

Rapid Detection and Identification of Enterobacterio phage MS2 by Atmospheric Pressure MALDI Ion Trap Mass Spectrometry



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INTRODUCTION

Atmospheric-pressure (AP) matrix-assisted laser desorption/ionization (MALDI) has become a useful technique for detection and identification of bioagents [1-5]. With tandem MS (MS/MS) capabilities offered by ITMS, AP-MALDI becomes a powerful tool for confirmation of mass spectral identities. Sample preparation methods for AP-MALDI are similar to vacuum MALDI.

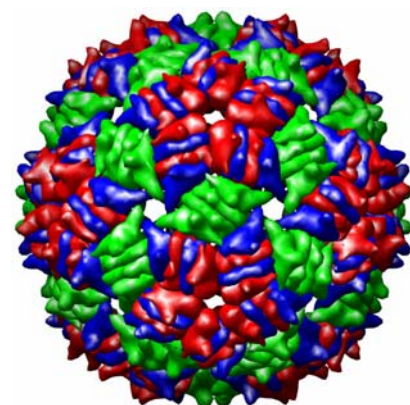


Figure 1. Bacteriophage MS2, 2ms2
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Bacteriophage MS2 belongs to a group of small RNA phages (lentiviruses) that infect F+ *E. coli* and are convenient model systems for the study of viral attachment to host cells, genetic control and virus assembly. The icosahedral protein shell is composed of 180 copies of the coat protein subunit with 129 amino acid residues and a molecular weight of 13,700 Da. The coat protein subunit exists as three conformers, (A, B and C), with the same amino acid sequence but with distinct tertiary structures. Each virus particle also has a copy of the A-protein (maturase protein) that is 393 amino acids long [6,7]. Several methods are currently available for the direct analysis of bacteriophage MS2, using electrospray ionization (ESI) or by vacuum MALDI time-of-flight (TOF) mass spectrometry [8-11].

The aim of the present study was to develop a rapid technique utilizing AP-MALDI MS that would reliably determine and identify the presence of MS2 as a stimulant for pathogenic viruses.

EXPERIMENTAL

Experiments were carried out on a Thermo Finnigan LCQ Deca XP ion trap mass spectrometer integrated with an AP/MALDI ion source equipped with a pulsed dynamic focusing (PDF) technology. AP-MALDI mass spectra and MS/MS spectra of selected peaks for Bacteriophage MS2 were acquired in positive ionization mode. Mascot database search software was used for protein/species identification. Bacteriophage MS2 was prepared with growth medium for the host bacteria, *E. coli*, by the addition of 10 g TryptoPeptone, 5 g BactoTM Yeast Extract, and 8 g NaCl to 1 liter of dd H₂O. *E. coli* Hfr+ strain (American Type Culture Collection, ATCC, No. 15597; Manassas, VA). When bacterial culture reached the optical density (OD) of 0.2 at 600 nm it was infected with a small aliquot of MS2 suspension, that had previously been propagated from purchased stock (ATCC, No.15597-B1) by a similar procedure and stored at 4° C. Propagation of the virus was carried out overnight at 37° C, to ensure complete lysis of the bacterial culture. Bacteriophage MS2 stock suspension was diluted to a final concentration of 2x10⁷ particles/mL and 1 µL sample was taken for AP-MALDI tests (2x10⁴ particles/sample).

RESULTS

Figure 1. AP MALDI mass spectrum of tryptic peptides of MS2 capsid protein in positive MS mode. Peaks corresponding to peptide ions of MS2 coat protein are indicated with stars.

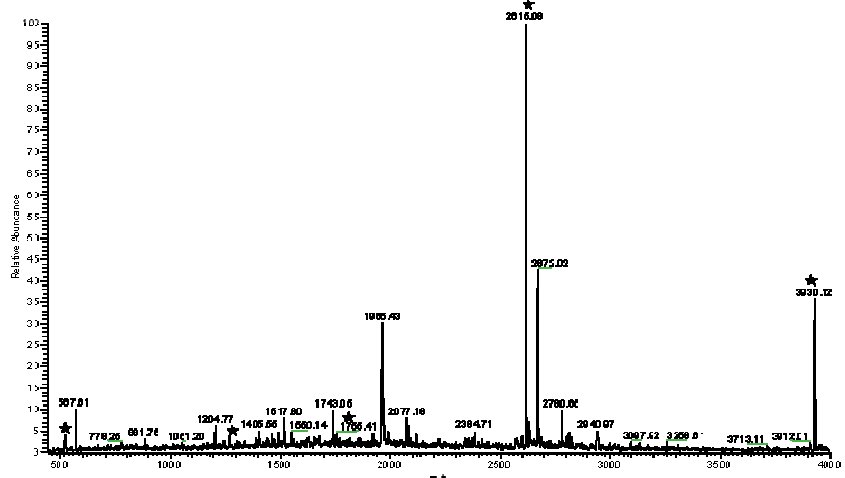
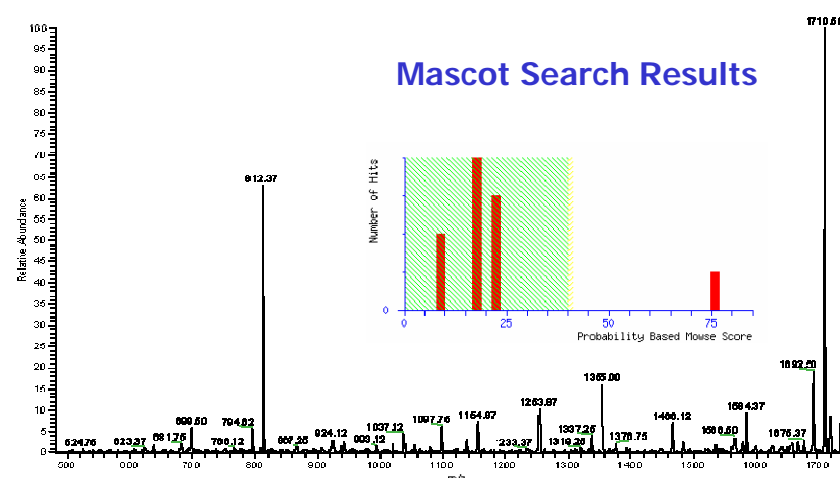


Table 1. Peptide fragments obtained from theoretical digestion of MS2 coat protein with trypsin. Fragments observed in AP-MALDI spectra are shown in red color.

Start - End	Peptide sequence (peptide length)	Calculated peptide mass
1 - 38	ASNFTQFLVDNGGTGDTVAPSINFANGVAEWISSNSR (38)	3930.22
39 - 43	SQAYK (5)	595.65
44 - 49	VTCSVR (6)	633.76
50 - 56	QSSAQR (7)	789.79
57	K (1)	146.19
58 - 61	YTIK (4)	523.62
62 - 66	VEVPK (5)	570.68
39-49	SQAYKVTCSVR (11)	1241.42
67 - 83	VATQTVGGVLPVAAWR (17)	1754.02
84 - 106	SYLNMLTIPIFATNSDCELIVK (23)	2615.03
107 - 113	AMQGLLK (7)	759.96
114 - 129	DGNPIPSAIAANSIGY (16)	1559.68

Figure 2. AP MALDI MS/MS spectrum of peptide ion m/z 1755 derived from the spectrum presented in Figure 1.



Protein View

Match to: **1AQ3A** Score: 76
ms2 protein capsid mutant T59S, chain A - phage ms2 Found in search of D:\MascotSearch\June_16_022.txt Nominal mass (M_r): 13714; Calculated pI value: 8.00 NCBI BLAST search of **1AQ3A** against nr Unformatted **sequence string** for pasting into other applications Taxonomy: **Enterobacterio phage MS2** Links to retrieve other entries containing this sequence from NCBI Entrez: **1AQ3B** from **Enterobacterio phage MS21AQ3C** from **Enterobacterio phage MS21MVBA** from **Enterobacterio phage MS21MVBB** from **Enterobacterio phage MS21MVBC** from **Enterobacterio phage MS2** Cleavage by Trypsin: cuts C-term side of KR unless next residue is P Sequence Coverage: 13% Matched peptides shown in **Bold Red**
1 ASNFTQFLV DNGGTGDTV APSNFANGVA EWISSNSRSQ AYKVTCSVRQ
51 SSAQNRKYSI KVEVPK **VATQ TVGGVLPVA AWR** SYLNML TIPIFATNSD
101 CELIVKAMQG LLKDGNPIS AIAANSIGY Start - End
Observed Mr(expt) Mr(calc) Delta **67 - 83** **1755.00**
1753.99 1754.00 -0.00 K.VATQTVGGVLPVAAWR.S (Ions score 76)

Protein View

Match to: **1AQ4A** Score: 191
ms2 protein capsid mutant T45A, chain A - phage ms2 Found in search of D:\MascotSearch\MS2.com.3.txt
Nominal mass (M_r): 13698; Calculated pI value: 8.00
NCBI BLAST search of **1AQ4A** against nr Unformatted **sequence string** for pasting into other applications
Taxonomy: **Enterobacterio phage MS2**
Links to retrieve other entries containing this sequence from NCBI Entrez: **1AQ4B** from **Enterobacterio phage MS2** **1AQ4C** from **Enterobacterio phage MS2** **1MWAA** from **Enterobacterio phage MS2** **1MWAB** from **Enterobacterio phage MS2** **1MWAC** from **Enterobacterio phage MS2**
Cleavage by Trypsin: cuts C - term side of KR unless next residue is P
Sequence Coverage: 43%
Matched peptides shown in **Bold Red**
1 ASNFTQFLV DNGGTGDTV APSNFANGVA EWISSNSRSQ AYKVTCSVRQ
51 SSAQNRKYSI KVEVPK **VAT Q TVGGVLPVA AWR SYLNML TIPIFATNSD**
101 **CELIVK** AMQG LLK **DGNPIS AIAANSIGY**
Start - End Observed Mr(expt) Mr(calc) Delta
67 - 83 1754.00 1752.99 1754.00
K.VATQTVGGVLPVAAWR.S (Ions score 87) -1.00
84 - 106 2614.70 2613.69 2615.03
R.SYLNMLTIPIFATNSDCELIVK.A (Ions score 48) -1.33
114 - 129 1559.80 1558.79 1559.68
K.DGNPIS AIAANSIGY. - (Ions score 86) -0.88

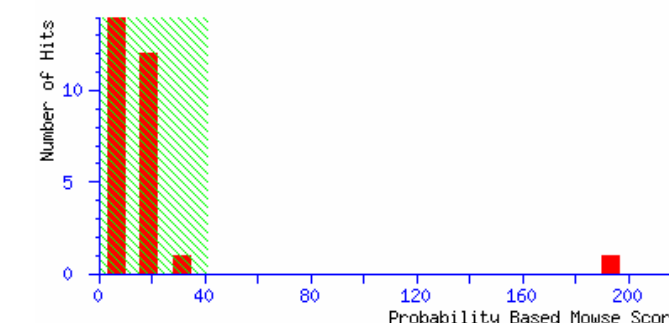


Table 2. The comparison of coat proteins of MS2, M12, JP501, R17 and F2 phages.

A: COAT_BPMS2 Coat protein.- Bacteriophage MS2;
B: Q9T1C7_9VIRU Coat protein.- Enterobacterio phage M12.
C: Q9MCD7_BPJP5 Coat protein.- Bacteriophage JP501.
D: VCBPR7 coat protein - phage R17
E: VCBPF2 coat protein - phage f2

A: ASNFTQFLVDNGGTGDTVAPSINFANGVAEWISSNSRSQAYKVTCSVRQSSAQRKYYTI 60
B: ASNFTQFLVDNGGTGDTVXPSNFANGVAEWISSNSRSQAYKVTCSVRQSSAQRKYYTI 60
C: ASNFTQFLVDNGGTGTVVAPSINFANGVAEWISSNSRSQAYKVTCSVRQSSAQRKYYTI 60
D: ASNFTQFLVDNGGTGNVTAPSINFANGVAEWISSNSRSQAYKVTCSVRQSSAQRKYYTI 60
E: ASNFTQFLVDNGGTGNVTAPSINFANGVAEWISSNSRSQAYKVTCSVRQSSAQRKYYTI 60

← Tryptic fragment → ↑ ↑ ↑ ↑
A: KVEVPKVATQTVGGVLPVAAWRSYLNMLTIPIFATNSDCELIVKAMQGLLKDGNPIS 120
B: KVEVPKVATQTVGGVLPVAAWRSYLNMLTIPIFATNSDCELIVKAMQGLLKDGNPIS 120
C: KVAVPKVATQTVGGVLPVAAWRSYLNMLTIPIFATNSDCELIVKAMQGLLKDGNPIS 120
D: KVEVPKVATQTVGGVLPVAAWRSYLNMLTIPIFATNSDCELIVKAMQGLLKDGNPIS 120
E: KVEVPKVATQTVGGVLPVAAWRSYLNMLTIPIFATNSDCELIVKAMQGLLKDGNPIS 120

A: AIAANSIGY 129
B: AIAANSIGY 129
C: AIAANSIGY 129
D: AIAANSIGY 129
E: AIAANSIGY 129

CONCLUSIONS

We have developed a simple protocol to identify bioagents that includes the use of: (a) fast (within a few minutes) *in situ* dissociation of viral capsid proteins and their proteolysis using trypsin immobilized on beads; (b) AP-MALDI ionization of the tryptic peptides without breaking the vacuum of a mass spectrometer; (c) tandem (or MS/MS) ion trap mass spectrometry analysis to get peptide sequencing information; and finally (d) the use of publicly available proteome/genome databases for reliable identification of viral species.

AP-MALDI is capable of achieving high-throughput screening and meets the requirements for MS/MS analysis on primary structures of proteins.

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