

## Complete Genome Sequence of *Vibrio anguillarum* M3, a Serotype O1 Strain Isolated from Japanese Flounder in China

## Guiyang Li,<sup>a</sup> Zhaolan Mo,<sup>a</sup> Jie Li,<sup>a</sup> Peng Xiao,<sup>b</sup> Bin Hao<sup>c</sup>

Laboratory of Maricultural Organism Disease Control and Molecular Pathology, Yellow Sea Fishery Research Institute, Chinese Academy of Fishery Sciences, Qingdao, China<sup>a</sup>; Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China<sup>b</sup>; State Key Laboratory of Microbial Resources, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China<sup>c</sup>

*Vibrio anguillarum* is an important bacterial pathogen that causes vibriosis in marine fish. We present the complete genome sequence of *V. anguillarum* M3, a serotype O1 clinical strain isolated from Japanese flounder (*Paralichthys olivaceus*) in Shandong, China.

Received 27 August 2013 Accepted 30 August 2013 Published 26 September 2013

Citation Li G, Mo Z, Li J, Xiao P, Hao B. 2013. Complete genome sequence of Vibrio anguillarum M3, a serotype O1 strain isolated from Japanese flounder in China. Genome Announc. 1(5):e00769-13. doi:10.1128/genomeA.00769-13.

Copyright © 2013 Li et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license.

Address correspondence to Zhaolan Mo, mozl@ysfri.ac.cn.

**V***ibrio anguillarum* causes vibriosis, a systemic disease of both wild and cultured marine fish characterized by hemorrhagic septicemia (1). There are 23 serotypes identified for this bacterium, and the O1 and O2 serotypes are the major causative agents of fish vibriosis (2). Recently, the genome sequences of three V. anguillarum strains, isolated from salmon (Oncorhynchus kisutch), striped bass (Morone saxatilis), and turbot (Scophthalmus maximus), were released (3). Here, we present the complete genome sequence of V. anguillarum M3, an O1 serotype clinical strain isolated from Japanese flounder (Paralichthys olivaceus) (4). This strain has been studied with respect to its *in vivo* antigen gene expression, metalloprotease and exopolysaccharide production, and biofilm formation (5–7).

The genome of V. anguillarum M3 was sequenced using the Roche 454 GS FLX Titanium system at SegWrite, Inc. (Houston, TX). A total of 23,745 reads were obtained from the sequencing (~20-fold coverage of the genome). The reads were assembled using DataAnalysis version 2.6 (Roche/454 Life Sciences), generating 59 contigs. Using the genome of V. anguillarum strain 775 as a reference, all gaps were filled by local assembly using the CLC Genomics Workbench (64-bit) 6.0.5 and ABI 3730 sequencing of the PCR products of the gap region. The coding sequences (CDSs) were predicted by the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) and the Rapid Annotations using Subsystems Technology (RAST) server (8). Noncoding RNAs were identified using tRNAscan-SE 1.21 (9) and RNAmmer 1.2 (10) by searching against the Rfam database (11). Functional assignment of the CDSs was performed on the InterProScan (12) and the Swiss-Prot and trEMBL databases (13).

The complete genome of *V. anguillarum* M3 contains two circular chromosomes and a plasmid. Chromosome 1 has 3,063,587 bp containing 2,842 CDSs, chromosome 2 has 988,134 bp containing 918 CDSs, and the plasmid has 66,164 bp containing 64 CDSs. Overall, the M3 and 775 genomes share high levels of similarity, as >90% of the CDSs have the same annotation, length, and relative position. With respect to potential virulence traits, a majority of the

virulence genes are conserved in both strains, such as those for hemolysins, metalloproteases, repeat in toxin (RTX) toxins, and vibriolysin (14). Both strains present two type VI secretion system loci on chromosome 1 and chromosome 2, and the genes in the two loci are organized identically in M3 but differently in 775 (15). In addition, M3 has 74 specific genes, encoding a transposase and 73 hypothetical proteins; 775 has 168 specific genes, including genes encoding mobile element proteins (30 genes), molybdopterin biosynthesis proteins (MoeA-MoeB), dGTPase, methylaccepting chemotaxis protein, tryptophanase, and ribonuclease E inhibitor RraB. Regarding the pathogenicity of M3, the complete genome sequence of this strain will increase the understanding of the evolution of *V. anguillarum* and will contribute new insights into virulence studies of this particular species.

Nucleotide sequence accession numbers. The sequence and annotation of the *V. anguillarum* M3 genome have been deposited in GenBank under accession no. CP006699 (M3 chromosome 1), CP006700 (M3 chromosome 2), and CP006701 (M3 plasmid).

## ACKNOWLEDGMENTS

This work was supported partially by the National Natural Science Foundation of China (grant no. 30871935 and 31372567), the Hi-tech Research and Development Program of China (grant no. 2012AA092203), and Special Scientific Research Funds for Central Non-Profit Institutes from Yellow Sea Fishery Research Institute (2013A0602).

## REFERENCES

- 1. Austin B, Austin DA. 2007. Bacterial fish pathogens: disease of farmed and wild fish. Springer Verlag, New York, NY.
- 2. Larsen JL, Pedersen K, Dalsgaard I. 1994. *Vibrio anguillarum* serovars associated with vibriosis in fish. J. Fish Dis. 17:259–267.
- 3. Naka H, Dias GM, Thompson CC, Dubay C, Thompson FL, Crosa JH. 2011. Complete genome sequence of the marine fish pathogen *Vibrio anguillarum* harboring the pJM1 virulence plasmid and genomic comparison with other virulent strains of *V. anguillarum* and *V. ordalii*. Infect. Immun. **79**:2889–2900.
- 4. Mo ZL, Tan XG, Xu YL, Zhang PJ. 2001. A *Vibrio anguillarum* strain associated with skin ulcer on cultured flounder, *Paralichthys olivaceus*. Chin. J. Oceanol. Limnol. 19:319–326.

- Zou YX, Mo ZL, Hao B, Ye XH, Guo DS, Zhang PJ. 2010. Screening of genes expressed *in vivo* after infection by *Vibrio anguillarum* M3. Lett. Appl. Microbiol. 51:564–569.
- Mo ZL, Guo DS, Mao YX, Ye XH, Zou YX, Xiao P. 2010. Identification and characterization of *prtV* gene in *Vibrio anguillarum*. Chin. J. Oceanol. Limnol. 28:55–61.
- Hao B, Mo ZL, Xiao P, Pan HJ, Lan X, Li GY. 2013. Role of alternative sigma factor 54 (RpoN) from *Vibrio anguillarum* M3 in protease secretion, exopolysaccharide production, biofilm formation, and virulence. Appl. Microbiol. Biotechnol. 97:2575–2585.
- 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. doi:10.1186/1471-2164-9-75.
- Lowe TM, Eddy R. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. 25: 955–964.
- Lagesen K, Hallin P, Rødland EA, Staerfeldt HH, Rognes T, Ussery DW. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res. 35:3100–3108.

- Griffiths-Jones S, Moxon S, Marshall M, Khanna A, Eddy SR, Bateman A. 2005. Rfam: annotating non-coding RNAs in complete genomes. Nucleic Acids Res. 33:D121–D124. doi:10.1093/nar/gki081.
- 12. Hunter S, Jones P, Mitchell A, Apweiler R, Attwood TK, Bateman A, Bernard T, Binns D, Bork P, Burge S, de Castro E, Coggill P, Corbett M, Das U, Daugherty L, Duquenne L, Finn RD, Fraser M, Gough J, Haft D, Hulo N, Kahn D, Kelly E, Letunic I, Lonsdale D, Lopez R, Madera M, Maslen J, McAnulla C, McDowall J, McMenamin C, Mi H, Mutowo-Muellenet P, Mulder N, Natale D, Orengo C, Pesseat S, Punta M, Quinn AF, Rivoire C, Sangrador-Vegas A, Selengut JD, Sigrist CJ, Scheremetjew M, Tate J, Thimmajanarthanan M, Thomas PD, Wu CH, Yeats C, Yong SY. 2012. InterPro in 2011: new developments in the family and domain prediction database. Nucleic Acids Res. 40:D306–D312. doi: 10.1093/nar/gkr948.
- 13. Magrane M, UniProt Consortium. 2011. UniProt Knowledgebase: a hub of integrated protein data. Database (Oxford) 2011:bar009. doi:10.1093 /database/bar009.
- 14. Naka H, Crosa JH. 2011. Genetic determinants of virulence in the marine fish pathogen *Vibrio anguillarum*. Fish Pathol. 46:1–10.
- Weber B, Hasic M, Chen C, Wai SN, Milton DL. 2009. Type VI secretion modulates quorum sensing and stress response in *Vibrio anguillarum*. Environ. Microbiol. 11:3018–3028.