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Use of the Bruker MALDI Biotyper for Identification of Molds in the Clinical Mycology Laboratory

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Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is increasingly used for the identification of bacteria and fungi in the diagnostic laboratory. We evaluated the mold database of Bruker Daltonik (Bremen, Germany), the Filamentous Fungi Library 1.0. First, we studied 83 phenotypically and molecularly well-characterized, nondermatophyte, nondematiaceous molds from a clinical strain collection. Using the manufacturer-recommended interpretation criteria, genus and species identification rates were 78.3% and 54.2%, respectively. Reducing the species cutoff from 2.0 to 1.7 significantly increased species identification to 71.1% without increasing misidentifications. In a subsequent prospective study, 200 consecutive clinical mold isolates were identified by the MALDI Biotyper and our conventional identification algorithm. Discrepancies were resolved by ribosomal DNA (rDNA) internal transcribed spacer region sequence analysis. For the MALDI Biotyper, genus and species identification rates were 83.5% and 79.0%, respectively, when using a species cutoff of 1.7. Not identified were 16.5% of the isolates. Concordant genus and species assignments of MALDI-TOF MS and the conventional identification algorithm were observed for 98.2% and 64.2% of the isolates, respectively. Four erroneous species assignments were observed using the MALDI Biotyper. The MALDI Biotyper seems highly reliable for the identification of molds when using the Filamentous Fungi Library 1.0 and a species cutoff of 1.7. However, expansion of the database is required to reduce the number of nonidentified isolates.

Molds are an important cause of morbidity and mortality among hospitalized patients, particularly among those who are immunocompromised or suffer from serious underlying disease (1–3). Traditionally, identification of molds in the diagnostic mycology laboratory is based on phenotypic traits (4–6). Sufficient growth and sporulation are required to assess macromorphological criteria, including growth on different media and at different temperatures, as well as micromorphological criteria, such as shape of conidia, spores, and mycelial structures. Conventional identification methods have important drawbacks: (i) conventional identification of molds requires a comparably long time to result, (ii) the enormous morphological variability of molds asks for extensive individual expertise of the laboratory personnel, and (iii) some mold isolates do not develop their characteristic structural features under laboratory conditions, preventing identification or even leading to misidentifications. Nucleic acid sequence analysis of the internal transcribed spacer (ITS) regions between the 18S and 28S rRNA genes has emerged as an alternative to conventional identification, especially for isolates with unusual phenotypic profiles and rare molds, including environmental contaminants (4, 7–9).

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is increasingly used in diagnostic bacteriology. Well-established commercial systems allow fast and reliable identification of bacteria (10–12). The use of MALDI-TOF MS in diagnostic mycology is limited so far, especially for the identification of molds, mainly due to the poor fungal coverage of the commercial databases and the requirement of extended sample preparation for molds to achieve good-quality mass spectra (13). Compared to that of bacteria, the cell wall of molds is more robust and rigid, making sample preparation more labor- and time-intensive (13, 14). Several studies reported the generation of in-house databases for the Bruker MALDI Biotyper (Bruker Daltonik, Bremen, Germany) covering mainly single genera, such as *Aspergillus* (15), *Fusarium* (15, 16), *Lichtheimia* (17), or the *Pseudallescheria/Scedosporium* complex (18); orders, such as the *Mucorales* (15); and individual groups of fungi, such as the dermatophytes (19, 20) or clinically important molds (14, 21–23). However, the species coverage of most of these fungus databases is limited, and many are neither publicly nor commercially available.

In this study, we have evaluated the first commercial mold database for the Bruker MALDI Biotyper, the Filamentous Fungi Library 1.0 (Bruker Daltonik), using liquid cultivation followed by ethanol-formic acid extraction for sample preparation. In the first part of the study, we analyzed a set of well-characterized, nondermatophyte, nondematiaceous molds from a clinical strain collection ($n = 83$) covering the most frequently occurring genera and species isolated at our laboratory (7). In a subsequent prospective study, we compared the identification of 200 clinical mold isolates by MALDI-TOF MS to the current identification algorithm used in our laboratory (4).

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MATERIALS AND METHODS

Mold isolates and culture conditions. A strain collection of 83 nondematiaceous, nondermatophyte molds was selected from our institute's strain collection covering the most commonly found genera ($n = 15$) and species ($n = 30$) at our laboratory (see Table S1 in the supplemental material) (7). When available, three isolates per genus/species were analyzed. The isolates were identified by phenotypic characterization and sequence analysis of the ITS region (4, 7). During the 6-month prospective study, 200 clinical mold isolates were identified in parallel by MALDI-TOF MS and by our conventional identification algorithm (4). This prospective cohort of isolates consisted of 33 unique species and 25 unique genera. As per our conventional algorithm, identification was based on phenotypic characteristics only for 195/200 isolates; for 5/200 isolates, additional ITS sequence analysis was done. Molds were cultivated on Sabouraud GMC agar (bioMérieux, Marcy l'Etoile, France) under aerobic conditions at 25°C. For MALDI-TOF MS measurement, isolates were grown in BBL Sabouraud liquid broth (BD, Franklin Lakes, NJ) at room temperature for 24 h to 48 h on a Stuart SB2 rotator (Bibby Scientific, Stone, United Kingdom).

Phenotypic and molecular identification. Phenotypic characterization and molecular identification of the isolates by ITS sequence analysis were done as previously described (4, 7). Sequence assignment to species and genus level was done according to guidelines published previously (4, 7). A sequence was assigned to a species if the best-matching reference sequence showed ≥98% homology and the next-best-matching reference species showed at least 0.8% less sequence homology. A sequence was assigned to genus level on the basis of 95% to 98% homology to the best-matching sequence or of ≥98% homology with sequence entries for several species from the same genus. "No identification" was defined as <95% homology with the best-matching reference sequence or as sequence homology of >95% with various genera present.

Sample preparation for MALDI-TOF MS. Preparation of mold isolates for MALDI-TOF MS measurement was done as previously described (14, 17). Briefly, 1 ml of fungus-containing medium was transferred to a 1.5-ml Eppendorf tube and centrifuged at 16,000 × *g* for 5 min. The supernatant was discarded, and the fungal pellet was washed twice with 1 ml distilled water. Finally, the pellet was suspended in 300 μl distilled water and 900 μl ethanol. The suspension was centrifuged at 16,000 × *g* for 2 min, and the pellet was dried in a Concentrator Plus (Eppendorf, Hamburg, Germany) at 30°C before being resuspended in 10 to 60 μl formic acid-water (70:30 [vol/vol]) depending on the fungal mass. After an incubation of 10 min at room temperature, an equal volume of acetonitrile was added. Samples were incubated again at room temperature for 10 min and subsequently centrifuged at 16,000 × *g* for 2 min. One microliter of the supernatant was transferred to a polished steel MSP 96 target (Bruker Daltonik) and allowed to dry at room temperature before being overlaid with 1 μl of a saturated *a*-cyano-4-hydroxycinnamic acid (HCCA) matrix solution in 50% acetonitrile-2.5% trifluoroacetic acid (Bruker Daltonik).

MALDI-TOF MS analysis. The acquisition and analysis of mass spectra were performed by a Microflex LT mass spectrometer (Bruker Daltonik) using the MALDI Biotyper software package (version 3.0) with the Filamentous Fungi Library 1.0 (Bruker Daltonik) and default parameter settings as published previously (24). The Bruker bacterial test standard (Bruker Daltonik) was used for calibration according to the instructions of the manufacturer. For each strain, two preparations of sample material were analyzed.

MALDI-TOF MS data interpretation. The Biotyper software compares each sample mass spectrum to the reference mass spectra in the database, calculates an arbitrary unit score value between 0 and 3 reflecting the similarity between sample and reference spectrum, and displays the top 10 matching database records. Standard Bruker interpretative criteria were applied (24). Briefly, scores of ≥2.0 were accepted for species assignment, and scores of ≥1.7 but <2.0 were accepted for an identification to the genus level. Scores below 1.7 were considered unreliable. In addition, consistency categories A (species consistency), B (genus consistency), and C (no consistency), which are assigned to the identifications by the Biotyper software, were considered for identification. Variations of the cutoff score value were done by reducing the species cutoff value to 1.9, 1.8, and 1.7 and the genus cutoff value to 1.6 and 1.5, followed by reinterpreting the top 10 matching database records.

Generation of in-house reference database. Reference spectra were created for 81 isolates of the retrospective study and added to the Bruker Filamentous Fungi Library 1.0. For each strain, a set of 24 spectra was measured and checked manually for flat-line, outlier, and single spectra with peaks differing from the other spectra. Questionable spectra were removed, and a total of 20 to 24 spectra were used to calculate a reference spectrum, using the automated function of the Biotyper software. For two *Scopulariopsis brevicaulis* isolates, the quality of the mass spectra was not sufficient for a reference entry.

Discrepancy analysis. Discrepancies between MALDI-TOF MS and phenotypic identification were resolved by ITS sequence analysis, which was considered the gold standard for identification (4).

Statistical analysis. Statistical calculations were done using IBM SPSS statistics software, version 20 (SPSS Inc., Chicago, IL). Overall differences among the tested cutoff score values for genus and species identification, respectively, were evaluated using the Friedman test. A P value of <0.05 was considered statistically significant. Follow-up tests were conducted using the Wilcoxon signed-rank test for pairwise comparison of the different cutoff score values. A Bonferroni correction was applied at the 0.0083 (0.05/6) level of significance across pairwise comparisons.

RESULTS

Strain collection. (i) Individual score cutoff values for the identification of molds. We used the mold database of Bruker Daltonik, the Filamentous Fungi Library 1.0, for the identification of 83 well-characterized, nondermatophyte, nondematiaceous molds by the MALDI Biotyper (Bruker Daltonik) (Table 1; also see Table S1 in the supplemental material). Applying the standard interpretation criteria of the manufacturer, i.e., a species cutoff score value of 2.0 and a genus cutoff score value of 1.7, the MALDI Biotyper correctly identified 65 (78.3%) of the 83 strains at genus level. Correct identification at species level was achieved for 45 (54.2%) of the 83 strains. Eighteen (21.7%) of the 83 strains were not identified by MALDI-TOF MS. Reducing the genus cutoff value from 1.7 to 1.6 and 1.5, respectively, had no influence on the genus identification rate (overall Friedman test, χ^2 [2, 83] = 4.67, $P = 0.097$). In contrast, a reduction of the species cutoff score value from 2.0 to 1.9, 1.8, and 1.7 significantly increased the species identification rate from 54.2% to 62.7% ($Z = -3.65$, $P = 0.0082$), 67.5% ($Z = -3.05$, $P = 0.002$), and 71.2% ($Z = -3.30$, $P = 0.001$), respectively, without introducing additional misidentifications.

(ii) Misidentifications by MALDI-TOF MS. At species level, one isolate each of *Penicillium olsonii* and *Trichoderma citrinoviride* was misidentified by the MALDI Biotyper as *Penicillium brevicompactum* and *Trichoderma koningii*, respectively (Table 1). No misidentification at genus level was observed for MALDI-TOF MS.

(iii) Low discrimination at species level by MALDI-TOF MS. Low discrimination at species level by the MALDI Biotyper was observed between *Aspergillus glaucus* (teleomorph: *Eurotium herbariorum*) and *Aspergillus amstelodami* (synanamorph: *Aspergillus hollandicus*; teleomorph: *Eurotium amstelodami*) (nomenclature according to the work of de Hoog et al. [5]). For two strains that were assigned to *A. glaucus* by phenotypic criteria, the MALDI Biotyper identification list showed scores above the species cutoff

TABLE 1 Strain collection of 83 clinical mold isolates analyzed by MALDI-TOF MS

Genus	No. of isolates	No. of isolates identified by MALDI-TOF MS[a]				No. of misidentifications[b]	No. of reference spectra[c]
		Genus level	Species level (cutoff, 2.0)	Species level (cutoff, 1.7)	No identification		
Acremonium	3	1	0	1	2	0	1
Aspergillus	24	24	17	21	0	0	90
Beauveria	3	1	0	1	2	0	1
Chrysosporium	3	3	2	3	0	0	3
Fusarium	6	6	6	6	0	0	36
Lichtheimia	3	3	3	3	0	0	11
Mucor	3	1	0	0	2	0	5
Paecilomyces	9	6	6	6	3	0	11
Penicillium	5	4	2	4	1	1	67
Rhizopus	3	3	1	3	0	0	15
Scedosporium	6	6	6	6	0	0	6
Schizophyllum	3	3	1	3	0	0	4
Scopulariopsis	6	0	0	0	6	0	9
Trichoderma	3	2	1	2	1	1	3
Trichurus	3	2	0	0	1	0	1
Total (%)	83 (100)	65 (78.3)	45 (54.2)	59 (71.1)	18 (21.7)	2 (2.4)	263

[a] MALDI-TOF MS identification applying a genus cutoff of 1.7 and species cutoffs of 2.0 and 1.7, respectively, using the Filamentous Fungi Library 1.0 (Bruker Daltonik).
[b] Misidentifications by MALDI-TOF MS: *Penicillium olsonii* (assignment by ITS sequence analysis) misidentified as *Penicillium brevicompactum* and *Trichoderma citrinoviride* (assignment by ITS sequence analysis) misidentified as *Trichoderma koningii*.
[c] Number of reference spectra in the Filamentous Fungi Library 1.0 (Bruker Daltonik).

of 2.0 for both *A. glaucus* and *A. amstelodami*, resulting in species inconsistency and in identification to the genus level only. In both cases, *A. glaucus* showed the highest score in the ranking list. ITS sequence analysis of these two strains showed sequence homology of >98% to different *Aspergillus* spp. and *Eurotium* spp., including *A. glaucus* and *A. amstelodami*; however, the sequences were closely related and no species assignment was possible. Lowering the species cutoff to 1.7 led to two additional species inconsistencies: for one isolate each of *Aspergillus versicolor* and *Phoma glomerata*, the MALDI Biotyper did not differentiate between *Aspergillus versicolor/unguis* and *Phoma glomerata/sorghina*, respectively.

(iv) Species coverage of the Filamentous Fungi Library 1.0. The strain collection investigated in this study included 30 species from 15 genera and covers the most frequently isolated genera and species of molds at our diagnostic laboratory (7). All genera within this strain collection were represented by at least one reference entry in the Filamentous Fungi Library 1.0 (Table 1). At the species level, seven species in this strain collection were not included in the database, i.e., *Mucor plumbeus*, *Mucor racemosus*, *Penicillium rolfsii*, *Scopulariopsis candida*, *Scopulariopsis cinerea*, *Trichoderma citrinoviride*, and *Trichoderma harzianum*. These isolates were not identified by MALDI-TOF MS, except for *T. citrinoviride*, which was erroneously assigned to the species *T. koningii*.

Prospective study. (i) Comparison of MALDI-TOF MS identification with the conventional identification algorithm in the routine diagnostic laboratory. In a prospective study, 200 clinical mold isolates including 33 species from 25 genera were identified by MALDI-TOF MS and compared to a conventional identification algorithm as previously published (4). As per this algorithm, identification was based on phenotypic characteristics only for 195/200 isolates, and for 5/200 isolates additional ITS sequence analysis was done. MALDI Biotyper spectra were analyzed using (i) the Filamentous Fungi Library 1.0 (Bruker Daltonik) and (ii) the Filamentous Fungi Library 1.0 amended with reference entries from 81 strains of the strain collection (our in-house database). Species cutoffs of 2.0 and 1.7, respectively, and a genus cutoff of 1.7 were applied for identification. The MALDI Biotyper identified 79% of the isolates at species level by applying the Filamentous Fungi Library 1.0 and a species cutoff of 1.7 (Table 2). Of the other isolates, 4.5% were assigned to a genus only, and for 16.5% of the isolates no identification was achieved. The conventional identification algorithm assigned 55% of the isolates to species level, 41% of the isolates were assigned to genus level only, and 4% of the isolates were not identified.

TABLE 2 Prospective study of 200 mold isolates with taxonomic assignment by conventional identification algorithm and MALDI-TOF MS

Identification system	No. (%) of isolates identified at taxonomic level:			
	Species	Genus	No identification	Total
Conventional identification algorithm[a]	110 (55.0)	82 (41.0)	8 (4.0)	200 (100)
MALDI-TOF MS[b]				
Fungi Library 1.0				
2.0 species cutoff	144 (72.0)	23 (11.5)	33 (16.5)	200 (100)
1.7 species cutoff	158 (79.0)	9 (4.5)	33 (16.5)	200 (100)
In-house database				
2.0 species cutoff	151 (75.5)	23 (11.5)	26 (13.0)	200 (100)
1.7 species cutoff	160 (80.0)	14 (7.0)	26 (13.0)	200 (100)

[a] Conventional identification algorithm combining phenotypic characteristics (done for all 200 isolates) with ITS sequence analysis (done for 5 isolates) according to the work of Ciardo et al. (4).
[b] Fungi Library 1.0, Filamentous Fungi Library 1.0 of Bruker Daltonik; in-house database, Filamentous Fungi Library 1.0 (Bruker) amended with 81 of our own reference entries from the strain collection part.

TABLE 3 Conventional identification algorithm versus MALDI-TOF MS identification for 165/200 clinical mold isolates of the prospective study

Conventional identification algorithm[a]	No. of isolates	No. (%) of concordant identifications by MALDI-TOF MS at[b]: Genus level (cutoff, 1.7)	Species level (cutoff, 1.7)	Discrepant identification by MALDI-TOF MS	Resolution of discrepancy
Aspergillus fumigatus	68	68 (100.0)	68 (100.0)		
Penicillium sp.	30	30 (100.0)			
Fusarium sp.	10	10 (100.0)			
Aspergillus flavus	10	10 (100.0)	10 (100.0)		
Aspergillus versicolor	9	9 (100.0)	7 (77.8)	*Aspergillus unguis* ($n = 2$)	*A. versicolor* and *A. unguis* are phenotypically closely related and not distinguished by the conventional identification algorithm
Aspergillus terreus	7	7 (100.0)	7 (100.0)		
Aspergillus sp.	4	4 (100.0)			
Cladosporium sp.	3	3 (100.0)			
Alternaria sp.	3	3 (100.0)			
Aspergillus glaucus	3	3 (100.0)	1 (33.3)	*Aspergillus* sp. ($n = 2$)	No differentiation of *A. glaucus* and *A. amstelodami* by MALDI-TOF MS and ITS sequence analysis
Botrytis sp.	3	3 (100.0)			
Rare isolates[c]	15	12 (80.0)	10 (66.7)		
Aspergillus nidulans				*Aspergillus fumigatus* ($n = 1$)	Phenotypic misidentification
Acremonium sp.				*Aspergillus fumigatus* ($n = 1$)	Contamination of liquid culture for MALDI-TOF MS identification with *A. fumigatus*
Beauveria sp.				*Beauveria bassiana* ($n = 1$)	ITS sequence analysis confirmation of *Beauveria bassiana*
Paecilomyces sp.				*Geosmithia argillacea* ($n = 1$)	Phenotypic misidentification
Phoma sp.				*Chaetomium globosum* ($n = 1$)	Phenotypic misidentification
Total	165	162 (98.2)	103 (62.4)		

[a] Identification algorithm according to the work of Ciardo et al. (4).
[b] MALDI-TOF MS identification applying a genus and species cutoff of 1.7 with the Filamentous Fungi Library 1.0 (Bruker).
[c] Isolates with *n* of ≤2, including *Acremonium* sp., *Arthrinium phaeospermum*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus ochraceus*, *Botrytis* sp., *Phoma* sp., *Scedosporium apiospermum*, *Chaetomium* sp., *Paecilomyces lilacinum*, *Paecilomyces* sp., and *Scopulariopsis brevicaulis*. Only those with discrepant identification of the conventional identification algorithm and MALDI-TOF MS are shown.

Overall, for 35 (17.5%) of the 200 isolates, no identification was achieved by either MALDI-TOF MS using the Filamentous Fungi Library 1.0 ($n = 27$ isolates), the conventional identification algorithm ($n = 2$ isolates), or both methods ($n = 6$ isolates) (see Table S2 in the supplemental material). A mass spectrum was measured for 29 of the 33 isolates that were not identified by MALDI-TOF MS, and for 4 of these 33 isolates, no mass peaks were detected. The corresponding genus of 13 of the 29 isolates that were not identified by MALDI-TOF MS but yielded a mass spectrum was represented by ≤1 reference spectrum in the Filamentous Fungi Library 1.0 (see Table S2). The genus of the remaining 16 isolates was covered by ≥6 reference entries. Among the isolates that were not identified by the MALDI Biotyper, *Penicillium* spp. ($n = 10$ isolates) and *Cladosporium* spp. ($n = 4$ isolates) were most frequent. Analysis by ITS sequence determination revealed 4 phenotypic misidentifications within the 27 isolates that were identified by conventional identification but not by MALDI-TOF MS (see Table S2). One *Arthrinium* sp. was erroneously assigned to the species *Arthrinium phaeospermum* by the MALDI Biotyper.

Identification at genus and/or species level by the MALDI Biotyper and the conventional identification algorithm was achieved for 165 (82.5%) of the 200 isolates. For 162 (98.2%) of these 165 isolates, concordant genus assignment was observed, and 101 (61.2%) of these 165 isolates yielded concordant species identifications, applying a species cutoff of 2.0 (Table 3; see also Tables S3 and S4 in the supplemental material). Decreasing the species cutoff to 1.7 increased the species identification rate of MALDI-TOF MS and increased the number of concordant species identifications to 103 (62.4%) of the 165 isolates.

(ii) Different levels of identification by MALDI-TOF MS and the conventional identification algorithm. Forty-seven (28.5%) of the 165 isolates were identified at species level by the MALDI Biotyper, while conventional phenotypic identification for these isolates yielded genus assignment only (see Table S4 in the supplemental material). For 18 of these 47 isolates, ITS sequence analysis confirmed the species identification by MALDI-TOF MS. Twenty-eight of 47 isolates could be assigned to genus level only by ITS sequence analysis due to a close genetic relatedness of several species. For these isolates, ITS reference sequences (SmartGene database 2013; SmartGene, Zug, Switzerland) of different species showed sequence homology above the threshold for species assignment (98%) (4). For 25 of the 28 isolates, the species as determined by MALDI-TOF MS was among the species exhibiting >98% sequence homology. Three of 28 isolates were misidentified by MALDI-TOF MS as *Penicillium discolor* (<98% ITS sequence homology to *P. discolor*). One of 47 isolates was misidentified as *Penicillium citrinum* by MALDI-TOF MS (*Penicillium*

sumatraense by ITS sequence analysis). The conventional identification algorithm obtained for two isolates a higher level of identification than did MALDI-TOF MS and ITS sequence analysis, i.e., two *A. glaucus* isolates.

Low discrimination at the species level was observed for two *A. glaucus* isolates (2/165, 1.2%) when applying a species cutoff of 2.0. This number was increased to 10/165 (6.0%) for a species cutoff of 1.7. Low discrimination at species level was observed for the following strains: five *Penicillium* strains (score of ≥1.7 for different *Penicillium* spp.), three isolates of *A. glaucus* (score of ≥1.7 for *A. glaucus/amstelodami*), one isolate of *A. unguis* (score of ≥1.7 for *A. unguis/versicolor*), and one *Fusarium* strain (score of ≥1.7 for *Fusarium proliferatum/verticillioides*).

(iii) Resolution of discrepancies between MALDI-TOF MS and the conventional identification algorithm. For 3 isolates, discrepancies of genus identification were observed (Table 3; see also Table S5 in the supplemental material). One *Geosmithia argillacea* strain and one *Chaetomium globosum* strain were misidentified by phenotypic identification as *Paecilomyces* sp. and *Phoma* sp., respectively. One *Arthrinium* sp. strain was identified as *Aspergillus fumigatus* by MALDI-TOF MS. However, a more detailed analysis revealed a contamination of the liquid culture used for MALDI-TOF MS sample preparation with *A. fumigatus*.

One *Aspergillus fumigatus* and two *Aspergillus unguis* strains were correctly identified by MALDI-TOF MS, which was confirmed by ITS sequence analysis, but were misidentified as *Aspergillus nidulans* and *Aspergillus versicolor*, respectively, by phenotypic characterization (Table 3; see also Table S5 in the supplemental material).

(iv) Increased identification rate by extended database. Applying our amended in-house database, i.e., an extended database combining the commercially available Filamentous Fungi Library 1.0 of Bruker with 81 in-house-generated reference spectra from strains of the strain collection, increased the rate of species identification from 72.0% to 75.5% and 80.0% for species cutoffs of 2.0 and 1.7, respectively (Table 2). Nonidentifications decreased from 16.5% to 13.0% due to the additional identification of 4 *Penicillium* spp., 2 *Beauveria* spp., and one *Acremonium* species (data not shown). The number of species inconsistencies caused by low discrimination at the species level did not change with the use of the in-house database compared to the Filamentous Fungi Library 1.0.

DISCUSSION

We here evaluated the first commercial database of Bruker Daltonik for the identification of molds, the Filamentous Fungi Library 1.0, by analyzing (i) the performance of the system, (ii) the impact of adaptions of the manufacturer's data interpretation algorithms, and (iii) the coverage of the database.

Interpretation algorithms. Using standard interpretation criteria recommended by Bruker Daltonik for the analysis of our strain collection (genus cutoff of 1.7 and species cutoff of 2.0) resulted in genus and species identification rates of 78.3% and 54.2%, respectively. Reducing the genus cutoff from 1.7 to 1.5 had no influence on genus identification. In contrast, species identification was significantly enhanced by reducing the species cutoff from 2.0 to 1.7 (from 54.2% to 71.1%) without increasing the rate of misidentifications. In the prospective study, the reduction of the species cutoff similarly increased the rate of species identification (Table 2). Our data support the findings of other studies that have evaluated in-house databases for molds in combination with the Bruker MALDI Biotyper and which recommended the application of a lower species cutoff (mostly 1.7) to increase identification rates (14, 20, 21).

The rate of species inconsistency due to low discrimination of different species of the same genus increased with decreasing species cutoffs. Species inconsistencies at a cutoff of 1.7 were observed especially for *Aspergillus glaucus*, *Aspergillus unguis*, *Fusarium* spp., and *Penicillium* spp. To minimize this problem, we suggest using a two-step interpretation as has previously been proposed for the identification of Gram-positive rod-shaped bacteria: in a first step, data interpretation is done according to the standard criteria (species cutoff of 2.0), and in a second step, a lower species cutoff (preferably 1.7) is applied for those isolates that did not yield species identification when using the standard species cutoff value of 2.0 (25).

Penicillium spp., *Fusarium* spp., and *Aspergillus* spp. were the most common species among those isolates of the prospective study that were identified only at genus level when applying a species cutoff of 2.0 but yielded a species identification when applying a cutoff of 1.7 (data not shown). These genera are among those that are covered by the highest number of reference entries in the Filamentous Fungi Library 1.0. This could suggest that the corresponding isolates achieved a low score (≤2.0) due to low spectral quality rather than a low database coverage. However, the isolates also included *Aspergillus fumigatus* and *Fusarium oxysporum*, two species broadly covered by 12 and 6 reference entries in the Filamentous Fungi Library 1.0, respectively. These were identified at species level by applying our in-house database but failed species identification using the Filamentous Fungi Library 1.0. Based on our data, it is therefore difficult to discriminate between low spectral quality and insufficient database coverage as potential reasons for low scores. Further studies are necessary to determine the number of reference entries that are needed to cover the diversity of single species and genera.

Coverage of the reference database. The Filamentous Fungi Library 1.0 contains 365 reference entries and covers 124 species from 30 genera. The prospective study showed that identification of the most commonly isolated molds, i.e., *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp., by the MALDI Biotyper was highly reliable. Considering all 200 isolates of the prospective study, the rate of nonidentification of isolates by MALDI-TOF MS was significantly higher than that by our conventional identification algorithm, i.e., 16.5% versus 4.0%. The corresponding genus of approximately 50% of the isolates not identified by the MALDI Biotyper was represented by one isolate or none (see Table S2 in the supplemental material). Applying our amended in-house database increased the overall identification rate in the prospective part of the study and reduced the rate of nonidentifications (Table 2). The impact of the in-house database on the species identification was, however, smaller than the impact of lowering the species cutoff to 1.7, i.e., a 3.5% versus 7% increase of the species identification rate for adding the in-house database and for lowering the cutoff, respectively. The high rate of nonidentifications by the MALDI Biotyper thus in part reflects limited database coverage. Proprietary in-house reference spectra were provided to Bruker Daltonik for integrating selected data sets and to enlarge the database.

In the analysis of the strain collection, discrepancies at species level were observed for *Penicillium olsonii* and *Trichoderma citri-*

noviride, which were misidentified by the MALDI Biotyper as *Penicillium brevicompactum* and *Trichoderma koningii*, respectively. Both pairs of species, *P. brevicompactum/olsonii* and *T. citrinoviride/koningii*, are phylogenetically closely related (5, 26). The Filamentous Fungi Library 1.0 contains five reference entries for *P. brevicompactum* and one reference entry for *P. olsonii*. *T. koningii* is represented by two isolates, the mass spectra of which only poorly match. *T. citrinoviride* is not included in the database.

In the prospective study, 47 isolates were assigned to species level by MALDI-TOF MS while conventional identification resulted in genus assignment only. For 28 of these 47 isolates, ITS sequence analysis allowed genus identification only due to the close relatedness of several species. However, for most of these isolates (25/28), the species as determined by MALDI-TOF MS was among the species exhibiting >98% sequence homology. Of note, 19 of these 25 isolates belonged to the genus *Penicillium*. The taxonomy of *Penicillium* spp. is complex, and species identification is difficult using current methods, often leading to misidentifications (27). Hettick et al. (28) report that MALDI-TOF MS may be a useful diagnostic tool for the identification of *Penicillium* spp. However, extension of the database with additional *Penicillium* spp. will be required to assess the discriminatory power of MALDI-TOF MS for this genus. *Penicillium* spp. are rarely correlated with infection, except for *Penicillium marneffei* (29, 30). Until further data are available, we suggest accepting the genus identification for isolates of *Penicillium* spp. assigned to species level by MALDI-TOF MS. Unfortunately, *P. marneffei* is not included in the Filamentous Fungi Library 1.0 and has to be identified by conventional morphological methods.

Taxonomic uncertainties. Low discrimination at species level by MALDI-TOF MS was observed for *Aspergillus glaucus* and *Aspergillus amstelodami*. In the literature, there exists a confusion of the taxonomic description of *A. amstelodami* and many isolates were misidentified (31). Bruker does not specify the origin of its isolate nor the species definition applied for assignment to *A. amstelodami*. Clarification of the taxonomic position and addition of further isolates of *A. amstelodami* to the database would be desirable.

Dematiaceous molds. Dematiaceous molds, such as *Cladosporium* spp., *Phoma* spp., or *Alternaria* spp., are poorly represented in the Filamentous Fungi Library 1.0. In our hands, mass spectra of dematiaceous molds had fewer peaks, and spectrum quality was often not sufficient to create reference entries, suggesting that sample preparation for these fungi is more difficult. In addition, fungal pigments may have inhibited the acquisition of MALDI-TOF mass spectra as described by Buskirk et al. (32). From 28 isolates of dematiaceous molds analyzed, 25.0% were identified at species level and 10.7% were identified at genus level (unpublished data). Dematiaceous molds are usually rarely found at our institution (7.5% in the prospective study, with *Cladosporium* sp. being found most commonly) and mostly represent contaminants or are considered to be of no pathogenetic significance (33, 34).

Implications for the clinical laboratory. The current identification algorithm of molds of our clinical laboratory combines phenotypic and genetic procedures (4). MALDI-TOF MS proved to be a reliable alternative tool for mold identification. However, simply replacing conventional methods by MALDI-TOF MS seems not possible at present due to a comparably high rate of nonidentifications (16.5% compared to 4% nonidentifications when using the conventional identification algorithm). In addition, optimal sample preparation of molds for identification by the Bruker MALDI Biotyper requires—according to the manufacturer—a liquid subculture and the ethanol-formic acid extraction procedure. This procedure is more time-consuming than the direct transfer preparation protocol that is suitable to identify most bacteria (24, 25). Collecting fungal material directly from solid medium (agar plates) instead of harvesting it from liquid subculture has been proposed by others and could simplify sample preparation and save time (14, 15, 19, 23, 28). In preliminary experiments, we observed significantly lower rates of identification when fungal material was scratched from agar plates before being extracted according to the ethanol-formic acid protocol. One major problem was to scratch fungal material from the plates without agar, which seemed to interfere with the fungal spectrum (R. Ledermann, unpublished data). Further studies are necessary to evaluate the time-to-result of conventional and MALDI-TOF MS identification and to define an optimal algorithm regarding time and workload for the identification of molds in the clinical laboratory combining MALDI-TOF MS with phenotypic and genetic methods.

In conclusion, this study showed that the identification of nondermatophyte, nondematiaceous molds by the Bruker MALDI Biotyper using a commercially available database, the Filamentous Fungi Library 1.0, is highly reliable. However, the database would benefit from additional species entries to elucidate the capacity of MALDI-TOF MS to differentiate between phylogenetically closely related species and to decrease the rate of nonidentified isolates. Identification rates can further be improved by reducing the species cutoff score value and by expanding the database to cover additional taxa.

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