



Antigenic Site Detection and Designing of a Potential Vaccine candidate against *Listeria monocytogenes* a Cause of Listeriosis using Reverse Vaccinology

Anuradha Parihar*, Alekhya Duggishetti¹

Bioaxis DNA Research Centre (P) Ltd. Hyderabad

Email ID: *anuparihar87@gmail.com, ¹alekhya.duggishetti@gmail.com

Received: June 12th 2012, Accepted: June 20th 2012, Published: July 1st 2012

Abstract:

“Listeriosis” is the recent pandemic caused by a bacterium named *Listeria monocytogenes* an intracellular pathogen causes very high mortality rates in the United States. In the Current study, an effort was taken to design a Potent Subunit vaccine against *L. monocytogenes* applying the reverse vaccinology approach. The method includes screening of complete proteome based on its antigenic property against Homo sapiens, identification of potential candidate vaccine, antigenic site prediction, peptide designing, and energy evaluation of the candidate vaccine is performed. A potent vaccine candidate has been selected which can be used as a vaccine against Listeriosis after required validation and clinical trials.

Keywords: Reverse vaccinology, Antigenic emboss, peptide designing.

Introduction:

Listeria monocytogenes is a Gram-positive, facultative intracellular bacterium that can live as a saprophyte, primarily in decaying vegetation in soil and as a pathogen in the tissues of mammals and birds, in which it can cause life-threatening disease⁽¹⁾. It infects a variety of phagocytic and nonphagocytic mammalian cells. Following internalization, the bacteria escapes from the vacuole/phagosome by membrane lysis into the cytosol, where it proliferates. *L. monocytogenes* has the capacity to cross three tight barriers, the intestinal, blood-brain, and fetoplacental barriers. These features are considered to be central to the pathophysiology of listeriosis. Symptoms of listeriosis may show up 2-30 days after exposure. Symptoms in pregnant women include mild flu-like symptoms, headaches, muscle aches, fever, nausea, and vomiting. If the infection spreads to the nervous system it can cause stiff neck, disorientation, or convulsions⁽²⁾. *Listeria* can infect

the placenta, the amniotic fluid, baby, and can cause miscarriage or stillbirth⁽³⁾. Infected babies who survive are likely to be born prematurely. Many will be born severely ill or get sick soon after birth, with problems that can include blood infection, difficulty breathing, fever, skin sores, lesions on multiple organs, and central nervous system infections such as meningitis. Infection can occur at any time during pregnancy. The genome size of *Listeria* is 2.94 mb with taxon ID 169963⁽⁴⁾. The “reverse vaccinology” approach is a genome based approach^{(5), (6)}. The conventional approach to vaccine development requires cultivation of the pathogenic microorganism & its dissection using biochemical, immunological and microbiological method in order to identify the components important for immunity. This method successful in many cases, failed to provide solution for many those pathogens for which a vaccine is not yet available.

Today the possibility of using genomic information by reverse vaccinology approach⁽⁶⁾ allows us to study vaccine development Insilco, without the need of cultivating the pathogen. It reduces the time required for the identification of candidate vaccine and new solution for those vaccines which have been difficult or impossible to develop. It is an improvement on vaccinology, pioneered by *RinoRappuoli*. The objective of this study was to identify immunogenic protein peptide that can serve as potential vaccine for listeriosis by using reverse vaccinology approach.

Materials and methods:

Protein sequence analysis

The proteome of *L. monocytogenes* was retrieved from CMR batch download (Comprehensive microbial resource by J. Craig Venter Institute)⁽⁴⁾. It is a freely available website which is used to

display publicly available information on complete prokaryotic genome and proteome.

Primary screening of proteome: In primary screening all the non required protein sequences are removed (i.e., hypothetical, putative, predicted). These sequences are removed because the role of these sequences in pathogenesis is not known clearly.

Secondary screening: In secondary screening PBLAST⁽⁷⁾ analysis is done against the *Homo sapiens* to identify the sequences that have no significant or less identical matches (<33%) with the protein sequences of the *Homo sapiens*.

Tertiary screening: In tertiary screening TFASTY⁽⁸⁾ is done to check the similarity with *Homo sapiens*, coding DNA sequences (CDS). TFASTY compares a protein sequence to a DNA sequence database, calculating similarities with frame shifts to the forward and reverse orientations. The sequences which showed no similarity or <33% similarity with human CDS were taken and then the further analysis was done.

Antigenic Site Determination:

To find out the antigenic sites within the proteins which we got after the tertiary screening Antigenic EMBOSS⁽⁹⁾ was performed with those sequences. Antigenic peptide prediction (Protein variability server)⁽¹⁰⁾ was used to verify the antigenic sites which give result in forms of peaks. Both finds out the potential antigenic regions of a protein sequence.

Calculation of Surface accessibility area:

It is the surface area of a biomolecule that is accessible to a solvent. Surface accessibility area of antigenic peptides which we got from the antigenic site determination was calculated to find out the protein having highest pathogenesis with specific sites.

Designing and Optimization of the vaccine:

The potential candidate vaccine was designed and optimized by using ArgusLab4.0.1.

Result and discussion:

Selection of the candidate vaccine:

The genome size of listeria is of 2.94 mb. After primary, secondary and tertiary screening of its proteome we got three proteins 1st was Imo0006

DNA gyrase subunit B (gyrB) 2nd was Imo0014 AA3-600 quinol oxidase subunit I (qoxB) and 3rd was Imo0527 transmembrane protein. These proteins showed no or <33% similarity with the known *Homo sapiens* CDS, Hence the protein was selected to design potential vaccine candidate against *Listeria monocytogenes* EGD-e strain.

Antigenic Site Determination:

Results from Antigenic Emboss gave the idea of the antigenic sites presents within these proteins along with antigenic score. Sites having highest score were considered (Table No.1) and further confirmation for the selection of antigenic sites was done with the Antigenic peptide prediction (Protein variability server) method {Table No. 2(i, ii, iii)}. Highlighted areas shows the same sequence as in Antigenic EMBOSS, which were taken for further analysis.

Table No-1: Antigenic sites obtained from Antigenic EMBOSS

Positions	Antigenic peptide site (EMBOSS-Antigenic)	Length	Score
6	RPTVEVIFTVLHAG	98-111	1.182
14	NFISTVGGFLMGVAFLV LCYNIYYSY	489-514	1.262
527	NAPIAVVIAVCGILVFFI	217-234	1.263

Table No-2: Antigenic sites obtained from Protein Variability Server

Table No. 2.i.6th protein

n	Start Position	Sequence	End Position
1	26	DQIQVLEGLEAVR	38
2	50	QRGLHHLVWE	59
3	65	IDEALAGFCTE	75
4	104	GRPTVEVIFTVLHA	117
5	125	GYKVSGLHGVGASVVNA LSTSLEVYVHR	153

Table No. 2.ii: 14th protein

	Start Position	Sequence	End Position
1	11	NEFIVTG	17
2	20	MILGAQISIVLVSIGVVALLT	40
3	52	WISSVDHK	59
4	456	FFWIFVVGFNVCFFPQYFLGL	476
5	482	RIYTYVQ	488
6	495	LN FISTVGGFLMGVAFLVLCYNIYYS	520
7	542	TSSAVPPKYNFAVLP	556
8	590	MVGFVMSVFFFIAGFGLVFY	609

Table No.2.iii:527th protein

	Start Position	Sequence	End Position
1	7	RKVKISMWLWHHLTPQIFAVICVFIIITIALFMP	41
2	68	YDALQFGHFVKEFGIYQ	84
3	89	NQVAIYSSQSIFIQMALLLNK	109
4	112	WSTTVFDVRFLGGLQLALLPAIYLLVAG	140
5	148	WPGYVVAALTVFIFADTAYTAYFN	171
6	174	FSEGLILIMMLYISAGFLLLYQHKY	198
7	202	AMLGLIFVASLILIT	216
8	220	Q NAPIAVVIAVCGILVFF	237

Table No-3: Peptide with Surface accessibility area (SAA)

Protein	Method used to predict antigenic site	Sequence	SAA
6 th	Antigenic Emboss	RPTVEVIFTVLHAG	28
	Protein Variability Server	GRPTVEVIFTVLHA	28
14 th	Antigenic Emboss	NFISTVGGFLMGVAFLVLCYNIYYS	26.93
	Protein Variability Server	LN FISTVGGFLMGVAFLVLCYNIYYS	30.7
527 th	Antigenic Emboss	NAPIAVVIAVCGILVFFI	33.3
	Protein Variability Server	QNAPIAVVIAVCGILVFF	33.3

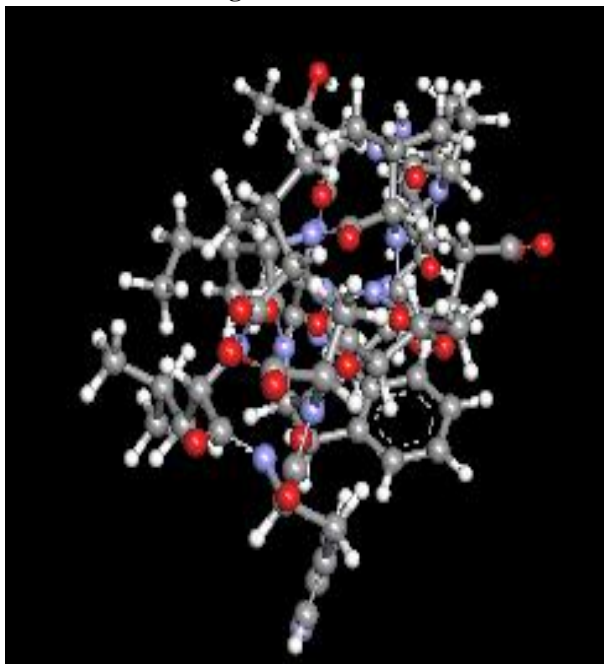
Designing and Energy Optimization:

The peptides i.e.6th RPTVEVIFTVLHAG, 14th LN**FISTVGGFLMGVAFLVLCYNIYYS** and 527th NAPIAVVIAVCGILVFFI were designed using ArgusLab4.0.1. After designing the peptides, their geometry was optimized and energy minimization was performed for the peptide with low energy to make the structures stable.

Table No-4: Geometry Optimization using Argus Lab (4.0.1) table showing the details:

Protein	Energy (au)	delE (au)	Grad Norm	Imax Grad(i)I
6 th	0.227390	-5.8888e-008	0.000384	0.000084
14 th	0.426351	-9.6854e-005	0.019730	0.005060
527	0.319271	-1.7033e-005	0.0025218	0.013875

Figure No. 1: Designed peptide (6th Protein's antigenic site) after energy minimization using ArgusLab4.0.1



7. <http://www.ufpel.edu.br/biotecnologia/gbiotec/site/content/paginadoprofessor/uploadsprofessor/975a6aff2d6cc346803e2c5582e9f72.pdf>.
8. <http://blast.ncbi.nlm.nih.gov/>
9. <http://seqtool.sdsc.edu>.
10. <http://150.185.138.86/cgi-bin/emboss/antigenic> Kolaskar AS, Tongaonkar PC. FEBS Letters 1990, 276:172-174.
11. <http://www.cdc.gov/listeria/statistics.html>.
12. "Listeria monocytogenes outbreak". CFIA. 2009-03-12. Retrieved 2009-03-20.
13. <http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm271899.htm>.
14. <http://www.cdc.gov/listeria/outbreaks/index.html>
15. "Colorado cantaloupes kill up to 16 in listeria outbreak". *BBC News*. September 28, 2011.
16. <http://abcnews.go.com/Health/listeria-found-lettuce/story?id=14641785>

Conclusion:

Through this work, an attempt has been done to design a potential peptide vaccine using the *in silico* approach "Reverse Vaccinology". The peptide RPTVEVIFTVLHAG of DNA gyrase subunit B (gyrB) protein can be used as a potential vaccine candidate against Listeriosis which can be further preceded for vaccine development and clinical trials.

References:

1. Alonzo F, Bobo LD, Skiest DJ, Freitag NE (April 2011). "Evidence for subpopulations of *Listeria monocytogenes* with enhanced invasion of cardiac cells". *J. Med. Microbiol.* 60 (Pt 4): 423–34. DOI: 10.1099/jmm.0.027185-0. PMID 21266727
2. <http://www.cdc.gov/listeria/statistics.html>
3. <http://www.americanpregnancy.org/pregnancycomplications/listeria.html>
4. <http://cmr.jcvi.org>
5. <http://www.ncbi.nlm.nih.gov/pubmed/11257410>
6. <http://www.ufpel.edu.br/biotecnologia/gbiotec/site/content/paginadoprofessor/uploadsprofessor/975a6aff2d6cc346803e2c5582e9f72.pdf>.