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Computational Approaches for Molecular Characterization and Functional Annotation of an Uncharacterized Protein of *Vibrio cholerae* Applying Bioinformatics Tools and Databases †

Md Yousuf [1,2], Abu Saim Mohammad Saikat [2,3,*] and Md. Ekhlas Uddin [2,4]

[1] Tejgaon College, Dhaka 1215, Bangladesh; mdyousuf5271@gmail.com
[2] Department of Computational Biology and Bioinformatics, Advanced Bioscience Center for Collaborative Research (ABSCCR), Rajshahi 6250, Bangladesh; ekhlasbt03@gmail.com
[3] Department of Biochemistry and Molecular Biology, Life Science Faculty, Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj 8100, Bangladesh
[4] Department of Biochemistry and Molecular Biology, Gono Bishwabidyalay, Dhaka 1344, Bangladesh
* Correspondence: asmsaikat.bmb@gmail.com
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Abstract: When inadequate water purification and sewage disposal systems, cholera poses a significant health threat in developing nations, *Vibrio cholerae* (*V. cholerae*) is one of the mindful microscopic organisms associated with cholera illness. If cholera is not treated, renal failure, shock, hypokalemia, and pulmonary edema can occur, resulting in death in a matter of hours. The machinery of bacterial virulence factors is what causes this disease. Among the various *V. cholerae* strains, *V. cholerae* O1 is the most prevalent and pathogenic strain. The total genome succession of *V. cholerae* unravels the presence of different genes and uncharacterized proteins whose capabilities are not yet perceived. Therefore, it is essential to comprehend *V. cholerae* by analyzing the structure and annotating the function of uncharacterized proteins. The NCBI sequence of uncharacterized *V. cholerae* O1 EET91795.1 proteins has been annotated for this study. Domain family, protein solubility, ligand binding sites, and other parameters have all been determined using a variety of databases and computational tools. The protein's ligand-binding sites were found, and its three-dimensional structure was modeled. According to the analysis, the hotdog family protein may play metabolic roles like thioester hydrolysis in the metabolism of fatty acids and the breakdown of two products such as phenylacetic acid and the pollutant 4-chlorobenzoate. The structural prediction of this protein and detection of binding sites would suggest a potential target to uncover promising inhibitors against the protein to treat infection caused by the target strain.

Keywords: *Vibrio cholerae*; uncharacterized proteins; characterization; microbes

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1. Introduction

V. cholerae is a gram-negative, facultatively anaerobic, highly motile, arch or comma-shaped with a single polar flagellum, gammaproteobacteria secrete enterotoxin, which induces severe diarrhea known as cholera [1]. *V. cholerae* is primarily found in unhealthy foods and polluted water and is transmitted by the fecal-oral route. An enzymatically active factor that raises cyclic adenosine 5-monophosphate (cAMP) production is secreted when the toxin binds to a specific receptor, monosialosyl ganglioside GM1, in the intestinal epithelial cells of the plasma membrane. Due to the cell's high cAMP levels, the intestinal lumen will overflow with electrolytes and water [2,3]. At an ever-increasing rate, structural genomics initiatives produce many uncharacterized protein structures. However, this enormous structural storage is only helpful with functional annotation for biologists interested in particular molecular systems [4].

We implement bioinformatic tools for function annotation for the EET91795.1 protein of *V. cholerae*, the causative agent of cholera, particularly in Southeast Asia [5]. This uncharacterized protein could be helpful as a pharmacological target and marker if annotated. According to the findings of various bioinformatics databases and studies, these proteins play a significant part in the pathogenesis of *V. cholerae*.

2. Materials and Methods

2.1. Protein Sequence Retrieval

The protein sequence was retrieved from the NCBI-protein database [6] with the accession number of EET91795 (version: EET91795.1).

2.2. Physicochemical Characterization

The amino acid sequence composition, the instability index, the aliphatic index, the GRAVY (the measurement of hydrophobicity or hydrophilicity of a protein), extinction coefficients, and the theoretical isoelectric point (pI) of the EET91795 protein was measure by the ExPaSy's ProtParam program [7,8].

2.3. Functional Annotation Prediction

The NCBI platform's CD search tool was applied to anticipate the conserved domain in the protein EET91795.1. Protein motif determination was implemented using the ScanProsite tool [9,10].

2.4. Subcellular Location Identification

The protein's subcellular location was documented using the PSORTb v.3.0.3, SOSUI assessment tool, PSLpred server, HMMTOP v.2.0, and TMHMM server v.2.0 [11–13].

2.5. Secondary Structural Assessment

The SOPMA server exploited the secondary structural elements' prediction following the default parameters of the protein EET91795.1 present in *V. cholerae*. Moreover, the PSIPRED v.4.0 tool was used to predict the secondary structure and the disordered areas [14].

2.6. Tertiary Structure Modeling and Validation

The three-dimensional structure of the protein was modeled by utilizing the Swiss-Model server. The anticipated tertiary structure obtained from the Swiss-Model server was subjected to structural quality evaluation experiments. The PROCHECK of the SAVES v.6.0 program was performed for Ramachandran plot calculation [15].

2.7. Active Site Determination

The CASTp v.3.0 server was used to predict the active sites of the modeled protein [16].

3. Results and Discussion

3.1. Protein Sequence Retrieval

Protein sequencing identifies a protein or peptide's amino acid sequence whole or segment. The NCBI Protein data bank is a big store of sequences from multiple sources, including GenBank, RefSeq SwissProt, PDB, etc. The protein obtained from the NCBI database with the accession number EET91795 (version: EET91795.1) is present in the locus EET91795 containing 161 amino acids (aa).

3.2. Physicochemical Characterization

By examining the properties of each amino acid in a protein, we can understand how proteins' physical and chemical properties are defined [17]. The ProtParam program on the ExPASy server was used to determine the physicochemical characteristics of the protein (EET91795.1). The protein consists of 161 amino acids, where Val (17) was an ample amount of amino acid followed by Ala (14), Arg (7), Asn (9), Asp (3), Cys (3), Gln (6), Glu (10), Gly (10), His (7), Ile (12), Leu (13), Lys (5), Met (5), Phe (4), Pro (6), Ser (13), Thr (12), Trp (2), Tyr (3). The time it takes for the radiolabeled target protein concentration to decrease by 50% from the level at the beginning of the chase is known as the protein half-life. The protein (EET91795.1) V. Cholerae has an estimated half-life of about 30 h (mammalian reticulocytes, in vitro), >20 h (yeast, in vivo), and >10 h (*Escherichia coli*, in vivo). The calculated isoelectric point (pI), molecular weight (MW), and the number of total atoms were 6.64, 17,568.20 Dalton, and 2476, respectively.

3.3. Functional Annotation Prediction

The CD search tool of NCBI predicted a conserved domain as a hot-dog superfamily protein (accession: cl00509) of the protein. The structure of *E. coli*'s FabA (beta-hydroxydecanoyl-acyl carrier protein (ACP)-dehydratase) and Pseudomonas' 4HBT (4-hydroxy benzoyl-CoA thioesterase) both contained the hot-dog fold. Various other inconsequential proteins additionally share the hot dog-fold. The metabolic functions of these proteins include thioester hydrolysis of fatty acids, degradation of phenylacetic acid, and the environmental pollutant 4-chlorobenzoate, among other related but distinct catalytic activities. FapR is a member of this superfamily involved in the transcriptional regulation of fatty acid biosynthesis. Moreover, the ScanProsite tool predicts four pattern sites of the protein EET91795.1. There are the Protein kinase C phosphorylation site (accession no.PS00005), N-glycosylation site (accession no. PS00001), Casein kinase II phosphorylation site (accession: PS00006), and N-myristoylation site (accession: PS00008).

3.4. Subcellular Location Determination

The PSORTb (v.3.0.2), SOSUIGramN, and PSLpred tools were used for subcellular location analysis of the protein (EET91795.1). The tools predicted the subcellular location of the protein as a cytoplasmic protein. The HMMTOP (v.2.0) and TMHMM (v.2.0) programs anticipated that there were no transmembrane helices in the protein (EET91795.1) and highlighted the cytoplasmic location of the protein present in *V. cholerae* (Table 1).

Table 1. Subcellular localization assessment.

Analysis	Results
PSORTb (v.3.0.2)	Cytoplasmic
SOSUIGramN	Cytoplasmic
PSLpred	Cytoplasmic
HMMTOP (v.2.0)	No transmembrane helices present
TMHMM (v.2.0)	No transmembrane helices present

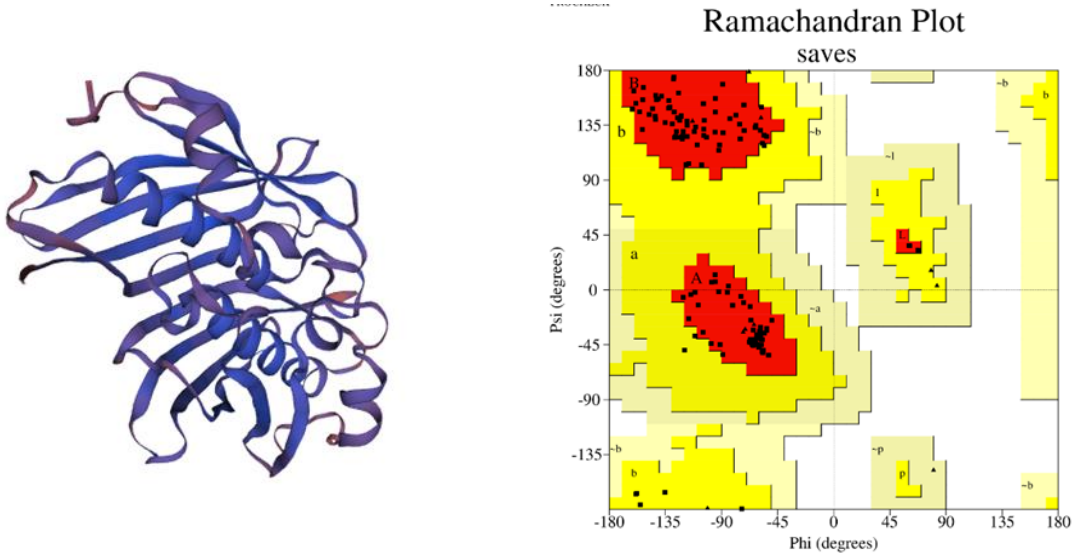
3.5. Secondary Structure Inquiry

Protein function and structure are vigorously connected. The secondary structural elements, e.g., helix, coil, sheet, and turn, have an excellent relationship with protein function, construction, and engagement. The SOPMA program predicted the secondary-structural component of the protein (EET91795.1) where the alpha-helix (Hh), extended-strand (Ee), and random-coils (Cc) were 49 (30.43%), 44 (27.33%), 68 (42.24%), respectively. The PSIPRED v.4.0 tool predicted the sequence plot and secondary structure. The sequence plot from the two-dimensional structure of the protein represents that most of the protein is cytoplasmic.

3.6. Tertiary Structure Modeling and Validation

The Swiss Model server was utilized for tertiary structure prediction of the protein EET91795.1. The Swiss Model server predicted the tertiary structure of the protein based on the most favored template (4qdb.1.A). This template bears the Global Model Quality Estimation (GMQE) values, QMEANDisCo Global, and sequence identity scores of 0.72, 0.80, and 46.48%, respectively. The Ramachandran plot analysis by PROCHECK was applied for structural assessment of the modeled tertiary structure obtained from the Swiss Model serve [18–21].

In the case of the anticipated tertiary structure by the Swiss Model, the assessment experiment executed by the Ramachandran Map (PROCHECK) indicated 93% of the total residues (227) found in the core; 7.0% of residues were found in the additional allowed regions; and there was no residue found in the abundantly authorized parts and the disallowed areas [22,23]. The number of non-glycine and non-proline residues was 244, which is 100%; the end residues were four; the glycine and proline residues were 20 and 8, respectively, among the 276 total residues (Figure 1).



(**a**) (**b**)

Figure 1. (**a**) structure of EET91795.1 protein predicted by Swiss Model (**b**) The Ramachandran plot statistics of the modeled three-dimensional structure validated by the PROCHECK program.

3.7. Active Site Determination

The CASTp version 3.0 program predicted 35 different active sites of the modeled protein. This server recognizes all surface pockets, internal cavities, and transversal channels in the protein structure and describes all atoms involved in their formation. It also calculates their exact size and area and the size of the mouth openings, if any. These dimensions are calculated analytically using a solvent-accessible surface model (Richards surface) and a molecular surface model (Connolly surface). CASTp could be utilized to investigate surface properties and protein operational zones. CASTp also helps study a protein's surface properties and functional parts. CASTp provides a graphical, versatile, dynamic user interface and user-provided constructs for rapid measurement. The top active sites of the modeled protein were identified between the area of 256.436 and the volume of 94.190.

4. Conclusions

In this study, the protein EET91795.1 of *V. Cholerae* was retrieved from the NCBI database and determined their physicochemical properties and identified domains and

families using various Bioinformatics tools and databases. This protein is hydrophobic and localized in the cytoplasm of the cell. Besides, it is a hot-dog superfamily protein that may act in metabolic roles. This protein has a coenzyme binding site and four more enzymatic pattern sites. The secondary structure verified that alpha-helix, random spiral, extended strand, and beta-turns were uppermost in most sequences. The Swiss-Model server was used to evaluate tertiary systems, and PROCHECK for Ramachandran Map Analysis showed that the protein residues in most favored regions were 93%. The CASTp program predicted 35 distinct functional areas of this modeled protein. The findings of this study may contribute to the modulation of new target identification and drug discovery for the control of cholera, thereby reducing this deadly global epidemic.

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