

AP-MALDI MS/MS and Proteomics based Rapid Detection of Food-borne Pathogens



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INTRODUCTION

Sensitive and accurate detection and identification of food-borne pathogens is a major concern for public health agencies and food industries. CDC estimates that 76 million people suffer from food-borne illnesses each year and more than 5000 deaths per year in the US. The economic impact of foodborne illnesses has been estimated to 5 to 6 billion dollars per year from direct medical expenses and lost productivity. Although traditional microbiological methods are sensitive and accurate, they are very time consuming (more than 2 to 4 days for obtaining confirmatory results) and can not be automated. Simultaneous screening and detection of multiple pathogens is also not possible by traditional methods, since each pathogen requires completely different sets of reagents and protocols. In this study we report a bioinformatics based approach using atmospheric pressure matrix assisted laser/desorption ionisation mass spectrometry (AP-MALDI MS) for rapid, automated detection of food-borne pathogens.

MATERIALS AND METHODS

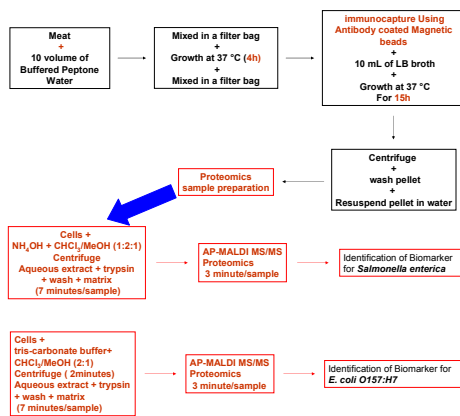
Reagents: α -cyano-4-hydroxycinnamic acid (4-CHCA) was from Fluka (Buch, Switzerland). Immobilized trypsin beads was from Applied Biosystems (Foster City, CA, USA). All other chemicals were purchased from Sigma Chemical Company (St. Louis, MO, USA).

Microrganisms: *Salmonella enterica subsp. Enterica* (ATCC 13076) and *E. coli* O157:H7(ATCC 43895) were purchased from ATCC (Manassas, VA) and grown using standard microbiological practices.

Mass Spectrometry:

All mass spectral experiments were carried out on a Thermo Finnigan (San Jose, CA, USA) LCQ Deca XP ion trap mass spectrometer fitted with an AP-MALDI ion source with pulsed dynamic focusing (MassTech, Inc., Columbia, MD, USA) using positive ionization mode. C-18 coated MALDI target plates were prepared according to published procedure.

Sample processing protocol:



Results

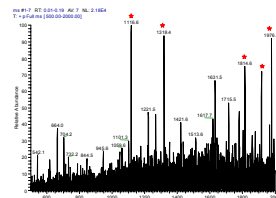


Figure 1. AP-MALDI MS of *Salmonella enterica subsp. Enterica*

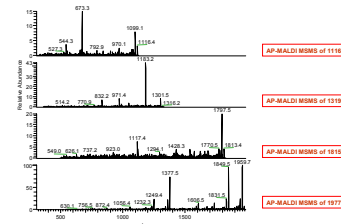


Figure 2. AP-MALDI MSMS of peptides with m/z = 1116, 1319, 1815 and 1977 (from Figure 1)



Figure 3. Mascot database search result using MSMS data from Figure 2.

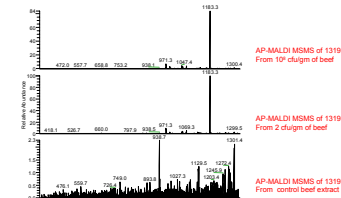


Figure 4. AP-MALDI MSMS of m/z = 1319 for processed spiked beef samples.

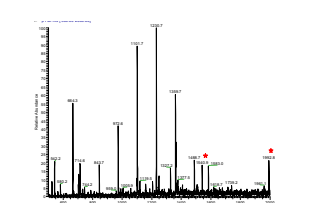


Figure 5. AP-MALDI MS of *E. coli* O157:H7 cells after proteomic sample processing.

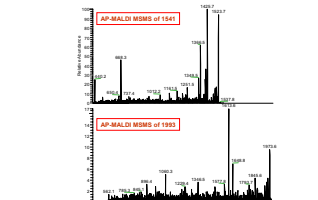


Figure 6. AP-MALDI MSMS of peptides with m/z = 1541 and 1993

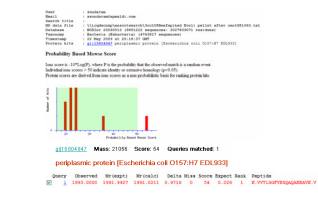


Figure 7. Database search result using MSMS data from Figure 6.

Salmonella Species
 Immunocapture-assisted protocol for *Salmonella* followed by AP-MALDI-MS/MS enabled us to detect less than 2 cfu per gram of ground beef in less than 24 hours.

Biomarker peptides used for AP-MALDI-MS/MS: Phase I Flagellin protein
 K.SIQDEIQQR.L (1116.18)
 K.IQGVADGETITDLQK.I (1814.98)
 R.SRIEDADYATEVSNMSK.V (1916.02)
 R.LEEIDRVSNQTFNGVK.V (1977.13)

E. coli O157:H7:
 Immunocapture-assisted protocol for *E. coli* O157:H7 followed by AP-MALDI-MS/MS enabled us to detect less than 10³ cfu per gram of ground beef in less than 24 h.

Biomarker peptides used for AP-MALDI-MS/MS: Periplasmic protein
 K.VVTLSGFVESAQAEEAVK.V (1991.99)
 Hypothetical protein Z4376
 K.DASGTINVVDIDHKR.V (1539.99)

CONCLUSIONS

Summary:
 Immunocapture followed proteomic sample preparation and AP-MALDI MSMS enabled us to detect food-borne pathogens in less than 24 hours with high sensitivity, and high specificity

This protocol and method can be easily automated

This method can be used for high throughput screening of food products

Further Research:

- Detection and identification of additional biomarkers (O157 antigen and H7 antigen based biomarkers for *E. coli* O157:H7)
- Optimization to improve LOD (< 1cfu/10 gm of beef)
- Simultaneous screening for multiple pathogens
- Complete automation of the sample analysis