PROKARYOTES



Draft Genome Sequences of Three Sub-Antarctic *Rhodococcus* spp., Including Two Novel Psychrophilic Genomospecies

Akhikun Nahar, a Anthony L. Baker, a Michael A. Charleston, b John P. Bowman, a Margaret L. Britz $^{\rm a,c}$

Tasmanian Institute of Agriculture, University of Tasmania, Hobart, Australia^a; School of Physical Sciences, University of Tasmania, Hobart, Australia^b; Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Melbourne, Australia^c

ABSTRACT The draft genome sequences of three sub-Antarctic *Rhodococcus* sp. strains—1159, 1163, and 1168—are reported here. The estimated genome sizes were 7.09 Mb with a 62.3% GC content for strain 1159, 4.45 Mb with a 62.3% GC content for strain 1163, and 5.06 Mb with a 62.10% GC content for strain 1168.

Rhodococcus, a genus in the order Actinomycetales, is ubiquitous in terrestrial ecosystems (1). Several species are biotechnologically important for their capability to biodegrade pollutants (2–4) and for their oleaginous nature (5–7). Here, we report the whole-genome sequences of *Rhodococcus* sp. strains 1159, 1163, and 1168.

To identify potential mycolic acid-producing bacteria, the University of Tasmania Antarctic culture collection was screened for lysozyme-resistant bacteria, which revealed these three strains originally isolated in 2001 from soil and detritus on Macquarie Island (54°36′S, 158°54′E). Strain 1159 is off-white to pale yellow when cultured on nutrient agar or minimal salt broth, and its optimal growth temperature is 30°C. The other two strains are yellow-orange when cultured similarly, with fastest growth occurring at 20°C.

High-molecular-weight genomic DNA was extracted following a modified extraction method originally described by Lévy-Frébault et al. (8) and whole-genome shotgun (WGS) sequenced using Illumina MiSeq technology by Macrogen (South Korea). Totals of 5,846,628, 6,354,712, and 6,661,398 reads with 1,752,144,208, 1,898,840,255, and 1,989,884,802 bases were obtained for strains 1159, 1163, and 1168, respectively. Raw sequences were *de novo* assembled using the AbySS sequence assembler (9), and 112, 43, and 97 contigs with lengths of \geq 200 bp were obtained for strains 1159, 1163, and 1168, respectively. Genome annotation was performed using RAST (10) and the NCBI Prokaryotic Genome Annotation Pipeline (11).

The estimated genome size of strain 1159 was 7.09 Mb with a 62.3% GC content and 6,546 coding sequences (CDSs), 6,385 coding genes, and 161 pseudogenes. These features are similar to those of *R. erythropolis* strain JCM 6824 (WGS, GenBank accession number BBLL00000000), which has a genome size of 7.02 Mb with a 62.3% GC content, encoding 6,608 genes and 6,372 proteins. However, the estimated genome sizes of strains 1163 and 1168 were 4.45 and 5.06 Mb with 62.3% and 62.10% GC contents, respectively. There were 4,042 CDSs, 3,956 coding genes, and 86 pseudogenes predicted for strain 1163 and 4,617 CDSs, 4,463 coding genes, and 154 pseudogenes predicted for strain 1168. These features most closely resembled those of *R. fasciens* F7 (NZ_LFDS00000000), which had a 5.24-Mb genome with a 64.7% GC content and 4,819 proteins.

Genetic similarities were calculated against the phylogenetically close species using the average nucleotide identity (ANI) tool of IMG/M (12). Strain 1159 was 98.7%, 95.6%,

Received 19 July 2017 Accepted 20 July 2017 Published 7 September 2017

Citation Nahar A, Baker AL, Charleston MA, Bowman JP, Britz ML. 2017. Draft genome sequences of three sub-Antarctic *Rhodococcus* spp., including two novel psychrophilic genomospecies. Genome Announc 5:e00898-17. https://doi.org/10 .1128/genomeA.00898-17.

Copyright © 2017 Nahar et al. This is an openaccess article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Akhikun Nahar, akhikun.nahar@utas.edu.au.

and 95.42% genetically similar to *R. erythropolis* 339MFSha3.1 (Ga0101996), *R. qingsh-engii* BKS 20-40 (Ga0032278), and *R. baikonurensis* JCM 18801 (Ga0128325), respectively.

As such, this strain was identified as *R. erythropolis*. Strains 1163 and 1168, however, form a separate phylogenetic clade in the 16S rRNA gene neighbor-joining tree, demonstrating, respectively, 78.43% and 78.62% similarities with *R. yunnanensis* (NZ_BCXH0000000) and 78.06% and 77.95% similarities with *R. fascians* 02-815 (Ga0125507). Strains 1163 and 1168 were calculated to be 93.7% similar to each other, below the ANI speciation point of 95%. These two strains are therefore suggested to represent novel species.

The RAST SEED Viewer identified a large number of genes involved in carbohydrate and lipid metabolism. Genes for aromatic compound metabolism were identified, indicating biotechnological potential.

Accession number(s). These whole-genome shotgun projects have been submitted to DDBJ/EMBL/GenBank under the accession numbers MJVD00000000, MKKX00000000, and MKKY00000000.

ACKNOWLEDGMENTS

We thank Joe Kocharunchitt, who initially screened the UTAS Antarctic collection for actinobacteria.

This work was supported through the UTAS Faculty of Science, Engineering and Technology Dean's support funds and the Tasmanian Institute of Agriculture.

REFERENCES

- McLeod MP, Warren RL, Hsiao WW, Araki N, Myhre M, Fernandes C, Miyazawa D, Wong W, Lillquist AL, Wang D, Dosanjh M, Hara H, Petrescu A, Morin RD, Yang G, Stott JM, Schein JE, Shin H, Smailus D, Siddiqui AS, Marra MA, Jones SJ, Holt R, Brinkman FS, Miyauchi K, Fukuda M, Davies JE, Mohn WW, Eltis LD. 2006. The complete genome of *Rhodococcus* sp. RHA1 provides insights into a catabolic powerhouse. Proc Natl Acad Sci U S A 103:15582–15587. https://doi.org/10.1073/pnas.0607048103.
- Alvarez PJ, Anid PJ, Vogel TM. 1991. Kinetics of aerobic biodegradation of benzene and toluene in sandy aquifer material. Biodegradation 2:43–51. https://doi.org/10.1007/BF00122424.
- van der Geize R, Dijkhuizen L. 2004. Harnessing the catabolic diversity of rhodococci for environmental and biotechnological applications. Curr Opin Microbiol 7:255–261. https://doi.org/10.1016/j.mib.2004.04.001.
- Grund E, Denecke B, Eichenlaub R. 1992. Naphthalene degradation via salicylate and gentisate by *Rhodococcus* sp. strain B4. Appl Environ Microbiol 58:1874–1877.
- Alvarez HM, Mayer F, Fabritius D, Steinbüchel A. 1996. Formation of intracytoplasmic lipid inclusions by *Rhodococcus opacus* strain PD630. Arch Microbiol 165:377–386. https://doi.org/10.1007/s002030050341.
- Kurosawa K, Boccazzi P, de Almeida NM, Sinskey AJ. 2010. High-celldensity batch fermentation of *Rhodococcus opacus* PD630 using a high glucose concentration for triacylglycerol production. J Biotechnol 147: 212–218. https://doi.org/10.1016/j.jbiotec.2010.04.003.

- Alvarez HM, Steinbüchel A. 2002. Triacylglycerols in prokaryotic microorganisms. Appl Microbiol Biotechnol 60:367–376. https://doi.org/10 .1007/s00253-002-1135-0.
- Lévy-Frébault V, Grimont F, Grimont PAD, David HL. 1984. Deoxyribonucleic acid relatedness study of *Mycobacterium fallax*. Int J Syst Bacteriol 34:423–425. https://doi.org/10.1099/00207713-34-4-423.
- Simpson JT, Wong K, Jackman SD, Schein JE, Jones SJ, Birol I. 2009. ABySS: a parallel assembler for short read sequence data. Genome Res 19:1117–1123. https://doi.org/10.1101/gr.089532.108.
- 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–DD214. https:// doi.org/10.1093/nar/gkt1226.
- 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.
- Chen IA, Markowitz VM, Chu K, Palaniappan K, Szeto E, Pillay M, Ratner A, Huang J, Andersen E, Huntemann M. 2017. IMG/M: integrated genome and metagenome comparative data analysis system. Nucleic Acids Res 45:D507–D516. https://doi.org/10.1093/nar/gkw929.