Type IV Secretion System Components in *Vibrio vulnificus*

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Vibrio vulnificus is a marine bacterium that is responsible for 95% of all seafood-related deaths in the US with a fatality rate of 50-60% (Jones and Oliver, 2009; Oliver, 2005). *V. vulnificus* is associated with three types of infection: gastroenteritis, primary septicemia following raw oyster consumption, and wound infections (Strom and Paranjpye, 2000; Oliver, 2005; Jones and Oliver, 2009). This organism can be separated into biotypes 1, 2, and 3, where biotype 1 is primarily associated with human disease. Biotype 1 strains can be further divided into C- and E-**genotypes**, easily determined by polymorphisms in the *vcg* (virulence correlated gene) region. C-genotypes are typically associated with clinical isolation and E-genotypes are associated with water/oyster isolation (Rosche *et al*, 2005; Warner and Oliver, 1998). It is also noteworthy that even within biotype 1 strains there is significant genetic variability (Hilton *et al*, 2006; Bogard and Oliver, 2007). *V. vulnificus* is associated with one definitive virulence factor, capsular polysaccharide, and several putative virulence factors such as flagella, proteases, lipopolysaccharide, and pili (Strom and Paranjpye, 2000; Oliver, 2005; Jones and Oliver, 2009).

There are several systems known to be associated with the transfer of virulence factors from bacteria to host cells. To date, there are 5 described bacterial secretion systems with the potential for a sixth (Ward *et al*, 2001; Boyer *et al*, 2009). Type IV secretion systems (T4SS) are transmembrane systems which are used to transfer substrates, including DNA and a variety of protein toxins, across the cell envelopes of gram positive and gram negative bacteria (Christie and Vogel, 2000; Christie *et al*, 2005). T4SS are composed of two components, the T-pilus and a membrane-associated complex. While it is known that there are exposed pilus proteins that interact with host cell surface proteins, it remains unclear how this system exports the macromolecules (Ward *et al*., 2001; Christie *et al*, 2005). The T4SS system is composed of a coupling protein (VirD4) and up to 11 VirB proteins which form the T-pilus and its membrane-associated complex. VirD4 and VirB4 are of particular interest as they are thought to be energizing components with ATPase abilities. VirD4 aids in recognition of the substrate and VirB4 interacts with several VirB proteins. It is thought to have a putative role in early assembly of the pilus structure as well as a structural role in transport (Ward *et al*, 2001; Christie and Vogel, 2000).

To date, only the complete sequences of two *V. vulnificus* genomes, both of the C-genotype, have been published. However, we report at this meeting the sequencing of three E-genotype strains. In comparing the E-sequences to the published C-genotype sequences, we found that only the C-genotype strains possessed the VirB4/VirD4 components of aT4SS. However, a discrepancy in the presence of the two genes between C- and E-genotypes was also observed**.**

The NCBI search engine**,** Entrez, was used to locate the sequence of the *virB4* and *virD4* genes in the C-genotype strain CMCP6 and their respective homologues (*traC* and *traD*) in the other published C-genotype strain YJ016. Primer BLAST, a basic alignment tool from NCBI, was used to create primers for each gene from CMCP6 and YJ016, creating four sets of primers that yielded no significant E score to the homologous gene of the other strain (i.e. the matches had high similarity to the strain designed from but not the homologue). Strains (n=40) of both genotypes of *V. vulnificus* were grown overnight shaking in HI broth at 30^{o}C. DNA was then extracted and stored at -20^{o}C. Using Promega GoTaq polymerase and buffer, multiplex PCR was performed according to standard protocol. While these primers yielded the appropriately sized bands for CMCP6 and YJ016, no amplification was observed for any of the other 38 strains.

The alignment program, ClustalW, was then utilized to create an alignment of *virB4* of CMCP6 and its YJ016 homologue, *traC*. An alignment was also created for *virD4* (CMCP6) and its YJ016 homologue, *traD*. Homologous regions in the *virB4/traC* and *virD4/traD* alignments were then chosen using OligoAnalyzer from Integrated DNA Technologies based on GC content, melting temperature, and ability to form primer dimers. The chosen sequences were then searched for a match to its homologue using E scores on BLAST, where an E score close to 0 is indicative of a high level of homology. Using sequences that displayed homology in the two strains, primers were designed (common sequence primers) which resulted in an E score of ~6e-05 for both primers against the two published strains, indicating an adequate match. This primer set contained primers for the *virB4/traC* homologues and for the *virD4/traD* homologues. A set of primers was also created using the non-homologous regions of both genes, i.e. regions that were unique in each published strain and their homologues in order to generate strain-specific primers. BLAST was again utilized to ascertain an appropriate E score which suggested a match to the desired gene but not to its homologue. Four sets of primers were individually designed from this process for *virD4, virB4, traD,* and *traC*. Multiplex PCR was again performed using both the common sequence and the strain-specific primer sets. The DNA was then visualized using gel electrophoresis.

The common sequence primer set yielded discrete bands at the appropriate product size for CMCP6 and YJ016, as expected. However, when tested against the 38 other *V. vulnificus* strains only one strain yielded bands at the appropriate size, a C-**genotype** strain (SREL278). The strain-specific primer sets also yielded appropriately sized discrete bands for the two published strains, as well as SREL278, however, bands were also detected for 5 other strains (Table 1).

Strain	Genotype	Isolate Source
JY1305	E	Oyster
LSU 763	C	Septicemia
C7184AV	C	Spontaneous avirulent mutant of C7184K2
C7184K2	C	Septicemia
SREL 116	E	Unknown

Table 1: Strains that returned distinct bands from strain-specific primer set.

This study has indicated that two of the main components of the T4SS system are present not only in C-**genotype strains, but in some E-genotype strains**. This could account for the occasional E-genotype strain which is associated with human infection.

The presence of these two genes suggests the presence of a type IV secretion system in *V. vulnificus*, which is often correlated with virulence. Type IV secretion systems are found in many organisms and are associated with the transport of many substrates. These substrates can vary from a plasmid or piece of single stranded DNA to components of a toxin in the case of *B. pertussis.* The T4SS is also capable of substrate transfer between not only other strains of a species, but also between different species. This system makes it possible for bacterial pathogens to transfer virulence factors and genetic material into the host organism, which is, in many cases, eukaryotic. The presence of these genes does not, however, indicate the presence of the complete T4SS system as it requires many other components in order to function properly. The studies by Hilton *et al* (2006) reveal that there is not only a great deal of variability encountered between the genotypes, but also within the genotypes and the individual strains, of *V. vulnificus*. There is indeed a possibility that, due to the large amount of genetic heterogeneity in this species, the primers we designed are not functional when used with DNA samples from other strains. However, it is also possible that all the necessary genes for a fully functional T4SS are present only in C-**genotypes** and not in most E-**genotypes.**

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