Microbiology Resource Announcements

| Applied and Industrial Microbiology | Announcement

Complete genome sequences of *Alkalilimnicola ehrlichii* strain SGAR-12 isolated from a pilot-scale haloalkaline biodesulfurization installation and type strain *A. ehrlichii* MLHE^T

Caroline M. Plugge,[1,2] Suyash Gupta,[1,3] Sarahi Garcia Villa,[1] Peer H. A. Timmers,[1,4] Gerard Muyzer[3]

AUTHOR AFFILIATIONS See affiliation list on p. 2.

ABSTRACT The sulfide-oxidizing bacterium *Alkalilimnicola ehrlichii* strain SGAR-12 was isolated from a pilot-scale haloalkaline biodesulfurization (BD) installation, and its genome was sequenced. Additionally, the genome of the type strain of the species, strain MLHE^T, was sequenced. In-depth genomic analysis may reveal differences in the sulfur utilization spectrum of both strains.

KEYWORDS *Alkalilimnicola*, sulfide, biodesulfurization

Only two *Alkalilimnicola* species have been isolated: *Alkalilimnicola halodurans* (1) and *A. ehrlichii* (2). *Alkalilimnicola* sp. was recently identified as a dominant player in a haloalkaline BD installation, converting sulfide to sulfur (3–6). We isolated the most dominant sulfide-oxidizing bacterium from a pilot-scale haloalkaline BD system located at Wageningen University Campus, the Netherlands. *Alkalilimnicola ehrlichii* SGAR-12 was enriched in serial dilution under oxygen-limiting conditions (12% v/v) in a haloalkaline mineral medium at pH 8.5 in serum bottles incubated at 35°C. The medium was prepared as described previously (7), i.e., with 20 mM sodium sulfide (Na_2S•9 H_2O) and without urea. The highest dilution with growth was streak plated on the same medium (7) with 2% (w/v) washed agar. SGAR-12 was obtained in pure culture and its genome sequenced. Additionally, we sequenced the genome of the type strain *A. ehrlichii* MLHE^T (DSM 17681^T) to facilitate comparative genome analysis.

SGAR-12 was grown in serum bottles under oxygen-limiting conditions (12% v/v) as described above. MLHE-1^T, from our culture collection, was cultured on medium 1457 as recommended by the DSMZ (https://www.dsmz.de/collection/catalogue/details/culture/DSM-17681).

The genomic DNA from both strains was extracted using the modified CTAB method (8, 9). The same extract was used for sequencing on both the Illumina NovaSeq 6000 and Oxford Nanopore platforms. Unless otherwise stated, default settings were applied for all analyzes.

For Illumina sequencing, the DNA concentration, purity, and integrity were analyzed using the Agilent 5400 fragment analyzer. Genomic DNA was randomly sheared into short fragments, which were then repaired, A-tailed, and ligated with Illumina adapters. Adapter-containing fragments were amplified by PCR, size-selected for ~350 bp, and purified using the Illumina Sequencing Analysis Viewer. Libraries were prepared using the NovaSeq 6000 SP Reagent Kit for the NovaSeq 6000 SP flow cell, producing 150-bp paired-end libraries. The libraries were checked using Qubit and real-time qPCR and then pooled. DNA quality checks, library preparation, and sequencing were carried out at Novogene Limited, Cambridge, UK. Raw read quality was assessed with the Illumina Sequencing Analysis Viewer. The raw data were cleaned for adapter sequences, reads with $N > 10\%$, and low-quality reads (Q-score ≤ 5).

Editor J. Cameron Thrash, University of Southern California, Los Angeles, California, USA

Address correspondence to Gerard Muyzer, g.muijzer@uva.nl.

Caroline M. Plugge and Suyash Gupta contributed equally to this article. Author order was determined alphabetically based on first names.

The authors declare no conflict of interest.

Received 26 May 2025
Accepted 19 August 2025
Published 18 September 2025

TABLE 1 Genome statistics of *Alkalilimnicola erhlichii* SGAR-12 and MLHE^T

	Strain SGAR-12	Strain MLHE^T
BioSample accession no	SAMN472143149	SAMN47214681
No. of Illumina raw reads	8,552,010	9,613,662
No. of Nanopore raw reads	33,508	123,456
Genome size (bp)	3,194,001	3,264,331
Genome coverage Illumina (×)	2.6	2.9
Genome coverage Nanopore (×)	1.2	4.3
Number of contigs	1 (circular)	1 (circular)
N_{50} value Nanopore (Kb)	21.13	20.89
CheckM completeness (%)	99.65	100
CheckM contamination (%)	1.12	0.59
G + C content (%)	67.12	67.54
Total genes	2,924	2,954
RNA genes	58	58
rRNA operons (5S, 16S, 23S)	2, 2, 2	2, 2, 2
Protein-coding genes	2,816	2,883
Pseudo genes	50	13
Genome accession no.	CP184336	CP183314

For Oxford Nanopore sequencing, the DNA library was prepared using the Nanopore Kit SQK-LSK109 (Oxford Nanopore Technologies, UK) without DNA shearing. To enrich DNA fragments larger than 3 kb, Long Fragment Buffer was used during the washing step before final elution. Sequencing was performed on the MinION platform using a FLO-FLG001 flow cell with 9.4.1 chemistry, followed by base calling with the MinKNOW v21.02.2 and Guppy v4.3.4 protocol. Raw read quality control was conducted using MinKNOW. Hybrid genome assembly was performed using Unicycler v0.4.9 under standard conditions for both strains (10). The quality of the assembled genomes was assessed with CheckM v1.4.0 (11). The assembly generated single circular contigs for both strains. The complete assembly size of SGAR12 and MLHE^T was 3,194,001 bp and 3,264,331 bp, respectively. Subsequently, the assembled genomes were annotated using the NCBI with the NCBI Prokaryotic Genome Annotation Pipeline (12). Further genome characteristics are in Table 1.

ACKNOWLEDGMENTS

This work was performed in the cooperation framework of Wetsus, European centre of excellence for sustainable water technology (www.wetsus.eu). Wetsus is co-funded by the Dutch Ministry of Economic Affairs and the Ministry of Infrastructure and Environment, the European Union Regional Development Fund, the Province of Fryslân, and the Northern Netherlands Provinces. This work is part of a project that has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement no. 665874. We thank Peter Kuperus (University of Amsterdam) for technical support.

AUTHOR AFFILIATIONS

[1]Wetsus, European Centre of Excellence for Sustainable Water Technology, Leeuwarden, the Netherlands
[2]Laboratory of Microbiology, Wageningen University and Research, Wageningen, the Netherlands
[3]Microbial Systems Ecology, Department of Freshwater and Marine Ecology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, the Netherlands
[4]Department of Environmental Sciences for Sustainability, IE University, Segovia, Spain

AUTHOR ORCIDs

Caroline M. Plugge http://orcid.org/0000-0002-3391-7742
Suyash Gupta http://orcid.org/0000-0002-7774-4636
Peer H. A. Timmers http://orcid.org/0000-0002-8049-9985
Gerard Muyzer http://orcid.org/0000-0002-2422-0732

AUTHOR CONTRIBUTIONS

Caroline M. Plugge, Conceptualization, Data curation, Formal analysis, Methodology, Supervision, Writing – original draft, Writing – review and editing | Suyash Gupta, Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review and editing | Sarahi Garcia Villa, Investigation, Methodology, Writing – review and editing | Peer H. A. Timmers, Writing – original draft, Writing – review and editing | Gerard Muyzer, Conceptualization, Data curation, Formal analysis, Methodology, Supervision, Writing – original draft, Writing – review and editing

DATA AVAILABILITY

Draft genome sequences were deposited at DDBJ/ENA/GenBank via BioProject PRJNA1231520 and PRJNA1231541 for SGAR-12 and *A. ehrlichii*, respectively. All sequence reads are available from NCBI Sequence Read Archive under SRP570540 and SRP570655, for SGAR-12 and MLHE^T, respectively.

REFERENCES

1. Yakimov MM, Giuliano L, Chernikova TN, Gentile G, Abraham WR, Lünsdorf H, Timmis KN, Golyshin PN. 2001. *Alcalilimnicola halodurans* gen. nov., sp. nov., an alkaliphilic, moderately halophilic and extremely halotolerant bacterium, isolated from sediments of soda-depositing Lake Natron, East Africa Rift Valley. Int J Syst Evol Microbiol 51:2133–2143. https://doi.org/10.1099/00207713-51-6-2133
2. Hoeft SE, Blum JS, Stolz JF, Tabita FR, Witte B, King GM, Santini JM, Oremland RS. 2007. *Alkalilimnicola ehrlichii* sp. nov., a novel, arsenite-oxidizing haloalkaliphilic gammaproteobacterium capable of chemoautotrophic or heterotrophic growth with nitrate or oxygen as the electron acceptor. Int J Syst Evol Microbiol 57:504–512. https://doi.org/10.1099/ijs.0.64576-0
3. de Rink R, Klok JBM, van Heeringen GJ, Sorokin DY, Ter Heijne A, Zeijlmaker R, Mos YM, de Wilde V, Keesman KJ, Buisman CJN. 2019. Increasing the selectivity for sulfur formation in biological gas desulfurization. Environ Sci Technol 53:4519–4527. https://doi.org/10.1021/acs.est.8b06749
4. de Rink R, Gupta S, Piccioli de Carolis F, Liu D, ter Heijne A, Klok JBM, Buisman CJN. 2021. Effect of process conditions on the performance of a dual-reactor biodesulfurization process. J Environ Chem Eng 9:106450. https://doi.org/10.1016/j.jece.2021.106450
5. Gupta S, Plugge CM, Klok JBM, Muyzer G. 2022. Comparative analysis of microbial communities from different full-scale haloalkaline biodesulfurization systems. Appl Microbiol Biotechnol 106:1759–1776. https://doi.org/10.1007/s00253-022-11771-y
6. Gupta S, de Rink R, Klok JBM, Muyzer G, Plugge CM. 2024. Process conditions affect microbial diversity and activity in a haloalkaline biodesulfurization system. Appl Environ Microbiol 90:e0186423. https://doi.org/10.1128/aem.01864-23
7. Kiragosyan K, Klok JBM, Keesman KJ, Roman P, Janssen AJH. 2019. Development and validation of a physiologically based kinetic model for starting up and operation of the biological gas desulfurization process under haloalkaline conditions. Water Res X 4:100035. https://doi.org/10.1016/j.wroa.2019.100035
8. Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19:11–15.
9. Doyle J. 1991. DNA protocols for plants, p 283–293. In Molecular techniques in taxonomy. Springer Berlin Heidelberg, Berlin, Heidelberg.
10. 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
11. 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
12. 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