



Complete Genome Sequence of *Sulfitobacter* sp. Strain D7, a Virulent Bacterium Isolated from an *Emiliana huxleyi* Algal Bloom in the North Atlantic

 Chuan Ku,^a  Noa Barak-Gavish,^a Mark Maienschein-Cline,^b Stefan J. Green,^c  Assaf Vardi^a

^aDepartment of Plant and Environmental Sciences, Weizmann Institute of Science, Rehovot, Israel

^bResearch Informatics Core, Research Resources Center, University of Illinois at Chicago, Chicago, Illinois, USA

^cSequencing Core, Research Resources Center, University of Illinois at Chicago, Chicago, Illinois, USA

ABSTRACT A *Rhodobacterales* bacterium, *Sulfitobacter* sp. strain D7, was isolated from an *Emiliana huxleyi* bloom in the North Atlantic and has been shown to act as a pathogen and induce cell death of *E. huxleyi* during lab coculturing. We report here its complete genome sequence comprising one chromosome and five low-copy-number plasmids.

The coccolithophore *Emiliana huxleyi* (Haptophyta) is a cosmopolitan marine microalga that plays an important role in global carbon and sulfur cycling by forming massive blooms and producing copious amounts of dimethylsulfoniopropionate (DMSP) and the atmospherically active compound dimethyl sulfide (1). Here, we report the complete genome sequence of *Sulfitobacter* sp. strain D7, a *Rhodobacterales* bacterium that, when cocultured with *E. huxleyi*, causes death of the alga in a DMSP-dependent manner (2). It was isolated from the microbiome of copepods collected during a natural *E. huxleyi* bloom in the North Atlantic, and its cooccurrence with the alga in the water column was confirmed by quantitative PCR (qPCR) (2).

Genomic DNA was extracted from a culture grown overnight in 1/2 yeast extract-tryptone-Sigma sea salts (YTSS) medium at 28°C under agitation (150 rpm) using the DNeasy blood and tissue kit (Qiagen, Hilden, Germany). A library with an insert size of ~500 bp was prepared using the Nextera XT kit (Illumina, San Diego, CA) and sequenced using the Illumina NextSeq 500 platform to generate 150-bp paired-end reads. A library of fragments longer than 10 kb was prepared following the PacBio (Menlo Park, CA) protocol, loaded with MagBeads, and sequenced on the RS II platform. Hybrid assembly of both PacBio (N_{50} , 10,282 bp) and Illumina reads was performed using the software package SPAdes v. 3.11 (3) with “31,51,71,91” for k-mers. After filtering for contig size (>500 bp) and Illumina read coverage (at least one-third of the coverage level at half the total assembly length) based on Bowtie2 v. 2.3.0 (4) mapping, six contigs longer than 80 kb were retained from the initial draft. Sequence errors, misassemblies, and gaps were corrected by mapping Illumina reads to the contigs with the Burrows-Wheeler Aligner (BWA) v. 0.7.12 (5) and visual inspection in Integrative Genomics Viewer (IGV) v. 2.3.90 (6), as in a previous study (7). The assembly was also checked by mapping the PacBio reads using Basic Local Alignment with Successive Refinement (BLASR) v. 5.3 (8) with the default settings for RS II reads. Completion and circularization of each contig were confirmed by Illumina read pairs and PacBio reads that joined the two ends. The NCBI Prokaryotic Genome Annotation Pipeline v. 4.1 (9) was used for gene predictions and annotations.

The complete genome sequence consists of six circular replicons (Table 1), including one 3,371,091-bp chromosome with an average GC content of 61.4% and five plasmids of between 81 and 193 kb that show low or single copy numbers characteristic of

Received 7 October 2018 Accepted 22 October 2018 Published 15 November 2018

Citation Ku C, Barak-Gavish N, Maienschein-Cline M, Green SJ, Vardi A. 2018. Complete genome sequence of *Sulfitobacter* sp. strain D7, a virulent bacterium isolated from an *Emiliana huxleyi* algal bloom in the North Atlantic. *Microbiol Resour Announc* 7:e01379-18. <https://doi.org/10.1128/MRA.01379-18>.

Editor J. Cameron Thrash, Louisiana State University

Copyright © 2018 Ku 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 Assaf Vardi, assaf.vardi@weizmann.ac.il.

TABLE 1 Features and accession numbers of the *Sulfitobacter* sp. D7 genome sequence

Replicon	GenBank accession no.	Size (bp)	GC content (%)	Illumina read coverage (fold)
Chromosome	CP020694	3,371,091	61.4	918
p1SUD7	CP020695	193,276	62.3	585
p2SUD7	CP020696	126,656	58.5	382
p3SUD7	CP020697	104,922	59.9	351
p4SUD7	CP020698	85,454	57.5	778
p5SUD7	CP020699	81,709	58.9	405

Rhodobacterales and other alphaproteobacteria (10, 11). In total, there are 4 complete rRNA operons, 47 tRNAs, 3,744 protein-coding genes, and 52 pseudogenes, with all RNA genes located on the chromosome. Consistent with observation of the production of methanethiol, a metabolic by-product of DMSP, during coculturing with *E. huxleyi*, the chromosome encoded *dmdA* for demethylation of DMSP (2). In addition, we identified type I and type II secretion system genes on the chromosome and type IV genes on the plasmids p2SUD7 and p3SUD7, which may be involved in the bacterium's interactions with the alga.

Data availability. The complete genome sequence of *Sulfitobacter* sp. strain D7 has been deposited in GenBank under the accession numbers [CP020694](#) to [CP020699](#). Raw Illumina and PacBio RS II sequence reads have been deposited in the NCBI Sequence Read Archive under the accession numbers [SRR7948486](#) and [SRR7948487](#), respectively.

ACKNOWLEDGMENTS

This work was supported by the European Research Council (ERC) CoG (VIROCELL-SPHERE grant number 681715) to Assaf Vardi. Chuan Ku was supported by an EMBO long-term fellowship (ALTF 1172-2016), and Noa Barak-Gavish was supported by a JNF fellowship for environmental studies from the Rieger Foundation.

Illumina library preparation was performed at the University of Illinois at Chicago Sequencing Core (UICSQC). PacBio sequencing was performed at the Great Lakes Genomics Center at the University of Wisconsin—Milwaukee. Genome assembly was performed at the Research Informatics Core (RIC) at the University of Illinois at Chicago.

REFERENCES

- Holligan PM, Fernández E, Aiken J, Balch WM, Boyd P, Burkill PH, Finch M, Groom SB, Malin G, Muller K, Purdie DA, Robinson C, Trees CC, Turner SM, van der Wal P. 1993. A biogeochemical study of the coccolithophore, *Emiliania huxleyi*, in the North Atlantic. *Global Biogeochem Cycles* 7:879–900. <https://doi.org/10.1029/93GB01731>.
- Barak-Gavish N, Frada MJ, Ku C, Lee PA, DiTullio GR, Malitsky S, Aharoni A, Green SJ, Rotkopf R, Kartvelishvily E, Sheyn U, Schatz D, Vardi A. 2018. Bacterial virulence against an oceanic bloom-forming phytoplankton is mediated by algal DMSP. *Sci Adv* 4:eau5716. <https://doi.org/10.1126/sciadv.aau5716>.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski 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>.
- Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. *Nat Methods* 9:357–359. <https://doi.org/10.1038/nmeth.1923>.
- Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler Transform. *Bioinformatics* 25:1754–1760. <https://doi.org/10.1093/bioinformatics/btp324>.
- Thorvaldsdóttir H, Robinson JT, Mesirov JP. 2013. Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. *Brief Bioinform* 14:178–192. <https://doi.org/10.1093/bib/bbs017>.
- Ku C, Lo W-S, Chen L-L, Kuo C-H. 2014. Complete genome sequence of *Spiroplasma apis* B31T (ATCC 33834), a bacterium associated with May disease of honeybees (*Apis mellifera*). *Genome Announc* 2:e01151-13.
- Chaisson MJ, Tesler G. 2012. Mapping single molecule sequencing reads using Basic Local Alignment with Successive Refinement (BLASR): application and theory. *BMC Bioinformatics* 13:238. <https://doi.org/10.1186/1471-2105-13-238>.
- 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>.
- Petersen J, Brinkmann H, Berger M, Brinkhoff T, Päuker O, Pradella S. 2011. Origin and evolution of a novel DnaA-like plasmid replication type in *Rhodobacterales*. *Mol Biol Evol* 28:1229–1240. <https://doi.org/10.1093/molbev/msq310>.
- Pinto UM, Pappas KM, Winans SC. 2012. The ABCs of plasmid replication and segregation. *Nat Rev Microbiol* 10:755–765. <https://doi.org/10.1038/nrmicro2882>.