

The complete genome of a copper-tolerant strain of *Rhodanobacter* from a Superfund site

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ABSTRACT A copper-tolerant, siderophore-producing bacterium, *Rhodanobacter* sp. BL-MT-08, was isolated from soil from the Carpenter Snow Creek Mining District Superfund Site in Montana, which has a legacy of heavy metal contamination. The *Rhodanobacter* have been investigated for heavy metal bioremediation, so BL-MT-08 was selected for whole-genome sequencing.

KEYWORDS *Rhodanobacter*, copper, Superfund

Rhodanobacter have been found to dominate some heavy metal-contaminated environments, with prior genomic studies investigating their systems for heavy metal transport/resistance (1–3). After encountering a copper-tolerant *Rhodanobacter* strain in soil from a heavy metal-contaminated Superfund site, this strain was selected for genome sequencing (4). The original soil sample was collected from the Haystack adit (46.971,483 N 110.719,064 W) on 25 May 2022. To briefly summarize the prior isolation (4), BL-MT-08 was isolated on 2.5 mM copper-treated agar plates of ×1/5 diluted lysogeny broth supplemented with D-amino acids, as described by Nguyen and colleagues (5), as well as trace metals, using the BG-11 trace metal mixture (6) abbreviated as 1/5 LB*. Colonies were restructured onto chrome azurol S dyed plates to identify siderophore-positive colonies, yielding BL-MT-08 as circular yellowish-white colored colonies. A frozen 25% (v/v) glycerol stock of a liquid culture was stored at –70°C. The prior preliminary partial 16S gene sequence was 1,209 bp (4).

Omega BioTek's E.Z.N.A. Bacterial Kit was used for cell lysis of a 5 mL liquid 1/5 LB* culture started from single-colony frozen stock and grown at 28°C for 3 days. Following the manufacturer's protocol, TE buffer (100 µL) and lysozyme (10 µL, 50 mg/mL) were added to the cell pellet, and this mixture was incubated at 37°C for 10 min. Then, TL buffer (100 µL) and proteinase K (20 µL, 20 mg/mL) were added for an hour-long incubation at 65°C. Subsequently, 5 µL of RNase (10 mg/mL) was added, and the mixture stood at room temperature for 5 min. After the lysis, the NucleoBond High Molecular Weight (HMW) Kit (Macherey-Nagel) was used to recover HMW DNA following the manufacturer's instructions. Nanopore DNA sequencing (PromethION with the latest primer-free v14 library prep chemistry) and basecalling (Dorado v4.3, Super-Accurate, with default Q10 quality filtering) by a commercial vendor (Plasmidsaurus, Louisville, Kentucky, USA; Standard Bacteria Genome service) gave 205,010 raw reads with an N50 of 10,419 bp. Reads were filtered with Filtlong (version 0.2.1) first to a minimum length of 500 bp keeping the top 98% of reads by quality score and separately filtering at 1,000 bp/90% (7). The 500 bp/98% filtered reads were assembled via Flye (version 2.9-b1778) with the Nanopore high-quality setting, resulting in one circular contig (252× mean coverage) (8). This draft was polished with Racon (version 1.5.0, default settings) using a minimap2-derived (version 2.17-r941) .sam file generated with the map-ont setting and the 1,000 bp/90% reads. This genome was polished again using the same reads with medaka (version 1.7.2, r941_min_sup_g507 configuration, batch size of 50) (9, 10).

Editor Simon Roux, DOE Joint Genome Institute, Berkeley, California, USA

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The authors declare no conflict of interest.

See the funding table on p. 3.

Received 1 April 2026

Accepted 28 April 2026

Published 18 May 2026

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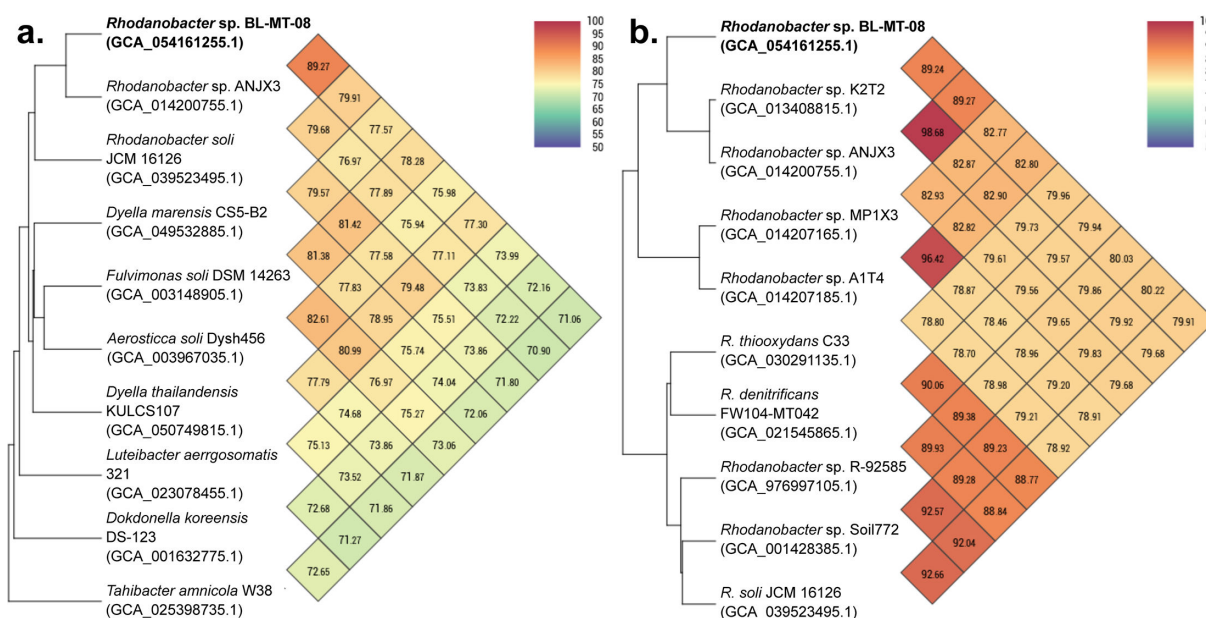


FIG 1 Sequence analysis of the *Rhodanobacter* sp. BL-MT-08 genome. The OrthoANI heatmaps were created with version 0.93.1 of the tool with four threads calculating the orthoANI in both directions with averaging of the result using a genome-to-genome-distance-calculator form of 2 (13). Initially, comparisons of many *Rhodanobacteraceae* strains were broadly made for the final figure, and a manual selection of strains was used comparing against: (a) representative strains across the *Rhodanobacteraceae* and (b) top hits found from the genus *Rhodanobacter*.

The final assembly was 4,233,771 bp with 62.9% guanine + cytosine (G + C) content. CheckM2 (version 1.1.0, default settings) scored the genome as 99.99% complete with 0.07% contamination (11). An Ortho Average Nucleotide Identity (orthoANI) Tool-based (version 0.93.1) comparison to other *Rhodanobacteraceae* showed orthoANI scores below 90% (Fig. 1a), but with top hits to *Rhodanobacter* spp. (Fig. 1b). A preliminary DFAST annotation (version 1.3.9, default settings) showed this genome had an *FeoAB* operon (3), but antiSMASH (version 8.0.4, relaxed strictness) did not identify any known metallophore pathways (12).

ACKNOWLEDGMENTS

The authors thank the Computational Chemistry and Bioinformatics Research Core within the University of Mississippi's Glycoscience Center of Research Excellence (NIH Project Number 5P20GM130460) for use of their computers and assistance with software installation for the genome assembly. The authors again thank Roger Hoogerheide at the Environmental Protection Agency and Colin McCoy, Chris Branson, and Kyle Paine at Tetra Tech for providing samples from the Carpenter Snow Creek site used in the original strain isolation. This research was conducted entirely in the United States of America.

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FUNDING

Funder	Grant(s)	Author(s)
National Institute of General Medical Sciences	5P20GM130460	Paul Davis Boudreau

AUTHOR CONTRIBUTIONS

Mohammed M. A. Ahmed, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft | Paul D. Boudreau, Conceptualization, Data curation, Formal analysis, Funding acquisition, Project administration, Supervision, Visualization, Writing – review and editing

DATA AVAILABILITY

The final version of the genome assembly for *Rhodanobacter* sp. BL-MT-08, as well as the Sequence Read Archive of the raw vendor reads and preliminary 16S partial gene sequence, are available on GenBank under BioProject number [PRJNA1140264](https://ncbi.nlm.nih.gov/bioproject/PRJNA1140264). The accession numbers for these data are [CP167872.1](https://ncbi.nlm.nih.gov/assembly/CP167872.1) for the genome, [SRR37849000](https://ncbi.nlm.nih.gov/sra/SRR37849000) for the sequence read archive, [PP868359](https://ncbi.nlm.nih.gov/seq/PP868359) for the 16S sequence, and [SAMN43036439](https://ncbi.nlm.nih.gov/samples/SAMN43036439) for the BioSample information on the collection site.

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