




Complete Genome Sequence of *Geobacter* sp. Strain FeAm09, a Moderately Acidophilic Soil Bacterium

Pooja Yadav,^a Sanjay Antony-Babu,^{a*} Erin Hayes,^a Olivia M. Healy,^a Donald Pan,^{a*} Wendy H. Yang,^{b,c} Whendee L. Silver,^d Christopher L. Anderson,^{a,e*} Adam Voshall,^a Samodha C. Fernando,^e Etsuko N. Moriyama,^{a,f}  Joshua R. Herr,^{f,g}  Karrie A. Weber^{a,h,i}

^aSchool of Biological Sciences, University of Nebraska—Lincoln, Lincoln, Nebraska, USA

^bDepartment of Plant Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

^cDepartment of Geology, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

^dDepartment of Environmental Science, Policy, and Management, University of California, Berkeley, Berkeley, California, USA

^eDepartment of Animal Science, University of Nebraska—Lincoln, Lincoln, Nebraska, USA

^fCenter for Plant Science Innovation, University of Nebraska—Lincoln, Lincoln, Nebraska, USA

^gDepartment of Plant Pathology, University of Nebraska—Lincoln, Lincoln, Nebraska, USA

^hDepartment of Earth and Atmospheric Sciences, University of Nebraska—Lincoln, Lincoln, Nebraska, USA

ⁱDaugherty Water for Food Institute, University of Nebraska—Lincoln, Nebraska, USA

ABSTRACT A moderately acidophilic *Geobacter* sp. strain, FeAm09, was isolated from forest soil. The complete genome sequence is 4,099,068 bp with an average GC content of 61.1%. No plasmids were detected. The genome contains a total of 3,843 genes and 3,608 protein-coding genes, including genes supporting iron and nitrogen biogeochemical cycling.

Microbial reduction of iron [Fe(III)] oxide minerals plays a significant role in coupled biogeochemical cycles in unsaturated soils (1, 2) and in saturated soils and sediments (3–6). To date, very few Fe(III)-reducing bacteria have been isolated from unsaturated soils. Here, we report the complete genome of *Geobacter* sp. strain FeAm09, which was isolated as an Fe(III)-reducing bacterium from moderately acidic unsaturated tropical forest soil in Puerto Rico (7) following enrichment in piperazine-*N,N'*-bis(3-propanesulfonic acid) (PIPPS)-buffered medium (pH 5.0) amended with synthetic ferrihydrite (8) under an anoxic atmosphere, transfer to medium containing Fe(III)-nitrilotriacetic acid (NTA), and isolation with anaerobic shake tubes (80:20 N₂/CO₂ atmosphere) (9, 10).

Cells of *Geobacter* sp. strain FeAm09 were cultivated with fumarate (40 mM) and acetate (20 mM) under an anoxic atmosphere (100% argon). Cultures were incubated at 37°C in the dark until mid-log phase (approximately 48 h), harvested by centrifugation (5 min at 12,000 × *g*), and resuspended in DNA extraction buffer (11). Cells were immediately lysed by heating (95°C for 15 min). Genomic DNA from the lysate was extracted using phenol-chloroform-isoamyl alcohol (25:24:1) followed by ethanol precipitation (12). PacBio 20-kb SMRTbell library preparation (P6-C4 chemistry) using the PacBio RS platform was completed by the University of Delaware DNA Sequencing and Genotyping Center. A total of 45,767 reads with a mean read length of 12,898 nucleotides were assembled into a single contig using the PacBio Hierarchical Genome Assembly Process (HGAP) pipeline version 3.0 with default parameters in single-molecule real-time (SMRT) Portal version 2.3.0 (13). The assembly revealed a single circular chromosome with a GC content of 61.1%. Genome annotation using PGAP version 4.8 predicted 3,843 total genes (14, 15). Totals of 6 rRNAs, 52 tRNAs, 1 transfer-messenger RNA, and 3,608 protein-coding sequences were identified (Fig. 1).

Geobacter sp. strain FeAm09 belongs to the *Geobacteraceae* family and is a Gram-

Citation Yadav P, Antony-Babu S, Hayes E, Healy OM, Pan D, Yang WH, Silver WL, Anderson CL, Voshall A, Fernando SC, Moriyama EN, Herr JR, Weber KA. 2021. Complete genome sequence of *Geobacter* sp. strain FeAm09, a moderately acidophilic soil bacterium. Microbiol Resour Announc 10:e00979-20. <https://doi.org/10.1128/MRA.00979-20>.

Editor John J. Dennehy, Queens College

Copyright © 2021 Yadav 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/).

Address correspondence to Karrie A. Weber, kweber@unl.edu.

* Present address: Sanjay Antony-Babu, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, Texas, USA; Donald Pan, Department of Ecology and Environmental Studies, The Water School, Florida Gulf Coast University, Fort Myers, Florida, USA; Christopher L. Anderson, Department of Civil and Environmental Engineering, Northeastern University, Boston, Massachusetts, USA.

Received 25 August 2020

Accepted 17 December 2020

Published 14 January 2021

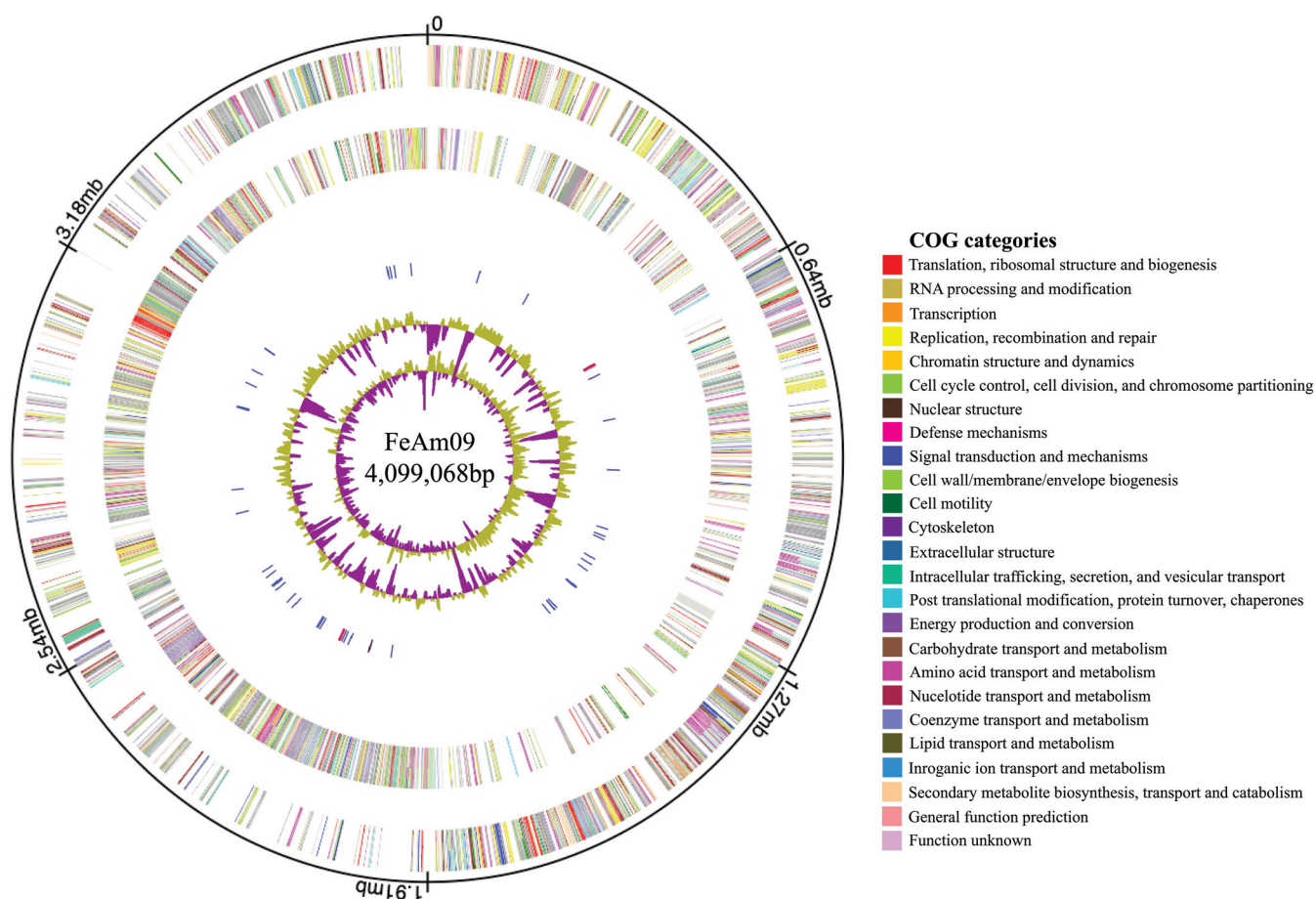


FIG 1 Circular genome map of *Geobacter* sp. strain FeAm09, generated by using DNAPlotter from Artemis version 18.1.0 (Wellcome Sanger Institute) (21). From the outside to the center: circle 1, DNA base position; circle 2, genes on the forward strand (color by Clusters of Orthologous Groups of proteins [COG] category); circle 3, genes on the reverse strand (color by COG category); circle 4, RNA genes (tRNAs, blue; rRNAs, red; other RNAs, black); circle 5, GC content plotted using a 10-kb window size (purple represents values below average, while olive represents values above average); circle 6, GC skew $[(G + C)/(G - C)]$ plotted using a 10-kb window size (purple indicates values below average, while olive represents values above average).

negative bacterium. Similar to other known Fe(III)-reducing *Geobacter* spp., the annotated genome of strain FeAm09 revealed putative genes for *c*-type cytochromes and type IV pili. The only nitrate reduction genes identified in the genome were for dissimilatory nitrate reduction to ammonium (nitrate reductase and nitrite reductase). Genes annotated for reduction of dimethyl sulfoxide (DMSO), thiosulfate, and sulfite were also identified, as were cytochrome *bd* ubiquinol terminal oxidase (*cydB* and *cydA*), similar to *Geobacter sulfurreducens* (16, 17). Hemerythrin and genes associated with oxidative stress tolerance (catalase peroxidase and rubrerythrin) were also identified. In addition to genes that support heterotrophy through the use of organic electron donors (formate dehydrogenase and citrate lyase), a NiFe hydrogenase (*hydB*) capable of converting H_2 to H^+ was found, which would enable lithotrophic growth. Genes associated with the reverse tricarboxylic acid (TCA) cycle (*mdh*, *fumB*, *frdA*, *frdB*, *sucC*, *sucD*, *korA*, *korB*, *icdI*, *acnB*, and *citC*) were identified, which could support autotrophy (18–20). Genes linked to assimilatory sulfate reduction and nitrogen fixation were also identified. Together, these physiological capabilities of *Geobacter* sp. strain FeAm09 indicate a diverse lifestyle that can adapt to fluctuating environmental conditions commonly found in soil systems.

Data availability. The genome sequence was deposited in GenBank under BioProject PRJNA555606, with the accession numbers CP042466 and SRX9278424.

ACKNOWLEDGMENTS

Import of soils and cultivation of Fe(III)-reducing bacteria were conducted under USDA Animal and Plant Health Inspection Service (APHIS) permit P526P-08-03-03546.

This research was supported by funds from the National Science Foundation (grants DEB-0841993 to K.A.W. and DEB-0842385 to W.L.S.) and the National Science Foundation Established Program to Stimulate Competitive Research (EPSCoR) Center for Root and Rhizobiome Innovation Award (grant OIA-1557471 to K.A.W., E.N.M., and J.R.H.). Computational analyses were performed with resources at the University of Nebraska—Lincoln Holland Computing Center.

REFERENCES

- Dubinsky EA, Silver WL, Firestone MK. 2010. Tropical forest soil microbial communities couple iron and carbon biogeochemistry. *Ecology* 91:2604–2612. <https://doi.org/10.1890/09-1365.1>.
- Yang WH, Liptzin D. 2015. High potential for iron reduction in upland soils. *Ecology* 96:2015–2020. <https://doi.org/10.1890/14-2097.1>.
- Weber KA, Achenbach LA, Coates JD. 2006. Microorganisms pumping iron: anaerobic microbial iron oxidation and reduction. *Nat Rev Microbiol* 4:752–764. <https://doi.org/10.1038/nrmicro1490>.
- Li X, Hou L, Liu M, Zheng Y, Yin G, Lin X, Cheng L, Li Y, Hu X. 2015. Evidence of nitrogen loss from anaerobic ammonium oxidation coupled with ferric iron reduction in an intertidal wetland. *Environ Sci Technol* 49:11560–11568. <https://doi.org/10.1021/acs.est.5b03419>.
- Zhang Z, Moon HS, Myneni SC, Jaffé PR. 2017. Effect of dissimilatory iron and sulfate reduction on arsenic dynamics in the wetland rhizosphere and its bioaccumulation in wetland plants (*Scirpus actus*). *J Hazard Mater* 321:382–389. <https://doi.org/10.1016/j.jhazmat.2016.06.022>.
- Guan Q, Cao W, Wang M, Wu G, Wang F, Jiang C, Tao Y, Gao Y. 2018. Nitrogen loss through anaerobic ammonium oxidation coupled with iron reduction in a mangrove wetland. *Eur J Soil Sci* 69:732–741. <https://doi.org/10.1111/ejss.12552>.
- Yang WH, Weber KA, Silver WL. 2012. Nitrogen loss from soil through anaerobic ammonium oxidation coupled to iron reduction. *Nat Geosci* 5:538–541. <https://doi.org/10.1038/ngeo1530>.
- Lovley DR, Phillips EJ. 1988. Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. *Appl Environ Microbiol* 54:1472–1480. <https://doi.org/10.1128/AEM.54.6.1472-1480.1988>.
- Widdel F, Bak F. 1992. Gram-negative mesophilic sulfate-reducing bacteria, p 3352–3378. In Balows A, Trüper HG, Dworkin M, Harder W, Schleifer KH (ed), *The prokaryotes*. Springer, New York, NY.
- Coates JD, Phillips E, Lonergan DJ, Jenter H, Lovley DR. 1996. Isolation of *Geobacter* species from diverse sedimentary environments. *Appl Environ Microbiol* 62:1531–1536. <https://doi.org/10.1128/AEM.62.5.1531-1536.1996>.
- Zhou J, Bruns MA, Tiedje JM. 1996. DNA recovery from soils of diverse composition. *Appl Environ Microbiol* 62:316–322. <https://doi.org/10.1128/AEM.62.2.316-322.1996>.
- Sambrook J, Russell DW. 2001. *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Chin C-S, 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>.
- Lagesen K, Hallin P, Rødland EA, Staerfeldt H-H, Rognes T, Ussery DW. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res* 35:3100–3108. <https://doi.org/10.1093/nar/gkm160>.
- Tatusova T, DiCuccio M, Badretdin A, Chetvermin 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>.
- Lin W, Coppi MV, Lovley D. 2004. *Geobacter sulfurreducens* can grow with oxygen as a terminal electron acceptor. *Appl Environ Microbiol* 70:2525–2528. <https://doi.org/10.1128/aem.70.4.2525-2528.2004>.
- Méthé BA, Nelson KE, Eisen JA, Paulsen IT, Nelson W, Heidelberg JF, Wu D, Wu M, Ward N, Beanan MJ, Dodson RJ, Madupu R, Brinkac LM, Daugherty SC, DeBoy RT, Durkin AS, Gwinn M, Kolonay JF, Sullivan SA, Haft DH, Selengut J, Davidsen TM, Zafar N, White O, Tran B, Romero C, Forberger HA, Weidman J, Khouri H, Feldblyum TV, Utterback TR, Van Aken SE, Lovley DR, Fraser CM. 2003. Genome of *Geobacter sulfurreducens*: metal reduction in subsurface environments. *Science* 302:1967–1969. <https://doi.org/10.1126/science.1088727>.
- Mall A, Sobotta J, Huber C, Tschirner C, Kowarschik S, Bačnik K, Mergelsberg M, Boll M, Hügler M, Eisenreich W, Berg IA. 2018. Reversibility of citrate synthase allows autotrophic growth of a thermophilic bacterium. *Science* 359:563–567. <https://doi.org/10.1126/science.aao2410>.
- Nunoura T, Chikaraishi Y, Izaki R, Suwa T, Sato T, Harada T, Mori K, Kato Y, Miyazaki M, Shimamura S, Yanagawa K, Shuto A, Ohkouchi N, Fujita N, Takaki Y, Atomi H, Takai K. 2018. A primordial and reversible TCA cycle in a facultatively chemolithoautotrophic thermophile. *Science* 359:559–563. <https://doi.org/10.1126/science.aao3407>.
- Zhang T, Shi X-C, Ding R, Xu K, Tremblay P-L. 2020. The hidden chemolithoautotrophic metabolism of *Geobacter sulfurreducens* uncovered by adaptation to formate. *ISME J* 14:2078–2089. <https://doi.org/10.1038/s41396-020-0673-8>.
- Carver T, Thomson N, Bleasby A, Berriman M, Parkhill J. 2009. DNAPlotter: circular and linear interactive genome visualization. *Bioinformatics* 25:119–120. <https://doi.org/10.1093/bioinformatics/btn578>.