ORIGINAL RESEARCH

Outer membrane protein secretin of type III secretion system of *Vibrio vulnificus*: structure prediction and orientation

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Abstract: The marine organism *Vibrio vulnificus* causes seafood-borne infection and is a major cause of human mortality. Secretin, a major component of the type III secretion system (TTSS) virulence machinery, forms oligomeric rings in the outer membrane of many Gram-negative organisms. The secretin ring-shaped complexes possess pore-forming activity. The pores function as channels for transport of macromolecules across the complex. However, the TTSS secretin family has not been studied in *V. vulnificus*. The secretin of TTSS of *V. vulnificus* was identified and predicted to be homologous to secretin of Gram-negative organisms like *Yersinia* and *Escherichia coli*. It contained an amino-terminal signal peptide region for processing by the sec machinery. The homology model of secretin of *V. vulnificus* possessed the *E. coli* periplasmic domain specific to secretin of TTSS. Buried pore-lining residues in the homology model were identified by bioinformatics tools. Thus, secretin of *V. vulnificus* may function as channels to allow transport of molecules. The optimized pore axis with the biggest and longest cavity through the channel was detected which generated a guide to the orientation of secretin in *V. vulnificus*. Thus, the secretin of *V. vulnificus* has a conserved C-terminal domain enclosing a pore and a nonconserved lipolytic motif which may be involved in adherence to the chitinous surface.

Keywords: *Vibrio vulnificus*, secretin, virulence, pore, transport, bioinformatics, type III secretion system

Introduction

Vibrio vulnificus is a Gram-negative opportunistic pathogen that is highly invasive. Individuals who come in direct contact with the water infected with the organism or those who consume seafood such as fish, oysters, and so on are at risk of being infected. The systemic infection is characterized by fever and septicemia with very high mortality rates. The type III secretion system (TTSS) is used by pathogens to colonize the susceptible hosts. The TTSS is a needle-shaped apparatus which injects virulence effector proteins into the host cell, evading the immune response of the host cell.[1–3]

Out of 20 components of the TTSS apparatus proteins, secretin is the only component found in the outer membrane of Gram-negative organisms. It is part of a much larger structure, the needle complex containing a basal body and an external needle. The basal body of the TTSS complex consists of two stacked upper and lower rings which span the inner membrane to the outer membrane. Secretin is assembled as the upper ring which is linked externally by the protruding needle. Secretin oligomers in the ring possess pore-like activity and function in transporting large molecules through the outer membrane.[4] The secretin possesses a signal peptide region which is processed by the sec machinery and transported to the

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outer membrane. Members of the secretin family possess a C-terminal homology domain which function in oligomerization and pore formation.[5,6] The assembly of TTSS components in the membrane precedes the needle formation and is not dependent on secretion of the needle. Finally, the TTSS needle is used to pierce the host cell and serve as a conduit to allow the transport of effector proteins through the inner and outer membranes.[7] The structure and function of proteins have been studied on the basis of similarities to other proteins. The components of TTSS have also been studied by a similar approach. In this study, we aim to study the secretin of *V. vulnificus* with reference to the TTSS. We use bioinformatics tools and techniques to understand the role of the protein in virulence.
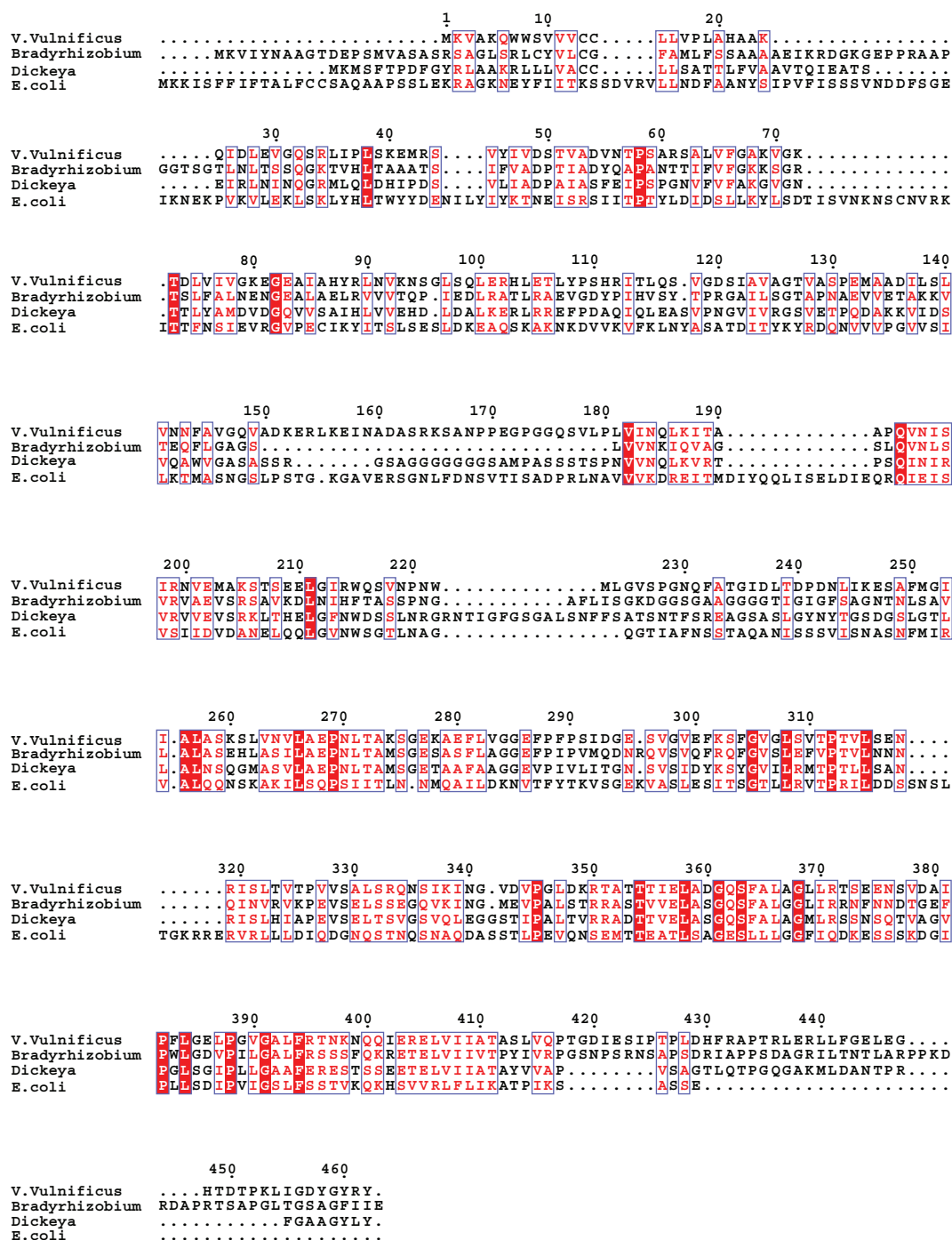
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                               1        10                20
V.Vulnificus   ......................MKVAKQWWSVVVCC.....LLVPLAHAAK................
Bradyrhizobium .....MKVIYNAAGTDEPSMVASASRSAGLSRLCYVLCG.....FAMLFSSAAAAEIKRDGKGEPPRAAP
Dickeya        ...............MKMSFTPDFGYRLAAKRLLLVACC.....LLSATTLFVAAVTQIEATS.......
E.coli         MKKISFFIFTALFCCSAQAAPSSLEKRAGKNEYFIITKSSDVRVLLNDFAANYSIPVFISSSVNDDFSGE

                     30        40        50        60        70
V.Vulnificus   .....QIDLEVGQSRLIPLSKEMRS....VYIVDSTVADVNTPSARSALVFGAKVGK...........
Bradyrhizobium GGTSGTLNLTSSQGKTVHLTAAATS....IFVADPTIADYQAPANTTIFVFGKKSGR...........
Dickeya        .....EIRLNINQGRMLQLDHIPDS....VLIADPAIASFEIPSPGNVFVFAKGVGN...........
E.coli         IKNEKPVKVLEKLSKLYHLTWYYDENILYIYKTNEISRSIITPTYLDIDSLLKYLSDTISVNKNSCNVRK

                     80        90        100       110       120       130       140
V.Vulnificus   .TDLVIVGKEGEAIAHYRLNVKNSGLSQLERHLETLYPSHRITLQS.VGDSIAVAGTVASPEMAADILSL
Bradyrhizobium .TSLFALNENGEALAELRVVVTQP.IEDLRATLRAEVGDYPIHVSY.TPRGAILSGTAPNAEVVETAKKV
Dickeya        .TTLYAMDVDGQVVSAIHLVVEHD.LDALKERLRREFPDAQIQLEASVPNGVIVRGSVETPQDAKKVIDS
E.coli         ITTFNSIEVRGVPECIKYITSLSESLDKEAQSKAKNKDVVKVFKLNYASATDITYKYRDQNVVVPGVVSI

                     150       160       170       180       190
V.Vulnificus   VNNFAVGQVADKERLKEINADASRKSANPPEGPGGQSVLPLVINQLKITA............APQVNIS
Bradyrhizobium TEQFLGAGS................................LVVNKIQVAG..............SLQVNLS
Dickeya        VQAWVGASASSR.......GSAGGGGGGSAMPASSSTSPNVVNQLKVRT...........PSQININR
E.coli         LKTMASNGSLPSTG.KGAVERSGNLFDNSVTISADPRLNAVVVKDREITMDIYQQLISELDIEQRQIEIS

               200       210       220              230       240       250
V.Vulnificus   IRNVEMAKSTSEELGIRWQSVNPNW.............MLGVSPGNQFATGIDLTDPDNLIKESAFMGI
Bradyrhizobium VRVAEVSRSAVKDINIHFTASSPNG..........AFLISGKDGGSGAAGGGGTIGIGFSAGNTNLSAV
Dickeya        VRVVEVSRKLTHELGFNWDSSLNRGRNTIGFGSGALSNFFSATSNTFSREAGSASLGYNYTGSDGSLGTL
E.coli         VSIIDVDANELQQLGVNWSGTLNAG.............QGTIAFNSSTAQANISSSVISNASNFMIR

               260       270       280       290       300       310
V.Vulnificus   I.ALASKSLVNVLAEPNLTAKSGEKAEFLVGGEFPFPSIDGE.SVGVEFKSFGVGLSVTPTVLSEN....
Bradyrhizobium L.ALASEHLASILAEPNLTAMSGESASFLAGGEFPIPVMQDNRQVSVQFRQFGVSLEFVPTVLNNN....
Dickeya        L.ALNSQGMASVLAEPNLTAMSGETAAFAAGGEVPIVLITGN.SVSIDYKSYGVILRMTPTLLSAN....
E.coli         V.ALQQNSKAKILSQPSIITLN.NMQAILDKNVTFYTKVSGEKVASLESITSGTLLRVTPRILDDSSNSL

                     320       330       340       350       360       370       380
V.Vulnificus   ......RISLTVTPVVSALSRQNSIKING.VDVPGLDKRTATTTIELADGQSFALAGLLRTSEENSVDAI
Bradyrhizobium ......QINVRVKPEVSELSSEGQVKING.MEVPALSTRRASTVVELASGQSFALGGLIRRNFNNDTGEF
Dickeya        ......RISLHIAPEVSELTSVGSVQLEGGSTIPALTVRRADTTVELASGQSFALAGMLRSSNSQTVAGV
E.coli         TGKRRERVRLLLDIQDGNQSTNQSNAQDASSTLPEVQNSEMTTEATLSAGESLLLGGFIQDKESSSKDGI

                     390       400       410       420       430       440
V.Vulnificus   PFLGELPGVGALFRTNKNQQIERELVIIATASLVQPTGDIESIPTPLDHFRAPTRLERLLFGELEG....
Bradyrhizobium PWLGDVPILGALRSSSFQKRETELVIIVTPYIVRPGSNPSRNSAPSDRIAPPSDAGRILTNTLARPPKD
Dickeya        PGLSGIPLLGAAERESTSSEETELVIIATAYVVAP........VSAGTLQTPGQGAKMLDANTPR....
E.coli         PLLSDIPVIGSLFSSTVKQKHSVVRLFLIKATPIKS........ASSE..................

                     450       460
V.Vulnificus   ....HTDTPKLIGDYGYRY.
Bradyrhizobium RDAPRTSAPGLTGSAGFIIE
Dickeya        ...........FGAAGYLY.
E.coli         ....................
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Figure 1 Multiple alignment of secretin of TTSS from *Vibrio vulnificus* (*V. vulnificus*), *Bradyrhizobium*, *E.coli* and *Dickeya zeae* (*Erwinia chrysanthemi*). Identical residues colored white in red background and similar residues colored red.

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Table 1 Secretin flanking locus showing proteins of TTSS(Hrp) similar to *Pseudomonas* spp

Locus	Secretin			
	VVI_2330 ⟹	**VVI_2331** ⟹	**VVI_2332** ⟹	**VVI_2333** ⟹
%GC Av G + C = 46.6	49.7	51	51	51
Protein length	279	462	180	358
Protein (similarity)	HrpQ(46%)	HrcC(42%)	HrcJ(50%)	HrpL(64%)
Organism	*Pseudomonas* sp	*Pseudomonas* sp	*Pseudomonas* sp	*Pseudomonas* sp

Materials and methods

Protein blast was carried out using National Center for Biotechnology Information (NCBI) Blast to find homologous proteins.[8] The sequence identified by blast was homology modeled using Swiss-model server automated mode (see http://swissmodel.expasy.org/).[9] The model was energy minimized using DeepView and validation of the structure was carried out using ANOLEA[10] and Verify3D.[11] It was compared by superimposing the structure of *Escherichia coli* TTSS secretin (PDB code: 3GR5) using Chimera[12] and displayed using Povray.[13] The protein PDB structure template of *E. coli* was obtained from http://www.PDB.org/. DaliLite Pairwise comparison of protein structures was used to obtain root mean square deviation (RMSD) between pairs of atoms obtained from http://www.ebi.ac.uk/Tools/dalilite/. The transmembrane regions and hydropathy plot were identified by SOSUI for classification and secondary structure prediction of membrane proteins from Nagoya University at the Web address http://www.tuat.ac.jp/~mitaku/sosui/.[14] Prediction of geometric pore centers and identification of pore-lining residues was carried out using the server PoreWalker (see http://www.dev.ebi.ac.uk/thornton-srv/software/PoreWalker/)[15] from EBI Tools. The pore dimension and orientation of the pore were also predicted using the server. The buried residues of the protein PDB was viewed in PyMOL (Delano Scientific LLC, San Francisco, CA).[16] Solvent accessibility studies were carried out using SARpred which is a neural network-based method available at http://www.imtech.res.in/raghava/sarpred/.[17]

Results and discussion

The outer membrane protein, secretin of the TTSS, was identified by NCBI Blast (accession number, NP_761179). It is 53% similar to secretin from *Bradyrhizobium*, a Gram-negative soil bacterium, 52% similar to EscC secretin from *E. coli*, 51% similar to secretin of *Burkholderia* (previously part of *Pseudomonas* genus), which is pathogenic to animals and plants, 51% similar to TTSS secretin of *Yersinia pseudotuberculosis*, 46% similar to TTSS outer membrane protein of *Photorhabdus*

spp, and 42% similar to secretin of *Pseudomonas* spp. Signal peptide analysis revealed that the protein was found to contain an mRNA signal at the N-terminal end, and the cleavage site was predicted to be located between position 22 and 23, in between two alanine residues of the sequence AHA|AKQ. Transmembrane region analysis revealed the protein was found to be a membrane protein with one transmembrane helix located at the N-terminal region. The hydropathy profile of the proteins yielded at least two peaks, one at the N-terminal and another at the C-terminal. The TTSS secretin of *V. vulnificus* possesses a dicysteine in the N-terminal transmembrane region and is similar to the secretin of *Dickeya zeae*, formerly *Erwinia chrysanthemi*, which is a plant pathogen. However,
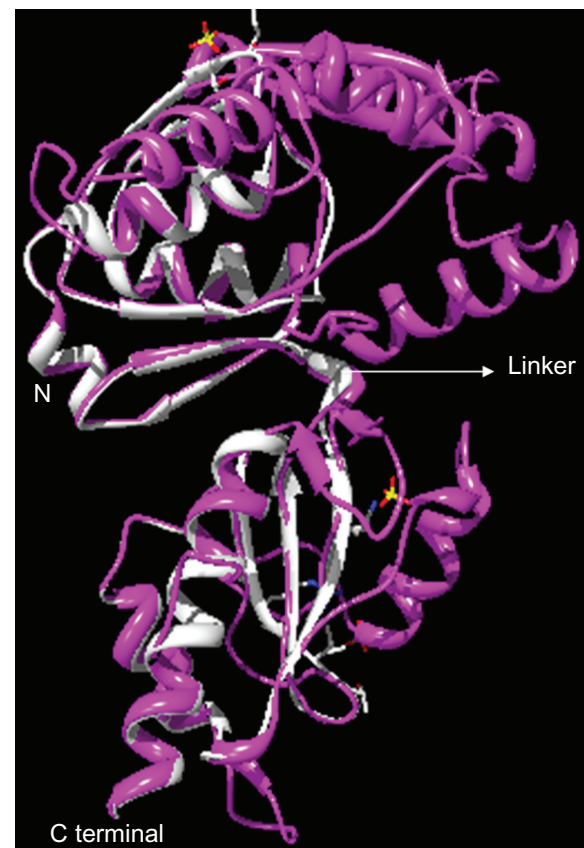

Figure 2 Secretin of *Vibrio vulnificus* superimposed with $EscC_{21-174}$ secretin of *Escherichia coli* showing the conserved fold-forming periplasmic domain.

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              1              11             21             31             41
_ene_pdb      L V N N F A V G Q V   A D K E R L K E I N   A D A S R K S A N P   P E G P G G Q S V L   P L V I N Q L K I T
pdb, chata A  . . . . . . . . . .   . . . . . . . . . .   M S S L E K R L . .   . . . . . G K N E Y   P I I T K . . . . .

              51             61             71             81             91
_ene_pdb      A A P Q V N I S I R   M V E M A K S T S E   E L G I R W Q S V N   P N W M L G V S P G   N Q F A T G I D L T
pdb, chata A  S S P . . . . . . .   . . . . V R A I L N   D F A A N . Y S I .   P . V F I S S S V N   D D F S G E I K N E

              101            111            121            131            141
_ene_pdb      D P D N L I K E S A   F M G I I D A L A S   K S L V N V L A E P   N L T A K S G E K A   E F L V G G E F P F
pdb, chata A  K P V K V L E K L S   K L Y H L T W Y Y D   E N I L Y I Y K T N   E I . S R S I I T P   T Y L D . . . . . .

              151            161            171            181            191
_ene_pdb      P S I D G E S E G V   E F K S F G V G L S   V T P T V L S E N R   I S . . . . . L T V   . . . . . . . . . .
pdb, chata A  . . I D S L L K Y L   S D T . . . . . I S   V . . . . . . . N K   N S C N V R K I T T   F N S I E V R G V P

              201            211            221            231            241
_ene_pdb      . . . . . . T P V V   S A L S R Q N S I K   I N G V D V P G L D   K R T A T T T I E L   A D G Q S F A L A G
pdb, chata A  E C I K Y I T S L S   E S L D K E A Q S K   . . . . . . . . . .   . . . . . . . . . .   . . . . . . . . . .
```

Figure 3 Structure-based alignment of model of *Vibrio vulnificus* (top) with EscC$_{21-174}$ (bottom).

the secretin of *Yersinia* spp has four cysteine residues located at the C-terminal end. The multiple alignment of secretin from the symbiotic bacterium of *Bradyrhizobium of Rhizobium* family and the plant pathogen *Dickeya zeae* and *E. coli* shows the proteins are homologous (Figure 1). The flanking region of secretin locus was found to contain proteins showing similarity to TTSS proteins of *Pseudomonas* sp (Table 1). The hydrophobic regions in the C-terminal were examined for the presence of any linear motifs. A nonconserved GDxG motif belonging to lipolytic enzyme family from the Blocks database was found in the extreme C-terminal of the protein from position 456 to 459 (Figure 1). The presence of the lipase motif led to assign the secretin as a lipolytic protein. The lipase motif was not conserved in secretin family of TTSS which led to the speculation of a different mode of action for secretin from *V. vulnificus*. Surface accessibility studies carried out by SARpred reveal the lipase motif (GDxG) is surface accessible with relative solvent accessibility up to 70%. *V. vulnificus* being a chitin colonizer, the presence of a lipase motif in the outer membrane ring-like structure may help in adherence of the membrane ring to the host. Similar activities have been hypothesized by Pruzzo et al where *Vibrio* spp adhere to chitin by lipase activity.[18]

Figure 4 Secretin showing secondary structure with hydrophobic C-terminal shown in red.
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Structure depiction of secretin

The model was energy minimized and validated using ANOLEA and Verify3D, and the model was found to be of good quality. Residues numbering from 62 to 400 were modeled. In order to study the domains of secretin, the modeled structure of *V. vulnificus* was superimposed with the structure of secretin from enteropathogenic *E. coli* (Pdb ID: 3GR5). The structure fragment from sequence 22 to 174 depicts the TTSS periplasmic domain located in the outer membrane of *E. coli* (Esc 22-174). The Dali score obtained for the structure superimposition was 1Å RMSD with an average RMSD z score of 16Å. This shows the presence of domain structure of TTSS secretin of *V. vulnificus* was structurally homologous to periplasmic domain secretin of *E. coli* (Figure 2). A structure-based sequence alignment
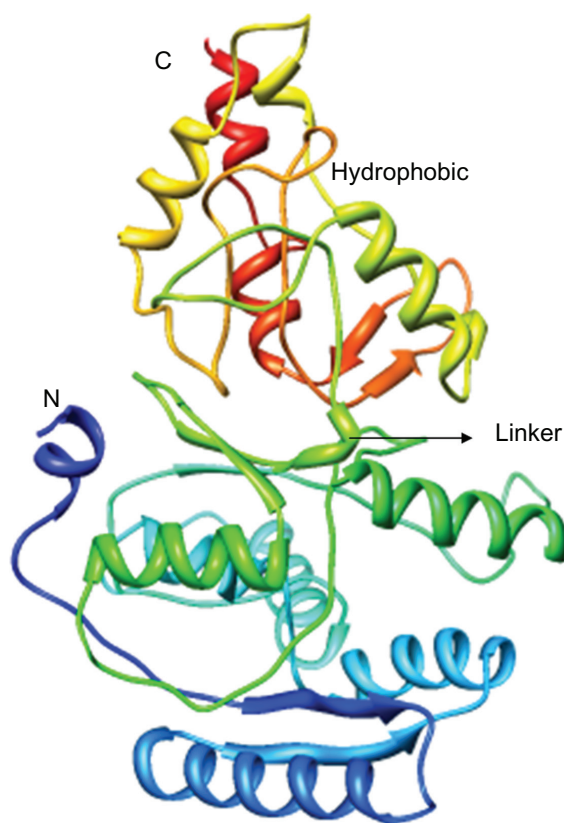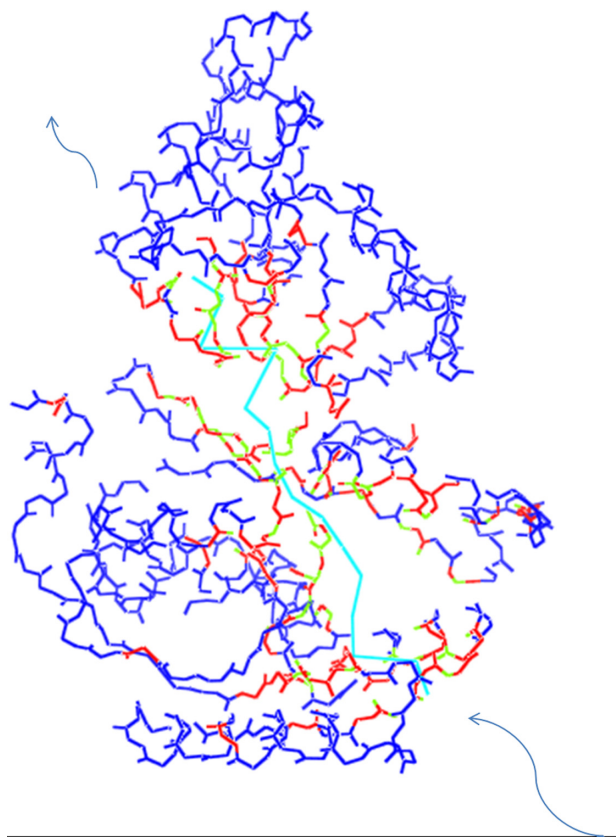
Pore diameter profile (3Å)
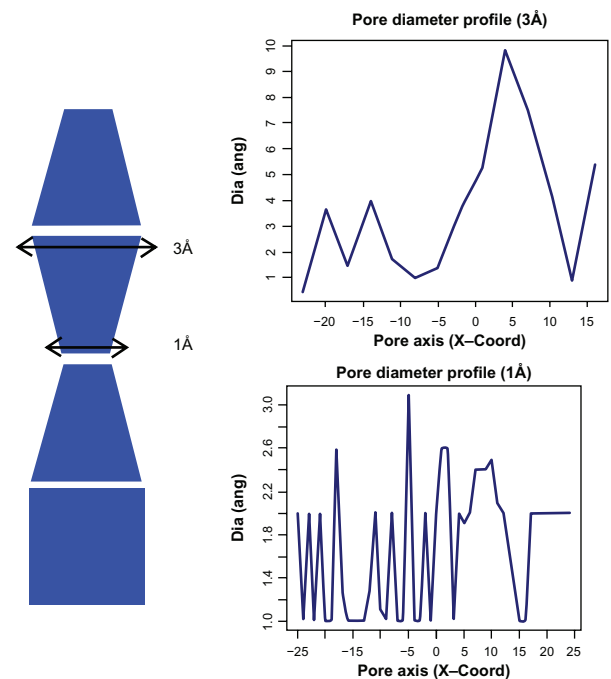
Pore diameter profile (1Å)

Figure 6 Conical pore with predicted pore size 3Å for secretin.
Notes: Identified shape of secretin: SDUD (*V. vulnificus*) software: PoreWalker.
D, Decreasing diameter conical frustum; U, Increasing diameter conical frustum;
S, Cylinder.

Figure 5 Central pore axis depicted in cyan. Red areas represent the buried pore. The conical domain at the top is the C-terminal.

yielded conservation of core residues (Figure 3). The amino domain and the carboxy domains are connected by a linker which is also a structurally conserved region functioning in regulation of the TTSS apparatus.[5] The linker region in the secretin of *V. vulnificus* was found to be conserved structurally but with glutamic acid in *E. coli* replaced by asparagine in *V. vulnificus*. The C-terminal region was also found to be hydrophobic as predicted by Chimera (Figure 4) suggesting that it may be interacting with another component of TTSS.

Analysis of secretin pore

The secretin family of TTSS whose primary function is to transport large molecules contains the pore-forming domain.[19] The pore analysis by PoreWalker revealed a pore-forming channel which was buried in the protein core (Figure 5). The server detected transmembrane protein channels from the 3D structure of the protein and detected the biggest and longest cavity through the channel. Optimization of the pore cavity was carried out by the server to identify the main axis, shape, and size of the pore. The predicted shape of the secretin pore was conical with the predicted pore dimension of 1Å and 3Å at the narrower and wider sections,

respectively (Figure 6). A total of 91 pore-lining residues were obtained for the homology modeled structure of secretin. The buried pore-lining residues led to the identification of the protein main axis which formed a conical-shaped pore. From the current study, the orientation of secretin molecule and the channel that it forms was predicted, which may facilitate the transport of toxic effector proteins through the TTSS. The prediction of channel for translocation of molecules may be important for any future pharmacological intervention that may be carried out.

Conclusion

The secretin outer membrane protein of TTSS of *V. vulnificus* was identified to be homologous to *Yersinia* spp, *E. coli*, and *Bradyrhizobium* spp. Superimposition studies helped identify the core periplasmic domain specific for secretin of TTSS. The pore-forming regions of the secretin in *V. vulnificus* were predicted to allow the transport of effector molecules through the needle of the TTSS. The shape of the pore was predicted to be conical with pore size dimensions from 1Å to 3Å. Thus, the highlights of the study are the secretin homology domain predicted to enclose a pore for translocation of virulence proteins and a lipolytic motif observed at the C-terminal end for adherence, which may govern virulence of the bacteria.

Acknowledgment

Disclosure

The authors report no conflicts of interest in this work.

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