REVOLUTIONARY DIAGNOSTICS – NONTRADITIONAL APPROACHES FOR DEVELOPING BREAKTHROUGH CAPABILITIES AGAINST EMERGING THREATS

Characterization Of Vocs Emitted From Pathogenic Bacteria Using Spme-gc-ms: Towards Non-invasive Diagnostics For Biological Agent Exposure

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A potential biosecurity-related application of breath analysis is rapid screening of subjects for exposure to a biological agent. This requires differentiation between the chemical markers from "healthy" exhaled breath and potential markers produced by proliferating biological agents in the lung of exposed individuals. We analyzed trace volatile organic compounds (VOCs) from Francisella tularensis novicida and Bacillus anthracis Sterne, both risk group 2 surrogates for potential biowarfare agents, as well as VOCs from the fully virulent risk group 3 B. anthracis Ames and F. tularensis SchuS4 grown under BSL-3 laboratory conditions. Volatile organic compounds (VOCs) emitted from bacterial cultures were sampled using solid-phase microextraction (SPME) at multiple time points and analyzed by gas chromatography-mass spectrometry (GC-MS). Individual compound identification was accomplished through comparison of chromatographically deconvoluted experimental spectra to library spectra (NIST14) and retention index matching. Control experiments included SPME VOC sampling of blank culture flasks and flasks with media only. For all for four species, characteristic VOCs were detected and attributed to the bacteria (not present in the controls). Bacterial VOC profiles evolved over time as a function of bacterial growth state. Differentiation of species was accomplished through comparisons of the species-specific VOC fingerprints, reproducibly derived from multiple biological replicates. VOC fingerprints for both Bacillus species were distinct from the markers for the two Francisella species. Markers for B.a. Sterne and B.a. Ames showed similarities, but also some notable differences. Biological functions were attributed to validated biomarkers. Our results indicate that breath analysis may have the potential to rapidly and non-invasively identify exposed individuals in a triage setting after a biological attack.

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