G8 Fatty Acid Methyl Ester Profiling of Bacterial Spores for Microbial Forensics

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After attending this presentation, attendees will be familiar with the use of fatty acid profiling to determine the source of bacterial spores grown on several different culture media and the forensic applications of gas chromatographic (GC) techniques for examining the fatty acid composition of the membranes of organisms isolated from biological evidence.

This presentation will impact the forensic community by introducing a technique that may assist investigators in determining the culture methods employed to produce a microbial agent used in a biocrimes or act of bioterrorism.

Fatty acids are components of bacterial membranes that can be regulated by the cell in response to the types of available nutrients present in the culturing media. Therefore, genetically identical bacteria that are grown on different media substrates can vary in their fatty acid composition. Previously, fatty acid methyl ester (FAME) profiling has been used in clinical settings for bacterial strain identification, but has not yet been applied for forensic applications.

In this research, three hypotheses were tested. First, whether a clinical method for FAME profiling of bacterial cells can be adapted for use with bacterial spores. Second, can reproducible FAME profiles be produced from minimal amounts of evidence (<3mg spores). Third, can a database of FAME profiles of spores grown under a variety of well-established conditions be used to reliably identify the medium used to grow the spores of an unknown sample.

For this work, 22 different culture formulations were used to prepare and process *Bacillus cereus* T-strain (BcT) cultures. Fatty acid extraction and GC profiling were performed on spores from each media preparation using two different analytical techniques: (1) the clinical FAME method ("Rapid Method") which requires approximately 30 mg of biological material and two hours to process, and (2) the more forensically relevant method ("Instant Method") which requires only 1 mg of biomaterial and approximately 15 minutes of processing time. The effect of media substrate on spore fatty acid composition was examined using Cluster Analysis (CA) and Principal Component Analysis (PCA) of all generated profiles.

FAME profiles from both methods and each media substrate were used to construct two BcT strain spore databases. Similarity indices calculated between FAME profiles with the Sherlock Microbial Identification System (MIDI) software were used to evaluate the variability and reproducibility of the spore database data.

The results of this research suggest that FAME profiles from spores grown on most of the surveyed media substrates can be statistically distinguished using CA and PCA. Oleic Acid appears to be specific for Columbia Blood Agar and Tryptic Soy Agar with Blood indicating that certain fatty acids may be diagnostic for specific media types. In addition, reproducible fatty acid profiles were generated from less than 1mg of dry spores using the "Instant Method." Results will be presented for comparison of 'blind' spore profiles against the profiles in the BcT spore-media databases. These studies will demonstrate the potential usefulness of FAME profiles for forensic microbiology.

Fatty Acid, Bacillus, Bioterrorism