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## ► To cite this version:

D. Dupont, A.-C. Normand, F. Persat, M. Hendrickx, R. Piarroux, et al.. Comparison of matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) systems for the identification of moulds in the routine microbiology laboratory. *Clinical Microbiology and Infection*, 2019, 25, pp.892 - 897. 10.1016/j.cmi.2018.10.013 . hal-03486261

**HAL Id: hal-03486261**

**<https://hal.science/hal-03486261>**

Submitted on 20 Dec 2021

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**Comparison of Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) systems for the identification of moulds in the routine microbiology laboratory**

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**Key words:** MALDI-TOF, moulds, identification, mass spectrometry, filamentous fungi

**Word count:** 3444 words

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## **Abstract (250 words)**

Objectives: The purpose of this study was to compare the efficiency of mould identification of two MALDI-TOF MS systems - Vitek MS and Microflex LT - and the MSI application.

Methods: Moulds were collected retrospectively and prospectively to display epidemiological diversity of a microbiology lab. All of them were identified via sequencing. Strains were then identified using the VMS v3.0, the MLT and the MSI software applied on MLT spectra. Rates of correct identifications to the species, to the complex level and to the genus level were compared to the molecular Gold Standard.

Results: A total of 102 isolates were collected. The rate of correct identification to the species level with the MLT was 42.2% (43/102) with a threshold of 1.7 (vs 16.7% (17/102) with a threshold of 2.0,  $p<0.05$ ). The VMS performed better than the MLT with a threshold of 1.7 for species (49.0% (50/102),  $p=0.33$ ) and complex level identifications (71.6% (73/102) vs 54.9% (56/102),  $p<0.05$ ). However the highest performances were observed when the MLT spectra were analysed via the MSI software reaching 90.2% (92/102) of correct identification to the species, 92.2% (94/102) to the species complex and 94.1% (96/102) to the genus level.

Conclusions: The VMS performed better than the MLT for mould identification. However, it remains of utmost importance to expand commercial databases, as performances of the MLT highly improved when using the MSI software and its extended database, reaching far above the VMS system. Thus the VMS could benefit from the use of this online tool.

## Introduction

During the last decade MALDI-TOF mass spectrometry (MALDI-TOF MS) has risen as one of the most cost-effective and accurate identification tools in microbiology laboratories. MALDI-TOF MS has earned universal acclaim for bacteria identification and is gradually taking over the traditional use of plates, however this method still suffers from different drawbacks in the field of mycology, especially for the identification of filamentous fungi. [1] Molecular identification remains the gold standard for fungal identification, but with high costs, long time to result, technical traps (contamination, amplification of paralog genes) and the need for experienced personnel, it has only been implemented in specialised centres or reference laboratories [2,3]. Although many studies [4–6] have already shown the usefulness of MALDI-TOF MS for yeast identification to the species level, mould identification rates remain insufficient for the medical mycology laboratory [2]. However, several studies have shown that MALDI-TOF MS could be adapted for the identification of moulds using properly designed reference spectrum libraries[7–9]. The purpose of this collaborative study was to compare the efficiency of clinically relevant mould identification between two MALDI-TOF MS systems, namely the VMS v3.0 (bioMérieux, Marcy l'Etoile, France) and the MLT (Bruker Daltonics GmbH, Bremen, Germany) in the routine clinical laboratory setting. Furthermore we aimed to evaluate the impact of applying the newly described MSI (Mass Spectrometry Identification) online application (<https://biological-mass-spectrometry-identification.com/msi/>) to the MLT spectra as compared to the VMS v3.0.

## Materials and methods

This work was done in collaboration between two French teaching hospitals in Marseille and Lyon. Trying to display the classical epidemiological diversity of a clinical microbiology lab of a university teaching hospital, we chose to retrospectively collect medically relevant moulds, isolated from proven or probable invasive fungal infections [10]. Furthermore, strains were collected prospectively over the course of October 2016. All strains were collected in Lyon teaching hospitals and were unrelated with those used to build MALDI-TOF libraries.

Strains were grown on standard fungal medias such as Can2 (bioMérieux, Marcy l'Etoile, France), Sabouraud Dextrose Agar with chloramphenicol and gentamicin (BioRad, Marnes-la-Coquette, France) associated or not with actidione (cycloheximide) which allowed morphological characterization. Additionally, they were stored at -20°C on CryoBeads before thawing and plating.

Molecular identification using multilocus gene sequencing was performed on a LightCycler 2.0 (Roche, Germany) after DNA extraction from a 1 cm<sup>2</sup> mould colony using the Qiagen DNA mini kit, as recommended by the manufacturer. The following primers were used: ITS1/ITS4 [11], NL1/NL4 for LSU [12], Bt2a/Bt2b for  $\beta$ -tubulin [13], and EF1/EF2 for TEF1 $\alpha$  [14].

The PCR assay was performed in 20  $\mu$ L, including 5  $\mu$ l of DNA; 0.5  $\mu$ M (each) primer and 1 $\times$  FastStart SybrGreen Master (Roche, Germany). The assay began at 95°C for 10 min and 45 amplification cycles of 95°C for 10 sec, 62°C for s (ITS) / 59°C for 10 sec (LSU) / 69°C for 10 sec (BT) / 57°C for 10 sec (TEF) and an extension step at 72°C for 30 sec. Amplimers produced by real-time PCR were sequenced on a ABI3730xl (Applied Biosystems). The sequences were aligned and compared with GenBank reference sequences using BLAST searches, CBS Knav Database (<http://www.cbs.knaw.nl/collections/BioloMICSSequences.aspx>), French National Reference

Center for Mycosis database for ITS sequences  
(<http://fungibank.pasteur.fr/BioloMICSSequences.aspx?file=MOLDseq&file=YEASTseq>)  
and FUSARIUM-ID for TEF sequences (<http://isolate.fusariumdb.org/>).

## **MALDI-TOF MS assays**

### **Vitek MS v3.0 system**

The strains were grown at 35°C on Can2 (bioMérieux, Marcy l'Etoile, France) for 24-48 hours, depending on growth. Fungal hyphae and spores were collected gently at the surface of the colony in order not to collect any agar. Extraction process using the new Vitek MS Mould Kit was used as recommended by the manufacturer (bioMérieux, Marcy l'Etoile, France). One µL of the supernatant was then deposited in four replicates on a target plate and air-dried. One µL of α-cyano-4-hydroxycinnamic acid (HCCA) matrix was added thus permitting recrystallization with the sample. For each acquisition group with a strain of *Escherichia coli* (ATCC8739) was used as calibrator in each experiment. Samples were processed in the VMS spectrometer using the manufacturer's standard settings. Spectrums were analysed using VMS automation control and Myla software. This included IVD database version 3.0. (bioMérieux, Marcy l'Etoile, France) containing 79 references.

According to the manufacturer, values between 60.0 and 99.9 indicate a reliable discrimination of species. However we chose to use a more discriminant cut off of 95% for identification as acknowledged by previous use with yeasts [15]. Only the deposit with the best identification score was taken into account by analogy with previously published literature about Bruker MALDI-TOF [16].

### **Microflex LT system**

The strains were grown at 35°C on Can2 (bioMérieux, Marcy l'Etoile, France) for 24-48 hours, depending on growth. Fungal hyphae and spores were collected gently at the surface of the colony, in order not to collect any agar. Extraction process was performed as described by Cassagne et al [17]. Briefly, the isolates were treated with a formic acid and acetonitrile protein extraction step. Four replicates of one µL of protein extract per isolate were deposited and the samples were then covered with one µL of HCCA matrix. Mass spectra were acquired with a MLT (Bruker Daltonics®) using the default acquisition parameters recommended by Bruker and using the new MBT Filamentous Fungi Library associated with the basic RUO library (332 official references in the Bruker Taxonomy-Eukaryota and 127 in the MBT Filamentous Fungi Library). Only the highest score of four spots was taken into account both with a 1.7 log(score) threshold and a 2.0 log(score) threshold as recommended by the manufacturer [16].

### **MSI online system**

The spectrums obtained with the MLT were analyzed using a newly described web application for the identification of moulds and yeasts available to medical mycologists (MSI software developed by Normand et al [18]) and using a threshold of 20 (938 references). Since its database was built using a MLT, spectra obtained with the VMS could not be analysed using MSI.

### **Statistical analysis**

All isolates were analysed using the MLT and the VMS systems, as well as the MSI software. The results obtained using these three libraries were then classified into five categories according to score, identification results and concordance/discordance with molecular typing: correct at the species level, correct at the complex level, correct at the genus level, false at the

genus level and identification criteria not met. “Correct” corresponds to concordant with the molecular identification at either the species, the complex or the genus level whereas “false” means discordant with the molecular identification at the genus level. “Identification criteria not met” corresponds to an identification score below the defined threshold.

Differences between spectral analysis results were analysed with the  $\chi^2$  test, and p values <0.05 were considered significant. The method to calculate confidence intervals is the Wilson score method without continuity correction.

## **Results**

### **Description of the species identified**

A total of 102 isolates were collected and included during the study. They represented 20 genera comprising of 45 taxa, all of which were identified via DNA sequencing. Details on the species variety included in this study are shown in Table 1. Twenty one taxa included in this study were not present in the VMS database, 21 taxa were not in the Bruker database, while only 6 taxa were not in the MSI database (Table 1). Overall 77 isolates were present in the VMS database, 65 in the Bruker database and 95 in the MSI database.

### **MALDI-TOF mass spectrometry based identification**

The results obtained using the three libraries are summarized in Table 2. Interestingly, no false identification was obtained for the isolates analysed using the MLT system whereas one false identification was obtained using the VMS.

### **Global results**

The percentage of isolates that did not meet the identification criteria differed greatly between the database/threshold association used, ranging from 81.4% (83/102) for the MLT system



using the Bruker library with a threshold of 2.0, to as low as 5.9% (6/102) for the MLT system associated with the MSI software.

When comparing the VMS to the MLT using the Bruker library with a 2.0 recommended threshold, we observed significant differences in favour of the VMS for all categories (genus level:  $p = 3.0 \cdot 10^{-14}$ , complex level:  $p = 9.4 \cdot 10^{-15}$ , species level:  $p = 2.2 \cdot 10^{-7}$ , identification criteria not met:  $p = 1.1 \cdot 10^{-14}$ ). Using a threshold of 1.7 increases performances of the MLT with the Bruker library for all categories as compared to using a threshold of 2.0 (genus level:  $p = 1.7 \cdot 10^{-9}$ , complex level:  $p = 3.1 \cdot 10^{-8}$ , species level:  $p = 6.5 \cdot 10^{-5}$ , identification criteria not met:  $p = 1.7 \cdot 10^{-9}$ ). However, the MLT using a threshold of 1.7 with the Bruker library still remains significantly inferior to the VMS at the complex level identification ( $p = 0.01$ ), whereas for the other categories there is no significant difference (genus level:  $p = 0.08$ , species level:  $p = 0.21$ , identification criteria not met:  $p = 0.05$ ).

Finally, the highest performances were observed when the MLT spectra were analysed via the MSI software (MSI vs Bruker library using a 2.0 threshold, genus level:  $p = 1.6 \cdot 10^{-27}$ , complex level:  $p = 1.1 \cdot 10^{-26}$ , species level:  $p = 6.5 \cdot 10^{-26}$ , identification criteria not met:  $p = 1.6 \cdot 10^{-27}$ ; MSI vs 1.7 threshold, genus level:  $p = 5.9 \cdot 10^{-9}$ , complex level:  $p = 1.6 \cdot 10^{-9}$ , species level:  $p = 4.1 \cdot 10^{-13}$ , identification criteria not met:  $p = 5.9 \cdot 10^{-9}$ ). Additionally, the use of MSI is significantly superior to the VMS (genus level:  $p = 1.9 \cdot 10^{-5}$ , complex level:  $p = 1.4 \cdot 10^{-4}$ , species level:  $p = 7.9 \cdot 10^{-10}$ , identification criteria not met:  $p = 3.6 \cdot 10^{-5}$ ).

### **Database related results**

Looking at database related results, VMS results are significantly better than the global results for all categories (genus level:  $p = 7.2 \cdot 10^{-5}$ , complex level:  $p = 7.2 \cdot 10^{-5}$ , species level:  $p = 0.03$ , identification criteria not met:  $p = 3.7 \cdot 10^{-5}$ ). Results for MLT using a 1.7 threshold are significantly better only to the species level identification (genus level:  $p = 0.15$ , complex level:  $p = 0.07$ , species level:  $p = 2.5 \cdot 10^{-3}$ , identification criteria not met:  $p = 0.15$ ). The results

obtained using a 2.0 threshold did not differ significantly when comparing database related results to global results ( $p < 0.05$  for all categories). However, database related results were significantly better than global results when using the MSI software for all categories ( $p = 0.02$  for all categories).

Comparing the VMS database related results to the MLT using the Bruker library with a 2.0 recommended threshold, we observed significant differences in favour of the VMS for all categories (genus level:  $p = 1.0 \cdot 10^{-16}$ , complex level:  $p = 2.7 \cdot 10^{-17}$ , species level:  $p = 8.9 \cdot 10^{-7}$ , identification criteria not met:  $p = 1.8 \cdot 10^{-18}$ ). Comparison of VMS results to the MLT using the Bruker library with a threshold of 1.7 showed significant differences in favour of the VMS in all categories but identification to the species level (genus level:  $p = 1.1 \cdot 10^{-4}$ , complex level:  $p = 5.1 \cdot 10^{-5}$ , species level:  $p = 0.86$ , identification criteria not met:  $p = 3.2 \cdot 10^{-5}$ ). Using a threshold of 1.7 still increases the performances of the MLT with the Bruker library for all categories as compared to using a threshold of 2.0 (genus level:  $p = 9.0 \cdot 10^{-7}$ , complex level:  $p = 8.8 \cdot 10^{-7}$ , species level:  $p = 4.8 \cdot 10^{-6}$ , identification criteria not met:  $p = 9.0 \cdot 10^{-7}$ ).

Finally the best results are obtained when using the MLT spectra with the MSI software (MSI vs VMS : genus level:  $p = 6.2 \cdot 10^{-4}$ , complex level:  $p = 3.3 \cdot 10^{-3}$ , species level:  $p = 5.2 \cdot 10^{-8}$ , identification criteria not met:  $p = 1.3 \cdot 10^{-3}$ ; MSI vs Bruker library using a 2.0 threshold, genus level:  $p = 6.1 \cdot 10^{-23}$ , complex level:  $p = 1.0 \cdot 10^{-22}$ , species level:  $p = 6.9 \cdot 10^{-22}$ , identification criteria not met:  $p = 6.1 \cdot 10^{-23}$  ; MSI vs 1.7 threshold, genus level:  $p = 2.0 \cdot 10^{-8}$ , complex level:  $p = 4.6 \cdot 10^{-8}$ , species level:  $p = 3.4 \cdot 10^{-8}$ , identification criteria not met:  $p = 2.0 \cdot 10^{-8}$ ).

## Discussion

In this study, we aimed to compare the two commercially available database/software associations for MALDI-TOF MS, namely the VMS from bioMérieux and the MLT from Bruker Daltonics. Yeast identification has been shown to be highly efficient and accurate

[19]. Conversely mould identification remains underevaluated particularly when using commercially available databases[19–21]. Regarding the VMS only a few studies have focused on specific taxon such as *Aspergillus* [22,23], or dermatophytes[24–26] and only one study, to our knowledge, have evaluated precisely this system on the species diversity observed in a medical mycology laboratory [27]. Previously lacking a reliable algorithm of spectral analysis for mould identification the VMS system has recently been updated to a newer version, namely v3.0 with a new algorithm and an enlarged database. This major update requires to be evaluation. Recently, the use of extensive reference spectrum databases has been widely encouraged [7,9]. Thus Rychert et al evaluated the performances of this new version reporting a rate of 91% of correct identification of moulds to the species level [28]. But as opposed to our study, they analysed only isolates that were known previously to be represented in the database. This highlights our results considering that in a clinical setting analysed isolates are not always present in the database.

As opposed to previous versions, the VMS has greatly improved (data not shown) considering mould identification. Results point out that VMS is superior to the MLT using the manufacturer recommended threshold of 2.0 at all taxonomic levels ( $p < 0.05$  for all four categories). As reported previously the use of a 1.7 threshold significantly increased the accuracy of the MLT system for all identification levels (identification to the species: 16.7% (17/102) to 42.2% (43/102),  $p < 0.05$ ) as previously reported [16,29]. However overall satisfactory identification seems in favour of the VMS as compared to the MLT system using a threshold of 1.7, with a rate of correct identification to the complex level significantly in favour of the VMS (71.6% (73/102) vs 54.9% (56/102),  $p < 0.05$ ). Indeed, looking at the other categories, the VMS system performs slightly better than the MLT – threshold of 1.7 – but with no significative differences ( $p > 0.05$ ). In order to evaluate more precisely the algorithm as opposed to the database, strains not belonging to the database were discarded and

identification accuracy results updated. VMS obtained better results than MLT using a 2.0 threshold for all identification levels. Moreover VMS performed better than MLT using a 1.7 threshold for all identification levels but to the species level.

Only one identification was false, at the genus level, and was reported with the VMS. This discrepancy occurred for a *Rhizopus oryzae* that was identified as an *Aspergillus brasiliensis*. Macroscopic and microscopic examination of the subculture used for MALDI-TOF was congruent with DNA sequencing of *R. oryzae*. New acquisition of this strain did not yield any results. *R. oryzae* identification using the VMS regularly does not give result in routine use (data not shown). Increasing the number of strains per taxa in the spectral database could be useful in preventing this kind of misidentification.

Considering isolates that did not meet the identification criteria, they spanned from 27.5% (28/102) for the VMS to as high as 81.4% (83/102) for the MLT using a threshold of 2.0. When discarding isolates not present in the different databases, these rates decreased to 2.9% (3/77) for VMS and to 72.3% (47/65) for MLT. This decrease is due to the use of limited reference spectrum database. However these 3 “no results” isolates with VMS (two *A. tubingensis* and one *A. flavus*) might be due to issues during the extraction process.

Looking at Bruker results, the high rates of identification criteria not met might be due to spectral analysis algorithm issues. Since MSI identifications were obtained from the same Bruker acquisition that yielded the MLT results, we can rule out extraction issues. Globally, the VMS has a lower rate of unidentified isolates as compared to the MLT system. The newly available update version of the VMS allowed the identification of more than 70% of isolates to the genus level. However it remains almost 49% of unsatisfactory identifications (correct at the genus but not at the species level or no identification or incorrect at the genus level). Most of these “correct at the genus or complex level but not at the species level” correspond to species complex issues. In fact the VMS database frequently relies on complex level reference

spectrum without specific intra-complex spectrum (13 *A. niger* complex, two *A. terreus* complex, two *Rasamsonia argillacea* complex, one *Rhizopus arrhizus* complex, one *Fusarium solani* complex). As only a few of these species complex have been investigated for antifungal susceptibility (eg *Aspergillus* section Fumigati and *Aspergillus lentulus*), it remains of the utmost importance to expand the number of reference spectra of species belonging to species complex. Looking at the Bruker database, intra-complex spectra are mostly used. Five strains were only identified at the genus level (two *Penicillium brasilianum* identified as *P. discolor*, a *P. sayulitensis* identified as *P. funiculosum*, a *P. chrysogenum* identified as *Penicillium* sp. and a *Curvularia spicifera* identified as *C. pallescens*). Only *P. chrysogenum* is present in the Bruker database. Using the MSI database, two strains were correctly identified only at the genus level (*F. oxysporum* identified as *F. ananatum* and *Penicillium sayulitensis* identified as *Talaromyces siamensis*), the latter being not present in the database. Looking at isolates not present in the database, MLT using a threshold of 1.7 was able to identify correctly 15 isolates to the genus or complex level (*Aspergillus mangaliensis*, *A. intermedius*, *A. tubingensis*, *A. calidoustus*, *Rasamsonia aegroticola*, *P. brasilianum*, *P. sayulitensis* and *C. pallescens*). All these identification errors involve closely related species and they should have no impact on therapeutic regimen. In order to reduce these misidentifications, and as supported by others, there is a considerable need to expand MALDI-TOF databases in order to increase the efficiency of these systems. This is possible using the Saramis system for research use only, but this is manageable only for specialized laboratories and is greatly time consuming.

Looking at the MLT system, it has been shown that its database could easily be enhanced by means of the addition of an in-house reference spectrum [7,8,16]. Recently, Normand et al showed the great potential of a new web application for the identification of yeasts and moulds available to medical mycologists (MSI software [18]). These results are

confirmed in our study in which MSI software greatly improves MLT system's performances (p<0.05) looking at correct identification at the species level (16.7% (17/102) to 90.2% (92/102)), at the complex (17.7% (18/102) to 92.2% (94/102)), at the genus (18.6% (19/102) to 94.1% (96/102)) or strains for which the identification criteria could not be met (81.4% (83/102) to 5.9% (6/102)). The use of this web software applied to MLT spectrums is greatly superior to the performances of the VMS. Accurate identification to the species is achieved in 90.2% (92/102) of isolates compared to 51.0% (52/102) when using the VMS (p<0.05). Moreover, "identification criterion not met" occurs less frequently when using the MSI software (5.9% (6/102) vs 27.5% (28/102), p<0.05). Only one isolate not present in the MSI database (*Aspergillus mangaliensis*) was correctly identified at the complex level as *A. iizukae*. Comparing database related results allowed us to compare the algorithm and the architecture of the database used. MLT database has been built on 1,158 references corresponding to 336 species (yeasts and molds) ranging from one to 31 strains per species. Only one reference was acquired for each strain. Whereas MSI and VMS databases were built using multiple spectrums per strains, giving far better results. VMS included only 79 species but its architecture is robust since it was constructed using two to 22 strains per species (75% represented by four or more isolates (D. Pincus, personal communication)), resulting in over 20 reference spectrums per species. MSI included 11,851 spectrums corresponding to 1,913 strains (938 species) ranging from two to eight spectrums per strain. This might explain the better performances of these two systems. Obviously, this study has some limitation, particularly its small size. Thus notable pathogens are missing and larger studies are required to confirm these results. However the relatively low number of isolates included in this study was largely enough to significantly highlight the flaws of commercial MALDI-TOF MS systems in the routine mycology laboratory. Our results show that original algorithms and enhanced database available online could compensate the flaws of commercial databases and

thus allow better identification of clinically relevant moulds. As did the MLT, the VMS could benefit from the use of this online software and further works should be prompted to enable the compatibility of VMS spectrums with the MSI software in order to reach maximum correct identifications to the species level.

**Transparency declaration:** no conflict of interest to declare, no external funding was received. Part of these results were presented as an oral presentation at the congress of the Société Française de Mycologie Médicale (SFMM, Medical Mycology French Society), in Nice, France, May 16<sup>th</sup> 2018.

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436    **List of tables**

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438    Table 1: Species variety included in this study

439    Table 2: Identification results obtained with the MALDI-TOF systems

Genus	Species	Number of strains	Presence in the database		
			VMS	MLT	MSI
<i>Alternaria</i>	<i>Alternaria alternata</i> complex	2	x	x	x
<i>Aspergillus</i>	<i>Aspergillus calidoustus</i>	1	x		x
	<i>Aspergillus flavus</i> - <i>oryzae</i>	6	x	x	x
	<i>Aspergillus floccosus</i>	1	x		x
	<i>Aspergillus fumigatus</i>	25	x	x	x
	<i>Aspergillus intermedius</i>	2			x
	<i>Aspergillus mangaliensis</i>	1			
	<i>Aspergillus niger</i> ( <i>welwitschiae</i> )	3	x	x	x
	<i>Aspergillus sydowii</i>	4	x	x	x
	<i>Aspergillus terreus</i>	1	x	x	x
	<i>Aspergillus tubingensis</i>	12	x		x
	<i>Emericella nidulans</i> ( <i>var echinulata</i> )	1			
	<i>Neosartorya pseudofischeri</i>	1		x	x
<i>Bjerkandera</i>	<i>Bjerkandera adusta</i> / <i>Thanetophorus cucumeris</i>	1		x	x
<i>Curvularia</i>	<i>Curvularia spicifera</i>	1	x		x
<i>Exophiala</i>	<i>Exophiala bergeri</i>	1			
<i>Fusarium</i>	<i>Fusarium oxysporum</i> complex	2	x	x	x
	<i>Fusarium proliferatum</i> / <i>Gibberella intermedia</i>	2	x	x	x
	<i>Fusarium petrophilum</i>	1	x		x
	<i>Gibberella fujikuroi</i> complex ( <i>Fusarium temperatum</i> )	1	x		x
<i>Galactomyces</i>	<i>Galactomyces candidum</i>	1	x	x	x
<i>Lichtheimia</i>	<i>Lichtheimia corymbifera</i>	1	x	x	x
<i>Lopharia</i>	<i>Lopharia spadicea</i>	1			x
<i>Metarhizium</i>	<i>Metarhizium robertsii</i>	3			
<i>Microsporum</i>	<i>Microsporum canis</i>	1	x	x	x
<i>Mucor</i>	<i>Mucor circinelloides</i> f. <i>circinelloides</i>	2		x	x
	<i>Mucor velutinosus</i>	1	x		x
<i>Neurospora</i>	<i>Neurospora sitophila</i>	1			x
<i>Penicillium</i>	<i>Penicillium brasilianum</i>	2			x
	<i>Penicillium chrysogenum</i>	3	x	x	x
	<i>Penicillium citrinum</i>	1	x	x	x
	<i>Penicillium glabrum</i>	1		x	x
	<i>Penicillium griseofulvum</i>	1			x
	<i>Penicillium purpurogenum</i>	1		x	x
	<i>Penicillium sayulitensis</i> / sp nov ?	1			
	<i>Talaromyces amestolkiae</i>	1			x
	<i>Talaromyces</i> sp.	1			x
<i>Phaeoacremonium</i>	<i>Phaeoacremonium parasiticum</i>	1		x	x
<i>Rasamsonia</i>	<i>Rasamsonia aegroticola</i>	2	x		
<i>Rhizomucor</i>	<i>Rhizomucor pusillus</i>	1		x	x
<i>Rhizopus</i>	<i>Rhizopus oryzae</i>	2	x	x	x
<i>Scedosporium</i>	<i>Scedosporium boydii</i>	1	x	x	x
	<i>Lomentospora prolificans</i>	1	x	x	x
<i>Schizophyllum</i>	<i>Schizophyllum commune</i>	1		x	x
<i>Scytalidium</i>	<i>Neoscytalidium dimidiatum</i>	1			x

**Table 1: Species variety included in this study**

VMS : Vitek MS; MLT : Microflex LT; MSI : in house online application

**Table 2: Identification results obtained with the MALDI-TOF systems**

Overall result for sequenced strains	No. (%) of strains identified, [ ] confidence intervals (95%)			
	Vitek-MS (threshold 95)	Bruker-MBT (threshold > 1.7)	Bruker-MBT (threshold > 2.0)	Bruker MSI-Community (threshold 20)
Correct at the species level	52 (51.0) [41.4 - 60.5]	43 (42.2) [33.0 - 51.9]	17 (16.7) [10.7 - 25.1]	92 (90.2) [82.9 - 94.6]
Correct at the complex level	73 (71.6) [ - 79.4]	56 (54.9) [45.2 - 64.2]	18 (17.7) [11.5 - 26.2]	94 (92.2) (85.3 - 96.0]
Correct at the genus level	73 (71.6) [62.2 - 79.4]	61 (59.8) [50.1 - 68.8]	19 (18.6) [12.3 - 27.3]	96 (94.1) [87.8 - 97.3]
False at the genus level	1 (1.0) [0.2 - 5.4]	0 (0) [0.0 - 3.6]	0 (0) [0.0 - 3.6]	0 (0) [0.0 - 3.6]
Identification criteria not met	28 (27.5) [19.7 - 36.8]	41 (40.2) [31.2 - 49.9]	83 (81.4) [72.7 - 87.7]	6 (5.9) [2.7 - 12.2]
Database related result for sequenced strains				
Correct at the species level	52 (67.5) [56.5 - 76.9]	43 (66.2) [54.0 - 76.5]	17 (26.2) [17.0 - 38.0]	93 (97.9) [92.7 - 99.4]
Correct at the complex level	73 (94.8) [87.4 - 98.0]	45 (69.2) [57.2 - 79.1]	17 (26.2) [17.0 - 38.0]	94 (99.0) [94.3 - 99.8]
Correct at the genus level	73 (94.8) [87.4 - 98.0]	46 (70.8) [58.8 - 80.4]	18 (27.7) [18.3 - 39.6]	95 (100.0) [96.1 - 100.0]
False at the genus level	1 (1.3) [0.2 - 7.0]	0 (0) [0.0 - 5.6]	0 (0) [0.0 - 5.6]	0 (0) [0.0 - 3.9]
Identification criteria not met	3 (2.9) [1.0 - 8.3]	19 (29.2) [19.6 - 41.2]	47 (72.3) [60.4 - 81.7]	0 (0) [0.0 - 3.9]

Correct, concordant with the molecular identification at either the species, the complex or the genus level; False, discordant with the molecular identification at the genus level; Identification criteria not met, score below the defined threshold.