



Draft Genome Sequence of *Rhodococcus ruber* Strain P25, an Active Polychlorinated Biphenyl Degrader

Ekaterina S. Shumkova,^{a,b} Björn E. Olsson,^c Anna V. Kudryavtseva,^d Elena G. Plotnikova^{b,e}

A.N. Bach Institute of Biochemistry, Russian Academy of Sciences, Moscow, Russia^a; Perm State University, Perm, Russia^b; University of Skövde, School of Bioscience, Skövde, Sweden^c; Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia^d; Institute of Ecology and Genetics of Microorganisms, Ural Branch of the Russian Academy of Sciences, Perm, Russia^e

E.S.S. and B.E.O. contributed equally to this work.

We report the 5,728,255-bp draft genome sequence of *Rhodococcus ruber* P25, isolated from a soil polluted with halogenated aromatic compounds in the city of Perm, Russia. The strain degrades polychlorinated biphenyls and a broad range of aromatic compounds. It possesses genes that mediate the degradation of biphenyls/polychlorinated biphenyls, naphthalene, and mono-aromatic compounds.

Received 21 July 2015 Accepted 24 July 2015 Published 3 September 2015

Citation Shumkova ES, Olsson BE, Kudryavtseva AV, Plotnikova EG. 2015. Draft genome sequence of *Rhodococcus ruber* strain P25, an active polychlorinated biphenyl degrader. Genome Announc 3(5):e00990-15. doi:10.1128/genomeA.00990-15.

Copyright © 2015 Shumkova et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license.

Address correspondence to Ekaterina S. Shumkova, ekaterinash80@mail.ru.

ctinobacterium Rhodococcus ruber P25 (=IEGM896) was isolated from soil polluted with the wastes of a chemical plant producing halogen-containing compounds in Perm (Russia) by enrichment in a liquid minimal medium containing biphenyl. Taxonomic assignment to R. ruber P25 was based on a 16S rRNA gene nucleotide sequence (100% similarity with R. ruber DSM 43338^T [GenBank accession no. X80625]), physiological, and biochemical features analysis (1). Strain P25 was capable of utilizing a broad range of aromatic compounds (biphenyl, phenol, toluene, naphthalene, salicylate, gentisate, orthophthalate, benzoate, 3-hydroxybenzoate, 4-hydroxybenzoate, and 3,4-dihydroxybenzoate), as well as their substituted derivatives (chlorinated biphenyls and chlorobenzoates, paramethylbenzoate, and 2,4-dichlorophenoxyacetate) as a sole carbon and energy source (2-4). R. ruber P25 is an active destructor of polychlorinated biphenyls, which are toxic and persistent organic pollutants (5).

To better understand the metabolic versatility of the strain P25, in particular biphenyl and polychlorinated biphenyl destruction pathways, analysis of its genome sequence was carried out. Strain P25 was grown in minimal medium with biphenyl as a sole carbon and energy source. DNA was prepared following a standard genomic DNA purification protocol (6). The draft genome sequence of P25 was prepared using the GS Junior (Roche) system, and the sequencing reads (194,173 reads with an average mean read length of 450 bp) were assembled using the GS De Novo Assembler v2.7. Contigs were ordered using the contig mover function in Mauve (7) using Rhodococcus pyridinivorans SB3094 (GenBank accession no. NC_023150.1) as the reference genome. Gene prediction was carried out using GeneMarkS (8). Ribosomal RNA genes were identified using RNAmmer 1.2 (9) and transfer RNAs by ARAGORN (10). Annotation of the predicted protein coding genes was carried out in the Blast2GO software (11).

The draft genome sequence has a total length of 5,728,255 bp

and is based on 73 contigs with an N_{50} length of 329,488, with the largest contig measuring 483.3 kb. Its analysis showed a G+C content of 70.5%. Contigs constituting 95.6% of the total length (5,479,412 bp) could be ordered by mapping to the reference genome. The draft sequence contains 5,319 coding sequences (CDSs), four rRNAs (5S, 16S, and 23S), and 64 tRNAs genes. The coding regions constitute 91.1% of the total sequence and the average gene length is 964. A total of 3,677 genes (69.6%) were annotated with gene ontology (GO) terms.

Functional annotation showed that strain P25 possesses *bph* genes, benzoate, protokatehoate-, gentisate-, and catechol-degrading genes, and genes of the phenol and naphtalen degradation pathways.

Organization of the *bph* gene cluster differed from other *bph* gene clusters known to date (12–15). It contained genes of the "upper" pathway, transcribed in the same direction: *bphAd-bphD-bphC-bphAa-bphAb-bphAc-bphB* (ferredoxin reductase, 2-hydroxy-6-oxo-6-phenylhexodienoat hydrolase and 2,3-dihydroxybiphenyl 1,2-dioxygenase, biphenyl 2,3-dioxygenase α - and β -subunites, ferredoxin, biphenyl 2,3-dihydrodiol de-hydrogenase, respectively). This order is not typical. The genes of the "lower" pathway of biphenyl degradation, encoding 4-hydroxy-2-oksovalerat aldolase, acetaldehyde dehydrogenase, and 2-keto-4-pentenoate hydratase, are located downstream of *bphB* and are transcribed in the opposite direction.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. LDUF000000000. The version described in this paper is version LDUF01000000.

ACKNOWLEDGMENT

This work was supported by the Ministry of Education and Science of the Russian Federation (project 2014-14-576-0058-020).

REFERENCES

- 1. Plotnikova EG, Rybkina DO, Demakov VA. 2005. Russian Federation patent no. 2262531.
- Shumkova ES, Solyanikova IP, Plotnikova EG, Golovleva LA. 2009. Degradation of *para*-toluate by the bacterium *Rhodococcus ruber* P25. Microbiology 78:376–378. http://dx.doi.org/10.1134/S0026261709030175.
- Shumkova ES, Egorova DO, Korsakova ES, Dorofeeva LV, Plotnikova EG. 2014. Molecular biological characterization of biphenyl degrading bacteria and identification of the biphenyl 2,3-dioxygenase α-subunit genes. Microbiology 83:160–168. http://dx.doi.org/10.1134/S0026261714010135.
- Solyanikova IP, Emelyanova EV, Shumkova ES, Egorova DO, Korsakova ES, Plotnikova EG, Golovleva LA. 2015. Peculiarities of the degradation of benzoate and its chloro- and hydroxy-substituted analogs by actinobacteria. Int Biodeterior Biodegrad 100:155–164. http://dx.doi.org/ 10.1016/j.ibiod.2015.02.028.
- Egorova DO, Demakov VA, Plotnikova EG. 2013. Bioaugmentation of a polychlorobiphenyl contaminated soil with two aerobic bacterial strains. J Hazard Mater 261:378–386. http://dx.doi.org/10.1016/ j.jhazmat.2013.07.067.
- Ausbel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K. 1995. Short protocols in molecular biology, 3rd ed. John Wiley and Sons, New York, NY.
- Rissman AI, Mau B, Biehl BS, Darling AE, Glasner JD, Perna NT. 2009. Reordering contigs of draft genomes using the Mauve aligner. Bioinformatics 25:2071–2073. http://dx.doi.org/10.1093/bioinformatics/btp356.
- Besemer J, Lomsadze A, Borodovsky M. 2001. GeneMarkS: a selftraining method for prediction of gene starts in microbial genomes. Implications for finding sequence motifs in regulatory regions. Nucleic Acids Res 29:2607–2618. http://dx.doi.org/10.1093/nar/29.12.2607.

- Lagesen K, Hallin P, Rødland EA, Staerfeldt HH, Rognes T, Ussery DW. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res 35:3100–3108. http://dx.doi.org/10.1093/ nar/gkm160.
- Laslett D, Canback B. 2004. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. Nucleic Acids Res 32:11–16. http://dx.doi.org/10.1093/nar/gkh152.
- Götz S, García-Gómez JM, Terol J, Williams TD, Nagaraj SH, Nueda MJ, Robles M, Talón M, Dopazo J, Conesa A. 2008. High-throughput functional annotation and data mining with the Blast2GO suite. Nucleic Acids Res 36:3420-3435. http://dx.doi.org/10.1093/nar/gkn176.
- 12. Yang X, Liu X, Song L, Xie F, Zhang G, Qian S. 2007. Characterization and functional analysis of a novel gene cluster involved in biphenyl degradation in *Rhodococcus* sp. strain R04. J Appl Microbiol 103:2214–2224. http://dx.doi.org/10.1111/j.1365-2672.2007.03461.x.
- Pieper DH, Seeger M. 2008. Bacterial metabolism of polychlorinated biphenyls. J Mol Microbiol Biotechnol 15:121–138. http://dx.doi.org/ 10.1159/000121325.
- Fujihara H, Yamazoe A, Hosoyama A, Suenaga H, Kimura N, Hirose J, Watanabe T, Futagami T, Goto M, Furukawa K. 2015. Draft genome sequence of *Pseudomonas abietaniphila* KF701 (NBRC110664), a polychlorinated biphenyl-degrading bacterium isolated from biphenylcontaminated soil. Genome Announc 3(3):e00473-15. http://dx.doi.org/ 10.1128/genomeA.00473-15.
- Li A, Qu YY, Pi WQ, Zhou JT, Gai ZH, Xu P. 2012. Metabolic characterization and genes for the conversion of biphenyl in *Dyella ginsengisoli* LA-4. Biotechnol Bioeng 109:609–613. http://dx.doi.org/10.1002/ bit.23333.