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

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17

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19

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24 **Abstract (250 words)**

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

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

32 Results: A total of 102 isolates were collected. The rate of correct identification to the species
33 level with the MLT was 42.2% (43/102) with a threshold of 1.7 (vs 16.7% (17/102) with a
34 threshold of 2.0, $p < 0.05$). The VMS performed better than the MLT with a threshold of 1.7
35 for species (49.0% (50/102), $p = 0.33$) and complex level identifications (71.6% (73/102) vs
36 54.9% (56/102), $p < 0.05$). However the highest performances were observed when the MLT
37 spectra were analysed via the MSI software reaching 90.2% (92/102) of correct identification
38 to the species, 92.2% (94/102) to the species complex and 94.1% (96/102) to the genus level.

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

43 **Introduction**

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

64

65 **Materials and methods**

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

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

77 Molecular identification using multilocus gene sequencing was performed on a LightCycler
78 2.0 (Roche, Germany) after DNA extraction from a 1 cm² mould colony using the Qiagen
79 DNA mini kit, as recommended by the manufacturer. The following primers were used:
80 ITS1/ITS4 [11], NL1/NL4 for LSU [12], Bt2a/Bt2b for β -tubulin [13], and EF1/EF2 for
81 TEF1 α [14].

82 The PCR assay was performed in 20 μ L, including 5 μ l of DNA; 0.5 μ M (each) primer and 1 \times
83 FastStart SybrGreen Master (Roche, Germany). The assay began at 95°C for 10 min and 45
84 amplification cycles of 95°C for 10 sec, 62°C for s (ITS) / 59°C for 10 sec (LSU) / 69°C for
85 10 sec (BT) / 57°C for 10 sec (TEF) and an extension step at 72°C for 30 sec. Amplimers
86 produced by real-time PCR were sequenced on a ABI3730xl (Applied Biosystems). The
87 sequences were aligned and compared with GenBank reference sequences using BLAST
88 searches, CBS Knaw Database
89 (<http://www.cbs.knaw.nl/collections/BioloMICSSequences.aspx>), French National Reference

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

93 **MALDI-TOF MS assays**

94 **Vitek MS v3.0 system**

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

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

111

112 **Microflex LT system**

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

125

126 **MSI online system**

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

132

133 **Statistical analysis**

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

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

142 Differences between spectral analysis results were analysed with the χ^2 test, and p values
143 <0.05 were considered significant. The method to calculate confidence intervals is the Wilson
144 score method without continuity correction.

145 **Results**

146

147 **Description of the species identified**

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

154

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

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

159 **Global results**

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

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

164 When comparing the VMS to the MLT using the Bruker library with a 2.0 recommended
165 threshold, we observed significant differences in favour of the VMS for all categories (genus
166 level: $p = 3.0 \cdot 10^{-14}$, complex level: $p = 9.4 \cdot 10^{-15}$, species level: $p = 2.2 \cdot 10^{-7}$, identification
167 criteria not met: $p = 1.1 \cdot 10^{-14}$). Using a threshold of 1.7 increases performances of the MLT
168 with the Bruker library for all categories as compared to using a threshold of 2.0 (genus level:
169 $p = 1.7 \cdot 10^{-9}$, complex level: $p = 3.1 \cdot 10^{-8}$, species level: $p = 6.5 \cdot 10^{-5}$, identification criteria not
170 met: $p = 1.7 \cdot 10^{-9}$). However, the MLT using a threshold of 1.7 with the Bruker library still
171 remains significantly inferior to the VMS at the complex level identification ($p = 0.01$),
172 whereas for the other categories there is no significant difference (genus level: $p = 0.08$,
173 species level: $p = 0.21$, identification criteria not met: $p = 0.05$).

174 Finally, the highest performances were observed when the MLT spectra were analysed via the
175 MSI software (MSI vs Bruker library using a 2.0 threshold, genus level: $p = 1.6 \cdot 10^{-27}$,
176 complex level: $p = 1.1 \cdot 10^{-26}$, species level: $p = 6.5 \cdot 10^{-26}$, identification criteria not met: $p = 1.6$
177 $\cdot 10^{-27}$; MSI vs 1.7 threshold, genus level: $p = 5.9 \cdot 10^{-9}$, complex level: $p = 1.6 \cdot 10^{-9}$, species
178 level: $p = 4.1 \cdot 10^{-13}$, identification criteria not met: $p = 5.9 \cdot 10^{-9}$). Additionally, the use of MSI
179 is significantly superior to the VMS (genus level: $p = 1.9 \cdot 10^{-5}$, complex level: $p = 1.4 \cdot 10^{-4}$,
180 species level: $p = 7.9 \cdot 10^{-10}$, identification criteria not met: $p = 3.6 \cdot 10^{-5}$).

181 **Database related results**

182 Looking at database related results, VMS results are significantly better than the global results
183 for all categories (genus level: $p = 7.2 \cdot 10^{-5}$, complex level: $p = 7.2 \cdot 10^{-5}$, species level: $p =$
184 0.03 , identification criteria not met: $p = 3.7 \cdot 10^{-5}$). Results for MLT using a 1.7 threshold are
185 significantly better only to the species level identification (genus level: $p = 0.15$, complex
186 level: $p = 0.07$, species level: $p = 2.5 \cdot 10^{-3}$, identification criteria not met: $p = 0.15$). The results

187 obtained using a 2.0 threshold did not differ significantly when comparing database related
188 results to global results ($p < 0.05$ for all categories). However, database related results were
189 significantly better than global results when using the MSI software for all categories ($p =$
190 0.02 for all categories).

191 Comparing the VMS database related results to the MLT using the Bruker library with a 2.0
192 recommended threshold, we observed significant differences in favour of the VMS for all
193 categories (genus level: $p = 1.0 \cdot 10^{-16}$, complex level: $p = 2.7 \cdot 10^{-17}$, species level: $p = 8.9 \cdot 10^{-7}$,
194 identification criteria not met: $p = 1.8 \cdot 10^{-18}$). Comparison of VMS results to the MLT using
195 the Bruker library with a threshold of 1.7 showed significant differences in favour of the VMS
196 in all categories but identification to the species level (genus level: $p = 1.1 \cdot 10^{-4}$, complex
197 level: $p = 5.1 \cdot 10^{-5}$, species level: $p = 0.86$, identification criteria not met: $p = 3.2 \cdot 10^{-5}$). Using a
198 threshold of 1.7 still increases the performances of the MLT with the Bruker library for all
199 categories as compared to using a threshold of 2.0 (genus level: $p = 9.0 \cdot 10^{-7}$, complex level: p
200 $= 8.8 \cdot 10^{-7}$, species level: $p = 4.8 \cdot 10^{-6}$, identification criteria not met: $p = 9.0 \cdot 10^{-7}$).

201 Finally the best results are obtained when using the MLT spectra with the MSI software (MSI
202 vs VMS : genus level: $p = 6.2 \cdot 10^{-4}$, complex level: $p = 3.3 \cdot 10^{-3}$, species level: $p = 5.2 \cdot 10^{-8}$,
203 identification criteria not met: $p = 1.3 \cdot 10^{-3}$; MSI vs Bruker library using a 2.0 threshold, genus
204 level: $p = 6.1 \cdot 10^{-23}$, complex level: $p = 1.0 \cdot 10^{-22}$, species level: $p = 6.9 \cdot 10^{-22}$, identification
205 criteria not met: $p = 6.1 \cdot 10^{-23}$; MSI vs 1.7 threshold, genus level: $p = 2.0 \cdot 10^{-8}$, complex level:
206 $p = 4.6 \cdot 10^{-8}$, species level: $p = 3.4 \cdot 10^{-8}$, identification criteria not met: $p = 2.0 \cdot 10^{-8}$).

207

208 **Discussion**

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

212 [19]. Conversely mould identification remains underevaluated particularly when using
213 commercially available databases[19–21]. Regarding the VMS only a few studies have
214 focused on specific taxon such as *Aspergillus* [22,23], or dermatophytes[24–26] and only one
215 study, to our knowledge, have evaluated precisely this system on the species diversity
216 observed in a medical mycology laboratory [27]. Previously lacking a reliable algorithm of
217 spectral analysis for mould identification the VMS system has recently been updated to a
218 newer version, namely v3.0 with a new algorithm and an enlarged database. This major
219 update requires to be evaluation. Recently, the use of extensive reference spectrum databases
220 has been widely encouraged [7,9]. Thus Rychert et al evaluated the performances of this new
221 version reporting a rate of 91% of correct identification of moulds to the species level [28].
222 But as opposed to our study, they analysed only isolates that were known previously to be
223 represented in the database. This highlights our results considering that in a clinical setting
224 analysed isolates are not always present in the database.

225 As opposed to previous versions, the VMS has greatly improved (data not shown)
226 considering mould identification. Results point out that VMS is superior to the MLT using the
227 manufacturer recommended threshold of 2.0 at all taxonomic levels ($p < 0.05$ for all four
228 categories). As reported previously the use of a 1.7 threshold significantly increased the
229 accuracy of the MLT system for all identification levels (identification to the species: 16.7%
230 (17/102) to 42.2% (43/102), $p < 0.05$) as previously reported [16,29]. However overall
231 satisfactory identification seems in favour of the VMS as compared to the MLT system using
232 a threshold of 1.7, with a rate of correct identification to the complex level significantly in
233 favour of the VMS (71.6% (73/102 vs 54.9% (56/102), $p < 0.05$). Indeed, looking at the other
234 categories, the VMS system performs slightly better than the MLT – threshold of 1.7 – but
235 with no significative differences ($p > 0.05$). In order to evaluate more precisely the algorithm
236 as opposed to the database, strains not belonging to the database were discarded and

237 identification accuracy results updated. VMS obtained better results than MLT using a 2.0
238 threshold for all identification levels. Moreover VMS performed better than MLT using a 1.7
239 threshold for all identification levels but to the species level.
240 Only one identification was false, at the genus level, and was reported with the VMS. This
241 discrepancy occurred for a *Rhizopus oryzae* that was identified as an *Aspergillus brasiliensis*.
242 Macroscopic and microscopic examination of the subculture used for MALDI-TOF was
243 congruent with DNA sequencing of *R. oryzae*. New acquisition of this strain did not yield any
244 results. *R. oryzae* identification using the VMS regularly does not give result in routine use
245 (data not shown). Increasing the number of strains per taxa in the spectral database could be
246 useful in preventing this kind of misidentification.

247 Considering isolates that did not meet the identification criteria, they spanned from
248 27.5% (28/102) for the VMS to as high as 81.4% (83/102) for the MLT using a threshold of
249 2.0. When discarding isolates not present in the different databases, these rates decreased to
250 2.9% (3/77) for VMS and to 72.3% (47/65) for MLT. This decrease is due to the use of
251 limited reference spectrum database. However these 3 “no results” isolates with VMS (two *A.*
252 *tubingensis* and one *A. flavus*) might be due to issues during the extraction process.

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

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

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

287 confirmed in our study in which MSI software greatly improves MLT system's performances
288 ($p < 0.05$) looking at correct identification at the species level (16.7% (17/102) to 90.2%
289 (92/102)), at the complex (17.7% (18/102) to 92.2% (94/102)), at the genus (18.6% (19/102)
290 to 94.1% (96/102)) or strains for which the identification criteria could not be met (81.4%
291 (83/102) to 5.9% (6/102)). The use of this web software applied to MLT spectrums is greatly
292 superior to the performances of the VMS. Accurate identification to the species is achieved in
293 90.2% (92/102) of isolates compared to 51.0% (52/102) when using the VMS ($p < 0.05$).
294 Moreover, "identification criteria not met" occurs less frequently when using the MSI software
295 (5.9% (6/102) vs 27.5% (28/102), $p < 0.05$). Only one isolate not present in the MSI database
296 (*Aspergillus mangaliensis*) was correctly identified at the complex level as *A. iizukae*.
297 Comparing database related results allowed us to compare the algorithm and the architecture
298 of the database used. MLT database has been built on 1,158 references corresponding to 336
299 species (yeasts and molds) ranging from one to 31 strains per species. Only one reference was
300 acquired for each strain. Whereas MSI and VMS databases were built using multiple
301 spectrums per strains, giving far better results. VMS included only 79 species but its
302 architecture is robust since it was constructed using two to 22 strains per species (75%
303 represented by four or more isolates (D. Pincus, personal communication)), resulting in over
304 20 reference spectrums per species. MSI included 11,851 spectrums corresponding to 1,913
305 strains (938 species) ranging from two to eight spectrums per strain. This might explain the
306 better performances of these two systems. Obviously, this study has some limitation,
307 particularly its small size. Thus notable pathogens are missing and larger studies are required
308 to confirm these results. However the relatively low number of isolates included in this study
309 was largely enough to significantly highlight the flaws of commercial MALDI-TOF MS
310 systems in the routine mycology laboratory. Our results show that original algorithms and
311 enhanced database available online could compensate the flaws of commercial databases and

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

316

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321

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435

436 **List of tables**

437

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 - 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 (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 (var echinulata)</i>	1		
	<i>Neosartorya pseudofischeri</i>	1		x	x
<i>Bjerkandera</i>	<i>Bjerkandera adusta/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/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 f. 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/ sp nov ?</i>	1			
	<i>Talaromyces amestolkiae</i>	1			x
	<i>Talaromyces sp.</i>	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.