Complete Genome Sequence of Vibrio vulnificus MO6-24/O[∇]

Jin Hwan Park, Yong-Joon Cho, Jongsik Chun, Yeong-Jae Seok, Jeong K. Lee, Kun-Soo Kim, Kyu-Ho Lee, Soon-Jung Park, and Sang Ho Choi **

National Research Laboratory of Molecular Microbiology and Toxicology, Center for Food Safety and Toxicology, and Department of Agricultural Biotechnology, School of Biological Sciences and Chunlab, Inc., Department of Biophysics and Chemical Biology and Institute of Microbiology, Seoul National University, Seoul 151-742, Department of Life Science and Interdisciplinary Program of Integrated Biotechnology, Sogang University, Seoul 121-742, Department of Environmental Science and Protein Research Center for Bio-Industry, Hankuk University of Foreign Studies, Yongin, Kyunggi-Do 449-791, and Department of Environmental Medical Biology, Institute of Tropical Medicine, and The Brain Korea 21 Project, Yonsei University College of Medicine, Seoul 120-752, South Korea

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Vibrio vulnificus is the causative agent of life-threatening septicemia and severe wound infections. Here, we announce the complete annotated genome sequence of V. vulnificus MO6-24/O, isolated from a patient with septicemia. When it is compared with previously known V. vulnificus genomes, the genome of this bacterium shows a unique genetic makeup, including phagelike elements, carbohydrate metabolism-related genes, and the superintegron.

Vibrio vulnificus, an opportunistic Gram-negative pathogen that commonly contaminates raw oysters, is the causative agent of food-borne diseases such as gastroenteritis and life-threatening septicemia in predisposed individuals. Mortality from septicemia is very high (>50%) and can occur within days from sepsis (4, 5, 6). Here, we report the complete genome sequence of V. vulnificus MO6-24/O, isolated from a patient with septicemia (8).

The draft genome sequence of MO6-24/O was determined by using a Roche 454 genome sequencer FLX. Shotgun libraries were prepared using both single-end (525,429 reads, 45-times coverage) and paired-end (494,133 reads, 4-times coverage) Titanium library preparation kits (Roche). De novo assembly was carried out using GS De Novo Assembler, version 2.0 (Roche), which resulted in 9 scaffolds containing 49 contigs. Gaps between contigs were closed by sequencing PCR products with a 3730xl DNA analyzer (Applied Biosystems). To correct homopolymer errors from the 454 sequencing, additional sequencing data (28,634,044 reads, 550-times coverage) with 100-bp read lengths were generated using an Illumina genome analyzer IIx. Gene prediction and annotation were carried out using Glimmer 3(2), the RAST annotation server (1), and the NCBI COG database. The MO6-24/O genome is composed of two circular chromosomes of 3,194,232 bp (46.7% G+C content) and 1,813,536 bp (47.4% G+C content). It does not contain any plasmids. The large chromosome (chromosome 1) contains 2,980 predicted coding sequences (CDS), 8 copies of 16S-23S-5S rRNA operons, and 100 tRNAs. The small chromosome (chromosome 2) contains 1,582 CDS, one rRNA operon, and 11 tRNAs.

Like the other completely sequenced *V. vulnificus* strains CMCP6 (GenBank accession numbers AE016795 and AE016796) and YJ016 (GenBank accession numbers BA000037, BA000038, and AP005352), MO6-24/O has the 125-kb-long superintegron in chromosome 1. This region has a lower G+C content (40.9%) than the average G+C content of the whole genome, and 82.5% (198/240) of predicted ORFs in this region are hypothetical genes. Although the integrase (*intI*) and multiple target-specific recombination sites (*attC*) were conserved in the superintegron, 133 predicted ORFs within this region showed no sequence homology to those found in the superintegrons of CMCP6 and YJ016.

In comparison with the genome sequences of both CMCP6 and YJ016, we found 272 MO6-24/O-specific genes, including phage-related genes, integrons, and transposons. The phage-related gene cluster contains ace (accessory cholera enterotoxin) and zot (zonula occludens toxin). Zot of Vibrio cholera increases the permeability of the small intestinal mucosa by affecting the structure of the intercellular tight junction (3), whereas Ace causes fluid secretion in ligated rabbit ileal loops (7). In addition, the MO6-24/O genome has a gene cluster that is required for the utilization of L-fucose; this is present in YJ016 but not in CMCP6. Interestingly, MO6-24/O has the PTS (phosphotransferase system) pathway for uptake of trehalose, which is absent in the two other V. vulnificus strains. The PTS genes show considerable homology with the PTS genes of other Vibrio spp. In conclusion, the complete genome sequence of MO6-24/O revealed genetic diversity of V. vulnificus resulting from extensive gene transfer.

Nucleotide sequence accession numbers. The genome sequence of *V. vulnificus* MO6-24/O has been deposited in GenBank under accession numbers CP002469 and CP002470.

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^{*} Corresponding author. Mailing address: Department of Agricultural Biotechnology, Seoul National University, Seoul 151-742, S. Korea. Phone: 82-2-880-4857. Fax: 82-2-873-5095. E-mail: choish@snu.ac.kr.

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REFERENCES

- 1. Aziz, R. K., et al. 2008. The RAST Server: rapid annotations using subsystems technology. BMC Genomics 9:75.
- 2. Delcher, A. L., D. Harmon, S. Kasif, O. White, and S. L. Salzberg. 1999. Improved microbial gene identification with GLIMMER. Nucleic Acids Res. **27:**4636–4641.
- 3. Fasano, A., et al. 1991. Vibrio cholerae produces a second enterotoxin, which
- affects intestinal tight junctions. Proc. Natl. Acad. Sci. U. S. A. 88:5242–5246. 4. Feldhusen, F. 2000. The role of seafood in bacterial foodborne diseases. Microbes Infect. 2:1651-1660.
- 5. Jones, M. K., and J. D. Oliver. 2009. Vibrio vulnificus: disease and pathogenesis. Infect. Immun. 77:1723-1733.
- Strom, M. S., and R. N. Paranjpye. 2000. Epidemiology and pathogenesis of Vibrio vulnificus. Microbes Infect. 2:177–188.
- 7. Trucksis, M., J. E. Galen, J. Michalski, A. Fasano, and J. B. Kaper. 1993. Accessory cholera enterotoxin (Ace), the third toxin of a Vibrio cholerae virulence cassette. Proc. Natl. Acad. Sci. U. S. A. 90:5267–5271.
- 8. Wright, A. C., L. M. Simpson, J. D. Oliver, and J. G. Morris, Jr. 1990. Phenotypic evaluation of acapsular transposon mutants of Vibrio vulnificus. Infect. Immun. 58:1769-1773.