Research Article

Pseudomonas aeruginosa vaccine - Search for cell surface-exposed proteins as a subunit vaccine candidate by reverse vaccinology approach

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ABSTRACT

Aim: Development of a vaccine to fight *Pseudomonas aeruginosa* infections is of prime concern as the pathogen has developed multiple drug resistance. In the present study, by reverse vaccinology approach, a number of cell surface-exposed proteins are reported as potential vaccine candidate. **Materials and Methods:** *P. aeruginosa* PA 38182 encoded 7279 proteins were screened as cell surface proteins using TMHMM, LipoP, PsortB, and Signal P server. 40 screened antigens were selected and screened for suitability as epitope candidates by human leukocyte antigen Pred epitope prediction server. **Results:** A total of 30 high score epitopes were recorded; amongst them based on binding energy calculation, protein "putative holin" epitope emerged as the most suitable candidate. **Conclusion:** *P. aeruginosa* PA 38182 encodes 30 cell surface-exposed epitopes which could be implemented in vaccine research to control *P. aeruginosa* infections successfully since no approved vaccine is available for them.

KEY WORDS: Antigen, Bioinformatics, Epitope, Reverse vaccinology, Vaccine

INTRODUCTION

Pathogen *Pseudomonas aeruginosa,* a deadly Gram-negative bacterium, causes a range of infections generally in immunocompromised patients and those with long hospital stay. The organism has developed antibiotic resistance and with number of failed clinical trials resultant, no approved vaccine is available.[1]

The current research is focused on the use of chimeric protein where chimeric PilQ/PilA proteins demonstrated an increase in anti-recombinant protein antibody titer and opsonophagocytosis of *P. aeruginosa* PA 01. The study recommended an anti-adhesion-based vaccination as a promising protocol for controlling *P. aeruginosa* infection.[2] Hegerle *et al*.[3] working on broad-spectrum glycol conjugate vaccine to control *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* infections used flagellin (FLB) as an antigen and showed successful immunization in rabbit. It is important to incorporate membrane protein in the vaccination program as illustrated by Liu *et al*.[4] while investigating the lipopolysaccharides heterologous *P. aeruginosa.* They emphasized the need for understanding the expression of all the proteins present on outer membrane and found that proteins such as FpvA, hasR, and foaA play key role in immune response and the same could be potential vaccine candidate. The study on *P. aeruginosa* recombinant PilQ and type b FLB protein was a success as increase in antisera titer was recorded in mice model. It also increased the titers of interleukin 4 (IL)-4 and IL17 which is necessary for the elimination of the bacterium.[5]

Along with proteins as a vaccine candidate, now DNA vaccine using oprL and oprF has also been investigated for *P. aeruginosa* in chickens. On vaccination, levels of IL-2 and interferon-gamma in chicken were found to be raised.[6] In the present study, reverse vaccinology has been applied to screen number of potential vaccine candidates by applying bioinformatics approach and future epitopes for *P. aeruginosa* vaccine have been proposed.

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MATERIALS AND METHODS

Collection of Proteome

To collect the protein sequences of *P. aeruginosa* PA 38182, database of KEGG was used. In a database

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information of organism was collected with code "paeu" and about 7279 protein sequences were retrieved successfully in a FASTA format.

Filtering of Cell Surface-associated Proteins

Based on the information published earlier by Gore *et al*.,[7] four servers are available online which are capable of predicting subcellular localization of the protein based on the signal sequence peptide, signal sequence cleavage site, and topology of transmembrane helices along with ability of protein to span intracellular region or to get secreted outside the cell membrane. The four programs named TMHMM, Lipo P, Signal P, and Psort B were used to select proteins which can span plasma membrane or cell wall. With TMHMM server, only those proteins were further evaluated whose transmembrane region was found to be spanning not more than twice in a plasma membrane while all other proteins even though recorded positive for cell surface spanning capabilities were not evaluated further as it is reported that such multitrans membrane helices proteins are difficult to express in cloning studies.

Filtering the Cell Surface Antigens

Once the identity of the cell surface proteins has been deduced through these programs, further filtering of proteins was carried out by proceeding with candidates which recorded positive in at least three softwares as this decision strengthens the possibility of a protein to span in cell surface region.

Epitope Prediction

Server "Human leukocyte antigen (HLA)Pred" helps in identification of epitope capable of binding HLA Class I alleles. Here in a server setting, epitope search has been kept as nonamer, with threshold 5% and searched for all alleles present in the database. The epitope and its allele were selected on the basis of their score.

Localization of Epitope Position

Once the position of the epitope has been recorded from HLAPred, it is exact position in cell structure such as ability to span plasma membrane structure, or to be in cytoplasm or exposed outside is predicted with the server TMHMM. The sequence of antigen was given as an input and exact position of the recorded epitope noted. Further, filtering of an epitope has been done based on the position, with outward exposure being taken as positive.

Binding Energy Analysis

Ability of binding MHC molecule with epitope has been recorded as binding energy at Kcal/mole with server T-cell epitope prediction. Server calculates the binding energy of MHC molecule with epitope, based on the information of HLA allele obtained from T-cell epitope prediction server.

Final Selection of Best-scored Epitopes

With the help of four servers, 7279 proteins of *P. aeruginosa* PA388182 were screened. Epitopes with lowest binding energy were scored positive and recorded accordingly.

RESULTS

With the total of 7279 proteins encoded by the genome of *P. aeruginosa* PA 38182, study recorded a number of cell surface proteins using Psort B, Lipo P, Signal P, and TMHMM. 40 cell surface proteins were found to be present in at least three servers. A majority of them were found to be encoding receptor proteins, few enzymes have also been detected like beta-lactamase, polyhydroxybutyrate depolymerase, a kinase, and synthase along with some hypothetical proteins as evident in Table 1.

Of these 40 antigenic proteins, epitopes from only 30 antigens were found to be exposed outside the cell surface [Table 1].

Based on the binding energy calculations for promising epitopes, protein BN889-00716 putative holing with an epitope "DKAGLGGWA" binding energy with HLA-A*0202 recorded the lowest binding energy at −2200.8 Kcal/mole, also BN889-01116 outer membrane lipoprotein SlyB with an epitope "AGSAVGGGK" and binding energy with HLA-A*202 as −2037.4 Kcal/mole holds promise as in Table 1.

DISCUSSION

In the present study, application of integrated genomics and proteomics approach enables us to predict number of potential immunogenetic surface-exposed epitopes in *P. aeruginosa.* Among the other predicted cell surface antigens such as metallo-beta-lactamase, type VI secretion systems protein, outer membrane receptor protein, and others, the most promising candidate is putative holing protein (BN889-00716) followed by outer membrane lipoprotein SlyB (BN 889-01116). A few uncharacterized proteins BN889-00507, BN889-00444, BN889-01313, and BN889-01429 are also potential vaccine candidate.

Rashid *et al*.[8] reported number of probable vaccine candidates such as surface component of antibiotic efflux pump, chaperone-usher pathway components, penicillin-binding protein of bacterial cell wall, extracellular component of Type 3 secretory systems, and three uncharacterized proteins. Similar to our study, this study also assumed that these proteins can act as a potential vaccine candidate and will find a place in subunit vaccine against *P. aeruginosa.*

Table 1: Vaccine antigens detected with epitopes for *Pseudomonas aeruginosa*

KEGG No					Type	SEQ	Score	Localization	Energy
BN889_00097	N	1	Y	SpI-1(7)	HLA-A*0202	EKAIFAVSS	15.65	O	−714.63
BN889_00126	CM 9.86	N	Y	SpI-1 (3), SpII 1 (1)	HLA-A*0202	ALTDNAQST	14.88	O	−489.81
BN889_00164	N	1	Y	SpI-1(8)	HLA-A*0202	RKVPALEFT	17.29	O	−644.08
BN889_00195	N	1	Y	SpI-1(8)	HLA-A*0202	LKPSLFKGK	15.07	O	−332.42
BN889_00198	N	1	Y	SpI-1 (7), SpII 1 (1)	HLA-A*0201	YKRDRNLGT	15.89	O	1384.94
BN889_00204	CM 10	N	Y	SpI-1 (9), SpII 1 (1)	HLA-A*0202	GGTQDAVMK	13.82	O	−1212.6
BN889_00213	N	1	Y	SpI-1 (9)	HLA-A*0202	RTEINAMNR	8.38	no sign sim	
BN889_00240	N	1	Y	SpI-1 (10)	HLA-A*0202	KSLFNATET	12.2	no sign sim	
BN889_00243	OM 10	N	Y	SpI-1 (6)	HLA-A*0201	FLGGQWQAT	13.65	O	−459.84
BN889_00424	CM 9.86	1	Y	SpI-1 (11)	HLA-A*0202	GKAGQALVV	14.46	I	−814.25
BN889_00444	N	1	Y	SpI-1 (4)	HLA-A*0201	GLEGQLGDK	12.81	O	−658.67
BN889_00446	CM 10	N	Y	SpI-1 (5)	HLA-A*0202	LLAAFFGFT	14.08	O	−1687.4
BN889_00507	CM 9.86	2	N	SpI-1 (7)	HLA-A*0202	ASVGLFCVA	9.22	O	−1568.11
BN889_00545	OM 10	N	Y	SpI-1 (7)	HLA-A*0202	LKVRGFKPT	19.55	O	−548.23
BN889_00627	CM 9.82	1	N	SpI-1 (5)	HLA-A*0201	WLQDEALRT	14.04	O	880.47
BN889_00716	CM 10	2	Y	N	HLA-A*0202	DKAGLGGWA	11.33	O	−2200.8
BN889_00779	CM 9.97	2	N	SpI-1 (7)	HLA-A*0201	TLETTAKNT	13.46	O	576.3
BN889_00817	N	1	Y	SpI-1 (8)	HLA-A*0202	RTEINAMRT	14.05	no sign. Simil	
BN889_00900	OM 10	N	Y	SpI-1 (11), SpII 1 (1)	HLA-A*0201	YLALPGDAK	11.77	O	−148.14
BN889_00996	CM 9.86	1	Y	SpI-1 (8)	HLA-A*0202	NKHYPTHET	11.86	no sign. Simil	
BN889_01010	OM 10	N	Y	SpI-1 (9)	HLA-A*0202	AKAGDISNT	20.19	O	−1662.77
BN889_01027	OM 10	N	Y	SpI-1 (12), SpII 1 (1)	HLA-A*0201	QLELVSYGK	12.54	O	97.81
BN889_01110	CM 10	N	Y	SpI-1 (12), SpII 1 (1)	HLA-A*0202	GHTDSTGSK	14.31	O	−1202.12
BN889_01116	OM 9.45	N	Y	SpI-1 (11), SpII 1 (1)	HLA-A*0202	AGSAVGGGK	12.38	O	−2037.4
BN889_01258	OM 10	N	Y	SpI-1 (13)	HLA-A*0202	DKVIDNTGT	16.9	O	−239.99
BN889_01313	CM 9.82	1	N	SpI-1 (8), SpII 1 (1)	HLA-A*0202	YKAGDYLEH	15.09	O	41.84
BN889_01358	OM 10	N	Y	SpI-1 (6), SpII 1 (1)	HLA-A*0202	TATIVENIT	11.14	no sign. Simil	
BN889_01407	OM 9.93	N	Y	SpI-1 (6)	HLA-A*0201	RWPLERAWK	10.65	I	312.39
BN889_01429	OM 9.93	N	Y	SpI-1 (6), SpII 1 (1)	HLA-A*0202	GKTITEGGT	21.53	O	−761.22
BN889_01536	CM 10	2	Y	SpI-1 (10), SpII 1 (1)	HLA-A*0201	GLVILWIGT	14.33	TM	−751.42
BN889_01575	CM 9.97	2	N	SpI-1 (6), SpII 1 (1)	HLA-A*0201	GLATRAMHT	12.98	no sign. Simil	
BN889_01607	CM 9.82	2	Y	SpI-1 (11), SpII 1 (1)	HLA-A*0202	RTEINAMSF	9.88	no sign. Simil	
BN889_01662	CM 9.82	2	N	SpI-1 (7)	HLA-A*0202	ASMWVNGIT	14	TM/O	−1249.43
BN889_01688	N	1	Y	SpI-1 (6)	HLA-A*0202	AKALRWVEE	11.6	O	−724.71
BN889_01716	CM 9.97	1	Y	SpI-1 (8)	HLA-A*0201	RLTAMDAAT	13.95	O	−34.51
BN889_01813	CM 9.82	1	N	SpI-1 (7)	HLA-A*0201	RLLRPVLET	13.07	I	−84.62
BN889_01875	N	1	Y	SpI-1 (11)	HLA-A*0201	QLLRDLAEK	13.71	O	−202.1
BN889_01892	OM 10	1	Y	SpI-1 (9), SpII 1 (1)	HLA-A*0202	EKKAHIVET	14.96	O	81.49
BN889_01905	OM 10	N	Y	SpI-1 (12)	HLA-A*0201	ILATRYNIT	11.93	no sign. Simil	
BN889_01952	CM 9.82	N	Y	SpI-1 (7), SpII 1 (2)	HLA-A*0202	AKRFNVEMK	14.83	O	−140.08
BN889_02025	OM 10	1	Y	SpI-1 (8)	HLA-A*0202	MLVLVASFT	13.41	TM	−611.46
BN889_02103	N	1	Y	SpI-1 (12)	HLA-A*0202	LKVLGNPEF	15.86	O	−276.2
BN889_02116	OM 9.52	N	Y	SpI-1 (6)	HLA-A*0202	FKTPVTNNA	15.04	O	65.54
BN889_02148	OM 10	N	Y	SpI-1 (7)	HLA-A*0201	SLLCRAALK	12.74	TM	−265.91

I: Inside, O: Outside, TM: Transmembrane

In a related study using reverse vaccinology approach, vaccine against ever-increasing multidrug-resistant *Mycobacterium abscessus* could be generated as Le Moigne *et al*.,[9] reported that *M. abscessus* phospholipase C could act as an antigen since an increase in antibody titer in an immunized mouse is seen. Similar to this study, our findings report a number of enzymes such as metallo-beta-lactamase, purine nucleosidase, depolymerase, sensor, histidine kinase, 3-hydrxyisobutyrate dehydrogenase, and 3-deoxy-7-phosphoheptalonate synthase as a potential vaccine candidate.

Applying reverse vaccinology approach, a number of epitopes have been reported for subunit vaccine preparation for *Streptococcus pneumonia*,[7] *Neisseria meningitides*,[10] *Haemophilus ducreyi, Ehrlichia ruminantium,*[11] and *Campylobacter fetus*.[12]

The present study suggests that by employing epitopes predicted to be vaccine candidate, a vaccine against *P. aeruginosa* could be developed and demands further investigation in clinical trials.

CONCLUSION

With the advancement in the field of genomics and proteomics, it is now possible to predict the success of any protein in vaccine research with confidence. In the present study by employing reverse vaccinology approach, *P. aeruginosa* genome was found to be encoding cell surface-exposed antigen capable of eliciting immune response. 30 epitopes were found to be the most suitable candidates capable of eliciting an immune response as per prediction tools available in bioinformatics domain.

REFERENCES

1. Merakou C, Schaefers MM, Priebe GP. Progress toward the elusive *Pseudomonas aeruginosa* vaccine. Surg Infect

(Larchmt) 2018. Doi: 10.1089/sur.2018.233.
2. Gholami M, Salimi Chirani A, Falak R, Moshiri M, Razavi S, Irajian G, *et al.* Induction of specific humoral immune response in mice against a *Pseudomonas aeruginosa* chimeric pilQ/PilA protein. Rep Biochem Mol Biol 2018;7:38-44.
3. Hegerle N, Choi M, Sinclair J, Amin MN, Ollivault-Shiflett M, Curtis B, *et al.* Development of a broad spectrum glycoconjugate vaccine to prevent wound and disseminated infections with *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. PLoS One 2018;13:e0203143.
4. Liu C, Pan X, Xia B, Chen F, Jin Y, Bai F, *et al.* Construction of a protective vaccine against lipopolysaccharide-heterologous *Pseudomonas aeruginosa* strains based on expression profiling of outer membrane proteins during infection. Front Immunol 2018;9:1737.
5. Bakht Azad S, Nikokar I, Faezi S, Rasooly S, Mahdavi M. Evaluation of the immune responses following co-administration of pilQ and type b-flagellin from *Pseudomonas aeruginosa* in the burn mouse model. Microb Pathog 2018;123:426-32.
6. Gong Q, Ruan MD, Niu MF, Qin CL, Hou Y, Guo JZ, *et al.* Immune efficacy of DNA vaccines based on oprL and oprF genes of *Pseudomonas aeruginosa* in chickens. Poult Sci 2018;97:4219-27.
7. Gore D, Gupta K, Shinde G. Computational identification of cell surface antigens in *Streptococcus pneumonia*. Biomirror 2011;2:1e5.
8. Rashid MI, Naz A, Ali A, Andleeb S. Prediction of vaccine candidates against *Pseudomonas aeruginosa*: An integrated genomics and proteomics approach. Genomics 2017;109:274-83.
9. Le Moigne V, Rottman M, Goulard C, Barteau B, Poncin I, Soismier N, *et al.* Bacterial phospholipases C as vaccine candidate antigens against cystic fibrosis respiratory pathogens: The *Mycobacterium abscessus* model. Vaccine 2015;33:2118-24.
10. Gore D, Reecha P. *In silico* identification of cell surface antigens in *Neisseria meningitidis*. Biomirror 2011;50:1e5.
11. Kale S, Gore D. *Ehrlichia ruminantium* vaccine epitope search by reverse vaccinology approach. J Pharm Res 2017;11:807-10.
12. Pande S, Gore D, Shanware A. Bioinformatics approach for screening the cell surface antigens of *Campylobacter fetus*. J Pharm Res 2012;5:3025-9.