AMERICAN SOCIETY FOR MICROBIOLOGY | genomeAnnouncements™

PROKARYOTES

Draft Genome Sequence of *Cyanobacterium* sp. Strain HL-69, Isolated from a Benthic Microbial Mat from a Magnesium Sulfate-Dominated Hypersaline Lake

J. M. Mobberley,[a]* M. F. Romine,[a] J. K. Cole,[a] Y. Maezato,[a]* S. R. Lindemann,[a]* W. C. Nelson[a]

[a]Biological Sciences Division, Pacific Northwest National Laboratory, Richland, Washington, USA

ABSTRACT The complete genome sequence of *Cyanobacterium* sp. strain HL-69 consists of 3,155,247 bp and contains 2,897 predicted genes comprising a chromosome and two plasmids. The genome is consistent with a halophilic nondiazotrophic phototrophic lifestyle, and this organism is able to synthesize most B vitamins and produces several secondary metabolites.

Cyanobacteria in phototrophic microbial mats are responsible for most of the primary production, provide fixed nitrogen and sulfur to the community, and contribute to the structural integrity of the mat (1, 2). We present here the complete genome sequence of the coccoid unicellular *Cyanobacterium* sp. strain HL-69 (CHL-69), which was derived from a microbial mat from the magnesium sulfate-dominated hypersaline Hot Lake in northern Washington (3, 4). CHL-69 was isolated from a Hot Lake mat enrichment culture by streaking until axenic on Hot Lake autotroph (HLA) medium, which is BG-11 amended to mimic Hot Lake water (5).

CHL-69 genomic DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) protocol (5) and was sequenced by the Department of Horticulture Genomics Lab at Washington State University in Pullman, WA, USA, on a PacBio RS II platform, which generated 60,773 reads with a mean length of 5,905 nucleotides (nt). *De novo* assembly with Hierarchical Genome Assembly Process (HGAP) (SMRT portal version 2.2.0) (Pacific Biosciences) (6) yielded 5 unique contigs. Gaps and sequence errors were resolved using assembled shotgun metagenome data (Illumina HiSeq) from the enrichment culture (https://github.com/jenmobberley/CyanobacteriumHL69). Gene prediction was performed with Prodigal (7) and through the Rapid Annotations using Subsystems Technology (RAST) server (8), and rRNAs and tRNAs were identified with Rfam (9). Genes were assigned functional annotation by use of information from the RAST server (8), BlastKOALA (10), and TIGRFAMs (11).

The genome of CHL-69 consists of a circular chromosome (3,155,247 bp) with an average G+C content of 37.8% and two plasmids, pCHL69-1 (86,432 bp) and pCHL69-2 (55,266 bp), with average G+C contents of 34.1% and 35.32%, respectively. Sequence analysis revealed 3,039 coding sequences, 9 rRNAs, and 44 tRNAs. The chromosome contained a putative prophage as well as a clustered regularly interspaced short palindromic repeat (CRISPR)-*cas* subtype I-D system. Each plasmid contained *parA* and toxin-antitoxin genes, which suggests that the plasmids are maintained at a low copy number. Average nucleotide identity (ANI) calculations showed that the HL-69 genome was 95.8% identical to that of the freshwater isolate *Cyanobacterium* sp. strain IPPAS B-1200 (3,410,249 bp) (GenBank accession no. LWHC00000000) (12) and 82.75% identical to that of the soda lake isolate *Cyanobacterium stanieri* PCC 7202 (3,163,381 bp) (GenBank accession no. CP003940) (13).

The nutritional dependencies of *Cyanobacterium* sp. HL-69 were revealed through metabolic reconstruction. HL-69 contains nitrate assimilation genes but lacks nitroge-

Received 3 January 2018 **Accepted** 9 January 2018 **Published** 8 February 2018

Citation Mobberley JM, Romine MF, Cole JK, Maezato Y, Lindemann SR, Nelson WC. 2018. Draft genome sequence of *Cyanobacterium* sp. strain HL-69, isolated from a benthic microbial mat from a magnesium sulfate-dominated hypersaline lake. Genome Announc 6:e01583-17. https://doi.org/10.1128/genomeA.01583-17.

Address correspondence to W. C. Nelson, william.nelson@pnnl.gov.

* Present address: J. M. Mobberley, Department of Chemistry and Biochemistry, University of California Santa Barbara, Santa Barbara, California, USA; Y. Maezato, Naval Research Lab, Chemistry Division, Washington, DC, USA; S. R. Lindemann, Department of Nutrition Science, Purdue University, West Lafayette, Indiana, USA.

nase, supporting experiments showing HL-69 grows on nitrate but not dinitrogen (Y.M. and J.K.C, unpublished data). The genome of HL-69 indicates it is auxotrophic for vitamin B_{12} and is capable of salvage through an ABC transporter (*btuBFCD*). HL-69 is prototrophic for B_2, B_6, B_7, and B_9; however, the presence of genes for uptake of B_7 (*bioY*) and B_9 (*folT*) suggests it might be conditionally syntrophic for those vitamins (14). Consistent with CHL-69 growing under a wide range of salinity and light conditions, stress response pathways were identified, such as biosynthesis of the osmolytes glucosyl-glycerol (*ggpS*) and choline (*glpQ*), as well as the UV protectant mycosporine, which may be induced by oxidative stress due to high light levels (15).

Accession number(s). This whole-genome shotgun project has been deposited in GenBank under the accession no. CP024912 (CHL-69), CP024913 (pCHL69-1), and CP024914 (pCHL69-2). The versions described in this paper are the first versions, CP024912.1, CP024913.1, and CP024914.1. The metagenome for the cyanobacterial enrichment culture is publically accessible in JGI's Integrated Microbial Genomes and Microbiomes (IMG) under IMG Genome ID 3300005412.

ACKNOWLEDGMENTS

We thank Mark Wildung at Washington State University for his assistance with PacBio sequencing and Beau Morton and Karl Dana for their assistance in handling cultures.

This research was supported by the U.S. Department of Energy (DOE), Office of Biological and Environmental Research (OBER), as part of OBER's Genomic Science Program (GSP). This contribution originates from the GSP Foundational Scientific Focus Area (FSFA) at the Pacific Northwest National Laboratory (PNNL). The work conducted by the U.S. Department of Energy Joint Genome Institute was supported by the Office of Science of the U.S. Department of Energy (under contract no. DE-AC02-05CH11231) and Community Sequencing Project 701.

REFERENCES

1. Wong HL, Ahmed-Cox A, Burns BP. 2016. Molecular ecology of hypersaline microbial mats: current insights and new directions. Microorganisms 4:6. https://doi.org/10.3390/microorganisms4010006.
2. Mobberley JM, Lindemann SR, Bernstein HC, Moran JJ, Renslow RS, Babauta J, Hu D, Beyenal H, Nelson WC. 2017. Organismal and spatial partitioning of energy and macronutrient transformations within a hypersaline mat. FEMS Microbiol Ecol 93. https://doi.org/10.1093/femsec/fix028.
3. Lindemann SR, Moran JJ, Stegen JC, Renslow RS, Hutchison JR, Cole JK, Dohnalkova AC, Tremblay J, Singh K, Malfatti SA, Chen F, Tringe SG, Beyenal H, Fredrickson JK. 2013. The epsomitic phototrophic microbial mat of Hot Lake, Washington: community structural responses to seasonal cycling. Front Microbiol 4:323. https://doi.org/10.3389/fmicb.2013.00323.
4. Zachara JM, Moran JJ, Resch CT, Lindemann SR, Felmy AR, Bowden ME, Cory AB, Fredrickson JK. 2016. Geo- and biogeochemical processes in a heliothermal hypersaline lake. Geochim Cosmochim Acta 181:144–163. https://doi.org/10.1016/j.gca.2016.02.001.
5. Cole JK, Hutchison JR, Renslow RS, Kim Y-M, Chrisler WB, Engelmann HE, Dohnalkova AC, Hu D, Metz TO, Fredrickson JK, Lindemann SR. 2014. Phototrophic biofilm assembly in microbial-mat-derived unicyanobacterial consortia: model systems for the study of autotroph-heterotroph interactions. Front Microbiol 5. https://doi.org/10.3389/fmicb.2014.00109.
6. Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat Methods 10:563–569. https://doi.org/10.1038/nmeth.2474.
7. Hyatt D, Chen GL, LoCascio PF, Land ML, Larimer FW, Hauser LJ. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics 11:119. https://doi.org/10.1186/1471-2105-11-119.
8. 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, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75. https://doi.org/10.1186/1471-2164-9-75.
9. Nawrocki EP, Burge SW, Bateman A, Daub J, Eberhardt RY, Eddy SR, Floden EW, Gardner PP, Jones TA, Tate J. 2015. Rfam 12.0: updates to the RNA families database. Nucleic Acids Res 43:D130–D137. https://doi.org/10.1093/nar/gku1063.
10. Kanehisa M, Sato Y, Morishima K. 2016. BlastKOALA and GhostKOALA: KEGG tools for functional characterization of genome and metagenome sequences. J Mol Biol 428:726–731. https://doi.org/10.1016/j.jmb.2015.11.006.
11. Haft DH, Selengut JD, White O. 2003. The TIGRFAMs database of protein families. Nucleic Acids Res 31:371–373. https://doi.org/10.1093/nar/gkg128.
12. Starikov AY, Usserbaeva AA, Sinetova MA, Sarsekeyeva FK, Zayadan BK, Ustinova VV, Kupriyanova EV, Los DA, Mironov KS. 2016. Draft genome sequence of *Cyanobacterium* sp. strain IPPAS B-1200 with a unique fatty acid composition. Genome Announc 4(6):e01306-16. https://doi.org/10.1128/genomeA.01306-16.
13. Stanier RY, Deruelles J, Rippka R, Herdman M, Waterbury JB. 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Microbiology 111:1–61. https://doi.org/10.1099/00221287-111-1-1.
14. Romine MF, Rodionov DA, Maezato Y, Osterman AL, Nelson WC. 2017. Underlying mechanisms for syntrophic metabolism of essential enzyme cofactors in microbial communities. ISME J 11:1434–1446. https://doi.org/10.1038/ismej.2017.2.
15. Wada N, Sakamoto T, Matsugo S. 2013. Multiple roles of photosynthetic and sunscreen pigments in cyanobacteria focusing on the oxidative stress. Metabolites 3:463–483. https://doi.org/10.3390/metabo3020463.