

Genome Sequence of *Novosphingobium lindaniclasticum* LE124^T, Isolated from a Hexachlorocyclohexane Dumpsite

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***Novosphingobium lindaniclasticum* LE124^T is a hexachlorocyclohexane (HCH)-degrading bacterium isolated from a high-dosage-point HCH dumpsite (450 mg HCH/g soil) located in Lucknow, India (27°00'N and 81°09'E). Here, we present the annotated draft genome sequence of strain LE124^T, which has an estimated size of 4.86 Mb and is comprised of 4,566 coding sequences.**

Received 10 August 2013 Accepted 19 August 2013 Published 12 September 2013

Citation 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.

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Sphingomonads represent a naturally selected taxon that can degrade and/or assimilate a wide range of xenobiotic compounds, including mono- and polycyclic aromatic compounds, chlorinated compounds, and pesticides (1). Members of this taxon are also known to be efficient degraders of hexachlorocyclohexane (HCH) isomers (1–3). In conformity with our primary objective, i.e., to elucidate the pangenomic variations across HCH-degrading genotypes, we have already sequenced two sphingomonads (4, 5). Both of their genomes represent species belonging to the genus *Sphingobium* (4, 5). We have sequenced the genome of yet another sphingomonad, belonging to the genus *Novosphingobium*, i.e., *N. lindaniclasticum* strain LE124^T, isolated from an HCH dumpsite (6).

The genomic DNA of strain LE124^T was sequenced by the Illumina genome analyzer Ix platform (paired-end library, 2 kb, $n = 4.96 \times 10^8$, 90 bp/read) and the 454 GS FLX Titanium platform (single reads, $n = 70,343,174$, >350 bp). The draft genome sequence (4.86 Mb) of strain LE124^T was assembled (150× coverage) into 156 contigs (>500 bp \pm 10 bp) using ABySS v.1.3.3 assembler (7), set at a k -mer size of 57. The final validated (based on paired-end criterion) assembly (N_{50} of contigs, 37.4 kb) (8) was annotated using RAST v.4.0 (9) and the NCBI Prokaryotic Genomes Automatic Annotation Pipeline v.2.1 (PGAAP) (<http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html>), which predicted 4,566 coding sequences (average G+C percentage, 64.6).

RAST annotation (9) predicted 414 subsystems and 30 putative metal resistance proteins. Nine rRNAs, 54 tRNAs, and 28 pseudogenes were found using PGAAP. Additionally, 52 transposases, 40 ABC transporters, and 90 transcriptional regulators were found in the genome. BLASTp-based (10) comparison with the ISfinder database (11) reported 10 insertion sequence (IS) elements (IS3/21) assigned to *Sphingobium* spp. (4). Plasmid-related genes, i.e., *repA*, *par*, and conjugative transfer genes, were detected on contig 113 (77,158 bp). Average nucleotide identity

(ANI) (12) analysis revealed that the draft genome of *N. lindaniclasticum* LE124^T is phylogenetically related to those of *Erythrobacter litoralis* (77.82%), *Novosphingobium aromaticivorans* (77.72%), and *Sphingopyxis alaskensis* (76.6%).

The HCH-degrading *lin* genes (13, 14) were found scattered throughout the draft genome assembly. A single copy of *linA* (dehydrochlorinase) was represented in the sequenced genome. Additionally, *linH*, *linK*, *linL*, *linM*, and two copies of *linG* were also present. In contrast to *Sphingobium indicum* B90A (4), *linB* (haloalkane dehalogenase) and *linDER* were absent from the genome of strain LE124^T; this was also confirmed by PCR amplification. We have recently reported that HCH selection pressure is responsible for bringing *lin* genes through horizontal gene transfer (HGT) into sphingomonads (15), and these findings reflect that strain LE124^T has yet to acquire these genes through HGT.

Interestingly, the draft genome of strain LE124^T also showed the presence of a benzoate-degrading gene cluster with the presence of 4-hydroxybenzoate 3-monooxygenase, benzoate transporter proteins, and genes for the chloroaromatic catechol branch of the β -ketoacid pathway. Additionally, genes encoding *ortho*-halobenzoate 1,2-dioxygenase α - and β -intracellular serine protease (ISP) protein (*ohbA*, *ohbB*), known to be involved in xenobiotic and benzoate degradation, were also found. The genetic compendium required to resolve the intergenus-level genetic divergence of the *lin* genes and degradation pathway can be analyzed by doing comparative analyses of genomes of sphingomonads that have now been sequenced.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. [ATHL000000000](https://www.ncbi.nlm.nih.gov/nuclink/ATHL000000000). The version described in this paper is version ATHL01000000.

ACKNOWLEDGMENTS

The work was supported by grants from the Department of Biotechnology (DBT), Government of India, under project no. 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 the National Bureau of Agriculturally Important Microorganisms (NBAIM) AMASS/2006–07/NBAIM/CIR, and the All India Network Project Soil Biodiversity-Biofertilizer (ICAR). A.S., N.N., N.S., and R.K. gratefully acknowledge the University Grants Commission (UGC) and the Council for Scientific and Industrial Research (CSIR), New Delhi, for providing research fellowships. This paper was finalized during the renewed visit under the Alexander von Humboldt fellowship (at the University of Freiburg, Germany) awarded to R.L.

REFERENCES

- 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.
- Lal R, Dadhwal M, Kumari K, Sharma P, Singh A, Kumari H, Jit S, Gupta SK, Nigam A, Lal D, Verma M, Kaur J, Bala K, Jindal S. 2008. *Pseudomonas* sp. to *Sphingobium indicum*: a journey of microbial degradation and bioremediation of hexachlorocyclohexane. *Indian J. Microbiol.* 48:3–18.
- Lal R, Dogra C, Malhotra S, Sharma P, Pal R. 2006. Diversity, distribution and divergence of *lin* genes in hexachlorocyclohexane-degrading sphingomonads. *Trends Biotechnol.* 24:121–130.
- 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.
- Niharika N, Sangwan N, Ahmad S, Singh P, Khurana JP, Lal R. 2013. Draft genome sequence of *Sphingobium chinhatense* IP26^T, isolated from a hexachlorocyclohexane dumpsite. *Genome Announc.* 1(4):e00680-13. doi:10.1128/genomeA.00680-13.
- Saxena A, Anand S, Dua A, Sangwan N, Khan F, Lal R. 2013. *Novosphingobium lindaniclasticum* sp. nov., a hexachlorocyclohexane (HCH)-degrading bacterium isolated from an HCH dumpsite. *Int. J. Syst. Evol. Microbiol.* 63:2160–2167.
- 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.
- Li H, Durbin R. 2010. Fast and accurate long-read alignment with Burrows-Wheeler Transform. *Bioinformatics* 26:589–595.
- 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: doi:10.1186/1471-2164-9-7575.
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215:403–410.
- 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.
- Konstantinidis KT, Tiedje JM. 2005. Towards a genome-based taxonomy for prokaryotes. *J. Bacteriol.* 187:6258–6264.
- 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.
- Malhotra S, Sharma P, Kumari H, Singh A, Lal R. 2007. Localization of HCH catabolic genes (*lin* genes) in *Sphingobium indicum* B90A. *Indian J. Microbiol.* 47:271–275.
- 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.