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GENOME SEQUENCES

# Complete Genome Sequence of *Vibrio coralliilyticus* RE22, a Marine Bacterium Pathogenic toward Larval Shellfish

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**ABSTRACT** *Vibrio coralliilyticus* RE22 is an indigenous marine pathogen that infects larval bivalve shellfish. This strain is particularly problematic in oyster hatcheries, where it causes high larval mortality. It contains two circular chromosomes and one megaplasmid. Annotation reveals multiple genes which may encode important virulence factors.

*Vibrio coralliilyticus* strain RE22 is a naturally occurring marine bacterium associated with intermittent outbreaks of larval oyster mortalities in U.S. West Coast shellfish hatcheries. It was first detected and isolated in larval oysters from a shellfish hatchery in Oregon in April 1999 (1) and was shown to be highly virulent to larval oysters (2). In the United States, laboratory studies have demonstrated the ability of RE22 to infect both Pacific oyster (*Crassostrea gigas*) and Eastern oyster (*Crassostrea virginica*) larvae (2–4). A draft genome of this bacterium, formerly thought to be *V. tubiashii*, was published (5), and it was confirmed to be *V. coralliilyticus*. This paper extends that work by providing the complete, circularized, genome sequence of RE22.

*Vibrio coralliilyticus* RE22 from the USDA culture collection was grown in Difco Luria-Bertani broth, Miller (Becton, Dickinson and Company, Sparks, MD) supplemented with 2% NaCl (3% total NaCl) at 26°C and 150 rpm overnight and centrifuged at 10,000 × *g* to pellet the cells. Genomic DNA was extracted and purified with Genomic-tip 100/G as per the manufacturer's protocol (Qiagen, Germantown, MD). A genomic library was prepared with the standard Pacific Biosciences (PacBio, Menlo Park, CA) protocol for 20-kb libraries, "20 kb Template Preparation Using Blue Pippin Size-Selection System." The genome was sequenced with the PacBio RS II system on a single-molecule real-time (SMRT) cell using PacBio P6-C4 chemistry. *De novo* assembly was performed with the Hierarchical Genome Assembly Process (HGAP, version 3) with a minimum seed read length of 17,000 bp. HGAP data processing consisted of BLASR (alignment), PacBio/dagcon (correction), Celera Assembler (version 8.1; overlap/layout), PacBio/utgcns (consensus), and Quiver (polishing) (6). Contigs were circularized with Circlator (version 1.1.3) (7). The average coverage was 185×. The fully assembled genome contains 5,784,972 bp, consisting of chromosome 1 (3,532,203 bp), chromosome 2 (1,915,494 bp), and a 337,275 bp megaplasmid (p337).

Genome annotation was obtained from the NCBI Prokaryotic Genome Annotation Pipeline (Bethesda, MD). The genome contains 5,317 genes, 73 pseudogenes, 5,094 coding sequences (CDS), 34 rRNAs (5S, 16S, and 23S), and 112 tRNAs and has a GC content of 45.81%. Chromosome 1 contains 3,309 genes, 3,123 CDS, 34 rRNAs, and 107 tRNAs and a GC content of 45.6% compared with the smaller chromosome 2, which contains 1,694 genes, 1,679 CDS, no rRNAs, and 5 tRNAs and a GC content of 45.4. The

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plasmid consists of 314 genes, 292 CDS, no rRNAs or tRNAs and a GC content of 50.3%. Annotation revealed the presence of 7 metalloproteases, 5 serine proteases, 6 hemolysins/cytolysins, and 5 toxins. Three of the 5 toxin genes are from the plasmid. The plasmid also contains the metalloprotease gene for the core-promoter binding protein (CPBP) family intramembrane metalloprotease (8). Together, this information should facilitate further study into virulence mechanisms associated with killing of larval shellfish induced by *V. coralliilyticus* RE22.

**Data availability.** The complete genome sequence of *V. coralliilyticus* RE22 has been deposited in GenBank under accession numbers CP031472 (chromosome 1), CP031473 (chromosome 2), and CP031474 (megaplasmid p337) and in the Sequence Read Archive (SRA) under accession number SRP156002.

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