An Epimerase Gene Essential for Capsule Synthesis in Vibrio vulnificus

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The extracellular capsule polysaccharide (CPS) of Vibrio vulnificus is a primary virulence factor which allows survival of the bacteria in the human host. To study the genes involved in expression of the capsule, we generated mutants that lost the ability to produce CPS following the insertion of a minitransposon into the genome of an encapsulated, clinical strain of V. vulnificus. A genomic region, from one nonencapsulated mutant, containing the transposon and flanking V. vulnificus DNA was cloned, and a probe complementary to the chromosomal DNA immediately adjacent to the transposon was used to locate this fragment in the genome of the encapsulated parent strain. The fragment, which contained a putative capsule gene, was cloned and, when supplied in trans, complemented the mutation in the nonencapsulated mutant to restore capsule production. In addition, virulence studies, using the 50% lethal dose assay, showed that the restoration of capsule production also restored the virulence of the organism. Sequence analysis of the gene disrupted by the transposon revealed that it matched a nucleotide-sugar epimerase of Vibrio cholerae O139, with 75 and 85% identities at the nucleotide and amino acid levels, respectively. In addition, computer analysis recognized epimerases of various organisms as highly similar to the putative epimerase of V. vulnificus. Finally, a combination of PCR amplification and Southern blotting showed that this epimerase is common to at least 10 strains of V. vulnificus that each express a serologically distinct CPS. Our results indicate that the epimerase gene is essential for capsule expression in V. vulnificus.

Vibrio vulnificus is well documented as the causative agent of sepsis and infectious disease following ingestion of raw seafood (8, 9). This gram-negative bacillus also causes wound infections from exposure of an open wound to contaminated seawater and may account for more than 50% of all vibrio-associated extraintestinal illnesses in the United States (9).

Asorted virulence factors which advance the pathophysiology of V. vulnificus, including the extracellular capsular polysaccharide (CPS), have been described (2, 30, 36, 41, 49, 57), and those isolates which do not express the CPS are considered avirulent (47, 58, 59). The capsule permits V. vulnificus to evade nonspecific host defense mechanisms such as activation of the alternative pathway of the complement cascade and complement-mediated opsonophagocytosis (34, 39, 55).

An opaque (designated by the suffix “O”) colony morphology on an agar surface is indicative of an encapsulated strain that is lethal to mice and capable of survival in normal human serum, as opposed to the nonencapsulated, translucent (T) strains (58, 59) which arise randomly during routine bacteriological manipulation of opaque strains (47). Once a strain is nonencapsulated, the incidence of reversion back to the encapsulated state, although rare, has been reported (58, 59), suggesting that the loss of CPS production may be reversible. The instance of reversion is also true for capsule expression in Pseudomonas atlantica (4) and Neisseria meningitidis (24).

The capsule gene locus of V. vulnificus has not been identified, leaving the gene arrangement to be speculated upon with examples of well-defined systems of Escherichia coli, Haemophilus influenzae, and Klebsiella pneumoniae as possibilities. Concurrent with the extensive serological identification of E. coli capsules (37), studies involving V. vulnificus capsules have focused solely on capsule types, resulting in the identification of at least 13 distinct serological CPS types (44) and 15 CPS chemotypes identified by high-performance anion-exchange chromatography and nuclear magnetic resonance spectroscopy (23).

Our laboratory set out to identify genes involved in capsule expression of V. vulnificus. Utilizing transposon mutagenesis, we generated mutants that no longer expressed a discernible polysaccharide capsule. DNA sequence of the cloned chromosomal region surrounding one transposon insertion revealed that the transposon had disrupted an open reading frame (ORF) that was found highly similar to the nucleotide-sugar epimerase gene of Vibrio cholerae O139.

MATERIALS AND METHODS

Bacterial strains and plasmids. The characteristics of selected bacterial strains and plasmids are shown in Table 1 for simplification. Additional strains and plasmids used in this study are briefly described herein. The encapsulated V. vulnificus clinical strain 1003(O) was used for transposon mutagenesis, from which the nonencapsulated transposon mutant ABZ1(T) was derived. The plasmid vector pEIS (constructed by Richard Cooper, Department of Veterinary Science, Louisiana State University) consists of pGPT704 (32, 53) with a mini-Tn5kan transposable element (26) cloned into its multiple cloning site (MCS) and was used for transposon delivery into V. vulnificus. E. coli SM10pir (32, 43) was used as the donor strain for conjugation experiments introducing the pEIS vector into V. vulnificus. The plasmid pBluescript SK– (Stratagene, La Jolla, Calif.) and the mobilizable vector pBBR1MCS (27) were used to clone V. vulnificus chromosomal DNA, and each vector was propagated in E. coli DH5α MCR (New England Biolabs Inc., Beverly, Mass.). E. coli MC1061 cells (54) and the transfer-proficient helper plasmid pRK2013 (17), which encodes all of the transfer genes necessary for successful mobilization of a plasmid, were used in triparental matings. Ten strains of V. vulnificus, each with a different capsular serotype (44), were examined for the epimerase gene. V. cholerae O139 was used in Southern hybridizations and was also examined for the epimerase gene.

**Growth conditions.** V. vulnificus and E. coli cells were propagated in heart infusion (HI) broth (Difco, Detroit, Mich.) supplemented with 2% NaCl for 18 h at 37°C with shaking at 200 rpm, unless otherwise stated. Colistin (118 μg/ml), kanamycin (50 μg/ml), ampicillin (50 μg/ml), and chloramphenicol (30 μg/ml) were used when appropriate.

Transposon mutagenesis. To disrupt genes essential for capsule synthesis in V. vulnificus, transposon mutagenesis was performed in the following manner. Sus-
pensions (1.5 ml of each) of *V. vulnificus* 1003(O) and *E. coli* SM10/αpir cells were separately pelleted, washed twice in HI broth, and resuspended in 15 μl of broth and then combined. Isopropyl-β-D-thiogalactopyranoside (Sigma Chemical Co., St. Louis, Mo.) was added to a final concentration of 1 mM to induce transposition (28). The bacteria were then spotted onto a nitrocellulose filter (Schleicher & Schuell, Keene, N.H.) placed on the surface of an HI agar plate and incubated at 30°C for 16 h. After incubation, the filter was submerged into 30 ml of HI broth to remove all cells, and 100-μl aliquots were spotted onto HI-kamycin-colistin agar and incubated at 37°C. Nonencapsulated, translucent colony phenotypes were selected and streaked onto HI-kamycin-colistin plates. Stable, translucent colonies were selected and stored at ~85°C in HI broth containing 15% glycerol.

DNA isolation. Genomic DNA was isolated by the method of Chan and Goodwin (13). This protocol was modified for nonencapsulated strains by omitting the addition of cetyltrimethylammonium bromide. Plasmid DNA was isolated by using a Perfect Prep plasmid isolation kit (5 Prime 3 Prime, Inc., Boulder, Colo.) according to the manufacturer's instructions.

*Probe synthesis.* To locate the transposon insertion in the chromosomes of each nonencapsulated mutant, a 980-bp digoxigenin (dig)-labeled probe specific for the putative capsule gene and not an artifact. The PCR products were electrophoresed on a 0.8% agarose gel, the DNA was transferred to a nitrocellulose filter on the surface of an HI agar plate and incubated overnight at 30°C. The filter was added to 5 ml of HI broth and vortexed to remove all discernible cells. The filter was dried, remoistened with 10 ml of HI broth and spotted onto a nitrocellulose filter. Following hybridization, the epimerase-probe A was allowed to hybridize at 68°C with the dig-labeled epimerase-probe A according to the suggested procedure for the Genius 3 kit (Boehringer Mannheim). The probe was synthesized by using the PCR DIG probe synthesis kit (Boehringer Mannheim). The protocol used to determine the 50% lethal doses (LD₅₀) of *V. vulnificus* strains was that of Wright et al. (57), involving intraperitoneal injections of *V. vulnificus* cells genetically immediately followed by intraperitoneal injections of ferric ammonium citrate into 6- to 8-week-old male mice. Following a 48-h period, mortalities were totaled and the method of Reed and Muench (38) was used to calculate the LD₅₀ for each strain tested.

*PCR amplification.* To determine the distribution of the *V. vulnificus* putative capsule gene among 10 cryptically diverse capsular types A PCR amplification approach was used for detection. The same primers as used to synthesize...
the epimerase-probe A (see above) were used to amplify the target region. Chromosomal DNA was prepared from 1.5-ml broth cultures of each capsular serotype. Pelleted cells were washed once with 1 M NaCl and once with 0.5 M NaCl, resuspended in 500 μl of water, and heated at 100°C for 15 min. Cell debris was removed by centrifugation, and the supernatant fluid, containing the chromosomal DNA, was used as the template. Ready-To-Go PCR beads (Pharmacia Biotech Inc., Piscataway, N.J.) were used, with the additions of 2.5 μl of DNA template, 1 μl of each 10 μM primer solution, and 20.5 μl of sterile, deionized water to each tube. Amplification was performed in a Perkin-Elmer 480 Thermal Cycler, using the parameters suggested for the Ready-To-Go PCR beads (Pharmacia) with a modification of the annealing temperature to 60°C. PCR products were electrophoresed on 8% polyacrylamide gels, stained with ethidium bromide, and visualized by using an Eagle Eye II (Stratagene).

**Computer analysis.** All nucleotide sequences obtained were entered into the Basic Localization Alignment Search Tool (BLAST) (1) for comparison to various similar nucleotide sequences. Translation from nucleotide sequence was also entered into BLAST and matched with proteins of similar sequence. Protein secondary structure was predicted by using the SSP program (48).

**RESULTS**

**Transposon mutagenesis and Southern blotting.** Transposon mutagenesis was used to disrupt genes involved in the capsule synthesis of *V. vulnificus*. Using the encapsulated *V. vulnificus* strain 1003(O) as the parent, successful insertion of the transposon in a target gene gave 23 nonencapsulated transposon mutants from eight separate experiments. The chromosomal DNA from each of the 23 nonencapsulated mutants was digested with seven restriction enzymes, chosen because their recognition sites are not present in the transposon and digestion yields the transposon flanked by the chromosomal DNA of the gene disrupted. Through Southern blot analysis, each mutant was examined for the presence of the transposon by hybridization with the kanamycin probe. Insertions were mapped to seven distinct chromosomal regions according to the fragment size recognized by the kanamycin probe, using all restriction enzymes listed above. Of interest in this study is one mutant, ABZ1(T), that contained a unique site of transposon insertion compared to the additional 22 mutants.

**Identification of a putative capsule gene.** To identify a gene disrupted by the transposon, the genomic region containing the transposon and flanking chromosomal DNA of an nonencapsulated mutant, ABZ1(T), was cloned into pBluescript. The insert, containing the 1.8-kb transposon, was 2.3 kb in size. Sequence analysis revealed 246 bp of *V. vulnificus* chromosomal DNA before the transposon was located. The derived nucleotide sequence was entered into BLAST (1) and found to be similar to 246 bases of a nucleotide-sugar epimerase gene, ORF9 of *Vibrio cholerae* O139 (14).

**Cloning and sequencing of the epimerase gene.** Southern blot analysis, using the dig-labeled epimerase-probe A, revealed the size of the EcoRI-digested fragments from *V. vulnificus* 1003(O) and *V. cholerae* O139 that contained the epimerase gene (Fig. 1). In *V. cholerae* O139, the probe recognized a fragment which was previously cloned by Comstock et al. and shown to contain genes responsible for capsule production of O139 that were not detected in O1 strains of *V. cholerae* (14). The probe also recognized a 6.5-kb fragment of *V. vulnificus* 1003(O) (Fig. 1), as well as 1003(T) (data not shown). The fragment from 1003(O) was extracted from an agarose gel and cloned into pBluescript, resulting in pEpiBS. The insert was partially sequenced, and the epimerase gene was located immediately adjacent to the T3 region of pBluescript, as shown in Fig. 2. Analysis of the nucleotide sequence showed that the epimerase of *V. vulnificus* matched that of *V. cholerae* O139, with 75 and 85% identity at the nucleotide and amino acid levels, respectively.

In addition to location of the epimerase gene, sequence analysis of the pEpiBS insert showed that immediately adjacent to the T7 region of pBluescript and approximately 5 kb upstream from the epimerase gene (Fig. 2) is a region of at least 250 bp that is 96% homologous to the rfbQRS sequence of *V. cholerae* O139 (7), indicating further homology between these two *Vibrio* species.

**Complementation.** Following several triparental matings, the pEpiBBR vector was successfully introduced into *V. vulnificus* mutant ABZ1(T), supplying the epimerase gene in trans. Capsule production, in the once-nonencapsulated strain, was restored, resulting in the encapsulated strain designated ABZ1(O).

**LD**

The LD** 50 of *V. vulnificus* 1003(O), 1003(T), ABZ1(T), and ABZ1(O) were determined by using the “iron

![FIG. 1. Southern blot of epimerase-probe A hybridized to a 6.5-kb fragment of *V. vulnificus* 1003 (lane 1) and a 21-kb fragment of *V. cholerae* O139 (lane 2), both digested with EcoRI. Lane MW contains molecular weight markers, sizes of which are shown in kilobases at the left.](http://iai.asm.org/)

![FIG. 2. Map of the pEpiBS vector, which consists of a 6.5-kb insert of *V. vulnificus* 1003(O) chromosomal DNA cloned into pBluescript at EcoRI restriction enzyme sites. Genes originating from the pBluescript vector itself are designated by open arrows. The positions and orientations of the epimerase gene and rfbQRS are shown by the striped and solid arrows, respectively.](http://iai.asm.org/)
V. vulnificus with a distinct CPS serotype. As shown in Fig. 3, all of the 10 epimerase gene in the genome of 10 determined. PCR was used to demonstrate the presence of the epimerase gene. Detection of the epimerase gene in 10 V. vulnificus capsular serotypes. The epimerase gene was shown to be present in strains 1003(O) and 1003(T), but its dissemination among additional encapsulated strains of V. vulnificus remained to be determined. PCR was used to demonstrate the presence of the epimerase gene in the genome of 10 V. vulnificus strains, each with a distinct CPS serotype. As shown in Fig. 3, all of the 10 V. vulnificus capsular serotypes and V. vulnificus 1003(O) show amplification of the 139-bp target region and were hybridized by epimerase-probe B that is specific for 83 bp of the epimerase gene. PCR amplification coupled with Southern blot analysis verified the presence of an epimerase gene in the chromosome of each strain.

**TABLE 2.** LD<sub>50</sub>s of encapsulated and nonencapsulated strains of V. vulnificus

<table>
<thead>
<tr>
<th>Strain</th>
<th>Phenotype</th>
<th>LD&lt;sub&gt;50&lt;/sub&gt; (cells)</th>
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<tbody>
<tr>
<td>1003(O)</td>
<td>Opaque</td>
<td>0.87</td>
</tr>
<tr>
<td>1003(T)</td>
<td>Translucent</td>
<td>&gt;6.50 × 10&lt;sup&gt;7&lt;/sup&gt;</td>
</tr>
<tr>
<td>ABZ1(T)</td>
<td>Translucent</td>
<td>&gt;4.90 × 10&lt;sup&gt;7&lt;/sup&gt;</td>
</tr>
<tr>
<td>ABZ1(O)</td>
<td>Opaque</td>
<td>9.70</td>
</tr>
</tbody>
</table>

* Highest amount of cells used to challenge mice. No deaths resulted from injections at this level.

**FIG. 3.** (a) PCR amplification of the 139-bp region of the putative epimerase gene in V. vulnificus C7184 (lane 1), 1010 (lane 2), 1014 (lane 3), 1007 (lane 4), 1657 (lane 5), 1866 (lane 6), 1002 (lane 7), 549 (lane 8), 938 (lane 9), 1456 (lane 10), and 1003 (lane 11). (b) Southern blot of the PCR products (see above) hybridized by an 83-bp probe (epimerase-probe B) specific for a region of the epimerase gene of V. vulnificus.

**DISCUSSION**

V. vulnificus expresses a negatively charged, extracellular CPS which serves to protect this pathogen in hostile environments of the human host. Capsule expression has been directly associated with virulence by mouse lethality studies (11, 47, 57) and transferrin-bound iron utilization (12, 33, 46). Kreger et al. (28) reported that the acidic surface polysaccharide was a major protective antigen by showing that mice vaccinated with surface antigen preparations were protected from overt infection when challenged with live encapsulated V. vulnificus cells. These findings suggest that protection from disease caused by V. vulnificus may be prominently humoral immunity targeting the capsular antigen and not cell-mediated immunity.

The direct association of capsule expression with virulence in assorted gram-negative pathogens has stimulated interest in the events leading to capsule expression and regulation. E. coli (15, 40), H. influenzae (29), N. meningitidis (20), Salmonella typhi (22), and K. pneumoniae (3) represent pathogens whose capsule genes are confined to a single chromosomal locus. This arrangement may permit a simple regulation of a large number of genes (39). The group II capsules of E. coli exemplify this arrangement, where three functional regions are collectively responsible for the assembly, translocation, and expression of the complete polysaccharide capsule (10). Because the capsule gene locus of V. vulnificus is not known, the gene arrangement can only be speculated upon when drawn from known systems such as those mentioned.

Our strategy for locating the gene locus was to disrupt a gene essential for capsule expression in V. vulnificus by transposon mutagenesis and then to identify that gene through sequence analysis. This preliminary analysis may lead to the identification of additional genes involved in CPS production.

In this study, sequence analysis has shown that one gene disrupted in the nonencapsulated mutant ABZ1(T) was very similar to a small segment of a 12-kb region believed to encode the surface polysaccharide of V. cholerae O139. Specifically, the V. vulnificus putative capsule gene product was found to be similar to the ORF9 protein of V. cholerae O139, which has been identified as a putative nucleotide-sugar epimerase (14). The BLAST (1) similarity search, using the amino acid sequence of the putative epimerase of V. vulnificus 1003(O), has shown it to also be highly similar to epimerases of several organisms in addition to V. cholerae O139. Those exhibiting the greatest identity to the putative epimerase of V. vulnificus are identified on Table 3. Orf2 of E. coli O111 has been described by Bastin et al. (5) and found to be homologous to epimerases of various organisms. This organism produces an O-antigen capsule so called because the sugar compositions of the O-

**TABLE 3.** Comparison of amino acid sequences found to be highly similar to the putative epimerase of V. vulnificus determined by BLAST

<table>
<thead>
<tr>
<th>Organism</th>
<th>Homologous protein</th>
<th>Amino acid identity (%)</th>
<th>Smallest sum probability (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. cholerae O139</td>
<td>Nucleotide-sugar epimerase</td>
<td>85</td>
<td>1.3 × 10⁻²⁴⁹</td>
</tr>
<tr>
<td>E. coli O111</td>
<td>Orf2 (hypothetical protein)</td>
<td>63.5</td>
<td>8.5 × 10⁻¹⁴⁹</td>
</tr>
<tr>
<td>S. aureus M</td>
<td>Cpl</td>
<td>56</td>
<td>1.5 × 10⁻¹²⁸</td>
</tr>
<tr>
<td>B. thuringiensis</td>
<td>Glucose epimerase</td>
<td>53.7</td>
<td>1.4 × 10⁻¹¹⁹</td>
</tr>
</tbody>
</table>

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Highly virulent, with an LD<sub>50</sub> of 9.7 × 10<sup>7</sup> cells, the greatest inoculum tested. The strain ABZ1(O), from which the transposon mutants were derived, gave an LD<sub>50</sub> of 0.87 cells. The spontaneously derived, nonencapsulated mutant ABZ1(T) also resulted in a high LD<sub>50</sub>, determined as &gt;4.9 × 10<sup>7</sup> cells, the greatest inoculum tested. The strain ABZ1(O), which acquired its ability to synthesize CPS through complementation, has proven to be highly virulent, with an LD<sub>50</sub> of 9.7 cells.

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antigen lipopolysaccharide (LPS) side chain and the capsule are identical (21). Cap1 of *Staphylococcus aureus* M is encoded by part of a capsule gene locus and has also been shown to be highly homologous with various epimerases. Cap1 has been described by Lin et.al. (31) as the NAD-dependent enzyme involved in the synthesis of N-acetylgalactosaminouronic acid due to its high homology to the VipB protein of *Salmonella typhi*, whose Vi polysaccharide is a homopolymer of N-acetylgalactosaminouronic acid. The putative glucose epimerase of *Bacillus thuringiensis* was identified by Dunn and Ellar (16) as part of a virulence locus consisting of *P1* and capsule genes.

Each of the epimerases identified possesses one common feature, the dependence on NAD as a cofactor for the formation of a ketose intermediate during enzymatic function (18). Wierenga et al. (56) have defined an amino acid “fingerprint” in addition to a βαβ fold that identifies the NAD binding domain of a protein. The epimerase of *V. vulnificus*, described in this study, contains the consensus amino acid sequence and the βαβ fold (Table 4), required for NAD binding, in its N terminus, as defined by computer analysis (48). The C-terminus domain of epimerases is generally responsible for binding to the substrate (52), with the nucleotide portion serving as the binding anchor (19). While various amino acid sequences have been suggested to be involved in substrate binding (52), a consensus sequence has not been identified for substrate specificity.

In addition to identifying the putative epimerase of *V. vulnificus* by nucleotide and amino acid sequence analysis, complementation was used to illustrate its role in capsule production. When introduced into the nonencapsulated ABZ1(T), pEpIBBR supplied the epimerase gene in *trans* and resulted in the restoration of capsule production. The presence of the epimerase gene allowed the once-nongencapsulated mutant to express a polysaccharide capsule, indicating its involvement in capsule production. In addition, complementation resulted in a lowering of the LD₅₀ of the nonencapsulated mutant from >4.9 × 10⁵ to 9.7 cells once capsule production was reestablished. The newly found ability of ABZ1(O) to express a polysaccharide capsule restored the virulence of the organism and indicates the requisite of CPS for virulence of *V. vulnificus*.

A region of *V. vulnificus* DNA upstream of the epimerase gene was found highly similar to *rfbQRS* of *V. cholerae* O139, which has been reported to be an insertion sequence that may be involved in DNA rearrangement (6). Insertion sequences, similar to the one mentioned here, that are linked to genes involved in polysaccharide synthesis are not unusual in *Vibrio* species (50), suggesting its position relative to the epimerase may indicate the presence of other neighboring genes involved in CPS or even LPS production. But because the epimerase and *rfbQRS* are separated by approximately 5 kb, they may not be labeled as necessarily linked until the adjoining DNA is identified as part of a capsule locus.

In summary, epimerases mediate for the interconversion of epimers, such as glucose and galactose, which differ in configuration at a single asymmetric center. Epimerization of activated monosaccharides generally occurs in the early stages, or precursor synthesis, of capsule formation (51). As shown in this study, the loss of a functional epimerase causes the disruption of capsule production, which may be due to the depletion of precursors required for capsule synthesis. An epimerase may be common to organisms that utilize the same precursors for capsule synthesis or quite possibly even express a common sugar in their capsules. Because the putative nucleotide epimerase described in this study was found to be common to all encapsulated *V. vulnificus* strains tested, it is most likely a gene involved in the synthesis of CPS conserved among strains to provide an essential element for the expression of all capsule types.

Further analysis of the capsule gene locus of *V. vulnificus* is imperative, but this study has proven to be a worthwhile endeavor of identification of a capsule gene and possibly the location of the capsule gene locus.

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Without the constant intuition and involvement of V. R. Srinivasan, this work would not have been possible. We are grateful for the continuous assistance of Richard Cooper, who provided the *P1* vector and the use of his automated sequencer. We also thank Eric Acherberger and Gregg Pettis for support throughout the study. We thank Jim Oliver and Debi Linkous, University of North Carolina at Charlotte, for their generosity in allowing the use of their facilities. Without their expertise and guidance, the virulence studies would not have been performed.

REFERENCES


### Table 4. Aligned NAD binding domains showing the fingerprint region and βαβ fold in the N termini of putative epimerases

<table>
<thead>
<tr>
<th>Organism</th>
<th>Sequence</th>
<th>β</th>
<th>α</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. vulnificus (Vv) 1003</td>
<td>KVLTVGAAGFGSI1TAELNAAGHVEGV1GD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V. cholerae (Vc) O139</td>
<td>KVLTVGAAGFGSI1TAELNAAGHVEGV1GD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli (Ec) O111</td>
<td>KVLTVGAAGFGSI1TAELNAAGHVEGV1GD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. aureus (Sa) M</td>
<td>KVLTVGAAGFGSI1TAELNAAGHVEGV1GD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. thuringiensis (Bt)</td>
<td>KVLTVGAAGFGSI1TAELNAAGHVEGV1GD</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Amino acid sequences shown: *V. vulnificus* (Vv) 1003 residues 2 to 31; *V. cholerae* (Vc) O139 residues 2 to 31; *E. coli* (Ec) O111 residues 2 to 31; *S. aureus* (Sa) M residues 2 to 31; and *B. thuringiensis* (Bt) residues 5 to 34. Residues identical in all sequences are in boldface.