EUROPEAN MICROBIAL COLLECTIONS

- a high added value for science -

Cantacuzino Institute, Bucharest, Romania, 8-9 March 2010

Selected Success Stories of Collection Exploitation

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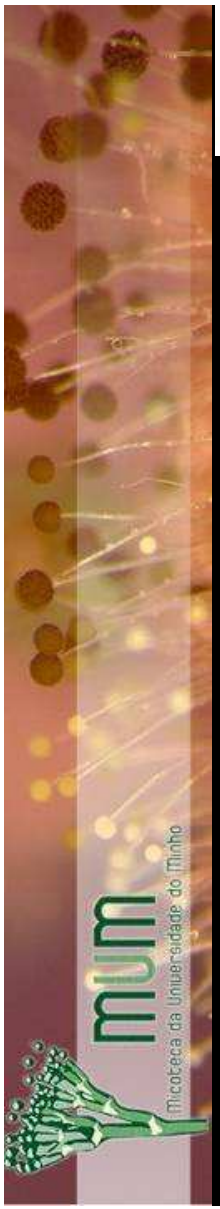

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mum
Micoteca da Universidade do Minho

MUM – A Fungal Culture Collection

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MUM is a filamentous fungal culture collection which was established in 1996. The purpose is to maintain and provide strains for research in biotechnology and in teaching laboratories, and to act as a centre of expertise, information and training complying with international quality standards.

Port Wine
Table Wine

The value generated by Portuguese wine exports represents:

1.0% of GDP

3.2% of export market share

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Ochratoxin A (OTA) was first detected as a wine contaminant in 1996 and the role of *Aspergillus* section *Nigri* in OTA production discovered in Europe in 1999.

2 µg/kg ochratoxin A (OTA) is the maximum limit in wine according the UE regulation 2005.

The latitude of production is an important factor in determining risk from OTA wine contamination. Some geographic regions in Southern Europe, like Portugal, are more prone to contamination with OTA.

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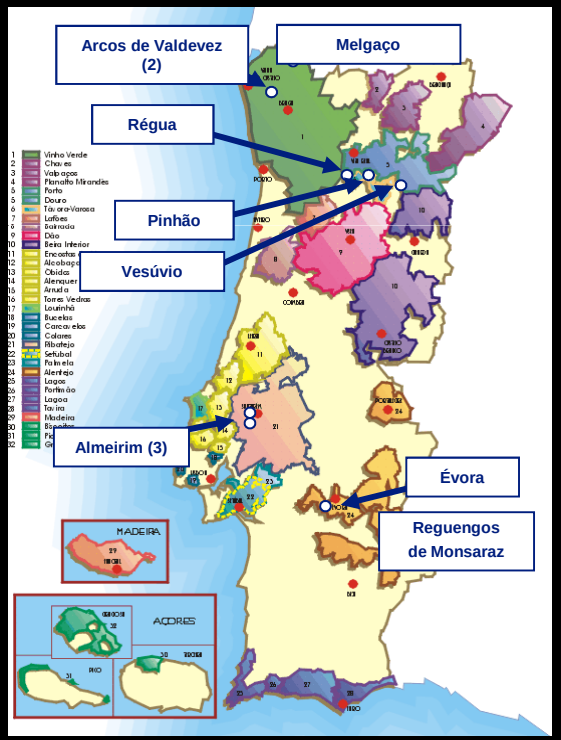
4 Wine Regions



Fungi Isolates

4450 grape berries studied

11138 fungal strains isolated

39 Genera found in the berries

56% of berries with *Cladosporium*

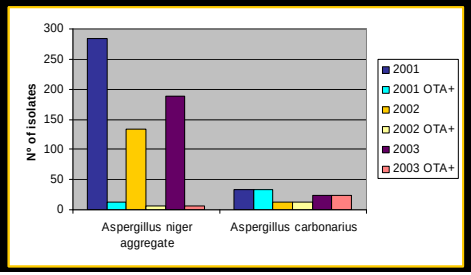
52% of berries with *Alternaria*

35% of berries with *Botrytis*

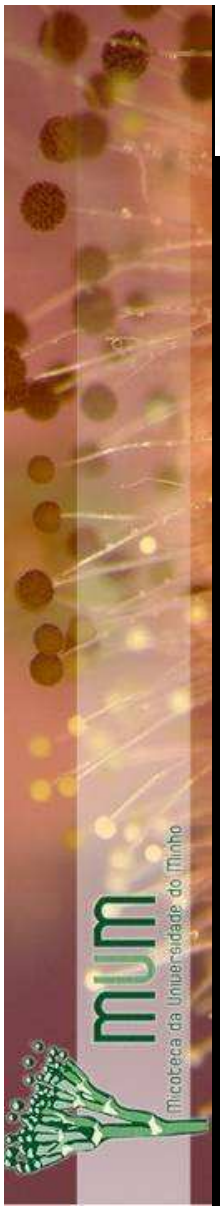
23% of berries with *Penicillium*

17% of berries with *Aspergillus*

Aspergillus niger 5% OTA^{+}



GRAS ??



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Aspergillus SECTION *Nigri* IDENTIFICATION USING POLYPHASIC APPROACH INCLUDING MALDI-TOF

(Matrix Assisted Laser Desorption Ionization – Time of Flight)

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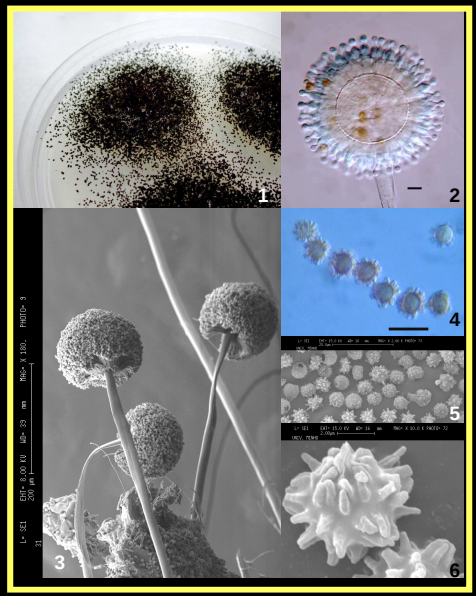
Species of the *Aspergillus* section *Nigri* have been extensively used for various biotechnological purposes and are among the fungi best studied causing biodeterioration of commodities and food spoilage.

Recently, *Aspergillus ibericus* was described as a new species in the section. This new species was not only separated from their relatives in the section by morphological distinction but also from molecular point of view: briefly, *A. ibericus* among other morphological differences has 5-7 µm conidia size which allows separate this *taxon* from *A. carbonarius* (7-9 µm) and *A. niger* and its aggregate species (3-5 µm).

Aspergillus ibericus

1. Colony grown in CZ (9 days).
2. Biseriate aspergilli of a 4 days old culture in CZ (bar = 10 µm).
3. Aspergilli at SEM (bar = 200 µm).
4. Conidia seen at Nomarski microscope (bar = 10 µm).
5. SEM picture of the conidia with variable ornamentation at different maturation stages (bar = 20 µm).
6. SEM picture of a mature conidium (bar = 2 µm).



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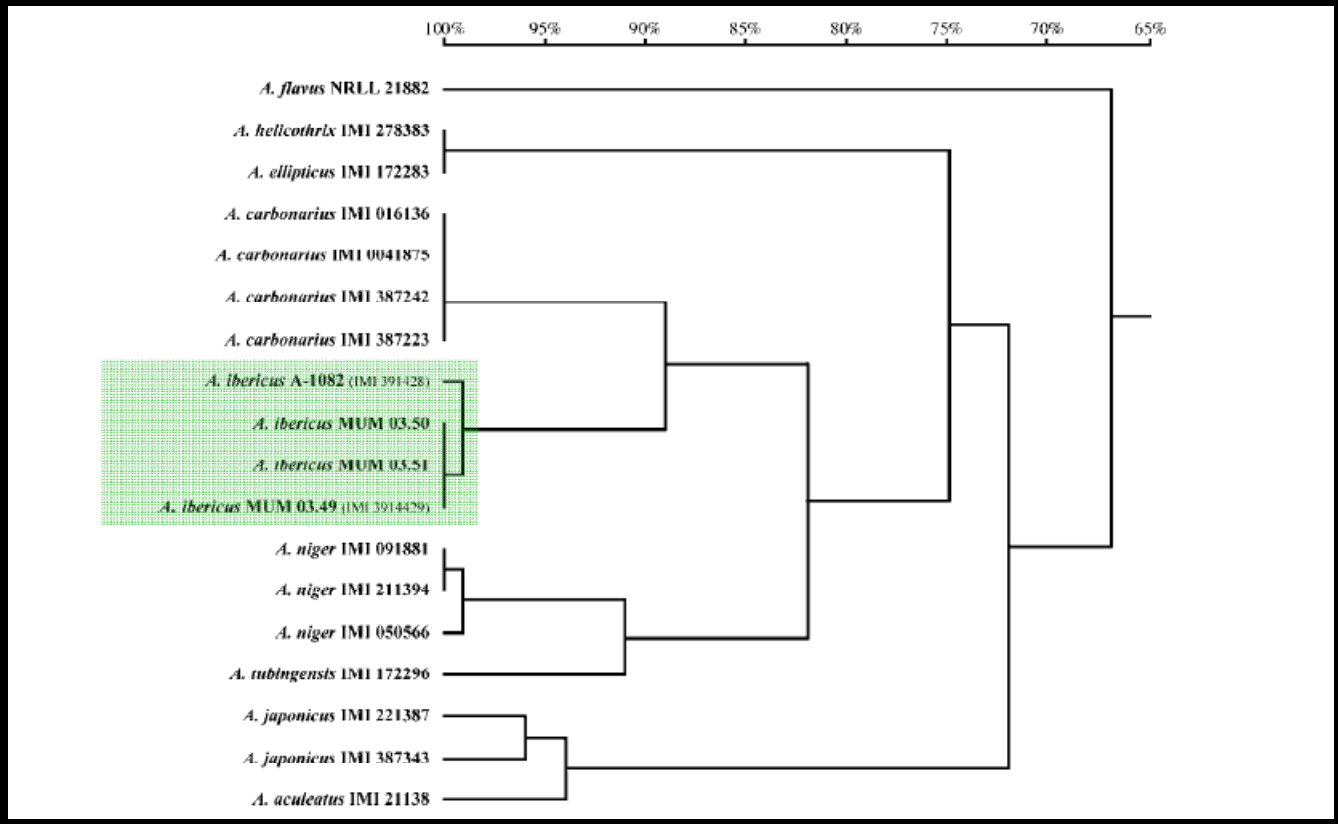
Homology tree obtained by comparison of partial calmodulin gene sequences. The dendrogram obtained clearly separated the four atypical strains (*A. ibericus*) from *Aspergillus carbonarius* strains and also from other closely related species.



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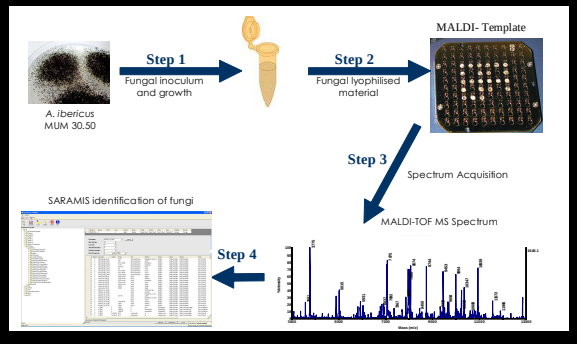
List of strains used for MALDI-TOF Mass Spectrometry analysis.

Species	Isolate number	Geographical origin	Source
A. ibericus	MUM 03.49 (IMI 391429, ITEM 4776) (T)	Portugal	Wine grapes
	MUM 03.50 (IMI 391430, ITEM 6601)	Portugal	Wine grapes
	MUM 03.51 (IMI 39143,1 ITEM 6602)	Portugal	Wine grapes
A. carbonarius	MUM 03.06 (IMI 016136, NRRL 369) (T)	Unknown	Paper
	MUM 05.18 (IMI 387223)	Portugal	Wine grapes
	MUM 03.59 (IMI 387242)	Portugal	Wine must
A. niger	MUM 03.01 (IMI 050566, NRRL 326) (T)	USA	Tannin-gallic acid fermentation
	MUM 03.57 (molecular pattern N)	Portugal	Wine grapes
	MUM 05.13 (molecular pattern T)	Portugal	Wine grapes
A. sclerotioniger	MUM 06.151 (CBS 115572) (T)	India	Arabic coffee, green
A. lacticoffeatus	MUM 06.150 (CBS 101883) (T)	Indonesia	Coffee robusta, surface sterlized beans
A. tubingensis	MUM 06.152 (CBS 134.48) (T)	Unknown	Unknown
A. vadensis	MUM 06.153 (CBS 113365) (T)	Unknown	Dead plant tissue
A. ellipticus	MUM 03.12 (IMI 172283, NRRL 5120) (T)	Costa Rica	Soil
A. japonicus	MUM 03.02 (ATCC 1042) (T)	Puorto Rico	Soil
A. aculeatus	MUM 03.11 (IMI 211388) (T)	Unknown	Tropical soil
A. phoenicis	MUM 03.05 (<NRRL 365)	Unknown	Unknown
A. flavus (outgroup)	MUM 00.06	Portugal	Cheese repining chamber

(T) Type strain.



1. Isolation of fungi, mycelium growth
2. Lyophilised mycelium, addition of matrix solution, transfer the material onto the MALDI sample plate
3. Air drying and transfer into the MALDI-TOF mass spectrometer and MALDI-TOF MS measurement
4. Editing of spectra (baseline correction, smoothing, peak detection), export of peak lists and import of peak lists to SARAMIS software
5. Automated identification / archiving / data storage / dendrogram calculation / search and comparison routines

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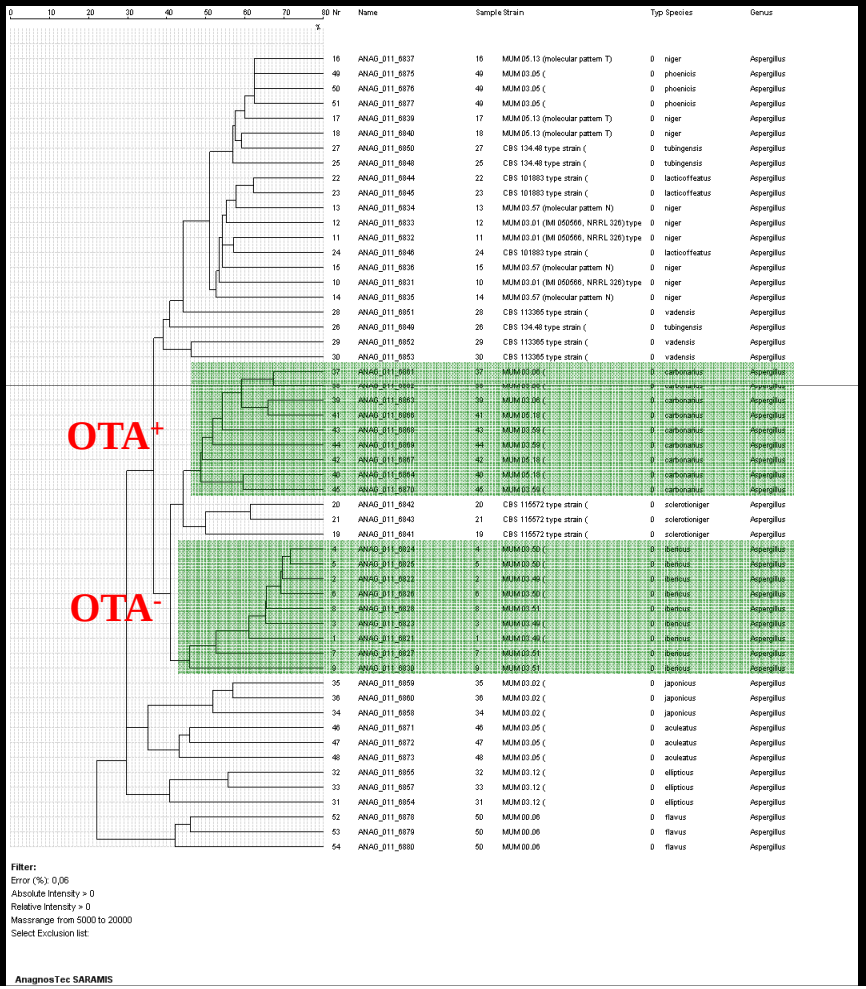

Dendrogram of relatedness between members of section *Nigri* based on MALDI-TOF MS analysis.

Success story

- Results of MALDI-TOF Mass Spectrometry analysis using mass range from 5000 – 20000 Da were similar to those of phylogenetic analysis giving a sound input for *A. ibericus* characterisation and showing the potentialities of this new method for taxonomic purposes
- To perform this study was necessary use related well characterised species deposit in different collections in order to compare their traits giving continuity to the taxonomic studies
- To have an informed decision and a right food risk assessment is absolutely necessary that the contaminants are correctly identified to the species level

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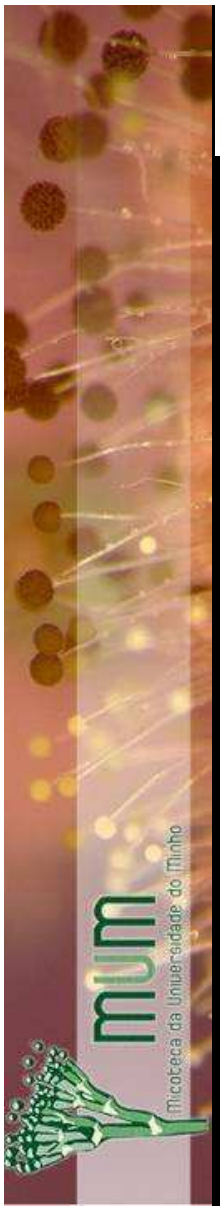

www.cabi.org

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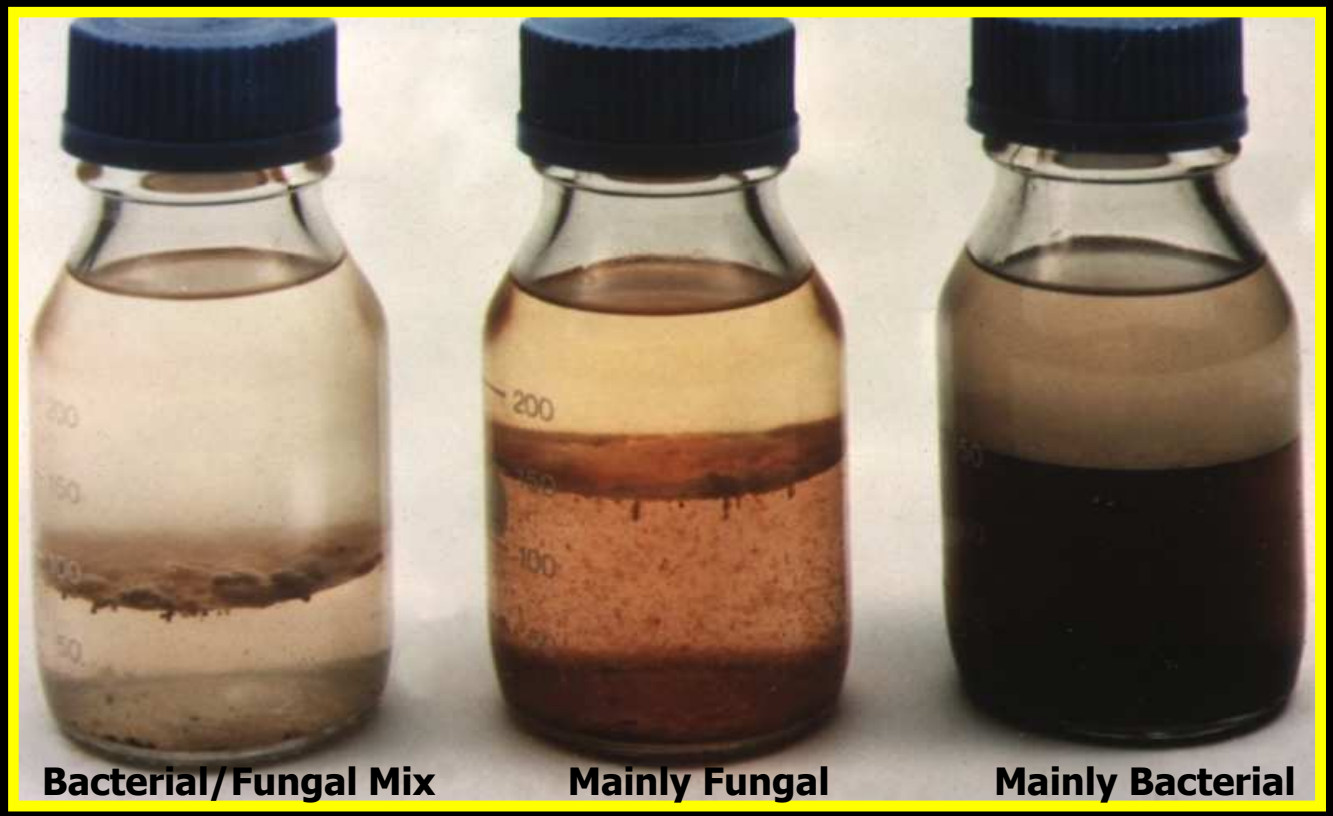
Solving problems: Microbial Fuel Contaminants



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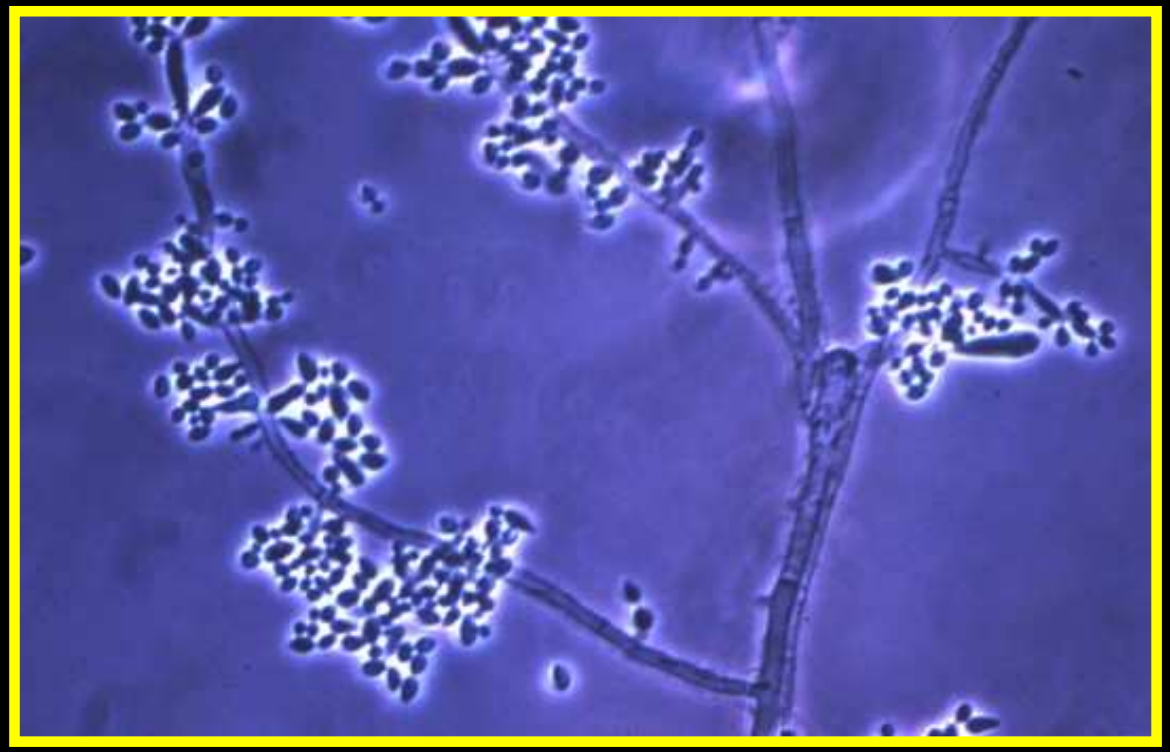
The fungal threat *Hormoconis resinae*



The solution: FUELSTAT™ resinae Detection Kit

Success story

- Isolates collected and stored from the 1960's
- Nan Onions asked to investigate
- Industrial laboratory established at CABI 1982
- Joan Kelley investigated detection kits to reduce time on the ground for aircraft while the fungus was detected via growth tests
- Fuelstat developed taking 10 minutes to determine if any fuel contamination and to what degree

The problem

- Desert locusts can invade 20% of the world land surface
- Their swarms can cover more than 100 km²

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Ultra-Low Volume (spraying) application of Green Muscle

Biopesticide based on spores of a naturally occurring entomopathogenic fungus *Metarhizium* in an oil formulation

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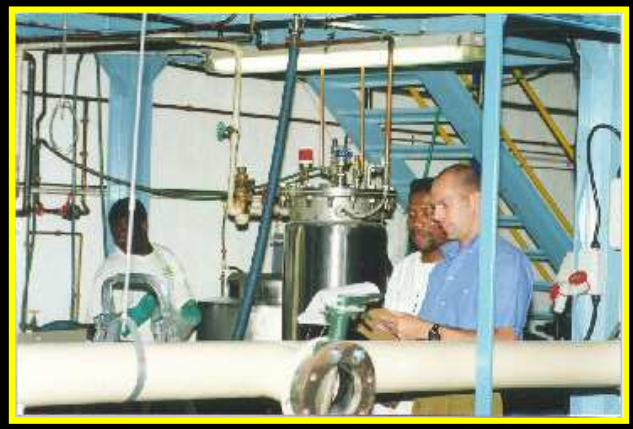

Biological Control Products SA (Pty) Ltd

PO Box 15132, Ashwood
South Africa 3605
Phone: +27 31 7004825
Fax: +27 31 7001338
mailto:info@biocontrol.co.za

Success story

- Partners got together to develop a control for African locust
- Fungal isolates examined from collections
- Most appropriate fungus selected and spray formulations developed
- The product “Green Muscle” seeing extended use in other areas of Africa and now Europe
- Profits from sale go into African Diversity Fund to fund African Biodiversity projects

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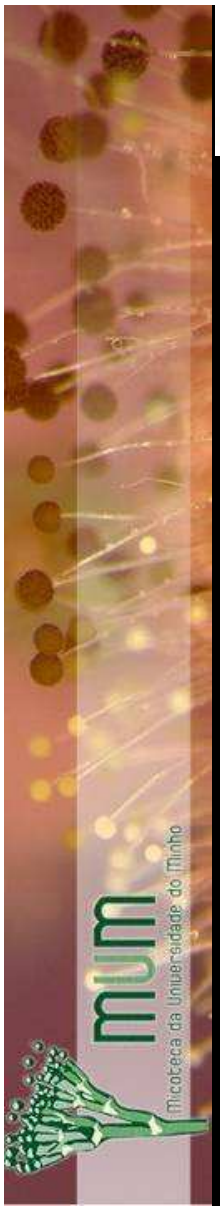

CAPACITIES

EMbaRC

INRA

CIRM
Centre International de Ressources Microbiennes

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Collecting biodiversity in tropical area

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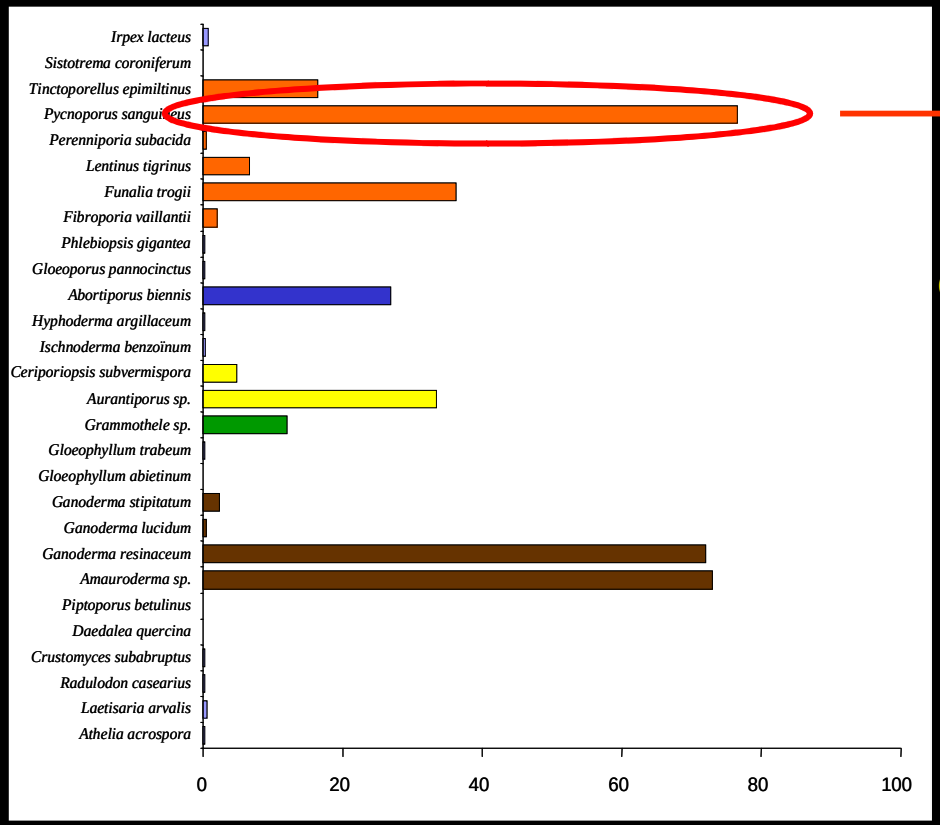
Screening of tropical species of basidiomycetes order Polyporales



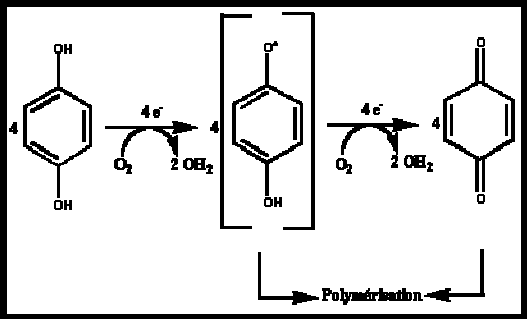
Pycnoporus sanguineus

Laccase

(Lignin oxidoreductase)

Using ABTS as chromogenic substrate

(2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)

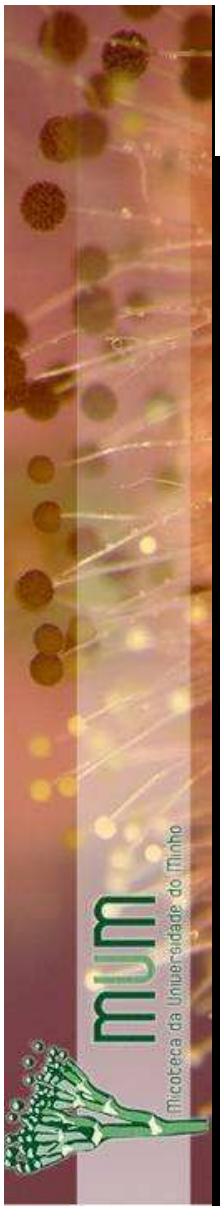


- Laccase purification
- Laccase characterization

Success story

- **New biochemical and biotechnological laccase properties**
- - High thermostability
- - High pH stability
- - Resistance to alcoholic solvents
- - Degradation of polyphenolic dyes
- - Oxidation of non-phenolic lignin model compounds (i.e.veratrylic alcohol)

Lesage-Meessen et al. (2008) 4th European Oxizymes Meeting 16-18 June, Helsinki
Uzan et al. (2010). Journal of Applied Microbiology (in press)



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BACTÉRIES D'INTÉRÊT ALIMENTAIRE

•Research programmes supported by French National Research Agency

ANR

Some examples of valorization for food related bacteria

Strategy:

Screening a collection of strains of food related bacteria the most diverse possible in terms of biotope and geographic origin.

Development of High throughput screening methods using specific equipment

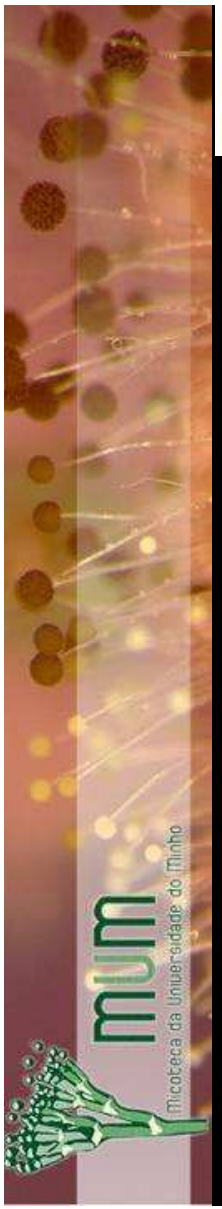
Success stories

Improved preservation of fermented dairy products

- development of antifungal bacterial cultures (bioprotective)

Non-antibiotic strategies against pathogenic bacteria

- exploration of inhibitory capabilities of natural ecosystems against contamination by *S. aureus* in dairy environment



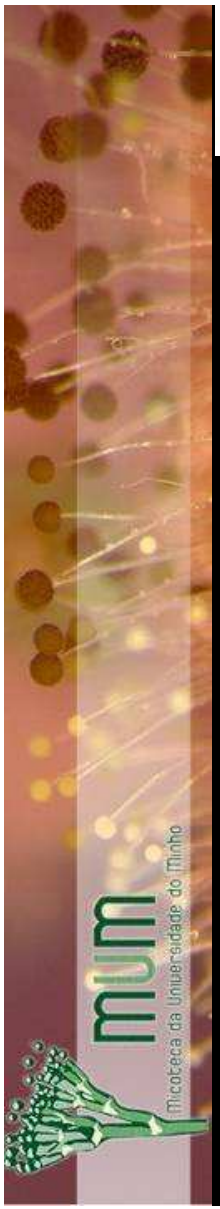
Keys features of Culture Collections

-Collect and preserve microbial cultures well identified and their associated information.

-Supply microbial strains with high quality and authenticity.

-Problem-solving oriented

-Engaged in the valorization of the chain-of-knowledge: *Research, Development & Innovation.*



Thank You For Your Attention