2016

Forensic Signatures for Production Conditions of Bacillus thuringiensis (str. HD1) Spore Cultures

Dani Jabado
Virginia Commonwealth University, jabadodb2@mymail.vcu.edu

Cristina Stanciu
Virginia Commonwealth University, stanciuce@vcu.edu

Christopher Ehrhardt
Virginia Commonwealth University, cehrhardt@vcu.edu

Forensic Signatures for Production Conditions of *Bacillus thuringiensis (str. HD1)* Spore Cultures

Dani Jabado, Cristina Stanciu, Christopher Ehrhardt;

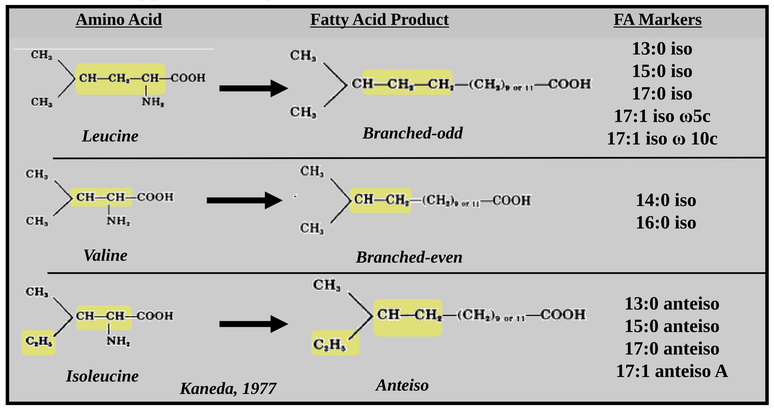
Department of Forensic Science, Virginia Commonwealth University, Richmond, VA

Abstract

Chemical signatures that can indicate growth medium recipes or other key aspects of the production conditions are an important goal for forensic and biodefense agencies. In this study, Fatty Acid Methyl Ester (FAME) profiles were analyzed from *Bacillus thuringiensis* spores grown five different published medium recipes. *B. thuringiensis* was chosen due to its biochemical, structural, and genetic similarity to *B. anthracis*, a Biosafety Level III select agent and the organisms used in the Amerithrax attacks in 2001. The relative abundance of 13 different fatty acid biomarkers, spanning four structure classes, was compared across all spore samples. Differences in the types and relative abundance of specific fatty acids was observed across each medium formulation, particularly within branched-odd and anteiso structure classes (e.g.15:0 iso, 17:0 iso, 15:0 anteiso). Spore cultures also varied in the proportion of unsaturated and saturated fatty acid biomarkers. When examining specific FAME biomarkers, CAD medium showed an average abundance of 30% for 15:0 iso, whereas LD 97 and Sch media showed average abundances of 23% and 27% respectively, and G-medium and G+Peptone exhibited average abundances of 22.5% and 23% respectively for the same fatty acid. Examination of the 17:0 iso fatty acid showed higher average abundances of 12% in CAD and 13.5% in G+Peptone, while showing lower average abundances of 10%, 8.5%, and 6% in LD, G-Medium, and Sch media respectively. Further differences in fatty acid content were also noted across the sporulation media, whereby CAD, G-Medium, and G+Peptone media exhibited an average abundance of 11.8%, 11.5%, and 11% respectively for 15:0 anteiso meanwhile lower abundances of the same fatty acid were noted in LD (8.5%) and Sch (9%) media. The results indicate that determining FAME profiles can be used to discriminate between a *Bacillus thuringiensis* species grown in different mediums, and that the amino acid content of each medium affects the FAME profile of the species in question.

Introduction

The Amerithrax case of 2001 necessitated the development of an identification system that can determine the taxonomy and production conditions of pathogenic strains recovered as evidence. Bacterial identification by FAME is one of such tools that can provide information about the microorganism's species/ strain and its culturing conditions. This approach offers many advantages among which is its efficiency and specificity for strain typing. The types and abundance of fatty acid structures present within phospholipids comprising the cytoplasmic membrane is indicative of the species/strain and the growth environment. Gas Chromatography (GC) analysis analysis can determine the lengths, bonds, rings and branches of the FAME. Each biomarker can provide information on the methods used to grow the microorganisms, particularly for Bacillus ACT (*anthracis*, *cereus*, *thuringiensis*) organisms where the abundance of branched-odd, branched-even, anteiso, and straight-chained fatty acids is largely determined by the concentrations and availability of amino acids leucine, valine, and isoleucine supplied in the growth medium.



Methods

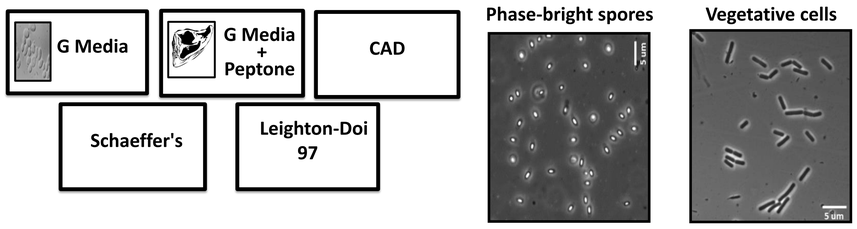


Figure 1. For this study, spores of *Bacillus thuringiensis* were grown and sporulated in one of five medium formulations (G Media, G Media + Peptone, Casein Acid Digest, Schaffer's, Leighton-Doi 97), followed by purification with ddH_2O. All final spore preparations were composed of at least 98% phase bright spores.

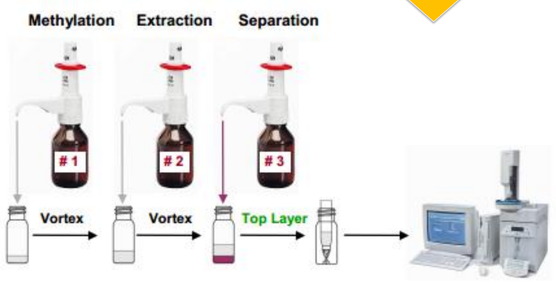
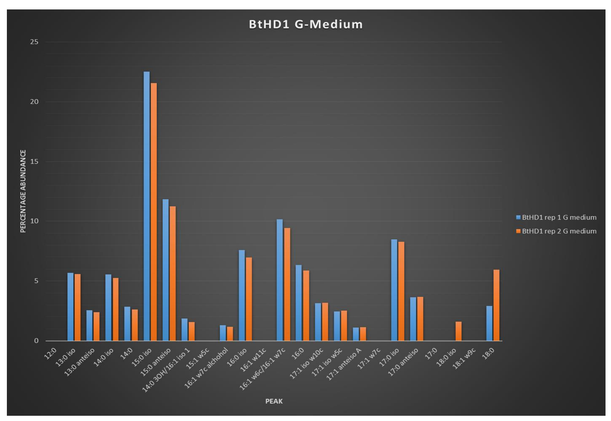
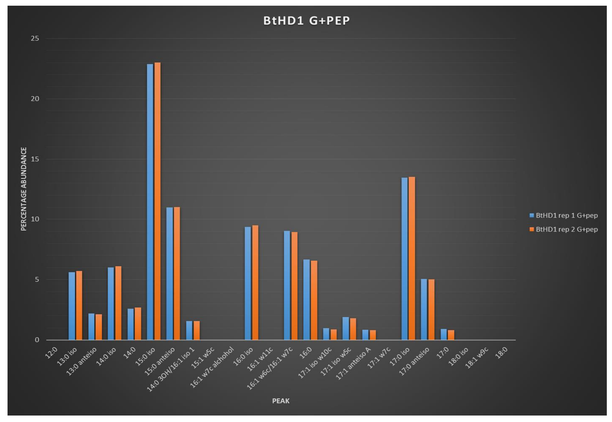
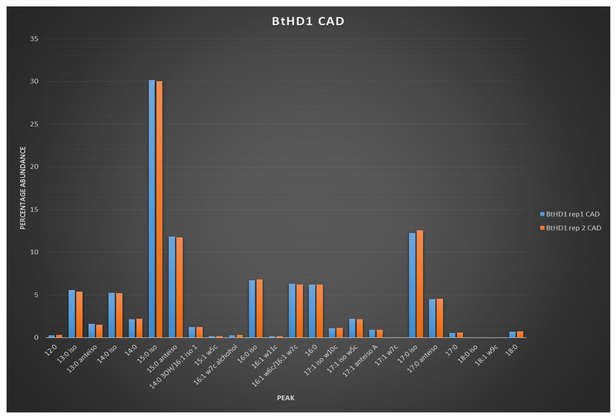


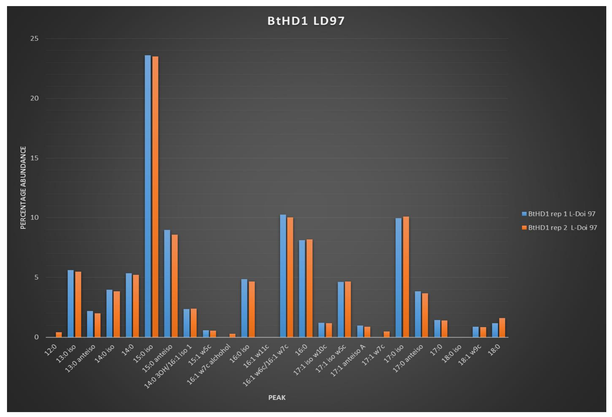
Figure 2. One milliliter of spore solution are extracted from each culture. The sample is centrifuged to produce a pellet. The supernatant is then discarded, and the pellet undergoes Fatty Acid Methyl Ester extraction. The final sample is analyzed by GC-FID, which provides a fatty acid profile for the examined spore culture.

GC FID Profiles Across Culturing Conditions for *BtHD1* Spores

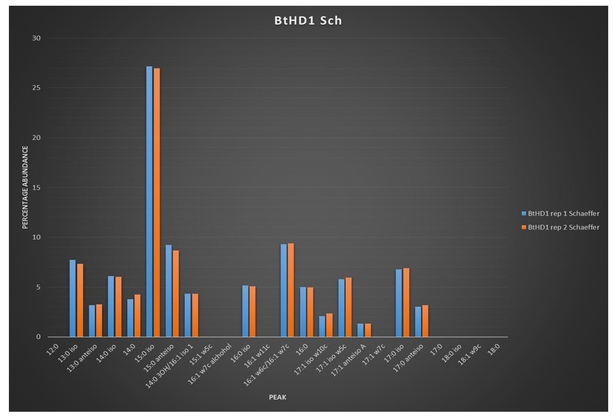








Figures 4. Fatty acid profiles for BtHD1 spore samples across five medium formulations. G-medium, G+Peptone, Casein Acid Digest, Leighton Doi 97, and Schaeffer's. Clear differences are observed in the relative proportion of branched-odd, branched-even, and straight-chained fatty acid biomarkers. Formulations with casein protein (CAD) showed higher relative abundances of branched-odd fatty acids compared to growth media with peptone supplements (G+Peptone, Schaeffer's, Leightion-Doi). Little to no variation was observed between replicates of the same culture suggesting that FAME profiles may be an effective chemical fingerprint for a specific culture or production run of an illicitly-produced pathogen.



Results and Conclusions

- *Bt* HDI fatty acid profiles vary across cultures produced with different growth medium formulations. The largest differences were observed between cultures grown with proteins and amino acids derived from casein and animal meat (peptone).
- Few differences were observed between replicates of the same spore culture. This suggests that FAME profiles may be useful biochemical 'fingerprints' for a single production or culturing run of a biothreat agent.
- Fatty acid signatures for growth medium are consistent with other *Bacillus* ACT organisms, suggesting that FAME profiles may be used for other virulent/pathogenic *Bacillus* strains (i.e. *B.anthracis*).
- Speed, sampling requirement, and simplicity of fatty acid profiling allows for this technique to be integrated with other forensic analyses (e.g., genetic or proteomic profiling) as either an investigative tool or a 'triage' method performed at the point of collection.