22nd ECCMID

Diagnosing fungal diseases: future perspectives

London, April 1st, 2012

MALDI-TOF in the clinical routine?

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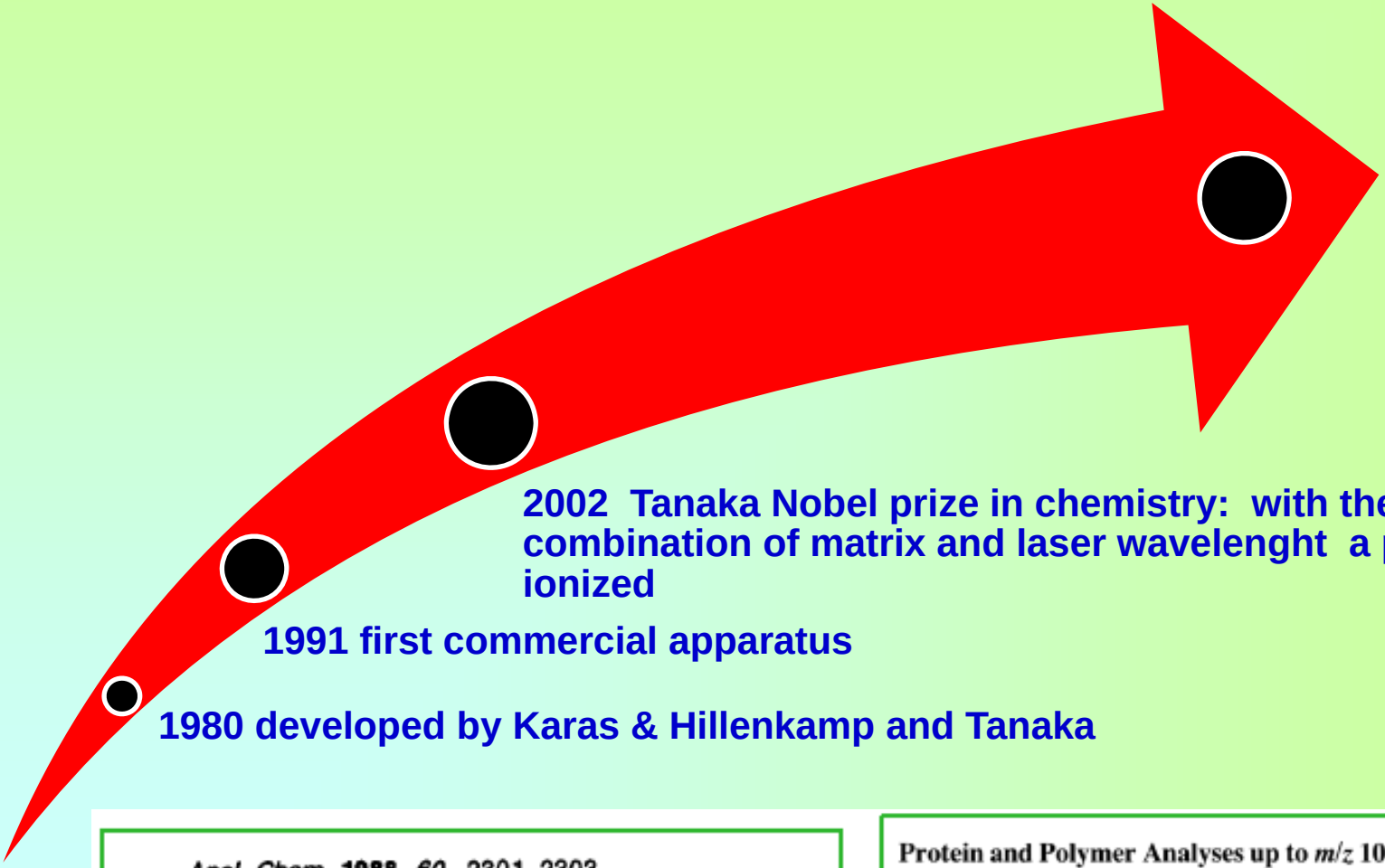
Mass spectrometry: old method…



New applications

2002 Tanaka Nobel prize in chemistry: with the proper combination of matrix and laser wavelenght a protein can be ionized

1991 first commercial apparatus

1980 developed by Karas & Hillenkamp and Tanaka

Anal. Chem. **1988**, *60*, 2301–2303

Laser Desorption Ionization of Proteins with Molecular Masses Exceeding 10 000 Daltons

Michael Karas*
Franz Hillenkamp

Protein and Polymer Analyses up to *m/z* 100 000 by Laser Ionization Time-of-flight Mass Spectrometry

Koichi Tanaka†, Hiroaki Waki, Yutaka Ido, Satoshi Akita, Yoshikazu Yoshida and Tamio Yoshida

Shimadzu Corporation, Nishinokyo-Kuwabaracho, Nakagyo-ku, Kyoto 604, Japan

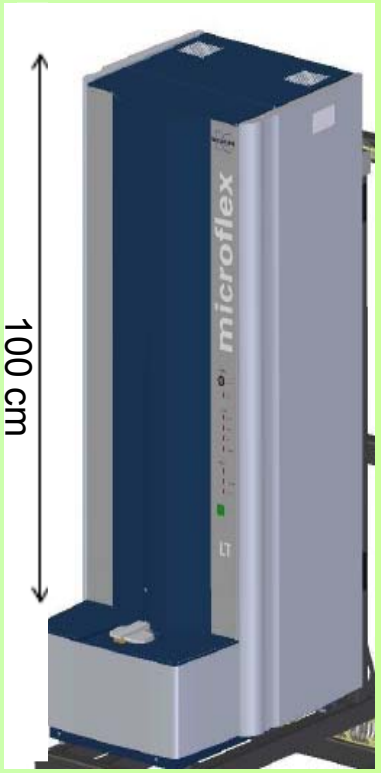
RAPID COMMUNICATIONS IN MASS SPECTROMETRY, VOL. 2, NO. 8, 1988 151

MALDI-TOF MS

- **Matrix-Assisted Laser Desorption Ionization (MALDI)**

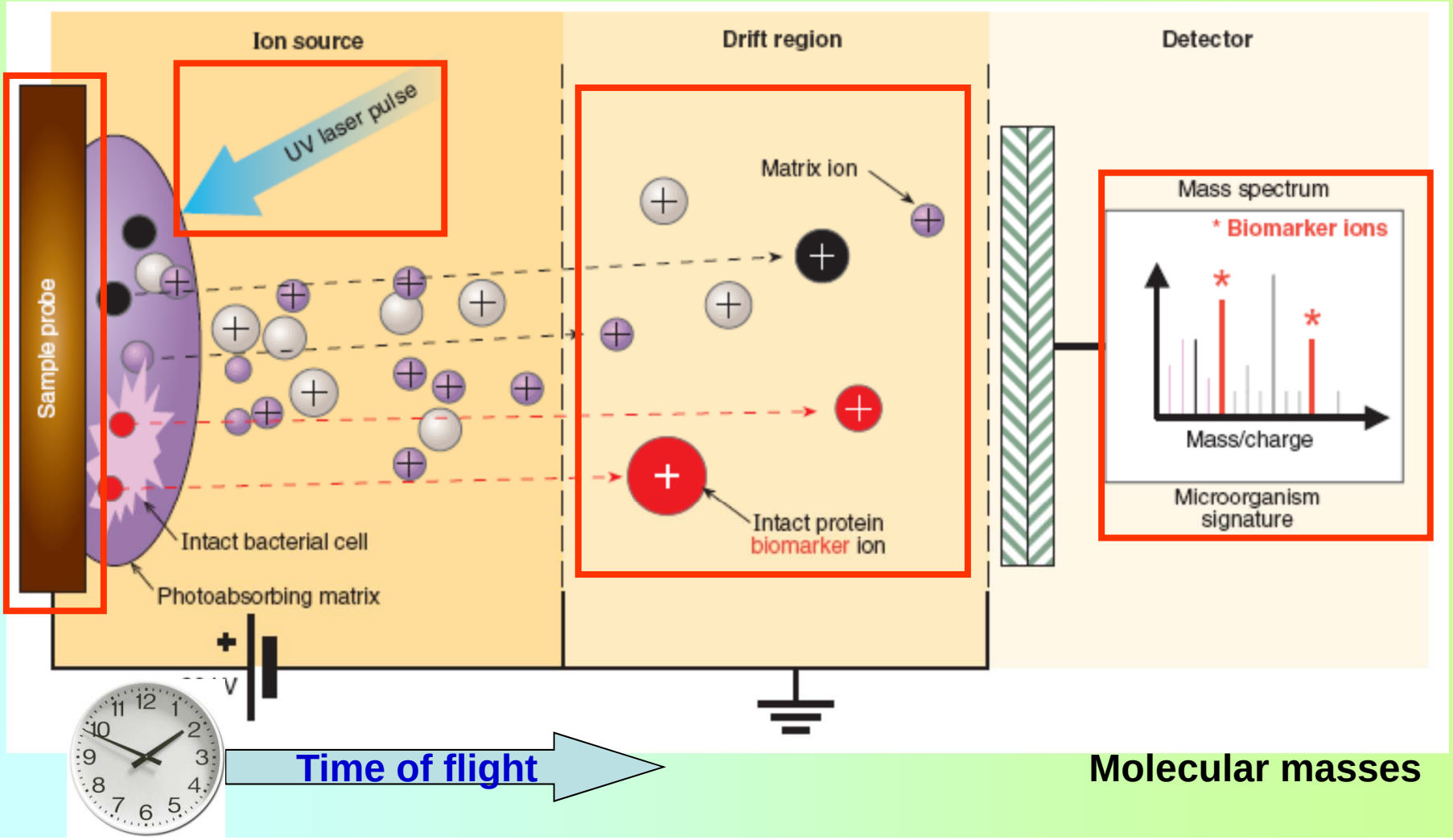
with analysis of

- **Time-Of-Flight (TOF)**



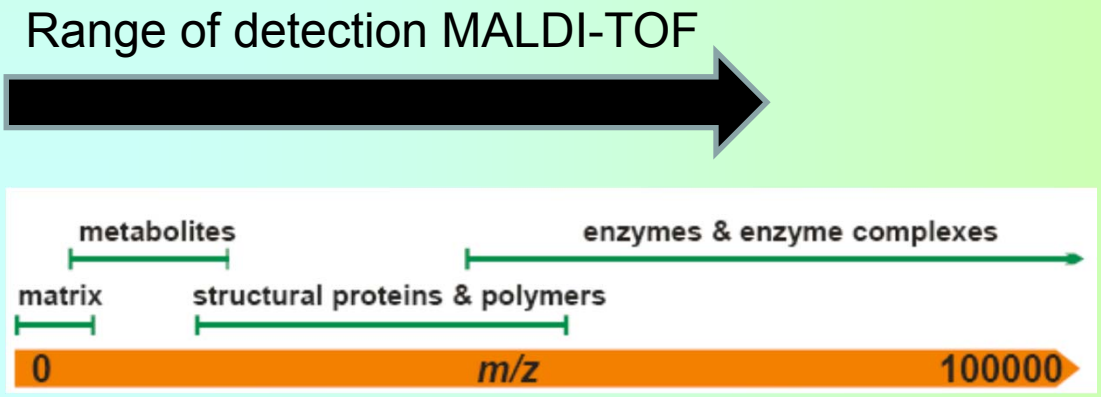
The advantage offered by the MALDI-TOF combination is the capability to easily desorb and analyze positive as well as negative ions from the same sample

Principle of operation of a MALDI-TOF spectrometer for microorganism identification



Biomarkers

- **Cellular compounds detected**
  - ✓ mostly ribosomal proteins or DNA-binding proteins, but also complex lipids and polysaccharides
- **Proteins detected**
  - ✓ extractable, soluble, moderately hydrophilic, stable, and abundant
- **Determination of protein mass signal intensities**
  - ✓ favored by abundance, stability, aminoacid composition (e.g. Arg and Lys)



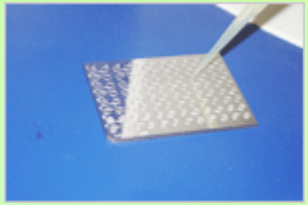
MALDI-TOF for the identification and classification of microorganisms needs dedicated software [e.g., BioTyper (Bruker Daltonics Inc.) or Saramis (AnagnosTec GmbH)] to enable comparisons of the unknown protein with reference molecular masses. Ribosomal proteins are used normally as reference molecular masses as they are most abundant in the cells.

In contrast to bacteria, fungal cell walls are mainly composed of polysaccharides (including chitin), but proteins, lipids, polyphosphates, and inorganic ions are also present. Proteins provide the most characteristics biomarkers available for the analysis of intact organisms without extraction, separation, or amplification.

“Intact” refers to microbial cells suspended in a solution and/or deposited directly onto a the sample holder. While exposure to water, organic solvent, and/or strong acid in the MALDI matrix lyses most vegetative bacteria, a lysis step, such as exposure of the fungus to a strong organic acid, is required to obtain a MALDI spectrum.

YEASTS protocol

Extraction mandatory



Formic acid 70%
Acetonitrile 100%

EtOH 70%

Direct deposition of samples on target plates at the bench

Processing by MALDI-TOF MS

Valid and accurate result

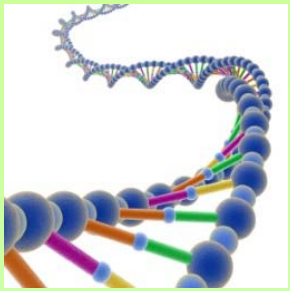
Interpretation and validation of identification

Clinical result

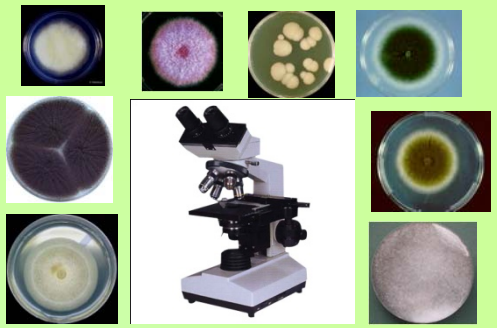
Non-reliable or doubtful result

Protein extraction under chemical hood

From: Bizzini and Greub, CMI, 2010



Molecular
(*ITS*, *β-TUB*, *CAM*, *EF-1α*)

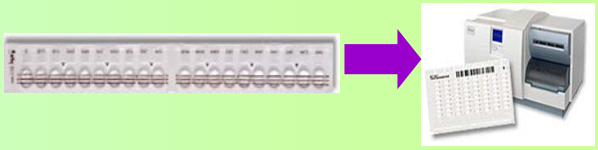


Morphology
(colony & micromorphology)

It could take 48 h to identification

SPECIES IDENTIFICATION

1990 2000



Biochemical metabolic capacities

MASS SPECTROMETRY

?

MALDI-TOF MS applications

- **Clinical isolates identification**
- **Direct identification of pathogens in clinical samples**
- **Subtyping**
- **Drug susceptibility testing ?**

MALDI-TOF Identification of Yeasts: Which is the Reality?

Study	No. of isolates/species	% of isolates identified
Marklein et al. 2009	267 isolates 25 species	92.5
Van Veen et al. 2010	61 isolates 12 species	85.2
Bizzini et al. 2010	24 isolates 4 species	100
Stevenson et al. 2010	194 isolates 23 species	87.1 (99)[a]
Bader et al. 2010	1192 isolates 36 species	97.6
Dhiman et al. 2011	138 isolates 14 species	92 .0 (96.3)[a]

[a]Using a score threshold of ≥1.8

- **Reproducible and accurate, with low consumable costs and minimal preparation time**
- **Several closely related species (e.g., *Candida* 'psilosis' or *Candida glabrata*/*bracarensis*) could be resolved by MALDI-TOF MS, but not by a biochemical approach**
- **5 min of hands-on time per identification**
- **$ 0.50 per sample**

A matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS)-based method for discrimination between molecular types of *Cryptococcus neoformans* and *Cryptococcus gattii*

Brunella Posteraro,[1†] Antonietta Vella,[2†] Massimo Cogliati,[3] Elena De Carolis,[2] Ada Rita Florio,[2] Patrizia Posteraro,[4] Maurizio Sanguinetti,[2*] and Anna Maria Tortorano[3]

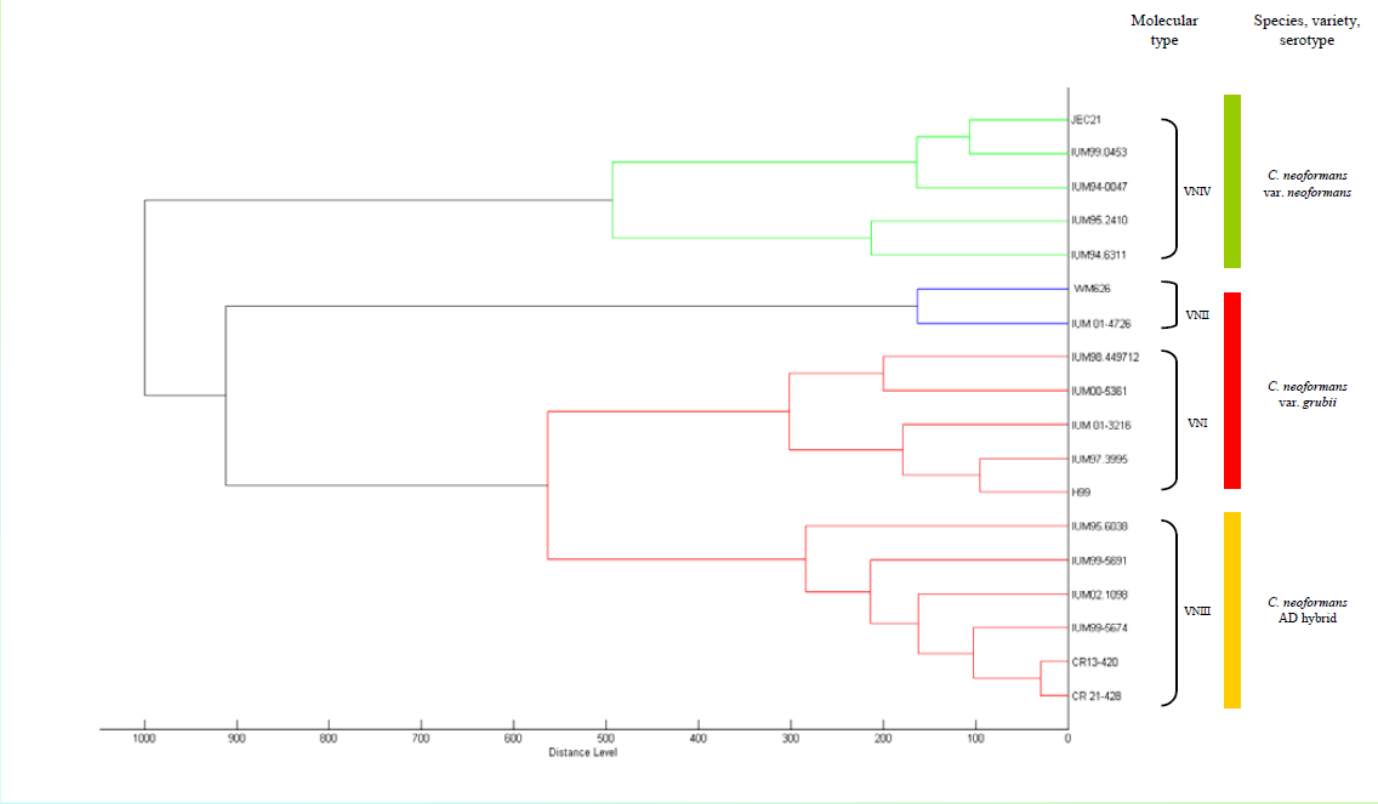
TABLE 1. Comparison between identification results obtained by MALDI-TOF MS analysis and DNA-based methods for 82 challenge and 25 reference isolates of *C. neoformans* and *C. gattii*

Isolate	Molecular characterization			MALDI-TOF MS		
	Species	Mating-type allele	Molecular type	Species	Molecular type	Log(score)
IUM[a] 97-4877	*C. neoformans*	αA	VNI	*C. neoformans*	VNI	2.559
IUM 98-3592	*C. neoformans*	αA	VNI	*C. neoformans*	VNI	2.355
IUM 97-4515	*C. neoformans*	αA	VNI	*C. neoformans*	VNI	2.308
IUM 98-0977	*C. neoformans*	αA	VNI	*C. neoformans*	VNI	2.437
IUM 98-2450	*C. neoformans*	αA	VNI	*C. neoformans*	VNI	2.489
IUM 98-4519	*C. neoformans*	αA	VNI	*C. neoformans*	VNI	2.237
IUM 98-4640	*C. neoformans*	αA	VNI	*C. neoformans*	VNI	2.350
IUM 99-1838	*C. neoformans*	αA	VNI	*C. neoformans*	VNI	2.271
CR 38	*C. neoformans*	αA	VNI	*C. neoformans*	VNI	2.664
CR 40	*C. neoformans*	αA	VNI	*C. neoformans*	VNI	2.498
CR 42	*C. neoformans*	αA	VNI	*C. neoformans*	VNI	2.509
IUM 93-3233	*C. neoformans*	αD	VNIV	*C. neoformans*	VNIV	2.520
IUM 94-2361	*C. neoformans*	αD	VNIV	*C. neoformans*	VNIV	2.613
IUM 93-3922	*C. neoformans*	αD	VNIV	*C. neoformans*	VNIV	2.444
CR 33	*C. neoformans*	αD	VNIV	*C. neoformans*	VNIV	2.382
CR 35	*C. neoformans*	αD	VNIV	*C. neoformans*	VNIV	2.568
IUM 93-4941	*C. neoformans*	αD	VNIV	*C. neoformans*	VNIV	2.173
IUM 91-1871	*C. neoformans*	αAαD	VNIII	*C. neoformans*	VNIII	2.155
IUM 92-6682	***C.gattii***	**αB**	**VGII**	***C.gattii***	**VGI**	**2.120**
IUM 91-6492	*C.gattii*	αB	VGI	*C.gattii*	VGI	2.298
WM[c] 163	*C.gattii*	αB	VGI	*C.gattii*	VGI	2.009
IUM 92-6957	*C.gattii*	αB	VGI	*C.gattii*	VGI	2.159
IUM 94-6315	*C.gattii*	αB	VGI	*C.gattii*	VGI	2.040
IP[d] 189	*C.gattii*	αB	VGIII	*C.gattii*	VGIII	2.199
WM 137	*C.gattii*	αC	VGIII	*C.gattii*	VGIII	2.438
NIMH[e] 155	*C.gattii*	αC	VGIV	*C.gattii*	VGIV	2.240
WM 779	*C.gattii*	αC	VGIV	*C.gattii*	VGIV	2.495
NIMH 103	*C.gattii*	αC	VGIV	*C.gattii*	VGIV	2.145

The only one isolate with discordant results is marked in bold.

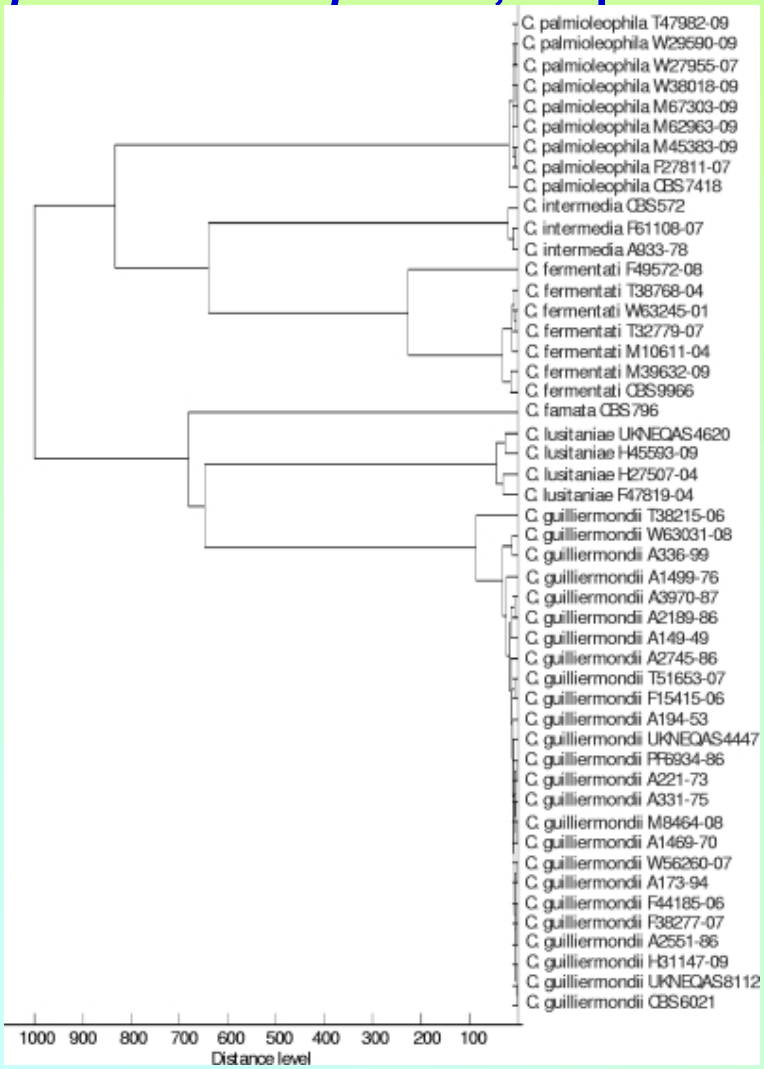
A matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS)-based method for discrimination between molecular types of *Cryptococcus neoformans* and *Cryptococcus gattii*

Brunella Posteraro,[1†] Antonietta Vella,[2†] Massimo Cogliati,[3] Elena De Carolis,[2] Ada Rita Florio,[2] Patrizia Posteraro,[4] Maurizio Sanguinetti,[2*] and Anna Maria Tortorano[3]



Cluster analysis of MALDI-TOF MS spectra of selected reference or challenge isolates of *C. neoformans*

MALDI-TOF for characterization of *Candida palmioleophila*, a previously overlooked patogen



Score-oriented dendrogram to cluster the MALDI-TOF mass spectra obtained for all included isolates. All samples were named based on molecular identification, illustrating the complete agreement between ITS sequencing and the MALDI-TOF spectra.

C. palmioleophila has previously been misidentified as *C. famata* or *C. guilliermondii.*

The susceptibility pattern for *C. palmioleophila* is unique, with low echinocandin MICs (range, 0.008 to 0.125 µg/ml) and high fluconazole MICs (range, 8 to >16 µg/ml). Thus, correct identification of *C. palmioleophila* is important.

Identification is possible yet laborious with conventional techniques, whereas MALDI-TOF MS easily separates the related species.

From: Jensen RH and Arendrup MC, JCM, 2011

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PLoS one

Rapid Species Diagnosis for Invasive Candidiasis Using Mass Spectrometry

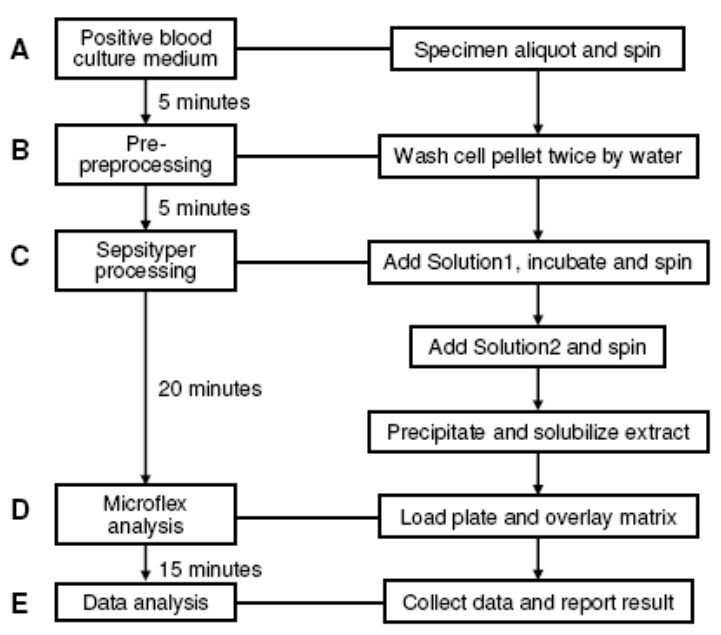
Carine Marinach-Patrice[1,2], Arnaud Fekkar[1,2,3], Ralitsa Atanasova[1,2], Johanna Gomes[1,2], Laura Djamdjian[3], Jean-Yves Brossas[1,2,4], Isabelle Meyer[3], Pierre Buffet[1,2,3], Georges Snounou[1,2,5], Annick Datry[1,2,3], Christophe Hennequin[1,2,6], Jean-Louis Golmard[7,8], Dominique Mazier[1,2,3,9]*

Table 2. Mean correlation coefficients of tested strains and clinical sample of patient against the seven-classes database.

	Class							MS identification	Conventional identification**
Tested strain	*C. albicans*	*C. glabrata*	*C krusei*	*C. lusitaniae*	*C. parapsilosis*	*C. tropicalis*	Yeast free control		
PS 569	0.312	**0.834**	0.549	0.311	0.379	0.140	0.489	*C. glabrata*	*C. glabrata*
PS 569*	0.366	**0.582**	0.510	0.352	0.422	0.178	0.428	*C. glabrata*	*C. glabrata*
PS 11188	−0.134	**0.571**	0.093	−0.097	−0.003	−0.211	0.181	*C. glabrata*	*C. glabrata*
PS 11189	0.203	**0.724**	0.334	0.244	0.254	0.108	0.348	*C. glabrata*	*C. glabrata*
PS 11594	0.215	**0.552**	0.315	0.332	0.292	0.093	0.226	*C. glabrata*	*C. glabrata*
PS 11597	0.337	**0.659**	0.473	0.442	0.384	0.184	0.445	*C. glabrata*	*C. glabrata*
PS 5282	0.201	**0.809**	0.544	0.293	0.354	0.025	0.520	*C. glabrata*	*C. glabrata*
PS 5176	0.398	0.167	0.357	**0.644**	0.348	0.290	0.247	*C. lusitaniae*	*C. lusitaniae*
PS 5176*	0.395	0.222	0.428	**0.525**	0.407	0.299	0.321	*C. lusitaniae*	*C. lusitaniae*
PS 922	0.415	0.044	0.075	**0.509**	0.083	0.129	0.050	*C. lusitaniae*	*C. lusitaniae*
PS 9230	0.576	0.451	0.475	**0.677**	0.341	0.330	0.550	*C. lusitaniae*	*C. lusitaniae*
PS 256	0.430	−0.047	0.042	**0.536**	0.118	0.181	0.014	*C. lusitaniae*	*C. lusitaniae*
PS 11242	0.526	0.316	0.337	**0.691**	0.291	0.324	0.336	*C. lusitaniae*	*C. lusitaniae*
PS 990	0.378	−0.119	0.026	**0.531**	0.137	0.146	−0.069	*C. lusitaniae*	*C. lusitaniae*
PS4667	**0.751**	0.466	0.502	0.615	0.326	0.401	0.614	*C. albicans*	*C. albicans*
PS4667*	**0.534**	0.103	0.117	0.381	0.082	0.293	0.145	*C. albicans*	*C. albicans*
PS 11802	**0.768**	0.486	0.524	0.730	0.544	0.523	0.566	*C. albicans*	*C. albicans*
PS 11862	**0.722**	0.415	0.354	0.616	0.367	0.391	0.396	*C. albicans*	*C. albicans*
PS 12355	**0.419**	0.022	−0.059	0.252	−0.030	0.238	−0.016	*C. albicans*	*C. albicans*
PS 12372	**0.657**	0.047	0.129	0.449	0.153	0.443	0.222	*C. albicans*	*C. albicans*
PS 5441	**0.506**	0.127	0.128	0.450	0.153	0.287	0.126	*C. albicans*	*C. albicans*
PS 9359	0.401	−0.149	−0.113	0.193	−0.059	**0.615**	0.039	*C. tropicalis*	*C. tropicalis*
PS 9359*	0.418	0.131	0.025	0.310	−0.030	**0.577**	0.103	*C. tropicalis*	*C. tropicalis*
PS 11185	0.460	0.082	0.079	0.350	0.093	**0.674**	0.217	*C. tropicalis*	*C. tropicalis*
PS 11186	0.512	0.011	0.031	0.392	0.056	**0.747**	0.144	*C. tropicalis*	*C. tropicalis*
PS 11187	0.410	−0.104	−0.067	0.263	−0.039	**0.637**	0.050	*C. tropicalis*	*C. tropicalis*
PS 11745	0.112	−0.167	−0.247	0.062	−0.034	**0.261**	−0.080	*C. tropicalis*	*C. tropicalis*
PS 10597	−0.014	−0.543	−0.588	−0.201	−0.314	**0.024**	−0.392	*C. tropicalis*	*C. tropicalis*

Identification of Yeast Species Directly from Positive Blood Culture Media

Sepsityper specimen processing



MALDI Biotyper system

Yeast-containing positive blood cultures Tested (n = 42)

No. of specimens	Species identified
28	*C. albicans*
8	*C. parapsilosis*
5	*C. tropicalis*
1	*C. neoformans*

- Limit of detection: 5.9×10^5 CFU
- Starting material: 1 ml of the blood culture fluid
- From specimen extraction to final result reporting: 1 hour

From. Yan *et al.* JCM 2011

JCM
Journals.ASM.org

Direct MALDI-TOF Mass Spectrometry Assay of Blood Culture Broths for Rapid Identification of *Candida* Species Causing Bloodstream Infections: an Observational Study in Two Large Microbiology Laboratories

Teresa Spanu,[a] Brunella Posteraro,[a] Barbara Fiori,[a] Tiziana D'Inzeo,[a] Serena Campoli,[a] Alberto Ruggeri,[a] Mario Tumbarello,[b] Giulia Canu,[a] Enrico Maria Trecarichi,[b] Gabriella Parisi,[c] Mirella Tronci,[c] Maurizio Sanguinetti,[a] and Giovanni Fadda[a]

Istituto di Microbiologia,[a] Istituto di Clinica delle Malattie Infettive,[b] Università Cattolica del Sacro Cuore, Rome, Italy, and Laboratorio di Microbiologia, Azienda ospedaliera San Camillo-Forlanini, Rome, Italy[c]

TABLE 1 Performances of Bruker Biotyper for direct identification of yeasts from blood culture bottles with culture-based identification as reference

Comparison method ID	No. of isolates: Total tested	No. of isolates: Concordant ID[a]	% Sensitivity (95% CI)[b]
Candida albicans	195	187	95.9 (91.8–98.1)
Candida famata	1	0	NT
Candida glabrata	26	22	84.6 (64.3–94.9)
Candida guilliermondii	10	6	60.0 (27.4–86.3)
Candida krusei	8	6	75.0 (35.6–95.5)
Candida lusitaniae	2	1	NT
Candida parapsilosis	69	65	94.2 (85.1–98.1)
Candida tropicalis	32	28	87.5 (70.1–95.9)
Rhodotorula glutinis	1	0	NT
Rhodotorula mucilaginosa	2	0	NT
Total	346	316	91.3 (87.7–93.9)

[a] Species identification furnished by the Bruker Biotyper was concordant with that of the comparison method.
[b] NT, not tested. Sensitivity was not calculated when <5 isolates were found.

- In house protocol extraction: detergent plus yeast protocol extraction
- Starting material: 6 ml of the blood culture fluid
- From specimen extraction to final result: 25 min

MALDI-TOF Identification of Filamentous Fungi: Which is the Reality?

- In contrast to bacteria or yeast, relatively few data are available regarding the identification of filamentous fungi
- This due to the lack of standardized protein extraction protocols for filamentous fungi and the poor number of spectra currently included in the database of commercially available devices

Filamentous fungi: state of the art

Study	No. of isolates/species	% of isolates identified
De Carolis *et al.* 2012	*Aspergillus*, *Fusarium*, and *Mucorales* 103 isolates 29 species	96.8
Coulibaly *et al.* 2011	*Scedosporium* 25 isolates 7 species	100
Alanio *et al.* 2010	*Aspergillus* 140 isolates 28 species	98.6
Marinach *et al.* 2009	*Fusarium* 62 isolates 9 species	92
Erhard *et al.* 2008	*Dermatophytes* 20 isolates 5 species	100

Identification of filamentous fungi remains difficult

- Biological complexity
- Changes in classification due to the introduction of nucleic-acid based techniques

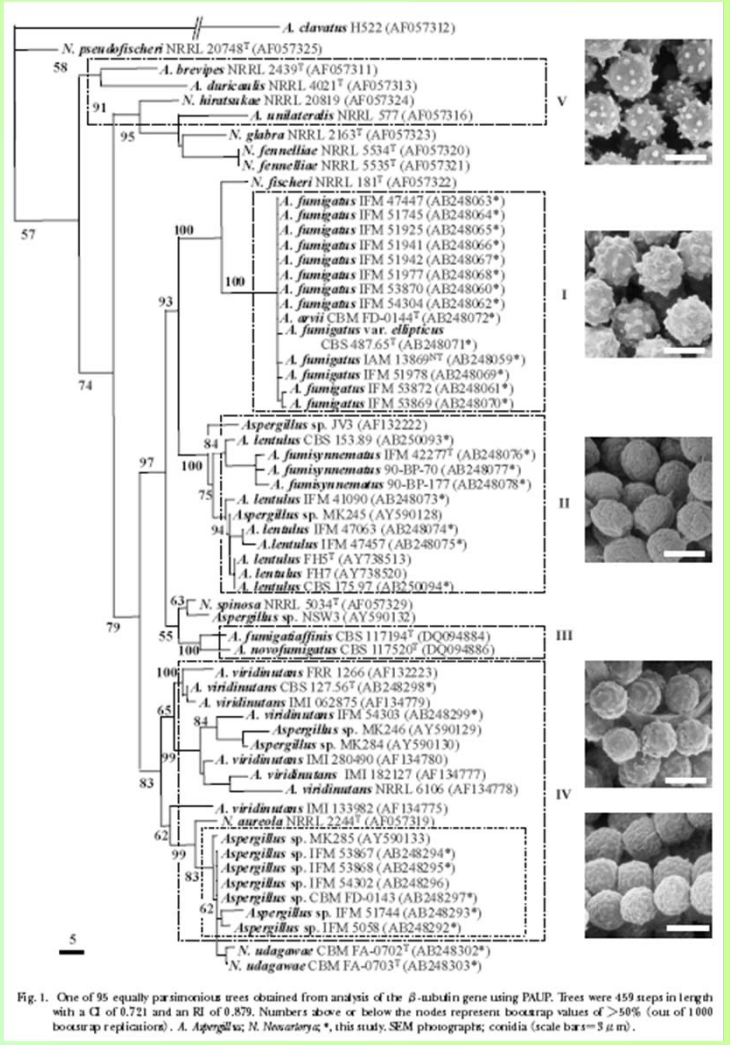


Fig. 1. One of 95 equally parsimonious trees obtained from analysis of the β-tubulin gene using PAUP. Trees were 459 steps in length with a CI of 0.721 and an RI of 0.879. Numbers above or below the nodes represent bootstrap values of >50% (out of 1000 bootstrap replications). *A. Aspergillus*; *N. Neosartorya*; *, this study. SEM photographs; conidia (scale bars=3 μm).

Identification of species within the *Aspergillus* species complex could drive appropriate therapy

- Existence of cryptic species implies differences in:
  - ✓ Clinical manifestations
  - ✓ Prognosis
  - ✓ Antifungal susceptibility profile

EUKARYOTIC CELL, Oct. 2006, p. 1705–1712 Vol. 5, No. 10
1535-9778/06/$08.00+0 doi:10.1128/EC.00162-06

Molecular Studies Reveal Frequent Misidentification of *Aspergillus fumigatus* by Morphotyping

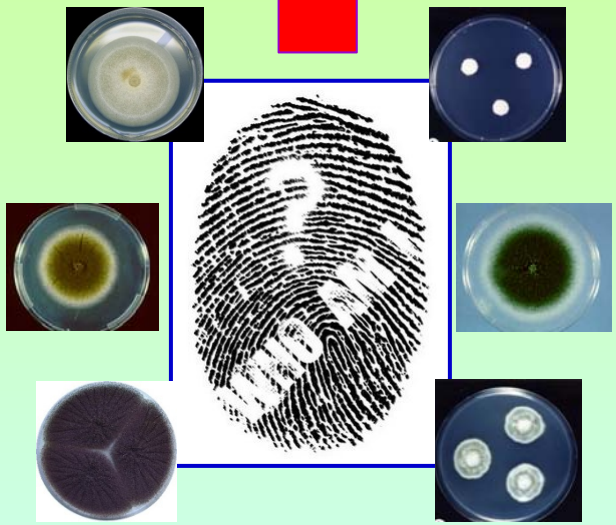
S. Arunmozhi Balajee,[1] David Nickle,[2] Janos Varga,[3]† and Kieren A. Marr[1,2,4]*

TABLE 2. Antifungal susceptibilities of *A. lentulus* and *A. udagawae* isolates

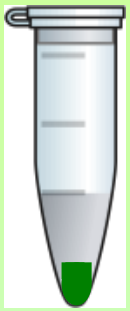
Isolate	Susceptibilities (μg/ml)[a]			
	AMB	ITZ	VRZ	CAS
A. lentulus				
FH265	1	0.5	1	2
FH278	1	1	1	2
CDC59	1	0.5	2	2
CDC61	2	0.5	2	16
CDC65	1	1	2	16
UT3351	2	1	2	2
UT1322	2	1	2	2
UT2411	2	1	1	2
A. udagawae				
CDC58	4	0.25	1	0.015
CDC57	0.5	0.125	1	0.03
CDC22	4	0.25	1	0.015
UT1561	2	0.125	0.25	0.03
FH103[b]	2	0.5	1	0.06
FH104[b]	2	0.5	1	0.015
FH105[b]	2	0.5	1	0.015
FH106[b]	2	0.5	1	0.03
Af293	0.5	0.25	0.25	0.125

[a] MICs are given for AMB, ITZ, VRZ; for CAS, the minimum effective concentration is given.
[b] Isolates recovered from a single patient.

Superficial scraping of mold colonies

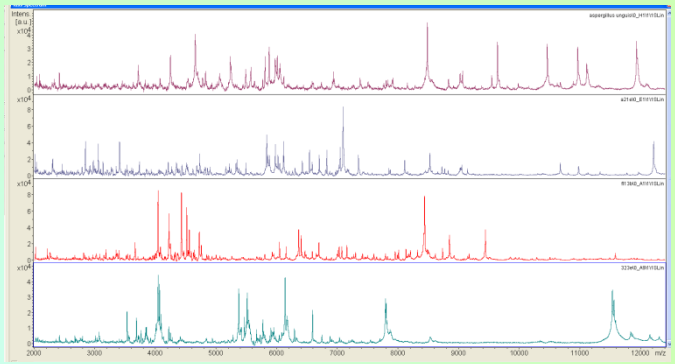


Unknown fungi



H_2O

Preparation onto a MALDI target plate



ETOH
Sample inactivation

HCCA matrix
(α-cyano-4-hydroxycinammic acid)

Generate MALDI-TOF profile spectrum

From: De Carolis *et al*., CMI 2012, in press

ORIGINAL ARTICLE MYCOLOGY

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry for fast and accurate identification of clinically relevant *Aspergillus* species

A. Alanio[1], J.-L. Beretti[1], B. Dauphin[1], E. Mellado[2], G. Quesne[1], C. Lacroix[3], A. Amara[1], P. Berche[1], X. Nassif[1] and M.-E. Bougnoux[1]

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Abstract

New *Aspergillus* species have recently been described with the use of multilocus sequencing in refractory cases of invasive aspergillosis. The classical phenotypic identification methods routinely used in clinical laboratories failed to identify them adequately. Some of these *Aspergillus* species have specific patterns of susceptibility to antifungal agents, and misidentification may lead to inappropriate therapy. We developed a matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS)-based strategy to adequately identify *Aspergillus* species to the species level. A database including the reference spectra of 28 clinically relevant species from seven *Aspergillus* sections (five common and 23 unusual species) was engineered. The profiles of young and mature colonies were analysed for each reference strain, and species-specific spectral fingerprints were identified. The performance of the database was then tested on 124 clinical and 16 environmental isolates previously characterized by partial sequencing of the β-tubulin and calmodulin genes. One hundred and thirty-eight isolates of 140 (98.6%) were correctly identified. Two atypical isolates could not be identified, but no isolate was misidentified (specificity: 100%). The database, including species-specific spectral fingerprints of young and mature colonies of the reference strains, allowed identification regardless of the maturity of the clinical isolate. These results indicate that MALDI-TOF MS is a powerful tool for rapid and accurate identification of both common and unusual species of *Aspergillus*. It can give better results than morphological identification in clinical laboratories.

Order	Genus	Strain[a]
Eurotiales	*Aspergillus*	
	Section *Fumigati*	*A. fumigatus* ATCC1028, CG221, CG230, CG274, CG287, CG295
		A. lentulus UCSC529, CBS117887
		A. novofumigatus CBS117519
		A. viridinutans CBS127.56
		Neosartorya udagawae CG283, CBS114217, UCSC298
		Neosartorya hiratsukae CBS294.93, UCSC194
		Neosartorya pseudofischeri CBS404.67, CG200
		A. unilateralis CBS126.56
	Section *Flavi*	*A. flavus* CBS110.45, UCSC387, FL16
		A. flavus var. *columnaris* CBS485.65
		A. oryzae CBS108.24, CBS819.72, FL13, FL22
		A. parasiticus CBS571.65
		A. parasiticus var. *globosus* CBS260.67
		A. alliaceus UCSC343
	Section *Terrei*	*A. terreus* UCSC405, UCSC431, FL3, FL9, FL52, FL64, FL67
		A. alabamensis FL109, FL110
	Section *Nigri*	*A. niger* FL6, FL17
		A. tubingensis CBS115.29, UCSC453, FL9, FL12
		A. awamori CBS113.33
		A. japonicus CBS568.65
		A. foetidus CBS119384, FL4
	Section *Nidulantes*	*Emericella nidulans* UCSC401, UCSC424
		Emericella quadrilineata CBS235.65
		A. sydowii UCSC344, UCSC438
		A. versicolor UCSC229, UCSC234, UCSC441
		A. unguis UCSC132, UCSC324
	Section *Usti*	*A. ustus* CBS239.90
		A. calidoustus CBS121601
	Section *Circumdati*	*A ochraceus* UCSC4, UCSC421
		A. melleus UCSC426
		A. sclerotiorum UCSC338
	Section *Aspergillus*	*A. glaucus* UCSC206, UCSC216
	Section *Candidi*	*A. candidus* UCSC175
Mucorales	*Mucor*	*M. racemosus* FL47
		M. plumbeus FL42
		M. fragilis FL71
		M. circinelloides FL84, UCSC161, UCSC167, IUM04-52007005
		M. hiemalis FL72
	Lichtheimia	*L. corymbifera* FL41, FL103
		L. ramosa FL76
	Rhizopus	*R. microsporus* FL15, FL28
		R. oryzae UCSC6, UCSC74, UCSC75, UCSC233
	Rhizomucor	*Rhizomucor pusillus* FL45, IUM07-0254
Hypocreales	*Fusarium*	*F. solani* species complex, FL94, FL97, UCSC447, UCSC448, UCSC490
		F. chlamydosporum species complex CBS145.25, FL94, FL96, FL97
		F. oxysporum species complex UCSC127, UCSC512
		F. proliferatum UCSC446
		F. subglutinans IUM02-0755
		F. brachygibbosum CBS121682, UCSC439
		F. incarnatum-equiseti species complex CBS150.25, UCSC490
		F. tricinctum species complex CBS449.67, UCSC463
		F. dimerum CBS366.73
		F. poae ATCC15654
		F. verticilloides CBS125.73
		Gibberella fujikuroi species complex UCSC464

ATCC, American Type Culture Collection (Manassas, VA, USA); CBS, Centraalbureau voor Schimmelcultures (Utrecht, The Netherlands); CG, culture strain collection of the Università La Sapienza (Rome, Italy); FL, culture strain collection of the University Hospital of Innsbruck (Innsbruck, Austria); IUM, culture strain collection of the Università degli Studi di Milano (Milan, Italy); UCSC, culture strain collection of the Università Cattolica del Sacro Cuore (Rome, Italy).

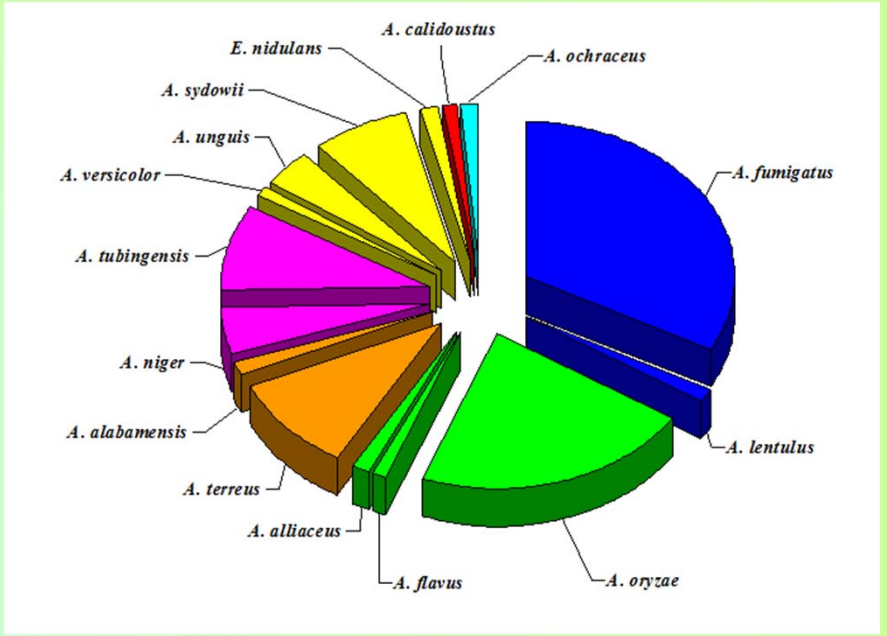
[a]A total of 109 fungal strains were used. With the exception of the ATCC-type and CBS-type strains, those from the other collections were identified molecularly, based on *β-TUB* and/or *CAM* (for *Aspergillus* strains), *EF-1α* (for *Fusarium* strains) and ITS1-5.8S-ITS2 (for *Mucorales* strains) gene sequence comparison.

- Reference spectra at different stages of maturation:
  - ✓ 109 from young colonies
  - ✓ 109 from mature colonies (diameter > 3 cm)
- 67 reference strains of *Eurotiales*
- 42 reference strains of *Mucorales* and *Hypocreales*

From: De Carolis *et al*., CMI 2012, in press

Challenge of 103 clinical isolates Identification by multilocus sequencing

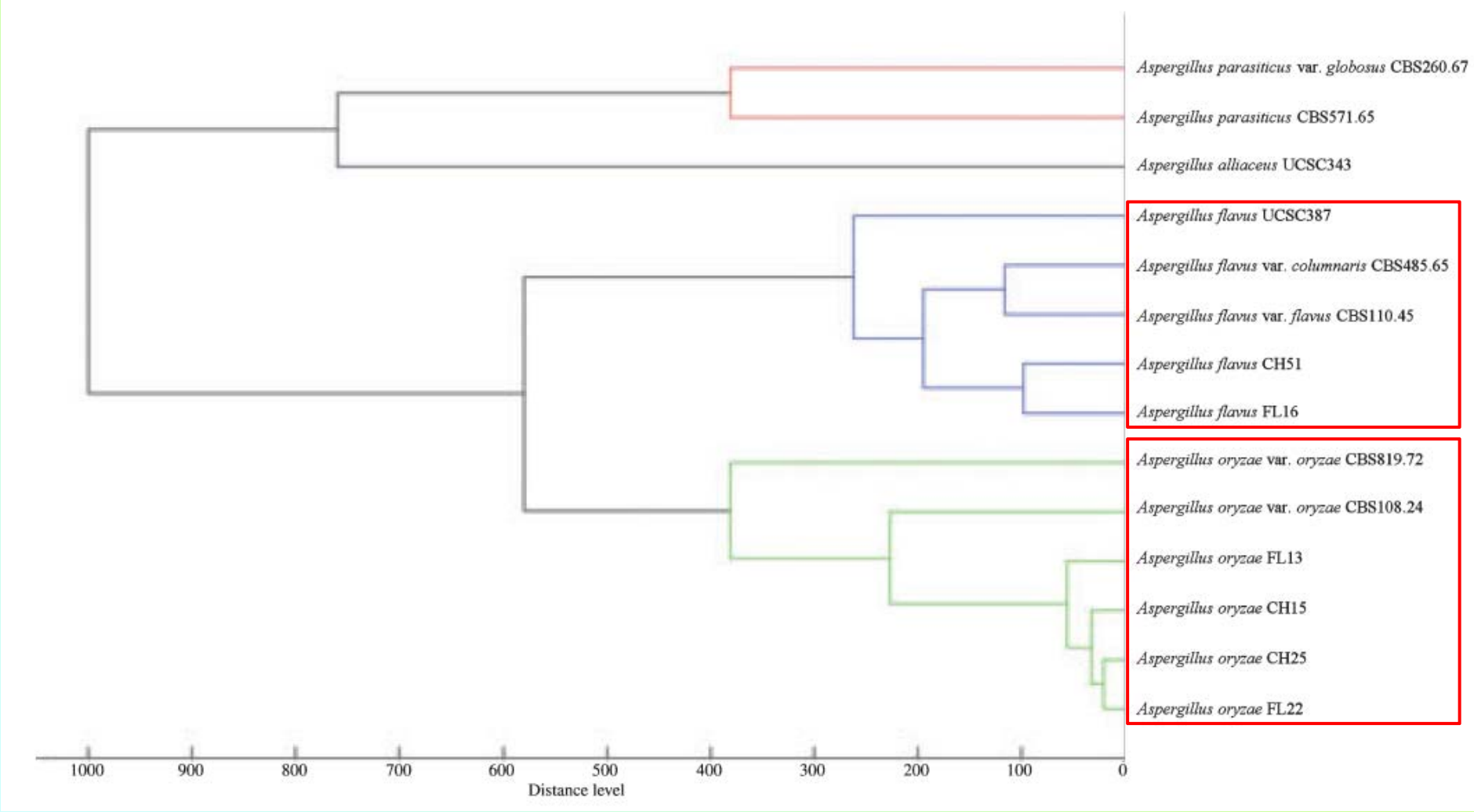
- Sequence analysis of the ITS1-5.8S-ITS2, β-*TUB*, *CAM*, and/or *EF-1α* gene regions was used as the reference identification method
- 85 isolates were unequivocally identified to the species level
- 18 isolates producing ambiguous results were initially identified to the genus level (*Aspergillus* section *Flavi*)
- Further molecular analysis assigned these isolates to the species *Aspergillus oryzae* (17 isolates) and *Aspergillus flavus* (1 isolate)



Challenge of 103 clinical isolates Identification by MALDI

- MALDI-TOF MS identified, according to their designated species, 91 of 94 clinical isolates (96.8%) of *Aspergillus*, *Fusarium* and *Mucorales*
- The log(-score) values of the 91 isolates with correct results were all higher than 2.0, whereas three isolates with a log(score) value of <2.0 (1.817, 1.874 and 1.796, respectively) could be identified only to the genus level, but had concordant species designations as compared with the results of the reference method
- By contrast, the clinical isolates that belonged to species not included in our database (9 isolates: 3 *Alternaria alternata*, 2 *Scedosporium prolificans*, 2 *Curvularia*, 1 *Beauveria bassiana*, 1 *Cladosporium*) had all log(score) values of <1.7, thereby confirming the specificity of MALDI-TOF MS identification

Cluster analysis of MALDI-TOF spectra of selected reference strains and challenge isolates (CH15, CH25 and CH51) identified as *Aspergillus* section *Flavi* species



From: De Carolis *et al*., CMI 2012, in press

Medical Mycology Month 2012, Early Online, 1–5
informa healthcare

Case Report

First case of breakthrough pneumonia due to Aspergillus nomius in a patient with acute myeloid leukemia

MORENA CAIRA*, BRUNELLA POSTERARO†, MAURIZIO SANGUINETTI†, ELENA DE CAROLIS†, GIUSEPPE LEONE* & LIVIO PAGANO*
*Institutes of *Hematology and †Microbiology, Università Cattolica del Sacro Cuore, Rome, Italy*

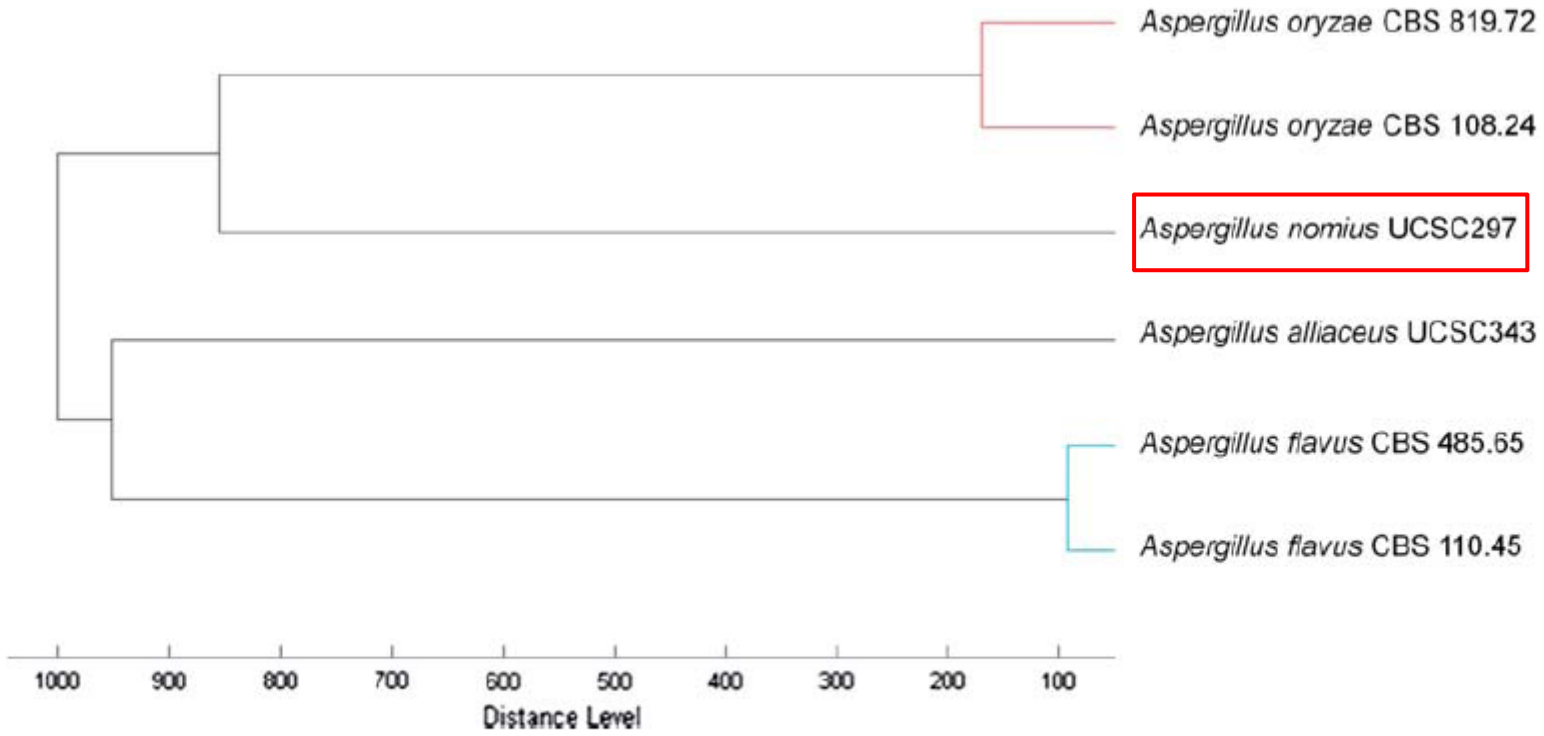
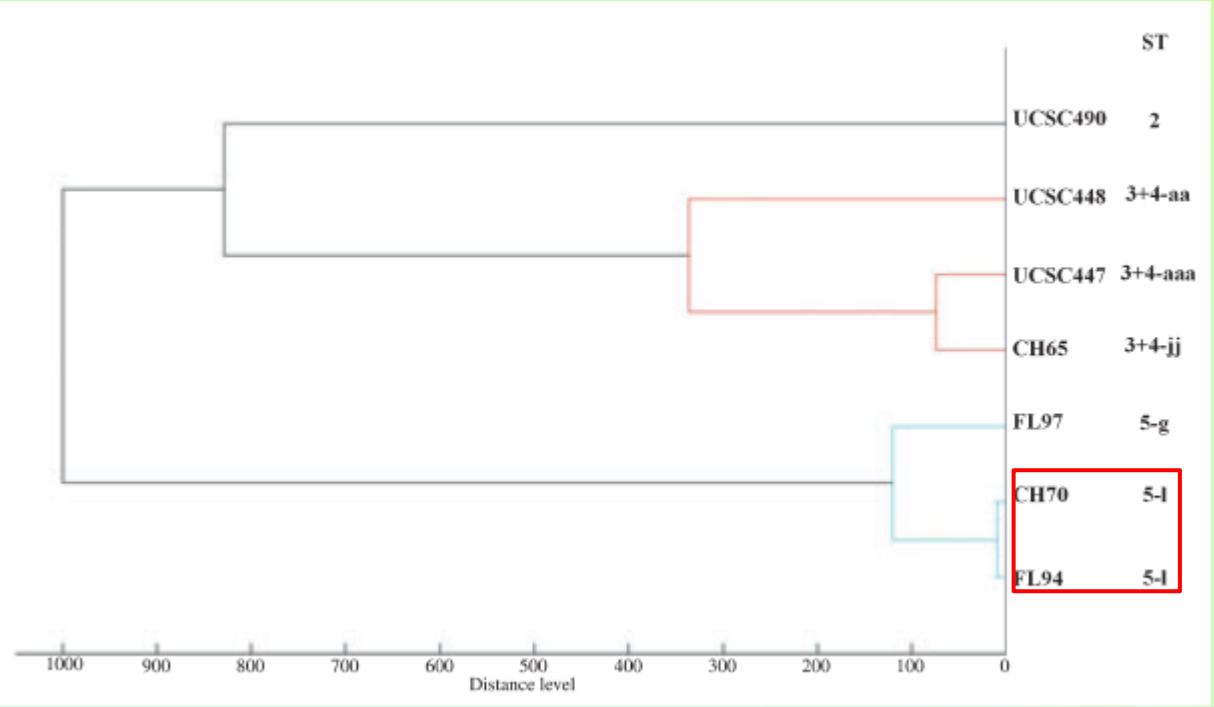


Fig. 3 Cluster analysis of matrix-assisted laser desorption ionization time-of-flight mass spectrometry spectra of selected strains identified as *Aspergillus* section *Flavi* species. Distance is displayed in relative units. *Aspergillus nomius* (UCSC297; this study) and *Aspergillus alliaceus* (UCSC343) are clinical isolates.

Fusarium solani species complex subtyping



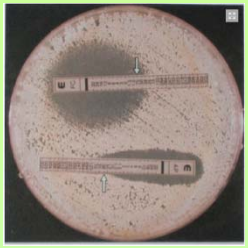
FUSARIUM-ID database SEQUENCE TYPE

Mass spectrometry-based dendrogram VS MLST

The strains were clustered in separate groups according to their phylogenetic species designation

- Each MSP is compared with the other in a matrix of cross-wise identification values. The matrix is used to calculate the distance values for each pair
- Based on the protein mass patterns, strains can be clustered hierarchically

MIC determination



Microdilution methods

E-Test

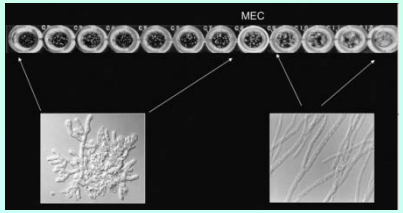
Disk diffusion

It could take 48 h

End-point

subjective

SUSCEPTIBILITY DETERMINATION



MEC

MASS SPECTROMETRY

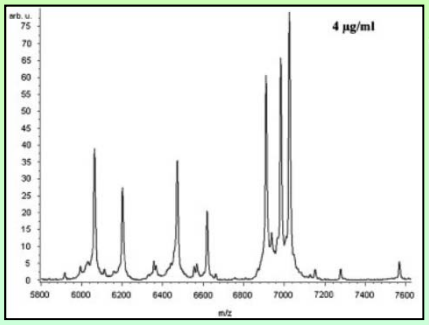
New generation of susceptibility tests

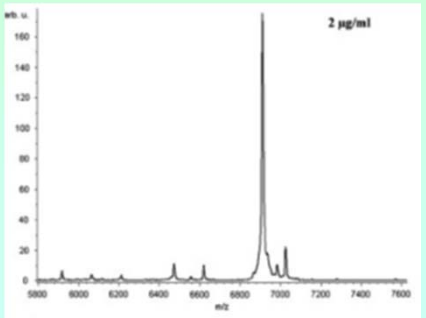
?

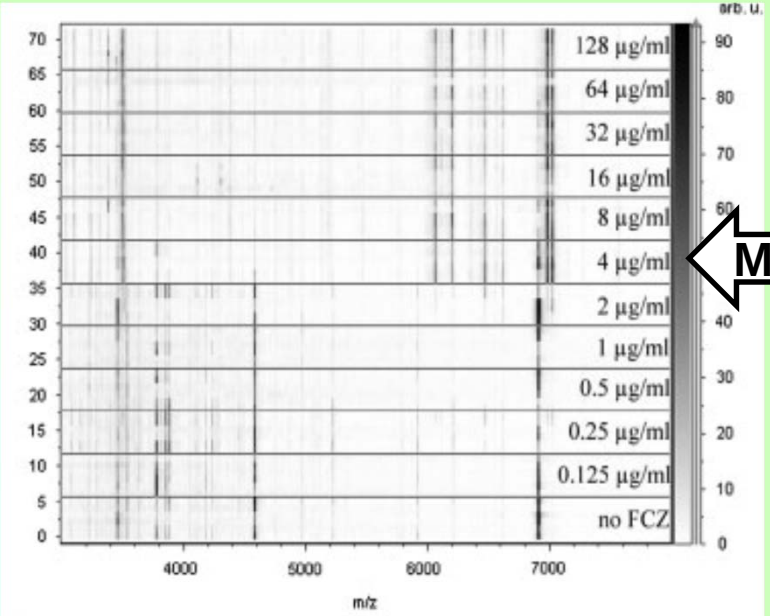
MPCC, the minimal profile change concentration

New endpoint:

a value defined as the lowest drug concentration at which a mass spectrum profile change can be detected







MPCC

Proteomics 2009, *9*, 4627–4631 DOI 10.1002/pmic.200900152

RAPID COMMUNICATION

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

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

- Inoculum 10^6 yeast/ml
- Turnaround time 15 h
- Acid extraction
- End-point reading objective
- Cost less than 1 euro

MPCC determinations were concordant with MIC BDM irrespective of the type of drug resistance mechanism

Use of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) for caspofungin susceptibility testing of *Candida* and *Aspergillus* species

Elena De Carolis,[1#] Antonietta Vella,[1#] Ada R. Florio,[1] Patrizia Posteraro,[2] David S. Perlin,[3] Maurizio Sanguinetti,[1*] and Brunella Posteraro[4]

TABLE 1. *In vitro* caspofungin susceptibility of 44 isolates of *Candida* and *Aspergillus* species as determined by the CLSI reference test and MALDI-TOF MS method[a]

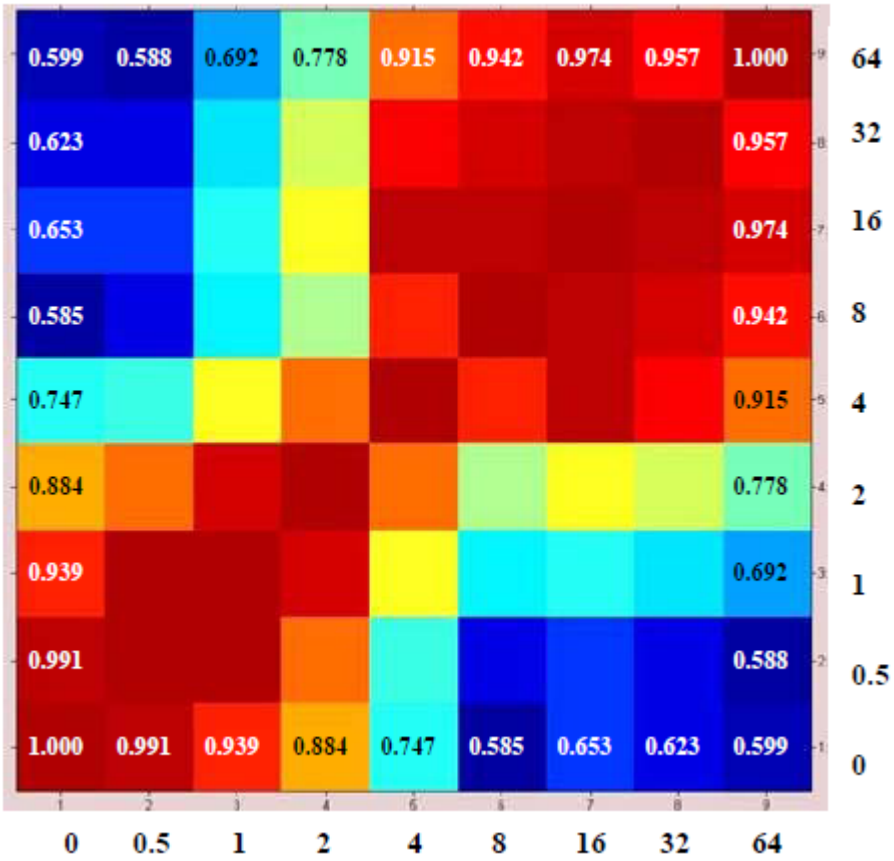
Species (n[b])	Strain designation	Phenotype[c]		MIC (or MEC)[d]	MPCC[d]
		Fks1	Fks2		
C. albicans (14)	UCSC13	WT	-	0.12	0.12
	UCSC69	WT	-	0.06	0.12
	UCSC70	WT	-	0.12	0.12
	UCSC131	WT	-	0.12	0.12
	DPL1012	D648Y	-	2.67	1
	DPL1006	F641L	-	2	2
	DPL1007	F641S	-	4	4
	DPL1010	S645F	-	4	1
	DPL1011	S645F plus R1361R/H		4	4
	DPL21	S645P	-	8	4
	DPL1009	S645F	-	4	8
	DPL1013	P649H	-	4	1
	DPL1040	R1361H	-	2	1
	DPL1014	R1361R/H	-	1	1
C. glabrata (12)	UCSC91	WT	WT	0.03	0.03
	UCSC92B	WT	WT	0.03	0.03
	UCSC103	WT	WT	0.06	0.06
	UCSC104	WT	WT	0.06	0.06
	DPL38	F625S	WT	8	2
	DPL155	WT	F659V	4	4
	DPL41	D632G	WT	4	16
	DPL33	WT	D666E	4	2
	DPL32	WT	D666G	4	8
	DPL34	WT	P667T	2	2
	DPL39	S629P	WT	8	16
	DPL30	WT	S663P	16	4

[a]Isolates include clinical (n = 40), reference (n = 3), and laboratory mutant (n = 1) strains.
[b]n, no. of isolates tested.
[c]WT, wild-type at mutational hot-spot regions of Fks1 and Fks2. Otherwise, the specific amino acid substitutions harbored by mutant strains are indicated.
[d]Geometric means values in μg/ml (three repetitions from separate preparations) are given.

Use of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) for caspofungin susceptibility testing of *Candida* and *Aspergillus* species

Elena De Carolis,[1#] Antonietta Vella,[1#] Ada R. Florio,[1] Patrizia Posteraro,[2] David S. Perlin,[3] Maurizio Sanguinetti,[1*] and Brunella Posteraro[4]

CCI matrix



MPCC (µg/ml)	Null CCI	Maximum CCI
64	0.599	1.000
32	0.623	0.957
16	0.653	0.974
8	0.585	0.942
4	0.747	0.915
2	0.884	0.778
1	0.939	0.692
0.5	0.991	0.588
0	1.000	0.599

MALDI-TOF intact cell MS (Pros)

- Rapidity
- Inexpensiveness in terms of labor and consumables
- High discriminatory power, accuracy, and superiority over morphological analysis and conventional identification
- Ability to easily differentiate species that are morphologically and phylogenetically similar to each other

MALDI-TOF intact cell MS (Cons)

- MALDI-TOF MS equipment is not cheap
- Molecular diagnostic techniques are still required in cases for which no reference spectra are present in the MALDI-TOF MS databases at the time of analysis
- Apart from positive blood cultures, MALDI-TOF cannot yet used directly on patient samples
- Also, the system is not able to identify the presence of several different pathogens in a sample

CONCLUSIONS (1)

- MALDI-TOF identification still requires a growth step in order to obtain fungal colonies for acquisition of spectra
- Apart from positive blood cultures, MALDI-TOF cannot yet used directly on patient samples
- Also, the system is not able to identify the presence of several different pathogens in a sample

CONCLUSIONS (2)

- In addition to the identification process, other aspects of microorganism analysis, such as the search for virulence factors and drug resistance determinants, and typing, will be expanded enormously
- This, in combination with the potential of each laboratory to create its own reference database to be widely used and shared, will help to extend MALDI-TOF analysis in clinical mycology laboratories

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