JCM
Journals.ASM.org

Comparison between the Biflex III-Biotyper and the Axima-SARAMIS Systems for Yeast Identification by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry

Caroline Lohmann,[a] Marcela Sabou,[b] Wardi Moussaoui,[c] Gilles Prévost,[c] Jean-Marie Delarbre,[a] Ermanno Candolfi,[b] Alain Gravet,[a] Valérie Letscher-Bru[b]

Laboratoire de Microbiologie, Centre Hospitalier de Mulhouse, Mulhouse Cedex, France[a]; Université de Strasbourg—CHRU Strasbourg, Dynamique des Interactions Hôte-Pathogène (EA-7292), Institut de Parasitologie et de Pathologie Tropicale, Laboratoire de Mycologie Médicale, Strasbourg, France[b]; Université de Strasbourg—CHRU Strasbourg, Physiopathologie et Médecine Translationnelle (EA-4438), Institut de Bactériologie, Strasbourg, France[c]

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is emerging in laboratories as a new diagnostic tool for microorganism identification. We prospectively compared the performances of the Biflex III-Biotyper (Bruker Daltonics) and the Axima (Shimadzu)-SARAMIS (AnagnosTec) systems for the identification of 312 yeasts isolated from clinical specimens (249 *Candida* spp., including 19 *C. albicans* and 230 non-*albicans* species and 63 isolates belonging to different species of the genera *Saccharomyces* [20 isolates], *Rhodotorula* [8 isolates], *Cryptococcus* [8 isolates], *Trichosporon* [7 isolates], *Pichia* [7 isolates], *Geotrichum* [12 isolates], and *Sporopachydermia cereana* [1 isolate]). Species were identified by using routine conventional phenotypical methods and internal transcribed spacer (ITS) sequencing in case of discrepancy. We used expanded thresholds for species identification (log score of ≥1.7 with 3 identical consecutive propositions and no discrepancy between the duplicates for the Bruker Daltonics system and similitude of ≥40% with 5 successive identical propositions and no discrepancy between the duplicates for the Shimadzu system). Of the 312 isolates, 272 (87.2%) and 258 (82.7%) were successfully identified by the Bruker Daltonics and Shimadzu systems, respectively. All isolates were successfully identified within the most frequent and clinically relevant *Candida* species by the two systems. Nonvalid results corresponded mainly to species not or poorly represented in the databases. Major misidentifications were observed for 2 isolates (0.6%) by the Bruker Daltonics system and 4 isolates (1.3%) by the Shimadzu system. In conclusion, the performances of the Bruker Daltonics and the Shimadzu systems for yeast identification were good and comparable under routine clinical conditions, despite their differences in sample preparation, database content, and spectrum analysis.

Over the past few years, the incidence of fungal infections has considerably increased, especially in critically ill and immunocompromised patients. Yeast infections are the most frequent and are a major cause of morbidity and mortality. Between 1979 and 2000, the number of annual cases of fungemia increased by 207% in the United States (1). *Candida* species are the 4th cause of nosocomial bloodstream infection and are associated with a high mortality rate (2). Many studies demonstrated that rapid initiation of appropriate antifungal therapy is crucial to reducing mortality (3, 4). The susceptibility to antifungal molecules varies depending on the genus and the species. If *Candida albicans* remains the most frequent bloodstream fungal pathogen, but non-*albicans* species are increasing and are associated with variable susceptibility profiles to antifungal drugs (5). Recently, new cryptic *Candida* species were described (i.e., *C. metapsilosis* and *C. orthopsilosis* in the *C. parapsilosis* complex or *C. nivariensis* and *C. bracarensis* in the *C. glabrata* complex), species that conventional phenotypic identification methods cannot identify (6, 7). Additionally, invasive infections due to the less frequent and naturally resistant to echinocandins species of the genera *Cryptococcus*, *Trichosporon*, *Malassezia*, and *Rhodotorula* have been observed (8). Except for *C. albicans*, which can be directly identified on chromogenic media, and *Candida glabrata* and *Candida krusei*, which can be identified by rapid enzymatic and latex agglutination methods, conventional phenotypic methods used for routine identification of other species are time-consuming, costly, and lack accuracy for less frequent or cryptic species.

Recently, matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) was applied to microorganism identification and emerged in laboratories as a large-scale retail and new diagnostic tool. Several studies have reported good performances of MALDI-TOF MS for *Candida* identification, allowing accurate, rapid, and cost-effective results (9–12).

Among the systems available, the Shimadzu system (Shimadzu-Biotech Corp., Kyoto, Japan) and the Bruker Daltonics system (Bruker, Bremen, Germany) are the most frequently used. We prospectively compared the performances of the Bruker Daltonics Biflex III spectrometer with its database, Biotyper, to the Shimadzu Axima spectrometer with its database, SARAMIS (AnagnosTec, Potsdam-Golm, Germany), for identification of

Received 12 December 2012 **Returned for modification** 7 January 2013
Accepted 1 February 2013
Published ahead of print 6 February 2013
Address correspondence to Valérie Letscher-Bru, letscher@unistra.fr.
C.L., M.S., A.G., and V.L.-B. contributed equally to this article.

doi:10.1128/JCM.03268-12

yeasts isolated from clinical specimens with a large range of species, including less common non-*albicans Candida* species and non-*Candida* species.

MATERIALS AND METHODS

Yeast isolates. All isolates were prospectively selected from positive cultures obtained from routine clinical specimens (blood samples, urine and stool specimens, mouth washes, respiratory tract samples, vaginal swabs, catheters, and peritoneal and pleural fluids) analyzed in the Mycological Laboratory of the Strasbourg University Hospital, Strasbourg, France, between November 2009 and June 2010. Altogether, this study included 312 isolates corresponding to 249 isolates of *Candida* spp. (19 *C. albicans* and 230 non-*albicans* species) and 63 isolates of non-*Candida* species (20 *Saccharomyces cerevisiae*, 8 *Rhodotorula mucilaginosa*, 8 *Cryptococcus neoformans*, 7 isolates of the *Trichosporon* genus, 7 isolates of the *Pichia* genus, 6 *Geotrichum candidum*, 6 *G. capitatum*, and 1 *Sporopachydermia cereana*). *Geotrichum* isolates were included as they may present yeast-like colonies and are therefore initially identified using laboratory methods applied to yeast identification.

Conventional identification. The isolates, primary isolated on the chromogenic medium chromID *Candida* (bioMérieux), were prospectively identified using phenotypic and, if necessary, molecular methods according to the routine diagnostic flow of the laboratory: *C. albicans* was directly identified by its shiny blue colonies on the chromogenic medium; colonies with presumptive identification of *Candida glabrata* (shiny white colonies) or *Candida krusei* (flat and dried white colonies) were tested using the enzymatic test Glabrata RTT (Fumouze) and the latex test Krusei-color (Fumouze), respectively. The other isolates were identified using the Auxacolor 2 (Bio-Rad) yeast identification system in combination with a microscopic morphological study on potato-carrot-bile medium (Bio-Rad). In case of failure or discrepancy between phenotypic and morphological characters, the isolates were retested using the phenotypic API ID 32C system (bioMérieux). If necessary, identification was confirmed by molecular identification as described below.

Molecular identification. Double-strand sequencing of the ribosomal internal transcribed spacer (ITS) DNA 5.8S region using ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) primers was adapted from reference 13. Sequences were analyzed with BLAST software against the GenBank EMBL, DDBJ, and PDB databases, and the results were confirmed against the CBS database.

Subculture before MALDI-TOF analysis. All of the isolates were subcultured on Sabouraud chloramphenicol dextrose agar (Oxoid) and/or on chromID *Candida* medium at 35°C, except for *Rhodotorula* and *Cryptococcus* isolates, which were incubated at 27°C. After 48 h of incubation, all isolates were simultaneously identified using the two spectrometers. In case of nonidentification, a second analysis was done after a new extraction, in order to proceed under conditions comparable to those in routine diagnostic flow.

MALDI-TOF Biflex III (Bruker Daltonics). According to the manufacturer's recommendations, one or two colonies were suspended in 1 ml of 70% ethanol (Carbo Erba reagents) in an Eppendorf microtube, briefly vortexed, and centrifuged for 2 min at 13,000 × *g*. The supernatant was removed, a second centrifugation of 30 s at 13,000 × *g* was performed, and the rest of supernatant was removed using a pipette. The pellet was resuspended in 50 μl of formic acid (70% [vol/vol]) (AnalaR Normapur), and 50 μl of acetonitrile (Sigma-Aldrich) was added. After 15 min of incubation at room temperature, the mixture was centrifuged for 2 min at 13,000 × *g* and 1 μl of the supernatant was applied in duplicate onto a steel target plate and allowed to dry at room temperature. Afterwards, 1 μl of matrix solution was applied to the spot and allowed to dry at room temperature. The matrix solution was freshly prepared with α-cyano-4-hydroxyl-cinnaminic (CHCA) powder in 500 μl of acetonitrile (Sigma-Aldrich) and 500 μl of 5% trifluoroacetic acid solution. Each specimen was analyzed in duplicate.

Measurements were performed with a MALDI-TOF Biflex III system (Faculty of Medicine, University of Strasbourg, France). Protein mass fingerprints were recorded in linear positive mode at a laser frequency of 20 Hz within a mass range from 2 to 20 kDa. The ionization source was fitted out with a delayed extraction time of 400 ns. Each spectrum was obtained from 240 shots in 40-shot steps from different positions on the target plate. The system was calibrated before each analysis using a strain of *Escherichia coli* DH5. Each spectrum was transferred to the Biotyper software and compared to the Maldi Biotyper DB database version 2.0.4.0, analyzed by the software-defined algorithm, resulting in a logarithm score from 0 to 3. According to the manufacturer's recommendations, species identification is valid for a score superior to 2.0, while a score of 1.7 to 2.0 identifies the genus. We used a lower threshold and considered species identification for scores from 1.7 with 3 identical successive propositions of identification and no discrepancy between two replicates. These expanded thresholds were previously validated in preliminary experiments conducted on 65 isolates belonging to 9 different common species that provided 92% of correct species identifications using the manufacturer's threshold recommendations and 100% of correct species identifications without any misidentification using the expanded threshold.

MALDI-TOF Axima (Shimadzu). One colony was suspended in 20 μl of 25% formic acid (Sigma-Aldrich). After 2 to 3 min at room temperature, the mixture was vortexed and 1 μl was applied in duplicate onto a steel target plate and allowed to dry at room temperature. Then, 1 μl of CHCA matrix solution (AnagnosTec) was added to the spot and allowed to dry at room temperature.

Measurements were performed using a MALDI-TOF Axima system (Microbiology Laboratory of the Mulhouse Hospital, France). The laser frequency was 50 Hz, the acceleration voltage was 20 kV, and the extraction delay time was 200 ns. The spectra were recorded in the linear positive mode within a mass range of 3 to 20 kDa. Each plate was calibrated and validated with a control strain of *E. coli* CGU 10979, tested at the beginning and at the end of the use of each plate. All mass fingerprints were analyzed by the SARAMIS software, which first compares them to the superspectra and in a second step to the individual spectra of the database AnagnosTec version 4.07. The results are expressed in percentages of similitude. Superspectra contain peaks common to different strains of the same species. Individual spectra correspond to spectra of each strain cultured in specific conditions. The manufacturer recommends validation of only the superspectra identifications with percentages comprising between 75 and 99.9%. We expanded the species identification to individual spectra with percentages of similitude above 40%, 5 successive identical propositions, and no discrepancy between the duplicates. These expanded thresholds were previously validated in preliminary experiments conducted on 65 isolates belonging to 9 different common species that provided 88% of correct species identifications using the manufacturer's threshold recommendations and 100% of correct species identifications without any misidentification using the expanded threshold.

Analysis. Definite species identification was considered when routine identification methods and the two MALDI-TOF systems gave the same result. In the case of discrepant results, ITS sequencing was performed and this result was considered the reference identification.

RESULTS

Conventional methods. Based on the criteria defined above, first-line routine methods gave a definite species identification for 289 of the 312 isolates (92.6%). Twenty-three (7.4%) were misidentified, mainly concerning species closely related, and then were re-identified by ITS sequencing (Table 1).

MALDI-TOF Biflex III-BioTyper (Bruker Daltonics). Of the 312 isolates included, 272 (87.2%) were successfully identified (Table 2). All isolates were successfully identified within the clinically most common *Candida* species (*C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, *C. kefyr*, *C. guilliermondii*, and

TABLE 1 Misidentifications by the conventional phenotypic methods

Molecular identification	No. of isolates	Conventional phenotypic identification		
		Misidentification	Method[a]	Cause of misidentification[b]
Candida pararugosa	7	*Candida rugose*	PCB/Auxa/ID32C	ND + no phenotypic differences
Pichia carpophila	2	*Candida guilliermondii*	PCB/Auxa/ID32C	ND + no phenotypic differences
Pichia caribbica	1	*Candida guilliermondii*	PCB/Auxa/ID32C	ND + no phenotypic differences
Candida orthopsilosis	2	*Candida parapsilosis*	PCB/Auxa	ND + no phenotypic differences
C. dubliniensis	1	*Candida albicans*	chromID *Candida* media	Similar to *C. albicans* on chromID *Candida* media
Pichia guilliermondii	1	*Candida famata*	PCB/Auxa/ID32C	Error
Candida sphaerica	1	*Candida lusitaniae*	PCB/Auxa	Error
Candida utilis	1	*Candida pelliculosa*	PCB/Auxa/ID32C	Error
Pichia burtonii	1	*Candida sake*	PCB/Auxa/ID32C	ND
Candida aaseri	1	*Candida sake*	PCB/Auxa/ID32C	ND
Pichia fabianii	1	*Candida utilis*	PCB/Auxa/ID32C	ND
Trichosporon asahii	1	*Trichosporon inkin*	PCB/Auxa	Error
Trichosporon inkin	1	*Trichosporon asahii*	PCB/Auxa	Error
T. cutaneum	1	*Trichosporon mucoides*	PCB/Auxa	ND
Sporopachydermia cereana	1	*Candida boidinii*	PCB/Auxa/ID32C	ND

[a] PCB, microscopic morphology on potato-carrot-bile medium; Auxa, Auxacolor 2 system; ID32C, API ID32C system.
[b] ND, species not present in the phenotypic database. "No phenotypic differences" indicates there are no phenotypic differences between the species according to macroscopic, microscopic, and phenotypic characteristics. "Error" indicates misidentification despite the presence of the species in the phenotypic database.

C. lusitaniae) and *S. cerevisiae*. MALDI-TOF enabled identification of some very closely related species, indistinguishable by conventional phenotypic methods, including, *C. pararugosa* (7 of the 8 isolates) and *C. orthopsilosis* (2 isolates), misidentified by conventional phenotypic methods as *C. rugosa* and *C. parapsilosis*, respectively. All 7 isolates of *C. dubliniensis* were successfully identified. Eight isolates were identified as *P. cactophila*, which is considered conspecific with *C. inconspicua* (14); such identification results were also observed in other studies (15, 16). These 8 isolates were reidentified by ITS sequencing and were confirmed as *C. inconspicua* species, so we considered them valid identifications.

Among the remaining 40 isolates detailed in Table 3, the correct identification was proposed by the system for 18 isolates, but with an insufficient score, <1.7. All of these isolates were species poorly represented in the database (less than 5 spectra per species). Twenty isolates were not identified because the species were either absent from the database (18 isolates) or poorly represented (2 isolates). Finally, 2 (0.6%) *Trichosporon* isolates were really misidentified: one *T. asahii* isolate and one *T. inkin* isolate, both identified as *T. cutaneum*.

MALDI-TOF Axima-SARAMIS (Shimadzu-AnagnosTec). Of the 312 isolates analyzed, 258 (82.7%) were successfully identified (Table 2). Again, all isolates of *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, *C. kefyr*, *C. guilliermondii*, *C. lusitaniae*, *C. dubliniensis*, and *S. cerevisiae* were successfully identified. Fifty-five isolates (17.3%) did not have a valid result (Table 4). Among them, the correct identification but with insufficient score (percentage of <40%) was proposed by the system for 9 isolates, despite a significant representation in the AnagnosTec database. Forty-one isolates were not identified, 37 belonging to species absent from (28 isolates, including *C. inconspicua* and *C. pararugosa*) or poorly represented in (less than 2 superspectra and less than 10 spectra per species) (9 isolates) the database. Four isolates were not identified despite a correct representation in the database. Finally, 4 isolates were misidentified, 3 of them corresponding to species not present in the database—*C. orthopsilosis* (2 isolates identified as *C. parapsilosis*) and *P. carpophila* (1 isolate identified as *C. guilliermondii*).

DISCUSSION

The aim of this study was to evaluate and compare the performances of the Biflex III-Biotyper (Bruker Daltonics) system to the Axima-SARAMIS (Shimadzu-AnagnosTec) system for MALDI-TOF prospective identification of a large panel of yeasts isolates from clinical specimens, including frequent and rare species and *Candida* and non-*Candida* species. The percentages of correctly identified isolates were comparable between the two MALDI TOF systems (Bruker Daltonics system, 87.2%; Shimadzu system, 82.7%). The majority of the isolates with no identification or with a correct identification associated with an insufficient score corresponded to species either absent from or poorly represented in the databases at the time of the study. Updated versions of the databases now available have been completed for some but not all of the nonidentified isolates of our study.

The numbers of major errors (misidentifications) were low for the two MALDI-TOF systems (0.6% for the Axima-SARAMIS and 1.3% for the Biflex III-Biotyper) compared to conventional methods (7.4%). With the Axima-SARAMIS system, 3 of the 4 errors concerned closely related species not or poorly represented in the database (2 *C. orthopsilosis* isolates and 1 *P. carpophila* isolate identified as *C. parapsilosis* and *C. guilliermondii*, respectively). Santos et al. have obtained good results for the *C. parapsilosis* complex by adding spectra of *C. orthopsilosis* and *C. metapsilosis* to the AnagnosTec database (11). *P. carpophila* is a recently described species very close to *C. guilliermondii* and *C. caribbica* (17).

The identification performance was lower for *Basidiomycetes* of the genera *Cryptococcus* and *Trichosporon* and for *Geotrichum capitatum*, perhaps related to the relatively low representation of these genera and species in the two databases or due to the particular composition of their cell wall, very rich in external sugars, making protein extraction more difficult. These dif-

TABLE 2 Distribution of MALDI-TOF results for species identifications obtained by the Biflex III-Biotyper and Axima-SARAMIS systems

Species	No. of isolates	No. of isolates with result by: Biflex III-Biotyper: Valid ID[a]	Biflex III-Biotyper: Correct species ID score of <1.7	Biflex III-Biotyper: No ID	Biflex III-Biotyper: False species ID	Axima-SARAMIS: Valid ID	Axima-SARAMIS: Correct species ID score of <40%	Axima-SARAMIS: No ID	Axima-SARAMIS: False species ID
Candida									
C. aaseri	1	0	0	1	0	1	0	0	0
C. albicans	19	19	0	0	0	19	0	0	0
C. blankii	1	0	0	1	0	0	0	1	0
C. boidinii	1	1	0	0	0	1	0	0	0
C. catenulata	2	0	2	0	0	2	0	0	0
C. ciferrii	1	0	1	0	0	1	0	0	0
C. colliculosa	1	0	1	0	0	0	0	1	0
C. dubliniensis	7	7	0	0	0	7	0	0	0
C. famata	2	0	1	1	0	1	0	1	0
C. glabrata	26	26	0	0	0	26	0	0	0
C. guilliermondii	16	16	0	0	0	16	0	0	0
C. haemulonii	1	1	0	0	0	0	0	1	0
C. holmii	1	0	0	1	0	0	0	1	0
C. inconspicua	8	8	0	0	0	0	0	8	0
C. intermedia	2	1	1	0	0	0	0	2	0
C. kefyr	23	23	0	0	0	23	0	0	0
C. krusei	22	22	0	0	0	22	0	0	0
C. lipolytica	9	9	0	0	0	9	0	0	0
C. lusitaniae	23	23	0	0	0	23	0	0	0
C. norvegensis	9	9	0	0	0	9	0	0	0
C. orthopsilosis	2	2	0	0	0	0	0	0	2
C. parapsilosis	28	28	0	0	0	28	0	0	0
C. pararugosa	8	7	1	0	0	0	0	8	0
C. pelliculosa	1	1	0	0	0	0	1	0	0
C. pulcherrima	2	2	0	0	0	1	1	0	0
C. sphaerica	3	0	0	3	0	0	0	3	0
C. tropicalis	24	24	0	0	0	24	0	0	0
C. utilis	6	6	0	0	0	6	0	0	0
Cryptococcus neoformans	8	5	3	0	0	3	5	0	0
Pichia									
P. burtonii	1	0	0	1	0	0	0	1	0
P. caribbica	1	0	0	1	0	0	0	1	0
P. carpophila	2	0	0	2	0	0	0	1	1
P. fabianii	1	0	0	1	0	0	0	1	0
P. farinosa	1	0	1	0	0	1	0	0	0
P. ohmeri	1	1	0	0	0	0	0	1	0
Rhodotorula mucilaginosa	8	7	1	0	0	8	0	0	0
Saccharomyces cerevisiae	20	20	0	0	0	20	0	0	0
Sporopachydermia cereana	1	0	0	1	0	0	0	1	0
Trichosporon									
T. asahii	2	1	0	0	1	0	0	2	0
T. cutaneum	1	0	0	1	0	0	0	1	0
T. inkin	1	0	0	0	1	0	0	0	1
T. mucoides	3	3	0	0	0	3	0	0	0
Geotrichum									
G. candidum	6	0	6	0	0	4	2	0	0
G. capitatum	6	0	0	6	0	0	0	6	0
Total no. (%)	312 (100)	272 (87.2)	18 (5.8)	20 (6.4)	2 (0.6)	258 (82.7)	9 (2.9)	41 (13.1)	4 (1.3)

[a] ID, identification.

TABLE 3 Distribution of the discrepancies observed in the Biflex III-Biotyper system

Nature of discrepancy (no. of isolates)	Representation of species in database (no. of isolates)	Isolates (no.)
False species (2)	Well represented (2)	*T. inkin* (1),[a] *T. asahii* (1)[a]
Correct species with insufficient score (18)	Weakly represented (18)	*G. candidum* (6), *C. neoformans* (3), *C. catenulata* (2), *C. ciferii* (1), *C. colliculosa* (1), *C. famata* (1), *C. intermedia* (1), *C. pararugosa* (1), *P. farinosa* (1), *R. mucilaginosa* (1)
No identification (20)	Not represented (18)	*G. capitatum* (6), *C. sphaerica* (3), *P. carpophila* (2), *P. caribbica* (1), *C. aaseri* (1), *C. blankii* (1), *C. holmii* (1), *P. burtonii* (1), *P. fabianii* (1), *S. cereana* (1)
	Weakly represented (2)	*C. famata* (1), *T. cutaneum* (1)

[a] Identified as *T. cutaneum*.

ficulties in identification were reported by other studies using the manufacturers' standard databases (9, 10, 18, 19). Good performances were obtained for *Cryptococcus* by enriching the databases (20, 21). Of the 6 *Trichosporon* isolates tested, 3 isolates of *T. mucoides* were correctly identified by both systems, but *T. asahii*, *T. inkin*, and *T. cutaneum* posed problems of nonidentification or misidentification as other species of the genus *Trichosporon*. All of the species tested were present in both databases, but only *T. mucoides* and *T. asahii* were well represented. For the latter, the identification problems could be related to the drier texture of the colonies, which makes it difficult to achieve homogeneous spots for analysis.

In the literature, a few studies evaluated the use of the MALDI-TOF method for yeast identification, reporting performances from 87 to 97.5% by using the manufacturers' extraction and validation recommendations on the Bruker Daltonics system (9, 10, 12, 15, 19, 22) and from 94.5 to 96.1% with the Shimadzu system (15, 23), depending on the panel of yeasts tested (especially for the presence of *Cryptococcus*, *Geotrichum*, and *Trichosporon* in the panel), the version of the database, and on the analysis's inclusion or noninclusion of the nonrepresented species in the database. In the Bruker Daltonics systems, these percentages increased to 96.3 to 98.4% without more misidentifications when a validation score of 1.8 or 1.7 was used (12, 16, 19, 22).

Bader et al. compared the two systems on 1,192 isolates representing 32 species and also described comparable performances between the two systems, with 97.6 and 96.1% of correct identifications on the Bruker Daltonics system and the Shimadzu system, respectively (15). These performances are higher than those in our study, probably due to the yeast panel included, since 93% of the isolates were common species (*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, *C. kefyr*, *C. lusitaniae*, and *S. cerevisiae*) for which the identification performances are very good. In our study, these species represented only 59% of the isolates tested and were all correctly identified by the two systems.

In conclusion, the performances of the Bruker Daltonics and the Shimadzu MALDI-TOF systems for yeast identification were good when performed under routine clinical conditions, despite their differences in sample preparation, database content, and spectrum analysis. Compared to biochemical techniques, mass spectrometry is easier, faster, cheaper, and more accurate, allowing identification of closely related species indistinguishable by conventional phenotypic methods. In the future, enrichment of databases should improve identification of rare species and *Basidiomycetes*. In parallel, works are in progress for the preanalytical extraction step, and improved and shorter protocols have been recently proposed as alternatives to the standard procedures recommended by the manufacturers (16, 24, 25).

TABLE 4 Distribution of the discrepancies observed in the Axima-SARAMIS system

Nature of discrepancy (no. of isolates)	Representation of species in database (no. of isolates)	Isolates (no.)
False species (4)	Not represented (3)	*C. orthopsilosis* (2),[a] *P. carpophila* (1),[b]
	Well represented (1)	*T. inkin* (1)[c]
Correct species with insufficient score (9)	Well represented (9)	*C. neoformans* (5), *C. pulcherrima* (1), *C. pelliculosa* (1), *G. candidum* (2)
No identification (41)	Not represented (28)	*C. inconspicua* (8), *C. pararugosa* (8), *C. sphaerica* (3), *C. blankii* (1), *C. colliculosa* (1), *C. holmii* (1), *P. burtonii* (1), *P. caribbica* (1), *P. carpophila* (1), *P. fabianii* (1), *P. ohmeri* (1), *Sporopachydermia cereana* (1)
	Weakly represented (9)	*G. capitatum* (6), *C. intermedia* (2), *C. haemulonii* (1)
	Well represented (4)	*T. asahii* (2), *T. cutaneum* (1), *C. famata* (1)

[a] Identified as *C. parapsilosis*.
[b] Identified as *C. guilliermondii*.
[c] Identified as *T. asahii*.

REFERENCES

1. **Martin GS, Mannino DM, Eaton S, Moss M.** 2003. The epidemiology of sepsis in the United States from 1979 through 2000. N. Engl. J. Med. **348**:1546–1554.
2. **Pfaller MA, Diekema DJ.** 2010. Epidemiology of invasive mycoses in North America. Crit. Rev. Microbiol. **36**:1–53.
3. **Morrell M, Fraser VJ, Kollef MH.** 2005. Delaying the empiric treatment of Candida bloodstream infection until positive blood culture results are obtained: a potential risk factor for hospital mortality. Antimicrob. Agents Chemother. **49**:3640–3645.
4. **Taur Y, Cohen N, Dubnow S, Paskovaty A, Seo SK.** 2010. Effect of antifungal therapy timing on mortality in cancer patients with candidemia. Antimicrob. Agents Chemother. **54**:184–190.
5. **Pfaller MA, Moet GJ, Messer SA, Jones RN, Castanheira M.** 2011. Candida bloodstream infections: comparison of species distributions and antifungal resistance patterns in community-onset and nosocomial isolates in the SENTRY Antimicrobial Surveillance Program, 2008–2009. Antimicrob. Agents Chemother. **55**:561–566.
6. **Cuenca-Estrella M, Gomez-Lopez A, Isla G, Rodriguez D, Almirante B, Pahissa A, Rodriguez-Tudela JL.** 2011. Prevalence of Candida bracarensis and Candida nivariensis in a Spanish collection of yeasts: comparison of results from a reference centre and from a population-based surveillance study of candidemia. Med. Mycol. **49**:525–529.
7. **Garcia-Effron G, Canton E, Peman J, Dilger A, Roma E, Perlin DS.** 2012. Epidemiology and echinocandin susceptibility of Candida parapsilosis sensu lato species isolated from bloodstream infections at a Spanish university hospital. J. Antimicrob. Chemother. **67**:2739–2748.
8. **Miceli MH, Diaz JA, Lee SA.** 2011. Emerging opportunistic yeast infections. Lancet Infect. Dis. **11**:142–151.
9. **Marklein G, Josten M, Klanke U, Muller E, Horre R, Maier T, Wenzel T, Kostrzewa M, Bierbaum G, Hoerauf A, Sahl HG.** 2009. Matrix-assisted laser desorption ionization-time of flight mass spectrometry for fast and reliable identification of clinical yeast isolates. J. Clin. Microbiol. **47**:2912–2917.
10. **Stevenson LG, Drake SK, Shea YR, Zelazny AM, Murray PR.** 2010. Evaluation of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) for the identification of clinically important yeast species. J. Clin. Microbiol. **48**:3482–3486.
11. **Santos C, Lima N, Sampaio P, Pais C.** 2011. Matrix-assisted laser desorption/ionization time-of-flight intact cell mass spectrometry to detect emerging pathogenic Candida species. Diagn. Microbiol. Infect. Dis. **71**: 304–308.
12. **Dhiman N, Hall L, Wohlfiel SL, Buckwalter SP, Wengenack NL.** 2011. Performance and cost analysis of matrix-assisted laser desorption ionization-time of flight mass spectrometry for routine identification of yeast. J. Clin. Microbiol. **49**:1614–1616.
13. **White TJ, Bruns T, Lee S, Taylor J.** 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, p 315–322. *In* Innis MA, Gelfland DH, Sninsky TJ (ed), PCR protocols. Academic Press, San Diego, CA.
14. **Kurtzman CP, Robnett CJ, Basehoar-Powers E.** 2008. Phylogenetic relationships among species of Pichia, Issatchenkia and Williopsis determined from multigene sequence analysis, and the proposal of Barnettozyma gen. nov., Lindnera gen. nov. and Wickerhamomyces gen. nov. FEMS Yeast Res. **8**:939–954.
15. **Bader O, Weig M, Taverne-Ghadwal L, Lugert R, Gross U, Kuhns M.** 2011. Improved clinical laboratory identification of human pathogenic yeasts by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin. Microbiol. Infect. **17**:1359–1365.
16. **Van Herendael BH, Bruynseels P, Bensaid M, Boekhout T, De Baere T, Surmont I, Mertens AH.** 2012. Validation of a modified algorithm for the identification of yeast isolates using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS). Eur. J. Clin. Microbiol. Infect. Dis. **31**:841–848.
17. **Vaughan-Martini A, Kurtzman CP, Meyer SA, O'Neill EB.** 2005. Two new species in the Pichia guilliermondii clade: Pichia caribbica sp. nov., the ascosporic state of Candida fermentati, and Candida carpophila comb. nov. FEMS Yeast Res. **5**:463–469.
18. **Pinto A, Halliday C, Zahra M, van Hal S, Olma T, Maszewska K, Iredell JR, Meyer W, Chen SC.** 2011. Matrix-assisted laser desorption ionization-time of flight mass spectrometry identification of yeasts is contingent on robust reference spectra. PLoS One **6**:e25712. doi:10.1371/journal.pone.0025712.
19. **Sendid B, Ducoroy P, Francois N, Lucchi G, Spinali S, Vagner O, Damiens S, Bonnin A, Poulain D, Dalle F.** 2013. Evaluation of MALDI-TOF mass spectrometry for the identification of medically-important yeasts in the clinical laboratories of Dijon and Lille hospitals. Med. Mycol. **51**:25–32.
20. **Firacative C, Trilles L, Meyer W.** 2012. MALDI-TOF MS enables the rapid identification of the major molecular types within the Cryptococcus neoformans/C. gattii species complex. PLoS One **7**:e37566. doi:10.1371/journal.pone.0037566.
21. **McTaggart LR, Lei E, Richardson SE, Hoang L, Fothergill A, Zhang SX.** 2011. Rapid identification of Cryptococcus neoformans and Cryptococcus gattii by matrix-assisted laser desorption ionization–time of flight mass spectrometry. J. Clin. Microbiol. **49**:3050–3053.
22. **van Veen SQ, Claas EC, Kuijper EJ.** 2010. High-throughput identification of bacteria and yeast by matrix-assisted laser desorption ionization-time of flight mass spectrometry in conventional medical microbiology laboratories. J. Clin. Microbiol. **48**:900–907.
23. **Martinez-Lamas L, Perez del Molino ML, Pardo F, Varela E, Regueiro BJ.** 2011. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry vs conventional methods in the identification of Candida non-albicans. Enferm. Infecc. Microbiol. Clin. **29**: 568–572. (In Spanish.)
24. **Rosenvinge FS, Dzajic E, Knudsen E, Malig S, Andersen LB, Lovig A, Arendrup MC, Jensen TG, Gahrn-Hansen B, Kemp M.** 26 August 2012. Performance of matrix-assisted laser desorption-time of flight mass spectrometry for identification of clinical yeast isolates. Mycoses [Epub ahead of print.] doi:10.1111/myc.12000.
25. **Goyer M, Lucchi G, Ducoroy P, Vagner O, Bonnin A, Dalle F.** 2012. Optimization of the preanalytical steps of matrix-assisted laser desorption ionization-time of flight mass spectrometry identification provides a flexible and efficient tool for identification of clinical yeast isolates in medical laboratories. J. Clin. Microbiol. **50**:3066–3068.