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Structural and Functional Characterization of a Unique Hypothetical Protein (Wp_000373624.1) Of Shigella Flexneri: A Computational Approach

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Abstract | **Original Research Article**

Shigella is one of the most common reason for morbidity and mortality all through the world, causing around 125 million Shigellosis per year and an expected 14,000 deaths, generally among children's of <5 years of age. Bacillary dysentery, a very common bowl disease is caused by *Shigella flexneri*. The hypothetical protein of *Shigella flexneri* (>WP_000373624.1) was taken and analyzed for the determination of its molecular and structural characteristics. Additionally, we attempt to forecast a worthy model of >WP_000373624.1 by utilizing in silico techniques related to protein homology and prediction of active sites to develop effective treatment against *Shigella flexneri*. Pyre2 software was used for the 3D structural analysis through homology modeling. The functional annotation was also performed by CDD Blast. This in silico hypothetical protein analysis can be additionally exploited in molecular drug strategy for other clinically important pathogens.

Keywords: Bacillary dysentery, Hypothetical protein, CDD Blast, Shigellosis.

INTRODUCTION

The innovation of the genomics field particularly subsequent generation sequencing logical strategies is being achieved by the gathering of huge quantity of gene sequencing attained from distinctive microbial strains [1-3]. In spite of the fact that about half of the total genes inside a genome are grouped in the classification of 'hypothetical', 'conserved' or 'unknown' or the 'orphans' genes, obliging our perception of the pathogenicity of the different types of microbes [4, 5], the genome sequence of *Shigella flexneri* is available in the NCBI database.

Nucleic acid predicted sequences are termed as hypothetical proteins. Human's proteomes is characterized by huge percentage of hypothetical proteins [6]. Various ways for the prediction of protein functions are developed by many researchers which can be achieved from the data derived from structure similarity, interaction between proteins, interactions between protein and ligand, similarity of the active sites, region of phosphorylation, gene expression etc. Though, the traditional method of the assuming function of protein lies on the similarity of the sequence using software's such as BLAST [7], FASTA [8]. There is no chemical existence of hypothetical protein and are predicted through nucleic acid sequences. Furthermore, these proteins are considered to be of low identity to be recognized as annotated proteins. Hypothetical proteins are not recognized and described at chemical level, however they represents a large portion in the sequenced microbial genomes [9]. Two classes of the hypothetical protein are known to exist. Of which one class is the class of uncharacterized family whereas the additional class is the class of domains having un-known function. There is no linkage between an unknown protein and a known gene as confirmed through experimental techniques.

There are many benefits on analyzing the protein which have no known function including 3D conformational structure which makes it happens to asses new domains and subjects, protein pathways and cascades. Pharmacological targets may also be offered through these new domains.

Numerous proteins have obscure functions; though, these protein domains contribute in the metabolism of the microbes and may results in contrary effects. Occasionally the protein function may change because of transformations, for example, insertion, substitutions and deletions of amino acids [10]. The principle target of the research is to recognize a protein domain whose function is un-known and to predict its

classification utilizing bioinformatics softwares. This examination will end up being valuable in indulgent to the mechanism involved in bacteria; helpful to the detection of new medications and could fundamentally affect infection control and antibody advancement methodologies.

Shigella is one of the most common reason for morbidity and mortality all through the world, causing around 125 million Shigellosis per year and an expected 14,000 deaths, generally among children's of <5 years of age [11]. In the United States, Shigella is the 3rd most common reasons for gastroenteritis, with no less than 500,000 cases of shigellosis connected diarrheal occasions in the USA yearly. General side effects of shigellosis includes bloody diarrhea, queasiness, and tenesmus (torment in the gut), whereas complications include post-disease joint inflammation, sepsis, seizures and hemolytic-uremic disorder. The two principle courses of transmission include (i) through contaminated food, and (ii) water tainted with human waste; Shigella is effectively transmitted through human contact because of its low irresistible dose of 10±200 cells.

Shigella flexneri, intracellular pathogen, is the major cause of bowl disease "bacillary dysentery" in people [12]. The infection is described by bacterial intrusion of the duodenal cells, direct spread from one cell to other by scattering inside the epithelium of the large intestine and also the inflammation of the intestinal mucosa. The disease spread procedure fundamentally depends on the assembly of the actin at the bacterial shaft, which moves the bacteria all through the cytoplasm of the infected cell. Polar actin get together is upheld by polar articulation of the bacterial auto transporter relative IcsA, which selects the N-WASP/ARP2/3 actin gathering machinery. As motile microscopic organisms experience cell to cell interactions, they form plasma film distensions that venture into neighboring cells [13].

The experimental ways to deal with allocating the function and also of prediction of three dimensional structures of non-characterized proteins are arduous but costly as well. "In silico" characterization that consolidates the utilization of numerous databases and calculations is another option, and we utilized this methodology in this study. The in silico techniques are outlined based on trial results and writing reports proposing their effective applicability [14, 15]. In this article, the hypothetical protein of *Shigella flexneri* (>WP_000373624.1) was taken and analyzed for the determination of its molecular and structural properties. Additionally, we attempt to forecast a worthy model of >WP_000373624.1 by utilizing in silico techniques related to protein homology and prediction of active sites to develop effective treatment against *Shigella flexneri*.

MATERIAL AND METHODS

Sequence retrieval

The unknown proteins were looked in NCBI, protein database, by means of keywords, "hypothetical protein," and then the subsequent hits were arbitrarily chosen to contemplate the close relative protein by exploiting the program 'blast'. To foresee the purpose of inquiry protein, a similar exploration was done by utilizing 'NCBI blast' to distinguish proteins that might have auxiliary resemblance with that of the theoretical protein. *Shigella flexneri* was retrieved for annotation from website (http: //www.ncbi.nlm.nih. gov/) for the analysis of various properties including physiochemical properties, functional and structural properties.

Physicochemical classification of the unknown protein

The theoretical protein in crude categorization design was assessed for the physicochemical cataloging utilizing the 'ProtParam' online software [16]. The properties figured by the software comprise the sub-atomic weight, hypothetical isoelectric point (pI), composition of amino-acids, grand average of hydropathicity (GRAVY), the coefficient of extinction, instability index and the aliphatic index. The coefficient of extinction shows the quantity of light absorbed by a certain protein at a specific wave-length. The instability catalog gives a gauge of the strength of a certain protein. Whereas the instability index of less than 40 is interpreted to be steady whereas above 40 is predicted to be unstable. The side chain amino acids possess some relative volume which is characterized through the aliphatic index. The GRAVY of a protein is ascertained as the entirety of the hydropathy estimations of the greater part of the amino acids separated by quantity of deposits in the arrangement [17].

Subcellular localization

Subsequent to assessing the physicochemical characteristics of the unknown proteins, protein localization analysis will be done. Subcellular localization is more useful in predicting drug and target for vaccine. Hypothetical proteins can be located at anyplace in cell. Cytoplasmic proteins are essential in determining drug target whereas external or internal proteins functions as vaccine target. The two databases commonly used for subcellular localization of protein are PSORTb [18] and CELLO [19]. When contrast with CELLO, PSORTb gives more exact location of protein in the cell. The software PSORTb needs a protein sequence to be entered in FASTA format and then organism and Gram stain was chosen. In this case we have taken bacteria *Shigella flexneri* and Gram negative was selected as *S. flexneri* is a Gram negative bacterium.

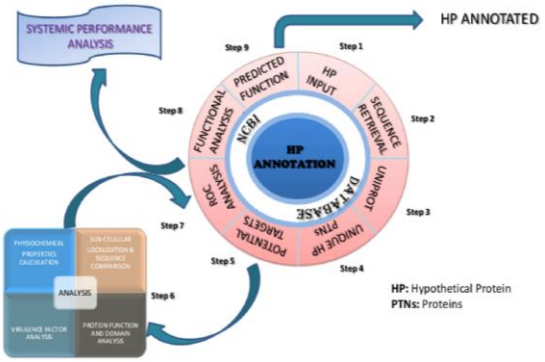

Fig-1: Representation of the different steps involved in annotation of hypothetical proteins

Homology modelling

The three dimensional protein structures can give exact data about in what way proteins associate and restrict in its steady structure. Without test points of interest of the three dimensional structure, homology displaying is the absolute most encouraging technique for 3D structure prediction. Different online web servers are accessible for structural demonstration of protein [20-22]. Regardless of insignificant change, one essential phase that is common to all demonstrating softwares is to predict the best-match tentatively demonstrated layout.

Virulence factor analysis

Virulent factor assumes to be a vital part in drug target prediction. It provides a portrayal about more dynamic proteins which play vital parts for pathogens survival. VICMPred software [23] and VirulentPred software [24] were utilized for the investigation of virulence. The VICMPred permits the expectation based on three diverse methodologies, i.e., designs based; designs based and composition of amino acids; designs based, amino acid sequence and dipeptide structure. For this situation, we have chosen design based approach. The software virulentPred provides a more precise prediction of virulence when contrasted with VICMPred [25].

SOSUI server

SOSUI software determines that the hypothetical protein is a cytoplasmic protein or a membrane protein and also segregates insoluble proteins from the soluble ones. This software utilizes four physio chemical characteristics, the hydropathy index, the length of every single sequence, an amphiphilic index and a record of amino acid charges. The outcomes of proteins which are forecast of trans-membrane helices for the membrane proteins, a helical turn and graph of the hydropathy plot [10, 26]

PROTEIN FUNCTION ANALYSIS

Proteins are divided into families and superfamily based on their function, structure and amino acid sequence by different protein classification software's like CATH, SCOP, CDD BLAST and so on. In view of amino acid sequence similarity, CDD-Blast database was utilized to foresee the protein function [27]. This approach was utilized using different software's including RPSBLAST, an altered form of PSI-BLAST, to rapidly check an arrangement of predetermined position-particular scoring matrices with a hypothetical protein.

RESULTS AND DISCUSSION

The database was sought with the word 'Hypothetical protein', which brought about millions of records that were illustrative of numerous species. To decrease the amounts, '*Shigella flexneri*' was utilized to sieve the results, and 127494 passages were appeared. From the outcomes, every unknown protein grouping with greater than 190 amino-acids were arbitrarily chosen and subjected to NCBI blastp investigation to get the preparatory information. From the outcome, the protein sequence of >WP_000373624.1 was chosen to predict its various useful and structural characteristics utilizing different computational techniques. The fasta classification of the selected protein is given underneath.

>WP_000373624.1 MULTISPECIES: hypothetical protein [Bacteria]
MDRFPRSDSIVQPRAGLQTYMAQVYGWMTVGL
LLTAFVAWYAANSAAVMELLFTNRVFLIGLIIAQ
LALVIVLSAMIQKLSAGVTTMLFMLYSALTGLTL
SSIFIVYTAASIASTFVVTAGMFGAMSLYGYTTKR
DLSGFGNMLFMALIGIVLASLVNFWLKSEALMW
AVTYIGVIVFVGLTAYDTQKLKNMGEQIDTRDTS
NLRKYSILGALTLYLDFINLFLMLLRIFGNRR

In this article the physiochemical characteristics of unknown protein of *Shigella flexneri* is described which are enlisted inTable 1. The number of amino acid was 234 and the molecular weight was 25901.87. Isoelectric point was 9.62. The predicted isoelectric point will be helpful as solvency is least and

in an electric field movement is zero at this point. In addition proteins end up stable and conservative at isoelectric pH, thus registered isoelectric point will be beneficial for building up a buffer system for filtration by isoelectric focusing strategy. The extinction coefficient at 280nm was 38390 M-1 cm-1 figured by Expasy's Protparam. The quantitative investigation of protein to protein and protein to ligand connections could be possible by utilizing this figured extinction coefficients [28]. The instability index was observed to be 17.96 which showed that the protein is stable. The aliphatic index is the comparative size of a protein possessed by aliphatic side chains which influence a positive aspect in rasing the thermostability of a globular protein. The AI for the HP is 120.90. The proteins with high aliphatic index may indicate steadiness in a wide temperature whereas proteins will low AI index are not thermally stable and show greater adaptability [29]. The GRAVY of hypothetical protein is -0.815. Normal range of GRAVY is from negatige 0.103 to negative 0.874. The enhanced collaboration of protein and water happens at a lower GRAVY. The GRAVY result is calculated by dividing hydropathy of aminoacids by the residues of aminoacids [30].

Table-1: Physicochemical properties of hypothetical protein

Properties	**Values**
No. of amino-acids	234
Molar mass	25901.87
Isoelectric point	9.62
No. of negatively charged residue	10
No. of positively charged residue	16
Extinction Coefficient at 280nm	38390 M^{-1} cm^{-1}
Instability index	17.96
Aliphatic index	120.90
Grand Average of Hydropathicity	0.815

Determining localization of the query proteins can provide data about their cell function. This data could be used in considering mechanism of disease and drug production. It was examined by CELLO and validated by PSORTb version 3.0 [18]. The prediction of the sub-cellular localization of the HP was of cytoplasmic locale. The subcellular localization commended that the inquiry protein was restricted in the cytoplasmic locale with a relating P-estimation of 10.0. There are a few reports showing that cytoplasmic proteins are associated with metabolic pathways and act about as pharmacological medication target [31, 32]. The outcome of PSORTb is illustrated in Table 3 which recommends the reasonableness of the hypothetical protein as drug target.

Table-2: The sub-cellular localization likelihood of the hypothetical protein of *Shigella flexneri* using software PSORTb

SUBCELLULAR LOCALIZATION	**SCORES**
Cytoplasmic	10.0
Extracellular	0.00
Outer Membrane	0.00
Periplasmic	0.00
Cytoplasmic Membrane	0.00
FINAL PREDICTION Cytoplasmic	 10.0

3D structure of proteins gives essential bits of knowledge about the molecular function and in this way allows a successful outline of analyses. Homology demonstrating of the chosen hypothetical protein was performed by utilizing Phyre2 keeping in mind the end goal to get 3D structure. The model obtained is given in figure 2. Furthermore, secondary structure and protein disorder were anticipated through phyre2. It was anticipated that secondary structure hypothetical protein model dimensions includes X=38.644 Y=56.551 Z=41.241 with alpha helix 82%, beta helix 0%, TM helix 62% and disordered 16%. Structural disorder is the area in the protein where some of the amino acids are missing shown in figure 3. Phyre2 likewise foresee the topology of transmembrane helices (figure 4). Transmembrane proteins are those proteins that are embedded into the lipid bilayer of cell membrane. In the HP, there were six transmembrane B-plated sheets with N-end and C-end [33].

Fig-2: 3D structure of Hypothetical protein

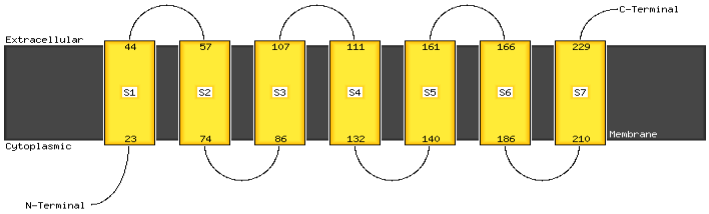

Fig-3: Transmembrane helical structure

Fig-4: Secondary structure and disorder prediction

VICMPred and VirulentPred was used to depict the virulence of the query protein >WP_000373624.1 [*Shigella flexneri*]. Determining the sequence of the bacterial virulent protein gives suggestions regarding the characterization and identifications of the factors involved in virulence and thus helps in discovering novel medications/antibody targets against pathogens, and also understanding the mechanism of action of the virulent pathogen [34]. The outcomes in this study demonstrated that the protein is non virulent with a prediction score of -0.667 as illustrated in table 3.

Table-3

SCORES OF DIFFERENT FUNCTIONAL CLASS	
FUNCTION	SCORES
Cellular process	0.67641024
Information Molecule	-6.2709436
Metabolism	0.99949743
Virulence factors	-0.66713171

The SOSUI server is used for the segregation of the membrane bound proteins and cytoplasmic proteins along with the determination of transmembrane helices, in which the accurateness of arrangement of the proteins were 99%, whereas the predicted value for trans-membrane helix was 97% [26]. The server SOSUI is accessible on site: http://www.tuat.ac.jp/~mitaku/sosui/. In this study the results of SOSUI server proposes that the hypothetical protein is a transmembrane protein which have seven transmembrane helices as illustrated in table 4.

Table-4: Trans-membrane Helices

NO	N-TERMINAL	TRANSMEMBRANE REGION	C-TERMINAL	TYPE	LENGTH
1	23	QVYGWMTVGLLLTAFVAWYAANS	45	PRIMARY	23
2	51	LLFTNRVFLIGLIIAQLALVIVL	73	PRIMARY	23
3	83	GVTTMLFMLYSALTGLTLSSIFI	105	PRIMARY	23
4	107	YTAASIASTFVVTAGMFGAMSL	128	SECONDARY	22
5	138	SGFGNMLFMALIGIVLASLVNFW	160	PRIMARY	23
6	165	ALMWAVTYIGVIVFVGLTAYDTQ	187	PRIMARY	23
7	208	SILGALTLYLDFINLFLMLLRIF	230	SECONDARY	23

Functional analysis of the query protein was done by using CDD Blast software which demonstrated bacterial BAX inhibitor (BI)-1/ YccA-like proteins. This family contains bacterial relatives of the mammalian individuals from the BI-1 like family having low number of transmembrane proteins, which have been appeared to have an antiapoptotic impact either by empowering the anti-apoptotic property of Bcl-2 which is a well described oncogene, or by inhibition of the proapoptotic function of Bax which also belongs to Bcl-2 family. In plants, BI-1 like proteins plays important role in portection against pathogen. BI-1 has been appeared to be related with calcium levels, responsive oxygen species (ROS) generation, cytosolic fermentation, and autophagy. In other diseases, BAX inhibitor has likewise been appeared to control insulin protection, adipocyte separation and hepatic disorder [35]. Moreover, BI-1 action is essential in a large number of tumors, promoting metastasis by balancing act in flow, a procedure subordinate upon the BI-1 C-end and BI-1: actin cooperation [36]. Control of BI-1 along these lines has the potential for critical remedial advantage in an extensive range of human infections

CONCLUSION

The event of HP in genomes establishes a crucial issue for both the relative and functional genomics examinations. Particularly for pathogenic microorganisms, these unknown proteins deter the search for novel and more intense medication targets which lead to progression to the improvement of scientific investigation on these living organisms and upgrade of our comprehension of their abilities to cause disease. Investigations of the function, family and structure of the hypothetical protein were done by using different software's and the function prediction of query protein was made. The data accumulated from this investigation would be useful in understanding the function of uncharacterized proteins exhibit in *Shigella flexneri*. Moreover, an in silico computational approach for functional characteristics of hypothetical proteins can encourage additional researches on these hypothetical proteins as novel putative medication target for other clinically critical pathogens.

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