



Complete Genome Sequence of *Ferrigenium kumadai* An22, a Microaerophilic Iron-Oxidizing Bacterium Isolated from a Paddy Field Soil

Takeshi Watanabe,^a Ashraf Khalifa,^{b,c} Susumu Asakawa^a

^aLaboratory of Soil Biology and Chemistry, Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya, Japan
^bBiological Sciences Department, College of Science, King Faisal University, Al-Ahsa, Saudi Arabia
^cBotany and Microbiology Department, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt

ABSTRACT Ferrigenium kumadai $An22^{T}$ (= JCM 30584^{T} = NBRC 112974^{T} = ATCC TSD- 51^{T}) is a microaerophilic iron oxidizer isolated from paddy field soil and belongs to the family *Gallionellaceae*. Here, we report the complete genome sequence of *F. kumadai* $An22^{T}$, which was obtained from the hybrid data of Oxford Nanopore long-read and Illumina short-read sequencing.

I icroaerophilic iron-oxidizing bacteria, which are capable of oxidizing ferrous iron (Fe) under circumneutral pH and microoxic conditions, are a key player in the Fe redox cycle in environments (1). However, only a few strains of microaerophilic Fe(II) oxidizers have been identified thus far from freshwater environments (2), and their genome information has been limited. In our previous study, a novel microaerophilic Fe(II) oxidizer, *Ferrigenium kumadai*, isolated from paddy soil has been described (3). Here, we report the complete genome sequences of *F. kumadai* An22^T (= JCM 30584^T = NBRC 112974^T = ATCC TSD-51^T).

Cultivation of strain An22^T and DNA preparation were described previously (3). Genome sequencing analysis was performed on the Illumina MiSeq platform (San Diego, CA) with paired-end libraries (<500 bp) and GridION with R9.4.1 flow cell (Oxford Nanopore Technologies [ONT], Oxford, UK). DNA libraries for MiSeq and ONT sequencing were prepared with a KAPA HyperPlus kit (Kapa Biosystems, Wilmington, MA) and FastGene adapter kit (Nippon Genetics, Tokyo, Japan) and with a rapid barcoding kit (ONT), respectively. Basecalling of ONT sequences was performed with Albacore v2.3.1 (ONT). Default parameters were used for all software in this study unless otherwise specified. In total, 151,075,784 (read 1, 74,493,341; read 2, 76,582,443) and 1,812,690,122 bp were obtained from MiSeq and ONT sequencing (= 58.7-fold and 704.6-fold genome coverage), respectively. The N_{50} value of raw ONT reads was 1,790 bp with a maximum length of 222,018 bp. Low-guality MiSeg reads (Q score, <20; single read, <127 bp) were removed with Sickle v1.33 (https://github.com/ najoshi/sickle). Short ONT reads (<1,000 bp) were filtered out with Filtlong v0.2.0 (https:// github.com/rrwick/Filtlong), and then error correction of the reads was carried out on FMLRC v0.1.2 (4) with the MiSeq reads as references. The quality-controlled MiSeq and ONT reads were assembled on MaSuRCA v3.2.8 (5). The assembled sequences were polished with Pilon v1.22 (6), followed by circularization of the contigs with Circlator v1.5.5 (7). The assembly quality was confirmed with BUSCO v5 (8) on gVolante v2.0.0 (9), and there was 98.63% completeness when examined with the ortholog set of Betaproteobacteria. Genes were annotated with DFAST v10 (10). Genes for fundamental metabolic pathways were searched on BlastKOALA v2.2 (11). The orthologous genes for Fe(II) oxidation, encoding MtoAB (12), PioAB (13), and Cyc2 (14, 15), were searched on the MBGD update 2018 (16) with the genome sequences of Sideroxydans lithotrophicus ES-1 (GenBank accession number CP001965.1), Rhodopseudomonas palustris TIE-1 (CP058907.1), Mariprofundus ferrooxydans

Citation Watanabe T, Khalifa A, Asakawa S. 2021. Complete genome sequence of *Ferrigenium kumadai* An22, a microaerophilic iron-oxidizing bacterium isolated from a paddy field soil. Microbiol Resour Announc 10:e00346-21. https://doi.org/10.1128/MRA.00346-21.

Editor J. Cameron Thrash, University of Southern California

Copyright © 2021 Watanabe et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Takeshi Watanabe, watanabe@agr.nagoya-u.ac.jp.

Received 2 April 2021 **Accepted** 11 June 2021 **Published** 8 July 2021 PV-1^T (DS022294.1), and Acidithiobacillus ferrooxidans ATCC 23270^T (CP001219.1). An orthologous gene cluster table among the genome sequences used was created (17), after which candidate genes were searched with the homology search program in MBGD based on BLASTP (18).

The genome size of strain $An22^{T}$ was 2,572,603 bp with a G+C content of 60.6%. The genome contains 2 sets of rRNA operons, 50 tRNA sequences, and 2,428 protein-coding genes, including a gene for Rubisco and a gene set for nitrogenase. A gene encoding putative Cyc2 (BBI98728.1) was found as a sole candidate gene of Fe(II) oxidation.

Data availability. The genome sequence of *F. kumadai* An22^T was deposited to the DDBJ database under the accession number AP019536.1. The raw sequencing data were deposited under BioProject accession number PRJDB7995 and SRA accession numbers DRR168795 and DRR168796.

ACKNOWLEDGMENTS

This study was supported in part by grants-in-aid from the JSPS KAKENHI (18K05372 and 21K05326) and a research grant from the Institute for Fermentation, Osaka (G-2015-1-012 and G-2021-1-004). The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

Genome sequencing was outsourced to the Bioengineering Lab Ltd. (Sagamihara, Japan).

REFERENCES

- Kappler A, Bryce C, Mansor M, Lueder U, Byrne JM, Swanner ED. 2021. An evolving view on biogeochemical cycling of iron. Nat Rev Microbiol 19:360–374. https://doi.org/10.1038/s41579-020-00502-7.
- Kato S, Krepski S, Chan C, Itoh T, Ohkuma M. 2014. Ferriphaselus amnicola gen. nov., sp. nov., a neutrophilic, stalk-forming, iron-oxidizing bacterium isolated from an iron-rich groundwater seep. Int J Syst Evol Microbiol 64:921–925. https://doi.org/10.1099/ijs.0.058487-0.
- Khalifa A, Nakasuji Y, Saka N, Honjo H, Asakawa S, Watanabe T. 2018. Ferrigenium kumadai gen. nov., sp. nov., a microaerophilic iron-oxidizing bacterium isolated from a paddy field soil. Int J Syst Evol Microbiol 68:2587–2592. https://doi.org/10.1099/ijsem.0.002882.
- Wang JR, Holt J, McMillan L, Jones CD. 2018. FMLRC: hybrid long read error correction using an FM-index. BMC Bioinformatics 19:50. https://doi .org/10.1186/s12859-018-2051-3.
- Zimin AV, Marçais G, Puiu D, Roberts M, Salzberg SL, Yorke JA. 2013. The MaSuRCA genome assembler. Bioinformatics 29:2669–2677. https://doi .org/10.1093/bioinformatics/btt476.
- Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One 9:e112963. https://doi.org/10.1371/journal.pone.0112963.
- Hunt M, Silva ND, Otto TD, Parkhill J, Keane JA, Harris SR. 2015. Circlator: automated circularization of genome assemblies using long sequencing reads. Genome Biol 16:294. https://doi.org/10.1186/s13059-015-0849-0.
- Seppey M, Manni M, Zdobnov EM. 2019. BUSCO: assessing genome assembly and annotation completeness. Methods Mol Biol 1962:227–245.
- Nishimura O, Hara Y, Kuraku S. 2017. gVolante for standardizing completeness assessment of genome and transcriptome assemblies. Bioinformatics 33:3635–3637. https://doi.org/10.1093/bioinformatics/btx445.
- Tanizawa Y, Fujisawa T, Kaminuma E, Nakamura Y, Arita M. 2016. DFAST and DAGA: web-based integrated genome annotation tools and resources. Biosci Microbiota Food Health 35:173–184. https://doi.org/10.12938/bmfh.16-003.
- 11. Kanehisa M, Sato Y, Morishima K. 2016. BlastKOALA and GhostKOALA:

KEGG tools for functional characterization of genome and metagenome sequences. J Mol Biol 428:726–731. https://doi.org/10.1016/j.jmb.2015 .11.006.

- Liu J, Wang Z, Belchik SM, Edwards MJ, Liu C, Kennedy DW, Merkley ED, Lipton MS, Butt JN, Richardson DJ, Zachara JM, Fredrickson JK, Rosso KM, Shi L. 2012. Identification and characterization of MtoA: a decaheme *c*-type cytochrome of the neutrophilic Fe(II)-oxidizing bacterium *Sideroxydans lithotrophicus* ES-1. Front Microbiol 3:37. https://doi.org/ 10.3389/fmicb.2012.00037.
- Jiao Y, Newman DK. 2007. The *pio* operon is essential for phototrophic Fe(II) oxidation in *Rhodopseudomonas palustris* TIE-1. J Bacteriol 189:1765–1773. https://doi.org/10.1128/JB.00776-06.
- Valdés J, Pedroso I, Quatrini R, Dodson RJ, Tettelin H, Blake R, II, Eisen JA, Holmes DS. 2008. Acidithiobacillus ferrooxidans metabolism: from genome sequence to industrial applications. BMC Genomics 9:597. https://doi.org/ 10.1186/1471-2164-9-597.
- Barco RA, Emerson D, Sylvan JB, Orcutt BN, Meyers MEJ, Ramírez GA, Zhong JD, Edwards KJ. 2015. New insight into microbial iron oxidation as revealed by the proteomic profile of an obligate ion-oxidizing chemolithoautotroph. Appl Environ Microbiol 81:5927–5937. https://doi.org/10 .1128/AEM.01374-15.
- Uchiyama I, Mihara M, Nishide H, Chiba H, Kato M. 2019. MBGD update 2018: microbial genome database based on hierarchical orthology relations covering closely related and distantly related comparisons. Nucleic Acids Res 47:D382–D389. https://doi.org/10.1093/nar/gky1054.
- Watanabe T, Khalifa A, Asakawa S. 2021. Orthologous gene cluster table among the genome sequences of Fe-oxidizing bacteria. https://doi.org/ 10.6084/m9.figshare.14754372.v1.
- Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402. https://doi.org/10.1093/nar/ 25.17.3389.