Analysis of Laboratory Grown Bacillus Spores Using Direct Analysis in Real Time-Mass Spectrometry (DART-MS): Rapid Taxonomic Identification and Forensic Attribution of Bacterial Threat Agents

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Abstract

Direct Analysis in Real Time-Mass Spectrometry (DART-MS) is a promising technique for rapid characterization of unidentified bacterial agents. Because there is little to no sample preparation and ionization occurs under ambient conditions, DART-MS may be used to analyze Bacillus spore preparations by identifying the species present and any compounds that are uniquely associated with the culturing method. Bacillus cereus spores were cultured and then purified by centrifugation through a Renografin (Meglumine Diatrizoate) gradient. DART-MS was then used on whole spore suspensions and mass spectra collected in the range of 50-1000 m/z.

Results showed that mass spectral profiles of whole spore suspensions consisted of complex lipid assemblages that included branched-chain odd, branched-chain even, and straight-chain fatty acids. The relative ratios of fatty acid structures were indicative of the species. Lastly, meglumine ion (m/z 196) was detected on all spores purified with a Renografin gradient. Both fatty acids and meglumine were detected in bacterial concentrations as low as 1x10^5 cells/mL. Taken together, these results suggest that DART-MS can be a powerful tool for taxonomic characterization of unidentified spores as well as detecting compounds that are specific for a spore’s production process.

Introduction

The Amerithrax case highlighted the utility of having information on both the taxonomy and the culturing conditions for a biological agent collected as evidence. While certain techniques are ideal for taxonomically identifying the organism and others are good for determining the growth medium, there are no signature systems that are capable of performing both of these tasks simultaneously on spore evidence. An alternative technique is DART-MS, which requires no sample preparation and shows no effects of sample carryover, which allows for samples to be analyzed quickly one after the next. Unlike DESI-MS, DART-MS does not require a solvent spray and instead uses a charged gas flow to introduce the sample to the mass spectrometer at atmospheric pressure.

There is little to no research found on analysis of lipid and protein compositions of spores and simultaneous determination of preparation conditions using DART-MS. In this study, Bacillus cereus spores were grown on three different medium types as well as purified through specialized density gradients to determine if both Bacillus biomarkers and organic molecules from the purification processes can be detected using DART-MS at the same time. This could allow for taxonomic identification and characterization of the culturing environment during a forensic investigation.

Preparation and Purification of Bacillus Spores

Preparation of Bacillus cereus T-strain and Bacillus cereus 14579 were grown on three different growth medium recipes. Each culture was purified using a Renografin-60 (m/z 196.1 and 208.1) gradient. However, changes in the relative abundance of Rg-60 (m/z 196.1 and 208.1) were observed in all spore cultures purified through a Renografin gradient. Because changes in the relative abundance of Rg-60 were observed across tryptone and peptone preparations, it is unclear whether these differences are artifacts of DART-MS ionization or reflect true compositional differences of the spore surface. In addition, several low molecular weight peaks were observed in both tryptone and peptone cultures. These likely are attributed to various amino acids. Changes in these peak abundances may be related to changes in the nutritional composition of each medium supplement (tryptone or peptone).

In this study, DART-MS provided both taxonomic information (fatty acid structures and their relative abundance) and culturing information (presence of megumine from Renografin, ratio of small MW compounds) for Bacillus cereus spores. While this data is preliminary, DART-MS is a promising new tool for forensic analysis of biological threat agents. Today, complete attribution of biocrime evidence can include a multitude of tests that are costly, laborious, and require significant amounts of sample to analyze. However, DART-MS provides comparable levels of information and requires virtually no sample preparation or consumption of evidence. With the capability to perform both the taxonomic characterization and analysis of forensically relevant compounds, this technique can significantly improve our ability to detect and attribute organisms used in a biocrime.

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