



Universitat  
Autònoma  
de Barcelona

*Aspergillus* systematics in the genomic era  
CBS, Utrecht, The Netherlands, 2007

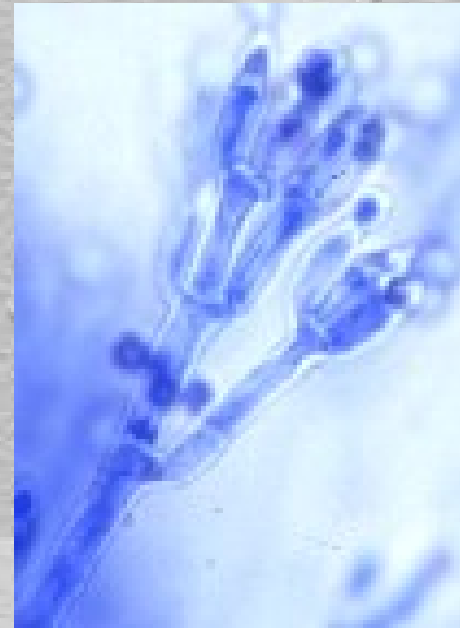
# Ochratoxin A in profiling and speciation

**F. Javier Cabañes**

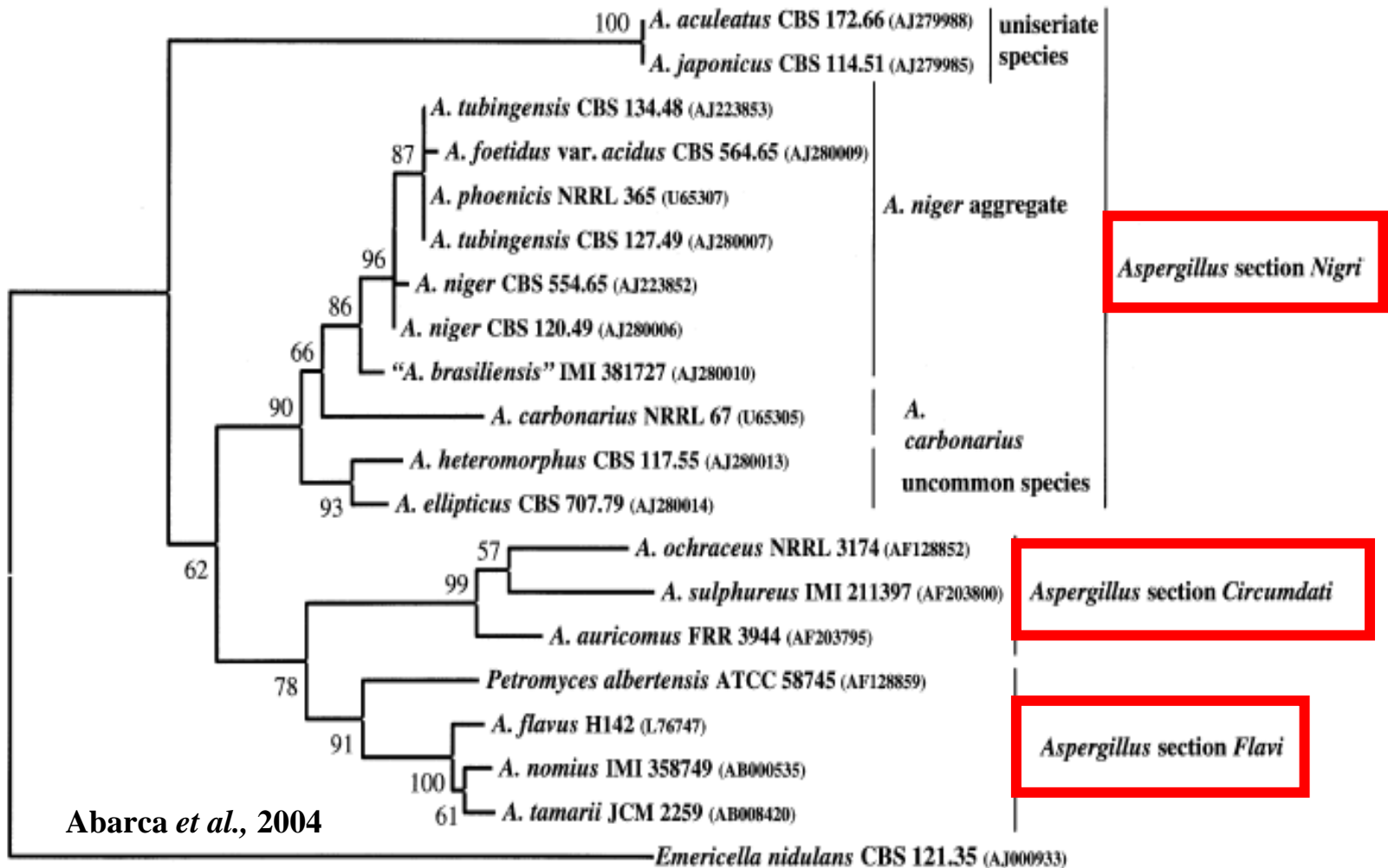


# OTA-producing fungi: *Penicillium*

- *P. verrucosum* (Van Walbeek *et al.*, 1969, *P. viridicatum*)
- *P. nordicum*



# OTA-producing fungi: *Aspergillus* sections



# OTA-producing fungi: *Aspergillus*

## Section *Circumdati*

- *A. ochraceus* (Van der Merwe *et al.*, 1965) \*
- *A. melleus*
- *A. ostianus*
- *A. petrakii*
- *A. persii*
- *A. sclerotiorum*
- *A. sulphureus*
- *A. cretensis*
- *A. flocculosus*
- *A. pseudoelegans*
- *A. steynii*
- *A. westerdijkiae* \*
- *A. roseoglobulosus*
- *N. muricatus*



## Section *Flavi*

- *P. alliaceus*, *P. albertensis*

## Section *Nigri*

- *A. niger* (Abarca *et al.*, 1994)
- *A. carbonarius*
- *A. lacticoffeatus*
- *A. sclerotioniger*

(Samson *et al.*, 2004)

(Frisvad *et al.*, 2004)



# OTA-producing fungi: in foods

- *P. verrucosum*
  - Cereals (wheat, barley, oats), ...



- *P. nordicum*
  - Meat products (salami, ham), ...



# OTA-producing fungi: in foods

- *A. ochraceus*, *A. westerdijkiae*
  - Beans (coffee, cocoa), nuts, rice, spices, ...



- *A. carbonarius*, *A. niger* aggregate,
  - Grapes (wine, dried vine fruits), coffee, ...



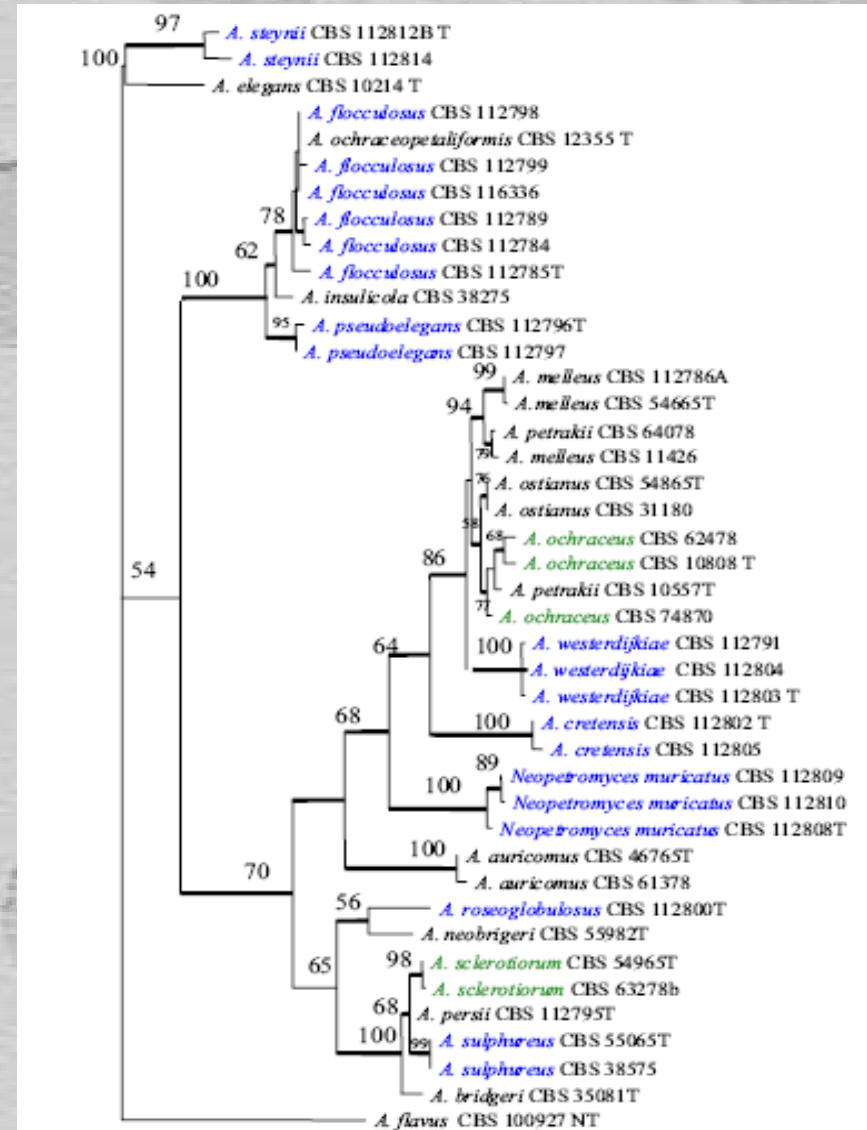
# New OTA-producing species:

## section *Circumdati*

- *A. cretensis*
- *A. flocculosus*
- *A. pseudoelegans*
- *A. roseoglobulosus*
- *A. steynii*
- *A. westerdijkiae*

✓ Apparently rare

✓ Not important in foods



$\beta$ -tubulin sequences

Grup de Micologia

Departament de Sanitat i d'Anatomia Animals

Frisvad *et al.*, 2004



# New OTA-producing species: section *Circumdati*

- *A. westerdijkiae* (Frisvad & Samson, 2004)
  - *A. ochraceus* (Van der Merwe *et al.*, 1965)
  - Sorghum, rice, coffee,...
- *A. steynii* (Frisvad & Samson, 2004)
  - NRRL 3519 (Hesseltine *et al.*, 1972)
  - Coffee, soil

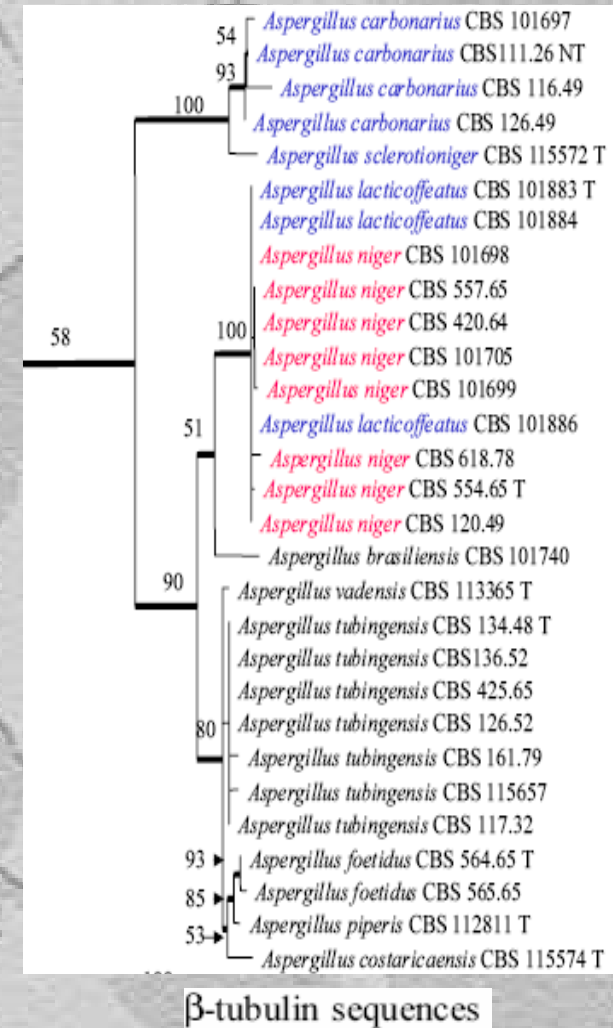
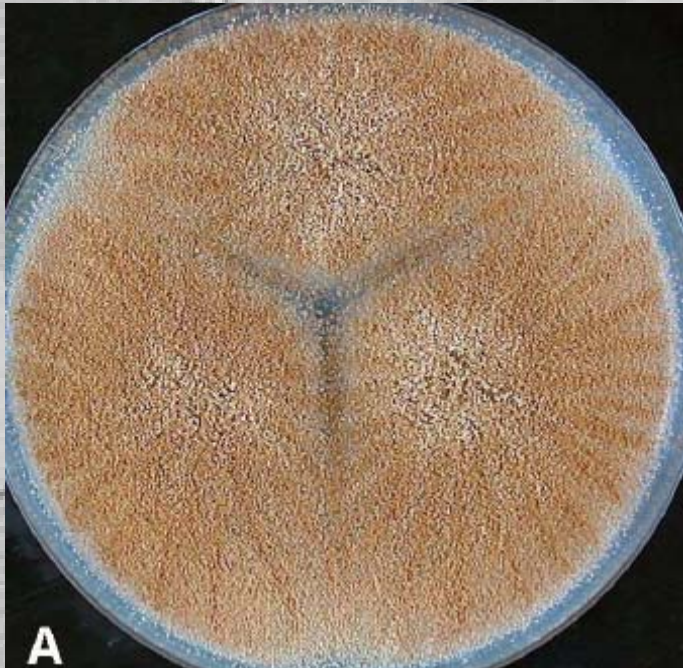


Frisvad *et al.*, 2004

# New OTA-producing species: section *Nigri*

- *A. lacticoffeatus* (Frisvad & Samson, 2004)

- 3 strains
- Coffee

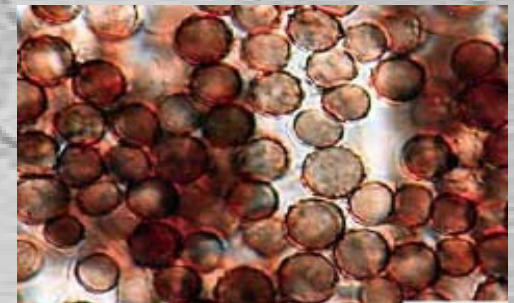
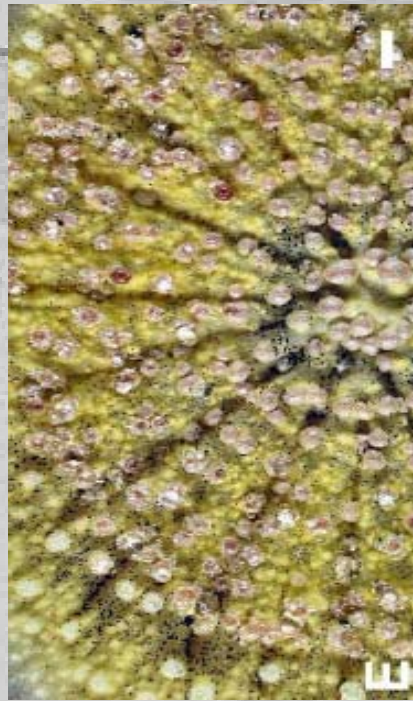


Samson *et al.*, 2004

# New OTA-producing species: section *Nigri*

- *A. sclerotioniger* (Samson & Frisvad, 2004)

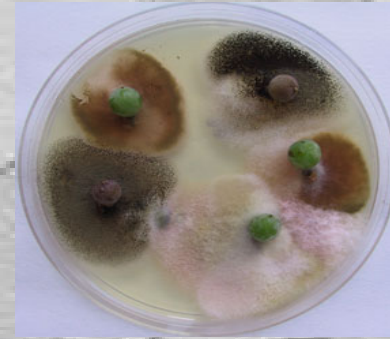
- 1 strain
- Coffee



Samson *et al.*, 2004

# OTA-producing fungi: detection

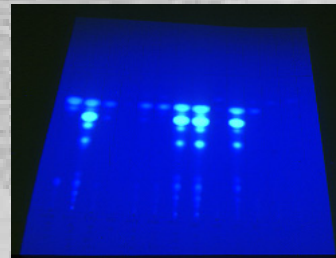
- **Traditional methods**
  - Isolation/Axenic culture
    - Identification
    - OTA production, TLC, HPLC, ...



✓ **Requires expertise:**

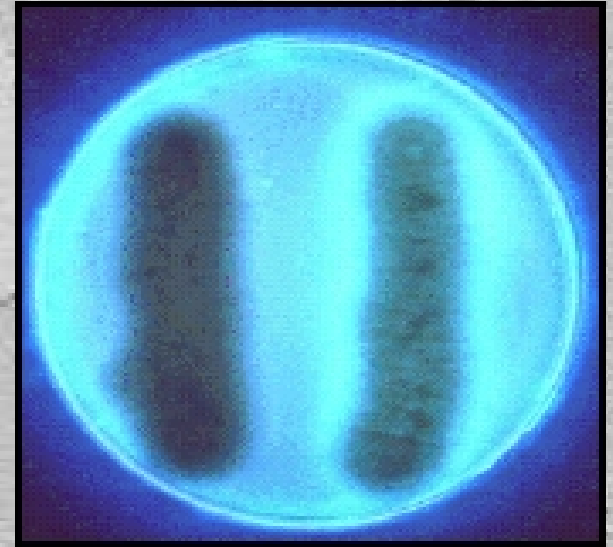
✓ fungal taxonomy, chromatography, ...

✓ **Time-consuming**



# OTA-producing fungi: detection

- Differential culture media
- Coconut Cream Agar



- Designed for detection of aflatoxin-producing fungi  
(Lin & Dianese, 1976; Dyer & McCammon, 1994)

Colony reverse under UV light:

- ✓ Green-blue fluorescence
- ✓ Turned purple-blue when exposed to ammonia



# Differential culture media:

## Coconut cream agar

- OTA producers: *A. niger*, *A. carbonarius*
  - 91% (30/33) produced fluorescence
  - 26/27 TLC OTA + (Heenan *et al.*, 1998)
  - Good correlation
- ✓ Not sure:
- ✓ To detect OTA producers
  - ✓ To differentiate between toxigenic and non toxigenic



# *Aspergillus* spp. section *Circumdati*

## % OTA producing isolates/strains

- *A. ochraceus*
- *A. sclerotiorum* • several strains OTA -
- *A. melleus*
- *A. ostianus*
- *A. petrakii* • inconsistent, OTA traces
- *A. persii*
- *A. sulphureus*
- *A. cretensis*
- *A. flocculosus*
- *A. pseudoelegans*
- *A. steynii*
- *A. westerdijkiae* • Approx. 100% OTA +
- *A. roseoglobulosus*
- *N. muricatus*

- ✓ HPLC
- ✓ Alkylphenone retention indices
- ✓ DAD UV-VIS



# *Aspergillus* spp.

% OTA producing isolates/strains

- *A. ochraceus* (3-54%) Abarca *et al.* 2001
- *A. carbonarius* (25-100%) Abarca *et al.* 2004
  - High % OTA producers
- *A. niger* (0.6-50%) Abarca *et al.* 2004
  - Low % OTA producers

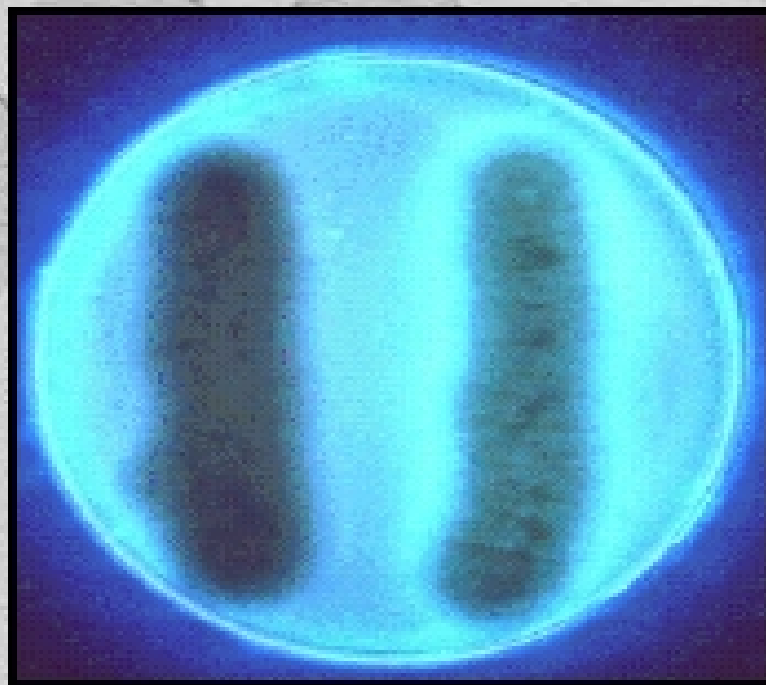


# Differential culture media: CCA

Coconut cream agar

*A. ochraceus*

NUIC118  
Non-OTA- producing  
mutant



Wild-type  
producer

O'Callaghan *et al.*, 2003

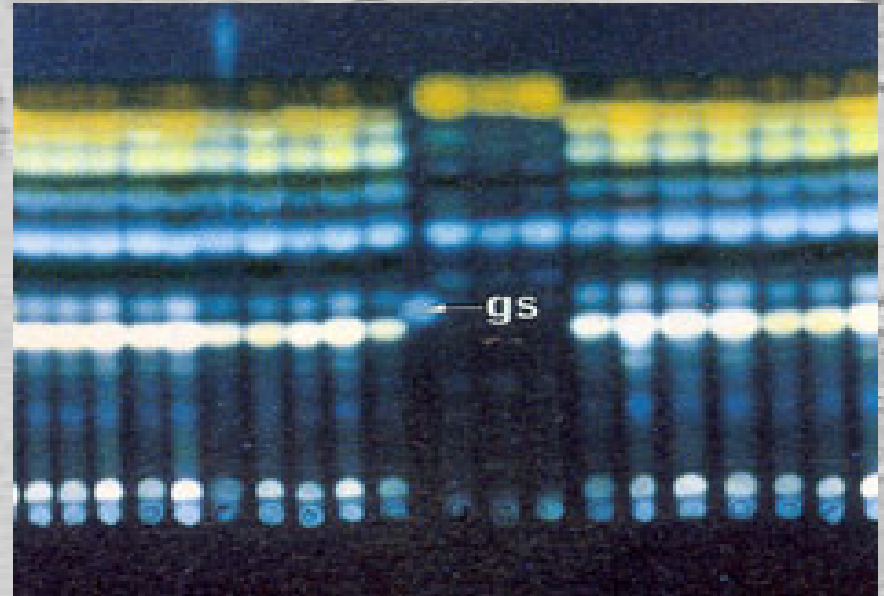
✓ Used in OTA biosynthesis studies in *A. ochraceus* (*pks*)



# Rapid chromatographic methods

## TLC

### Agar plug sampling technique



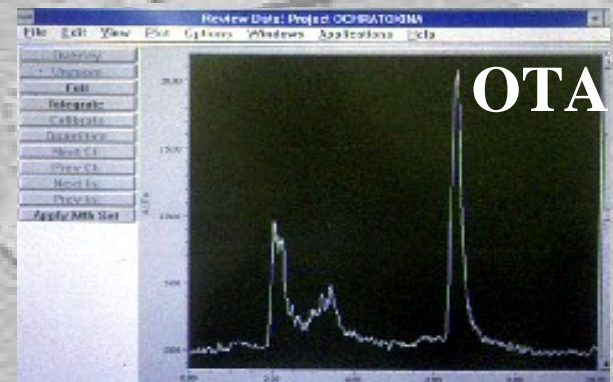
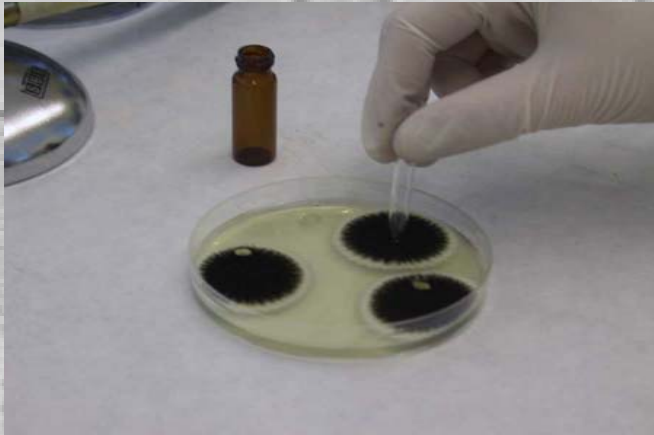
- ✓ **Agar plugs:**
  - ✓ Removed from the cultures
  - ✓ Placed on the TLC plates
  - ✓ Extraction on the plate

Filtenborg & Frisvad, 1980



# Rapid chromatographic methods

## Agar plug HPLC



**Extraction:**  
methanol, 0.5 ml  
1 hour

Bragulat *et al.*, 2001

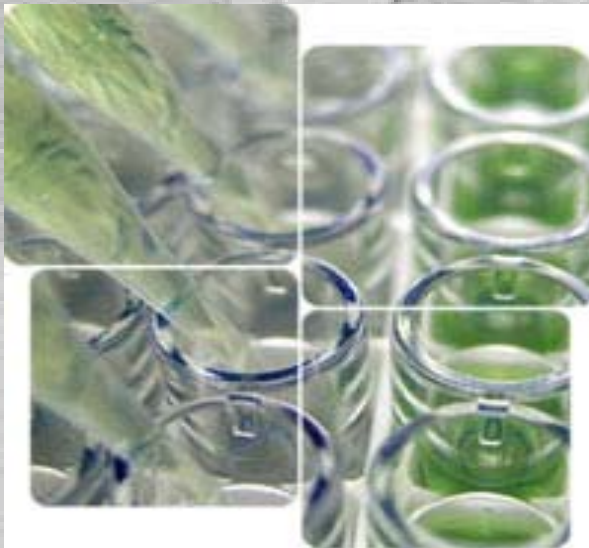
- ✓ Specifically designed for OTA-producing fungi
- ✓ Used in the screening of the project **WINE-OCHRA RISK**



# Rapid immunological methods

## ELISA

- ✓ Different commercial kits
- ✓ Microtiter plate format



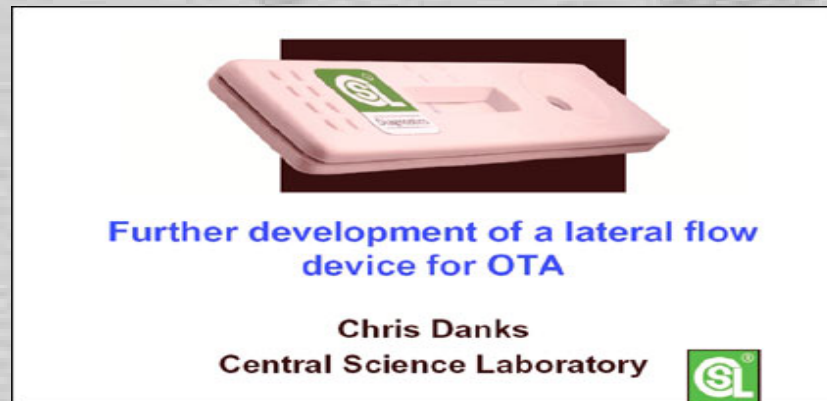
# Rapid immunological methods

## ELISA

✓ **Screening cards:** ochracard



✓ **Lateral flow devices:** agar plug



Further development of a lateral flow device for OTA

Chris Danks  
Central Science Laboratory



# Methodological problems : OTA producers

Characterization tests may vary depending on different conditions:

- ✓ **Growth conditions:**

- ✓ Temperature, water activity, pH,

- ✓ **Length of incubation period**

- ✓ **Composition of the medium**

- ✓ **Detection technique:** TLC, HPLC, ELISA,...

- ✓ Detection limit/Quantification



# *A. niger* aggregate: grapes

% OTA producing isolates/strains

- **0% (0/473) Spain** Bau *et al.* 2005
- **1.6% (20/173) Europe/Israel** Bau *et al.* 2006
- **2.4% (2/84) Tunisia** Lasram *et al.* 2007
- **2.6% (3/113) Australia** Leong *et al.* 2007
  
- **16.7% (8/48) Argentina** Da Rocha *et al.* 2002
- **30.2% (16/53) Brazil** Da Rocha *et al.* 2002
- **28% (37/132) Argentina** Ponsone *et al.* 2007



## ***A. carbonarius***: % OTA producing isolates/strains

- **100% (101/101) Spain** Bau *et al.* 2005 (CYA)
- **100% (119/119) Europe/Israel** Bau *et al.* 2005 (CYA)
- **93.6% (44/47) Tunisia** Lasram *et al.* 2007 (CYA)
- **100% (32/32) Australia** Leong *et al.* 2007 (CCA)
  
- **(not isolated) Argentina** Da Rocha *et al.* 2002
- **25% (8/32) Brazil** Da Rocha *et al.* 2002 (YES)
- **0% (0/7) Argentina** Ponsone *et al.* 2007 (YES)

**CYA better than YES**

(Bragulat *et al.* 2001)



# *A. niger*: first detected producers

Abarca *et al.* 1994

**YES agar better than CYA (Bragulat *et al.* 2001)**

(ppm)	YES agar	CYA
A 75	50.7	1.6
A136	51.7	12.3



# *A. aculeatus/A. japonicus*: grapes

% OTA producing isolates/strains

- 0% (0/5) **Spain** Bau *et al.* 2005
- 0% (0/66) **Europe/Israel** Bau *et al.* 2005
- 0% (0/1) **Tunisia** Lasram *et al.* 2007
- (not tested) **Australia** Leong *et al.* 2007
- (not isolated) **Argentina/Brazil** Da Rocha *et al.* 2002
  
- 26.2% (28/107) **Argentina** Ponsone *et al.* 2007
  - 1.2-8.6 ppm (D.L. 1 ppm)
- 25.4% (16/63) **Italy** Battilani *et al.* 2003
  - <1 ppb (D.L. 0.7 ppb)

**D.L./Quantification  
problems??**



# *Aspergillus* spp.: from figs

% OTA producing isolates/strains

- *A. ochraceus* (n:7) (0%)
- *A. melleus* (n:7) (0 %)
- *A. sclerotiorum* (n:2) (0 %)
- *A. alliaceus* (n:6) (100 %)
  - PDB: 4.2 ppm
  - YES: 11 ppm
  - HPLC-FD, D.L. = 10 ppb

**Bayman *et al.*, 2002**



# *Penicillium* spp. : endophytic from coffee plants OTA Detection limit??

Vega *et al.* 2006 (D.L. (apparatus) 0.01 ng)

- *A. ochraceus* 5,000-25,000 ppb
- *P. brevicompactum* 0.037 ppb ?
- *P. crustosum* 0.074 ppb ?
- *P. olsonii* 0.025 ppb ?
- *P. oxalicum* 0.037 ppb ?

D.L./Quantification  
problems??

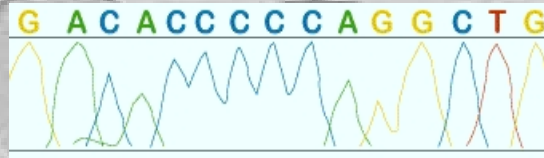
YMA (3 months)

Method: Bragulat *et al.* 2001 D.L. (10-50 ppb)



# Molecular diagnostic methods

## Main approaches

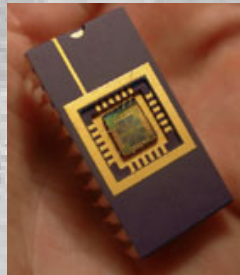
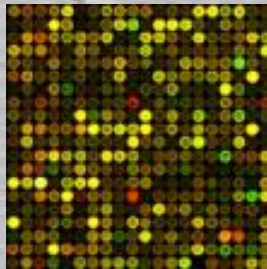


- ✓ Detection of genes involved in OTA biosynthesis
- ✓ Species specific DNA fragments of OTA producers



# Detection OTA-producing fungi

- ✓ Traditional and molecular methods are used
- ✓ Differentiate between toxigenic and non toxigenic
- ✓ Little is know about OTA genes



Future standardized methods:

- biochips, biosensors, electronic noses,...
- good accuracy, specificity, sensitivity & reproducibility

