A36 Determination of Forensic Signatures for Growth Media in Bacillus Spores Using Matrix Assisted Laser Desorption Ionization (MALDI) Spectrometry and Microbial Adherence to Hydrocarbon (MATH) Assays

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After attending this presentation, attendees will understand the significance of the effect that different media compositions have in the derivation of protein profiles of Bacillus spores and its utility in the characterization of pathogens recovered as evidence.

This presentation will impact the forensic science community by providing potential tools to establish protein signatures of Bacillus spores grown on different media which can be used to provide leads in microbial forensic investigations.

The variety and abundances of proteins found either inside Bacillus spores or on its membrane surfaces can vary significantly with the types of metabolic substrates available to the cell during vegetative cell growth and sporulation. To investigate the relationship between spore protein profiles and growth medium, *Bacillus cereus* T-strain spore cultures were grown on 12 different media containing a range of different protein and amino acid substrates. Each spore culture was then analyzed with Matrix Assisted Laser Desorption Ionization (MALDI) spectrometry and Microbial Adherence to Hydrocarbon (MATH) assays to characterize the whole-cell and cell-surface proteins, respectively.

Results suggest that protein signatures do vary among spore cultures grown on different protein/amino acid substrates. Whole-cell protein profiles detected by MALDI suggest that eight spore cultures can be reproducibly differentiated (G, BHI, LL, NSM, CDSM, BE, PepBE, G4TN). In addition, biomarkers m/z 5724, 6278, 6982, and 7514 are exclusively associated with spore cultures grown in BHI, CDSM, PepBE, and G4TN respectively. Results from MATH assays also suggested that many of the spore cultures have unique protein profiles that are related to

the medium used for growth. Profiles for spore cultures grown in media with higher concentrations of complex protein sources (CAB, BHI, and Sch) showed consistently higher hydrophobicity values (~40%-64% in 200µl hexadecane) compared to cultures grown in nutrient limited media (G, BE, G4TN, PepBE, GPN, CDSM; 10%-32% in 200µl hexadecane). This suggests that the concentration of protein used in the culturing media has a significant effect on the concentration of hydrophobic proteins associated with the spore membrane. However, as in the MALDI profiles, differences in cell surface hydrophobicity could not be directly correlated with specific protein/amino acid components in any of the media suggesting that the relationship between substrates and protein signatures in *Bacillus cereus* T-strain spores depends on more complex metabolic relationship between substrate and biochemical phenotypes. Nevertheless, both MALDI spectrometry and MATH assays show promise as analytical tools to analyze spore cultures and could be combined with other types of biomolecular phenotyping, such as Fatty Acid Methyl Ester Analysis, to build larger databases useful for microbial attribution in biocrime investigations.

Protein Profiles, Growth Media, Bacteria

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