Identification of molds with MALDI-TOF mass spectrometry: performance of the newly developed MSI-2 application in comparison with the Bruker filamentous fungi database and MSI-1

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Abstract.

Matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) represents a promising tool for the rapid and efficient identification of molds, but improvements are still necessary to achieve satisfactory results when identifying cryptic species. Here, we aimed to validate a new web application, MSI-2, which replaces MSI-1, an application that was built and deployed online in 2017. For the evaluation, we gathered 633 challenging isolates obtained from daily hospital practice that were first identified with DNA-based methods, and we submitted their corresponding mass spectra to three identification programs (Bruker, MSI-1 and MSI-2). The MSI-2 application had a better identification performance at the species level than MSI-1 and Bruker, reaching 83.25% correct identifications compared with 63.19% (MSI-1), 38.07% (Bruker with 1.7 threshold) and 21.8% (Bruker with 2.0 threshold). The MSI-2 application performed especially well for *Aspergillus* and *Fusarium* species, including for many cryptic species, reaching 90% correct identifications for *Aspergillus* species and 78% for *Fusarium* species compared to 69% and 43% with MSI-1. Such improvement may have a positive impact on patient management by facilitating the identification of cryptic species potentially associated with a specific antifungal resistance profile.

Keywords.

MALDI-TOF mass spectrometry, mold, online identification, MSI, database

Introduction

Molds are saprophytic environmental fungi that are widely used in biotechnologies, but that can also be responsible for infections in plants, animals and humans. They also cause food spoilage and indoor damages. Phytopathogens can destroy hectares of agricultural crops, and fungal infections in livestock herds can result in the loss of many animals. Mold infections in humans are diverse, ranging from skin or nail infections to invasive or disseminated forms, mostly described in immunocompromised patients. Fungal species do not all have the same antifungal susceptibility or the same pathogenicity in humans and animals. Consequently, their precise identification is essential to diagnose and manage the infections they provoke. The identification of fungi is usually based on complementary approaches: morphological identification and molecular biology. For a long time, molecular biology approaches were mainly based on the comparison of DNA sequences to reference sequences in internet databases or on sequence alignments with reference data on phylogenetic trees. This technique is still considered the gold standard for identification, but it is expensive and time-consuming. Since the beginning of 2000, matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry (MS) has provided a faster and less expensive method to identify bacteria, yeasts and molds by analyzing the protein profiles of microorganisms and comparing them to an available database. Hence, MALDI-TOF MS has been used for a decade to identify filamentous fungi, mainly those implicated in clinical human or veterinary pathology (1–6). MALDI-TOF MS suppliers propose fungal spectra databases that include the species implicated in the most frequent fungal infections encountered in medical practice. However, these databases cover only a portion of the hundreds already recognized human, animal and plant pathogens. In 2017, the first web application was built and deployed online (MSI, for Mass Spectral Identification) to allow mycologists around the world to identify fungal MALDI-TOF mass spectra against a larger database (7). However, this application presented problems regarding the curation of the references that could hardly be deleted or modified due to iterative updates in the Java language that was used. Since 2019, a new application has been developed and coded in Python, a less sensitive language. The MSI-2 application is currently available at https://msi.happy-dev.fr. The performance of this application has not yet been formally evaluated in the identification of molds of medical interest. In this study, we assess the identification results obtained with a panel of 633 DNA-based identified mold isolates with the new MSI application (hereafter, "MSI-2") and compare them to those obtained with the current version of the Bruker database and with the first MSI application (MSI-1), which was available until 2019.

Materials and Methods

Isolates

All the mold isolates available at the Mycology Laboratory of La Pitié-Salpêtrière Hospital (Paris, France) for which DNA sequence-based identification was available were included in the study.

Gold standard identification of the selected isolates

As the fungal taxonomy is always evolving, the DNA sequences of the selected isolates were searched against updated DNA databases to confirm their identities in October 2020 (NCBI-blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi), MycoBank-blast (https://www.mycobank.org/page/Pairwise_alignment)). For each of the selected isolates, with the exception of the *Aspergillus* and *Fusarium* isolates, identification relied on the sequencing of the internal transcribed spacer (ITS) sequence (8, 9). Isolates were included in this study only if the obtained sequences were sufficiently discriminant to support identifications at the species level. As large international databases such as NCBI-blast or MycoBank-blast are difficult to maintain updated with the constant evolution of *Aspergillus* and *Fusarium* taxonomy, we considered identifications through the usual blast programs not accurate enough to discriminate between the cryptic species inside species complexes. Therefore, we performed DNA identifications by submitting the sequences to locally built phylogenetic trees that included reference strain sequences obtained using their published accession numbers (10–14). For most *Aspergillus* isolates, the sequence of beta-tubulin was required to ensure the correct identification of cryptic species. However, in some cases, where paralogous genes exist, calmodulin sequences were also used for identification (10). For *Fusarium* isolates, the ITS sequences were not sufficiently discriminant. Hence, the transcription elongation factor 1α sequence was used to perform the identification at the species level (9).

<u>Mass spectrum preparation</u>

For all isolates, protein extracts were obtained following the previously published protocol for culture on solid media. Briefly, approximately one cubic millimeter of the fungal culture was gently scraped with a scalpel blade, with extra care not to collect any agar. The samples were suspended in 70% HPLC ethanol for inactivation. After a 2- to 5-minute centrifugation step at 13,000 x g (depending on the sporulation of the fungal colony, a longer centrifugation might be required to fix the pellet on the microtube), the hydroalcoholic solution was removed, and the pellets were suspended in at least 10 µl of 70% formic acid (or enough volume to cover the pellet). The fungal samples were homogenized in formic acid by pipetting up and down. After a 5-minute incubation step that allowed the cell walls to be destroyed by contact with the formic acid, an equal volume of HPLC acetonitrile was added, and the two reagents were mixed by pipetting up and down. After 5 minutes of incubation at ambient temperature for neutralization of the acid and precipitation of the proteins, the sample was centrifuged for 2 minutes at 13,000 x g, and 1-µl drops of the supernatant were deposited onto the polished steel targets in two to four replicates. Each deposit was covered with 1 µl of HCCA matrix (α-cyano-4-hydroxycinnamic acid) and dried at room temperature before processing on a Microflex mass spectrometer (Bruker Daltonics).

<u>MSI-2 application</u>

MSI-2 is an online mass spectrum identification application developed by Sorbonne University (Paris, France) and available at https://msi.happy-dev.fr. Original algorithms, different from those utilized in MSI-1, have been applied to optimize identification performances and to shorten the duration of calculations. After cleaning the spectrum from noise and applying a baseline subtraction, the most intense peaks are selected and compared to a set of reference spectra. The most resembling spectrum is identified and a resemblance score is calculated. The new application is coded in Python and framed in a Django web environment that allows an easier management of modifications among the reference spectra (addition of references, correction or deletion of incorrect references). New functions for diagnostic and epidemiological purposes has been developed. The application contains several spectral databases, and the main database, which is used for the identification of fungal species, was jointly developed by Sorbonne University and Sciensano (Brussels, Belgium). The list of the 1301 fungal species and the 9969 references included in the corresponding reference database is available at https://msi.happy-dev.fr/identification/bankspecieslist/10/. The strains whose spectra are used for the reference library have been extensively identified and stored in the BCCM/IHEM collection at Sciensano. This collection has successfully undergone audits for ISO 17025 accreditation and ISO 9001 certification.

Mass spectra identification

After confirmation of the identification of the isolates, spectra were compared to the local MSI-1 version (7) (no longer available online; the list of reference species is available in Supplementary Table 1), to the newly developed MSI-2 application and to the Bruker Research Use Only (RUO)-database (BDAL revision 9) coupled with the filamentous fungi database (revision 3) containing a total of 1450 fungal references (yeasts, molds (535 references) and dermatophytes (101 references) included). A graphical description of the content of the MSI-2 mold database can be found in Figure 1. For the MSI-2 application identifications, as some of the isolates of the panel have been previously used to build up the library of reference spectra, we discarded the results corresponding to self-recognition of isolates present in both the panel and the library. The three compared databases differed in several parameters, both in the identification algorithms and in the references composition. References parameters are compared in table 1.

Table 1: comparison of the references parameters between the three different databases (Bruker RUO, MSI-1 and MSI-2)

	Type of references	Number of subcultures per strain	Number of spectra per strain	Number of references per strain	Number of species	Number of genera	Origin of the strains
Bruker RUO	Meta-spectra	1	24	1	411	111	Collections
MSI-1	Spectra	1 to 4	10 to 40 (10 per subculture)	1 to 4 (one per subculture)	818	209	Collections and individual spectra from various hospitals
MSI-2	Spectra	1 to 4	2 to 40 (2 to 10 per subculture)	1 to 4 (one per subculture)	1301	358	Collections

Performance of the MS databases

For each isolate, 2 to 4 extraction replicates were deposited onto the steel target, but only the identification corresponding to the highest score was considered. As previous publications proposed lowering the Bruker identification threshold from 2.0 to 1.7 (15–18), we considered the performance of the Bruker database with both thresholds. For each isolate, the retained identification was compared to the DNA-based identification, and 5 categories of identification accuracy were established: 1/ Correct at the species level, when the MS identification was identical to the DNA-based identification; 2/ Correct at the complex level, when the MS identification was different from the DNA-based identification at the species level but belonged to the same complex of species, 3/ Correct at the genus level, when both species and complex of species were incorrect but the identification belonged to the same genus as the DNA-based identification, 4/ Incorrect at the genus level, when different genera were identified by the MS and

DNA-based identifications (with the exception of closely related genera such as *Paecilomyces* and *Byssochlamys,* for which the taxonomy is still doubtful, and for which we considered an identification correct at the genus level when it occurred), and 5/ Under the defined identification threshold, when the score was lower than the 1.7 or 2.0 thresholds for Bruker and lower than 20 for the two MSI applications.

Statistical analyses

The identification performances using each of the databases were compared based on the proportions and 95% exact binomial confidence intervals (http://statpages.info/confint.html) of the categorical identifications for each database. Significances of the differences between the contingencies tables were calculated using the Pearson's Chi-squared test (http://biostatgv.sentiweb.fr/?module=tests/chideux).

Results

Fungal diversity of the study panel

Analyses were performed on 633 isolates corresponding to 124 species and 26 genera of nondermatophytic filamentous fungi. A description of the species, complexes of species and genera of the isolates included in this study is shown in Table 2.

Table 2: DNA-based identification of the 633 isolates included in the selected panel for the comparison of the mass spectrometry databases.

GENUS	Complex	SPECIES	NB OF ISOLATES
Acrophialophora	-	*Acrophialophora levis*	1
Alternaria	-	*Alternaria abundans*	1
	-	*Alternaria alternata*	4
Aphanocladium	-	*Aphanocladium album*	1
Arthrinium	-	*Arthrinium arundinis*	3
Arthrographis	-	*Arthrographis curvata*	1
Aspergillus	Aspergillus	*Aspergillus montevidensis*	2
		Aspergillus pseudoglaucus	1
	Circumdati	*Aspergillus affinis*	1
		Aspergillus insulicola	3
		Aspergillus ochraceopetaliformis	4
		Aspergillus ochraceus	6
		Aspergillus persii	5
		Aspergillus sclerotiorum	16
		Aspergillus subramanianii	1
		Aspergillus westerdijkiae	14
	Flavi	*Aspergillus alliaceus*	1
		Aspergillus flavus	38
		Aspergillus parasiticus	1
		Aspergillus tamarii	1
	Fumigati	*Aspergillus felis*	3
Fusarium/Acremonium	Oxysporum	*Fusarium carminascens*	1
		Fusarium contaminatum	1
		Fusarium cugenangense	1
		Fusarium curvatum	7
		Fusarium elaeidis	1
		Fusarium gossypinum	1
		Fusarium languescens	1
		Fusarium nirenbergiae	13
		Fusarium oxysporum	3
		Fusarium triseptatum	1
		Fusarium veterinarium	53
	Redolens	*Fusarium redolens*	1
	Sambuccinum	*Fusarium culmorum*	1
	Solani	*Fusarium brevicona*	1
		Fusarium falciforme	8
		Fusarium keratoplasticum	11
		Fusarium lichenicola	1
		Fusarium metavorans	1
		Fusarium petroliphilum	14
		Fusarium solani	2
		Fusarium solani.FSSC5	8

		Aspergillus fischeri	3
		Aspergillus fumigatus	26
		Aspergillus hiratsukae	13
		Aspergillus lentulus	9
		Aspergillus thermomutatus	18
		Aspergillus tsurutae	1
		Aspergillus udagawae	3
	Nigri	*Aspergillus niger*	13
		Aspergillus tubingensis	5
	Terrei	*Aspergillus floccosus*	1
		Aspergillus terreus	18
	Unguis	*Aspergillus unguis*	2
	Usti	*Aspergillus calidoustus*	5
	Nidulantes	*Aspergillus nidulans*	5
		Aspergillus spinulosporus	4
		Aspergillus sublatus	18
		Aspergillus amoenus	1
		Aspergillus creber	9
		Aspergillus hongkongensis	1
		Aspergillus jensenii	10
		Aspergillus protuberus	3
		Aspergillus puulaauensis	3
		Aspergillus sydowii	5
Bipolaris	-	*Bipolaris hawaiiensis*	1
Ceriporia	-	*Ceriporia lacerata*	3
Cladosporium	-	*Cladosporium cladosporioides*	4
Engyodontium	-	*Engyodontium album*	1
Eutypella	-	*Eutypella scoparia*	2
Fomes	-	*Fomes fomentarius*	3
Fomitopsis	-	*Fomitopsis pinicola*	1
Fusarium/Acremonium	Acremonium	*Acremonium sclerotigenum*	3
	Dimerum	*Bisifusarium dimerum*	5
	Fujikuroi	*Fusarium acutatum*	1
		Fusarium andiyazi	2
		Fusarium lactis	1
		Fusarium proliferatum	48
		Fusarium sacchari	3
		Fusarium verticillioides	8
	Incarnatum	*Fusarium bubalinum*	1
		Fusarium equiseti	2
		Fusarium flagelliforme	1
		Fusarium incarnatum	2
		Fusarium tanahbumbuense	1
		Fusarium solani.FSSC9	2
		Fusarium solani.new.sp.1	8
		Fusarium solani.new.sp.2	2
		Fusarium solani.sp.robiniae	2
Geosmithia	-	*Rasamsonia argillacea*	1
Geotrichum	Candidum	*Gallactomyces candidum*	2
		Gallactomyces geotrichum	5
	Capitatum	*Geotrichum capitatum*	6
Mucor	-	*Mucor circinelloides*	1
Paecilomyces	Lilacinum	*Paecilomyces lilacinus*	2
	Variotii	*Paecilomyces formosus*	1
		Paecilomyces variotii	2
Penicillium/Talaromyces	Aspergilloides	*Penicillium glabrum*	19
		Penicillium palmense	1
	Canescentia	*Penicillium canescens*	1
	Chrysogena	*Penicillium chrysogenum*	20
	Citrina	*Penicillium citrinum*	3
	Exilicaulis	*Penicillium corylophilum*	1
	Fasciculata	*Penicillium crustosum*	1
	Islandici	*Talaromyces rugulosus*	1
	Lanata-divaricata	*Penicillium oxalicum*	1
	Penicillium	*Penicillium expansum*	1
	Talaromyces	*Penicillium crateriforme*	1
		Talaromyces amestolkiae	1
		Talaromyces pinophilus	1
	Trachyspermi	*Talaromyces diversus*	3
		Talaromyces minioluteus	1
Perenniporia	-	*Perenniporia tenuis*	1
Phanerochaete	-	*Phanerochaete sordida*	1
Rhizopus	-	*Rhizopus microsporus*	1
	-	*Rhizopus oryzae*	3
Scedosporium	Apiospermum	*Scedosporium apiospermum*	7
		Scedosporium boydii	8
	Aurantiacum	*Scedosporium aurantiacum*	4
	Prolificans	*Lomentospora prolificans*	1
Scopulariopsis	-	*Scopulariopsis brevicaulis*	5
Thanatephorus	-	*Thanatephorus cucumeris*	2
Trichoderma	-	*Trichoderma harzianum*	1
	-	*Trichoderma longibrachiatum*	1
TOTAL		124 species	633

Comparison of the identification performances of the mass spectrometry databases

The spectra obtained for the 633 isolates were subjected to three different MALDI-TOF MS identifications. Of the 124 species included in our panel, only 51 were represented in the three corresponding databases. The overall results are presented in Table 3.

Table 3: Identification performances of the different MALDI-TOF MS identification databases. Percentage of identification and 95% confidence interval for each of the categories between brackets. * MS identification was different from DNA-based identification at the species level but belonged to the same complex of species as the sequencing Gold Standard; ** both species and complexes of species were incorrect, but identification belonged to the same genus as the sequencing Gold Standard; *** genus difference.

	Confidence level of the identification	**Bruker (2.0)**	**Bruker (1.7)**	**MSI-1 (20)**	**MSI-2 (20)**
All isolates (n=633 isolates; 124 species)	Correct at the species level	21.8% [18.64-25.22]	38.07% [34.27-41.98]	63.19% [59.3-66.96]	83.25% [80.11-86.08]
	Correct at the complex level*	14.22% [11.59-17.18]	25.12% [21.78-28.69]	30.17% [26.62-33.91]	12.95% [10.44-15.82]
	Correct at the genus level**	0.32% [0.04-1.14]	1.74% [0.87-3.09]	3.95% [2.57-5.78]	0.16% [0-0.88]

	Incorrect at the genus level***	0% [0-0]	0.32% [0.04-1.14]	0.16% [0-0.88]	0% [0-0]
	Under the defined identification threshold	63.67% [59.78-67.42]	34.76% [31.05-38.61]	2.53% [1.45-4.07]	3.63% [2.32-5.4]

***Aspergillus* spp. (n=273 isolates; 39 species in 10 complexes)**	**Confidence level of the identification**	**Bruker (2.0)**	**Bruker (1.7)**	**MSI-1 (20)**	**MSI-2 (20)**
	Correct at the species level	27.11% [21.92-32.79]	46.89% [40.85-53]	69.23% [63.39-74.65]	89.74% [85.52-93.08]
	Correct at the complex level*	18.32% [13.91-23.42]	24.54% [19.56-30.09]	30.04% [24.66-35.85]	8.42% [5.42-12.37]
	Correct at the genus level**	0% [0-0]	0% [0-0]	0% [0-0]	0% [0-0]
	Incorrect at the genus level***	0% [0-0]	0% [0-0]	0% [0-0]	0% [0-0]
	Under the defined identification threshold	54.58% [48.47-60.59]	28.57% [23.29-34.33]	0.73% [0.09-2.62]	1.83% [0.6-4.22]

***Fusarium* spp. (n=223 isolates; 38 species in 8 complexes)**	**Confidence level of the identification**	**Bruker (2.0)**	**Bruker (1.7)**	**MSI-1 (20)**	**MSI-2 (20)**
	Correct at the species level	11.21% [7.39-16.1]	21.97% [16.72-27.99]	42.6% [36.02-49.38]	77.58% [71.53-82.88]
	Correct at the complex level*	16.59% [11.96-22.14]	37.67% [31.29-44.38]	46.19% [39.51-52.97]	21.52% [16.32-27.51]
	Correct at the genus level**	0% [0-0]	2.69% [0.99-5.76]	9.87% [6.29-14.56]	0.45% [0.01-2.47]
	Incorrect at the genus level***	0% [0-0]	0.9% [0.11-3.2]	0% [0-0]	0% [0-0]
	Under the defined identification threshold	72.2% [65.82-77.97]	36.77% [30.43-43.47]	1.35% [0.28-3.88]	0.45% [0.01-2.47]

Species represented in all databases (n=365 isolates; 51 species)	**Confidence level of the identification**	**Bruker (2.0)**	**Bruker (1.7)**	**MSI-1 (20)**	**MSI-2 (20)**
	Correct at the species level	37.81% [32.81-43]	66.03% [60.92-70.88]	83.56% [79.35-87.21]	91.51% [88.16-94.16]
	Correct at the complex level*	2.74% [1.32-4.98]	5.48% [3.38-8.34]	14.25% [10.83-18.26]	4.93% [2.95-7.68]
	Correct at the genus level**	0.55% [0.07-1.96]	1.92% [0.77-3.91]	0.55% [0.07-1.96]	0% [0-0]
	Incorrect at the genus level***	0% [0-0]	0% [0-0]	0.27% [0.01-1.52]	0% [0-0]
	Under the defined identification threshold	58.9% [53.66-64]	26.58% [22.11-31.42]	1.37% [0.45-3.17]	3.56% [1.91-6.01]

The identification performance at the species level of the MSI-2 application was significantly better than that of either the MSI-1 application (p-value <0.001) or the Bruker software (p-value <0.001).

The Bruker software showed better identification performances for the 51 species represented in the three databases than for the whole panel (66% for the 51 species vs 38% for all species, at a 1.7 threshold).

Both of the MSI applications allowed the identification of more than 80% of the isolates corresponding to these 51 species, marking large improvements compared to the Bruker software. Improvements made in the latest MSI-2 application regarding these 51 species were significant compared to the previous MSI-1 application, with approximately 92% correct identifications at the species level compared to approximately 84% with MSI-1. The percentages of identification at the species level per database and per submitted species for the 51 species represented in the three databases are shown in Supplementary Table 2.

Discussion.

Here, we performed a comparative study of three MALDI-TOF mass spectra identification systems. We selected 633 isolates for which we had already obtained DNA identifications and mass spectra acquisitions. In our laboratory, we do not perform systematic molecular identifications for every mold that we obtain in culture. Instead, we carry out DNA sequence analysis only for isolates belonging to unusual species or to formally confirm identifications proposed in scientific studies. Therefore, this panel consisted only of isolates obtained from cultures of human samples and was not representative of routine activity. The panel underrepresents the most common molds (*Aspergillus flavus* and *A. fumigatus,* for example) and over represents those molds that are rarer and more complicated to identify. With this panel of difficult isolates containing only molds, we challenged the most recent Bruker filamentous database, the now unavailable MSI-1 application, and the newly developed MSI-2 application.

The results show that the new MSI-2 application allows the identification of both the filamentous fungi usually identified in the daily activity of a clinical mycology laboratory and rarer cryptic mold species with high success rates at the species level, exhibiting better performances than either the previous MSI-1 application or the different Bruker databases.

The greatest improvements in identification were observed within the *Aspergillus* and *Fusarium* genera, which have been a particular focus for the implementation of the MSI database between the two versions. Indeed, in a previous study, the difficulty of identifying cryptic species of *Aspergillus* at the species level was stated (19). In the current study, we used a panel of mold species that was rigorously selected based on the availability and correctness of their DNA sequences (only isolates with sequences of genes that allowed reliable identification at the species level).

Obtaining an accurate and reliable identification, in addition to its obvious necessity for the development of knowledge and for epidemiology, may be of clinical interest for the management of patients suffering from a fungal infection. Indeed, there might be a difference in susceptibility to a particular antifungal drug among species complexes, such as those reported by Imbert et al. for *Aspergillus* species in section *Fumigati* (20) or by Carrara et al. for species in section *Nigri* (21). In these sections, species identification relates to the intrinsic antifungal susceptibility profile, suggesting the usefulness of accurate identification for disease management.

In the present study, we focused on molds because the main manufacturers' databases are exhaustive enough for yeast identification. In contrast, improvements are still necessary for dermatophyte identification, and we plan to set up a new database for these specific fungi. This database was not presented here, as the work is still in progress.

The best performances of the official Bruker RUO and filamentous fungal databases reached 66% correct identification at the species level when considering only species that are represented in the database (i.e., without most of the cryptic *Aspergillus* and *Fusarium* species). The percentages of identification at the species level within the *Fusarium* genus with the Bruker system remained low regardless of the threshold utilized.

Even when focusing on the 51 species (365 isolates) of our panel that are represented in the three databases, i.e., for isolates that are likely to be identified regardless of the database tested, the performances of the three applications were significantly different, indicating that mold identification is affected by not only the database content (i.e., the number of references per species) but also by the preparation method of the protein extract, the type of culture (the mold references by Bruker were acquired from liquid cultures), the spectra acquisition conditions, and the identification algorithms, which play important roles in the performances. We previously observed this while locally implementing the Bruker database and comparing the identification results with those produced with the same references but implemented on the MSI-1 application (7). In this panel of 365 isolates, only three isolates were correctly identified by the Bruker application while identified only at the complex of species level by MSI-2. Those three isolates were two *Alternaria alternata* that were identified as *Alternaria arborescens* by MSI-2 and one *Geosmithia argillacea* that was misidentified as *Geosmithia piperina* by MSI-2. Regarding the *Alternaria* mis-identifications, the two species are closely related and the introduction of the *A. arborescens* for phytopathology purposes into the database might be a source of confusion. The reference that mis-identified the *G. argillacea* into *G. piperina* (IHEM16128) has recently been renamed by the Belgian collection and was labelled *G. argillacea* until recently.

The references that were proposed in the MSI-1 application were thoroughly cleaned up for MSI-2. In some cases, this might lead to a decrease in performance with the new application (Supplementary Table 2), as MSI-1 contained references that have been deleted in MSI-2, even if some of them contributed to the performance of MSI-1. These references corresponded to spectra obtained in various collaborating centers and were obtained from isolates morphologically identified. However, those isolates were never stored in a collection nor sequenced. Hence, their identification could not be verified with a DNA-based method, and we decided to remove these uncertain references from the MSI-2 application. To further improve the MSI-2 application and to fill the gaps resulting from the removal of these references, as shown for nine species in supplemental table 2 (*Aspergillus flavus*, *Aspergillus parasiticus*, *Cladosporium cladosporioides*, *Fusarium incarnatum*, *Fusarium oxysporum*, *Geosmithia argillacea, Geotrichum capitatum*, *Penicillium glabrum* and *Lomentospora prolificans*), we encourage MSI-2 users to send us any isolates that could not be identified, when possible, so that we can perform in-depth identifications of the isolates prior to including them in the new reference database. In addition to these changes, some strains have been renamed due to changes in the fungal taxonomy, as it is the case for the *Geosmithia piperina* IHEM16128 reference, and others were deleted from the database, as they were excluded from the BCCM/IHEM collection. All those modifications globally improve the quality of the identifications obtained with MSI-2 even though a few isolates of our panel (16/365) were better identified with the former MSI-1 application.

Using the new application, we achieved approximately 78% correct identifications at the species level for the *Fusarium* isolates. This result is still unsatisfactory even if it is 35% better than the first MSI application and 65% better than the Bruker database with a threshold of 2.0. The new application comprises many references of new species to take into account new developments of the *Fusarium* taxonomy, especially regarding the *Fusarium solani* (FSSC) and the *Fusarium oxysporum* (FOSC) complexes (11, 22). This increase in the number of species made it more difficult to obtain accurate identification results. Moreover, as these new species are almost impossible to distinguish from each other and are rarely encountered in clinical practice, they have not been considered in clinical studies in terms of specific pathogenicity or antifungal susceptibility profiles, which raises the question of the significance of these new species in the context of routine practice.

In conclusion, the MSI-2 application constitutes a reliable identification tool for most filamentous species identified on a daily basis in a clinical mycology laboratory. The improvements of the reference database regarding the *Aspergillus* and *Fusarium* genera now allow us to identify clinical specimens from these genera at the species level. These improvements may have an impact on the treatment given to the patient, as the most effective treatment may vary from one cryptic species to another; moreover, the improvements may decrease the delay before the prescription of adequate antifungal treatment.

AUTHOR CONTRIBUTIONS. Conceptualization, A.C.N. and R.P.; Methodology, A.C.N. and R.P.; Software, A.C.N. and R.P.; Validation, A.C.N., M.B., and R.P.; Formal Analysis, A.C.N., M.B., and R.P.; Investigation, A.C.N., M.B., S.I., and R.P.; Data Curation, A.C.N.; Writing – Original Draft Preparation, A.C.N. and M.B.; Writing – Review & Editing, A.C.N., M.B., S.I., A.P., P.B., D.S., A.F., and R.P.; Visualization, A.C.N., M.B., S.I., A.P., P.B., D.S., A.F., and R.P.; Supervision, R.P.

CONFLICTS OF INTEREST. None

FUNDING. Development and maintenance of the MSI-2 application is funded by Sorbonne University (Paris, France)

ACKNOWLEDGMENTS. We thank Martine Piarroux, Sylvain Lebon and Simon Rouault for coding and uploading the new application, and for keeping it accessible for all users on a daily base.

FIGURE LEGEND

Figure 1: Graphical representation of the MSI-2 Molds database; inside the *Penicillium/Talaromyces, Aspergillus* and *Fusarium*/*Acremonium* taxa, representation of the most represented complexes and the number of species in each one. The "others" category groups 258 genera, represented by 1 (as is the case for 153 genera) to 28 species (*Trichoderma* genus).

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Supplemental table 1: List of species represented in the MSI-1 application database

genera	species
Absidia	Absidia corymbifera
	Absidia cylindrospora
	Absidia glauca
	Absidia pseudocylindrospora
	Absidia spinosa
Acremonium	Acremonium af.pteridii
	Acremonium breve
	Acremonium butyri
	Acremonium charticola
	Acremonium chrysogenum
	Acremonium falciforme
	Acremonium furcatum
	Acremonium fusidioides
	Acremonium hansfordii
	Acremonium implicatum
	Acremonium kiliense
	Acremonium longisporum
	Acremonium ochraceum
	Acremonium polychromum
	Acremonium roseolum
	Acremonium sclerotigenum
	Acremonium strictum
Acrodontium	Acrodontium crateriforme
	Acrodontium salmoneum
	Acrodontium simplex
Actinomucor	Actinomucor elegans
	Actinomucor elegans-var-meitauzae
Alternaria	Alternaria acalyphicola
	Alternaria alternata
	Alternaria calycipyricola
	Alternaria chlamydospora
	Alternaria citri
	Alternaria infectoria
	Alternaria malorum
	Alternaria roseogrisea
	Alternaria soliaegyptiaca
	Alternaria undulata
Amaurascopsis	Amaurascopsis perforata
	Amaurascopsis perforatus
Amauroascus	Amauroascus kuehnii
Anixiopsis	Anixiopsis biplanata
Anthopsis	Anthopsis deltoidea
Aphanoascus	Aphanoascus fulvescens
	Aphanoascus verrucosum
Aphanocladium	Aphanocladium album
Apophysomyces	Apophysomyces variabilis
Arachniotus	Arachniotus littoralis
Arthrinium	Arthrinium arundinis
	Arthrinium marii
	Arthrinium rasikravindrii
	Arthrinium sphaerospermum
Arthrobotrys	Arthrobotrys oligospora
Arthroderma	Arthroderma benhamiae
	Arthroderma borellii
	Arthroderma cookiellum
	Arthroderma corniculatum
	Arthroderma crocatum
	Arthroderma cuniculi
	Arthroderma curreyi
	Arthroderma fulvum
	Arthroderma gloriae
	Arthroderma grubyi
	Arthroderma gypseum
	Arthroderma incurvatum
	Arthroderma lenticulare
	Arthroderma multifidum
	Arthroderma persicolor
	Arthroderma racemosum
	Arthroderma simii
	Arthroderma vanbreuseghemii
Arthrographis	Arthrographis kalrae

genera	species
Arxula	Arxula adeninivorans
Ascochyta	Ascochyta pisi-var-pisi
Aspergillus	Aspergillus aculeatus
	Aspergillus af-flavipes
	Aspergillus af-viridinutans
	Aspergillus alabamensis
	Aspergillus allahabadi
	Aspergillus allahabadii
	Aspergillus alliaceus
	Aspergillus amylovorus
	Aspergillus ardalensis
	Aspergillus astellatus
	Aspergillus aureoterreus
	Aspergillus auricomus
	Aspergillus auroleatus
	Aspergillus austroafricanus
	Aspergillus avenaceus
	Aspergillus bombycis
	Aspergillus brasiliensis
	Aspergillus brevipes
	Aspergillus caelatus
	Aspergillus caesiellus
	Aspergillus caespitosus
	Aspergillus calidoustus
	Aspergillus candidus
	Aspergillus carbonarius
	Aspergillus carneus
	Aspergillus cervinus
	Aspergillus clavatoflavus
	Aspergillus clavatus
	Aspergillus coremiiformis
	Aspergillus creber
	Aspergillus cretensis
	Aspergillus dimorphicus
	Aspergillus duricaulis
	Aspergillus eburneocremeus
	Aspergillus ellipticus
	Aspergillus flaschentraegeri
	Aspergillus flavipes
	Aspergillus flavofurcatus
	Aspergillus flavus
	Aspergillus flavus-var-columnaris
	Aspergillus floccosus
	Aspergillus fumigatiaffinis
	Aspergillus fumigatus
	Aspergillus fumigatus-var-ellipticus
	Aspergillus giganteus
	Aspergillus heterocaryoticus
	Aspergillus heteromorphus
	Aspergillus hollandicus
	Aspergillus hortai
	Aspergillus iiukae
	Aspergillus insuetus
	Aspergillus insulicola
	Aspergillus japonicus
	Aspergillus jensenii
	Aspergillus lentulus
	Aspergillus melleus
	Aspergillus microcysticus
	Aspergillus neoellipticus
	Aspergillus nidulans
	Aspergillus niger
	Aspergillus niveus
	Aspergillus nomius
	Aspergillus ochraceus
	Aspergillus oryzae
	Aspergillus ostianus
	Aspergillus pallidus
	Aspergillus parasiticus
	Aspergillus persii
	Aspergillus proliferans

genera	species
Aspergillus	Aspergillus protuberus
	Aspergillus pseudoglaucus
	Aspergillus pseudoustus
	Aspergillus puniceus
	Aspergillus puulaauensis
	Aspergillus restrictus
	Aspergillus sclerotiorum
	Aspergillus sojae
	Aspergillus sparsus
	Aspergillus spelunceus
	Aspergillus speluneus
	Aspergillus stromatoides
	Aspergillus subolivaceus
	Aspergillus sydowii
	Aspergillus tabacinus
	Aspergillus tamarii
	Aspergillus terreus
	Aspergillus terreus-var-africanus
	Aspergillus thomii
	Aspergillus tubingensis
	Aspergillus unguis
	Aspergillus ustus-var-pseudodeflectus
	Aspergillus uvarum
	Aspergillus versicolor
	Aspergillus viridinutans
	Aspergillus wentii
	Aspergillus westerdijkiae
	Aspergilus parasiticus
Aureobasidium	Aureobasidium pullulans
	A. pullulans-var-melanogenum
Auxarthron	Auxarthron alboluteum
	Auxarthron californiense
	Auxarthron conjugatum
	Auxarthron reticulatum
	Auxarthron umbrinum
Beauveria	Beauveria bassiana
	Beauveria caledonica
Bipolaris	Bipolaris sorokiniana
	Bipolaris spicifera
	Bipolaris victoriae
Bjerkandera	Bjerkandera adusta
Blastobotrys	Blastobotrys proliferans
Botrytis	Botrytis anthophila
Candida	Candida africana
	Candida albicans
	Candida allociferrii
	Candida andamanensis
	Candida auris
	Candida blankii
	Candida boidinii
	Candida bovina
	Candida bracarensis
	Candida castellii
	Candida catenulata
	Candida ciferrii
	Candida deformans
	Candida dubliniensis
	Candida ernobii
	Candida etchelsii
	Candida famata
	Candida friedrichii
	Candida glabrata
	Candida guilliermondii
	Candida haemulonii
	Candida hellenica-var-hellenica
	Candida inconspicua
	Candida kefyr
	Candida krusei
	Candida laemsonensis
	Candida lambica
	Candida lipolytica

genera	species
Candida	Candida lusitaniae
	Candida maltosa
	Candida maris
	Candida melibiosica
	Candida melinii
	Candida metapsilosis
	Candida nivariensis
	Candida norvegensis
	Candida norvegica
	Candida oleophila
	Candida orthopsilosis
	Candida palmioleophila
	Candida parapsilosis
	Candida pararugosa
	Candida pintolopesii
	Candida pseudohaemulonii
	Candida pulcherrima
	Candida quercitrusa
	Candida ranongensis
	Candida rugosa
	Candida sake
	Candida sojae
	Candida sorbosa
	Candida sorbosivorans
	Candida sorboxilosa
	Candida spencermartinsiae
	Candida sphaerica
	Candida tenuis
	Candida tropicalis
	Candida utilis
	Candida valida
	Candida vini
	Candida zeylanoides
Chaetomium	Chaetomium globosum
Chalaropsis	Chalaropsis punctulata
Chrysonilia	Chrysonilia tetrasperma
Chrysosporium	Chrysosporium keratinophilum
	Chrysosporium lucknowense
	Chrysosporium merdarium
	Chrysosporium pannicola
	Chrysosporium queenslandicum
	Chrysosporium synchronum
	Chrysosporium tropicum
Citeromyces	Citeromyces matritensis
Cladiophiolophora	Cladiophiolophora carrionii
Cladobotryum	Cladobotryum mycophilum
Cladosporium	Cladosporium cladosporioides
	Cladosporium cucumerinum
	Cladosporium macrocarpum
	Cladosporium oxysporum
	Cladosporium pseudocladosporioides
	Cladosporium ramotenellum
	Cladosporium sphaeropermum
	Cladosporium sphaerospermum
	Cladosporium tenuissimum
	Cladosporium variabile
Claviceps	Claviceps purpurea
Clavispora	Clavispora lusitaniae
Cochliobolus	Cochliobolus hawaiiensis
Cokeromyces	Cokeromyces recurvatus
Colletotrichum	Colletotrichum crassipes
	Colletotrichum musae
Conidiobolus	Conidiobolus coronatus
Cryptococcus	Cryptococcus aerius
	Cryptococcus af-diffluens
	Cryptococcus albidosimilis
	Cryptococcus albidus
	Cryptococcus carnescens
	Cryptococcus curvatus
	Cryptococcus cyanovorans
	Cryptococcus difluens

genera	species
Cryptococcus	Cryptococcus flavescens
	Cryptococcus gattii
	Cryptococcus humicola
	Cryptococcus laurentii
	Cryptococcus luteolus
	Cryptococcus neoformans
	Cryptococcus neoformans-var-grubii
	Cryptococcus neoformans-var-neoformans
	Cryptococcus stepposus
	Cryptococcus terreus
	Cryptococcus terricola
	Cryptococcus uniguttulatus
	Cryptococcus victoriae
Cunninghamella	Cunninghamella bertholletiae
Curvularia	Curvularia affinis
	Curvularia geniculata
	Curvularia inaequalis
	Curvularia lunata
	Curvularia lunata-var-aeria
	Curvularia verruculosa
Debaryomyces	Debaryomyces etchellsii
	Debaryomyces hansenii
	Debaryomyces vanrijiae-var-vanrijiae
	Debaryomyces yamadae
Dekkera	Dekkera bruxellensis
Dicyma	Dicyma olivacea
Dothideomyces	Dothideomyces sp.
Emericella	Emericella desertorum
	Emericella echinulata
	Emericella heterothallica
	Emericella nidulans
	Emericella nidulans-var-acristata
	Emericella quadrilineata
	Emericella rugulosa
	Emericella striata
Emericellopsis	Emericellopsis synnematicola
Emmonsia	Emmonsia parva-var-crescens
	Emmonsia pasteuriana
Engyodontium	Engyodontium album
	Engyodontium parvisporum
Epicoccum	Epicoccum nigrum
Epidermophyton	Epidermophyton floccosum
Eupenicillium	Eupenicillium cinnamopurpureum
	Eupenicillium javanicum
	Eupenicillium limoneum
	Eupenicillium pinetorum
	Eupenicillium shearii
	Eupenicillium terrenum
Eurotium	Eurotium amstelodami
	Eurotium athecium
	Eurotium chevalieri
	Eurotium cristatum
	Eurotium echinulatum
	Eurotium herbariorum
	Eurotium intermedius
	Eurotium minus
	Eurotium montevidense
	Eurotium pseudoglaucum
	Eurotium repens
	Eurotium rubrum
Eutypella	Eutypella scoparia
Exophiala	Exophiala alcalophila
	Exophiala dermatitidis
	Exophiala jeanselmei
	Exophiala salmonis
	Exophiala sp.
	Exophiala spinifera
Exserohilum	Exserohilum rostratum
Fellomyces	Fellomyces polyborus
Fennellomyces	Fennellomyces linderi
Fissuricella	Fissuricella filamenta

genera	species
Fomitopsis	Fomitopsis palustris
	Fomitopsis pinicola
Fonsecaea	Fonsecaea pedrosoi
Fusarium	Fusarium acuminatum
	Fusarium acutatum
	Fusarium af-andiyazi
	Fusarium ananatum
	Fusarium andiyazi
	Fusarium annulatum
	Fusarium anthophilum
	Fusarium chlamydosporum
	Fusarium delphinoides
	Fusarium dimerum
	Fusarium equiseti
	Fusarium fujikuroi
	Fusarium incarnatum
	Fusarium lichenicola
	Fusarium musae
	Fusarium napiforme
	Fusarium nygamai
	Fusarium oxysporum
	Fusarium oxysporum-var-redolens
	Fusarium petroliphilum
	Fusarium poae
	Fusarium polyphialidicum
	Fusarium proliferatum
	Fusarium proliferatum-var-minus
	Fusarium proliferatum-var-proliferatum
	Fusarium sacchari
	Fusarium sambucinum
	Fusarium solani
	Fusarium sporotrochioides
	Fusarium subglutinans
	Fusarium thapsinum
	Fusarium trincinctum
	Fusarium verticillioides
Galactomyces	Galactomyces candidum
	Galactomyces citri-aurantii
	Galactomyces geotrichum
Ganoderma	Ganoderma resinaceum
Geomyces	Geomyces pannorum
Geosmithia	Geosmithia argillacea
	Geosmithia pallida
Geotrichum	Geotrichum candidum
	Geotrichum capitatum
	Geotrichum fermentans
	Geotrichum klebahnii
	Geotrichum marinum
Gilbertella	Gilbertella persicaria
Gliocladium	Gliocladium viride
Gliomastix	Gliomastix felina
	Gliomastix polychroma
Gloeophyllum	Gloeophyllum trabeum
Glomerella	Glomerella cingulata
Guehomyces	Guehomyces pullulans
Gymnascella	Gymnascella dankaliensis
	Gymnascella devroeyi
	Gymnascella hyalinospora
	Gymnascella marginispora
	Gymnascella udagawae
Gymnoascus	Gymnoascus reessii
	Gymnoascus udagawae
Hamigera	Hamigera fusca
	Hamigera insecticola
Hanseniaspora	Hanseniaspora guilliermondii
	Hanseniaspora uvarum
Hansfordia	Hansfordia pulvinata
Haplographium	Haplographium debellae
Hemicarpenteles	Hemicarpenteles acanthosporus
	Hemicarpenteles ornatus
	Hemicarpenteles paradoxus

genera	species
Hexagonia	Hexagonia hydnoides
Histoplasma	Histoplasma capsulatum
Hormoconis	Hormoconis resinae
Hormographiella	Hormographiella verticilliata
Hortaea	Hortaea werneckii
Humicola	Humicola grisea-var-thermoidea
Hyalodendron	Hyalodendron lignicola
Hyphozyma	Hyphozyma variabilis
Hypocrea	Hypocrea atrogelatinosa
	Hypocrea hunua
	Hypocrea parapilulifera
	Hypocrea schweinitzii
	Hypocrea vinosa
Hypoxylon	Hypoxylon howeanum
	Hypoxylon lividipigmentum
Isaria	Isaria farinosa
Kazachstania	Kazachstania pintolopesii
	Kazachstania wufongensis
Kloeckera	Kloeckera apiculata
Kluyveromyces	Kluyveromyces marxianus
Kodamaea	Kodamaea ohmeri
Kuraishia	Kuraishia molischiana
Lecanicillium	Lecanicillium fungicola
	Lecanicillium psalliotae
Lecythophora	Lecythophora sp.
Lipomyces	Lipomyces starkeyi
Lodderomyces	Lodderomyces elongisporus
Macrophomina	Macrophomina phaseolina
Madurella	Madurella pseudomycetomatis
Malassezia	Malassezia furfur
	Malassezia globosa
	Malassezia pachydermatis
	Malassezia restricta
	Malassezia sloofiae
	Malassezia sympodialis
Malbranchea	Malbranchea arcuata
Metschnikowia	Metschnikowia pulcherrima
	Metschnikowia sinensis
	Metschnikowia viticola
Meyerozyma	Meyerozyma guilliermondii
Microsporum	Microsporum audouinii
	Microsporum boullardii
	Microsporum canis
	Microsporum canis-var-distortum
	Microsporum cookei
	Microsporum equinum
	Microsporum ferrugineum
	Microsporum fulvum
	Microsporum gypseum
	Microsporum nanum
	Microsporum persicolor
	Microsporum praecox
	Microsporum racemosum
	Microsporum rivalieri
	Microsporum vanbreuseghemii
Monodictys	Monodictys castanae
Mucor	Mucor circinelloides
	Mucor durus
	Mucor ellipsoideus
	Mucor flavus
	Mucor fragilis
	Mucor indicus
	Mucor mucedo
	Mucor plumbeus
	Mucor racemosus-f-racemosus
	Mucor saturninus
	Mucor velutinosus
Myceliophthora	Myceliophthora thermophila
	Myceliophthora vellerea
Mycotypha	Mycotypha microspora
Myriodontium	Myriodontium keratinophilum

genera	species
Myrothecium	Myrothecium cinctum
	Myrothecium roridum
	Myrothecium verrucaria
Myxotrichum	Myxotrichum chartarum
	Myxotrichum deflexum
Neocosmospora	Neocosmospora vasinfecta
	Neocosmospora vasinfecta-var-africana
Neofabraea	Neofabraea malicorticis
Neosartorya	Neosartorya af.aureola
	Neosartorya aurata
	Neosartorya aureola
	Neosartorya fischeri
	Neosartorya glabra
	Neosartorya hiratsukae
	Neosartorya pseudofischeri
	Neosartorya quadricincta
	Neosartorya stramenia
	Neosartorya udagawae
Neoscytalidium	Neoscytalidium dimidiatum
	Neoscytalidium dimidiatumIHEM3489
Neotestudina	Neotestudina rosatii
Nigrospora	Nigrospora oryzae
	Nigrospora sphaerica
Nodulisporium	Nodulisporium griseobrunneum
	Nodulisporium melonis
	Nodulisporium verrucosum
Ochroconis	Ochroconis humicola
Ogataea	Ogataea allantospora
	Ogataea nitratoaversa
Onychocola	Onychocola canadensis
Oosporidium	Oosporidium margaritiferum
Ophidiomyces	Ophidiomyces ophiodiicola
Ophiostoma	Ophiostoma stenoceras
Pachysolen	Pachysolen tannophilus
Paecilomyces	Paecilomyces amoeneroseus
	Paecilomyces carneus
	Paecilomyces formosus
	Paecilomyces inflatus
	Paecilomyces lilacinus
	Paecilomyces niphetodes
	Paecilomyces pascua
	Paecilomyces saturatus
	Paecilomyces variotii
Parascedosporium	Parascedosporium putredinidis
Penicilium	Penicilium mariae-crucis
	Penicillium af.ochrochloron
	Penicillium af.toxicarum
	Penicillium af.verruculosum
	Penicillium allii
	Penicillium allii-sativi
	Penicillium asturianum
	Penicillium atrosanguineum
	Penicillium aurantiogriseum
	Penicillium bilaiae
	Penicillium brasilianum
	Penicillium brevicompactum
	Penicillium camemberti
	Penicillium canescens
	Penicillium capsulatum
	Penicillium cecidicola
	Penicillium cfr.simplicissimum
	Penicillium chermesinum
	Penicillium chrysogenum
	Penicillium chrysogenum-var-dipodomyis
	Penicillium citreonigrum
	Penicillium citrinum
	Penicillium copticola
	Penicillium coralligerum
	Penicillium corylophilum
	Penicillium crateriforme
	Penicillium crustosum

genera	species
Penicillium	Penicillium decumbens
Penicillium	Penicillium digitatum
Penicillium	Penicillium expansum
Penicillium	Penicillium fellutanum
Penicillium	Penicillium funiculosum
Penicillium	Penicillium georgiense
Penicillium	Penicillium glabrum
Penicillium	Penicillium griseofulvum
Penicillium	Penicillium helicum
Penicillium	Penicillium hirsutum-var-albocoremium
Penicillium	Penicillium isariiforme
Penicillium	Penicillium janthinellium
Penicillium	Penicillium lividum
Penicillium	Penicillium loliense
Penicillium	Penicillium madriti
Penicillium	Penicillium mallochii
Penicillium	Penicillium mariae-crucis
Penicillium	Penicillium marneffei
Penicillium	Penicillium melanoconidium
Penicillium	Penicillium melinii
Penicillium	Penicillium minioluteum
Penicillium	Penicillium montanense
Penicillium	Penicillium nalgiovense
Penicillium	Penicillium neoechinulatum
Penicillium	Penicillium olsonii
Penicillium	Penicillium oxalicum
Penicillium	Penicillium pancosmium
Penicillium	Penicillium piceum
Penicillium	Penicillium pinophilum
Penicillium	Penicillium polonicum
Penicillium	Penicillium purpurescens
Penicillium	Penicillium purpurogenum
Penicillium	Penicillium raistrickii
Penicillium	Penicillium ramulosum
Penicillium	Penicillium resedanum
Penicillium	Penicillium restrictum
Penicillium	Penicillium rolfsii
Penicillium	Penicillium roqueforti
Penicillium	Penicillium rubens
Penicillium	Penicillium rugulosum
Penicillium	Penicillium sanguifluum
Penicillium	Penicillium sizovae
Penicillium	Penicillium smithii
Penicillium	Penicillium sp.
Penicillium	Penicillium spinulosum
Penicillium	Penicillium steckii
Penicillium	Penicillium striatisporum
Penicillium	Penicillium subrubescens
Penicillium	Penicillium terrigenum
Penicillium	Penicillium thomii
Penicillium	Penicillium toxicarium
Penicillium	Penicillium ulaiense
Penicillium	Penicillium variabile
Penicillium	Penicillium viridicatum
Pestalotiopsis	Pestalotiopsis hainanensis
Petromyces	Petromyces alliaceus
Phaeoacremonium	Phaeoacremonium parasiticum
Phaeoacremonium	Phaeoacremonium scolyti
Phaeotheca	Phaeotheca triangularis
Phaffia	Phaffia rhodozyma
Phanerochaete	Phanerochaete chrysosporium
Phialemonium	Phialemonium globosum
Phialophora	Phialophora bubakii
Phialophora	Phialophora japonica
Phialophora	Phialophora olivacea
Phlebia	Phlebia acerina
Phlebia	Phlebia radiata
Phlebia	Phlebia tremellosa
Phoma	Phoma macrostoma-var-macrostoma
Phoma	Phoma sp
Phycomyces	Phycomyces blakesleeanus

genera	species
Pichia	Pichia angusta
Pichia	Pichia anomala
Pichia	Pichia burtonii
Pichia	Pichia caribbica
Pichia	Pichia carribbica
Pichia	Pichia fabianii
Pichia	Pichia jadinii
Pichia	Pichia koratensis
Pichia	Pichia manshurica
Pichia	Pichia membranifaciens
Pichia	Pichia rhodanensis
Pithomyces	Pithomyces chartarum
Pleospora	Pleospora herbarum
Pleurostomophora	Pleurostomophora richardsiae
Pochonia	Pochonia bulbillosa
Porostereum	Porostereum spadiceum
Pseudallescheria	Pseudallescheria angusta
Pseudallescheria	Pseudallescheria apiosperma
Pseudallescheria	Pseudallescheria boydii
Pseudallescheria	Pseudallescheria desertorum
Pseudallescheria	Pseudallescheria ellipsoidea
Pseudallescheria	Pseudallescheria minutispora
Pseudeurotium	Pseudeurotium bakeri
Pseudeurotium	Pseudeurotium zonatum
Pseudomicrodochium	Pseudomicrodochium fusarioides
Pseudozyma	Pseudozyma aphidis
Pseudozyma	Pseudozyma rugulosa
Radulidium	Radulidium subulatum
Rhizomucor	Rhizomucor miehei
Rhizomucor	Rhizomucor pusillus
Rhizomucor	Rhizomucor variabilis
Rhizopus	Rhizopus homothallicus
Rhizopus	Rhizopus microsporus
Rhizopus	Rhizopus oryzae
Rhizopus	Rhizopus schipperae
Rhizopus	Rhizopus sexualis
Rhizopus	Rhizopus stolonifer
Rhizopus	Rhizopus stolonifer-var-stolonifer
Rhodotorula	Rhodotorula glutinis
Rhodotorula	Rhodotorula laryngis
Rhodotorula	Rhodotorula minuta
Rhodotorula	Rhodotorula mucilaginosa
Rhodotorula	Rhodotorula rubescens
Saccharomyces	Saccharomyces cerevisiae
Saccharomyces	Saccharomyces servazzii
Saccharomyces	Saccharomyces transvaalensis
Saccharomycopsis	Saccharomycopsis fibuligera
Saccharomycopsis	Saccharomycopsis javanensis
Sagenomella	Sagenomella diversispora
Saprochaete	Saprochaete clavata
Scedosporium	Scedosporium apiospermium
Scedosporium	Scedosporium aurantiacum
Scedosporium	Scedosporium boydii
Scedosporium	Scedosporium minutispora
Scedosporium	Scedosporium prolificans
Schizoblastosporion	Schizoblastosporion starkeyihenricii
Schizophillum	Schizophillum commune
Schizosaccharomyces	Schizosaccharomyces octosporus
Schizosaccharomyces	Schizosaccharomyces pombe
Scopulariopsis	Scopulariopsis acremonium
Scopulariopsis	Scopulariopsis asperula
Scopulariopsis	Scopulariopsis atra
Scopulariopsis	Scopulariopsis brevicaulis
Scopulariopsis	Scopulariopsis brumptii
Scopulariopsis	Scopulariopsis candida
Scopulariopsis	Scopulariopsis cinerea
Scopulariopsis	Scopulariopsis fusca
Scopulariopsis	Scopulariopsis murina
Scytalidium	Scytalidium dimidiatum
Scytalidium	Scytalidium hyalinum
Scytalidium	Scytalidium lignicola

genera	species
Septofusidium	Septofusidium berolinense
Sordaria	Sordaria fimicola
Spicellum	Spicellum roseum
Sporobolomyces	Sporobolomyces roseus
	Sporobolomyces salmonicolor
Sporopachydermia	Sporopachydermia lactativora
Sporothrix	Sporothrix inflata
	Sporothrix insectorum
	Sporothrix schenckii
Sporotrichum	Sporotrichum aurantiacum
	Sporotrichum pruinosum
Stachybotrys	Stachybotrys chartarum
Sterigmatomyces	Sterigmatomyces elviae
Syncephalastrum	Syncephalastrum racemosum
Talaromyces	Talaromyces amestolkiae
	Talaromyces coalescens
	Talaromyces diversus
	Talaromyces echinosporus
	Talaromyces flavovirens
	Talaromyces helicus
	Talaromyces leycettanus
	Talaromyces minioluteus
	Talaromyces pinophilus
	Talaromyces purpurogenus
	Talaromyces radicus
	Talaromyces siamensis
	Talaromyces stollii
	Talatomyces allahabadensis
Thanatephorus	Thanatephorus cucumeris
Thielavia	Thielavia heterothallica
Tilletiopsis	Tilletiopsis minor
Torulaspora	Torulaspora delbrueckii
Trametes	Trametes trogii
	Trametes versicolor
Trichoderma	Trichoderma aureoviride
	Trichoderma citrinoviride
	Trichoderma ghanense
	Trichoderma harzianum
	Trichoderma koningii
	Trichoderma koningiopsis
	Trichoderma longibrachiatum
	Trichoderma parceramosum
	Trichoderma polysporum
	Trichoderma virens
	Trichoderma viride
	Trichoderma viridescens
Trichophyton	Trichophyton ajelloi
	Trichophyton concentricum
	Trichophyton eboreum
	Trichophyton indicum
	Trichophyton interdigitale
	Trichophyton kuryangei
	Trichophyton mentagrophytes
	Trichophyton mentagrophytes-var-batonrougei
	Trichophyton mentagrophytes-var-erinacei
	Trichophyton mentagrophytes-var-porcellae
	Trichophyton persicolor
	Trichophyton phaseoliforme
	Trichophyton quinckeanum
	Trichophyton rubrum
	Trichophyton schoenleinii
	Trichophyton sinnii
	Trichophyton soudanense
	Trichophyton terrestre
	Trichophyton tonsurans
	Trichophyton vanbreuseghemii
	Trichophyton verrucosum
	Trichophyton violaceum
Trichosporiella	Trichosporiella cerebriformis
	Trichosporiella ornithopoda

genera	species
Trichosporon	Trichosporon aquatile
	Trichosporon asahii
	Trichosporon asteroides
	Trichosporon brassicae
	Trichosporon coremiiforme
	Trichosporon cutaneum
	Trichosporon debeurmannianum
	Trichosporon dohaense
	Trichosporon domesticum
	Trichosporon dulcitum
	Trichosporon faecale
	Trichosporon gracile
	Trichosporon inkin
	Trichosporon jirovecii
	Trichosporon laibachii
	Trichosporon loubieri
	Trichosporon moniliiforme
	Trichosporon montevideense
	Trichosporon mucoides
	Trichosporon ovoides
	Trichosporon terricola
Trichothecium	Trichothecium roseum
Tyromyces	Tyromyces fissilis
Udeniomyces	Udeniomyces piricola
Ulocladium	Ulocladium oudemansii
Ustilago	Ustilago cynodontis
Venturia	Venturia inaequalis
Verticillium	Verticillium tenerum
Westerdykella	Westerdykella sp.
Wickerhamia	Wickerhamia fluorescens
Wickerhamiella	Wickerhamiella domercqiae
Wickerhamomyces	Wickerhamomyces onychis
Williopsis	Williopsis pratensis
Wolfiporia	Wolfiporia cocos
Ybotromyces	Ybotromyces caespitosus
Zygosaccharomyces	Zygosaccharomyces bailii
	Zygosaccharomyces bisporus
	Zygosaccharomyces rouxii

Supplementary Table 2: Identification performance of the three databases for each of the 51 species represented in the three databases.

species	Confidence level of identification	Bruker 2,0	Bruker 1,7	MSI1	MSI2
Alternaria alternata (N=4)	**correct at the species level**	**100**	**100**	**25**	**50**
	discordant at the species level	0	0	75	50
	under defined threshold	0	0	0	0
Arthrinium arundinis (N=3)	**correct at the species level**	**33,3**	**33,3**	**100**	**100**
	discordant at the species level	33,3	33,3	0	0
	under defined threshold	33,3	33,3	0	0
Aspergillus calidoustus (N=5)	**correct at the species level**	**20**	**20**	**60**	**100**
	discordant at the species level	60	60	40	0
	under defined threshold	20	20	0	0
Aspergillus flavus (N=38)	**correct at the species level**	**36,8**	**76,3**	**100**	**94,7**
	discordant at the species level	2,6	2,6	0	2,6
	under defined threshold	60,5	21,1	0	2,6
Aspergillus fumigatus (N=26)	**correct at the species level**	**50**	**76,9**	**100**	**100**
	discordant at the species level	0	0	0	0
	under defined threshold	50	23,1	0	0
Aspergillus lentulus (N=9)	**correct at the species level**	**77,8**	**100**	**44,4**	**100**
	discordant at the species level	0	0	55,6	0
	under defined threshold	22,2	0	0	0
Aspergillus montevidensis (N=2)	**correct at the species level**	**0**	**50**	**0**	**100**
	discordant at the species level	0	0	100	0
	under defined threshold	100	50	0	0
Aspergillus nidulans (N=5)	**correct at the species level**	**60**	**100**	**100**	**100**
	discordant at the species level	0	0	0	0
	under defined threshold	40	0	0	0
Aspergillus niger (N=13)	**correct at the species level**	**30,8**	**46,2**	**53,8**	**61,5**
	discordant at the species level	0	0	46,2	30,8
	under defined threshold	69,2	53,8	0	7,7
Aspergillus ochraceus (N=6)	**correct at the species level**	**83,3**	**100**	**33,3**	**100**
	discordant at the species level	0	0	66,7	0
	under defined threshold	16,7	0	0	0
Aspergillus parasiticus (N=1)	**correct at the species level**	**0**	**0**	**100**	**0**
	discordant at the species level	0	0	0	0
	under defined threshold	100	100	0	100
Aspergillus pseudoglaucus (N=1)	**correct at the species level**	**0**	**0**	**0**	**100**
	discordant at the species level	0	0	100	0
	under defined threshold	100	100	0	0
Aspergillus sclerotiorum (N=16)	**correct at the species level**	**87,5**	**93,75**	**12,5**	**100**
	discordant at the species level	0	0	87,5	0
	under defined threshold	12,5	6,25	0	0
Aspergillus sydowii (N=5)	**correct at the species level**	**0**	**0**	**100**	**100**
	discordant at the species level	20	20	0	0
	under defined threshold	80	80	0	0
Aspergillus tamarii (N=1)	**correct at the species level**	**0**	**0**	**100**	**100**
	discordant at the species level	0	0	0	0
	under defined threshold	100	100	0	0
Aspergillus terreus (N=18)	**correct at the species level**	**22,2**	**77,8**	**88,9**	**100**
	discordant at the species level	0	0	11,1	0
	under defined threshold	77,8	22,2	0	0
Aspergillus thermomutatus1 (N=18)	**correct at the species level**	**11,1**	**61,1**	**83,3**	**100**
	discordant at the species level	0	22,2	16,7	0
	under defined threshold	88,9	16,7	0	0

Aspergillus unguis (N=2)	**correct at the species level**	**50**	**100**	**100**	**100**
	discordant at the species level	0	0	0	0
	under defined threshold	50	0	0	0
Aspergillus westerdijkiae (N=14)	**correct at the species level**	**42,9**	**64,3**	**85,7**	**100**
	discordant at the species level	0	0	14,3	0
	under defined threshold	57,1	35,7	0	0
Bisifusarium dimerum (N=5)	**correct at the species level**	**60**	**100**	**100**	**100**
	discordant at the species level	0	0	0	0
	under defined threshold	40	0	0	0
Cladosporium cladosporioides (N=4)	**correct at the species level**	**0**	**0**	**75**	**25**
	discordant at the species level	0	0	25	50
	under defined threshold	100	100	0	25
Fusarium equiseti (N=2)	**correct at the species level**	**0**	**50**	**100**	**100**
	discordant at the species level	0	50	0	0
	under defined threshold	100	0	0	0
Fusarium incarnatum (N=2)	**correct at the species level**	**0**	**0**	**50**	**0**
	discordant at the species level	0	50	50	100
	under defined threshold	100	50	0	0
Fusarium oxysporum (N=4)	**correct at the species level**	**0**	**33,3**	**100**	**33,3**
	discordant at the species level	0	0	0	66,7
	under defined threshold	100	66,7	0	0
Fusarium petroliphilum (N=14)	**correct at the species level**	**14,3**	**21,4**	**85,7**	**100**
	discordant at the species level	21,4	57,1	14,3	0
	under defined threshold	64,3	21,4	0	0
Fusarium proliferatum (N=48)	**correct at the species level**	**37,5**	**68,75**	**91,7**	**100**
	discordant at the species level	0	4,2	8,3	0
	under defined threshold	62,5	27,1	0	0
Fusarium solani (N=2)	**correct at the species level**	**0**	**50**	**100**	**100**
	discordant at the species level	0	0	0	0
	under defined threshold	100	50	0	0
Fusarium verticillioides (N=8)	**correct at the species level**	**25**	**62,5**	**100**	**100**
	discordant at the species level	0	0	0	0
	under defined threshold	75	37,5	0	0
Gallactomyces candidum (N=2)	**correct at the species level**	**100**	**100**	**100**	**100**
	discordant at the species level	0	0	0	0
	under defined threshold	0	0	0	0
Gallactomyces geotrichum (N=5)	**correct at the species level**	**60**	**100**	**100**	**100**
	discordant at the species level	0	0	0	0
	under defined threshold	40	0	0	0
Geosmithia argillacea (N=1)	**correct at the species level**	**0**	**100**	**100**	**0**
	discordant at the species level	0	0	0	100
	under defined threshold	100	0	0	0
Geotrichum capitatum (N=6)	**correct at the species level**	**16,7**	**16,7**	**66,7**	**33,3**
	discordant at the species level	0	0	0	0
	under defined threshold	83,3	83,3	33,3	66,7
Mucor circinelloides (N=1)	**correct at the species level**	**100**	**100**	**100**	**100**
	discordant at the species level	0	0	0	0
	under defined threshold	0	0	0	0
Paecilomyces lilacinus (N=2)	**correct at the species level**	**100**	**100**	**100**	**100**
	discordant at the species level	0	0	0	0
	under defined threshold	0	0	0	0
Paecilomyces variotii (N=2)	**correct at the species level**	**0**	**50**	**100**	**100**
	discordant at the species level	50	50	0	0
	under defined threshold	50	0	0	0
Penicillium chrysogenum (N=20)	**correct at the species level**	**20**	**55**	**100**	**100**

	discordant at the species level	0	5	0	0
	under defined threshold	80	40	0	0
Penicillium citrinum (N=3)	**correct at the species level**	**0**	**0**	**100**	**100**
	discordant at the species level	66,7	100	0	0
	under defined threshold	33,3	0	0	0
Penicillium corylophilum (N=1)	**correct at the species level**	**100**	**100**	**100**	**100**
	discordant at the species level	0	0	0	0
	under defined threshold	0	0	0	0
Penicillium crustosum (N=1)	**correct at the species level**	**0**	**0**	**100**	**100**
	discordant at the species level	0	0	0	0
	under defined threshold	100	100	0	0
Penicillium expansum (N=1)	**correct at the species level**	**0**	**0**	**0**	**0**
	discordant at the species level	0	0	100	100
	under defined threshold	100	100	0	0
Penicillium glabrum (N=19)	**correct at the species level**	**42,1**	**73,7**	**94,7**	**73,7**
	discordant at the species level	0	0	5,3	21,1
	under defined threshold	57,9	26,3	0	5,3
Penicillium oxalicum (N=1)	**correct at the species level**	**0**	**0**	**100**	**100**
	discordant at the species level	0	0	0	0
	under defined threshold	100	100	0	0
Rhizopus microsporus (N=1)	**correct at the species level**	**100**	**100**	**100**	**100**
	discordant at the species level	0	0	0	0
	under defined threshold	0	0	0	0
Rhizopus oryzae (N=3)	**correct at the species level**	**66,7**	**100**	**100**	**100**
	discordant at the species level	0	0	0	0
	under defined threshold	33,3	0	0	0
Scedosporium apiospermum (N=7)	**correct at the species level**	**42,9**	**71,4**	**71,4**	**71,4**
	discordant at the species level	0	0	0	0
	under defined threshold	57,1	28,6	28,6	28,6
Scedosporium aurantiacum (N=4)	**correct at the species level**	**100**	**100**	**100**	**100**
	discordant at the species level	0	0	0	0
	under defined threshold	0	0	0	0
Scedosporium prolificans (N=1)	**correct at the species level**	**0**	**0**	**100**	**0**
	discordant at the species level	0	0	0	0
	under defined threshold	100	100	0	100
Scopulariopsis brevicaulis (N=5)	**correct at the species level**	**20**	**80**	**100**	**100**
	discordant at the species level	0	0	0	0
	under defined threshold	80	20	0	0
Talaromyces rugulosus (N=1)	**correct at the species level**	**0**	**100**	**0**	**100**
	discordant at the species level	0	0	0	0
	under defined threshold	100	0	100	0
Thanatephorus cucumeris (N=2)	**correct at the species level**	**50**	**100**	**100**	**100**
	discordant at the species level	0	0	0	0
	under defined threshold	50	0	0	0
Trichoderma longibrachiatum (N=1)	**correct at the species level**	**0**	**0**	**0**	**100**
	discordant at the species level	0	0	100	0
	under defined threshold	100	100	0	0