

Draft genome sequence of *Halobacillus campisalis* strain ASL-17

Anushree Srivastava,¹ Michael Christopher Macey,¹ Terry J. McGenity,² Karen Olsson-Francis¹

AUTHOR AFFILIATIONS See affiliation list on p. 2.

ABSTRACT We report here the genome sequence of moderately halophilic *Halobacillus campisalis* ASL-17, isolated from hypersaline sediment from the Yellow Sea, Korea. The bacterium was Gram variable, oval or coccoid, and mesophilic. The genome of *H. campisalis* ASL-17 has 3.8 Mbp, with 3,910 coding sequences, 76 RNAs, and 41.3% G + C content.

KEYWORDS bacteria, halophiles, genomics

Spring et al. (1) first described the genus *Halobacillus*, which belongs to the family *Bacillaceae* (2, 3) within the phylum *Firmicutes* (synonym *Bacillota*, 3). Genus *Halobacillus* comprises 27 species: *Halobacillus halophilus*, *Halobacillus litoralis*, *Halobacillus trueperi* (1), *Halobacillus thailandensis* (4), *Halobacillus salinus* (5), *Halobacillus karajensis* (6) *Halobacillus localis* (7), *Halobacillus styriensis* (8), *Halobacillus aidingensis* and *Halobacillus dabanensis* (9), *Halobacillus yeomjeoni* (10), *Halobacillus profundus* and *Halobacillus kuroshimensis* (11), *Halobacillus campisalis* (12), *Halobacillus faecis* (13), *Halobacillus mangrovi* (14), *Halobacillus alkaliphilus* (15), *Halobacillus seohaensis* (16), *Halobacillus naozhouensis* and *Halobacillus salsuginis* (17), *Halobacillus hunanensis* (18), *Halobacillus sediminis* (19), *Halobacillus andaensis* (20), *Halobacillus salicampi* (21), *Halobacillus massiliensis* (22), *Halobacillus marinus* (23), *Halobacillus ihumii* (24), and *Halobacillus fulvus* (25). Members of the genus *Halobacillus* are moderate to highly halophilic (13, 26), which makes them ideal candidates for biotechnological (27) as well as for astrobiological investigations (28). Many halophiles produce pigments (or carotenoids) to prevent photooxidative damage (29), endospores to survive a wide variety of physicochemical stresses [e.g., (30)], and synthesize osmoprotective compounds (31).

H. campisalis strain ASL-17 was isolated from hypersaline sediment from the Yellow Sea, Korea, by Yoon et al. (12). The polyphasic characterization revealed that *H. campisalis* strain ASL-17 is Gram-positive or variable, cocci or oval-shaped, and light-yellow in color. It grew optimally in the presence of approximately 8% (wt/vol) NaCl, at pH 7.0–8.0 and 37°C (12). The most distinguishing feature of the strain ASL-17 is that the cell wall peptidoglycan is composed of meso-diaminopimelic acid, unlike other *Halobacillus* (12).

For genome sequencing, freeze-dried cells of *H. campisalis* strain ASL-17 (strain CCUG 54360) were purchased from the Culture Collection University of Gothenburg Sweden (<https://www.ccug.se>). To culture, freeze-dried cells were first rehydrated using 0.5 mL of marine broth liquid medium marine broth (or DIFCO 2216), as recommended by the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (<https://www.dsmz.de/>) for the type species *H. halophilus* and mixed well. Then aliquots of the broth were transferred onto agar plates. The plates were then incubated at 30°C until growth appeared. A pure culture was obtained *via* repeated streaking, confirmed based on the uniformity of colony morphology. For genome sequencing, cultures were removed from the plate and resuspended into the barcoded bead tube provided by MicrobesNG (<https://microbesng.com/>) using the sterile loop. The tube was inverted 10 times, sealed, and returned to MicrobesNG at room temperature, with a guarantee that

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Address correspondence to Anushree Srivastava, anushree.srivastava1@open.ac.uk, or Michael Christopher Macey, michael.macey@open.ac.uk.

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the delivery would arrive within 2 days of making the stock. Genomic DNA extraction and whole-genome sequencing were carried out by MicrobesNG. Genomic DNA libraries were prepared by MicrobesNG using the Nextera XT Library Prep Kit (Illumina, San Diego, USA) following the manufacturer's protocol with the following modifications: input DNA was increased twofold, and PCR elongation time was increased to 45 s. DNA quantification and library preparations were carried out on a Hamilton Microlab STAR automated liquid handling system (Hamilton Bonaduz AG, Switzerland). Pooled libraries were quantified using the Kapa Biosystems Library Quantification Kit for Illumina. The genome libraries were sequenced by Illumina HiSeq technology and a 250 bp paired-end protocol. The assembly metrics were calculated using QUAST (Quality Assessment Tool for Genome Assemblies). Trimmed reads were produced using Trimmomatic (v0.30) with a sliding window quality cutoff of Q15. *De-novo* assembly was performed using SPAdes (v3.7; default settings) (32, 33). Coverage of 53-fold was achieved, calculated using BWA, SAMtools (v0.1.19), and BEDTools genomcov (v2.2.7) with default settings (34–36). Functional annotation of genes was performed using the RAST server (37) with the SEED database (38).

The assembled genome sequence of *H. campisalis* ASL-17 yielded 3,828,543 bp distributed in 43 contigs (scaffold N50 size = 4,52,793 bp). The G + C content was 41.3%, with 448 subsystems, 3,910 CDS, 67 tRNAs, and 1 tmRNA. The most represented RAST subsystem features, and the respective gene clusters (>100 gene clusters) were carbohydrates (430), amino acids and derivatives (429), protein metabolism (234), cofactors, vitamins, prosthetic groups, pigments (214), fatty acids, lipids, and isoprenoids (158), RNA metabolism (143), cell wall and capsule (128), stress response (116), nucleosides and nucleotides (112), and DNA metabolism (104). Notably, the genome of strain ASL-17 possesses a number of anaerobic respiratory reductases that potentially act as terminal electron acceptors in anaerobic respiration, including thiosulfate reductase, anaerobic dimethyl sulfoxide reductase, anaerobic sulfite reductase, dissimilatory sulfite reductase, and ferric and arsenate reductases.

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AUTHOR AFFILIATIONS

¹AstrobiologyOU, The Open University, Milton Keynes, United Kingdom

²School of Life Sciences, University of Essex, Colchester, United Kingdom

AUTHOR ORCID*s*

Anushree Srivastava  <http://orcid.org/0009-0008-3713-5490>

Michael Christopher Macey  <http://orcid.org/0000-0002-1800-0469>

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AUTHOR CONTRIBUTIONS

Anushree Srivastava, Conceptualization, Data curation, Investigation, Methodology, Writing – original draft | Michael Christopher Macey, Conceptualization, Supervision, Writing – review and editing | Terry J. McGenity, Conceptualization, Supervision, Writing – review and editing | Karen Olsson-Francis, Conceptualization, Supervision, Writing – review and editing

DATA AVAILABILITY

The draft genome sequence of *Halobacillus campisalis* type strain ASL-17 was deposited to GenBank under WGS accession [JAPVRC000000000](https://doi.org/10.1093/jbcr/abaa000).

REFERENCES

- Spring S, Ludwig W, Marquez MC, Ventosa A, Schleifer K-H. 1996. *Halobacillus* gen. nov., with descriptions of *Halobacillus litoralis* sp. nov. and transfer *Sporosarcina halophila* to *Halobacillus halophilus* comb. nov. *Int J Syst Bacteriol* 46:492–496. <https://doi.org/10.1099/00207713-46-2-492>
- Cohn F. 1872. Untersuchungen Über bakterien. beitrage zur biologie der pflanzen studies on bacteria. Contributions to the Biology of Plants 1:127–224.
- Oren A, Garrity GM. 2021. Notification that new names of prokaryotes, new combinations, and new taxonomic opinions. *Int J Syst Evol Microbiol* 71:2. <https://doi.org/10.1099/ijsem.0.004766>
- Chaiyanan S, Chaiyanan S, Mauget T, Huq A, Robb FT, Colwell RR. 1999. Polyphasic taxonomy of a novel *Halobacillus*, *Halobacillus thailandensis* sp. nov. isolated from fish sauce. *Syst Appl Microbiol* 22:360–365. [https://doi.org/10.1016/S0723-2020\(99\)80043-5](https://doi.org/10.1016/S0723-2020(99)80043-5)
- Yoon J-H, Kang KH, Park Y-H. 2003. *Halobacillus salinus* sp. nov., isolated from a salt lake on the coast of the east sea in Korea. *Int J Syst Evol Microbiol* 53:687–693. <https://doi.org/10.1099/ijms.0.02421-0>
- Amoozegar MA, Malekzadeh F, Malik KA, Schumann P, Sproer CH. 2003. *Halobacillus karajensis* sp. nov., a novel moderate halophile. *Int J Syst Evol Microbiol* 53:1059–1063. <https://doi.org/10.1099/ijms.0.02448-0>
- Yoon J-H, Kang KH, Oh T-K, Park Y-H. 2004. *Halobacillus locisalis* sp. nov., a halophilic bacterium isolated from a marine solar saltern of the yellow sea in Korea. *Extremophiles* 8:23–28. <https://doi.org/10.1007/s00792-003-0352-5>
- Ripka K, Denner EBM, Michaelsen A, Lubitz W, Piñar G. 2006. Molecular characterisation of *Halobacillus* populations isolated from different medieval wall paintings and building materials. *Int Biodet Biodeg* 58:124–132. <https://doi.org/10.1016/j.ibiod.2006.05.004>
- Liu WY, Zeng J, Wang L, Dou YT, Yang SS. 2005. *Halobacillus dabanensis* sp. nov. and *Halobacillus aidingensis* sp. nov., isolated from salt lakes in Xinjiang, China. *Int J Syst Evol Microbiol* 55:1991–1996. <https://doi.org/10.1099/ijms.0.63787-0>
- Yoon J-H, Kang S-J, Lee C-H, Oh HW, Oh T-K. 2005. *Halobacillus yeomjeoni* sp. nov., isolated from a marine solar saltern in Korea. *Int J Syst Evol Microbiol* 55:2413–2417. <https://doi.org/10.1099/ijms.0.63801-0>
- Hua NP, Kobayashi F, Iwasaka Y, Shi G-Y, Naganuma T. 2007. Detailed identification of desert-originated bacteria carried by Asian dust storms to Japan. *Aerobiologia* 23:291–298. <https://doi.org/10.1007/s10453-007-9076-9>
- Yoon J-H, Kang S-J, Jung Y-T, Oh T-K. 2007. *Halobacillus campisalis* sp. nov., containing meso-diaminopimelic acid in the cell-wall peptidoglycan, and emended description of the genus *Halobacillus*. *Int J Syst Evol Microbiol* 57:2021–2025. <https://doi.org/10.1099/ijms.0.65188-0>
- Sun-Young A, Kanoh K, Kasai H, Goto K, Yokota A. 2007. *Halobacillus faecis* sp. nov., a spore-forming bacterium isolated from a mangrove area on Ishigaki Island, Japan. *Int J Syst Evol Microbiol* 57:2476–2479. <https://doi.org/10.1099/ijms.0.64896-0>
- Soto-Ramírez N, Sánchez-Porro C, Rosas-Padilla S, Almodóvar K, Jiménez G, Machado-Rodríguez M, Zapata M, Ventosa A, Montalvo-Rodríguez R. 2008. *Halobacillus mangrovi* sp. nov., a moderately halophilic bacterium isolated from the black mangrove *Avicennia germinans*. *Int J Syst Evol Microbiol* 58:125–130. <https://doi.org/10.1099/ijms.0.65008-0>
- Romano I, Finore I, Nicolaus G, Huertas FJ, Lama L, Nicolaus B, Poli A. 2008. *Halobacillus alkaliphilus* sp. nov., a halophilic bacterium isolated from a salt lake in Fuente de Piedra, Southern Spain. *Int J Syst Evol Microbiol* 58:886–890. <https://doi.org/10.1099/ijms.0.65457-0>
- Yoon J-H, Kang S-J, Oh T-K. 2008. *Halobacillus seohaensis* sp. nov., isolated from a marine solar saltern in Korea. *Int J Syst Evol Microbiol* 58:622–627. <https://doi.org/10.1099/ijms.0.65341-0>
- Chen YG, Liu ZX, Zhang YQ, Zhang YX, Tang SK, Borrahybay E, Li WJ, Cui XL. 2009. *Halobacillus naozhouensis* sp. nov., a moderately halophilic bacterium isolated from a sea anemone. *Antonie Van Leeuwenhoek* 96:99–107. <https://doi.org/10.1007/s10482-009-9340-9>
- Peng QZ, Peng QJ, Zhang YQ, Liu ZX, Wang YX, Li WJ, Cui XL, Chen YG. 2019. *Halobacillus hunanensis* sp. nov., a moderately halophilic bacterium isolated from a subterranean brine. *Antonie Van Leeuwenhoek* 96:497–504. <https://doi.org/10.1007/s10482-009-9365-0>
- Kim S-J, Lee J-C, Han S-I, Whang K-S. 2015. *Halobacillus sediminis* sp. nov., a moderately halophilic bacterium isolated from a solar saltern sediment. *Int J Syst Evol Microbiol* 65:4434–4440. <https://doi.org/10.1099/ijsem.0.000595>
- Wang K, Zhang L, Yang Y, Pan Y, Meng L, Liu H, Hong S, Huang H, Jiang J. 2015. *Halobacillus andaensis* sp. nov., a moderately halophilic bacterium isolated from saline and alkaline soil. *Int J Syst Evol Microbiol* 65:1908–1914. <https://doi.org/10.1099/ijms.0.000198>
- Kim S-J, Lee J-C, Han S-I, Whang K-S. 2016. *Halobacillus salicampi* sp. nov., a moderately halophilic bacterium isolated from a solar saltern sediment. *Antonie Van Leeuwenhoek* 109:713–720. <https://doi.org/10.1007/s10482-016-0672-y>
- Senghor B, Seck EH, Khelaifia S, Bassene H, Sokhna C, Fournier PE, Raoult D, Lagier JC. 2017. Description of '*Bacillus dakarensis*' sp. nov., '*Bacillus sinesaloumensis*' sp. nov., '*Gracilibacillus timonensis*' sp. nov., '*Halobacillus massiliensis*' sp. nov., '*Lentibacillus massiliensis*' sp. nov., '*Oceanobacillus senegalensis*' sp. nov., '*Oceanobacillus timonensis*' sp. nov., '*Virgibacillus dakarensis*' sp. nov. and '*Virgibacillus marseillensis*' sp. nov., nine halophilic new species isolated from human stool. *New Microbes new infect* 17:45–51. <https://doi.org/10.1016/j.nmni.2017.01.010>
- Panda AN, Mishra SR, Das L, Rastogi S, Pattanaik G, Suar M, Raina V. 2018. Taxonomic description and genome sequence of *Halobacillus marinus* sp. nov., a novel strain isolated from Chilika lake, India. *J Microbiol* 56:223–230. <https://doi.org/10.1007/s12275-018-7387-x>
- Konate S, Lo CI, Kuete E, Sarr M, Amsrtong N, Levasseur A, Caputo A, Thera MA, Raoult D, Million M. 2020. *Halobacillus ihmii* sp. nov., a new bacterium isolated from stool of healthy children living in Mali. *New Microbes New Infect* 37:100708. <https://doi.org/10.1016/j.nmni.2020.100708>
- Booncharoen A, Visessanguan W, Kuncharoen N, Yiamsombut S, Santiyanont P, Mhuantong W, Rojsitthisak P, Tanasupawat S. 2021. *Halobacillus fulvus* sp. nov., a moderately halophilic bacterium isolated from shrimp paste (ka-pi) in Thailand. *Int J Syst Evol Microbiol* 71. <https://doi.org/10.1099/ijsem.0.005054>
- Kushner DJ, Kamekura M. 1988. Physiology of Halophilic Eubacteria, p 109–138. In Rodriguez-Valera F (ed), *Halophilic bacteria*, 1st ed. CRC Press, Boca Raton, FL.
- Dutta B, Bandopadhyay R. 2022. Biotechnological potentials of halophilic microorganisms and their impact on mankind. *Beni Suef Univ J Basic Appl Sci* 11:75. <https://doi.org/10.1186/s43088-022-00252-w>
- Ramírez Jimenez SI, Cardona C, Izquierdo R, Rodriguez EC, Figueroa PA. 2019. Halophilic bacteria strategies help to understand the Habitability aspects of the solar system. *MEMORIE della Società Astronomica Italiana* 90:597. <http://sait.oat.ts.astro.it/MSAIt900419/PDF/2019MmSAI..90..597I.pdf>
- Köcher S, Müller V. 2011. The nature and function of carotenoids in the moderately halophilic bacterium *Halobacillus halophilus*, p 303–317. In Ventosa A, Oren A, Ma Y (ed), *Halophiles and hypersaline environments*, 1st ed. Springer, Berlin, Heidelberg.
- Nicholson WL, Fajardo-Cavazos P, Rebeil R, Slieman TA, Riesenman PJ, Law JF, Xue Y. 2002. Bacterial endospores and their significance in stress resistance. *Antonie Van Leeuwenhoek* 81:27–32. <https://doi.org/10.1023/a:1020561122764>
- Ventosa A, Nieto JJ, Oren A. 1998. Biology of moderately halophilic aerobic bacteria. *Microbiol Mol Biol Rev* 62:504–544. <https://doi.org/10.1128/MMBR.62.2.504-544.1998>

32. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski 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>
33. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinform* 30:2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>
34. Li H, Durbin R. 2009. Fast and accurate short read alignment with burrows-wheeler transform. *Bioinform* 25:1754–1760. <https://doi.org/10.1093/bioinformatics/btp324>
35. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup. 2009. The sequence alignment/map format and SAMtools. *Bioinform* 25:2078–2079. <https://doi.org/10.1093/bioinformatics/btp352>
36. Quinlan AR, Hall IM. 2010. BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinform* 26:841–842. <https://doi.org/10.1093/bioinformatics/btq033>
37. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, et al. 2008. The RAST server: rapid annotations using subsystems technology. *BMC Genomics* 9:75. <https://doi.org/10.1186/1471-2164-9-75>
38. Disz T, Akhter S, Cuevas D, Olson R, Overbeek R, Vonstein V, Stevens R, Edwards RA. 2010. Accessing the SEED genome databases via web services API: tools for programmers. *BMC Bioinform* 11:319. <https://doi.org/10.1186/1471-2105-11-319>