Polyphasic Taxonomy of Toxigenic Fungi

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Taxonomy

- Separation into genus and/or species:
- Morphology: colony colour, size and conidia format, presence of sclerocia, microscopic characters.
- Physiology: growth temperature, water activity, resistance to chemical compounds.
- Quimio-taxonomy: production of extrolites (acids, mycotoxins and others).
- Molecular techniques (DNA sequences, ITS region, b – tubulin, fingerprints and others).

Polyphasic Taxonomy Concept

Morphology

Physiology

Extrolites

DNA

Species

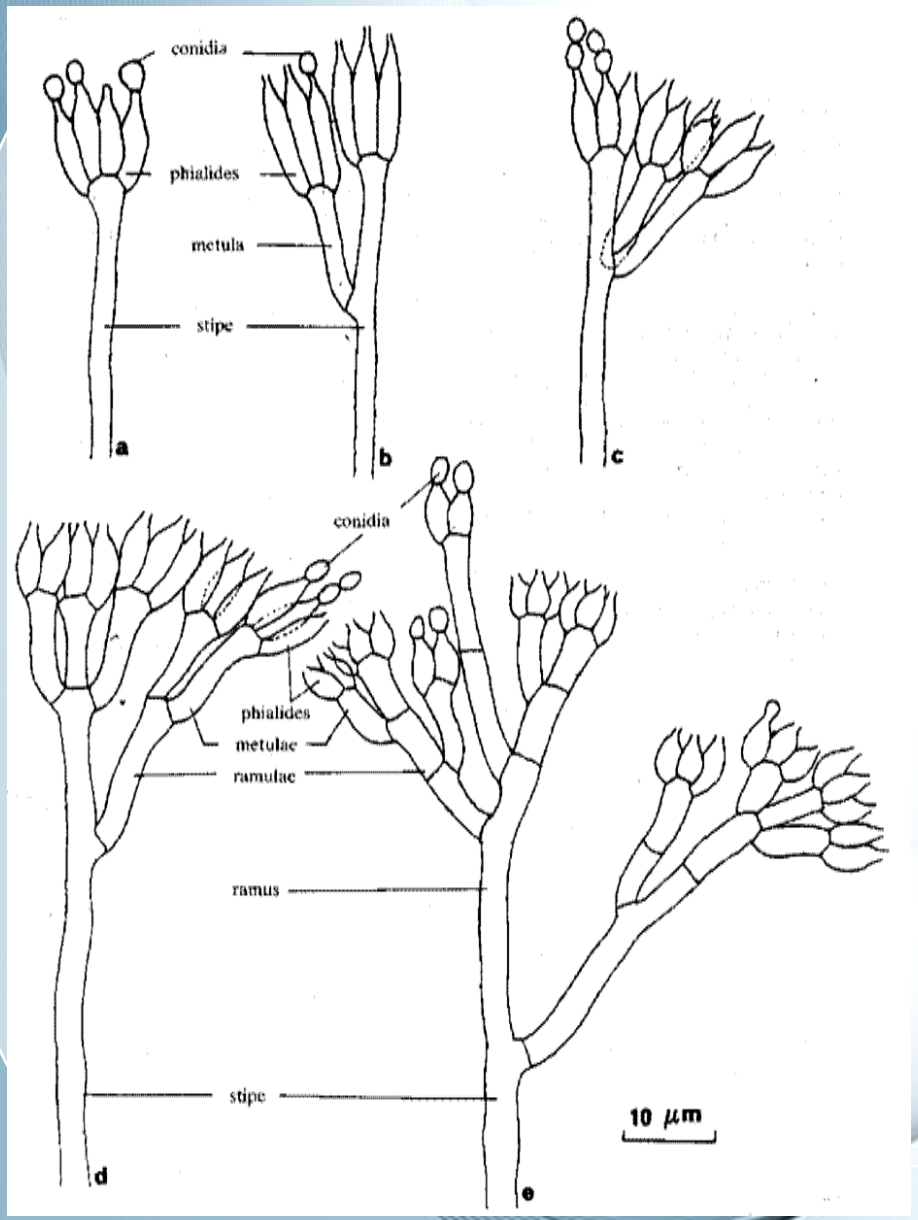
Main genus of filamentous fungi in food

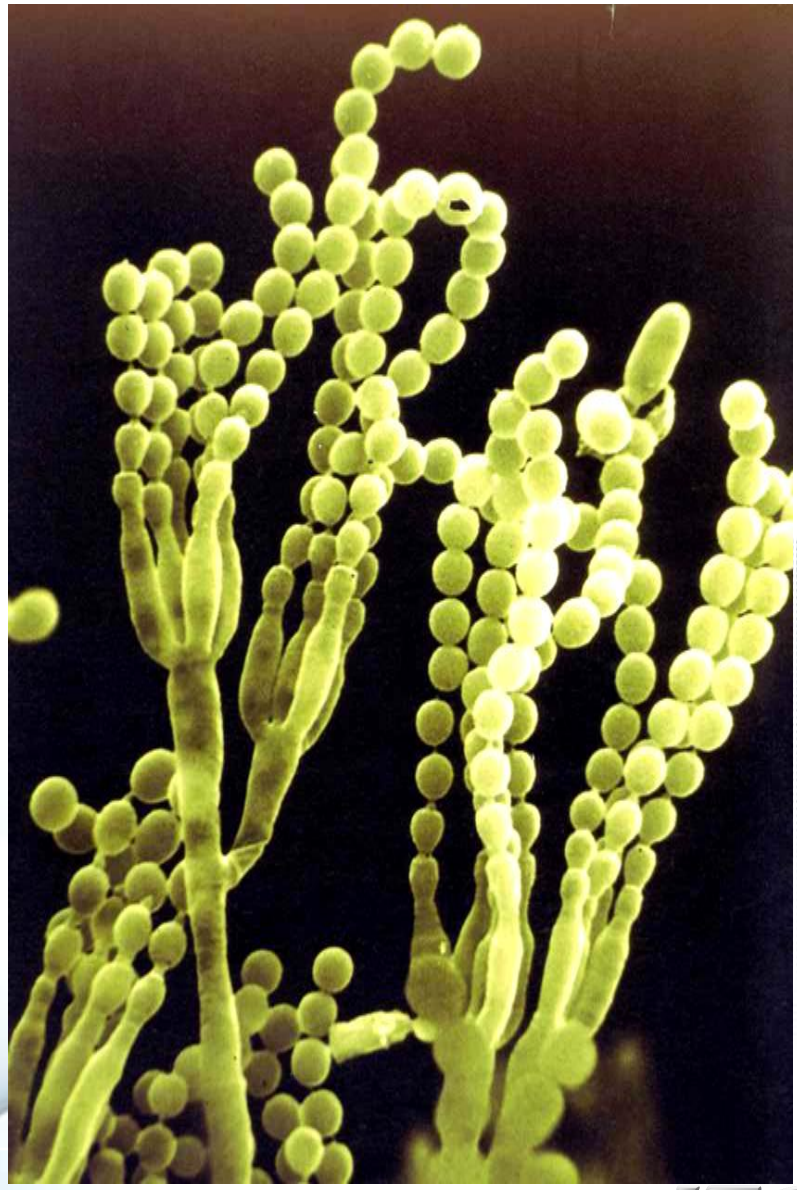
- *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria* are the principal genera concerning mycotoxins and widespread occurrence.
- *Aspergillus* is the most common in the tropics and subtropics.
- *Penicillium* is the most common in the temperate and polar regions, but certain species are also common in the tropics.
- *Fusarium* and *Alternaria* are common world-wide.

Penicillium toxins

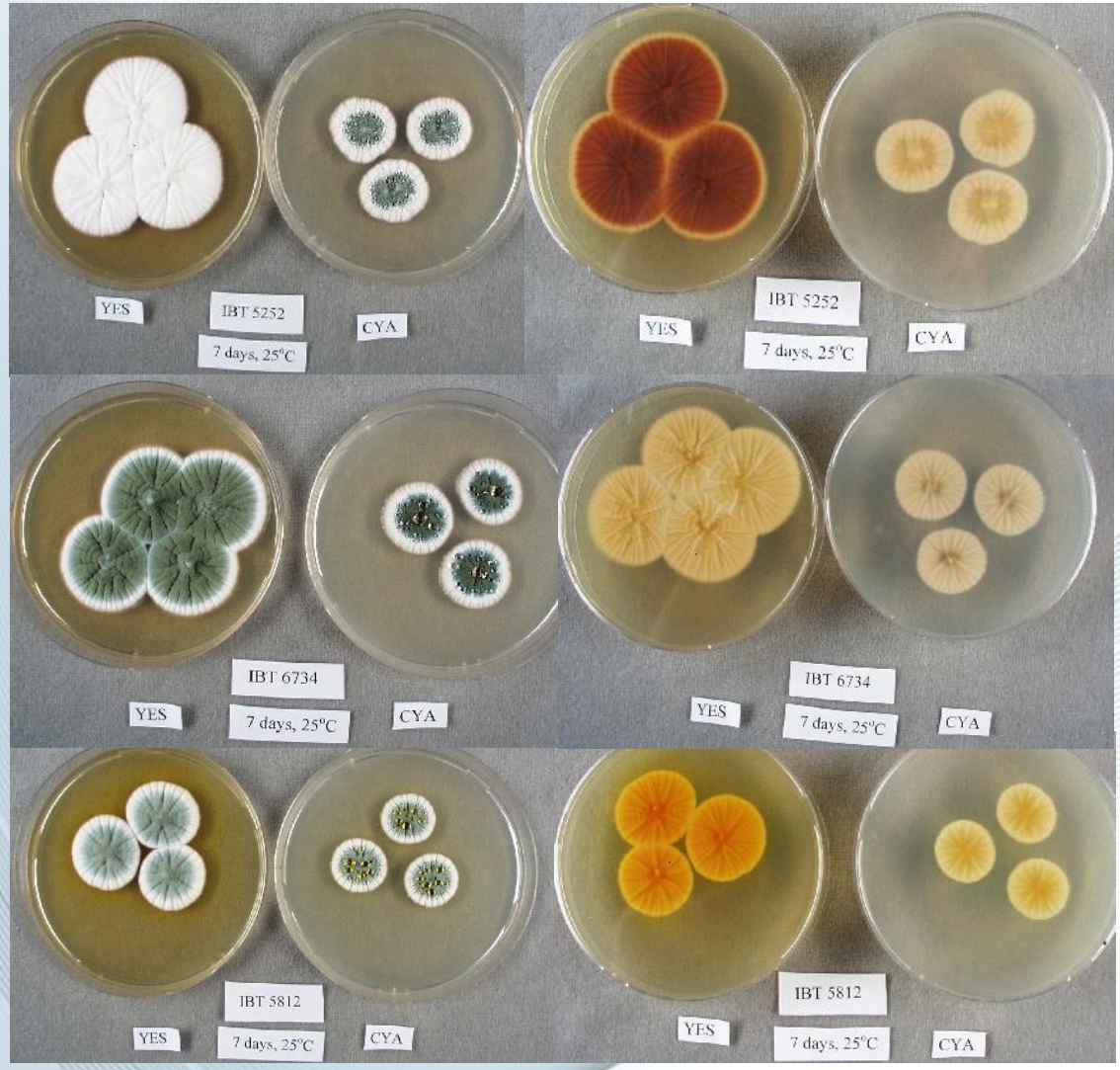
Species	Toxins
verrucosum, nordicum	Ochratoxins
expansum	Patulin
commune *camembertii*	Cyclopiazonic acid
citrinum, verrucosum	Citrinin
crustosum	Penitrem A
islandicum	Islanditoxin

Penicillium





Ochratoxin A producing Penicillia



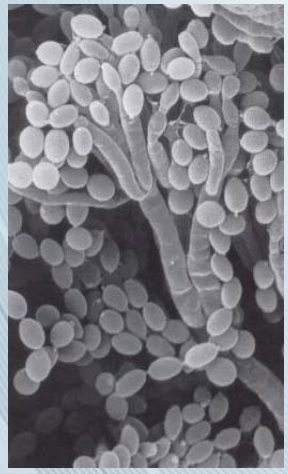
Fungi producing patulin

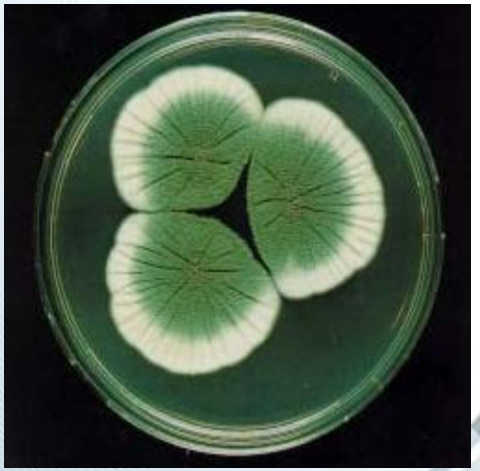
Produced by *Penicillium, Aspergillus, Byssochlamys*

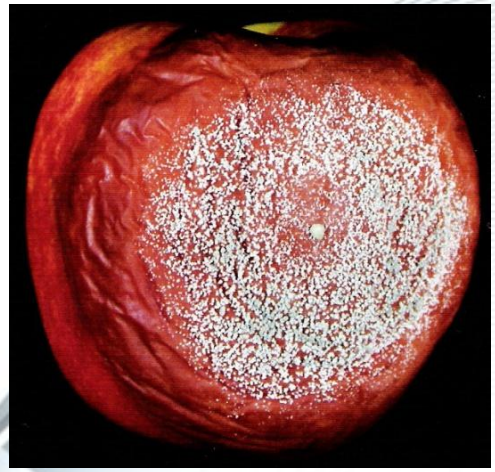
Soluble in water

Patulin can be present in apples, apple juice and products.

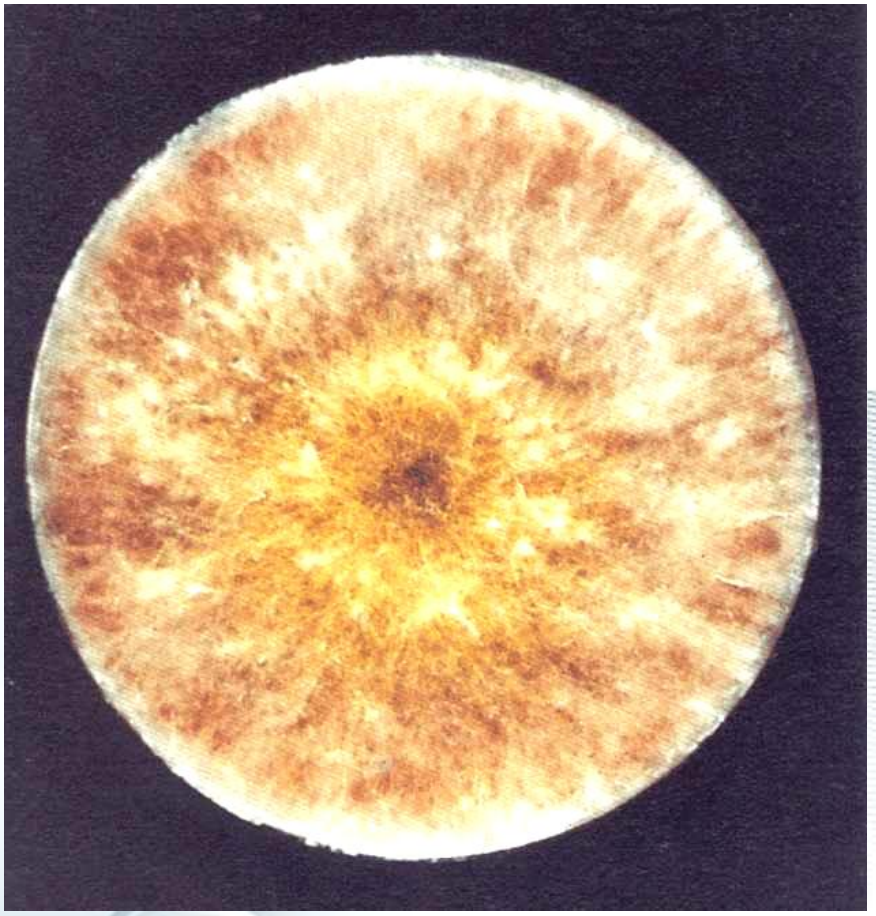
Penicillium expansum







Fusarium



Fusarium toxins

Species	Toxins
graminearum, culmorum	Zearalenone DON
nivale	Nivalenol DON
equiseti	T-2 toxin DAS
verticillioides *proliferatum*	Fumonisins
sporotrichioides	T-2 Toxin

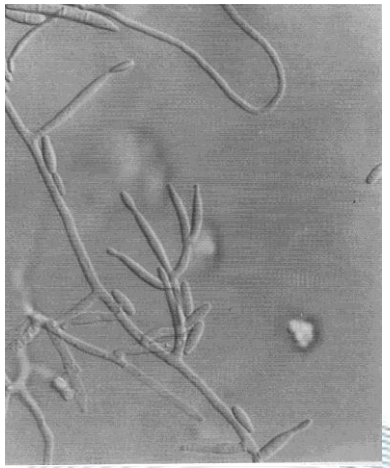
Fusarium Producing Fumonisins

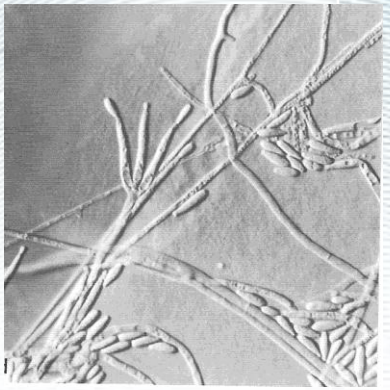
Main species:

Fusarium verticillioides (*F. moniliforme*) and *F. proliferatum*.

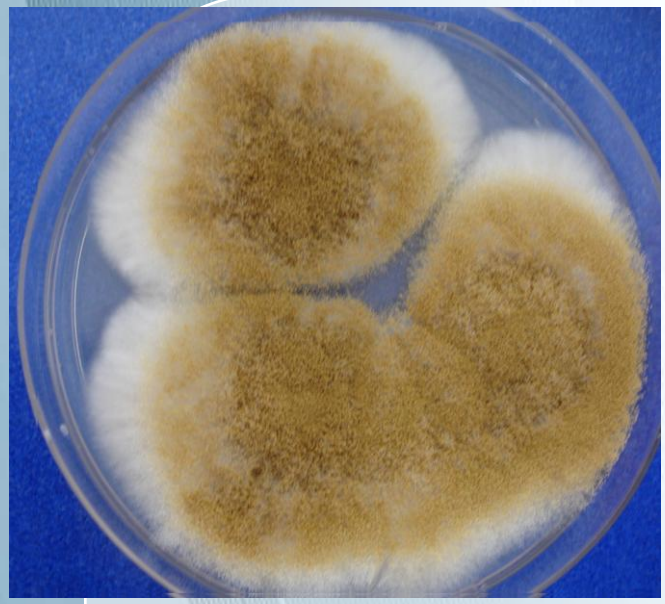
F. verticillioides is systemic in maize and is present in plants, even in healthy kernels.

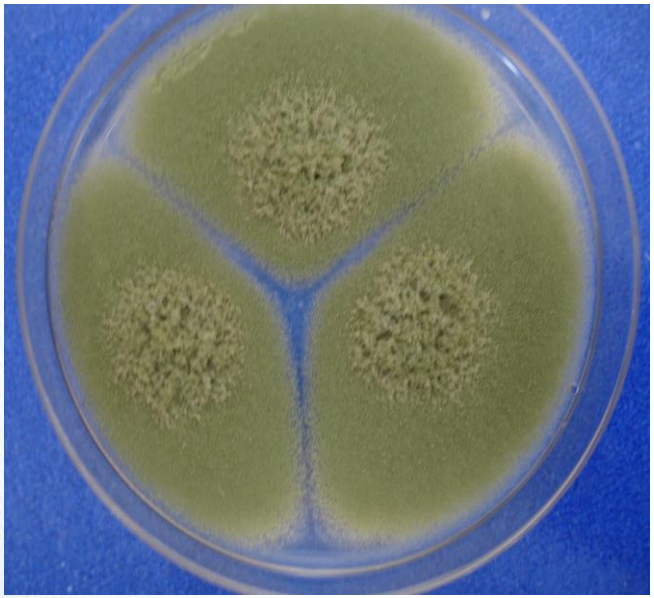
Fumonisins are produced when drought stress or other unfavourable conditions disturb the balance between fungus and plant.

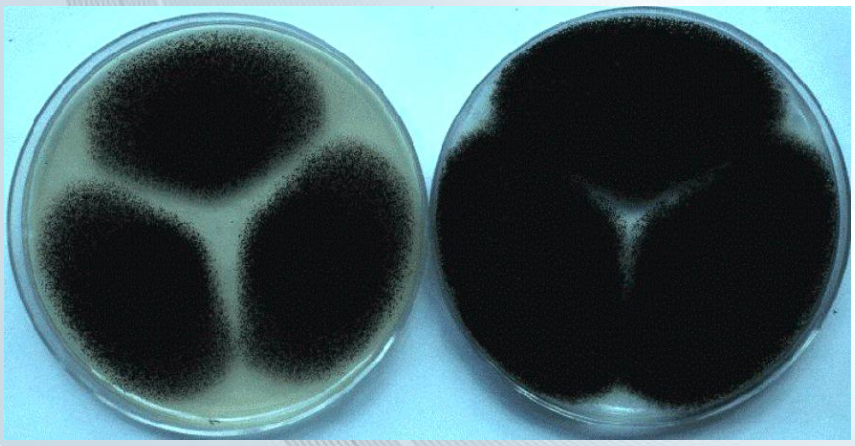


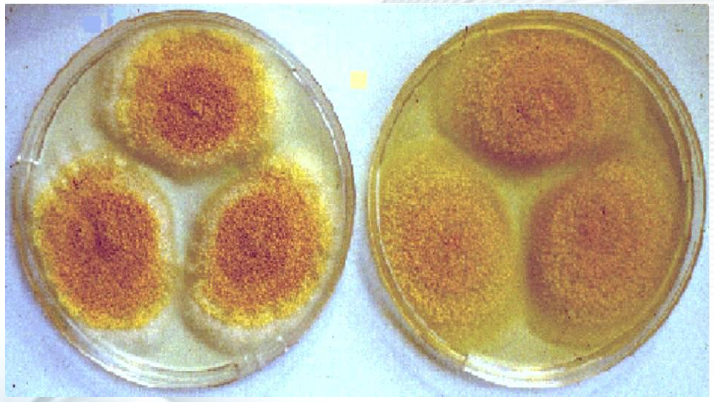


Aspergillus

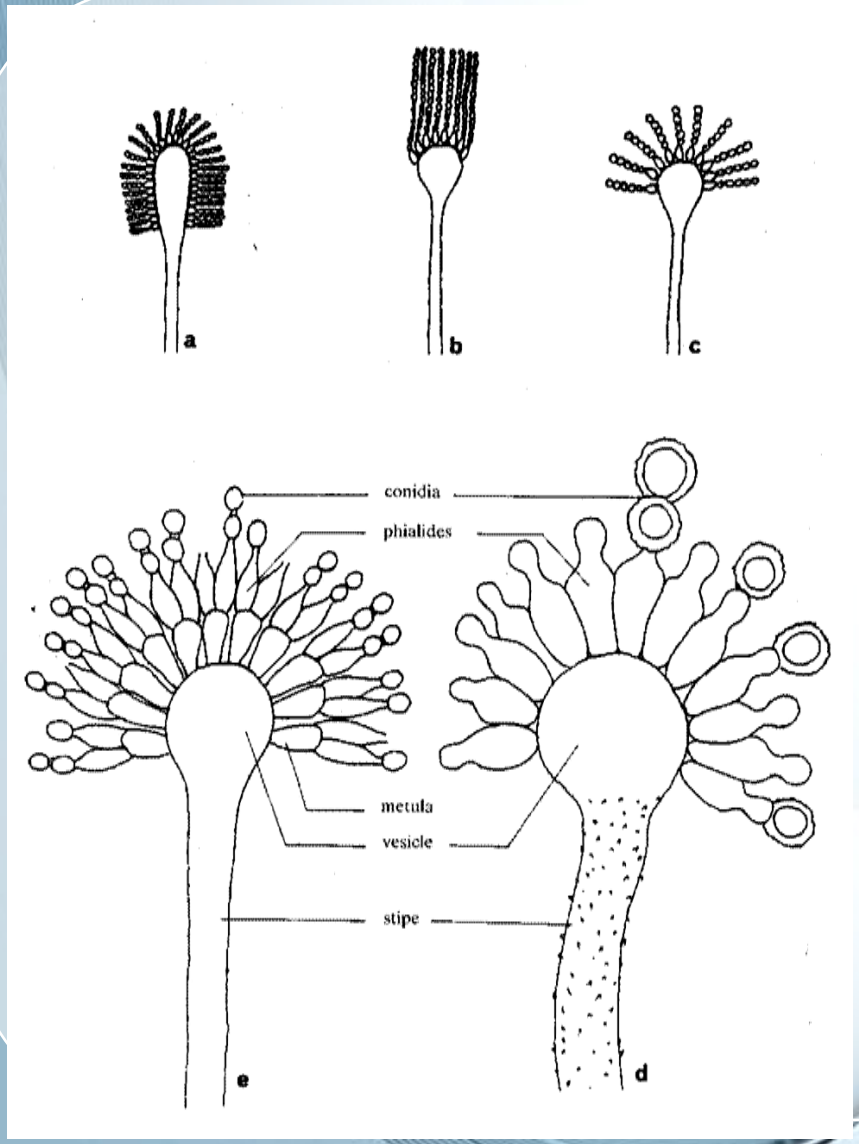


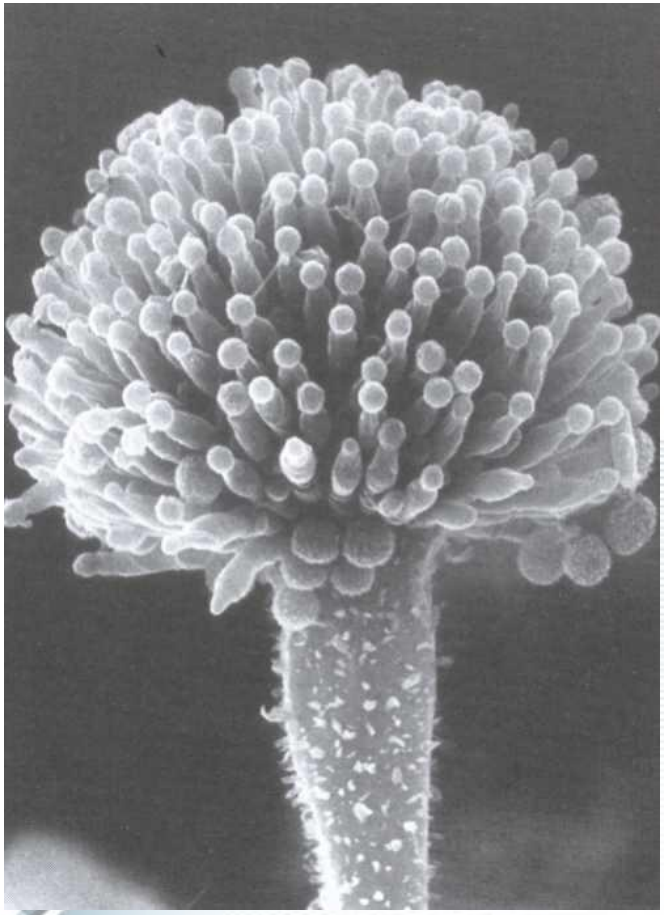






Aspergillus





Toxigenic *Aspergillus*

- *Aspergillus* section *Flavi* (*A. flavus* group)
- *Aspergillus* section *Nigri* (*A. niger* group)
- *Aspergillus* section *Circumdati* (*A. ochraceus* group)
- *Aspergillus* section *Versicolores* (*A. versicolor* group)
- *Aspergilus* section *Clavati* (*A. clavatus* group)
- *Aspergillus* section *Fumigati* (*A. fumigatus* group)

Aflatoxin producing *Aspergilli*

Main species:

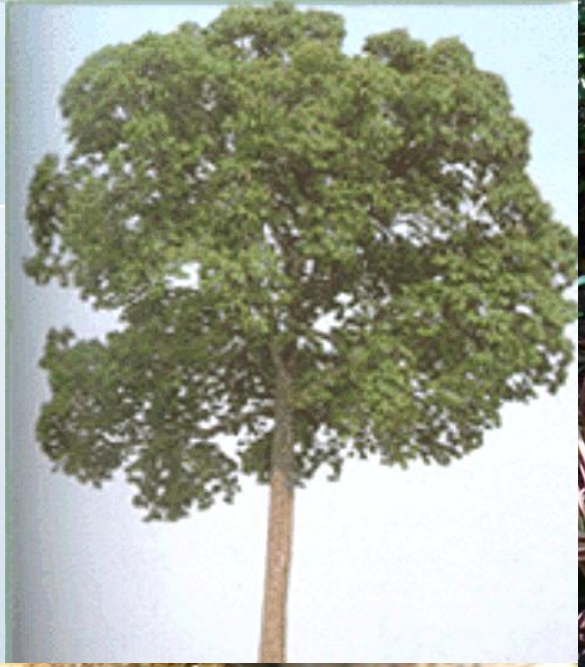
Aspergillus flavus, A. parasiticus, A. nomius

Other species:

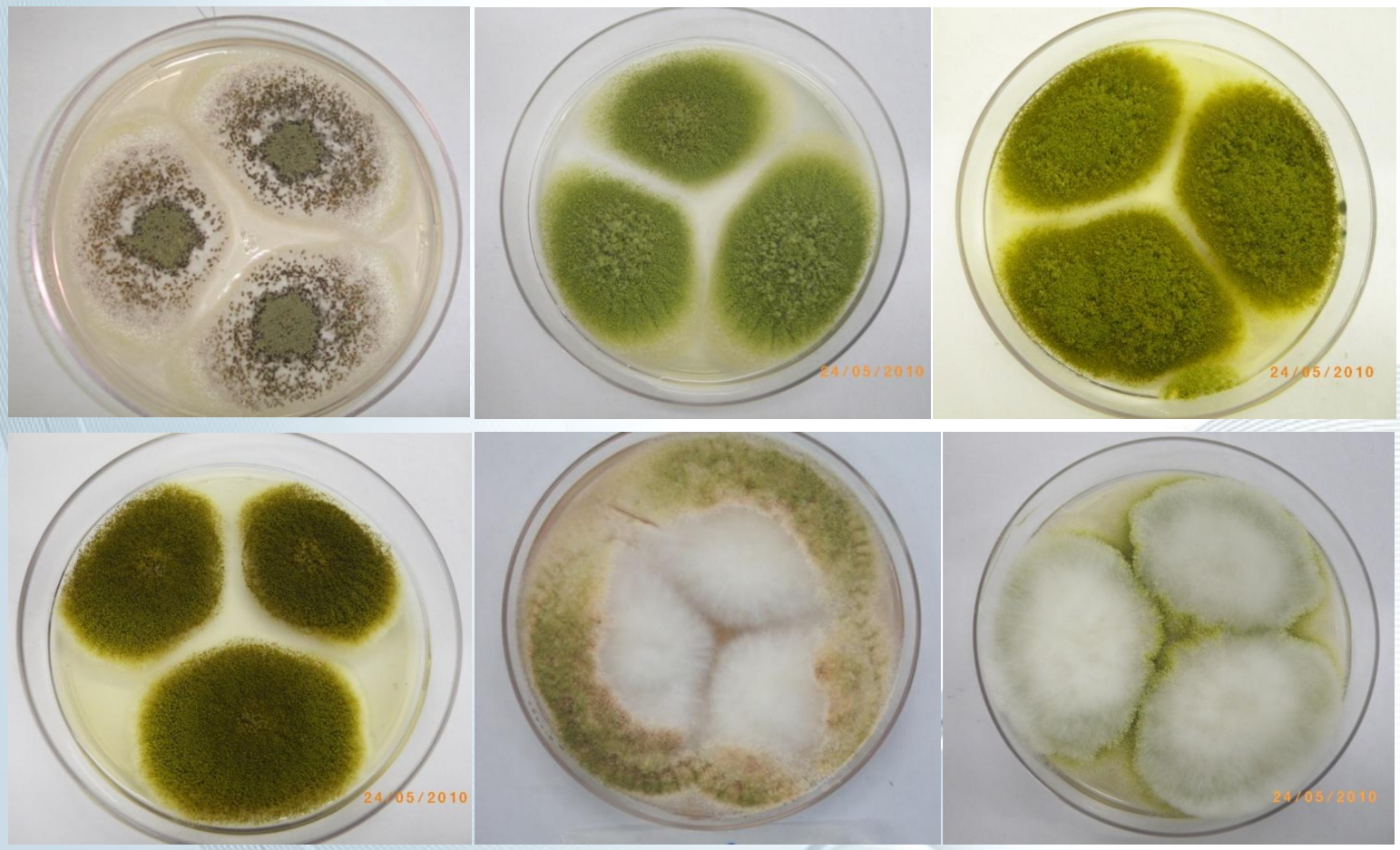
A bombycis, A pseudotamarii, A toxicarius, A parvisclerotigenus, A ochraceoroseus, A rambellii, Emericella astellata, E. venezuelensis.

Species	Heads	Conidia	Esclerotia	Ocurrence	Mycotoxins
Aspergillus flavus	Mostly biseriate	Spherical to ellipsoidal Smooth to rough wall	Large, spherical	Tropics and subtropics	40% afla B 50% CPA
A parasiticus	Rarely biseriate	Spherical Rough	Large, spherical (uncommon)	South America, USA, Australia	Nearly 100% afla B and G
A nomius	Mostly biseriate	Spherical to ellipsoidal Smooth to rough	Small, Bullet shape	Brazil, USA, Thailand	Usually afla B and G
A bombycis	Mostly biseriate	Spherical to subspheroidal, Rough	Not reported	Japan, Indonesia	Afla B and G
A pseudotamarii	Biseriate	Spherical to subspheroidal, Very rough	Large spherical	Japan, Argentina	Afla B, CPA
A toxicarus	Rarely biseriate	Spherical Rough	Large, Spherical	USA, Uganda	Afla B and G
A parvisclerotigenus	Mostly bisseriate	Spherical Rough	Small, Spherical	USA, Argentina, Japan, Nigeria	Afla B and G, CPA
A ochraceroseus	Biseriate	Subspherical to ellipsoidal, smooth	Not reported	Ivory Coast	Afla B, esterigmatocystin
A rambellii	Biseriate	Ellipsoidal, smooth	Not reported	Ivory Coast	Afla B, Sterigmatocystin
Emericella astellata	Biseriate	Spherical Rough	Ascomata and hulle cells	Ecuador	Afla B, Sterigmatocystin
E. venezuelensis	Biseriate	Spherical Rough	Ascomata and hulle cells	Venezuela	Afla B, Sterigmatocystin

Biodiversity of *Aspergillus* section *Flavi* in brazil nuts



Biodiversity of *Aspergillus* section *Flavi* isolated from brazil nuts



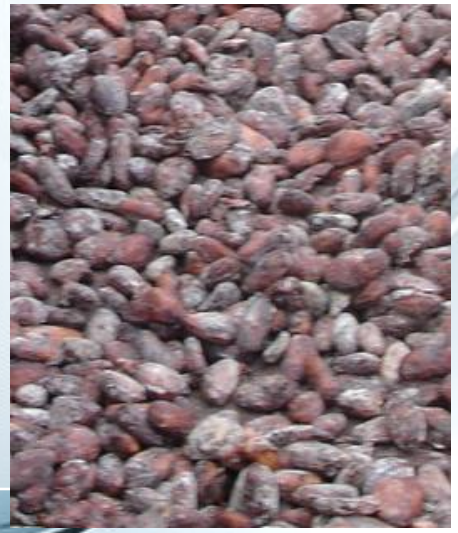
Percentage (%) of infection of toxigenic fungi in brazil nuts

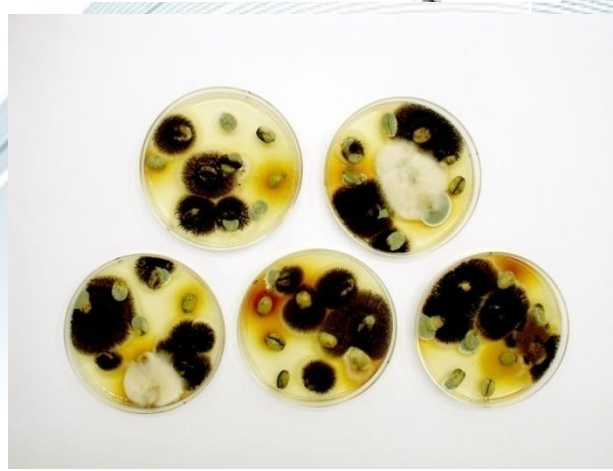
Toxigenic species	Nº of isolates	%	Average of Infection (%)	Variation (%)
Fungi in general	7,642		83.1	0 – 100
A. flavus	632		12.1	0 – 100
A. flavus producing aflatoxins	173	27.4		
A nomius	225		4.6	0 – 36
A nomius producing aflatoxins	225	100		
Group *Flavi*	416		11.5	0 – 100
Group *Flavi* producing aflatoxins	80	19.2		
Group *Nigri*	339		8.1	0 – 78
Group Nigri producing ochratoxin A	11	3.2		

Aspergillus section *Nigri*

These species are common in many types of foods, feeds, raw materials for beverages and others.

- Grapes, raisins
- Green coffee beans
- Green tea, black tea, fermented tea
- Cocoa
- Maize
- Onions
- Peanuts
- Brazil nuts





Aspergillus niger

- GRAS status for the following processes
  - amyloglucosidase, α-amylase, glucoamylase
  - Lipase
  - Maltase
  - Citric acid
  - Ethanol
  - Mono-oxygenase and others

- However, some *A. niger* strains (3- 10%) can produce the carcinogenic nephrotoxin ochratoxin A

  More than 60% of isolates produce the carcinogenic mycotoxin fumonisin B2.

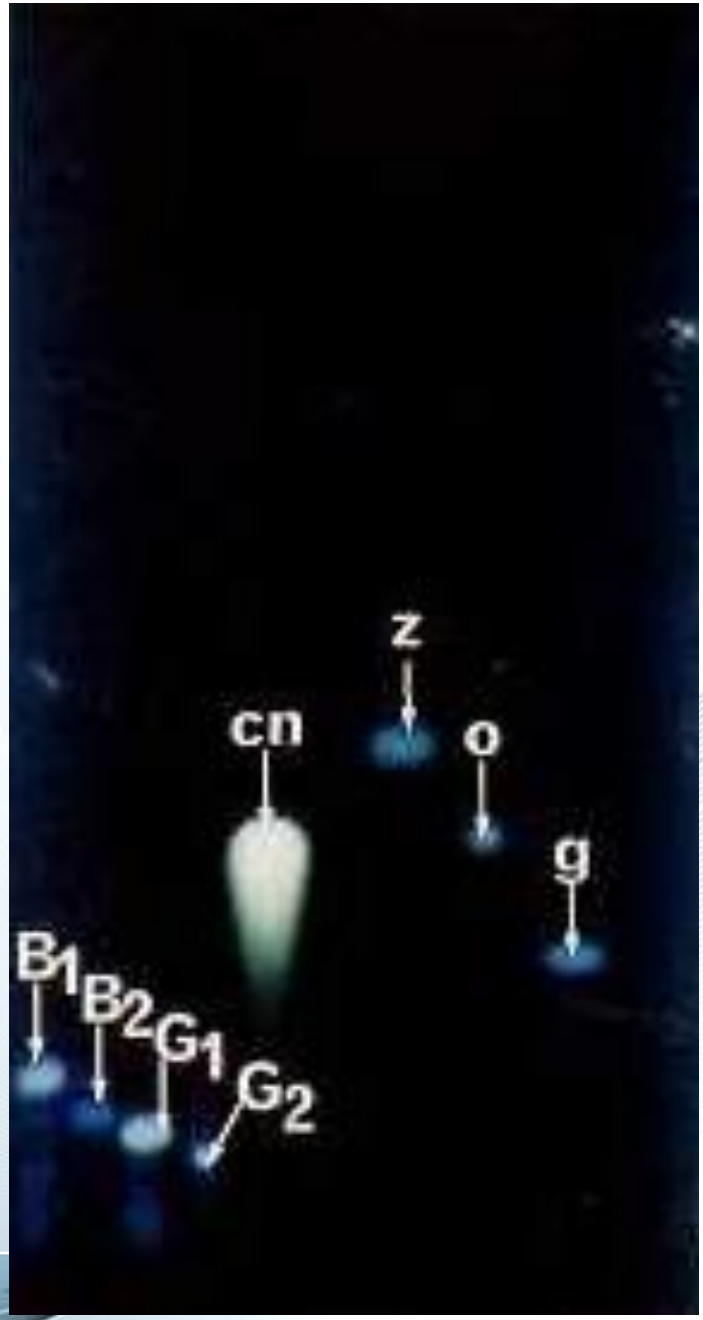
Aspergillus section Nigri

- 108 taxas in literature
- Samson et al. (2004) accepted 15 species:
  - Morphology
  - Physiology
  - Extrolite profiles
  - Molecular techniques
- Frisvad (2007): 20 species

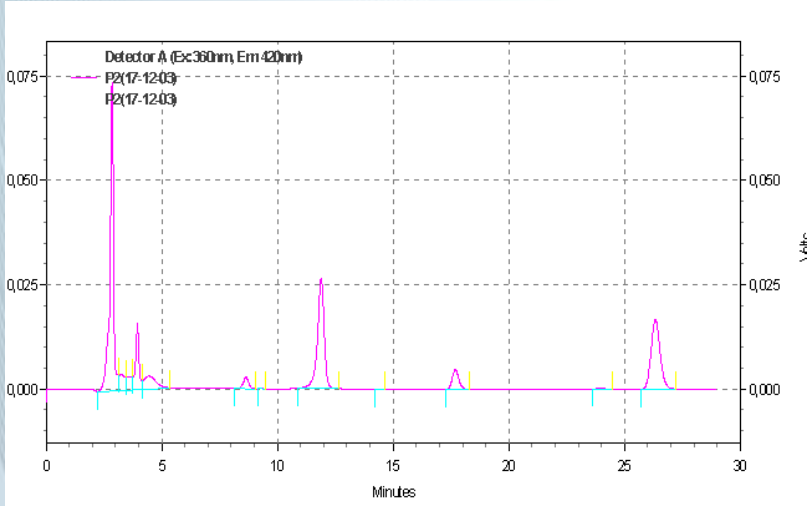
Analysis of extrolites (including mycotoxins)

- (Paper chromatography)
- (HP)TLC-Fluorescence-DAD-MS-chemical reactions
- GC-FID-MS-FTIR
- CE-DAD-Fluorescence-MS
- HPLC-LSD-DAD-MS-NMR

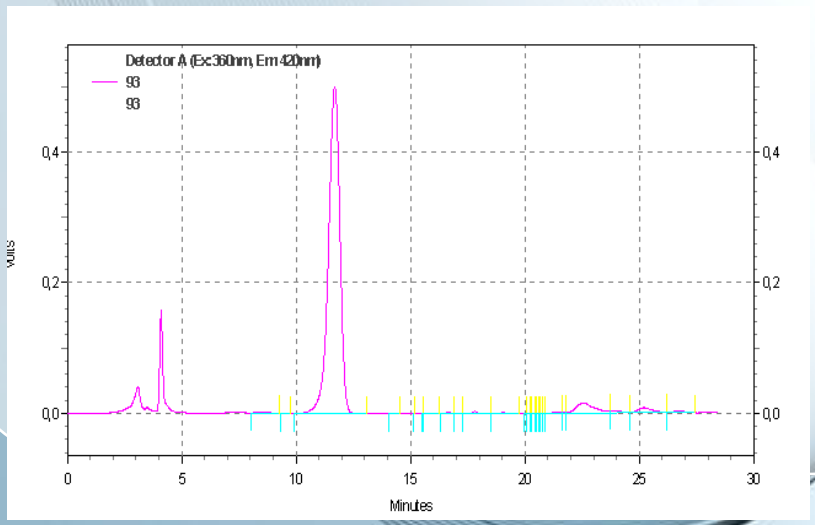
Thin Layer Chromatography



High Performance Liquid Chromatography



Aflatoxins $B_1B_2G_1G_2$



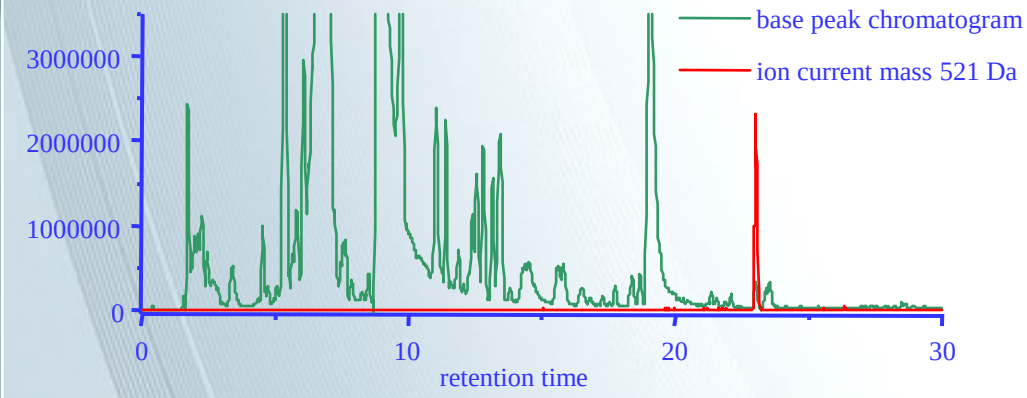
Aflatoxin B_1

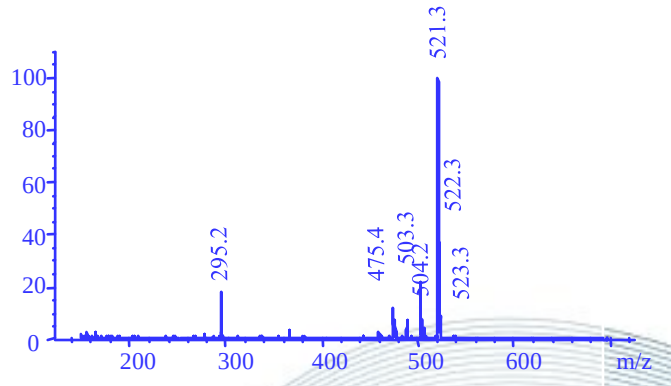
Hyphenated techniques

LC- UV - MS analysis of plug extract...

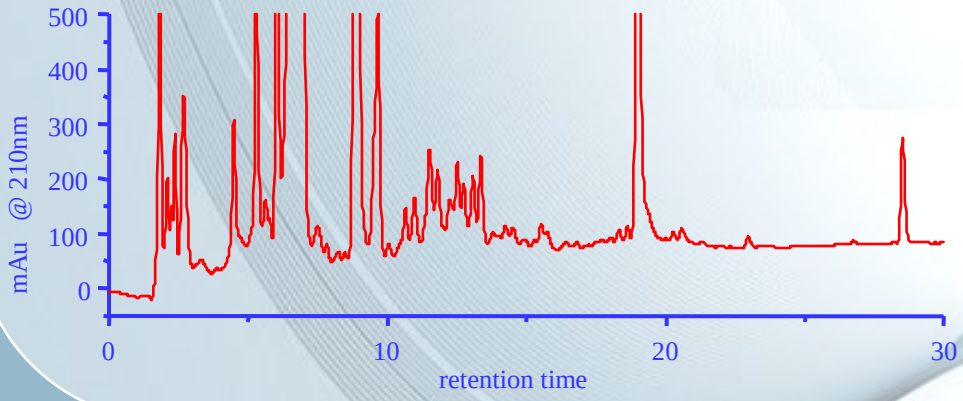
...hunting the unknown metabolite in *A. niger*

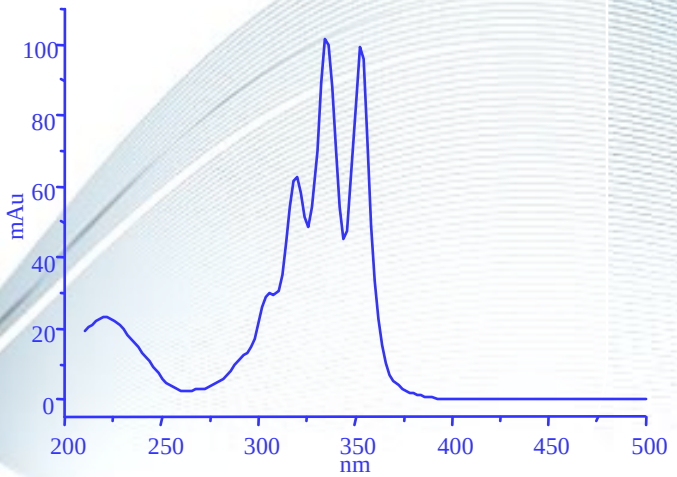
APCI MS data



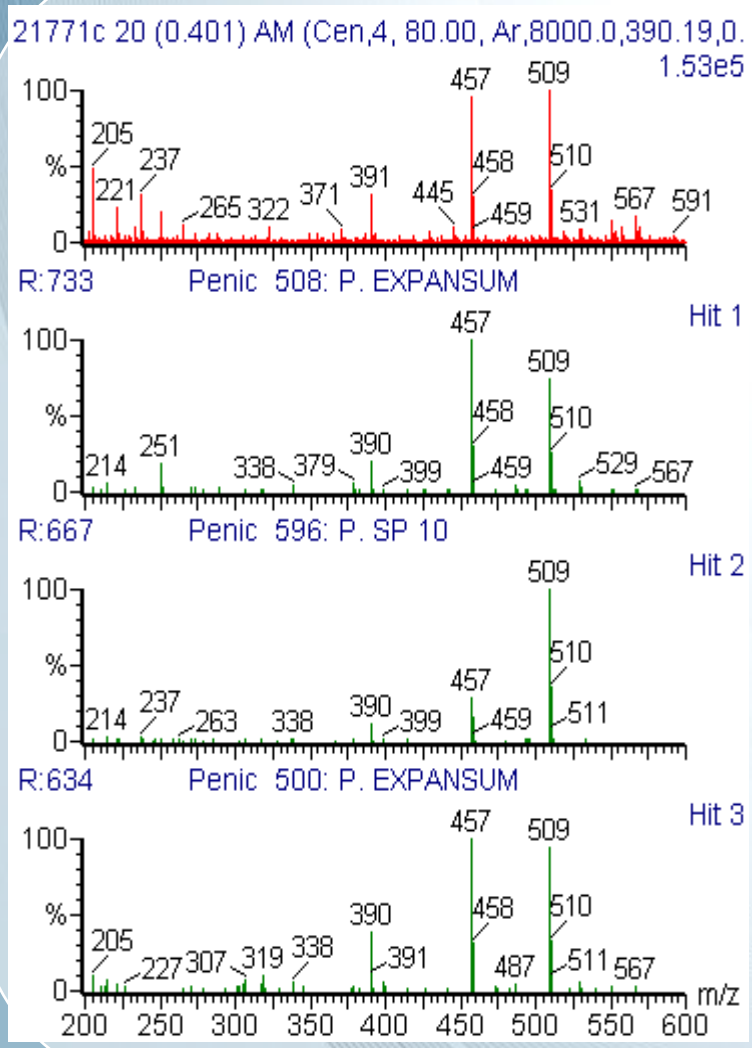


Diode array data





Mass profile library searching

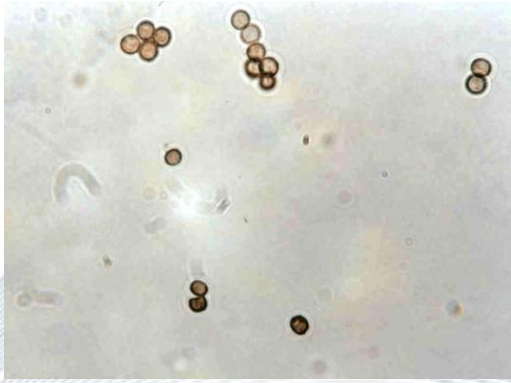


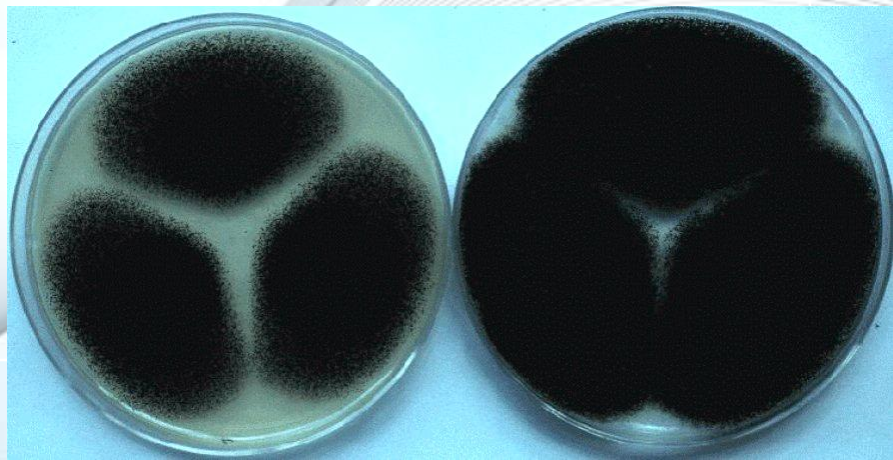
Hit	Compound Name	Rev	CAS
Hit code	**Species**	**similarity**	**IBT-media-**
1	P. expansum	733	15717-10-0
2	P. marinum	667	16712-10-9
3	P. expansum	634	15658-10-0
4	P. marinum	622	16715-10-9
5	P. marinum	604	16716-10-9
6	P. expansum	603	15598-10-0
7	P. expansum	592	16943-10-0
8	P. expansum	564	16705-10-0

Aspergillus section *Nigri I*

Series *Nigri*:

- Subseries *Nigri*
  - *Aspergillus niger*: ochratoxin A, fumonisin B2
  - *Aspergillus lacticoffeatus*: ochratoxin A, fumonisin B2
  - *Aspergillus brasiliensis*
- Subseries *Tubingensis*
  - *A. tubingensis*
  - *A. coreanus*
  - *A. citricus (foetidus)*
  - *A. vadensis*
  - *A. piperis*
  - *A. costaricaensis*





Species in *Nigri* II

Series *Carbonaria*:

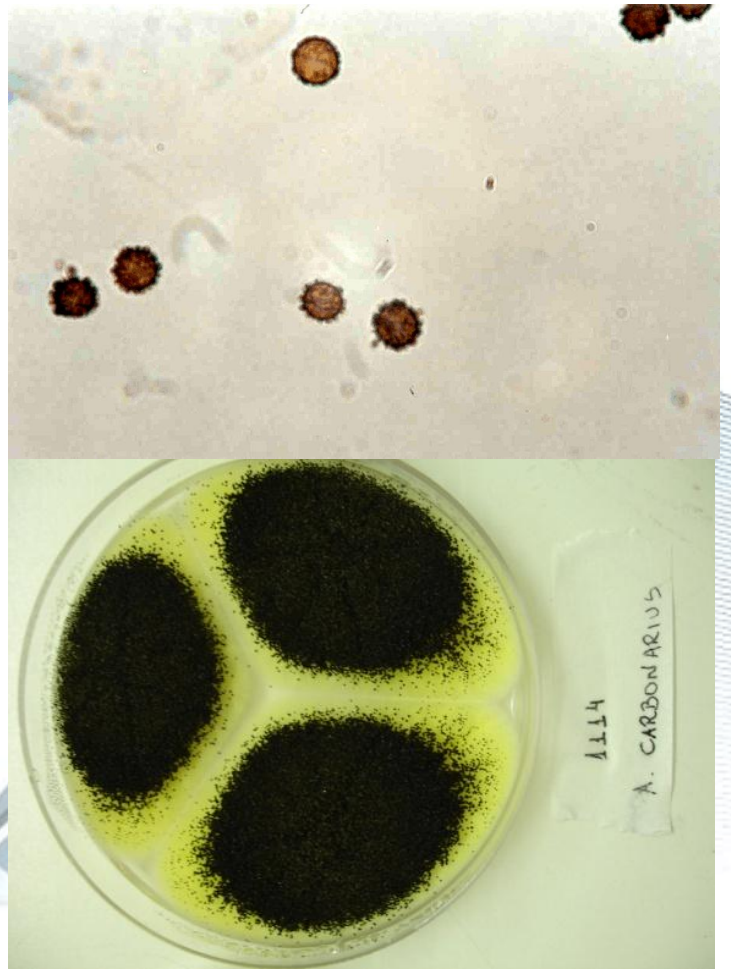
- *Aspergillus carbonarius*: ochratoxin A
- *Aspergillus sclerotioniger*: ochratoxin A
- *Aspergillus sclerocarbonarius*
- *Aspergillus ibericus*

Series *Heteromorpha*:

- *Aspergillus heteromorphus*
- *Aspergillus ellipticus*

Series *Homomorpha*:

- *Aspergillus homomorphus*



Species in *Nigri* III

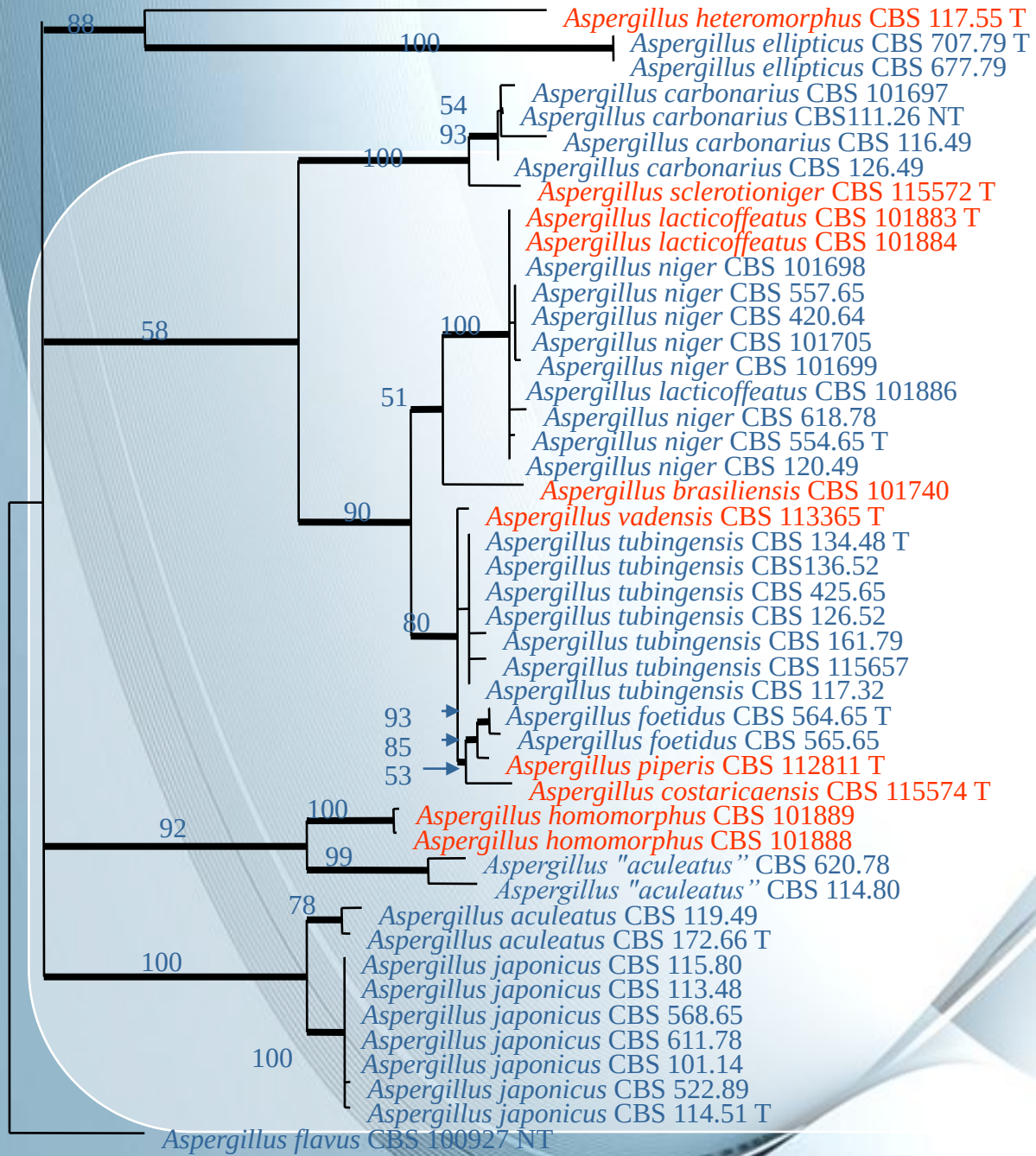
Series *Aculeata*:

Aspergillus aculeatus: secalonic acid

Aspergillus aculeatinus: secalonic acid

Aspergillus uvarum: secalonic acid

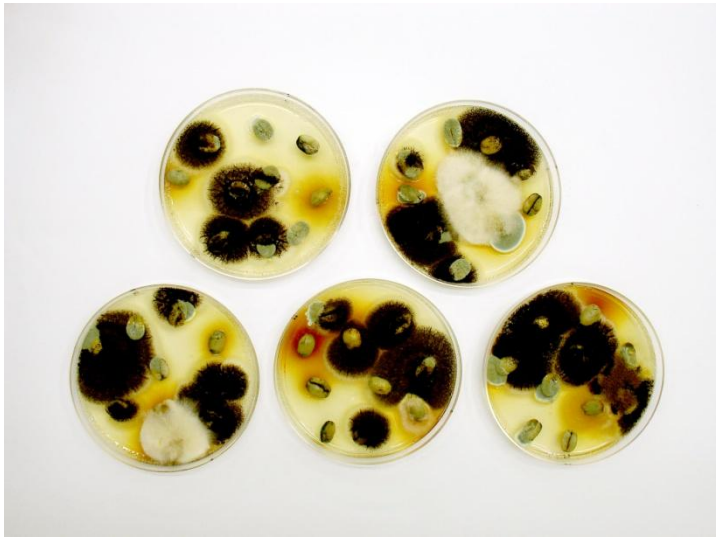
Aspergillus japonicus: cycloclavine, festuclavine

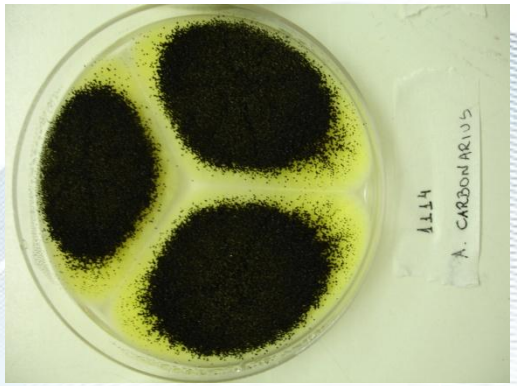


β-tubulin sequenced based cladogram

Potential OTA producer fungi in coffee:

- ✓ *Aspergillus westerdjikiae*
- ✓ *Aspergillus ochraceus*
- ✓ *Aspergillus carbonarius*
- ✓ *Aspergillus niger*

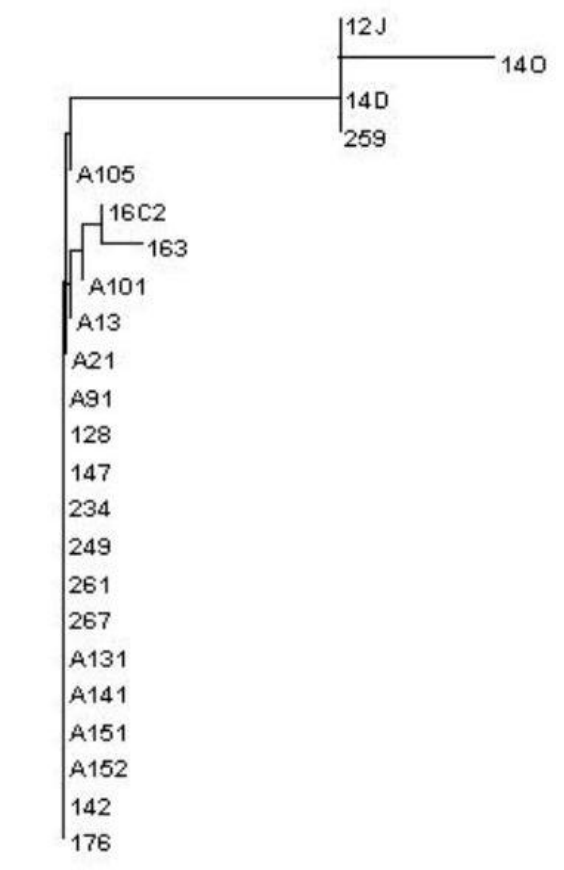


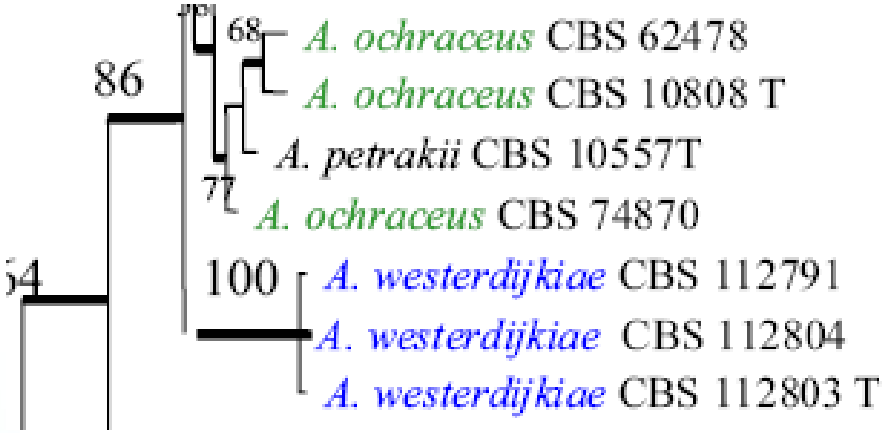


Taniwaki, M.H.; Pitt, J.I.; Teixeira, A.A. & Iamanaka, B.T. 2003. The source of ochratoxin A in Brazilian coffee and its formation in relation to processing methods. **International Journal of Food Microbiology, 82 (2):** 173-179.

iTAL

Aspergillus section *Circumdati*

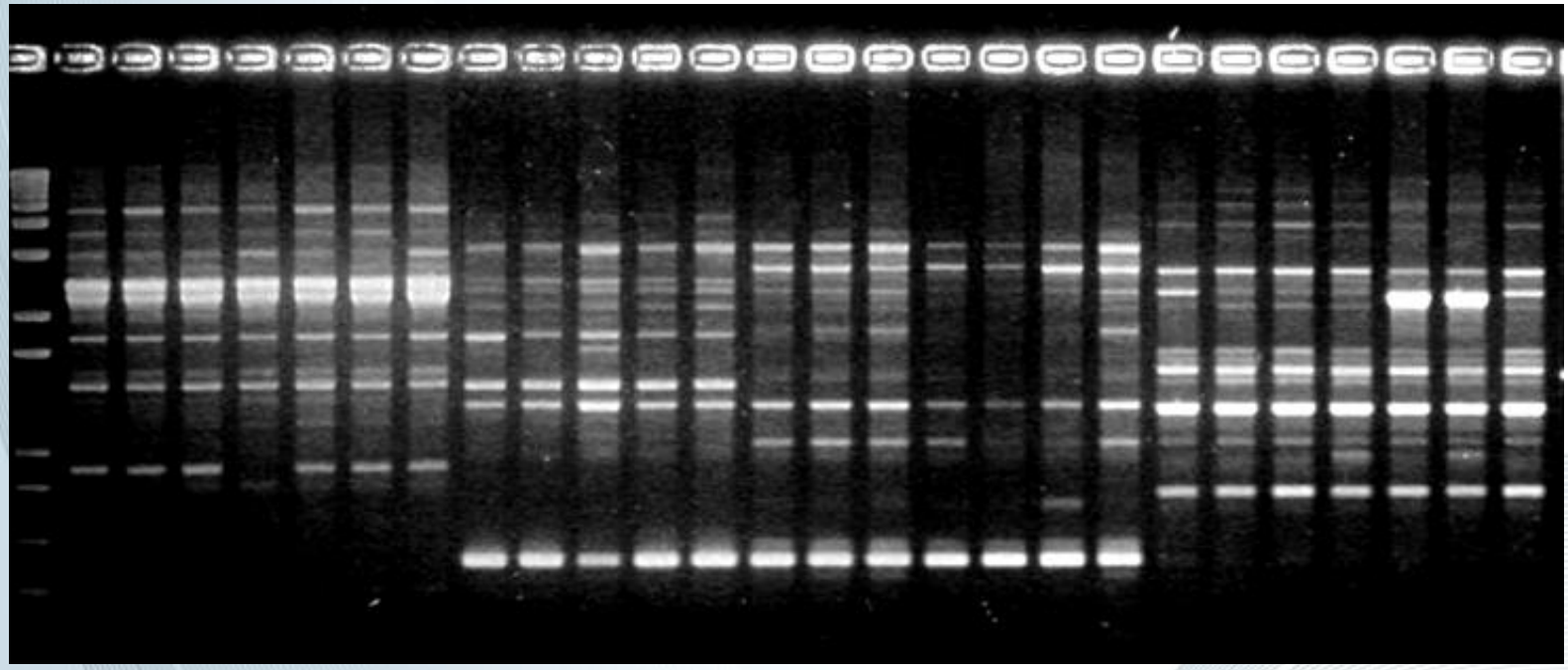




Frisvad et al. 2004. New ochratoxin A producing species of *Aspergillus* section *Circumdati.* **Studies in Mycology 50:** 23-43

Fungaro, M.H.P., Magnani, M., Vilas-Boas, L.A., Vissotto, P.C., Furlaneto, M.C., Vieira, M.L.C.& Taniwaki, M. H. 2004. Genetic relationships among Brazilian strains of *Aspergillus ochraceus* based on RAPD and ITS sequences. **Canadian Journal of Microbiology 50:** 985-988.

RAPD *Aspergillus* section *Nigri*



A. niger *A. tubingensis* *A. carbonarius*

Fungaro, M.H.P.; Vissotto, P.C.; Sartori, D.; Vilas-Boas, L.A.; Furlaneto, M.C. & Taniwaki, M.H. 2004. A molecular method for detection of *Aspergillus carbonarius* in coffee beans. **Current Microbiology** 49: 123-127.

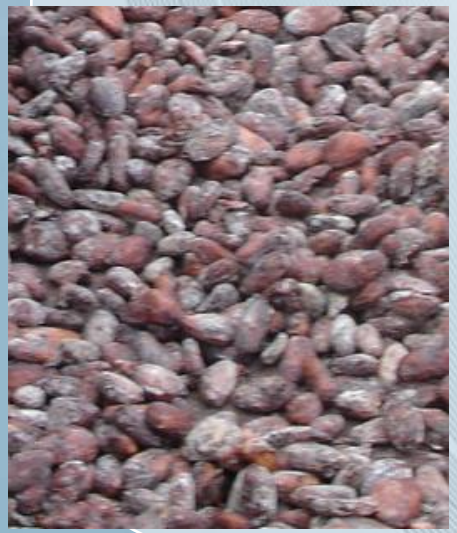
Aspergillus niger and fumonisin

- Baker (2006) and Pel et al. (2007) indicated the there was a putative fumonisin gene cluster in *Aspergillus niger* (in two different full genome sequenced strains).
- *A. niger* produced fumonisin B2 in CYA, YES, CY20S and CYAS, media with high sugar or NaCl concentration, but not in MEA, PCA, PDA and OAT.
- The regulation of fumonisin production in *Fusarium* and *Aspergillus niger* is completely different.

Frisvad et al., 2007, Production of fumonisin B2 by *Aspergillus niger* *Journal of Agricultural and Food Chemistry*, , 55: 9727-9732.

Ochratoxin A and fumonisin production by *Aspergillus* section *Nigri* in food from different origins

- Dried fruits (117
- Green coffee beans (408)
- Cocoa (226)
- Brazil nuts (84)
- 1,246 *Aspergillus* section *Nigri*



Ochratoxin A and fumonisin production by *Aspergillus* section *Nigri* in food from different origins

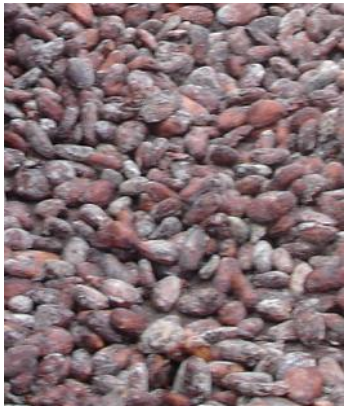
Cocoa:

A. Section *Nigri*: 87.7% FB2

25% FB2 + OTA

12.5% non producers

A. carbonarius: 100% OTA



Fungi	ITAL cc	Pyranonigrin A	Nafto-y-pyrones	NOE	CAR	Kotanin	Funalenone	asperazine	DERH	Tensidol B	DEDO	Xant	Fumonisins	OTA	Malformin
A. niger	632	+	+	0	1	+	+	0	+	+	+	0	+	+	0
A. niger	1240	+	+	0	1	+	+	0	+	+	+	0	+	+	+
A. niger	1244	+	+	0	1	+	+	0	+	+	+	0	+	0	+
A. niger	1469	+	+	0	1	+	+	0	+	+	+	0	+	0	+
A. niger	1242	+	+	0	0	+	+	0	+	+	+	0	+	0	
A. niger	1246	+	+	0	0	+	+	0	+	+	+	0	+	0	
A. niger	777	+	+	0	0	+	+	0	0	+	+	0	0	0	
A. niger	1121	+	+	0	?	+	+	0	+	+	+	0	+	0	
A. tubingensis	264	+	+	0	+	0	+	+	+	0	0	0	0	0	+
A. tubingensis	1250	+	+	+	+	0	+	+	+	0	0	0	0	0	+
A. tubingensis	1633	+	+	+	+	0	+	+	+	0	0	0	0	0	+
A. tubingensis	1001	+	+	+	+	0	+	+	0	+	0	0	0	0	
A. carbonarius	792	+	+	+	+	0	0	0	+	0	0	+	0	+	0
A. carbonarius	1375	+	+	+	+	0	0	0	+	0	0	+	0	+	0
A. carbonarius	2153	+	+	+	+	0	0	0	+	0	0	+	0	0	0
A. carbonarius	1160	+	+	0	+	0	0	0	+	0	0	+	0	1	0
A. carbonarius	791	+	+	+	+	0	0	0	+	0	0	+	0	1	0
A. carbonarius	1150	+	+	+	+	0	0	0	+	0	0	+	0	1	0

Polyphasic Taxonomy Manager

Morphology

Physiology

Extrolites

DNA

It is an exciting time to be a mycologist!

Species

Acknowledgements

Dr Jens Frisvad from Denmark Technical University (DTU) for his collaboration in this presentation.

Thank you very much for your attention!

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