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Single Circular Chromosome Identified from the Genome Sequence of the *Vibrio cholerae* O1 bv. El Tor Ogawa Strain V060002

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ABSTRACT We report here the complete genome sequence of the *Vibrio cholerae* O1 bv. El Tor Ogawa strain V060002, isolated in 1997. The data demonstrate that this clinical strain has a single chromosome resulting from recombination of two prototypical chromosomes.

Vibrio cholerae is a waterborne pathogen that causes the fatal diarrheal disease cholera. Of the more than 200 serogroups of *V. cholerae*, O1 and O139 are associated with epidemic and pandemic cholera and with the major virulence determinant cholera toxin (1, 2) produced by the filamentous bacteriophage CTXϕ (3). Serogroup O1 comprises two biotypes, classical and El Tor. The classical biotype caused the sixth and probably earlier cholera pandemics, whereas the El Tor biotype is responsible for the current seventh cholera pandemic (4).

The genome of *V. cholerae* is split into two circular chromosomes (chr1 and chr2) (5, 6), a feature common in the family *Vibrionaceae* (7, 8). However, recent genomic studies on *V. cholerae* isolates have revealed two non-O1/non-O139 strains, each with a single chromosome (9–11). It has also been reported that *V. cholerae* O1 strains with single chromosomes can be generated by genome engineering (12) or spontaneously isolated as suppressors of lethal mutations that disrupt the replication of chr2 (13, 14).

The sequenced O1 biovar El Tor Ogawa strain V060002 was isolated in 1997 from a patient who traveled to Indonesia, and the strain has been used in our laboratory as a model for studying regulatory mechanisms of chitin-induced natural transformation (15–18). Genomic DNA was extracted with the DNeasy blood and tissue kit (Qiagen) following the manufacturer's instructions. A 20-kbp library for P6-C4 chemistry was prepared using the RS II SMRTbell template preparation kit version 1.0 (PacBio) and sequenced with the P6 version 2 single-molecule real-time (SMRT) sequencing platform (PacBio). Sequencing reads were assembled *de novo* using the Hierarchical Genome Assembly Process version 3 (HGAP3) (19) with a mean sequence coverage of 196.55-fold. This assembly was corrected with the Quiver consensus algorithm to obtain a high-accuracy genome assembly (19). The contig was further corrected using Pilon version 1.22 (20), and paired-end short reads (300-mer $\times$ 2) were obtained from the MiSeq platform (Illumina).

The generated sequence assembly unexpectedly yielded a single circular chromosome with a genome size of 4,057,041 bp and a GC content of 47.5%. The size and number were verified by pulsed-field gel electrophoresis of the intact chromosome of strain V060002 (data not shown). Comparison of the genome sequences of V060002 and the O1 model strain N16961 (5) revealed that a single chromosome of V060002 was generated by recombination of highly homologous insertion sequence elements shared by chr1 and chr2 (99% identity, corresponding to *vc1789* to *vc1790* on chr1 and *vca0791* to *vca0792* on chr2 of N16961). It should be noted that these recombination

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sites are identical to those of a representative chromosome fusion spontaneously isolated from N16961 with a null mutation of the *dam* gene (14), which is essential for chr2 replication (21).

Annotation of the V060002 genome using the DDBJ Fast Annotation and Submission Tool (DFAST) (22) identified 3,560 coding sequences, 28 rRNA sequences, and 98 tRNA sequences. Strain V060002 also carried well-known gene clusters associated with pathogenesis (23–26), as well as two copies of the CTXφ prophage.

More detailed genomic and phenotypic analyses of this naturally occurring *V. cholerae* O1 strain with a single chromosome will be presented in future publications.

Accession number(s). The annotated chromosome has been deposited in DDBJ/GenBank under the accession number AP018677.

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