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Campylobacter jejuni ATCC 700819: An *in silico* Approach to Identify and Categorize Probable Drug Targets by Subtractive Genome Analysis

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Campylobacter jejuni, **the causative agent of common gastroenteritis in human, is a global public health concern. Antimicrobial resistance of this bacterium to fluroquinolones, macrolides, tetracyclines, aminoglycosides and other antibiotics is a major challenge in controlling campylobacteriosis that led to a search for novel drug targets. For efficient remedial advancement, this experiment was aimed to identify potential drug targets of *C. jejuni* ATCC 700819 using computer-aided protein data analysis via subtractive genomics approach. In this study, 3 vital membrane bound unique metabolic proteins: preprotein translocase subunit SecD (T1), ccoN:cbb3-type cytochrome c oxidase subunit I (T2) and preprotein translocase subunit SecE (T3) were disclosed by analyzing 228 essential proteins of *C. jejuni*. Post modeling analysis excluded T3 from study site. After analyzing the active sites and druggable pockets of these selected proteins, the highest drug scores: 0.82 and 0.81 were obtained for P0 pocket of T1 and T2 respectively. In addition, protein-protein interaction study revealed other proteins including preprotein translocase subunit SecF and cbb3-type cytochrome c oxidase subunit II were highly interacted with T1 and T2 respectively. Our findings suggest that, these two important proteins might be considered as potent curative targets against *C. jejuni* mediated gastroenteritis.**

Keywords: *Campylobacter jejuni*, *in silico* approach, Subtractive genome analysis, Drug targets, Essential proteins, Protein data analysis.

Campylobacter jejuni is one of the most pathogenic bacterium amongst 21 *Campylobacter species, which is associated with* acute bacterial gastroenteritis in humans (with the symptoms of abdominal pain, fever and vomiting), causing millions of cases of diarrheal illness per year[1]. *Campylobacter mediated illness is known as* campylobacteriosis, which is also an important precondition for a rare type of neurological immunopathological disorder namely, Guillain-Barré syndrome[2]. As a zoonotic enteric pathogen, *C. jejuni* is a Gram-negative, non-spore forming, micraerophilic organism that can inhabit in the intestinal tracts of wide range of domestic animals counting poultry, cattle and swine as well as wild birds and animals[3]. Human transmission of *C. jejuni* is carried out by the consumption of impure water and foods including undercooked poultry meat, unpasteurized milk and dairy products[4].

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Transmission between human to human has also been observed at low frequencies[5].

Though the majority of *Campylobacter* mediated infections are often acute, self-limiting does not require antibiotic treatment and generally enduring within 3-5 days[6].Rigorous or persisting enteritis such as septicemia or other invasive forms of the disease with immunocompromised individuals may require antibacterial treatment[7]. For treating *Campylobacter infections*, erythromycin (a macrolide) is considered as the drug of choice but ciprofloxacin and levofloxacin (fluoroquinolone), due to their broad spectrum of activity, can also be used frequently[8]. Conversely systemic campylobacteriosis is treated with tetracyclines and gentamicin[9]. The worldwide resistance of *C. jejuni isolates to several useful antibiotics especially* fluoroquinolone*, is* emerging gradually that limits its usefulness and multiple resistance blueprints to numerous classes of antibiotics has also been observed[10,11].Therefore, increasing resistance pattern as well as the development and transmission of antibiotic resistant *C. jejuni* is an alarming issue in public health sector.

Since several antibiotics are no longer efficient in the clinical management of campylobacteriosis, new generation of antibiotics with novel treatment regime should be initiated. Hence modern molecular advances, such as computational genomics and proteomics were employed in this study to identify, characterize and analyze putative drug targets in *C. jejuni* by investigating human nonhomologous essential metabolic proteins in unique pathways.

MATERIALS AND METHODS

The chronological diagram for the identification and categorization of alleged drug targets in *Campylobacter jejuni* ATCC 700819 is illustrated in Fig. 1.

Identification of Feasible Target Proteins in *C. jejuni* ATCC 700819

At first, 228 crucial genes of *C. jejuni* were retrieved from the National Center for Biotechnology Information (NCBI)[12,13] in FASTA format according to the Database of Essential Genes (DEG)[14].

Identification of human nonhomologous indispensable proteins

The retrieved 228 crucial proteins were subjected to BlastP, where default constraints were kept unchanged at the NCBI server against *Homo sapiens*. By using a protein database named Refseq, human nonhomologous essential proteins of *C. jejuni* were identified with dissimilarities in threshold expectation values greater than 10,000[15]. Thus, the human nonhomologous essential proteins of *C. jejuni* were screened, as they form the bacterium's 30 crucial proteins.

Metabolic pathway analysis of human nonhomologous essential proteins

The 30 screened human nonhomologous essential proteins of *C. jejuni* were then subjected to metabolic pathway analysis, carried out by KAAS (KEGG Automatic Annotation Server) server at KEGG (Kyoto Encyclopedia of Genes and Genomes). This well-organized annotation of the genes was performed by this server through BLAST comparison of the genes aligned with physically pastured KEGG GENES Database[16].

Determination of unique metabolic pathways

Afterward, unique metabolic pathways were determined in *C. jejuni* which were absent in *H. sapiens*. This was accomplished by comparing metabolic pathways of both *C. jejuni* and *H. sapiens* using KEGG GENOME Database and finally, unique metabolic pathways of *C. jejuni* were identified[17].

Determination of membrane bound protein in unique metabolic pathways

To mark the human nonhomologous membrane bound proteins in unique metabolic pathways of *C. jejuni*, subcellular localization was determined by PSORTb[18]. This technique can be used to discover alleged therapeutic targets against microbes.

Characterization of uncharacterized membrane bound proteins

Uncharacterized membrane bound hypothetical proteins in *C. jejuni* were depicted by using SVM-PROT[19].

Characterization of Feasible Target Proteins in *C. jejuni* ATCC 700819

3D and 2D model development of target proteins

For each of the three membrane bound target protein in *C. jejuni,* 3D (three-dimensional)

model was developed by using Phyre2[19], which detects homologues of known 3D structures for generating homology models and is called template-based homology modeling. Furthermore, 2D (two dimensional) models for the three membrane bound target proteins were developed by PRED-TMBB[20], which predicts the transmembrane beta strands of the Gram negative bacterial membrane bound proteins and differentiates them from water soluble ones by screening large datasets.

3D model validation of target proteins

To find out best 3D models, structure and stereo chemical analysis were performed by using evaluation and validation tools with ProSA, PROCHECK, ERRAT and Verify 3D[21]. After that, Protein Data Bank (pdb) files of the best protein models were downloaded and used for the refinement of models by using ModRefiner[22,23].

Active sites prediction of target proteins

To forecast the active sites of target proteins, an online tool CASTp was utilized which identify and measure the volume of surface manageable pockets as well as accessible interior cavities of protein. Along with their volumes, it calculates both the molecular surface (Connolly's surface) and the solvent accessible surface (Richards' surface) area for each cavity and pocket[24].

Druggable pocket recognition of target proteins

To predict druggable pockets a web-based tool -DoGSiteScorer was used, which provides qualitatively and quantitatively valuable data for druggability assessment based on size, shape and chemical features[25]. Moreover, DoGSiteScorer is helpful to assess amino acid composition, functional groups, and elements that can be present on the targeted pocket. Considering all descriptors, DoGSiteScorer provides a drug score value (0–1) for a selected pocket. The higher the score, the more druggable pocket is estimated to be.

Functional properties analysis of target proteins

The Network Portal is a database of gene transcription regulatory networks and also enables exploration, annotation and comparative analysis. The version 0.0.1 of the portal was used for the functional properties analysis of these target proteins[26].

Prediction of protein–protein interactions of target proteins

To predict protein-protein interaction of target proteins, a reliable database -STRING (Search Tools for the Retrieval of Interacting Genes/Proteins) was used. To envisage protein-protein interaction, both physical and functional traits were assembled from four sources: genomic context; high-throughput experiments; (conserved) co-expression; and previous knowledge. The association of STRING quantitatively consolidates data interaction from these sources for a large number of organisms and sends information between these organisms when it is pertinent[27].

RESULTS AND DISCUSSION

Subtractive genome analysis is a strategy which is employed on a genome of a microorganism to find out few nonhomologous essential proteins as potential drug targets. The fundamental principle of this approach is, "a good therapeutic target is a gene/protein essential for the bacterial survival, which cannot be found in host"[28]. Essential genes are the minimal set of genes which are ample for

Table 1. Metabolic pathways of human nonhomologous essential proteins in *C. jejuni* ATCC 700819

Name of the pathways	KEEG entry
1. Energy metabolism	
a. Methane metabolism	cje00680
2. Biosynthesis of other secondary metabolites	
a. Carbapenem biosynthesis	cje00332
b. Novobiocin biosynthesis	cje00401
c. Streptomycin biosynthesis	cje00521
d. Biosynthesis of secondary metabolites	cje01110
3. Carbohydrate metabolism	
a. C5-Branched dibasic acid metabolism	cje00660
4. Glycan biosynthesis and metabolism	
a. Peptidoglycan biosynthesis	cje00550
b. Lipopolysaccharide biosynthesis	cje00540
5. Metabolism of other amino acids	
a. D-Alanine metabolism	cje00473
6. Xenobiotics biodegradation and metabolism	
a. Beta-lactam resistance	cje01501
b. Vancomycin resistance	cje01502
7. Membrane transport	
a. Bacterial secretion system	cje03070
8. Others	
a. Two-component system	cje02020
b. Bacterial chemotaxis	cje02030
c. Flagellar assembly	cje02040
d. Microbial metabolism in diverse environments	cje01120

the continued existence of a cellular life form under favorable condition[29]. Since the majority of antibacterial agents aimed at vital cellular processes in microbes, essential gene products of microbial cells are guaranteed novel targets for antibacterial therapeutics[30]. Therefore, evaluation of species specific drug targets can be done by identifying essential genes which are unique to the cellular processes of a particular microbe[31].

For alternative treatment of *Campylobacter jejuni* mediated gastroenteritis, the present study was aimed to determine, categorize and analyze potential drug targets. In this study, Database of Essential Genes (DEG) was used for retrieving essential proteins of *C. jejuni* ATCC 700819. Agreement with the experimental determination of 150 essential genes in the enterobacteria *E. coli*[32], this current experiment discovered a total of 228

Table 2. Uniquemetabolic pathways of human nonhomologous essential proteins in C. jejuni ATCC 700819

Metabolic Pathway	Gene Reference	DEG Code	KO Number	Membrane Bound
Bacterial secretion system	218562127	DEG10310051	K03073	Yes
Biosynthesis of secondary metabolites	218562198	DEG10310071	K03182	No
Flagellar assembly	218562397	DEG10310101	K02386	No
Peptidoglycan biosynthesis	218562670	DEG10310136	K01924	No
Bacterial secretion system	218562707	DEG10310147	K03072	Yes
Two component system	218563091	DEG10310196	K00404	Yes
Peptidoglycan biosynthesis	218563264	DEG10310215	K00075	No

Abbreviation: KO, KEEG Organism

Table 3. Molecular functions, biological processes and cellular components of target proteins in *C. jejuni* ATCC 700819

Target protein	Molecular function	Biological process	Cellular component
T1 (Preprotein translocase subunit SecD)	Protein transmembrane transporter activity	Protein transport	Peptidoglycan-based cell wall
	P-P-bond-hydrolysis-driven transmembrane transporter activity	Intracellular transport	Protein complex
		Protein secretion	Cell part
		Protein transmembrane transport	T2 (ccoN: cbb3-
Heme-copper terminal oxidase type cytochrome c oxidase subunit I)	Electron transport activity	Intrinsic to	membrane
	Hydrogen ion transmembrane transporter activity	Cellular respiration	Plasma membrane part
	Oxidoreductase activity, acting on a heme group of donors, oxygen as acceptor		Respiratory chain complex IV
	Transition metal ion binding		
	aa3-type cytochrome c oxidase		
	ba3-type cytochrome c oxidase		
	caa3-type cytochrome c oxidase		
	cbb3-type cytochrome c oxidase		
	Iron ion binding		
	Tetrapyrrole binding		

Table 4. Recognized drug targetable pockets of target proteins

Target protein	Pocket ID	Volume (Å^3)	Surface (Å^2)	Liposurface (Å^2)	Depth (Å)	Drug score
T1 (preprotein translocase subunit SecD)	P0	1146.13	1021.70	669.45	23.55	0.82
	P1	1017.93	849.42	592.95	24.53	0.81
	P2	709.97	841.75	552.18	16.68	0.80
	P3	524.57	721.54	635.21	24.50	0.78
T2 (ccoN: cbb3-type cytochrome c oxidase subunit I)	P0	3269.44	2924.08	2184.61	41.43	0.81
	P1	734.44	942.63	818.06	18.84	0.80
	P2	454.67	419.03	306.95	18.95	0.80
	P3	259.26	266.82	153.82	11.45	0.49
	P4	166.40	279.83	267.34	8.52	0.32

Table 5. Amino acid composition of selected pockets

Target protein	Targeted pocket	Apolar amino acid ratio	Polar amino acid ratio	Positive amino acid ratio	Negative amino acid ratio
T1 (preprotein translocase subunit SecD)	P0	0.61	0.19	0.07	0.13
	P1	0.41	0.41	0.10	0.08
T2 (ccoN: cbb3-type cytochrome c oxidase subunit I)	P0	0.52	0.31	0.13	0.03
	P1	0.61	0.32	0.07	0.00

essential proteins in *C. jejuni* ATCC 700819, of which 30 were human nonhomologous. Among those human nonhomologous essential proteins, 16 were found to be involved in metabolic pathways and classified into 8 classes. Of these 16 proteins, only three of them were identified as membrane bound human nonhomologous essential proteins engaged in unique metabolic pathways, thus can be used as alleged therapeutic target.

To circumvent any cross reactivity of the conventional drugs with the human host, these 228 essential proteins were analyzed with BlastP, which resulted 30 human nonhomologous essential proteins with a threshold expectation value larger than 10^4. For further characterization of these human nonhomologous essential proteins, metabolic pathway scrutinization was performed by using KAAS server at KEGG. The consequence was a set of 16 proteins classified into 8 classes that were involved in different metabolic pathways (Table 1). To find out possible therapeutic targets among these 16 metabolic proteins, KEGG GENOME Database was utilized for comparative analysis of the metabolic pathways of human host and *C. jejuni*. This assessment exposed seven different proteins involved in five unique metabolic pathways that are only present in *C. jejuni* but absent in human host. In the middle of seven unique metabolic proteins, two (DEG10310051 and DEG10310147) were present in bacterial secretion system and two (DEG10310136 and DEG10310215) were found to involved in peptidoglycan biosynthesis pathway. At the same time single metabolic protein of DEG10310071, DEG10310101 and DEG10310196 were found to be engaged in biosynthesis of secondary metabolites, flagellar assembly and two component system respectively (Table 2). Determination of subcellular localization and depiction of uncategorized proteins revealed three of the seven unique metabolic proteins in cytoplasmic membranes, which were involved in bacterial secretion and two component system. These findings arrange a quicker approach in recognizing cell surface drug targets[33].

In this study, obligatory nonhuman homologous proteins in the unique metabolic pathways of *C. jejuni* were identified as therapeutic target. Previous experiment showed that, microbial growth can be suppressed efficiently by designing effective inhibitors that target these exclusive metabolic proteins. Motive behind hunting the drug target is that microbes are getting resistant

Table 6. Predicted interacting partners with target proteins

Target protein	Predicted functional partners	Score
T1 (Preprotein translocase subunit SecD)	Preprotein translocase subunit, secF; Part of the Sec protein translocase complex	0.999
	Preprotein translocase subunit,yajC	0.990
	Preprotein translocase subunit, secA; Part of the Sec protein translocase complex	0.988
	Preprotein translocase subunit, secY; The central subunit of the protein translocation channel S	0.987
	Preprotein translocase subunit, secG	0.918
	Leucyl-tRNA synthetase, leuS	0.898
	folylpolyglutamate synthase/dihydrofolate synthase, folC	0.895
	ATP/GTP-binding protein, Cj1084c	0.895
	Peptidase,Cj1087c	0.894
	Lipoprotein,Cj1090c	0.891
T2 (ccoN: cbb3- cbb3-type type cytochrome c oxidase subunit I)	cb-type cytochrome C oxidase subunit III, ccoP; C-type cytochrome. Part of the cbb3-type cytochrome c reductase	0.999
	cb-type cytochrome C oxidase subunit IV, ccoQ	0.997
	Ubiquinol-cytochrome Creductase cytochrome B subunit, petB; component of the ubiquinol-cytochrome c reductase	0.978
	Integral membrane protein, Cj1493c	0.965
	Ferredoxin domain-containing integral membrane protein, Cj0369c	0.961
	Periplasmic protein, Cj1486c	0.950
	Ubiquinol-cytochrome Creductase iron-sulfur subunit, petA; component of the ubiquinol-cytochrome c reductase	0.943
	Cation-transporting ATPase, Cj1155c	0.932
	Cation-transporting ATPase, Cj1161c	0.872

to currently used antibiotics which are capable of targeting just four pathways (cell wall synthesis, protein synthesis, nucleic acid synthesis and folate synthesis)[34]. Macrolide antibiotics such as azithromycin and erythromycin inhibits the growth of the growing polypeptide chain by combining to and interfering with the assembly of 50S large ribosomal subunit, thus inhibits bacterial protein biosynthesis. Molecular mechanism of these antibiotics is associated with their binding to a conservative target called polypeptide exit tunnel (PET) close to the peptidyl transferase center (PTC) on 23S rRNA where the progressing peptide is chiefly formed, hence hinder the activity of ribosomal peptidyl transferase[35,36]. Unluckily microbial resistance to macrolides is conferred by point mutations in 23r DNA[37]. Similarly fluoroquinolones for example- ciprofloxacin and levofloxacin are broad spectrum antibiotics acting on both Gram-positive and Gram-negative bacterium by restraining the activity of two type II topoisomerase enzyme, namely topoisomerase IV and DNA gyrase. Prior to bacterial cell division, topoisomerase IV and DNA gyrase are required for the separation of replicated DNA and supercoiling respectively, consequently vital for bacterial growth[38,39]. Resistances toward fluoroquinolones are attributed by *gyrA* and *gyrB* mutations in Gram negative and *parC* mutation in Gram-positive bacteria[40]. Thus crucial microbial proteins involved in exclusive metabolic pathways have the potential for antimicrobial agents and can be used as possible therapeutic targets. Due to hazardous outcomes for both humans and microbes, this approach is not utilized in human cases. This experiment identifies proteins in *C. jejuni*, which are human nonhomologues and can, therefore, be used as drug targets[41].

In Gram-negative bacterial pathogenesis, outer membrane has significant purpose in the interaction with hosts, adherence, uptake of nutrients and countering host defense mechanism. This may possibly be the protective place for antigens because the components of the outer

membrane are straightly recognized as foreign substances by host immunesystem[42]. Among all the different drug targets, 60% are membrane bound proteins and received particular consideration due to their ease of exploration by means of *in silico* approaches rather than experimental methods[43]. This investigation unearthed seven essential human nonhomologous metabolic proteins, of which three were membranous proteins including preprotein translocase subunit SecD (T1, gene reference 218562707), cbb3-type cytochrome c oxidase subunit I (T2, gene reference 218563091) and preprotein translocase subunit SecE (T3, gene reference 218562127). These three proteins might be act as potential targets against *C. jejuni*.

3D and 2D model development of target proteins

The 3D structures were generated by Phyre2, where homology modeled structures of T1 (Fig. 2.A1), T2 (Fig. 2.B1) and T3 (Fig. 2.C1), were developed based on template c3k07A, d3dtua and c2zjsE respectively. For both T1 and T2 the sequence coverage was found 80% and level of confidence was found 100% with c3k07A and d3dtua respectively. While T3 had a sequence coverage of 93% at a confidence level of 83.6% with c2zjsE. 2D structures for T1 (Fig. 3A) and T2 (Fig. 3B) were developed by PRED-TMBB, whereas model for T3 could not be depicted due to high sequence score than threshold value.

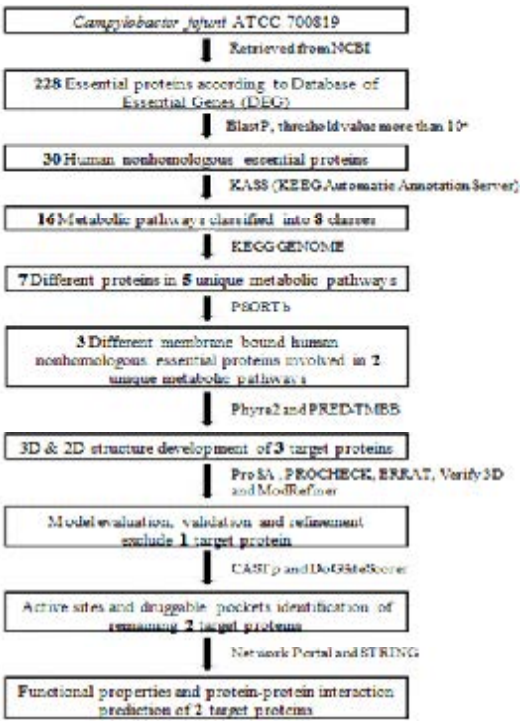

Fig. 1. Schematic illustration showing the process of analysis and interpretation for the identification and categorization of alleged drug targets in *C. jejuni* ATCC 700819

3D model validation of target proteins

Post modeling analysis revealed the Z score of model for T1 (Fig. 2.A2), T2 (Fig. 2.B2) and (Fig. 2.C2) was -12.59, -5.35 and -0.07 respectively. PROCHECK analysis of the model for T1 exposed that, at resolution 3.52 Å and R-factor of 0.239, Psi-Phi pairs in Ramachandran plot (Fig. 2.A3) had 86.7% residues in most favored region [A, B, L]. Likewise, at resolution 2.15 Å and R-factor of 0.184, Psi-Phi pairs in Ramachandran plot of model for T2 (Fig. 2.B3) had91.2% residues in the most favored region [A, B, L], at the same time Psi-Phi pairs in Ramachandran plot of model for T3 (Fig. 2.C3) had 88% residues in the most favored region [A, B, L] at a resolution of 3.20 Å and R-factor of 0.246. For the three models no amino acid residues were found to present in disallowed regions. Further verification of crystallographic protein model was done by using ERRAT and Verify 3D programs. ERRAT express as the percentage of protein for which the calculated error value falls below 95% of rejection limit. High resolution (3-4 Å) structures generally produce values around 95% or higher, though for lower resolution (2.5-3 Å) the overall quality factor is around 91%. According to ERRAT the overall quality factor of model for T1, T2 and T3 was found 88.38, 94.72 and 80% respectively. To pass a model through Verify 3D, 80% of its amino acids must have a score at least >=0.2 in 3D-1D profile. Verify 3D results showed that, 84.20 and 92.94% residues had an average 3D-1D score >=0.2 for the models of T1 and T2 respectively, though none of the residues for model of T3 had an average 3D-1D score >=0.2. After analyzing the results model of T3 (c2zjsE) was found unsuitable for further study and expelled from the scene. Following that, remaining two models of T1 (c3k07A) and T2 (d3dtua) were refined with ModRefiner which disclosed that, refined model of c3k07A had RMSD-1.229 and TM score-0.9915 to initial model, whereas refined model of d3dtua had RMSD-0.707 and TM score-0.9941 to initial model.

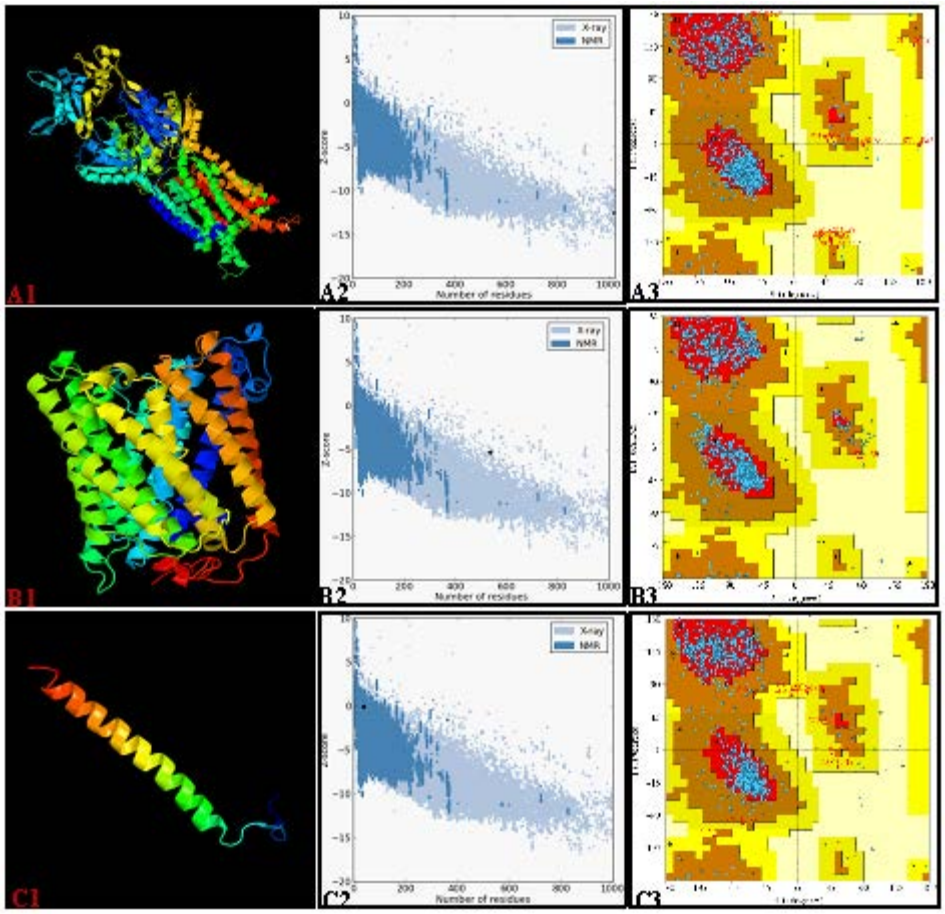

Fig. 2. (A1) Homology-based 3D model of T1 (c3k07A), (A2) Ramachandran plot of c3k07A, (A3) Z score plot of c3k07A; (B1) Homology-based 3D model of T2 (d3dtua), (B2) Ramachandran plot of d3dtua, (B3) Z score plot of d3dtua; (C1) Homology-based 3D model of T3 (c2zjsE), (C2) Ramachandran plot of c2zjsE and (C3) Z score plot of c2zjsE

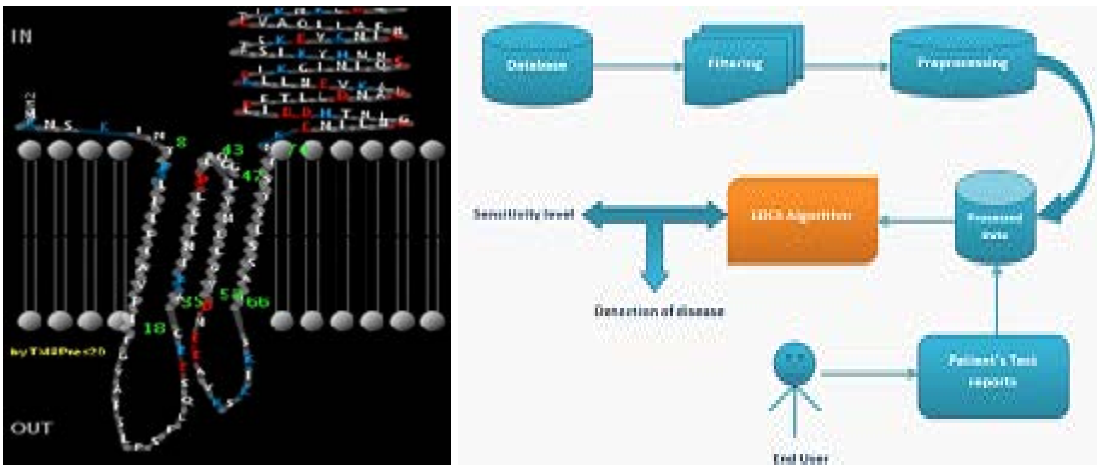

Fig. 3. Predicted 2D structure of (A) Model for T1 (c3k07A) and (B) Model for T2 (d3dtua). Active sites forecasting of target proteins

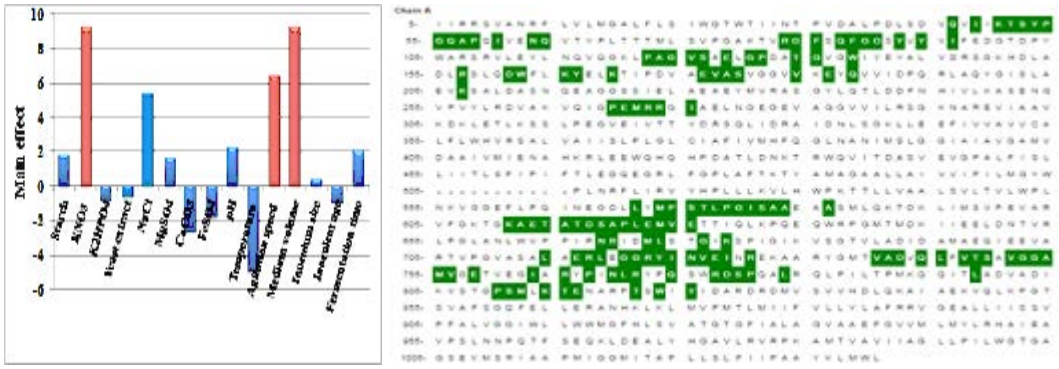

Fig. 4.(A). The 3D structure of best active site of T1 (preprotein translocase subunit SecD) by CASTp and (B) Active site information of T1 that is most highly positioned in area 3981.1 and volume 9114.4, where green color shows the active site position from 46 to 826

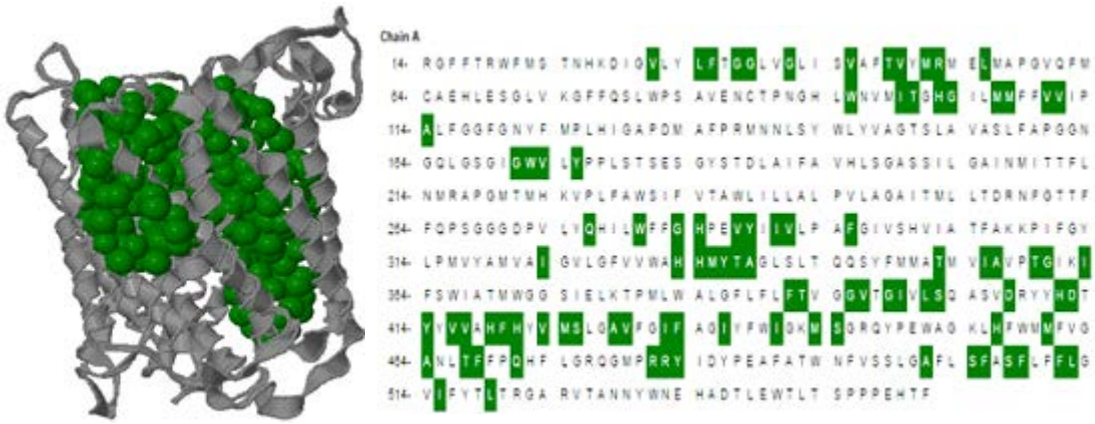

Fig. 5.(A). The 3D structure of best active site of T2 (ccoN:cbb3-type cytochrome c oxidase subunit I) by CASTp and (B) Active site information of T2that is most highly positioned in area 2397.1 and volume 3270.3, where green color shows the active site position from 32 to 520

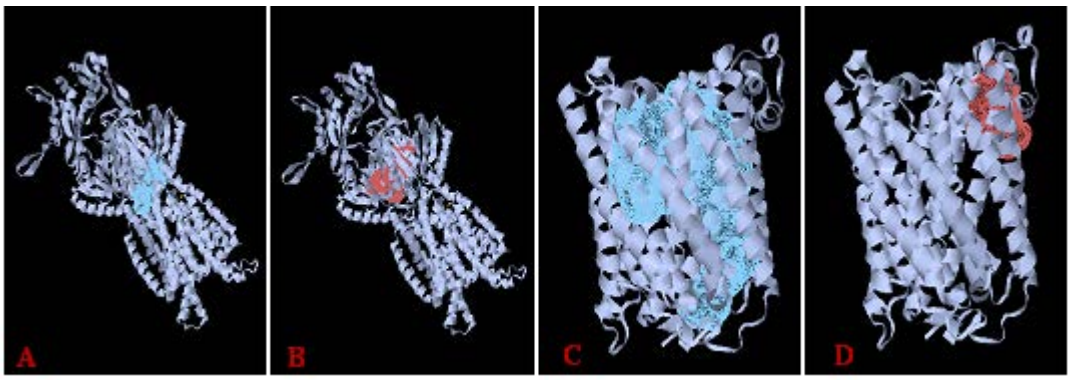

Fig. 6. Druggable pocket structure predicted by DoGSiteScorer. (A) T1 (preprotein translocase subunit SecD) protein, pocket 0. (B) T1 (preprotein translocase subunit SecD) protein, pocket 1. (C) T2 (ccoN:cbb3-type cytochrome c oxidase subunit I) protein, pocket 0. (D) T2 (ccoN:cbb3-type cytochrome c oxidase subunit I) protein, pocket 1

Active sites forecasting of target proteins

The active sites of T1 and T2 were determined by CASTp server, which assessed paramount active site regions of target proteins along with the number of amino acids occupied in that site. In *C. jejuni,* T1 (preprotein translocase subunit SecD) is a human nonhomologous essential protein which plays crucial role in bacterial secretion system and protein transmembrane transport. Its cellular components include peptidoglycan based cell wall, protein complex and cell part; while molecular functions include protein transmembrane transporter activity and P-P-bond-hydrolysis-driven transmembrane transporter activity (Table 3 and Fig. 4) [44].Concurrently T3 (ccoN: cbb3-type cytochrome c oxidase subunit I) involved in two-components system is also a human nonhomologous protein that plays vital roles in electron transport and respiration system. Beside the biological functions, its cellular components include respiratory chain complex IV, intrinsic to membrane and plasma membrane part; whereas its molecular functions include heme-copper terminal oxidase activity, transition metal ion binding, hydrogen ion transmembrane transporter activity etc. (Table 3 and Fig. 5) [44].

Druggable pockets recognition of target proteins

In proteins, binding of substrate to a pocket with high affinity is called druggability. The druggability assessment of a protein has been an extensively discussed area in pharmaceutical community due to high attenuation and failure rate in drug discovery and development. The DoGSiteScorer program was used to determine the precise pockets of a protein that are superlative for drug binding. In this study, 34 pockets were determined for T1 (preprotein translocase subunit SecD) while T2 (ccoN:cbb3-type cytochrome c oxidase subunit I) had only seven pockets. Among these, four pockets were chosen; two from each protein that have the highest druggability (Fig. 6). For the assessment of protein's druggability pocket volume, surface area, liposurface area, depth and drug scores were considered. According to this investigation, T1 protein had two best pockets whose druggability scores were 0.82 and 0.81; likewise T3 protein also had two pockets whose druggability scores were 0.83 and 0.81. Previous research showed that, pockets having large volume, high depth and a high apolar amino acid ratio are helpful factors to determine druggability[45]. Pocket volumes of preferred pockets, namely P1 and P0 of T1 were 1017.9 and 1146.13 respectively, whereas selected pockets, P1 and P0 of T2 had a pocket volume of 734.44 and 3269.44 respectively. Again in both cases, the depth of the pockets were more than 18 Å (Table 4) with high apolar amino acid ratio (Table 5) which suggests that, those pockets could be prospective therapeutic targets.

Prediction of protein–protein interactions of target proteins

Interactions between proteins are much essential for their proper functioning. Protein–protein interactions are immensely vital

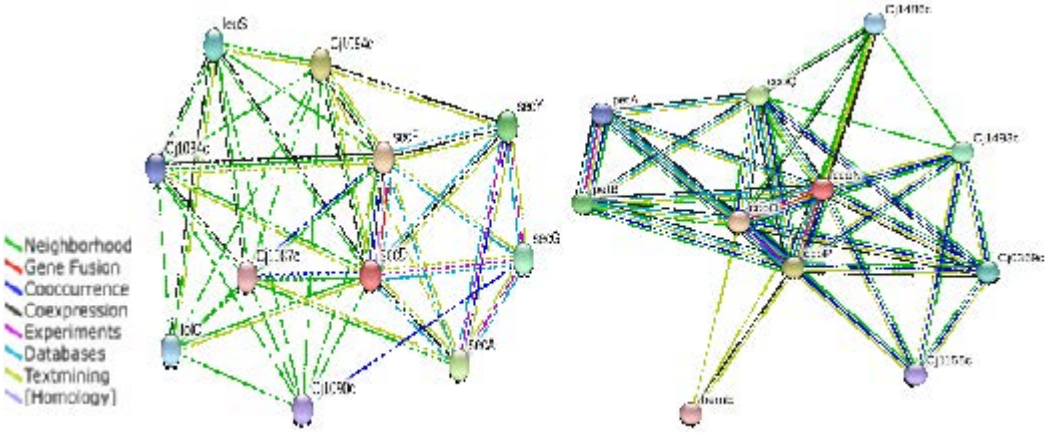

Fig. 7. STRING shows the interacting networks as evidence view of (A) T1 (preprotein translocase subunit SecD) and (B) T2 (ccoN:cbb3-type cytochrome c oxidase subunit I). Here different line colors represent the types of evidence for the association

in biological pathways, cellular network and structural organization of a cell. Nowadays, one of the foremost challenges in bioinformatics is the computational analysis of the complex networks created by interacting proteins which facilitate to understand the cellular activities, drug target and whole cell engineering[46]. Interactions of T1 and T2 target proteins with their partners were established through STRING (Fig. 7) at medium confidence (0.400). The target protein T1 was found to interact with the SecYEG preprotein conducting channel and SecDF which uses the proton motive force (PMF) to complete protein translocation after the ATP-dependent function of SecA. On the other hand T2 target protein was found to interact with cbb3-type cytochrome c oxidase subunit II (ccoO) involved in the transfer of electron from cytochrome c to catalytic subunit I and ubiquinol-cytochrome C reductase cytochrome B subunit (petB) involved in ATP generation (Table 6).

In these days, *in silico* identification and characterization of novel therapeutic target is the riding light for the treatment of various diseases. The current experiment showed that, target proteins T1 and T2 have higher associations and functions in diverse and imperative metabolic pathways of *C. jejuni*. Therefore, these proteins may exceedingly be suitable for therapeutic targets at battle against *C. jejuni*.

CONCLUSION

Identification and categorization of alleged drug targets is an innovative tendency in genomics, which becomes possible due to the accessibility of whole genome sequences and computer aided software. The present study is the first report of subtractive genome analysis of *Campylobacter jejuni* and its human host to identify probable drug targets. Due to subtractive genomics approach, drugs designed to these targets would be highly specific for particular pathogen and nonlethal to its host. With that intention, essential human nonhomologous metabolic membrane bound proteins were identified as potential drug targets along with their best active sites for which an effective drug can be designed. *C. jejuni* is a multidrug resistant infectious agent that causes gastroenteritis and other infections in human host. Novel chemical compounds targeting these possible drug targets would extremely be helpful in conquering the harmful consequences of *C. jejuni* mediated infection.

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