



Draft Genome Sequences of Three Terrestrial Isoprene-Degrading *Rhodococcus* Strains

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ABSTRACT Isoprene is produced in abundance by plants and constitutes a carbon source for microbes. The genomes of three isoprene degraders isolated from tree leaves or soil from the campus of the University of East Anglia were sequenced. These high-GC-content isolates are actinobacteria belonging to the genus *Rhodococcus*.

The emissions of isoprene to the atmosphere from terrestrial plants, principally trees, are similar in magnitude to those of methane (approximately 550 Tg per year). Some bacteria are capable of using isoprene as a sole source of carbon and energy, but their diversity and contribution to cycling of this climate-active trace gas have not been intensively studied until recently (1, 2). So far, genome sequences for a relatively small number of isoprene-degrading strains have been published (3–5).

Rhodococcus sp. strains ACPA1 and ACPA4 were isolated from the leaves of a white poplar tree (*Populus alba*) and *Rhodococcus* sp. strain ACS1 was isolated from soil in the vicinity of willow trees (*Salix fragilis*) located on the campus of the University of East Anglia, Norwich, United Kingdom. Isolates were grown in liquid culture supplied with isoprene, as described previously (3). Genomic DNA was extracted using a conventional phenol-chloroform method (3). For each strain, genomic DNA was sequenced by Edinburgh Genomics (Edinburgh, UK), following the construction of three libraries with inserts of 330, 550, and 4,500 bp, on an Illumina MiSeq instrument generating 300-nucleotide (nt) paired-end reads. Reads were trimmed using Cutadapt version 1.8.3 (6) using parameters -q 30 and -m 50, assembled using SPAdes version 3.7.0 (7) (removing contigs shorter than 200 bp), and annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). The basic genome statistics are shown in Table 1.

The large genome sizes (7 to 11 Mbp) are typical of metabolically versatile rhodococci (8), although the genome of *Rhodococcus* sp. ACPA4 is significantly smaller and of lower GC content than those of the other two strains. Based on analysis of the 16S rRNA genes, *Rhodococcus* sp. strains ACPA1 and ACPA4 are most closely related to the isoprene degraders *Rhodococcus opacus* PD630 (9) and *Rhodococcus* sp. strain AD45 (3), respectively, and *Rhodococcus* sp. strain ACS1 is related most closely to a non-isoprene-degrading *Rhodococcus koreensis* strain (10). All three genomes contain high-similarity homologues (>80% amino acid identity) of the isoprene metabolic genes described in *Rhodococcus* sp. AD45 (3, 11), including those encoding the soluble diiron center isoprene monooxygenase (*isoABCDE*), glutathione-S-transferase (*isoI*), dehydrogenase (*isoH*), and genes for enzymes predicted to perform subsequent metabolic steps (*isoG* and *isoJ*). As in other isoprene degraders, *isoGHIJ* are duplicated nearby, while *Rhodococcus* sp. ACPA4 also contains a third copy of *isoH* and *isoJ*. The glutathione biosynthesis genes *gshA* and *gshB* are also present in all three strains, consistent with the observation that conjugation of isoprene epoxide with glutathione appears to be

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TABLE 1 Genome statistics and accession numbers

Isolate	Genome size (Mbp)	Coverage (×)	No. of contigs	N_{50} (Mbp)	G+C content (%)	No. of rRNA operons	No. of tRNAs	No. of CDSs ^a	GenBank accession no.
ACPA1	10.06	238	47	1.38	66.9	1	68	9,193	NSDX00000000
ACPA4	7.07	296	9	5.07	61.6	3	55	6,473	NSDY00000000
ACS1	10.89	172	40	1.74	67.1	1	70	10,062	NSDZ00000000

^aCDSs, coding sequences.

universal among isoprene degraders, despite the uncommon usage of this small thiol in Gram-positive bacteria (12, 13).

Interestingly, the genomes of *Rhodococcus* sp. strains ACPA1 and ACS1 contain an additional soluble diiron center monooxygenase in a different region of the genome, with high similarity (>90% amino acid identity) to propane monooxygenase from *Gordonia* TY-5 (14), indicative of the ability of many isoprene-degrading strains to grow on short-chain alkanes in addition to isoprene (5, 15).

These genome sequences extend the diversity of known *iso* genes and will enable the development of improved gene probes and molecular ecology methods for the detection of isoprene degraders in the environment.

Accession number(s). This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under the accession numbers shown in Table 1. The versions described in this paper are the first versions.

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REFERENCES

- Guenther AB, Jiang X, Heald CL, Sakulyanontvittaya T, Duhl T, Emmons LK, Wang X. 2012. The Model of Emissions of Gases and Aerosols from Nature version 2.1:(MEGAN2.1): an extended and updated framework for modeling biogenic emissions. *Geosci Model Dev* 5:1471–1492.
- Fall R, Copley SD. 2000. Bacterial sources and sinks of isoprene, a reactive atmospheric hydrocarbon. *Environ Microbiol* 2:123–130. <https://doi.org/10.1046/j.1462-2920.2000.00095.x>.
- Crombie AT, Khawand ME, Rhodius VA, Fengler KA, Miller MC, Whited GM, McGenity TJ, Murrell JC. 2015. Regulation of plasmid-encoded isoprene metabolism in *Rhodococcus*, a representative of an important link in the global isoprene cycle. *Environ Microbiol* 17:3314–3329. <https://doi.org/10.1111/1462-2920.12793>.
- El Khawand M, Crombie AT, Johnston A, Vavlline DV, McAuliffe JC, Latone JA, Primak YA, Lee SK, Whited GM, McGenity TJ, Murrell JC. 2016. Isolation of isoprene degrading bacteria from soils, development of *isoA* gene probes and identification of the active isoprene-degrading soil community using DNA-stable isotope probing. *Environ Microbiol* 18:2743–2753. <https://doi.org/10.1111/1462-2920.13345>.
- Johnston A, Crombie AT, El Khawand M, Sims L, Whited GM, McGenity TJ, Colin Murrell JC. 2017. Identification and characterisation of isoprene-degrading bacteria in an estuarine environment. *Environ Microbiol* 19:3526–3537. <https://doi.org/10.1111/1462-2920.13842>.
- Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J* 17:10–12. <https://doi.org/10.14806/ej.17.1.200>.
- 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>.
- Bell KS, Philip JC, Aw DWJ, Christofi N. 1998. The genus *Rhodococcus*. *J Appl Microbiol* 85:195–210. <https://doi.org/10.1046/j.1365-2672.1998.00525.x>.
- Chen Y, Ding Y, Yang L, Yu J, Liu G, Wang X, Zhang S, Yu D, Song L, Zhang H, Zhang C, Huo L, Huo C, Wang Y, Du Y, Zhang H, Zhang P, Na H, Xu S, Zhu Y, Xie Z, He T, Zhang Y, Wang G, Fan Z, Yang F, Liu H, Wang X, Zhang X, Zhang MQ, Li Y, Steinbüchel A, Fujimoto T, Cichello S, Yu J, Liu P. 2014. Integrated omics study delineates the dynamics of lipid droplets in *Rhodococcus opacus* PD630. *Nucleic Acids Res* 42:1052–1064. <https://doi.org/10.1093/nar/gkt932>.
- Yoon JH, Cho YG, Kang SS, Kim SB, Lee ST, Park YH. 2000. *Rhodococcus koreensis* sp. nov., a 2,4-dinitrophenol-degrading bacterium. *Int J Syst Evol Microbiol* 50:1193–1201. <https://doi.org/10.1099/00207713-50-3-1193>.
- van Hylckama Vlieg JET, Leemhuis H, Spelberg JHL, Janssen DB. 2000. Characterization of the gene cluster involved in isoprene metabolism in *Rhodococcus* sp. strain AD45. *J Bacteriol* 182:1956–1963. <https://doi.org/10.1128/JB.182.7.1956-1963.2000>.
- van Hylckama Vlieg JET, Kingma J, Kruijzinga W, Janssen DB. 1999. Purification of a glutathione S-transferase and a glutathione conjugate-specific dehydrogenase involved in isoprene metabolism in *Rhodococcus* sp. strain AD45. *J Bacteriol* 181:2094–2101.
- Allocati N, Federici L, Masulli M, Di Ilio C. 2012. Distribution of glutathione transferases in Gram-positive bacteria and archaea. *Biochimie* 94:588–596. <https://doi.org/10.1016/j.biochi.2011.09.008>.
- Kotani T, Yamamoto T, Yurimoto H, Sakai Y, Kato N. 2003. Propane monooxygenase and NAD⁺-dependent secondary alcohol dehydrogenase in propane metabolism by *Gordonia* sp. strain TY-5. *J Bacteriol* 185:7120–7128. <https://doi.org/10.1128/JB.185.24.7120-7128.2003>.
- Acuña Alvarez LA, Exton DA, Timmis KN, Suggett DJ, McGenity TJ. 2009. Characterization of marine isoprene-degrading communities. *Environ Microbiol* 11:3280–3291. <https://doi.org/10.1111/j.1462-2920.2009.02069.x>.