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.

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. The secretin possesses a signal peptide region which is processed by the sec machinery and transported to the
outer membrane. Members of the secretin family possess a C-terminal homology domain which function in oligomerization and pore formation. 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. 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.

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

<table>
<thead>
<tr>
<th>Locus</th>
<th>Secretin</th>
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<tbody>
<tr>
<td></td>
<td>VV1_2330</td>
</tr>
<tr>
<td>%GC Av G + C = 46.6</td>
<td>49.7</td>
</tr>
<tr>
<td>Protein length</td>
<td>279</td>
</tr>
<tr>
<td>Protein (similarity)</td>
<td>HrpQ(46%)</td>
</tr>
<tr>
<td>Organism</td>
<td>Pseudomonas sp</td>
</tr>
</tbody>
</table>

Materials and methods

Protein blast was carried out using National Center for Biotechnology Information (NCBI) Blast to find homologous proteins. The sequence identified by blast was homology modeled using Swiss-model server automated mode (see http://swissmodel.expasy.org/). The model was energy minimized using DeepView and validation of the structure was carried out using ANOLEA10 and Verify3D.11 It was compared by superimposing the structure of Escherichia coli TTSS secretin (PDB code: 3GR5) using Chimera12 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/) 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 EscC21–174 secretin of Escherichia coli showing the conserved fold-forming periplasmic domain.
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

**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

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**Figure 3** Structure-based alignment of model of *Vibrio vulnificus* (top) with EscC<sub>22–174</sub> (bottom).

**Figure 4** Secretin showing secondary structure with hydrophobic C-terminal shown in red. Copyright © 2011, Chimera. Reproduced with permission from the Regents of the University of California.
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. 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 hydro-
phobic 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. The pore analysis by PoreWalker revealed a pore-
forming channel which was buried in the protein core (Fig-
ure 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 secre-
tin 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 dimen-
sions 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
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Disclosure
The authors report no conflicts of interest in this work.

References