INNOVATING CROSS-DOMAIN SOLUTIONS TO DETECT EMERGING BIOLOGICAL THREATS

Location-specific Volatile Organic Compounds Detected In Bacillus Anthracis Delta Sterne Spore Preparations.

Charles DeSanti Battelle **Megan Cruz** Battelle **Erich Strozier** Battelle **Cedric Neuman** Battelle

The overall objective of the IARPA-funded B24IC Bloodhound program was to determine the feasibility of novel modalities of attribution for biologically derived materials through the collection and analysis of volatile organic compounds (VOCs). Specifically, the program conducted experimentation to test the hypothesis that process location-specific VOCs would associate with and could be detected in the headspace profiles of bioproducts that were prepared using identical starting materials and comparable equipment and procedures, but different processing locations. During the effort, the program team established, practiced, and then implemented a standard operating procedure for bioproduct generation at two bioprocessing facilities: one in Columbus, OH and the other in West Jefferson, OH. A pure isolate of avirulent Bacillus anthracis Delta Sterne was generated by curing B. anthracis Sterne of the pXO1 plasmid, verified by nucleic acid analysis methods, and then used to prepare a seed bank of single-use inocula for nearly identical bioprocessing schemes that were conducted in both locations. The resulting B. anthracis Delta Sterne spore powder samples were then aliquoted into VOC collection vials, headspace VOCs were collected using solid Phase Micro Extraction (SPME) and analyzed by GCxGC-TOFMS. After each B. anthracis Delta Sterne bioprocess at each location, a sham fermentation, starting with 10 g of diatomaceous earth at the seed culture stage, was conducted using the SOP to generate an abiotic powder product to test for exogenous VOCs of potentially non-biogenic origin. A stochastic block-model clustering data analysis approach was devised and implemented to identify candidate VOC signatures that associated with only one of the two processing locations, and the candidate VOCs were subject to spectral verification. The results of this study reveal that at least eight unique, tentatively identified VOCs were detected to associate with products from only one of the two bioprocessing laboratories. This study provides evidence that biological products can harbor trace VOC signatures that are potentially revelatory of their individual processing locations. These results are relevant to DTRA JSTO and the biological defense community in general because they establish the basis for new forensic exploitation avenues applicable to biological products in general, and biological threats in particular.

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