



Draft Genome Sequence of *Brevundimonas* sp. Strain T2.26MG-97, Isolated from a Rock Core Sample from 492.6 Meters Deep on the Subsurface of the Iberian Pyrite Belt

 E. Rodríguez-Robles,^a J. M. Martínez,^a T. Leandro,^{a,b}  R. Amils^{a,c}

^aCentro de Biología Molecular Severo Ochoa (CBMSO, CSIC-UAM), Universidad Autónoma de Madrid, Cantoblanco, Madrid, Spain

^bCentre for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal

^cCentro de Astrobiología (CAB, CSIC-INTA), Carretera de Torrejón a Ajalvir, Torrejón de Ardoz, Madrid, Spain

ABSTRACT The draft genome of *Brevundimonas* sp. strain T2.26MG-97, isolated at a depth of 492.6 m in the subsurface of the Iberian Pyrite Belt, is reported here. It consists of 262 scaffolds with a total genome length of 3.68 Mbp, where 3,549 coding DNA sequences have been annotated.

The genus *Brevundimonas* is classified within the class *Alphaproteobacteria* and family *Caulobacteraceae*. *Brevundimonas* species are characterized as Gram-negative, aerobic or facultative anaerobic, rod-shaped, non-spore-forming bacteria (1) and have been isolated from diverse environments. These include extreme environments of astrobiological interest like the Antarctic Dry Valleys (2), Everest (3), ice glaciers in Greenland (4), and the Arctic (5).

Brevundimonas sp. strain T2.26MG-97 was isolated under strict anaerobic conditions from a 492.6-m-below-surface rock core sample from the Iberian Pyrite Belt (IPB), in the framework of the Iberian Pyritic Belt Subsurface Life (IPBSL) drilling project (6). The aim of this project was to characterize the subsurface microbial diversity in the IPB, a geological formation in southwest Spain considered a terrestrial geochemical and mineralogical Mars analogue (7). Drilling and sampling were performed as described by Puente-Sánchez et al. (8). Culturing, isolation, and taxonomic identification of *Brevundimonas* sp. strain T2.26MG-97 were carried out as described by Leandro et al. (9).

For whole-genome sequencing, genomic DNA was extracted by the cetyltrimethylammonium bromide (CTAB)-based method (10), and its concentration was determined with a Qubit v.2.0 fluorometer (Invitrogen, USA). It was submitted to the MicrobesNG sequencing facility (University of Birmingham, UK) for Illumina MiSeq sequencing with 27× coverage. A Nextera XT kit (Illumina) was used to prepare the genomic libraries following the manufacturer's protocol. The run resulted in 242,736 2 × 250-bp paired-end reads with a length between 36 and 251 bp. The reads were trimmed by Trimmomatic v.0.36 (11), and quality analyses of the reads were performed using FastQC v.0.11.8 software (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). *De novo* assembly was carried out using SPAdes v.3.12.0 (“-careful” option) (12), and the assembly of extrachromosomal genetic elements was performed using Recycler, which uses the read coverage of contigs to distinguish between plasmids and chromosomes (13). Plasmid contigs were aligned against the chromosomal assembly with Mauve Aligner v.2.4.0 (14) to separate chromosomal and plasmid contigs. Contigs were extended and merged into scaffolds using SSPACE software (15). Gaps created with SSPACE were closed with GapFiller v.1-10 software (16). This resulted in a chromosome in 262 scaffolds, with an N_{50} value of 35.42 kb, a GC content of 67.20%, and a total size of 3,683,793 bp, and one plasmid of 19,563 bp in two contigs with a N_{50} value of 17.27 kb. Default parameters were used for all software unless otherwise specified.

Citation Rodríguez-Robles E, Martínez JM, Leandro T, Amils R. 2019. Draft genome sequence of *Brevundimonas* sp. strain T2.26MG-97, isolated from a rock core sample from 492.6 meters deep on the subsurface of the Iberian Pyrite Belt. *Microbiol Resour Announc* 8: e00375-19. <https://doi.org/10.1128/MRA.00375-19>.

Editor Vincent Bruno, University of Maryland School of Medicine

Copyright © 2019 Rodríguez-Robles 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 R. Amils, ramils@cbm.csic.es.

Received 30 March 2019

Accepted 16 July 2019

Published 8 August 2019

The complete genome was annotated with Prokka v1.12 software (17), RAST (18), and BlastKOALA v2.1 (19), which predicted a total of 3,549 coding DNA sequences, 1 rRNA operon, and 53 tRNA genes. This allowed the identification of genes involved in dissimilatory nitrate reduction to ammonium, denitrification, aerobic respiration control protein ArcA, arsenate reductase, and resistance to heavy metals (Cu, Co, Zn, Cd, As, and Cr). Regarding the plasmid, 16 coding DNA sequences have been annotated and multiple hypothetical proteins detected.

The comparative analysis of the genome of *Brevundimonas* sp. strain T2.26MG-97 with those from other microbial subsurface isolates should provide insights on the mechanisms used by microorganisms to inhabit deep terrestrial anaerobic environments under oligotrophic conditions and in the absence of light.

Data availability. Reads have been deposited at DDBJ/ENA/GenBank under the accession numbers [ERR2864313](#), [ERR2864314](#), and [ERR2864315](#). The complete genome sequences and annotations have been deposited under the accession numbers [UXHF00000000](#) for the chromosome and [UXHD00000000](#) for the plasmid, and they can be found under the BioProject accession number [PRJEB29440](#). The versions described in this paper are the first versions, UXHF01000000 and UXHD01000000.

ACKNOWLEDGMENTS

We thank all of the IPBSL project team members for facilitating access to the sample. Genome sequencing was provided by MicrobesNG (<http://www.microbesng.uk>). Next-generation sequencing (NGS) analysis was performed by the Genomics and NGS Core Facility at the CBMSO (CSIC-UAM).

This work was supported by MINECO grant CGL2015-66242-R.

REFERENCES

- Abraham WR, Rohde M, Bennisar A. 2014. The family *Caulobacteraceae*, p 179–205. In Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (ed), *The prokaryotes*. Springer, Berlin, Germany.
- Dartnell LR, Hunter SJ, Lovell KV, Coates AJ, Ward JM. 2010. Low-temperature ionizing radiation resistance of *Deinococcus radiodurans* and Antarctic Dry Valley bacteria. *Astrobiology* 10:717–732. <https://doi.org/10.1089/ast.2009.0439>.
- Liu Y, Yao T, Jiao N, Kang S, Huang S, Li Q, Wang K, Liu X. 2009. Culturable bacteria in glacial meltwater at 6,350 m on the East Rongbuk Glacier, Mount Everest. *Extremophiles* 13:89–99. <https://doi.org/10.1007/s00792-008-0200-8>.
- Sheridan PP, Miteva VI, Brenchley JE. 2003. Phylogenetic analysis of anaerobic psychrophilic enrichment cultures obtained from a Greenland glacier ice core. *Appl Environ Microbiol* 69:2153–2160. <https://doi.org/10.1128/AEM.69.4.2153-2160.2003>.
- Schuergler AC, Lee P. 2015. Microbial ecology of a crewed rover traverse in the Arctic: low microbial dispersal and implications for planetary protection on human Mars missions. *Astrobiology* 15:478–491. <https://doi.org/10.1089/ast.2015.1289>.
- Amils R, Fernández-Remolar D, Parro V, Rodríguez-Manfredi JA, Timmis K, Oggerin M, Sánchez-Román M, López FJ, Fernández JP, Puente F, Gómez-Ortiz D, Briones C, Gómez F, Omeregíe E, García M, Rodríguez N, Sanz JL, the IPBSL Team. 2013. Iberian Pyrite Belt Subsurface Life (IPBSL), a drilling project of bihydrometallurgical interest. *Adv Mat Res* 825: 15–18. <https://doi.org/10.4028/www.scientific.net/AMR.825.15>.
- Amils R, Fernández-Remolar D, IPBSL Team. 2014. Río Tinto: a geochemical and mineralogical terrestrial analogue of Mars. *Life (Basel)* 4:511–534. <https://doi.org/10.3390/life4030511>.
- Puente-Sánchez F, Arce-Rodríguez A, Oggerin M, Carcía-Villadangos M, Moreno-Paz M, Blanco Y, Rodríguez N, Bird L, Lincoln SL, Tornos F, Prieto-Ballesteros O, Freeman KH, Pieper DH, Timmis KN, Amils R, Parro V. 2018. Viable cyanobacteria in the deep continental subsurface. *Proc Natl Acad Sci U S A*. <https://doi.org/10.1073/pnas.1808176115>.
- Leandro T, Rodríguez N, Rojas P, Sanz JL, da Costa MS, Amils R. 2018. Study of methanogenic enrichment cultures of rock cores from the deep subsurface of the Iberian Pyritic Belt. *Heliyon* 4:e00605. <https://doi.org/10.1016/j.heliyon.2018.e00605>.
- Wilson K. 2001. Preparation of genomic DNA from bacteria. *Curr Protoc Mol Biol* Chapter 2:Unit 2.4. <https://doi.org/10.1002/0471142727.mb0204s56>.
- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
- 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>.
- Rozov R, Brown Kav A, Bogumil D, Shterzer N, Halperin E, Mizrahi I, Shamir R. 2017. Recycler: an algorithm for detecting plasmids from *de novo* assembly graphs. *Bioinformatics* 33:475–482. <https://doi.org/10.1093/bioinformatics/btw651>.
- Darling AC, Mau B, Blattner FR, Perna NT. 2004. Mauve: multiple alignment of conserved genomic sequence with rearrangements. *Genome Res* 14:1394–1403. <https://doi.org/10.1101/gr.2289704>.
- Boetzer M, Henkel CV, Jansen HJ, Butler D, Pirovano W. 2011. Scaffolding pre-assembled contigs using SSPACE. *Bioinformatics* 27:578–579. <https://doi.org/10.1093/bioinformatics/btq683>.
- Nadalin F, Vezzi F, Policriti A. 2012. GapFiller: a *de novo* assembly approach to fill the gap within paired reads. *BMC Bioinformatics* 13:58. <https://doi.org/10.1186/1471-2105-13-514-58>.
- Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30:2068–2069. <https://doi.org/10.1093/bioinformatics/btu153>.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: rapid annotations using subsystems technology. *BMC Genomics* 9:75. <https://doi.org/10.1186/1471-2164-9-75>.
- 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>.