

Atmospheric pressure MALDI facilitates proteomics-based analysis of *Bacillus* spore mixtures.

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There is a great deal of emphasis on the development of methods and instrumentation for the rapid identification of microorganisms, individually and in mixtures. Protein biomarkers serve as excellent candidates for species-specific identification of bacteria using mass spectrometry. Recently we have reported the use of microsequences obtained from a limited set of proteins in the rapid identification of spores of the genus *Bacillus* by MALDI-TOF mass spectrometry (Analytical Chemistry 75, 5608 and 5618). Small acid-soluble proteins (SASPs) are a conserved family of proteins that are abundant in *Bacillus* spores. We report here the analysis of SASP proteins from several different *Bacillus* species, individually and in mixtures, using an ion trap mass spectrometer with an atmospheric-pressure (AP) MALDI source. A comprehensive list of known SASP sequences from different *Bacillus* species was compiled from a search of protein databases and *de novo* sequencing in our laboratory. *In silico* trypsin digestion was carried out to generate lists of species-specific candidate tryptic peptides for use in species identification. SASP proteins were solubilized from *Bacillus* spore samples (including mixtures) and digested with immobilized trypsin *in situ* on the MALDI target plate. Species-specific peptides were directly selected and subjected to collisionally-induced dissociation for sequence analysis. Interpretation of the resulting spectra and Mascot Sequence Query searches of protein databases were used to confirm the identities and sources of the peptides. This strategy offers improved speed of analysis based on direct selection of targeted peptides and the exclusion of pressure reduction as a sample processing step.

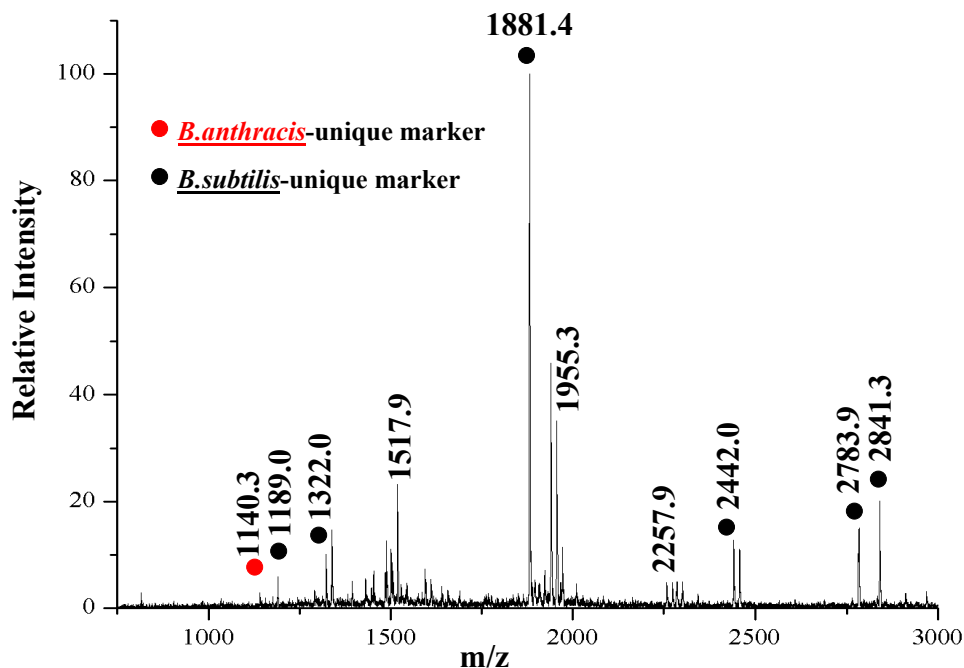
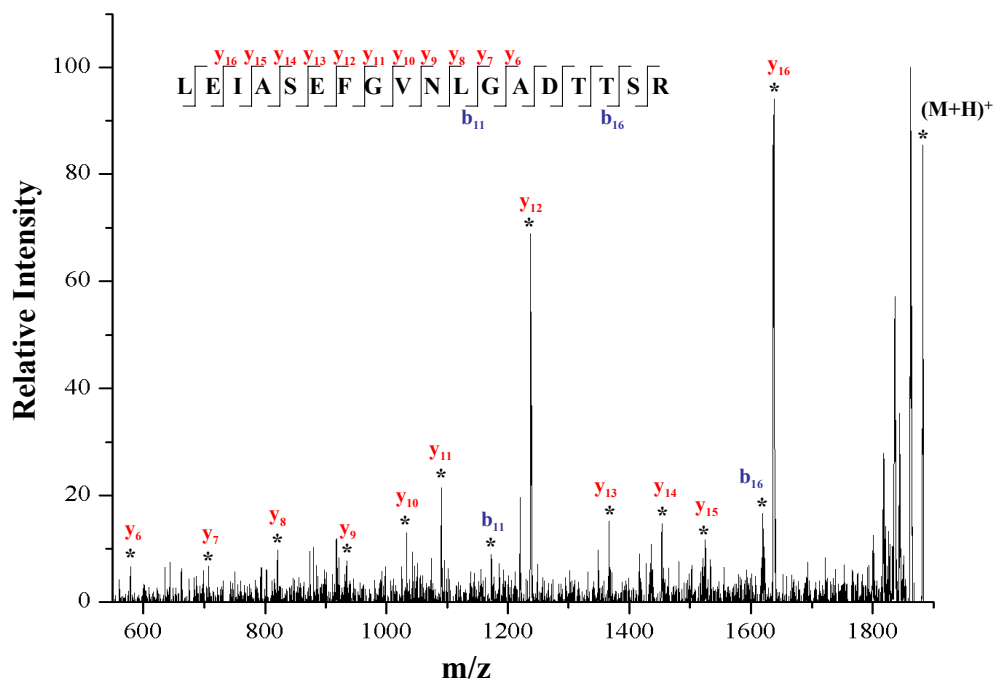


Figure 1. Example tryptic digest of a 1:1 mixture of *B. anthracis* Sterne:*B. subtilis* 168.



MASCOT Search Results : SASP α/β (*B.subtilis*, gi | 16080009), score = 233. (search carried out against NCBI nr database, taxonomy set to Eubacteria, protein and peptide tolerances set to ± 1.5 Da. Scores higher than 41 are considered significant).

Figure 2. MS/MS analysis of *B.subtilis*-specific peptide with m/z=1881Da.