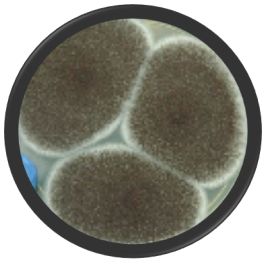
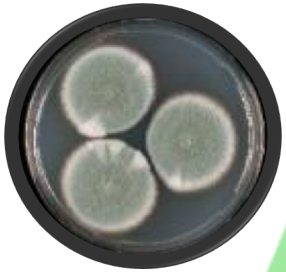
National Research Council
INSTITUTE OF SCIENCES OF FOOD PRODUCTION

CNR-ITEM Collection: Microbial Resources For Food Bio-Economy

Giancarlo Perrone, Antonia Susca and Antonio F. Logrieco





ECCO 2019

University of Turin

12-14 June 2019

Key topics

- **ITEM – Collection**

- **Biodiversity studies' applications:**
  (from ITEM - Microbial Resource)
  - **Biodiversity of toxigenic fungi**
  - ***Penicillium salamii* in dry cured meat**
  - **Autochtonous yeast in wine and olive fermentation**
  - ***Lactobacillus:* probiotics and antagonistic strains**
  - **Fungal strains in biological control**
  - **Bioactive metabolites**

ITEM - Agro-Food Microbial Culture Collection: Structure and Services for Biodiversity Preservation

Importance of Biodiversity

Nowadays a wide spectrum of direct and indirect values has been recognized as tightly correlated to biodiversity.

Microorganisms and in particular fungi play an important role in natural ecosystems and could represent an extraordinary source of:

New compounds of great ecological relevance

- Antibiotics
- Antitumoral
- Enzymes
- Insecticides
- Fungicides
- Mycotoxin

Pharmaceutic, biotechnology, fermentation

Food spoilage, plant and human disease

Preservation

CULTURE COLLECTIONS

Store and preserve the biodiversity and viability of microorganisms, cells, plant for research community, institutions, companies, ect.

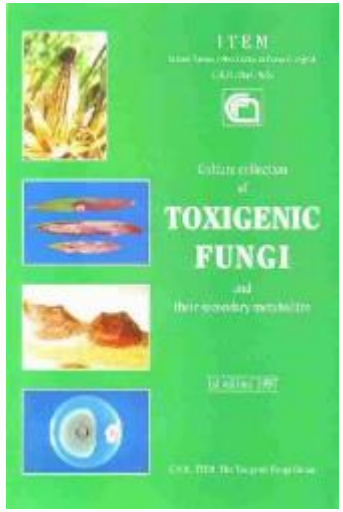
ISPA

ITEM - Agro-Food Microbial Culture Collection: Structure and Services for Biodiversity Preservation

1997 **Collection of Toxigenic Fungi,** in Institute of Toxins and Mycotoxins from plant parasites-CNR published its first printed catalogue, with about 5,000 strains

2001 **Agro-Food Microbial Culture Collection,** in Institute of Sciences of Food Production-CNR, the Collection, 12,000 strains (fungi, bacteria and yeasts)

2003 dynamic web – site was developed through the combination of PHP, Apache and MySQL www.ispa.cnr.it/Collection



2012-2015 : Involved as associated partner in the **European Project on Microbial Resource Research Infrastructure** - MIRRI (www.mirri.org/)

From 2015 Member of :

Agro-Food Microbial Culture Collection ITEM

ecco European Culture Collection Organization

WFCC World Federation for Culture Collection

In September 2017 joined within CNR the JRU MIRRI-IT

From 2018 ITEM is Certified ISO 9001:2015 35674/17/S

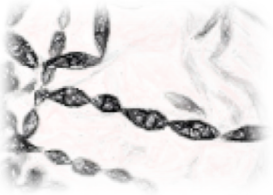
ISPA

Turin, 14 June 2019

ITEM - Agro-Food Microbial Culture Collection: Structure and Services for Biodiversity Preservation

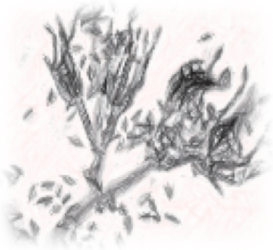
Now ITEM includes about 12,000 microbial strains (of which 7,000 are public) belonging mainly to fungal toxigenic and phytopatogenic genera:

Alternaria

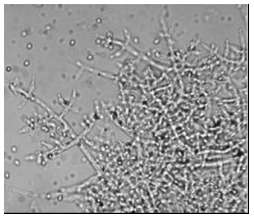


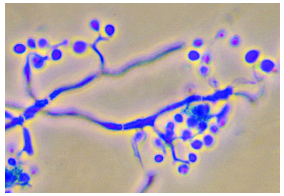
Aspergillus

Penicillium



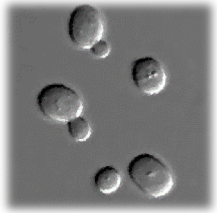
Fusarium

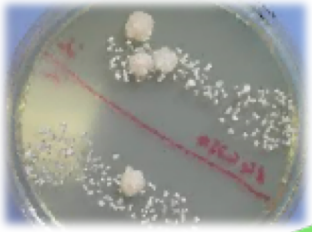




…but also fungal genera useful in biological control like *Trichoderma, Beauveria, Aerobasidium….*

…as well as yeasts (~ 300) and bacteria (~ 400) useful for biotechnological application in agro-food sector.





ITEM - Agro-Food Microbial Culture Collection: Structure and Services for Biodiversity Preservation

ITEM - STRUCTURE

ITEM – Director

Dr. Antonio F. Logrieco
Director at ISPA –CNR (Bari)

Responsible of Management of the Collection

ITEM – CURATOR

Dr. Giancarlo Perrone
Researcher at ISPA –CNR (Bari)

Scientific Responsible of the Collection

ITEM – Technical Staff

Dr. Filomena Epifani
Technician at ISPA –CNR (Bari)

Responsible for culture collection preservation and transfer. Web data-base management.

Dr. Giuseppe Cozzi
Technician at ISPA –CNR (Bari)

Responsible for administrative aspects of the collection (orders, deposition, expeditions).

ITEM – Scientific Staff

Dr. Antonio F. Logrieco
Director at ISPA –CNR (Bari)
Expert in ecophysiology and morpho-phenotypic features of toxigenic fungi; culture collection management

Dr. Antonia Susca
Researcher at ISPA –CNR (Bari)

Responsible for molecular identification and characterization of culture collection. Molecular data-base management.

Dr. Antonio Moretti
Senior research at ISPA –CNR (Bari)

Expert in ecophysiology and morpho-phenotypic features of *Fusarium* and *Alternaria* species

Dr. Giancarlo Perrone
Researcher at ISPA –CNR (Bari)

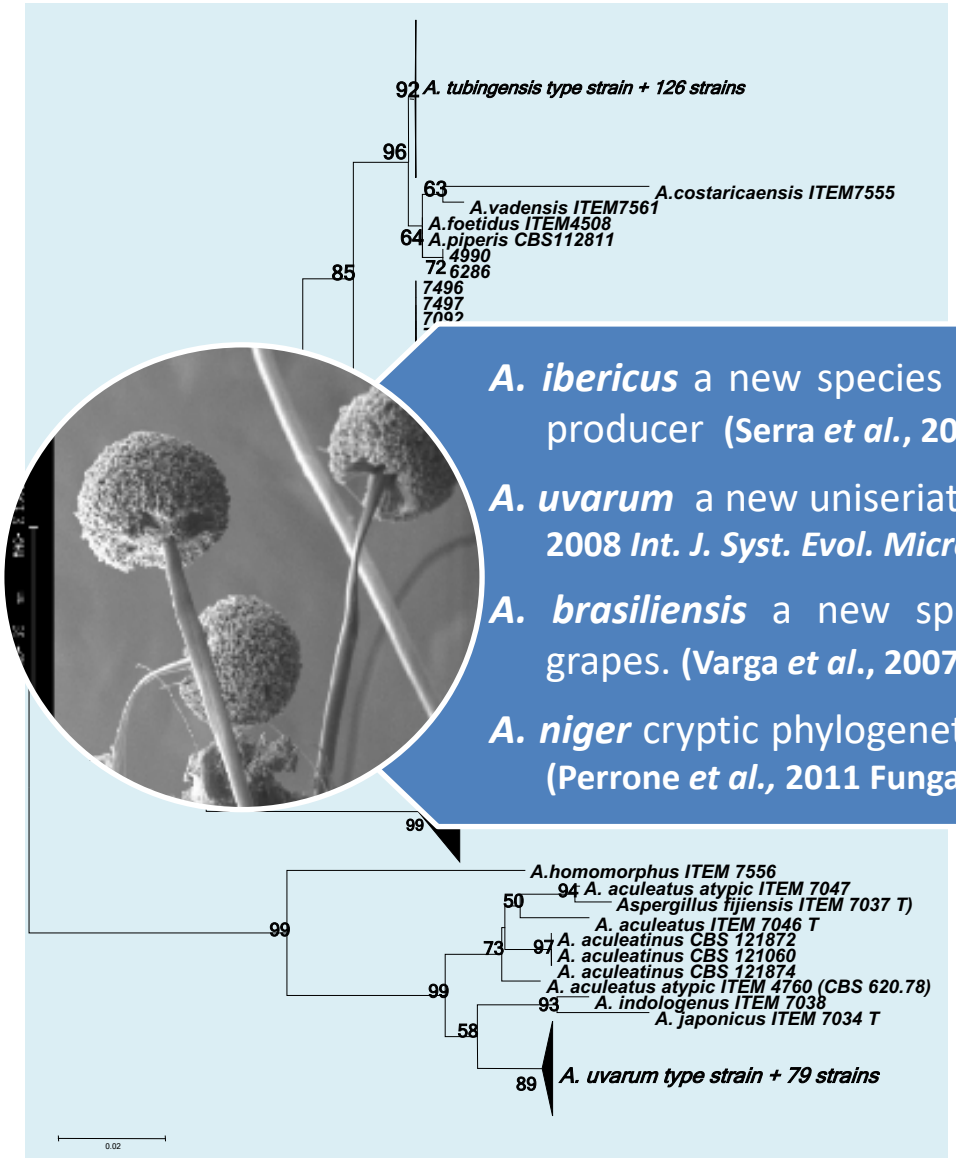
Expert in ecophysiology and morpho-phenotypic features of *Aspergillus* and *Penicillium* species

Key topics

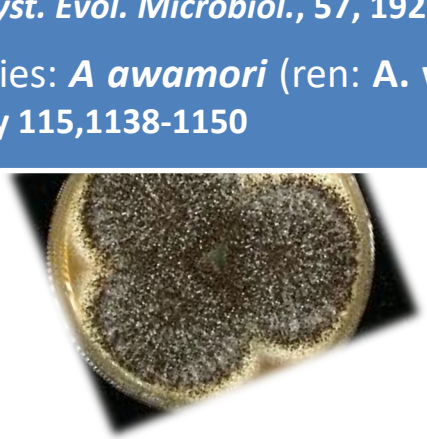
- ITEM – Collection

- **ITEM - Microbial Resource**
  - **Biodiversity of toxigenic fungi**
  - ***Penicillium salamii* in dry cured meat**
  - **Autochtonous yeast in wine and olive fermentation**
  - ***Lactobacillus:* probiotics and antagonistic strains**
  - **Fungal strains in biological control**
  - **Bioactive metabolites**

BIODIVERSITY OF TOXIGENIC FUNGI (1)



Aspergillus section *Nigri* from **grapes and raisins** world-wide

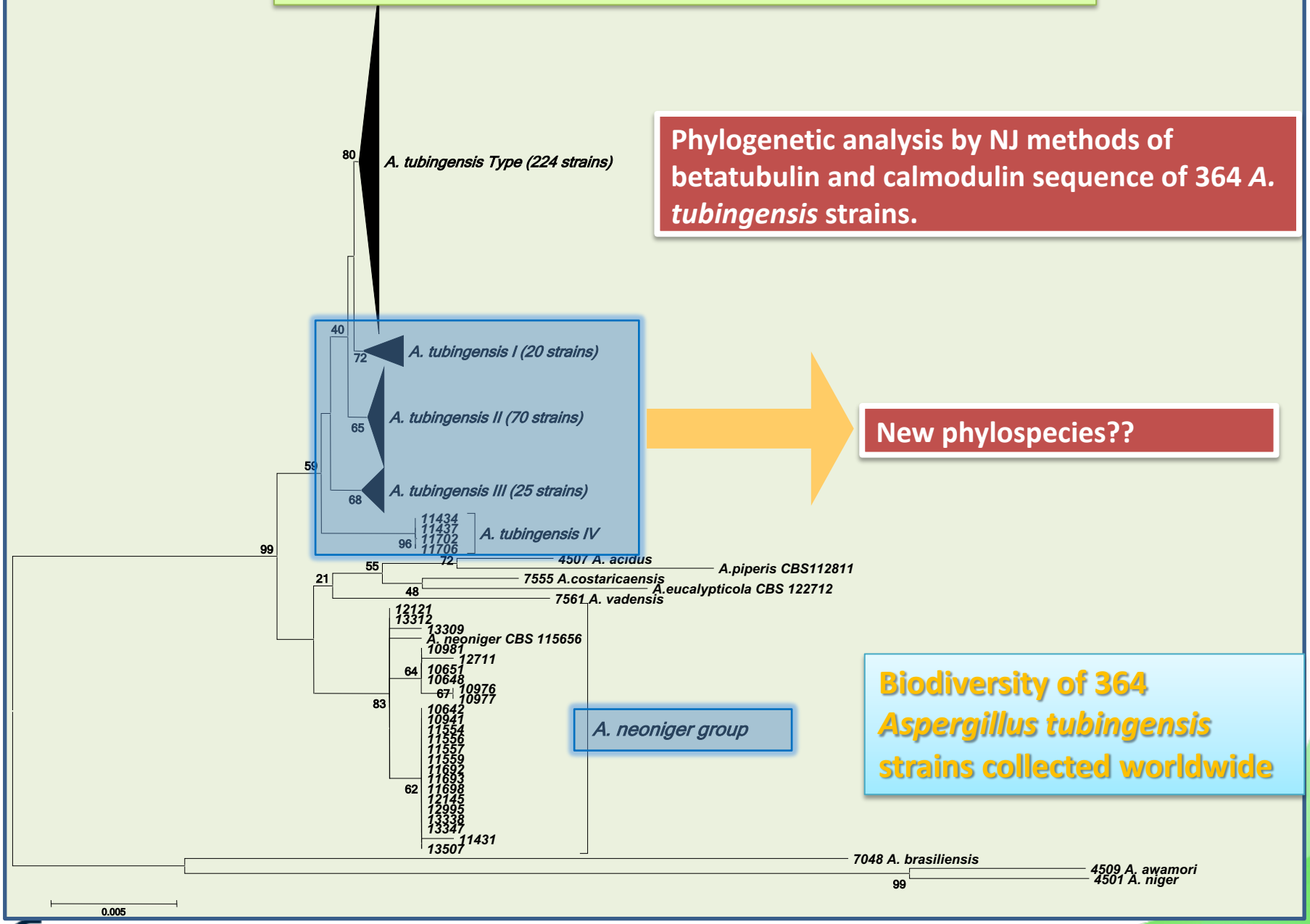


- ***A. ibericus*** a new species from grapes close to *A. carbonarius*, no OTA producer **(Serra *et al.*, 2006 *Mycologia,* 98:295-306)**
- ***A. uvarum*** a new uniseriate species from grapes in Europe **(Perrone *et al.*, 2008 *Int. J. Syst. Evol. Microbiol.*, 58, 1032-1039)**
- ***A. brasiliensis*** a new species worldwide distributed and found on grapes. **(Varga *et al.*, 2007 *Int. J. Syst. Evol. Microbiol.*, 57, 1925-1932)**
- ***A. niger*** cryptic phylogenetic species: ***A awamori*** (ren: **A. welwitschiae**) **(Perrone *et al.*, 2011 Fungal biology 115,1138-1150**

Perrone G., et al. 2007, Studies in Mycology 59: 53-66.
Susca et al. 2013, Int. J. Food Microb. 165:163-168.

ISPA

BIODIVERSITY OF TOXIGENIC FUNGI (2)



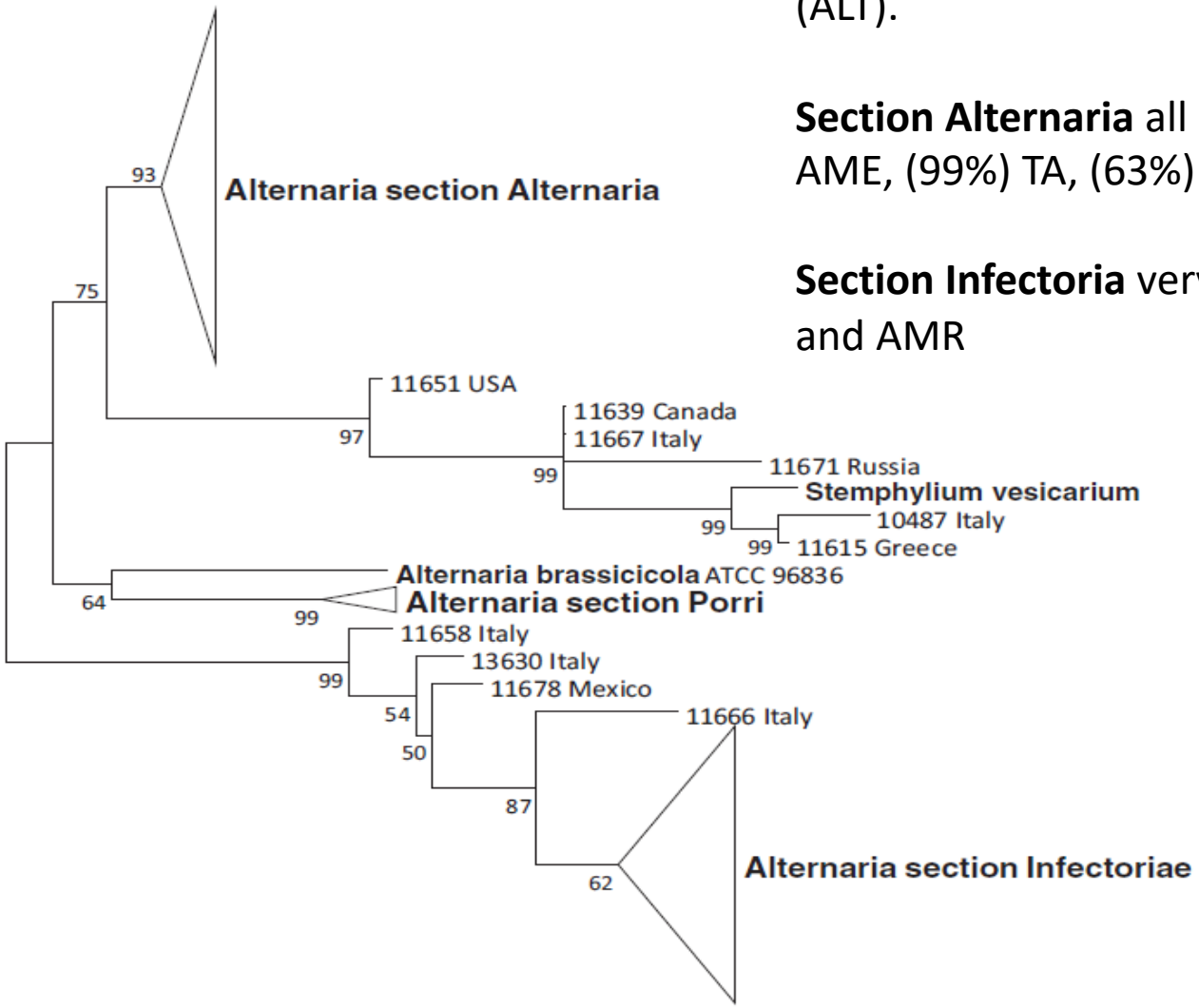
Phylogenetic analysis by NJ methods of betatubulin and calmodulin sequence of 364 *A. tubingensis* strains.

New phylospecies??

Biodiversity of 364 *Aspergillus tubingensis* strains collected worldwide

BIODIVERSITY OF TOXIGENIC FUNGI (3)

Worldwide *Alternaria* species associated to wheat black point



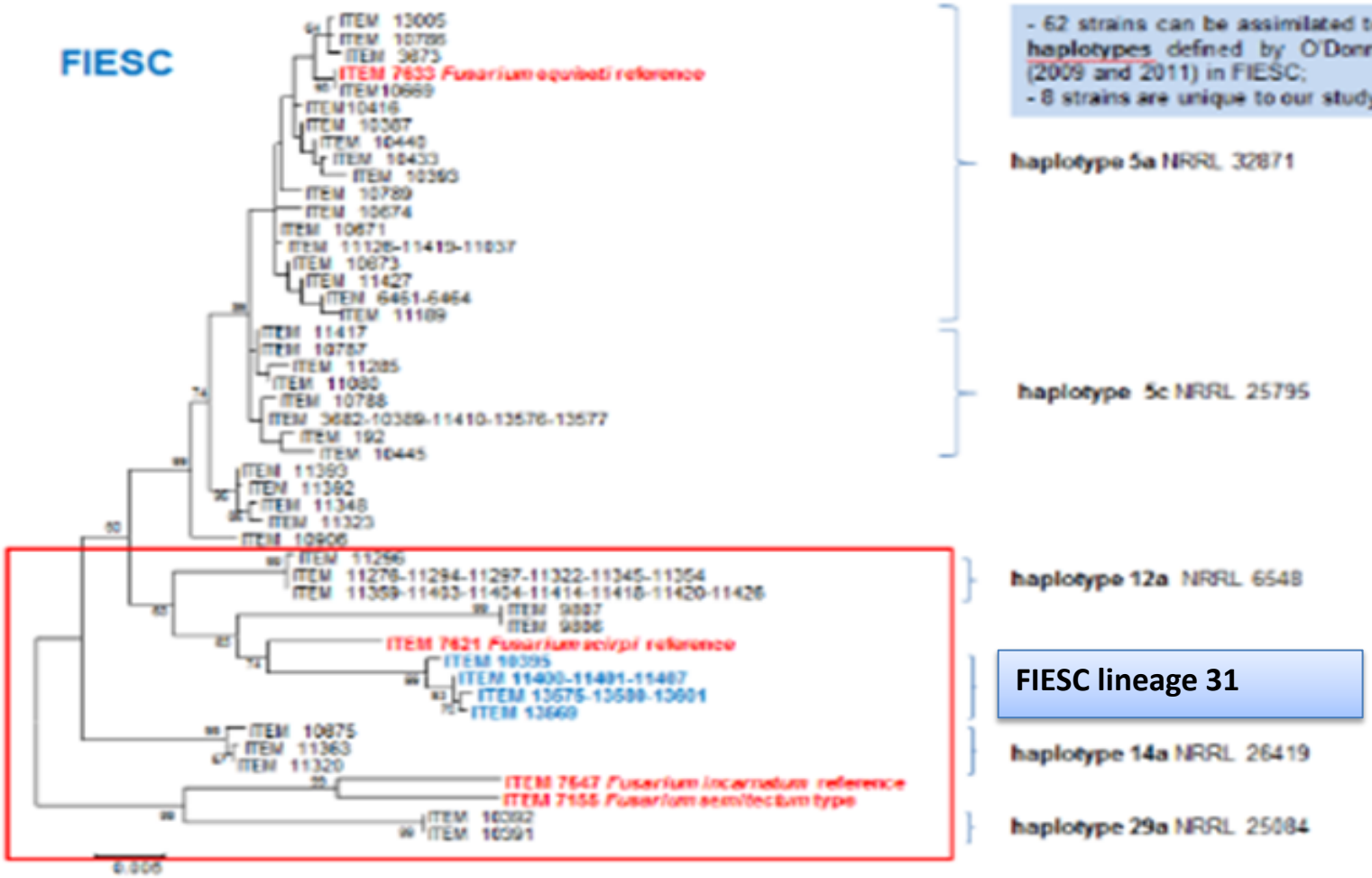
Several mycotoxins such as (TA), (AOH), (AME), (ALT).

Section Alternaria all strains produced AOH and AME, (99%) TA, (63%) ALT.

Section Infectoria very low producer of AOH and AMR

Somma et al. 2019 Int.J.Food Microbiol 293 34–43

BIODIVERSITY OF TOXIGENIC FUNGI:



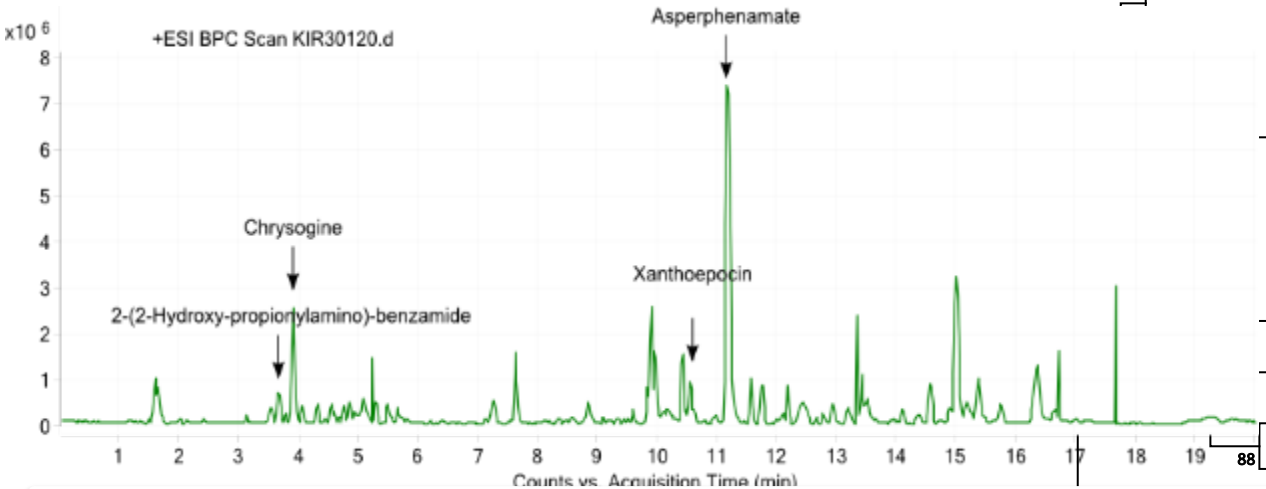
FIESC 31 produced a new chemical profile NIV in addition to DAS, NEO and FUS-X.

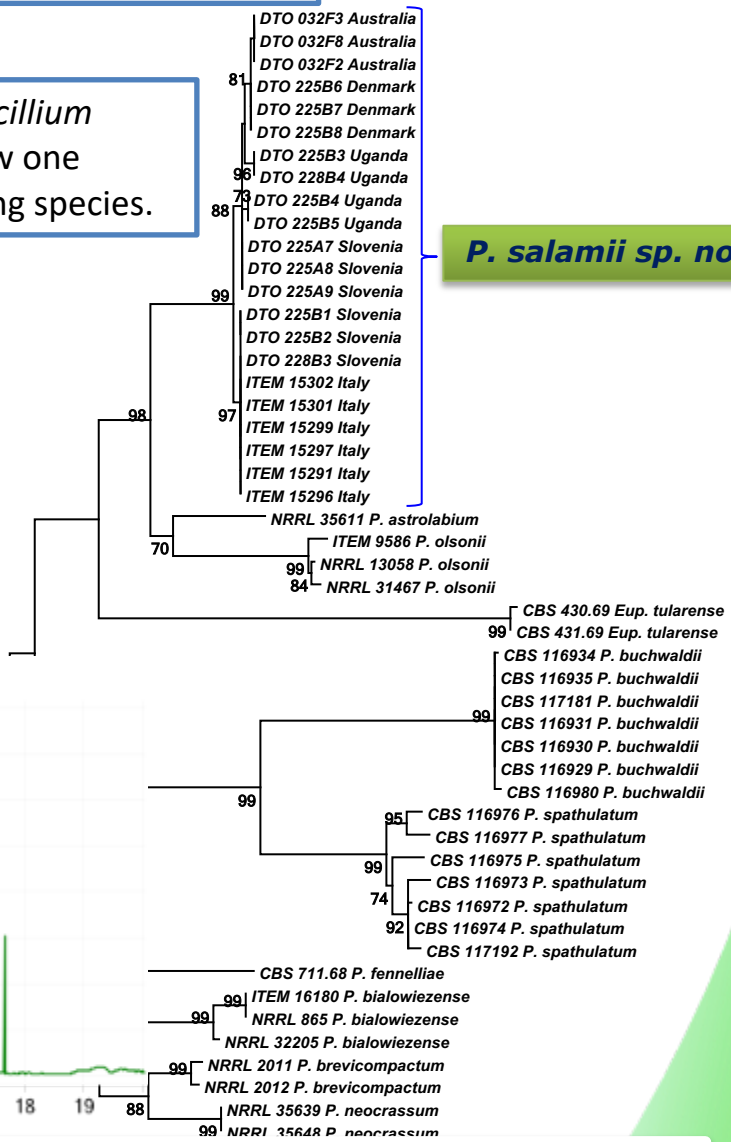
P. salamii, a new species occuring in dry-cured meat

Perrone G. et al IJFM 2015, 193:91-98.

Isolated in a survey of fungal in a salami plant (Calabria, Italy), two *Penicillium* species were predominantly present: *Penicillium nalgiovense*, and a new one similar, but distinct from, *P. olsonii*, it was identified as aa new interesting species.

Extrolites profile





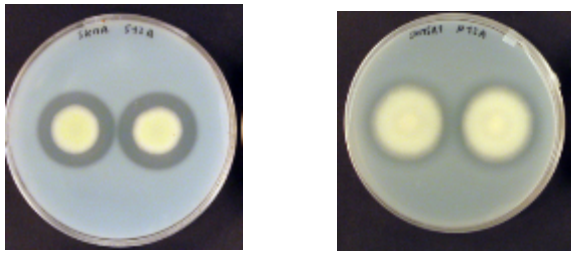
The extrolites profile of all strains of *P. salami* are clear different because they typically produced **asperphenamate, chrysogine, xanthoepocin and derivatives, and indolalkaloids.**

P. salamii as promising fungal starter

P. salamii was tested for its proteolytic activity and lipolytic activity and for sensory analysis in a lab scale and industrial scale experiment.

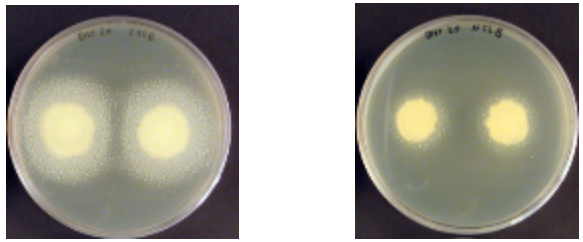
Proteolytic activity

P. salamii ITEM 15302	P. nalgiovense ITEM 15292



Lypolitic activity

P. salamii ITEM 15302	P. nalgiovense ITEM 15292



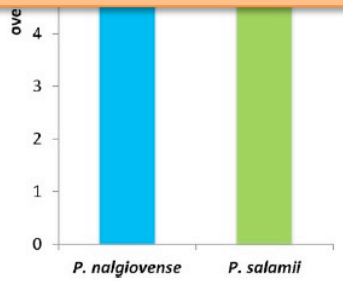
P. salamii after the publication of the scientific papers got some (digital) press:

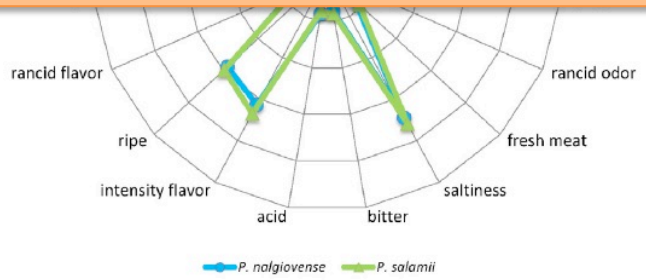
http://www.popsci.com/scientists-discover-new-fungal-species-salami

http://microbialfoods.org/science-digested-new-penicillium-species-discovered-salami/

http://www.latimes.com/food/dailydish/la-dd-scientists-new-mold-salami-20150129-story.html

http://theviewfromcullingworth.blogspot.it/2015/02/friday-fungus-penicillium-salamii.html





Magistà D. et al. IJFM 2016, 231:33–41.

ISPA

Turin, 14 June 2019

Autochthonous yeast – bacteria in olives fermentation

Because of their worldwide economic impact, table olives are the most important fermented vegetables.

The study of the microbial dynamics during fermentations of different olive cultivars brings to characterize moulds, yeasts and LAB associated to the fermentation processes

Yeasts and LAB activities was described by analysis of sugars, organic acids, alcohols profiles, mono and polyphenols profiles, sensory analysis of olives and brines.

Selected yeast and LAB has been used as starters to inoculate pilot-scale fermentation of olives.

Photos from Dr. Bleve
ISPA-Lecce

Autochtonous yeast – bacteria in olives fermentation



Fermented table olives with improved organoleptic characteristics, longer shelf-life, produced by drastically reduced time of fermentation, maintaining or enhancing nutritional traits.

YEAST and BACTERIA identification
[Patent MI 2013A002063]
European Deposit Application [Nr. 14197402.2.]

DSMZ Collection as Patent Deposit:

- *Saccharomyces cerevisiae* DSMZ 27800 for Leccino table olives
- *Lactobacillus plantarum* DSMZ 27925 for Leccino table olives
- *Saccharomyces cerevisiae* DSMZ 27801 for Kalamata table olives
- *Leuconostoc mesenteroides* DSMZ 27926 for Kalamata table olives

(Deposited as no public in the ITEM collection)

Bleve G., Tufariello M., Durante M., Perbellini E., Mita G., Ramires A.F., Grieco F., Logrieco A.F. Metodo per la produzione di olive da tavola fermentate. Brevetto MI 2013A002063. Dep. 11/12/2013. European Patent Deposit Nr. 14197402.

- **Bleve et al. (2015).** Food Microbiology, 46:368-382
- **Bleve et al. (2014)**. Frontiers in Microbiology 5:570.
- **Tufariello et al. (2015)**. Frontiers in Microbiology, 6:1007.

ISPA

Autochthonous yeast fermentation to valorize regional wines properties

Wine productions are typical because they are based on selected varieties of grapes and a peculiar fermentation process that determines the quality of the wine.

Recently the research activity of CNR-ISPA has been directed to the exploitation of autochthonous microbiota to enhance the quality of regional wines.

Natural fermentations of Negroamaro, Primitivo and Susumaniello musts allowed the identification of four indigenous *S. cerevisiae* strains candidate as autochthonous fermentation starters.

920 yeast strains
325 bacteria from malolactic fermentation

ISPA

Autochthonous yeast fermentation to valorize regional wines properties

The enological properties of the above strains have been evaluated during the vintages 2006-2012, by performing more than 40 large scale vinification trials in 21 different industrial cellars in Apulia.

The selected strains demonstrated that they were always able to dominate the fermentation process and to produce a final product characterized by excellent oenological and organoleptic features.

ITEM 6993 and 6920 selected for Negroamaro wine production

ITEM 9502 and 9520 selected for Susumaniello wine production

- **Grieco F. et al (2011).** Exploitation of autochthonous micro-organism potential to enhance the quality of Apulian wine. Annals of Microbiology 61, 67-73.
- **Tristezza M. et al. (2012).** Autochthonous fermentation starters for the industrial production of Negroamaro wines. Journal of Industrial Microbiology & Biotechnology 39, 81-92.
- **Tristezza M., et al. (2014).** Molecular and technological characterization of *Saccharomyces cerevisiae* strains isolated from natural fermentation of Susumaniello grape must in Apulia, Southern Italy. International Journal of Microbiology, Volume 2014, Article ID 897428, 11 pages,
- **Tufariello M. et al. (2014).** Influence of autochthonous *Saccharomyces cerevisiae* strains on volatile profile of Negroamaro wines. LWT - Food Science and Technology, 58, 35–48

Probiotics *Lactobacillus* strains

MICROBIAL BIOTECHNOLOGY to realize non conventional ready-to-eat probiotic vegetables and fish fillets for a *functional diet*

Biotechnology

Conventional Foods → Functional foods

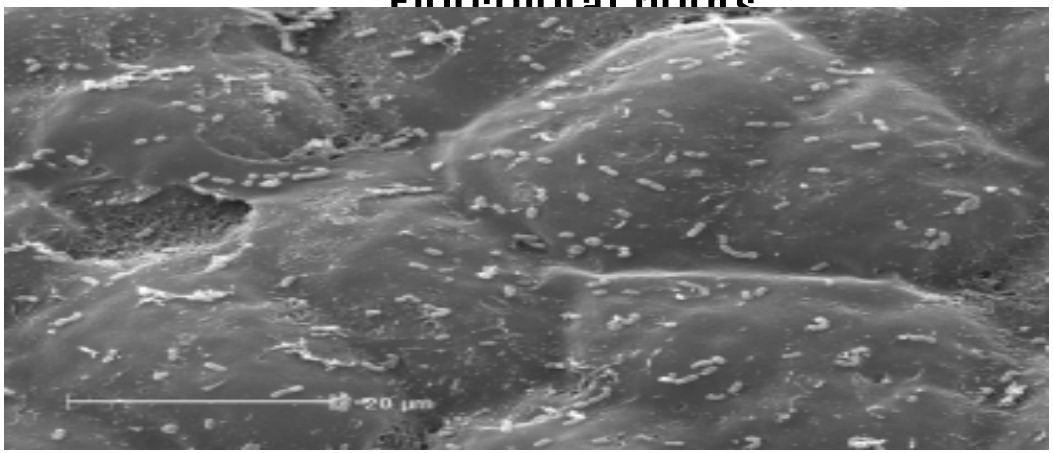


Table olive surface carrying live populations of
LACTOBACILLUS PARACASEI ITEM 17146 (IMPC2.1; **EU Patent B1**).
A portion of olives or artichokes or cabbage or fish fillet can carry more than **1 BILLION LIVE AND ACTIVE** cells, amount comparable or greater than those of milk- based products

Nutritional Trials demonstrated ***gut microbiota manipulation***

Approval by the Italian Ministry of Health

Riezzo, …Lavermicocca et al. 2013. Alimentary, Pharmacology & Therapeutics 35:441
Orlando A., Lavermicocca, et al. 2012 Nutrition and Cancer, 64 : 1103.
De Bellis et al. 2010. Int J Food Microbiol. 140:6-13.
Valerio et al. 2015. J Functional Foods, 17, 468–475
Lavermicocca et al. 2015. Bioactive Foods in Health Promotion. In: Probiotics, Prebiotics, and Synbiotics: Eds Watson & Preedy,Elsevier
Sarvan et al. 2013. Food Res Int, 54, 706–710.0

ISPA

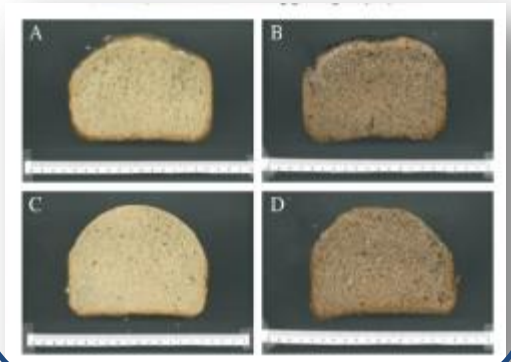
Turin, 14 June 2019

Bacteria strains in bread fermentation

MICROBIAL BIOTECHNOLOGY for process and product innovation

Application of **L. brevis ITEM 17147** (18F)and **L. plantarum ITEM 17148** (21B) bioingredients in bread-making process (lab-scale and industrial-scale)

- Improves the **technological properties** of bread
- Delays the **bread staling**



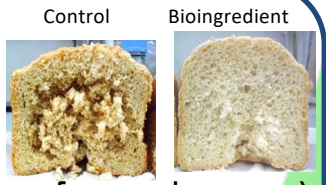
Photos from Dr. Lavermicocca ISPA-Bari

Improves the **health properties**:

- *Free-yeast bread "*
- *Low-salt bread* (Salt reduction **by70%**)

Control Bioingredient

Improves the **microbiological quality**:



- Reduces the "bread rope" (caused by sporeformer bacteria)
- Delays the bread contamination by moulds

De Bellis P. Use of a selected Leuconostoccitreum strain as a starter for making a "yeast-free" bread. Foods, 2019, 8(2), 70

Lavermicocca et al. 2016. Spore-forming bacteria associated with bread production: spoilage and toxigenic potential (Chapter 5). In: Food Hygiene and Toxicology in Ready to Eat Foods, (Ed. P. Kotzekidou) Elsevier

De Bellis et al. 2015. Int J Food Microbiol 197, 30–39

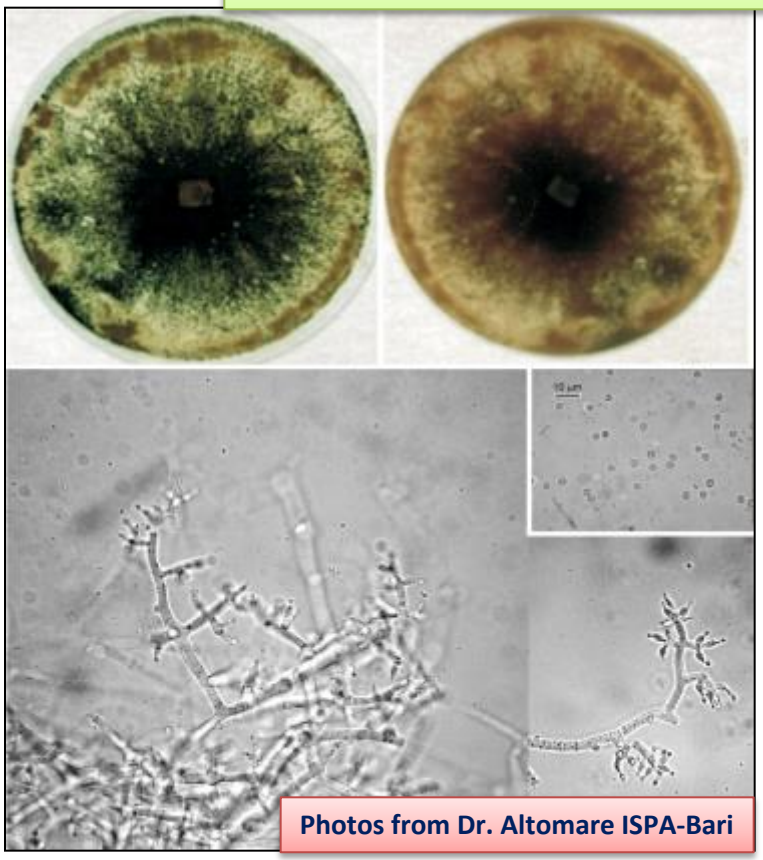
Valerio et al 2015 Food Microbiology 197: 30

Di Biase et al. 2014. It J Agron. Vol 9 (614): 146-151.

Valerio et al. 2014. Inn Food Sci Emerg Technol. 25: 2

ISPA

Antagonistic strains in biological control



Photos from Dr. Altomare ISPA-Bari

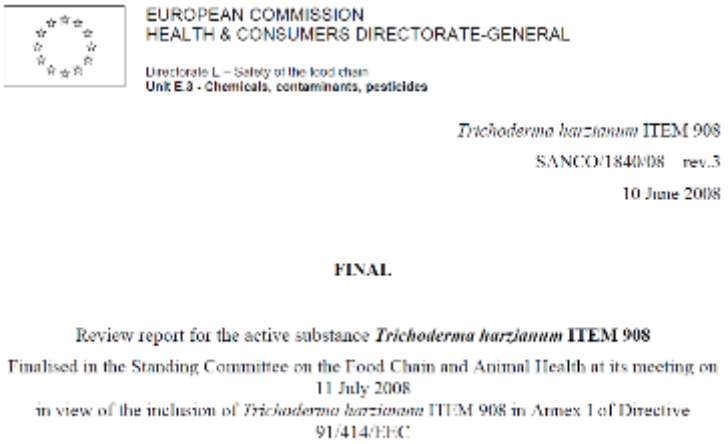
Trichoderma harzianum Rifai ITEM 908

Effective biocontrol agent of crown, stem and root rot diseases caused by *Rhizoctonia*, *Sclerotinia* and *Pythium* in tomato and other vegetable crops

Included in Annex I of the EU Directive 91/414/CEE concerning the placing of plant protection products on the market

It has been developed into a commercial biopesticide by an Italian private company under a license from ISPA.

Marzano M, et al. 2013. Improvement of biocontrol efficacy of *Trichoderma harzianum* vs. *Fusarium oxysporum* f. sp. *lycopersici* through UV-induced tolerance to fusaric acid. Biological Control 67, 397–408



EUROPEAN COMMISSION
HEALTH & CONSUMERS DIRECTORATE-GENERAL

Directorate E – Safety of the food chain
Unit E.3 - Chemicals, contaminants, pesticides

Trichoderma harzianum ITEM 908
SANCO/1840/08 rev.3
10 June 2008

FINAL

Review report for the active substance *Trichoderma harzianum* ITEM 908

Finalised in the Standing Committee on the Food Chain and Animal Health at its meeting on 11 July 2008
in view of the inclusion of *Trichoderma harzianum* ITEM 908 in Annex I of Directive 91/414/EEC

+ ITEM 908 **Control**

MICOVER® KELIDOR® RADIFORCE®

Bioactive metabolites

Toxins produced by *Sphaeropsidales* studied by our groups

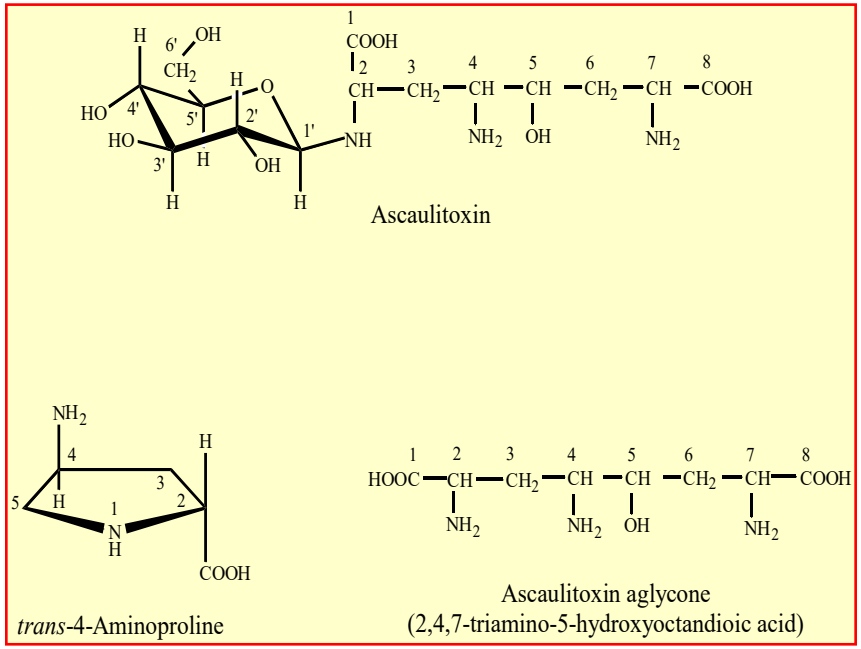
Pathogen	ITEM	Host	Toxin
Ascochyta agropyrina	*12530*	*Elitrigia repens*	agropyrenol
Ascochyta caulina	*1058*	*Chenopodium album*	ascaulitoxin; aglycone; aminoproline
Ascochyta pinodes	*1059*	*Pisum sativum*	pinolidoxins
Ascochyta pisi	*1050*	*Pisum sativum*	ascosalitoxin
Ascochyta sonchi	*6217*	*Sonchus arvensis*	ascosonchine
Phoma exigua var. *het.*	*330*	*Nerium oleander*	cytochalasins A, B, T, U, W, Z,
Phoma putaminum	*2472*	*Erigeron annuus*	putaminoxins
Phomopsis sp.	*13496*	*Carthamus lanatus*	phomentrioloxin
Phyllosticta cirsii	*8964*	*Cirsium arvense*	phyllostictines A-D; phyllostoxin

Bioactive metabolites

Ascaulitoxin, trans-aminoproline and ascaulitoxin aglycone three fungal metabolites with herbicidal properties from *Ascochyta caulina*

ITEM 1058 – CBS 344.78

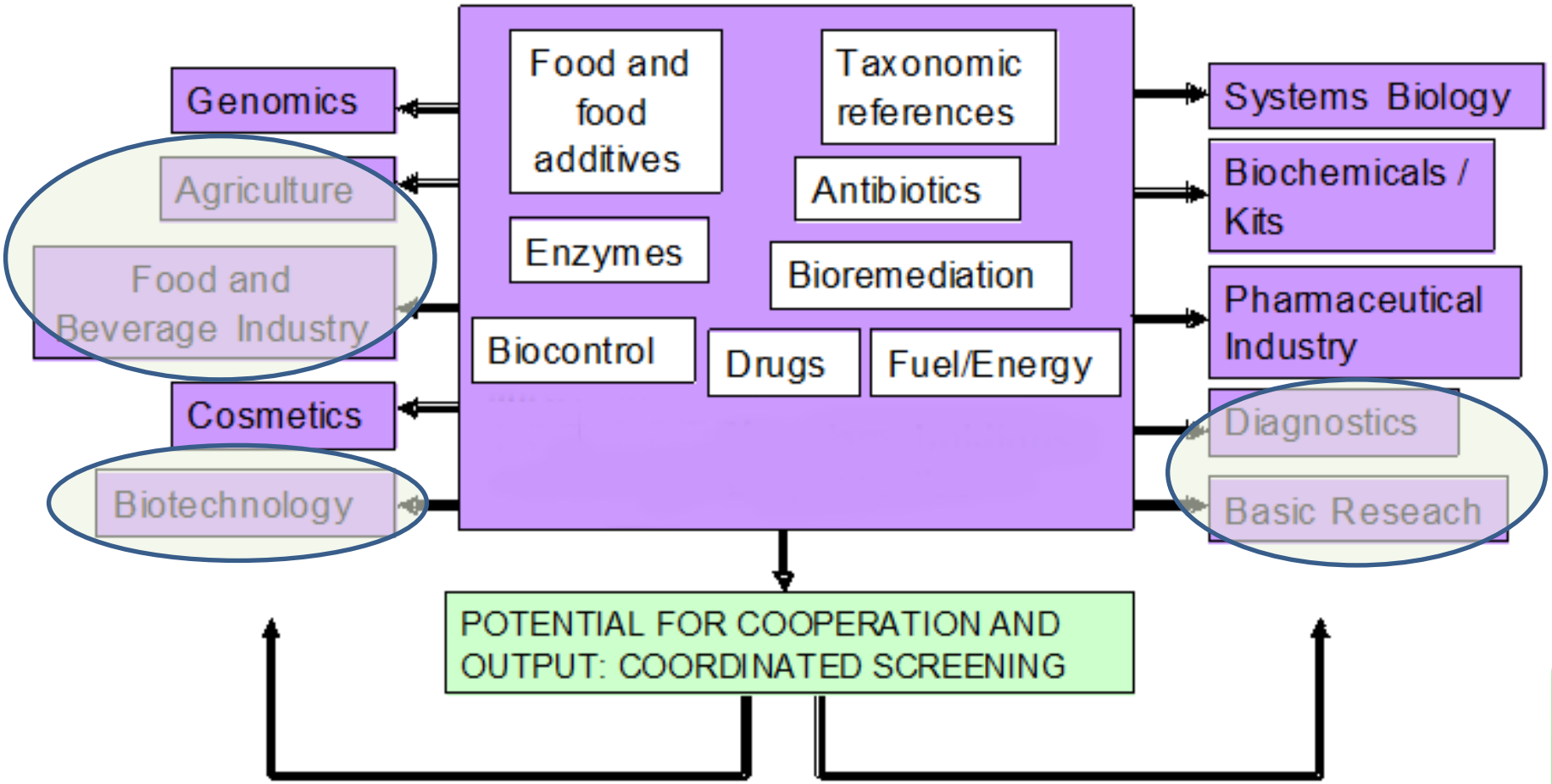
(**Vurro M.** et al, 2012 Biological Control 60 (2) 192–198)



Conyza canadensis

Photos from Dr. Vurro ISPA-Bari

Examples for Uses and Applications of Microorganisms



Bari - City

ISPA - CNR

THANKS FOR YOUR ATTENTION