A157 Forensic Discrimination of Bacillus Spores Grown With Different Media Based on Cellular Fatty Acid Composition: Implications for Biocrime Investigations

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After attending this presentation, attendees will be familiar with fatty acid profiling of bacteria, the diagnostic phenotypic signatures associated with different growth substrates and culturing conditions of spore cells, and the potential applications of fatty acid profiling for microbial forensic investigations. In addition, attendees will be exposed to statistical techniques that can aid in analyzing complex phenotypic systems and differentiating closely related forensic samples.

This presentation will impact the forensic science community by introducing novel applications of accepted microbiologic techniques that can assist forensic investigators in identifying the culture methods used to produce microbial bioterrorism agents.

Fatty acids are the main components of bacterial membranes that protect the cell from its environment. The types and relative proportions of different fatty acids present in a laboratory grown bacterial culture is in large part determined by the nutrients available in the culturing media and the environmental conditions present during growth. Fatty acid profiling is a common technique for bacterial characterizations of environmental samples and species identification of unknown microbial agents in academic, industrial, and clinical settings. However, the potential for fatty acid profiling to assist in biocrime investigations by identifying phenotypic markers for different growth media within spores of a forensically relevant organism has not been explored.

In this study, cellular Fatty Acid Methyl Ester (FAME) profiling was investigated as a method to resolve the differences in membrane composition among spore cultures of *Bacillus cereus* T-strain each grown on different substrates. Ten media formulations were chosen that varied in the sources and concentrations of protein/amino acids, sugars, carbohydrates, and inorganic salts. *B. cereus* was used as a forensically relevant surrogate for *B. anthracis* because of the genetic, structural, and biochemical similarities between these two organisms. To analyze FAME profiles and identify biomarkers that were diagnostic for the growth media used to culture *Bacillus* spores, total profile dissimilarities were assessed with non-metric multidimensional scaling (nMDS) and analysis of similarities (ANOSIM). Discriminant Function Analysis (DFA) was subsequently used to isolate a subset of fatty acids that maximized profile differences among spore groups and determined which variables are contributing most to sample discrimination. In addition, profiles for each spore sample were characterized either by the relative abundances of the four structure classes of *Bacillus* fatty acids (iso-odd, iso-even, anteiso, and straight-chained) as well as the abundance of individual FAME biomarkers.

Results showed that FAME profile differences were most pronounced among spore cultures grown on media with varying sources of proteins and amino acids in their formulations ($R>0.8$, $p<<0.01$ for ANOSIM). Organisms grown on chemically defined media with exogenous protein sources either absent or in low concentrations were easily differentiated. In addition, spore cultures grown on media supplemented with disparate protein sources (i.e., tryptone versus peptone) exhibited the largest variation in FAME composition. One FAME biomarker, oleic acid (18:1 ω9c), was found exclusively in spore cultures grown on Columbia Agar supplemented with sheep blood. DFA indicated that the proportion of anteiso fatty acids (15:0 anteiso, 17:1 anteiso A) contributed significantly to the discrimination of spores grown in yeast extract media whereas branched-even (14:0 iso, 16:0 iso) and branched-odd (17:1 iso ω5c, 17:1 iso ω10c) fatty acids drove differentiation of spores grown on media supplemented with singular sources of supplemental protein (peptone or tryptone). For spores prepared on media containing multiple protein sources, discrimination functions were influenced most by branched odd variables 17:1 iso ω5c and 17:1 iso ω10c. The fact that different sets of FAME markers are needed for various group comparisons indicates that forensic differentiation of spores may require a hierarchical classification system based on dicriminant functions. These results suggest that FAME profiling can detect individual biomarkers and multivariate differences among spore cultures that are diagnostic for the protein/amino acid components of growth media and that this technique may be a promising tool in biocrime investigations.

Biocrime, Microbiology, Fatty Acid

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