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Bioinformatics based analysis of Type III secretion system effector protein of *Vibrio vulnificus*

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ABSTRACT

Pathogens use the needle shaped Type III secretion system to inject effector proteins into the host cell. The effector proteins evade the immune system of the host and help in bacterial colonization. Many effector proteins alter the host cellular target like the cytoskeleton or membrane and bring about necrosis. Thus the identification of effectors is a crucial step in understanding the pathogenesis of the organism. The Type III secretion system of Vibrio vulnificus, which is a marine bacteria causing infection in human beings, has not been studied. The motive of the present study was to identify the effector protein by its amino terminal signal peptide and study its predicted 3-D structure by bioinformatics tools. Homology modeling was carried out by SWISS MODEL server to reveal a helix coiled-coil domain which are associated with the Type III secretion system. Motif analysis revealed probable tyrosine phosphorylation with Phosphoinositide 3-kinase binding motif (MxxY motif) usually occurring in Toll-Like Receptors signaling pathway. The functional annotation of the type III secreted effector protein of V. vulnificus may throw some light on the mechanism of virulence of the organism.

Keywords: *Vibrio vulnificus*; effector protein; virulence; seafood; marine.

INTRODUCTION

V. vulnificus is a gram-negative marine pathogen. Individuals who come in direct contact with the water infected with the organism through soft tissue exposure are at risk. It can also be transmitted to humans by infected sea-food like fish, oysters etc. The symptoms include high fever, gastroenteritis with high mortality rates caused by septicemia [1]. The organism occurs in many parts of the world and has been isolated from coastal waters of India [2]. The Type III Secretion System (TTSS) is a common mode of infection employed by gram-negative bacteria.

The TTSS is a needle shaped apparatus comprising many components and a large number of effector proteins. The toxic effector proteins secreted by the bacteria are transported directly through the TTSS into the host to establish infection.

The effector proteins collectively attack the immune system of the host. As a result of the entry of the virulence effector proteins, the bacteria is able to persist in the host organism inspite of the host immune response[2,3].

The role of the effector proteins has been studied in *Yersinia* and *Salmonella* [4]. The host targets of only a few pathogens are known and they may vary from host cytoskeleton to host ubiquitin. Derrangement of the actin cytoskeleton leads to cell necrosis.

The TTSS of many gram-negative bacteria of animal and plant origin have been studied. However, the TTSS and the effectors of *V. vulnificus* has not been studied. We aim to identify and study the TTSS effector protein by bioinformatics tools. The effector proteins are identified on the basis of the mRNA signal that they possess at the amino terminal end. Since no consensus sequence has been observed in the signal, the general characteristics of the signal are used to detect the presence of the signal peptide in effector proteins [5,6]. In addition, the TTSS effector proteins possess coiled-coil domain for protein-protein interaction [7]. Our first objective was the identification of the effector protein of *V. vulnificus* by bioinformatics tools. A study of the amino terminal signal peptide and the coiled-coil domain was proposed. The second objective was to predict the 3D structure of the protein which would also help to detect and visualize the coiled-coil domain.

MATERIALS AND METHODS

Sequence identification: The effector protein of type III secretion system of *V.vulnificus* was identified by using the server http://www.effectors.org/ set up by the University of Vienna [8]. The interactive module performs a signal peptide prediction for gram-negative organisms against a standard set.

NCBI sequences: (http://www.ncbi.nlm.nih.gov/) The identified sequences were downloaded from NCBI (Genbank ID: NP_934212 and NP_761660 from strain YJ016 and strain CMCP6 respectively).

Sequence analysis: The potential phosphorylation sites of effectors were predicted by NetPhos 2.0 Server from technical university of Denmark at the web address http://www.cbs.dtu.dk/services/NetPhos/ [9]. Coils server was used for identifying coiled-coil domain at http://www.ch.embnet.org/software/COILS_form.html [10].Transmembrane helix was used as a guide for the presence of any transmembrane helix http://www.cbs.dtu.dk/services/TMHMM/.

Structure analysis: Homology modeling was performed by Swiss Model server http://swissmodel.expasy.org/. [11]. The template selected had the PDB ID 1R6F. Multiple alignment of the sequence with the template was obtained from EBI clustal W to be used for submission to the Swiss Model server. SwissPdbViewer was used for energy minimization of the

theoretical model and correcting the stereochemistry of the model. Validation was done by Ramachandran plot from Ramachandran plot assessement server, Rampage (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) [12] and Swiss-model server for Anolea [13] and Procheck [14]. Dalilite server http://www.ebi.ac.uk/Tools/dalilite/index.html was used for structural superpositioning [15].

RESULTS AND DISCUSSION

Sequence analysis :

Identification of the type III secretion system effector protein of *V.vulnificus* was achieved by bioinformatics server effectors.org. The accession number identified was NP_934212 for *V. vulnificus* strain YJ016 and NP_761660 for strain CMCP6 and had 443 amino acid residues each. Analysis of the N-terminal signal peptide of the effector protein revealed that it possessed the typical characteristics of the TTSS effectors. (i) The characteristic feature was a high proline and serine content of 22% within the first 50 amino acid. Cunnac and his coworkers observed a positive bias for serine and proline residues in the N-terminal signals of *Ralstonia* effectors of TTSS. (ii) The second criteria was the absence of negatively charged residues in the first 12 residues. This criteria was also fulfilled because of the absence of negatively charged amino acids within the first 12 residues. The identified effector fulfilled two of the criteria and was placed in the class II effector category based on the studies done by Guttman coworkers and Schechter coworkers [16]. Analysis of the identified effector protein was done using the coiled-coil server to study the presence of coiled–coil domain. A coiled coil structural motif occurs when 2-7 alpha-helices are coiled together to form a rope like structure. They serve important function like sensing and regulating secretion to enzymatic activity and are also associated with the TTSS [7]. The prediction of coiled-coil domain helped identify the presence of coiled-coil in the TTSS effector. At least two coiled-coil region were detected in the effector protein of *V.vulnificus* identified (Fig 1).

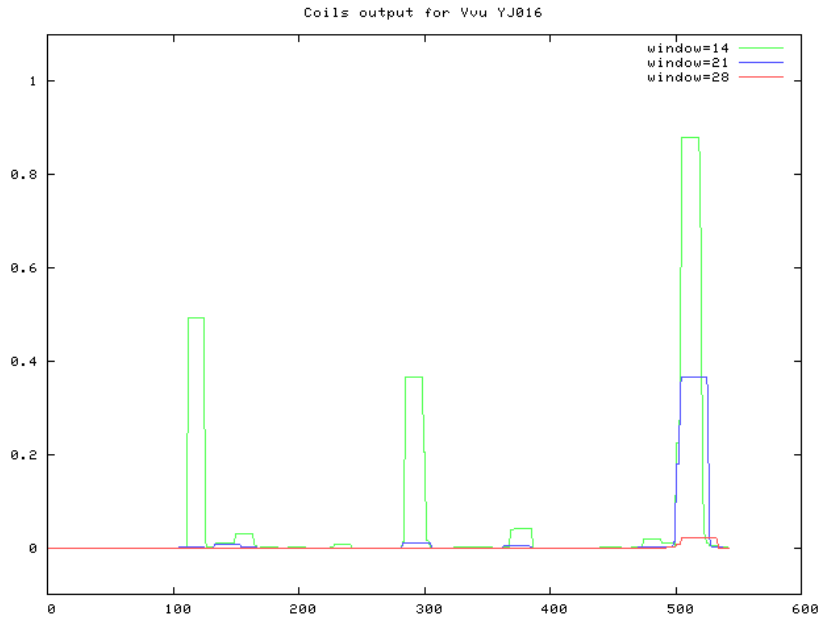

Fig1: Coils server detected coiled-coil domain for the protein.

The prediction for phosphorylation by done by Netphos server which showed the probable sites of phosphorylation for the protein at serine and tyrosine residues. The prediction results for residue serine position 271 had the probability score of 0.97 for phosphorylation. The prediction for tyrosine phosphorylation (Fig 2) was located at position Y ^{173} and Y ^{265} with scores 0.84 and 0.87 respectively (Table1). Further motif analysis was carried out for the protein.

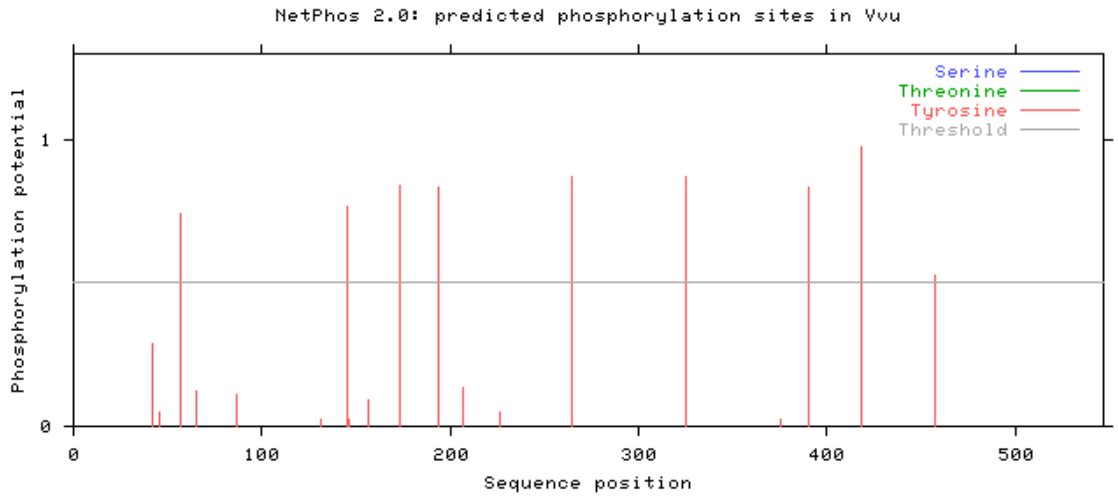

Fig2: NetPhos server detected probable tyrosine phosphorylation sites

Tyrosine predictions

Pos	Context	Score	Pred
146	LTHYYDMLL	0.026	.
157	AFGNYRDLL	0.092	.
173	V**MGDY**LSMM	0.842	*Y*
194	PDENYAREV	0.836	*Y*
207	SIGLYQLNQ	0.136	.
226	LLPTYSQDD	0.052	.
265	A**MAPY**ADKH	0.873	*Y*
325	PSPQYVERV	0.868	*Y*

Table 1: Tyrosine phosphorylation prediction at residue positions indicated. It involved MxxY motif at position 173 and 265.

Motif analysis

Motifs were analysed at the location of the residue tyrosine at position 173 and 265. Analysis of motif yielded two MxxY motif at both positions (Table 1). Arancibia and his coworkers demonstrated that phosphoinositide 3-kinase binding motif is exemplified by MxxY motif signaling the toll-like receptor pathway [17]. This suggests the effector protein may bind to phosphoinositide 3-kinase by the two MxxY motifs. The involvement of phosphoinositide 3-kinase interaction. suggests that toll-like receptors signaling pathway may exist in the regulation of Type III secretion effector of *V.vulnificus*.

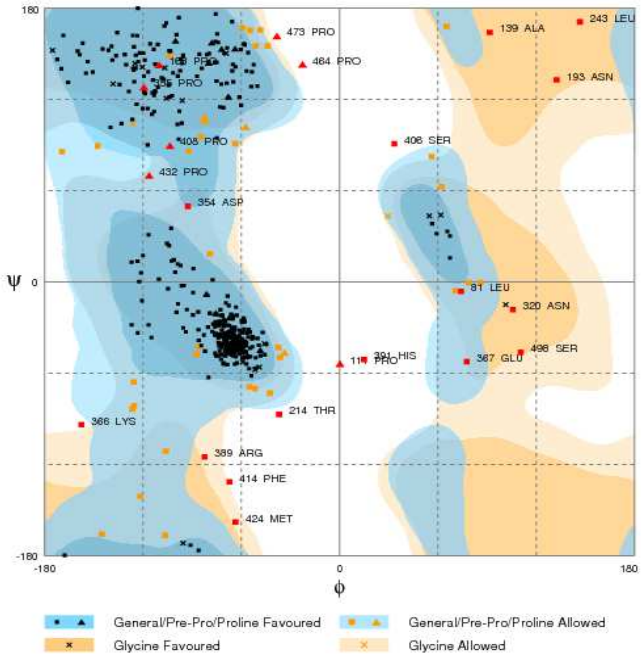

Fig 3 A

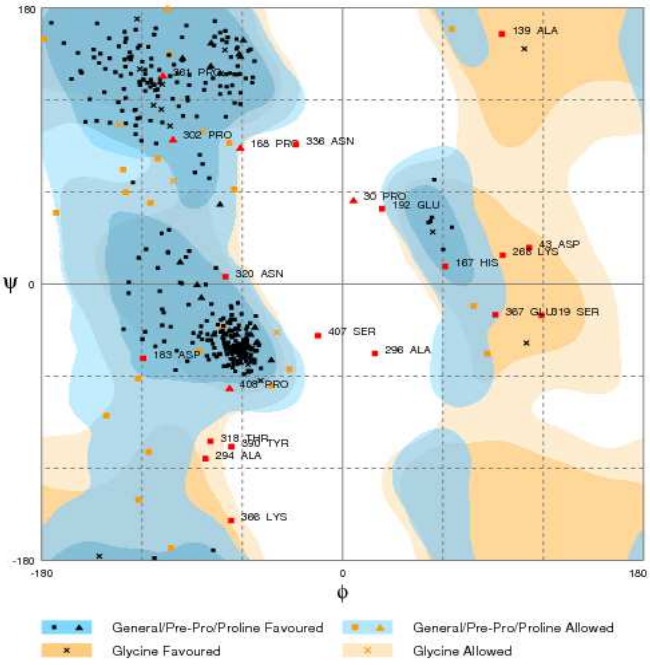

Fig 3B

Fig 3 A: Ramachandran plot analysis of effector protein of *V.vulnificus* strain YJ016 B: Ramachandran plot analysis of effector protein of *V.vulnificus* strain CMCP6

Structure modeling and validation

Homology Modeling of the sequence was carried out from the sequence. The type III secretion system protein, LcrV of *Yersinia pestis* (PDB code: 1R6F) was selected as the template to model the protein. It was 57% similar to LcrV, a TTSS pore component *Yersinia* spp.. The structure modeled after energy minimization and validation by Anolea server and Procheck was found to be acceptable. Ramachandran plot values for *V.vulnificus* YJ016 were within limits with 87% in favoured region, 8.3% in allowed region and only 4% in outlier whereas it was 88.4% in favoured region, 6.4 % in allowed region and only 5.2% in outlier. Thus the homology modeled structures were found to be have acceptable conformations (Fig 3 A, B).

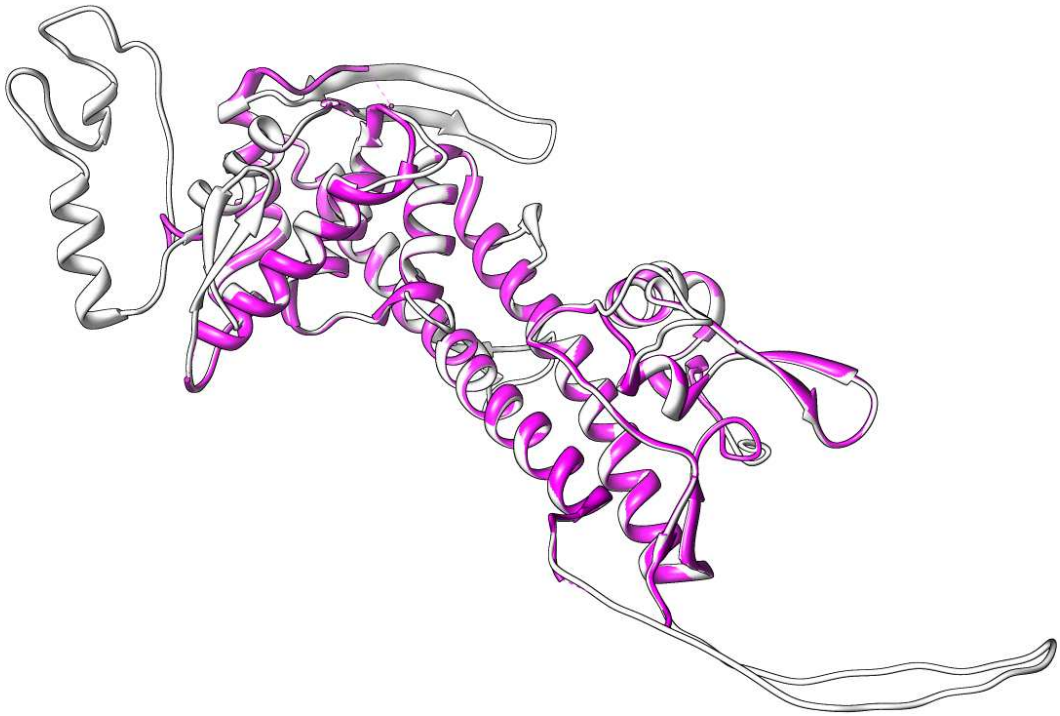

Fig.4 Structural superimposition of TTSS effector of *V.vulnificus* (colored white) with LcrV of *Yersinia* (colored magenta)

Superimposition of coiled-coiled region in both the TTSS proteins is shown.

The homology modeled structure of effector protein was superimposed with the template of LcrV protein from *Yersinia pestis* (Fig 4). Dalilte program aligned 267 residues with a Dalilite score of 0.7 °A which was a good score. Thus, the modeled structure was predicted to be a TTSS effector protein with coiled-coil domain and having a predicted Phosphoinositide 3-kinase activity. The structure was found suitable for further work in wet lab.

CONCLUSION

The identification of type III secretion system effector protein and its structural and predicted functional annotation may throw some light on the mechanism of action of the protein. The presence of amino terminal signal peptide and the coiled-coil domain are the results of sequence analysis. The potential for phosphorylation of the protein may attach virulence functions to the protein and subsequent motif analysis may suggest phosphoinositide 3-kinase activity. The study

also revealed the predicted 3D structure and functional annotation of the protein for possible future pharmacological intervention.

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REFERENCES

[1] P. A.Gulig, K. L. Bourdage, A. M. Starks, *J. Microbiol.*, **2005**, 43, 118-131.
[2] A. Parvathi, H. S. Kumar, I.Karunasagar, I.Karunasagar, Appl. Environ. Microbiol., **2004**, 70(11), 6909-6913.
[3] U.Gophna, E.Z. Ron, D. Graur. *Gene*, **2003**, 312, 151-63.
[4] P. Ghosh, **2004.** *Mol. Biol. Rev*., 68, 771–795.
[5] C.M. Shaver, and A. R. Hauser, *Microbiology*., **2006**, 152, 143–152.
[6] S.Cunnac, A. Occhialini, P. Barberis, C. Boucher, and S. Gennin, *Mol Microbiol.*, 2004, 53, 115–128.
[7] D.S.Guttman, B. A. Vinatzer, S. F. Sarkar, M. V. Ranall, G. Kettler, and J. T. Greenberg, *Science*, **2002**, 295,1722-1726.
[8] R. M. Delahay and G. Frankel, *Mol. Microbiol.*, **2002** , 45 , 905–916.
[9] R. Arnold, M.A. Jehl, T. Rattei, *Microbes Infect*., **2010**, 12, 346-58.
[10] N. Blom, S. Gammeltoft, and S. Brunak, *J. Mol. Biol*., **1999**, 294, 1351-1362.
[11] A.Lupas, M.V. Dyke and J. Stock, *Science*, **1991**, 252,1162-1164.
[12] K. Arnold, L.Bordoli, J. Kopp and T. Schwede, *Bioinformatics*, **2006**, 22 , 195–201.
[13] S.C.Lovell, I.W. Davis, W.B. Arendall III, P.I.W. de Bakker, J.M. Word, M.G. Prisant, J.S. Richardson, D.C. Richardson, *Proteins: Struct. Funct. Genet.*, **2003**, 50, 437-450.
[14] Melo F, Feytmans E. *J. Mol. Biol*., **1997**,267, 207-222.
[15] Luthy R, James UB, Eisenberg D. *Nature*, **1992**, 356,83-85.
[16] L.Holm, S. Kääriäinen, P. Rosenström, A. Schenkel, *Bioinformatics*, **2008**, 24, 2780-2781.
[17] L.M. Schechter, K. A., Roberts, Y. Jamir, J. R. Alfano, and A. Collmer, *J. Bacteriol.* **2004**,186, 543-555.
[18] S.Arancibia, D. Benítez, L. E. Núñez, C.M. Jewell, P. Langjhar , E. Candia, G. Z Torres., J.A. Cidlowski, M.J.González, M.A. Hermoso, *J. Cell. Mol. Med*., **2009** doi: 10.1111/j.1582-4934.2009.00958.x 2009