

Differentiation of Human Introduction vs. Natural Occurrence of Biothreat Agents using Exposome Analyses

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Introduction

What is an Exposome?

- Measure of all the exposures of an individual in a lifetime and how those exposures relate to health.
- Utilization of “omics” techniques to determine biomarkers of relevance to exposure factors.

Why Study Exposomes?

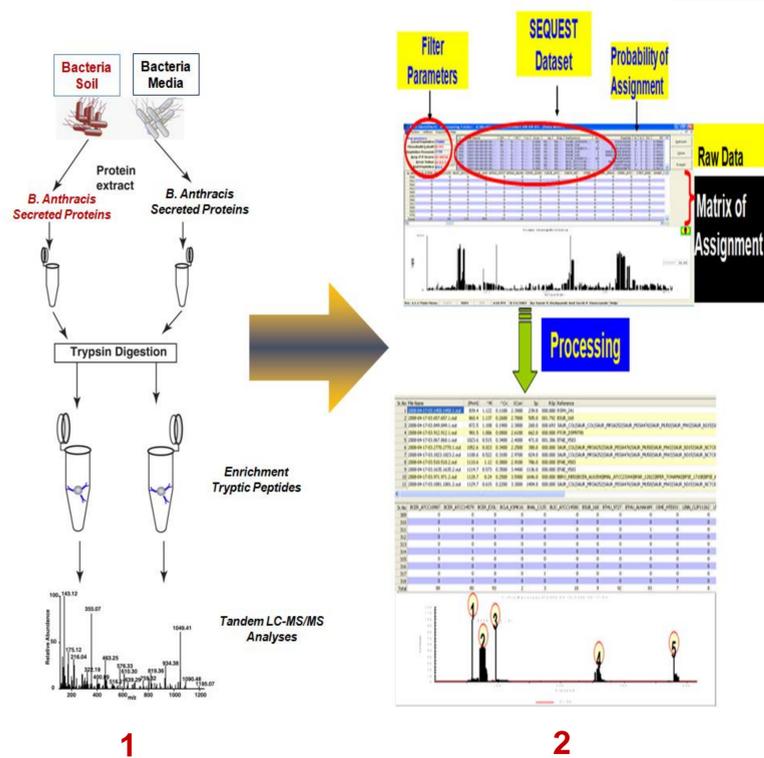
- More than 90% of mutations in microorganisms are based on environmental exposure.
- Rapid changes in protein expression as a function of environmental factors is well documented.

What are the challenges?

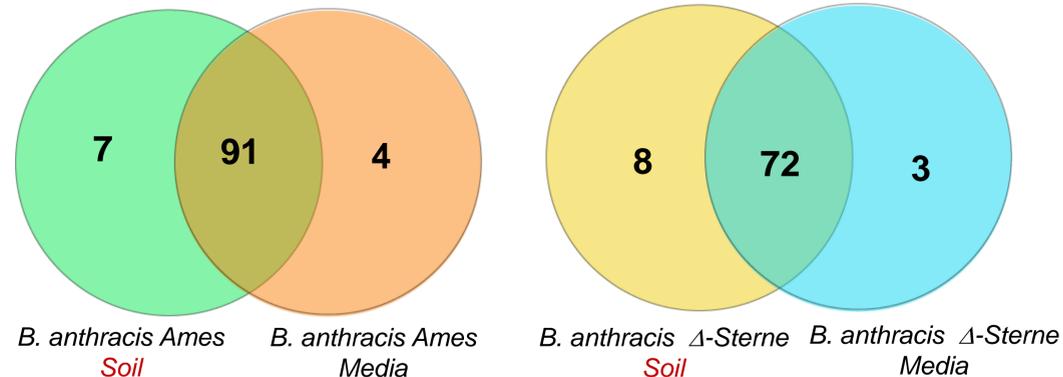
- Establish a correlation between media and protein expression.
- Ability of LC-MS to provide specific protein(s) responsible for possible unique presence (exposome omics).

Methods

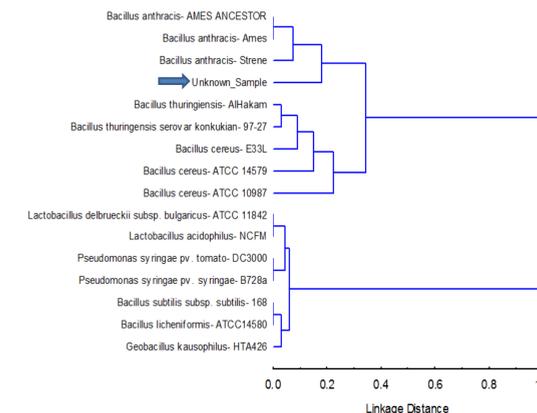
- The *Bacillus anthracis* strains were cultured in minimal media and Soil Agar.
- Bacterial cells were harvested and the bacterial cells were pelleted using centrifugation.
- Bacterial pellets were washed with 1X phosphate-buffered saline and then lysed by sonication using 4% SDS lysis buffer at pH 8.00.
- 20 mg of protein lysate from *B. anthracis* Ames and Sterne from each biological replicate of above strains was reduced, alkylated and subjected to filter-aided sample prep-based sample processing. It was also digested using trypsin and the tryptic digest was further cleaned using solid-phase extraction columns and lyophilized.
- The enriched tryptic peptides were analyzed on a nano-flow reverse phased liquid chromatography interfaced with a LTQ-Orbitrap Elite mass spectrometer system (1).
- Tandem mass spectral data were processed using a patented and in-house developed biological identification and classification algorithm, ABOid® (2).



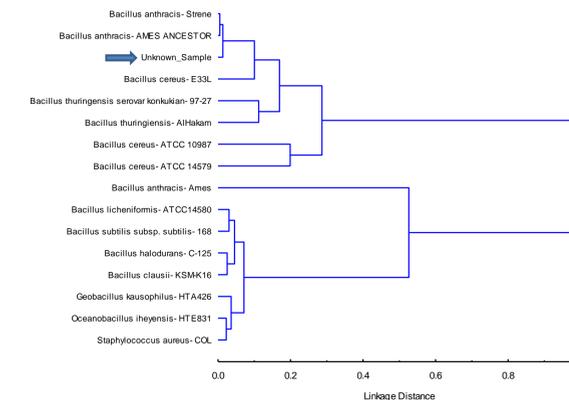
Results



B. anthracis Ames in SOIL



B. anthracis Δ-Sterne in Soil



B. anthracis Ames In SOIL Unique Proteins

NP_845633.1 hypothetical protein BA3347
 NP_845449.1 hypothetical protein BA3126

B. anthracis Δ-Sterne In SOIL Unique Proteins

YP_030705.1 small acid-soluble spore protein SspI
 YP_030570.1 forespore-specific protein putative

Comparative Proteomic Results of Soil Media (n=3)

Teller Sandy (TSL)	Sassafras Sandy Loam (SSL)	Kirkland Loam (KL)	Annessex loam (AL)	Webster clay loam (WCL)
<i>Pseudomonas fluorescens</i> SBW25	<i>Paracoccus denitrificans</i> PD1222	<i>Burkholderia thailandensis</i> E264	<i>Thermus thermophilus</i>	<i>Frankia EAN1</i>
<i>Sinorhizobium meliloti</i>	<i>Burkholderia multivorans</i>	<i>Streptomyces coelicolor</i> A32	<i>Streptomyces cattleya</i>	<i>Streptomyces cattleya</i>
<i>Bacteroides fragillis</i>	<i>Burkholderia thailandensis</i>	<i>Xanthomonas oryzae</i>	<i>Bradyrhizobium</i>	<i>Mycobacterium smegmatis</i>
<i>Clostridium saccharolyticum</i>	<i>Streptomyces flavogriseus</i>	<i>Pseudomonas fluorescens</i>	* <i>Dechloromonas aromatica</i>	<i>Rhizobium etli</i>
<i>Streptomyces cattleya</i>	<i>Rhodopseudomonas palustris</i>	<i>Bordetella petrii</i>	<i>Aeromonas veronii</i>	<i>Ralstonia solanacearum</i>
<i>Frankia EAN1</i>	<i>Hahella chejuensis</i>	<i>Myxococcus fulvus</i>		<i>Xanthomonas campestris</i>
<i>EAN1;Burkholderia 383</i>		<i>Rhodopirellula baltica</i>		<i>Colwellia psycherthryaea</i>
<i>B. anthracis</i> (2 unique peptides)				

Conclusions

- We established an accurate and rapid detection and identification method for bacteria in soil.
- Biomarkers dataset to distinguish soil and laboratory-grown bacteria for pathogenic bacillus anthracis strains.
- Loci map of bacteria under various growth conditions for studied bacteria.

Transition Potential:

1. Biosurveillance monitoring (JPM-CBD)
2. Biothreat detection and sample source identification (JPM-CBD)
3. Biothreat detection and ID (DoD & DHS)
4. Bioforensic profile (DoJ-FBI)

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