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Original Article

Evaluation of Bruker Biotyper and Vitek MS for the identification of *Candida tropicalis* on different solid culture media

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Culture media

Abstract *Background:* The aim of this study was to investigate the performance of the Bruker Biotyper matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and Vitek MS systems for identification of genetically-confirmed blood isolates of *Candida tropicalis* that had been grown on several types of culture media commonly used for primary fungal isolation.
Methods: Isolates included 105 from the National China Hospital Invasive Fungal Surveillance Net program (CHIF-NET) and 120 from National Taiwan University Hospital (NTUH). Culture media tested for CHIF-NET isolates included trypticase soy agar supplemented with 5% sheep blood (BAP), Sabouraud dextrose agar (SDA-C), CHROMagar, China blue agar (CBA), chocolate agar supplemented with vancomycin (CAP-VA), and MacConkey agar (MAC). Culture media used for NTUH isolates included BAP, SDA, CHROMagar, eosin methylene blue (EMB), inhibitory mold agar (IMA), Mycosel agar, and cornmeal agar (CMA).
Results: The Bruker Biotyper correctly identified all CHIF-NET isolates to the species level on all six agar media tested and correctly identified the majority of NTUH isolates with the

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exception of isolates grown on SDA (85.8%) and CMA (52.5%). The Vitek MS system correctly identified all CHIF-NET isolates to the species level with the exception of isolates grown on CHROMagar (84.8%), and correctly identified the majority of NTUH isolates with the exception of isolates grown on SDA (51.7%), Mycosel agar (57.5%), and CMA (9.2%) for NTUH isolates.
Conclusion: Clinical microbiologists should be aware that different culture media can affect the performance of the Bruker Biotyper MALDI-TOF MS and Vitek MS systems in identifying *C. tropicalis*.

Introduction

Candidemia is associated with high rates of mortality and morbidity among immunocompromised and critically ill patients.[1–5] Although the most important cause of candidemia is *Candida albicans*, the incidence of candidemia due to *Candida* species other than *C. albicans* is increasing.[2,3,5–10] Of those species, *C. tropicalis* has become an important cause of bloodstream infections (BSIs) in seriously ill patients, those with hematological malignancy and HIV-infection, and recipients of organ transplantation.[6,9,11,12] In Mainland China, the most prevalent *Candida* species associated with nosocomial bloodstream infection are *C. albicans* (38.9%), followed by *C. parapsilosis* (23.2%) and *C. tropicalis* (20.5%).[9] A multicenter, prospective, observational study of adult patients in 67 ICUs across China revealed that *C. tropicalis* (17.6%) was the second most common cause of BSI due to Candida species [7]. In Taiwan, *C. tropicalis* was the most common *Candida* species (46%) associated with BSI among 111 patients with hematological malignancies, followed by *C. albicans* (32%), *C. parapsilosis* (12%), and *C. glabrata* (7%).[6] Importantly, 7–40% of clinical isolates of *C. tropicalis* have recently been reported to be resistant to azoles, particularly fluconazole.[6,9,11,12] In the Asia–Pacific region, the highest rates of resistance to fluconazole and voriconazole among *C. tropicalis* isolates were found in China, with 8.2% of isolates demonstrating resistance to fluconazole and 5.7% being resistant to voriconazole.[12,13] The percentages of *C. tropicalis* isolates with the non-wide type phenotype for susceptibility to posaconazole have been reported to be 31.7% in Australia and 62.2% in Taiwan.[13] Consequently, timely and accurate identification of *C. tropicalis* is crucial, particularly for immunocompromised patients with BSI.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is an accurate, rapid, and inexpensive method for identifying clinically relevant *Candida* species.[14–25] However, most studies on the accuracy of MALDI-TOF MS have been conducted on colonies grown on trypticase soy agar supplemented with 5% sheep blood (blood agar plate, BAP), Sabouraud's dextrose agar (SDA), or CHROMagar *Candida* medium (CHROMagar).[14,16–26] In our previous study, we found that the Bruker Biotyper MALDI-TOF MS (Bruker Daltonics GmbH, Germany) and Vitek MS (bioMérieux, France) systems were able to correctly identify more than 90% of *C. tropicalis* isolates grown on BAP and SDA.[17]

The purpose of this study was to investigate the performance of the Bruker Biotyper and Vitek MALDI-TOF MS systems for identification of *C. tropicalis* isolates grown on several types of agar media that are commonly used in clinical mycology laboratories for primary isolation of fungi (yeasts and molds).

Methods

C. tropicalis isolates

A total of 225 non-duplicate isolates of *C. tropicalis* were evaluated in this study. Among these isolates, 120 were recovered from patients with candidemia who were treated at National Taiwan University Hospital (NTUH) from 2013 to 2014. The rest of the isolates (n = 105) were obtained from patients with BSI at 48 hospitals situated in 24 provinces across China and were tested at the Peking Union Medical College Hospital (PUMCH) as part of the National China Hospital Invasive Fungal Surveillance Net (CHIF-NET) program in 2010–2014.[24]

Agar media

For MALDI-TOF MS analysis, colonies were grown on different types of culture agar media commonly used for primary isolation of fungi at the PUMCH and NTUH. The six culture media commonly used for fungal isolation at the PUMCH for CHIF-NET isolates included CHROMagar *Candida* (Becton Dickinson Microbiology Systems, Sparks, MD, USA), BAP, SDA supplemented with chloramphenicol (SDA-C), China blue agar (CBA), chocolate agar supplemented with vancomycin (CAP-VA), and MacConkey agar (MAC). All media used at the PUMCH were obtained from Thermo Fisher Biochemicals, Beijing, China. The seven different culture media used for NTUH isolates included BAP, SDA, CHROMagar *Candida*, Eosin methylene blue (EMB), inhibitory mold agar (IMA), Mycosel agar and cornmeal agar (CMA). All media used at the NTUH were purchased from Becton Dickinson Microbiology Systems. For NTUH isolates, isolates on all six types of media were incubated at 35 °C with 5% CO_2 and colonies were collected for MALDI-TOF MS analysis after incubation for 18–24 h. For CHIF-NET isolates, isolates on all types of media with the exception of SDA-C-28 were incubated at 35 °C with 5% CO_2 and colonies

were chosen after 24—48 h of incubation. Isolates grown on SDA-C-28 were incubated at 28 °C.

Sequencing analysis

All isolates were identified by DNA sequencing of the fungal ITS region. Primers ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) were used to amplify the ITS region.[24] Amplification of the ITS region was carried out under the following conditions: denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 90 s, and elongation at 72 °C for 75 s, with a final extension step of 10 min at 72 °C. The determined sequences were compared to reference data available in GenBank, which was searched using the nucleotide BLAST tool (blast.ncbi.nlm.nih.gov). Results were considered acceptable if homology with other entries in the databases was >99.5% [24]. All *C. tropicalis* isolates obtained from the NTUH and the CHIF-NET program were confirmed as *C. tropicalis* with an identification probability of >99.5% by sequencing of the fungal ITS region.

Vitek MS analysis

For the Vitek MS system, proteins were extracted as recommended by the manufacturer. Briefly, a small portion of a single colony was directly spotted onto a target plate and covered with 0.5 μl formic acid (bioMérieux). All mass profiles were analyzed using the Vitek MS database (MS-ID version v.2.0). A single identification is displayed (green), with a confidence value (% probability) from 60.0 to 99.9 (good confidence level), when one significant yeast or yeast group is retained. ''Low-discrimination'' identifications are displayed (red) when two to four significant yeasts or yeast groups are retained. Isolates that were reported as no identification by the Vitek MS system indicated no peaks or bad spectra were found or no specific single species was demonstrated. When more than four yeasts or yeast groups are found, or when no match is found, the yeast is considered unidentified (orange). Freshly prepared *Escherichia coli* ATCC 8739, *Enterococcus faecalis* ATCC 19433, and *C. glabrata* ATCC MYA2950 were used as control strains. An isolate was considered correctly identified with an acceptable confidence value of 99.9% [17, 24]. If isolates exhibited discrepant identification results or produced low matches by Vitek MS and ITS sequencing analysis, identification by Vitek MS for the isolates was repeated.

Bruker Biotyper MALDI-TOF MS analysis

For the Bruker Biotyper MALDI-TOF MS system, pure fungal isolates (each from a single colony) were directly smeared onto the target plate (Bruker Daltonics, GmbH) and overlaid with 1 μl of 70% formic acid (Sigma Aldrich). The Bruker Biotyper MS system database DB 5627, v.3.1 was used. Identification scores of ≥2.000 indicated species-level identification, scores of 1.700—1.999 indicated genus-level identification, and scores of <1.700 indicated no identification. A modified species-level identification cutoff value of ≥1.700 was also applied for the Bruker Biotyper MALDI-TOF MS system.[24,27] If isolates exhibited discrepant identification results or produced low matches by Bruker Biotyper MALDI-TOF MS analysis and ITS sequencing analysis, identification by Bruker Biotyper MALDI-TOF MS analysis for the isolates was repeated.

Statistical analysis

The chi-square test was used to evaluate the performance of Bruker Biotyper MALDI-TOF MS and Vitek MS systems for the identification of *C. tropicalis* isolates obtained from PUMCH and NTUH. Identification results by the two MALDI-TOF MS systems on *C. tropicalis* colonies that grown on the same culture media from the same manufacturer (only CHROMagar *Candida*) were evaluated. *P* values <0.05 are considered statistically significant.

Results

Identification of CHIF-NET isolates by two MALDI-TOF MS systems

Fig. 1 shows the performance of both MALDI-TOF MS systems for identifying the 105 CHIF-NET isolates of *C. tropicalis* after incubation for 24 h (Fig. 1A and C) or 48 h (Fig. 1B and D) on different culture media. Among isolates that were repeatedly tested by either MALDI-TOF MS systems due to discrepant identification results between MALDI-TOF MS systems and sequencing analysis results, the original and repeated identification results were similar.

The rates of species-level identification (score values of ≥2.000) for CHIF-NET isolates by the Bruker Biotyper MALDI-TOF MS system after incubation for 24 h were 87.6% on SDA-C-28, followed by 84.8% on CAP-VA, 83.8% on SDA-C-35, 81.9% on BAP, 78.1% on CHROMagar *Candida*, 76.2% on MAC media, and 42.9% on CBA (Fig. 1A). However, at a species-level identification cutoff value of ≥1.700, the accurate identification rates after incubation for 24 h were 100% on BAP, DSA-C-35, SDA-C-28, CHROMagar *Candida*, and MAC, 98.1% on CAP-VA and 93.4% on CBA. All isolates were identified as *C. tropicalis* by the Bruker Biotyper MALDI-TOF MS system regardless of the identification score.

The rates of species-level identification (confidence values of 99.9%) by the Vitek MS system for CHIF-NET isolates after incubation for 24 h were highest (100%) on BAP, followed by 99.0% on MAC, 98.0% on both CBA and CAP-VA, and lowest (84.8%) on CHROMagar *Candida* (Fig. 1C). The rates of correct identification for CHIF-NET isolates after incubation for 24 and 48 h were similar between the two MALDI-TOF MS systems. All isolates with identification confidence values of 65%—99.9% were identified as *C. tropicalis*.

Identification of NTUH isolates by two MALDI-TOF MS systems

Fig. 2 demonstrates the performance of the Bruker Biotyper MALDI-TOF MS (Fig. 2A) and Vitek MS systems (Fig. 2B) for identification of 120 NTUH blood isolates of *C. tropicalis* that were grown on different culture media. Among

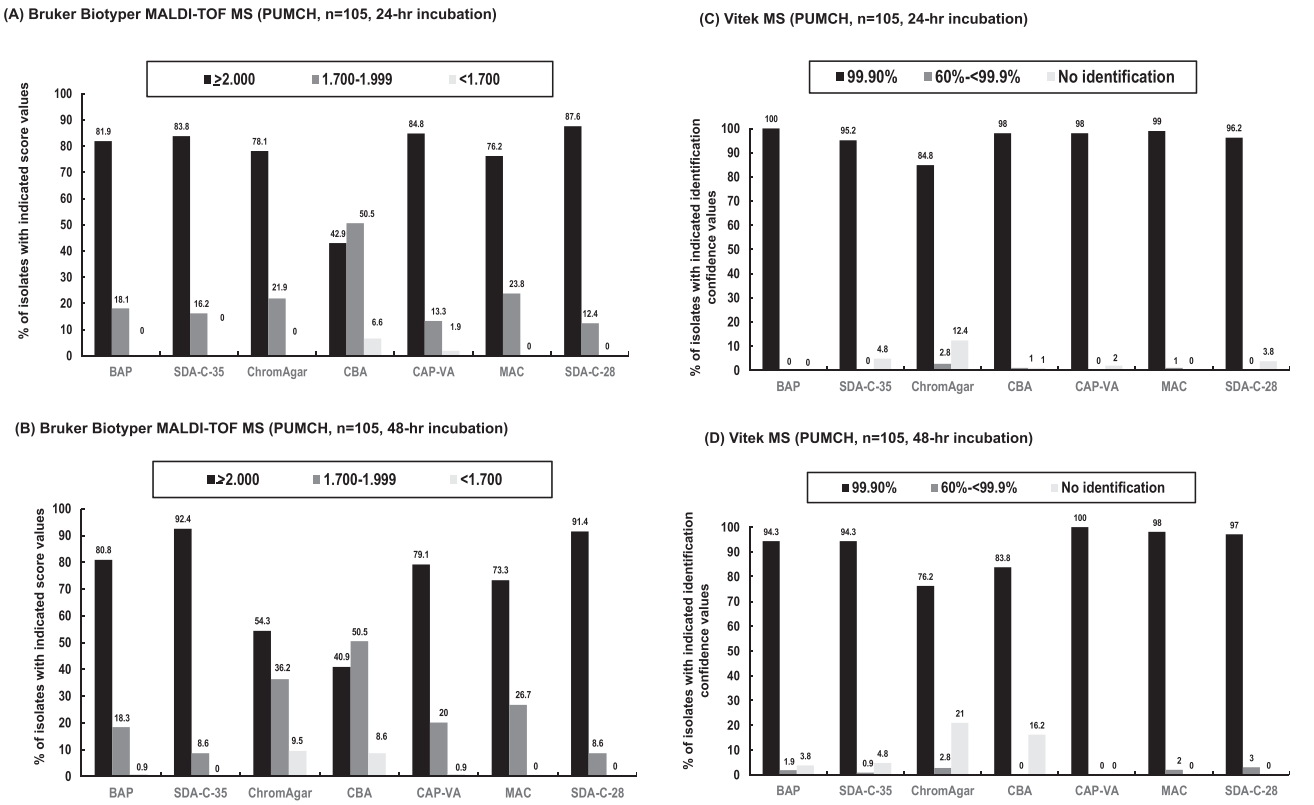

Figure 1. Performance of the Bruker Biotyper MALDI-TOF MS (A and B) and Vitek MS (C and D) systems for identification of 105 clinical isolates of *C. tropicalis* that were grown for 24 h (A and C) and 48 h (B and D) on different culture media. All *C. tropicalis* isolates were recovered from patients with invasive candidiasis or were obtained from the yeast collection as part of the National China Hospital Invasive Fungal Surveillance Net (CHIF-NET) program in China from 2013 to 2014 and were identified to the species level at the Peking Union Medical College Hospital (PUMCH). The currently available databases of the two systems (the Vitek MS system; version v.2.0 knowledge base clinical use and Bruker Biotyper MALDI-TOF MSMS system; DB 5627, v.3.1) were applied. All isolates were identified as *C. tropicalis* by the Bruker Biotyper MALDI-TOF MS regardless of the identification score. All isolates with identification confidence values of 65%—99.9% were identified as *C. tropicalis*. Isolates that were reported as no identification by the Vitek MS system indicated no peaks or bad spectra were found or no specific single species was demonstrated. BAP = trypticase soy agar supplemented with 5% sheep blood; SDA-C-35 = Sabouraud dextrose agar with chloramphenicol at 35 °C incubation; CHROMagar = CHROMagar *Candida* media; SDA-35 = Sabouraud dextrose agar at 35 °C incubation; CB = China Blue agar; CAP-VA = Chocolate agar plate with vancomycin; MAC = MacConkey agar; SDA-C-28 = Sabouraud dextrose agar with chloramphenicol at 28 °C incubation.

isolates that were repeatedly tested by either MALDI-TOF MS systems due to discrepant identification results between MALDI-TOF MS systems and sequencing analysis results, the original and repeated identification results were the same.

The rates of species-level identification (score values of ≥2.000) by Bruker Biotyper MALDI-TOF MS system after incubation for 24 h were 97.5% on BAP, 60.8% on SDA, 69.2% on CHROMagar *Candida*, 48.3% on EMB, 83.3% on IM agar, 73.3% on Mycosel agar, and 2.5% on CMA. However, at a species-level identification cutoff value of ≥1.700, the species-level identification rates were 100% on BAP, 85.8% on SDA, 99.2% on CHROMagar *Candida*, 95.8% on EMB, 99.1% on IM agar, 92.5% on Mycosel agar, and 52.5% on CMA (Fig. 1A). Several isolates with score values of <1.700 were identified as *C. tropicalis* on EMB (n = 6), IM agar (n = 1), Mycosel agar (n = 2), SDA (n = 3), and CMA (n = 9).

The rates of correct identification (confidence values of 99.9%) of NTUH isolates by the Vitek MS system were >90% on BAP (99.2%), CHROMagar *Candida* (95.0%), EMB (96.7%), and IM agar (92.5%). However, the rates of correct identification were <60% on SDA (51.7%), Mycosel agar (57.5%), and CMA (9.2%). Although the identification confidence values were 99.9%, the Vitek MS system misidentified two isolates as *Malassezia pachydermatis* and one isolate as *Candida dubliniensis* on SDA, one isolate as *C. albicans* on CHROMagar, one isolate as *C. dubliniensis* (n = 1) on IM agar, and one isolate as *C. albicans* (n = 1) on Mycosel agar.

When comparing the results on the identification of *C. tropicalis* that grew after incubation for 24 h on the same agar media from same manufacturer (i.e. CHROMagar *Candida*), significant difference ($P < 0.05$) was noted for isolates collected from CHIF-NET collection (84.8%) and NTUH (95.0%) by the Vitek MS system with confidence values of 99.9%. For Bruker Biotyper MALDI-TOF MS system, there was no significant difference on the identification performance (score values of ≥1.700) between isolates from CHIF-NET collection (100%) and NTUH (99.2%).

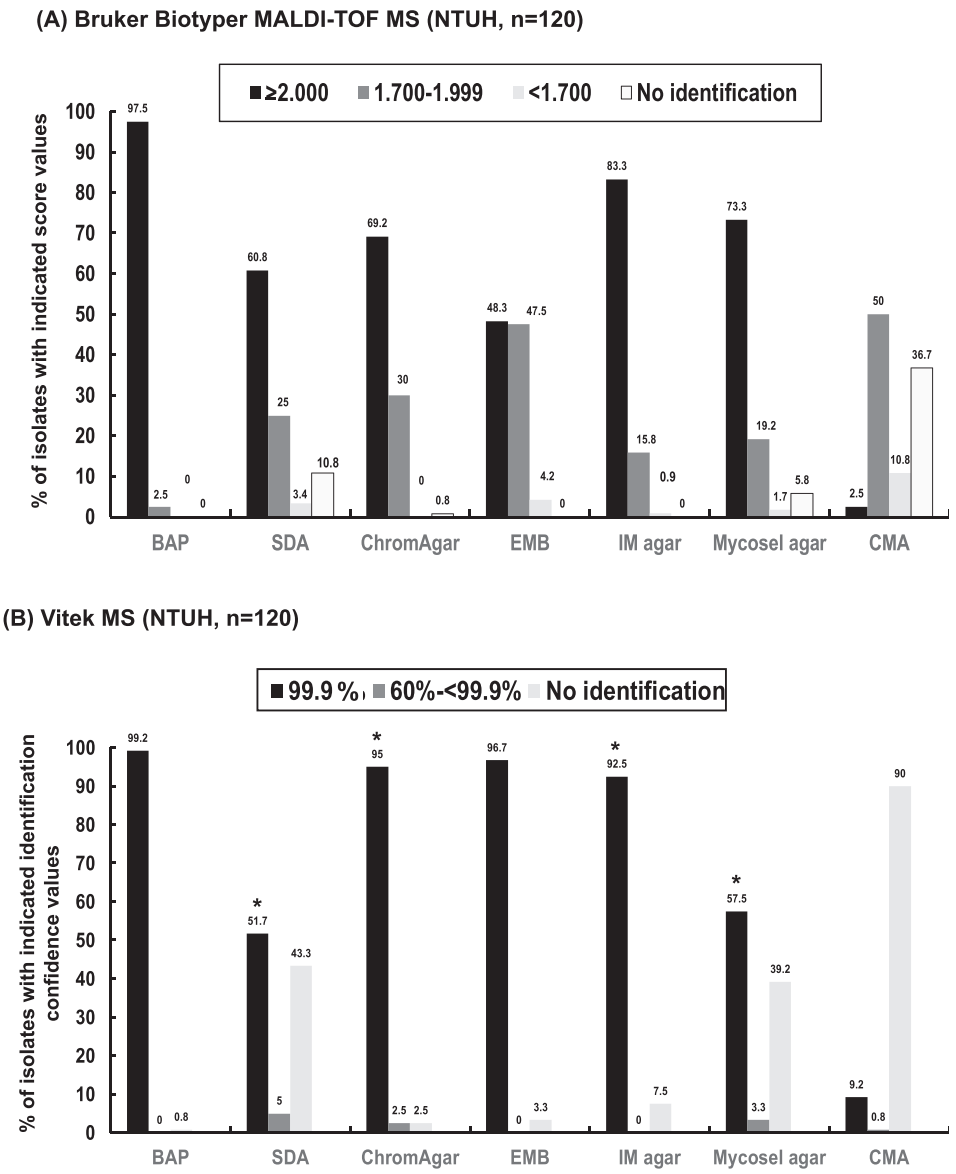

Figure 2. Performance of the Bruker Biotyper MALDI-TOF MS (A) and Vitek MS (B) systems for the identification of 120 blood isolates of *C. tropicalis* that were grown on different culture media. All *C. tropicalis* isolates were recovered from patients with candidemia who were treated at National Taiwan University Hospital (NTUH) from 2013 to 2014. The direct on-plate smear method was used to prepare all specimens for analysis by MALDI-TOF MS. The currently available databases of the two systems (the Vitek MS system; version v.2.0 knowledge base clinical use and Bruker Biotyper MS system; DB 5627, v.3.1) were applied. Isolates that were reported as no identification by the Vitek MS system indicated no peaks or bad spectra were found or no specific single species was demonstrated. * indicates that although the identification confidence values were 99.9%, the Vitek MS system misidentified two isolates as *Malassezia pachydermatis* and one isolate as *Candida dubliniensis* on SDA, one isolate as *C. albicans* on CHROMagar *Candida* media, one isolate as *C. dubliniensis* on IM agar, and one isolate as *C. albicans* on Mycosel agar. BAP = trypticase soy agar supplemented with 5% sheep blood; SDA = Sabouraud dextrose agar; CHROMagar = CHROMagar *Candida* media; EMB = Eosin methylene blue; IM agar = inhibitory mold agar; CMA = cornmeal agar.

Discussion

The rapid and accurate identification of yeasts is essential for the optimization of antifungal therapy.[1,4] This is especially important for yeast pathogens isolated from blood and other sterile body fluids in immunocompromised or critically-ill patients and for fungal isolates with potential resistance to commonly used antifungal agents.[1,4,28] The direct smear method with on-plate formic acid extraction has been demonstrated to be useful for yeast identification by both the Bruker Biotyper MALDI-TOF MS and Vitek MS systems.[24,29] A total of 15 culture media for bacteria and yeasts have been validated by Bruker Daltonik GmbH. For yeasts, cultures should be incubated for 18–48 h at 29 °C (±2 °C) and culture plates can be stored for up to 12 h at room temperature (18–25 °C) after incubation. For Vitek MS, eight culture media obtained from three suppliers have been evaluated and validated for analysis of bacteria and

Table 1 Summary of identification results by Bruker Biotyper MALDI-TOF MS and Vitek MS for *C. tropicalis* isolates from colonies that were grown on different agar media.[14–24]

No.	Year of report (reference)	Country or region (no of isolates tested)	Agar media (incubation condition)	No. (%) of isolates with correct identification to species level	
				Bruker Biotyper MALDI-TOF MS	Vitek MS
1.	2011 (14)	Germany (n = 88)	SDA (24 h at 30 °C or 35 °C)	88 (100)	88 (100)
2.	2013 (15)	Hong Kong (n = 7)	SDA (48–96 h at 30 °C)	7 (100)	7 (100)
3.	2013 (16)	Italy (n = 17)	SDA (24 h at 35 °C)	17 (100)	17 (100)
4.	2013 (17)	Demark (n = 10)	SDA (24 h at 35 °C)	9 (90)	–
5.	2014 (18)	Taiwan (n = 17)	BAP (24 h at 35 °C)	17 (100)	17 (100)
6.	2014 (19)	France (n = 81)	CHROMagar (48 h at 35 °C)	81 (100)	–
7.	2014 (20)	Germany (n = 33)	SDA (24 h at 37 °C)	33 (100)	33 (100)
8.	2014 (21)	Kuwait (n = 33)	SDA (48–96 h at 30 °C)	30 (90.0)	33 (100)
9.	2014 (22)	Mainland China (218)	SDA (24 h or 48 h at 35 °C)	–	214 (98.1)
10.	2015 (23)	Australia (n = 11)	SDA (18–24 h at 35 °C)	11 (100)	11 (100)
11.	2016 (24)	Mainland China (n = 412)	CHROMagar (48 h at 35 °C)	411 (99.8)	407 (98.8)

SDA = Sabouraud dextrose agar; BAP = trypticase soy agar supplemented with 5% sheep blood; CHROMagar = CHROMagar *Candida* media.

yeasts (www.accessdata.fda.gov/cdrh_docs/reviews/k124067.pdf). For species-level detection by the Vitek MS system, bacteria and yeast must be allowed to grow for 24–72 h. It has been suggested that organisms that grow on media other than those that have been validated may exhibit different metabolic activity or color changes that can affect species-level detection by the Bruker Biotyper and Vitek MALDI-TOF-MS systems (U.S. Food and Drug Administration, http://www.accessdata.fda.gov/cdrh_docs/reviews/K124067.pdf). Several culture media contain a variety of differential and selective components, including antibiotics, pH indicators, and salts (some are well known inhibitors of MALDI-TOF MS), and different media can also induce changes in protein expression of organisms.[30] However, limited studies have been conducted on the effect of various agar media on MALDI-TOF MS identification for yeasts.[26,31] To address this issue, we investigated the effect of commonly used agar media on the performance of yeast identification by two MALDI-TOF MS systems.

The reason why we chose only *C. tropicalis* in this study was that, in our preliminary study, we found that five of the 412 isolates of genetically-confirmed *C. tropicalis* failed to obtain correct identification (no identification) from colonies grown on CHROMagar *Candida* by the Vitek MS system.[24] Clinical microbiologists from PUMCH (CHIF-NET collection) and NTUH also experienced that some blue colonies (*C. tropicalis*) grown on CHROMagar *Candida* were identified as *C. albicans* by the Vitek MS or Bruker Biotyper MALDI-TOF system (personal communication). These scenarios were rarely found by our colleagues among other common *Candida* species, like *C. albicans*, *C. glabrata*, *C parapsilosis*, and *C. guilliermondii*. During the time of this study, Bruker Biotyper MALDI-TOF MS and Vitek MS systems were starting to be introduced into clinical mycology laboratories of both hospitals for yeast identification. Consequently, there was an urgent need for clinical microbiology laboratories to evaluate the performance of yeast identification for all pure colonies that grown on various types of agar media that are commonly used for primary isolation of fungi.

Among the culture media evaluated in this study, three (BAP, SDA, and chocolate agar) have been validated by the manufacturers of the Bruker Biotyper and Vitek systems. In general, colonies for MALDI-TOF MS analysis should be cultivated on culture media that have been validated by the manufacturers of the detection systems used. However, given the importance of rapid species-level identification, isolates of yeast that grow in the laboratory are commonly subjected to MALDI-TOF MS analysis regardless of the media on which the isolates are grown. For example, CMA is a strong inducer of pseudohyphal growth in several *Candida* species, it would therefore not be surprising if the spectra looked different than those from, e.g., SDA.

The findings of 11 studies that used MALDI-TOF MS systems for identification of *C. tropicalis* are summarized in Table 1. Of them, colonies were obtained for analysis from SDA in 8 studies, CHROMagar *Candida* in 2 studies and BAP in one study (Table 1).[14,16–25] The number of *C. tropicalis* isolates tested in those studies ranged from seven to 412.[14,16–25] Failure to identify *C. tropicalis* most commonly occurred in colonies grown on SDA and CHROMagar *Candida*, although the number of isolates investigated was small in most studies.[21,23–25] In Mainland China, 0.2% (n = 2) and 1.2% (n = 5) of *C. tropicalis* isolates grown on CHROMagar *Candida* were not correctly identified by the Bruker Biotyper MALDI-TOF MS and Vitek MS systems, respectively.[24] In Kuwait, the Bruker Biotyper MALDI-TOF MS system failed to correctly identify 10% (n = 3) of *C. tropicalis* isolates that were grown on SDA media.[21]

A recent study conducted by Cassagne et al. clearly indicated the significant impact on identification log score values when different culture media were used for yeast identification.[26] Among the 6192 isolates identified by the Bruker Biotyper MALDI-TOF MS system on various solid culture media, including Sabouraud gentamicin chloramphenicol agar (SGC, n = 2412), Columbia blood agar (n = 1667), chocolate agar (n = 1365), CHROMagar *Candida* (n = 654),

and other miscellaneous culture media (n = 94), they found that the log score values varied significantly from the colonies grown on different culture media. The log score values acquired from colonies grown on SGC (mean, 2.204) were significantly lower than those grown on chocolate agar, blood agar or CHROMAgar *Candida*, although the spectra included in the spectral reference library had been acquired on SGC.[26] In this study, using the thread-hold of log score vale of 1.8, the overall accurate identification rates of all fungal isolates tested was 99.9%, regardless of the culture media used and only resulted in nine misidentifications, including one *C. tropicalis* (n = 331) misidentified as *C. dubliniensis* (log score value of 1.83).[26]

In Taiwan, Chao et al. reported that all 17 isolates of *C. tropicalis* that had been grown on BAP (one of the validated culture media) were correctly identified by both the Bruker Biotyper and Vitek MALDI-TOF MS systems.[17] However, a recent study conducted in Mainland China revealed that of 412 *C. tropicalis* isolates that had been grown on CHROMagar, only one isolate (0.2%) was misidentified using the Bruker Biotyper system whereas five isolates (1.2%) were either misidentified or were not identifiable using the Vitek MS system.[24] CHROMagar has not been validated by the manufacturers of the two MALDI-TOF MS systems; however, ChromID CPS agar, a culture medium similar to CHROMagar, has been approved by the manufacturer of the Vitek MS system.

Ramos et al. found that the Vitek MS system could detect carbapenem hydrolysis in bacterial colonies grown on Mueller Hinton agar, BAP and ChromID CPS agar media but not on MacConkey agar.[31] However, MacConkey agar has been validated as an appropriate culture media for the detection of yeasts by the Bruker Biotyper and Vitek MS systems. Moreover, use of MAC agar from some suppliers has been demonstrated to result in less than optimal detection performance by the Vitek MS system (www.accessdata.fda.gov/cdrh_docs/reviews/k124067.pdf). Pence et al. first evaluated the performance of the Bruker Biotyper and Vitek MS systems for the detection of medically relevant yeast species grown on five different media commonly used in mycology laboratories, namely BAP (Remel, Lenexa, KS, USA), brain heart infusion agar (BHI) with blood (Remel), CandiSelect4 chromogenic Agar (Bio-Rad, Hercules, CA, USA), IMA (Remel), and SDA with chloramphenicol (Remel).[29] Isolates were tested after 24 and 48 h of incubation at 35 °C with 5% CO_2, with the exception of isolates grown on CandiSelect4, which were incubated at 35 °C in air as per the manufacturer's instructions. They demonstrated that isolates grown on all five media types could be accurately detected by MALDI-TOF MS. For growth on media other than SDA, they recommend performing MALDI-TOF MS analysis after 24 h. If identification is not achieved, the isolate should be subcultured on SDA for analysis the following day. However, their results are limited by the small number of isolates tested and by the fact that only 10 of the isolates were *C. tropicalis*.[29] Among the 10 isolates of *C. tropicalis* that were prepared with 25% formic acid on-plate extraction for Bruker Biotyper MALDI-TOF MS, seven (all $\geq$1.7000) failed to achieve a score value of $\geq$2.000. However, Vitek MS analysis of samples prepared with 25% formic acid on-plate extraction resulted in the correct identification of all 10 isolates with confidence values of 99.9%.[29]

The strengths of this study are the large number of *C. tropicalis* isolates and the large variety of culture media evaluated. The limitations include the lack of investigation of factors contributing to the poor performance of the MALDI-TOF MS systems for identification of *C. tropicalis* isolates from growth on several types of culture media.

In conclusion, MALDI-TOF users should be aware about which culture media and incubation conditions are recommended by the manufacturer to have adequate performance for species identification. This study clearly demonstrated that the performance of the Bruker Biotyper MALDI-TOF MS and Vitek MS systems for the identification of *C. tropicalis* isolates varied when different culture media were used, even though the standard protein extraction procedure recommended by the manufacturers was applied. These differences of performances in identifying *C. tropicalis* isolates from CHIF-NET and NTUH collections might be related to the agar media produced by different manufacturers (all agar media tested in this study except CHROMagar *Candida*), particularly those not validated by the manufacturers of the two MALDI-TOF MS systems. The difference in the geographical sources of *C. tropicalis* isolates might also result in different identification performance on colonies obtained from the same agar medium purchased from the same manufacturer (i.e. CHROMagar *Candida*). To achieve better performance of identification of *C. tropicalis* isolates by an individual MALDI-TOF MS system, the colonies for analysis should be collected from agar media recommended by the respective manufacturer and validation of the identification accuracy with these agar media is mandatory before the system is introduced into clinical mycology laboratories for routine identification of fungal isolates.

Conflicts of interest

None declared.

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