



Metagenome-Assembled Genome Sequence of *Kapabacteriales* Bacterium Strain Clear-D13, Assembled from a Harmful Algal Bloom Enrichment Culture

Shaikha Al-Saud,^a Kyra M. Florea,^a  Eric A. Webb,^a  J. Cameron Thrash^a

^aDepartment of Biological Sciences, University of Southern California, Los Angeles, California, USA

ABSTRACT Metagenomic sequencing of a *Dolichospermum circinale* enrichment culture resulted in the assembly of several cocultured metagenome-assembled genomes (MAGs). One MAG was affiliated with the class *Kapabacteriales* and included 5,724,991 bp in 127 contigs with a GC content of 48.4%.

Cyanobacterial harmful algal blooms (cyanoHABs) are a significant environmental threat in freshwater environments globally (1), and *Dolichospermum* is a prominent cyanoHAB genus (2), prompting interest in the organisms that constitute these blooms. Metagenomic sequencing and assembly of a *Dolichospermum* enrichment culture yielded a metagenome-assembled genome (MAG) from a cooccurring organism belonging to the class *Kapabacteriales*. We present the details of the sequencing and reconstruction of this MAG to aid in understanding organisms that coexist and potentially interact in cyanoHABs.

We hand-isolated *Dolichospermum* colonies from samples collected via surface tow at Clear Lake, CA (lat 38.973166, long 122.72809), in August 2019. Clear Lake is included on the California 303(d) list of impaired waters because of nutrients and experiences frequent cyanoHABs, including those formed by the cyanobacterial genus *Dolichospermum* (3). We identified *Dolichospermum* colonies morphologically (4) using an Axiostar epifluorescence microscope (Zeiss, Oberkochen, Germany). Multiple colonies were cultured in 50% BG-11₀ medium at 25°C and 100 μmol Q/m²/s on a 12:12-h light/dark cycle for roughly 7 months. We maintained growth by adding medium every 2 weeks. BG-11₀ medium lacks a nitrogen source in order to selectively grow diazotrophs. To isolate DNA, we filtered 50 ml of a single enrichment culture onto 25-mm-diameter 8-μm polycarbonate filters. The collected solids were then rinsed into 2-ml bead-beating tubes using the lysing solution from the DNeasy PowerBiofilm kit (Qiagen, Hilden, Germany). We subjected the tubes to five freeze-thaw cycles in liquid nitrogen, followed by an overnight incubation in a proteinase K solution (25 μl of a 25-mg/ml stock solution) at 55°C to induce cell lysing. Genomic DNA was then extracted via the Qiagen PowerBiofilm kit following the manufacturer's instructions. DNA was quantified with NanoDrop UV-visible (UV-Vis) spectroscopy and Qubit spectrofluorometry (Thermo Fisher Scientific, Waltham, MA). Illumina 150-bp paired-end (PE) sequencing (1 Gbp) with 300-bp inserts was performed by Novogene (Nanjing, China) after library preparation with a NEBNext DNA library preparation kit according to the manufacturer's recommendations. This resulted in 19,844,532 reads. We performed quality control with FastQC v0.11.5 (5) and Trimmomatic v0.36 (6), assembly with metaSPAdes v3.13.0 (7), and binning with MaxBin v2.2.4 (8) on KBase (9). The genome was annotated via PGAP (10). We completed taxonomic identification with GTDB-tk v1.1.1, run with "classify_wf" using the release 95 database. Functional annotation was estimated using FuncSanity as part of the MetaSanity v1.2 wrapper (11). Default settings were used for all software unless otherwise noted.

Clear-D13 had 5,724,991 bp in 127 contigs, an N_{50} value of 90,289 bp, and a GC

Citation Al-Saud S, Florea KM, Webb EA, Thrash JC. 2020. Metagenome-assembled genome sequence of *Kapabacteriales* bacterium strain Clear-D13, assembled from a harmful algal bloom enrichment culture. *Microbiol Resour Announc* 9:e01118-20. <https://doi.org/10.1128/MRA.01118-20>.

Editor Frank J. Stewart, Georgia Institute of Technology

Copyright © 2020 Al-Saud 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 J. Cameron Thrash, thrash@usc.edu.

Received 24 September 2020

Accepted 24 September 2020

Published 3 December 2020

content of 48.4%. CheckM v1.0.18 (12) estimated the MAG as 96.2% complete with 2% contamination. The genome is predicted to contain 4,434 genes. GTDB-tk identified Clear-D13 as belonging to the class *Kapabacteriales* (formerly *Ignavibacteria*), in the provisional genus PH2015. Genome analysis revealed that this strain contains a cluster for sulfur assimilation and metabolism, including sulfite dehydrogenase and sulfide oxidation, which indicates putative chemolithotrophic capabilities. Since no carbon fixation pathways were observed, this organism is likely heterotrophic. Transporters for cobalt (*corA*), copper (*copA*), ferrous iron (*feoB*), Fe-Mn (*mntH*), phosphate (*pst*), phosphonate (*phn*), and ammonia (*amt*) were also identified. The presence of the glyoxylate shunt pathway indicates that this MAG yields the potential to use small carbon sources like acetate.

Data availability. This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number [JACVZY000000000](https://www.ncbi.nlm.nih.gov/assembly/JACVZY000000000/). The version described in this paper is version [JACVZY010000000](https://www.ncbi.nlm.nih.gov/assembly/JACVZY010000000/). The BioProject number is [PRJNA657201](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA657201/), and the reads are available in the SRA under the accession number [SRX8961729](https://www.ncbi.nlm.nih.gov/sra/SRX8961729/).

ACKNOWLEDGMENTS

This work was funded by the University of Southern California and was part of the laboratory component of BISC419, Environmental Microbiology.

We thank Elaina Graham, Ben Tully, and John F. Heidelberg for assistance with the data analysis.

S.A., K.M.F., E.A.W., and J.C.T. wrote the paper, and K.M.F. and E.A.W. are the sources of the cultures.

REFERENCES

- Hudnell HK (ed). 2008. Cyanobacterial harmful algal blooms: state of the science and research needs. Springer, New York, NY.
- Li X, Dreher TW, Li R. 2016. An overview of diversity, occurrence, genetics and toxin production of bloom-forming *Dolichospermum* (*Anabaena*) species. *Harmful Algae* 54:54–68. <https://doi.org/10.1016/j.hal.2015.10.015>.
- State Water Resources Control Board. 2018. Final 2014/2016 California integrated report (Clean Water Act Section 303(d) list/305(b) report). California Water Boards, Sacramento, CA. https://www.waterboards.ca.gov/water_issues/programs/tmdl/integrated2014_2016.shtml.
- Komárek J, Zapomilová E. 2008. Planktic morphospecies of the cyanobacterial genus *Anabaena* = subg. *Dolichospermum*, 2. part: straight types. *Fottea* 8:1–14. <https://doi.org/10.5507/fot.2008.001>.
- Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. Babraham Bioinformatics, Cambridge, United Kingdom.
- 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>.
- Nurk S, Meleshko D, Korobeynikov A, Pevzner PA. 2017. MetaSPAdes: a new versatile metagenomic assembler. *Genome Res* 27:824–834. <https://doi.org/10.1101/gr.213959.116>.
- Wu Y-W, Simmons BA, Singer SW. 2016. MaxBin 2.0: an automated binning algorithm to recover genomes from multiple metagenomic datasets. *Bioinformatics* 32:605–607. <https://doi.org/10.1093/bioinformatics/btv638>.
- Arkin AP, Cottingham RW, Henry CS, Harris NL, Stevens RL, Maslov S, Dehal P, Ware D, Perez F, Canon S, Sneddon MW, Henderson ML, Riehl WJ, Murphy-Olson D, Chan SY, Kamimura RT, Kumari S, Drake MM, Brettin TS, Glass EM, Chivian D, Gunter D, Weston DJ, Allen BH, Baumohl J, Best AA, Bowen B, Brenner SE, Bun CC, Chandonia JM, Chia JM, Colasanti R, Conrad N, Davis JJ, Davison BH, Dejongh M, Devoid S, Dietrich E, Dubchak I, Edirisinghe JN, Fang G, Faria JP, Frybarger PM, Gerlach W, Gerstein M, Greiner A, Gurtowski J, Haun HL, He F, Jain R, Joachimiak MP, Keegan KP, Kondo S, Kumar V, Land ML, Meyer F, Mills M, Novichkov PS, Oh T, Olsen GJ, Olson R, Parrello B, Pasternak S, Pearson E, Poon SS, Price GA, Ramakrishnan S, Ranjan P, Ronald PC, Schatz MC, Seaver SMD, Shukla M, Sutormin RA, Syed MH, Thomason J, Tintle NL, Wang D, Xia F, Yoo H, Yoo S, Yu D. 2018. KBase: the United States Department of Energy Systems Biology Knowledgebase. *Nat Biotechnol* 36:566–569. <https://doi.org/10.1038/nbt.4163>.
- 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>.
- Neely CJ, Graham ED, Tully BJ. 2020. MetaSanity: an integrated microbial genome evaluation and annotation pipeline. *Bioinformatics* 36:4341–4344. <https://doi.org/10.1093/bioinformatics/btaa512>.
- Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 25:1043–1055. <https://doi.org/10.1101/gr.186072.114>.