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SCREENING AND IDENTIFICATION OF DRUG TARGETS AND VACCINE CANDIDATES FOR *HELICOBACTER PYLORI* STRAIN *Hp*26695

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ABSTRACT

Helicobacter pylori, a class 1 carcinogen colonizes stomach causing gastric carcinoma. Rising antibiotic resistance and reinfections are drawbacks of antibiotic therapies. Alternating drugs and vaccination may be the promising approach to prevent and treat reoccurring infections. Therefore, there is a need for discovery of drug targets, drugs and vaccine candidates for the treatment of *H. pylori*. An objective of this current study is to identify potential drug targets and suitable vaccine candidates for *H. pylori* strain *Hp*26695 by *insilico* genome and proteome analysis.

Drug targets were identified initially by comparing the genomes between *H. pylori* and *Homosapien sapiens* using RAST. RAST identified a total of 569 unique genes. These unique genes later were subjected to non-homology and gene property analysis to identify the potential drug targets. BLASTpfollowed by gene property analysis of 569 unique genes identified seven potential drug targets.

Vaccine candidates were identified initially by screening protein sequences for pathogenic factors. These pathogenic factors were screened to identify non-homologous molecules and secondary structure patterns (helices). The proteins ≤ 3 helices are subjected to screening of antigenic nature followed by allergenicity. The proteins qualifying the above criteria were screened for antigens, B-cells and T-cell epitopes. Proteins showing positive predictions for antigenic, B-cell, T-cell activities are thus shortlisted as vaccine candidates for vaccine designing. Analysis identified 16 immunogenic proteins contributing to immune-response. These methods have enabled rapid identification of potential drug targets and vaccine candidates for strain *Hp*26695 with possible therapeutic implications for gastric cancer. cancer.

INTRODUCTION

Gastric cancer is caused by infection of class 1 carcinogen *Helicobacter pylori* (Zhang, 1994). Treatment for *H. pylori* infection includes drugs to relieve from pain and acidity, but not for gastritis, peptic ulcers, and gastric cancer. Carcinogenic activity of *H. pylori* suggests the need for discovery of new drug targets and drugs for prevention of *H. pylori*. Laboratory techniques and bioinformatics approaches are used to identify drug targets which can influence growth, colonization and virulence of *H. pylori*(Neelapu*et al.*, 2014). Availability of the complete *H. pylori* genome sequence of pathogens provides us the platform and opportunity to mine the genome and harness the potential drug targets. Comparative genomic analysis between host and pathogen would provide us with a tremendous amount of information that can be useful in drug target identification (Neelapu*et al.*, 2013).Comparative genomics analysis of host with pathogens revealed potential drug targets in *Staphylococcus aureus* (Uddin*et al.*, 2014), *H. pylori* (Neelapu and Pavani, 2013; Neelapu*et al.*, 2015;Nammi*et al.*, 2016)*Listeria monocytogenes*(Hossain*et al.*, 2013), *Leishmaniainfantum*(Suthar*et al.*, 2009), *L. major* (Florez*et al.*, 2010), *Mycobacterium leprae*(Wiwanitkit, 2014), *Pseudomonas aeruginosa*(Sakharkar*et al.*, 2004), *Schistosomamansomi*(Caffrey*et al.*, 2009).Metabolic pathway analysis (Sarkar*et al.*, 2012), reverse docking (Cai*et al.*, 2006) and screening for essential genes (Dutta*et al.*, 2006) are used to identify drug targets in *H. pylori*. However, there are no specific reports to date, on comparing genomes of *H. pylori* strain *Hp*26695 with host *Homosapiens* to identify drug targets in *H. pylori*. Therefore, comparing genome of host and pathogen may provide novel drug targets for *H. pylori* strain*Hp*26695.

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Rising antibiotic resistance due to efflux pumps (Rauws and Tytgat, 1990; Graham *et al.*, 1991), potential reinfection of *H. pylori* even after successful eradication therapy (Arora and Czinn, 2005), inhibition of T-cell stimulation by vacuolatingcytotoxin (Vac A) (Molinari, 1998; Reyrat, 1999), and the highly inflammatory nature by the constituents of the cell wall suggests vaccination as an alternative for protection against *H. pylori*. For better immunization the half-life of the antimicrobial agents should be long enough to be effective and also penetrate mucosal barrier (Arora and Czinn, 2005). Hence, the use of vaccines with appropriate immunogens may provide immune protection against *H. pylori*. Therefore, mining the genomic sequences via bioinformatics approaches for immunological data would provide suitable vaccine candidates. The objective of the current paper is to screen and identify novel drug targets and vaccine candidates for therapeutic intervention of *H. pylori*.

MATERIAL AND METHODS

Sample

Complete genomeof *H. pylori* strain *Hp*26695 with the geographical origin of Europe has the following accession number and genome length NC_018939 and 1,667,867 bp respectively (Manolov*et al.*, 2014). In our study, identification of novel drug targets and vaccine candidates for *H. pylori* has been accomplished for the first time in *H. pylori* strain *Hp*26695. Novel drug targets were screened and identified using an integrated approach of genome, proteome and metabolic pathway analysis followed by primary property analysis of the genes/proteins using computational resources. Novel vaccine candidates were screened and identified by searching for pathogenic factors, followed by non-homology, secondary patterns and subsequent analysis for antigens, non-allergens and epitopes using computational resources.

Screening and Identification of Drug Targets for H. pylori

The following protocol was followed for screening and identification of novel drug targets in *H. pylori* (Figure 1)

Drug target screening for identification of unique molecules in H. pylori

Comparative genome analysis was performed to screen the drug targets for pathogen *H. pylori.*Genomeof *H. pylori* strain *Hp*26695 was initially annotated and further reconstructed for metabolic pathways using Rapid Annotation Subsystem Technology (RAST) server (Aziz *et al.*, 2008). Comparative genome analysis between pathogen *H. pylori* and host *Homo sapiens sapiens*was performed using RAST to screen unique genes that are only present in pathogen and not present in the host (Table 1). Genes which are unique to *H. pylori* in the above method were filtered and catalogued.

Drug target screening for confirmation of unique molecules in H. pylori

Bacterial genes which are non-homologous to humans are essential for pathogen. To identify the non-homologous molecules in *H. pylori*, homology at the level of sequence and structure of molecules were used as the parameters. BLASTp (Altschul*et al.*, 1990) which is based on principle of homology was used to confirm the uniqueness of the catalogued genes in *H. pylori* by comparing genes against *Homosapiens sapiens* (Table 1).

Drug target identification

A set of computational resources were used to analyse the characteristic features of the genes, to identify the potential drug targets.BTXpred (Saha and Raghava, 2007), SRTpred (Garg and Raghava, 2008), VGIchan (Saha *et al.*, 2007) and VICMpred (Saha and Raghava, 2006) are the potential targets servers (Table 1) to identify the potentiality of the drug targets. Catalogued genes were verified for their potentiality as drug targets using the above list of servers.

Screening and Identification of Vaccine Candidates for H. pylori

The following protocol was followed for screening and identification of vaccine candidates in *H. pylori* (Figure 2).

Screening of proteome for identification of pathogenic factors in H. pylori

The bacterial genome was retrieved and the translated protein sequences of the pathogen are screened for pathogenic factors. Virulence factors, secretory proteins, outer-membrane proteins, bacterial toxins are the pathogenic factors. VirulentPred, EffectiveDB, CELLO, BTXpred (Table 1) are used to screen virulence factors, secretory proteins, outer-membrane proteins and bacterial toxins respectively. Further, the pathogenic factors of the bacteria are screened for non-homologous proteins as per the procedure described above in "Drug target screening for confirmation of unique molecules in *H. pylori"*.

Screening of non-homologous proteins for identification of secondary patterns in H. pylori

The non-homologous proteins of the bacteria are screened for secondary patterns – helices using Chou Fasman method by CFSSP: Chou and Fasman Secondary Structure Prediction Server (Ashok Kumar, 2013) (Table 1). Proteins with alpha-helices ≤ 3 are selected for further analysis.

Predicting function of proteome

Metabolic pathway analysis using RAST (Aziz *et al.*, 2008), BLASTp (Altschul*et al.*, 1990), NCBI Conserved Domain Database (Marchler-Bauer*et al.*, 2004), ProtFun 2.2 Server (Jensen *et al.*, 2002) (Table 1) and literature-search are used for identifying the function of proteins.

Screening of proteome for antigens

The proteins fulfilling the above criteria are screened for antigens using Antigenic Emboss Server (Kolaskar and Tongaonkar, 1990) (Table 1). These proteins are catalogued and subjected to further analysis.

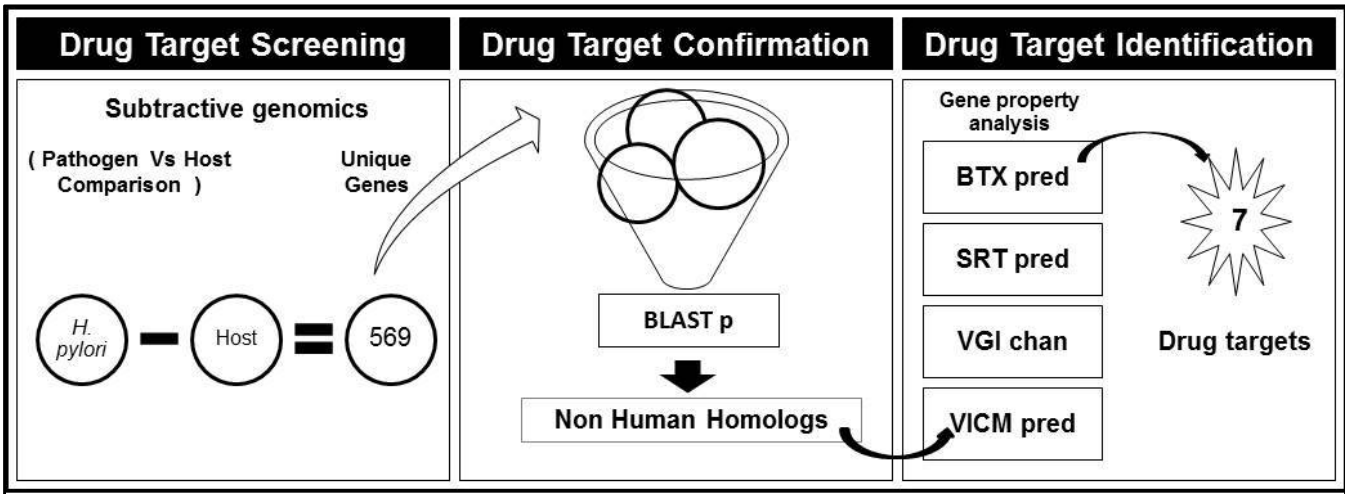
Screening of proteome for non-allergenicity

The proteins which are antigenic in nature are screened for allergenicity using server Allergen Online (Maria *et al.*, 2006) (Table 1). The non-allergens are shortlisted and catalogued for further analysis.

Table 1 Computational resources used for identification of potential drug targets and suitable vaccine candidates in *Helicobacter pylori*

S. No	Server Name	Server Function	Reference
1	RAST	Rapid Annotation Subsystem Technology Server is used for prediction of unique genes based on metabolic pathways.	Aziz *et al*., (2008)
2	BTXpred	Server is for prediction of bacterial toxins and its function from primary amino acid sequence.	Saha and Raghava, (2007)
3	SRTpred	Server classifies protein sequence as secretory or non-secretory proteins.	Garg and Raghava, (2008)
4	VGIchan	Server predicts voltage gated ion-channels and classifies them into sodium, potassium, calcium and chloride ion channels from primary amino acid sequences.	Saha*et al*., (2007)
5	VICM pred	Server aids in broad functional classification of bacterial proteins into virulence factors, information molecule, cellular process and metabolism molecule.	Saha and Raghava, (2006)
6	VirulentPred	VirulentPred predicts virulence proteins using reliable Support Vector Machine (SVM) algorithm. This server has a prediction accuracy of 65%.	Garg*et al*., (2008)
7	EffectiveDB	EffectiveDB predicts putative effectors by identifying eukaryotic-like protein domains and by detecting the 2 known types of signal peptides. This server has a prediction accuracy of 80%.	Jehl*et al*., (2011)
8	CELLO-V	CELLO (subcellular localization predictor) predicts protein present in outer membrane directly from protein sequences. The server uses two-level support vector machine (SVM) system: the first level contains SVM classifiers and the second level SVM classifier function to generate the probability distribution of decisions for possible localizations. This server has a prediction accuracy of 90%.	Yu *et al*., (2004)
9	CFSSP Server	Chou &Fasman Secondary Structure Prediction (CFSSP) server predicts protein conformation like helices, beta sheets, random coils based on Chou &Fasman algorithm. This server has a prediction accuracy of 88%.	Ashok Kumar, (2013)
10	Antigenic	Antigenic server predicts potentially antigenic sites of a protein sequence. The server uses semi-empirical method consisting physicochemical properties of amino acids and their frequencies of occurrence in experimentally known epitopes. This server has a prediction accuracy of 75%.	Kolaskar and Tongaonkar, (1990)
11	ABCpred	ABCpred server predicts B-cell epitope using Recurrent Artificial Neural Network-(ANN-) based algorithm. This server has a prediction accuracy of 65.93%.	Saha and Raghava, (2006)
12	HLApred	HLApred server identifies the experimentally proven binders taken from MHCBN database based on quantitative matrices HLA alleles which were obtained from literature. This server has a prediction accuracy of 80%.	http://www.imtech.res.in/raghava/hlapred/index.html
13	Allergen Online	Cross reactive allergens are predicted using server Allergen Online based on BLOSUM50 scoring matrix algorithm. This Server has a prediction accuracy of 70%.	Maria *et al*., (2006)

Figure1 Screening and identification of novel drug targets for *H. pylori*



Screening of non-allergenic proteome for identification of antigenic and epitope regions in H. pylori

The proteins fulfilling the above criteria are screened for promising epitopes which include both B cell & T cell epitopes. B-cells epitopes are screened and identified using ABCpred Server (Saha *et al*., 2006) (Table 1), whereas T-cell epitopes are screened and identified using HLApred (Table 1). Finally, proteins satisfying the above three criteria's i.e. proteins showing positive predictions for antigenic, B-cell, T-cell activities are short listed as vaccine candidates for vaccine designing.

RESULTS

Genome Wide InsilicoAnalysis for Screening of Drug Targets in H. pylori

Genome wide *in silico* analysis for screening drug targets identified 569 unique genes in *H. pylori* strain *Hp*26695 (Table 2). These molecules fall under 24 metabolic categories as shown in Table 2. Proteome analysis followed by gene property analysis of 569 unique genes identified seven potential drug targets in *H. pylori* (Table 3). These molecules fall under five metabolic categories as shown in Table 3.

Proteome Wide InsilicoAnalysis for Screening of Vaccine Candidates in H. pylori

Screening of 1469 proteins in *H. pylori* strain *Hp*26695 identified 643 pathogenic factors.VirulentPred, EffectiveDB, CELLO, BTXpredidentified 399, 291, 197, 18 proteins respectively (Table 4). Analysis of 643 pathogenic factors identified 146 non-homologous proteins (Table 4). Screening of 146 non-homologous proteins for secondary structure patterns identified 46 proteins with ≤ 3 helices (Table 4). Analysis of these 46 proteins identified 44 proteins which are antigenic in nature. Further analysis of 44 antigenic proteins identified 29 non-allergenic proteins (Table 4). Analysis of these 29 non-allergenic proteins revealed antigens (99), B-cell (198) and T-cell (419) epitopes shortlisting to 16 proteins with antigenic regions (26) and potential epitopes (52) (Table 5, 6, 7, 8). These 16 immunogenic proteins contribute to immune-response and are well-suited for vaccine designing (Table 8). *Insilico*screening of peptides have helped examining the molecular properties, further *in vivo*-studies would be most helpful in bringing out potentially specific vaccine candidates.

Table 2 Total number of unique genes identified for each metabolic category using RAST in *H. pylori* strain *Hp*26695 by comparing genomes of pathogen *H. pylori* and Host*Homosapiens sapiens*

S. No	Metabolic category	*Hp*26695
1	Amino Acids and Derivatives	49
2	Carbohydrates	37
3	Cell Division and Cell Cycle	3
4	Cell Wall and Capsule	43
5	Clustering-based subsystems	82
6	Cofactors, Vitamins, Prosthetic Groups, Pigments	50
7	DNA Metabolism	31
8	Fatty Acids, Lipids, and Isoprenoids	29
9	Membrane Transport	22
10	Miscellaneous	14
11	Motility and Chemotaxis	38
12	Nucleosides and Nucleotides	2
13	Phosphorus Metabolism	3
14	Potassium metabolism	3
15	Protein Metabolism	84
16	RNA Metabolism	18
17	Regulation and Cell signalling	7
18	Respiration	28
19	Stress Response	15
20	Sulfur Metabolism	2
21	Virulence, Disease and Defense	9
22	Iron acquisition and metabolism	0
	Total Number of Genes	569

Figure 2 Screening and identification of vaccine candidates for *H. pylori*

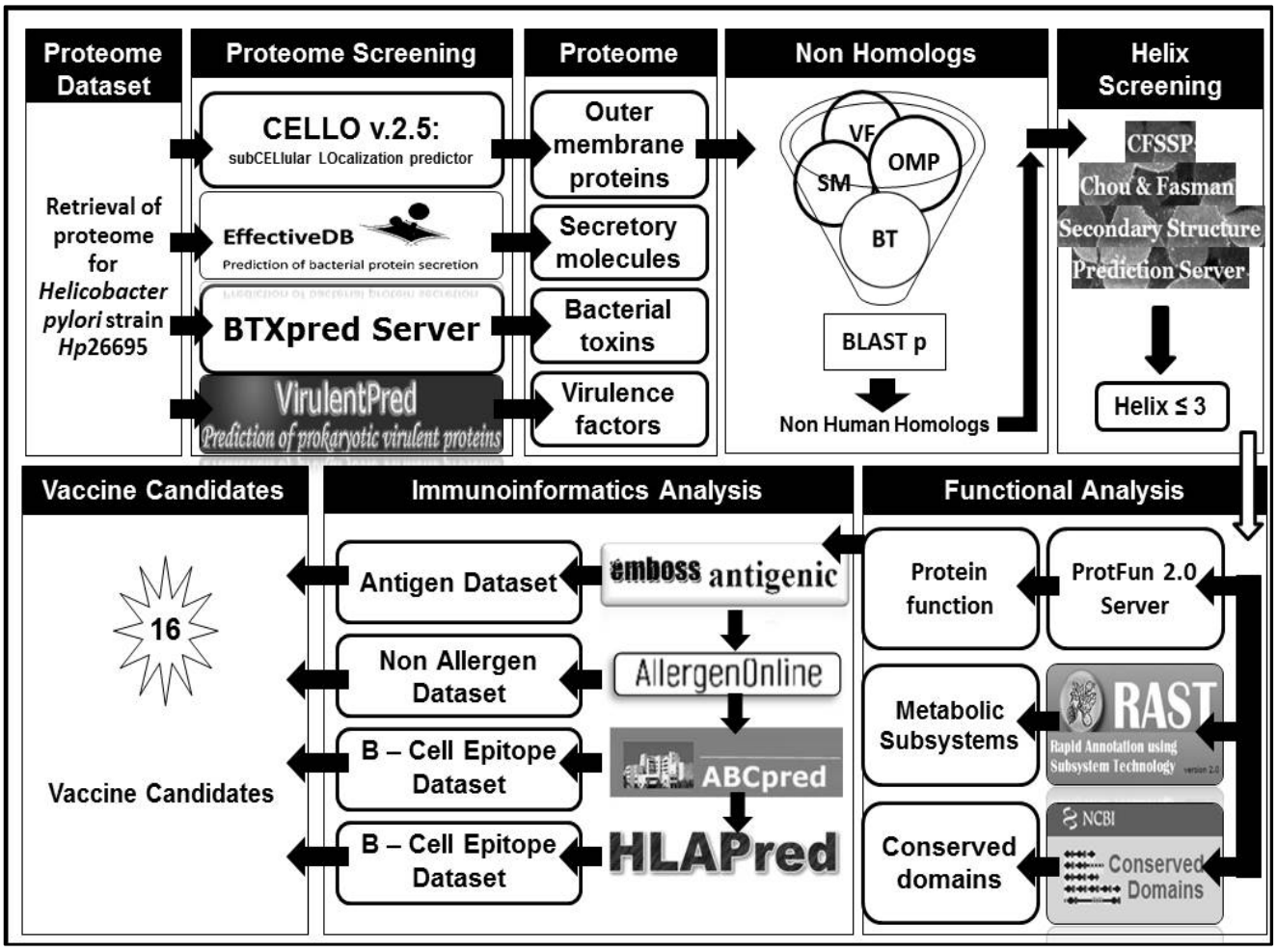


Table 3 Drug targets identified in the *H. pylori* strain *Hp*26695 by comparing genomes of pathogen *H. pylori* and Host*Homosapiens sapiens*

S. No	Drug Target	Metabolic Category	Gene ID	Validated[1]	Novel[2]
1	Menaquinone via futalosine step 1	Cofactors, Vitamins, Prosthetic Groups, Pigments	GI:15645397	+	
2	Type III restriction-modification system methylation subunit	DNA Metabolism	GI:15645218	+	
3	Dipeptide transport system permease protein DppB	Membrane Transport	GI:15644927	+	
4	Dipeptide transport system permease protein DppC	Membrane Transport	GI:15644928	+	
5	Ferric siderophore transport system, biopolymer transport protein ExbB	Membrane Transport	GI:15646054		+
6	Ribonuclease BN	RNA Metabolism	GI:15646017		+
7	NADH-ubiquinone oxidoreductase chain J	Respiration	GI:15645883		+

[1]*Experimentally validated drug targets either by genetically or biochemically*
[2]*Novel drug targets identified in this study*

Table 4 Pathogenic factors, non-homology proteins, helices, antigens and non-allergens identified in *H. pylori* strain *Hp*26695

Analysis Type	Virulence Factor	Secretory Proteins	Outer Membrane Proteins	Bacterial Toxin	Total Proteins
Pathogenic factors	399	291	197	18	643
Non-homology to humans	88	71	57	3	146
Proteins with ≤3 helices	37	17	4	0	46
Antigenic proteins	35	16	4	0	44
Non-allergenic proteins	24	9	3	0	29

DISCUSSION

Insilico methods helped in identifying novel drug targets and vaccine candidates for *H. pylori*. This was the first report on implementation of *insilico* subtractive genomics and *insilico* reverse vaccinology to identify novel drug targets and vaccine candidates respectively for *H. pylori* strain *Hp*26695.

Drug Targets for H. pylori

Subtractive genomics, metabolic pathway analysis, essential gene analysis and reverse docking were earlier used to identify drug targets for *H. pylori*. Cai *et al.* (2006) used reverse docking to identify a drug target in *H. pylori*. Dutta *et al.* (2006) identified 40 essential genes as drug targets in *H. pyloriHp*AG1 strain. Sarkhar *et al.* (2012) identified lipopolysaccharide biosynthesis pathway as a source of potential drug targets in *H. pylori* using metabolic pathway analysis. Neelapu and Pavani (2013) identified 17 novel drug targets in *Hp*B38, *Hp*P12, *Hp*G27, *Hp*Shi470, *Hp*SJM180 strains of *H. pylori* using genomics and proteomics. Neelapu *et al.* (2015)using genomics and proteomics identified 29 novel drug targets in *Hp*AG1 strain of *H. pylori*. Nammi *et al*., (2016) using comparative genomics identified 29 novel drug targets. In this present study subtractive genomics was used to identify novel drug targets in addition to the existing drug target's pool of *H. pylori* (Neelapu*et al*., 2016). Mining the genome of pathogen has identified nearly seven potential drug targets for *H. pylori.* These novel drug targets fall under the following categories of functions such as Cofactors, Vitamins, Prosthetic Groups, and Pigments; DNA Metabolism; Membrane Transport; RNA Metabolism; and Respiration.

Drug targets influencing cofactors, vitamins, prosthetic groups, pigments of the pathogen

Menaquinone via futalosine step 1, is identified as the drug target in *H. pylori.* This drug target influences metabolic pathway of cofactors, vitamins, prosthetic groups, pigments of the pathogen. Menaquinone is an important component of the electron transfer pathway. An alternative pathway is present in the human commensal intestinal bacteria *H. pylori* and *Campylobacter jejuni*. Disruption of menaquinone via futalosine pathway had shown inhibition of bacteriostatic growth (Arakawa *et al*., 2011). Therefore, designing an inhibitor for menaquinone via futalosine step 1 would affect the growth of *H. pylori*.

Drug targets influencing DNA metabolism of the pathogen

Type III restriction-modification system methylation subunit of restriction–modification (R-M) systems, is identified as the drug target in *H. pylori.* This drug target influences metabolic pathway DNA metabolism of the pathogen. *H. pylori* are naturally competent and prone to take DNA from the environment (Dorer *et al*., 2010) and bacteriophages also infect *H. pylori* (Heintschel *et al*., 1993). Missense and frameshift mutations can accumulate and inactivate genes when bacteriophages or free DNA or plasmids enter into other cells.

Evidence is there that sometimes even both endonuclease and methylase genes of R-M systems have to be turned off. However, *H. pylori* in a population have a very good defensive system, where R-M systems protect the genome of *H. pylori* from accumulated mutations when bacteriophages or free DNA or plasmids enter into other cells. Mutant strains lacking this display a pleiotropic phenotype, including increased mutability, hyper recombination, and increased sensitivity to DNA-damaging agents. Therefore, designing an inhibitor for type III restriction-modification system methylation subunit decreases the rate of survival of *H. pylori* due to gross changes occurring in the genetic material.

Drug targets influencing DNA metabolism of the pathogen

Dipeptide transport system permease protein DppB, dipeptide transport system permease protein DppC and ferric siderophore transport system, biopolymer transport protein ExbB are identified as drug targets in *H. pylori.* These drug targets influence membrane transport of the pathogen.

Dipeptide*DppABCDF* and oligopeptide*oppABCD* genes are a class of ABC-type transporter in *H. pylori*. Dipeptide transport system permease protein - DppBC are responsible for transporting dipeptides. Dipeptide and oligopeptide system

Table 5 Antigens predicted in the proteome of *H. pylori* strain *Hp*26695 using Antigenic server

S. No	Protein ID	Protein Name	Antigen No	Antigen Sequence
1	NP_206874.1	Lipoprotein signal peptidase	AG1	KSLLVFMGVFFLIFGVDQAIKYAILEG
			AG2	YESLMIDIVLVFNKGVAFSLLSFLEGGLKYLQILLILGLFIFLM
			AG3	GAGVSNVLDRFVHGGVVDYVYYHYGFDFAIFNFADVMIDVGVGVLL LKQFFFK
2	NP_206881.1	Membrane Protein	AG4	ALKSKAFRVSIQWNALVRKLLALE
3	NP_206885.1	Cytochrome c oxidase VI a.	AG5	IAKKAVKIVFFLGLVVVLLMMI
4	NP_206945.1	CBB3-type cytochrome c oxidase Q	AG6	LRGFAYAFFTILFTLFLYAYIFSM
			AG7	YGYLALND
5	NP_206947.1	DUF4006 superfamily	AG8	GNLLIVVILLCVAVFFTLKAIHIQK
			AG9	YELVNQ
6	NP_207071.1	Translation Protein	AG10	LHFSHL
			AG11	DVGFIIKNLVFLGVFSLLGW
			AG12	FLWPSMLELKKILLE
			AG13	KSVLEYAQR
			AG14	ESLLKI
			AG15	LEKILKKCFDAYKIKPLLSQNS
			AG16	KTQFFIMA
			AG17	KTYLFFTLINKYLPSAQSQLPLKIS
			AG18	KLLVLEFR
7	NP_207125.1	Flagellar protein FlaG	AG19	WMYSTFISLKTHLQFIE
			AG20	HRYFLF
			AG21	EEGVYLGVGSI
			AG22	KHGYLGIYKNP
			AG23	ILKALEFI
			AG24	FEEFQLHSLHLEV
8	NP_207138.1	Translation Protein	AG25	FIDVLLYYK
			AG26	LKLEGCEKHCKKKYAIEKVIKEVGLELKSKSVMPY
			AG27	RSQIISILMK
9	NP_207241.1	Transporter Protein	AG28	SLAILMPSFLLAAPDYK
			AG29	KFTQILDFI
			AG30	IKAIGGLIIVGTCIYAY
10	NP_207255.1	Translation Protein	AG31	WKCVGIIIITAAIS
			AG32	VGISVSNL
			AG33	FLWLNAKSFLLSGFVPFIMIPWLDILNSFVLYVCFLLIFSIAE
			AG34	SDILIAHSK
			AG35	SLIFKKVRIYSKMLVALGLSSVLIGCAM
			AG36	SSEHVTPLDFNYPIHIVQAPQNHHVVGILTPRIQVSDNLKPYID
11	NP_207289.1	putative paralog of *Hp*aA	AG37	FQDALINQIQT
			AG38	RGYQVLR
			AG39	KIFSVLDLKGWVGILE
			AG40	LDTLVDQSSGSVWF
			AG41	SNRVVHDFAVEVGT
			AG42	NRMYAVVMK
12	NP_207332.1	Cag pathogenicity island protein cag15	AG43	ISKLKQNFLQFKH
			AG44	LDKYSLYYRLFNISSIVIGFLVALFSYGAGVILVYPILFLFALIIKPSFFYY TTYLLLLVSLSIISKYYLLSHA
			AG45	KLIILMT
			AG46	HPEYIPCKVTCVTSG
13	NP_207346.1	50S ribosomal protein L31	AG47	EIEVLST
			AG48	ISSFCHPFY
14	NP_207357.1	30S ribosomal protein S21	AG49	RNLVVTEC
			AG50	KKKVLKRLYML
15	NP_207389.1	Transport and binding protein	AG51	LGLILSLAAILIAFK
16	NP_207531.1	Integral membrane protein	AG52	SLRACFLTLFFSGY
			AG53	IGSLVALLLGLPVLIFSANTLFLGAVFVGLIAIAQI
			AG54	SSYIVIDEL
			AG55	AMAISGLSLAGVILSFIFFRIYDITKPSLIGK
17	NP_207549.1	Translation Protein Enzyme	AG56	YKNVYDDD
18	NP_207582.1	Type I restriction-modification system	AG57	STVVAEF
19	NP_207692.1	Hydrogenase expression/formation protein HypC	AG58	AIPSKVIAIKDNVVLLETLGVQRE
			AG59	GESVKVGDYVLLHIGYVMSK
			AG60	LESIELYQE
			AG61	VYLVQSD
20	NP_207710.1	TsaC protein (YrdC domain) required for threonylcarbamoyladenosine t(6)A37 modification in tRNA	AG62	IGLLSK
			AG63	NQSVLIESA
			AG64	FSTLKSLVRAP
			AG65	TTFIYPNSKAVRVIRG
			AG66	TLYSTS
			AG67	LTQCAYDKE
			AG68	ASNLADVIVSDE
21	NP_207792.1	Translation Protein	AG69	SKIFRLY
			AG70	AHTLYISE
			AG71	SVFVQDAIIFYLEY

22	NP_207808.1	Chaperone/ Serine/ HtrA protease	AG72	TLFISLALALSLN
			AG73	KERVSVPSK
			AG74	VSLVIVFCCFLRAVELPGIY
			AG75	TQEFLYMKSSFVEFF
23	NP_207818.1	DUF2147 superfamily	AG76	KFYAYGISDV
			AG77	KGVVFLSDLIKVGKR
			AG78	KTYYVRVT
			AG79	LDEVLKTI
			AG80	SNLLELLQEALASL
			AG81	LNSLSVTKVECSKGKHHAYVFVLSSDHKILSKL
24	NP_207838.1	Ribosome-binding factor A	AG82	LIRQFVLQAS
			AG83	WFKCPKLSFVSDN
			AG84	EKQLRLDAI
25	NP_207888.1	Translation Protein	AG85	KSALLGVRRILGEV
			AG86	IAFYFFAILTLSMALVVITTTNILYAITALASSMVFISAFFFLLDAEFLGV VQITVYVGAVIVMYA
26	NP_208061.1	NADH dehydrogenase subunit J	AG87	AAEVVERK
			AG88	PKILCILSFGVALLLTLILSAPS
			AG89	DAQIPNIKAIGYVLFTNYLIPFEAAALMLLVAMVGGI
			AG90	TGIQKI
			AG91	EGIVIDDN
			AG92	HIKVISI
27	NP_208233.1	Carbon storage regulator	AG93	RGSVRLGFE
			AG94	ESTLILRAE
			AG95	KEAIVSEN
			AG96	KASVCVDES
28	NP_208314.1	Cell envelope Protein	AG97	ITHFIAISFVLSLFSACKD
29	NP_208381.1	Ubiquinol-cytochrome C chaperone	AG98	DLEFLKRL
			AG99	LKDLFDALVYD

Table 6 B-cell, T-cell and Consensus epitopes predicted in the proteome of *H. pylori* strain *Hp*26695

Antigen No	Antigen Sequence	B-cell Epitopes (Threshold > 0.60)	T-cell Epitopes (Threshold > 0.70)	Consensus Epitopes
AG1	KSLLVFMGVFFLIFGVDQAIKYAILEG	1	3	–
AG2	YESLMIDIVLVFNKGVAFSLLSFLEGGLKYLQILLILGLFIFLM	1	8	1
AG3	GAGVSNVLDRFVHGGVVDYVYYHYGFDFAIFNFADVMIDVG VGVLLLKQFFFK	–	7	–
AG4	ALKSKAFRVSIQWNALVRKLLALE	1	4	2
AG5	IAKKAVKIVFFLGLVVVLLMMI	–	5	–
AG6	LRGFAYAFFTILFTLFLYAYIFSM	1	4	2
AG7	YGYLALND	–	–	–
AG8	GNLLIVVILLCVAVFFTLKAIHIQK	1	6	1
AG9	YELVNQ	–	1	–
AG10	LHFSHL	1	–	–
AG11	DVGFIIKNLVFLGVFSLLGW	–	–	–
AG12	FLWPSMLELKKILLE	–	–	–
AG13	KSVLEYAQR	–	–	–
AG14	ESLLKI	1	–	–
AG15	LEKILKKCFDAYKIKPLLSQNS	–	1	–
AG16	KTQFFIMA	–	–	–
AG17	KTYLFFTLINKYLPSAQSQLPLKIS	–	4	–
AG18	KLLVLEFR	–	–	–
AG19	WMYSTFISLKTHLQFIE	2	3	3
AG20	HRYFLF	1	–	–
AG21	EEGVYLGVGSI	–	–	–
AG22	KHGYLGIYKNP	1	–	–
AG23	ILKALEFI	1	–	–
AG24	FEEFQLHSLHLEV	1	2	1
AG25	FIDVLLYYK	–	–	–
AG26	LKLEGCEKHCKKKYAIEKVIKEVGLELKSKSVMPY	–	5	–
AG27	RSQIISILMK	1	–	–
AG28	SLAILMPSFLLAAPDYK	1	–	–
AG29	KFTQILDFI	–	–	–
AG30	IKAIGGLIIVGTCIYAY	1	3	1
AG31	WKCVGIIIITAAIS	–	5	–
AG32	VGISVSNL	–	2	–
AG33	FLWLNAKSFLLSGFVPFIMIPWLDILNSFVLYVCFLLIFSIAE	–	8	–
AG34	SDILIAHSK	–	–	–
AG35	SLIFKKVRIYSKMLVALGLSSVLIGCAM	2	3	2
AG36	SSEHVTPLDFNYPIHIVQAPQNHHVVGILTPRIQVSDNLKPYID	2	2	2
AG37	FQDALINQIQT	–	–	–
AG38	RGYQVLR	–	–	–
AG39	KIFSVLDLKGWVGILE	–	–	–

AG40	LDTLVDQSSGSVWF	1	–	–
AG41	SNRVVHDFAVEVGT	1	1	1
AG42	NRMYAVVMK	–	–	–
AG43	ISKLKQNFLQFKH	–	–	–
AG44	LDKYSLYYRLFNISSIVIGFLVALFSYGAGVILVYPILFLFALIIK PSFFYYTTYLLLLVSLSIISKYYLLSHA	2	5	2
AG45	KLIILMT	–	1	–
AG46	HPEYIPCKVTCVTSG	1	1	1
AG47	EIEVLST	1	–	–
AG48	ISSFCHPFY	1	–	–
AG49	RNLVVTEC	–	–	–
AG50	KKKVLKRLYML	–	–	–
AG51	LGLILSLAAILIAFK	–	–	–
AG52	SLRACFLTLFFSGY	–	–	–
AG53	IGSLVALLLGLPVLIFSANTLFLGAVFVGLIAIAQI	2	8	3
AG54	SSYIVIDEL	–	–	–
AG55	AMAISGLSLAGVILSFIFFRIYDITKPSLIGK	3	6	6
AG56	YKNVYDDD	1	–	–
AG57	STVVAEF	1	–	–
AG58	AIPSKVIAIKDNVVLLETLGVQRE	3	3	1
AG59	GESVKVGDYVLLHIGYVMSK	2	4	4
AG60	LESIELYQE	–	–	–
AG61	VYLVQSD	–	–	–
AG62	IGLLSK	2	–	–
AG63	NQSVLIESA	–	–	–
AG64	FSTLKSLVRAP	1	–	–
AG65	TTFIYPNSKAVRVIRG	–	–	–
AG66	TLYSTS	–	1	–
AG67	LTQCAYDKE	1	–	–
AG68	ASNLADVIVSDE	–	–	–
AG69	SKIFRLY	1	–	–
AG70	AHTLYISE	1	–	–
AG71	SVFVQDAIIFYLEY	–	1	–
AG72	TLFISLALALSLN	–	2	–
AG73	KERVSVPSK	1	1	1
AG74	VSLVIVFCCFLRAVELPGIY	2	5	4
AG75	TQEFLYMKSSFVEFF	2	2	1
AG76	KFYAYGISDV	1	1	1
AG77	KGVVFLSDLIKVGKR	–	3	–
AG78	KTYYVRVT	–	–	–
AG79	LDEVLKTI	–	–	–
AG80	SNLLELLQEALASL	–	–	–
AG81	LNSLSVTKVECSKGKHHAYVFVLSSDHKILSKL	2	4	4
AG82	LIRQFVLQAS	–	1	–
AG83	WFKCPKLSFVSDN	1	1	1
AG84	EKQLRLDAI	1	–	–
AG85	KSALLGVRRILGEV	1	2	–
AG86	IAFYFFAILTLSMALVVITTTNILYAITALASSMVFISAFFFLLDA EFLGVVQITVYVGAVIVMYA	3	11	6
AG87	AAEVVERK	1	–	–
AG88	PKILCILSFGVALLLTLILSAPS	1	2	1
AG89	DAQIPNIKAIGYVLFTNYLIPFEAAALMLLVAMVGGI	1	1	1
AG90	TGIQKI	1	–	–
AG91	EGIVIDDN	–	–	–
AG92	HIKVISI	1	1	1
AG93	RGSVRLGFE	–	–	–
AG94	ESTLILRAE	1	–	–
AG95	KEAIVSEN	–	–	–
AG96	KASVCVDES	–	–	–
AG97	ITHFIAISFVLSLFSACKD	–	5	–
AG98	DLEFLKRL	1	–	–
AG99	LKDLFDALVYD	1	–	–

mutant's in *H. pylori* lacked the ability to use certain dipeptides, hexapeptides, and nonapeptides due to compromisation of either substrate binding domain or permease domains (Weinberg, 2007). Therefore, designing an inhibitor to dipeptide transport system permease protein DppBC would affect the growth and survival of *H. pylori*.

Ferric transport system, biopolymer transport protein ExbB is a member of transporter proteins in *H. pylori*. All bacterial pathogens have developed highly sophisticated iron assimilation systems as a response to iron-limiting conditions encountered in environment and host's body fluids. Production of siderophores, small nonproteinaceous molecules with extremely high affinity for iron (III), is one of the most successful and widely utilized strategies of iron assimilation (Merrell *et al.*, 2003). Common components of both siderophore-dependent and host iron-binding protein-dependent iron acquisition systems are receptor proteins involved in binding of siderophores and interacting with the host iron-binding proteins. These large outer membrane proteins are responsible for the transport of iron or iron-containing

Table 7 Consensus epitopes identified in *H. pylori* strain *Hp*26695

Antigen No	Antigen Sequence	Epitope No	Consensus Epitopes
AG2	YESLMIDIVLVFNKGVAFSLLSFLEGGLKYLQILLILGLFIFLM	EPC5	LLILGLFIF
AG4	ALKSKAFRVSIQWNALVRKLLALE	EPC37	LVRKLLALE
		EPC38	VRKLLALER
AG6	LRGFAYAFFTILFTLFLYAYIFSM	EPC66	FLYAYIFSM
		EPC67	LFLYAYIFS
AG8	GNLLIVVILLCVAVFFTLKAIHIQK	EPC81	FTLKAIHIQ
AG19	WMYSTFISLKTHLQFIE	EPC132	LKTHLQFIE
AG24	FEEFQLHSLHLEV	EPC140	FQLHSLHLE
AG30	IKAIGGLIIVGTCIYAY	EPC185	LIIVGTCIY
AG35	SLIFKKVRIYSKMLVALGLSSVLIGCAM	EPC224	FKKVRIYSK
		EPC225	LIFKKVRIY
AG36	SSEHVTPLDFNYPIHIVQAPQNHHVVGILTPRIQVSDNLKPYID	EPC228	VVGILTPRI
		EPC229	VGILTPRIQ
AG41	SNRVVHDFAVEVGT	EPC235	VVHDFAVEV
AG44	LDKYSLYYRLFNISSIVIGFLVALFSYGAGVILVYPILFLFALIIKPSFFYYTTYLLLLVSL SIISKYYLLSHA	EPC262	ILVYPILFL
		EPC263	VILVYPILF
AG46	HPEYIPCKVTCVTSG	EPC300	YIPCKVTCV
AG53	IGSLVALLLGLPVLIFSANTLFLGAVFVGLIAIAQI	EPC337	IFSANTLFL
		EPC338	IGSLVALLL
		EPC339	VALLLGLPV
AG55	AMAISGLSLAGVILSFIFFRIYDITKPSLIGK	EPC346	FFRIYDITK
		EPC347	FRIYDITKP
		EPC348	FIFFRIYDI
		EPC349	ILSFIFFRI
		EPC350	VILSFIFFR
		EPC351	LSLAGVILS
AG58	AIPSKVIAIKDNVVLLETLGVQRE	EPC382	VVLLETLGV
		EPC385	LHIGYVMSK
AG59	GESVKVGDYVLLHIGYVMSK	EPC386	VLLHIGYVM
		EPC387	YVLLHIGYV
		EPC388	VGDYVLLHI
AG73	KERVSVPSK	EPC454	KERVSVPS
AG74	VSLVIVFCCFLRAVELPGIY	EPC464	VSLVIVFC
		EPC466	IVFCCFLRA
		EPC467	LVIVFCCFL
		EPC468	VIVFCCFLR
AG75	TQEFLYMKSSFVEFF	EPC470	YMKSSFVEF
AG76	KFYAYGISDV	EPC472	FYAYGISDV
AG81	LNSLSVTKVECSKGKHHAYVFVLSSDHKILSKL	EPC500	FVLSSDHKI
		EPC501	YVFVLSSDH
		EPC502	LSSDHKILS
		EPC503	VLSSDHKIL
AG83	WFKCPKLSFVSDN	EPC505	WFKCPKLSF
		EPC528	FYFFAILTL
		EPC529	ITVYVGAVI
AG86	IAFYFFAILTLSMALVVITTTNILYAITALASSMVFISAFFFLLDAEFLGVVQITVYVGAV IVMYA	EPC530	VVQITVYVG
		EPC531	VYVGAVIVM
		EPC532	LVVITTTNI
		EPC533	VVITTTNIL
AG88	PKILCILSFGVALLLTLILSAPS	EPC540	LTLILSAPS
AG89	DAQIPNIKAIGYVLFTNYLIPFEAAALMLLVAMVGGI	EPC542	YVLFTNYLI
AG92	HIKVISI	EPC572	HIKVISI

responsible for the transport of iron or iron-containing compounds throughthe otherwise impermeable outer membrane (Ye *et al*., 2003). Ferric transport system Exb B biopolymer transport protein in *H. pylori* is responsible for the transport of iron or iron-containing compounds through the impermeable outer membrane. Sequence analysis in *E. coli, Haemophilusinfluenzae, Neisseria meningitides* and *Pseudomonas putida* provided information on existence mechanism that utilizes Ton-independent heme. Knockout mutant and complementation studies in *Neisseria meningitides* established this fact (Sarangi *et al*., 2009). Designing an effective inhibitor to the existing multiple proteins for the utilization of heme-containing compounds effects the survival of *H. pylori* in their natural habitat, human mucosal surfaces.utilization of heme-containing compounds effects the

Drug targets influencing RNA metabolism of the pathogen

Ribonuclease BN is identified as a drug target in *H. pylori*. This drug target influences RNA metabolism of the pathogen. Ribonuclease, BN, lacking RNase H and RNase D activity was identified in *E. coli* and it is different from other exoribonucleases known till date in *E. coli*. RNase BN is a substrate specific with specificity towards C-C-A sequence in tRNA than other types of tRNA and substrate specificity was proved both *in vitro* and *in vivo*. Mutants of these proteins affect the processing of tRNA's and ultimately synthesis of protein (Asha *et al*., 1983). Hence, an effective inhibitor for Ribonuclease BN can block the function of protein synthesis.

Table 8 Vaccine Candidates for *H. pylori* strain *Hp*26695

S. No	Protein ID	Protein Name
1	NP_206874.1	lipoprotein signal peptidase
2	NP_206881.1	Membrane Protein
3	NP_206945.1	Cbb3-type cytochrome c oxidase subunit Q CcoQ
4	NP_206947.1	DUF4006 superfamily
5	NP_207125.1	Flagellar protein FlaG
6	NP_207241.1	Transporter Protein
7	NP_207289.1	Putative paralog of *Hp*aA
8	NP_207332.1	cag pathogenicity island protein cag15
9	NP_207346.1	50S ribosomal protein L31
10	NP_207531.1	Integral membrane protein
11	NP_207692.1	Hydrogenase expression/formation protein HypC
12	NP_207808.1	HtrA protease/chaperone protein / Serine protease
13	NP_207818.1	DUF2147 superfamily
14	NP_207838.1	Ribosome-binding factor A
15	NP_208061.1	NADH dehydrogenase subunit J
16	NP_208233.1	Carbon storage regulator

Drug targets influencing respiration of the pathogen

NADH-ubiquinone oxidoreductase chain J is identified as a drug target in *H. pylori*. This drug target influences metabolic pathway and effect respiration of the pathogen. The NADH ubiquinone oxidoreductase (Complex I), provides the input to the respiratory chain from the NAD-linked dehydrogenases of the citric acid cycle. The complex couples the oxidation of NADH and the reduction of ubiquinone, to the generation of a proton gradient which is then used for ATP synthesis. The complex occurs in the mitochondria of eukaryotes and in the plasma membranes of purple photosynthetic bacteria, and the closely related respiratory bacteria. All inhibitors affect the electron-transfer step from the high-potential iron-sulphur cluster to ubiquinone. Class I inhibitors appear to act directly at the ubiquinone-catalytic site which is related in complex I and glucose dehydrogenase (Friedrich.*et al*., 1994). Inhibitors designed to bind to NADH-ubiquinone oxidoreductase chain J competitively inhibit the protein from functioning which results in chemical asphyxiation of cells.

Vaccine Candidates for H. pylori

Constructive screening protocol was implemented to identify suitable vaccine candidates for *H. pylori.* Choosing such a conservative way to face vaccine design inevitably implies missing some pathogen antigens, but still this is a small price to reach a valuable compromise. Forwarding further bioinformatics analyses on selected ones, may prove successful (Sandro*et al.*, 2006). Bioinformatics approach has helped us in shortlisting and in identifying pathogenic factors from the proteome of the pathogen. Screening of pathogen factors for non-homology would shortlist the proteins which have the potential to cross react when vaccine is administered. Usually a protein has high probability of failure to cloning and express in experiment when it is likely to have more helices. Hence, proteins with alpha-helices < 3 are selected for further analysis. (Sandro*et al.*, 2006; Capecchi*et al.*, 2004; Pizza *et al.*, 2000).Screening of proteome for allergenicity would avoid the proteins which can elicit undesirable reaction during vaccination. Further, proteins showing positive predictions for antigenic, B-cell, T-cell activities are characterized as potential immunogens which are suitable for vaccine candidates.

CONCLUSION

Comparative genomics of *H. pylori* and *Homosapien sapiens* identified seven bacterial genes which are non-homologous to humans and are essential for pathogen. Four genes of the 7 predicted drug targets are already experimentally validated lending credence to our approach. These novel drug targets may have possible therapeutic implications for gastric cancer. Systematic *insilico* analysis approach identified 16 immunogenic proteins which are suitable vaccine candidates for *H. pylori.* Thus, bioinformatics approaches helped in rapid identification of novel drug targets and vaccine candidates for *H. pylori* strain *Hp*26695.

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References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990): Basic local alignment search tool. *J. Mol. Biol*., 215(3): 403-410.

Arakawa, C., Kuratsu, M., Furihata, K., Hiratsuka, T., Itoh, N., Seto, H. and Dairi, T. (2011): Diversity of the early step of the futalosine pathway. *Antimicrob. Agents Chemother*., 55(2): 913-916.

Arora, S. and Czinn, S.J. (2005): Vaccination as a method of preventing *Helicobacter pylori*associated gastric cancer.*CancerEpidemiol. Biomarkers Prev.,*14: 1890-1891.

Asha, P.K., Blouin, R.T., Zaniewski, R. and Deutscher, M.P. (1983): Ribonuclease BN: Identification and partial characterization of a new tRNA processing enzyme. *Proc. Natl. Acad. Sci. USA*, 80: 3301-3304.

Ashok, K.T. (2013): CFSSP: Chou and Fasman secondary structure prediction server. *Wide Spect.*, 1(9): 15-19.

Aziz, R.K., Bartels, D., Best, A.A., DeJongh, M., Disz, T., Edwards, R.A., Formsma, K., Gerdes, S., Lass, E.M., Kubal, M., Meyer, F., Olsen, G.J., Olson, R., Osterman, A.L., Overbeek, R.A., McNeil, L.K., Paarmann, D., Paczian, T., Parrello, B., Pusch, G.D., Reich, C., Stevens, R., Vassieva, O., Vonstein, V., Wilke, A. and Zagnitko, O. (2008): The RAST server: rapid annotations using subsystems technology. *BMC Genomics*, 9: 75.

Caffrey, C.R., Rohwer, A., Oellien, F., Marhöfer, R.J., Braschi, S., Oliveira, G., McKerrow, J.H. and Selzer, P.M. (2009): A comparative chemogenomics strategy to predict potential drug targets in the metazoan pathogen. *Schistosomamansoni.PLoS ONE*, 4(2): e4413.

Cai, J., Han, C., Hu, T., Zhang, J., Wu, D., Wang, F., Liu, Y., Ding, J., Chen, K., Yue, J., Shen, X. and Jiang, H.

(2006): Peptide deformylase is a potential target for anti-*Helicobacter pylori* drugs: reverse docking, enzymatic assay, and x-ray crystallography validation. *Protein Sci.,* 15(9): 2071-2081.

Capecchi, B., Serruto, D., Adu-Bobie, J., Rappuoli, R. and Pizza, M. (2004): The genome revolution in vaccine research. *Curr.Issues Mol. Biol.,* 1(6): 17-28.

Chou, P.Y. and Fasman, G.D. (1978): Empirical predictions of protein conformation.*Annu. Rev. Biochem.,* 47: 251-276.

Dorer, M.S., Fero, J. and Salama, N.R. (2010): DNA damage triggers genetic exchange in *Helicobacter pylori*. *PLoSPathog.*, 6(7): e1001026.

Dutta, A., Singh, S.K., Ghosh, P., Mukherjee, R., Mitter, S. and Bandyopadhyay, D. (2006):*Insilico*identification of potential therapeutic targets in the human pathogen *Helicobacter pylori*. *In Silico Biol.*, 6(2): 43-47.

Florez, A.F., Park, D., Bhak, J., Kim, B.C., Kuchinsky, A, Morris, J.H., Espinosa, J. and Muskus, C. (2010): Protein network prediction and topological analysis in *Leishmania major* as a tool for drug target selection. *BMC Bioinformatics,* 11(1): 484.

Friedrich, T., Heek, P., Leif, H., Ohnishi, T., Forche, E., Kunze, B., Jansen, R., Trowitzsch-Kienast, W., Höfle, G., Reichenbach, H. and Weiss, H. (1994): Two binding sites of inhibitors in NADH: ubiquinone oxidoreductase (complex I). *Eur. J. Biochem.,* 219(1-2): 691-698.

Garg, A. and Gupta, D. (2008):VirulentPred: a SVM based prediction method for virulent proteins in bacterial pathogens.*BMC Bioinformatics*, 9(1): 62.

Garg, A. and Raghava, G.P.S. (2008): A machine learning based method for the prediction of secretory proteins using amino acid composition, their order and similarity-search. *In Silico Biol.*, 8(2): 129-140.

Graham, D.Y., Lew, G.M., Evans, D.G., Evans, D.J. and Klein, P.D. (1991): Effect of triple therapy (antibiotics plus bismuth) on duodenal ulcer healing. A randomized controlled trial. *Ann. Intern. Med.,* 115(4): 266-269.

Heintschel V.H.E., Nalik, H.P. and Schmid, E.N. (1993)**:** Characterisation of a *Helicobacter pylori* phage (HP1). *J. Med. Microbiol.*, 38: 245-249.

*Henikoff, S. and Henikoff, J.G. (1992):*Amino acid substitution matrices from protein blocks. *Proc. Natl. Acad. Sci. USA*,*89(22):* 10915-10919.

Hossain, M.M., Mosnaz, A.T.M.J., Mohin, S.A., Kumar, P.R., Kabir, S.S., Ullah, S.M. and Prodhan, S.H. (2013): Identification of putative drug targets of *Listeria monocytogenes*F2365 by subtractive genomics approach. *J. Biosci. Biotechnol.,*2(5): 63-71.

Jehl, M.A., Arnold, R. and Rattei, T. (2011): Effective-a database of predicted secreted bacterial proteins. *Nucleic Acids Res.*, 39: D591-D595.

Jensen, L.J., Gupta, R., Blom, N., Devos, D. Tamames, J., Kesmir, C., Nielsen, H., Stærfeldt, H.H., Rapacki, K., Workman, C. and Andersen, C.A. (2002): Prediction of human protein function from post-translational modifications and localization features. *J. Mol. Biol.,* 319(5): 1257-1265.

Kolaskar, A.S. and Tongaonkar, P.C.(1990): A semi-empirical method for prediction of antigenic determinants on protein antigens.*FEBS Lett.*, 276(1-2): 172-4.

Manolov, A., Prihodko, E., Larin, A., Karpova, I., Semashko, T., Alexeev, D., Kostrjukova, E. and Govorun, V. (2014): Direct submission byBioinformatics, Research Institute for Physico-Chemical Medicine,MalayaPirogovskaya 1A, Moscow 119992, Russia. Availablefrom: http://www.ncbi.nlm.nih.gov/nuccore/GI:410024832.(AccessedJuly 15, 2014).

Marchler-Bauer A. and Bryant S.H. (2004): CD-search: protein domain annotations on the fly. *Nucleic Acids Res.*, 32(2): W327-31.

Maria, A., Scalab, E., Palazzob, P., Ridolfib, S., Zennarob, D. and Gabriele, C. (2006):Bioinformatics applied to allergy: allergen databases, from collecting sequence information to data integration. The Allergome platform as a model.*Cell Immunol.*, 244(2): 97-100.

Merrell, D.S., Thompson, L.J., Kim, C.C., Mitchell, H., Tompkins, L.S., Lee, A. and Falkow, S. (2003): Growth phase-dependent response of *Helicobacter pylori* to iron starvation. *Infect. Immun.*, 71(11): 6510-6525.

Molinari, M, Galli C, Norais N, Telford, J.L., Rappuoli, R. and Luzio, J.P. (1997):Vacuoles induced by *Helicobacter pylori* toxin contain both late endosomal and lysosomal markers. *J. Biol. Chem.,* 272: 25339-25344.

Nammi, D., Srimath-Tirumala-Peddinti, R.C.P.K. and Neelapu, N.R.R. (2016): Identification of drug targets in *Helicobacter pylori* by *insilico* analysis: possible therapeutic implications for gastric cancer. *Curr. Cancer Drug Targets*, 16(1): 79-98.

Neelapu N.R., Nammi, D., Pasupuleti, A.C., Surekha, C. (2014): *Helicobacter pylori* induced gastric inflammation, ulcer, and cancer: A pathogenesis perspective. *Interdiscip. J. Microinflamm.*, 1(2): 113.

Neelapu, N.R.R. and Pavani, T. (2013): Identification of novel drug targets in *Hp*B38*, Hp*P12*, Hp*G27*, Hps*hi470*, Hp*SJM180strains of *Helicobacter pylori*: An *insilico*approach for therapeutic intervention. *Curr. Drug Targets*, 14(5): 601-611.

Neelapu, N.R.R., Mutha, V.R.N. and Akula, S. (2015): Identification of potential drug targets in *Helicobacter pylori* strain *Hp*AG1 by *insilico* genome analysis. *Infect. Disorders Drug Targets*, 15(2): 106-117.

Neelapu, N.R.R., Nammi, D., Pasupuleti, A.M.C. and Challa, S. (2016): Targets against *Helicobacter pylori* and other tumor-producing bacteria. In: Villa T.G. and Vinas M. (eds) New Weapons to Control Bacterial Growth, Springer International Publishing, pp. 239-279.

Neelapu, N.R.R.,Srimath-Tirumala-Peddinti, R.C.P.K., Deepthi, N. and Pasupuleti, A.C.M (2013): New strategies and paradigm for drug target discovery: a special focus on infectious diseases tuberculosis, malaria, leishmaniasis, trypanosomiasis and gastritis. *Infect. Disorders Drug Targets*, 13(5): 352-364.

Pizza, M., Scarlato, V., Masignani, V., Giuliani, M.M., Arico, B., Comanducci, M., Jennings, G.T., Baldi, L., Bartolini, E., Capecchi, B., Galeotti, C.L., Luzzi, E., Manetti, R., Marchetti, E., Mora, M., Nuti, S., Ratti, G., Santini, L., Savino, S., Scarselli, M., Storni, E., Zuo, P.,

Broeker, M., Hundt, E., Knapp, B., Blair, E., Mason, T., Tettelin, H., Hood, D.W., Jeffries, A.C., Saunders, N.J., Granoff, D.M., Venter, J.C., Moxon, E.R., Grandi, G. and Rappuoli, R.(2000):Identification of vaccine candidates against serogroup B meningococcus by whole-genome sequencing. *Science*, 287(5459): 1816-1820.

Rauws, E.A. and Tytgat, G.N. (1990): Cure of duodenal ulcer associated with eradication of *Helicobacter pylori*. *Lancet*, 335(8700): 1233–1235.

Reyrat, J.M., Pelicic, V., Papini, E., Montecucco, C., Rappuoli, R. and Telford, J.L. (1999): Towards deciphering the *Helicobacter pylori* cytotoxin. *Mol. Microbiol.*, 34: 197-204.

Saha, S. and Raghava G.P.S. (2006): Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. *Proteins*, 65(1): 40-48.

Saha, S. and Raghava, G.P.S. (2006): VICMpred: SVM-Based method for the prediction of functional proteins of gram-negative bacteria using amino acid patterns and composition. *Geno. Prot. Bioinfo.*, 4(1): 42-47.

Saha, S. and Raghava, G.P.S. (2007): Prediction of bacterial proteins. *In Silico Biol.*, 7(5): 0028.

Saha, S., Zack, J., Singh, B. and Raghava, G.P.S. (2007): VGIchan: Prediction and classification of voltage-gated ion channels. *Geno. Prot. Bioinfo.,* 4(4): 253-258.

Sakharkar, K.R., Sakharkar, M.K. and Chow, V.T.K. (2004): A novel genomics approach for the identification of drug targets in parasites, with special reference to *Pseudomonas aeruginosa. In Silico Biol.,* 4(3): 355-360.

Sandro, V., Filippo, B. and Francesco, F. (2006): Nerve: new enhanced reverse vaccinology environment. *BMC Biotechnol.*,6: 35.

Sarangi, A.N., Aggarwal, R., Rahman, Q. and Trivedi, N. (2009): Subtractive genomics approach for *insilico*identification and characterization of novel drug targets in *Neisseria meningitidis*serogroup B. *J. Comput. Sci. Syst. Biol.*, 2(5): 255-258.

Sarkar, M., Maganti, L., Ghoshal, N. and Dutta C. (2012): *Insilico* quest for putative drug targets in *Helicobacter pyloriHp*AG1: Molecular modeling of candidate enzymes from lipopolysaccharide biosynthesis pathway. *J. Mol. Model.,* 8(5): 1855-1866.

Suthar, N., Goyal, A. and Dubey, V.K. (2009): Identification of potential drug targets of *Leishmaniainfantum*by *insilico*genome analysis. *Lett.Drug Des.Discov.,* 6(8): 620-622.

Uddin, R. and Saeed, K. (2014): Identification and characterization of potential drug targets by subtractive genome analyses of methicillin resistant *Staphylococcus aureus*. *Comput. Biol. Chem.*, 63(4): 48-55.

Weinberg, M.V. and Maier, R.J. (2007): Peptide transport in *Helicobacter pylori*: roles of Dpp and Opp systems and evidence for additional peptide transporters. *J. Bacteriol.,* 189(9): 3392-3402.

Wiwanitkit, V. (2005): Analysis of *Mycobacterium lepra*genome: *Insilico*searching for drug targets. *Southeast Asian J. Trop. Med. Public Health,* 36: 225-227.

Ye, S., Von Delft, F., Brooun, A., Knuth, M.W., Swanson. R.V. and McRee, D.E. (2003): The crystal structure of shikimate dehydrogenase (AroE) reveals a unique NADPH binding mode. *J. Bacteriol.*, 185(14): 4144-4151.

Yu, C.S., Lin, C.J. and Hwang, J.K. (2004):Predicting subcellular localization of proteins for Gram-negative bacteria by support vector machines based on *n*-peptide compositions. *Protein Sci. J.*,13: 1402-1406.

Zhang, Z.W. (1994): Schistosomes. liver flukes and *Helicobacter pylori*. IARC working group on the evaluation of carcinogenic risks to humans. Lyon, 7-14 June 1994. *IARC Monogr.Eval.Carcinog. Risks Hum.,* 61:1-241.

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