A163 Single Cell Characterization of *Bacillus* Spores: Novel Forensic Signatures for Biothreat Agents

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After attending this presentation, attendees will understand the effect laboratory preparation methods have on the phenotypic characteristics of *Bacillus* spores, the forensic issues associated with microbial evidence characterization, and the use of Atomic Force Microscopy (AFM) for the characterization of the surfaces of single bacterial spores.

This presentation will impact the forensic science community by introducing a new signature system for determining forensically relevant aspects of a single organism's culturing environment. Since an organism's phenotype can be influenced by laboratory production processes (i.e., culturing conditions and purification processes), knowledge of these processes may provide investigative leads and/or exclude suspects during a forensic investigation.

Phenotypic profiling of bacterial threat agents can be a valuable tool for investigating bioterrorism. By analyzing the non-genetic features of an organism grown in a laboratory, it is possible to reconstruct key aspects of culturing medium and/or purification process. Recently, several forensic phenotypic signatures have been developed, but most of these methods require a significant number of bacterial cells (i.e., 10^6 – 10^{10}) which may be unlikely in microbial evidence. New signature systems are needed for the detection and characterization of samples with trace quantities of cells recovered during a forensic investigation.

AFM has emerged as a versatile tool to visualize biological molecules ranging from single proteins and DNA molecules to whole cells, with sub-nanometer resolution. In the context of forensic analysis, the AFM can provide rapid, high-resolution, 3D imaging of cellular samples at the single cell level under a variety of environmental conditions. Additionally, AFM has been used to differentiate between bacterial species and strains; however, the effect of various culturing conditions and purification processes has yet to be fully investigated.

The purpose of this research was to use AFM to identify forensically relevant features of the cell surface that are created during culturing in the laboratory. More specifically, the morphology of the cell surface of *Bacillus cereus* spores was investigated as an indicator of the composition of the growth medium and the purity of the cell culture. The research focused on two strains, T and 14579, of *B. cereus* in four culture media: G medium (G); Peptone G medium (PGM); Tryptone G medium (TGM); and Brain Heart Infusion G medium (BGM). Four purification steps were also studied: zero; one; three; and five washes in nanopure water. For each culture preparation, surfaces of individual cells were analyzed at the micron to nanometer scale. As a result, the spore surface roughness (RMS) was measured.

The results from the effects of culturing medium on the cell surface showed that the roughness of the *Bacillus* spores were consistent between the different growth conditions. Although medium components have been shown not to affect the RMS, results revealed that RMS is affected by purification processes. As the number of washes increased, the topography of the spore changed. Without any washes (i.e., crude purification), both strains of *B. cereus* had several observable small features (<100nm) on the surface and the spores' natural shape was not visible due to the adherence of molecules. However, after one wash, there were fewer features and the spores were ellipsoid in shape. As the number of washes increased, the roughness decreased as a result of the features being removed. Overall, *B. cereus* showed an average RMS value of 15.49nm for zero washes and 11.36nm for five washes. Comparing crude (zero washes) to pure (five washes) spore preparations, the RMS values were significantly different (P=0.0012) and could represent a statistically robust signature for differentiating these two types of cells.

Overall, the results of this research suggest that the surface of single spores is affected by the extent of purification and that AFM can be a tool for processing microbial evidence. Since forensic evidence often contains trace quantities of material, the use of AFM in detecting phenotypic differences in *Bacillus* spores from batched cultures treated with various laboratory processes can be a potential technique for the attribution of microbial biocrimes.

***Bacillus Cereus*, Atomic Force Microscopy (AFM), Microbial Forensics**

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