Application Note: 1201

# Analysis of Microorganisms by Atmospheric Pressure Matrix Assisted Laser Desorption/Ionization and MT Explorer 100 Ion Trap Mass Spectrometer

Appavu Sundaram, Jane Razumovski, Berk Oktem,

Jianhua Tang, Vladimir Doroshenko, MassTech Inc, Columbia, MD, USA

## Overview

The use of an AP MALDI ion source coupled to the field deployable MassTech MT Explorer 100 ion trap mass spectrometer enables fast and specific analysis of microorganisms.

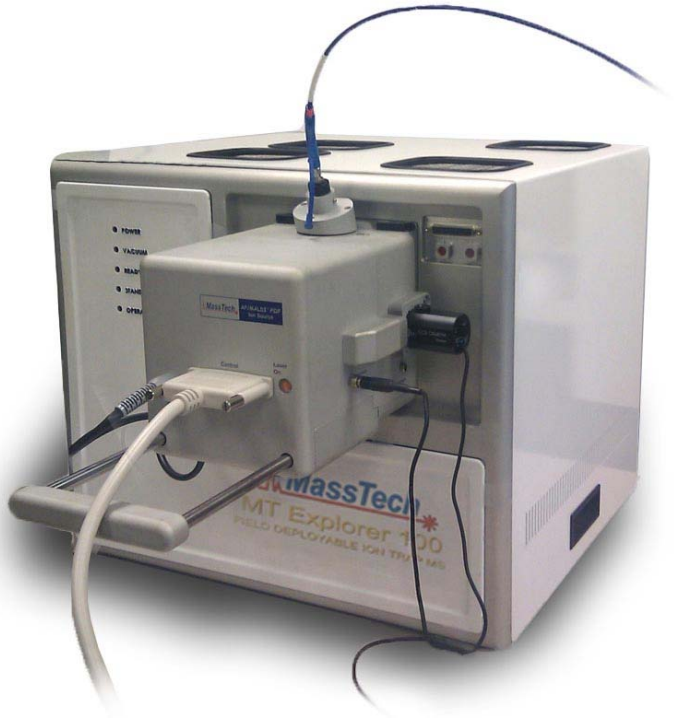


Figure 1: AP MALDI PDF$^{+}$ ion source coupled to MT Explorer 100 MS.

**Keywords:**

AP MALDI
MT Explorer 100
Microorganisms

## Introduction:

Mass spectrometry has become an important analytical tool in biological work, as it provides high throughput, sensitive and specific analysis of microorganisms[1]. Development of soft ionization techniques, such as Electrospray Ionization (ESI) and Matrix Assisted Laser Desorption/Ionization mass spectrometry (MALDI) has significantly improved mass spectrometric characterization and quantification of thermally labile biomolecules such as peptides and proteins[1-3]. Mass spectrometric "Top-down" and "Bottom-Up" approaches have greatly enhanced proteomic research, especially in microbial proteomic. Mass spectrometric methods have the ability to provide taxonomically relevant information in a rapid fashion, using proteomics based approaches allowing either rapid fingerprinting of microbial proteins or high-throughput micro sequencing of protease (trypsin) digested microbial peptides. Fragmentation patterns of each peptide provide peptide's sequence information and are employed by bioinformatics tools which search protein databases to match the proteins. With the tandem mass spectrometry (MS/MS) capabilities of the ion trap mass spectrometer, Atmospheric Pressure Matrix Assisted Laser Desorption/Ionization (AP MALDI) has become a powerful tool to confirm peptide/protein identities which in turn can be used for rapid identification of microbial samples in a number of applications including, environmental sample analysis, food safety concerns and clinical diagnosis of diseases[4, 6-8]. In this application note we show two examples of microbial analysis by AP MALDI source coupled to field deployable MT Explorer 100 ion trap mass spectrometer using "Bottom Up" approach.

## Methods

### Preparation of *Salmonella enterica* enteritidis

1 µL of *Salmonella* sample on a MALDI plate incubated at 42 °C, an equal volume of tris-carbonate-methanol reagent (50 mM trizma and 250 mM ammonium carbonate in 50% methanol) was added. 1 µL of trypsin was then added to the sample mixture and allowed to dry out. During this process certain *Salmonella* proteins (especially Flagellin proteins) are selectively extracted and digested. Once the sample dries out, the sample spot is then rapidly cleaned by adding 3 µL of water and removing after 5 seconds. This rapid water wash helps remove salts, detergents and certain cell debris. The digested peptides present in the sample spot are then co-crystallized with MALDI matrix by adding 1 µL of α-cyano 4-hydroxy cinnamic acid (CHCA, 10 mg/mL in 70% acetonitrile/0.1% trifluoroacetic acid) and allowing the spot to dry out. The processed sample spot is then analyzed by AP MALDI and MT Explorer 100 ion trap mass spectrometer in positive ion mode.

### Preparation of *Bacillus globigii* spores

We have modified the protocol developed by Fenselau and co-workers to extract small acid soluble proteins (SASPs) from spores, to suit the rapid analysis of microbial samples for analysis by AP MALDI MS[5, 7-9]. To 1 µL of bacterial spores on a MALDI target plate incubated at 50 °C, an equal volume of 50% acetonitrile in 1% trifluoroacetic acid was added and the sample was allowed to dry out. As the sample dries out, SASPs were extracted out from the spores. The extracted SASPs were then digested by adding 1 µL of trypsin and incubating at 50 °C until the sample dried out. At this stage the tryptic peptides were purified by the on-probe (C18 coated target plate) sample clean up method by washing with water. The purified tryptic peptides were then co-crystallized with CHCA and then analyzed by AP MALDI and MT Explorer 100 ion trap mass spectrometer in positive ion mode.

## Mass Spectrometry

All mass spectrometry experiments were carried out on a MT Explorer 100 (MassTech Inc., Columbia, MD, USA) ion trap mass spectrometer integrated with an AP MALDI source with pulsed dynamic focusing (MassTech Inc., Columbia, MD, USA) using positive ionization mode. A high repetition rate (up to 200 Hz) solid state Nd:YAG laser ($\lambda$ = 355 nm) was used. Laser pulse duration was approximately 5 nanoseconds, and the laser beam was focused to approximately 500 μm size spot. During analysis, the laser energy was attenuated to about 60 μJ/pulse for MS/MS data collection and to about 50 μJ/pulse for MS data collection. Sample spots and laser pulse were observed using a CCD camera. Experiments were carried out in a repetitive laser shot mode (frequency 30 Hz). Operating conditions for MT Explorer 100 ion trap mass spectrometer were as follows: automatic gain control (AGC) was turned off, ion injection time was 300 ms, and the temperature of the capillary was held at 180 °C. Spectra were acquired over a 1 min span and then averaged.

## Results and Discussion:

### Analysis of *Salmonella enterica* enteritidis:

*Salmonella* is a Gram negative, non-spore forming enterobacteria, often associated with food-borne illnesses. Treatment of vegetative cells with different chemicals will yield different set of proteins, depending on the extent of cell lysis and pH dependant solubility of the proteins. It should be noted that unlike those in spores, a number of different proteins can be extracted from the cells due to their active metabolic status. Sample preparation of *Salmonella* cells for analysis by AP MALDI MS includes treatment with tris-carbonate-methanol reagent to extract mostly flagellin proteins, although other proteins such as acyl carrier proteins (ACP) may also be extracted. After extracting the flagellin proteins, trypsin is then added to digest these proteins. After a rapid washing step, the digested peptides are then co-crystallized with CHCA for further analysis by AP MALDI MS. The washing step helps remove any salt and detergent that might interfere with the MALDI process. An AP MALDI MS spectrum of *Salmonella enteritidis* prepared as described above is shown in Figure 2. The tryptic peptides obtained from *Salmonella* proteins are indicated in the spectrum. An AP MALDI MS/MS spectrum of *Salmonella*-specific biomarker peptide with m/z value of 2008 (from Figure

2) is shown in Figure 3. When this AP MALDI MS/MS data for the peptide ion with an m/z value of 2008 was used for searching against public protein data base (NCBInr) using MASCOT Search engine (Matrix Science), the corresponding peptide sequence (FNSAITNLGNTVNNLTSAR) and the information about the associated proteins (flagellar proteins from *Salmonella enterica* species) were obtained.

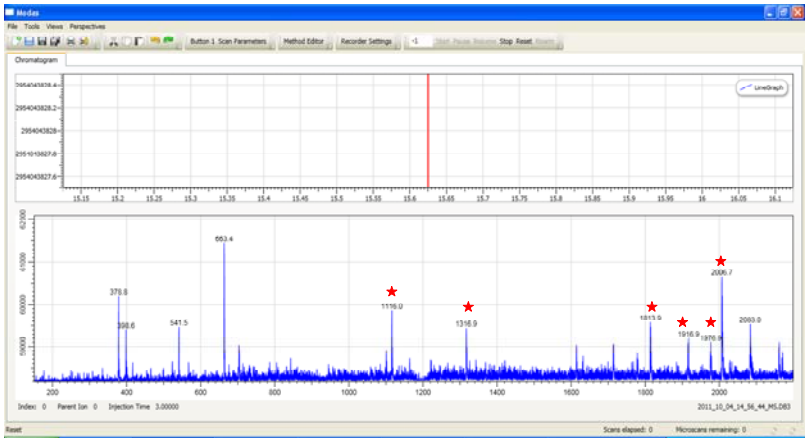

Figure 2: AP MALDI MS spectrum of *Salmonella enterica* enteritidis ($10^7$ cfu) after chemical processing and digestion by trypsin. *Salmonella* biomarker peptides are indicated.

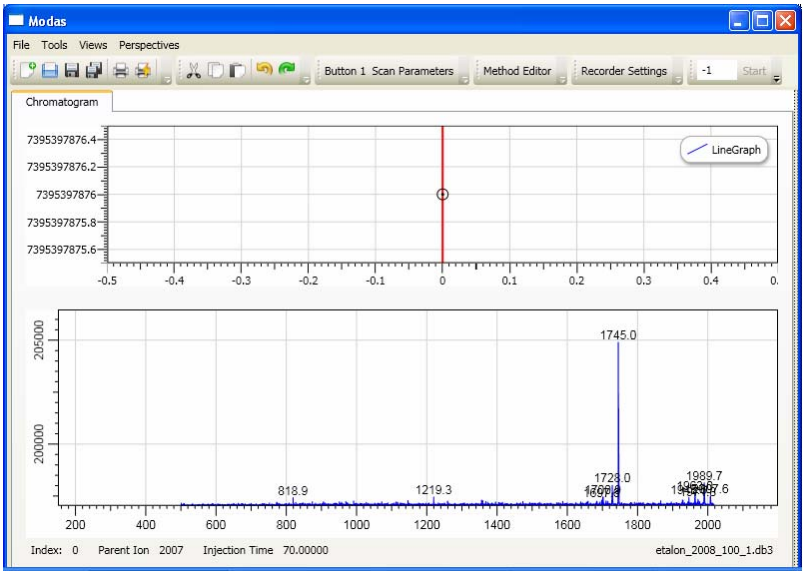


Figure 3: AP MALDI MS/MS spectrum of peptide ion with m/z value of 2008 in Figure 2.

## Analysis of *Bacillus globigii* spores

*Bacillus globigii* is a Gram positive, spore forming non-pathogenic bacteria that is normally used as a simulant for *B. anthracis* because its particle size and dispersal characteristics are similar to that of *B. anthracis*. Extraction of small acid soluble spore proteins (SASPs) from various *Bacillus* spores by treating with 10% trifluoroacetic acid followed by analysis using mass spectrometry has been reported by Fenselau and co-workers[8]. SASPs are present only in spores and are consumed by enzymatic hydrolysis during the spore germination process. Therefore, SASPs serve as reliable targets for detecting the presence of spores. We have since modified this extraction protocol, as described in the experimental section, to suit the rapid analysis of microbial samples by AP MALDI MS. Figure 4 shows the AP MALDI MS spectrum recorded for the trypsin digested SASPs from *B. globigii* spores, using MT Explorer 100 ion trap mass spectrometer. Peptides with m/z values of 1584 and 1928 indicated in Figure 4 are tryptic peptides of SASPs from *B. globigii* spores. AP MALDI MS/MS spectrum of peptides with m/z values of 1584 and 1928 (from Figure 4) are shown in Figures 5 and 6 respectively. When these AP MALDI MS/MS data were used for searching against public protein data bases using MASCOT Search engine, the corresponding peptide sequences (**LVSFAQQNMSGQQF** for the peptide with m/z = 1585 and **FEIASEFGVNLGAETTSR** for the peptide with m/z = 1928)and the information about the associated proteins (small acid soluble spore proteins from *B. globigii*) were obtained. From the database search analysis, the peptide ion with m/z value of 1585 was identified as the unique biomarker for *B. globigii* while the peptide ion with m/z value of 1928 was present in SASPs of other species as well.

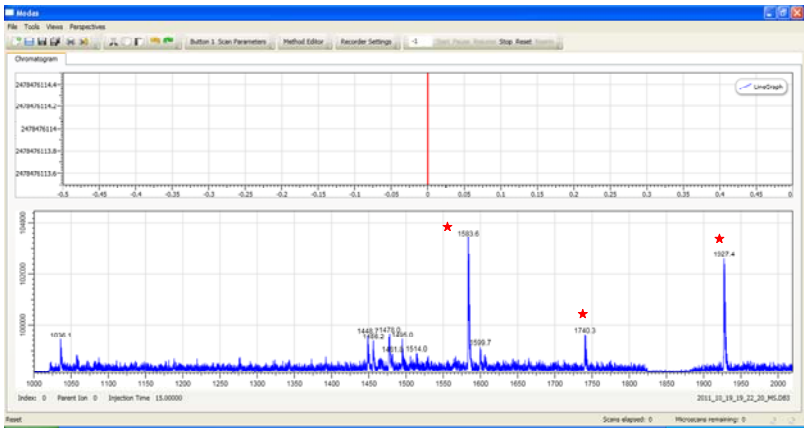

Figure 4: AP MALDI MS spectrum of *Bacillus globigii* spores ($3 \cdot 10^6$ cfu) after chemical processing and digestion by trypsin. *Bg* spores biomarker peptides are indicated.

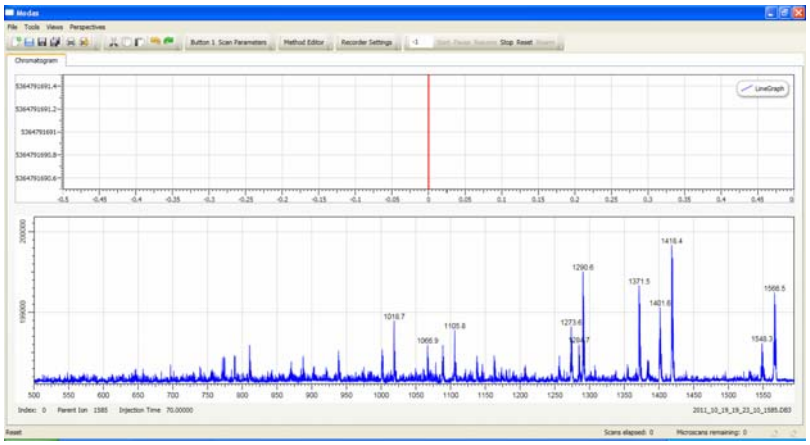

Figure 5: AP MALDI MS/MS spectrum of peptide ion with m/z value of 1584 in Figure 4.

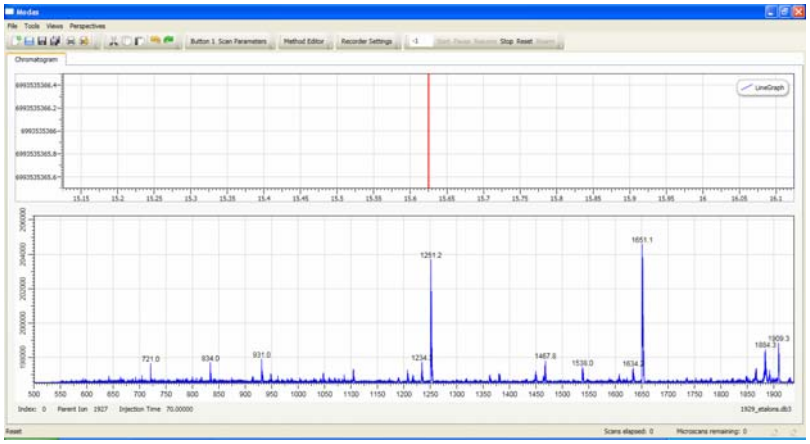

Figure 6: AP-MALDI MS/MS spectrum of peptide ion with m/z value of 1928 in Figure 4.

**Summary**:

AP MALDI MS/MS data for the tryptic peptides of microorganisms obtained using MT Explorer 100 ion trap mass spectrometer can be successfully applied for rapid detection and identification of microorganisms as shown in this application note. Sample preparation protocols required for microorganisms analysis by AP MALDI MS using MT Explorer 100 ion trap mass spectrometer are simple and can be completed in less than 10 minutes.

**References:**

1. Demirev, P. A., J. Ramirez, and C. Fenselau, *Tandem mass spectrometry of intact proteins for characterization of biomarkers from Bacillus cereus T spores.* Anal Chem, 2001. **73**(23): p. 5725-31.
2. Demirev, P. A. and C. Fenselau, *Mass spectrometry in biodefense.* J Mass Spectrom, 2008. **43**(11): p. 1441-57.
3. Doroshenko, V. M. and R. J. Cotter, *Pulsed gas introduction for increasing peptide CID efficiency n a MALDI/quadrupole ion trap mass spectrometer.* Anal Chem, 1996. **68**(3): p. 463-72.
4. Doroshenko, V. M., V. V. Laiko, N. I. Taranenko, V. D. Berkout, and H. S. Lee, *Recent Developments in Atmospheric Pressure MALDI MS.* J. Mass Spectrom, 2002. **221**: p. 39-58.
5. Hathout, Y., P. A. Demirev, Y. P. Ho, J. L. Bundy, V. Ryzhov, L. Sapp, J. Stutler, J. Jackman, and C. Fenselau, *Identification of Bacillus spores by matrix-assisted laser desorption ionization-mass spectrometry.* Appl Environ Microbiol, 1999. **65**(10): p. 4313-9.
6. Laiko, V. V., S. C. Moyer, and R. J. Cotter, *Atmospheric pressure MALDI/ion trap mass spectrometry.* Anal Chem, 2000. **72**(21): p. 5239-43.
7. Sundaram, A. K., B. Oktem, J. Razumovskaya, S. N. Jackson, A. S. Woods, and V. Doroshenko, *Sample Preparation in Biological Analysis by Atmospheric Pressure Matrix Assisted Laser/Desorption Ionization (AP-MALDI) Mass Spectrometry*, in *Sample Preparation in Biological Mass Spectrometry*, Ivanov, A. R. and A. V. Lazarev, Editors. 2011, Springer. p. 749 - 764.
8. Warscheid, B. and C. Fenselau, *Characterization of Bacillus spore species and their mixtures using postsource decay with a curved-field reflectron.* Anal Chem, 2003. **75**(20): p. 5618-27.
9. Warscheid, B., K. Jackson, C. Sutton, and C. Fenselau, *MALDI analysis of Bacilli in spore mixtures by applying a quadrupole ion trap time-of-flight tandem mass spectrometer.* Anal Chem, 2003. **75**(20): p. 5608-17.