

Draft Genome Sequence of *Sphingobium* sp. Strain HDIPO4, an Avid Degrader of Hexachlorocyclohexane

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Sphingobium sp. strain HDIPO4 was isolated from a hexachlorocyclohexane (HCH) dumpsite and degraded HCH isomers rapidly. The draft genome sequence of HDIPO4 (~4.7 Mbp) contains 143 contigs and 4,646 coding sequences with a G+C content of 65%.

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Sphingobium sp. strain HDIPO4 was originally isolated in 2008 (1) from the hexachlorocyclohexane (HCH)-contaminated dumpsite at Ummari Village, Lucknow, India. Phylogenetic analysis using 16S rRNA gene sequences showed that strain HDIPO4 is most closely related to another HCH-degrading strain, *Sphingobium francense* Sp+ (with 98% sequence similarity). Strain Sp+ was previously isolated from French soil contaminated with γ -HCH (2, 3). In contrast to strain Sp+, which degrades HCH isomers slowly, strain HDIPO4 was found to degrade HCH isomers at a high rate (1).

The genome sequencing of strain HDIPO4 was carried out by using the Illumina genome analyzer platform and the 454 GS FLX Titanium platform. For this purpose, paired-end libraries of 2 kb were constructed that led to the generation of 761 Mbp of raw data, which generated 561 Mbp of clean data.

The draft genome sequence of HDIPO4 consists of 4,741,576 bp with 90× genome coverage. The draft genome was assembled into 143 contigs by using the ABySS 1.3.3 assembler (4) at a k-mer of 53 with an average G+C content of 65%. The validation of the final assembly was done based on the paired-end information. The annotations were done by using RAST version 4.0 (5) and the NCBI Prokaryotic Genomes Annotation Pipeline (PGAP) version 2.0 (http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html).

A total of 4,646 open reading frames (ORFs) and 54 tRNA and 13 rRNA genes were identified. In addition, 3 copies of the 16S rRNA gene were also detected. Interestingly, this strain contained two copies each of *linB* (upper degradation pathway genes) and *linDER* (lower degradation pathway genes) (6), in contrast to previously reported sphingomonads that contained only one copy each of these genes (7, 8). The presence of two copies each of *linB* and *linDER* may be responsible for the faster degradation of HCH isomers by this strain.

Besides the HCH degradation pathway genes, strain HDIPO4 showed the presence of phenol-, chlorophenol-, toluene-, and homogentisate-degrading gene clusters. Eighteen copies of IS6100 were found that are known to play active roles in horizontal gene

transfer of *lin* genes among sphingomonads (3). The TonB-dependent receptor, which mediates transport of iron siderophore complexes in Gram-negative bacteria (11), was also detected in abundance, in the vicinity of the *lin* genes. A total of 29 insertion sequence (IS) elements, including IS6100, ISSp5, ISGbe1, and ISSpma1, were also present in the draft genome (12).

Such myriad *lin* genes, transposons, and other catabolic genes were also reported in the genomes of HCH-degrading sphingomonads (7–10) and as products of enrichment and colossal horizontal gene transfer in the recent metagenomic analyses of the HCH dumpsite (13, 14). The genome sequence of this strain, especially the presence of multiple copies of *lin* genes, clearly indicates that it may be useful in the development of a consortium for bioremediation of HCH at the HCH dumpsite.

Nucleotide sequence accession numbers. The genome sequence of *Sphingobium* sp. HDIPO4 has been assigned GenBank accession number ATDO000000000. The version described in this paper is the first version, ATDO01000000.

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REFERENCES

- 1. Dhadwal M, Singh A, Prakash O, Gupta SK, Kumari K, Sharma P, Verma M, Holliger C, Lal R. 2008. Proposal of biostimulation for hexachlorocyclohexane (HCH)-decontamination and characterization of culturable bacterial community from high-dose point HCH-contaminated soils. J. Appl. Microbiol. 106:381–392.
- 2. Pal R, Bala S, Dadhwal M, Kumar M, Dhingra G, Prakash O, Prabagaran

- SR, Shivaji S, Cullum J, Holliger C, Lal R. 2005. Hexachlorocyclohexane-degrading bacterial strains *Sphingomonas paucimobilis* B90A, UT26 and Sp+, having similar *lin* genes, represent three distinct species, *Sphingomonas indicum* sp. nov., *Sphingobium japonicum* sp. nov., and *Sphingobium francense* sp. nov., and reclassification of *Sphingomonas chungbukensis* as *Sphingobium chungbukense* comb. nov. Int. J. Syst. Evol. Microbiol. 55:1965–1972.
- Dogra C, Raina V, Pal R, Suar M, Lal S, Gartemann KH, Holliger C, van der Meer JR, Lal R. 2004. Organization of *lin* genes and IS6100 among different strains of hexachlorocyclohexane-degrading *Sphingomonas* paucimobilis: evidence for horizontal gene transfer. J. Bacteriol. 186: 2225–2235.
- Simpson JT, Wong K, Jackman SD, Schein JE, Jones SJ, Birol I. 2009. ABySS: a parallel assembler for short read sequence data. Genome Res. 19:1117–1123.
- 5. 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.
- 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.
- 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.

- 8. Niharika N, Sangwan N, Ahmad S, Singh P, Khurana JP, Lal R. 2013. Draft genome sequence of *Sphingobium chinhatense* strain IP26^T, isolated from a hexachlorocyclohexane dumpsite. Genome Announc. 1(4): e00680-13. doi:10.1128/genomeA.00680-13.
- Saxena A, Nayyar N, Sangwan N, Kumari R, Khurana JP, Lal R. 2013. Genome sequence of *Novosphingobium lindaniclasticum* LE124^T, isolated from a hexachlorocyclohexane dumpsite. Genome Announc. 1(5): e00715-13. doi:10.1128/genomeA.00715-13.
- Singh AK, Sangwan N, Sharma A, Gupta V, Khurana JP, Lal R. 2013. Draft genome sequence of *Sphingobium quisquiliarum* P25^T, a novel hexachlorocylohexane (HCH)-degrading bacterium isolated from an HCH dumpsite. Genome Announc. 1(5):e00717-13. doi:10.1128/genomeA.00717-13.
- 11. Miller TR, Delcher AL, Salzberg SL, Saunders E, Detter JC, Halden RU. 2010. Genome sequence of the dioxin-mineralizing bacterium *Sphingomonas wittichii* RW1. J. Bacteriol. 192:6101–6102.
- 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. doi:10.1093/nar/gkj014.
- 13. 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 Ü, 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. doi:10.1371/journal.pone.0046219.
- 14. Sangwan N, Verma H, Kumar R, Negi V, Lax S, Khurana P, Khurana JP, Gilbert JA, Lal R. Reconstructing an ancestral genotype of two hexachlorocyclohexane degrading *Sphingobium* species using metagenomic sequence data. ISME J., in press.