G13 Forensic Identification of Microbial Mixtures Via ESI-TOF Mass Spectrometry

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After attending this presentation, attendees will learn the basics of ESI MS, the basic problems with current identification methods of unknown microbes, and how ESI-TOF can correctly identify microbes based on DNA base composition.

This presentation will impact the forensic community by explaining a novel method of identifying unknown microbes in a complex sample.

The growing threat of bioterror events is a significant problem for the security of individuals worldwide. When an unknown biological agent is released, identification can be delayed due to complexity and number of samples required. Whole genome sequencing (WGS) is a possible solution; however this can be costly for complex mixtures. Targeted methodologies search for specific bacterial agents and can be limited by the requirement to determine if the agent of interest is present within the sample. An alternative to WGS and targeted methodologies was developed by Ibis BioSciences using mass spectrometry (MS). This new MS-based method allows an analyst to determine initially which species are present in the sample, rather than asking if a certain species of bacteria is present. Further delineation is then possible by fine-tuning the assay. The DNA base composition can be determined by mass measurements using high resolution MS, which can detect differences in DNA and allow strains of bacteria to be identified. A primary goal of this research is to determine the level of strain delineation possible amidst other strains of a select microbe using this MS-based method.

Broadly conserved genes in bacteria were selected for amplification with specifically designed forward and reverse primers to *Bacillus subtilis*. Genomic DNA was extracted from *B. subtilis* and amplified using PCR. These selected amplicons were analyzed via Electrospray Ionization Time of Flight (ESI-TOF) MS. Using an integrated fluidics system, DNA samples could be introduced to the ESI source at a high flow rate but then electrosprayed at a slower flow rate to improve resolution. After deconvoluting the information from the mass spectrometer, the organism can be identified by comparison to a library using abundance estimation, joint maximum likelihood, and base composition analysis. The molecular weights from multiple strands, when combined, provide a unique molecular fingerprint which allows an organism to be identified down to the species and strain-level.

A binary set of strains from *B. subtilis* were mixed at various concentration levels to evaluate this MS-based approach in terms of speed and accuracy. An internal mass standard sequence of DNA was used to allow the concentrations of microbial DNA to be calculated after amplification. When using single-stranded oligonucleotides, more than 1200 base compositions could be reported. However, using the complement strand at low concentrations has shown to reduce complexity and error in the data, improving the accuracy of the result.

An expansion and variation of the number of bacterial species and strains tested will occur as time permits for this presentation. As a bioterror event could result in thousands of organisms present in a sample, there will continue to be a need for methods which can select the correct organism, especially in the case of a novel strain for forensic studies.

ESI-TOF, Unknown Microbes, Base Composition

 ** Presenting Author*