

Complete Genome Sequences of a Clinical Isolate and an Environmental Isolate of *Vibrio parahaemolyticus*

Catharina H. M. Lüdeke,^{a,b} Nguyet Kong,^{c,d} Bart C. Weimer,^{c,d} Markus Fischer,^b Jessica L. Jones^a

Gulf Coast Seafood Laboratory, Division of Seafood Science and Technology, Food and Drug Administration, Dauphin Island, Alabama, USA^a; Hamburg School of Food Science, University of Hamburg, Hamburg, Germany^b; School of Veterinary Medicine^c and 100K Pathogen Genome Project,^d University of California, Davis, California, USA

***Vibrio parahaemolyticus* is the leading cause of seafood-borne infections in the United States. We report complete genome sequences for two *V. parahaemolyticus* strains isolated in 2007, CDC_K4557 and FDA_R31 of clinical and oyster origin, respectively. These two sequences might assist in the investigation of differential virulence of this organism.**

Received 13 February 2015 Accepted 18 February 2015 Published 26 March 2015

Citation Lüdeke CHM, Kong N, Weimer BC, Fischer M, Jones JL. 2015. Complete genome sequences of a clinical isolate and an environmental isolate of *Vibrio parahaemolyticus*. *Genome Announc* 3(2):e00216-15. doi:10.1128/genomeA.00216-15.

Copyright © 2015 Lüdeke et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](https://creativecommons.org/licenses/by/3.0/).

Address correspondence to Jessica L. Jones, jessica.jones@fda.hhs.gov.

Vibrio parahaemolyticus is a halophilic Gram-negative bacterium naturally occurring in estuarine environments (1). Through consumption of raw or undercooked seafood, or contact with contaminated seawater, *V. parahaemolyticus* can cause infections in humans; gastroenteritis is typical, but rarely, wound and/or sepsis infections occur. *V. parahaemolyticus* is the leading cause of seafood-borne gastroenteritis in the United States (2), and there has been an increase in reported illnesses in the last two decades (3).

V. parahaemolyticus can carry the thermostable direct hemolysin (*tdh*) and *tdh*-related hemolysin (*trh*) genes, which are generally associated with pathogenicity and are used in outbreak investigations and assessing risk (4, 5). Clinical isolates more frequently carry the *tdh* and/or *trh* genes than environmental isolates (6). However, in recent studies, clinical isolates lacking both of these genes have been identified (7). Tissue culture studies have revealed that the presence of *tdh* had no effect on cytotoxicity (8, 9). Hence, additional virulence factors likely exist for *V. parahaemolyticus*.

We sequenced two *V. parahaemolyticus* isolates, CDC_K4557 and FDA_R31, to better understand the pathogenic potential of these isolates and eventually improve risk assessment. CDC_K4557 was isolated from the stool of a patient in Louisiana in 2007 and submitted to the Centers for Disease Control and Prevention (CDC). FDA_R31 was isolated by the Food and Drug Administration (FDA) from an oyster sample harvested in Louisiana in 2007. As the clinical isolate is *tdh*⁻ *trh*⁻ and the oyster isolate is *tdh*⁺ *trh*⁺ by PCR, these strains are ideal for identifying new and/or additional virulence markers.

The genomes were sequenced within the University of California at Davis 100K Pathogen Genome Project using the PacBio RSII sequencing platform (Pacific Biomarkers, Menlo Park, CA, USA). High-molecular-weight gDNA was extracted from overnight cultures grown on Trypticase soy agar, lysed with an enzyme cocktail, purified with the QIAamp DNA minikit (Qiagen, Valencia, CA, USA), and analyzed on a 2200 TapeStation system with the Genomic DNA ScreenTape (Agilent Technologies, Santa Clara,

CA, USA) assay for integrity of high molecular weight gDNA (10). After evaluation of gDNA size and quantity, 10 μg was used for fragmentation using the Covaris g-TUBE device (Covaris, Woburn, MA, USA) following the manufacturer's instructions (11). The fragmented gDNA was used for library construction with the PacBio SMRTbell 10kb Library preparation kit, which was normalized to 1 to 5 μg input. Libraries were sequenced utilizing PacBio RSII and C2 chemistry with 100× coverage per the manufacturer's instructions. For each isolate, the genomic sequence single-pass reads were *de novo* assembled using the Hierarchical Genome Assembly Process (HGAP) version 1.4 software (Pacific Biosciences) and were then annotated using the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (http://www.ncbi.nlm.nih.gov/genome/annotation_prok) (12). Through the annotation process, 4,771 and 4,937 genes for the clinical and oyster isolates, respectively, as well as 4,579 and 4,731 coding regions were identified. The presence or absence of the *tdh* and *trh* genes was confirmed in both isolates.

Nucleotide sequence accession numbers. The closed genome sequences of the two *V. parahaemolyticus* isolates are available in GenBank under the accession numbers CP006004 and CP006005 for chromosomes I and II of FDA_R31, respectively, and CP006008 and CP006007 for CDC_K4557. The versions described in this paper are the first versions.

ACKNOWLEDGMENTS

This project was supported by an appointment to the Research Fellowship Program for the Center for Food Safety and Applied Nutrition administered by the Oak Ridge Associated Universities through a contract with the FDA. The 100K Pathogen Genome Project was supported by the FDA and Agilent Technologies to produce these sequences.

We thank Whitney Ng and Kao Thao for their effort in the isolate logistics.

REFERENCES

1. Kueh CS, Chan KY. 1985. Bacteria in bivalve shellfish with special reference to the oyster. *J Appl Bacteriol* 59:41–47. <http://dx.doi.org/10.1111/j.1365-2672.1985.tb01773.x>.

2. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM. 2011. Foodborne illness acquired in the United States—major pathogens. *Emerg Infect Dis* 17:7–15. http://www.ncbi.nlm.nih.gov/eid/article/17/1/p1-1101_article.
3. Newton A, Kendall M, Vugia DJ, Henao OL, Mahon BE. 2012. Increasing rates of vibriosis in the United States, 1996–2010: review of surveillance data from 2 systems. *Clin Infect Dis* 54(Suppl 5):S391–S395. <http://dx.doi.org/10.1093/cid/cis243>.
4. Broberg CA, Calder TJ, Orth K. 2011. *Vibrio parahaemolyticus* cell biology and pathogenicity determinants. *Microbes Infect* 13:992–1001. <http://dx.doi.org/10.1016/j.micinf.2011.06.013>.
5. Depaola A, Jones JL, Woods J, Burkhardt W III, Calci KR, Krantz JA, Bowers JC, Kasturi K, Byars RH, Jacobs E, Williams-Hill D, Nabe K. 2010. Bacterial and viral pathogens in live oysters: 2007 United States market survey. *Appl Environ Microbiol* 76:2754–2768. <http://dx.doi.org/10.1128/AEM.02590-09>.
6. Depaola A, Kaysner CA, Bowers J, Cook DW. 2000. Environmental investigations of *Vibrio parahaemolyticus* in oysters after outbreaks in Washington, Texas, and New York (1997 and 1998). *Appl Environ Microbiol* 66:4649–4654.
7. Jones JL, Lüdeke CHM, Bowers JC, Garrett N, Fischer M, Parsons MB, Bopp CA, DePaola A. 2012. Biochemical, serological, and virulence characterization of clinical and oyster *Vibrio parahaemolyticus* isolates. *J Clin Microbiol* 50:2343–2352. <http://dx.doi.org/10.1128/JCM.00196-12>.
8. Lynch T, Livingstone S, Buenaventura E, Lutter E, Fedwick J, Buret AG, Graham D, DeVinney R. 2005. *Vibrio parahaemolyticus* disruption of epithelial cell tight junctions occurs independently of toxin production. *Infect Immun* 73:1275–1283. <http://dx.doi.org/10.1128/IAI.73.3.1275-1283.2005>.
9. Park KS, Ono T, Rokuda M, Jang MH, Iida T, Honda T. 2004. Cytotoxicity and enterotoxicity of the thermostable direct hemolysin-deletion mutants of *Vibrio parahaemolyticus*. *Microbiol Immunol* 48:313–318. <http://dx.doi.org/10.1111/j.1348-0421.2004.tb03512.x>.
10. Jeannotte RE, Kong N, Ng W, Weimer BC. High-throughput analysis of foodborne bacterial genomic DNA using Agilent 2200 TapeStation and Genomic DNA ScreenTape system. Agilent Technologies, Santa Clara, CA. <http://www.chem.agilent.com/Library/applications/5991-4003EN.pdf>. Accessed 11 February 2015.
11. Kong N, Thao K, Ng W, Kim KS, Korlach J, Hickey L, Kelly L, Lappin S, Weimer BC. Automation of PacBio SMRTbell 10 kb template preparation on an Agilent NGS Workstation. Agilent Technologies, Santa Clara, CA. <http://www.chem.agilent.com/Library/applications/5991-4482EN.pdf>. Accessed 11 February 2015.
12. Klimke W, Agarwala R, Badretdin A, Chetvernin S, Ciufu S, Fedorov B, Kiryutin B, O'Neill K, Resch W, Resenchuk S, Schafer S, Tolstoy I, Tatusova T. 2009. The National Center for Biotechnology Information's Protein Clusters Database. *Nucleic Acids Res* 37:D216–D223. <http://dx.doi.org/10.1093/nar/gkn734>.