

Genome Sequence of *Ralstonia* sp. Strain PBA, a Bacterium Involved in the Biodegradation of 4-Aminobenzenesulfonate

Han Ming Gan,^a Teong Han Chew,^b Yea-Ling Tay,^a Siew Fen Lye,^a and Adibah Yahya^c

ScienceVision SB, Shah Alam, Selangor, Malaysia,^a and Department of Biological Sciences^b and Department of Industrial Biotechnology,^c Faculty of Biosciences and Bioengineering, Universiti Teknologi Malaysia, Skudai, Johor, Malaysia

***Ralstonia* sp. strain PBA was isolated from textile wastewater in a coculture with *Hydrogenophaga* sp. strain PBC. Here we present the assembly and annotation of its genome, which may provide further insights into the mechanism of its interaction with strain PBC during 4-aminobenzenesulfonate degradation.**

Ralstonia is a recently designated genus which is comprised of former *Burkholderia* species. Members of the genus *Ralstonia* were ubiquitous inhabitant of soil, freshwater, and even ultrapure water in industrial systems. Currently, the study of the *Ralstonia* genus focuses on three main species, namely, *Ralstonia solanacearum* (a plant pathogen), *Ralstonia eutropha* (a bioplastic producer), and *Ralstonia pickettii* (a versatile pollutant degrader) (8–10).

Ralstonia sp. strain PBA was isolated as a coculture with *Hydrogenophaga* sp. strain PBC during the selection of textile wastewater bacteria for biodegradation of 4-aminobenzenesulfonate (4-ABS), a sulfonated aromatic amine which is recalcitrant to biodegradation but is frequently used in the production of textile dyes, sulfonamide, optical brighteners, and pesticides (2). The ability of strain PBC to degrade 4-ABS axenically with the supplementation of *p*-aminobenzoate and biotin raised the possibility of strain PBA being a growth factor provider in the coculture. Based on 16S rRNA gene sequence analysis, strain PBA was more closely related to an uncultured bacterium clone, 300A-F12 (99.1% identity), than to other common *Ralstonia* strains (<97.6% identity).

Recent advancements in the study of 4-ABS biodegradation have been made possible owing to the syntropic interaction between strains PBA and PBC. However, the mechanism of this interaction at the genetic level remains unclear due to the channeling of concerted efforts toward the identification of genes involved in the first step of 4-ABS biodegradation in strain PBC. The critical questions which we hope to answer via analysis of the draft genome of strain PBA are the following. (i) What is the intermediate from 4-ABS biodegradation which can be utilized by strain PBA for its survival in minimal medium? (ii) Is the genome of strain PBA enriched in the genes involved in biotin and *p*-aminobenzoate biosynthesis?

The genome sequencing of *Ralstonia* sp. PBA was performed using the Illumina Genome Iix analyzer (100-bp paired-end reads). The paired-end reads were trimmed and assembled *de novo* using the software program CLC Genomics Workbench 4.8 (CLC Bio, Denmark). The programs Prodigal 2.50, tRNAscan-SE 1.3, and RNAmmer 1.2 (5–7) were used to predict open reading frames (ORFs), tRNAs, and rRNAs, respectively. Subsequent genome annotation was performed using the software program Blast2GO 2.5.0 (1, 3). A total of 48 contigs were produced from the *de novo* assembly, with an accumulated length of 3,829,108 bp and an average GC content of 63.66%. The contig N50 was 256,706 bp in length, and the largest assembled contig was 509,421 bp. Three thousand five hundred seventeen ORFs, forty-five tRNAs, and three rRNAs were predicted from the draft genome.

Ralstonia sp. PBA contains not one but two *pcaIJ* homologs (contigs 5 and 24) which encode beta-ketoadipate succinyl-coenzyme A transferase for the penultimate step in the bioconversion of various aromatic compounds to the tricarboxylic acid cycle intermediates (4). Having an additional homolog of this gene set may give strain PBA an evolutionary edge in the competition for beta-ketoadipate. Two homologs of genes involved in the synthesis of *p*-aminobenzoate were also identified (contigs 21 and 27). These features in the genome of *Ralstonia* sp. PBA may grant it an exclusive partnership with *Hydrogenophaga* sp. PBC to enable the biodegradation of 4-ABS.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. [AJWL000000000](http://ajwl000000000). The version described in this article is the first version, [AJWL010000000](http://ajwl010000000).

ACKNOWLEDGMENTS

This work was supported by Universiti Teknologi Malaysia and ScienceVision SB.

We thank Lian Shien Lee for assistance in this project.

REFERENCES

1. Conesa A, et al. 2005. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics* 21: 3674–3676.
2. Gan HM, Shahir S, Ibrahim Z, Yahya A. 2011. Biodegradation of 4-aminobenzenesulfonate by *Ralstonia* sp. PBA and *Hydrogenophaga* sp. PBC isolated from textile wastewater treatment plant. *Chemosphere* 82: 507–513.
3. Gan HM, Shahir S, Yahya A. 18 May 2012. Cloning and functional analysis of the genes coding for 4-aminobenzenesulfonate 3,4-dioxygenase from *Hydrogenophaga* sp. PBC. *Microbiology*. [Epub ahead of print.] doi:10.1099/mic.0.059550-0.
4. Harwood CS, Parales RE. 1996. The β -ketoadipate pathway and the biology of self-identity. *Annu. Rev. Microbiol.* 50:553–590.
5. Hyatt D, et al. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* 11:1–11.
6. Lagesen K, et al. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res.* 35:3100–3108.

Received 29 June 2012 Accepted 5 July 2012

Address correspondence to Han Ming Gan, hmg2760@gmail.com.

Copyright © 2012, American Society for Microbiology. All Rights Reserved.

doi:10.1128/JB.01165-12

7. **Lowe TM, Eddy SR.** 1997. tRNA-scan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res.* **25**:955–964.
8. **Pohlmann A, et al.** 2006. Genome sequence of the bioplastic-producing “Knallgas” bacterium *Ralstonia eutropha* H16. *Nat. Biotechnol.* **24**:1257–1262.
9. **Remenant B, et al.** 2010. Genomes of three tomato pathogens within the *Ralstonia solanacearum* species complex reveal significant evolutionary divergence. *BMC Genomics* **11**:379.
10. **Ryan MP, Pembroke JT, Adley CC.** 2007. *Ralstonia picketti* in environmental biotechnology: potential and applications. *J. Appl. Microb.* **103**:754–764.