



# Draft Genome Sequence of *Pseudomonas* sp. Strain T2.31D-1, Isolated from a Drilling Core Sample Obtained 414 Meters below Surface in the Iberian Pyrite Belt

J. M. Martínez,<sup>a</sup> C. Escudero,<sup>b</sup> T. Leandro,<sup>a,c</sup> G. Mateos,<sup>a</sup>  R. Amils<sup>a,b</sup>

<sup>a</sup>Centro de Biología Molecular Severo Ochoa, Universidad Autónoma de Madrid, Madrid, Spain

<sup>b</sup>Centro de Astrobiología, Madrid, Spain

<sup>c</sup>Institute for Bioengineering and Bioscience, Instituto Técnico, Lisbon, Portugal

**ABSTRACT** We report the draft genome of *Pseudomonas* sp. strain T2.31D-1, which was isolated from a drilling core sample obtained 414 m below surface in the Iberian Pyrite Belt. The genome consists of a 4.7-Mb chromosome with 4,428 coding sequences, 1 rRNA operon, 59 tRNA genes, and a 31.8-kb plasmid.

*Pseudomonas stutzeri* is a Gram-negative gammaproteobacterium that has been isolated from a wide variety of environments (1). *P. stutzeri* is described as a facultative anaerobe that is able to denitrify (2), fix nitrogen (3), and biodegrade aromatic compounds (4). *Pseudomonas* sp. strain T2.31D-1 was isolated, using the Hungate roll tube method (5), from a strict anaerobic denitrification enrichment culture (6) of a powdered core sample (~6 g) that was obtained 414 m below surface during the development of the Iberian Pyritic Belt Subsurface Life (IPBSL) drilling project (7) (January to March 2012) in Peña de Hierro (Iberian Pyrite Belt), Spain (37°43'45.42"N, 6°33'23.57"W).

Genomic DNA extraction from the T2.31D-1 strain was performed from a culture in Reasoner's 2A (R2A) liquid medium using the cetyltrimethylammonium bromide (CTAB)-based method (8), and the 16S rRNA gene was amplified and sequenced as described previously (6). Quality-based editing and assembly of 16S rRNA gene reads were carried out as described previously (9). Comparison of the complete 16S rRNA gene sequence with the GenBank database (10) of the NCBI using BLAST (11) showed that the closest sequence corresponded to *P. stutzeri* ATCC 17588<sup>T</sup> (99.93% similarity).

MicrobesNG prepared the library with the Nextera XT library preparation kit (Illumina, San Diego, CA, USA) by following the manufacturer's protocol with the following modifications: 2 ng of DNA instead of 1 ng was used as the input, and the PCR elongation time was increased from 30 s to 1 min. The library was then sequenced using the Illumina MiSeq technology, yielding a mean coverage of 61.75× and 659,784 paired-end 2 × 250-bp reads. Trimming and quality analysis were performed using Trimmomatic v0.36 (12) and FastQC v0.11.9 (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc>) software, respectively. *De novo* assembly was completed using SPAdes v3.14.1 (13). Extrachromosomal genetic elements were assembled using Recycler (14). Mauve Aligner v2.4.0 (15) was used to align plasmid contigs against the chromosomal assembly. Contigs were ordered in scaffolds using SSPACE software (16). Unless otherwise specified, default parameters were used for all software. A 4,762,555-bp chromosome was obtained in 47 scaffolds with an  $N_{50}$  value of 282.20 kb and a GC content of 63.90%, similar in size and GC content to other sequenced *P. stutzeri* strains (1, 4, 17–19). In addition, this strain harbored one plasmid of 31,837 bp assembled in two scaffolds, similar to other strains of this species (20).

Prokka v1.14.5 software (21) and the RAST platform (22) were used for gene

**Citation** Martínez JM, Escudero C, Leandro T, Mateos G, Amils R. 2021. Draft genome sequence of *Pseudomonas* sp. strain T2.31D-1, isolated from a drilling core sample obtained 414 meters below surface in the Iberian Pyrite Belt. *Microbiol Resour Announc* 10:e01165-20. <https://doi.org/10.1128/MRA.01165-20>.

**Editor** Catherine Putonti, Loyola University Chicago

**Copyright** © 2021 Martínez 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](mailto:ramils@cbm.csic.es).

**Received** 8 October 2020

**Accepted** 23 November 2020

**Published** 7 January 2021

prediction and annotation, using *P. stutzeri* ATCC 17588 as a genome reference. A total of 4,428 coding DNA sequences, 1 rRNA operon, a second copy of a 5S rRNA gene, 59 tRNAs, and 1 transfer-messenger RNA were identified. Genes involved in denitrification, thiosulfate oxidation, phosphite oxidation, catabolism of aromatic compounds, fermentation, and heavy metal (Cd, Co, Cr, Cu, and Zn) resistance were detected in the chromosome.

**Data availability.** Reads were deposited in DDBJ/ENA/GenBank under the accession number [ERR3773731](https://doi.org/10.1093/bioinformatics/btu170) and the complete genome sequences and annotations under the accession number [CAJFAG010000000](https://doi.org/10.1093/bioinformatics/btu170) for the chromosome (annotation under the accession number [GCA\\_903995555](https://doi.org/10.1093/bioinformatics/btu170)) and the accession number [CAJFAF010000000](https://doi.org/10.1093/bioinformatics/btu170) for the plasmid (annotation under the accession number [GCA\\_903995525](https://doi.org/10.1093/bioinformatics/btu170)). All of the reads and sequences are included under the study number [PRJEB35933](https://doi.org/10.1093/bioinformatics/btu170).

## ACKNOWLEDGMENTS

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

This work was supported by MICINN grant PID2019-1048126GB-I00. Genome sequencing was provided by MicrobesNG (Birmingham, UK). Data submission to the ENA repository was performed by the Genomics and NGS Core Facility at the Centro de Biología Molecular Severo Ochoa (CSIC-UAM).

## REFERENCES

- Lalucat J, Bennisar A, Bosch R, García-Valdés E, Palleroni NJ. 2006. Biology of *Pseudomonas stutzeri*. *Microbiol Mol Biol Rev* 70:510–547. <https://doi.org/10.1128/MMBR.00047-05>.
- Carlson CA, Ingraham JL. 1983. Comparison of denitrification by *Pseudomonas stutzeri*, *Pseudomonas aeruginosa*, and *Paracoccus denitrificans*. *Appl Environ Microbiol* 45:1247–1253. <https://doi.org/10.1128/AEM.45.4.1247-1253.1983>.
- Desnoues N, Lin M, Guo X, Ma L, Carreño-Lopez R, Elmerich C. 2003. Nitrogen fixation genetics and regulation in a *Pseudomonas stutzeri* strain associated with rice. *Microbiology (Reading)* 149:2251–2262. <https://doi.org/10.1099/mic.0.26270-0>.
- Brunet-Galmés I, Busquets A, Peña A, Gomila M, Nogales B, García-Valdés E, Lalucat J, Bennisar A, Bosch R. 2012. Complete genome sequence of the naphthalene-degrading bacterium *Pseudomonas stutzeri* AN10 (CCUG 29243). *J Bacteriol* 194:6642–6643. <https://doi.org/10.1128/JB.01753-12>.
- Hungate RE. 1969. A roll tube method for cultivation of strict anaerobes. *Methods Microbiol* 3B:117–132. [https://doi.org/10.1016/S0580-9517\(08\)70503-8](https://doi.org/10.1016/S0580-9517(08)70503-8).
- 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. *Microbiol Resour Announc* 7:e01098-18. <https://doi.org/10.1128/MRA.01098-18>.
- 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>.
- Wilson K. 2001. Preparation of genomic DNA from bacteria. *Curr Protoc Mol Biol* 56:2.4.1–2.4.5. <https://doi.org/10.1002/0471142727.mb0204s56>.
- Marián 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>.
- Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL. 2008. GenBank. *Nucleic Acids Res* 36:D25–D30. <https://doi.org/10.1093/nar/gkm929>.
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J Mol Biol* 215:403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2).
- 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, 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* 27:578–579. <https://doi.org/10.1093/bioinformatics/btq683>.
- Chen M, Yan Y, Zhang W, Lu W, Wang J, Ping S, Lin M. 2011. Complete genome sequence of the type strain *Pseudomonas stutzeri* CGMCC 1.1803. *J Bacteriol* 193:6095–6095. <https://doi.org/10.1128/JB.06061-11>.
- Rainey PB, Thompson IP, Palleroni NJ. 1994. Genome and fatty acid analysis of *Pseudomonas stutzeri*. *Int J Syst Bacteriol* 44:54–61. <https://doi.org/10.1099/00207713-44-1-54>.
- Yu H, Yuan M, Lu W, Yang J, Dai S, Li Q, Yang Z, Dong J, Sun L, Deng Z, Zhang W, Chen M, Ping S, Han Y, Zhan Y, Yan Y, Jin Q, Lin M. 2011. Complete genome sequence of the nitrogen-fixing and rhizosphere-associated bacterium *Pseudomonas stutzeri* strain DSM4166. *J Bacteriol* 193:3422–3423. <https://doi.org/10.1128/JB.05039-11>.
- Haefeli C, Franklin C, Hardy K. 1984. Plasmid-determined silver resistance in *Pseudomonas stutzeri* isolated from a silver mine. *J Bacteriol* 158:389–392. <https://doi.org/10.1128/JB.158.1.389-392.1984>.
- Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30:2068–2069. <https://doi.org/10.1093/bioinformatics/btu153>.
- Aziz RK, Bartels D, Best A, DeJongh M, Disz T, Edwards RA, Formosa 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* 9:75–15. <https://doi.org/10.1186/1471-2164-9-75>.