Evaluation and Optimization of MALDI-TOF Mass Spectrometry for Identification of Filamentous Fungi during Environmental Monitoring

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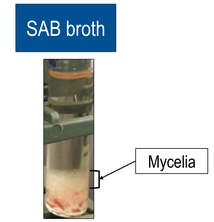
1 ABSTRACT

Accurate and rapid identification (ID) of filamentous fungi is an important part of tracking and trending a facility's microflora and can aide during investigations related to contamination events. Traditional ID methods are not enough, thus we evaluated cultivation and sample preparation methods, in addition to library coverage, for the identification of filamentous fungi using MALDI-TOF mass spectrometry (MALDI). The study cohort included 130 isolates spanning 27 genera and 67 species that represent the broad phylogenetic diversity frequently recovered from manufacturing facilities. All isolates were identified by sequencing the ITS2 ribosomal region. MALDI IDs were generated with the Bruker Biotyper library and an in-house supplemental library. Cultivating filamentous fungi in SAB broth or specialized agar plates (ID-Fungi Plates, CONIDIA, France) for up to 72 h and harvesting the mycelial growth using the extraction method were the optimal conditions to generate high-quality spectra. The ID rate was greater than 90% for both cultivation options. Nearly 91% of the identifications by MALDI were concordant with the ITS2 sequence-based IDs. ID-Fungi plates (IDFP) agar cultivation is a rapid and reproducible alternative to SAB broth. These results demonstrate that MALDI can be an effective platform for ID of filamentous fungi but supplementing the library with a diversity of isolates is critical to improve performance.

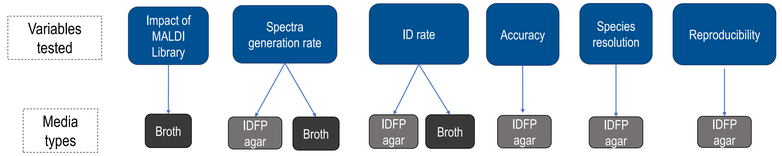
2 INTRODUCTION

- Presence of fungal spores in sterile and non-sterile manufacturing facilities can cause product contamination. Monitoring, identifying, and trending of fungal contamination is crucial to minimize risk.
- During 2002-2012, fungal contamination in pharmaceutical products was the second highest microbial reason for recalls (Reference: Sandle T., Issue 1, Feb 2014, EPR).
- Product contaminated by fungi could pose a life-threatening risk to immunocompromised patients and/or reduce product's shelf-life.
- Filamentous fungi (molds) have a greater morphological diversity compared to bacteria, making species identification using MALDI challenging, and nearly impossible by traditional morphological or phenotypic methods.
- The goal of this study was to evaluate the feasibility of identifying filamentous fungi using MALDI-TOF.



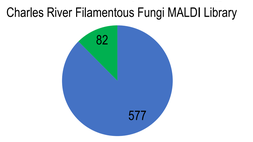
3 STUDY DESIGN

- Study cohort consisted of 130 unique isolates tested with SAB broth cultivation followed by extraction. These strains represented 67 species and 27 genera of frequently encountered organisms at Accugenix®.
  - A subset of 63 isolates were tested by cultivation with IDFP agar followed by extraction. These strains represented 47 species and 24 genera.
- To test the reproducibility of results from IDFP agar, three technicians independently cultivated and performed routine extraction on 28 isolates representing 28 species and 19 genera.
- All isolates were identified by sequencing the ITS2 ribosomal region.



4 RESULTS

Impact of Targeted Library Development



Fil. Fungi MALDI Library	Entries	Species	Genera
Bruker supplemental v3.0	577	180	62
Accugenix® (in-house)	82	61	25

Figure 1. Distribution of supplemental Filamentous Fungi MALDI Entries

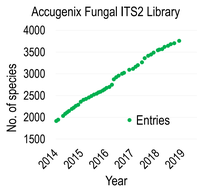
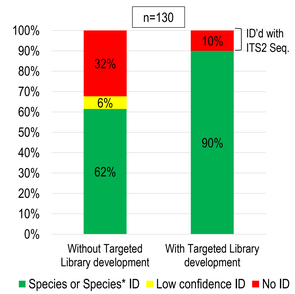


Figure 2. Accugenix Fungal ITS2 Library update history



MALDI Score Key	Without library development	With library development
Species or Species* ID	≥1.8	≥1.75
Low confidence ID	≥1.6 and <1.8	NA
No ID	<1.6	<1.75

Figure 3. Impact of Accugenix® (in-house) library of 82 entries on ID rate. Targeted library development is required to improve the ID rate of the assay. Note: Species* ID implies two or more closely related species are the final ID call.

Spectra generation on Bruker MALDI microflex instrument

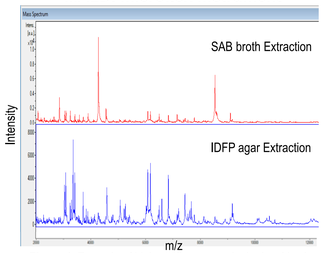


Figure 4. Example of high-quality spectra by both cultivation methods of species *Alternaria alternata.*

MALDI spectra generation rate

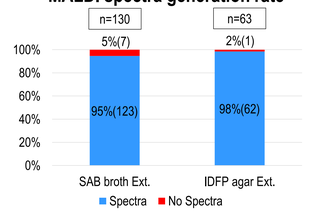


Figure 5. Spectra generation is possible using the extraction method for both cultivation methods (SAB broth and IDFP agar).

MALDI ID rate

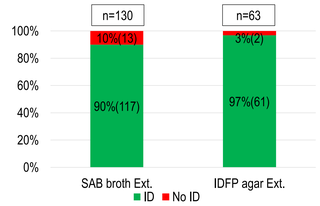


Figure 6. Score threshold of ≥1.75 was Species ID and <1.75 was No ID. Both cultivation methods generated a high ID rate (>90%) when the combined Bruker supplemental v3.0 and Accugenix® library was used.

Accuracy of MALDI ID

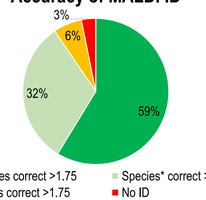


Figure 7. Accuracy was determined by comparing MALDI results of 63 isolates tested from IDFP agar with ITS2 gene reference results. 91% of the IDs were correct to either Species or Species* level. Note: Species* ID implies two or more closely related species are the final ID call.

ID Resolution of MALDI

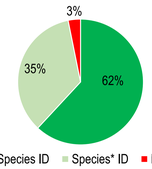


Figure 8. MALDI results of 63 isolates tested from IDFP agar based on pre-defined reporting criteria for ID. 35% of IDs are at the Species* level (Species* ID implies two or more closely related species are the final ID call due to overlapping spectral profiles).

Reproducibility of IDFP agar

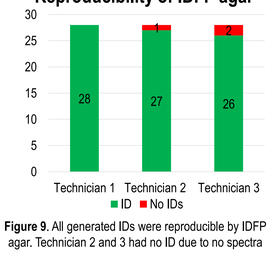


Figure 9. All generated IDs were reproducible by IDFP agar. Technician 2 and 3 had no ID due to no spectra generation.

5 CONCLUSIONS

- Sampling mycelia allows MALDI to identify the organism accurately and reproducibly.
- SAB broth or IDFP agar cultivation followed by the Extraction produce high-quality spectra.
- IDFP agar is a rapid alternative to SAB broth cultivation.
- MALDI library development of fungal isolates found in manufacturing facilities was critical to increase the ID rate.
- Many closely related fungi remain unresolvable to Species level by routine MALDI. Identification with ITS2 gene sequencing will play a prominent role for critical needs.
- The results of this phylogenetically diverse study cohort shows that MALDI is a feasible platform for the identification of filamentous fungi.

Workflow of MALDI backed by Sequencing