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IN SILICO ANALYSIS OF *BURKHOLDERIA PSEUDOMALLEI* PROTEOME TO PREDICT POTENTIAL VACCINE CANDIDATE PROTEINS

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ABSTRACT

Burkholderia pseudomallei is a Gram-negative pathogen that is the causative agent of melioidosis predominantly in South East Asia and tropical Australia. There is currently no vaccine available against Burkholderia species and available prophylactic measures have limitations. In this study, potential vaccine candidates against *B. pseudomallei* were predicted by *in silico* analysis of its proteome using Vaxign, the bioinformatic tool. Proteins localized extracellular and in outer membrane with adhesion probability of 0.5 or more, number of trans-membrane helices less than 2 and dissimilar with human and mouse proteome were selected. 58 proteins were predicted as potential vaccine candidates that may help in designing effective vaccines.

KEYWORDS: Vaccine, Outer membrane proteins, Melioidosis, Proteome, *In silico.*

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INTRODUCTION

Burkholderia pseudomallei is a facultative intracellular bacterium causing melioidosis in humans[1]. Percutaneous inoculation of bacteria followed by exposure to surface water or muddy soil, particularly in rice paddy fields, in endemic area is the main reason of infection[2]. Certain environmental conditions such as inhalation, cyclones and tropical storms are considered as the main mode of infection[3]. Also, incidences of melioidosis increase during monsoon season in association with pneumonia leading to high mortality rate[3]. *B. pseudomallei* is intrinsically resistant to a number of antibiotics, hence treatment options for melioidosis are limited[4]. Sub unit vaccine development is the prime concern for researchers to discover treatment options against this pathogen. Isolation and characterization of individual components of bacterium to mount an immune response in host is the conventional way to find effective vaccine candidates. These purified components from pathogens are generally safe as they are individual and toxin free sub units. Although their production is costly due to large scale cultivation and downstream processing but sub unit vaccines are the most promising tools to fight the diseases. Lipopolysaccharides and capsular polysaccharides of *B. pseudomallei* were found to induce immune response resulting in the survival of mice after bacterial challenge[5]. Similarly, Chen et al (2006) have reported the immunoprotective efficacy of flagellin[6]. Outer membrane proteins play crucial role in virulence of pathogen. Immunization with OMPs could generate significant antibody titre and survival of mice after bacterial challenge. Omp85, Omp3, Omp7 and LolC have been reported to improve survival rate in mice[7, 8, 9]. Identification and development of novel vaccines require screening of components of pathogen capable of evoking immune system of the host. Conventional vaccinology, in which individual components are isolated from the pathogen and investigated for immunoprotection in animal models, could be a long and laborious process. Another limitation is in difficulty of cultivation of the microorganism in the laboratory. Availability of the genomic sequences and advancements in bioinformatics has resulted in exponential growth of vaccine development against a number of pathogens. In reverse vaccinology (RV), bioinformatic analysis of the proteome of pathogen was carried out and on the basis of certain parameters, potential vaccine candidates are predicted[10]. This *in silico* analysis leads to discovery of novel antigens irrespective of their abundance in cell, *in vitro* expression and immunogenicity during infection. RV methodology starts with the retrieving the proteomic data from protein database and apply bioinformatic tools to scrutinize proteins on the basis of criteria for potential vaccine candidate: 1. Sub-cellular localization, 2. Number of trans-membrane helices, 3. Adhesion probability, 4. Ability to bind to MHCs, 5. Dissimilarity with human and mouse proteome. Protein localized in outer membrane ensures its interaction with the immune system of host. In this work, we have predicted novel and efficient vaccine candidates using reverse vaccinology.

MATERIALS AND METHODS

Sequence retrieval

Burkholderia pseudomallei MSHR 305 was used for the analysis. Complete proteome of this pathogen was downloaded from UniProt database.

Prediction of vaccine candidates using Vaxign

Whole proteome was subjected to Vaxign online tool to predict potential vaccine candidates. Vaxign uses various online tools PSORTb, TMHMM, SPAAN, Vaxitop and OrhtoMcl to analyze the protein sequences for sub-cellular localization, number of trans-membrane helices, adhesion probability, ability to bind to MHC molecules and dissimilarity with human and mouse proteome respectively[11].

RESULTS

The total numbers of proteins in Burkholderia pseudomallei MSHR 305 proteome were 3495.

After Vaxign analysis, total 143 proteins were found in outer membrane and 97 were extracellular (table 1). Outer membrane and extracellular proteins were further categorized according to their adhesion probability (Table 2 and table 3 respectively). Out of these 143 outer membrane proteins, 58 proteins were having adhesion probability more than 0.5. These include 27 porin family proteins and other proteins with molecular weight from 218 to 3107 kDa.

Table 1

Number of proteins with their sub cellular localization and adhesion probability

Sub cellular localization	Total proteins	Adhesion probability (p>0.5)	Adhesion probability (p<0.5)
Extracellular	97	42	55
Outer membrane	143	85	58

Table 2

Proteins localized in outer membrane and having adhesion probability more than 0.5

S. No.	Protein Accession	Protein Note	Outer Membrane Probability	Adhesin Probability	Trans-membrane helices	Protein Length (amino acids)
1	tr|S5P316	VacJ like lipofamily protein	0.992	0.538	0	324
2	tr|S5NR69	OmpW family protein	0.993	0.603	0	243
3	tr|S5NYL7	Gram-negative porin family protein	0.992	0.613	0	307
4	tr|S5PA01	Protein CyaE	0.971	0.558	0	482
5	tr|S5NMZ2	Gram-negative porin family protein	0.993	0.583	0	355
6	tr|S5NQ16	Uncharacterized protein	0.886	0.717	0	347
7	tr|S5NNQ1	Gram-negative porin family protein	1	0.670	0	391
8	tr|S5PFQ6	Outer membrane protein assembly factor BamA	1	0.640	0	768
9	tr|S5NP62	Gram-negative porin family protein	1	0.723	0	374
10	tr|S5NQA4	Gram-negative porin family protein	1	0.564	0	358
11	tr|S5NRP6	OmpW family protein	0.993	0.648	0	274
12	tr|S5NJL2	Gram-negative porin family protein	0.993	0.589	0	380
13	tr|S5NL84	Gram-negative porin family protein	1	0.622	0	384
14	tr|S5NCZ3	Gram-negative porin family protein	1	0.631	0	384
15	tr|S5NK95	Gram-negative porin family protein	0.992	0.685	0	357
16	tr|S5PG94	Gram-negative porin family protein	1	0.630	0	366
17	tr|S5NKH0	Uncharacterized protein	0.993	0.614	0	362
18	tr|S5NY84	Gram-negative porin family protein	1	0.567	1	419
19	tr|S5NMT2	Coiled stalk of trimeric autotransporter adhesin family protein	0.886	0.560	0	303
20	tr|S5NSD9	Uncharacterized protein	1	0.536	0	811
21	tr|S5P3C2	Outer membrane porin, OprD family protein	0.993	0.609	1	486
22	tr|S5PG25	Outer membrane protein assembly factor BamE	0.992	0.626	0	261
23	tr|S5NHQ0	Gram-negative porin family protein	1	0.555	0	379
24	tr|S5NJ05	Flagellar L-ring protein	0.993	0.521	0	218
25	tr|S5P472	Hemolysin secretion/activation ShlB/FhaC/HecB family protein	1	0.663	0	477
26	tr|S5NLG7	Serine carboxypeptidase family protein	0.952	0.514	0	585
27	tr|S5NK89	Gram-negative porin family protein	1	0.619	0	400
28	tr|S5NMZ5	Peptidase propeptide and YPEB domain protein	0.952	0.640	1	567
29	tr|S5NTE4	Gram-negative porin family protein	1	0.599	0	386
30	tr|S5NM91	Gram-negative porin family protein	1	0.589	0	377
31	tr|S5P5B0	Autotransporter beta-domain protein	1	0.611	0	1120
32	tr|S5NTB5	OmpW family protein	0.993	0.575	0	276
33	tr|S5NMW0	Peptidase M23 family protein	0.886	0.590	0	414
34	tr|S5NZ96	Gram-negative porin family protein	1	0.591	1	363
35	tr|S5NJY3	LPS-assembly protein LptD	1	0.626	0	787
36	tr|S5NY15	Gram-negative porin family protein	0.993	0.608	0	375
37	tr|S5PBS5	Uncharacterized protein	0.995	0.769	0	3107
38	tr|S5NPV0	MltA-interacting MipA family protein	0.949	0.629	0	248
39	tr|S5NXZ2	TonB-dependent siderophore receptor family protein	1	0.589	0	737
40	tr|S5NIH8	Gram-negative porin family protein	1	0.526	1	384
41	tr|S5NKC7	TonB dependent receptor family protein	0.995	0.631	0	685
42	tr|S5NP68	Coiled stalk of trimeric autotransporter adhesin	0.995	0.675	0	771

		family protein				
43	tr|S5NX28	Gram-negative porin family protein	0.998	0.526	0	363
44	tr|S5P777	Gram-negative porin family protein	1	0.605	0	399
45	tr|S5P1E1	Gram-negative porin family protein	1	0.680	0	391
46	tr|S5NIE5	Gram-negative porin family protein	1	0.640	2	357
47	tr|S5NRA3	OmpA family protein	0.993	0.597	0	312
48	tr|S5NHH6	Hemolysin secretion/activation ShlB/FhaC/HecB family protein	0.992	0.650	0	257
49	tr|S5NLS8	Gram-negative porin family protein	1	0.645	1	382
50	tr|S5NW31	Efflux transporter, outer membrane factor (OMF) lipo, NodT family protein	0.999	0.569	1	538
51	tr|S5NRH3	Gram-negative porin family protein	1	0.588	0	357
52	tr|S5NXD5	Gram-negative porin family protein	0.995	0.744	0	362
53	tr|S5NXN6	Gram-negative porin family protein	1	0.619	0	358
54	tr|S5NS57	Carbohydrate-selective porin, OprB family protein	0.993	0.590	0	504
55	tr|S5P1L9	TonB-dependent siderophore receptor family protein	1	0.625	0	748
56	tr|S5P8C4	VacJ like lipofamily protein	0.993	0.534	0	319
57	tr|S5NJK2	Gram-negative porin family protein	0.992	0.721	0	363
58	tr|S5NMD0	Gram-negative porin family protein	1	0.630	0	379

Table 3
Extracellular proteins having adhesion probability more than 0.5

S. No.	Protein Accession	Protein Note	Probability	Adhesin Probability	Trans-membrane helices	Protein Length
1	tr|S5NNJ1	BNR repeat-like domain protein	0.965	0.698	1	479
2	tr|S5P6D1	Flagellar hook-basal body family protein	1	0.629	0	395
3	tr|S5P716	Flagellar hook-associated family protein	1	0.535	0	441
4	tr|S5NNP6	Fimbrial family protein	0.972	0.707	1	181
5	tr|S5NCP0	Uncharacterized protein	0.965	0.585	1	524
6	tr|S5NR99	Uncharacterized protein	0.964	0.647	0	385
7	tr|S5P0G9	SMP-30/Gluconolaconase/LRE-like region family protein	0.965	0.604	0	638
8	tr|S5NPW3	Uncharacterized protein	0.944	0.758	0	141
9	tr|S5NSF9	Chitin binding domain protein	0.971	0.512	0	214
10	tr|S5PEU1	Type-1 fimbrial protein, A chain	1	0.511	0	170
11	tr|S5PGU9	Esterase, PHB depolymerase family protein	1	0.668	1	492
12	tr|S5PAS5	GDSL-like Lipase/Acylhydrolase family protein	0.946	0.518	0	379
13	tr|S5PD46	Glycosyl hydrolases 16 family protein	0.964	0.612	0	389
14	tr|S5NR04	Flagellar hook-length control FliK family protein	0.971	0.560	0	466
15	tr|S5P5K6	Type VI secretion system effector, Hcp1 family protein	0.971	0.608	0	167
16	tr|S5P3P6	Putative bpaA	0.995	0.775	1	5431
17	tr|S5P4Z9	Uncharacterized protein	0.964	0.605	0	416
18	tr|S5P5Z4	Pentapeptide repeats family protein	0.971	0.521	0	354
19	tr|S5NI08	Uncharacterized protein	0.964	0.675	0	655
20	tr|S5NJ03	Spore Coat Protein U domain protein	0.965	0.665	0	186
21	tr|S5NN56	Putative transmembrane protein	0.964	0.598	0	354
22	tr|S5NS87	Serine carboxypeptidase family protein	0.965	0.534	0	615
23	tr|S5NQB1	Uncharacterized protein	0.964	0.590	0	345
24	tr|S5P3V1	Uncharacterized protein	0.965	0.692	0	431
25	tr|S5PDC7	Flagellar basal-body rod protein FlgF	1	0.548	0	262
26	tr|S5NC88	Peptidase M66 family protein	0.972	0.624	0	644
27	tr|S5PD87	Spore Coat U domain protein	0.965	0.635	0	172
28	tr|S5PG18	Fimbrial family protein	1	0.708	0	171
29	tr|S5NKY5	Uncharacterized protein	0.965	0.585	1	171
30	tr|S5NUL1	Serine carboxypeptidase family protein	0.965	0.533	0	544
31	tr|S5NYB1	Fimbrial family protein	0.971	0.640	0	323
32	tr|S5NSL1	Flagellar hook-associated protein 3	1	0.669	0	410
33	tr|S5NLL1	Uncharacterized protein	0.964	0.633	0	457
34	tr|S5NY45	Glycine zipper 2TM domain protein	0.965	0.539	1	241
35	tr|S5P672	Lipase	1	0.524	1	364
36	tr|S5P358	Uncharacterized protein	0.971	0.508	0	175
37	tr|S5NYP4	Uncharacterized protein	1	0.717	0	388
38	tr|S5NLV0	Putative fimV C-domain protein	0.944	0.532	0	888
39	tr|S5P4L8	Flp pilus-assembly TadE/G-like family protein	0.964	0.675	1	418

40	tr|S5P1D7	Uncharacterized protein	1	0.547	0	296
41	tr|S5PFJ4	Uncharacterized protein	0.965	0.749	0	71
42	tr|S5P9T6	Thermolysin metallopeptidase, catalytic domain protein	0.844	0.618	0	565
43	tr|S5P0U1	Sporulation related domain protein	0.964	0.629	1	261
44	tr|S5P848	Uncharacterized protein	0.964	0.550	0	304
45	tr|S5NWS4	Uncharacterized protein	0.944	0.769	0	506
46	tr|S5NLZ1	Uncharacterized protein	0.964	0.696	0	686
47	tr|S5PAE0	Uncharacterized protein	1	0.583	0	280
48	tr|S5NWI8	Bacterial Ig-like domain family protein	0.999	0.763	0	3204
49	tr|S5NNG6	Flagellar hook-associated family protein	0.995	0.688	0	506
50	tr|S5P3P1	Flagellar hook-associated family protein	1	0.505	0	507
51	tr|S5P120	Right handed beta helix region family protein	0.965	0.590	0	420
52	tr|S5P3Z0	Levansucrase	1	0.508	0	494
53	tr|S5NZK6	Flagellar hook-basal body family protein	1	0.642	0	413
54	tr|S5NKY5	Flagellar hook-associated protein FlgK	0.995	0.680	0	662

DISCUSSION

Burkholderia pseudomallei causes melioidosis in humans and animals (Cheng and Currie, 2005). It is multi drug resistant pathogen associated with pneumonia (Currie and Jacups, 2003). Vaccination has become the choice of clinicians to combat this MDR pathigen. Although a number of efforts have been tried in the vaccine development against this pathogen, but more accurate and effective vaccine candidates are yet to be explored. Flagellin protein (FliC) has been investigated for its immunoprotective efficacy in mice model. Immunization with flagellin was able to elicit both humoral and cellular immune response in Balb/c mice. Additionally, flagellin immunization reduced bacterial load and resulted in 83% survival rate in mice[12]. Srilunchang et al (2009) reported the importance of AroC protein in immunoprotection. AroC catalyzes the formation of chorismate from 5-O-(1-carboxyvinyl)-3-phosphoshikimate in aromatic amino acid biosynthesis. The constructed unmarked aroC mutant of *B. pseudomallei* was able to confer significant protection in C57Bl/6 mice[13]. LolC, a putative lipoprotein releasing system transmembrane protein, has been reported to provide protection against *B. pseudomallei* infection. This protein stimulated humoral and cellular immune responses when delivered with monophosphoryl lipid A-trehalose dicorynomycolate[14]. Similarly, phosphoribosylglycinamide formyltransferase or PurN and phosphoribosylaminoimidazole synthetase or PurM provided protection against this pathogen[15]. We have predicted 58 proteins as potential vaccine candidates after analyzing whole proteome of *B. pseudomallei* MSHR 305. These candidate proteins include mostly porin family proteins, Ton b receptors, Bam (β-barrel assembly proteins: BamA and BamE), outer membrane proteins and uncharacterized proteins. Porins are β- barrel outer membrane proteins facilitating diffusion of molecules such as drugs. Immunization with porin OmpD from nontyphoidal *Salmonella* has been reported to evoke antibody titre and reduced bacterial load in mice lung[16]. Bam proteins (BamA and BamE) belong to Omp85 protein family and are highly conserved among the Gram negative bacteria. *In silico* analysis of BamA has shown its vaccine potential against *A. baumannii* [17]. Such genomic and proteomic analysis could help to identify potential vaccine targets in lesser time as compared to conventional vaccinology[18].

CONCLUSION

Reverse vaccinology is an emerging technology for prediction of vaccine candidates against pathogens. It is a time saving and highly productive approach. Bioinformatic analysis of *B. pseudomallei* proteome showed 58 potential vaccine candidates that could be useful in vaccine development against this pathogen.

CONFLICT OF INTEREST

Conflict of interest declared none.

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