Evaluation of a New Protein Extraction Method Using an Advanced Acoustic Technology for Identification of Filamentous Fungi by MALDI-TOF MS

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ABSTRACT

Background

While matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) has become a mainstay for clinical labs to identify most bacteria and yeast, its application for filamentous fungi (mold) identification (ID) is still limited. In this study, we assessed a new protein extraction method that generated spectra suitable for MALDI-TOF MS using Adaptive Focused Acoustics with a Focused-ultrasonicator (Covaris® M220, Woburn, MA, USA).

METHODS

Aspergillus fumigatus, A. terreus, F. solani, S. apiospermum, P. lilacinus, A. alternata, E. dermatitidis, M. canis were grown in Sabouraud Dextrose (SAB-DEX) broth (Becton Dickinson) and rotated at room temperature to generate a hyphal mass. In addition to Bruker’s extraction protocol, each sample was extracted using Covaris’ ultrasonication with beads (75 PIP 25 DF that included the hyphal mass and 70% Formic Acid incubation.

RESULTS

No Peaks= NP, No Reliable Identification= NRI

NP, NP, NP, NP

NP, NRI 1.312, NRI 1.089, NP

NRI 1.218, NRI 0.998, NRI1.403, NRI 1.295

NRI 1.65, 1.832, 1.747, NRI 1.688

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INTRODUCTION

In this study, we assessed a new protein extraction method by Covaris that generated spectra suitable for MALDI-TOF MS using Adaptive Focused Acoustics with a Focused-ultrasonicator (Covaris® M220, Woburn, MA, USA).

RESULTS

Phase 1

• Place the SAB broth onto the rotator for 24 hours to allow the filamentous to stay in its hyphal phase.
• Centrifuge at 2 minutes at maximum speed (13,000-15,000 rpm)
• Pipet 1 uL of the supernatant onto the MALDI target

Phase 2

• Place the SAB broth onto the rotator for 24 hours to allow the filamentous to stay in its hyphal phase.
• Centrifuge at 2 minutes at maximum speed (13,000-15,000 rpm)
• Pipet 1 uL of the supernatant onto the MALDI target

CONCLUSION

• The first phase of the Covaris study showed that Aspergillus fumigatus required increased contact time with 70% Formic Acid which was seen by the increased scores obtained in the final phase.
• The second phase of the Covaris study showed as ultrasonication time increased, MALDI identification scores decreased.
• In the final phase of the Covaris study, the micro-TUBES with fiber at 40 PIP 50 DF at 2 min, achieved comparable MALDI scores to Bruker’s 30 min protocol using fewer steps and less hands-on time.
• Although further research is required to investigate ways to increase the correct ID rate, Covaris ultrasonication extraction method was a simple, rapid, and efficient tool for mold identification.

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