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GENOME SEQUENCES

Draft Genome Sequences of Nine *Vibrio* sp. Isolates from across the United States Closely Related to *Vibrio cholerae*

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ABSTRACT We are reporting whole-genome sequences of nine *Vibrio* sp. isolates closely related to the waterborne human pathogen *Vibrio cholerae*. These isolates were recovered from sources, including human samples, from different regions of the United States. Genome analysis suggests that this group of isolates represents a highly divergent basal *V. cholerae* lineage or a closely related novel species.

The genus *Vibrio* is one of the most diverse and ubiquitous groups of marine bacteria, including species with significant clinical importance, such as *Vibrio cholerae*, which is the causative agent of the pandemic diarrheal disease cholera (1). Surveillance conducted under the Cholera and Other *Vibrio* Illness Surveillance (COVIS) program (https://www.cdc.gov/vibrio/surveillance.html) revealed nine isolates indistinguishable from *V. cholerae* by traditional phenotypic tests but phylogenetically and genotypically divergent from that species.

Cultivation and laboratory identification of these strains based on *rpoB* sequence determination and phylogenetic reconstruction were done following standard procedures (2). Genomic DNA was extracted from the isolates using an ArchivePure DNA cell/tissue kit (5 PRIME) according to the manufacturer's instructions. Sequencing libraries were prepared from the genomic DNA using the Nextera XT DNA library preparation kit (Illumina, San Diego, CA, USA) and sequenced using Illumina MiSeq sequencing platforms (2 × 250-bp paired-end reads). Quality control and *de novo* assembly of the reads were done using default parameters in CLC Genomics workbench 7 (Qiagen). The average genome size was 3.9 Mbp with an average G+C content of 47.3%. Detailed isolate information is outlined in Table 1. Genome annotations were done using RAST 2.0 (3) and the Prokaryotic Genome Annotation Pipeline (PGAP) (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/). Average nucleotide identity (ANI) and *in silico* DNA-DNA hybridization (dDDH) values in comparison to those of the reference strains were calculated using JSpecies v1.2.1 (4) and Genome-to-Genome Distance Calculator (GGDC), respectively. Whole-genome alignment was performed using Mugsy v1.2.3 (5) with default parameters, and a maximum likelihood tree was built from this alignment using RaxML v8 (6) under the GTR+GAMMA model with 1,000 bootstrap replicates. The core genome phylogenetic tree and strains used for dDDH and ANI have been deposited in publicly available databases.

Species delineation values for prokaryotes have been considered 95% or higher for ANI and 70% or higher for dDDH (7). The nine sequenced strains shared 96.5% to 99% ANI and 82% to 85% dDDH values. On the other hand, in comparisons with representative *V. cholerae* strains, ANI ranged from 94% to 96% and dDDH from 65% to 70%. Core genome phylogeny shows that these strains form a strongly supported monophyletic clade basal to *V. cholerae*. Taken together, the tree topology and ANI and

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TABLE 1 Demographic and genomic features of the isolates

Isolate	Source	State	Genome size (bp)	G+C content (%)	No. of contigs	N_{50} (bp)	GenBank accession no.	NCBI SRA accession no.
2017V-1110	Wound	CO	3,994,199	47.1	168	48,914	QKKU00000000	SRR7962202
2016V-1091	Stool	NE	3,814,331	47.3	93	156,947	QKKQ00000000	SRR7962186
2017V-1176	Animal feed	IA	3,896,770	47.2	110	134,986	QKKW00000000	SRR7962200
2016V-1114	Stool	IL	3,873,695	47.3	92	150,142	QKKS00000000	SRR7962196
2016V-1111	Stool	ND	3,883,261	47.3	92	118,713	QKKR00000000	SRR7962199
2017V-1105	Wound	FL	4,080,989	47.2	90	167,992	QKKT00000000	SRR7962197
2014V-1107	Stool	IA	3,988,159	47.4	116	114,877	QKKP00000000	SRR7962194
2017V-1144	Stool	TX	3,869,006	47.2	131	124,156	QKKV00000000	SRR7962203
07-2425	NA[a]	NA	3,826,909	47.4	103	112,361	QKKO00000000	SRR7962192

[a] NA, not applicable.

dDDH values suggest that these nine isolates could belong to a new species representing the closest relative of *V. cholerae* known to date or a highly divergent lineage within the species.

All nine strains contain the toxin transcriptional regulator gene (*toxR*), which is the master regulator of virulence genes in *V. cholerae*, but lack major virulence factor and related genetic elements and/or genes typical of toxigenic *V. cholerae*, such as *ctxAB*, *tcpA*, and the two *Vibrio* pathogenicity islands VPI-1 and VPI-2. However, eight out of nine strains possessed RTX toxin (8), and two strains possessed genes for the type III secretion system, which is an established virulence factor for *V. cholerae* (9). The presence of these virulence-related genes, in addition to their isolation from clinical cases, underscores the pathogenic potential of this monophyletic group of strains.

Data availability. The complete genome sequences were deposited in DDBJ/GenBank and raw reads were deposited in the SRA depository under the accession numbers listed in Table 1. Phylogenetic tree, ANI, and dDDH values were deposited in a publicly available repository under the DOI 10.7939/DVN/OZHLC2 (https://doi.org/10.7939/DVN/OZHLC2).

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