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Evaluation of matrix-assisted laser desorption ionization–time-of-flight mass spectrometry to differentiate between *Candida albicans* and *Candida dubliniensis*

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ABSTRACT

Matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF-MS) analysis in conjunction with the direct formic acid (FA) sample processing method was evaluated for the ability to differentiate the closely related species of *Candida albicans* and *Candida dubliniensis*. The results showed that MALDI-TOF-MS, using the direct FA method, was reliable to differentiate between these species.

Candida dubliniensis is an opportunistic *Candida* species that, when identified to the species level, has been associated with recurrent oral candidiasis as well as invasive disease in immunocompromised patients infected with human immunodeficiency virus (Sullivan and Coleman, 1998; Sullivan et al., 1995). This species has since been isolated from multiple body sites in critically ill patients, although the true epidemiological prevalence of this species in causing human disease is unknown due to the close phenotypic and genetic relatedness to *Candida albicans* (Ells et al., 2009; Lockhart, 2011; Sebti et al., 2001; Sullivan and Coleman, 1998).

Phenotypic assays such as growth at a higher temperature, quantity of chlamydoconidia formed, and rapid biochemical strip-based (API 20C AUX, RapID Yeast Plus; ID32C), as well as automated identification systems (Vitek 2ID-YST card), have been used with some success to separate *C. albicans* from *C. dubliniensis* (Ells et al., 2009; Gutierrez et al., 2002; Loreto et al., 2010; Sullivan et al., 1995). In addition, genomic methods using sequence analysis of target areas within the 16S and internal transcribed spacer (ITS) regions have been shown to be reliable for differentiation (Ells et al., 2009; Pfaller et al., 2012; Sebti et al., 2001). These gold standard diagnostic methods are not only expensive but also time consuming, taking up to 48–72 hours to differentiate these species after visible growth occurs.

Matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF-MS) has been introduced to the microbiology laboratory for the rapid identification of bacteria, mycobacteria, and fungi (Bizzini et al., 2010; Huang et al., 2013; Klein et al., 2012; Marklein et al., 2009). A previous study by Hof et al. (2012) showed that the MALDI-TOF-MS was able to discriminate 14 well-characterized isolates of *C. dubliniensis* from *C. albicans* using the ethanol extraction preparation method. In contrast, the present study used a well-characterized set of isolates for the ability of MALDI-TOF-MS to identify and differentiate between *C. albicans* and *C. dubliniensis* using the direct formic acid (FA) preparation method. Thirty clinical isolates composed of 20 *C. dubliniensis* (11 isolates from Creighton University Medical Center, Omaha, NE, USA; 5 isolates from Nebraska Medicine, Omaha, NE, USA; and 4 isolates from the University of Iowa, Iowa City, IA, USA) were included in this evaluation along with 10 *C. albicans* (Creighton University Medical Center). All isolates were identified to species using either the API 20 C AUX system (bioMérieux, Durham, NC, USA) or the Vitek 2 YST ID card system (bioMérieux) along with microscopic chlamydoconidia formation on cornmeal agar and growth or no growth at 42 °C ambient air. These standard phenotypic methods were performed per manufacturer's package insert, previously described chlamydoconidia formation and arrangement method, or for temperature dependence (Ells et al., 2009; Sebti et al., 2001; Sullivan and Coleman, 1998; Sullivan et al., 1995). Gene amplification and sequencing of the complete ITS-1 and ITS-2 regions of the ribosomal DNA complex as described previously were used to confirm the species identifications (Iwen et al., 2004;

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Southern et al., 2015). ITS1-5.8S-ITS2 was amplified using ITS1 forward primer, 5′-TCCGTAGGTGAACCTGCGG-3′, and ITS 4 reverse primer, 5′-TCCTCCGCTTATTGATATGC-3′ (Iwen et al., 2004; Southern et al., 2015). The 500-bp product was assembled into contigs using the Chromas pro software version 6.5 (Oxford Molecular Group, Campbell, CA, USA) and compared with known organism sequences in the NCBI GenBank (National Center for Biotechnology Information, Washington, DC, USA), using a nongapped advanced BLAST search.

For MALDI-TOF-MS analysis, the isolates were grown on Sabouraud dextrose agar (Remel, Thermo Scientific, Lenexa, KS, USA) for 1 day at 35 °C, ambient air. Isolates were subsequently spotted in duplicate onto the steel target plate, immediately overlaid with 1 μL of FA (Sigma-aldrich, St Louis, MO, USA), allowed to air dry, and finally overlaid with 1 μL of HCCA matrix (Bruker Daltonics, Billerica, MA, USA). Matrix material consisted of HCCA portioned (alpha-cyano-4-hydroxycinnamic acid) matrix, each tube contains 2.5 ± 0.3 mg of HCCA, and 250 μL of organic standard solvent (acetonitrile 50%, water 47.5%, trifluoroacetic acid 2.5%) (#19182; Sigma-Aldrich, St Louis, MO, USA) was utilized for solubilization of the HCCA to provide a final concentration of 10 mg/mL. The target plate was analyzed according to manufacturer's instructions using the Bruker Biotyper software (Bruker Daltonic MALDI Biotyper, Version 3.1) paired with the Bruker Microflex LT MS. Bruker IVD Bacterial Test Standard (extract of *Escherichia coli* DH5 alpha and spiked with 2 additional proteins and range 3.6–17 kDa) solubilized in standard organic solvent was used as the calibrating material. The Autoxecute MBT_AutoX calibration program in quadratic mode was utilized with automatic sum spectra of 6 × 40 laser shot. FlexControl scanning and analysis were performed with the MBT_FC.par program with the detection medium mass range 2–20 kDa and a laser (337-nm Nitrogen laser with variable repetition rate) frequency of 60 Hz.

Using the MALDI-TOF Biotyper Version 3.1 software database library (BDAL) with the direct FA method, *C. dubliniensis* isolates were organism level (best match) identified as *C. dubliniensis* with a median score of 1.826 (range: 1.59–1.99) (Table 1). All *C. albicans* isolates identified correctly with organism best match of *C. albicans* (Table 1). A potential explanation for the low score results, yet still correct organism (best match) for *C. dubliniensis*, is the limited number of isolate reference mean spectrum projection (MSP) spectra of *C. dubliniensis* included in the manufacturer's database. At the time of the study, there were 3 MSP spectra for *C. dubliniensis* (RV 2 2006 VML; 99 PSB; 20 UKE) and 16 MSP spectra for *C. albicans* in the BDAL (Biotyper Version 3.1).

To further optimize the standard score values for *C. dubliniensis* species, the 20 well-characterized *C. dubliniensis* MSP spectra were added to the Biotyper database. After the addition of these isolates, the *C. dubliniensis* isolates were reanalyzed. The scores improved by 83.64% (average 1.798 initial analysis to 2.197 postspectra addition), indicating that the narrow database of 3 strains limits the score. Fig. 1 shows representative annotated MSP for *C. dubliniensis* and *C. albicans*, which are highly similar. Post-MSP spectra addition data results of this study, with the direct FA method, were comparable to the ethanol extraction processed results of Hof et al. (2012). Another study noted that a low score of <1.7 tended to lead to inappropriate database assignment for *Candida* species but ≥1.7 was associated with species accuracy (Marklein et al., 2009). Since completion of the study, the manufacturer has added an additional 11 MSP spectra for *C. dubliniensis* to the BDAL database.

Table 1
Evaluation of MALDI-TOF-MS for the ability to differentiate between *C. albicans* and *C. dubliniensis*.

Strain code	*Candida* species ID by:		Score[b]		Score[c]		Score[d]		Score[e]	
	Conventional[a]	MALDI	1	2	1	2	1	2	1	2
B7560-08	*albicans*	*albicans*	2.023	2.019	ND	ND	2.395	2.330	2.012	2.135
B7706-08	*albicans*	*albicans*	2.030	2.026	ND	ND	2.319	2.238	2.114	2.108
B8627-09	*albicans*	*albicans*	2.004	1.983	ND	ND	2.365	2.407	2.022	2.055
B9005-09	*albicans*	*albicans*	2.116	2.112	ND	ND	2.349	2.312	2.183	2.055
B9010-09	*albicans*	*albicans*	2.014	2.011	ND	ND	2.398	2.340	2.005	2.028
B9028-09	*albicans*	*albicans*	1.971	2.024	ND	ND	2.349	2.065	2.145	2.105
B9299-09	*albicans*	*albicans*	2.095	2.084	ND	ND	2.449	2.521	2.129	2.123
B9029-09	*albicans*	*albicans*	2.079	2.069	ND	ND	2.306	2.249	2.127	2.088
B0208-12	*albicans*	*albicans*	2.066	2.049	ND	ND	2.356	2.493	2.123	2.098
F38983-12	*albicans*	*albicans*	2.019	2.006	ND	ND	2.312	2.309	2.081	2.034
CU001	*dubliniensis*	*dubliniensis*	1.893	1.726	2.148	2.080	2.448	2.373	2.128	2.112
CU002	*dubliniensis*	*dubliniensis*	1.932	1.788	2.024	2.324	2.408	2.458	2.076	2.114
CU003	*dubliniensis*	*dubliniensis*	1.825	1.770	2.200	2.051	2.368	2.362	2.140	2.075
CU004	*dubliniensis*	*dubliniensis*	1.867	1.822	2.142	2.079	2.350	2.389	2.104	2.050
CU005	*dubliniensis*	*dubliniensis*	1.827	1.787	2.245	2.133	2.238	2.111	2.079	2.161
CU006	*dubliniensis*	*dubliniensis*	1.910	1.853	2.174	2.165	2.233	2.203	2.086	2.151
CU007	*dubliniensis*	*dubliniensis*	1.739	1.651	2.172	2.086	2.436	2.428	2.159	2.175
CU008	*dubliniensis*	*dubliniensis*	1.814	1.746	2.184	2.182	2.396	2.380	2.139	2.164
CU009	*dubliniensis*	*dubliniensis*	1.928	1.752	2.306	2.161	2.291	2.257	2.101	2.120
CU010	*dubliniensis*	*dubliniensis*	1.950	1.743	2.257	2.248	2.289	2.388	2.107	2.043
CU011	*dubliniensis*	*dubliniensis*	1.831	1.713	2.093	2.017	2.439	2.370	2.026	2.096
20-070-029	*dubliniensis*	*dubliniensis*	1.763	1.730	2.340	2.215	2.332	2.235	2.168	2.249
20-070-031	*dubliniensis*	*dubliniensis*	1.618	1.760	2.225	2.124	2.307	2.332	2.072	2.063
20-070-032	*dubliniensis*	*dubliniensis*	1.652	1.519	2.216	2.111	2.345	2.354	2.111	2.051
20-070-033	*dubliniensis*	*dubliniensis*	1.919	1.842	2.332	2.307	2.216	2.054	2.210	2.161
NE-TNMC-120905	*dubliniensis*	*dubliniensis*	1.590	1.585	1.908	1.891	2.241	2.275	2.011	2.144
NE-TNMC-072407	*dubliniensis*	*dubliniensis*	1.702	1.647	2.005	2.358	2.367	2.304	2.137	2.075
NE-TNMC-121007	*dubliniensis*	*dubliniensis*	1.713	1.700	1.580	1.562	2.365	2.280	2.071	2.080
NE-TNMC-012608	*dubliniensis*	*dubliniensis*	1.640	1.631	2.192	2.164	2.281	2.185	2.132	2.212
NE-TNMC-041108	*dubliniensis*	*dubliniensis*	1.871	1.774	2.251	2.197	2.428	2.509	2.054	2.058

ND = not determined; 1 = spot 1; 2 = spot 2.

[a] Species identifications were verified by both phenotypic and genotypic analyses.

[b] Analysis based on MALDI Biotyper Version 3.1 database containing 3 strains of *C. dubliniensis*.

[c] Analysis based on MALDI Biotyper Version 3.1 database with the addition of ICD-20CD spectra.

[d] Analysis based on MALDI Biotyper Version 3.1.66 database with traditional Ethanol Extraction method; performed after addition of 16 MSP spectra of *C. albicans* and 11 MSP spectra of *C. dubliniensis* by the manufacturer.

[e] Analysis based on MALDI Biotyper Version 3.1.66 database based on direct FA method, performed after addition of 16 MSP spectra of *C. albicans* and 11 MSP spectra of *C. dubliniensis* by the manufacturer.

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