Journal of Engineering and Applied Sciences 12 (6): 1603-1611, 2017
ISSN: 1816-949X

Recombinant OprF-OprL-OprI as a Vaccine Against *Pseudomonas aeruginosa* Infections

[1]Farnoush Farjam, [2]Mitra Fakhar Khorasgani, [3]Mojtaba Kiany Boroujeni,
[1]Hooriyeh Ranjbaran, [1]Tayebeh Abdpoursogh,
[1]Navid Farahmandian and [1]Fateme Sefid
[1]Departeman of Biology, Science and Art University, Yazd, Iran
[2]Department of Biology, Ashkezar Branch, Islamic Azad University, Tehran, Iran
[3]Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran

Abstract: *Pseudomonas aeruginosa* is an important cause of nosocomial infection and may lead to septicemia and death. *P. aeruginosa* septicaemia is associated with the highest mortality rate of all gram-negative infections. Because of the general resistance of the organism to antibiotics, research has been focused on immunotherapy. There are several bacterial cell components incorporated into subunit vaccines. Vaccine studies have often focussed on Lipo Poly Saccharide (LPS) and the outer membrane proteins (OPRs) due to its potent stimulation of the immune response. The pathophysiological nature of LPS and its serotype-specific immunological activities has limited LPS using for Pseudomona infection control whereas using major Outer Membrane Proteins of cell walls (mOMPs) of *Pseudomonas aeruginosa* and other gram-negative bacteria, not only is non-toxic and actively stimulate the immune system but also shows immunological cross-reactivity with mOMPs of other serotypes belonging to same species. Today, the protection of mOMPs in many pathogenic gram-negative bacteria against the corresponding etiologic factors have completely been approved. The major OPRs, OprF, OprL and OprI interested in the potential of OPRs as vaccines. Determination of these tertiary structure and theoretical methods for epitope prediction has been led to synthesis of such peptides that are important for immunodiagnostic tests and vaccines. Bioinformatic tools to better understanding and characterizing the OprF, OprL and Oprl structure of *P. aeruginosa* was used. For homology modeling, BLAST was run on the sequence in order to find the best template. The template was then served to model the 3D structure. Also, secondary structure of the proteins was predicted. Moreover, topology, signal peptide and B-cell epitopes of proteins were predicted.

Key words: *Pseudomonas aeruginosa*, vaccine candidate, OprF, OprL, Oprl

INTRODUCTION

Pseudomonas aeruginosa, one of the most important hospital pathogens is an opportunistic gram-negative bacilli. The mortality resulted from infections caused by this bacteria is very common due to its inherent resistance to most common antibiotics. *Pseudomonas aeruginosa* continues to be a cause of life-threatening infections in patients treated in intensive care units (Wessel *et al*., 2013; Glaser *et al*., 2003). Whereas healthy individuals are not in general susceptible to infection by this organism, the risk to granulocytopenic patients such as cancer patients undergoing cytostatic therapy or transplant patients receiving immunosuppressive (with extensive bums, for instance or recovering from major surgery) is dramatically increased (Worgall *et al*., 2005; Lim *et al*., 1997; Glaser *et al*., 2003). The origin of the invading microorganisms is most frequently the patient's own microflora. Damage to the first line of defence such as the skin or the mucous membranes, enables the colonizing bacteria to enter the bloodstream and cause septicemia (Ochs *et al*., 1999; Sugawara *et al*., 1996; Glaser *et al*., 2003). *P. aeruginosa* septicaemia is associated with because of the general resistance of the organism to antibiotics, research has been focused on immunotherapy (Doring and Pier, 2008; Glaser *et al*., 2003).

Virulence factors of this bacteria including toxins, enzymes, flagella, lipopolysaccharide, pili and secretory proteins create severe and fatal infections. The two major antigenic surface-associated components of *P. aeruginosa* are the Lipo Poly Saccharides (LPS) and the Outer Membrane Proteins (OPRs). LPS-based vaccines have been successfully tested in animal models as well as in clinical trials. However, the severe side

Corresponding Author: Fateme Sefid, Departeman of Biology, Science and Art, University,Yazd, Iran

effects observed in vaccinated individuals has made it necessary to develop subunit vaccines (Qian *et al*., 2007; Glaser *et al*., 2003).

The major OPRs, OprF interested in the potential of OPRs as vaccines because OPRs have been shown to be highly conserved and antigenically related in all 17 serogroups of the international antigenic typing scheme (Glaser *et al*., 2003). Due to the accessibility on the bacterial surface, LPS and OM proteins of *P. aeruginosa* are particularly important targets for vaccine studies (Tamber and Hancock, 2004; Glaser *et al*., 2003).

The pathophysiological nature of LPS and its serotype-specific immunological activities has limited LPS using for Pseudomona infection control whereas using major outer membrane proteins of cell walls (mOMPs) of *Pseudomonas aeruginosa* and other gram-negative bacteria not only is non-toxic and actively stimulate the immune system but also shows immunological cross-reactivity with mOMPs of other serotypes belonging to same species. Today, the protection of mOMPs in many pathogenic gram-negative bacteria against the corresponding etiologic factors have completely been approved. OMP-F vesicles with molecular weight of 35-37 kDa are the major outer membrane proteins of *Pseudomonas aeruginosa* cell wall. So, they can be considered useful as vaccine candidate against serotypes of *P. aeruginosa*-infected patients. Also, Oprl and OprL are lipoprotein constituent of secretory pumps in outer membrane of *P. aeruginosa*. Oprl is a lipoprotein with low molecular weight which is always expressed in pseudomonas and its number is high in outer membrane of this genus. Recent studies have shown that the amino acid sequence of the Oprl gene is 3-23% similar to major lipoprotein sequence of *E. coli* OprL. Also called H_2 protein is attached to the peptidoglycan through fatty acyl chain so called peptidoglycan-associated lipoproteins. This protein is highly conserved in pseudomonas. Operl and OprL are used to identify the genus Pseudomonas and genes *Pseudomonas aeruginosa* specie, respectively. These two encode the major lipoproteins of outer membrane involved in antibiotic resistance of bacteria (Priebe and Goldberg, 2014; Sherman *et al*., 2001).

The property of an antigen to bind specifically complementary antibodies is known as the antigen's antigenicity; likewise, the ability of an antigen to induce an immune response is called its immunogenicity. Attempts should be made to discover peptides that could mimic protein epitopes and possess the same immunogenicity as the whole protein. Subsequently, theoretical methods for epitope prediction have been developed leading to synthesis of such peptides that are important for development of immunodiagnostic tests and vaccines (Glaser *et al*., 2003). The present study was designed to in silico resolving the major obstacles in the control or in prevention of the diseases caused by *P. aeruginosa*. We exploited bioinformatic tools to better understanding and characterizing the OprF, OprL and OprI structure of *P. aeruginosa* and select appropriate regions as effective B-cell epitops.

MATERIALS AND METHODS

Sequence availability and homology search: The OprF, OprL and OprI protein sequences with accession No. WP_004885687.1, NP_249664.1 and NP_251543.1 respectively acquired from NCBI at http://www.ncbi.nlm.nih.gov/protein were saved in FASTA format for further analyses. The sequences served as a query for protein BLAST at http://blast.ncbi.nlm.nih.gov/Blast.cgi against non redundant protein database. Probable putative conserved domains of the query protein were also searched for at the above address.

Template search: The query protein sequences were used as an input data for the PSI-BLAST against Protein Data Bank (PDB) at http://blast.ncbi.nlm.nih.gov/Blast.cgi to identify its homologous structures.

Primary sequence analysis: Protparam online software at http://expasy.org/tools/protparam.html was employed for estimation and determination of properties such as molecular weight, theoretical pl, amino acid composition, total number of negatively and positively charged residues, instability index and aliphatic index.

Subcellular localization: Subcellular localization of the proteins was predicted by CELLO at http://cello.life.nctu.edu.tw/.

Topology and signal peptide prediction: SignalP 4.1 server at http://www.cbs.dtu.dk/services/SignalP/ was invoked to predict the presence and location of signal peptide cleavage sites in amino acid sequences from different organisms. The method incorporates a prediction of cleavage sites and a signal peptide/non-signal peptide prediction based on a combination of several artificial neural networks. SPOCTOPUS at http://spoctopus.cbr.su.se/ was also employed to determine membrane protein topology and signal peptides.

Secondary structure prediction: Secondary structure of the proteins was predicted by PSIPRED at http://bioinf.cs.ucl.ac.uk/psipred/.

The PSIPRED Protein Sequence Analysis Workbench aggregates several UCL structure prediction methods into one location. Phyre2 server at 2http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index employed to validate PSIPRED predictions.

3D structure prediction: The SWISS-MODEL workspace at http://swissmodel.expasy.org/ is a web-based integrated service dedicated to protein structure homology modelling. It assists and guides the user in building protein homology models at different levels of complexity. Building a homology model comprises four main steps: identification of structural template (s) alignment of target sequence and template structure (s) model building and model quality evaluation.

These steps can be repeated until a satisfying modelling result is achieved. Each of the four steps requires specialized software and access to up-to-date protein sequence and structure databases.

Models evaluations: All 3D models of the proteins built were qualititatively estimated by GMQE and QMEAN4 scores.

Identification of functionally and structurally important residues: InterProSurf at 9http://curie.utmb.edu/pattest 9.html predicting functional sites on protein surface using patch analysis was employed. The 3D structures determined in a previous step, served as input files for this server.

Single-scale amino acid properties assay: IEDB at http://tools.immuneepitope.org/tools/bcell/iedb_input computed single-scale amino acid properties. Parameters such as hydrophilicity, flexibility, accessibility, turns and antigenic propensity of polypeptide have been correlated with the location of B-cell epitopes. This has led to a search for empirical rules that would allow the position of B-cell epitopes to be predicted from certain features of the protein sequence.

B-cell epitope prediction: Ellipro at http://tools. immuneepitope.org/tools/ElliPro/tutorial.jsp predicts linear and discontinuous antibody epitopes.

RESULTS AND DISCUSSION

Sequence availability and homology search: The OprF sequence with 344 residues, OprL sequence with 168 residues and OprI sequence with 83residues obtained from NCBI and saved in FASTA format. Protein sequence serving as query for BLAST produced a set of sequences as the highest similar sequence. BLAST search revealed numerous hits to the OprF and OprL and OprI subunit sequence. All hits were of Pseudomonas. Putative conserved domains were detected within this sequence.

Template search: PSl-BLAST against Protein Data Bank (PDB) results displayed several hits as homologous structures. The first hit possessing the highest score was selected as a template for homology modelling. The first hit possessing the highest score was selected as a template and show in Table 1.

Table 1: First hits possessing the highest score selected as templates

PDB blast	Accession	Max score	Query coverage (%)	Max ident (%)
OprF	4RLC-A	148.0	44	50
OprL	4G4V-A	134.0	59	63
Oprl	3SLU-A	28.5	83	34

Table 2: OprF, OprL and OprI various physical and chemical parameters

Model	Residue number	Molecular weight	Theoretical pI	Instability index	Aliphatic index	GRAVY
OprF	344	36549.1	4.69	30.77 (stable)	69.74	-0.448
OprL	168	17925.0	5.95	25.79 (stable)	73.27	-0.432
Oprl	83	8834.8	7.90	17.95 (stable)	72.29	-0.541

Table 3: Attribution of secondary structure components in the proteins

Models	Alpha helix (%)	Beta strand (%)	Random coil (%)
OprF	19	35	46
OprL	42	8	50
OprI	89	0	11

Primary sequence analysis: The protein sequence served as input for the computation of various physical and chemical parameters. The computed parameters included the molecular weight, theoretical pI, instability index, aliphatic index and grand average of hydropathicity (indicates the solubility of the proteins: positive GRAVY (hydrophobic), negative GRAVY (hydrophilic)) are summarized in Table 2.

Subcellular localization: OprF and OprL Subcellular localization predicted by CELLO was outer membrane with the highest reliability index (4.848 and 4.016, respectively). OprI subcellular localization was Periplasmic with the highest reliability index (1.930).

Topology and signal peptide prediction: OprF and OprL signal peptide cleavage site was predicted by SPOCTOPUS and SignalP server and shown in Fig. 1.

Secondary structure prediction: Secondary structure of the proteins was predicted by PSIPRED and Phyre2 server. Coil, helix and strands are components constituting secondary structure of the proteins. The secondary structure could be used to validate the tertiary structures. Attribution of secondary structure components in the proteins is listed in Table 3.

3D structure prediction: Building a homology model comprises four main steps: identification of structural template(s), alignment of target sequence and template structure(s), model building and model quality evaluation. These steps can be repeated until a satisfying modelling result is achieved. Each of the four steps

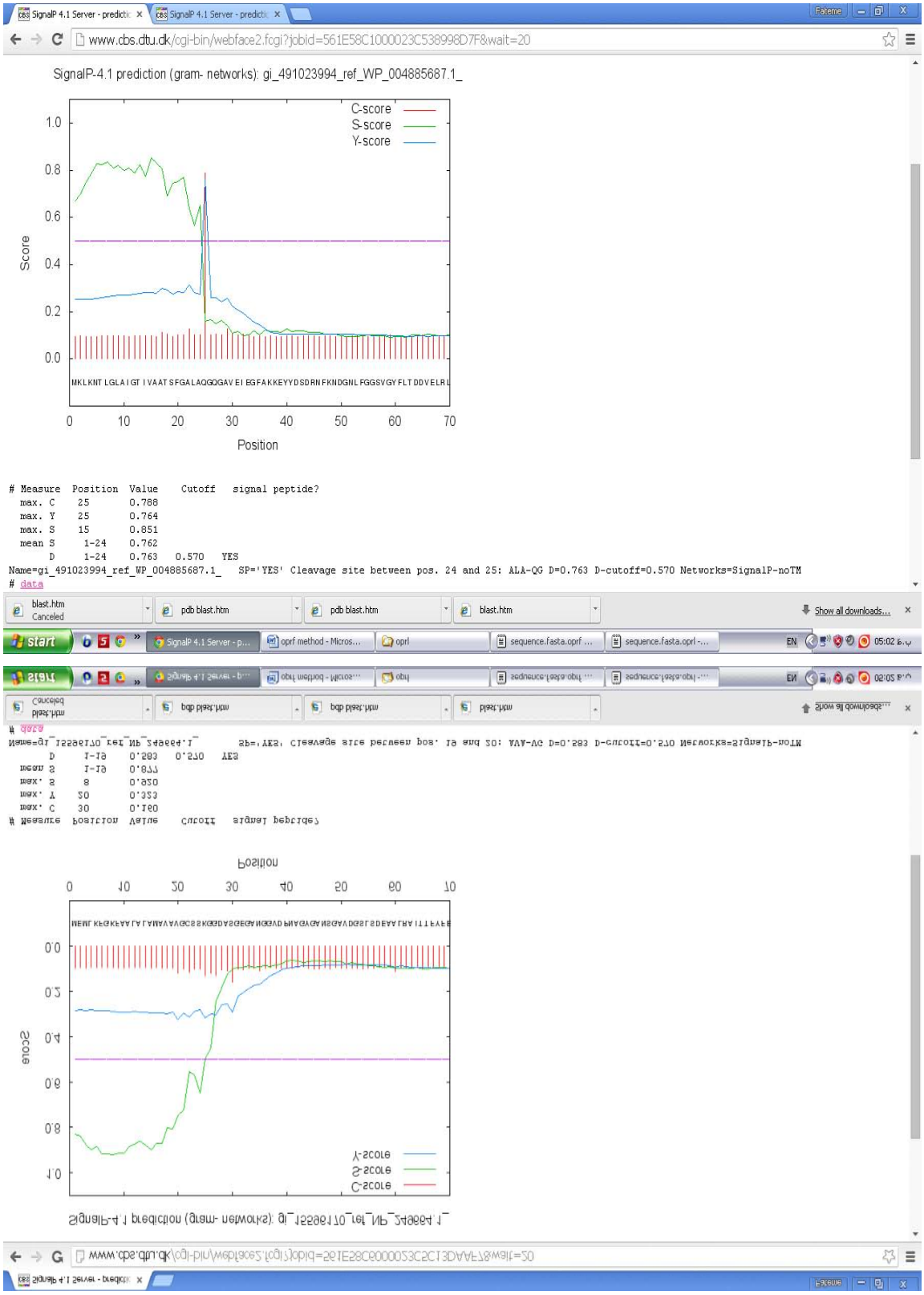


Fig. 1: Continue

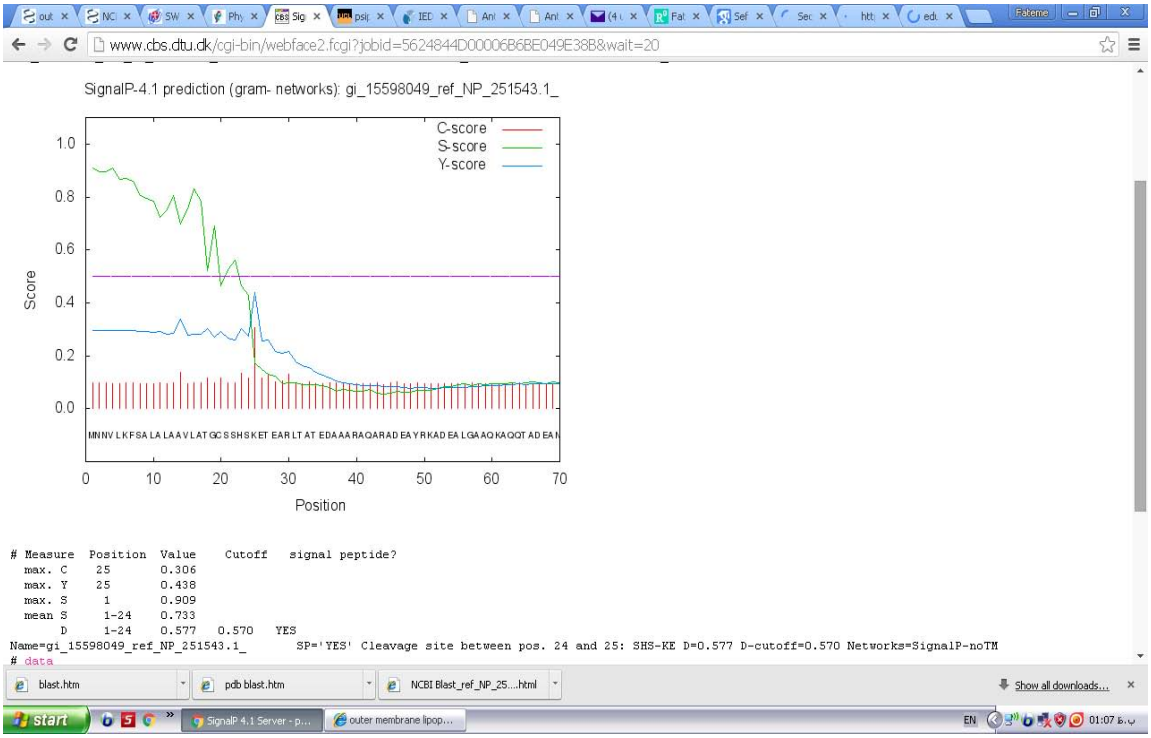
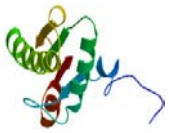


Fig. 1: OprF, OprL and Oprl signal peptid prediction of SignalP server. Cleavage site for OprF and Oprl sequence predicted between position 24 and 25. Cleavage site for OprL sequence predicted between position 19 and 20

Table 4: OprF, OprL and OprI predicted 3D models estimated qualititatively

Models	Figure	Seq. identity (%)	Seq. similarity	Coverage	GMQE	QMEAN4
OprF 1		53.33	0.46	0.44	0.29	-3.60
OprF 2		50.99	0.45	0.44	0.26	-4.23
OprL 1		42.86	0.41	0.75	0.58	-3.39
OprL 2		50.40	0.43	0.74	0.57	-1.97
Oprl 1		30.77	0.36	0.31	0.17	-2.30

requires specialized software and access to up-to-date protein. Swiss model software recruited for homology modeling introduced 3 model. All the models were selected for further analyses.

Models evaluations: The 3D models estimated qualititatively by tow servers revealed that there was a consensus on a single model. Results are shown in Table 4.

QMEAN is a composite scoring function for the estimation of the global and local model quality. QMEAN consisting of four structural descriptors: the local geometry is analyzed by a torsion angle potential over three consecutive amino acids. Two pairwise distance-dependent potentials are used to assess all-atom and C-beta interactions. A solvation potential describes the burial status of the residues. The pseudo energies returned from the four structural descriptors and the final QMEAN4 score get directly related to what we would expect from high resolution X-ray structures of similar size using a Z-score scheme. The score of a model in also shown in relation to a set of high-resolution PDB structures (Z-score). The plot relates the obtained global QMEAN4 value to scores calculated from a set of high-resolution X-ray structures. Local estimates of the model quality based on the QMEAN scoring function are shown as per-reside plot. Each residue is assigned a reliability score between 0 and 1, describing the expected similarity to the native structure. Higher numbers indicate higher reliability of the residues.

GMQE (Global Model Quality Estimation) is a quality estimation which combines properties from the target-template alignment. The resulting GMQE score is expressed as a number between zero and one, reflecting the expected accuracy of a model built with that alignment and template. Higher numbers indicate higher reliability. Once a model is built, the GMQE gets updated for this specific case by also taking into account the QMEAN4 score of the obtained model in order to increase reliability of the quality estimation.

Identification of functionally and structurally important residues: Interprosurf annotated functional residues on the 3D structure of OprF, OprL and Oprl. Results are shown in Fig. 2. Figure 3 and 4 functional residues on the

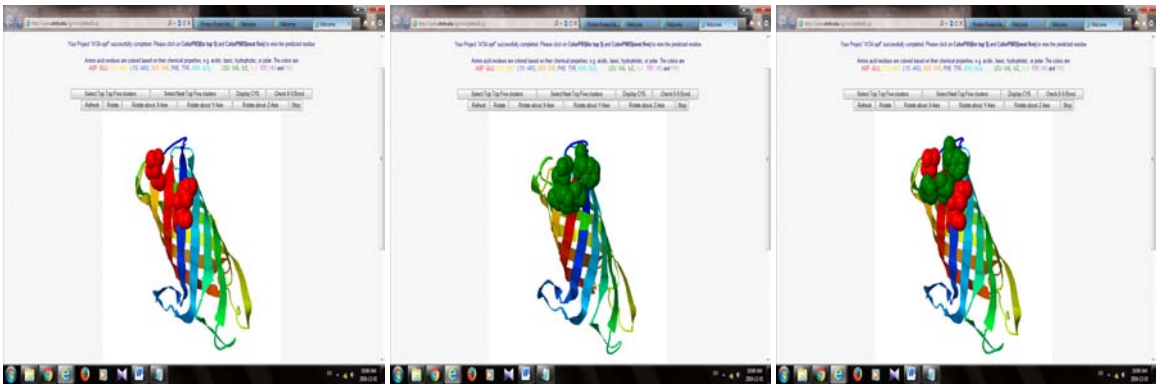


Fig. 2: Residues predicted by auto patch analysis are: 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 55, 56

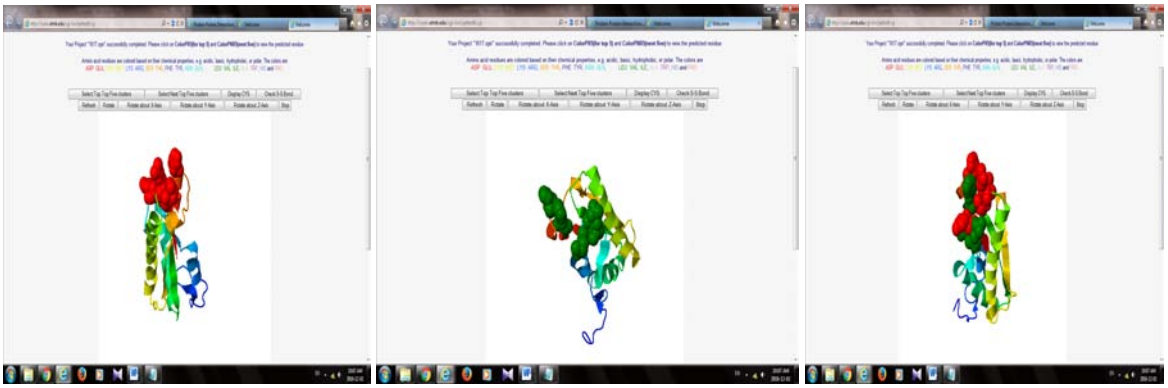


Fig. 3: Residues predicted by auto patch analaysis are: 70, 71, 72, 73, 105, 106, 107, 153, 158

3D structure of OprF, OprL and Oprl, respectively from top to down. The structure display figure provides a 3D view of the functional residues in the context of the protein structure using the Jmol java applet. Protein structures are shown in cartoons and the predicted functional residues are shown in space filled model.

Single-scale amino acid properties assay: IEDB server predict several properties such as hydrophilicity, accessibility, antigenicity, flexibility and beta turn secondary structure in the protein sequence. Propensity scale methods assign a propensity value to each amino acid which measures the tendency of an amino acid to be part of a B-cell epitope (as compared to the background). To reduce fluctuations, the score for each target amino acid residue in a query sequence is computed as the average of the propensity values of the amino acids in a sliding window centered at the target residue. hydrophilicity, accessibility, antigenicity, flexibility and secondary structure properties have fundamental role in B-cell epitope prediction. Relying on just one of these properties, reliable results could not be achieved.

Prediction of B-cell epitopes by integrated strategy: Four linear along with 11 discontinuous B-cell epitopes were predicted for OprF protein. Five linear along with 8 discontinuous B-cell epitopes were predicted for OprL protein. Five linear along with 5 discontinuous B-cell epitopes were predicted for OprF protein. Best 3 linear and conformational epitopes with the highest Pl score are shown in Table 5 and 6.

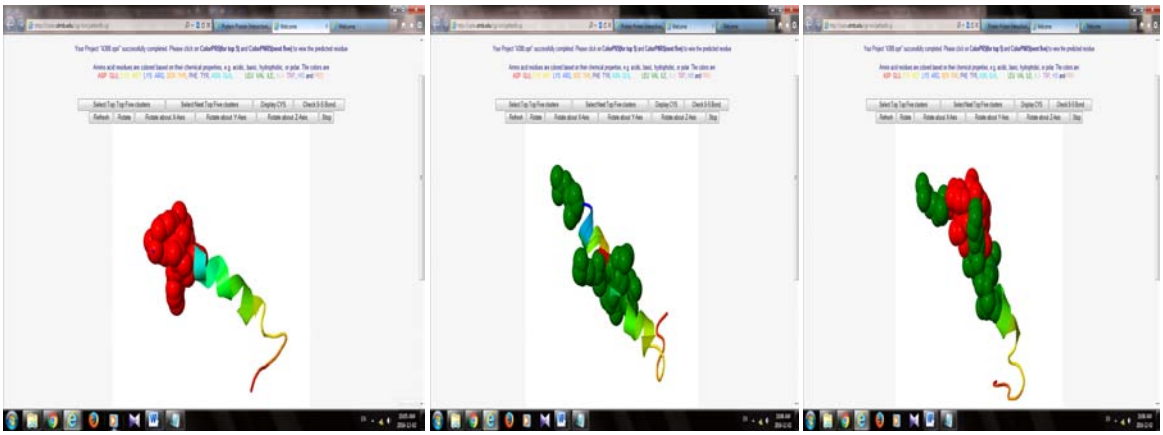


Fig. 4: Residues predicted by auto patch analaysis are: 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 55, 56

Table 5: OprF, OprL and OprI linear epitopes predicted by ellipro

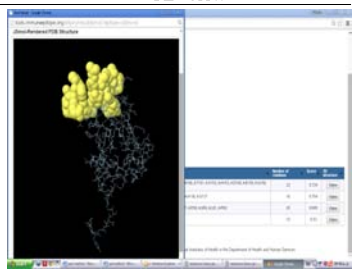
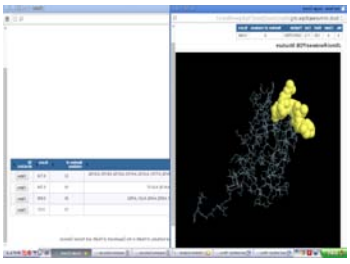
Protein	No.	Start	End	Peptide	No. of residues	Score	3D view
OprF	1	209	344	CPDTPANVTVDADGCPAVAEV VRVEL DV FDKSVVKPSS YGDIK NL AD KFD FMQ AY QYPQTSTTVEGHT DSVGPD NQKLSERRA NA VKQVLVNQYGVGASRVNSV GYGESRPVADNATESGRAV NRRVEAEVEAQAK	136	0.792	
OprF	2	1	7	MKLKNTL	7	0.592	

Table 5: Continue

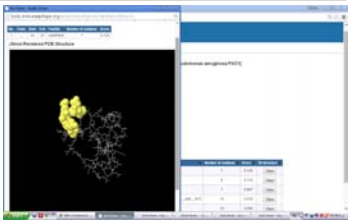
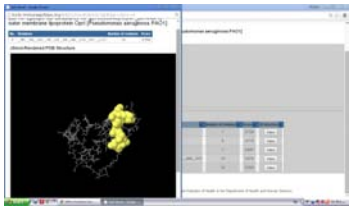
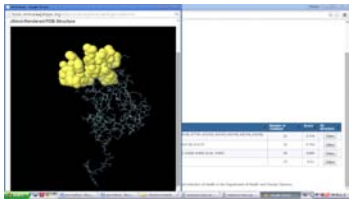
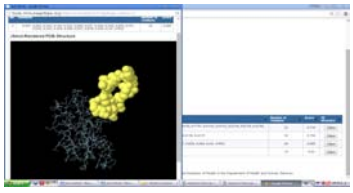
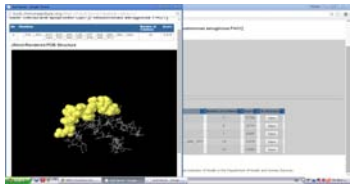
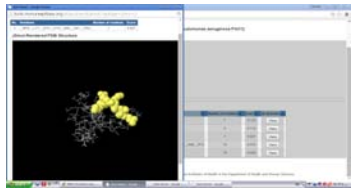
Protein	No.	Start	End	Peptide	No. of residues	Score	3D view
OprF	3	156	165	QYNIDQGNTE	10	0.583	
OprL	1	138	168	LELVSYGKERPVATGHDEQSWA QNRRVELKK	31	0.837	
OprL	2	93	111	GSGQRVVLEGHTDERGTRE	19	0.682	
OprL	3	75	85	DLKPEAMRALD	11	0.665	
OprI	1	41	47	AQARADE	7	0.728	
OprI	2	13	21	AAVLATGCS	9	0.719	
OprI	3	75	83	RMLEKASRK	9	0.647	

Table 6: OprF, OprL and OprI linear epitopes predicted by Elipro

Protein	No.	Residues	No. of residues	Score	3D view
OprF	1	_:R334, _:V335, _:E336, _:A337, _:E338, _:V339, _:E340,_:A341, _:Q342, _:A343,_:K344	11	0.983	
OprF	2	_:R318, _:P319, _:V320, _:A321, _:D322, _:N323,_:A324, _:T325, _:E326, _:S327,_:G328, _:R329,_:A330, _:V331, _:N332	15	0.942	
OprF	3	_:Y302, _:G303, _:V304, _:G305, _:A306, _:S307, _:R308, _:V309, _:N310, _:S311,_:V312, _:G313, _:Y314, _:G315, _:E316, _:S317	16	0.862	
OprI	1	_:R163, _:V164, _:E165, _:L166	4	0.772	
OprI	2	_:W158, _:A159, _:Q160, _:N161	4	0.759	
OprI	3	_:E155, _:Q156, _:S157	3	0.75	
OprI	1	_:A41, _:Q42, _:A43, _:R44, _:A45, _:D46, _:E47	7	0.728	
OprI	2	_:A13, _:A14, _:V15, _:L16, _:A17, _:T18,_:G19, _:C20, _:S21	9	0.719	
OprI	3	_:M76, _:L77, _:E78, _:K79, :A80, :S81, :R82	7	0.687	

CONCLUSION

In conclusion, proteins epitopes were selected as vaccine candidates. These regions contain functional exposed amino acids with higher properties score of B-cell epitopes. In these regions, the majority of amino acids are hydrophile, flexible, accessible and favorable for B-cells with a view to point of secondary structure.

REFERENCES

Doring, G. and G.B. Pier, 2008. Vaccines and immunotherapy against *Pseudomonas aeruginosa*. Vaccine, 26: 1011-1024.

Glaser, F., T. Pupko, I. Paz, R.E. Bell, D. Bechor-Shental, E. Martz and N. Ben-Tal, 2003. ConSurf: Identification of functional regions in proteins by surface-mapping of phylogenetic information. Bioinformatics, 19: 163-164.

Lim, J.A., D.D. Vos, M. Brauns, D. Mossialos and A. Gaballa *et al*., 1997. Molecular and immunological characterization of OprL, the 18 kDa outer-membrane Peptidoglycan-Associated Lipoprotein (PAL) of *Pseudomonas aeruginosa*. Microbiol., 143: 1709-1716.

Ochs, M.M., M.M.P. Cusker, M. Bains and R.E. Hancock, 1999. Negative regulation of the Pseudomonas aeruginosa outer membrane porin OprD selective for imipenem and basic amino acids. Antimicrob. Agents Chemother., 43: 1085-1090.

Priebe, G.P. and J.B. Goldberg, 2014. Vaccines for *Pseudomonas aeruginosa*: A long and winding road. Expert Rev. Vaccines, 13: 507-519.

Qian, F., Y. Wu, O. Muratova, H. Zhou and G. Dobrescu *et al*., 2007. Conjugating recombinant proteins to *Pseudomonas aeruginosa* ExoProtein a: A strategy for enhancing immunogenicity of malaria vaccine candidates. Vaccine, 25: 3923-3933.

Sherman, N.E., B. Stefansson, J.W. Fox and J.B. Goldberg, 2001. *Pseudomonas aeruginosa* and a proteomic approach to bacterial pathogenesis. Dis. Markers, 17: 285-293.

Sugawara, E., M. Steiert, S. Rouhani and H. Nikaido, 1996. Secondary structure of the outer membrane proteins OmpA of Escherichia coli and OprF of Pseudomonas aeruginosa. J. Bacteriol., 178: 6067-6069.

Tamber, S. and R.E. Hancock, 2004. The Outer Membranes of Pseudomonads. In: Pseudomonas, Ramo, J.L. (Ed.). Springer, Berlin, Germany, ISBN: 978-1-4613-4788-0, pp: 575-601.

Wessel, A.K., J. Liew, T. Kwon, E.M. Marcotte and M. Whiteley, 2013. Role of *Pseudomonas aeruginosa* peptidoglycan-associated outer membrane proteins in vesicle formation. J. Bacteriol., 195: 213-219.

Worgall, S., A. Krause, M. Rivara, K.K. Hee and E.V. Vintayen *et al*., 2005. Protection against *P. aeruginosa* with an adenovirus vector containing an OprF epitope in the capsid. J. Clin. Invest., 115: 1281-1289.