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GENOME ANNOUNCEMENT

Complete Genome Sequence of a Sucrose-Nonfermenting Epidemic Strain of *Vibrio cholerae* O1 from Brazil

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We report the genome sequence of *Vibrio cholerae* strain IEC224, which fails to ferment sucrose. It was isolated from a cholera outbreak in the Amazon. The defective sucrose phenotype was determined to be due to a frameshift mutation, and a molecular marker of the Latin American main epidemic lineage was identified.

The *Vibrio cholerae* O1 strain IEC224 fails to ferment sucrose on thiosulfate-citrate-bile salt-sucrose (TCBS) agar. It was isolated from a patient's stool specimen in November 1994 at the city of Belém, Brazil, in the context of the Amazon cholera epidemic that occurred in the 1990s. Cholera was introduced in Brazil through the state of Amazonas and spread quickly following the riverine travel routes. This epidemic was driven by an El Tor lineage that caused approximately 169,000 cholera cases between 1991 and 2001 (6). During this main epidemic, a local outbreak was caused by an El Tor strain that failed to ferment sucrose on TCBS agar (4). This variant accounted for the majority of late cholera cases in the Amazon (80.4%) (4). Sequencing provided the opportunity to elucidate the genetic background responsible for its altered sucrose metabolic pathway.

Genomic DNA was extracted by a previously described method (5). The whole-genome sequence was achieved in a hybrid sequencing strategy using the GS FLX 454 (Roche, Applied Science) platform, combined with the SOLiD 3 Plus platform (Life Technologies). The data generated by the GS FLX 454 yielded a total of 319,825 reads (125 Mb) with a coverage of 30.5×, which formed 92 contigs. The SOLiD 3 Plus data yielded a total of 23 million short reads (1.1 Gb) with a genome coverage of 268×. The reads were ordered against the N16961 reference sequence (2), and the merging of both strategies resulted in a closed genome of two scaffolds, corresponding to chromosomes I and II. The final genome was annotated using the RAST pipeline to identify orthologous genes and predict gene functions (1). The annotated genome was manually curated using the *V. cholerae* Genome BLAST as a parameter.

The sequence of the IEC224 strain comprises two circular chromosomes, in which chromosome I contains 3,007,450 bp, while chromosome II contains 1,072,136 bp. The overall GC content is of 47.5%. There are 3,901 predicted CDS, of which 2,989 had a homologous function predicted, and 900 were annotated as hypothetical proteins. The strain's genome contained 98 tRNA genes and 25 rRNA genes.

For analysis, we used the complete genome of the N16961 strain (2) and INDRE 91/1—a genomic draft from a Mexican epidemic strain. In the IEC224 genome, we found a frameshift mutation on the gene encoding the phosphotransferase system (PTS) sucrose-specific IIB component. This mutation truncates the protein structure, accounting for the defective sucrose-fermenting phenotype. Furthermore, we found an altered *Vibrio* seventh pandemic island II (VSP-II) region like that in the already described alleles of Latin American strains (3) and a 49.2-kb bacteriophage that proved to be a molecular marker of the main Latin American epidemic lineage.

Nucleotide sequence accession numbers. The sucrose-nonfermenting strain of *Vibrio cholerae* O1 (IEC224) has been deposited in the Bacterial Collection of the Evandro Chagas Institute (Ananindeua-Pará, Brazil). The results of the whole-genome project have been deposited in GenBank under accession no. CP003330 and CP003331, respectively, for chromosomes I and II. The version described in this paper is the first version.

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