

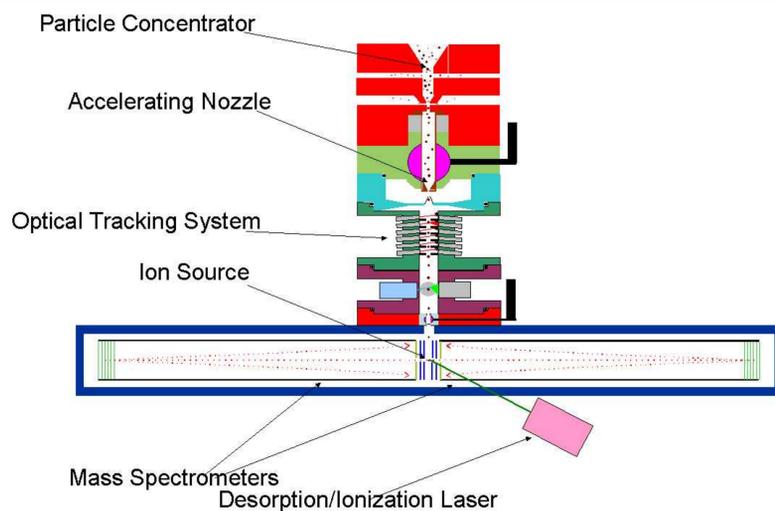
The Analysis of Seven Species of Bacillus Spores by

BioAerosol Mass Spectrometry, David P. Fergenson, Maurice E. Pitesky, Herbert J. Tobias, Paul T. Steele, Greg A. Czerwieniec, Scott C. Russell, Carlito B. Lebrilla, Joanne M. Horn, M. Frank, Eric E. Gard*, BioSecurity and Nanosciences Laboratory/Chemical Biology and Nuclear Sciences Division, CMS Directorate, Lawrence Livermore National Laboratory, Livermore CA, 94551

Introduction:

BioAerosol Mass Spectrometry (BAMS) was created to analyze individual bioaerosol particles in real time for biodefense and public health applications. BAMS has been demonstrated capable of identifying *Bacillus* spores and other microorganisms from within a background of suspicious white powders. Here we demonstrate the ability of BAMS to distinguish *Bacillus* spores of seven species from one another with varying levels of specificity.

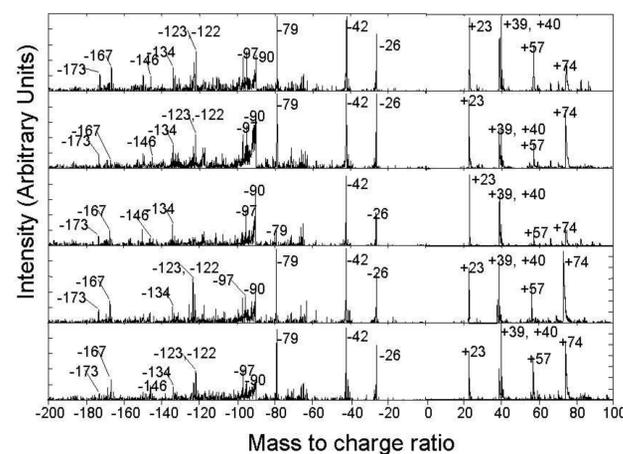
Instrumental Methods:



The BAMS is operated under vacuum. Ambient atmospheric pressure sends particles into the instrument where they are tracked by a series of lasers. The tracking lasers determine the particles' terminal velocities, which are related to their sizes. The tracking lasers are also used to calculate the time of arrival for the particles in the source region of a unique dual-polarity time of flight mass spectrometer. A high-powered Nd:YAG laser operated at 266 nm is fired to desorb and ionize the particles for mass spectral analysis.

BAMS Data:

To the right are five spectra from individual bacillus spores. The fact that they each resulted from a single spore was proven by the sizes of the particles that produced them. Notice that each spectrum has substantially the same peaks but that the intensities vary from one to the other. An automated data analysis method must be robust against these minor variations between spectra while still rejecting non-spore spectra.

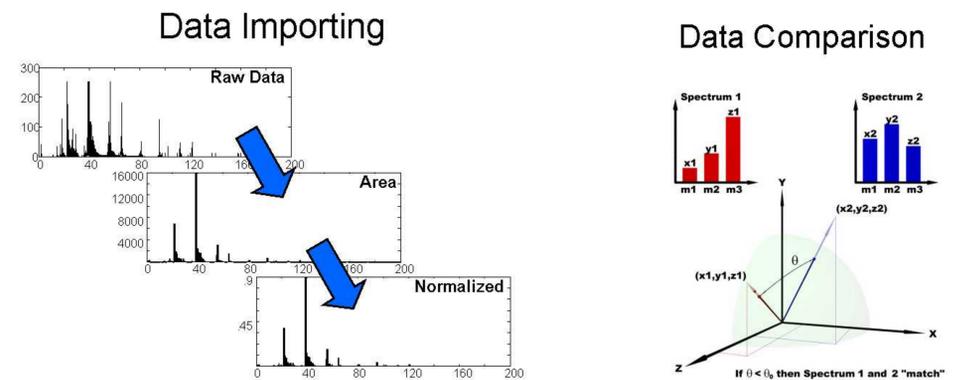


Experimental:

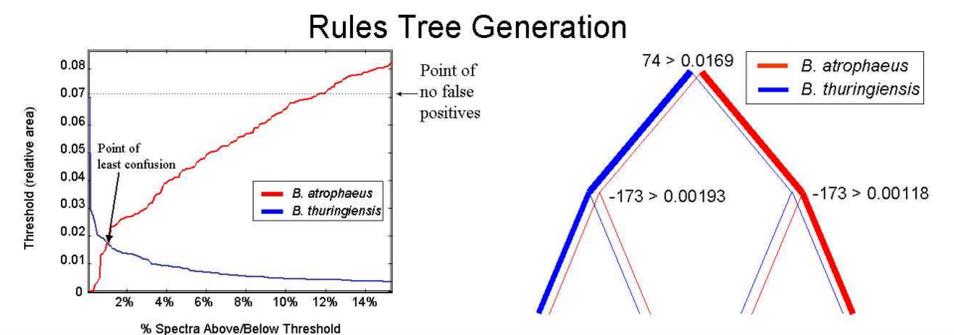
Two batches each of the seven species of *Bacillus* shown in the figure below were grown under identical conditions—1/4 TY Medium shaken/incubated at 32°C for 10 days. Three blind-coded unknowns were also grown under the same conditions. The samples were analyzed in random order by BAMS operating at a laser fluence of 0.22 mJ/pulse over the course of several weeks.

Data Treatment:

The data was analyzed in two stages. First, a simple pattern recognition algorithm based on the ART-2a neural network was applied to the data to remove any non-spore spectra. This was effected by converting the spectra to a vector of areas at each mass-to-charge ratio for the first 350 positive and negative whole mass units and then comparing the direction of the vector to vectors derived from known *Bacillus* spores, as shown below.

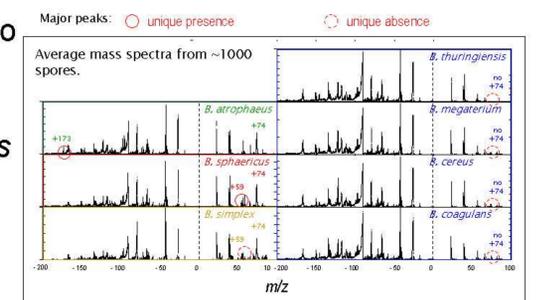


The species were differentiated from one another by a rules-induction algorithm whereby each mass-to-charge ratio was scanned to find the threshold that differentiated the most species of one type from those of all others. The most effective mass-to-charge ratio/threshold combination (rule) was used to divide the groups and then a new best rule was induced for each group. Once trained, the rules were applied to the other data sets for the same species.



Results:

The spores could be identified as belonging to one of the four categories to the right, with *Bacillus thuringiensis* being indistinguishable from *Bacillus cereus*, among others. *Bacillus anthracis* is commonly considered to be of the same species as each other and as *Bacillus anthracis*. It is believed that a wider signature space enabled by a higher maximum mass would enable more specific resolution of species.



Acknowledgments:

This work was performed under the auspices of the U. S. Department of Energy by the University of California, Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48. The Lawrence Livermore National Laboratory contributed financially to the experiments through Laboratory Directed Research and Development grant No. 02-ERD-002. This project was also funded by the Technical Support Working Group through the Department of Defense.