestic and industrial wastewaters. Due to the threat these compounds pose to the environment and particularly to the ecologic equilibrium, the degradation of these compounds is a priority research area. Among the different degradation strategies explored so far, phytoremediation, bioremediation with fungi and the use of purified enzymes, are the most common. The most efficient enzymes reported, so far, for their transformation are manganese peroxidase and laccase obtained from ligninolitic fungi. In this work, we explore the transformation of different EDCs by the laccase produced by the white-rot fungi *Coriolopsis gallica* UAMH 8260. We analyzed the *in vitro* transformation of 4 estrogenic compounds: 17-beta-estradiol, beta-sitosterol, bisphenol-A (BPA) and nonylphenol (NP). Laccase is able to transform the compounds 17-beta-estradiol, BPA and NP with similar catalytic efficiencies (37, 21 42 s^{-1} mM^{-1}); this indicates that this laccase could be able to efficiently transform the different EDCs in a complex mixture. The transformation of BPA produced a dimer and the compound 4-

isopropenylphenol. Analyzing the mechanism followed in this transformation, it is very likely that polymers of high molecular weight are produced. For NP the formation of an insoluble precipitate was observed, also indicating the production of a polymer of high molecular weight. The polymerization of the EDCs would probably reduce their estrogenic activity because they would not be able to interact with the estrogen receptor given the increase in size. In conclusion, the laccase produced by the fungus *C. gallica* UAMH 8260 is an interesting alternative for the elimination of the estrogenic activity of diverse compounds present in wastewaters.

125. MOLECULAR CHARACTERIZATION OF HYDROCARBON DEGRADING FUNGI.

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Microbial population present in soil is one of the most complex cooperation ecosystems in the world, and its role has generate big expectations in the last few years, in many different areas such as ecology, metagenomics, agronomy and many branches of the industrial sector. At contaminated areas during the process of bioremediation, microorganism can play a major role, unfolding and assimilating very complex xenobiotics and metabolizing these toxic molecules into ordinary products. This activity can be used to restore natural equilibrium. Oil industry has always had a mayor problem: pollution of soil by hydrocarbons, and clearly the strategies provided by a biological approach such as bioremediation gives a big hope to solve this issue. Over the time, the main alternative to hydrocarbon biodegradation has been bacteria, but recent efforts in the search for new and more efficient microorganisms has lead to the study of different fungal organisms such as the well documented lingnolitic white rot fungi (*Phanerochaete*) and several *Ascomycota*. We have analyzed by microbiological methods several samples of soil taken from heavily polluted areas tempered for over 15 years in the state of Veracruz, Mexico. These studies have given us a wide number of fungal isolates that were identified taxonomically. And by a restrictive screening we were able to obtain hydrocarbon degrading species by culturing them in a selective solid medium (using BTEX as only source of carbon).We selected 6 different strains of fungi that were able to grow even when they were inoculated at low cellular concentrations and could use hydrocarbons as the only source of carbon making them suitable for biodegradation strategies. We performed a genomic DNA isolation and purification from these fungi, trying different protocols such a chemical lyses and physical lyses (using liquid nitrogen) depending on its morphological characteristics. Then by amplification and sequencing of the intergenic spacer region (ITSR) of the 18S ribosomal gene by using a pair of degenerated primers which showed a 800bp we realized a multiple alignment using the NCBI tool BLASTn. By this method we could select the more similar sequences and identify the genus of the selected strains. We have isolated and characterized 6 different fungi that correspond with most cited genus in literature but with the difference that this strains are form the most important area for the oil industry in Mexico.
Fungal kingdom posses a much more complex metabolism if we compare it with bacteria the great amounts of biomass that can be created by them in the form of mycelium make them great candidates for bioremediation.

126. PRODUCTION OF LECTINS FROM BASIDIOMYCETES: THEIR RELEVANT PROPERTIES FOR BIOTECHNOLOGICAL APPLICATIONS

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Basidiomycetes play an important role in the ecosystem as they are able to biodegrade agricultural wastes and several xenobiotic molecules. Their fruiting bodies are appreciated for their flavour, nutritional characteristics and used in traditional medicine. Mushroom extracts have been reported to have antibacterial, antiviral, antitumor, hypotensive and hepatoprotective effects and several bioactive molecules could be useful for biotechnological purposes. In particular, fungal lectins play important physiological roles participating in the process of forming fruiting body primordial, the creation of mycelium structures easing the penetration of parasitic fungi into the host organism, as well as the identification of appropriate partners during the early stage of mycorrhization. Their biological activity includes lymphomitogenic activity and immunomodulatory properties, suppression of cell proliferation, and antitumor activity, among others. Their high binding affinity towards specific oligosaccharides also converts them in powerful tools to be applied in production processes for mammalian glycoprotein purification.
We have recently detected the presence of lectins in several basidiomycetes, including *Gymnopillus spectabilis* and *Pycnoporus sanguineous,* and their carbohydrate specificity have been determined.
In this work we evaluated the fermentation conditions of the fungi for the production of these lectins. Different carbon sources and stirring conditions were assayed. The lectin content (measured as hemmaglutination activity related to protein concentration) was also determined in extracts prepared from the fruiting bodies. The highest production of the *P. sanguineous* lectin from mycelium was found under static culture conditions and using malt extract broth. Although the lectin activity was detected in mycelium extracts from *G. spectabilis,* the highest level of the lectin was found in the fruiting bodies extracts.
The lectin from *Gymnopilus spectabilis* was partially purified from fruiting bodies extracts using a protocol previously optimized in our laboratory and their *in vitro* antimicrobial activity was evaluated against four different microorganisms. Under these conditions the lectin was able to clearly inhibit the growth of *Staphylococcus aureus*. Extracts from *P. sanguineous* containing lectin activity was found to inhibit the growth of *Escherichia coli*. The purification of the lectin is under progress, in order to later determine its participation in the inhibition process.
Financial support: ANII, PDT (74/12) and PEDECIBA-Química.

127. MICROARRAY STUDY OF A SET OF GENES IMPORTANT FOR THE SURVIVAL OF THE BASIDIOMYCETE *Bjerkandera adusta* IN THE PRESENCE OF PETROL.

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Oil pollution is one of the most important environmental problems. Bioremediation offers a good alternative for treatment of polluted soil and water because it usually can be done *in situ*, it is environmentally friendly and its costs are low. However, environmental conditions of polluted sites are often very different from laboratory conditions, so treatment *in situ* can be hampered by this fact. Basidiomycete fungi, offer good perspectives for this process, because, they are able to degrade all wood components and many xenobiotic compounds, since they produce oxidative extracellular enzymes with activity over a broad range of compounds.

In this study we constructed a cDNA library of the basidiomycete *B. adusta* grown in the presence of 1% crude Mayan oil and using differential subtraction, we isolated a set of genes specifically induced in the presence of petrol. From the 382 originally isolated clones, 77 proved to be false positives and only 105 sequences showed homologies with known functions, while the rest were classified as "hypothetical proteins" or showed no significant similitude with known proteins or genes. Many clones showed homology to the same sequence so were classified as "abundant" clones. ClustalW multiple alignments were used to determine if these sequences derived form the same transcript or belonged to gene families. Finally, we selected 40 sequences that showed the most reliable parameters of homology to known genes (Blast score, e value, etc.). Among the differentially expressed genes, we found some related to the ligninolytic degradation system that could act together with cell wall re-modelling activities and detoxification enzymes, signal transduction genes, protein metabolism genes, and gene expression regulators. A lipase was also found that could be involved in the degradation of the aliphatic fraction of petroleum.
Using a satisfiability constraints solving approach, an algorithm was developed to design probes for microarrays; the algorithm selects unique sequences of similar Tm that do not form secondary structures. Therefore, we were able to design and validate a set of 40 probes for the microarray experiments.
Microarray profiling was carried out with this set of genes comparing different culture conditions that have been previously shown to be important for lignocellulolytic regulation. Temperature regulation was assessed by comparing cultures grown at 34ºC or 28ºC; nitrogen source and carbon source regulation (peptone vs. ammonia and glucose vs. wheat straw, respectively) were also studied. Our results indicate that some of the genes necessary to grow in the presence of oil are also regulated by temperature and nutrients.

128. THE FUNGAL GLUTATHIONE-*S*-TRANSFERASE SYSTEM OF THE WOOD-DEGRADING BASIDIOMYCETE *Phanerochaete chrysosporium*.

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The recent release of several basidiomycete genome sequences allows an improvement of the classification of fungal glutathione transferases (GSTs). GSTs are well known detoxification enzymes which can catalyze the conjugation of glutathione to non-polar compounds that contain an electrophilic carbon, nitrogen or sulphur atom. Following this mechanism, they are able to metabolize drugs, pesticides, and many other xenobiotics and peroxides. A genomic and phylogenetic analysis of GST classes in various sequenced fungi –zygomycetes, ascomycetes and basidiomycetes- revealed some particularities in GST distribution, in comparison with previous analyses with ascomycetes only. By focusing essentially on the wood-degrading basidiomycete *Phanerochaete chrysosporium*, this analysis highlighted a new fungal GST class named GTE, which is related to bacterial etherases and two new subclasses of the omega GSTs.
Moreover, our phylogenetic analysis suggests a relationship between the saprophytic behaviour of some fungi and the number and distribution of some GST isoforms within specific classes.

129. **CHARACTERIZATION OF THE MAJOR LACCASE FROM *Trametes maxima* CU1 AND DECOLORIZATION OF COMMERCIALLY SIGNIFICANT DYES BY THE ENZYME**

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T. maxima CU 1 is a native basidiomycete with an outstanding ability to degrade model synthetic dyes in plate and submerged cultures associated to the production of extracellular laccases. In this work we report the partial purification and characterization of the major laccase isoform produced by this autochthonous strain and the results of the evaluation of its capability to degrade industrially important dyes from different chemical classes. A filtrate containing the laccase activity was obtained from submerged cultures carried out on modified Tien & Kirk media amended with inductors (350 µM $CuSO_4$ and 3% ethanol). Enzyme was concentrated through a 10 kDa cut-off cartridge and purified by anion-exchange chromatography using a DEAE-sepharose column and gel filtration chromatography with a Sephadex G-200 column. After gel filtration laccase eluted as a sole chromatographic peak with an isoelectric point of 4.4 by chromatofocusing, but two monomeric laccase isoforms with apparent molecular masses of 62 and 71 kDa were revealed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Deglycosylation and LC-MS/MS analysis of both isoforms strongly suggested they were glycoforms, since they migrated as a single band of 61 kDa on SDS-PAGE and share eight peptides with identical sequences, including the N-terminal (GIGPVADLTIT-NAAVSPDGFSRQ). Purified laccase (ratio $A_{280/605}$ of 18) had an optimum pH of 3.0 for 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) and 2,4-dimethoxyphenol (DMOP), and 4.5 for both syringaldazine (SYR) and the pair 3-methyl-2-benzothiazoline hydrazone (MBTH) + 3-dimethylaminobenzoic acid (DMAB). The optimum temperature was 50°C at pH 3 (ABTS, DMOP) or 4.5 (SYR) and retained 82% of its activity after 5 h at this temperature, but only 10% at 60°C. It retained 98% of its activity at pH 7 after 4 h. The Km values were 20 and 74 µM, for SYR and DMOP respectively with Vmax values of 1.5×10^{-5} and 8.5×10^{-6} $M \cdot min^{-1}$. The laccase activity was completely inhibited by sodium azide (0.1 mM), was partially inhibited by thioglicolic acid and sodium fluoride and only slightly by dimethylsulfoxide. The capacity to degrade synthetic dyes was tested on a panel of nine of the most extensively used by the Mexican textile industry. The dyes, belonging to five different chemical classes (monoazo, diazo, triazo, tetrakisazo and anthraquinonic), were kindly provided by PYOSA. Dye decolorization varied individually and was limited by their solubility, however a mixture of the nine dyes (total concentration of 450 ppm) was efficiently decolorized (67%) in 24 h with 10 UI of laccase.

130. **ENETIC MODIFICATION OF *Claviceps purpurea* FOR INCREASING THE CONTENTS OF ERGOT ALKALOIDS**

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Claviceps purpurea is an organism producing ergot alkaloids used for industrial preparation of various therapeutic substances. Genetically modified *Claviceps* with enhanced biosynthesis of these alkaloids can significantly reduce costs of production. Three genes have been selected and characterized in wild type and alkaloid producing strains of *Claviceps purpurea*. The overexpression of these genes might lead to an increase in the final alkaloid content. The genes in question are the *dmaW* gene encoding tryptophan dimethyllallyltransferase, the first enzyme of ergot alkaloid biosyntetic pathway; the *easC* gene, the gene for a hypothetical catalase, that could regulates the expression of other genes of ergot alkaloid cluster; and the easG gene, putative transcription factor.

The method for the protoplast transformation of *C. purpurea* has been modified and the conditions for transformation of Gal 404 strain were optimized by means of osmotic pressure and form of recombinant DNA. Success of transformation was checked by qPCR of genomic DNA and cDNA isolated from transformants. Constructs bearing gene for resistance to phleomycin, as a selection marker, was prepared where the three above mentioned genes are under the control of a strong constitutive promoter of fungal glyceraldehyde 3-phosphate dehydrogenase. Inoculums of modified strain Gal 404 will be applied on sterile flowers of susceptible cultivar of *Secale cereale* and levels and composition of produced alkaloids in matured sclerotia will be determined in near future.
Project is supported by IVAX Pharmaceutical, Ltd.

131. *IN SILICO* IDENTIFICATION OF GPI AND PIR GENES IN THE *Mycosphaerella fijiensis* GENOME: PREDICTING THE FUNCTION IN PATHOGENESIS

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The hemibiotrophic ascomycete fungus *Mycosphaerella fijiensis* is the causal agent of black Sigatoka, a disease that affects bananas production of (*Musa* spp.) worldwide. This disease is controlled by fungicides that account for about 40-60% of the total cost of banana culture production. In Mexico, bananas producers invest annually $1100 USD by hectare in fungicides an economic resource that only big companies can face. In addition, the fungus has developed resistance to some fungicides, making *M. fijiensis* a serious agronomic problem. The identification of genes related to fundamental aspects of this fungus (essential genes or pathogenesis related) is a promissory field of study to the development of new control strategies. Little is known about the genes which are important for *M. fijiensis* pathogenesis. During the interaction with the host (banana), the plant cell wall is the first barrier of defense protecting its cells. The fungal cell wall is different in composition, basically containing β-glucans, chitin and mannanproteins, this is an attractive target site to design antifungal drugs since it is essential and specific fungal (mammalian cells don't have cell wall). In ascomycete fungi there are descriptions of some cell wall proteins playing a role in virulence: GPI proteins (glycosyl phosphatidylinositol) and Pir (protein with internal repeats). In 2007, the Joint Genome Institute of the US Department of Energy finished sequencing the genome of *M. fijiensis,* making it publicly available. Using this resource, we are interested in 1) the *in silico* identification of genes encoding GPI and Pir proteins of *M. fijiensis* and, 2) perform functional analysis for some candidate genes, selected by its potential role in pathogenesis. In the *M. fijiensis* genome, we identified 50 putative GPI-anchored proteins: 44 predicted GPI-cell wall anchored and 6 GPI-membrane anchored. Likewise, we identified 4 Pir proteins, which have at least one tandem repeat consensus sequence. All these sequences were screened in a database prepared from *M. fijiensis* unigenes (Dr. Steve Goodwin, Purdue University, USA) finding one of the predicted Pir proteins and 7 GPI proteins; the function for most of them is unknown. It is largely unknown if Pir proteins have roles in pathogenesis, reason why we are selecting them to analyze their expression during *M. fijiensis* life cycle (stages of mycelium and conidia) and during the infection process of banana. In addition, a unique GPI putative adhesin was also selected. For functional analysis, we are selecting genes with homology (blastp, $e \geq 10^{-10}$) to important cell wall or cell surface proteins of phytopathogenic fungi. Currently, we are designing the construction for silencing the selected genes.

132. EXPRESSION IN YEAST OF A NEW TYPE OF EXPANSIN-LIKE PROTEIN OF THE BASIDIOMYCETE *Bjerkandera adusta* AND QUANTIFICATION OF EXPANSIN ACTIVITY.

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A group of proteins called expansins with the ability to relax cell wall components and promote cell enlargement was identified originally in plants. These proteins participate in different processes such as organogenesis, abscission, initiation of leaves, fruit ripening, pollen tube penetration of the stigma and other developmental processes in which cell wall modification occurs. Expansin molecular mechanism remains unknown, however it has been proposed that expansins could disrupt the hydrogen bonds between cellulose fibrils or between cellulose and other polysaccharides through a non enzymatic process. Expansin classification is based on their phylogenetic relationships; the expansin superfamily comprises four groups: α-expansin (EXPA), β-expansin (EXPB), expansin like A (EXLA) and expansin like B (EXLB). Expansin like proteins have been identified in molds, nematodes, mussels, moss, bacteria and in the ascomycete fungus *Trichoderma reesei* but this type of proteins from basidiomycetes have not been reported yet. In this work, a clone (EXL*Ba*) from a *Bjerkandera adusta* cDNA library grown on wheat straw was isolated and expressed in yeast. The expansin like protein of 13.4 kDa was purified by ultrafiltration and used in qualitative and quantitative activity assays showing that EXL*Ba* relaxed crystalline structure of cellulose from cotton fibers observed as swollen areas along the fibers. Although this protein did not show hydrolytic activity on cellulose fibers, a significant amount of reducing sugars was released after incubation of cotton fibers with EXL*Ba* and subsequent treatment with a commercial endo cellulase. Phylogenetic analysis showed that EXL*Ba* is part of a new group of proteins with expansin activity along with expansins from another basidiomycete fungus, *Laccaria bicolor*. In pH stability assays EXL*Ba* retains 50% expansin activity after 8 h of incubation at pH 3 and pH 7, being its optimum pH 5.
This is the first report of a protein with expansin activity from a basidiomycete fungus expressed in yeast. Gene expression analysis by real time PCR are currently under process. Since expansins are capable of loosening cell wall structure under acidic conditions they could be used as low cost additives to facilitate enzymatic saccharification of cellulosic biomass to improve its solubility and subsequent use in processes such as biofuel production.

133. HETEROLOGOUS EXPRESION OF A PEPTIDE FROM THE FUNGUS *Bjerkandera adusta* (Basidiomycota) WITH EXPANSIN ACTIVITY

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Introduction. The expansins comprise a family of proteins previously described in plants. They can bind at the interface between the cellulose microfibrils and matrix polysaccharides of cell walls and induce their extension by reversibly disrupting noncovalent bonds within this polymeric network. Expansins represent a very promising tool for biofuel generation from agricultural leftovers. They could help to improve and optimize the process of lignocellulose breakdown, since they display their wall loosening activity in conditions similar to those observed for cellulases.
We have identified a gene from the basidiomycota fungus *Bjerkandera adusta* with sequence homology to expansins. The product of the expression of this gene in *S. cerevisiae* showed expansin-like activity, relaxing cotton fibers and favoring the release of sugars when those fibers were thereafter treated with endoglucanases.
The generation of antibodies with specificity for this peptide would greatly aid in the monitoring of its expression in *B. adusta* and other heterologous systems. Antibodies capable of neutralizing the expansin activity would allow us to study the relevance of this peptide in the metabolism and growth of *B.*

adusta. Up to date, attempts to express plant expansins in heterologous systems have been unfruitful. We are dealing with the heterologous expression of the *B. adusta* peptide in *E.coli*. We plan to use the purified peptide as antigen for the antibody generation process.
Methods. We will employ the protocols previously developed in our group for the heterologous expression of small polypeptides. Concisely, the sequence encoding the polypeptide with expansin activity is amplified with primers bearing the suitable restriction sites for cloning in the expression vector. A Factor Xa protease recognition site is also introduced within the primers, in such a way that the polypeptide will be expressed as a fusion protein containing a His_6 tag -which will aid in its purification- separated from the main sequence by the Factor Xa site. The amplified construction is blunt-end cloned into pBluescriptKS(+), sequenced, and then subcloned into pQE30 via the inserted restriction sites. The plasmid is transformed into *E.coli* M15 strain and the expression is induced with IPTG after the cells have achieved the log phase. This expression system directs the overexpressed polypeptide to inclusion bodies, which are then solubilized and purified via ion-metal affinity chromatography (IMAC) with Ni-NTA columns. The His_6 tag can then be removed by proteolytic cleavage with Factor Xa, leaving the native polypeptide ready for use in mice immunizations and/or antibody library screening.
Results and discussion. Thus far, we have successfully amplified the *B. adusta* expansin gene, which has been subsequently cloned into pBluescript and subcloned into pQE30, our expression vector of choice. The sequence of the final construct has been verified. We have now proceeded to small-scale expression experiments, in order to optimize the expression conditions. We have already detected the expressed protein by Western blot experiments with an anti-His_6 antibody. Specific bands at the expected molecular mass have been observed.

134. INDUSTRIAL WASTEWATER TREATMENT BY A CONTINUOUS SYSTEM: ELECTROCOAGULATION - AQUATIC FUNGUS WITH *Chytryomyces sp*.

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The *Chytryomyces* genus includes several species of aquatic fungus capable of absorbing pollutants from wastewater. The industrial wastewater treatment, coupling the chemical systems with biological treatment, increases the efficiency on pollutants reducing. Electrocoagulations oxides the wastewater pollutants using iron and aluminium ions from the electrodes and electrochemical cells. A continuous system electrocoagulation-aquatic fungus using *Chytryomyces sp.* was applied to industrial effluents. 8 samples from the industrial wastewater from to Lerma river in Mexico State were characterized before and after continuous treatment. The parameters considered in the characterization were pH, conductivity, color, tubidity and COD (Chemical Oxygen Demand). The continuous system contained a electrochemical and biological reactor with a flow of 50 ml min^{-1} and treatment time of 15 min. The electrochemical reactor was a column of 2.5 L with aluminium electrodes of 0.14 A cm^{-2} of current density and 3.4 A of electrical current. The biological reactor was a glass of 2.5 L with a *Chytryomyces sp* culture, the treatment time was 45 minutes, food and air controled. The pH and conductivity decreased during the treatment time. The efficiency for color and turbidity (90%) decreased after 45 minutes of (from 2700 ± 300 to 170 ± 30 Pt-Co and from 120 ± 50 to 10 ± 2 FAU, respectively) electrochemical treatment and held the same values after the biological treatment. The COD declined to an efficiency of 59% after 45 min of (2100 ± 200 to 860 ± 50 mg L^{-1}) electrochemical treatment and increased the efficiency up to 80% (from 860 ± 50 to 500 ± 5 mg L^{-1}) with the biological treatment using *Chytryomyces sp*. These results showed that the culture of *Chytryomyces sp.* is efficient and increases the removal of the pollutants from industrial wastewater. The efficiencies reported in the literature on COD are around 60% using common biological treatments. The efficiency using a continuous system and the *Chytryomyces sp* culture increases because the pollutants were more available to the aquatic fugus after the treatment in the electrochemical reactor.

135. ISOLATION AND CHARACTERISTICS OF A LOW-MOLECULAR-WEIGHT COMPOUND WHICH COULD PROMOTE THE ACTIVITY OF MnP FROM *Phanerochaete chrysosporium*

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A low-molecular-weight compound which could promote the activity of MnP, named MnP promoter, was purified from the liquid culture of *Phanerochaete chrysosporium*. The molecular weight of MnP promoter was about 1400 Da and may have a π conjugated structure of five-membered or six-membered ring with a molecular weight of 98 Da according ESI/MS and UV spectra. MnP promoter had well thermostability. It could chelate metal ions like Fe^{3+} and reduce Fe^{3+} to Fe^{2+}. MnP promoter promoted the activity of MnP markedly. It is speculated that MnP promoter acted as a chelator of Mn^{3+} and formed certain stable complex with high redox potential so that it can improve the oxidative ability of MnP. It is significant to make these mechanisms clear in order to ascertaining the exact function of MnP in the natural lignin biodegradation and elucidating the principle of lignin degrading.

136. VALIDATION OF A SPECIFIC DIAGNOSTIC METHOD TO DETECT THE FUNGUS *Sclerotium cepivorum* Berk IN SOIL AND GARLIC SEED

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The central region of Mexico, especially the states of Guanajuato, Querétaro, Aguascalientes and Zacatecas, is the main area for production of garlic (*Allium sativum*). Despite the crop's economic advantages, there is no guarantee of yield and production due to high incidence of the disease known as "white rot," whose causal agent is the fungus *Sclerotium cepivorum* Berk. This disease impacts negatively on production of this vegetable, causing significant economic losses for producers in the region.
The resistance and reproduction structures of *Sclerotium cepivorum* Berk are the sclerotia, which can remain in soil for up to 20 years and can spread from one crop field to another very easily.
We intend to develop a specific molecular detection system for *S. cepivorum* Berk and validate it using soil and seed samples, in order to provide producers with the possibility of fast, reliable detection, and thus allow them to develop their planting strategies.
We have seven primers that have proven to be specific for the amplification of genomic DNA from *S. cepivorum* Berk. We have modified protocols already described in the literature for extraction of DNA from soil (Zhou modified, 1996), in order to amplify the obtained DNA with specific primers for the DNA of *Sclerotium cepivorum* Berk.
This study was supported by grant from the DAIP, U. de Guanajuato and CONACYT-SAGARPA 2005-11919

137. COMPARISON BETWEEN OF *Penicillium chrysogenum* AND *Penicillium sp* ISOLATED TWO DIFFERENT SOURCES FOR HYDROCARBONS REMOVAL IN LIQUID MEDIUM AND SOLID CULTURE

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The various species of *Penicillium* can colonize many different environments, they are common in soils, in foods, in drinks and in indoor air. In general, fungi are less efficiently than bacteria for xenobiotic degradation, but on the other hand, fungi are more versatile and more suitable in what concerns

to the breadth of substrates they can use. Unlike fungi, bacteria are unable to degrade efficiently polycyclic aromatic hydrocarbons (PAHs) that contain more than four aromatic rings.
In this work it was established a treatment test for a hydrocarbon contaminated soil, with an initial concentration of 1200 mg of media fraction hydrocarbons (MFH) (aliphatic chains of $C_{10} - C_{28}$) for Kg of soil added with green coffee beans, a removal of 90% of MFH was observed in 12 days.
Different fungal species were isolated, and we were able to identify the presence of two different species of *Penicillium* that were identified by molecular biology techniques, *Penicillium chrysogenum,* that was isolated and identified from green coffee beans and *Penicillium sp*, isolated from the contaminated soil.
To determine the hydrocarbon removal capacity of *Penicillium* strains, we used a liquid medium with the following composition: Dextrose (10g/L), polypeptone (2 g/L), $(NH_4)_2SO_4$ (1 g/L), $MgSO_4 .7H_2O$ (0.5 g/L), KH_2PO_4 (0.875 g/L), K_2HPO_4 (0.125 g/L) plus trace elements, additionally, we add an extract hydrocarbons from soil with a concentration of 280 mg media fraction hydrocarbons per liter of medium, after 15 days of *Penicillium* sp and *Penicillium chrysogenum* removed 83 %.
The objective of this work was to compare the capability of the two strains of *Penicillium* for MFH removal.

138. IDENTIFICATION OF INTERMEDIARIES FORMATED FOR DEGRADATION OF PAHs BY A STRAIN *Aspergillus niger* MODIFICATED GENETICALLY ABLE TO PRODUCE MANGANESE PEROXIDASE OF *P. chrysosporium*

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Polycyclic aromatic hydrocarbons (PAHs) are common environmental pollutants that arise from industrial operations as well as from some natural events such as forest fires. Some of these PAHs and/or their metabolites even at low concentrations can be toxic or carcinogenic to humans and other forms of life, which emphasizes the importance of their removal from the environment. Many of the remediation technologies currently being used for contaminated soil involve not only physical and chemical treatment also biological processes, where microbial activity is the responsible for pollutant removal and/or recovery. White rot fungi are likely to be involved in the biodegradation of PAH in soil. The majority of studies have focused on *Phanerochaete chrysosporium* because this has a efficient extracellular enzymatic system for degradation of xenobiotic; therefore, there is a clear disadvantage of these is their preference to colonize wood and not soil. There are other non-ligninolytic fungi with capacities of removing PAHs from soil by cytochrome P-450 monooxygenase enzyme, theses fungi do not produce extracellular peroxidases. The first steps of PAH oxidation result in the formation of diphenols, dihydrodiols, and quinines, these metabolites have higher water solubility and reactivity than the parent PAH and this fact is important because for example, Pyrene (Pi) is known as not genotoxic and is commonly used as an indicator for monitoring PAHs. However, its metabolites are mutagenic and more toxic than the parent compound and for this reason it is important to know the pathways by which these products could be further degraded.
Particularly *Aspergillus niger* is a good host for the heterologous expression of peroxidases to increase PAHs removal capacity. In our laboratory a strain was isolated and was identified by the sequences of the fragments ITS as *A. niger* SCB2 and was able to tolerate (800 ppm) and remove 50% of the initial Phe. This strain was co-transformed by electroporation, one of the plasmids was the pTAAMnP1, with the gene *mnp*1 encoding a MnP from *P. chrysosporium*. Transformant was able to remove 95% of the initial Phe (400 ppm) more efficiently that the wild strain, suggesting that MnP production is one of the most effective ways to activate PAHs degradation by fungi.
The aim of this study is identify intermediaries formed during the PAHs (Phenantrene and Pyrene) removal in liquid culture by a strain of *A. niger* can produce MnP by LC/MS and compare with the intermediaries formed by the wild strain for know the to understand the metabolic changes obtained by genetic transformation.

Different intermediaries were formed by the two strains during degradation because the transformant was able to produced quinines and wild-type produced dihydrodiols, which are carcinogenic. Transformant strain was able to remove more Phe and Pi that the wild-type strain.

139. IMMOBILIZATION OF ENZYMATIC EXTRACT FROM *Penicillium camemberti* WITH LIPOXYGENASE ACTIVITY ONTO A HYBRID LAYERED DOUBLE HYDROXIDE.

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A Zn/Al layered double hydroxide was synthesized by alkaline co-precipitation with azelate ions (-OOC(CH2)7COO-). The interlayer space of the layered material is occupied by organic ions as confirmed by X-ray diffraction and FTIR spectroscopy. The resulting hybrid material was tested as support for *Penicillium camemberti* enzymatic extract, containing lipoxygenase (LOX) activity.
The optimal condition for LOX immobilization is done with 0.6 mol.L-1 potassium phosphate buffer and pH 6.0. The enhanced thermostability was evident with incubation at 60 °C where the immobilized enzyme retains 92% of specific activity while the free enzyme loss the total activity. The affinity for the substrate linoleic is kept after immobilization; however the specific activity slightly decreases.

140. BIOLOGICAL SYNTHESIS OF COPPER AND PALLADIUM NANOPARTICLES USING THE FUNGUS *Neurospora crassa*.

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The study of nanoparticles is of great importance for science and industry. Nowadays there is an increasing interest in their production by biological methods because physical and chemical procedures need elaborate methods to produce them and the resulting waste could damage the environment. *Neurospora crassa* is a model fungus in many fields of biological research. In this work we explore the ability of *N. Crassa*, to form copper (Cu) and palladium (Pd) nanoparticles using the ion solutions of copper chloride (II) ($CuCl_2$) and palladium acetate (II) $(C_2H_3O_2)_2Pd$. The mycelium was exposed to aqueous solutions of 10^{-3} M of copper chloride and palladium acetate for 24 h of incubation. The color of the fungal biomass changed from pale yellow to light greenish when exposed to copper chloride (II) and to dark brown in the case of palladium acetate (II), suggesting the formation of nanoparticles. Analysis by UV-vis spectroscopy, Scanning Electron Microscopy (SEM), Energy Dispersive X-ray Spectroscopy (EDS) and Transmission Electron Microscopy (TEM) were carried out to characterize the nanoparticles. We found that *N. crassa* can be used for the production of copper and palladium nanoparticles and preliminary results show that for both metals the biosynthesis was mainly intracellular.

141. FUNGUS-MEDIATED BIOSYNTHESIS OF SILVER AND GOLD NANOPARTICLES USING *Neurospora crassa*

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The development of reliable and environment-friendly synthesis of metallic nanoparticles is an important area of research in nanotechnology. Metallic nanoparticles will play a key role in many technologies of the future and have several important applications in the field of biolabelling, sensors, antimicrobial agents and filters among others. Therefore the production of metallic nanoparticles using natural processes such as biological systems is the subject of current investigation in many countries. The nanoparticles of noble metals like silver and gold are found to have potential applications in various fields including biomedicine, where they can be used for drug delivery systems and treatment of some cancers. In this work we have investigated the biosynthesis of silver and gold nanoparticles using the filamentous fungus *Neurospora crassa*. The formation of nanoparticles using this fungus was found to be successful, when the fungal biomass was exposed to the aqueous solutions of 10^{-3} M of $AgNO_3$ and $HAuCl_4$, the color of the biomass turned from pale yellow to brown and purple respectively. Analysis by transmission electron microscopy (TEM) was carried out to characterize the formation of metallic nanoparticles. TEM photographs of extracted nanoparticles were used to determine the shape and average size of nanoparticles. The shape of nanoparticles was found to be mainly spherical with 11.14±4.36 nm and 12.86±5.86 nm diameter for silver and for gold, respectively. TEM images of thin sections of the Ag and Au nano-*N. crassa* cells confirmed the intracellular formation of silver and gold nanoparticles. The results obtained indicate that *N. crassa* can be a potential "nanofactory" for the synthesis of various metallic nanoparticles. The use of *N. crassa* for this purpose will offer several advantages since it is considered as a non-pathogenic organism, has a fast growth rate and easy and economic biomass handling.

142. BIOSYNTHESIS OF PLATINUM NANOPARTICLES BY THE FILAMENTOUS FUNGUS *Neurospora crassa*.

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In recent years, the development of biosynthetic processes to generate nanomaterials is of great interest. In addition to the high costs, the use of physical and chemical methods to produce metallic nanoparticles generates toxic waste that could potentially damage the environment. The synthesis of inorganic materials by using biological systems is on the other hand characterized by processes generally run at ambient conditions (pressure and temperature) and considered as eco-friendly. In this work, we report for the first time the biosynthesis of platinum nanoparticles using the filamentous fungus *Neurospora crassa*. The experiment was carried out using a source of salts of hexacloroplatinic acid (H_2PtCl_6) at 10^{-3} M for an incubation period of 24 h. A change of color of the mycelia from pale yellow to dark brown was the first indication of the possible formation of nanoparticles by the fungus. Analysis by UV-vis spectroscopy, scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDS) and transmission electron microscopy (TEM) confirm the biosynthesis of nanoparticles by the fungus.

143. **APOCAROTENOID BIOSYNTHESIS IN *Phycomyces blakesleeanus***
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The interaction between strains of opposite sex of *Phycomyces blakesleeanus* and other Mucorales begins with the development of specialized structures, the zygophores, on hyphal tips and an increase in ß-carotene synthesis that is exploited by industry. These processes are triggered by an exchange of signals derived from ß-carotene (apocarotenoids). An 18-carbon fragment of ß-carotene would give rise to trisporic acids and other trisporoids, and some of these would be cut down to 15-carbon apotrisporoids. We assumed that the initial cleavage of ß-carotene is mediated by carotene cleavage oxygenases similar to those converting ß-carotene to retinal. The genome of *P. blakesleeanus* encodes five members of the carotenoid cleavage oxygenase family, three of which contain the four histidin residues thought to be essential for activity. Based on expression in ß-carotene accumulating *Escherichia coli* cells and on in vitro assays, we conclude that two of these genes code for carotene oxygenases mediating sequential cleavage of ß-carotene (C_{40}). The first enzyme catalyzes the formation of a C_{15}- and a C_{25} -apocarotenal, the latter is then converted into fragments of 18 and 7 carbons by the second enzyme. Thus, the C_{15}-compounds are apocarotenoids directly derived from ß-carotene and cannot longer be considered as apotrisporoids. Sexual interaction increases the concentration of mRNA of oxygenases, as determined by quantitative RT-PCR in single and mated cultures.

144. **BIOSINTHESIS OF SILVER/GOLD BIMETALLIC NANOPARTICLES USING THE FILAMENTOUS FUNGUS *Neurospora crassa.***
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The study of metal nanosized system has attracted considerable attention in recent years due the wide range of applications in which metallic nanostructures are important, thus better control of its properties would have deep economical implications. The physicochemical and optoelectronic properties of metallic nanoparticles are strongly dependent on the size and shape. Wide varieties of physical and chemical procedures have been developed in order to synthesize nanoparticles of different composition, size, shape and controlled polydispersity. Nevertheless, the routinely physicochemical techniques for nanoparticle production remain expensive or employ hazardous substances. Green chemistry methods offer opportunities to design nanomaterial production processes that can reduce the environmental impact, offer waste reduction and increase the energy efficiency. The biological synthesis of Au/Ag bimetallic nanoparticles fungi-assisted is discussed in this report. We demonstrate the bioreduction of Au/Ag nanoparticles employing the fungus *Neurospora crassa*. TEM images of thin sections of the Ag/Au nanoparticles-*N. crassa* cells confirmed the intracellular formation of silver-gold bimetallic nanoparticles. TEM studies demonstrated that nanoparticles have dimensions between 20 and 50 nm. SAED and EDS results confirm the formation of alloy-type Au/Ag bimetallic nanoparticles. Therefore, *N. crassa* has been probed successfully as "nanofactory" in order to obtain bimetallic Au/Ag nanoparticles in a greener process.

145. OXIDATIVE STRESS AND CELL WALL PROTEINS IN THE OPORTUNISTIC FUNGAL PATHOGEN *Candida glabrata*.

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Candida glabrata is an opportunistic fungal pathogen capable of invasive infections in inmunocompromised individuals. *C. glabrata* is extremely resistant to oxidative stress generated by H_2O_2. The single catalase, Cta1p, and the transcription factors Yap1p, Skn7p, Msn2p and Msn4p mediate the response to oxidative stress. However, *in vivo* experiments showed that Cta1p seemed not to be important for virulence. To further understand the oxidative stress response in *C. glabrata*, we found an interesting relation between silencing, regulation of adhesions and response to oxidative stress. *EPA1, EPA2* and *EPA3*, GPI-anchored cell wall proteins of the *EPA* gene family, are localized at the end of chromosome E and subject to chromatin-based subtelomeric silencing. We found that *EPA2* which is not expressed *in vitro*, is induced upon exposure to H_2O_2 in stationary phase. This induction is detected after 5 minutes of exposure and remains constant up until 2 hours. Furthermore, this induction is regulated at least by Yap1p, Skn7p, Msn2p and/or Msn4p. We are currently establishing which other *EPA* are induced by oxidative stress and where are these cell wall proteins expressed in the host.

146. USEFULNESS OF THE Hcp100 MOLECULAR MARKER TO DEMOSTRATE *Histoplasma capsulatum* INFECTION IN WILD AND CAPTIVE BATS FROM FRANCE

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Histoplasma capsulatum (*H.c.*) is a dimorphic fungus distributed worldwide that produces infection in mammalian hosts. Infection is caused by the inhalation of aerosolized propagules of its saprobe mycelial-phase (infective-phase), growing in confined environments associate with accumulated bat and bird droppings. This fungus develops a facultative intracellular parasitic yeast-phase within phagocytes of susceptible hosts (virulent-phase). Previous studies in our group indicate that bats have a high risk of infection with this pathogen and represent a natural model for epidemiologic studies. Using a pathogen-specific molecular marker, the fungus presence was revealed in host tissues. *H. capsulatum* infection, in humans, has been successfully monitored in several tissues sampled using nested-PCR with a 100-kDa protein coding gene (Hcp100). This marker is highly sensitive and specific to detect infection in wild and captive mammals´ tissue, as demonstrated in a snow leopard (*Uncia uncia*), two maras (*Dolichotis patagonum*), and in wallabies (*Macropus* sp.). Taking into account the abovementioned findings, the Hcp100 gene fragment was used to reveal the presence of *H.c.* infection in lung of infected bats from two different regions from France, “La Palmyre” (south) and “Bourges” (central). A total of 61 bats were screened at “La Palmyre”, belonging to the following species: *Pteropus rodricensis* (45) and *Rousettus aegyptiacus* (16). From Bourges 22 bats were processed, including three species: *Plecotus* sp. (1); *Pipistrellus pipistrellus* (3); and *Nyctalus noctula* (18). Out of 83 bat lung samples studied, six amplified the Hcp100 marker, three from “La Palmyre” (one of *P. rodricensis* and two of *R. aegyptiacus*) and three from Bourges (one *P. pipistrellus* and two *Nyctalus noctula*). *P. rodricensis* (from Rodrigues islands, Indian Ocean) and *R. aegyptiacus* (from Africa and Asia) specimens were born in “La Palmyre” zoo; in contrast all other bat specimens studied were naturally captured in wild life. Although Europe is still considered a non-endemic area of histoplasmosis, *H.c.* infection was found in infected bats from two French regions studied. Although the presence of *H.c.* iso-

lates in wild mammals from Europe was previously reported, our findings emphasize the relevance of *H.c*. geographic distribution in the old continent.

147. NEW *Candida* INHIBITORS TARGETTING OF RATE-LIMITING STEP OF ERGOSTEROL SYNTHESIS

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The 3-Hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) catalyses the conversión of HMG-CoA to mevalonate and this enzyme is the statin target. The statins has been reported as inhibitor of growth in *Candida* and *A. fumigatus*. This growth inhibition is likely to be due to lower levels of ergosterol and this indicate that statins have a potential role as antifungal agents. In addition, statins combined with fluconazol has synergistic antifungal activities. In contrast to their beficial effects, the statins cause hepatotoxicity and by this need search new drugs and the HMGR could be to therapeutic target to treatment of candidosis. Strains*:* In this study *Candida albicans* (ATCC, CAL18 and CAL30) and *C. glabrata* (CBS, CGL24 and CGL43) were used. *In vitro* susceptibility testing: Guidelines for antifungal susceptibility testing (M27-A). Sterol quantification method: The ergosterol from yeast in presence or absence of HMGR inhibitors was obtained by Ethanol-KOH extraction and quantified by spectrophotometric scans ranging from 240to 300 nm. Viability assay: Strains were treated for 3, 6, 9 and 12h in presence or absence of compounds. Percent of survival was determined as the number of yeast colonies divided by the number of the untreated culture. mtDNA damage: Strains were treated for 12h in presence of each compound, then were growth on YPD and the colonies with *petite* phenotype were selected and grown on YPG and YPE. mtDNA damage was confirmed by amplified of mitochondrial genes. RESULTS: *MIC's:* We selected one strain phenotype representative of sensitive, SDD and resistant *C. albicans* and *C. glabrata*. With the compound 1a the $MIC_{50\%}$ on CBS138 was 32 μg/mL, 1c on ATCC1035 and CAL18 0.5 μg/mL, 7b on CBS138, CGL24, CGL43, ATCC, CAL18 and CAL30 was 64 μg/mL and 7b on CAL30 was 64 μg/mL. However, the other strains with others compounds the phenotype was resistant o inhibition below 50%. *Sterol biosynthesis inhibition:* All compounds presented decrease in the mean amount of ergosterol produced compared to non-treated strains. *Growth inhibition:* All compounds presented growth inhibition, however fluconazole and simvastatin showed highest inhibition. Also HMGR inhibitors caused major inhibition on *C. albicans* than *C. glabrata* since the compound 6g on *C. albicans* was better than fluconazole and simvastatin. *Petite mutants induction:* Only four compounds inducted *petite* mutants, this results caught our attention since it has been described a direct correlation between *petite* mutants and toxicity. *Sinergysm on growth:* The strains were treated with 1a, 1c, 4c, 5a, 7a and 7b along with fluconazole and the inhibition was higher than that obtained with compounds alone. CONCLUSION: All compounds inhibited the growth of *C. glabrata* and *C. albicans* and the ergosterol synthesis. Compounds 1a, 1c and 6g showed the highest inhibition. It is likely that the pharmacophore groups are mainly the acetic moiety and the methoxy or the nitro group in the ortho position; and the polar groups as NO_2 and NH_2 in the C-5 carbon of the benzene ring. The low frequency of *petite* mutant induction by HMGR inhibiting compounds might suggest low toxicity. The tested compounds showed synergism with fluconazole, reducing the viability of *C. albicans* and *C. glabrata*. The HMGR enzyme is a good target for the design of antifungal compounds, evidenced by the fact that the new drugs were effective on *Candida.*

148. RELATION BETWEEN AFLP AND FLUCONAZOLE RESISTANCE AMONG DIFFERENT *Candida albicans* CLINICAL ISOLATES

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Although the list of opportunistic fungi causing serious, life-threatening infection increases every year, without question the single most important cause of opportunistic mycoses worldwide remains *Candida* species. *Candida albicans* is the main cause of invasive fungal infections. The attributable mortality of these infections is as high as 38%, whereas crude mortality rates exceed 50%. In recent years, the frequency of fungal infections has steadily grown and although these infections are generally less frequent than bacterial infections, opportunistic infections in immunocompromised patients represent an increasingly common cause of mortality and morbidity. Amplified fragment length polymorphism (AFLP) analysis is a relatively new technique. This method has proved to be robust and reproducible and is an almost ideal high-resolution genotyping method suitable for clinical use. Little is known about the etiological importance of *Candida* colonization and infection among Mexican people. Also environmental conditions in northern Mexico favor vaginal infections with *C. albicans*. In this work we determine the correlation using AFLP patterns of *C. albicans* between clinical isolates and fluconazole resistance.
A total of 90 clinical isolates of *C. albicans* from vulvovaginitis and blood cultures, 2 isolates of non-*C. albicans*, 1 *C. albicans* ATCC 14053 and 1 *Candida sp* ATCC 204276 were included in the study. Their fluconazole resistance was evaluated by the Drop Test technique, sorting the population into 3 groups: resistant, susceptible dose dependent and susceptible (16, 13 and 65 isolates, respectively). Subsequently, DNA extraction was performed using the rapid isolation of yeast DNA method. Conditions of AFLP were established. The analysis of identification, presence and absence of bands was done with the SAGA^{MX} program. Phylogenetic analysis was performed using PAUP version 4.0b10 using Fitch parsimony. *C. parapsilosis* was designated as outgroup The results grouped 8 of the 16 resistant samples to one clade which could mean a possible relationship or a common origin of the strains that form this clade, the rest of the resistant isolates did not group so their resistance may have different origins. This would indicate that clinical isolates and their resistance from the Hospital Universitario of the UANL may have different origins and represents to our knowledge the first epidemiological study of this kind in northern Mexico.

149. FUNGAL DIVERSITY IN SOIL ISOLATES FROM MYCOSES-ENDEMIC AREAS.

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The soil constitutes the main reservoir of fungi. Majority of disease-producing fungi for men and animals exist freely in nature as soil saprophytes or plant pathogens and gain entrance into the body through abrasion, implantation or inhalation. The potentially pathogenic fungi and allied geophilic species are widespread worldwide. Huauchinango (Puebla, México) corresponds to an endemic area for some mycoses. In this work, our main goal was to determine the fungal population isolated from soil samples recovered from different locations within this municipality. In order to accomplish this, soil samples were collected from ten different locations and the associated plant was registered for each sample. Fungi isolation for phenotypic identification was performed. At the same time, total DNA was isolated from soil and PCR-amplification using specific oligos that target fungal intergenic regions was performed. Molecular tools allowed the identification of otherwise uncultured species. Overall, more than 31 different fungal species were identified, including some agents of superficial,

subcutaneous and opportunist mycoses. Diversity and conservation degree (including phylogenetic studies) for the different species isolated was determined. These results, joined to the environmental conditions for each soil could suggest new insights regarding the specific conditions that favor the development of potentially pathogen and opportunist fungi for the inhabitants of the region studied.

150. ANTIFUNGAL RESISTANCE OF *Candida spp.* ISOLATED FROM VAGINAL SAMPLES.

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The widespread use of antifungal agents has produced a change in the epidemiologic patterns of *Candida spp*. *Candida albicans,* the most common specie has been replaced by other species as *Candida glabrata, Candida dublinensis, Candida tropicalis* and *Candida parapsilosis.* These species have become important fungal pathogens in the last two decades. The changes in the distribution of species, contribute in the selection pressure that is important for the development of resistant *Candida spp*. from previously susceptible yeast. In this study we determined the *in vitro* susceptibility of the three most important Candida species isolated from vaginal samples. We used Agar Diffusion Method with Neo-Sensitab (Rosco Diagnostica). The 93% of the isolates were resistant to 5 Fluorocytocine (5FC), 46% to Nystatin, 46% to ketoconazole and 23% to Itraconazole. *Candida albicans* isolates were sensible to azoles, resistant to 5FC, and 15%, resistant to polyene. *Candida tropicalis* were resistant to the polyene and 5FC and the 50 % *Candida glabrata* isolates had azole cross resistance to Ketoconazole (83%) and Itraconazole, 33% is resistant to de polyene, and 83% is resistant to 5FC. The prevalence on polyene resistance on the three *Candida spp*. is interesting because previously reports of polyene resistance are less than 5 %.

151. *Candida spp.* DIVERSITY IN VAGINAL ISOLATES FROM GYNECOLOGIC PATIENTS IN ENSENADA, B. C.

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In the last years, *Candida spp.* have emerged as major opportunistic pathogen, *C. albicans* is the most frequent species, nevertheless species such as *C. glabrata* , *C. guilliermondii* , *C. krusei* , *C. parapsilosis* and *C. tropicalis* have increasingly been isolated from people with wide distribution. Our goal was to study the diversity and distribution of *Candida spp*. among gynecologic patients from the Ensenada General Hospital, 140 women were included, the vaginal swabs were first inoculated in YPD and the recovered colonies were analyzed using a PCR-based method with primers ITS1 and ITS4 and subsequently by RFLP using MspI as a restriction enzyme.
The 42.8% of the women included in this study were positive for *Candida* (PCR). By comparing the PCR-RFLP of the vaginal isolates, all could be identified to the species level, except in two cases, one that produced a no recognizable pattern. *C. albicans* was the predominant species (55.0 %), followed by *C. glabrata* (11.6%), and *C. tropicalis* (5.0 %). It was possible to differentiate two species in 15 % of the vaginal samples.
As other studies, *C. albicans* was present in more than the half of the positive samples, however it is very important to identify the species throughout simple molecular based methods that make easy to know the responsible of the infection in order to recommend an adequate antifungal treatment.

152. **MOLECULAR IDENTIFICATION OF *Coccidioides* spp. IN ENVIRONMENTAL SAMPLES: EXPLORING THE ECOLOGICAL NICHE OF THE VALLEY FEVER FUNGUS IN BAJA CALIFORNIA.**

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Coccidioidomycosis (CM) is an emerging human fungal infection of North American deserts, with highly endemic areas distributed along the U. S.-Mexico border. In the last 20 years the incidence and prevalence rates in endemic regions have increased notably, with estimates of more than 200,000 cases annually only in the U. S. Most epidemiological and environmental studies have searched for correlations between this important fungal disease, and patterns of drought and rain in arid lands. Despite the high prevalence rates of the disease, reports of positive isolations of the causal agent, *Coccidioides* spp. from environmental samples have been scarce. To resolve this paradox, we have evaluated the potential ecological niche of this fungus, and subsequently used improved available molecular tools to identify *Coccidioides* spp. in soil samples collected from areas of Baja California, Mexico, previously predicted as endemic "hotspots".
Soil samples from heteromyids' active burrows, latrines and paths were collected using a randomized sampling method. A fungal ITS region of 900 bp was amplified by nested PCR. The resulting amplicon was used as template for diagnostic PCR of *Coccidioides* spp. We succeeded to obtain 170 bp amplicons in 65% of the samples (25 of 38) collected in Valle de las Palmas and 30% of the samples (3 of 10) collected in San Jose de la Zorra. Whereas no positive isolations were obtained from 10 samples collected in San Vicente. Sequence analysis identified isolates as *C. posadasii*, which agrees with the biogeographical distribution of *Coccidioides* spp. in the endemic areas. We characterized the vegetation, edaphology and heteromid communities associated to the positive isolation points. Most of the positive detections were found in riparian areas in stream beds and dry watercourses and corresponded to the entrances to heteromyids' burrows of two identified species: *Chaetodipus fallax* and *Dipodomys simulans*. The soils of the sampled regions were recently deposited eutric fluvisols, characterized by non-consolidated material and with a thin A horizon layer containing scarce organic matter. We suggest some clues of the spatial distribution and potential hotspots for this important fungal disease agent and review potential changes in its incidence and distribution associated to regional climate warming in endemic Western North American deserts.

POLARITY AND CYTOSKELETON

153. **REGULATION OF LOCALIZATION OF THE *Aspergillus nidulans* ORTHOLOGUE OF PROTEIN KINASE C TO SITES OF SEPTUM FORMATION**

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We have shown that the *Aspergillus nidulans* orthologue of protein kinase C (PkcA) participates in regulating cell wall integrity (CWI) and localizes at sites of cell wall synthesis, including growing hyphal tips and septa. A strain bearing the *calC*2 mutation in *PkcA* is hypersensitive to wall-inhibiting chemicals but does not exhibit growth or cytokinesis abnormalities, indicating that PkcA's role in CWI is regulated separately from its role in growth/cytokinesis. To better understand the mechanisms by which PkcA localizes to tips and septa, we have used temperature-sensitive mutants and *AlcA* promoter replacement to observe the effect of reduced function of several proteins necessary for septation

upon formation of PkcA cortical rings at sites of future septation. We find that PkcA cortical rings do not form in hyphae having reduced function of MobA (Mob1p orthologue), TpmA (tropomyosin), SepA (formin), SepD, SepG, or proteins encoded by two other not-yet-cloned *Sep* loci. This places localization of PkcA to septation sites "downstream" of the functions of these proteins. PkcA localization lies "upstream" of MyoB (myosin II orthologue), the *A. nidulans* orthologue of Bud4p (a bud site selection marker in yeast), and a protein encoded by a third not-yet-cloned *Sep* locus. This conclusion is based upon the fact that PkcA cortical rings still form in the absence of function of these proteins, though septa do not then develop. In addition, we have co-imaged PkcA and other septation proteins bearing complementary fluorescent tags. SepA, TpmA, MyoB, and MobA all appear to colocalize with PkcA during normal septum formation. Studies with other septation-related proteins are ongoing.

154. THE *Aspergillus nidulans* CORTICAL MARKER TeaA MEDIATES MICROTUBULE-CORTEX INTERACTIONS AND REGULATES MICROTUBULE POLYMERASE ACTIVITY OF XMAP215 FAMILY PROTEIN AlpA

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In eukaryotic cells microtubule (MT) length is determined by polymerization and depolymerization phases. One important parameter for length determination is the contact with the cell cortex. Here, we show in the model organism *Aspergillus nidulans* that the contact of MT plus ends with the cortex is mediated through interaction between a putative MT polymerase (XMAP215, *A. nidulans* homologue AlpA), and a cortical cell end marker protein, TeaA. AlpA-TeaA interaction was observed in a bimolecular fluorescence complementation assay only after MT plus ends contacted the cortex. In the absence of TeaA, MT plus ends contacted random places along the tip cortex and occasionally continued to grow after reaching the cortex. To determine if AlpA has MT polymerase activity and if the activity is directly controlled by the interaction with TeaA, we developed an *in vitro* assay system. Fast MT polymerization depended on the presence of AlpA and was inhibited by TeaA.

155. PHEROMONE RECEPTOR AND RAS PROTEIN IN SIGNAL TRANSDUCTION PATHWAYS OF *Schizophyllum commune*

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The white rot basidiomycete *Schizophyllum commune* has been used as a model organism to study mating and sexual development as well as analysis of cell development. The mating system depends on two independ genetic factors, *A* and *B*. The *B*-locus encodes a pheromone/receptor system composed of the pheromone receptor and different lipopeptide pheromones. The seven transmembrane receptor Bar2 was visualized by fluorescence microscopy of HA-EGFP-tagged Bar2 transformants. The receptor could be localized in mating interactions.
Subsequent to pheromone recognition, intracellular signal transduction leads to a specific phenotype involving nuclear migration and clamp fusion. A role of the small G-protein Ras had been postulated in pheromone response in addition to MAPK signalling. To investigate the role of Ras constitutively active Ras alleles (Ras^{G12V} and Ras^{Q61L}) as well as a □RasGap1 mutant (Gap1 = GTPase activating protein) were analyzed. They show phenotypes with disorientated growth pattern, reduced growth rates and hyperbranching. The fungal cytoskeleton, composed of actin and microtubules has been investigated by immunofluorescence microscopy to reveal whether Ras signaling has an influence on the cytoskeleton

156. NUCLEAR MOVEMENTS IN *Schizophyllum commune*: REVISITED IN THE LIGHT OF THE SEQUENCED GENOME

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In the homobasidiomycete *S. commune* intracellular nuclear migration occurs in haploid hyphae when the telophase nuclei quickly separate after nuclear division and in interphase when the nucleus maintains its position in respect to the apex of the growing hypha. Intercellular nuclear migration is associated with the mating of haploid strains with different B-mating-type complexes and it involves passage of fertilizing nuclei between mates through hyphal bridges formed mainly by fusion of small side branches. In dikaryotic hyphae with clamp connections the intercellular nuclear migration also plays an important role in the movement of the nucleus from the clamp connection into subapical cell after the fusion of the clamp connection with the subapical cell. Depolymerizing of actin filaments with Latrunculin A in homokaryotic or dikaryotic hyphae lead to swelling of hyphal tips, reorganization of microtubule cytoskeleton and fast cessation of hyphal growth but not to the arrest of nuclear divisions. Depolymerization of microtubules with Nocodazole causes strong branching of hyphal tip and a slow cessation of apical growth and nuclear divisions (Raudaskoski et al. 1994, Eur. J. Cell Biol. 64: 131). Both treatments inhibit the intercellular nuclear migration and fertilization. These observations suggest that the normal growth pattern of fungal hyphae is dependent on well-organized actin and microtubule cytoskeleton. The *S. commune* total genome sequence now available permits the identification of putative components, in addition to actin and tubulin, regulating the function of intra- and intercellular nuclear migration. Along this line we have screened the expression of the 10 kinesin genes of *S. commune* genome by qRT-PCR during the fungal life cycle. The actual role of kinesins as well as actin associated motor molecules in hyphal growth and nuclear movements are under investigation.

157. INVESTIGATIONS OF THE DYNEIN HEAVY CHAIN IN THE BASIDIOMYCETE *Schizophyllum commune*

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The filamentous basidiomycete *S. commune* is an interesting model organism to study sexual development because of its tetrapolar mating-system with multi allelic incompatibility factors. A big strain collection, the recently published genome sequence and proteome analyzes allow the investigation of mating in more detail. During the mating action two partners exchange nuclei which underlie a fast transport along microtubule tracks where the motorprotein dynein is required. The dynein heavy chain (dhc) of *S. commune* is in focus of our study, which is encoded by two genes, *dhc1* and *dhc2*, both located on the same chromosome. The disjunction of both genes is a phenomenon in basidiomycetes although the heterobasidiomycete *Ustilago maydis* shows a different split point between the two proteins as compared to mushroom-forming homobasidiomycetes. Dhc1 and Dhc2 could be localized using immunfluorescence staining. The deletion of *dhc2* is viable. The phenotype of these knock-out mutants is characterized by a reduced growth rate and a defect in nuclear positioning compared to wildtype strains. The aim of this work is to breed strains deleted for *dhc1*. Comparisons of the resulting phenotypes of delta*dhc1* and delta*dhc2* will allow statements about possible functions of both genes. Specifically, a possible function of Dhc1 in spindle assembly and spindle orientation during cell division is assessed. In combination with 2D electrophoresis studies, specific functions are to be assigned to each Dhc protein.

158. CORONIN: A SMOOTH REGULATOR OF THE ACTIN CYTOSKELETON

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Coronin is a protein that binds to F-actin and the Arp2/3 complex. It localizes at sites of dynamic actin remodeling, playing an important role in endocytosis. Recently, a role in acting turnover has been elucidated, showing the other feature of coronin in promoting selective disassembly of older ADP-actin filaments. In *Neurospora crassa*, the mutation of the coronin gene affected hyphal polarized growth. The lack of coronin affected actin and ABPs patch organization at sites of endocytosis as well as in the apex during cell growth. The internalization of the lypophilic dye FM4-64 was slower in the coronin mutant than the WT strain. Spitzenkörper size and dynamics were notoriously compromised. In the coronin mutant, the Spk was unable to support continuous hyphal polarized directionality. Mitochondrial and nuclear distribution was also affected. Coronin-GFP localized at the subapex, where endocytosis is suggested to take place and co-localized with the Arp2/3 complex. Coronin is a protein important for "smooth calibration" of actin-based polarized cell growth and actin-tubulin mediated roles, but is not an essential gene for development.

159. LIFEACT AS A REPORTER FOR THE ACTIN CYTOSKELETON IN *Neurospora crassa*

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The actin cytoskeleton plays a crucial role in hyphal growth and morphogenesis, hence the importance of visualizing its localization and dynamics in growing hyphae. The visualization of the actin cytoskeleton in *N. crassa* has proven to be difficult by GFP-tagging of the actin monomer (G-actin). Recent strategies have consisted in the utilization of actin binding proteins (ABPs) fused to fluorescent proteins. The latest reporter developed is called Lifeact, which consists of the first 17 aminoacids of the actin binding protein Abp140 of *Saccharomyces cerevisiae*. Here we present Lifeact-GFP distribution in *N. crassa* hyphae. Lifeact-GFP labels presumably all F-actin present in *N. crassa*, in the form of cables, patches and a contractile ring involved in septum formation. This idea is supported by the observations that Lifeact tags structures previously imaged using other ABPs such as, tropomyosin-GFP labeling actin cables, and fimbrin-GFP as well as components of the Arp2/3 complex for the actin patches.

160. THE MICROTUBULE PLUS END ASSOCIATED PROTEIN MTB-3 IN *Neurospora crassa*

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Microtuble (Mt) plus end proteins (TIPs +) regulate the polymerization and depolymerization of the Mts during dynamic instability. End binding proteins (e.g., EB I) are a highly conserved family of Mt plus end stabilizer proteins. MTB-3 represents the member of this family in S. *pombe,* which sequence was used to identify the encoding gene in *Neurospora crassa.* MTB-3 was molecularly tagged by the

fusion of the *mtb-3* gene with the *sgfp* gene (*mtb-3::sgfp*). Using different fluorescent microscopy techniques (wide field epifluorescence microscopy, laser scanning confocal microscopy and total internal reflection fluorescence microscopy) we analyzed the distribution and dynamics of MTB-3-sGFP in *N. crassa.* We observed abundant fluorescent comet-like structures in all hyphal regions. The comets had a length of 1.6 ± 0.4 µm (mean ± standard deviation), they moved in an anterograde manner, and had an average motility rate of 1.9 $\mu m\ s^{-1}$, almost 5-folds faster than the cell growth (0.2 $\mu m\ s^{-1}$). In the subapical region, the MTB-3 comets displacement was bidirectional and we also observed a few instances of comets moving in retrograde direction from the apex. The latter, suggests that MTB-3 supports the polymerization of Mts that are nucleated in the Spk. Membrane organelles stained with FM4-64 did not colocalized with MTB-3-sGFP. Cells treated with 5 µg ml of Benomyl did not show fluorescent comets, only a punctuated distribution of the fluorescence in the subapical region and some accumulations around the nuclei. The similarity between MTB-3 displacement rate and the Mts polymerization rate and the localization ofthis protein along the Mts indicate that MTB-3 participate in the regulation ofrescue during dynamic instability.

161. CHARACTERIZATION OF THE MUTANTS *Lis1-1*, *Lis1-2* AND DOUBLE MUTANT *Lis1-1 Lis1-2* IN THE FILAMENTOUS FUNGUS *Neurospora crassa*

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The *Lis1-1*and *Lis1-2* genes of *Neurospora crassa* have a high identity with the *nudF* gene of *Aspergillus nidulans*, the *pac1* gene of *Saccharomyces cerevisiae* and the human gene *lis1*. The proteins encoded by these genes appear to interact with the dynein/dynactin complex at the plus end of microtubules (Mts) and are required for proper nuclear distribution, in regulating Mts dynamics and cell growth. In *A nidulans*, lack of NUDF may cause dynein to be kept at its inactive state and inhibit its transport of bound cargo, resulting in an over-accumulation at the plus end. The mammalian homolog of NUDF, LIS1, is product of a gene whose mutations cause brain malformation characterized by a disorganization of the neurons by a nuclear migration defect. To determine whether the lack of *Lis1-1*and *Lis1-2* genes can affect cell growth, we characterized *Lis1-1*, *Lis1-2* null mutants and the double mutant *Lis1-1 Lis1-2* in *Neurospora crassa*. The growth rate of *Lis1-1* mutant and double mutant *Lis1-1;Lis1-2* were 62% and 75% less than the wt, in contrast to *Lis1-2* mutant that had the same growth rate as the wt. Conidia production was affected in the mutants the strongest effect was in the double mutant (99% conidial production reduction). Branching rate in *Lis1-1* mutant and double mutant *Lis1-1;Lis1-2* were 3-folds higher than the wt ($p<0.05$), and the *Lis1-2* mutant showed similar branching rate than the wt. The biomass production was the same in all mutants. The mutation in the *Lis1-1*and *Lis1-2* gene in *N. crassa* are not essential but in *Lis1-1* affects strongly the growth rate, increase the frequency of branching and reduce the production of conidia, although *Lis1-2* mutation does not affect the phenotype and growth rate, the lack of both *Lis1-1* and *Lis1-2* have a synergic effect.

162. DYNAMICS OF MICROTUBULE RELATED MOTOR PROTEINS IN *Neurospora crassa*

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Dynein/Dynactin and Kinesin are motors proteins associated to microtubules (Mts). Both proteins play an important role in the intracellular trafficking of vesicles and organelles. Dynein transport the cargo from the plus end to the minus end of Mts and the kinesin moves from the minus end to the plus end. In order to determine the localization and dynamic of microtubule related motor proteins dyne-

in/dynactin complex and conventional kinesin in the filamentous fungus *Neurospora crassa*, we tagged the dynein light chain (DLIC), P-150^{glued} subunit of dynactin and nkin with the green fluorescent protein (GFP). By laser scanning confocal microscopy, we observed that dynein, dynactin and nkin are localized mainly at the hyphal apex. Dynein and dynactin are organized as short filaments while nkin is distributed in the cytoplasm in a gradient concentration pattern. The major concentration of dynein and dynactin are localized at the first 15 µm from the apex of the hyphae, the short-filaments are 2.22±0.55 µm and 1.6±0.40 µm of length for dynein and dynactin respectively (n=180). This filaments are highly dynamics, them move in anterograde direction with a velocity of 0.42±0.14 µm s^{-1} and 0.48 ± 0.20 µm s^{-1} (n=30) for dynein and dynactin respectively.

RHYTHMS AND PHOTOBIOLOGY

163. IDENTIFICATION AND CHARACTERIZATION OF BLUE LIGHT RESPONSE ELEMENTS ON *Trichoderma atroviride* GENES.

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Trichoderma atroviride is a soil fungus commonly used as a biocontrol agent against a wide range of phytopathogenic fungi, as well as a photomorphogenetic model due to its ability to conidiate upon exposure to light. In total darkness, *T. atroviride* grows indefinitely as a mycelium, while a short pulse of blue light induce conidiation in a discrete ring located at the colony perimeter where the light pulse was given. It has been proposed that the BLR-1 and -2 proteins are the responsible of blue light perception in *T. atroviride*. Mutants in either *blr*-1 or *blr*-2 did not conidiate and the expression of blue light responsive gene *phr*-1 was abolished. Likewise, expression analysis by cDNA microarrays showed that 2% and 1% of genes were induced and repressed respectively in response to blue light. Besides, light response elements (LRE) similar to those identified in *Neurospora crassa*, were found on promoters of blue light responsive genes in *Trichoderma reesei*. In base to this, our goal was to define the minimal promoter responsive to blue light on the *phr*-1 and *bld*-2 genes, which are induced and repressed by the stimulus respectively. To this purpose, several versions of *phr*-1 and *bld*-2 promoters were fused to the *LacZ* reporter gene. These constructs were used to transform the wild type strain to further select and analyze stable transformants. We determined the integrity of the corresponding endogenous *phr*-1 and *bld*-2 genes and the integration of the complete constructions into the genome, taken those with an intact endogenous gene and those that showed the integration of the constructs.

Beta-galactosidase activity assays under different dark and light conditions were conducted for the selected transformants. Control strain transformed with the empty vector showed no activity, while the 1026 bp promoter version was responsive to blue light. On the other hand, the shorter versions bearing just the predicted LREs did not show beta-galactosidase activity. By mutating the consensus bases of the putative LRE, we observed a slight increase on the beta-galactosidase activity, suggesting a possible repressor on this promoter. Concerning the *bld*-2 transformants we were unable to detect □-galactosidase activity in any strain probed. We are analyzing the β-galactosidase transcript to determine if the *bld*-2 untranslated region is affecting the β-galactosidase translation. Taken into account our result we performed a bioinformatic analysis on three orthologous promoters to *phr*-1, confirming the predicted LRE and a conserved region, which could function as a transcriptional enhancer. With this information, we generated a chimera that included these two regions fused to a minimal promoter to further analyze the gene expression in the transformants. Our results suggest that *i*) the minimal promoter inducible by light may consist of several regulatory elements and *ii*) the sequences that constitute the LRE in *T. atroviride* could be different to the consensus reported for *N. crassa*.

<u>164.</u> EPIGENETICS OF BLUE LIGHT PERCEPTION IN *Trichoderma atroviride,* A MYCO-PARASITIC FUNGUS.

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Light is a key environmental signal for living organisms on Earth. This cue regulates a wide variety of physiological and development processes in living organisms. *Trichoderma atroviride*, a common soil fungus widely used as a biocontrol agent against a wide range of phytopathogens, responds to blue light by developing a ring of green conidia in the colony perimeter where the light pulse was given. In addition, blue light induces and represses transcription of genes in this fungus. Upon a blue light pulse, *phr*-1 gene is upregulated, while *bld*-2 gene is downregulated. Transcriptional regulation of genes is conducted at several levels including epigenetic regulation. Genomic DNA in eukaryotic organisms is compacted by an octamer of conserved basic proteins called histones, which together conform the highly organized structure known as chromatin, which contains genetic information to direct cell functions. Several research groups have shown that covalent modifications of N-terminal tails of the core histones affect nucleosomes positioning and compaction. Acetylation and deacetylation of histones play important roles in activation or repression of genes respectively. We are interested in elucidate the epigenetic mechanisms involved in blue light perception in *T. atroviride*. To achieve this, we analyzed the effect of Trichostatine A (TSA) and Cyclopentylidene-[4-(4′-chlorophenyl)thiazol-2-yl)hydrazone (CPTH2), inhibitors of histone deacetylases (HDACs) and histone acetyl transferases (HATs) respectively in conidiation and gene expression of *phr*-1 and *bld*-2 in *T.atroviride*. We found that TSA induced conidiation even in the dark, while CPTH2 repressed sporulation in presence of light. On the other hand, the *phr*-1 gene was constitutively expressed after a light pulse in presence of TSA. Chromatin Immuno Precipitation (ChIP) analysis on *phr*-1 and *bld*-2 promoters, showed an increased acetylation level on the *bld*-2 promoter in dark conditions, however its acetylation was reduced after a blue light pulse coinciding with a reduction in the transcription of this gene. Taken into account these results, we mutated the *Saccharomyces cerevisiae* orthologous genes *gcn*-5 and *rpd3* in *T. atroviride*, which encode for a HAT and a HDAC respectively. *gcn*-5 mutant showed slow growth, conidiates poorly and did not conidiate after a blue light pulse when compared with the wild type (WT) strain. On the other hand, *hd*-1 mutant showed an exacerbated conidiation and grew faster when compared with the WT strain. In addition, we overexpressed the *gcn*-5p and *hd*-1 genes in WT, observing the opposite phenotypes when compared with those showed by the corresponding mutants. Finally, expression analysis of *phr*-1 and *bld*-2 in Δ*gcn*-5, Δ*hd*-1, OE*gcn*-5, OE*hd*-1 revealed that epigenetic processes in this fungus regulate such genes. Taken together, our findings we can conclude that chromatin modification plays an important role in the epigenetic of blue light perception in *T. atroviride*.

<u>165.</u> A STRESS RESPONSE MAP-KINASE PATHWAY IS REGULATED BY LIGHT IN *Trichoderma atroviride*

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Light is an environmental signal that impacts on the behavior of almost all living forms, regulating growth, development, reproduction, pigmentation and circadian rhythms. In *T. atroviride*, light regulates asexual reproduction, growth and metabolism. The BLR1/BLR2 proteins, homologous to photoreceptor complex of *Neurospora crassa* WC1/WC2, regulate these responses to blue light. These PAS domain proteins have a DNA binding domain and their activity as a transcription factor is mainly light dependent. BLR1 has a LOV domain, which contains all amino acids necessary to bind FAD as chromophore and in analogy to WC1 and phototropins, light impacts upon the flavin activating the BLR

complex, consequently, regulating many physiological responses to light. A large number of light regulated genes have been identified, however, the signaling cascade triggered by light downstream of these type of photoreceptors is still poorly understood. It has become evident that light regulates genes encoding DNA-repair enzymes, and enzymes implicated in the synthesis of photoprotective pigments, suggesting that light is seen as stress signal in living forms. Here, we show that the mitogen-activated protein kinases pathway involved in stress responses is activated by light in *T. atroviride*. Expression of the MAPKK Tapbs2 and the MAPK Tahog1 is regulated by light through the photoreceptor complex BLR1/BLR2. Interestingly, TaHog1 is phosphorylated rapidly upon light exposure and this post-translational regulation is apparently independent of the BLR proteins. This MAP-Kinases pathway is indispensable for tolerance to osmotic stress and to a lesser extent to oxidative stress in vegetative growth; however, in conidia it plays key protective roles to different kinds of stress.

166. FUNCTIONAL CHARACTERIZATION OF *cry*1 GENE IN *Trichoderma reesei*

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In fungi, light regulates physiological processes such as phototropism, circadian rhythms, morphogenesis, and secondary metabolism, among others. Three light perception systems have been described in fungi, one for red light, one for blue light and recently one for green light. Specific chromoproteins perceive light of the different wavelengths. Phytochromes perceive red light, cryptochrome and phototropins blue light, and opsins are involved green light perception. The most studied light perception system is the one involved in blue light responses of *Neurospora crassa*, where white collar genes (*wc-1* and *wc-2*) control virtually all known functions in response to blue light, such as carotenogenesis , induction of protoperithecium, phototropism, induction of hyphal growth, asexual spore formation and regulation of the circadian clock, but in other models such as *Mucor circinelloides* and *Aspergillus nidulans*, apparently the blue-light response system is more complex than that of *N. crassa.*
In *Trichoderma* the blue light responses are mediated by the regulators blr-1 and blr-2, homologous to *wc-1* and *wc-2* respectively, which have some of the functions described in *N. crassa.* However, recently our working group, demonstrated the existence a set of genes that are induced in response to light and are not controlled by genes *blr-1* and *blr-2*. These data indicate the existence of yet another regulator of the light response that controls the expression of this set of genes. These data coupled with the fact that in the a gene with great similarity to cryptochrome (*cry1*) was found in the genome of *T. reesei*, and the scarce information on the role of cryptochromes in fungi, has led to a great interest on the study of the role of cryptochrome like genes. Here we report the functional characterization of the *T. reesei cry*1 gene, Gene replacement mutants showed phenotypic changes, as well as its over expression. Finally, we report heterologous expression in *E. coli* and the production of antibodies.
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OTHERS

167. THE EVOLUTION OF PHEROMONAL SIGNAL SYSTEM IN HOMOTHALLIC SPECIES OF *Neurospora*

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The genus *Neurospora* (Ascomycota) is especially suitable for studies on reproductive system evolution, since the genus has representatives for all known reproductive systems known in the fungal kingdom (heterothallism, homothallism, and pseudohomothallism).
In heterothallic species of *Neurospora* strains of opposite mating type (*mat a* and *mat A*) must interact, for the sexual cycle to occur. Two strains of the same type result in a heterokaryon incompatibility reaction. The *mat* genes in *Neurospora* control the downstream targets governing the whole expression cascade of mating-type-specific expressed genes, including the pheromone receptor genes *pre-1* and *pre-2* and the pheromone precursor genes (*ccg-4* and *mfa-1*). Pheromone and pheromone receptor genes have previously been investigated in heterothallic species, and the *pre-1* and *pre-2* genes evolve rapidly. Both genes evolve under low selective constraints, and in additon, *pre-1* contains sites under positive selection.
In homothallic species, all information necessary for sexual reproduction is contained in one haploid nucleus. Previously, signs of genetic decay of the mating type genes in homothallic *Neurospora* have been described. In this study we are interested to investigate the downstream targets of the mating type genes, to see if they also show signs of genetic decay or are conserved. We study the molecular evolution of the pheromone receptor (*pre-1* and *pre-2*), and pheromone precursor (*ccg-4* and *mfa-1*) genes in several homothallic taxa of the genus *Neurospora*. We perform Q-PCR at different time point in the sexual cycle and measure the level of pheromone and receptors. In addition, we perform cDNA sequencing of the pheromone receptor and pheromone precursor genes.

168. INHIBITORY EFFECT OF *Bacillus subtilis* ON *Fusarium circinatum* GROWTH

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Fusarium circinatum is a known pathogen that produces pitch canker on conifers. In Uruguay, its presence was recently detected on *Pinus taeda* seedlings in nurseries mainly affecting stem collar. In order to avoid the propagation to field plantations where its presence has not been yet found a possible biocontrol mechanism was evaluated. *Bacillus subtilis* has been identified as a potent antagonist against several fungal pathogens due to the production of antifungal compounds, antibiotics and proteases, hence is extensively used in agricultural systems. On the other hand, little work has been performed of antifungal activities on forest pathogens. In this study, the effect of four strains of *Bacillus subtilis* on mycelia growth rate and hyphal morphology of *Fusarium circinatum* were assayed. Direct antagonistic effect of live bacteria on fungal growth was evaluated placing a 5-day-old disk with pathogen mycelium in the centre of a Petri dish containing Potato-Dextrose-Agar. Bacterial inocula were placed at four equidistant points of the fungal inoculum. Plates inoculated with a disk containing fungal mycelium were used as control. These plates were incubated at 25ºC. Five replicates were performed for each trial. The effect on mycelial growth was recorded and microscopic observations of the hyphal morphology from the advancing zone were carried on. Another assay to analyze the effect of termostable metabolites was performed. Autoclaved bacterial culture was incorporated to Potato Dextrose Agar and 20 ml was used to fill each Petri dish. A disk containing mycelium of *F. circinatum*

was inoculated at the centre of the Petri dish. The control was the pathogen growing on PDA. The mycelial growth rate was evaluated and the percentage of inhibition was calculated.
Inhibition of fungal growth rate was observed. Some differences of the effect among the four bacteria were observed. The production of thermostable inhibitory metabolites was confirmed. Moreover, the mycelia microscopic examination revealed morphological modifications including lysis of mycelial fragment, abnormal swelling, abundant vacuolation and other deformities. The production of abundant pigment was also observed in the interface zone.
Since biocontrol seems to be a good alternative to the use of chemicals for disease control, the identification and the mode of action of antifungal compounds produced by antagonist bacteria need to be studied.

169. THE *Neurospora crassa chrA* GENE CONFERS CHROMATE RESISTANCE IN *Saccharomyces cerevisiae*

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ChrA is a membrane protein that confers resistance to chromate through a detoxification mechanism that expels the ion from the cytoplasm. ChrA belongs to the CHR superfamily of chromate ion transporters. This includes numerous homologs in diverse bacterial species as well as in fungi (including *Neurospora crassa, Aspergillus nidulans* and *Ustilago maydis*). It is noteworthy that the genome of *Saccharomyces cerevisiae* does not contain CHR homologs. The *chrA* gene of filamentous fungi *N. crassa* of 1745 bp, has two introns and encodes a protein of 507 amino acid residues. The objective of this work was to elucidate the function of the *N. crassa chrA* gene. The analysis of gene expression by RT-PCR showed that under varying conditions of cultivation the *chrA* gene is expressed in *N. crassa* only in the presence of chromate, suggesting that the gene is inducible. To determine the function of the protein, the *N. crassa chrA* gene was amplified by PCR from genomic DNA of *N. crassa*, cloned into the pYES2 vector and the recombinant plasmid was used to transform *S. cerevisiae*. Chromate susceptibility tests in raffinose/galactose medium showed a slight increase in resistance to chromate of the transformants compared to the sensitive strain. These results show that the *N. crassa* gene *chrA* confers resistance to chromate in *S. cerevisiae*. Prediction of the secondary structure of *N. crassa* ChrA protein showed it to contain 9-10 transmembrane segments. A potential promoter region was identified 366 nt upstream of the *chrA* gene initiation codon. Perspectives are to determine the type of promoter identified and to silence the *N. crassa chrA* gene by RNA interference to elucidate the role of the gene in this organism.

170. OBTAINING THE HOMOLOGUE OF HUMAN DYSKERIN PROTEIN IN *Ustilago maydis.*

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The telomere is a specialized structure that protects the extremity of linear chromosomes, prevents degradation and aberrant recombination and allows the complete replication of the chromosomal end. Without this protection,different mechanisms could trigger cell cycle arrest, senescence and apoptosis. The mammalian telomeric sequence corresponds to TTAGGG repeated in tandem with a 5'-3' orientation. Elongation of telomeres is mediated by telomerase, an enzyme composed by a ribonucleoprotein complex. It has two essential components: the telomerase enzyme with transcriptase reverse activity (TERT) and a catalytic RNA subunit (TERC), joined to other associated proteins. The catalytic subunit TERT uses the short sequence of TERC as a template. TERC has also an H/ACA motif that serves as the interaction site with a protein called dyskerin. This protein is a putative pseudouridine

synthase with a possible role in ribosomal processing due to its binding ability to different snoRNAs (small nucleolar RNA's) which are responsible for ribosomal maturation. The evidence relating dyskerin and telomere maintenance isa rare genetic disorder called dyskeratosis congenita (DKC, or Zinsser-Engman-Cole Syndrome). This Syndrome causes abnormal skin pigmentation, nail dystrophy and mucosal leucoplakia; with bone marrow failure as the principal cause of death.
Ustilago maydis is a biotrophic pathogen specific for maize and teozintle. In this plants, it causes a worldwide-distributed disease known as common smut or "cuitlacoche" in México. *U. maydis* has been a classic model for the study of different molecular mechanisms, including DNA recombination and repair. Surprisingly, the telomeric sequence of this fungus is the same of mammals (TTAGGG). The relationship between *U. maydis* and human has recently become evident with the discovery that human shares more conserved proteins with this fungus that with the classical model *Saccharomyces cerevisae*. This observation suggests that the same machinery and similar pathways may be common for both organisms. For these reasons, *U. maydis* could be considered a good and reliable model for the study of telomeres and its maintenance.
We identified a putative Dyskerin sequence in *U. maydis*, which showed 66% identity with the human Dyskerin protein. Surprisingly, the conservation level between them is high, suggesting that the function might also be conserved in both organisms. We designed primers to amplify the complete coding sequence for Dyskerin from *U. maydis*, and PCR was performed. We obtained a PCR product of approximately 1500bp (the expected size was 1491bp). After subcloning, we obtained the recombinant protein. Functional analyses will show if the putative protein identified maintains telomeres in *Ustilago* and the mechanism involved in this protection might be uncovered.

171. IDENTIFICATION AND CLONING OF THE HUMAN Pot1 HOMOLOGUE IN *U. maydis*.

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Ustilago maydis is a bacydiomicete that causes the corn disease known in Mexico as "cuitlacoche". Besides its phytopathogenic activity, the importance of *U. maydis* as a model system increased recently, as bioinformatic and proteomic studies revealed that important processes are conserved between *U. maydis* and mammalians, like cytoskeleton rearrangement and mitosis. Interestingly, the telomeric sequence is also conserved between these two organisms, consisting in short DNA repeats (TTAGGG repeated in tandem with 5'-3' orientation) that recruit specific proteins at the chromosome termini. The function of telomeres is to protect chromosomal endings against DNA repair and degradation activities, and it also serves as substrate for telomerase, the ribonucleoproteic enzyme that synthesizes the telomeric repeats.
A six-protein complex associates to the telomeric sequence to protect chromosome ends. This complex is called shelterin, and shows three subunits (TRF1, TRF2 and Pot1) that directly recognize TTAGGG, associated to three aditional proteins (TIN2, TPP1 Rap1). However, the most important protein of the shelterin complex is Pot1 (protection of telomere), given its ability to bind single-stranded DNA overhangs at human chromosome ends, to protect chromosomal termini and to help regulating telomeric length. Human Pot1 is a 634 amino-acid protein with an N-terminal evolutionarily conserved pair of oligonucleotide/oligosacharide (OB) folds responsible for telomeric single-stranded DNA binding, while the remaining C terminus is able to bind other proteins of the shelterin complex. Unfortunately, the understanding of how Pot1 protects chromosomal ends is still incomplete. Specifically, it remains to be determined which of the functions of Pot1 is responsible for the protection of chromosome ends.
In this investigation, we localized a putative Pot1 to the locus UM05117 in *Ustilago maydis*, we examined the coding sequence for this UPot1 protein, designed the primers to amplify the complete ORF, cloned and induced the production of the recombinant UPot1 to finally obtain the pure protein.
The comparison between UPot1 and Human Pot1 showed an overall identity of 27% when the protein sequences were compared. UPot1 also showed an important degree of evolutionary conservation,

when compared with the homologue in different species. Surprisingly, UPot1 seems to be more related to mammalian Pot1 than to other fungal homologs.

172. TRACES OF ALTERNATIVE SPLICING AND NMD IDENTIFIED IN THE BASIDIOMYCETE *Ustilago maydis.*

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Tight control of eukaryotic gene expression is absolutely required to ensure cell survival. Alternative splicing is responsible for the multiplicity of protein isoforms that can be generated from a single pre-mRNA in most eukaryotic organisms. During splicing, removal of non-coding sequences is followed by exon-ligation to generate a message that will be translated into a functional protein. This reaction is catalyzed by the spliceosome, a complex assembly of five small nuclear ribonucleoprotein (snRNP) particles (U1, U2, U4, U5 and U6) associated with a large number of additional proteins. Most alternative splicing events affect the coding sequence, with half of these altering the reading frame and a third apparently leading to nonsense-mediated mRNA decay (NMD), which not only degrades faulty transcripts but also regulates the steady state level of many physiological mRNAs involved in a variety of different cellular processes. The NMD core factors Upf1p, Upf2p and Upf3p are present in all higher eukaryotes analyzed to date and they are believed to function at the heart of NMD. The molecular mechanisms regulating alternative splicing and NMD are still not fully understood, but recent data suggest that these processes are highly conserved in different organisms. Smuts belong to the order *Ustilaginales* and are pathogenic basidiomycete fungi responsible for significant agricultural losses worldwide. *Ustilago maydis* is perhaps the best-known smut and has become a model for this group of fungi. As a result of the extensive studies with *U. maydis*, a vast number of molecular tools are available including the genome sequence. Using these tools, we have identified in *Ustilago maydis* the putative UPF1, UPF2 and UPF3 NMD factors as well as several core and auxiliary proteins involved in splicing regulation. When comparing *Ustilago* and human, identity for most putative splicing factors ranges between 40% and 60%. Surprisingly, UPF1 shows 65% identity between *Ustilago* and human. Other factors are highly conserved as well. This high homology could suggest an important degree of conservation between human and fungal control of alternative splicing and NMD.

173. PARTICIPATION OF THE SHO1 BRANCH IN THE RESPONSE TO HYPEROSMOTIC STRESS *Kluyveromyces lactis*.

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In their natural environment, yeast cells have to adapt to a variety of fluctuating stress conditions such as nutrient depletion, changes in ambient temperature high and low external osmolarity, etc. When exposed to high solute concentrations in their environment (hyperosmotic stress), eukaryotic cells respond by rapidly activating a conserved mitogen activated protein kinase cascade, known in the budding yeast *Saccharomyces cerevisiae* as the high osmolarity glycerol (HOG) pathway. The HOG pathway consist of a MAP kinase signaling module composed by the MAPK Hog1p, the MAPKK Pbs2p, and three different MAPKKK proteins, Ssk2p, Ssk22p and Ste11p . Once activated, Hog1p is translocated to the nucleus and stimulates expression of several high osmolarity-induced genes. The Ssk2 and Ssk22 proteins are activated by Ssk1p, which is the response regulator of a histidine kinase signaling system consisting of Sln1p, the putative osmosensing histidine kinase, and the phosphorelay protein, Ypd1p. Alternatively, the third MAPKKK, Ste11p, can be triggered by the putative osmosensor Sho1p, through the activity of Ste20p and Ste50p. Pbs2p was shown to act as a scaffold protein bringing Sho1p and Ste11p together in a signaling complex.

We have cloned several genes encoding components of the HOG pathway in the budding yeast *Kluyveromyces lactis*. Disruption studies have indicated that the SHO1 branch may operate differently to that of *S. cerevisiae*. All disruption mutants were constructed integrating the *URA3* cassette by homologous recombination in wild type cells. The mutant strains Δ*Klsho1*, Δ*Klste20 and* Δ*Klste11* were assayed in conditions that produced osmotic stress (0.5M and 0.75M KCl and 1M Sorbitol). The Δ*Klste11* mutant showed strong sensitivity to high osmolarity, which indicate that this protein is very important for hyperosmotic stress response. In these experiments we found that mutants Δ*Klsho1*, Δ*Klste20* shown moderate sensitivity to hyperosmotic stress, indicating that KlSho1p KlSte20p are not essential for adaptation to hyperosmotic conditions however, they are important to establish an appropriate response. We hypothesized that lack of an active SHO1 branch can be masked by the phosphorelay SLN1 branch which acts positively over Hog1p in the hyperosmotic response. In order to answer this question, mutants in both the SHO1 and the SLN1 branches were constructed, and we found that sensitivity to hyperosmotic stress was increased significantly.
Supported by CONACYT (Grant number: 80343), México and PAEP, UNAM.

174. EVOLUTIONARY FATE OF BRANCHED CHAIN AMINO ACID TRANSAMINASES GENES (*BAT1* AND *BAT2*) OF *Saccharomyces cerevisiae*

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Saccharomyces cerevisiae has an average of 550 duplicated genes and it has been proposed that gene duplicates arose from a whole genome duplication event. Some of the retained duplicated genes are involved in nodes that connect glucose catabolism and tri-carboxylic acid cycle with amino acid biosynthesis, and the amino acid catabolism with glucose pathway and Krebs' cycle. We have special interest in these duplicated genes, because we would like to infer their evolutionary fate using comparative analysis and understand why they were maintained in the *S. cerevisiae* genome.
The branched chain amino acid transaminase activity in *S. cerevisiae* is performed by two isozymes encoded by the paralogous genes *BAT1* and *BAT2*. A mutant yeast strain lacking both genes is a valine, leucine and isoleucine auxotroph. It has been observed that Bat1p (mitochondrial enzyme) is involved in biosynthetic roles; and Bat2p (cytosolic enzyme) in catabolic roles.
A comparative analysis using the upstream regulatory region of *sensu stricto Saccharomyces* yeasts showed us that *BAT1* gene conserved more regulatory motifs associated with amino acid biosynthesis regulators than *BAT2* gene did, although both genes shared a regulatory motif that recognize the specific leucine pathway transcriptional factor Leu3p. In addition, *BAT2* share regulatory motifs recognized by transcription factors involved in the regulation of catabolic genes.
Complementation analysis results done with the *BAT* orthologue gene of *Saccharomyces kluyveri*, a yeast to have become a species before the whole genome duplication that occurred in the *Saccharomyces* lineage, will be presented in order to answer if the duplicated genes (*BAT1* and *BAT2*) have split a possible dual function of its ancestral gene.

175. OBTAINING THE HOMOLOGUE OF HUMAN DYSKERIN PROTEIN IN *Ustilago maydis.*

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The telomere is a specialized structure that protects the extremity of linear chromosomes, prevents degradation and aberrant recombination and allows the complete replication of the chromosomal end. Without this protection,different mechanisms could trigger cell cycle arrest, senescence and apoptosis. The mammalian telomeric sequence corresponds to TTAGGG repeated in tandem with a 5'-3' orientation. Elongation of telomeres is mediated by telomerase, an enzyme composed by a ribonucleoprotein complex. It has two essential components: the telomerase enzyme with transcriptase reverse activity (TERT) and a catalytic RNA subunit (TERC), joined to other associated proteins. The catalytic subunit TERT uses the short sequence of TERC as a template. TERC has also an H/ACA motif that serves as the interaction site with a protein called dyskerin. This protein is a putative pseudouridine synthase with a possible role in ribosomal processing due to its binding ability to different snoRNAs (small nucleolar RNA's) which are responsible for ribosomal maturation. The evidence relating dyskerin and telomere maintenance isa rare genetic disorder called dyskeratosis congenita (DKC, or Zinsser-Engman-Cole Syndrome). This Syndrome causes abnormal skin pigmentation, nail dystrophy andmucosal leucoplakia; with bone marrow failure as theprincipal cause of death.
Ustilago maydis is a biotrophic pathogen specific for maize and teozintle. In these plants, it causes a worldwide-distributed disease known as common smut or "cuitlacoche" in México. *U. maydis* has been a classic model for the study of different molecular mechanisms, including DNA recombination and repair. Surprisingly, the telomeric sequence of this fungus is the same of mammals (TTAGGG). The relationship between *U. maydis* and human has recently become evident with the discovery that human shares more conserved proteins with this fungus that with the classical model *Saccharomyces cerevisae*. This observation suggests that the same machinery and similar pathways may be common for both organisms. For these reasons, *U. maydis* could be considered a good and reliable model for the study of telomeres and its maintenance.
We identified a putative Dyskerin sequence in *U. maydis*, which showed 66% identity with the human Dyskerin protein. Surprisingly, the conservation level between them is high, suggesting that the function might also be conserved in both organisms. We designed primers to amplify the complete coding sequence for Dyskerin from *U. maydis*, and PCR was performed. We obtained a PCR product of approximately 1500bp (the expected size was 1491bp). After subcloning, we obtained the recombinant protein. Functional analyses will show if the putative protein identified maintains telomeres in *Ustilago* and the mechanism involved in this protection might be uncovered.

176. EXOGENEOUS DNA IS MAINLY INCORPORATED INTO *Trichoderma spp.* BY HOMOLOGOUS RECOMBINATION.

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The genus *Trichoderma* encompasses many species with great biotechnological interest, such as good enzymes producers, biological control agents, and in some cases pathogens of Basidiomycetes used in the food industry. Genetic transformation has been a powerful tool to understand these processes and implement new technological advances. However, our knowledge about transformation in fungi has been taken mainly from yeasts and the processes easily explained in other fungi by analogy; without, sometimes, a good understanding. In *Trichoderma spp*. as well as in other fungi very similar integration patterns of the transformant DNA have been found. That behavior has been explained as tandem

ectopic or illegitimate integrations and these events proposed to occur in recombination hotspots. Furthermore, there is evidence of genetic instability in fungi. However, an explanation about it is scarce. In this work, we show evidences at the molecular and physiological level that explain such behavior. We found that similar integration patterns are associated to incorporation of DNA by homologous recombination at least in three species of *Trichoderma*. Curiously, we found the genetic instability associated to an autoreplicative behavior of the plasmids used for transformation. Finally, we demonstrate the homologous recombination as the main mechanism of DNA incorporation into *T. atroviride*, by producing a number of targeted insertional mutants in the *blr* genes.

177. CHARACTERIZING THE FUNGAL BIODIVERSITY OF ARID ECOSYSTEMS OF BAJA CALIFORNIA, MEXICO

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Fungi play an important role in soil ecosystems. They are involved in primary processes such as nutrient cycling and organic matter degradation. They can exist under any environmental conditions, including those in arid ecosystems (<245 mm of annual precipitation). Even though fungi are the dominant microbiota in desert lands, they have been scarcely studied. This lack of information is due to the small fraction of culturable fungi present in soils. Therefore the conventional identification techniques yield limited information about the composition and dynamics of soil fungal communities.
Nowadays, the possibility to extract and amplify DNA directly from soil samples has provided the means to characterize fungal biodiversity without the need to cultivate the organisms. Many studies on fungal biodiversity in environmental samples aim to amplify the internal transcribed spacer (ITS) of the ribosomal DNA (rDNA) since it has highly variable non-coding sequences that accumulate neutral mutations, adequate to discern between closely related species.
As Baja California has arid ecosystems in more than 50% of its territory in which the local soil fungal biodiversity is unknown, soil samples were collected from well-represented arid ecosystems along the state. These ecosystems have been categorized as "arid" by the National Institute of Statistics and Geography of Mexico (INEGI). Although they have similar annual precipitation, its vegetation and edaphology, as well as other climate conditions vary in each area. We selected five locations, which based in the conditions mentioned before, have been classified into different arid ecosystems: La Salada and Cataviña belong to the ecosystem categorized as desert shrub, Santa Catarina as chaparral, Valle de las Palmas as transition chaparral and El Rosario as transition desert shrub. Genomic DNA was extracted from soil samples collected at these locations and the ITS region was amplified by nested PCR using specific primers (NSA3-NLC2 and NSI1-NLB4) for the Dikarya subkingdom, obtaining 900 bp amplicons from all the samples. Subsequently, the characterization of fungal biodiversity is being carried out through the construction and analysis of an ITS gene library. Thus, we expect to find high rates of fungal biodiversity, since we know that in other North American deserts high fungal biodiversity has been found.

178. **UNEVEN DISTRIBUTION OF H^+-ATPASE (PMA-1-GFP) ALONG THE PLASMA MEMBRANE IN LIVING CELLS OF *Neurospora crassa***

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One of the main features of filamentous fungi is their apical mode of growth. The highest concentration of secretory vesicles occurs at the apex, where they aggregate temporarily at the Spitzenkörper before they fuse with the plasma membrane. Accordingly, most models for fungal growth have proposed a directional traffic of secretory vesicles to the hyphal apex. As part of an ongoing project to better understand and characterize the secretory pathway in filamentous fungi, we have studied the distribution of the plasma membrane H^+-translocating ATPase (PMA-1) in *Neurospora crassa* cells. PMA-1 is delivered via the classical secretory pathway (ER to Golgi) to the cell surface, where it pumps H^+ out of the cell, generates a large electrochemical gradient and supplies energy to H^+-coupled nutrient uptake systems. We have fused the H^+-ATPase encoding gene *pma-1* from *N. crassa* (NCU01680), with *gfp* and studied its expression in living cells of *N. crassa*.
We observed that PMA-1-GFP localized at the plasma membrane in hyphal distal regions (>500 µm) and in already formed septa. Whereas it did not accumulate at the Spitzenkörperor at the plasma membrane in the apical and proximal subapical hyphal regions. In conidia, PMA-1-GFP was evenly distributed in the plasma membrane. During germination no fluorescence was observed at the emerging tube. In young germlings (12-49 µm) PMA-1-GFP accumulated at the plasma membrane of the germinated conidia and extended to the base of the germ tube (5.15-18.34 µm). To test the functionality of the recombinant protein, *N. crassa* SMRP99 (PMA-1-GFP) was crossed to a heterokaryon *pma-1* KO mutant strain (FGSC#11519). These results demonstrate that the H^+-ATPase is deficient at the apex during early development and suggest that it helps generate the electrochemical gradient that drives apical nutrient uptake once polarity has been established.

179. **TREHALOSE ACCUMULATION IN RESPONSE TO STRESS IN *Ustilago maydis.***

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Ustilago maydis is the agent responsible for corn smut. The fungus exhibits a dimorphic lifestyle. It has a nonpathogenic haploid phase and pathogenic filamentous dikaryon phase. The ability of *U. maydis* to adapt to high environmental osmolarity and exposition to higher temperatures are process that subsequent loss viability. Trehalose is a disaccharide which serves as a carbohydrate reserve and stress protector.
Haploid strain FB2 of *U.maydis.* Growth conditions: yeast cells were cultured in minimal medium, at pH 7.0, in the presence of sodium chloride (0.5 and 1.0 M), D-sorbitol (1M), or exposed at three different temperatures (35, 37 and 40ºC). The control condition corresponds to growth at 28ºC. Cell growth was followed by measuring the absorbance at 600 nm.
Trehalose quantification: after 20h of cultivation the yeast cells were collected and harvested by centrifugation at 4°C, the cell pellets were re-suspended in 10 mM MES. The samples (250 mg wet weight) were extracted by boiling at 95°C in a water bath for 10 min and then centrifuged. Trehalose was measured by enzymatic method. The resulting glucose was determined with glucose-oxidase method.
Measurement of trehalase activity: The reaction mixtures for trehalase activity contained 0.05% digitonin, 100 mM trehalose, 50 mM HEPES pH 7.0 and cell suspension. After incubation at 30°C the reaction tubes were immersed in a boiling water bath at 95°C for 2 min, cooled and centrifuged at 5000 x g for 10 min. Glucose was determined. Protein content was determined by the Lowry method.

Oxygen consumption was measured using an assay medium of 50 mM HEPES pH 7.0, cell suspension (10 µl), when indicated, 1M glucose, 1M sodium chloride or 1M sorbitol in a final volume 1 mL at 26°C.
Cells cultivated in the presence of 0.5M NaCl or 35°C showed similar growth rates as control cells (28°C). However, with 1M NaCl or 37°C, cell growth was lower than the control one. At 40°C cell growth was inhibited.
When yeast cells were exposed to high temperature (37°C) or a high concentration of sodium chloride (1M), trehalose accumulated in the first 15 min of the stress, and then its concentration decreased, even below the concentration in control cells. However, the larger accumulation of trehalose was found under osmotic stress by D-sorbitol. We tested the effect of these conditions on trehalase activity. The osmotic stress induced by 1M NaCl did not increase this activity, but there was a 2-fold increase with 1M Sorbitol. At 37°C there is a decrease in trehalase activity after 30 min incubation. The activity of control cells was constant during the 2h incubation. We studied the effect of osmolarity on oxygen uptake. In the absence of glucose, there was a time dependent decrease in oxygen consumption; subsequent addition of glucose after 400 sec in the respiratory medium re-established the respiratory activity. With 1M NaCl the cells showed a behavior in oxygen uptake which was similar to the cells without glucose.

180. CHARLES BRACKER'S ELECTRON MICROSCOPY OF CHITOSOMES – A FORERUNNER OF FUNGAL NANOTECHNOLOGY

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The emergence of Nanotechnology as a field of study provides renewed impetus, tools and opportunities to revisit the subject of chitin microfibril formation, and cell morphogenesis. In a tripartite collaborative study initiated in 1976 with Jose Ruiz-Herrera and the present author, Bracker's high resolution electron micrographs were the key to disclosing the fine structure and other unique properties of chitosomes. These microvesicles (or nanovesicles according to their nanoscale dimensions: 40-60 nm) are responsible for synthesizing *in vitro* chitin microfibrils (or nanofibrils had they been named by their 10-15 nm width). Chitosomes function as fungal nanoreactors capable of assembling chitin fibrils with different morphologies from tight coils to long extended fibrils. Although published studies have described the basic morphology and properties of chitosomes in different fungi, important features and clues about their structure, function and genesis were left largely unexplored. These now offer intriguing opportunities for future nanobiological studies. Foremost is the unique capacity of chitosomes to disassemble into a uniform population of spheroid particles (7-12 nm diameter; 16 S according to their sedimentation constant) upon digitonin treatment and reassemble into membranous structures with vesicular morphologies after removal of digitonin. High-resolution electron micrographs showed these processes most vividly. The self-assembly of a biological membrane from spheroid protein particles of ~500 kDa is likely to disclose novel features of organelle biogenesis. How chitosomes are positioned to function *in vivo* is another subject with promising potential to help understand a fundamental aspect of fungal cell wall formation. On the other hand, the ability to produce fibrils of nanoscale dimensions, and to control the process by substrate supply and/or zymogen activation, offers promising possibilities for nanotechnological applications.

Social Program

SOCIAL PROGRAM

SUNDAY Dec 6, 2009

20:00 - 23:00 **WELCOME PARTY**
CASINO ROOM, CENTRO SOCIAL CIVICO Y CULTURAL RIVIERA.
Transportation will be provided

MONDAY Dec 7, 2009

19:00 - 22:00 **MEXICAN BUFFET - DRINKS – MUSIC**
THIRD FLOOR OF THE BIOLOGY DIVISION (DBEA) BUILDING. CICESE.
Transportation will be provided

TUESDAY Dec 8, 2009

F R E E A F T E R N O O N

Tour 1- Wine Tasting
Tour 2- Beach walk
Tour 3- Bar hopping
Sign up – Registration desk
Group 1
Group 2
Group 3
Group 4
Group 5

WEDNESDAY Dec 9, 2009

19:00 - 22:00 **MEXICAN BUFFET - DRINKS – MUSIC**
TALENT NIGHT
FEATURING SPITZ & KORPER AND MANY MORE
Sign up-Registration desk
THIRD FLOOR OF THE BIOLOGY DIVISION (DBEA) BUILDING. CICESE.
Transportation will be provided

THURSDAY Dec 10, 2009

19:30 - ???? **GALA DINNER & D A N C E**
Ensenada Hall
Hotel Coral & Marina

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(GGP) glucose-6-phosphate, 10.
(HMGR) 3-Hydroxy-3-methylglutaryl coenzime A reductase, 147.
(PrA) Acid Aspartyl proteinase, 65.
(PrB) Neutral Serine proteinase, 65.
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Cover drawings:

Mendocino codex

Neurocihuatl

One of our enterprising students, Sinsan Diego Slender, a budding paleo-anthropomycologist, made a sensational discovery in the caves north of Loreto, Baja California Sur. The details of this well preserved hieroglyph, dated ca. 1200 AC, reveal a surprising knowledge of filamentous fungi among ancient Mexicans. It is believed to be a masterful representation of the goddess “Neurocihuatl”, divine regulator of daily rhythms in the world.
Notable features are the shield decorated with a perithecium-like figure and bodies resembling asci and ascospores adorning her headpiece and face; the sun, the moon and the bands on her face and body emphasize her circadian power.

Design by ***Diego Luis Delgado-Alvarez*** and ***Karla Alcocer-Soto***.

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