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GENOME SEQUENCES

Complete Genome Sequence of *Rhodococcus opacus* Strain MoAcy1 (DSM 44186), an Aerobic Acetylenotroph Isolated from Soil

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ABSTRACT We report the genome of *Rhodococcus opacus* strain MoAcy1 (DSM 44186), an aerobic soil isolate capable of using acetylene as its primary carbon and energy source (acetylenotrophy). The genome is composed of a single circular chromosome of ~8 Mbp and two closed plasmids, with a G+C content of 67.3%.

Acetylenotrophic microbes use acetylene (C_2H_2) as their primary carbon and energy source (1), and one such organism is *Rhodococcus opacus* strain MoAcy1 (DSM 44186). This strain was isolated by Rosner et al. from soil in Tübingen, Germany, on a mineral medium under an air headspace containing 10% acetylene (2). Strain MoAcy1 has a biochemically distinct acetylene hydratase (AH) enzyme, compared to that of *Syntrophotalea acetylenica* (2–5). To better understand aerobic acetylenotrophs and the versatility of this species, we report the complete genome of *R. opacus* strain MoAcy1, which was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ) (DSM 44186).

Strain MoAcy1 was propagated on both Trypticase soy broth (TSB) (6) and acetylenotrophic medium (2) at 28°C. Genomic DNA (gDNA) for Pacific Biosciences (PacBio) sequencing was extracted from a culture grown on TSB using the U.S. Department of Energy Joint Genome Institute cetyltrimethylammonium bromide (CTAB) procedure for isolating high-molecular-weight gDNA (http://jgi.doe.gov/wp-content/uploads/2014/02/JGI-Bacterial-DNA-isolation-CTAB-Protocol-2012.pdf). gDNA for Illumina sequencing was isolated from a culture grown on acetylenotrophic medium using a modified phenol-chloroform extraction procedure (7). gDNA concentrations and purity were determined as described by Sutton et al. (8), and the gDNA was then sent to the University of California, Davis, Genome Center (http://genomecenter.ucdavis.edu) for sequencing on an RS II system (PacBio, Menlo Park, CA) and to the Microbial Genome Sequencing Center (https://www.migscenter.com) for sequencing on a NextSeq 2000 system (Illumina, Inc., San Diego, CA). A PacBio SMRTbell library was prepared with 20-kb inserts via BluePippin size selection and then sequenced with P6-C4 chemistry on the PacBio RS II platform (9). PacBio sequencing yielded 122,352 reads, with an N_{50} value of 15,836 bp. An Illumina standard shotgun library was constructed and sequenced on the Illumina NextSeq 2000 platform. Illumina sequences were quality filtered and trimmed for Phred scores of >Q10, resulting in a total of 3,234,702 paired-end 150-bp sequence reads. PacBio libraries were assembled using NextDenovo v. 2.4.0 (https://github.com/Nextomics/NextDenovo), which filtered out reads of <1,000 bp. Default parameters were used for all software unless otherwise specified. The assembly was polished with both PacBio and Illumina libraries using NextPolish v. 1.3.1 (https://github.com/Nextomics/NextPolish). The final assembly yielded 3 contigs based on 1.57 Gbp of PacBio sequences, which provided an average coverage of 315×.

The contigs were run through Circlator v. 1.5.5 (10), which revealed that the largest contig was a complete, circular chromosome and provided no evidence that the smaller contigs were part of the larger circular chromosome. We conclude that the complete genome

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of *R. opacus* strain MoAcy1 consists of an 8,044,513-bp chromosome, with a G+C content of 67.3%, and two plasmids, i.e., pRop44186_a (745 kb; G+C content, 74.5%) and pRop44186_b (52 kb; G+C content, 66.7%). Genome annotation by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (11) predicted 7,415 genes, of which 7,351 are protein-coding genes. The genome contained a total of 64 RNA genes, including 12 rRNAs (5S rRNA, 4 copies; 16S rRNA, 4 copies; 23S rRNA, 4 copies), 49 tRNAs, 1 transfer-messenger RNA, and 2 noncoding RNAs.

Data availability. The complete genome sequence of *R. opacus* strain MoAcy1 (DSM 44186) was deposited in the NCBI database under the following accession numbers: BioProject, PRJNA561397; BioSample, SAMN12617337; SRA, SRR15616890 (PacBio reads) and SRR15616889 (Illumina reads); and GenBank, CP082160 (chromosome), CP082159 (pRop44186_a), and CP082158 (pRop44186_b).

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