



Complete Genome Sequence of *Tessaracoccus* sp. Strain T2.5-30 Isolated from 139.5 Meters Deep on the Subsurface of the Iberian Pyritic Belt

Tânia Leandro,^{a,b} Milton S. da Costa,^c Jose L. Sanz,^d Ricardo Amils^a

Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Universidad Autónoma de Madrid, Cantoblanco, Madrid, Spain^a; Centre for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal^b; Department of Life Sciences, University of Coimbra, Coimbra, Portugal^c; Department of Molecular Biology, Universidad Autónoma de Madrid, Madrid, Spain^d

ABSTRACT Here, we report the complete genome sequence of *Tessaracoccus* sp. strain T2.5-30, which consists of a chromosome with 3.2 Mbp, 70.4% G+C content, and 3,005 coding DNA sequences. The strain was isolated from a rock core retrieved at a depth of 139.5 m in the subsurface of the Iberian Pyritic Belt (Spain).

The genus *Tessaracoccus* was described in 1999 and is classified within the family Propionibacteriaceae, phylum Actinobacteria (1). The genus contains nine validly published species, namely, *T. bendigoensis* (1), *T. flavescentis* (2), *T. lubricantis* (3), *T. lapidicaptus* (4), *T. oleagri* (5), *T. rhinocerotis* (6), *T. flavus* (7), *T. massiliensis* (8), *T. defluvii* (9), and one species, *T. profundi* (10), that has not been validly published. *Tessaracoccus* species have been isolated from diverse environments and are characterized as Gram-positive non-spore-forming facultative anaerobic bacteria (1). The type strains of *T. profundi* and *T. lapidicaptus* have also been isolated from deep subsurface environments (4, 10).

Tessaracoccus sp. strain T2.5-30 was isolated from a core sample from 139.5 m in the subsurface of the Iberian Pyritic Belt (IPB, Peña de Hierro, Spain). The IPB is characterized as one of the largest sulfide ore deposits known (11).

For whole-genome sequencing, DNA was extracted using a cetyltrimethylammonium bromide (CTAB)-based extraction method (12). The quantity of extracted genomic DNA was determined with the Qubit version 2.0 fluorometer (Invitrogen, USA), and quality was analyzed by electrophoresis on an agarose gel, as well as on a NanoDrop 2000 (Thermo Scientific, USA) for measurement of the A_{260}/A_{280} ratio. Genomic DNA was submitted to the Norwegian Sequencing Centre (University of Oslo, Norway) for PacBio single-molecule real-time (SMRT) sequencing (13). One SMRT cell was used for sequencing on a Pacific Biosciences RSII instrument using P6-C4 chemistry, with a 360-min movie time. The generated reads were then assembled using the Hierarchical Genome Assembly Process (HGAP) (SMRT Analysis software version 2.3.0; Pacific Biosciences) (14). The final assembly resulted in four contigs. The Minimus2 software (Amos package) was used to circularize the contigs (15). Circularization of contig 0 by joining and trimming of the overlapping 3' and 5' ends resulted in a circular closed chromosome. Contigs 1, 2, and 4 correspond to direct repeats of sequences contained in contig 0 and were excluded from our analysis.

The total genome length was approximately 3.2 Mbp, with a G+C content of 70.4%. The genome did not contain plasmids. PacBio SMRT sequencing resulted in approximately 175% coverage. The complete genome was annotated with NCBI Prokaryotic Genome Annotation Pipeline (PGAP) and with Rapid Annotations using Subsystems Technology (RAST), as incorporated in the PATRIC server (16, 17). RAST predicted a total of 3,005 DNA coding sequences, six rRNA genes, and 46 tRNA genes.

Received 3 March 2017 Accepted 9 March 2017 Published 27 April 2017

Citation Leandro T, da Costa MS, Sanz JL, Amils R. 2017. Complete genome sequence of *Tessaracoccus* sp. strain T2.5-30 isolated from 139.5 meters deep on the subsurface of the Iberian Pyritic Belt. *Genome Announc* 5:e00238-17. <https://doi.org/10.1128/genomeA.00238-17>.

Copyright © 2017 Leandro et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](#).

Address correspondence to Tânia Leandro, tjt.leandro@gmail.com.

The comprehensive analysis of the complete genome sequence of *Tessaracoccus* sp. strain T2.5-30 will provide insights into the genetic potential of this strain to elucidate the mechanisms used by life to inhabit deep terrestrial environments in the absence of light as well as under oligotrophic conditions.

Accession number(s). The nucleotide sequence for the *Tessaracoccus* sp. strain T2.5-30 complete genome has been deposited at DDBJ/ENA/GenBank under the accession number [CP019229](#). The version described in this paper is the first version.

ACKNOWLEDGMENTS

This work was supported by the Foundation for Science and Technology (FCT), Portugal (doctoral fellowship SFRH/BD/87076/2012 to T.L.) and by ERC Advanced grant IPBSL-250350.

We acknowledge María José López Sánchez and the rest of the team from the Genomics and Massive Sequencing Service of the Centro de Biología Molecular “Severo Ochoa” for their assistance in the bioinformatics analysis of the genome.

REFERENCES

- Maszenan AM, Seviour RJ, Patel BKC, Schumann P, Rees GN. 1999. *Tessaracoccus bendigoensis* gen. nov., sp. nov., a Gram-positive coccus occurring in regular packages or tetrads, isolated from activated sludge biomass. *Int J Syst Bacteriol* 49:459–468. <https://doi.org/10.1099/00207713-49-2-459>.
- Lee DW, Lee SD. 2008. *Tessaracoccus flavescent*s sp. nov., isolated from marine sediment. *Int J Syst Evol Microbiol* 58:785–789. <https://doi.org/10.1099/ijss.0.04868-0>.
- Kämpfer P, Ludders N, Warfolomeiw I, Busse HJ. 2009. *Tessaracoccus lubricantis* sp. nov., isolated from a metalworking fluid. *Int J Syst Evol Microbiol* 59:1545–1549. <https://doi.org/10.1099/ijss.0.006841-0>.
- Puente-Sánchez F, Sánchez-Román M, Amils R, Parro V. 2014. *Tessaracoccus lapidicaptus* sp. nov., an actinobacterium isolated from the deep subsurface of the Iberian pyrite belt. *Int J Syst Evol Microbiol* 64: 3546–3552. <https://doi.org/10.1099/ijss.0.060038-0>.
- Cai M, Wang L, Cai H, Li Y, Wang YN, Tang YQ, Wu XL. 2011. *Salinimonas ramus* sp. nov. and *Tessaracoccus oleigri* sp. nov., isolated from a crude oil-contaminated saline soil. *Int J Syst Evol Microbiol* 61: 1767–1775. <https://doi.org/10.1099/ijss.0.025932-0>.
- Li GD, Chen X, Li QY, Xu FJ, Qiu SM, Jiang Y, Jiang CL. 2015. *Tessaracoccus rhinocerotis* sp. nov., isolated from the faeces of *Rhinoceros unicornis*. *Int J Syst Evol Microbiol* 66:922–927. <https://doi.org/10.1099/ijsem.0.000812>.
- Kumari R, Singh P, Schumann P, Lal R. 2016. *Tessaracoccus flavus* sp. nov., isolated from the drainage system of a lindane-producing factory. *Int J Syst Evol Microbiol* 66:1862–1868. <https://doi.org/10.1099/ijsem.0.00958>.
- Seck E, Traore SI, Khelaifia S, Beye M, Michelle C, Couderc C, Brah S, Fournier PE, Raoult D, Dubour G. 2016. *Tessaracoccus massiliensis* sp. nov., a new bacterial species isolated from the human gut. *NEW Microbes and NEW Infections* 13:3–12. <https://doi.org/10.1016/j.nmni.2016.05.002>.
- Srinivasan S, Sundaraman A, Lee SS. 2017. *Tessaracoccus defluvii* sp. nov., isolated from an aeration tank of a sewage treatment plant. *Antonie Leeuwenhoek* 110:1–9. <https://doi.org/10.1007/s10482-016-0766-6>.
- Finster KW, Cockell CS, Voytek MA, Gronstal AL, Kjeldsen KU. 2009. Description of *Tessaracoccus profundus* sp. nov., a deep-subsurface actinobacterium isolated from a Chesapeake impact crater drill core (940 m depth). *Antonie Leeuwenhoek* 96:515–526. <https://doi.org/10.1007/s10482-009-9367-y>.
- 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, Omorregie EO, García M, Rodríguez N, Sanz JL. 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>.
- Wilson K. 2001. Preparation of genomic DNA from bacteria. *Curr Protoc Mol Biol Chapter 2:Unit 2.4*.
- Eid J, Fehr A, Gray J, Luong K, Lyle J, Otto G, Peluso P, Rank D, Baybayan P, Bettman B, Bibillo A, Bjornson K, Chaudhuri B, Christians F, Cicero R, Clark S, Dalal R, Dewinter A, Dixon J, Foquet M, Gaertner A, Hardenbol P, Heiner C, Hester K, Holden D, Kearns G, Kong X, Kuse R, Lacroix Y, Lin S, Lundquist P, Ma C, Marks P, Maxham M, Murphy D, Park I, Pham T, Phillips M, Roy J, Sebra R, Shen G, Sorenson J, Tomaney A, Travers K, Trulson M, Vieceli J, Wegener J, Wu D, Yang A, Zaccarin D. 2009. Real-time DNA sequencing from single polymerase molecules. *Science* 323:133–138. <https://doi.org/10.1126/science.1162986>.
- Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Non-hybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat Methods* 10:563–569. <https://doi.org/10.1038/nmeth.2474>.
- Sommer DD, Delcher AL, Salzberg SL, Pop M. 2007. Minimus: a fast, lightweight genome assembler. *BMC Bioinformatics* 8:64. <https://doi.org/10.1186/1471-2105-8-64>.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formmska 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>.
- Wattam AR, Abraham D, Dalay O, Disz TL, Driscoll T, Gabbard JL, Gillespie JJ, Gough R, Hix D, Kenyon R, Machi D, Mao C, Nordberg EK, Olson R, Overbeek R, Pusch GD, Shukla M, Schulman J, Stevens RL, Sullivan DE, Vonstein V, Warren A, Will R, Wilson MJC, Yoo HS, Zhang C, Zhang Y, Sobral BW. 2014. PATRIC, the bacterial bioinformatics database and analysis resource. *Nucleic Acids Res* 42:D581–D591. <https://doi.org/10.1093/nar/gkt1099>.