



Complete Genome Sequence of Vibrio coralliilyticus OCN008

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ABSTRACT Here, we report the complete genome sequence of *Vibrio corallilityticus* OCN008, a marine bacterium that infects reef-building coral. Previous sequencing efforts yielded an incomplete sequence (210 contigs). We used Nanopore and Illumina sequencing data to obtain complete sequences of the two circular chromosomes (3.48 and 1.91 Mb) and one megaplasmid (244.69 kb).

V*ibrio coralliilyticus* is a marine bacterium that infects multiple genera of coral and marine shellfish species (1–3). Recent experiments with the OCN008 strain have investigated its basic physiology, pathogenesis, and responses to changes in environmental signals (3–7). Strain OCN008 was isolated from the coral *Porites compressa* and subsequently discovered to infect *Montipora capitata* from the fringing reef of Moku o Lo'e in Kāne'ohe Bay, HI; its cultivation methods have been previously reported (5, 6). A draft genome sequence of 210 contigs was published and is available in GenBank (accession number AVOO0000000.1) (5). To complete a new, whole sequence of this genome and enable genetic and transcriptomic data analyses of OCN008, we resequenced the genome and *de novo* assembled the genome using new sequencing data.

Genomic DNA was extracted using the GeneJET genomic DNA purification kit (Thermo Scientific). The DNA integrity was analyzed using a 4200 TapeStation system (Agilent Technologies) and quantified using the Qubit double-stranded DNA (dsDNA) high-sensitivity (HS) assay kit (catalog number Q32854; Invitrogen). The Illumina Nextera DNA sequencing (DNA-seq) library was prepared with a Nextera DNA Flex library prep kit (Illumina); the standard manufacturing protocol was followed with one-fifth the standard reaction volume for all of the steps. The final Nextera DNA Flex library was performed using an Illumina NextSeq 500/550 system (v2 kits) with a 75-bp sequencing module, generating 42-bp paired-end reads. Additionally, a Nanopore genomic DNA-seq library was prepared using a Nanopore genomic DNA by ligation kit (catalog number SQK-LSK109). The final library was quantified by using a Qubit dsDNA HS assay kit, and sequencing was performed using a MinION R9.4.1 flow cell (catalog number FLO-MIN106).

The Nanopore data were basecalled using the graphics processing unit (GPU)enabled Guppy algorithm (version 3.4.4+a296acb) (8). The primary assembly was performed with Canu (version 1.8) (9) with the following parameters: genomeSize, 5m; maxThreads, 20; and -nanopore-raw combined.fastq. Illumina reads (6,127,597 read pairs) were trimmed with Trimmomatic (version 0.38) (10) with the following parameters: ILLUMINACLIP:adapters.fa:3:20:6, LEADING:3, TRAILING:3, SLIDINGWINDOW:4:20, and MINLEN:40. Only 2.53% of the reads were dropped. The quality of the reference was tested and corrected using breseq (version 0.33.2) (11). The breseq output was used with custom Perl scripts to iteratively correct the reference (custom scripts are available on Github at https://github.com/Juliacvk/OCN008_ASM). After 6 iterations, the consensus converged and no longer had errors. The overall contiguity was verified by examining the alignment of the Nanopore reads to the corrected reference (minimap2 Citation Lydick VN, Rusch DB, Ushijima B, van Kessel JC. 2020. Complete genome sequence of *Vibrio coralliilyticus* OCN008. Microbiol Resour Announc 9:e00323-20. https://doi.org/ 10.1128/MRA.00323-20.

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Received 30 March 2020 **Accepted** 30 June 2020 **Published** 23 July 2020 version 2.17-r943-dirty) (12). The final genome assembly was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (13, 14).

The resulting genome assembly consists of two chromosomes and one megaplasmid. The assembly is full length with 3 contigs and a total size of 5.63 Mb (chromosome I [Chr I], 3.48 Mb; Chr II, 1.91 Mb; plasmid, 244.69 kb). The OCN008 genome has a G+C content of 45.7%, and those of individual molecules are 45.7% (Chr I), 45.3% (Chr II), and 49.7% (plasmid). The closest relative using RefSeq genomes compared with chromosome I of *Vibrio coralliilyticus* OCN008 in GenBank is *Vibrio coralliilyticus* SNUTY-1 chromosome I (97.5% nucleotide identity). The NCBI annotation revealed 4,989 proteincoding sequences, 12 55 rRNAs, 11 165 RNAs, 11 235 rRNAs, and 116 tRNAs. The contributions of this complete genome sequence assembly encourage further research into the functional roles of quorum response and virulence interaction among various vibrio and host species.

Data availability. The complete genome sequence was deposited in GenBank under the accession numbers CP048693, CP048694, and CP048695. Versions CP048693.1, CP048694.1, and CP048695.1 are described in this paper. The raw reads are available under BioProject number PRJNA605822 and Sequence Read Archive (SRA) Run Selector study accession number SRP262515.

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REFERENCES

- Richards GP, Watson MA, Needleman DS, Church KM, Häse CC. 2015. Mortalities of Eastern and Pacific oyster larvae caused by the pathogens Vibrio coralliilyticus and Vibrio tubiashii. Appl Environ Microbiol 81: 292–297. https://doi.org/10.1128/AEM.02930-14.
- Zhou Z, Zhao S, Tang J, Liu Z, Wu Y, Wang Y, Lin S. 2019. Altered immune landscape and disrupted coral-symbiodinium symbiosis in the scleractinian coral Pocillopora damicornisby Vibrio coralliilyticus challenge. Front Physiol 10:366. https://doi.org/10.3389/fphys.2019.00366.
- Ushijima B, Videau P, Burger AH, Shore-Maggio A, Runyon CM, Sudek M, Aeby GS, Callahan SM. 2014. Vibrio corallilyticus strain OCN008 is an etiological agent of acute montipora white syndrome. Appl Environ Microbiol 80:2102–2109. https://doi.org/10.1128/AEM.03463-13.
- Guillemette R, Ushijima B, Jalan M, Häse CC, Azam F. 2020. Insight into the resilience and susceptibility of marine bacteria to T6SS attack by Vibrio cholerae and Vibrio coralliilyticus. PLoS One 15:e0227864. https:// doi.org/10.1371/journal.pone.0227864.
- Ushijima B, Videau P, Aeby GS, Callahan SM. 2013. Draft genome sequence of Vibrio coralliilyticus strain OCN008, isolated from Kāne'ohe Bay, Hawai'i. Genome Announc 1:e00786-13. https://doi.org/10.1128/ genomeA.00786-13.
- Ushijima B, Häse CC. 2018. Influence of chemotaxis and swimming patterns on the virulence of the coral pathogen Vibrio corallilyticus. J Bacteriol 200:e00791-17. https://doi.org/10.1128/JB.00791-17.
- Ushijima B, Videau P, Poscablo D, Stengel JW, Beurmann S, Burger AH, Aeby GS, Callahan SM. 2016. Mutation of the toxR or mshA genes from Vibrio coralliilyticus strain OCN014 reduces infection of the coral Acropora cytherea. Environ Microbiol 18:4055–4067. https://doi.org/10.1111/ 1462-2920.13428.

- Wick RR, Judd LM, Holt KE. 2019. Performance of neural network basecalling tools for Oxford Nanopore sequencing. Genome Biol 20:129. https://doi.org/10.1186/s13059-019-1727-y.
- Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. 2017. Canu: scalable and accurate long-read assembly via adaptive κ-mer weighting and repeat separation. Genome Res 27:722–736. https://doi .org/10.1101/gr.215087.116.
- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi .org/10.1093/bioinformatics/btu170.
- Deatherage DE, Barrick JE. 2014. Identification of mutations in laboratory-evolved microbes from next-generation sequencing data using breseq. Methods Mol Biol 1151:165–188. https://doi.org/10.1007/978 -1-4939-0554-6_12.
- Li H. 2018. minimap2: pairwise alignment for nucleotide sequences. Bioinformatics 34:3094–3100. https://doi.org/10.1093/bioinformatics/ bty191.
- Tatusova T, Dicuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44: 6614–6624. https://doi.org/10.1093/nar/gkw569.
- Haft DH, DiCuccio M, Badretdin A, Brover V, Chetvernin V, O'Neill K, Li W, Chitsaz F, Derbyshire MK, Gonzales NR, Gwadz M, Lu F, Marchler GH, Song JS, Thanki N, Yamashita RA, Zheng C, Thibaud-Nissen F, Geer LY, Marchler-Bauer A, Pruitt KD. 2018. RefSeq: an update on prokaryotic genome annotation and curation. Nucleic Acids Res 46:D851–D860. https://doi.org/10.1093/nar/gkx1068.