Evaluation of MALDI-TOF Mass Spectrometry for Identification of Yeasts Commonly Found During Environmental Monitoring

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Introduction

An ideal environmental monitoring (EM) program should have the ability to detect and identify all categories of microbes – bacteria, yeasts and molds. The following study focuses on identifying yeasts commonly found during environmental monitoring. Yeasts belong to the kingdom of fungi and are placed in the phyla of Ascomycota and Basidiomycota. They are typically single-celled organisms that reproduce by fission or budding. Yeasts have been central to one of the earliest biological process developed for human use due to their ability to ferment carbohydrates into alcohol and carbon dioxide. Their widespread application in products related to beverages, food and pharmaceuticals – to name a few – have raised their commercial relevance. Alternatively, yeasts can be opportunistic pathogens causing mild to life-threatening infections[1] from varied sources, such as a human commensal (e.g., *Candida albicans* in the human gut) or the environment (e.g., *Cryptococcus neoformans* in soil or bird droppings). Therefore, both in beneficial and harmful ways, yeasts are important organisms for humans. Whether it is for diagnosing a patient with a potential yeast infection, or for an EM program in a manufacturing facility, accurate identification (ID) is key to guiding appropriate therapy or remediation activities, respectively. For an EM program, this information can be used to track and trend the facility's microflora, and establish microbial alert and action limits[2].

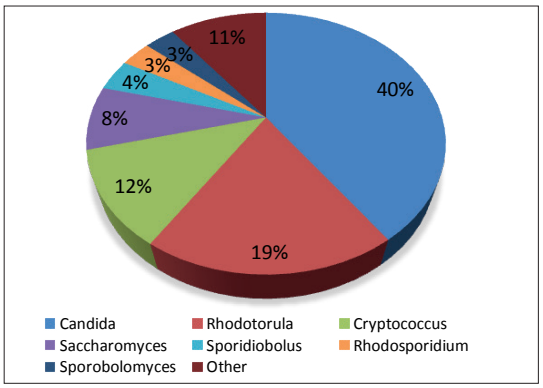
There are several technologies available for yeast identification; however, the use of ITS2 gene sequences has emerged as an important approach over the past decade[3-5]. In recent years, profiling cellular proteins using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) as the basis for identification has shown promise[6]. The process of using MALDI-TOF MS for microbial ID involves ionizing abundant cellular proteins, predominantly ribosomal[7], with a low-mass organic compound (α-Cyano-4-hydroxycinnamic acid) and a UV laser as the energy source. Gas phase ions in the molecular mass range of 2K to 20K Daltons are separated in the time-of-flight analyzer by applying an electrical field. The detected ions are transformed into a spectrum that displays the relationship between various masses and their abundance. In most cases, this spectral protein profile is unique to a species, which forms the rationale for identification. The spectra from an unknown organism is searched against the library to calculate a numerical score value that signifies the "closeness" of the unknown spectra to the reference library entry. The magnitude of the score value determines the confidence for identification – a greater value indicating a superior match. Although identification of yeasts commonly found in clinical settings by this technology is mainstream[8-11], its performance is less understood for yeast isolates commonly found during environmental monitoring in manufacturing processes.

Based on our experience at Charles River of identifying yeasts by DNA sequencing for our customers with EM programs, and a review of the literature in clinical microbiology, it is apparent that many yeasts pertinent to manufacturing processes are likely to belong to different species, and possess intraspecific diversity that is greater than that found in clinical settings. The goal of this study was to optimize and evaluate performance of MALDI-TOF MS for yeasts commonly found during environmental monitoring.

Materials and Methods

Study isolates — Yeast isolates (152) representing 65 unique species and 19 unique genera were selected based on their frequency of occurrence of testing at Charles River. Reference species identification of these isolates was performed by sequencing the ITS2 portion of the ribosomal RNA gene region and comparing the sequences to the validated Charles River reference library. A phylogenetic analysis of the sequences was performed to determine the reference ID of the test isolates.

Figure 1. cohort by genus (n=152)



MALDI-TOF MS — Isolates were cultivated as pure cultures at 32 °C on Sabouraud dextrose agar and tested by MALDI-TOF MS at 24 hours of growth. The direct smear method with 0.4 µL 100% formic acid overlay was tested for a subset of isolates (n=51). All samples were processed by the formic acid/acetonitrile extraction method recommended by Bruker Daltonics. Mass spectra were acquired for two spots per isolate. Data were collected between 2,000 and 20,000 m/z in linear positive ionization mode using the Real Time Classification Biotyper 4.0 software with library version 5989 (Bruker Daltonics) and autoflex™ instrument (Bruker Daltonics). Each spectrum was a sum of 1000 shots collected in increments of 250.

MALDI-TOF MS Charles River library development — Isolates that failed to identify and additional isolates of the same species were used to generate MALDI-TOF MS library entries as per the cGMP-compliant procedures at Charles River. A supplemental MALDI library database containing 41 entries was developed. These 41 entries represented 32 unique species spanning 14 unique genera when compared to the species entries in the Bruker database.

Data analysis — The spot (out of two) resulting in the higher score value was used for analysis. Concordance was calculated after applying scoring thresholds (2.0-3.0 for high confidence identification and 1.7-1.999 for low confidence identification) as per Bruker's recommendation and using Charles River's internally validated thresholds for bacterial identification (≥1.75 for species-level and <1.75 for no identification). In addition, concordance and performance were calculated with and without Charles River's supplemental MALDI library entries in the reference database.

Results

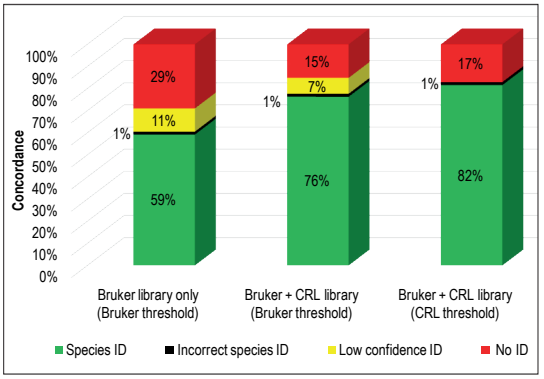
Charles River yeast library development — The Bruker Daltonics MALDI-TOF MS library (version 5989) had 690 fungi, of which approximately 95% are yeasts. During this study, Charles River added 41 new MALDI-TOF MS library entries (Table 1).

Table 1. Number of species by genus that were added to the Charles River library to improve coverage (n=41).

Genus	No. unique species	No. of library entries
Bullera	1	1
Candida	12	15
Citeromyces	1	1
Coniochaeta	1	1
Cryptococcus	3	4
Filobasidium	1	1
Hannaella	1	1
Pseudozyma	1	1
Rhodosporidium	2	3
Rhodotorula	4	7
Saccharomyces	1	2
Sporidiobolus	1	1
Sporobolomyces	1	1
Trichosporon	2	2

Performance of MALDI-TOF MS — Concordance was calculated by applying score thresholds and comparing IDs by MALDI-TOF MS with the IDs obtained by sequencing the ITS2 portion of the ribosomal RNA gene region. With Bruker's MALDI-TOF MS library and score thresholds, identification rates were 59% (90/152) to species (score ≥2.0), 11% (16/152) were low confidence scores (≥1.7 to <2.0), and 29% (44/152) were not a reliable ID (<1.7) (Figure 2). Several published studies have shown the relationship between optimizing MALDI-TOF MS score thresholds to increase identification rates without significantly compromising accuracy[12-14]. Identification rates improved to 82% (124/152) to species (score ≥1.75), and 17% (26/152) were not a reliable ID (<1.75), when Charles River's supplemental MALDI-TOF MS library and score thresholds were applied (Figure 2). There was a 1% (2 of 152) species-level misidentification rate using both Bruker's and Charles River's score thresholds. It is important to note that the number of incorrect IDs did not increase when the species-level score threshold was lowered to ≥1.75, indicating that the incorrect IDs occurred at scores >2.0. Of the two discrepant IDs, the first was *Rhodotorula slooffiae* identified as *Rhodotorula minuta*, and the second was *Cryptococcus albidus* identified as *Cryptococcus liquefaciens*, by MALDI-TOF MS. In both cases, the species in question are closely related and may either require new library entries for accurate species resolution, or may end up as "species group" level IDs. Overall, these results show that MALDI-TOF MS is an effective platform for identification of yeast commonly found during environmental monitoring, but supplementing the library with the diversity of isolates relevant to manufacturing processes (Table 1) and optimizing score thresholds is critical to improved performance (Figure 2).

Figure 2. Performance of MALDI-TOF MS for yeast identification (n=152)



Conclusions

Several clinical validation studies published since 2009 have shown identification rates using MALDI-TOF that range from 80% to 100% [8-11]. Based on the results of this study, 98.4% (124/126) of the species IDs that were generated were concordant with the IDs obtained by the ITS2 sequence based IDs. With Bruker's MALDI-TOF MS library and score thresholds, identification rates were 59% (90/152) to species (score ≥2.0), 11% (16/152) were low confidence scores (≥1.7 to <2.0), and 29% (44/152) were not a reliable ID (<1.7) (Figure 2). As expected, identification rates improved to 82% (124/152) to species (score ≥1.75), and 17% (26/152) were not a reliable ID (<1.75), when Charles River's supplemental MALDI-TOF MS library and score thresholds were applied (Figure 2) with equivalent accuracy of the results. These data with isolates primarily from manufacturing environments, and numerous studies with isolates from clinical settings[1, 8-11] show that MALDI-TOF MS can provide yeast identifications with high accuracy. This system becomes an even more favorable option for routine yeast identifications when its other advantages, like low reagent cost and rapid sample processing time, are considered. Nevertheless, it has been noted in several studies with clinical isolates that the performance of a MALDI-TOF MS system depends on the breadth of library coverage[1, 11, 15]. Similarly, our study highlights the necessity of expanding the MALDI-TOF MS library with new entries to capture strain and species diversity that is present in environmental samples so that the operational performance can be improved.

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