IDENTIFICATION OF UNCOMMON CLINICALLY IMPORTANT YEASTS AND MOULDS BY THE BRUKER

BIOTYPER MATRIX-ASSISTED LASER DESORPTION IONIZATION-TIME OF FLIGHT MASS SPECTROMETRY

(MALDI-TOF MS) SYSTEM IN A GLOBAL ANTIFUNGAL SURVEILLANCE PROGRAM

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Abstract:

We report the evaluation of the Bruker Biotyper MS (BMS) system for the identification (ID) of 437 isolates of uncommon species of *Candida* (106 isolates; 17 species), non-*Candida* yeasts (100 isolates, 16 species), *Aspergillus* (164 isolates; 11 species) and non-*Aspergillus* moulds (57 isolates; 36 species) collected from 68 laboratories in 2012. Using confidence scores of ≥1.7 to <2.0 for genus only ID and scores of ≥2.0 for species-level ID, BMS correctly IDed 89.8% of yeasts and 85.5% of moulds to the genus level and 71.3% and 73.3%, respectively, to the species-level. Applying a lower threshold of >1.7 for species-level ID to isolates included in the BMS database improved the accuracy of species ID from 88.7% to 99.0% for *Candida*, from 65.4% to 91.3% for non-*Candida* yeasts and from 78.6% to 85.4% for moulds. BMS gave a result of no identification to those species not included in the database and generated very few (5 total, all moulds) mis-IDs, all of which were correctly IDed at the genus-level.

***Keywords**: MALDI-TOF, yeasts, moulds, surveillance*

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INTRODUCTION

The frequency of occurrence of invasive fungal infections (IFI) coupled with an ever-increasing diversity of opportunistic yeasts and moulds capable of causing serious IFIs significantly impact the ability to diagnose and treat these infections even in the present era of echinocandins and mould-active triazole antifungal agents (Alastruey-Izquierdo et al., 2013; Arendrup et al., 2013; Azie et al., 2012; Brown et al., 2012; Pfaller et al., 2009; Pfaller and Diekema, 2010; Pfaller et al., 2012). Serious life-threatening infections are being reported with an expanding array of pathogens including the well-known opportunists *Candida albicans, Cryptococcus neoformans* and *Aspergillus fumigatus* (Azie et al., 2012; Brown et al., 2012; Pfaller and Diekema, 2004; 2010.) New and emerging fungal pathogens include species of *Candida* and *Aspergillus* other than *C. albicans* and *A. fumigatus*; opportunistic yeast-like fungi such as *Cryptococcus gattii*, *Trichosporon* spp., *Dipodascus capitatus, Rhodotorula* spp., and *Saceharomyces cerevisiae*; the mucormycotes; hyaline moulds such as *Fusarium, Acremonium, Scedosporium, Paecilomyces (Purpureocillium)* and *Trichoderma* species; and a wide variety of demateaccores moulds (Alastruey-Izquierdo et al., 2013; Brown et al., 2012; Miceli and Lee, 2011; Miceli et al., 2011; Pfaller et al., 2012; Pfaller et al., 2013). Accurate identification (ID) of these organisms is important in guiding therapy and determining prognosis in these IFIs as well as in epidemiological surveys (Balajee et al., 2009; Borman et al., 2012; Brandt and Park, 2013; Linton et al., 2007; Miceli and Lee, 2011; Pounder et al., 2007). Conventional methods of yeast and mould ID take into account biochemical and morphological characteristics and are both slow and labor intensive requiring considerable mycological expertise (Alastruey-Izquierdo et al., 2013; Balajee et al., 2009; Borman et al., 2012; Cendejas-Bueno et al., 2010; Latouche et al., 1997; Meletiadis et al., 2011; Sanguinetti et al., 2007). Furthermore, classical methods of ID that rely on phenotypic characteristics increasingly have been shown to mis-ID the less common yeasts and moulds with potentially deleterious effects on informed therapeutic choices and patient management (Alastruey-Izquierdo et al., 2013; Borman et al., 2012; Castanheira et al., 2013; Pfaller et al., 2012). Whereas molecular methods for ID of yeasts and moulds represent improvements in timelines and accuracy of fungal ID, even DNA sequence-based fungal ID has several limitations (Cassagne et al., 2011). Issues with DNA extraction, cost and the need for highly trained personnel put molecular ID methods out of reach for most clinical microbiology laboratories (Balajee et al., 2009; Cassagne et al., 2011). Indeed, in 2007 only 17% of clinical laboratories in the United States performed molecular analysis of fungi (Balajee et al., 2007).

In light of these concerns the introduction of a new proteomic technology, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has revolutionized the ID of microorganisms, including yeasts and moulds (Buchan and Ledeboer, 2013; Cassagne et al., 2011; Patel, 2013). MALDI-TOF MS is a technique that generates a protein-based spectrial profile that is unique to a given species of microorganism and has been shown to be highly accurate, rapid and

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cost-effective in the ID of bacteria and most yeasts of clinical importance (Buchan and Ledeboer, 2013; Patel, 2013). The application of MALDI-TOF MS to the ID of moulds has been more limited to date with the greatest experience having to do with the ID of *Aspergillus* species (Alanio et al., 2011; Cassagne et al., 2011; De Carolis et al., 2012).

In the present study, we assessed the utility of the Bruker Daltonics MALDI Biotyper (Freemont, CA) for the ID of yeasts and moulds submitted during the course of the SENTRY Antifungal Surveillance Program (2012) that presented significant challenges to conventional methods of ID: rare species of *Candida* (other than *C. albicans, C. glabrata, C. parapsilosis, C. tropicalis* or *C. krusei*), non-candidal yeasts, *Aspergillus* spp., and other moulds. The reference ID for each isolate was determined using DNA sequence-based methods as described previously (Pfaller et al., 2012).

MATERIALS AND METHODS

Organisms. Among the 2,190 viable fungal isolates received during the SENTRY Surveillance Program in 2012, 437 moulds and uncommon yeast strains from 68 hospitals worldwide were IDed by MALDI-TOF MS and DNA sequencing methods. The SENTRY Program collects consecutive clinically significant fungal isolates from blood cultures (all fungi) as well as from respiratory tract specimens of patients diagnosed with pneumonia caused by *Aspergillus* spp. or other moulds from each participating medical center. Isolates were previously identified at participant institutions using methods routinely employed at the submitting laboratory. Purity of isolates was confirmed by subculturing the isolates in appropriate media followed by visual inspection. All *Candida* species isolates that were not confirmed upon receipt at JMI Laboratories (North Liberty, IA USA) by morphology on CHROMagar (Becton-Dickinson, Sparks, MD, USA) (*C. albicans, C. tropicalis* and *C. krusei*) growth at 45°C (*C. albicans, C. dubliniensis*), or assimilation of trehalose (*C. glabrata*) as well as all non-*Candida* yeasts and all moulds were submitted to molecular ID. In an effort to provide a more rigorous challenge to ID by MALDI-TOF MS, we did not include the more common species of *Candida* (*C. albicans, C. glabrata, C. parapsilosis, C. tropicalis* or *C. krusei*) in this analysis. All experiments were carried out in the Mycology and Molecular Research Sections of JMI Laboratories.

DNA sequencing-based identification. Isolates were subcultured from storage and IDed to species or species complex (SC) level by molecular methods. DNA extractions were performed using QIAquick Extraction kit (Qiagen, Hilden, Germany; yeasts only) or UltraClean Microbial DNA Isolation kit (MO BIO Laboratories, Carlsbad, California, USA) and amplification and sequencing of the following genes; internal transcribed spacer (ITS) region, 28S ribosomal subunit, β-tubulin (*Aspergillus* only), translation elongation factor (TEF: *Fusarium* only) and IGS (*Trichosporon* spp. and *C. neoformans* SC) were carried out as described previously (Balajee et al., 2009; Castanheira et al., 2013) (Lew et al 2006; Gilgado et al 2005; O'Donnell et al 2008). Nucleotide sequences were analyzed using Lasergene® software (DNAStar, Madison, WI, USA) and compared with available sequences using

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BLAST. TEF sequences were analyzed using the *Fusarium*-II database (http://www.isolate.fusariumdb.org/index.php) and the *Fusarium* multilocus sequence typing (MLST) database (http://www.chs.knaw.nl/fusarium/.) Results were considered acceptable if homology was >99.5% with other entries in the databases used for comparison. Available sequences that were considerably different from the majority of entries for one species were considered outliers and discarded in the analysis. Additionally, if no match was found in the database, the ID was based on species complex, genus, family, or order, according to the most current classification system.

MALDI-TOF MS. All isolates were tested via MALDI-TOF MS using the Burker Daltronics MALDI Biotyper (Freemont, CA, USA) by following the manufacturer's instructions and previously described guidelines for yeast and mould ID (Cassagne et al., 2011; Cassagne et al., 2013; Van Herendael et al., 2012). All yeasts were extracted from cultures on Sabouraud dextrose agar (Remel, Lenexa, KS, USA) by the complete extraction procedure as recommended by the manufacturer. Moulds were grown on potato dextrose agar slants (Remel) until mature and transferred to Sabouraud dextrose broth (Becton Dickinson, Maryland, USA). These tubes were placed on a rotator and incubated for 24 to 48 hours at 35°C or room temperature, as appropriate. Fungal material was centrifuged, and the pellet was washed twice with sterile water. The pellet was then suspended in water and ethanol (proportions??), centrifuged and decanted; 50 µl of 70% formic acid was added, mixed and allowed to incubate at room temperature (?) for at least 10 minutes (?range?). Acetonitrile (Sigma Aldrich, St. Louis, MO, USA) was then added and following centrifugation the supernatant (?volume?) was applied on a spot of polished steel target (Bruker) and air dried. Each spot was then covered by a matrix solution of 2-cyano-4-hydroxycinnamic acid (Sigma) in 50% acetonitrile and ___________________ (scan got cut off!) whether an isolate ID was classified as species-level, genus-level or unidentified was based on confidence scores as determined by the manufacturer: confidence scores of ≥2.0 are considered secure species-level ID, scores of 1.7 to <2.0 are considered genus-only ID, and scores of <1.7 are considered unreliable ID and termed unidentified.

RESULTS AND DISCUSSION

Fungal isolates. Among the 437 isolates IDed by molecular methods in this study, 106 were *Candida* spp. (17 species), 110 were non-*Candida* yeasts (16 species), 164 were *Aspergillus* spp (11 species) and 57 were non-*Aspergillus* moulds (36 species) (Tables 1-3). Overall the ID provided by the submitting laboratory was confirmed by DNA sequence analysis in 83.5% of the isolates (84.3% of yeasts and 82.8% of moulds).

MALDI-TOF MS identification of *Candida* spp. Among the 106 isolates of uncommon species of *Candida*, 93.4% were IDed correctly to genus and 81.1% were IDed correctly to species by MALDI-TOF MS (Table 1). There were seven isolates (6.6%) for which the MALDI-TOF MS result was unidentified (no reliable identification; confidence score <1.7) of which *C. bracarensis* (1 isolate), *C. fabianii* (2 isolates), *C. fluviatilis* (1

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isolate), *C. thasaensis* (1 isolate) and *C. thermophia* (1 isolate) were not included in the Bruker database. One isolate of *C. guilliermondii* was not IDed due to a failure of extraction (no peaks detected) despite three different attempts. Among the 11 species (97 isolates) of *Candida* that were represented in the Bruker database, 88.7% were IDed correctly to species and 100.0% were IDed correctly to genus. Notably, of the 13 isolates that were IDed to genus but not to species, 10 isolates were *C. dubliniensis* for which the Bruker MS ID was correct to species but the confidence scores ranged from 1.803 to 1.996 (Table 4). The 3 isolates of *C. fermentati*, a species not included in the database, were all IDed as *C. guilliermondii*, but with confidence scores <1.7 (Table 4). It should be noted that *C. fermentati* is included within the *C. guilliermondii* species complex. MALDI-TOF MS did not produce any incorrect IDs among the 106 isolates of *Candida* in this challenging collection.

MALDI-TOF MS identification of non-*Candida* yeasts. Among the 110 isolates of non-*Candida* yeasts, 86.4% were IDed correctly to genus and 61.8% were IDed correctly to species by MALDI-TOF MS (Table 2). There were 15 isolates (13.6%) that were unidentified by MALDI-TOF MS, 6 of which were not contained in the Bruker database and 9 of which were isolates of *C. neoformans* where ID was not possible due to a lack of detectable peaks despite multiple attempts at extraction. Among the 53 isolates of *C. neoformans* for which extraction was successful, all were correctly IDed at the genus level, 33 of which were IDed correctly to species with a confidence score of ≥2. The remaining 20 isolates of *C. neoformans* were also correctly IDed to species but with confidence scores ranging from 1.731 to 1.913 (Table 4). Similarly, the 2 isolates of *C. gattii* were IDed correctly to species but with confidence scores of 1.802 and 1.970 (Table 4).

Among the remaining 9 species (40 isolates) of non-*Candida* yeasts that were represented in the Bruker database, 87.5% were IDed correctly to species and 100.0% were IDed correctly to genus. As with *Candida* spp., of the 5 isolates that were IDed to genus but not to species, 4 isolates of *R. mucilaginosa* and 1 isolate of *S. cerevisiae* were correctly IDed to species but with confidence scores ranging from 1.875 to 1.995 (Table 4). None of the non-*Candida* isolates were misidentified by the Bruker system.

MALDI-TOF MS identification of moulds. Among the 221 mould isolates from the 2012 SENTRY survey, 164 (74.2%) were species of *Aspergillus* (Table 3). The Bruker Biotyper correctly IDed 95.7% of these isolates to the genus level and 90.9% to the species level. There were seven isolates that were not identified either due to the lack of representation in the database (*Asp. candidus* and *Asp. udagawae* [1 isolate each]) or due to failed extraction (*Asp. flavus* SC and *Asp. nidulans* [1 isolate each]). An additional 3 isolates (*Asp. nidulans* [1 isolate] and *Asp. terreus* SC [2 isolates]) represented species included in the Bruker database that were unidentified due to confidence scores of <1.7. Among the eight species of *Aspergillus* that were represented in the Bruker database (161 isolates), 96.9% were IDed correctly by genus and 92.5% were IDed correctly by species. Of the eight isolates of *Aspergillus* that were correctly IDed by genus but not by species, one isolate each of *Asp. clavatus*, *Asp. niga* SC, *Asp. nomius* and *Asp. sydowii* and three

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isolates of *Asp. flavus* SC were correctly IDed to species but with confidence scores ranging from 1.752 to 1.996 (Table 4). The sole misidentification among the *Aspergillus* spp. in the 2012 SENTRY collection was an isolate of *Asp. tubingensis* that was misidentified as *Asp. niger* with a confidence score of 2.251 (Table 4). *Asp. tubingensis* is in the *Aspergillus* section Nigri.

There were 57 non-*Aspergillus* moulds representing 36 different genera or species in the collection (Table 3): 56.1% were IDed correctly by genus and 22.8% were IDed correctly by species. This diverse group of organisms was underrepresented in the Bruker database and 25 isolates were unidentified either due to a lack of inclusion in the database or a confidence score of <1.7 without an accompanying ID (19 isolates) or due to a failed extraction (six isolates). Among the 15 genera or species (45 isolates) that were included in the Bruker database, 72.7% were IDed correctly by genus and 29.5% were IDed correctly by species. Of the 19 isolates of rare moulds that were correctly IDed by genus but not by species, one isolate each of *F. solari* SC and *P. lilacinum*, 2 isolates of *R. microsporus*, and 3 isolates of *F. fujikuroi* SC were correctly IDed to species or species complex but with confidence scores ranging from 1.761 to 1.994 (Table 4). It should be noted that *F. moniliforme* and *F. proliferatum* are species within the *F. fujikuroi* SC and thus may be considered to be correctly IDed to species by the Bruker Biotyper (Table 4). There were 2 isolates that were IDed by the reference method as *Cochliobolus* without a species designation that were IDed by the Bruker system as *Curvularia pallescens* with confidence scores of 1.940 and 2.028 (Table 4). Given that *Cochliobolus* is the teleomorphic state of *Curvularia*, these isolates were considered to be correctly IDed by genus. Since there was no reference ID to species, these isolates could not be counted as an accurate species ID nor were they misidentified.

Among this diverse array of rare moulds, 5 (8.8%) were mis-IDed by the Bruker Biotyper: one isolate of *M. circinelloides* was mis-IDed as *M. ramosissiomus* (confidence score, 2.037), one isolate of *Penicillium* subgenus *aspergilloides* was mis-IDed as *P. glabrum* (confidence score, 2.253), one isolate of *Penicillium* subgenus *terverticillata* was mis-IDed as *P. chrysogenum* (confidence score, 2.005) and one isolate of *S. aurantiacum* was mis-IDed as *S. apiospermum* (confidence score, 2.454). In addition, one isolate of *T. veruculosus* was mis-IDed as *T. (Penicillium) funiculosus* but with a confidence score of only 1.761 (Table 4).

The results of this study both confirm and extend the findings of other investigators who have assured the ability of the Bruker Biotyper to ID clinically relevant yeasts and moulds (Alanio et al., 2011; Buchan and Ledeboer, 2013; Cassagne et al., 2011; Cassagne et al., 2013; Chen et al., 2013; De Carolis et al., 2012). The collection of 80 different species of clinically relevant yet rare yeasts and moulds all IDed by reference DNA sequencing methods presented a rigorous challenge to the Bruker MS system. Overall, 89.8% of yeasts and 85.5% of moulds were correctly IDed to the genus level; 71.3% and 73.3%, respectively were correctly IDed to the species level. Whereas, these figures may be lower than those reported by others, this is to expected given the challenging nature of this

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organism collection.

It should be noted that whereas the confidence scores of ≥2.0 used for secure species level ID in the present study are those recommended by the manufacturer, other investigators have suggested that this breakpoint may be overly conservative and that lowering the secure species threshold to 1.8 improved species level ID of yeasts from 87%-92% to >99% of isolates while preserving 100% accuracy of IDs (Buchan and Ledeboer, 2013; Dhiman et al., 2011; Stevenson et al., 2010). In another study VanHerendael et al (2012) found that 100% of isolates with lower confidence scores indicating genus only ID were also correctly IDed to species level. Applying the lower threshold of >1.7 for species level ID to isolates included in the Bruker database in the present study improves the accuracy of species ID from 88.7% to 99.0% for *Candida*, from 65.4% to 91.3% for non-*Candida* yeasts and from 78.6% to 85.4% for moulds. Overall the accuracy of the Bruker Biotype for species level ID of rare yeasts and moulds represented in the database improved from 77.6% to 90.2% when a confidence score of >1.7 was used as the secure species threshold.

In conclusion we have demonstrated a high degree of accuracy for the Bruker Daltronics MS Biotyper in the ID to both genus and species of a challenging set of uncommon yeasts and moulds collected in the context of a global fungal surveillance program. The Bruker system was most accurate for the species level ID of uncommon species of *Candida* and of *Aspergillus* but the accuracy improved for both yeasts and moulds when the lower threshold of >1.7 was used. As seen by other investigators the Bruker system gave a result of no identification to those species not included in the database and generated very few (5 total) mis-IDs. In considering the rare mis-IDs, all were correctly IDed at the genus level. The Bruker Biotyper should prove to be a major advance for clinical microbiology laboratories providing rapid and accurate ID of common and uncommon species of yeasts and moulds in addition to bacteria.

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Table 1. Performance characteristics of the Bruker MS Biotyper in identifying uncommon species of *Candida*

		No. (%) of isolates			
Organism	Total	Identified correctly to genus	Identified correctly to species	Unidentified	Misidentified
C. bracarensis	1			1 (100.0)	
C. catenulato	1	1 (100.0)	1 (100.0)		
C. duobushhaemubii	1	1 (100.0)	1 (100.0)		
C. dubliniensis	23	23 (100.0)	13 (56.5)		
C. fabianii	2			2 (100.0)	
C. fermentati	3	3 (100.0)			
C. fluviatilis	1			1 (100.0)	
C. guilliermondii	13	12 (92.3)	12 (92.3)	1 (7.7)[a]	
C. inconspicua	2	2 (100.0)	2 (100.0)		
C. kefyr	14	14 (100.0)	14 (100.0)		
C. lipolytica	2	2 (100.0)	2 (100.0)		
C. lusitaniae	29	29 (100.0)	29 (100.0)		
C. orthopsilosis	3	3 (100.0)	3 (100.0)		
C. pelliculosa	8	8 (100.0)	8 (100.0)		
C. rugosa	1	1 (100.0)	1 (100.0)		
C. thasaensis	1			1 (100.0)	
C. thermophia	1			1 (100.0)	
Total	106	99 (93.4)	86 (81.1)	7 (6.6)	

a. No peaks found

Table 2. Performance characteristics of the Bruker MS Biotyper in identifying non-*Candida* yeast species

		No. (%) of isolates			
Organism	Total	Identified correctly to genus	Identified correctly to species	Unidentified	Misidentified
Cr. gattii	2	2 (100.0)			
Cr. laurentii	1			1 (100.0)	
Cr. neoformans	62	53 (85.5)	33 (53.2)	9 (14.5)[a]	
Cr. cyanovonans	1			1 (100.0)	
Cryptococcus sp.	1			1 (100.0)	
D. capitatus	3	3 (100.0)	3 (100.0)		
G. clavatum	2			2 (100.0)	
L. elongisporus	1	1 (100.0)	1 (100.0)		
M. pachydermatis	1	1 (100.0)	1 (100.0)		
P. manshurica	1	1 (100.0)	1 (100.0)		
R. minuta	1	1 (100.0)	1 (100.0)		

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R. mucilaginosa	8	8 (100.0)	4 (50.0)		
S. cerevisiae	10	10 (100.0)	9 (90.0)		
Sp. nylandii	1			1 (100.0)	
T. asahi	14	14 (100.0)	14 (100.0)		
T. mycotoxinivorans	1	1 (100.0)	1 (100.0)		
Total	110	95 (86.4)	68 (61.8)	15 (13.6)	

a. No peaks found for one isolate

Table 3. Performance characteristics of the Bruker MS Biotyper in identifying clinically relevant and rare moulds

		No. (%) of isolates			
Organism	Total	Identified correctly to genus	Identified correctly to species	Unidentified	Misidentified
Acremonium sp.	1			1 (100.0)	
Alternaria spp.	3	2 (66.7)	2 (66.7)	1 (33.3)[a]	
Aspergillus spp.	164	157 (95.7)	149 (90.9)	7 (4.3)	1 (0.6)
Asp. candidus	1			1 (100.0)	
Asp. clavatus	1	1 (100.0)			
Asp. flavus SC	18	17 (94.4)	14 (77.8)	1 (5.6)[a]	
Asp. fumigatus	107	107 (100.0)	107 (100.0)		
Asp. niger SC	13	13 (100.0)	12 (92.3)		
Asp. nidulans	6	4 (66.7)	4 (66.7)	2 (33.3)[a]	
Asp. nomius	1	1 (100.0)			
Asp. terreus SC	10	8 (100.0)	8 (80.0)	2 (20.0)	
Asp. tubingusis	1	1 (100.0)			1 (100.0)
Asp. sydowii	5	5 (100.0)	4 (80.0)		
Asp. udagane	1			1 (100.0)	
Arthrographis kalrae	1	1 (100.0)	1 (100.0)		
Cadophora malorum	1			1 (100.0)	
Cochliobolus spp.	3	2 (66.7)		1 (33.3)[a]	
Coprinellus sp.	1			1 (100.0)	
Cunninghamella sp.	1			1 (100.0)	
Curvalana spp.	4	1 (25.0)		3 (75.0)[a]	
Epicoccum nigrum	1			1 (100.0)	
Exophiala sp.	1			1 (100.0)	
Fonsecaea sp.	1			1 (100.0)	
Fusarium spp.	10	6 (60.0)	1 (10.0)	4 (40.0)	
F. fujikuroi SC	4	4 (100.0)	1 (25.0)		
F. incarnatumequiseti SC	1			1 (100.0)	
F. oxysporum SC	1			1 (100.0)	
F. solani SC	3	1 (33.3)		2 (66.7)	
Fusarium sp.	1	1 (100.0)			
Hamigera sp.	1			1 (100.0)	
Leptosphaerulina chartarum	1			1 (100.0)	
Lichtheimia ramosa	1	1 (100.0)			
Microascus sp.	1			1 (100.0)	
Mucor circinelloides	1	1 (100.0)			1 (100.0)

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Ochroconis sp.	1			1 (100.0)	
Penicillium spp.	7	3 (42.9)	1 (14.3)	4 (57.1)	2 (28.6)
P. subgenus *aspergilloides*	1	1 (100.0)			1 (100.0)
P. subgenus *biverticillata*	1			1 (100.0)	
P. subgenuss *terverticillata*	4	1 (25.0)		3 (75.0)[b]	1 (25.0)
P. citrinum	1	1 (100.0)	1 (100.0)		
Phoma sp.	1	1 (100.0)			
Purpureocillium lilacinum	2	2 (100.0)	1 (50.0)		
Rhizopus microsporus	4	4 (100.0)	2 (50.0)		
Rhizopus oryzae SC	1	1 (100.0)	1 (100.0)		
Sarocladium kiliense	1	1 (100.0)			
Scopulariopsis brevecaulis	1			1 (100.0)	
Scedosporium spp.	5	5 (100.0)	4 (80.0)		
S. auantiacum	1	1 (100.0)			1 (100.0)
S. apiospermum	3	3 (100.0)	3 (100.0)		
S. prolificans	1	1 (100.0)	1 (100.0)		
Talaromyces verruculasas	1	1 (100.0)			
Total	221	189 (85.5)	162 (73.3)	32 (14.5)	5 (2.3)

a. No peaks found for one isolate each.

b. No peaks found for three isolates.

Table 4. Discrepant species identification of yeasts and moulds obtained by the Bruker MS Biotyper in comparison with reference molecular methodology.

Reference identification	No. of isolates	Bruker MS identification (confidence score)
C. dubliniensis	10	*C. dubliniensis* (1.996)
		C. dubliniensis (1.975)
		C. dubliniensis (1.975)
		C. dubliniensis (1.958)
		C. dubliniensis (1.940)
		C. dubliniensis (1.845)
		C. dubliniensis (1.834)
		C. dubliniensis (1.827)
		C. dubliniensis (1.815)
		C. dubliniensis (1.803)
C. fermentati[a]	3	*C. guilliermondii* (1.635)
		C. guilliermondii (1.576)
		C. guilliermondii (1.520)
Cr. gattii	2	*Cr. gattii* (1.970)
		Cr. gattii (1.802)
Cr. neoformans	20	*Cr. neoformans* (1.996)
		Cr. neoformans (1.995)
		Cr. neoformans (1.981)
		Cr. neoformans (1.977)
		Cr. neoformans (1.972)
		Cr. neoformans (1.952)
		Cr. neoformans (1.936)

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		Cr. neoformans (1.933)
		Cr. neoformans (1.913)
		Cr. neoformans (1.902)
		Cr. neoformans (1.881)
		Cr. neoformans (1.880)
		Cr. neoformans (1.860)
		Cr. neoformans (1.833)
		Cr. neoformans (1.820)
		Cr. neoformans (1.813)
		Cr. neoformans (1.813)
		Cr. neoformans (1.790)
		Cr. neoformans (1.765)
		Cr. neoformans (1.731)
R. mucilaginosa	4	*R. mucilaginosa* (1.986)
		R. mucilaginosa (1.977)
		R. mucilaginosa (1.958)
		R. mucilaginosa (1.875)
S. cerevisiae	1	*S. cerevisiae* (1.995)
Asp. clavatus	1	*Asp. clavatus* (1.757)
Asp. flavus SC	3	*Asp. flavus* (1.996)
		Asp. flavus (1.943)
		Asp. flavus (1.815)
Asp. niger SC	1	*Asp. niger* (1.903)
Asp. nomius	1	*Asp. nomius* (1.828)
Fusarium fujikuroi SC[d]	3	*F. moniliforme* (verticilloides) (1.994)
		F. proliferatum (1.930)
		F. proliferatum (1.899)
F. solani SC	1	*F. solani* (1.791)
Lichtheimis ramosa	1	*Lichtheimia corymbifera* (1.913)
Mucor circinelloides	1	*Mucor ramosissimus* (2.037)
Penicillium subgenus *aspergilloides*	1	*Penicillium glabrum* (2.253)
Penicillium subgenus *terverticillata*	1	*Penicillium chrysogenum* (2.005)
Purpureocillium lilacinum	1	*Paecilomyces lilacinus (Purpureorillium lilacinum)* (1.954)
Rhizopus microsporus	2	*Rhizopus microsporus* (1.858)
		Rhizopus microsporus (1.792)
Sarocladium kiliense	1	*Acremonium (Sarocladium) strictum* (1.731)
Scedosporium aurantiacum	1	*Scedosporium apiospermum* (2.454)
Talaromyces verruculosus	1	*Penicillium funiculosum* (*Talaromyces funiculosus*) (1.761)

a. C. fermentati is in the C. guilliermondii species complex (SC).

b. Asp. tubingensis is in Aspergillus Section Nigii.

c. The teleamorphic state of Curvularia spp. is Cochliobolus spp.

d. F. moniliforme (syn. verticilloides) and F. proliferatum are species within the F. fujikuroi SC

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