

Comparison of Atmospheric Pressure MALDI IT and Vacuum MALDI TOF-TOF MS Analysis of Bacterial Proteins from *Bacillus* Spores

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Novel Aspect: Comparison of MS-MS analysis of atmospheric pressure MALDI IT and vacuum MALDI TOF-TOF MS using *Bacillus* spores.

Introduction: Mass spectrometric analysis of peptides generated by enzymatic digestion of proteins is a routine method for identification. Sequence information can also be obtained by combining MALDI ionization with MS-MS analysis. This study focuses on MS-MS analysis of proteins isolated from *Bacillus anthracis* and *Bacillus cereus* analyzed by both atmospheric pressure (AP) MALDI-ion trap (IT) and MALDI-TOF-TOF mass spectrometry. Originally the intent of this study was to use a gel digestion procedure, however; the proteins of interest could not be digested using trypsin, due to the lack of cleavage sites. Thus a new enzyme, pepsin, was selected for use. Unfortunately, there was limited success with pepsin; therefore, a new procedure using a simple TFA extraction was applied to the analysis of whole spores to see if both species and strain specificity could be determined with the above mentioned analysis.

Methods: Spores from strains of *Bacillus anthracis* and *Bacillus cereus* were grown on agar plates. After harvesting, the vegetative cells were removed by freeze-thaw method to induce autolysis. Then, the spore suspension were lyophilized and stored at -80°C. Approximately, 1 mg of the lyophilized spores were weighed and 1 mL of purified water was added. Next, the spores were analyzed with a Bruker Daltonics MALDI TOF MS to observe the intact small acid-soluble proteins, using a simple TFA extraction procedure. Following this analysis the same samples were subjected to a quick analysis by both a Finnigan Classic IT equipped with a MassTech AP MALDI source and with a Bruker Daltonics MALDI TOF-TOF MS, using immobilized trypsin in order to digest the intact small acid-soluble proteins.

Data: Currently there is great interest in developing techniques that allow for forensic identification of microorganisms using mass spectrometry. One method that has been used focuses on the analysis of whole cells directly from culture and matching fingerprint spectra generated from MALDI TOF MS analysis. This method has shown promise at the species level, but at the strain level the technique has had difficulties. By employing a simple separation procedure, such as one-dimensional gel separation, one can focus on proteins that are known to exhibit strain variability. Two such proteins are the glycoproteins BclA and BclB found in *Bacillus anthracis* spores. However, there is difficulty in digesting these two proteins of interest, since there are no tryptic sites present, then an enzyme other than trypsin is needed. Pepsin was selected as an alternate enzyme, but there has been only limited success with developing this protocol.

Alternatively, a new procedure has been developed that employs the use of a simple TFA extraction that allows for small acid-soluble proteins to be observed. MALDI TOF MS spectra of several strains of both *B. cereus* and *B. anthracis* spores were obtained. This data indicated that it was not possible to differentiate between the *Bacillus* species. Next, a series of experiments used another procedure where immobilized trypsin was added to the spores and analyzed by AP MALDI IT MS. Clearly, figure 1 shows a difference between the *Bacillus* species with two different masses of 1518 and 1534. Figure 2 is an MS-MS spectra of each respective mass. This data clearly shows that two different proteins exist between the two species. Several strains of each *Bacillus* species were analyzed and all of the data was consistent with the figures shown.

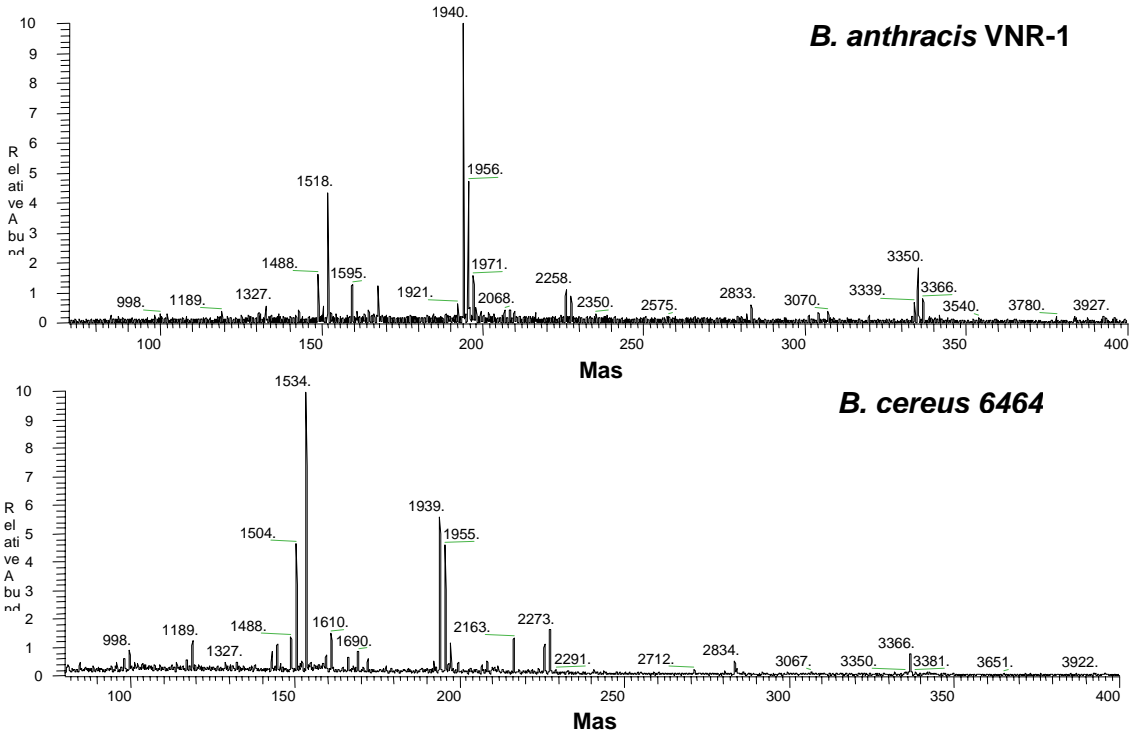


Figure 1: Atmospheric Pressure MALDI Iontrap MS Spectrum from a Tryptic digest of *Bacillus anthracis* VNR-1 (Top) and *Bacillus cereus* 6464 (Bottom)

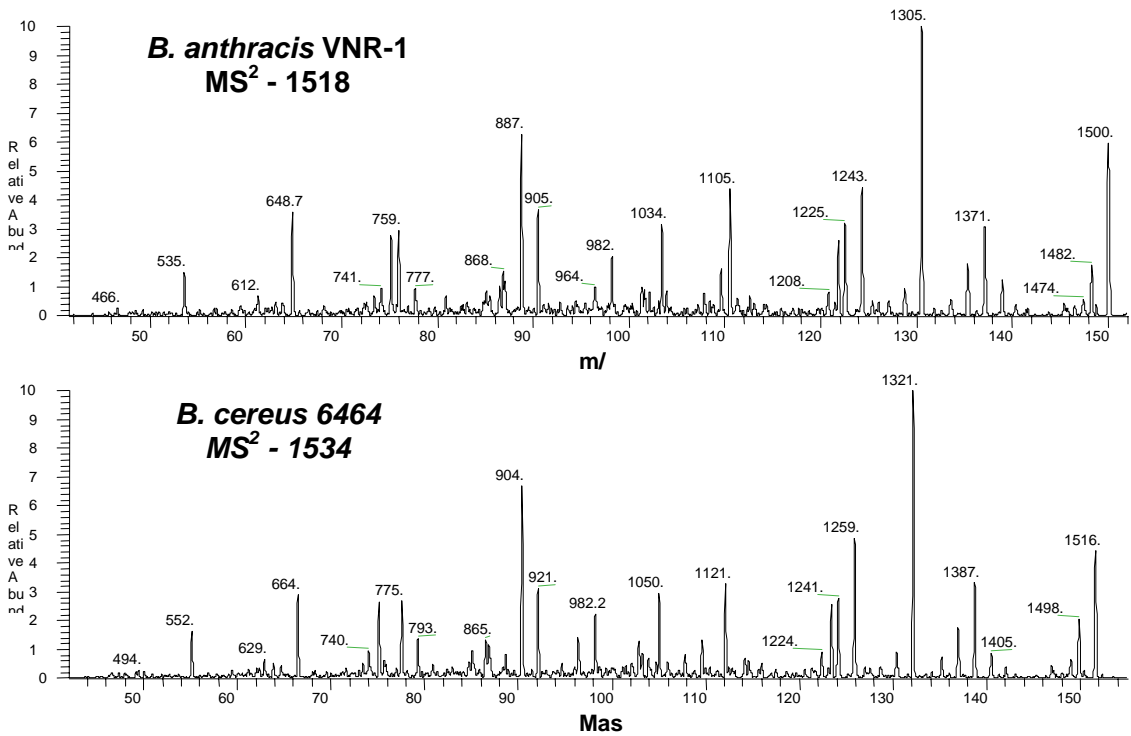


Figure 2: MS-MS of Figure 1, (Top) *B. anthracis* VNR-1 of m/z 1518 and (Bottom) *B. cereus* 6464 of m/z 1534.