Entire genome sequence of E-genotypes of *Vibrio vulnificus* biotype 1 strains

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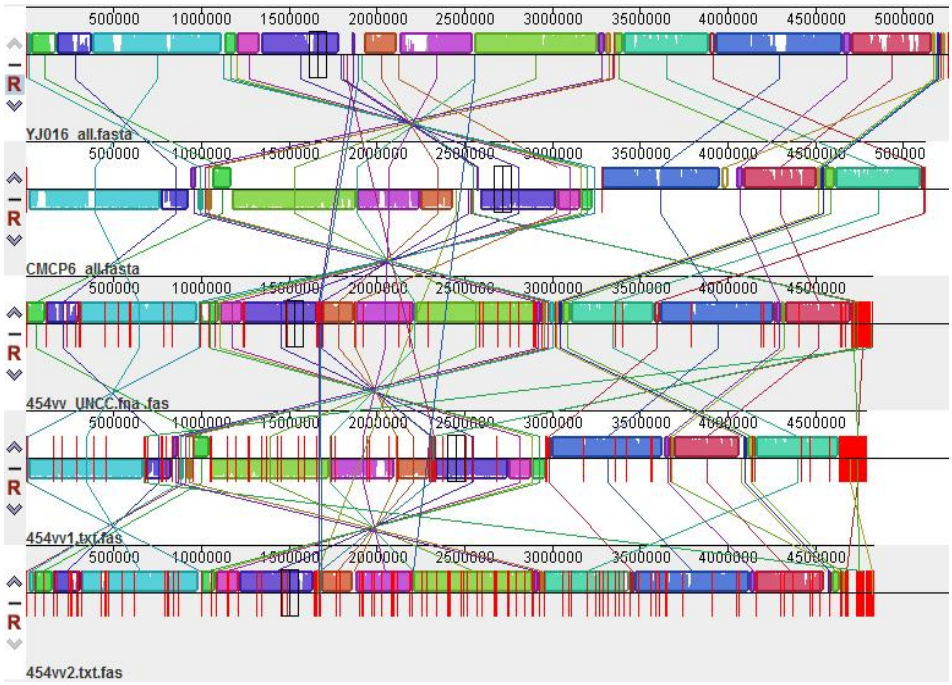
The Gram-negative bacterium, *V. vulnificus*, is a ubiquitous inhabitant of estuarine waters and a serious human pathogen (Oliver, 2006). Numerous *V. vulnificus* isolates have been studied, and it has been shown that two genetically distinct subtypes, distinguished by 16SRNA as well as numerous other gene polymorphisms (Rosche et al., 2005), are associated specifically with either environmental or clinical isolation. While local genetic differences between the subtypes have been probed, only the genomes of two clinical isolates (YJ016 and CMCP6) have so far been completely sequenced (Chen et al., 2003; Kim et al., 2003). In order to better understand *V. vulnificus* as an agent of disease and to identify the molecular components of its pathogenesis, we have completed draft sequence assemblies of three diverse environmental isolates using a pyrosequencing approach. All three strains were characterized as being "E-genotype" strains, based on the "virulence correlated gene", *vcg*, as described by Rosche *et al*. (2005). *V. vulnificus* strain JY1305, an oyster isolate, was sequenced to a depth of 32x, resulting in a complete coverage of that genome. Strains E64MW, a clinical (wound) isolate and JY1701, a second oyster isolate, were sequenced to lesser depth, covering approximately 99% of these genomes, respectively. We have performed a comparative analysis of these sequences using the previously published sequences of the two published *V. vulnificus* clinical isolates as a reference.

We found that the genome size of the E-genotypes of this pathogen to be approximately 4.7-4.8Mbp, compared to approximately 5.1-5.2Mbp for the published C-genotypes. We used a plasmid isolation kit and observed an electrophoretic band indicating the existence of such an extrachromosomal element. Further, our genomic isolation protocol would be expected to extract all DNA, including any plasmid DNA. However, our analysis of the full genomic sequences of the three E-genotype strains revealed no significant alignment to the YJ016 plasmid.

The genomes we studied are dynamic, with 1.4% of the genes in the C-strain genomes not found in the E genomes. Key differences identified in a preliminary comparison among C-strains and the three un-closed E genomes comprise 53 genes. These genes are components of the common core genome of the clinical (C-genotype) but are not found in any of the environmental (E-genotype) strains. Most significant may be components of the Type IV secretory pathway, known to be a major virulence factor in several bacteria, including *V. cholerae* (Alvarez-Martinez and Christie, 2009). Type IV secretion systems (T4SS) are transmembrane transfer 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. T4SS is composed of two components, the T-pilus (comprised of up to 11 VirB proteins) and a membrane-associated complex which includes the VirD4 coupling protein.

Comparative Genome Analysis of C- and E-types of *V. vulnificus*



YJ016 ("C")

CMCP6 ("C")

JY1305 ("E")

E64MW ("E")

JY1701 ("E")

Figure 1. A comparison of overall genomic structure of the five sequenced *Vibrio vulnificus* strains. The comparison was performed using Mauve. Similarly colored blocks indicate LCBs ("Locally Collinear Blocks") that are internally free from rearrangements. Red ticks indicate gaps in the alignment.

We postulate that the significant observed differences in genome structure and content between analyzed E and C-type strains contribute directly to observed patterns of pathogenicity and virulence in this important human pathogen.

Literature Cited

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