

High Throughput Sample Preparation for Atmospheric Pressure MALDI-MS for Rapid Detection and Identification of Microorganisms

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INTRODUCTION

Rapid detection and identification of pathogenic microorganisms in contaminated food and environmental samples is critical for treatment of the infected patients as well as for necessary decontamination efforts.

Traditional microbiological methods to identify pathogens are time consuming, taking 24 to 48 hours for the confirmatory results to arrive.

MALDI-MS has often been successfully used for rapid characterization of microorganisms. However due to introduction of the samples into vacuum, this often requires human intervention.

In this work, we report development of a high throughput atmospheric pressure (AP) MALDI-MS based assay. It offers important benefits such as seamless integration of sample preparation with MS and fast overall analysis, on the order of minutes.

EXPERIMENTAL METHOD

For initial studies, we modified a Multiprobe II laboratory workstation (Perkin Elmer LAS, Shelton, CT) which was modified to include customized liquid handling by BioHIT (Helsinki, Finland). This used for sample preparation on C-18 functionalized AP-MALDI target probe at 50-60°C. (Murphy et. al., 2006 54th ASMS Conference, Seattle WA). Then a custom system is designed to include:

- ✓ Automated plate transfer from sample processing workstation to the mass spectrometer.
- ✓ Fast actuators to reduce sample preparation time.
- ✓ Compact cooling mechanism to increase duration of unattended operation.
- ✓ Expansion possibility to include
 - Different types of liquid handling.
 - Alternative sample introduction possibilities.

Commercial liquid handling workstations typically lack possibility to use μ l amounts of liquid when used with disposable tips.

A Varian 500-MS mass spectrometer is used in integrated system. (See Figure 1) A Thermo LCQ DecaXP is used otherwise.

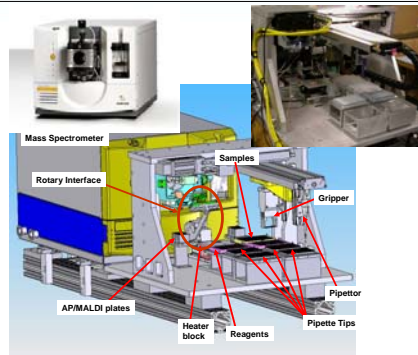


Figure 1. 3-D Model and actual picture of the automated system

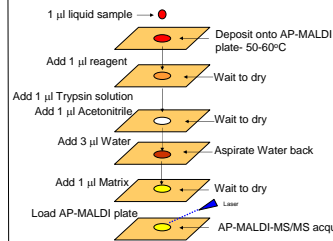


Figure 2. On-Probe sample processing protocol. Agent specific biomarker protein extraction reagents are: Spores: 10% trifluoro acetic acid (TFA); Virus: 50% aqueous ammonium hydroxide; Vegetative cells: 1%TFA in 50% acetonitrile; Toxins: None

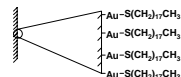


Figure 3. C18 coated gold plate showing the magnified surface. Sample processing on C-18 functionalized plates enabled automation of sample clean up on the AP/MALDI plate.

RESULTS AND DISCUSSION

Automation Considerations

- ✓ Solution stability
- ✓ Reagent stability
- ✓ Reagent containment/access
- ✓ Speed
- ✓ Reproducibility



Figure 4. Picture of processed plate. 0.5 μ l PBS spotted.

Analysis of Microorganisms

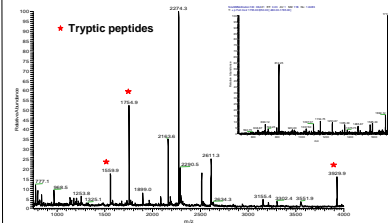


Figure 5. AP-MALDI MS and MS/MS spectrum (inset) of MS2 bacteriophage capsid protein after on-probe sample processing. Concentration of MS2 was 2.5×10^6 pfu/sample.

Mascot Search Results

Peptide m/z	Sequence	Protein Match
MS2 1754.5	K.VATQTVGGVLPVAAWR.S	Coat protein (COAT_BPF2)
BG 1584.5	R.LVYFAQQNMSGQGF-	Small Acid-soluble spore protein 2 (SASP-2)

Figure 6. Table of Peptides for BG and MS2

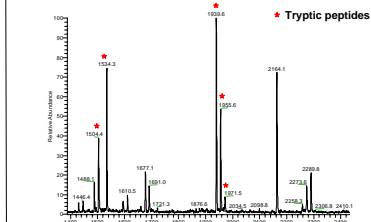


Figure 7. AP-MALDI MS spectrum of spores of *Bacillus thuringiensis* (Small acid soluble protein) after on-probe sample processing. 4×10^6 spores/sample

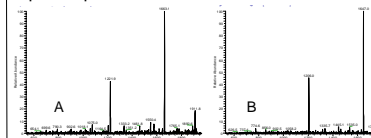


Figure 8. AP-MALDI MS/MS spectra of peptide ions with m/z value of A) 1955 and B) 1939 from Figure 7.

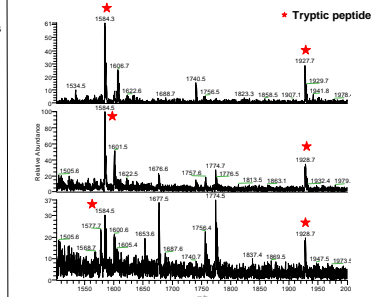


Figure 9. AP-MALDI MS spectra of spores of *Bacillus globigii* (Small acid soluble protein) A) 5×10^6 spores/sample, B) 10^6 spores/sample and C) 5×10^4 spores/sample

Automated Analysis

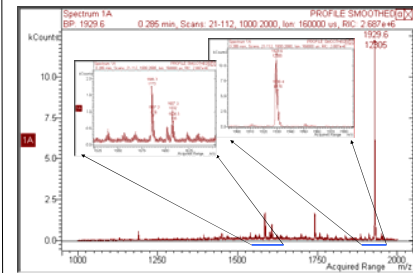


Figure 10. Mass spectrum obtained from a BG sample with a concentration of 1.5×10^6 spores/sample using the fully automated system.

Data Analysis/automated database search discussed in another presentation at this conference: "Microorganism Identification by MS/MS Typing Using Spectral Correlation Methods" (ThPJ 159) by Razumovskaya et al.

CONCLUSIONS

- ✓ AP-MALDI MS/MS assay takes less than ten minutes per sample to get the confirmatory results.
- ✓ Since the detection is proteomics based, design of any species specific binding protein such as antibodies are not needed, allowing for detection of any pathogen as long as the protein has been sequenced and available in the database
- ✓ AP-MALDI MS based detection system can detect and identify any number of pathogens

ACKNOWLEDGEMENTS

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