Draft Genome Sequence of the Shellfish Bacterial Pathogen Vibrio sp. Strain B183

Harold J. Schreier, a,b Eric J. Schott c

Department of Marine Biotechnology, Institute of Marine and Environmental Technology, University of Maryland, Baltimore County, Baltimore, Maryland, USA a; Department of Biological Sciences, University of Maryland, Baltimore County, Baltimore, Maryland, USA b; University of Maryland Center for Environmental Science, Institute of Marine and Environmental Technology, Baltimore, Maryland, USA c

We report the draft genome sequence of Vibrio sp. strain B183, a Gram-negative marine bacterium isolated from shellfish that causes mortality in larval mariculture. The availability of this genome sequence will facilitate the study of its virulence mechanisms and add to our knowledge of Vibrio sp. diversity and evolution.

Vibrio sp. strain B183 is a marine bacterium isolated from diseased bay scallop (Argopecten irradians) larvae and shown to cause mortality of oyster (Crassostrea virginica) larvae under mariculture conditions (1). Here we announce the genome sequence of strain B183 in order to facilitate identification of processes involved in pathogenesis and to add to our knowledge of Vibrio sp. diversity and evolution.

A single colony of strain B183 was grown in marine broth 2216 (Difco) at 28°C and DNA was extracted using the Wizard genomic DNA purification kit (Promega). Sequencing was done with an Illumina MiSeq benchtop sequencer. The read library comprised 5,580,583 (2 × 250-bp) fragments, representing one of the largest Vibrio sp. genomes to date, with average coverage of 840X. De novo assembly of the paired reads was done using the CLC Genomics Workbench assembly tool (CLC Bio/Qiagen), yielding 52 contigs with an average length of 107,309 bp. The N50 is 292,693 bp with a G+C composition of 45.2%. Gene prediction and annotation using the RAST (RAPid Annotation using Subsystem Technology) server (2) generated 5,143 protein encoding genes and 81 transfer and ribosomal RNA genes. The closest relative analyzed by the SEED viewer 2.0 program (3) was coral pathogen Vibrio coralliilyticus strain ATCC BAA-450 (score = 526).

The B183 genome carries quorum-sensing and biofilm production-associated genes including luxU, luxO, luxT, luxN, luxR, and hapR regulators (4), the gene for N-(3-hydroxybutanoyl)-L-homoserine lactone (autoinducer-1) synthase, genes for mannose-sensitive hemaglutinin (MSHA) biogenesis and pilin proteins (5), and gene clusters associated with capsule polysaccharide production (cps, vps, and eps) (6). The syp gene cluster for symbiotic colonization (7) was identified and genes for ABC and siderophore transporters, receptors, and the Fur regulator were found, which may also contribute to colonization (8).

While Vibrio CTX phage (9) and zona occludens toxin genes appear to be absent in the B183 genome, the RTX toxin was identified and the PHAST search tool (10) revealed an intact phage genome related to the Vibrio cholerae K139 lysogenic phage (11).

Virulence-related secretory HlyD, at least seven hemolysins, the toxRS virulence regulator, and genes encoding types I, II, III, and V1 secretion system components were found. Genes for proteases important for Vibrio pathogenicity (12) were identified, including metalloproteases, collagensases, and four vibriolysins, as well as ten chitinase-encoding genes, a virulence inventory that is comparable to that found for V. coralliilyticus (13).

The genome encodes 1,484 hypothetical proteins (from 113 to 4451 aa) with no significant similarity to any protein in GenBank (28.8% of the open reading frames [ORFs]). Studies focusing on these unknown ORFs as well as the investigation of specific pathways defined by the genes mentioned above will provide insight to their contribution to the pathogenicity of B183. Development of molecular tools to track and enumerate B183 in in vivo challenges of oysters and other hosts is being conducted to assist in these investigations.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number JPQB00000000. The version described in this paper is the first version, JPQB01000000.

ACKNOWLEDGMENTS

We thank Diane Kapareiko for providing strain B183 and technical support, Sabeena Nazar and Ryan McDonald for assistance with genome sequencing, and Jeanette Davis for help with the genome assembly and submission.

Support was provided by Dr. Gary Wikfors, NOAA Fisheries Northeast Fisheries Science Center Milford Laboratory, and grant number NA11SEC4810002 from the NOAA-EPP Living Marine Resources Cooperative Research Center.

REFERENCES

1. Lim HJ, Kapareiko D, Schott E, Hanif A, Wikfors GH. 2011. Isolation and evaluation of a new probiotic bacteria for use in shellfish hatcheries: I. isolation and screening for bioactivity. J. Shellfish Res. 30:609–615. http://dx.doi.org/10.2983/035.030.0303.
2. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA,
Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GI, Olson R, Osterman AL, Overbeek RA, McNeil IK, Paarmann D, Paczian T, Parrrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: rapid annotations using subsystems technology. BMC Genomics 9:75. http://dx.doi.org/10.1186/1471-2164-9-75.

3. Overbeek R, Begley T, Butler RM, Choudhuri JV, Chuang H-Y, Chooho M, de Crécy-Lagard V, Diaz N, Disz T, Edwards R, Fonstein M, Frank ED, Gerdes S, Glass EM, Goesmann A, Hanson A, Iwata-Reuyl D, Jensen R, Jamshidi N, Krause L, Kubal M, Larsen N, Linke B, McHardy AC, Meyer F, Neuweger H, Olsen G, Olson R, Osterman A, Portnoy V, Pusch GD, Rodionov DA, Rückert C, Steiner J, Stevens R, Thiele I, Vassieva O, Ye Y, Zagnitko O, Vonstein V. 2005. The subsystems approach to genome annotation and its use in the project to annotate 1000 genomes. Nucleic Acids Res. 33:5691–5702. http://dx.doi.org/10.1093/nar/gki866.

4. Waters CM, Bassler BL. 2005. Quorum sensing: cell-to-cell communication in bacteria. Annu. Rev. Cell Dev. Biol. 21:319–346. http://dx.doi.org/10.1146/annurev.cellbio.21.012704.131001.

5. Ariyakumar DS, Nishiguchi MK. 2009. Characterization of two host-specific genes, mannose-sensitive hemagglutinin (mshA) and uridyl phosphate dehydrogenase (UDPDH) that are involved in the *Vibrio fischeri*–*Euprymna tasmanica* mutualism. FEMS Microbiol. Lett. 299:65–73. http://dx.doi.org/10.1111/j.1574-6968.2009.01732.x.

6. Jiang P, Li J, Han F, Duan G, Lu X, Gu Y, Yu W. 2011. Antibiofilm activity of an exopolysaccharide from marine bacterium *Vibrio* sp. QY101. PLoS One 6:e18514. http://dx.doi.org/10.1371/journal.pone.0018514.

7. Yip ES, Geszvain K, DeLoney-Marino CR, Visick KL. 2006. The symbiosis regulator RscS controls the *sp* gene locus, biofilm formation and symbiotic aggregation by *Vibrio fischeri*. Mol. Microbiol. 62:1586–1600. http://dx.doi.org/10.1111/j.1365-2958.2006.05475.x.

8. Septer AN, Wang Y, Ruby EG, Stabb EV, Dunn AK. 2011. The haem-uptake gene cluster in *Vibrio fischeri* is regulated by Fur and contributes to symbiotic colonization. Environ. Microbiol. 13:2855–2864. http://dx.doi.org/10.1111/j.1462-2920.2011.02558.x.

9. Johnson JA, Morris JG, Kaper JB. 1993. Gene encoding zona occludens toxin (*sot*) does not occur independently from cholera enterotoxin genes (*ctx*) in *Vibrio cholerae*. J. Clin. Microbiol. 31:732–733.

10. Zhou Y, Liang Y, Lynch KH, Dennis JJ, Wishart DS. 2011. PHAST: a fast phage search tool. Nucleic Acids Res. 39:W347–W352. http://dx.doi.org/10.1093/nar/gkr485.

11. Kapfhammer D, Blass J, Evers S, Reidl J. 2002. *Vibrio cholerae* phage K139: complete genome sequence and comparative genomics of related phages. J. Bacteriol. 184:6592–6601. http://dx.doi.org/10.1128/JB.184.23.6592-6601.2002.

12. Miyoshi S-I. 2013. Extracellular proteolytic enzymes produced by human pathogenic *Vibrio* species. Front Microbiol. 4:339. http://dx.doi.org/10.3389/fmicb.2013.00339.

13. de Oliveira Santos E, Alves N, Dias GM, Mazotto A, Vermeilho A, Vora GJ, Wilson B, Beltran VH, Bourne DG, Le Roux F, Thompson FL. 2011. Genomic and proteomic analyses of the coral pathogen *Vibrio corallilyticus* reveal a diverse virulence repertoire. ISME J. 5:1471–1483. http://dx.doi.org/10.1038/ismej.2011.19.