A64 Cell Surface Hydrophobicity of Biothreat Agents: A Novel Forensic Signature for the Attribution of Microbial Biocrimes

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After attending this presentation, attendees will understand the significance of the growth medium composition on bacterial signatures, the analytical considerations for microbial hydrophobicity assays, and the forensic issues associated with microbial threat agent identification and characterization.

This presentation will impact the forensic science community by introducing a new technique that can assist future biocrime investigations. Because an organism's hydrophobicity phenotype is influenced by taxonomy and the laboratory conditions used for culturing, analyzing these signatures may help provide leads and/or exclude suspects during a forensic investigation.

The Amerithrax investigation in 2001 highlighted the need for forensic signatures that can rapidly identify a threat agent and determine the laboratory in which it was cultured. Since 2001, many methods have been developed to identify the species or strain of organisms in evidence recovered from a biocrime; however, few forensic signatures exist that indicate the culturing conditions of a threat agent and, therefore, the lab of origin. The few signatures that do exist require significant amounts of sample (~$1x10^6$ cells) that is often prohibitive in a forensic investigation. Most strikingly, there are no analytical techniques that simultaneously provide both types of information.

One promising strategy for addressing this need is analyzing an organism's cell surface hydrophobicity. The surface hydrophobicity of microorganisms is influenced by the proteins, lipids, and carbohydrates present on the membrane. The presence of these extracellular substances is affected by the nutrients in the growth medium and varies between culturing environments (i.e., different laboratories). Procedures for measuring bacterial hydrophobicity are well documented and include microbial adhesion to hydrocarbons (MATH) and hydrophobic interaction chromatography (HIC). These techniques are rapid (<10min per sample), relatively inexpensive, and simple to execute. Despite these advantages, MATH and HIC have never been applied biothreat agents in a forensic context.

The goal of this study was to test whether the cell surface hydrophobicity could be used to determine the species/strain of an organism, the cell type (vegetative or spore cell), and the growth conditions used to cultivate an unknown organism (*e.g.,* peptone- or tryptone-containing medium). The following organisms were used for all experiments: *Bacillus cereus* T-strain (*BcT*) vegetative cells, *BcT* spores, and *E.coli*. The cell surface hydrophobicity of each sample was measured using MATH and HIC techniques. For the MATH assay, spectrophotometric absorbance was measured for the initial and final cell concentrations of the bacteria suspended in 1xPBS that had been mixed with equal volumes of hexadecane. The HIC assay involved applying the bacterial suspension (in dH_2O) to a HP Ion Exchange column and measuring the proportion of microbes adhering in the column.

Results from the MATH assay showed that *E.coli* has a higher surface hydrophobicity than *BcT* vegetative cells (30% and 16% adhesion, respectively). In addition, *BcT* spores had a higher affinity for hexadecane than *BcT* vegetative cells (57% and 16% adhesion, respectively). *BcT* spores grown in six different medium formulations showed little variation in hydrophobicity with all samples ranging between 60% and 70% adhesion to hexadecane. ANOVA analysis of the MATH data confirmed that there were no significant differences in hydrophobicity across the six growth media types.

Results from the HIC assay also showed *Escherichia coli* and *Bacillus cereus* vegetative cells had different levels of hydrophobicity, 25% and 50% retention in the column, respectively. In addition, *BcT* spores had an affinity for the HP Ion Exchange approximately 20% higher than *BcT* vegetative cells (51% and 61% retention, respectively). An increase of ionic concentration of the suspension increased the affinity of the *BcT* spores by approximately 25%. This suggests *BcT* spores are sensitive to ionic interactions on cell surface and functionalized substrates. Given that sporulation media differs in nutrient and metal availability, the HIC assay may be a good indicator of culturing conditions. Since the MATH assay was demonstrated useful for species identification and discrimination between cell types, the combination of these two hydrophobicity assays may be a powerful technique for forensic characterization of unknown organisms in a suspected biocrime.

Bacillus Cereus, Biocrime, Hydrophobicity

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