

Genome of *Lamprobacter modestohalophilus* ShNLb02, a moderate halophilic photosynthetic purple bacterium of the Chromatiaceae family

John A. Kyndt,¹ Irina A. Bryantseva,² Vladimir M. Gorlenko,² Johannes F. Imhoff³

AUTHOR AFFILIATIONS See affiliation list on p. 3.

ABSTRACT The genome sequence of the moderately halophilic *Lamprobacter modestohalophilus* ShNLb02 was compared to those of other *Lamprobacter* and *Halochromatium* species. It revealed an average nucleotide identity of 94% to *Lpb. modestohalophilus* DSM 25653 and of 89.7% to *Halochromatium roseum* DSM 18859, underscoring their close phylogenetic relationship.

KEYWORDS *Lamprobacter*, Lake Shunet, *Halochromatium*, whole-genome sequence

The genus *Lamprobacter* contains a single species of halophilic purple sulfur bacteria. Previous morphological, physiological, and 16S rRNA studies have shown a close relationship of *Lamprobacter modestohalophilus* to *Halochromatium roseum* and more distantly to *Halochromatium salexigens* and *Halochromatium glycolicum*. These studies have led to the proposal to move *Hch. roseum* to the genus *Lamprobacter* (1). Strain ShNLb02 is the only *Lamprobacter* strain that does not require vitamin B₁₂ for growth (1). The genome sequence of *Lpb. modestohalophilus* DSM 25653 had previously been determined (2). We now report the sequence of strain ShNLb02, which will allow a refined phylogenetic comparison of the genus.

Strain ShNLb02 was isolated from the meromictic saline Lake Shunet (Russia, Siberia; 54°25'07"N; 90°13'41"E; salinity 65 g/L) by serial dilutions after repeated transfers of well-isolated colonies from 0.5%–0.7% (wt/vol) agar medium as described in references (1, 3). Genomic DNA was prepared from frozen cells stored in anaerobic vials at –80°C, using the GeneJET DNA purification kit (Thermo Scientific), giving an A_{260/280} of 1.81. The sequencing library was prepared using the Illumina DNA Library Prep kit. The genome was sequenced by an Illumina MiniSeq using 500 µL of a 1.8 pM library. Paired-end (2 × 150 bp) sequencing generated 2,472,406 reads and 193 Mbps. Quality control of the reads was performed using FASTQC (v1.0.0), using a k-mer size of 5 and contamination filtering for overrepresented sequences against the default contamination list. Oxford Nanopore library prep was performed following the Ligation Sequencing Kit (SQK-LSK110) without size selection, on a FLO-MIN106D flow cell with a MinION-Mk1B instrument (4). No DNA shearing was performed. Read QC and “superaccuracy basecalling” were performed using Guppy (v6.5.7) (5). We obtained 52,757 reads (74.4 Mbps), with a mean read length of 1,883 bp. A combined *de novo* genome assembly with the Illumina sequencing was performed using Unicycler (v0.5.0) (6) within BV-BRC (7). The resulting contig, CDS, and N50 values are in (Table 1). The coarse and fine consistency were 94% and 90%, respectively (8). The final assembled genome was 100% complete according to CheckM (v1.1.6) (9) with less than 2% contamination. The genome was annotated by the NCBI PGAP (v6.6) (10). Default parameters were used unless otherwise specified.

Editor Elinne Becket, California State University San Marcos, San Marcos, California, USA

Address correspondence to John A. Kyndt, jkyndt@bellevue.edu.

The authors declare no conflict of interest.

Received 8 February 2024

Accepted 10 March 2024

Published 25 March 2024

Copyright © 2024 Kyndt 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/).

TABLE 1 Overview of genome features of all of the sequenced *Lamprobacter* and *Halochromatium* species^a

Species	Size	% GC	Contigs	Coverage	N50	CDS	tRNAs	ANI	Reference	Genbank acc. #
<i>Lamprobacter modestohalophilus</i> ShNLb02	6.1 Mb	61.8	606	44x	80,713	5,935	43	–	This study	JAXUF0100000000
<i>Lamprobacter modestohalophilus</i> DSM 25653	5.7 Mb	62	209	126x	90,783	5,899	47	94.4	(2)	NRRY0000000000
<i>Halochromatium roseum</i> DSM 18859 [T]	5.2 Mb	61.9	88	50x	1,43,744	5,208	44	89.7	(2)	NHSH0000000000
<i>Lamprobacter</i> sp. SM2E	6.2 Mb	61.4	787	55x	34,435	6,915	56	84	(11)	JAYESN000000000000
<i>Halochromatium salexigens</i> DSM 4395 [T]	3.8 Mb	63.7	100	61x	66,939	3,831	46	81.5	(2)	NHFS0000000000
<i>Halochromatium glycolicum</i> DSM 11080 [T]	5.3 Mb	64.6	147	75x	84,551	5,291	45	78.1	(2)	NBSJ0000000000

^aANI percentage is based on bidirectional ANIb values to strain ShNLb02, calculated using *JSpecies*.

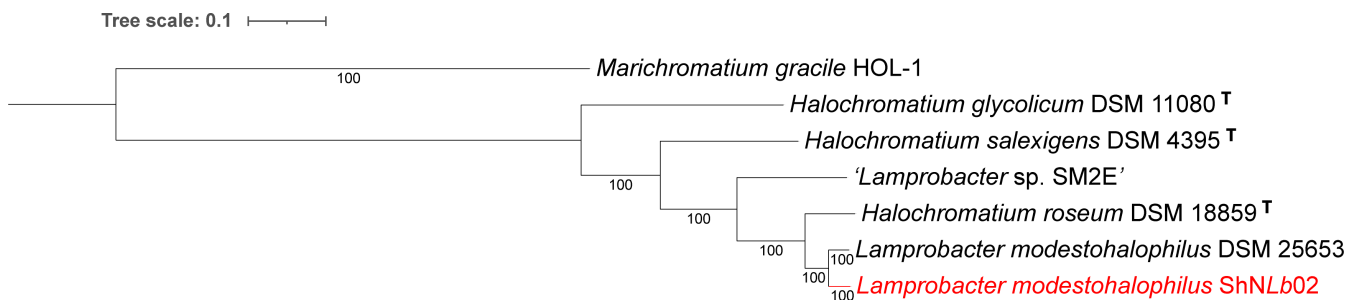


FIG 1 Phylogenetic tree of *Lamprobacter* whole-genome comparison to its closest relatives. The phylogenetic tree was generated using the codon tree method within BV-BRC (7), which used PGFams as homology groups and analyzed 500 aligned proteins and coding DNA from single-copy genes using RAXML (V8) (13, 14). The support values for the phylogenetic tree are generated using 100 rounds of the "Rapid bootstrapping" option of RaxML. *Marichromatium gracile* HOL-1 (17) was used as an outgroup. iTOL was used for the tree visualization (18).

A JSpecies comparison [12; v4.1.1] of average percentage nucleotide identity (ANI) gave 94.4% identity of *Lpb. modestohalophilus* ShNLb02 to *Lpb. modestohalophilus* DSM 25653 and 89.7% identity to *Hch. roseum* DSM 18859. Phylogenetic analysis of the *Lpb. modestohalophilus* ShNLb02 genome using RAXML (13, 14) showed *Lpb. modestohalophilus* DSM 25653 as the closest relative (Fig. 1) and more distantly *Hch. roseum* DSM18859 from salt pans in India (11) and "*Lamprobacter* sp. SM2E" from the Nebraska Salt Marshes (15). Both the ANI and phylogenetic comparisons support the earlier proposition, based on morphological and physiological data, of including *Hch. roseum* DSM 18859 into the *Lamprobacter* genus (1). All genomes from *Lpb. modestohalophilus* and *Hch. roseum* contain a complete gas vesicle operon, which is absent from *Hch. salexigens*. The ShNLb02 genome contains three distinct gene clusters for cobalamin biosynthesis, with an AAI of 64% using LALIGN (16), consistent with the observation that ShNLb02 does not require vitamin B₁₂ supplement for growth (1).

ACKNOWLEDGMENTS

This work was sponsored by the Wilson Enhancement Fund for Applied Research in Science at Bellevue University.

AUTHOR AFFILIATIONS

¹College of Science and Technology, Bellevue University, Bellevue, Nebraska, USA

²Winogradsky Institute of Microbiology, Research Center of Biotechnology, Russian Academy of Sciences, Moscow, Russia

³GEOMAR Helmholtz Centre for Ocean Research Kiel, Kiel, Germany

AUTHOR ORCIDs

John A. Kyndt  <http://orcid.org/0000-0001-7168-8015>

AUTHOR CONTRIBUTIONS

John A. Kyndt, Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Resources, Software, Validation, Writing – original draft, Writing – review and editing | Irina A. Bryantseva, Conceptualization, formal analysis, Investigation, Resources, Validation, Writing – review and editing | Vladimir M. Gorlenko, Conceptualization, formal analysis, Investigation, Resources, Validation, Writing – review and editing | Johannes F. Imhoff, Conceptualization, formal analysis, Investigation, project administration, Resources, Validation, Writing – review and editing

DATA AVAILABILITY

This Whole-Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession [JAXUF1000000000](https://www.ncbi.nlm.nih.gov/nuccore/JAXUF1000000000). The version described in this paper is version

JAXUF101000000. The raw sequencing reads have been submitted to SRA, and the corresponding accession number for the Illumina data is [SRR27256383](https://doi.org/10.1134/S0026261714060071). The accession numbers for the Oxford Nanopore data are [SRR27887957](https://doi.org/10.1134/S0026261714060071), [SRR27887954](https://doi.org/10.1134/S0026261714060071), [SRR27887951](https://doi.org/10.1134/S0026261714060071), [SRR27887959](https://doi.org/10.1134/S0026261714060071), [SRR27887956](https://doi.org/10.1134/S0026261714060071), [SRR27887953](https://doi.org/10.1134/S0026261714060071), [SRR27887950](https://doi.org/10.1134/S0026261714060071), [SRR27887958](https://doi.org/10.1134/S0026261714060071), [SRR27887952](https://doi.org/10.1134/S0026261714060071), [SRR27887949](https://doi.org/10.1134/S0026261714060071), [SRR27887948](https://doi.org/10.1134/S0026261714060071), [SRR27887947](https://doi.org/10.1134/S0026261714060071), [SRR27887946](https://doi.org/10.1134/S0026261714060071).

REFERENCES

- Gorlenko VM, Bryantseva IA, Lunina ON, Tourova TP. 2014. Phylogenetic position of the purple sulfur bacterium *Lamprobacter modestohalophilus* determined based on the data on new strains of the species. *Microbiology* 83:829–837. <https://doi.org/10.1134/S0026261714060071>
- Imhoff JF, Rahn T, Künzel S, Keller A, Neulinger SC. 2020. Osmotic adaptation and compatible solute biosynthesis of phototrophic bacteria as revealed from genome analyses. *Microorganisms* 9:46. <https://doi.org/10.3390/microorganisms9010046>
- Lunina ON, Bryantseva IA, Akimov VN, Rusanov II, Rogozin DYU, Barinova ES, Lysenko AM, Pimenov NV. 2007. Seasonal changes in the structure of the anoxygenic photosynthetic bacterial community in Lake Shunet, Khakassia. *Microbiology* 76:368–379. <https://doi.org/10.1134/S0026261707030149>
- Jain M, Olsen HE, Paten B, Akeson M. 2016. The Oxford Nanopore MinION: delivery of nanopore sequencing to the genomics community. *Genome Biol* 17:239. <https://doi.org/10.1186/s13059-016-1103-0>
- Wick RR, Judd LM, Holt KE. 2019. Performance of neural network basecalling tools for Oxford Nanopore sequencing. *Genome Biol* 20:129. <https://doi.org/10.1186/s13059-019-1727-y>
- Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 13:e1005595. <https://doi.org/10.1371/journal.pcbi.1005595>
- Wattam AR, Davis JJ, Assaf R, Boisvert S, Brettin T, Bun C, Conrad N, Dietrich EM, Disz T, Gabbard JL, et al. 2017. Improvements to PATRIC, the all-bacterial bioinformatics database and analysis resource center. *Nucleic Acids Res* 45:D535–D542. <https://doi.org/10.1093/nar/gkw1017>
- Parrello B, Butler R, Chlenski P, Olson R, Overbeek J, Pusch GD, Vonstein V, Overbeek R. 2019. A machine learning-based service for estimating quality of genomes using PATRIC. *BMC Bioinformatics* 20:486. <https://doi.org/10.1186/s12859-019-3068-y>
- 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>
- 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>
- Anil Kumar P, Srinivas TNR, Sasikala C, Ramana CV. 2007. *Halochromatium roseum* sp. nov., a non-motile phototrophic gammaproteobacterium with gas vesicles, and emended description of the genus *Halochromatium*. *Int J Syst Evol Microbiol* 57:2110–2113. <https://doi.org/10.1099/ijs.0.65034-0>
- Richter M, Rosselló-Móra R, Glöckner OF, Peplies J. 2016. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics* 32:929–931. <https://doi.org/10.1093/bioinformatics/btv681>
- Stamatakis A, Hoover P, Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML web servers. *Syst Biol* 57:758–771. <https://doi.org/10.1080/10635150802429642>
- Stamatakis AJB. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>
- Athen SR, Dubey S, Kyndt JA. 2021. The Eastern Nebraska salt marsh microbiome is well adapted to an alkaline and extreme saline environment. *Life* 11:446. <https://doi.org/10.3390/life11050446>
- Madeira F, Pearce M, Tivey ARN, Basutkar P, Lee J, Edbali O, Madhusoodanan N, Kolesnikov A, Lopez R. 2022. Search and sequence analysis tools services from EMBL-EBI in 2022. *Nucleic Acids Res* 50:W276–W279. <https://doi.org/10.1093/nar/gkac240>
- Kyndt JA, Dubey S, Frazier N, Meyer TE. 2022. Genome sequences of *Marichromatium gracile* HOL-1 and its purple photosynthetic co-isolate *Affifella* sp. H1R. *Microbiol Resour Announc* 11:e0003322. <https://doi.org/10.1128/mra.00033-22>
- Letunic I, Bork P. 2019. Interactive tree of life (iTOL) v4: recent updates and new developments. *Nucleic Acids Res* 47:W256–W259. <https://doi.org/10.1093/nar/gkz239>