A10 Forensic Signatures of Laboratory Grown Bacillus Spores Based on Cellular Lipid Composition: Implications for the Analysis and Attribution of Microbial Evidence Collected From a Bio-Crime

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After attending this presentation, attendees will become familiar with lipid composition of Bacillus cells, the effect that laboratory growth recipes have on the relative abundance of lipid biomarkers in cells, and multivariate signatures that may help provide investigative leads in a microbial forensic investigation.

This presentation will impact the forensic science community by establishing new biochemical markers that potentially can be used to analyze evidence collected at the scene of a biocrime and, consequently, can assist an investigation.

Lipids are dynamic features of the bacterial cell membrane. Synthesis and incorporation of different lipid structures can reflect the nutritional substrates available to an organism during growth. Within the laboratory, the particular recipe of growth nutrients used during batch culturing is often unique to the host facility or an individual scientist conducting the research. Previous studies have demonstrated that biochemical signatures are associated with certain growth recipe constituents (e.g., brain-heart infusion), but no study to date has systematically examined a comprehensive complement of complex nutrient additives and its effect on the forensic signatures of *Bacillus* spores.

In this study, lipid composition was investigated as an informative phenotype for the types of nutrients that were used during batch growth of *Bacillus cereus* T-strain (BcT) spores. BcT was chosen as a forensic surrogate because of its genetic, structural, and biochemical similarity to pathogenic strains of *Bacillus anthracis*. Five protein sources were examined: tryptone (enzymatic digest of casein protein), peptone (enzymatic digest of meat protein), soy protein, brain-heart infusion, and gelatin. BcT spores were grown in media containing the same base formulation but supplemented with 8g/L of each of the five nutrient types. After growth, lipids were extracted from spore cultures using a saponification-hexane separation technique. Structural characterization and determination of 11 relative abundance for each lipid was determined with gas chromatography (GC). Composition was characterized by the relative abundances of the four structure classes of *Bacillus* fatty acids (iso-odd, iso-even, anteiso, and straight-chained) and the abundance of individual fatty acid methyl ester (FAME) biomarkers.

Results showed that spores grown on each of the nutrient sources possessed significant differences in the abundance of certain lipid markers. Specifically, spores grown on tryptone containing media were enriched in branched-odd (15:0 iso and 17:1 ω10c) and depleted in straight-chained lipids (14:0, 16:0, 16:1 ω7c). The converse was true for organisms grown on peptone-containing media. Spores grown on soy and gelatin additives showed lipid compositions intermediate between tryptone and peptone cultures. Spores harvested from brain-heart media showed proportions of branched-odd lipids that were similar to tryptone cultures, but were also enriched in one straight-chained lipid, 15:1 ω5c suggesting that it may be an informative biomarker for brain-heart additive. Discriminant function analysis (DFA) was used to model the variation among each nutrient type and to generate a mathematical framework for systematically discriminating each of the five spore cultures based on lipid composition. DFA results showed excellent discrimination with linear equations based on eight lipid biomarkers. In addition, examination of the canonical variable coefficients identified promising stand alone biomarkers (14:0, 17:1 ω10c) for nutrient source identification.

Historic research of bacterial fatty acids and spores led to the hypothesis that fatty acid content of bacterial spores could provide insight into the growth formulations and process by which the spores were produced. The results of the work reported here support the hypothesis. Thus, by comparing the fatty acid profiles of the evidence obtained at a bio-crime with those of collected evidence from facilities under investigation, meaningful inclusions and exclusions might be made. While FAME analysis alone cannot constitute a forensic investigation, it can provide useful information regarding the provenance of a threat agent.

Fame, Microbial Forensics, Sample Provenance

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