

BCCM • IHEM

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

GREAT AT SMALL THINGS

MALDI-TOF MS & Fungi

ECCO

Marijke Hendrickx 13/06/2019



The BCCM Consortium

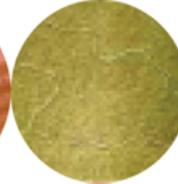
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BCCM/LMG
Bacteria
Ghent University



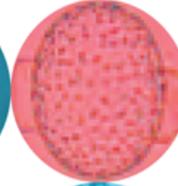
BCCM/CC
Coordination Cell
Belgian Science Policy Office

BCCM/IHEM
Fungi: human and animal health
Sciensano



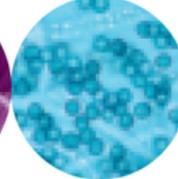
BCCM/Genecorner
Plasmids
Ghent University

BCCM/ITM
Mycobacteria
Institute of Tropical Medicine,
Antwerp



BCCM/DCG
Diatoms
Ghent University

BCCM/MUCL
Agro-Food and Industrial Fungi
collection
Université Catholique de Louvain



BCCM/ULC
Cyanobacteria
University of Liège

In our collection...

Impact on human and animal health

- Isolates from human and animal infections
- Allergy causing species
- Mycotoxin producing species
- Isolates from patient environment

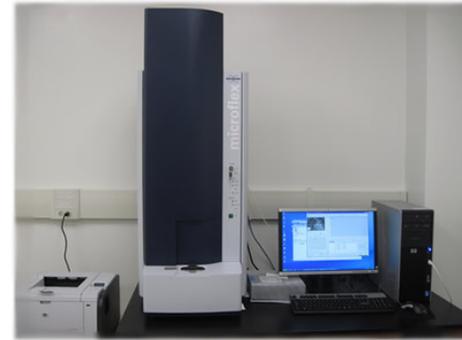
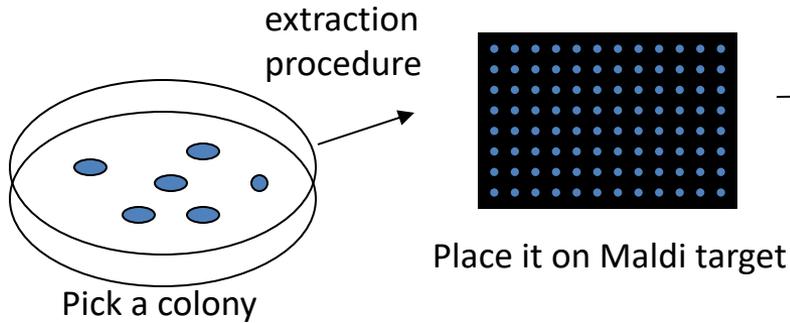


Maldi-Tof MS: Matrix Assisted Laser Desorption Ionisation – Time of Flight Mass Spectrometry



MALDI-TOF MS Principle:

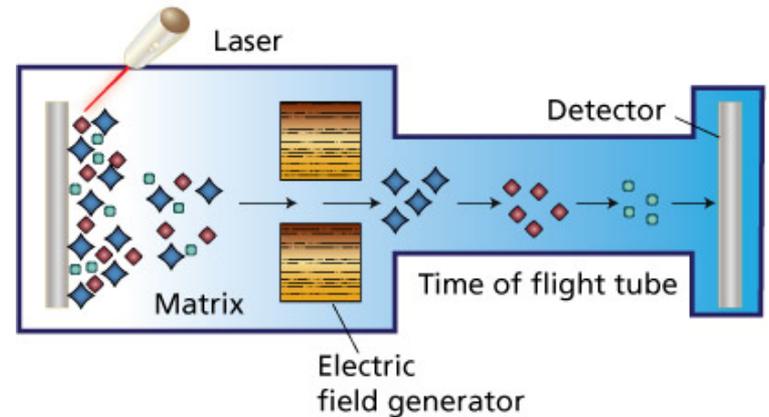
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Spectrum <> protein content

↓
'unique' for
the micro-organism

↓
Compare it to reference
database

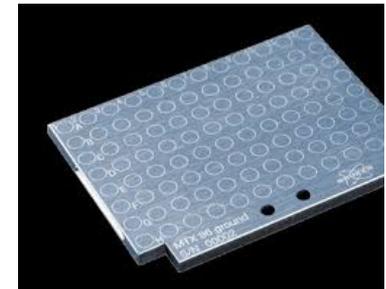
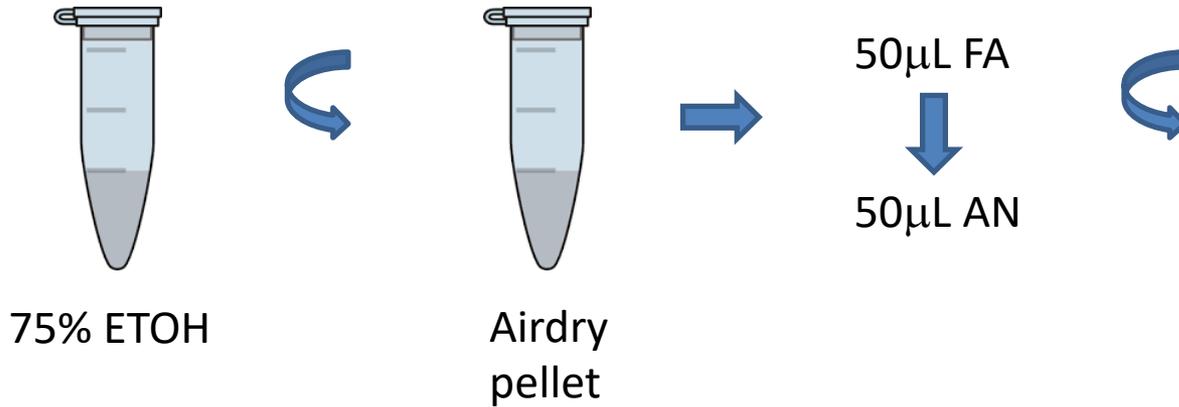


Species specific fingerprints

Some particularities for fungi...

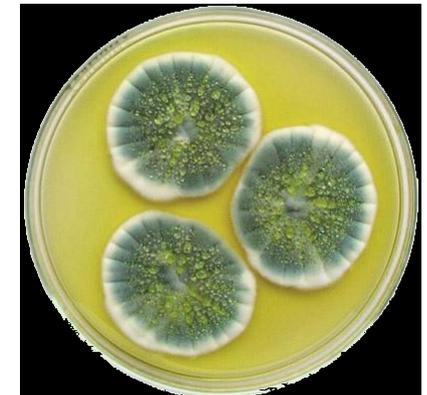
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- Protein extraction procedure



Spot supernatant

- Heterogeneity of Fungal cultures
 - Spores <> Mycelium
 - Centre <> Periphery
- Growth into agar plate

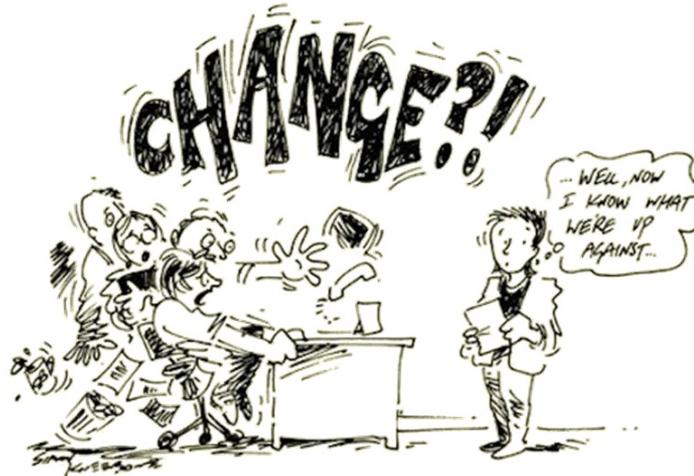




- Databases limited



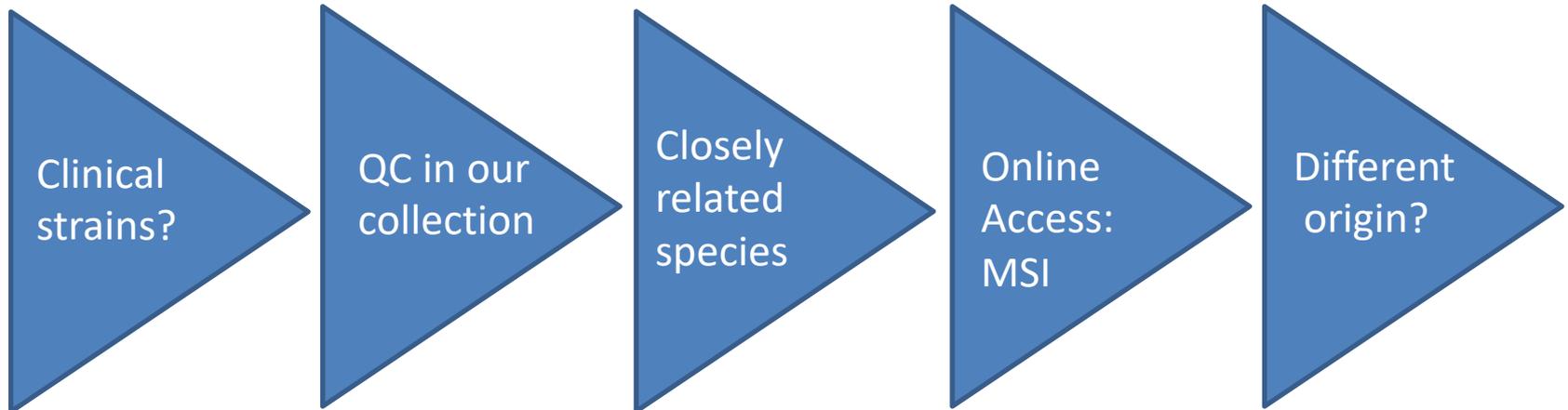
- Species complexes & taxonomic changes



- Intra- species variability

- ✓ More precise than microscopy
- ✓ More cost efficient than MLGS
- ✓ Low error rate

- Very low error rate
- Importance of the database
- Created in house:
 - sequenced/available strains from our collection
 - >3 strains when possible



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Universitair
Ziekenhuis
Brussel

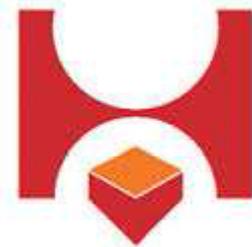


Clinical ID
(morphology):
1: *A. fumigatus*
2: *S. prolificans*
3: *A. niger*
4: *F. oxysporum*
5: *A. nidulans*
Etc.

In our lab
(MALDI-TOF MS):
1: *A. fumigatus*
2: *S. prolificans*
3: *A. niger*
4: *F. oxysporum*
5: *A. versicolor*
Etc.



Molecular Identification as gold standard



CHU St-Pierre | UMC St-Pieter

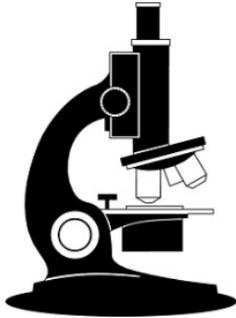


Clinical ID
(morphology)

In our lab
(MALDI-TOF MS)

Clinical
strains?

390 isolates over 1 year period



- 61,5% correct on species level
- 33,1% on genus level only
- 5,4% incorrect

- 95,4% correct on species level
- 1% on genus level only
- 3,3% not identified => Database
- 0,3% incorrect

Identification of filamentous fungi

QC in our collection

Gold standard

Multilocus
gene
sequencing

Quality control of preserved batches

ISO 9001 certification
ISO 17025 accreditation



MALDI-TOF MS analysis in parallel to QC

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- 446 strains (112 species, 40 genera)
 - 35 double batches
- => 481 identity controls

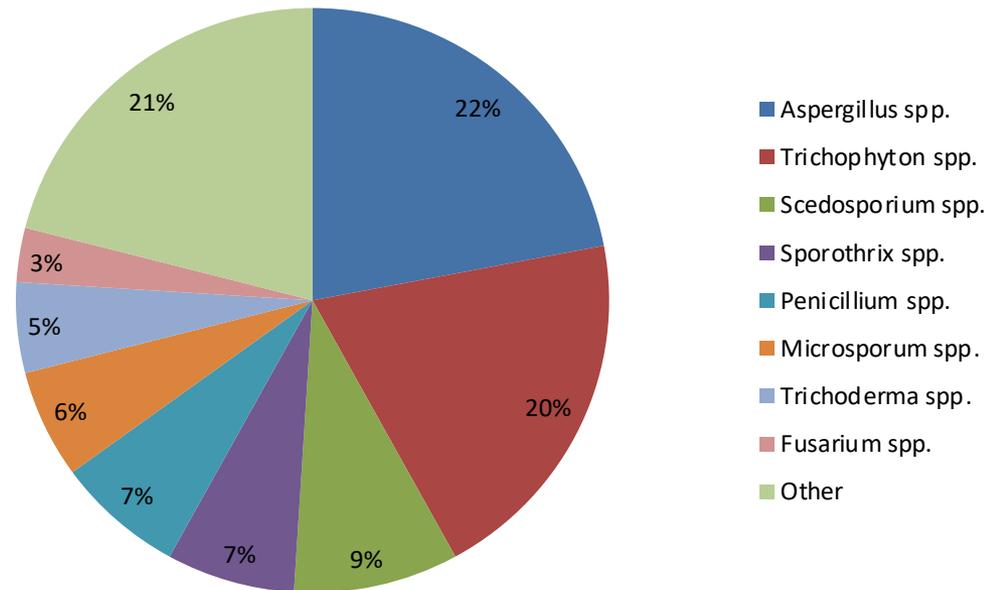


Table 2 – Strains correctly identified by MALDI-TOF MS during identity controls.

Species	No. of controls	Score range
<i>Aphanoascus fulvescens</i>	1	2.09
<i>Arthroderma benhamiae</i>	8	1.84–2.08
<i>Arthroderma corniculatum</i>	1	1.83
<i>Arthroderma fulvum</i>	1	2.30
<i>Aspergillus carbonarius</i>	2	2.26–2.39
<i>Aspergillus clavatus</i>	2	2.42–2.45
<i>Aspergillus flavus</i>	11	1.88–2.53
<i>Aspergillus fumigatus</i>	57	1.82–2.47
<i>Aspergillus melleus</i>	1	1.99
<i>Aspergillus nidulans</i>	2	1.72–2.24
<i>Aspergillus niger</i>	3	2.24–2.32
<i>Aspergillus niveus</i>	1	1.74
<i>Aspergillus ochraceus</i>	1	1.83
<i>Aspergillus rugulosus</i>	2	2.24–2.41
<i>Aspergillus scerotiorum</i>	2	2.01–2.22
<i>Aspergillus sojae</i>	2	2.05–2.39
<i>Aspergillus sydowi</i>	1	2.01
<i>Aspergillus tamaris</i>	2	2.19–2.30
<i>Aspergillus terreus</i>	3	1.82–2.30
<i>Aspergillus unguis</i>	1	2.14
<i>Aspergillus wentii</i>	1	1.91
<i>Aureobasidium pullulans</i>	1	2.22
<i>Beauveria bassiana</i>	1	2.10
<i>Bipolaris hawaiiensis</i>	1	2.37
<i>Cladosporium bruhnei</i>	1	1.95
<i>Exophiala dermatitidis</i>	1	2.20
<i>Exophiala spinifera</i>	1	2.09
<i>Fusarium oxysporum</i>	2	1.90–2.03
<i>Fusarium proliferatum</i>	5	1.90–2.22
<i>Fusarium solani</i>	5	1.85–2.13
<i>Fusarium tricinctum</i>	1	1.94

<i>Fusarium verticillioides</i>	2	1.74–1.76
<i>Geosmithia pallida</i>	1	2.50
<i>Gymnascella dankaliensis</i>	8	1.77–2.21
<i>Microsporium audouinii</i>	1	2.37
<i>Microsporium canis</i>	22	1.96–2.58
<i>Microsporium fulvum</i>	2	1.83–2.41
<i>Microsporium gypseum</i>	2	2.27–2.50
<i>Mucor circinelloides</i>	2	2.02–2.43
<i>Myxotrichum deflexum</i>	1	1.87
<i>Paecilomyces carneus</i>	1	2.02
<i>Penicillium chermesinum</i>	1	2.13
<i>Penicillium chrysogenum</i>	2	2.20–2.23
<i>Penicillium citreonigrum</i>	2	1.88–2.30
<i>Penicillium citrinum</i>	3	1.89–2.02
<i>Penicillium crustosum</i>	3	2.19–2.33
<i>Penicillium funiculosum</i>	3	1.92–1.96
<i>Penicillium glabrum</i>	1	1.84
<i>Penicillium griseofulvum</i>	2	2.50–2.63
<i>Penicillium helicum</i>	1	1.84
<i>Penicillium hirsutum</i>	1	2.20
<i>Penicillium roqueforti</i>	1	2.29
<i>Penicillium variabile</i>	1	2.11
<i>Phialophora olivacea</i>	1	2.01
<i>Phoma herbarum</i>	1	1.93
<i>Rasamsonia argillacea</i>	1	2.00
<i>Rhizopus microsporus</i>	2	2.28–2.36
<i>Scedosporium apiospermum</i>	34	1.70–2.63
<i>Scedosporium prolificans</i>	2	2.08–2.40
<i>Schizophyllum commune</i>	1	1.72
<i>Scopulariopsis asperula</i>	2	1.80–2.22
<i>Scopulariopsis brevicaulis</i>	5	2.00–2.37
<i>Scopulariopsis candida</i>	1	2.21
<i>Sporothrix schenckii</i>	35	1.72–2.39
<i>Syncephalastrum racemosum</i>	1	2.41
<i>Trichoderma citrinoviride</i>	1	1.73
<i>Trichoderma longibrachiatum</i>	5	1.71–2.30
<i>Trichoderma polysporum</i>	1	2.13
<i>Trichoderma pseudokoningii</i>	1	1.92
<i>Trichoderma reesei</i>	3	1.79–2.30
<i>Trichoderma virens</i>	1	2.19
<i>Trichophyton concentricum</i>	6	2.02–2.26
<i>Trichophyton interdigitale</i>	21	2.22–2.70
<i>Trichophyton mentagrophytes</i>	13	1.94–2.57
<i>Trichophyton rubrum</i>	31	1.70–2.61
<i>Trichophyton terrestre</i>	1	2.35
<i>Trichophyton tonsurans</i>	2	2.41–2.59
<i>Trichophyton violaceum</i>	6	2.03–2.42
<i>Trichothecium roseum</i>	3	1.76–2.39

Results:

- 354: identity confirmed (81%)
- 44: no reference in database (9,8%)
- 60: uninterpretable (13,7%)
- 23: identified as different species (5,3%)

} Microscopy + sequencing

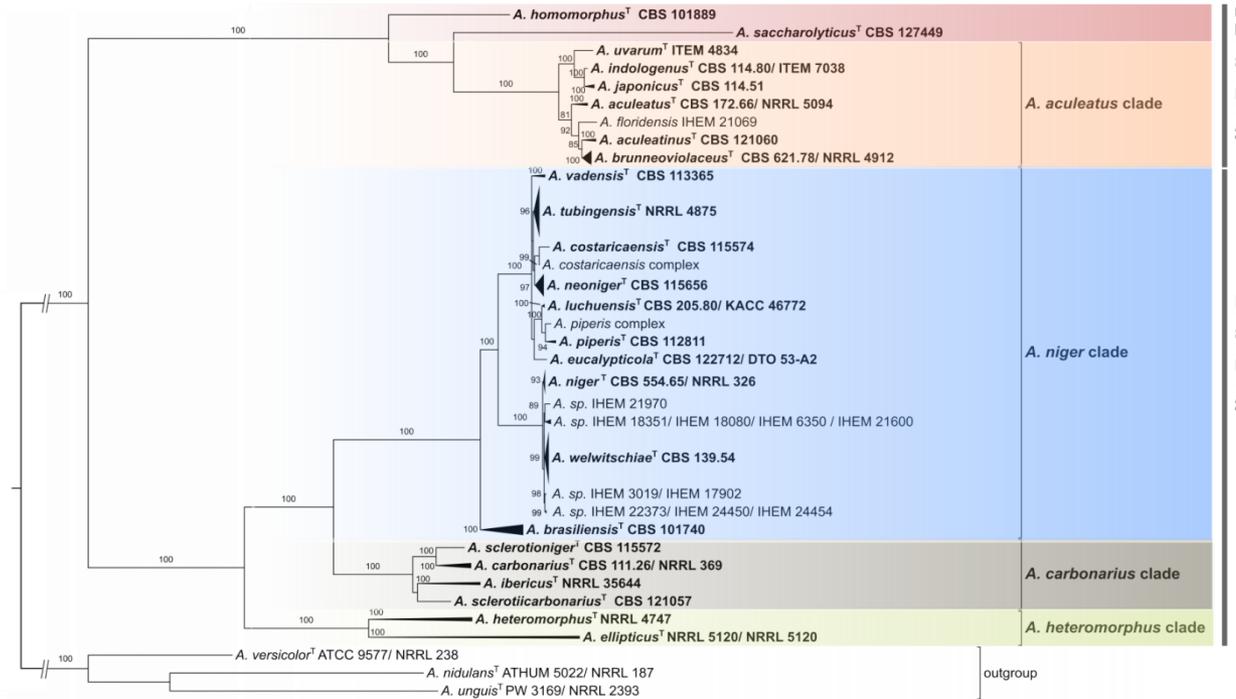


Multilocus
gene
sequencing

- 14 correct
- 9 incorrect

- When uninterpretable or false: low number of reference strains

ISO 9001 certification
ISO 17025 accreditation



Closely related species

18 species in our collection

MALDI-TOF MS
135 strains

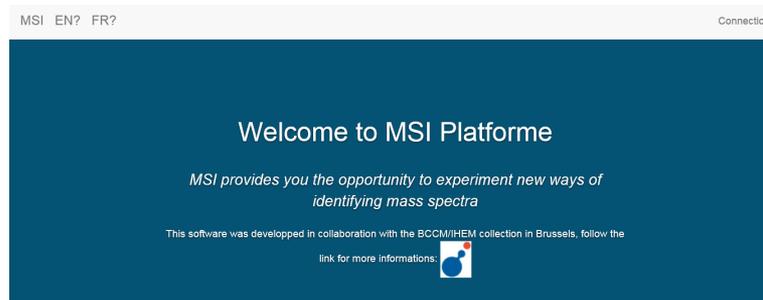


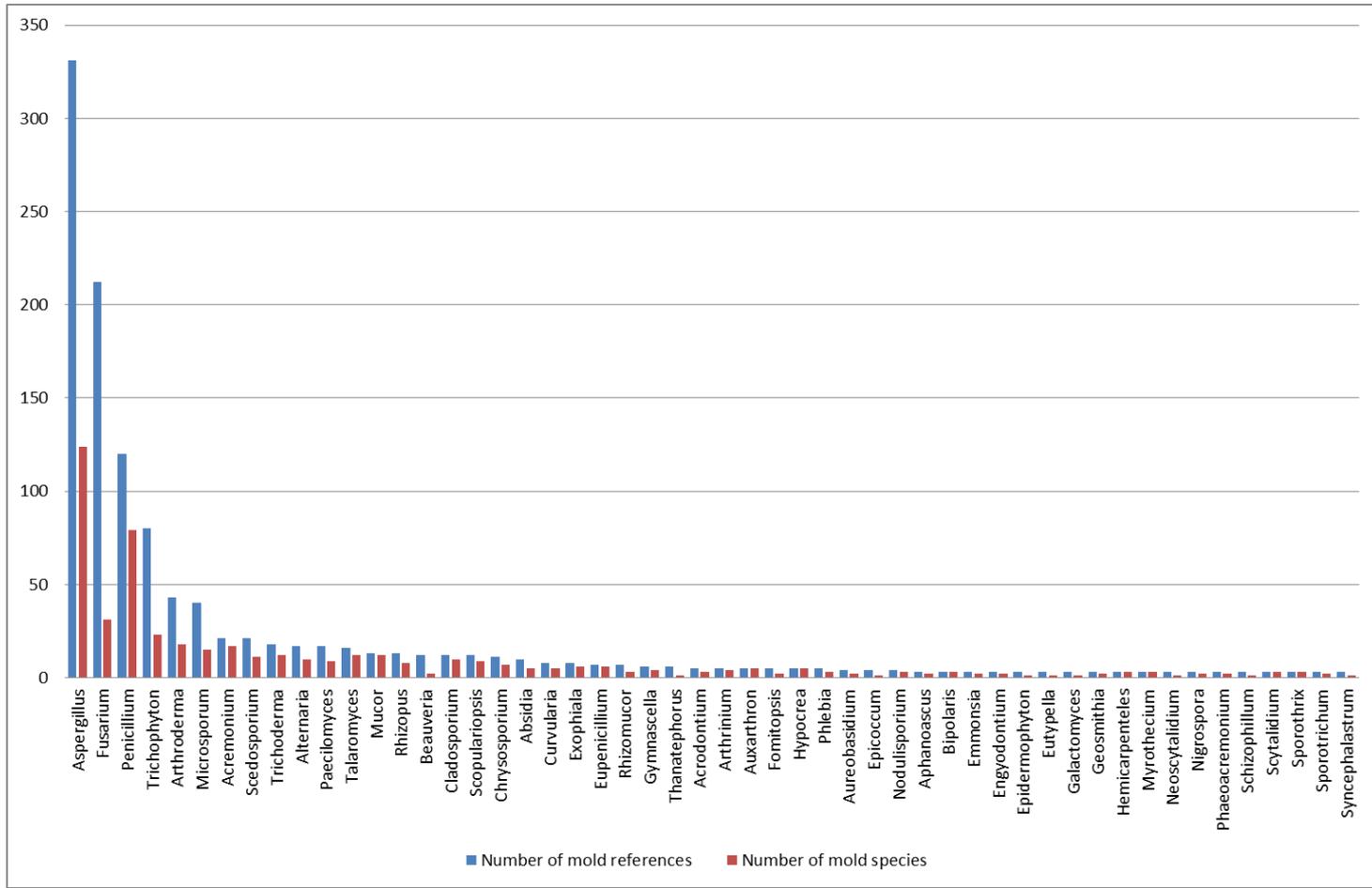
Correct clade
9% no ID



Online
Access:
MSI

- BCCM/IHEM fungi collection & team of Renaud Piarroux
=> In house database
 - Based upon reference strains from BCCM/IHEM collection: sequenced & available
 - Intra-species variability (>3 strains, multiple spectra per strain)





1913 strains, 938 species, 246 genera

Validation of a New Web Application for Identification of Fungi by Use of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry

A. C. Normand,¹ P. Becker,² F. Gabriel,³ C. Cassagne,⁴ I. Accoceberry,⁵ M. Gari-Toussaint,⁶ L. Hasseine,⁷ D. De Geyter,⁸ D. Pierard,⁹ I. Surmont,¹⁰ F. Djenad,¹¹ J. L. Donnadieu,¹² M. Piarroux,¹³ S. Ranque,¹⁴ M. Hendrickx,¹⁵ and R. Piarroux¹⁶

TABLE 3 Panel 2 identification results obtained with the five identification systems

Result for panel 2 sequenced strains ^a	No. (%) of strains identified				
	IHEM/MRS-MSI (threshold = 20)	IHEM/MRS-MBT (threshold = 1.7)	IHEM/MRS-MBT (threshold = 2.0)	Bruker-MBT (threshold = 1.7)	Bruker-MBT (threshold = 2.0)
Correct at the species level	435 (87.35)	411 (82.53)	312 (62.65)	259 (52.01)	119 (23.9)
Correct at the genus level	26 (5.22)	34 (6.83)	12 (2.41)	41 (8.23)	9 (1.81)
False at the genus level	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Identification criteria not met	37 (7.43)	53 (10.64)	174 (34.94)	198 (39.76)	370 (74.3)

^aFor each database/software combination, the number (%) of strains is specified. Correct, concordant with the molecular identification at either the species or the genus level. False, discordant with the molecular identification at the genus level. Identification criteria not met, score below the defined threshold.

Normand et al.

Journal of Clinical Microbiology

TABLE 4 Performance of the five identification systems per participating center

City	Parameter	% Identification (threshold value)				
		IHEM/MRS-MSI (20)	IHEM/MRS-MBT (1.7)	IHEM/MRS-MBT (2.0)	Bruker-MBT (1.7)	Bruker-MBT (2.0)
Bordeaux	% identification overall	88.16	84.58	48.98	66.81	41.23
	% identification at the species level	85.96	82.53	48.32	66.08	40.79
	% identification at the genus level	88.16	84.50	48.98	66.81	41.23
Bruges	% identification overall	80.65	84.95	43.55	54.30	19.89
	% identification at the species level	79.57	81.18	42.47	47.31	19.35
	% identification at the genus level	80.65	84.95	43.55	54.30	19.89
Brussels	% identification overall	90.18	80.36	49.36	38.14	7.78
	% identification at the species level	85.20	75.51	47.58	32.78	7.53
	% identification at the genus level	90.18	80.23	49.36	38.14	7.78
Marseille	% identification overall	94.63	93.61	71.23	59.70	23.80
	% identification at the species level	94.12	93.44	71.12	59.02	23.80
	% identification at the genus level	94.63	93.61	71.23	59.70	23.80
Nice	% identification overall	82.00	75.51	50.41	52.26	18.21
	% identification at the species level	80.66	72.53	49.28	49.49	17.90
	% identification at the genus level	81.89	75.21	50.41	51.95	18.21

Evaluation of three MALDI-TOF mass spectrometry libraries for the identification of filamentous fungi in three clinical microbiology laboratories in Manitoba, Canada.

Stein M^{1,2}, Tran V¹, Nichol KA², Laqacé-Wiens P^{1,2}, Pieroni P³, Adam HJ^{1,2}, Turenne C^{1,2}, Walkty AJ^{1,2}, Normand AC⁴, Hendrickx M⁵, Piarroux R⁴, Karlowsky JA^{1,2}.

Different origin?

TABLE 2 MALDI-TOF MS library mould species and genus identification rates and accuracy^a

Cumulative data from all three testing laboratories	MALDI-TOF MS Library					Conventional identification
	Bruker	NIH	Bruker +NIH	MSI	Bruker +NIH+MSI	
Number of isolates with no identification	143	108	84	57	45	0
Number of isolates with species-level identification	30	43	64	159	162	70
Number of isolates with genus-level identification	44	68	68	N/A ^b	9	121
Number of isolates with species- or genus-level identification	74	111	132	159	171	191
Number of isolates that were misidentified	4	2	5	5	4	30
Total number of identifications	78	113	137	164	175	221
Total number of isolates tested	221	221	221	221	221	221
Percentage of total number of identifications that were misidentifications	5.1%	1.8%	3.7%	3.1%	2.3%	13.6%
Species identification rate for the total number of isolates tested	13.6%	19.5%	29%	72%	73.3%	31.7%
Genus identification rate for the total number of isolates tested	33.5%	50.2%	59.7%	72%	77.4%	86.4%
Percentage of the total number of identifications made that agreed with the DNA sequencing result	94.9%	98.2%	96.4%	97%	97.7%	86.4%

^aSpecies identification referred to a MALDI-TOF MS score of ≥2.0 (Bruker Filamentous Fungi Library 1.0 or NIH library database) or ≥17 (MSI database) and genus identification referred to a MALDI-TOF MS score of ≥1.7- <2 (Bruker Filamentous Fungi Library 1.0 or NIH library database).

^bN/A, not applicable.



March 2017

September 2017

Isolation of 290 strains

ZOO: strains from turtle and penguin

Private Lab: practitioners treating pets

Public Lab: farm animals (miscarriages in cattle)



Gold standard

Multilocus gene sequencing



60%

10%

89% / 99%

1%

Conclusion

GREAT AT SMALL THINGS

- Reliable, fast & objective identification tool
- Gold standard remains MLGS
- Implemented in QC in our collection + identification service
- ↗ accuracy ↘ TAT

BCCM/IHEM team

Sam Roesems

Pierre Becker

Elizabet D'hooge

Dirk Stubbe

Renaud Piarroux

Anne Cécile Normand

Ann Packeu



