



A135 Principles and Applications of Fatty Acid Profiling in Microbial Forensics Investigations

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After attending this presentation, attendees will be familiar with fatty acid profiling of bacteria, the effects of different growth substrates and culturing conditions on fatty acid composition of microorganisms, and the potential applications of fatty acid profiling for microbial forensic investigations. In addition, attendees will be exposed to statistical packages and techniques that can aid in the differentiation of closely related forensic samples.

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

Fatty acids are the main components of bacterial membranes that protect the cell from its environment. Cellular fatty acid profiles are determined by the genetic makeup of the organism, the nutrients available in the culturing media, and the environmental conditions present during growth. Previously, fatty acid profiling has been used for species and strain identification of unknown microbial agents in a variety of academic, industrial, and clinical settings. However, the potential for fatty acid profiles to yield forensically relevant information about the culturing conditions of microorganisms has not been explored.

In this research, three hypotheses were tested. First, can microbes grown on different media formulations be distinguished by their fatty acid profiles. Second, do changes in environmental conditions such as oxygen concentration, temperature, and pH induce significant differences in a microorganism's fatty acid profile. Third, can a post-processing statistical technique be developed that minimizes the effect of varying environmental conditions on fatty acid profiles and provide leads towards identification of media substrates that were used to grow microbes.

For this work, 12 different culture formulations were used to prepare and process sporulating cultures of *Bacillus cereus* T-strain (BcT). Fatty acid extraction and GC profiling were performed on 1-2mg of dried spore material from each media preparation using the "Instant Method" developed by MIDI, Inc. In addition, sporulating BcT cultures were grown under different oxygen concentrations, temperatures, and pH levels. The effect of media substrates and environmental conditions on spore fatty acid composition was examined using non-metric multidimensional scaling (nMDS) and Principal Component Analysis (PCA) of all generated profiles. Multivariate statistical comparisons between each of the 12 media groups were conducted using multivariate analysis of variance (MANOVA) and Discriminant Function Analysis (DFA). The latter technique was used to generate classification functions that tested how often spores were correctly identified in their corresponding media group.

Results suggest that fatty acid profiles from spores grown on most of the surveyed media substrates can be statistically distinguished with PCA and nMDS analyses. Spores grown on Casein Acid Digest, G Media, Brain Heart Infusion, and Chemically Defined Sporulation Medium showed distinct fatty acid profiles that could be easily resolved from other media types. DFA-derived classification functions showed that almost 93% of all the spore samples (n=132) represented by all 12 media groups could be correctly identified with the growth media on which they were cultured.

In addition, changes in the oxygen concentration, temperature, and pH levels during growth and sporulation of BcT cultures caused fatty acid profile differences in certain fatty acid markers (15:0iso, 16:0, 16:1ω7c) that reduced the efficacy of spore identification with the correct media group. However, analyzing the data set with variables derived from synthesis pathways in *Bacillus* rather than individual fatty acid markers was found to minimize the effect of environmental factors and increased the likelihood that growth media for each BcT spore sample was correctly identified.

Microbial Forensics, Fatty Acid, Statistics