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RESEARCH ARTICLE

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Evaluation of a new matrix-assisted laser desorption/ionization time-of-flight mass spectrometry system for the identification of yeast isolation

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Background: Currently, three commercial in vitro diagnostic matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) systems are widely used in clinical laboratories. The ASTA MicroIDSys system (ASTA Inc, South Korea) is a newly developed MALDI-TOF MS system used for the identification of pathogenic microorganisms. In the present study, we assessed the performance of the ASTA MALDI-TOF MS system for the identification of pathogenic yeast from clinical samples.

Methods: We tested 284 clinical yeast isolates from various clinical specimens using ASTA MALDI-TOF MS, and the results were compared with those using molecular sequencing of the ITS or D1-D2 regions of rDNA and biochemical assays.

Results: A total of 284 isolates were tested and found to be distributed across 14 species including *Candida albicans* (n = 100) and other yeast species (n = 184). ASTA MALDI-TOF MS correctly identified 95.1% (270/284) of the yeast species compared to molecular sequencing. Among them, 262 isolates showed acceptable MALDI-TOF MS scores (≥140), and 98.1% (257/262) isolates were identified correctly. In addition, among 22 isolates with a MALDI-TOF MS score <140, 59.1% (13/22) of the isolates showed concordance with molecular typing at the species level. Clustering analysis revealed the effectiveness of the new MALDI-TOF MS system for the identification of yeast species.

Conclusions: ASTA MALDI-TOF MS showed high accuracy in the identification of yeast species; it involves facile sample preparation and extraction procedures. ASTA MALDI-TOF MS is expected to be useful for yeast identification in clinical microbiology laboratories due to its reliability and cost-effectiveness.

KEYWORDS
identification, MALDI-TOF MS, sequencing, yeast

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1 | INTRODUCTION

With the increasing number of immunosuppressed patients and significant emergence of opportunistic pathogens,[1-3] invasive fungal infections caused by *Candida* species and other yeasts have continuously increased over the last decade.[4-6] The treatment guideline for candida infection including candidemia, candiduria, vulvovaginal candidiasis, and invasive infections have been revised in 2016.[7] Because antifungal drug susceptibility or resistance varies among species, accurate identification and rapid identification play an important role in selecting antifungal treatment options.[8] Because of the limited treatment choices and high rate of therapeutic failures, novel strategies are needed to improve patient outcomes.[9,10] Conventional identification of yeast is based on morphology and biochemical tests using its ability to metabolize different sugars and related compounds. Such methods take approximately 24-72 hours and often fail to accurately identify emerging or cryptic species.[11] Molecular identification by sequencing of genomic regions is considered a standard method for identifying yeast to the species level.[12] However, molecular identification remains expensive, time-consuming, and relatively labor-intensive to perform in clinical microbiology laboratories.[13,14] Due to the above limitations, the introduction of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been currently in the spotlight and has greatly improved the routine identification of yeast as well as bacteria at species level in the last few years.[4,8,11,13-21] There are three commercially available MALDI-TOF MS systems, the Microflex Biotyper (Bruker Daltonics, Bremen, Germany), the VITEK MS (bioMérieux, Marcy l'Etoile, France), and the Andromas (Andromas, Paris, France).[16,17,22-24] Recently, a new MALDI-TOF MS system (ASTA Inc, Suwon, Korea) has been developed for the identification of clinically important pathogens including yeast isolates. The new MALDI-TOF MS system involves a simplified extraction method allowing rapid identification of yeast isolates, and its database (ver 1.2) contains 407 species (894 strains) of yeast.

In the present study, we assessed the performance of the ASTA MALDI-TOF MS system in the identification of yeast species from a collection of 284 isolates, in comparison with the performance of molecular identification and biochemical assays.

2 | MATERIALS AND METHODS

2.1 | Clinical samples

A total of 284 yeast isolates in the form of various clinical specimens were obtained from the microbiology laboratory of International St. Mary's hospital in Korea between 2016 and 2017. These specimens consisted of sputum (93, 32.7%), urine (93, 32.7%), vaginal swabs (27, 9.5%), blood (18, 6.3%), bronchial washing fluid (14, 4.9%), ear (12, 4.2%), catheter tips (9, 3.2%), body fluid (7, 2.5%), wounds (7, 2.5%), stool (3, 1.1%), and tissue (1, 0.04%). All specimens were from immunocompromised patients diagnosed as malignancy (n = 83), chronic respiratory disease (n = 60), neurologic disease (n = 38), diabetes mellitus (n = 30), infectious diseases (n = 21), chronic kidney disease (n = 9), organ transplantation (n = 5), liver disease (n = 5), autoimmune disease (n = 4), HIV (n = 1), and those who underwent major surgery within the prior 30 days (n = 28) with symptom and clinical suspicion of yeast infection. We obtained only one isolate per patient. These specimens were inoculated onto media. The media used in this study included sheep blood agar (BAP; Synergy Innovation, Seongnam, Korea), chocolate agar (CHOCOLATE; Synergy Innovation), and Sabouraud dextrose agar (SDA; Synergy Innovation). Yeast-like colonies in the primary isolation media or SDA were subcultured in SDA for 24 hours at 35°C. All strains were identified using conventional phenotypic methods including the API 20C system (bioMérieux), the API ID 32C system (bioMérieux), or the VITEK 2 YST Card system (bioMérieux), in addition to morphologic examination on corn meal agar with Tween 80 and chromogenic agar culture. Then, the strains were frozen at −70°C in glycerol broth (20%) before the present study.

The study was approved by the institutional review board of International St. Mary's Hospital, the Catholic Kwandong University College of Medicine in Korea (IRB035, IS16EISI0043).

2.2 | MALDI-TOF analysis

Before being processed for MS identification, each isolate was thawed and subcultured in SDA for 24 hours at 35°C. A single colony from a pure agar culture was directly smeared onto the target plate (ASTA Inc) with wooden stick. After the colony spot was completely dry, 1.5 μL of 70% formic acid was added to it and dried. Then, 1.5 μL of a α-cyano-4-hydroxycinnamic acid (CHCA) matrix solution was overlaid on the spot on the MALDI plate and dried again. MALDI-TOF analysis for peptide profiling-based identification was performed using MALDI-TOF MS (ASTA Inc) in a positive linear mode with a mass range of 2-20 kDa. The MALDI-TOF MS database (ver. 1.2) contains reference MALDI spectra for 407 species (894 strains) of yeast. The acceptable cutoff score for identification was set at ≥140 for all microorganisms. If the test provided a score <140 or invalid results, we immediately repeated the test with other colonies from the same agar plate using the on-plate method. The AMS (ASTA MicroID Standard; ASTA) was used for instrument calibration, and *Candida krusei* ATCC 6258, *Candida parapsilosis* ATCC 22019, and *Candida albicans* ATCC 64548 were used as control strains.

2.3 | Sequencing

All isolates were further identified by sequencing the internal transcribed spacer (ITS) using ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) primers[25] and/or the D1/D2 domains of 28S ribosomal DNA using LR0R (5′-GTA CCC GCT GAA CTT AAG C-3′)[26] and LR5 (5′-ATC CTG AGG GAA ACT TC-3′) primers.[27] The sequences obtained were compared with GenBank data using the BLAST alignment software (blast.ncbi.nlm.nih.gov) and the CBS yeast database (www.cbs.

knaw.nl). A threshold of ≥99% homology was used for identification to the species level.

2.4 | Interpretation of the results

Molecular sequencing was considered the gold standard method in our evaluation. The final identification using ASTA MALDI-TOF MS was done as follows. Identification to the species level was done when the results had good confidence levels (score ≥140) or duplicate results showed the same species-level identification although the score was <140. When one of the results was invalid, or duplicate results showed discrepancies, we considered the identification as a failure.

2.5 | Statistical analysis

Chi-square test or Fisher's exact test was used for analyzing differences in the identification rate. Receiver operating characteristic (ROC) curve analysis was used to verify the efficiency of MALDI-TOF MS All P values were two-sided, and the statistical significance threshold was set at $P < 0.05$. Statistical analysis was performed with MedCalc version 15.5 (MedCalc, Mariakerke, Belgium).

3 | RESULTS

The total of 284 isolates was identified as belonging to 14 different species comprising *C. albicans* (n = 100) and other yeast species (*Candida tropicalis* [n = 64], *Candida glabrata* [n = 58], *C. parapsilosis* [n = 21], *Cyberlindnera fabianii* [n = 11], *C. krusei* [n = 8], *Saccharomyces cerevisiae* [n = 6], *Candida guilliermondii* [n = 3], *Candida Pelliculosa* [n = 3], *Candida lusitaniae* [n = 3], *Trichosporon asahii* [n = 2], *Candida auris* [n = 2], *Candida dubliniensis* [n = 2], and *Candida orthopsilosis* [n = 1]).

Compared to molecular sequencing, ASTA MALDI-TOF MS showed concordant identification in 270 (95.1%) cases. For yeast species that were frequently found in clinical specimens, the concordance rates were as follows: *C. albicans* (100%, 100/100), *C. tropicalis* (98.4%, 63/64), *C. glabrata* (87.9%, 51/58), and *C. parapsilosis* (95.2%, 20/21).

Among the 262 isolates with an acceptable MALDI-TOF MS score of ≥140, 257 (98.1%) were identified in concordance with molecular sequencing and five *C. glabrata* isolates showed disconcordant results (Table 1). Further, among 22 isolates with a MALDI-TOF MS score <140, 13 isolates (*C. glabrata* [n = 5], *C. albicans* [n = 2], *Cy. fabianii* [n = 2], *C. lusitaniae* [n = 2], *C. Tropicalis* [n = 1], and *C. dubliniensis* [n = 1]) showed concordance with molecular typing at the species level. ROC analysis revealed that using a cutoff score of 128, ASTA MALDI-TOF MS could perform correct identification with a sensitivity of 98.2% and a specificity of 64.3% (area under the curve, 0.848; $P < 0.001$).

A total of 14 isolates were recorded as identification failures using ASTA MALDI-TOF MS (Table 2). Five *C. glabrata* isolates were identified as *C. albicans*, and one *C. orthopsilosis* isolate was identified as *C. parapsilosis*. The other cases resulted in invalid identification including two *C. auris* isolates misidentified as other yeast species called *Pichia alni*.

To assess the effectiveness of ASTA MALDI-TOF MS-based identification, we performed clustering analysis with correctly identified isolates (Figure 1). Clustering analysis based on mass bin profiles of the isolates indicated species-specific mass profiles. The pattern and intensity of each mass bin were mostly similar within the same species, while most of them were different between the

TABLE 1 Summary of identification results of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

		Score ≥140			Score <140		
Molecular identification	No. of isolates	Concordant	Invalid/ disconcordant	Subtotal	Concordant	Invalid/ disconcordant	Subtotal
Candida albicans	100	98	0	98	2	0	2
Candida tropicalis	64	62	0	62	1	1	2
Candida glabrata	58	46	5	51	5	2	7
Candida parapsilosis	21	20	0	20	0	1	1
Cyberlindnera fabianii	11	8	0	8	2	1	3
Candida krusei	8	8	0	8	0	0	0
Saccharomyces cerevisiae	6	6	0	6	0	0	0
Candida guilliermondii	3	3	0	3	0	0	0
Candida pelliculosa	3	2	0	2	0	1	1
Candida lusitaniae	3	1	0	1	2	0	2
Candida dubliniensis	2	1	0	1	1	0	1
Candida auris	2	0	0	0	0	2	2
Trichosporon asahii	2	2	0	2	0	0	0
Candida orthopsilosis	1	0	0	0	0	1	1
Total (%)	284	257 (90.5)	5 (1.8)	262 (92.3)	13 (4.6)	9 (3.2)	22 (7.7)

TABLE 2 List of isolates whose identification showed discrepancies or errors by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (N = 14)

Molecular identification	MALDI-TOF MS				Biochemical assay
	Spot 1		Spot 2		
	Identification	Score	Identification	Score	
Candida glabrata	*Candida albicans*	184	Not tested		*Candida albicans*
Candida glabrata	*Candida albicans*	158	Not tested		*Candida albicans*
Candida glabrata	*Candida albicans*	207	Not tested		*Candida albicans*
Candida glabrata	*Candida albicans*	154	Not tested		*Candida albicans*
Candida glabrata	*Candida albicans*	193	Not tested		*Candida albicans*
Candida glabrata	*Nocardioides daejeonensis*	119	*Cryptococcus neoformans*	128	*Candida glabrata*
Candida glabrata	*Sphingomonas yunnanensis*	117	*Sphingomonas yunnanensis*	117	*Candida glabrata*
Candida orthopsilosis	*Candida parapsilosis*	124	*Candida parapsilosis*	111	*Candida parapsilosis*
Candida auris	Invalid	89	Invalid	95	*Rhodotorula glutinis*
Candida auris	*Pichia alni*	121	*Pichia alni*	117	*Rhodotorula glutinis*
Candida parapsilosis	*Staphylococcus simulans*	114	*Dichelobacter nodosus*	120	*Candida parapsilosis*
Candida pelliculosa	Invalid	101	Invalid	109	*Candida pelliculosa*
Candida tropicalis	Invalid	101	*Solirubrobacter ginsenosidimutans*	123	*Candida tropicalis*
Cyberlindnera fabianii	Invalid	102	*Clostridium sporogenes*	118	*Candida pelliculosa*

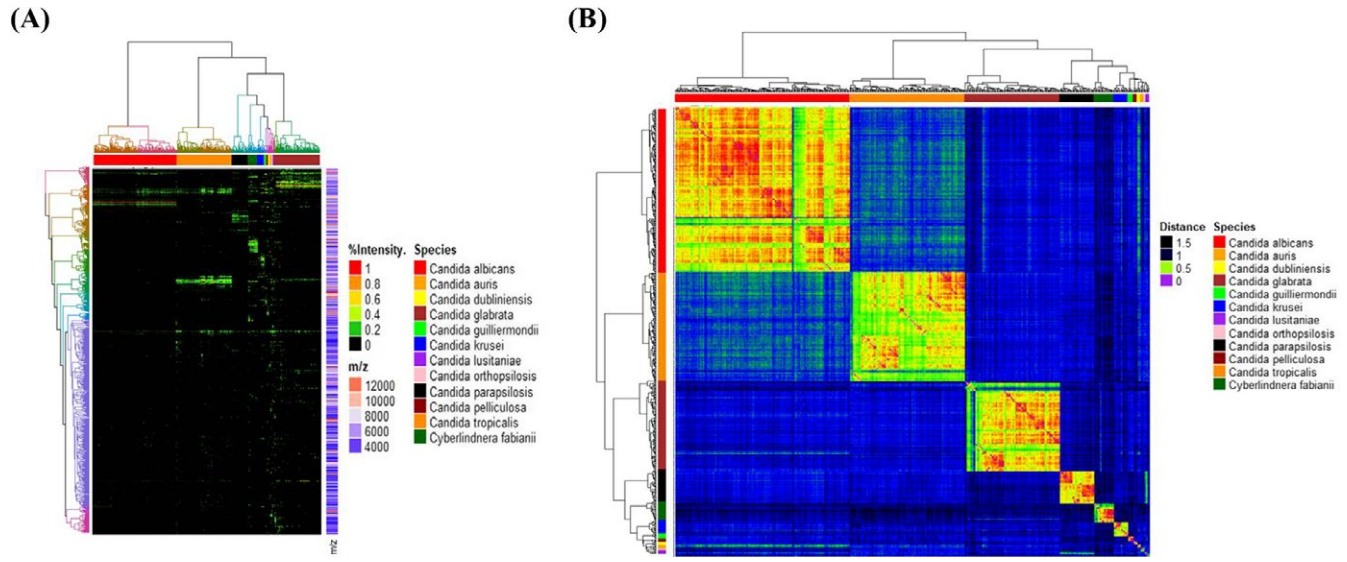

FIGURE 1 Assessment of the effectiveness of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)-based identification of yeast species using composite correlation index (CCI) generated using MALDI-TOF MS A, Clustering analysis was performed based on mass bin profiles. B, A heat map was generated using the Spearman correlation method, and hierarchical clustering was performed by Ward.d2, using the software R version 3.4.3 (R Foundation for Statistical Computing, Vienna, Austria)

species. To visualize the distances between intra- and interspecies isolates, heat map analysis was performed. Intraspecies isolates showed strong correlations. In interspecies comparison, *C. orthopsilosis* and *C. parapsilosis* exhibited the strongest correlation with each other. In addition, *C. albicans* showed a weak similarity with *C. tropicalis* and *C. dubliniensis*. Taken together, our results show that mass bin profiling based on MALDI-TOF MS enabled high resolution in the identification of yeasts.

4 | DISCUSSION

In the present study, we tested the diagnostic performance of a newly developed commercially available ASTA MALDI-TOF MS system for the identification of yeast species, in comparison with that of molecular sequencing. In previous reports comparing MALDI-TOF MS systems with molecular sequencing, the sequencing with only using yeast isolates showed the results inconsistent with those of

other MALDI-TOF MS systems or biochemical tests.[4,14,18-21,24,28-32] Such a strategy could result in reducing the rate of misidentification of cryptic *Candida* species or the rate of disconcordance. Therefore, we designed our study to directly compare MALDI-TOF MS data of all isolates with those of ITS and/or D1/D2 region sequencing.

The overall species-level identification rate of MALDI-TOF MS relative to that of molecular sequencing was 95.1% in the present study. Previously, the accuracy of the VITEK MS and Bruker Biotyper MS systems in yeast identification based on ITS sequencing was found to range from 76.5% to 99.3% and 89.8% to 99.8%, respectively.[4,14,18-21,24,28-32] For the *C. albicans* complex (*C. albicans*, *C africana*, and *C. dubliniensis*), ASTA MALDI-TOF MS showed 100% accuracy in identification of *C. albicans* and *C. dubliniensis* isolates in the present study, comparable to a previous report using Bruker Biotyper showing an identification accuracy of 98.5% for the *C. albicans* complex.[33]

Using the cutoff score per the manufacturer's recommendation, identities of 90.5% (257/284) of the isolates were concordant with those obtained via molecular sequencing. Moreover, out of the 22 isolates with scores lower than the cutoff (<140), 13 (59.1%) were identified in agreement with molecular sequencing. So far, only one study has compared the identification performance of the ASTA MALDI-TOF MS with that of Bruker Biotyper using *Candida* species. According to Lee et al, 98.3% concordant identification was observed for five common *Candida* species (*C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *and C. tropicalis*). However, only 39.5% of isolates with concordant identification met the cutoff score in both systems; scores ≥2.0 using Bruker Biotyper and scores ≥140 using ASTA MALDI-TOF MS. On the other hand, 58.6% of isolates with concordant identification showed scores between 1.7 and 2.0 using Bruker Biotyper and ≥140 using ASTA MALDI-TOF MS.[24] These findings suggested that the performance of ASTA MALDI-TOF MS in distinguishing *Candida* species was better than that of Bruker Biotyper. In ROC analysis, we found that a cutoff score of 128 could increase the sensitivity of ASTA MALDI-TOF MS to 98.2%. This indicates the possibility of applying a lower cutoff score in clinical laboratories.

On the other hand, 14 isolates showed disconcordant/invalid results compared with molecular sequencing, of which five isolates of *C. glabrata* with a score ≥140 were misidentified as *C. albicans*. In addition, some species appear to be poorly represented in databases. Among nine isolates showing disconcordant/invalid results with a score <140, the newly emerging, multidrug-resistant *C. auris* was incorrectly represented as *Pichia alni* or showed invalid results. Therefore, the databases need to be updated with information regarding *C. auris*, because it has recently received attention around the world due to challenges involving antifungal resistance and identification associated with it.[34] ASTA MALDI-TOF MS also failed to identify one *C. orthopsilosis* isolate, reporting it as *C. parapsilosis*. However, such results are also often seen with the VITEK MS system which has shown problems in distinguishing the *C. parapsilosis* complex.[4,21] These two species are thought to be challenging to distinguish using MALDI-TOF MS systems because these systems are based on ribosomal protein spectra.[35] For species with extreme similarity in ribosomal protein sequences, protein mass profiles are also too similar to be effectively distinguished using MALDI-TOF MS systems. In such cases, additional molecular data are eventually required for correct identification.

The specimens we used in this study were those obtained from patients with clinical suspicion of yeast infection. Especially, urine sample was from patients with fever and symptom of urinary tract infection or vulvovaginal candidiasis.[7,36] The presence of candiduria in the asymptomatic patient usually presents colonization, but our patients had symptom and almost all of them underwent urinary tract instrumentation.[7] However, it was hard to distinguish respiratory specimens from colonization or pathogens as *Candida* pneumonia is very rare.[7]

Using clustering analysis with heat map representation, we demonstrated that the new ASTA MALDI-TOF MS system is an effective method for the identification of yeast species. The MALDI-TOF MS mass profiles exhibited species-specific patterns with signature mass peaks and intensities. Because most of the peaks represent ribosomal proteins or housekeeping proteins, clustering analysis of the MALDI-TOF MS profile might be informative in distinguishing yeast species. Commercial MALDI-TOF MS systems have operational differences in terms of database systems and matching processes. For example, the VITEK MS requires input data regarding whether bacterial or yeast isolates are being analyzed. The Bruker Biotyper system and the new ASTA MALDI-TOF MS system automatically query spectra without the need for prior input regarding sample information.[20] Therefore, the latter two systems have the probability of misidentification of a yeast isolate as a bacterium, especially if the spectrum of a yeast sample is poorly obtained or is not registered in the database. Unlike bacteria, yeast has a thick and chitinous cell wall, which might interfere with direct smear analysis using MALDI-TOF MS. Therefore, formic acid/acetonitrile extraction is recommended before analysis.[8] For repeated tests, the Biotyper system recommends in-tube extraction instead of on-plate extraction, while the MALDI-TOF MS and VITEK MS systems recommend only the on-plate method. However, the strategy of yeast identification using ASTA MALDI-TOF MS is simplified based on the on-plate testing method using 70% formic acid. The procedure (70% formic acid and CHCA matrix solution addition followed by smearing with the organism) is a standard procedure in the preparation of all microorganisms. In fact, the identification process involves matching of the acquired spectrum with the previously generated reference database, and sample preparation also requires the same procedure as that used to generate the reference spectrum to ensure accurate identification. Because its turnaround time for sample preparation and spectra acquisition is <10 minutes, the on-plate extraction method using formic acid would be a simpler and time-saving method in clinical microbiology laboratories to minimize additional prescreening of microorganisms.

In conclusion, ASTA MALDI-TOF MS has proven to be a rapid, highly accurate, and cost-effective method for the identification of most yeast species isolated from clinical specimens. The

new MALDI-TOF MS is a reliable and convenient system for yeast identification and is expected to be useful in clinical microbiology laboratories.

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