

# Pox proteomics: Mass Spectrometric Analysis and Identification of Vaccinia Virion Proteins

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## Overview:

Variola virus and/or genetically-engineered orthopoxviruses are considered one of the most significant Category A pathogenic threats for malevolent use as potential agents of bioterrorism. Because of the close homology at the nucleotide level, it is believed that a comprehensive proteomic analysis of the vaccinia virion will provide detail knowledge of variola and/or genetically engineered orthopoxviruses. Hence, we have conducted a comprehensive proteomic analysis of the vaccinia virion using different analytical strategies such as sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) or reverse phase high performance liquid chromatography (HPLC) combined with tandem mass spectrometry. In addition, a “shotgun” approach with no further separation was evaluated. In this study, sixty three unique vaccinia proteins were identified by searching uninterpreted tandem mass spectra against the NCBI and an in house VV database with MASCOT and SEQUEST software, of which two were predicted gene products that have not been shown to be expressed before: E6R and L3L. So we believe that these results will help to develop new antiviral drugs/vaccines to treat or prevent the infectious human diseases.

## Introduction:

Poxviruses, such as vaccinia are amongst the largest and most complex of the eukaryotic DNA viruses. Vaccinia virus regulates the expression of more than 250 viral gene products in a temporal fashion during the replication process. This results in the formation of two main infectious forms of vaccinia virion called intracellular mature virus (IMV) and extracellular enveloped virus (EEV). Some membrane and core proteins of the VV virion were identified previously and denoted by their corresponding open reading frames (ORF). The conventional designation of VV ORF consists of a Hind III DNA fragment (A-O), followed by the number of the ORF in that fragment (numbered left to right), and finally by the direction of the ORF (L or R).

## Methods:

- Vaccinia virus (VV) was purified, fractionated into membrane and core-enriched fractions, and further separated by SDS-PAGE or HPLC.
- Beta-octylglucopyranoside (OG) was chosen as the detergent for dissolving the membrane.
- The soluble and insoluble fractions were also analyzed directly with no further separation by shotgun approach.
- The samples were digested with trypsin and analyzed using different mass spectrometers.
- The mass-spectrometry based methods used were:
  - SDS-PAGE combined with LC-ESI-Q-TOF MS/MS
  - SDS-PAGE combined with LC-ESI-QIT MS/MS
  - HPLC combined with LC-ESI-QIT MS/MS
  - LC-ESI-Q-TOF MS/MS
  - MALDI-TOF/TOF MS/MS
- Proteins were identified using following criteria for “positive” matches: greater than 5% of the protein sequence and more than one peptide needed to be identified in a single MS method, or a single peptide needed to be identified at least with two different MS methods.

## Results:

A peptide from the L4R protein using SDS-PAGE + LC-ESI-Q-TOF MS Method

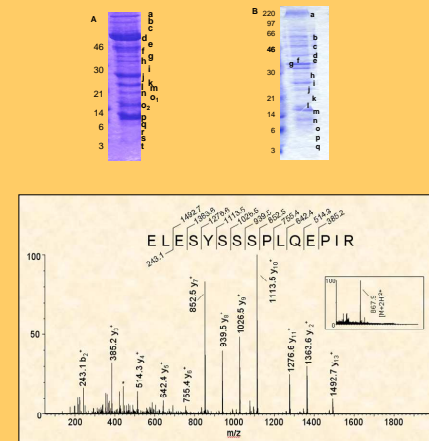


Fig 1: SDS-PAGE gel of core and membrane fractions. Mass spectrum (inset) displays a doubly charged parent ion at m/z 867.9, reveals a peptide of the L4R protein

A peptide from the L1R protein using HPLC + LC-ESI-QIT MS Method

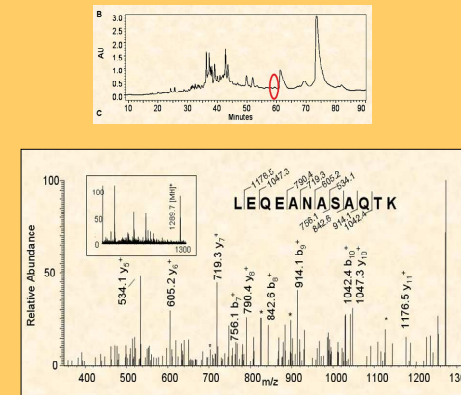


Fig 3: Mass spectrum of fraction 59-60 (marked in red) from HPLC produced from a singly charged precursor ion (inset, m/z 1289.7), yielded fragment ions which corresponded to a peptide of the L1R protein

A peptide from the L3L protein using a shotgun approach i.e; LC-ESI-Q-TOF MS Method

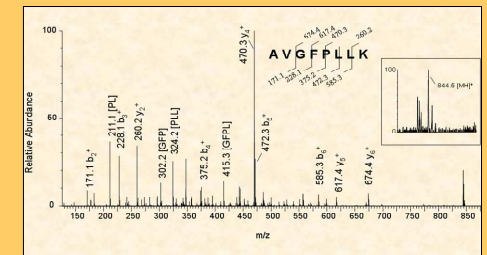


Fig 4: The full scan mass spectrum displays a peak at m/z 844.5 (inset), and corresponding tandem mass spectrum identifies a peptide of the L3L protein.

## Vaccinia virion proteins identified in this study

ORF	Function/location	Methods	# peptides	% Coverage
A3L	Major core protein	1,2,3,4,5	39	71.6
A4L	IMV/P4a associated protein	1,2,3,4,5	16	49.1
A5R	RNA pol. subunit	1,4,5	3	29.9
A7L	Early transcription factor	1,2,4,5	7	12.7
A10L	Major core protein	1,2,3,4,5	62	64.3
A12L	Viral structural protein	1,3,4	4	14.6
A13L	Membrane phosphoprotein	1,2,3,4,5	6	91.4
A14L	Membrane phosphoprotein	1,2,4,5	4	61.1
A15L	Core assoc. protein	1,2,3	5	60.2
A16L	Myristoylprotein; entry/fusion	1,2,5	4	12.2
A17L	IMV membrane prtn	1,2,4,5	4	32.0
A24R	RNA pol. subunit	1,2,4,5	26	30.9
A27L	IMV membrane prtn	1,2,3,4,5	17	70.0
A29L	RNA pol. subunit	2,5	2	8.2
A30L	Virion component	2,3,4,5	3	58.4
A33R	EEV glycoprotein	1,4,5	2	21.6
A34R	EEV glycoprotein	1,2,4	2	23.2
A42R	Profilin homolog	1,2,3,4,5	6	51.1
A46R	Interact with host IL-1	1	2	12.6
A56R	EEV glycoprtn, hemagglutinin	1,4,5	3	12.4
B5R	EEV glycoprotein	4	2	10.4
B22R	Serpin (C16L)	1,2,4,5	3	19.9
D1R	Capping enzyme subunit	1,2,4,5	15	22.7
D2L	virion component	1,2,4,5	9	63.0
D3R	virion component	1,2,4,5	8	50.6
D6R	Early transcription factor	2,5	7	11.9
D8L	IMV membrane protein	1,2,3,4,5	26	89.1
D11L	DNA-dependent ATPase	1,2,5	9	17.3
D12L	Capping enzyme subunit	1,2,4,5	8	40.4
E1L	PolyA polymerase	2,4,5	4	11.1
E3L	dsRNA dep. protein kinase	1,5	1	13.2
E4L	RNA polymerase	1,2,4,5	5	27.8
E6R	unknown	1,2,4,5	20	43.1
E8R	Virion component	1,2,4,5	11	57.1
E10R	Oxidase	2,3,4,5	2	17.9
E11L	Viral core protein	1,2,4,5	2	26.4
F8L	Cytosolic protein	3,4,5	4	60.0
F9L	Mem. prtn.; similarity to L1R	1,2,3,4	4	22.6
F10L	Protein kinase	1,2,5	4	15.7
F13L	EEV membrane protein	1,2,4,5	9	32.0
F17R	DNA binding phosphoprotein	1,2,3,4,5	9	55.4
G1L	metalloproteinase	1,2,4,5	10	19.1
G3L	Entry/fusion complex	2,3,4,5	6	41.4
G4L	glutaredoxin	2,3,4,5	11	77.4
G7L	Core cmpnt, partners w/A30L	1,2,3,4,5	20	59.8
H1L	Protein phosphatase	1,2,3,4,5	10	67.3
H3L	Immunodominant protein	1,2,3,4,5	31	79.0
H4L	RNA pol. associated protein	1,2,4,5	5	10.6
H5R	Membrane phosphoprotein encapsidated DNA-binding protein	1,2,3,4,5	8	49.3
I1L	DNA binding phosphoprotein	2,5	3	18.6
I5L	Virion component	1,4	5	94.9
I7L	Core protein proteinase	2	9	18.4
I8R	RNA/DNA-dependant NTPase	4,5	4	8.7
J1R	IMV membrane protein	1,2,4,5	5	30.1
J3R	Poly(A) polymerase, RNA methyltransferase	1,2,4,5	14	47.4
J4R	RNA polymerase	1,2,4	6	38.4
J6R	RNA polymerase	1,2,4,5	34	33.9
K4L	Homolog to VP37, phoshoplipase D	3,4	4	8.5
L1R	IMV membrane protein	2,3,4,5	8	40.8
L3L	unknown	1,2,4,5	7	22.9
L4R	Major core protein	1,2,3,4,5	25	77.7
O2L	Glutaredoxin	1,2,3,4,5	7	70.4

A peptide from the E6R protein using SDS-PAGE + LC-ESI-QIT MS Method

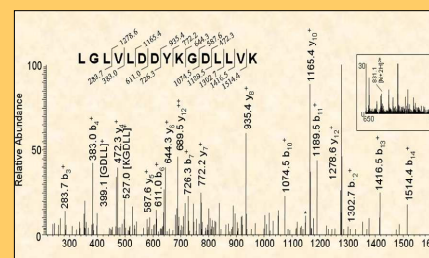


Fig 2: The tandem mass spectrum data, correlating to the full scan mass spectrum (inset, doubly charged parent ion at m/z 831.1), reveals a peptide of the E6R protein.

## Conclusions:

Sixty-three (63) vaccinia virion proteins were identified using five complementary mass-spectrometry-based proteomics strategies

Peptides identified for each protein ranged from 1 to 62 and the sequence coverage of the proteins ranged from 8.2% to 94.9%.

Two new proteins were identified, E6R and L3L using SDS-PAGE combined with LC-ESI-QIT MS and a shotgun approach i.e; LC-ESI-Q-TOF MS methods respectively.

E6R protein has a predicted molecular mass of 66,670 Da and pI of 6.16 and 19 peptides were observed covering 43.1% of the sequence.

The L3L protein has a predicted molecular mass of 40.6 kDa (350 amino acids), and a predicted pI of 8.91 and 7 peptides were observed covering 22.9% of the sequence.

## Acknowledgements:

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