

# Draft Genome Sequence of *Sphingobium quisquiliarum* Strain P25<sup>T</sup>, a Novel Hexachlorocyclohexane (HCH)-Degrading Bacterium Isolated from an HCH Dumpsite

Amit Kumar Singh,<sup>a</sup> Naseer Sangwan,<sup>a</sup> Anukriti Sharma,<sup>a</sup> Vipin Gupta,<sup>a</sup> J. P. Khurana,<sup>b</sup> Rup Lal<sup>a</sup>

Department of Zoology, University of Delhi, Delhi, India<sup>a</sup>; Interdisciplinary Centre for Plant Genomics & Department of Plant Molecular Biology, University of Delhi South Campus, New Delhi, India<sup>b</sup>

Here, we report the draft genome sequence (4.2 Mb) of *Sphingobium quisquiliarum* strain P25<sup>T</sup>, a natural *lin* (genes involved in degradation of hexachlorocyclohexane [HCH] isomers) variant genotype, isolated from a heavily contaminated (450 mg HCH/g of soil) HCH dumpsite.

Received 12 August 2013 Accepted 19 August 2013 Published 12 September 2013

Citation Kumar Singh A, Sangwan N, Sharma A, Gupta V, Khurana JP, Lal R. 2013. Draft genome sequence of *Sphingobium quisquiliarum* strain P25<sup>T</sup>, a novel hexachlorocyclohexane (HCH)-degrading bacterium isolated from an HCH dumpsite. *Genome Announc.* 1(5):e00717-13. doi:10.1128/genomeA.00717-13.

Copyright © 2013 Kumar Singh et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](http://creativecommons.org/licenses/by/3.0/).

Address correspondence to Rup Lal, [ruplal@gmail.com](mailto:ruplal@gmail.com).

The disposal of hexachlorocyclohexane (HCH) waste in the past has resulted in the pangenomic enrichment of various sphingomonad genotypes at HCH dumpsites (1, 2). In order to continue our efforts to sequence genomes of sphingomonads from the HCH dumpsite located near Lucknow, India (27°00'N and 81°09'E) (3, 4), we sequenced the genome of another sphingomonad strain, P25<sup>T</sup> (4.2 Mb).

The draft genome sequence of strain P25<sup>T</sup> was obtained by use of an Illumina Genome Analyzer II platform. The sequencing data ( $n = 3,882,670$ ; 90 bp/read) were assembled into contigs ( $n = 181$ , >500 bp) using ABySS 1.3.3 (5) set at a k-mer size of 47. Contigs ( $N_{50}$ , 45 kb) were further validated (paired-end criterion) using *bwa*-0.5.9 (6). Glimmer-3.02 (7) was used to predict the protein-encoding genes, whereas tRNA and rRNA genes were identified using ARAGORN (8) and RNAmmer (9), respectively. A total of 4,033 coding sequences (CDS), 70 pseudogenes, 54 tRNA genes, and 1 rRNA operon were observed, with an average G+C content of 64%. Validated (paired-end criterion) genome assembly was annotated using RAST version 4.0 (10) and the NCBI Prokaryotic Genomes Automatic Annotation pipeline (PGAAP) (<http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html>). Average nucleotide identity (ANI) (11) analysis revealed that *Sphingobium japonicum* UT26S (83.3%) (12), *Sphingobium indicum* B90A (83.0%) (4), and *Sphingomonas* sp. SKA58 (80.8%) are the closest phylogenetic neighbors of *S. quisquiliarum* P25<sup>T</sup>.

The mechanisms of acquisition of *lin* genes in sphingomonads under HCH stress at these dumpsites are still not clearly understood (2). The *lin* genes were first reported in *S. japonicum* UT26 (12) and subsequently from *S. indicum* B90A (4). Many more sphingomonads have been isolated recently from the HCH dumpsite (2, 14). All of these strains by and large share the same pathway for the degradation of HCH isomers that requires the *linA* through *linF* genes (2). Interestingly, the analysis of the draft genome of strain P25<sup>T</sup> revealed the presence of one copy each of *linA*, *linH*, *linK*, *linL*, *linM*, *linN*, and *linX*, and the IS FINDER database (13) (<http://www-is.biotoul.fr>) predicted the occurrence of IS6 ( $n =$

21), IS1380 ( $n = 4$ ), IS3 ( $n = 1$ ), and IS256 ( $n = 1$ ) as the major transposon families. However, *linB*, which encodes haloalkane dehalogenase, was absent, indicating that this strain has yet to acquire *linB* through horizontal gene transfer.

In comparison with the whole-genome sequence of *S. japonicum* UT26 (12), P25<sup>T</sup> showed the presence of phenol- and toluene-degrading gene clusters, whereas homogentisate-, chlorophenol-, and anthranilate-degrading pathways were clearly absent in *S. quisquiliarum* P25<sup>T</sup>. Reciprocal smallest distance (RSD) analysis ( $e$  value,  $10^{-15}$ ; distance, 0.125) revealed that *S. quisquiliarum* P25<sup>T</sup> and *S. japonicum* UT26 share 1,650 orthologous genes. Data and information assimilation from the complete genome of this species and a comparative analysis with other sphingomonad genomes (3) are under way to expand our understanding of HCH degradation, especially the rapid evolution and acquisition of *lin* genes in sphingomonads under HCH selection pressure.

**Nucleotide sequence accession number.** The draft genome sequence of *Sphingobium quisquiliarum* P25<sup>T</sup> has been deposited in GenBank under the accession number [ATHO00000000](https://www.ncbi.nlm.nih.gov/nuclseq/ATHO00000000). The version described in this paper is the first version.

## ACKNOWLEDGMENTS

The work was supported by grants from the Department of Biotechnology (DBT), Government of India (under project BT/PR3301/BCE/8/875/11), the University of Delhi/Department of Science and Technology Promotion of University Research and Scientific Excellence DU-DST-PURSE grant and National Bureau of Agriculturally Important Microorganisms (NBAIM) AMASS/2006-07/NBAIM/CIR, and the All India Network Project Soil Biodiversity-Biofertilizer (ICAR). A.K.S., N.S., V.G., and A.S. gratefully acknowledge the Department of Biotechnology (DBT), Council for Scientific and Industrial Research (CSIR), and National Bureau of Agriculturally Important Microorganisms (NBAIM) for providing research fellowships. This paper was finalized during the renewed visit under Alexander von Humboldt Fellowship (at the University of Freiburg, Germany) awarded to R.L.

## REFERENCES

1. Sangwan N, Lata P, Dwivedi V, Singh A, Niharika N, Kaur J, Anand S, Malhotra J, Jindal S, Nigam A, Lal D, Dua A, Saxena A, Garg N, Verma M, Kaur J, Mukherjee U, Gilbert JA, Dowd SE, Raman R, Khurana P, Khurana JP, Lal R. 2012. Comparative metagenomic analysis of soil microbial communities across three hexachlorocyclohexane contamination levels. *PLoS One* 7:e46219.
2. Lal R, Pandey G, Sharma P, Kumari K, Malhotra S, Pandey R, Raina V, Kohler HP, Holliger C, Jackson C, Oakeshott JG. 2010. Biochemistry of microbial degradation of hexachlorocyclohexane and prospects for bioremediation. *Microbiol. Mol. Biol. Rev.* 74:58–80.
3. Niharika N, Sangwan N, Ahmad S, Singh P, Khurana JP, Lal R. 2013. Draft genome sequence of *Sphingobium chinhatense* IP26<sup>T</sup>, isolated from a hexachlorocyclohexane dumpsite. *Genome Announc.* 1(4):e00680-13. doi:10.1128/genomeA.00680-13.
4. Anand S, Sangwan N, Lata P, Kaur J, Dua A, Singh AK, Verma M, Kaur J, Khurana JP, Khurana P, Mathur S, Lal R. 2012. Genome sequence of *Sphingobium indicum* B90A, a hexachlorocyclohexane-degrading bacterium. *J. Bacteriol.* 194:4471–4472.
5. Simpson JT, Wong K, Jackman SD, Schein JE, Jones SJ, Birol B. 2009. ABySS: a parallel assembler for short read sequence data. *Genome Res.* 19:1117–1123.
6. Li H, Durbin R. 2010. Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics* 26:589–595.
7. Delcher AL, Bratke KA, Powers EC, Salzberg SL. 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. *Bioinformatics* 23:673–679.
8. Laslett D, Canback B. 2004. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. *Nucleic Acids Res.* 32:11–16.
9. 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.
10. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: rapid annotations using subsystems technology. *BMC Genomics* 9:75. doi:10.1186/1471-2164-9-75.
11. Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandame P, Tiedje JM. 2005. DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. *Int. J. Syst. Evol. Microbiol.* 57: 81–91.
12. Nagata Y, Ohtsubo Y, Endo R, Ichikawa N, Ankaï A, Oguchi A, Fukui S, Fujita N, Tsuda M. 2010. Complete genome sequence of the representative  $\gamma$ -hexachlorocyclohexane-degrading bacterium *Sphingobium japonicum* UT26. *J. Bacteriol.* 192:5852–5853.
13. Siguier P, Perochon J, Lestrade L, Mahillon J, Chandler M. 2006. ISfinder: the reference centre for bacterial insertion sequences. *Nucleic Acids Res.* 34:D32–D36.
14. Böltner D, Moreno-Morillas S, Ramos JL. 2005. 16S rDNA phylogeny and distribution of *lin* genes in novel hexachlorocyclohexane-degrading *Sphingomonas* strains. *Environ. Microbiol.* 7:1329–1339.