



# Draft Genome Sequence of a Harveyi Clade Bacterium Isolated from *Lolliguncula brevis* Squid

Alecia N. Septer,<sup>a</sup>  Lauren Speare,<sup>a</sup> Collin K. Coleman,<sup>b</sup> Stephanie Smith,<sup>a</sup> Coby Dorsey,<sup>a</sup> Travis Wilson,<sup>a</sup>  Scott M. Gifford<sup>a</sup>

<sup>a</sup>Department of Marine Sciences, University of North Carolina, Chapel Hill, North Carolina, USA

<sup>b</sup>Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, North Carolina, USA

**ABSTRACT** *Vibrio* species of the Harveyi clade are commonly found in free-living and host-associated marine habitats. Here, we report the draft genome sequence for a Harveyi clade bacterium, *Vibrio* sp. strain LB10LO1, which was isolated from the Atlantic brief squid *Lolliguncula brevis*.

Bacteria within the genus *Vibrio* are widely distributed throughout marine systems where they inhabit both nutrient-rich and oligotrophic environments (1). Within the *Vibrio* genus, members of the Harveyi clade include 11 closely related species that are commonly found in surface waters, in marine sediments, and as pathogens and commensals of both vertebrates and invertebrates (2–13). Moreover, these species serve as important model organisms for studying biofilm formation, bioluminescence, and quorum sensing (14).

Here, we introduce the genome sequence of a Harveyi clade bacterium, *Vibrio* sp. strain LB10LO1, which was isolated from a wild-caught Atlantic brief squid, *Lolliguncula brevis*. In the summer of 2017, *L. brevis* squid were collected from the bycatch of a trawl off the coast of Morehead City, North Carolina. A deceased animal was immediately washed with filter-sterilized instant ocean. Tissue within the mantle cavity was removed, homogenized, and plated directly onto Luria-Bertani with added salt (LBS) agar (15). Cultivation plates were incubated at 24°C overnight, and a brightly luminescent colony was picked and restreaked for purification, resulting in strain LB10LO1. The initial phylogeny of this isolate was determined using analysis of the *hsp60* and *toxR* sequences (16, 17), which suggested that LB10LO1 is a *Vibrio campbellii* species within the Harveyi clade. Because recent studies have shown that whole-genome comparisons are the best way to confirm phylogeny among Harveyi clade members (2), we sought to sequence the genome of LB10LO1 to determine how this isolate relates to other species within the Harveyi clade.

A single LB10LO1 colony was picked and streaked onto LBS agar plates and incubated overnight at 24°C. Genomic DNA was extracted from this clonal bacterial growth with a Zymo DNA miniprep kit, and the quantity and quality were determined using an Eppendorf BioSpectrometer. Library preparation was performed using a TruSeq DNA kit (Illumina, San Diego, CA, USA), following the manufacturer's protocol. The library was sequenced using the MiSeq Illumina platform and 300-bp paired-end reads at the University of North Carolina (UNC) High-Throughput Genomic Sequencing Facility, resulting in a total of 1,798,287 paired reads. Raw reads were trimmed using Trimmomatic (18) using a 10-bp sliding window average, Phred score threshold of 20, and minimum read length of 50 nucleotides (nt). Paired reads were assembled using PEAR (default settings) (19). The remaining sequences were assembled using SPAdes (default settings) (20); sequences were annotated via the Prokka Prokaryotic Genome Annotation Pipeline, BlastKoala within the KEGG platform, and BioCyc (21).

The final draft genome of LB10LO1 is 5,515,790 bp long in 90 contigs (>1,000 bp),

**Citation** Septer AN, Speare L, Coleman CK, Smith S, Dorsey C, Wilson T, Gifford SM. 2020. Draft genome sequence of a Harveyi clade bacterium isolated from *Lolliguncula brevis* squid. *Microbiol Resour Annu* 9:e00078-20. <https://doi.org/10.1128/MRA.00078-20>.

**Editor** Frank J. Stewart, Georgia Institute of Technology

**Copyright** © 2020 Septer et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Alecia N. Septer, [asepter@email.unc.edu](mailto:asepter@email.unc.edu), or Scott M. Gifford, [sgifford@email.unc.edu](mailto:sgifford@email.unc.edu).

**Received** 27 January 2020

**Accepted** 29 January 2020

**Published** 20 February 2020

with a G+C content of 45.45%, 97-fold genome coverage, and an  $N_{50}$  score of 165,319 bp. A total of 4,995 DNA coding regions were identified, including 4,891 encoding proteins and 104 encoding RNAs. Prokka produced annotated functions for 3,127 of the proteins, while the other 1,764 proteins were assigned as hypothetical. Finally, a MiGA (22) and Genome Taxonomy Database (GTDB) (23) analysis of the LB10LO1 genome determined that the most closely related genomes in the database were *Vibrio campbellii* isolates, with an average nucleotide identity (ANI) of >96%.

**Data availability.** This genome sequence is available in GenBank under the Bio-Project number [PRJNA602499](https://ncbi.nlm.nih.gov/bioproject/PRJNA602499); the Illumina reads are available in the SRA under accession number [SRX7614634](https://ncbi.nlm.nih.gov/sra/SRX7614634). Cultures of LB10LO1 are available upon request.

### ACKNOWLEDGMENTS

This work was supported by a UNC Course-based Undergraduate Research Experience (CURE) award to S.M.G. C.D. was supported by the UNC Institute for the Environment's IDEA program (NSF number 1600506). C.K.C. was supported in part by a grant from the National Institute of Environmental Health Sciences (T32ES007018).

We thank Acacia Zhao for technical assistance and Joel Fodrie, Martin Benavides, and the crew of the R/V *Capricorn* for assistance in the field.

### REFERENCES

- Thompson JR, Polz MF. 2006. Dynamics of *Vibrio* populations and their role in environmental nutrient cycling, p 190–203. In Thompson FL, Austin B, Swings J (eds), *Biology of vibrios*. ASM Press, Washington, DC.
- Urbanczyk H, Ogura Y, Hayashi T. 2013. Taxonomic revision of Harveyi clade bacteria (family Vibrionaceae) based on analysis of whole genome sequences. *Int J Syst Evol Microbiol* 63:2742–2751. <https://doi.org/10.1099/ijs.0.051110-0>.
- Thompson JM, Polz MF. 2005. Diversity, sources, and detection of human bacterial pathogens in the marine environment, p 29–68. In Belkin S, Colwell RR (ed), *Oceans and health: pathogens in the marine environment*. Springer, New York, NY.
- Austin B, Zhang X-H. 2006. *Vibrio harveyi*: a significant pathogen of marine vertebrates and invertebrates. *Lett Appl Microbiol* 43:119–124. <https://doi.org/10.1111/j.1472-765X.2006.01989.x>.
- Guerrero-Ferreira RC, Nishiguchi MK. 2007. Biodiversity among luminescent symbionts from squid of the genera *Uroteuthis*, *Loliolus* and *Euprymna* (Mollusca: Cephalopoda). *Cladistics* 23:497–506. <https://doi.org/10.1111/j.1096-0031.2007.00155.x>.
- Liu PC, Lin JY, Chuang WH, Lee KK. 2004. Isolation and characterization of pathogenic *Vibrio harveyi* (*V. carchariae*) from the farmed marine cobia fish *Rachycentron canadum* L. with gastroenteritis syndrome. *World J Microbiol Biotechnol* 20:495–499. <https://doi.org/10.1023/B:WIBI.0000040402.44340.0e>.
- Mera H, Bourne DG. 2018. Disentangling causation: complex roles of coral-associated microorganisms in disease. *Environ Microbiol* 20: 431–449. <https://doi.org/10.1111/1462-2920.13958>.
- Chimetto LA, Brocchi M, Gondo M, Thompson CC, Gomez-Gil B, Thompson FL. 2009. Genomic diversity of vibrios associated with the Brazilian coral *Mussismilia hispida* and its sympatric zoanthids (*Palythoa caribaeorum*, *Palythoa variabilis* and *Zoanthus solanderi*). *J Appl Microbiol* 106: 1818–1826. <https://doi.org/10.1111/j.1365-2672.2009.04149.x>.
- Vezzulli L, VibrioSea Consortium, Pezzati E, Moreno M, Fabiano M, Pane L, Pruzzo C, Consortium V. 2009. Benthic ecology of *Vibrio* spp. and pathogenic *Vibrio* species in a coastal Mediterranean environment (La Spezia Gulf, Italy). *Microb Ecol* 58:808–818. <https://doi.org/10.1007/s00248-009-9542-8>.
- Jesser KJ, Noble RT. 2018. *Vibrio* ecology in the Neuse River Estuary, North Carolina, characterized by next-generation amplicon sequencing of the gene encoding heat shock protein 60 (*hsp60*). *Appl Environ Microbiol* 84:e00333-18. <https://doi.org/10.1128/AEM.00333-18>.
- Hundenborn J, Thurig S, Kommerell M, Haag H, Nolte O. 2013. Severe wound infection with *Photobacterium damsela* ssp. *damsela* and *Vibrio harveyi*, following a laceration injury in marine environment: a case report and review of the literature. *Case Rep Med* 2013:610632. <https://doi.org/10.1155/2013/610632>.
- Wilkins S, Millar M, Hemsworth S, Johnson G, Warwick S, Pizer B. 2008. *Vibrio harveyi* sepsis in a child with cancer. *Pediatr Blood Cancer* 50: 891–892. <https://doi.org/10.1002/pbc.21356>.
- Del Gigia-Aguirre L, Sánchez-Yebra-Romera W, García-Muñoz S, Rodríguez-Maresca M. 2017. First description of wound infection with *Vibrio harveyi* in Spain. *New Microbes New Infect* 19:15–16. <https://doi.org/10.1016/j.nmni.2017.05.004>.
- Ng W-L, Bassler BL. 2009. Bacterial quorum-sensing network architectures. *Annu Rev Genet* 43:197–222. <https://doi.org/10.1146/annurev-genet-102108-134304>.
- Stabb EV, Reich KA, Ruby EG. 2001. *Vibrio fischeri* genes *hvnA* and *hvnB* encode secreted NAD(+) glycohydrolases. *J Bacteriol* 183:309–317. <https://doi.org/10.1128/JB.183.1.309-317.2001>.
- Pang L, Zhang X-H, Zhong Y, Chen J, Li Y, Austin B. 2006. Identification of *Vibrio harveyi* using PCR amplification of the *toxR* gene. *Lett Appl Microbiol* 43:249–255. <https://doi.org/10.1111/j.1472-765X.2006.01962.x>.
- Goh SH, Potter S, Wood JO, Hemmingsen SM, Reynolds RP, Chow AW. 1996. HSP60 gene sequences as universal targets for microbial species identification: studies with coagulase-negative staphylococci. *J Clin Microbiol* 34:818–823. <https://doi.org/10.1128/JCM.34.4.818-823.1996>.
- 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>.
- Zhang JJ, Kobert K, Flouri T, Stamatakis A. 2014. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics* 30:614–620. <https://doi.org/10.1093/bioinformatics/btt593>.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
- Karp PD, Billington R, Caspi R, Fulcher CA, Latendresse M, Kothari A, Keseler IM, Krummenacker M, Midford PE, Ong Q, Ong WK, Paley SM, Subhraveti P. 2019. The BioCyc collection of microbial genomes and metabolic pathways. *Brief Bioinform* 20:1085–1093. <https://doi.org/10.1093/bib/bbx085>.
- Rodríguez-R LM, Gunturu S, Harvey WT, Rossello-Mora R, Tiedje JM, Cole JR, Konstantinidis KT. 2018. The Microbial Genomes Atlas (MiGA) webserver: taxonomic and gene diversity analysis of Archaea and Bacteria at the whole genome level. *Nucleic Acids Res* 46:W282–W288. <https://doi.org/10.1093/nar/gky467>.
- Chaumeil P-A, Mussig AJ, Hugenholtz P, Parks DH. 2019. GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database. *Bioinformatics*. <https://doi.org/10.1093/bioinformatics/btz848>.