





Complete Genome Sequence of Acute Hepatopancreatic Necrosis Disease-Causing Vibrio campbellii LA16-V1, Isolated from Penaeus vannamei Cultured in a Latin American Country

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ABSTRACT We report here the complete genome sequence of *Vibrio campbellii*, isolated from *Penaeus vannamei* cultured in a Latin American country. The Tn3-like transposon and *pirAB* genes were encoded on the plasmid pLA16-2. These data support the geographical variations in the virulence plasmid found among acute hepatopancreatic necrosis disease (AHPND)-causing *Vibrio* isolates from Latin America and Asia.

A cute hepatopancreatic necrosis disease (AHPND), which is caused by *Vibrio* spp. carrying a virulence plasmid encoding the *pirAB* gene, has caused significant economic losses in the shrimp-farming industry (1). Initially, *V. parahaemolyticus* was the only species known to cause this disease; later, other AHPND-causing *Vibrio* spp., such as *V. owensii* and *V. campbellii*, were also reported (2–5). Recent studies have also shown that there are geographical variations in the virulence plasmid among AHPND isolates (6). There is limited information for other AHPND-causing *Vibrio* spp., and so we present here the complete genome of *V. campbellii* LA16-V1, isolated from *Penaeus vannamei* cultured in a Latin American country.

Genomic DNA was isolated using a DNeasy blood and tissue kit (Qiagen). Sequencing was performed by Macrogen, Inc., using a hybrid approach with the PacBio RS II (Pacific Biosciences) (20-kb SMRTbell template library) and Illumina HiSeq 2000 (pairedend short-read data) platforms. The PacBio long-read data (1,787,793,760 bp, 255,476 reads) were *de novo* assembled by FALCON version 0.2.1, and the Illumina paired-end reads (1,053,525,915 bp, 10,439,662 reads) were mapped to the assembled contigs to improve the accuracy of the genome. Annotation was carried out using the NCBI's Prokaryotic Genome Annotation Pipeline (http://www.ncbi.nlm.nih.gov/books/NBK174280).

The fully assembled genome contained 6,124,368 bp consisting of two chromosomes, designated Chr I (3,579,637 bp) and Chr II (2,230,177 bp), and a total of four plasmids, designated pLA16-1 (109,793 bp), pLA16-2 (73,461 bp), pLA16-3 (67,308 bp), and pLA16-4 (63,992 bp). The two chromosomes showed similar G+C contents (45.6% and 45.4%) and percentages of coding regions (87.1% and 86.8%). Moreover, most of the predicted tRNAs (n=133), rRNA (n=37), and noncoding RNAs (n=4) were encoded on Chr I, except 15 tRNAs and 3 rRNAs on Chr II and one tRNA on pLA16-1. Overall genome similarities among LA16-V1 and other completely sequenced V. campbellii strains were assessed using the orthologous average nucleotide identity algorithm

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(7), and the results indicated 96.7% and 97.9% genome similarities to those of V. campbellii ATCC BAA-1116 (8) and V. campbellii 20130629003S01 (9), respectively.

The pirAB-containing region that comprises the homologue counterparts of the pirAB and pirAB-Tn903 composite transposons was encoded on the plasmid pLA16-2. The pirAB-containing region was almost identical (>99%) to those from the AHPNDrelated plasmids pVPE61a (10), pVA1 (11), and pVPA3-1 (1) identified in V. parahaemolyticus, plasmid pVHvo (12) in V. owensii, and plasmid pVCGX1 (8) in V. campbellii. Similar to the previous report (6), the pLA16-2 contained a 3-kb Tn3-like transposon that is absent in pVCGX1 in V. campbellii 20130629003S01 isolated from China, as well as other plasmids in V. parahaemolyticus from southeast Asia, thus supporting the geographical variations in the virulence plasmid found among AHPND-related Vibrio isolates from Latin America and Asia. The genome of V. campbellii LA16-V1 provides important insights for the study of virulence plasmids to facilitate AHPND control in shrimp aquaculture.

Accession number(s). The genome of V. campbellii strain LA16-V1 has been deposited at DDBJ/ENA/GenBank under the accession numbers CP021145 (Chr I), CP021146 (Chr II), CP021147 (pLA16-1), CP021148 (pLA16-2), CP021149 (pLA16-3), and CP021150 (pLA16-4).

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REFERENCES

- 1. Han JE, Tang KFJ, Tran LH, Lightner DV. 2015. Photorhabdus insectrelated (Pir) toxin-like genes in a plasmid of Vibrio parahaemolyticus, the causative agent of acute hepatopancreatic necrosis disease (AHPND) of shrimp. Dis Aquat Organ 113:33-40. https://doi.org/10.3354/dao02830.
- 2. Dong X, Wang H, Xie G, Zou P, Guo C, Liang Y, Huang J. 2017. An isolate of Vibrio campbellii carrying the pirVP gene causes acute hepatopancreatic necrosis disease. Emerg Microbes Infect 6:e2. https://doi.org/10 .1038/emi.2016.131.
- 3. Han JE, Tang KFJ, Aranguren LF, Piamsomboon P. 2017. Characterization and pathogenicity of acute hepatopancreatic necrosis disease natural mutants, $pirAB_{vp}$ (-) V. Parahaemolyticus, and $pirAB_{vp}$ (+) V. Campbellii strains. Aquaculture 470:84-90. https://doi.org/10.1016/j.aquaculture .2016.12.022.
- 4. Kondo H, Van PT, Dang LT, Hirono I. 2015. Draft genome sequence of non-Vibrio parahaemolyticus acute hepatopancreatic necrosis disease strain KC13.17.5, isolated from diseased shrimp in Vietnam. Genome Announc 3(5):e00978-15. https://doi.org/10.1128/genomeA.00978-15.
- 5. Liu L, Xiao J, Xia X, Pan Y, Yan S, Wang Y. 2015. Draft genome sequence of Vibrio owensii strain SH-14, which causes shrimp acute hepatopancreatic necrosis disease. Genome Announc 3(6):e01395-15. https://doi .org/10.1128/genomeA.01395-15.
- 6. Han JE, Tang KF, Lightner DV. 2015. Genotyping of virulence plasmid from Vibrio parahaemolyticus isolates causing acute hepatopancreatic necrosis disease in shrimp. Dis Aquat Organ 115:245-251. https://doi .org/10.3354/dao02906.
- 7. Lee I, Kim YO, Park SC, Chun J. 2015. OrthoANI: an improved algorithm

- and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 66:1100-1103. https://doi.org/10.1099/ijsem.0.000760.
- 8. Lin B, Wang Z, Malanoski AP, O'Grady EA, Wimpee CF, Vuddhakul V, Alves Jr N, Thompson FL, Gomez-Gil B, Vora GJ. 2010. Comparative genomic analyses identify the Vibrio harveyi genome sequenced strains BAA-1116 and HY01 as Vibrio campbellii. Environ Microbiol Rep 2:81-89. https://doi.org/10.1111/j.1758-2229.2009.00100.x.
- 9. Dong X, Wang H, Zou P, Chen J, Liu Z, Wang X, Huang J. 2017. Complete genome sequence of Vibrio campbellii strain 20130629003S01 isolated from shrimp with acute hepatopancreatic necrosis disease. Gut Pathog 9:31. https://doi.org/10.1186/s13099-017-0180-2.
- 10. Theethakaew C, Nakamura S, Motooka D, Matsuda S, Kodama T, Chonsin K, Suthienkul O, lida T. 2017. Plasmid dynamics in Vibrio parahaemolyticus strains related to shrimp acute hepatopancreatic necrosis syndrome (AHPNS). Infect Genet Evol 51:211-218. https://doi.org/10.1016/j.meegid .2017.04.007
- 11. Lee CT, Chen IT, Yang YT, Ko TP, Huang YT, Huang JY, Huang MF, Lin SJ, Chen CY, Lin SS, Lin SS, Lightner DV, Wang HC, Wang AH, Wang HC, Hor LI, Lo CF. 2015. The opportunistic marine pathogen Vibrio parahaemolyticus becomes virulent by acquiring a plasmid that expresses a deadly toxin. Proc Natl Acad Sci U S A 112:10798-10803. https://doi.org/10 .1073/pnas.1503129112.
- 12. Xiao J, Liu L, Ke Y, Li X, Liu Y, Pan Y, Yan S, Wang Y. 2017. Shrimp AHPND-causing plasmids encoding the PirAB toxins as mediated by pirAB-Tn903 are prevalent in various Vibrio species. Sci Rep 7:42177. https://doi.org/10.1038/srep42177.