



## Draft Genome Sequence of a New *Pseudomonas* sp. Strain, ef1, Associated with the Psychrophilic Antarctic Ciliate *Euplotes focardii*

Kesava Priyan Ramasamy,<sup>a</sup> Andrea Telatin,<sup>b</sup> Matteo Mozzicafreddo,<sup>a</sup> Cristina Miceli,<sup>a</sup> Sandra Pucciarelli<sup>a</sup>

<sup>a</sup>School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, MC, Italy <sup>b</sup>Quadram Institute Bioscience, Gut Microbes and Health Institute Strategic Program, Norwich Research Park, Norwich, United Kingdom

**ABSTRACT** We announce here the draft genome sequence of a new *Pseudomonas* strain, named *Pseudomonas* sp. strain ef1, associated with the cold-adapted Antarctic ciliate *Euplotes focardii*. The genome sequence is 6,228,167 bp long with a G+C content of 59.7%.

he genus Pseudomonas is ubiquitous in aquatic and terrestrial environments and metabolically diverse, including the capacity to remove toxic heavy metals (1) and to degrade various aromatic hydrocarbons (2, 3). The bacterial strain reported here was isolated from a consortium associated with the cold-adapted ciliate Euplotes focardii maintained in the laboratory at 4°C (4, 5). The present study aimed to isolate and characterize metal-resistant bacteria that can be useful for biosorption. For strain isolation, the logarithmically growing E. focardii cultures were harvested by centrifugation at 3,000 rpm for 10 min, and the pellet was suspended with sterile seawater. The suspension was sonicated for 5 to 10 seconds at a pulse rate of 6 V. The total cell extract (200  $\mu$ l) was inoculated directly into lysogeny broth (LB) agar medium (1% tryptone, 0.5% yeast extract, 1% NaCl, and 1.5% agar) supplemented with the final concentration of filter-sterilized 2 mM copper (II) chloride (Sigma-Aldrich) solution and incubated at 4°C for 1 week. Morphologically different colonies were picked and routinely subcultured onto LB agar plates to obtain a pure culture. The isolated strain was stored as a stock in 25% glycerol at -80°C for further use. Prior to DNA extraction, a single colony was grown in LB broth, and genomic DNA was extracted using the commercial PureLink genomic DNA isolation kit (Invitrogen) according to the manufacturer's instructions. Genomic DNA integrity was checked with gel electrophoresis and then quantified with the Qubit fluorometer (double-stranded DNA [dsDNA]) assay. A whole-genome shotgun library was prepared, starting from 1 ng of genomic DNA, using the Illumina Nextera XT kit. Whole-genome sequencing was performed by Illumina MiSeq 2 imes300-bp sequencing at BMR Genomics, Padua, Italy, and generated a total of 1,358,254 reads (12-fold coverage). All sequence reads were quality checked using FastQC 0.11.1 (6) and assembled using SPAdes 3.6 (7) with the following parameters: k-mer values of 21, 33, 55, 77, 99, and 127 and -careful. Default parameters were used for all software unless otherwise specified. Genome sequences were annotated with Prokaryotic Genome Annotation Pipeline (PGAP) version 4.8 (8). The assembly consisted of 72 contigs (N<sub>50</sub>, 242 kbp), 5,587 predicted coding DNA sequences (CDSs), 6 rRNA operons (5S, 16S, and 23S), 62 tRNAs, and 4 noncoding RNAs. Compared with the reference Pseudomonas koreensis D26 (GenBank accession number CP014947), 71% of the assembly bases were aligned (4.5 Mbp) with OrthoANI (9) with a value of 99.94%. This genome harbored a set of copper resistance and copper transporting genes which may be important for the survival of Pseudomonas sp. strain ef1 in the presence of copper. Genome analyses revealed the presence of aromatic hydrocarbon degradation genes, such as homogen-

**Citation** Ramasamy KP, Telatin A, Mozzicafreddo M, Miceli C, Pucciarelli S. 2019. Draft genome sequence of a new *Pseudomonas* sp. strain, ef1, associated with the psychrophilic Antarctic ciliate *Euplotes focardii*. Microbiol Resour Announc 8:e00867-19. https://doi.org/10.1128/MRA.00867-19.

**Editor** Catherine Putonti, Loyola University Chicago

**Copyright** © 2019 Ramasamy et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Kesava Priyan Ramasamy, kesavanlife@gmail.com, or Sandra Pucciarelli, sandra.pucciarelli@unicam.it.

Received 23 July 2019 Accepted 21 September 2019 Published 10 October 2019 tisate 1,2-dioxygenase (locus tag FEE99\_00455), protocatechuate 3,4-dioxygenase (locus tag FEE99\_02300), and salicylate hydroxylase (locus tag FEE99\_04700) (10). Based on the functional annotation in the Rapid Annotations using Subsystems Technology (RAST) 2.0 server (11), we predicted a total of 107 genes potentially involved in the metabolism of aromatic compounds. The draft genome sequence of *Pseudomonas* sp. strain ef1 provides new insights for a better understanding of ecologically important degradation genes. Furthermore, it provides the knowledge of enzymes and other protein sequences able to function at a constant temperature of 4°C.

**Data availability.** The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Pseudomonas* sp. strain ef1 is MH177769. This whole-genome project for *Pseudomonas* sp. strain ef1 has been deposited in GenBank under the accession number VAUR000000000. Raw reads are available under the SRA accession number SRR9712345.

## **ACKNOWLEDGMENTS**

This work was funded by the European Commission Marie Sklodowska-Curie actions H2020 RISE Metable 645693 grant. Bioinformatics analyses were partly performed on the MRC CLIMB cloud computing environment supported by grant MR/L015080/1.

## REFERENCES

- Pardo R, Herguedas M, Barrado E, Vega M. 2003. Biosorption of cadmium, copper, lead and zinc by inactive biomass of *Pseudomonas putida*. Anal Bioanal Chem 376:26–32. https://doi.org/10.1007/s00216-003 -1843-z.
- Krell T, Lacal J, Guazzaroni ME, Busch A, Silva-Jiménez H, Fillet S, Reyes-Darías JA, Muñoz-Martínez F, Rico-Jiménez M, García-Fontana C, Duque E, Segura A, Ramos JL. 2012. Responses of *Pseudomonas putida* to toxic aromatic carbon sources. J Biotechnol 160:25–32. https://doi.org/10 .1016/j.jbiotec.2012.01.026.
- Ma Y, Wang L, Shao Z. 2006. Pseudomonas, the dominant polycyclic aromatic hydrocarbon-degrading bacteria isolated from Antarctic soils and the role of large plasmids in horizontal gene transfer. Environ Microbiol 8:455–465. https://doi.org/10.1111/j.1462-2920.2005.00911.x.
- Valbonesi A, Luporini P. 1993. Biology of *Euplotes focardii* an Antarctic ciliate. Polar Biol 13:489–493. https://doi.org/10.1007/BF00233140.
- Pucciarelli S, Devaraj RR, Mancini A, Ballarini P, Castelli M, Schrallhammer M, Petroni G, Miceli C. 2015. Microbial consortium associated with the antarctic marine ciliate *Euplotes focardii*: an investigation from genomic sequences. Microb Ecol 70:484–497. https://doi.org/10.1007/s00248-015 -0568-9.
- 6. Andrews S. 2010. FastQC: a quality control tool for high throughput

sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc.

- 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.
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44:6614–6624. https://doi .org/10.1093/nar/gkw569.
- Yoon SH, Ha S, Lim J, Kwon S, Chun J. 2017. A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie Van Leeuwenhoek 110:1281–1286. https://doi.org/10.1007/s10482-017-0844-4.
- Pérez-Pantoja D, González B, Pieper DH. 2010. Aerobic degradation of aromatic hydrocarbons, p 799–837. *In* Handbook of hydrocarbon and lipid microbiology. Springer-Verlag, Berlin, Germany.
- Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Res 42:D206–D214. https://doi.org/10.1093/nar/gkt1226.