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BELGIAN CO-ORDINATED COLLECTIONS OF MICRO-ORGANISMS  
BIOMEDICAL FUNGI AND YEASTS COLLECTION

# The use of MALDI-TOF MS in mycology

P. Becker & A. Packeu

Service of Mycology & Aerobiology, WIV-ISP

BSHAM annual meeting 19/11/2015

New Technologies in Mycology

Belgian  
Science  
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Office



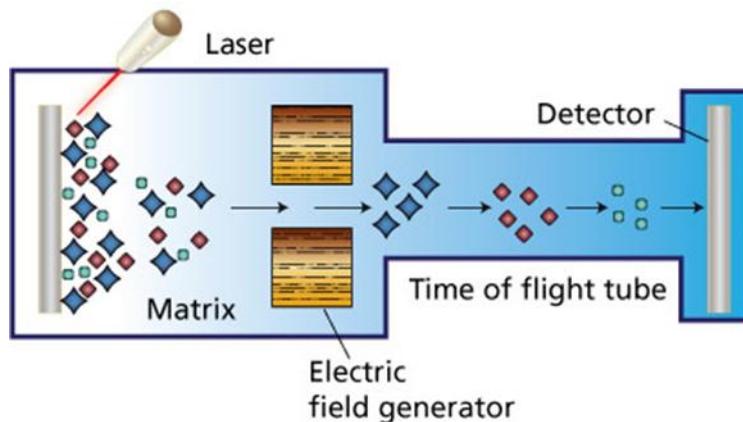
# Matrix Assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry

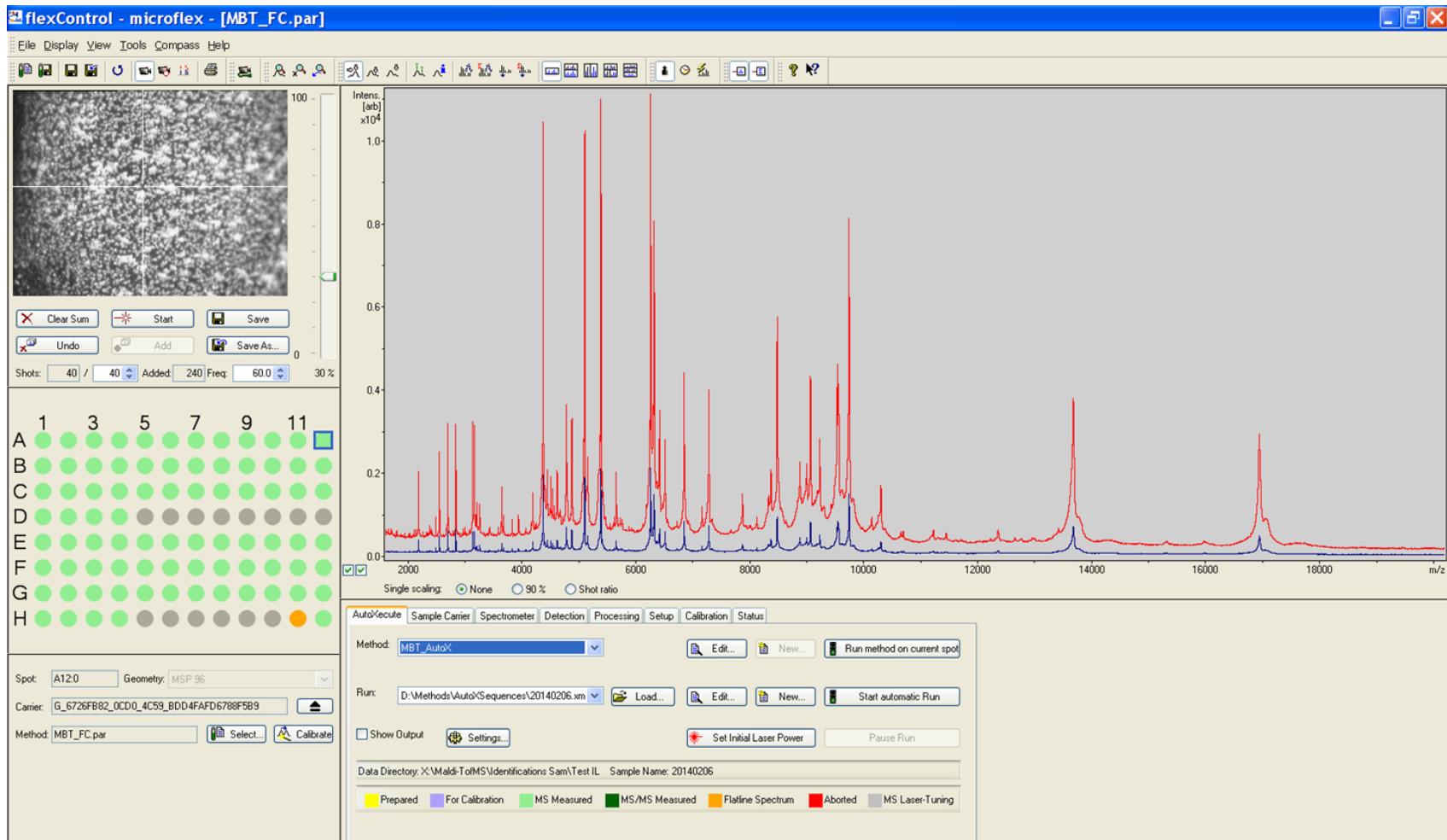


Direct deposit

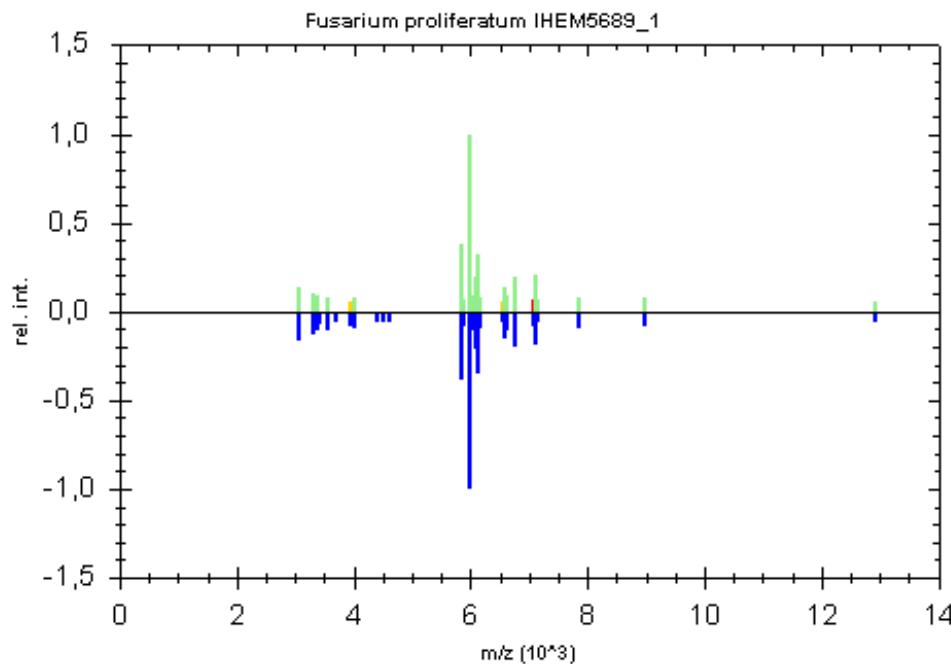


Sabouraud + Ab, 3 jours





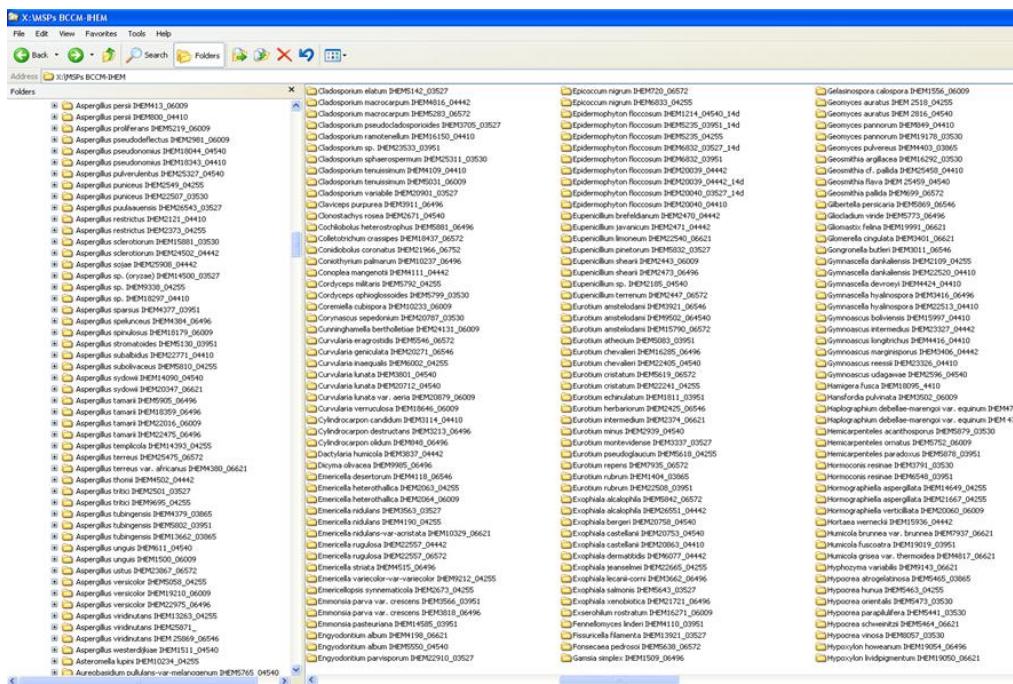
# 1. IDENTIFICATION



Detected Species	Log(Score)
Fusarium proliferatum IHEM5689_1	2,876
Fusarium proliferatum IHEM5689_3	2,580
Fusarium proliferatum IHEM5689	2,538
Fusarium proliferatum IHEM5689_4	2,372
Fusarium proliferatum IHEM9573_3	2,277
Fusarium proliferatum IHEM5689_2	2,211
Fusarium proliferatum IHEM9573	2,203
Fusarium proliferatum IHEM9880	2,094
Fusarium proliferatum IHEM9573_2	2,087
Fusarium proliferatum IHEM25667_3	2,050



# **Building of the BCCM/IHEM MALDI-TOF MS spectra database.** In collaboration with the Aix-Marseille University, CHU Timone (Prof. R. Piarroux)



Containing ca. 1200 reference spectra/strains representing ca. 600 species of moulds (including dermatophytes)



Original Article

## Identification of filamentous fungi isolates by MALDI-TOF mass spectrometry: clinical evaluation of an extended reference spectra library

Pierre T. Becker<sup>1,\*</sup>, Annelies de Bel<sup>2</sup>, Delphine Martiny<sup>3,4</sup>,  
Stéphane Ranque<sup>5,6</sup>, Renaud Piarroux<sup>5,6</sup>, Carole Cassagne<sup>5,6</sup>,  
Monique Detandt<sup>1</sup> and Marijke Hendrickx<sup>1</sup>

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Received 4 April 2014; Revised 12 August 2014; Accepted 2 September 2014

Universitair  
Ziekenhuis  
Brussel**Clinical ID****(morphology)**

- #1: *A. fumigatus*
- #2: *A. fumigatus*
- #3: *A. niger*
- #4: *S. prolificans*
- #5: *F. oxysporum*
- #6: *A. fumigatus*
- #7: *A. nidulans*

Etc.

**MALDI-TOF ID**

- #1: *A. fumigatus*
- #2: *A. fumigatus*
- #3: *A. niger*
- #4: *S. prolificans*
- #5: *F. oxysporum*
- #6: *A. fumigatus*
- #7: *A. versicolor*

Etc.

**DNA sequencing**

CHU St-Pierre | UMC St-Pieter

**Clinical ID****(morphology)**

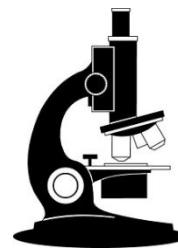
- #1: *A. fumigatus*
- #2: *A. flavus*
- #3: *R. arrhizus*
- #4: *A. versicolor*
- #5: *F. oxysporum*
- #6: *A. fumigatus*
- #7: *A. fumigatus*

**MALDI-TOF ID**

- #1: *A. fumigatus*
- #2: *A. flavus*
- #3: *R. arrhizus*
- #4: *A. versicolor*
- #5: *F. oxysporum*
- #6: *A. fumigatus*
- #7: *A. fumigatus*



390 isolates analysed



ID at genus level	94.6% correct	96.7% correct
ID at species level	61.5% correct	85.6% correct

if score  
 $>1.70$



Unless 95.4%  
correct!!!

ID limited to genus: *Fusarium* sp., *Penicillium* sp., *Mucorales*, etc.

- MALDI-TOF MS shows similar robustness than microscopy but is more accurate
- Misidentifications due to lack of discrimination for some species, quality of the spectra, etc.



## Full paper

## Quality control in culture collections: Confirming identity of filamentous fungi by MALDI-TOF MS



Pierre T. Becker<sup>a,\*</sup>, Dirk Stubbe<sup>a</sup>, Jessie Claessens<sup>a</sup>, Sam Roosens<sup>a</sup>, Yves Bastin<sup>b</sup>, Chantal Planard<sup>b</sup>, Carole Cassagne<sup>b,c</sup>, Renaud Piarroux<sup>b,c</sup>, Marijke Hendrickx<sup>a</sup>

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Mass spectrometry  
Preservation

## ABSTRACT

In culture collections, strains are controlled after preservation to guarantee their viability, purity and identity. For filamentous fungi, the identity is traditionally verified by performing morphological analyses with the support of DNA sequencing if required. These methods are particularly time consuming and require extensive knowledge of mycology. In this study, matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) was evaluated as an alternative method for fast, robust and objective identity controls in the routine work of the BCCM/IHEM fungal collection. A total of 481 controls were carried out using mass spectrometry and compared to the results obtained by the conventional methods. The overall performance of the MALDI-TOF MS reached 84% correct identification at species level. Moreover, a reference database in the collection was put in evidence for 14 strains by mass spectrometry and confirmed by DNA sequencing. Out of these, only eight were detected by the traditional method. Considering these results, a workflow combining MALDI-TOF MS, microscopy and genetic analyses is proposed to speed up and objectify identity controls in fungal culture collections.

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## Matrix-assisted laser desorption ionization time-of-flight mass spectrometry: revolutionizing clinical laboratory diagnosis of mould infections

M. Gautier<sup>1</sup>, S. Ranque<sup>1,2</sup>, A.-C. Normand<sup>1</sup>, P. Becker<sup>3</sup>, A. Packeu<sup>1</sup>, C. Cassagne<sup>1</sup>, C. L'Offillier<sup>1</sup>, M. Hendrickx<sup>2</sup> and R. Piarroux<sup>1,2</sup>  
<sup>1</sup> Parasitology & Mycology, Assistance Publique-Hôpitaux de Marseille, CHU Timone-Adults, <sup>2</sup> Aix-Marseille University, UMR MDS IP-TFT, Marseilles, France and <sup>3</sup> Mycology and Aerobiology Section, BCCM/IHEM, Scientific Institute of Public Health, Brussels, Belgium

## Abstract

The clinical diagnosis of mould infections currently involves complex species identification based on morphological criteria, which is often prone to error. Employing an extensive mould species reference spectral library (up to 2832 reference spectra, corresponding to 708 strains from 347 species), we assessed the extent to which matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) enhanced the accuracy of species identification. MALDI-TOF MS data were validated against morphology-based and DNA sequence-based results with 262 clinical isolates collected over a 4-month period in 2013. The implementation of MALDI-TOF MS resulted in a dramatic improvement in mould identification at the species level (from 78.2% to 98.1%) and a marked reduction in the misidentification rate (from 9.8% to 1.2%). We then compared the mould identification results obtained before (i.e. 2011) and after (i.e. 2013) the implementation of MALDI-TOF MS in routine identification procedures, which showed an improvement from 64.5% to 100%. Reassessment of a set of isolates from 2011 with this procedure, including MALDI-TOF MS, yielded an increase in species diversity from 16 to 42 species. Finally, application of this procedure during a 16-month period (2012–2013) enabled the identification of 1094 of 1107 (96.8%) clinical mould isolates corresponding to 107 distinct species. MALDI-TOF MS-based mould species identification may soon challenge traditional techniques in the clinical laboratory, as patient prognosis is largely contingent on rapid and accurate diagnosis.

Keywords: Fungi, humans, mass, matrix-assisted laser desorption ionization time-of-flight, microbiological techniques, mycoses, spectrometry  
Original Submission: 6 March 2014; Revised Submission: 20 May 2014; Accepted: 27 June 2014  
Editor: E. Rollides  
Article published online: 4 July 2014  
Clin Microbiol Infect 2014; 20: 1366–1371  
10.1111/1469-0591.12750



## Fast and Accurate Identification of Dermatophytes by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry: Validation in the Clinical Laboratory

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Scientific Institute of Public Health, Service of Mycology and Aerobiology, Brussels, Belgium<sup>a</sup>; Department of Microbiology and Infection Control, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel (VUB), Brussels, Belgium<sup>b</sup>; Aix Marseille Université, IP-TPT UMR MDS, Marseille, France<sup>c</sup>; Parasitology and Mycology Laboratory, CHU Timone-adultes, APHM, Marseille, France<sup>d</sup>; BCCM/IHEM Fungal Collection, Scientific Institute of Public Health, Mycology and Aerobiology Section, Brussels, Belgium<sup>e</sup>

The performance of a matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) workflow using an extensive reference database for dermatophyte identification was evaluated on 176 clinical strains. Using a direct-deposit procedure after 3 incubation days yielded 40% correct identification. Both increasing incubation time and using an extraction procedure resulted in 100% correct identification.

Dermatophytes, which invade and infect keratinized tissues, affect a large proportion of the population, and over \$500,000,000 per year is spent on antifungal drugs (1). This specific group of filamentous fungi can be separated in three anam-

scribed for molds (27) was applied and main spectra (MSP) were created using the MSP creation function of the Maldi Biotype software. Instrument calibration was performed with a bacterial test standard (BTS; Bruker Daltonics) on a MicroFlex (Bruker



Original article

## MALDI-TOF mass spectrometry identification of filamentous fungi in the clinical laboratory

Stéphane Ranque,<sup>1,2</sup> Anne-Cécile Normand,<sup>1</sup> Carole Cassagne,<sup>1,2</sup> Jean-Benjamin Murat,<sup>3,4</sup> Nathalie Bourgeois,<sup>5</sup> Frédéric Dalle,<sup>6</sup> Martine Gari-Toussaint,<sup>7</sup> Patrick Fourquet,<sup>8</sup> Marijke Hendrickx<sup>9</sup> and Renaud Piarroux<sup>1,2</sup>

<sup>1</sup> UMR MDS, Aix-Marseille Université, Marseille, France, <sup>2</sup>Laboratoire de Parasitologie-Mycologie, APHM Timone, Marseille, France, <sup>3</sup>Parasitologie-Mycologie, CHU de Grenoble, Grenoble, France, <sup>4</sup>UMR 5163 CNRS, Université Joseph Fourier Grenoble I, Grenoble, France, <sup>5</sup>Parasitologie-Mycologie, CHU de Montpellier et de CHU de Nîmes, Université Montpellier I, Montpellier, France, <sup>6</sup>Parasitologie-Mycologie, Hôpital du Bocage, Université de Bourgogne, Dijon, France, <sup>7</sup>Parasitologie-Mycologie, Hôpital Archet 2, Nice, France, <sup>8</sup>Service Protéomique, Centre d'Immunologie de Marseille Luminy, Marseille, France and <sup>9</sup>Mycologie & Aerobiologie, BCCM/IHEM, WIV-ISP, Brussels, Belgique

## Summary

This study aimed to validate the effectiveness of a standardised procedure for the MALDI-TOF mass spectrometry (MS)-based identification on a large sample of filamentous fungi routinely identified in university hospitals' laboratories. Non-dermatophyte filamentous fungi prospectively isolated in the routine activity of five teaching hospitals in France were first identified by conventional methods in each laboratory and then by MS in one centre. DNA sequence-based identification resolved discrepancies between both methods. In this study, of the 625 analysed filamentous fungi of 58 species, 501 (80%) and 556 (89%) were correctly identified by conventional methods and MS respectively. Compared with the conventional method, MS dramatically enhanced the performance of the identification of the non-*Aspergillus* filamentous fungi with a 31–61% increase in correct identification rate. In conclusion, this study on a large sample of clinical filamentous fungi taxa demonstrates that species identification is significantly improved by MS compared with the conventional method. The main limitation is that MS identification is possible only if the species is included in the reference spectra library. Nevertheless, for the routine clinical laboratory, MS provides the means to attain markedly accurate results in filamentous fungi identification, which was previously restricted to only a few reference laboratories.



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## Espace de gestion

[Identification d'échantillons](#)[Mes rapports d'identification](#)[Statistiques sur les identifications](#)[Matrice de distances \(Asp. F\)](#) IDENTIFICATION D'ÉCHANTILLONS

## Sélectionner l'organisme

- Leishmanies
- Filamenteux
- Levures
- Bactéries

## Sélectionner le fichier zip

 Parcourir ...

La taille du fichier doit être inférieure à 50 Mo

Cette plateforme a été conçue et réalisée par:

Le Professeur **Renaud PIARROUX**, Directeur du Laboratoire de Parasitologie et Mycologie de l'Assistance Publique-Hôpitaux de Marseille

Anne-Cécile NORMAND, PhD, Ingénierie de recherche en Mycologie

Farid DJENAD, Ingénieur en Informatique

Pour plus d'informations veuillez contacter le Professeur PIARROUX à l'adresse suivante:

[Renaud.PIARROUX@ap-hm.fr](mailto:Renaud.PIARROUX@ap-hm.fr)

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Espace de gestion

Identification d'échantillons

Mes rapports d'identification

Statistiques sur les identifications

Matrice de distances (Asp. F)

## Q IDENTIFICATION D'ÉCHANTILLONS

Sélectionner l'organisme

- Leishmanies
- Filamenteux
- Levures
- Bactéries

**Un organisme doit être sélectionné**

Sélectionner le fichier zip

 13\_908F.zip  Supprimer  Démarrer  Parcourir ...

La taille du fichier doit être inférieure à {0} Mo

Cette plateforme a été conçue et réalisée par:  
Le Professeur **Renaud PIARROUX**, Directeur du Laboratoire de Parasitologie et Mycologie de l'Assistance Publique-Hôpitaux de Marseille  
**Anne-Cécile NORMAND**, PhD, Ingénierie de recherche en Mycologie  
**Farid DJENAD**, Ingénieur en Informatique

Pour plus d'informations veuillez contacter le Professeur PIARROUX à l'adresse suivante:  
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Espace de gestion

Identification d'échantillons

Mes rapports d'identification

Statistiques sur les identifications

Matrice de distances (Asp. F)

## RAPPORT D'IDENTIFICATION

Service	BCCM
Crée Le	November 6, 2015 9:27:43 AM CET
Fichier	13_908F.zip
Critères	Filamenteux

Rechercher les mélanges  Nouvelle Identification

### LISTE DES RÉSULTATS

ÉCHANTILLON	PICS	RÉFÉRENCE	S(%)	RÉFÉRENCE PROCHE	S(%)	RÉFÉRENCE NON PROCHE	S(%)	ACTIONS
20130617:0_H5	177	Scopulariopsis brevicauli ...	47.18	Scopulariopsis candida	15.27	Fusarium anthophilum	14.68	
20130617:0_H6	177	Scopulariopsis brevicauli ...	47.83			Fusarium anthophilum	16.03	
20130617:0_H7	176	Scopulariopsis brevicauli ...	45.74	Scopulariopsis candida	16.49	Fusarium anthophilum	16.33	
20130617:0_H8	180	Scopulariopsis brevicauli ...	44.92	Scopulariopsis acremonium ...	16.08	Trichophyton tonsurans-Tr ...	15.11	



## 2. Drug resistance detection

### MALDI-TOF MS-based drug susceptibility testing of pathogens: The example of *Candida albicans* and fluconazole

Marinach et al. 2009

Carine Marinach<sup>1,2\*</sup>, Alexandre Alanio<sup>1,2,3\*</sup>, Martine Palous<sup>3</sup>, Stéphanie Kwasek<sup>1,2</sup>, Arnaud Fekkar<sup>1,2,3</sup>, Jean-Yves Brossas<sup>1,2,4</sup>, Sophie Brun<sup>1,2,3</sup>, Georges Snounou<sup>1,2,5</sup>, Christophe Hennequin<sup>1,2</sup>, Dominique Sanglard<sup>6</sup>, Annick Datury<sup>3</sup>, Jean-Louis Golmard<sup>7\*</sup> and Dominique Mazier<sup>1,2,3\*</sup>

#### MPCC: minimal profile change concentration

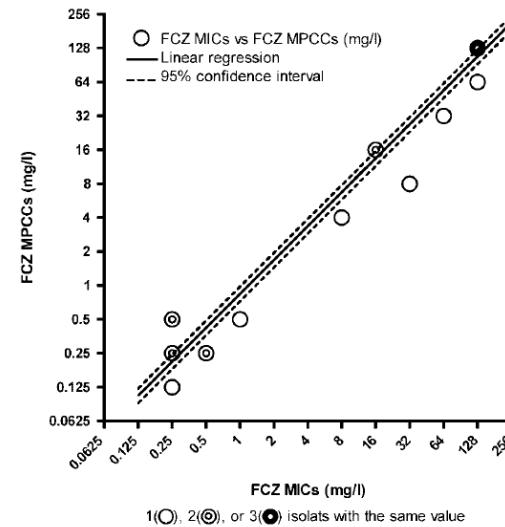
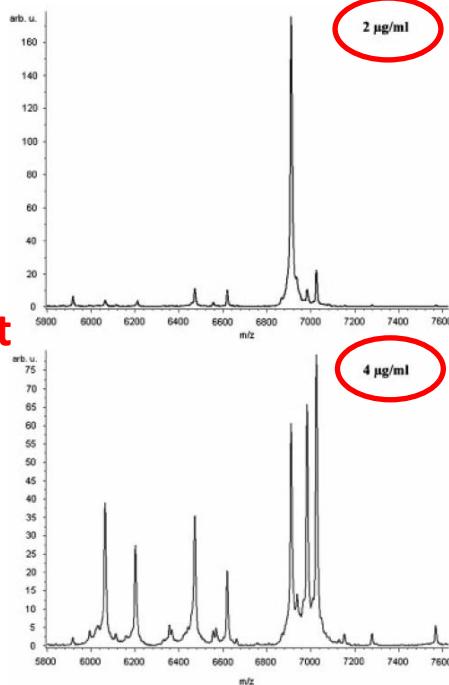


Figure 2. Correlation between MICs evaluated by the CLSI methodology and MPCCs determined by our MALDI-TOF-MS method (regression line). Corresponding values and characteristics of each strain are listed in Table 1.

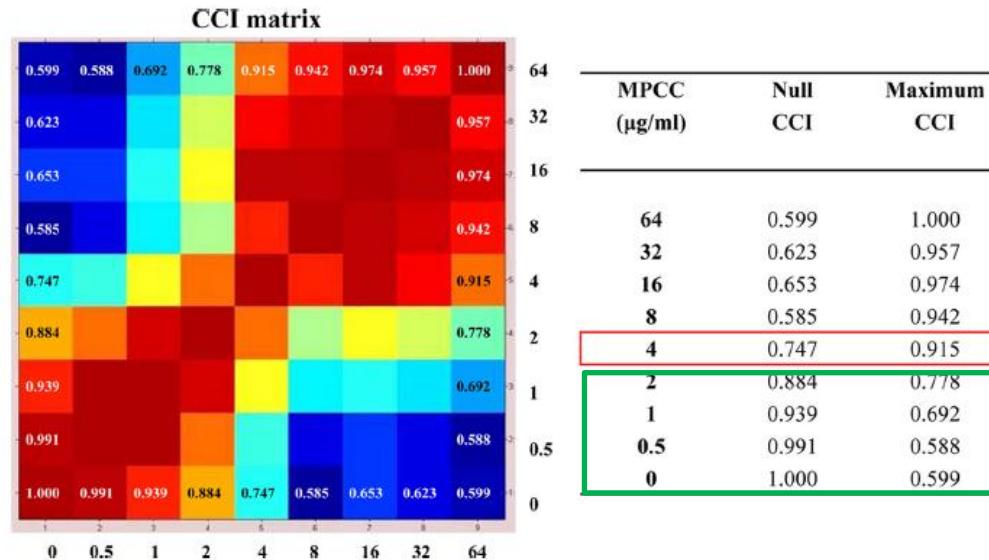
## Use of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for Caspofungin Susceptibility Testing of *Candida* and *Aspergillus* Species

De Carolis et al. 2012

Elena De Carolis,<sup>a</sup> Antonietta Vella,<sup>a</sup> Ada R. Florio,<sup>a</sup> Patrizia Posteraro,<sup>b</sup> David S. Perlin,<sup>c</sup> Maurizio Sanguinetti,<sup>a</sup> and Brunella Posteraro<sup>d</sup>

Institute of Microbiology<sup>a</sup> and Institute of Hygiene<sup>d</sup> Università Cattolica del Sacro Cuore, Rome, Italy; Clinical Laboratory, Ospedale San Carlo, Rome, Italy<sup>b</sup>; and Public Health Research Institute, New Jersey Medical School, UMDNJ, Newark, New Jersey, USA<sup>c</sup>

**CCI = composite correlation index**



More related  
to Maximum  
CCI

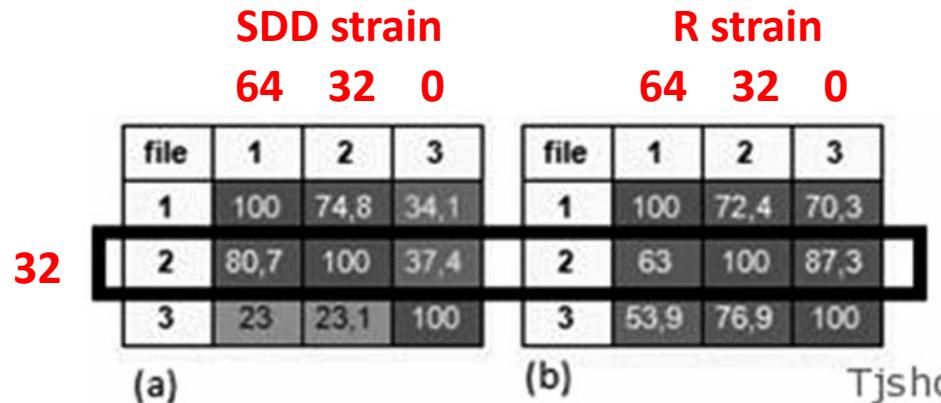


Original Article

## Detection of triazole resistance among *Candida* species by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS)

Saracli et al. 2015

Mehmet A. Saracli<sup>1,\*</sup>, Annette W. Fothergill<sup>2</sup>, Deanna A. Sutton<sup>2</sup>  
and Nathan P. Wiederhold<sup>2</sup>



**Figure 1.** Representative composite correlation index (CCI) matrixes derived from selected mass spectra. (a) A fluconazole (FLU) susceptible (MIC of 16 µg/ml) *C. glabrata* strain. The CCI value of the spectra at 64 and 32 µg/ml was higher than the CCI value of the spectra at 32 µg/ml and 0 µg/ml (i.e., 80.7 > 37.4). (b) A fluconazole (FLU) resistant (MIC of >64 µg/ml) *C. glabrata* strain. The CCI value of the spectra at 64 and 32 µg/ml was lower than the CCI value of the spectra at 32 and 0 µg/ml (i.e., 63 < 87.3). In the matrixes, the numbers 1, 2, and 3 denote the highest drug concentration (64 µg/ml), mid-range drug concentration (32 µg/ml), and "null" concentration (0 µg/ml) of FLU, respectively.

## Drug resistance detection by MALDI-TOF MS



Technically  
possible



High workload

Interesting compared to standard reference methods  
(EUCAST, CLSI)???

Eur J Clin Microbiol Infect Dis (2012) 31:2919–2928  
DOI 10.1007/s10096-012-1642-6

ARTICLE

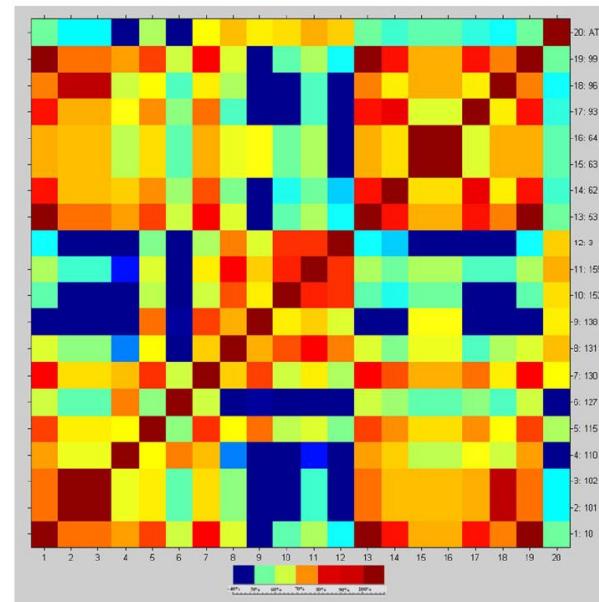
## 3. Strain typing

**MALDI-TOF mass spectrometry and microsatellite markers to evaluate *Candida parapsilosis* transmission in neonatal intensive care units**

G. Pulcrano • E. Rossetto • V. D. Iula • D. Panellis •  
F. Rossano • M. R. Catania

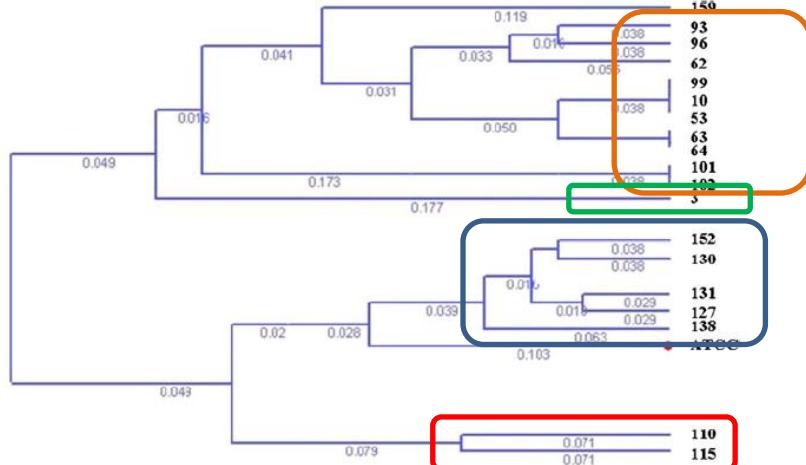
Fig. 3 Composite correlation index (CCI) of the 19 *C. parapsilosis* isolates and the ATCC strain. Different colors indicate the correlation distance

**19 strains over a 4 years period**

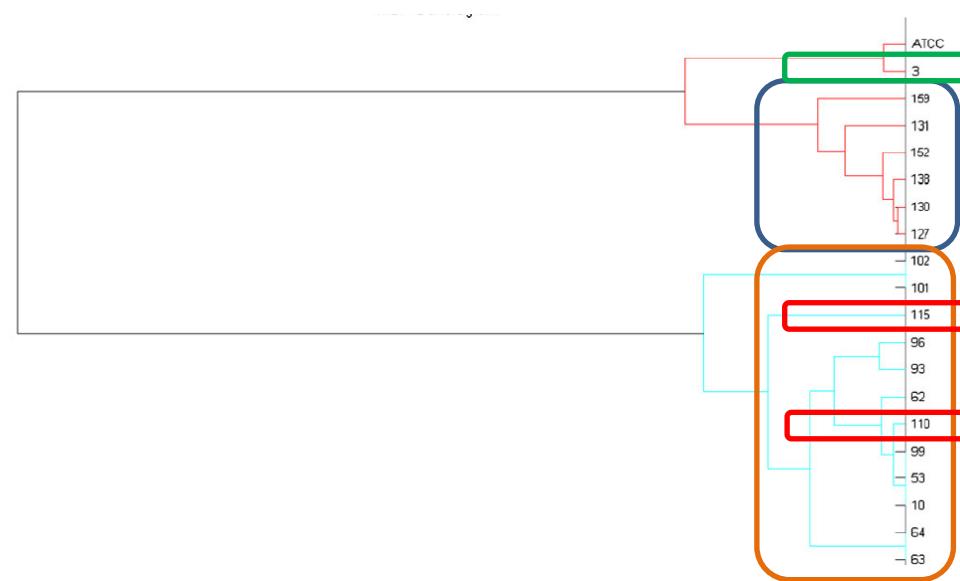


Pulcrano et al. 2012

## Microsatellite markers

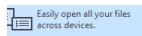


## MALDI-TOF MS



2007-2009

2010-2011



## Research article

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**Journal of  
MASS  
SPECTROMETRY**

Published online in Wiley Online Library

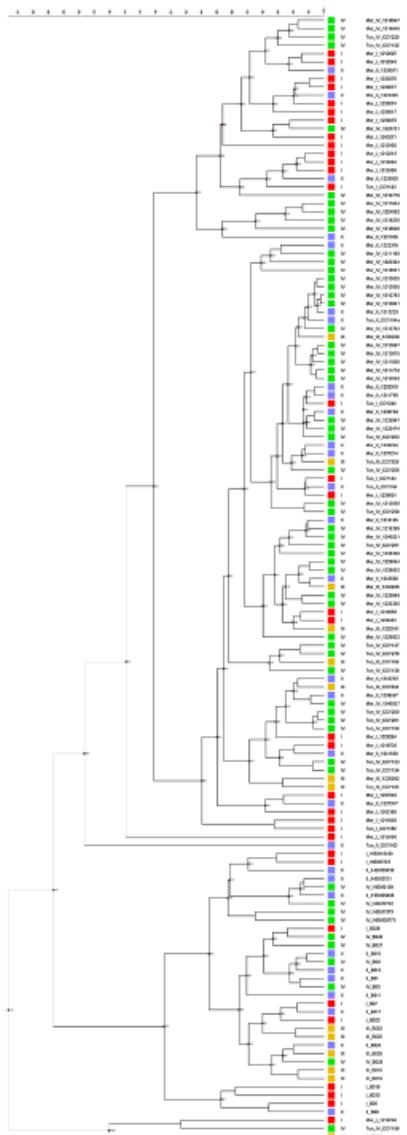
(wileyonlinelibrary.com) DOI 10.1002/jms.3538

**Comparison of MALDI-TOF mass spectra with microsatellite length polymorphisms in *Candida albicans***

C. Dhibe,<sup>a</sup> A. C. Normand,<sup>b</sup> C. L'Ollivier,<sup>b,c</sup> M. Gautier,<sup>b,c</sup> K. Vranckx,<sup>d</sup>  
D. El Euch,<sup>e</sup> E. Chaker,<sup>f</sup> M. Hendrickx,<sup>g</sup> F. Dalle,<sup>h</sup> N. Sadfi,<sup>a</sup> R. Piarroux<sup>b,c</sup>  
and S. Ranque<sup>b,c,\*</sup>

**Dhibe et al. 2014**

- Reference database (32 strains) with representatives of the 4 genetic clades (I, II, III, IV)
- 102 clinical isolates compared to the reference database

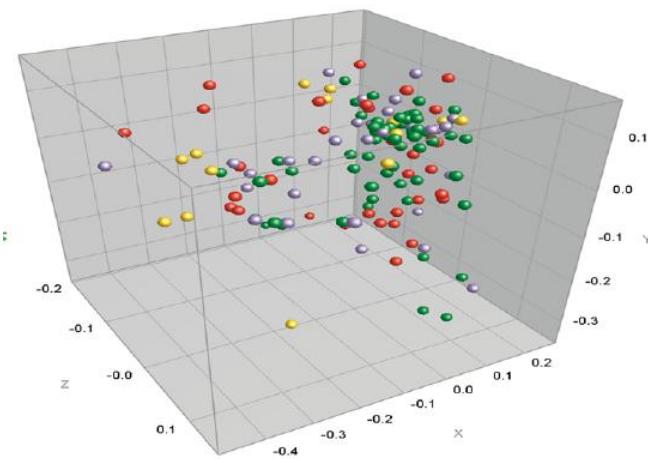


Clade I

Clade II

Clade III

Clade IV



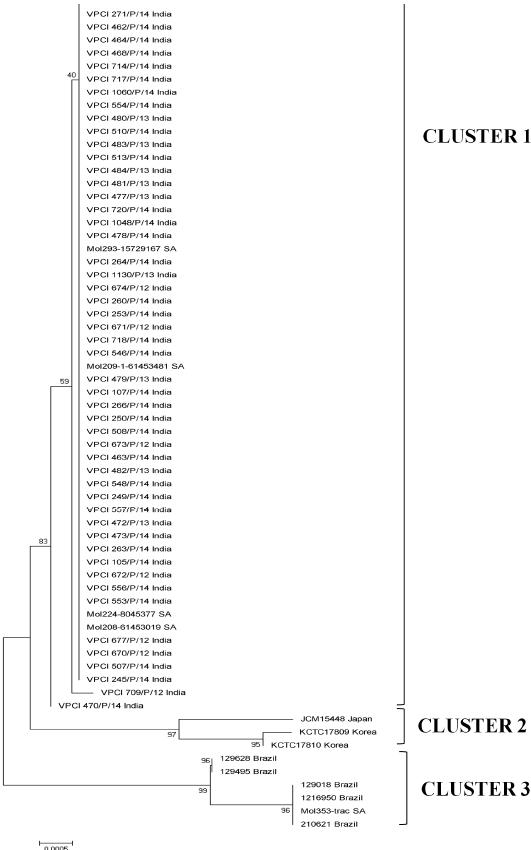
**Table 4.** Distribution of the tested isolates according to genetic clade and best match reference strain clade

Clade n (%)	<i>Best match references</i>				Total
	I	II	III	IV	
Tested isolates					
I	10 (38.5)	13(50.0)	1 (3.9)	2 (7.7)	26 (25.5)
II	4 (20.0)	7 (35.0)	0 (0.0)	9 (45.0)	20 (19.61)
III	3 (37.5)	2 (25.0)	0 (0.0)	3 (37.5)	8 (7.8)
IV	11 (22.9)	7 (14.6)	2 (4.2)	28 (58.3)	48 (47.1)
Total	28 (27.5)	7 (28.4)	3 (2.9)	42 (41.2)	102 (100.0)

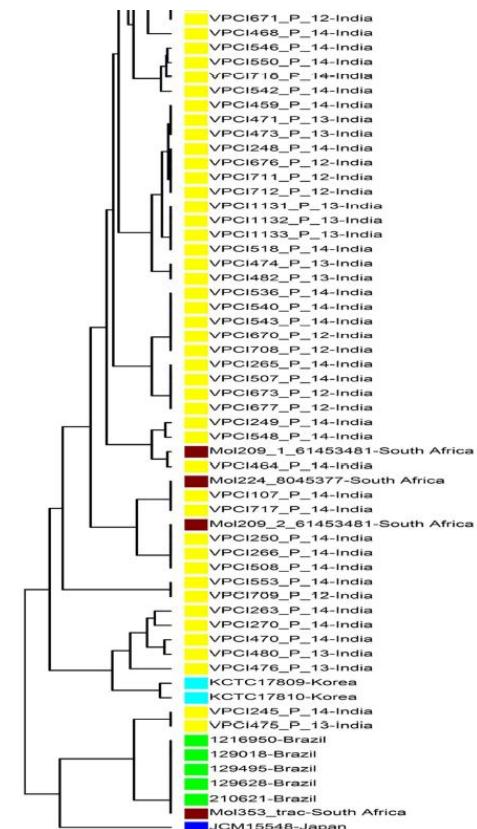
45% matching between isolates and the reference spectra

A. Prakash et al. 2015, Clinical Microbiology and Infection, in press  
 doi: 10.1016/j.cmi.2015.10.022

## 104 *Candida auris* isolates from India, Brazil and South Africa, Korea, Japan



AFLP



MALDI-TOF MS

## Take home message

### The use of MALDI-TOF MS in mycology:

- For species identification



- For drug resistance detection



- For strain typing



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