

Molecular Signatures of *Bacillus anthracis* Spores for Microbial Forensics

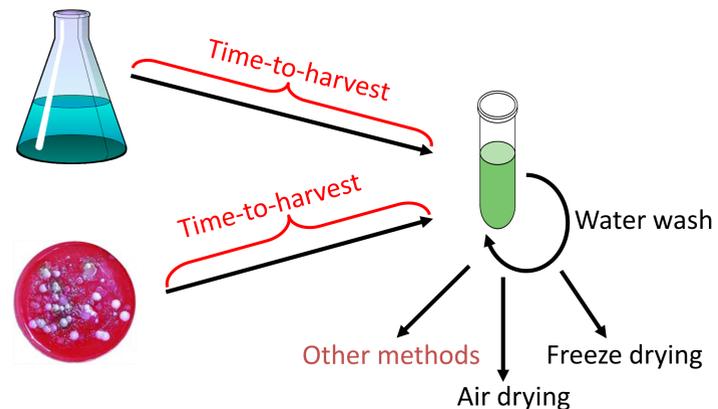
¹Dan Schabacker, ²Adam Driks, ³Tasha Pravecek, ⁴Tim Barder, ²Joe Conway, and ⁴Sara Forrester (¹Argonne National Laboratory, Argonne IL; ²Loyola University Medical Center, Maywood IL; ³United States Air Force; ⁴Eprogen Inc., Downers Grove IL)

Capability Gap

There is a critical need for forensic methodologies to identify the source of a biological threat agent (BTA). These techniques must provide a BTA signature that is detailed enough for attribution and sufficiently reliable to withstand legal challenges in court. While genetic analysis can identify the strain of the organisms, it may not provide sufficient detail for attribution. Since *Bacillus anthracis* spores can readily be used as a biological weapon, and the expertise to do this is known to a significant number of individuals potentially hostile to the United States, forensic methods to analyze *B. anthracis* spores released in an attack is an urgent need.

Solution

The protein composition of the *B. anthracis* spore varies with growth conditions and spore purification protocols. Therefore, the spore preparation method results in a distinct protein signature. We have identified changes in this signature that correspond to medium composition, temperature, duration of culture, and purification methods.



Culturing variables

- media
- temperature
- Inoculation conditions

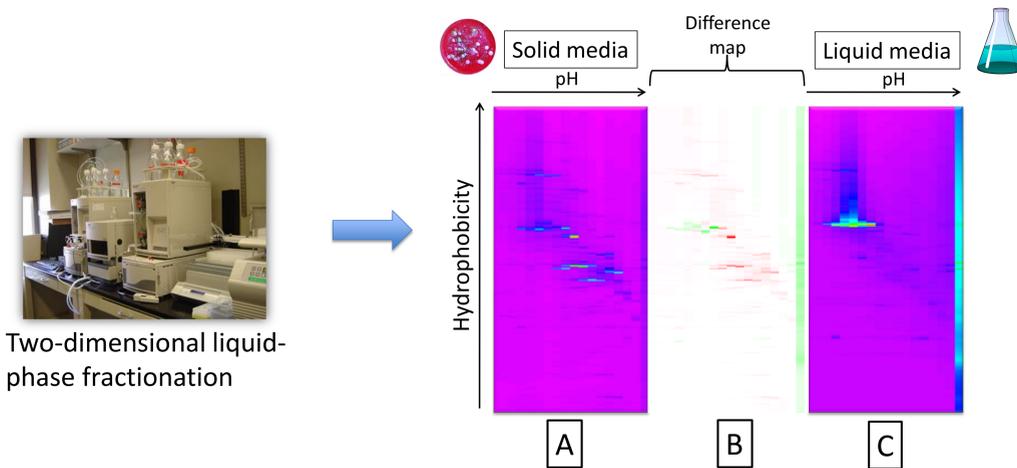
Preparation variables

- treatment preventing germination
- purification
- drying method

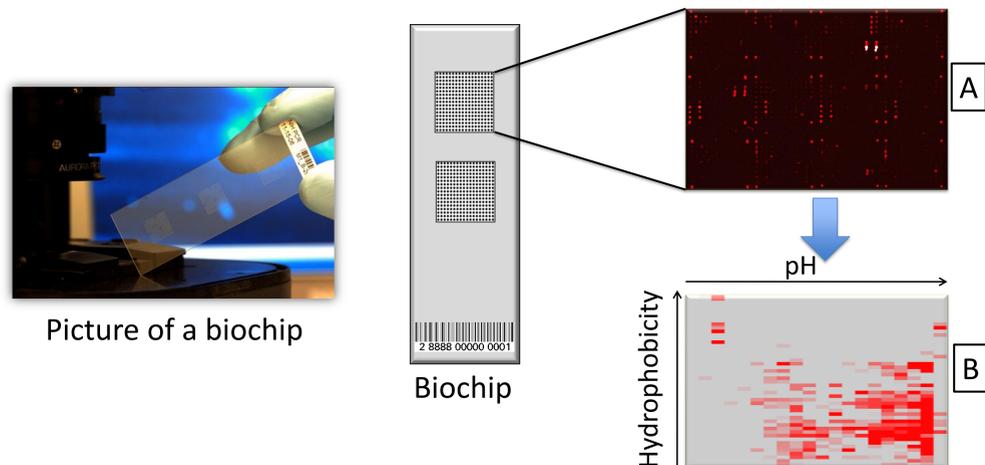
Potentially, Argonne could determine not only the preparation method, but the sophistication of the operator and his/her resources.

What We've Achieved

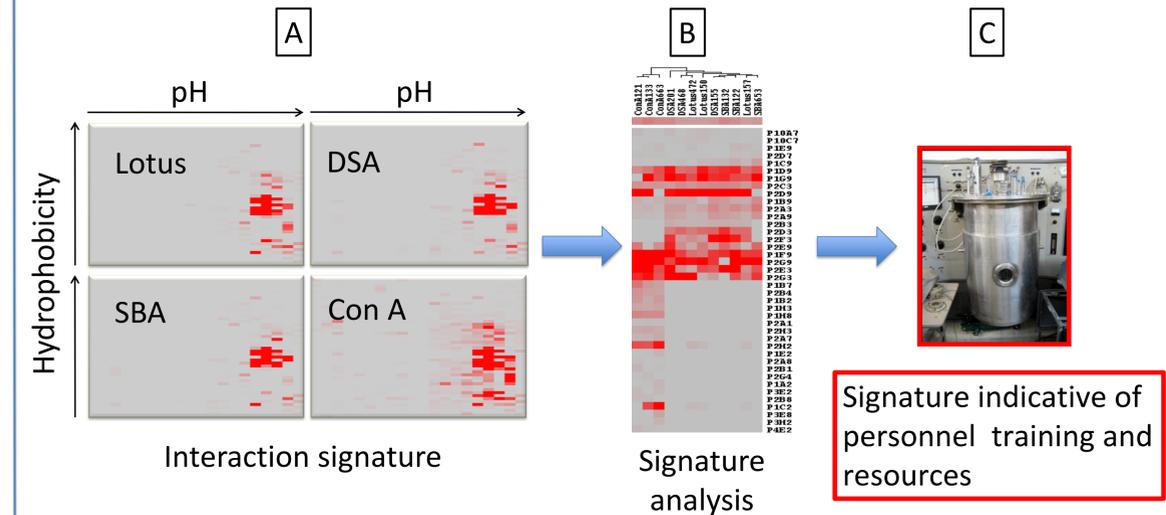
We have successfully resolved >1000 molecular species from *B. anthracis* spores of the Sterne strain using liquid-phase fractionation. Images below on the right show protein signatures from spores grown on a liquid (A) or solid medium (C), and a difference map (B).



Using biochip technology we then characterize all proteins present within a sample, identifying slight changes in protein structure. Figure (A) below displays an image from a biochip scanner. A heat map (B) is then generated producing an interaction signature for comparative analysis identifying various growth conditions and/or preparation methods.



Application of any of a variety of sugar-binding reagents (lectins) results in staining patterns (A) that can be analyzed by hierarchical clustering (B) enhancing attribution (C). Application of lectins (and potentially other reagents) provides an alternate way of visualizing the signature that can be more robust and more easily automated than analysis of total spore protein.



Benefits

- Automated signal analysis
- Reproducible, robust, high-resolution molecular characterization
- In-depth molecular characterization can support attribution
- Easily deployed, economical
- Signatures generated through standard biochemical techniques

Contact

Daniel S. Schabacker, Bio-Detection Technologies, Energy Systems Division, Argonne National Laboratory, 9700 South Cass Ave., Argonne IL 60439. Office 630-252-5191, Cell 630-251-4926; email: dschabacker@anl.gov.