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Insilico Search for Potential Vaccine Candidates in *Helicobacter Pylori* Genome

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Abstract*— Availability of genome sequences of pathogens has provided a tremendous amount of information that can be useful in drug target and vaccine target identification. The proteins/ peptides vaccines that could elicit the mucosal immune response are of great interest as potential vaccines. Recent new developments in the field of bioinformatics, genomics and proteomics have triggered the development of the insilico approach of vaccine design. This study follows the approach of 'reverse vaccinology' which employs the whole genome sequencing and advances in bioinformatics to identify vaccine candidates. The completely sequences genome of Helicobacter pylori 26695 (NC_000915) comprising of 1576 electronically annotated ORFs were analyzed in silico using a multi step computational screen to identify potential vaccine candidates. The selection parameters used were cellular localization, sequence similarity to known virulence factors and additional filtering criteria such as size. These screening criteria resulted in the selection of 316 ORFs with known and hypothetical proteins as potential vaccine candidates.

***Index Terms*— in silico, reverse vaccinology, vaccine candidates, virulence factors**

I. INTRODUCTION

Helicobacter pylori is a gram-negative, microphilic spiral shaped bacterium that chronically infects the gastric mucosa of more than half of all humans worldwide and is a major cause of gastritis and peptic ulcer disease and an early risk factor for gastric cancer (Eck *et al*., 1997). The *colonization* or virulence of *Helicobacter pylori* is due to prominent gene products of the bacteria (Kostrzynska *et al*., 1991, Graham *et al*., 1992, Cover *et al*., 1992.Censini *et al*., 1996 ,Tomb *et al***.,** 1997). Acid lowering drugs such as are generally safe, but some patients have developed transient Candida infection after antibiotic use. Cure rates have been less with shorter therapies but longer therapies have not been shown to result in greater cure rates (Chen *et al*., 1983). The immune response to *H. pylori* is remarkably diverse. Evidence from human and animal studies has shown that the immune system expends substantial energy in response to *H. pylori*. Yet, the infection is commonly lifelong, and the immune response activated against this organism does not affect clearance or prevent reinfection after successful antimicrobial treatment (Ramirez *et al*., 1997). The greatest problem for vaccine developers is the selection of an effective method for presenting antigens to the host's immune system in such a way that protective or therapeutic immune responses are elicited in the gastric mucosa.

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Since the mechanisms by which *H pylori* evades immunity and the roles of T and B cells in effector responses are poorly understood, purely empirical approaches have been applied to screen antigens, adjuvants, and delivery systems One vaccine that has been attempted was a recombinant vaccine using *Salmonella typhi* to express UreA and UreB, *H. pylori* urease genes (Kreiss, 1996). This vaccine produces no side effects, but there was also no immune response to urease. Another vaccine containing inactivated whole killed cells plus an adjuvant was practised (Kotloff, 2001). This trial vaccine contained formalin killed *H. pylori* cells with varying doses of an adjuvant, LTR192G. Unfortunately the vaccine raised IFN-γ levels, but it did not raise levels in infected individuals. When this vaccine was given orally *H. Pylori* specific antibody-secreting cells were induced in gastric tissues of uninfected volunteers with a high response in the duodenum (Losonsky, 2003). Comparative genomics and bioinformatics provide new opportunities for finding optimal targets among previously unexplored cellular functions based on the understanding of their related biological processes in bacterial pathogens and their hosts. (Itaya, 1995) The entire approach is built on the assumption that the potential target must play an essential role in the pathogen's survival and constitute a critical component in its metabolic pathway.
(Tatusov *et al*., 1997).

The computational genomics approach [Sakharkar *et al.*, 2004] is likely to speed up drug discovery process by removing hindrances like dead ends or toxicity that are encountered in classical approaches. Presumably, screening against such novel targets for functional inhibitors will result in discovery of novel therapeutic compounds active against bacteria, including the increased number of antibiotic resistant clinical strains [Thanassi *et al.*, 2002]. Till date there is no specific drug to be administered for *H. pylori* infection. Identification of non-human homologs in the essential genes of *H. pylori* with subsequent screening of the proteome to find the corresponding protein product are likely to lead to development of drugs that specifically interact with the pathogen. The non-human homologs of the surface proteins would represent ideal vaccine targets. Inactivation of these surface protein through vaccines would likely result in inactivation of the pathogen (Anirban *et al*., 2006**).**

The aim of the research work is to search for vaccine candidates in *Helicobacter pylori* by *in silico* analysis of the genome. By comparing the ORFs of the bacteria through BLAST analysis and selecting the proteins that have no homologous to host protein and serve as virulence factors and By screening the ORFs through psortb and sosui servers from the proteins selected through BLAST analysis for being present as extracellular, outer membrane or secreted proteins.

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II. PROCEDURE

A. *Tools used*

The online tools that have been used for the screening and selection of vaccine candidates in the genome of *H.pylori* are

- BLAST – To select the proteins that have no homology to the proteins present in humans for the next step of selection criteria
- PSORTB – To find the site of location of the proteins
- SOSUI – To find whether the protein is a membrane protein or a soluble protein.

B. *Methodology*

The *in silico* selection strategy employed to identify potential vaccine candidates was based on a rationale approach comprising of the following rationales:

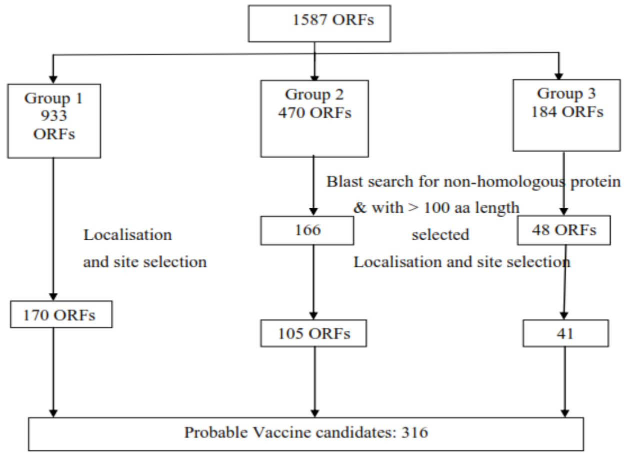
1) Proteins with significant sequence similarity to previously documented virulence factors may play a key role in the pathogenesis of *H.pylori* and hence are potential targets for vaccine control

2) Proteins that are secreted, membrane bound or surface exposed are easily accessible by the immune system and hence are potential antigens

The completely sequenced genome of *Helicobacter pylori* 26695 (NC_000915) comprising of 1576 electronically annotated ORFs (open reading frames) was screened using certain selection/filtering parameters to arrive at a limited subset of proteins that could serve as potential vaccine candidates.

Amino acid sequences of the 1576 ORFs of *Helicobacter pylori* 26695 (NC_000915) were downloaded from the Comprehensive Microbial Resource (CMR) database at TIGR (www.tigr.org). Initially the 1576 ORFs were categorized into three broad categories as proteins with known and putative function (group 1), and hypothetical proteins (group 2) and conserved hypothetical proteins (group 3).

The batch downloaded sequences were screened based on sequence similarity. Searches were performed by blast analysis against non-redundant database (NCBI) for group 2 and group 3 proteins. The proteins that have no homology to the proteins present in human beings and those proteins that are Transmembrane proteins, Membrane Proteins, Adhesions/ Flagellar proteins, Secretory Proteins, Lipoproteins, Regulatory Proteins, Multidrug resistance protein, Hypothetical proteins and Proteins with multiple functions were selected. The selected proteins are then screened for their sub-cellular localisation using the online localisation prediction tool, psortb (www.psortb.org/psortb). The outermembrane, extracellular, cytoplasmic membrane proteins were selected. The proteins whose sub- cellular localisation was predicted as unknown was screened for the presence of transmembrane helix using the sosui membrane protein prediction server (http://bp.nuap.nagoya-u.ac.jp/sosui). The extracellular, outer membrane and cytoplasmic membrane proteins having amino acid sequence length greater than 100 that were selected by psortb server and sosui server were selected as probable vaccine candidates.



Overview of selection process

III. RESULTS

A.Selection based on similarity search

Owing to the significantly large proportion of hypothetical and conserved hypothetical proteins, an initial screening for sequence similarity on group 2 and group 3 reduced the number of hypothetical and conserved hypothetical to 166 and 48 in which the proteins are classified into 11 categories as listed in table 1 and 2 for hypothetical and conserved hypothetical proteins respectively.

S. No.	Category	Proteins
1	Transmembrane Proteins	3
2	Adhesions/ Flagellar proteins	13
3	Secretory Proteins	4
4	Lipoproteins	5
5	Regulatory Proteins	1
6	Membrane proteins	9
7	Integral membrane proteins	10
8	Outer membrane proteins	29
9	Multidrug resistance protein	2
10	Proteins with multiple functions	9
11	Hypothetical	81

Table:1 Broader criteria for selection of hypothetical proteins

S. No.	Category	Proteins selected
1	Transmembrane Proteins	3
2	Adhesions/ Flagellar proteins	1
3	Secretory Proteins	4
4	Lipoproteins	1
5	Regulatory Proteins	3
6	Membrane proteins	7
7	Integral membrane proteins	7
8	Outer membrane proteins	6
9	Multidrug resistance protein	1

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10	Proteins with multiple functions	6
11	Hypothetical	9

Table:2 Broader criteria for selection of conserved hypothetical proteins

B.Selection based on localization

The proteins in group 1 are selected based on their site of location in which the membrane proteins and extracellular proteins are selected. The group 2 and group 3 proteins from table 1 and table 2 were screened for their localization for being as the membrane protein or extracellular protein or secreted. Table 3 shows the number of proteins selected based on their localization.

Group	ORF function	Number of proteins taken for screening	Number of proteins selected by localization
1	Known proteins	933	170
2	Hypothetical proteins	166	105
3	Conserved hypothetical proteins	48	41

Table:3 ORFs selected based on localization in the pathogen

S. No.	TIGR Locus	Gene	Name
Cell Envelope - Surface structures			
1	HP0410	hpaA	putative neuraminyllactose-binding hemagglutinin
Cell Envelope- Biosynthesis and degradation of murein sacculus and peptidoglycan			
2	HP0160		conserved hypothetical secreted protein
3	HPO493	mraY	phospho-N-acetylmuramoyl-pentapeptide-transferase
4	HP0567	PBP-1A	penicillin-binding protein 1A
5	HP0740	murF	UDP-MurNac-pentapeptide presynthetase
6	HPO743	mreB	rod shape-determining protein
7	HP1155	murG	transferase, peptidoglycan synthesis
8	HP1372	mreC	rod shape-determining protein
9	HP1543	tagE	toxR-activated gene
10	HP1544	tagE	toxR-activated gene
11	HP1565	pbp2	penicillin-binding protein 2
Cell Envelope- Biosynthesis and degradation of surface polysaccharides and			
12	HP0208	rfaJ	lipopolysaccharide 1,2-glucosyltransferase, authentic
13	HP0279	rfaC	lipopolysaccharide heptosyltransferase-1
14	HP0855	algI	alginate O-acetylation protein
15	HP0957	kdtA	3-deoxy-d-manno-octulosonic-acid transferase
16	HP1191	rfaF	ADP-heptose-lps heptosyltransferase II
17	HP1581	Llm	methicillin resistance protein
Cell envelope-other			
18	HP0009	omp1	outer membrane protein
19	HP0018		lipoprotein, putative
20	HP0025	omp2	outer membrane protein
21	HP0057		lipoprotein, putative
22	HP0079	omp3	outer membrane protein
23	HP0087		lipoprotein, putative
24	HP0122		lipoprotein, putative
25	HP0127	omp4	outer membrane protein
26	HP0135		lipoprotein, putative
27	HP0174		membrane protein, putative
28	HP0175		cell binding factor 2
29	HP0180	cute	apolipoprotein N-acyltransferase
30	HP0227	omp5	outer membrane protein
31	HP0229	omp6	outer membrane protein

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32	HP0252	omp7	outer membrane protein
33	HP0254	omp8	outer membrane protein
34	HP0289		toxin-like outer membrane protein
35	HP0317	omp9	outer membrane protein
36	HP0324	omp10	outer membrane protein
37	HP0350		membrane protein, putative
38	HP0472	omp11	outer membrane protein
39	HP0477	omp12	outer membrane protein
40	HP0492		lipoprotein, putative
41	HP0511		lipoprotein, putative
42	HP0567		membrane protein
43	HP0596		lipoprotein, putative
44	HP0610		toxin-like outer membrane protein
45	HP0637		lipoprotein, putative
46	HP0638	omp13	outer membrane protein
47	HP0655		protective surface antigen D15
48	HP0671	omp14	outer membrane protein
49	HP0706	omp15	outer membrane protein
50	HP0722	omp16	outer membrane protein
51	HP0725	omp17	outer membrane protein
52	HP0746		lipoprotein, putative
53	HP0762		lipoprotein, putative
54	HP0771		membrane protein, putative
55	HP0796	omp18	outer membrane protein
56	HP0833		lipoprotein, putative
57	HP0836		lipoprotein, putative
58	HP0838		lipoprotein, putative
59	HP0839	ompP1	outer membrane protein P1
60	HP0861		membrane protein, putative
61	HP0863		lipoprotein, putative
62	HP0896	omp19	outer membrane protein
63	HP0912	omp20	outer membrane protein
64	HP0913	omp21	outer membrane protein
65	HP0922		toxin-like outer membrane protein
66	HP0923	omp22	outer membrane protein
67	HP0931		lipoprotein, putative
68	HP0955	lgt	prolipoprotein diacylglyceryl transferase
69	HP1002		lipoprotein, putative
70	HP1039		membrane protein, putative
71	HP1081		lipoprotein, putative
72	HP1107	omp23	outer membrane protein
73	HP1113	omp24	outer membrane protein
74	HP1125	omp18	peptidoglycan associated lipoprotein precursor
75	HP1156	omp25	outer membrane protein
76	HP1157	omp26	outer membrane protein
77	HP1177	omp27	outer membrane protein
78	HP1243	omp28	outer membrane protein

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79	HP1342	omp29	outer membrane protein
80	HP1395	omp30	outer membrane protein
81	HP1424		lipoprotein, putative
82	HP1450		60 kDa inner-membrane protein
83	HP1456	lpp20	membrane-associated lipoprotein
84	HP1469	omp31	outer membrane protein
85	HP1501	omp32	outer membrane protein
86	HP1564		outer membrane protein
87	HP1571	rlpA	rare lipoprotein A
Cellular Process- Chemotaxis and motility			
88	HP0082	tlpC	methyl-accepting chemotaxis transducer
89	HP0099	tlpA	methyl-accepting chemotaxis protein
90	HP0103	tlpB	methyl-accepting chemotaxis protein
91	HP0173	fliR	flagellar biosynthetic protein
92	HP0232		secreted protein involved in flagellar motility
93	HP0246	flgI	flagellar basal-body P-ring protein
94	HP0295	Fla	flagellin B homolog
95	HP0325	flgH	flagellar basal-body L-ring protein
96	HP0327	flag	flagellar protein G
97	HP0351	fliF	flagellar basal-body M-ring protein
98	HP0392	cheA	histidine kinase
99	HP0601	flaA	flagellin A
100	HP0752	fliD	flagellar hook-associated protein 2
101	HP0770	flhB	flagellar biosynthetic protein
102	HP0870	flgE	flagellar hook
103	HP1035	flhF	flagellar biosynthesis protein
104	HP1041	flhA	flagellar biosynthesis protein
105	HP1119	flgK	flagellar hook-associated protein 1 (HAP1)
106	HP1192		secreted protein involved in flagellar motility
107	HP1274	pflA	paralysed flagella protein
108	HP1419	fliQ	flagellar biosynthetic protein
Cellular Processes- Toxin production and resistance			
109	HP0887		vacuolating cytotoxin
110	HP1165		tetracycline resistance protein tetA(P), putative
Cellular processes-Pathogenesis			
111	HP0459	virB4	virB4 homolog
112	HP0520	cag1	cag pathogenicity island protein
113	HP0521	cag2	cag pathogenicity island protein
114	HP0522	cag3	cag pathogenicity island protein
115	HP0523	cag4	cag pathogenicity island protein
116	HP0524	cag5	cag pathogenicity island protein
117	HP0526	cag6	cag pathogenicity island protein
118	HP0527	cag7	cag pathogenicity island protein
119	HP0528	cag8	cag pathogenicity island protein
120	HP0529	cag9	cag pathogenicity island protein
121	HP0530	cag10	cag pathogenicity island protein
122	HP0531	cag11	cag pathogenicity island protein

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123	HP0532	cag12	cag pathogenicity island protein
124	HP0533	cag13	cag pathogenicity island protein
125	HP0534	cag14	cag pathogenicity island protein
126	HP0535	cag15	cag pathogenicity island protein
127	HP0536	cag16	cag pathogenicity island protein
128	HP0537	cag17	cag pathogenicity island protein
129	HP0538	cag18	cag pathogenicity island protein
130	HP0539	cag19	cag pathogenicity island protein
Cellular processes-Pathogenesis			
131	HP0540	cag20	cag pathogenicity island protein
132	HP0541	cag21	cag pathogenicity island protein
133	HP0542	cag22	cag pathogenicity island protein
134	HP0543	cag23	cag pathogenicity island protein
135	HP0544	cag24	cag pathogenicity island protein
136	HP0545	cag25	cag pathogenicity island protein
137	HP0546	cag26	cag pathogenicity island protein
138	HP0547	cag27	cag pathogenicity island protein
139	HP0885	mviN	virulence factor mviN protein
Cellular processes- Adaptations to atypical conditions			
140	HP0280	ibpB	heat shock protein B
141	HP0927	htpX	heat shock protein
Cellular processes- Other			
142	HP0599	hylB	hemolysin secretion protein precursor
Central intermediary metabolism- Other			
143	HP1186		carbonic anhydrase
Energy metabolism- Electron transport			
144	HP0144	fixN	cytochrome c oxidase, heme b and copper-binding
145	HP0146	CcoQ	cbb3-type cytochrome c oxidase subunit Q
146	HP0147	fixP	cytochrome c oxidase, diheme subunit, membrane-
147	HP0265	ccdA	cytochrome c biogenesis protein
148	HP0378	ycf5	cytochrome c biogenesis protein
Energy metabolism-Electron transport			
149	HP0631	hydA	quinone-reactive Ni/Fe hydrogenase, small subunit
150	HP0632	hydB	quinone-reactive Ni/Fe hydrogenase, large subunit
151	HP0633	hydC	quinone-reactive Ni/Fe hydrogenase, cytochrome b
152	HP1227		cytochrome c553
153	HP1461		cytochrome c551 peroxidase
154	HP1508		ferrodoxin-like protein
155	HP1538	fbcH	ubiquinol cytochrome c oxidoreductase, cytochrome c1 subunit
156	HP1539	fbcH	ubiquinol cytochrome c oxidoreductase, cytochrome
157	HP1540	fbcF	ubiquinol cytochrome c oxidoreductase, Rieske 2Fe-
Protein fate-Protein and peptide secretion and trafficking			
158	HP0074	lspA	signal peptidase II
159	HP0576	lepB	signal peptidase I
160	HP1152	ffh	signal recognition particle protein
161	HP1300	secY	preprotein translocase subunit
162	HP1549	secF	protein-export membrane protein

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163	HP1550	secD	protein-export membrane protein
Regulatory functions- Other			
164	HP0224	msrA	peptide methionine sulfoxide reductase
165	HP0244	atoS	signal-transducing protein, histidine kinase
166	HP1168	cstA	carbon starvation protein
167	HP1364		signal-transducing protein, histidine kinase
168	HP1572		regulatory protein DniR
Unknown function-General			
169	HP0322		poly E-rich protein
170	HP0377		thiol:disulfide interchange protein (dsbC), putative

Table:4 Group 1 proteins selected as probable vaccine candidates

S. No.	TIGR LOCUS
Membrane proteins	
1	HP0118
2	HP0129
3	HP0149
4	HP0181
5	HP0342
6	HP0554
7	HP0565
8	HP0583
9	HP0708
Transmembrane proteins	
10	HP0097
11	HP0185
12	HP1479
Adhesions/Flagellar proteins	
13	HP0114
14	HP0245
15	HP0272
16	HP0433
17	HP0573
18	HP0809
19	HP0817
20	HP0820
21	HP1154
22	HP1265
Secretory proteins	
23	HP0038
24	HP0040
25	HP0336
Lipoproteins	
26	HP0150
27	HP0287
28	HP0837
29	HP1457
Regulatory Proteins	
30	HP1173

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Outermembrane proteins	
31	HP0101
32	HP0209
33	HP0253
34	HP0358
35	HP0424
36	HP0486
37	HP0487
38	HP0605
39	HP0609
40	HP0694
41	HP0726
42	HP0744
43	HP0782
44	HP0788
45	HP0914
46	HP0953
47	HP0971
48	HP1055
49	HP1056
50	HP 1057
51	HP1083
52	HP1167
53	HP1327
54	HP1408
55	HP1411
56	HP 1568
Integral membrane	
57	HP0158
58	HP0249
59	HP0288
60	HP0342
61	HP0427
62	HP1454
63	HP1569
Multi drug resistance protein	
64	HP1502
Proteins with multiple functions	
65	HP0560
66	HP0622
67	HP1467
Hypothetical proteins	
68	HP0063
69	HP0080
70	HP0120
71	HP0130
72	HP0137
73	HP0170
74	HP0204
75	HP0241
76	HP0256

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77	HP0292
78	HP0311
79	HP0345
80	HP0579
81	HP0721
82	HP0778
83	HP0806
84	HP0812
85	HP0852
86	HP0856
87	HP0994
88	HP0996
89	HP1029
90	HP1065
91	HP1074
92	HP1076
93	HP1089
94	HP1143
95	HP1187
96	HP1188
97	HP1288
98	HP1333
99	HP1390
100	HP1396
101	HP1397
102	HP1409
103	HP1451
104	HP1520
105	HP1524

Table:5 Group 2 (Hypothetical) proteins selected as probable vaccine candidates

Membrane proteins	
1	HP0248
2	HP0575
3	HP0946
4	HP1080
5	HP1486
6	HP1509
Transmembrane proteins	
7	HP1185
8	HP1321
9	HP1487
Adhesions/Flagellar proteins	
10	HP0465
Secretory proteins	
11	HP1117
12	HP1286
13	HP1551
Lipoproteins	
14	HP0785
Outermembrane proteins	

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15	HP0506
16	HP0710
17	HP1066
18	HP1285
19	HP1453
Integral membrane proteins	
20	HP0571
21	HP0920
22	HP1044
23	HP1162
24	HP1235
25	HP1343
Multidrug resistance protein	
26	HP0759
Proteins with multiple functions	
27	HP0677
28	HP1075
29	HP1175
30	HP1484
31	HP1488
32	HP1570
Hypothetical proteins	
33	HP0100
34	HP0162
35	HP0189
36	HP0226
37	HP0274
38	HP0374
39	HP0395
40	HP0709
41	HP1234

Table:6 Probable vaccine candidates from conserved hypothetical proteins

IV. DISCUSSION

The *in silico* approach has resulted in 385 proteins based on the various criteria chosen. The proteins that are selected do not have any homology with the host bacteria. As the proteins were screened down for being present in the outer membrane, extracellular and secreted proteins, the targeting becomes much easier.

A. Selection based on sequence similarity search

In the blast analysis, the hypothetical and conserved hypothetical proteins that have no homology with the host proteins and the proteins those were homologous to membrane proteins, lipoproteins, transmembrane proteins, adhesions/flagellar proteins, secreted proteins, regulatory proteins, outer membrane proteins, integral membrane proteins, multi drug resistance protein, proteins with multiple functions and strictly hypothetical proteins were chosen as each of those selected have maximum probability of being a vaccine candidate.

B. Membrane proteins/ integral membrane proteins/ outer membrane proteins

These are the proteins that are present on the cell membrane of the pathogen. Vaccines based on these proteins make the immune system of the host to elicit immune responses effectively by targeting the membrane of the pathogen and phagocyte the pathogen.

C. Adhesins/Flagellar proteins

Adhesions/Flagellar proteins represent one of the immunologically significant category, since adhesions are surface proteins that aid in the attachment of the pathogen thereby facilitating infection. Adhesions are potent antigens eliciting secretory IgA antibody responses in the mucosal routes.

D. Secretory proteins

The secretory proteins are interesting target in developing vaccines since interfering with protein secretion can block the translocation of virulence factors out of the pathogen.

E. Lipoproteins

Lipoproteins are the major component of the outer membrane of bacteria. The lipoproteins are the immunostimulatory molecules.

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F. Selection based on localisation of the proteins

The membrane proteins are exposed to the immune responses of the host more effectively than the soluble proteins. Hence by the use of psortb and sosui analysis, membrane proteins are selected as probable vaccine candidates.

V. CONCLUSION

The *in silico* approach in identifying vaccine targets is a preliminary approach towards narrowing down the search space for the vaccine candidates. The choice of selection parameters and stringency allowed in the selection process are very important in the determining the usefulness of the result. The availability of the whole sequence data for many pathogens has opened new avenues in the research for vaccine development. Nevertheless, genome data alone cannot accurately predict the *in vivo* immunogenicity of proteins and their usefulness as vaccine candidates. Therefore, vaccine candidates selected on the basis of *in silco* approach need to be validated using genomic, proteomic, genetic, biochemical and bioinformatic approaches, in addition to appropriate animal models. The selected 316 proteins may be further screened down by motif analysis and other approaches towards specificity.

REFERENCS

1. Anirban Dutta, Shashi Kr. Singh, Payel Ghosh, Runni Mukherjee, Sayak Mitter and Debashis Bandyopadhyay, *In silico* identification of potential therapeutic targets in the human pathogen *Helicobacter pylori, In Silico* Biology 6, 0005 (2006); ©2006, Bioinformation Systems e.V.
2. Bumann D, Metzger WG, Mansouri E, Palme O, Wendland M, Hurwitz R, Haas G, Aebischer T, von Specht BU, Meyer TF. Safety and immunogenicity of live recombinant *Salmonella enteric* serovar *typhi* Ty21a expressing urease A and B from *Helicobacter pylori* in human volunteers. Vaccine, 20: 845-52, 2001.
3. Blaser M. J., Kirschner D. 1999. Dynamics of *Helicobacter pylori* colonization in relation to the host response. *Proc. Natl. Acad. Sci. USA* 96: 8359-8364.
4. Censini, S., C. Lange, Z. Xiang, J. E. Crabtree, P. Ghiara, M. Borodovsky, R.Rappuoli, and A. Covacci. 1996. cag, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. Proc. Natl. Acad. Sci. USA 93:14648–14653
5. Chen, M., A. Lee, S. Hazell, P. Hu, and Y. Li. 1993. Immunisation against gastric infection with *Helicobacter* species: first step in the prophylaxis of gastric cancer? Int. J. Med. Microbiol. Virol. Parasitol. Infect. Dis. 280:155–165.
6. Chiba N., Rao B. V., Rademaker J. W., Hunt R. H. 1992. Meta-analysis of the efficacy of antibiotic therapy in eradicating *Helicobacter pylori*. *Am. J. Gastroenterol.* 87: 1716-1727
7. Cover, T. L., and M. J. Blaser. 1992. Purification and characterization of the vacuolating toxin from *Helicobacter pylori*. J. Biol. Chem. 267:10570–10575.
8. Davies GR, Banatvala N, Collins CE, Sheaff MT, Abdi Y, Clements L & Rampton DS (1994) Relationship between infective load of Helicobacter pylori and reactive oxygen metaboliteproduction in antral mucosa. Scand J Gastroenterol 29: 419–424.
9. Debets-Ossenkopp Y. J., Herscheid A. J., Pot R. G. J., Kuipers E. J., Kusters J. G., Vandenbroucke-Grauls C. M. J. E. 1999. Prevalence of *Helicobacter pylori* resistance to metronidazole, clarithromycin, amoxycillin, tetracycline and trovafloxicin in The Netherlands. *J. Antimicrob. Chemother.* 43: 511-515
10. D'Elios MM, Manghetti M, De Carli M, Costa F, Baldari CT, Burroni D, Telford JL, Romagnani S & Del Prete G (1997) Th1 effector cells specific for Helicobacter pylori in the gastric antrum of patients with peptic ulcer disease. J Immunol 158:962–967.
11. DiPetrillo MD, Tibbetts T, Kleanthous H, Killeen KP. Safety and immunogenicity of phoP/phoQ-deleted *Salmonella typhi* expressing *Helicobacter pylori* urease in adult volunteers. Vaccine, 18: 449-59, 1999.
12. Eck, M., B. Schmausser, R. Haas, A. Greiner, S. Czub, and H. Muller-Hermelink.1997. MALT-type lymphoma of the stomach is associated with *Helicobacter pylori* strains expressing the CagA protein. Gastroenterology 112:1482–1486.
13. El-Omar E. M., Carrington M., Chow W.-H., McColl K. E. L., Bream J. H., Young H. A., Herrera J., Lissowska J., Yuan C.-C., Rothman N., Lanyon G., Martin M., Fraumeni J. F., Rabkin C. S. 2000. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature* 404: 398-402 Infect Med 15(8):534-546, 1998. © 1998 Cliggott Publishing, Division of SCP Communications
14. Fox J. G., Beck P., Dangler C. A., Whary M. T., Wang T. C., Shi H. N., Nagler- Anderson C. 2000. Concurrent enteric helminth infection modulates inflammation and gastric immune responses and reduces helicobacter-induced gastric atrophy. *Nat. Med.* 6: 536-542
15. Galmiche A,Rassow J,Doye A,Cagnol S,Chambard JC,Contamin S,de Thillot V,Just I,Ricci V,Solcia E,Van Obberghen E,Boquet P., The N-terminal 34 kDa fragment of Helicobacter pylori vacuolating cytotoxin targets mitochondria and induces cytochrome c release. EMBO J. 2000 Dec 1; 19(23):6361-70.
16. Gerhard M,Lehn N,Neumayer N,Boren T,Rad R,Schepp W,Miehlke S,Classen M,Prinz C. Clinical relevance of the Helicobacter pylori gene for blood-group antigen-binding adhesin. Proc Natl Acad Sci U S A. 1999 Oct 26; 96(22):12778-83
17. Graham, D. Y., M. F. Go, and D. J. Evans, Jr. 1992. Urease, gastric ammonium/ ammonia, and *Helicobacter pylori*—the past, the
18. present, and recommendations for future research. Aliment. Pharmacol. Ther. 6:659–669.
19. Graham D. Y. 1998. Antibiotic resistance in *Helicobacter pylori*: implications for therapy. *Gastroenterology* 115: 1272-1277.
20. Hamlet A, Thoreson AC, Nilsson O, Svennerholm AM & Olbe L (1999) Duodenal Helicobacter pylori infection differs in cagA genotype between asymptomatic subjects and patients with duodenal ulcers. Gastroenterology 116: 259–268
21. Itaya, M. (1995). An estimation of the minimum genome size required for life. FEBS Lett. 362, 257-260.
22. Kelly SM, Pitcher MC, Farmery SM, Gibson GR. Isolation of *Helicobacter pylori*from feces of patients with dyspepsia in the United Kingdom. Gastroenterol,107:16771-4, 1994.
23. Kreiss C., Buclin T, Cosma M, Corthesy-Theulaz I, Michetti P. Safety of oral immunization with recombination urease in patients with *Helicobacter pylori* infection. Lancet, 347: 1630-1, 1996.
24. Kostrzynska, M., J. D. Betts, J. W. Austin, and T. J. Trust. 1991. Identification, characterization, and spatial localization of two flagellin species in *Helicobacter pylori* flagella. J. Bacteriol. 173:937–946.
25. Losonsky GA, Kotloff KL, Walker RI. B-cell responses in gastric antrum and duodenum following oral-inactivated *Helicobacter pylori* whole-cell (HWC) vaccine and LT (R192G) in *H. pylori*-seronegative individuals. Vaccine, 2: 562-5,2003.
26. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet*, 1: 1311-15, 1984.
27. Marshall BJ (1994) Helicobacter pylori. Am J Gastroenterol 89:116–128.
28. Mathijs Bergman, Gianfranco Del Prete, Yvette van Kooyk & Ben Appelmelk., Helicobacter pylori phase variation, immune modulation and gastric autoimmunity. Nature Reviews Microbiology 4, 151-159 (February 2006)
29. Nurse Pract. Helicobacter pylori: an emerging infectious disease, 2000 Aug; 25(8):40,43-4,47-8 passim; quiz 54-5.
30. Odenbreit S. Adherence properties of *Helicobacter pylori*: impact on pathogenesis and adaptation to the host. Int J Med Microbiol, 295:317-24, 2005.
31. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N & Sibley RK (1991) Helicobacter pylori infection and the risk of gastric cancer. New Engl J Med 325:1127–1131
32. Ramirez-Ramos A., Gilman R. H., Leon-Barua R., Recavarren-Arce S., Watanabe J., Salazar G., Checkley W., Mc-Donald J., Valdez Y., Cordero L., Carrazco J.1997. Rapid recurrence of *Helicobacter pylori* infection in Peruvian patients after successful eradication. *Clin. Infect. Dis.* 25: 1027-1031.

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33. Ruggiero P, Peppoloni S, Rappuoli R, Del Giudice G. The quest for a vaccine against *Helicobacter pylori*: how to move from mouse to man? Microbes Infect,5: 749-56, 2003.
34. Sakharkar, K. R., Sakharkar, M. K. and Chow, V. T. K., (2004). A novel genomics approach for the identification of drug targets in pathogens, with special reference to *Pseudomonas aeruginosa*. In Silico Biol. 4, 0028
35. Tatusov, R. L., Koonin, E. V. and Lipman, D. J. (1997). A genomic perspective of protein families. Science 278, 631-637.
36. Thanassi, J. A., Hartman-Neumann, S. L., Dougherty, T. J., Dougherty, B. A. and Pucci, M. J. (2002). Identification of 113 conserved essential genes using a high- throughput gene disruption system in *Streptococcus pneumoniae*, Nucleic Acid Res. 30, 3152-3162
37. Tomb, J.-F., *et al*. 1997. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. Nature 388:539–547.
38. Tomita T,Jackson AM,Hida N,Hayat M,Dixon MF,Shimoyama T,Axon AT,Robinson PA,Crabtree JE., Expression of Interleukin-18,a Th1 cytokine,in human gastric mucosa is increased in Helicobacter pylori infection. J Infect Dis.2001 Feb 15; 183(4):620-7. Epub 2001 Jan 24.
39. Tsuji S, Kawai N, Tsujii M, Kawano S, Hori M (2003). "Review article: inflammation-related promotion of gastrointestinal carcinogenesis - a perigenetic pathway". *Aliment Pharmacol Ther* 18 (Suppl 1): 82–9.
40. Wotherspoon AC, Ortiz-Hidalgo C, Falzon MF & Isaacson PG (1991) Helicobacter pylori associated gastritis and primary B-cell lymphoma. Lancet 338:1175–1176.

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