Draft Genome Sequence of the Oyster Larval Probiotic Bacterium Vibrio sp. Strain OY15

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We report the draft genome sequence of Vibrio sp. strain OY15, a Gram-negative marine bacterium isolated from an oyster (Crassostrea virginica) digestive tract and shown to possess probiotic activity. The availability of this genome sequence will facilitate the study of the mechanisms of probiotic activity as well as virulence capacity.

CONTREllting microbial pathogens in aquaculture using probiotic bacteria is becoming increasingly preferred over the use of chemical treatments, such as disinfectants or antibiotics (1). The marine Vibrio sp. OY15 is a naturally occurring bacterium isolated from the digestive gland of an adult oyster (Crassostrea virginica) and has been shown to significantly improve survival of oyster larvae to metamorphosis when challenged with a pathogenic Vibrio strain (2). Here we announce the genome sequence of strain OY15 in order to facilitate identification of processes involved in probiotic activity and to ascertain virulence potential. A single colony of strain OY15 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 using an Illumina MiSeq benchtop sequencer. The read library contained 9,221,838 paired-end reads with 284 average read length and average coverage of 430×. De novo assembly of the paired reads was done using CLC Genomics Workbench (CLC Bio/Qiagen) yielding 45 contigs with an average length of 115,533 bp and 5,198,998 total bp. The N50 is 544,789 bp with a G+C composition of 44.6%. Gene prediction and annotation using RAST (Rapid Annotation using Subsystem Technology) (3), generated 4,814 open reading frames. The closest neighbors identified by SEED viewer 2.0 (4) were Vibrio sp. EX25 (score = 534) and V. alginolyticus 40B (score = 489).

The genome carries genes for regulators luxU, luxO, and hapR and a homoserine lactone efflux pump—suggesting that quorum sensing is likely important for colonization (5)—as well as scrABC systems involved in Vibrio swarming (6). A ferric siderophore transporter, vibrioferin/ferrichrome siderophores, and the Fur and IrgB regulators may also play roles in colonization of the digestive tract (7). A cluster of 17 genes for mannose-sensitive hemagglutinin (MSHA) biogenesis proteins were identified, which may also play roles in adhesion and colonization (8). Candidates for probiotic activity include several Rhs family genes, which mediate intercellular competition (9) and may stimulate host immunity (10), and three alginate lyase precursors, which disrupt biofilms (11). Gene clusters associated with exopolysaccharide production—rbm and vps—are present, which may play a role in antibiofilm activity (12). Bacteriocin (colicin V) and bacteriocin tolerance genes were also found.

While phage-related genes encoding putative RTX and zona occludens toxins were identified in the OY15 genome, genes encoding Vibrio CTX phage (13) appear to be absent. Virulence-related secretory HlyD, several hemolysins, the toxRS and vieSB virulence regulators, and genes encoding types I, III, and VI secretion system components were found. On the other hand, tdh and trh genes associated with virulent V. parahaemolyticus (14, 15) were not identified, which is consistent with the finding that OY15 tested negative in a mammalian-cell bioassay for cytotoxicity (J. Jones, U.S. FDA, personal communication). Genomic and phenotypic studies of OY15 will improve our understanding of probiosis and pathogenesis mechanisms of Vibrio spp.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under accession number JPII00000000. The version described in this paper is the first version, JPII01000000.

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REFERENCES
1. Pérez-Sánchez T, Ruiz-Zarzuela I, Blas I, Balcázar JL. 2013. Probiotics in aquaculture: a current assessment. Reviews in Aquaculture. http://dx.doi.org/10.1111/raq.12033.
2. Kapareiko D, Lim HJ, Schott EJ, Hanif A, Wikfors GH. 2011. Isolation and evaluation of a new probiotic bacteria for use in shellfish hatcheries. II.
Effects of a *Vibrio* sp. probiotic candidate upon survival of oyster larvae (*Crassostrea virginica*) in pilot-scale trials. J. Shellfish Res. 30:617–625. http://dx.doi.org/10.2983/035.030.0304.

3. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello 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.

4. Overbeek R, Begley T, Butler RM, Choudhuri JV, Chuang H-Y, Coelho M, de Creey-Lagard V, Diaz N, Disz T, Edwards R, Fonstein M, Frank ED, Gerdes S, Goesmann A, Hanson A, 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.

5. 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.

6. Boles BR, McCarter LL. 2002. *Vibrio parahaemolyticus scrABC*, a novel operon affecting swimming and capsular polysaccharide regulation. J. Bacteriol. 184:5946–5954. http://dx.doi.org/10.1128/JB.184.21.5946-5954.2002.

7. 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.

8. 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.

9. Koskiniemi S, Lamoureux JG, Nikolakakis K, ‘t Kint de Roodenbeke C, Kaplan MD, Low DA, Hayes CS. 2013. Rhs proteins from diverse bacteria mediate intercellular competition. Proc. Natl. Acad. Sci. U. S. A. 110:7032–7037. http://dx.doi.org/10.1073/pnas.1300627110.

10. Kung VL, Khare S, Stehlik C, Bacon EM, Hughes AJ, Hauser AR. 2012. An *rhs* gene of *Pseudomonas aeruginosa* encodes a virulence protein that activates the inflammasome. Proc. Natl. Acad. Sci. U. S. A. 109:1275–1280. http://dx.doi.org/10.1073/pnas.1109285109.

11. McDougald D, Rice SA, Barraud N, Steinberg PD, Kjelleberg S. 2012. Should we stay or should we go: mechanisms and ecological consequences for biofilm dispersal. Nat. Rev. Microbiol. 10:39–50. http://dx.doi.org/10.1038/nrmicro2695.

12. 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.*. PLoS ONE 6:e18514. http://dx.doi.org/10.1371/journal.pone.0018514.

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

14. Lida T, Park K-S, Suthienkul O, Koza Y, Yamaichi Y, Yamamoto K, Honda T. 1996. Close proximity of the *tdh*, *trh* and *ure* genes on the chromosome of *Vibrio parahaemolyticus*. Microbiology 142:2517–2523. http://dx.doi.org/10.1099/00221287-144-9-2517.

15. Gutierrez West CK, Klein SL, Lovell CR. 2013. High frequency of virulence factor genes *tdh*, *trh*, and *thl* in *Vibrio parahaemolyticus* strains isolated from a pristine estuary. Appl. Environ. Microbiol. 79:2247–2252. http://dx.doi.org/10.1128/AEM.03792-12.