

GENOME SEQUENCES



Draft Genome Sequence of *Rhodoplanes* sp. Strain T2.26MG-98, Isolated from 492.6 Meters Deep on the Subsurface of the Iberian Pyrite Belt

N. Mariñán,^a DJosé 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 ^bCenter for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal ^cCentro de Astrobiología (CAB, CSIC-INTA), Torrejón de Ardoz, Madrid, Spain

ABSTRACT *Rhodoplanes* sp. strain T2.26MG-98 was isolated from the deep subsurface of the Iberian Pyrite Belt. We report its draft genome, consisting of 214 contigs with a chromosome of \sim 5.6 Mb and a 53.7-kb plasmid. The chromosome annotation identified 4,994 coding DNA sequences, 1 rRNA operon, and 57 tRNA genes.

Rhodoplanes is a facultative anoxygenic phototroph genus classified within the family *Hyphomicrobiaceae*. Its growth is possible both photoheterotrophically under anoxic conditions and chemotrophically under oxic conditions in the dark or under anoxic conditions by denitrification (1). *Rhodoplanes* sp. strain T2.26MG-98 was isolated from a strictly anaerobic methanogenic enrichment culture using a 492.6-m-deep core sample obtained from a drilling project designed to identify the microbially diverse populations existing in the deep subsurface of the Iberian Pyrite Belt (southwestern Spain) (2). The borehole coordinates were 37°43′45.42″N, 6°33′23.57″W. Drilling was performed as described in reference 3. A powdered rock sample (~5 g) was used as inoculum. The isolation and growth of *Rhodoplanes* sp. strain T2.26MG-98 were done under the conditions described in reference 4.

Genomic DNA of strain T2.26MG-98 was extracted and the 16S rRNA gene amplified under the conditions described in reference 5. Reads were edited and assembled by Mega X (6) and Bioedit 7.0.4 (7). The complete 16S rRNA gene sequence was compared with sequences on other GenBank databases on NCBI using BLAST (8). The closest sequence was found to belong to *Rhodoplanes piscinae* JA266 (98.96%), a facultative anaerobic microorganism (9).

Genomic DNA was sequenced using the Illumina MiSeq platform, with 39.6× mean coverage. The sequencing run generated 533,028 2 × 250-paired-end reads with a mean length of 503 bp. The reads were trimmed by Trimmomatic 0.36, and quality analyses were performed for the reads using FastQC v.0.11.8 software (http://www .bioinformatics.babraham.ac.uk/projects/fastqc/). *De novo* assembly was carried out using the software SPAdes 3.9.0 (10), and the assembly of extrachromosomal genetic elements was carried out using Recycler (11). Plasmid contigs were aligned against the chromosomal assembly with Mauve Aligner 2.4.0 (12). Contigs were ordered and new scaffolds were created by means of SSPACE software (13) using the genome of *Rhodoplanes piscinae* DSM 19946 as the reference (GenBank accession number NPEW00000000). This yielded a chromosome in 214 scaffolds, with an N_{50} of 50.61 kb, a GC content of 70.24%, one chromosome of 5,589,602 bp, and one plasmid of 53,767 bp in a single contig. Default parameters were used for all software unless otherwise specified.

Gene prediction and annotation were carried out using three methods, PROKKA v1.12 software (14), BlastKOALA v2.1 (15), and the RAST platform (16), using *Rhodo*-

Citation Mariñán N, Martínez JM, Leandro T, Amils R. 2019. Draft genome sequence of *Rhodoplanes* sp. strain T2.26MG-98, isolated from 492.6 meters deep on the subsurface of the Iberian Pyrite Belt. Microbiol Resour Announc 8:e00070-19. https://doi.org/10.1128/ MRA.00070-19.

Editor David A. Baltrus, University of Arizona Copyright © 2019 Mariñán et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to R. Amils, ramils@cbm.csic.es.

Received 31 January 2019 Accepted 21 March 2019 Published 18 April 2019 *planes piscinae* DSM 19946 as a reference genome. The CRISPRCasFinder Web server was used to find clustered regularly interspaced short palindromic repeat (CRISPR) structures (17). A total of 4,994 coding DNA sequences, 57 tRNA genes, 1 rRNA operon, 1 transfer-messenger RNA (tmRNA), 445 signal peptides, and 4 CRISPR sequences were identified. A complete set of genes encoding nitrate ammonification, denitrification, and sulfur oxidation, as well as thiosulfate reduction, was detected. A gene encoding a periplasmic iron oxidase (UniProtKB accession number P50500) identified in *Acidithiobacillus ferrooxidans* (18) was also detected in the genome. The strain also presents genes encoding the reduction of perchlorate, an activity of astrobiological interest (19). Regarding annotation of the 53.7-kb plasmid, 69 coding genes were identified. Genes involved in the regulation of the plasmid copy number (*repA*), one signal peptide, and one tRNA were identified.

The analysis of the genome of *Rhodoplanes* sp. strain T2.26MG-98 should be helpful in identification of the mechanisms used for life to develop in the extreme oligotrophic conditions existing in the dark biosphere.

Data availability. Reads have been deposited at DDBJ/ENA/GenBank under the accession number ERR2843940, and the complete genome sequences and annotations have been deposited under the accession numbers UWOC00000000 for this chromosome, LR026982 for this plasmid, and GCF_900604295 for the annotation. The version described in this paper is the first version.

ACKNOWLEDGMENTS

We thank all of the IPBSL project team members for facilitating the access to the sample.

This work was supported by Ministerio de Economía y Competitividad (MINECO) grant GLC2015-66242-R.

Genome sequencing was provided by MicrobesNG. The next-generation sequencing (NGS) analysis has been performed by the Genomics and NGS Core Facility at the CBMSO (CSIC-UAM).

REFERENCES

- Oren A, Xue-Wei X. 2014. The family *Hyphomicrobiaceae*, p 247–281. *In* Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (ed), The prokaryotes, 4th ed. Springer, Berlin, Germany.
- 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, Omoregie EO, García M, Rodríguez N, Sanz JL, the IPBSL Team. 2013. Iberian Pyrite Belt Subsurface Life (IPBSL), a drilling project of biohydrometallurgical interest. Adv Mat Res 825:15–18. https://doi.org/10.4028/www.scientific.net/AMR .825.15.
- Puente-Sánchez F, Arce-Rodríguez A, Oggerin M, García-Villadangos M, Moreno-Paz M, Blanco Y, Rodríguez N, Bird L, Lincoln SA, 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 115:10702–10707. https://doi.org/10.1073/pnas .1808176115.
- Leandro T, Rodriguez 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.
- García R, Martínez JM, Leandro T, Amils R. 2018. Draft genome sequence of *Rhizobium* sp. strain T2.30D-1.1, isolated from 538.5 meters deep on the subsurface of the Iberian Pyrite Belt. Microbial Resour Announc 7:e01098-18. https://doi.org/10.1128/MRA.01098-18.
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol 35:1547–1549. https://doi.org/10.1093/molbev/msy096.
- Hall TA. 2011. BioEdit: an important software for molecular biology. GERF Bull Biosci 2:60–61.
- Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL. 2007. GenBank. Nucleic Acids Res 36:D25–D30. https://doi.org/10.1093/nar/ gkm929.

- Chakravarthy SK, Ramaprasad EVV, Shobha E, Sasikala C, Ramana CV. 2012. *Rhodoplanes piscinae* sp. nov. isolated from pond water. Int J Syst Evol Microbiol 62:2828–2834. https://doi.org/10.1099/ijs.0 .037663-0.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski 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, Kav AB, 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 ACE, 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 5:578–579. https:// doi.org/10.1093/bioinformatics/btq683.
- Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153.
- 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.
- 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.

- Grissa I, Vergnaud G, Pourcel C. 2007. CRISPRFinder: a Web tool to identify clustered regularly interspaced short palindromic repeats. Nucleic Acids Res 35:W52–W57. https://doi.org/10.1093/nar/gkm360.
- Kucera J, Pakostova E, Lochman J, Janiczek O, Mandl M. 2016. Are there multiple mechanisms of anaerobic sulfur oxidation with ferric iron in *Acidithiobacillus ferrooxidans*? Res Microbiol 167:357–366. https://doi .org/10.1016/j.resmic.2016.02.004.
- Hecht MH, Kounaves SP, Quinn RC, West SJ, Young SMM, Ming DW, Catling DC, Clark BC, Boynton WV, Hoffman J, DeFlores LP, Gospodinova K, Kapit J, Smith PH. 2009. Detection of perchlorate and the soluble chemistry of Martian soil at the Phoenix lander site. Science 325:64–67. https://doi.org/10.1126/science.1172466.