

Whole-Genome Sequencing of a *Vibrio cholerae* El Tor Strain Isolated in the Imported Cholera Focus in Siberia

S. V. Balakhonov, L. V. Mironova, E. A. Basov, A. S. Gladkikh, M. V. Afanasev, V. S. Ganin, L. Y. Urbanovich, E. A. Sidorova

Irkutsk Antiplague Research Institute, Irkutsk, Russia

The draft genome sequence of *Vibrio cholerae* O1 strain I-1263, isolated from a patient in the imported focus in Siberia, was determined. The established structural features of the mobile genetic elements indicate stage-by-stage formation of a highly pathogenic *V. cholerae* clone and promote understanding of the mechanisms of evolutionary pathogen transformations.

Received 10 February 2015 Accepted 13 February 2015 Published 26 March 2015

Citation Balakhonov SV, Mironova LV, Basov EA, Gladkikh AS, Afanasev MV, Ganin VS, Urbanovich LY, Sidorova EA. 2015. Whole-genome sequencing of a *Vibrio cholerae* El Tor strain isolated in the imported cholera focus in Siberia. *Genome Announc* 3(2):e01550-14. doi:10.1128/genomeA.01550-14.

Copyright © 2015 Balakhonov et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to L. V. Mironova, mironova-lv@yandex.ru.

Atypical variants of *Vibrio cholerae* El Tor appeared in the beginning of the 1990s and contained a cholera toxin subunit B gene of the classical type (*ctxB1*) (1) that caused all of the epidemic cholera complications registered during that period in Siberia and the Far East (2). Analysis of the genetic structure of atypical *V. cholerae* El Tor variants isolated in Siberia and the Far East in the 1990s demonstrated its differentiation to some genotypes (3). The *V. cholerae* El Tor O1 I-1263 strain isolated in the imported focus in Irkutsk city in 1997 from a patient followed from Uzbekistan was attributed to one of these genotypes (III b). Analysis of CTX prophage structure revealed both the presence of nucleotide replacements in the *ctxB* gene (C/T in the 115 and 203 positions) and also a smaller number of repeats in the P_{ctxAB} promoter region (3). In addition, detection of VSP-I and VSP-II in PCR showed deficiency of the pro496 locus VSP-II in this strain.

Deciphering of the nucleotide sequence of *V. cholerae* El Tor O1 I-1263 genome was performed for profound analysis of its genetic organization and for further phylogenetic analysis.

Genome libraries were constructed according to Roche's protocol with the reagents of the GS Junior Titanium series. Sequencing was implemented using Roche GS Junior. Primary processing of reads was performed using GS Reference software v. 2.8 by alignment on the genome of the *V. cholerae* El Tor O1 N16961 reference strain. The draft genome of the *V. cholerae* El Tor O1 I-1263 strain included 50 contigs, with a total length of 3,957,767 bp. The average length of a contig was 80,764 bp, with a maximal contig length of 656,754 bp and an N_{50} of 236,680 bp. The percentage of mapped reads was 96.7%. An average covering of the genome was 13, with maximal coverage of 41. Genome annotation was achieved using the RAST server. A total 3,985 protein-coding and 123 RNA-coding sequences were revealed. The draft genome revealed 29 genes associated with phages, prophages, and mobile elements, 7 additional genes of pathogenicity, and 60 genes providing resistance to antibiotics.

The analysis of the CTX prophage region revealed high homology with *V. cholerae* El Tor O1 N16961 genes. Two nucleotide

replacements in the 115/203 positions were identified in the *ctxB* gene sequence. The presence of three tandem repeats (5-TTTTGAT-3) was established in the structure of the P_{ctxAB} promoter area, which conformed to our earlier results (3). Deletion of the VC0496-to-VC0498 loci was revealed in the VSPII region. According to Taviani et al., the strains that appeared after 2004 with extended VSPII deletions covering the VC0496-to-VC0512 loci were characterized by increased epidemic potential, and in previous years these variants displaced earlier circulating atypical *V. cholerae* El Tor strains with intact VSPII (4). The tested *V. cholerae* El Tor O1 I-1263 strain with deletion of three VSPII loci may be an intermediate in the formation of a new clone of the highly pathogenic *V. cholerae* El Tor variant.

Nucleotide sequence accession number. The whole-genome shotgun project for *Vibrio cholerae* O1 El Tor strain I-1263 has been deposited at DDBJ/EMBL/GenBank under the accession no. [JPLT00000000](https://www.ncbi.nlm.nih.gov/nuccore/JPLT00000000).

ACKNOWLEDGMENT

This work has been performed with the financial support of the Russian Federal program "National System of Chemical and Biological Safety," state contract N22-D/4.

REFERENCES

- Safa A, Nair GB, Kong RY. 2010. Evolution of new variants of *Vibrio cholerae* O1. *Trends Microbiol* 18:46–54. [http://dx.doi.org/10.1016/j.tim.2009.10.003](https://doi.org/10.1016/j.tim.2009.10.003).
- Mironova LV, Balakhonov SV, Urbanovich L, Polovinkina VS, Kozhevnikova AS, Kulikalova ES, Afanasev MV. 2011. Detection of "hybrid" *Vibrio cholerae* El Tor strains at epidemic complications in Siberia and at the Far East. *Zh Mikrobiol Epidemiol Immunobiol* 5:12–18. (In Russian.)
- Mironova LV, Balakhonov SV, Urbanovich LY, Kozhevnikova AS, Polovinkina VS, Kulikalova ES, Afanasev MV. 2012. Molecular-genetic analysis of *Vibrio cholerae* El Tor strains of epidemic risk isolated in Siberian and Far East regions of Russia. *Mol Genet Microbiol Virol* 27:61–68. [http://dx.doi.org/10.3103/S0891416812020073](https://doi.org/10.3103/S0891416812020073).
- Taviani E, Grim CJ, Choi J, Chun J, Haley B, Hasan NA, Huq A, Colwell RR. 2010. Discovery of novel *Vibrio cholerae* VSP-II genomic islands using comparative genomic analysis. *FEMS Microbiol Lett* 308:130–137. [http://dx.doi.org/10.1111/j.1574-6968.2010.02008.x](https://doi.org/10.1111/j.1574-6968.2010.02008.x).