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Discrimination of *Penicillium* isolates by MALDI-TOF mass spectrometry fingerprinting

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Novel Aspect:

Improved fungal sample preparation allows MALDI-TOF MS fingerprinting with high accuracy.

Introduction:

Fungi are a diverse kingdom consisting of approximately 1.5 million species. The taxonomy of fungi is primarily based on time consuming and subjective identification of morphological colony features or by phylogenetic analysis. Fingerprinting techniques based on matrix-assisted laser desorption/ionization (MALDI) time-of-flight mass spectrometry (TOF MS) have been demonstrated to be a powerful tool for the analysis of microorganisms such as bacteria, however, relatively few studies have focused on fungi. Fungi of the genus *Penicillium* have been shown to produce infection, particularly in immunocompromised hosts. In this study, we investigate thirteen species of this medically-relevant genus.

Methods:

Thirteen fungal isolates (ATCC) were sub-cultured from stock sources and grown 14 days on malt extract agar. Conidia and hyphae of individual fungi were isolated from the surface of one plate and then transferred to a 2.0 ml microcentrifuge tube. 100 µL of 0.1 mm zirconium beads and 1 mL 50/50 acetonitrile/4% trifluoroacetic acid were added to each tube and the samples subjected to three one minute bead beating cycles. The resultant solution was centrifuged at 14,500 rpm for 10 minutes and the supernatant taken for MALDI-TOF analysis. Samples were mixed 1:1 with 10 mg/mL α-cyano-4-hydroxycinnamic acid (CHCA), and a 1 µL aliquot was spotted on a gold target plate and allowed to air dry.

Preliminary Results:

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was used to generate highly reproducible mass spectral "fingerprints" for thirteen species of fungi of the genus *Penicillium*. Prior to MALDI-TOF MS analysis, the fungi were subjected to three one-minute bead beating cycles in an acetonitrile/trifluoroacetic acid solvent. Although early attempts at MALDI-TOF MS fingerprinting of fungi were plagued by poor spectra containing few peaks, the mass spectra of bead-disrupted fungi contain abundant peaks in the range of 5-20 kDa, and may be used to unambiguously discriminate between species. In addition, a potential genus-specific biomarker for *Penicillium* is observed at m/z 13.9 kDa. To ensure reproducibility, each fungal species was independently cultured eight times, and the MALDI-TOF MS spectrum from each independent culture was added to the database. . The fungal isolates can be correctly identified at the species level with 100% accuracy using canonical discriminant analysis with stepwise variable selection. Both resubstitution and cross-validation error estimates result in 0% error for the dataset of 104 fungal fingerprint mass spectra. Although the fungal fingerprint mass spectra produced by the described methodology are highly reproducible, spectra of *Penicillium* species differ from others in the published literature, reiterating the importance of highly controlled culture, sample preparation, and mass spectrometry conditions for mass spectrometry-based chemotaxonomy of microbiological samples.

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TP 253 **Discrimination of *Penicillium* isolates by MALDI-TOF Mass Spectrometry Fingerprinting**; Justin M. Hettick; Amanda D. Buskirk; Brett J. Green; Michael L. Kashon; James E. Slaven; Erika Janotka; Detlef Schmechel; Donald H. Beezhold; *NIOSH, Morgantown, WV*

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