

## Genome Sequence of *Rhodococcus* sp. Strain R04, a Polychlorinated-Biphenyl Biodegrader<sup>∇</sup>

Xiuqing Yang,<sup>1\*</sup> Rui Xue,<sup>1</sup> Chong Shen,<sup>1</sup> Shuren Li,<sup>1</sup> Chong Gao,<sup>1</sup> Qi Wang,<sup>2</sup> and Xiaoxia Zhao<sup>3</sup>

Shanxi University, Institute of Biotechnology, Key Laboratory of Chemical Biology and Molecular Engineering of Ministry of Education, 030006 Taiyuan, People's Republic of China<sup>1</sup>; Shanxi University, School of Life Science, 030006 Taiyuan, People's Republic of China<sup>2</sup>; and Shanxi University, Institute of Applied Chemistry, 030006 Taiyuan, People's Republic of China<sup>3</sup>

Received 21 June 2011/Accepted 29 June 2011

**The genus *Rhodococcus* has proved to be a promising option for the cleanup of polluted sites and application of a microbial biocatalyst. *Rhodococcus* sp. strain R04, isolated from oil-contaminated soil, can biodegrade polychlorinated biphenyls. Here we report the draft genome sequence of *Rhodococcus* sp. strain R04, which could be used to predict genes for xenobiotic biodegradation and provide important insights into the applications of this strain.**

The genus *Rhodococcus* is a very diverse group of bacteria that possess the ability to degrade a large number of organic compounds, including some of the most difficult compounds with respect to recalcitrance and toxicity. Several strains belonging to the genus *Rhodococcus* have been isolated from various contaminated environments (1, 3, 10, 11, 12), which have proved to be ideal candidates for enhancing the bioremediation of contaminated sites and a wide range of biotransformations, such as steroid modifications, enantioselective synthesis, and the production of amides from nitriles (4, 14).

*Rhodococcus* sp. strain R04 was isolated from oil-contaminated soil in northern China. Strain R04 was able to biodegrade polychlorinated biphenyls (PCBs) not only via ring cleavage but also through dechlorination (15). In addition, it metabolized phenol, benzoate, and 2-nitropropane when provided as the sole source of carbon and energy and cometabolized pentachlorophenol, dibenzofuran, benzothiophene, and atrazine. It is suggested that strain R04 will potentially be useful in the biotreatment of wastewater and bioremediation of contaminated soils.

The genome sequence of R04 was determined by using the high-throughput Solexa sequencing technology (Illumina GA2x) in Shenzhen, China. Whole-genome shotgun (WGS) sequence data for 825 Mb, giving approximately 92-fold genome coverage, were generated and assembled into 2,548 contigs using SOAPdenovo v.1.04 (7). Furthermore, the contigs were joined into 110 scaffolds (>1 kb in size) using paired-end information. The genome sequence analysis of R04 showed a genome size of 9,125,386 bp, with a mean GC content of 69.62%. Annotation of the open reading frames was performed using Glimmer v.3.0 (2) and by comparison with the corresponding data from the COG, KEGG, Swiss-Prot, TrEMBL, and NR databases. tRNA and rRNA genes were

identified by tRNAscan and RNAmmer, respectively (6, 8). There were 9,318 coding sequences (CDSs) with an average length of 826 bp, 99 tRNAs, and 20 rRNAs.

At least 82 genes were found to be potentially involved in xenobiotic metabolism. Among these genes, four extradiol dioxygenase genes (*bphC*) and two hydrolase genes (*bphD*) were involved in PCB degradation by R04. In addition, the R04 genome contains at least six ring-hydroxylating dioxygenase genes and 15 cytochrome P450 genes, which enables strain R04 to adapt to catabolize a large number of substrates. Differing from *Rhodococcus jostii* RHA1 (formerly named by *Rhodococcus* sp. strain RHA1), whose genome holds two set of polychlorinated biphenyl transformation systems (5), *Rhodococcus* sp. strain R04 contains only a *bph* gene cluster consisting of *bphALA2A3A4*, *bphB*, *bphC*, and *bphD* (16). In strain R04, ketosteroid-9- $\alpha$ -hydrolase, 3-ketosteroid- $\delta$ -dehydrogenase, 3-ketosteroid-1-dehydrogenase, and steroid  $\delta$ -isomerase are involved in the transformation and metabolism of steroid compounds, and they share 69 to 96% of identity with those of *Rhodococcus jostii* RHA1, *Rhodococcus rhodochrous*, and *Rhodococcus erythropolis* (9, 10, 13). It has been suggested that *Rhodococcus* sp. R04 is a potential candidate for the industrial production of bioactive steroid compounds.

**Nucleotide sequence accession numbers.** This whole-genome shotgun project has been deposited into DDBJ/EMBL/GenBank under accession number AFAQ00000000. The version described in this paper is the first version, accession number AFAQ01000000.

This research was supported by the National Natural Science Foundation of China (grant 30800030), the Young Science Foundation of Shanxi Province (grant 207021030), and the Natural Science Foundation of Shanxi Province (grant 2007031003).

### REFERENCES

1. Curragh, H., et al. 1994. Haloalkane degradation and assimilation by *Rhodococcus rhodochrous* NCIMB 13064. *Microbiology* **140**:1433–1442.
2. Delcher, A. L., K. A. Bratke, E. C. Powers, and S. L. Salzberg. 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. *Bioinformatics* **23**:673–679.
3. Haroune, N., et al. 2004. Metabolism of 2-mercaptobenzothiazole by *Rhodococcus rhodochrous*. *Appl. Environ. Microbiol.* **70**:6315–6319.
4. Kim, B. Y., and H. H. Hyun. 2002. Production of acrylamide using immobi-

\* Corresponding author. Mailing address: Shanxi University, Institute of Biotechnology, Key Laboratory of Chemical Biology and Molecular Engineering of Ministry of Education, Wuchenglu 92, 030006 Taiyuan, People's Republic of China. Phone: 86 351 7017661. Fax: 86 351 7010215. E-mail: xiuqiang@sxu.edu.cn.

<sup>∇</sup> Published ahead of print on 8 July 2011.

- lized cells of *Rhodococcus rhodochrous* M33. *Biotechnol. Bioproc. Eng.* **7**:194–200.
5. **Kitagawa, W., et al.** 2001. Multiplicity of aromatic ring hydroxylation dioxygenase genes in a strong PCB degrader, *Rhodococcus* sp. strain RHA1 demonstrated by denaturing gradient gel electrophoresis. *Biosci. Biotechnol. Biochem.* **65**:1907–1911.
  6. **Lagesen, K., et al.** 2007. RNAmmer: consistent annotation of rRNA genes in genomic sequences. *Nucleic Acids Res.* **35**:3100–3108.
  7. **Li, R., et al.** 2010. De novo assembly of human genomes with massively parallel short read sequencing. *Genome Res.* **20**:265–272.
  8. **Lowe, T. M., and S. R. Eddy.** 1997. tRNAscan-SE: a program for improved detection of tRNA genes in genomic sequence. *Nucleic Acids Res.* **25**:955–964.
  9. **McLeod, M. P., et al.** 2006. The complete genome of *Rhodococcus* sp. RHA1 provides insights into a catabolic powerhouse. *Proc. Natl. Acad. Sci. U. S. A.* **103**:15582–15587.
  10. **Morii, S., et al.** 1998. 3-Ketosteroid-delta1-dehydrogenase of *Rhodococcus rhodochrous*: sequencing of the genomic DNA and hyperexpression, purification, and characterization of the recombinant enzyme. *J. Biochem.* **124**:1026–1032.
  11. **Prince, R. C., and M. J. Grossman.** 2003. Substrate preferences in biodesulfurization of diesel range fuels by *Rhodococcus* sp. strain ECRD-1. *Appl. Environ. Microbiol.* **69**:5833–5838.
  12. **Seto, M., et al.** 1995. A novel transformation of polychlorinated biphenyls by *Rhodococcus* sp. strain RHA1. *Appl. Environ. Microbiol.* **61**:3353–3358.
  13. **van der Geize, R. et al.** 2001. Unmarked gene deletion mutagenesis of *kstD*, encoding 3-ketosteroid delta1-dehydrogenase, in *Rhodococcus erythropolis* SQ1 using *sacB* as counter-selectable marker. *FEMS Microbiol. Lett.* **205**:197–202.
  14. **Wu, Z. L., and Z. Y. Li.** 2003. Highly enantioselective synthesis of  $\alpha,\alpha$ -disubstituted malonamic acids through asymmetric hydrolysis of dinitriles with *Rhodococcus* sp. CGMCC 0497. *Chem. Commun. (Camb.)* **68**:386–387.
  15. **Yang, X. Q., et al.** 2007. Characterization and functional analysis of a new gene cluster involved in biphenyl/PCB degradation in *Rhodococcus* sp. strain R04. *J. Appl. Microbiol.* **103**:2214–2224.
  16. **Yang, X. Q., Y. Sun, and S. J. Qian.** 2004. Biodegradation of seven polychlorinated biphenyls by a new isolated aerobic bacterial (*Rhodococcus* sp. R04). *J. Ind. Microbiol. Biotechnol.* **31**:415–420.