

Draft Genome Sequence of *Shewanella* sp. Strain CP20

Carla Lutz,^{a,*} Qi Xiang Martin Tay,^b Shuyang Sun,^{a,b} Diane McDougald^{a,b,c,d}

Centre for Marine Bio-Innovation, School of Biotechnology and Biomolecular Science, University of New South Wales, Sydney, Australia^a; Singapore Centre on Environmental Life Sciences Engineering, Nanyang Technological University, Singapore^b; School of Biological Sciences, Nanyang Technological University, Singapore^c; ithree Institute, University of Technology, Sydney, Sydney, Australia^d

* Present address: Carla Lutz, Singapore Centre on Environmental Life Sciences Engineering, Nanyang Technological University, Singapore.

***Shewanella* sp. CP20 is a marine bacterium that survives ingestion by *Tetrahymena pyriformis* and is expelled from the protozoan within membrane-bound vacuoles, where the bacterial cells show long-term survival. Here, we report the draft genome sequence of *Shewanella* sp. CP20 and discuss the potential mechanisms facilitating intraprotozoan survival.**

Received 20 February 2015 Accepted 23 February 2015 Published 9 April 2015

Citation Lutz C, Martin Tay QX, Sun S, McDougald D. 2015. Draft genome sequence of *Shewanella* sp. strain CP20. *Genome Announc* 3(2):e00256-15. doi:10.1128/genomeA.00256-15.

Copyright © 2015 Lutz 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 Diane McDougald, diane.mcdougald@uts.edu.au.

Shewanella spp. are Gram-negative aquatic bacteria, ranging in length from 2 to 3 μm , and are motile by a single polar flagellum (1). *Shewanella* sp. CP20 was isolated from coastal waters off the coast of Clovelly, Australia (33.9121°S, 151.2629°E). *Shewanella* sp. CP20 survives digestion by the bacterivorous ciliate *Tetrahymena pyriformis*. The bacterial cells that are packaged within food vacuoles are eventually expelled into the external milieu without being digested. The *Gammaproteobacteria*, to which *Shewanella* sp. CP20 belongs, contains many bacterial species that can survive intracellularly within a range of protozoa, e.g., *Salmonella* spp. (2), *Yersinia* spp. (3), *Vibrio* spp. (4), and *Legionella* spp. (5). The mechanisms that facilitate survival in the expelled vacuoles are not fully understood; therefore, it is important to investigate the genome of this isolate to gain insight into the genes that potentially facilitate this interaction.

The genome of *Shewanella* sp. CP20 was sequenced on the Illumina HiSeq 2000 platform at the Singapore Centre on Environmental Life Sciences Engineering (SCELSE), Nanyang Technological University, Singapore. A total of 1,502,730 paired-end reads (150 bp in length) were assembled into a draft genome consisting of 41 contigs with the use of Velvet sequence assembly software, version 1.2.10 (EMBL-EBI). The N_{50} score of the assembly was 743,057, with the length of the longest contig being 1,147,437 bp. The contigs were then submitted to the Rapid Annotation using Subsystem Technology server (RAST) (6). Initial prediction of open reading frames (ORFs) was performed by GLIMMER2, which provides identification of sequences universal in bacteria (7). Hierarchical clustering and analysis of the clusters of orthologous groups (COGS) with other members of the *Shewanella* genus available in the RAST database indicated that *Shewanella* sp. CP20 is most closely related to *Shewanella loihica* PV-4 (score 538) and *Shewanella piezotolerans* WP3 (score 506).

The *Shewanella* sp. CP20 genome is a circular chromosome of 4,475,402 bp with a total of 3,798 predicted coding sequences (CDSs). There was no evidence for a plasmid; however, phage DNA synthesis genes were present. The majority of the CDSs encoded genes for amino acid synthesis and degradation (354

genes), carbohydrate transport (276 genes), and the synthesis of cofactors, vitamins, prosthetic groups, and pigments (261 genes). There were 1,794 CDSs that were not assigned to a functional COG, with the majority of these being hypothetical proteins.

The annotation of the *Shewanella* sp. CP20 genome also identified genes associated with establishing and maintaining intraprotozoan survival. These properties included phospholipid metabolism, secretion systems for the translocation of effector proteins into the host, metal homeostasis, oxidative stress, and acid stress tolerance. The genome information obtained from *Shewanella* sp. CP20 will be an important addition to the understanding of intraprotozoan survival of bacteria in the marine environment.

Nucleotide sequence accession number. The genome sequence of *Shewanella* sp. CP20 has been deposited in the GenBank database under the accession number [JPII00000000](https://www.ncbi.nlm.nih.gov/nuccore/JPII00000000).

ACKNOWLEDGMENTS

This work was supported by SCELSE and the Australian Research Council.

We thank Stephan Schuster for helpful discussions regarding genome analysis.

REFERENCES

- Venkateswaran K, Moser DP, Dollhopf ME, Lies DP, Saffarini DA, MacGregor BJ, Ringelberg DB, White DC, Nishijima M, Sano H, Burghardt J, Stackebrandt E, Neelson KH. 1999. Polyphasic taxonomy of the genus *Shewanella* and description of *Shewanella oneidensis* sp. nov. *Int J Syst Bacteriol* 49:705–724. <http://dx.doi.org/10.1099/00207713-49-2-705>.
- Gaze WH, Burroughs N, Gallagher MP, Wellington EM. 2003. Interactions between *Salmonella* Typhimurium and *Acanthamoeba polyphaga*, and observation of a new mode of intracellular growth within contractile vacuoles. *Microb Ecol* 46:358–369. <http://dx.doi.org/10.1007/s00248-003-1001-3>.
- Ly TM, Müller HE. 1990. Ingested *Listeria monocytogenes* survive and multiply in protozoa. *J Med Microbiol* 33:51–54. <http://dx.doi.org/10.1099/00222615-33-1-51>.
- Abd H, Weintraub A, Sandström G. 2005. Intracellular survival and

- replication of *Vibrio cholerae* O139 in aquatic free-living amoebae. *Environ Microbiol* 7:1003–1008. <http://dx.doi.org/10.1111/j.1462-2920.2005.00771.x>.
5. Fields BS, Shotts EB, Feeley JC, Gorman GW, Martin WT. 1984. Proliferation of *Legionella pneumophila* as an intracellular parasite of the ciliated protozoan *Tetrahymena pyriformis*. *Appl Environ Microbiol* 47:467–471.
 6. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD. 2008. The RAST server: Rapid Annotations using Subsystems Technology. *BMC Genomics* 9:75. <http://dx.doi.org/10.1186/1471-2164-9-75>.
 7. Delcher AL, Bratke KA, Powers EC, Salzberg SL. 2007. Identifying bacterial genes and endosymbiont DNA with glimmer. *Bioinformatics* 23: 673–679. <http://dx.doi.org/10.1093/bioinformatics/btm009>.