

Optimization of Enzymatic Sample Processing for the Detection of Bioagents Using AP-MALDI-MS/MS



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OVERVIEW

Purpose
 Minimizing the time required for *in situ* sample processing on the MALDI plate without the loss of the qualitative information needed for bio-agent identification

Method
 Bottom up proteomic analysis of MS2 bacteriophage, *Bacillus globigii* spores, and ovalbumin including:

•Gold plated MALDI target with C-18 surface, prepared in house, see poster ThP08 no. 122

•*In situ* digest with immobilized trypsin

•AP/MALDI-MS/MS analysis on an ion trap mass spectrometer

•MASCOT MS/MS Ion Search

Results
 Sample processing at 50°C or 60°C reduced the *in situ* sample processing time by 50% or more, while providing tryptic fragments suitable for MS/MS analysis and subsequent bioagent identification using MASCOT MS/MS ion search; as demonstrated for the bioagent simulants MS2, *Bacillus globigii* spores & Ovalbumin.

INTRODUCTION

MALDI mass spectrometry has been found to be a viable technique for the analysis of intact microorganisms.¹ AP-MALDI MS/MS analysis in particular has proven to be a fast, effective method for the identification of bacteriophage, spores², and toxins. *In situ* protein digestion, using immobilized trypsin, serves as the basis of AP-MALDI detection and identification of these bio-molecules. However, the speed of analysis is limited by the time required for enzymatic digestion and sample evaporation. The aim of the present study was to optimize the *in situ* sample processing, including trypsin digestion and liquid evaporation conditions, so as to minimize sample processing time while retaining MS/MS spectral quality suitable for MASCOT database search.

REFERENCES

- Fenselau, C., and P. A. Demirev, "Characterization of Intact Microorganisms by MALDI Mass Spectrometry," *Mass Spectrom Rev* 20.4 (2001): 157-71.
- Pribil, P. A., E. Patton, G. Black, V. Doroshenko, and C. Fenselau, "Rapid Characterization of Bacillus Spores Targeting Species-Unique Peptides Produced with an Atmospheric Pressure Matrix-Assisted Laser Desorption/Ionization Source," *J Mass Spectrom* 40.4 (2005): 464-74.

MATERIALS & METHODS

Species analyzed
Bacillus globigii spores, 1 mg/mL (2 X10⁸ CFU/mL)
 MS2 viral phage, 10¹⁰ PFU/mL
 Ovalbumin, 1mg/mL

Reagents
 Immobilized Trypsin was purchased from Applied Biosystems(Foster City, CA). The following were purchased from Sigma-Aldrich(St. Louis, MO): alpha-cyano-4-hydroxycinnamic acid (CHCA), Dulbecco's Phosphate Buffered Saline, ammonium hydroxide, and trifluoroacetic acid. All reagents were ACS grade or better.

Sample Preparation
 C-18 MALDI sample plate heated to required temperature using hot plate

1 µL sample solution added to C-18 MALDI plate

Solution evaporated to dryness

Add 1 µL solubilizing reagent*

Solution evaporated to dryness

Add 1 µL of immobilized trypsin

Add 1 µL 100% acetonitrile

Trypsin digest proceeds

Solution evaporated to dryness

Wash MALDI plate with 3 µL D.I. water

Solution evaporated to dryness

Add 1 µL CHCA matrix solution

Solution evaporated to dryness

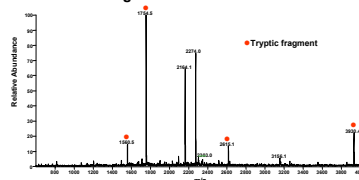
*Solubilizing Reagent
 Spores, 10% TFA
 Virus, 50% NH₄OH
 Toxin, distilled water

Hot Plate
 RT-elite (Model SP136425, Barnstead, Dubuque, Iowa)
 The following temperatures were used: 37, 50, 60, 70, & 80 °C ±2 °C. All determinations were made in triplicate.

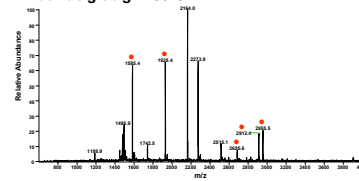
Mass Spectrometer
 All mass spectra were acquired using an LQC Deca XP ion trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA) fitted with an AP/MALDI ion source (MasTech Inc., Columbia, MD, USA) with pulsed dynamic focusing.
Database Searches
 MS/MS spectra were exported to MASCOT (Matrix Science, Boston, MA) as text files for an MS/MS Ion Search. One missed cleavage was allowed. SwissProt data base was searched.

RESULTS

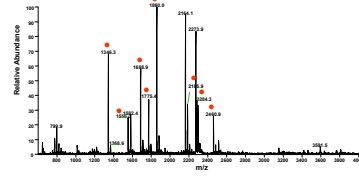
AP-MALDI MS of *In situ* Tryptic Digest
 MS2 Viral Phage: 60°C



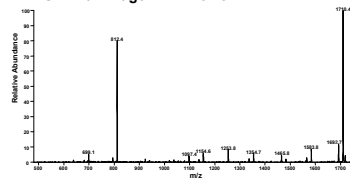
Bacillus globigii : 50°C



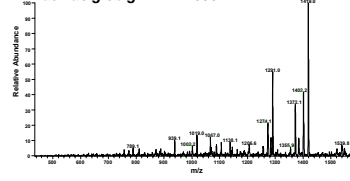
Ovalbumin : 50°C



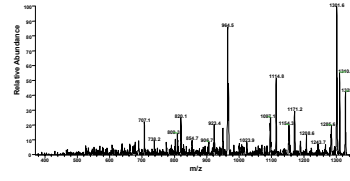
MS/MS of Tryptic Fragments
 MS2 Viral Phage: m/z 1754.5



Bacillus globigii : m/z 1585.4



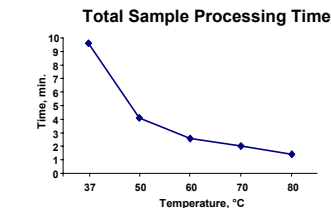
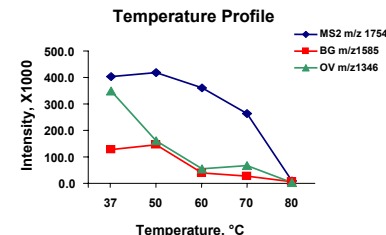
Ovalbumin : m/z 1346.3



MASCOT Results

	Peptide m/z	Sequence	Protein Match	Score ¹	Expected Rank
MS2	1754.5	K.VATQTVGGVLPVAAWR.S	Coat protein (COAT_BPF2)	69	0.00014
BG	1585.4	R.LVSFQAQNMMSGQQF.-	Small Acid-soluble spore protein 2 (SASP-2)	49	0.0055
Ovalbumin	1346.3	K.HIATNAVLFQGR.C	Ovalbumin (OVAL_CHICK)	65	0.00054

¹ The "ions score" is -10*Log(P), where P is the probability that the observed match is a random event.



CONCLUSIONS

Increasing the sample processing temperature from 37 °C to 50 °C resulted in a 57% decrease in the total sample processing time to 4.0 min for *Bacillus globigii* and ovalbumin.

Increasing the temperature of the C-18 MALDI plate from 37 °C to 60 °C resulted in a 73% decrease in the total sample processing time to 2.6 min for MS2.

Immobilized trypsin (Applied Biosystems) was still active up to 60 °C, providing tryptic fragments suitable for MS/MS analysis and subsequent bioagent identification using MASCOT MS/MS ion search; as demonstrated for the bioagent simulants MS2, *Bacillus globigii* spores & Ovalbumin.