

Draft Genome Sequence of Hexachlorohexane (HCH)-Degrading *Sphingobium lucknowense* Strain F2^T, Isolated from an HCH Dumpsite

Vivek Negi,^a Pushp Lata,^a Naseer Sangwan,^a Sanjay Kumar Gupta,^a Shreyasi Das,^a D. L. N. Rao,^b Rup Lal^a

Department of Zoology, University of Delhi, Delhi, India^a; Indian Institute of Soil Science Nabi Bagh, Bhopal, Madhya Pradesh, India^b

***Sphingobium lucknowense* F2^T, isolated from the hexachlorocyclohexane (HCH) dumpsite located in Ummari village, Lucknow, India, rapidly degrades HCH isomers. Here we report the draft genome of strain F2 (4.4 Mbp), consisting of 4,910 protein coding genes with an average G+C content of 64.3%.**

Received 16 July 2014 Accepted 21 July 2014 Published 7 August 2014

Citation Negi V, Lata P, Sangwan N, Kumar Gupta S, Das S, Rao DLN, Lal R. 2014. Draft genome sequence of hexachlorohexane (HCH)-degrading *Sphingobium lucknowense* strain F2^T, isolated from an HCH dumpsite. *Genome Announc.* 2(4):e00788-14. doi:10.1128/genomeA.00788-14.

Copyright © 2014 Negi 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.

To continue our previous efforts to characterize the microbial diversity at a hexachlorocyclohexane (HCH) dumpsite by using culture-dependent (1–6) and culture-independent (7, 8) approaches, we have isolated from an HCH dumpsite yet another bacterial strain, characterized as *Sphingobium lucknowense* F2^T (1). This strain was found to rapidly degrade all HCH isomers (α -, γ -, β -, and δ -HCH), as reported earlier for *Sphingobium* spp. (1, 9–12). This motivated our efforts to sequence the genome of F2^T and compare its draft genome with the already-sequenced draft genomes of sphingomonads isolated from a similar HCH dumpsite (13–19).

The draft genome sequence of strain F2 was generated by using Illumina HiSeq 2000 (~5,744,444 paired-end reads) and 454 GS FLX titanium platforms (~77,766 single reads). The sequence data were assembled using Velvet_1.2.03 (20) at a k-mer length of 59 (expected coverage, 50; coverage cutoff, 10; and minimum contig length cutoff, 500 bp), ABySS 1.3.4 (21) at a k-mer length of 49, and SPAdes 3.0 (22). The sets of contigs generated from these assemblers were subjected to hybrid assembly integrated by Contig Integrator for Sequence Assembly (CISA) (23). We obtained 103 contigs having a total size of 4.4 Mbp, with average GC content of 64.3%. RNAmmer 1.2 (24) and ARAGORN (25) were used to predict rRNA genes, i.e., 2S rRNAs, 1e 16S rRNA, 1 23S rRNA, 49 tRNA, and 1 transfer-messenger RNA (tmRNA) gene. The Rapid Annotations using Subsystems Technology (RAST) pipeline (26), the KEGG Automatic Annotation Server (KAAS) (27), and the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (<http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html>) were used for annotation. RAST annotation revealed 4,910 coding sequences (CDSs), of which 41.85% have hypothetical proteins. Four insertion elements (IS) (IS1247, IS6100, ISShsp3, and ISSsp5) were identified on the basis of their sequence length and different layouts. The draft genome was binned using a Hidden Markov model (HMM) profile of 107 essential single-copy genes conserved in 95% of all bacteria, and it was found to contain 97 such genes, suggesting more than 90.7% completion (28).

Sphingobium lucknowense F2^T contains one copy each of *linA*,

linB, *linC*, *linD*, *linE*, *linF*, *linI*, *linJ*, *linK*, *linL*, *linM*, *linN*, and *linR* and four copies of *linG* and *linH*. These *lin* genes have already been implicated in the degradation of HCH isomers (10, 29, 30). Three putative gene clusters involved in degradation of chlorophenol, homogentisate, and toluene/phenol in other sphingomonads (14, 19) were also identified in the draft genome of *Sphingobium lucknowense* F2^T.

Strikingly, strain F2^T was found to contain higher abundances of membrane transport proteins ($n = 228$), stress response proteins ($n = 117$), putative aromatic hydrocarbon pathways ($n = 30$), and transposable elements and proteins ($n = 12$) of phages and prophages. The draft genome sequence of F2^T, along with the genome sequences of seven HCH-degrading *Sphingobium* spp. (13–17, 30, 31) and metagenome data from the HCH dumpsite (7, 8) will now provide deeper insights into the evolution and acquisition of *lin* genes within this group of sphingomonads.

Nucleotide sequence accession numbers. The genome sequence of *Sphingobium lucknowense* F2^T has been assigned the GenBank accession number [JANF00000000](https://www.ncbi.nlm.nih.gov/nuccore/JANF00000000). The version described in this paper is version JANF02000000.

ACKNOWLEDGMENTS

The work was supported by grants from the Department of Biotechnology (DBT), Government of India, under project (BT/PR3301/BCE/8/875/11) and the All India Network Project on Soil Biodiversity-Biofertilizers (ICAR) XII Plan (12-A/2007-IA II).

V.N., P.L., N.S., S.K.G., and S.D. gratefully acknowledge the Council for Scientific and Industrial Research (CSIR), University Grant Commission (UGC), Department of Biotechnology (DBT), and Government of India and All India Network Project on Soil Biodiversity-Biofertilizer (ICAR) for providing research fellowships.

This paper was written during a visit under the DST-DAAD project to Helmholtz Zentrum für Umweltforschung-UFZ, (Leipzig, Germany) by R.L.

REFERENCES

1. Dadhwal M, Singh A, Prakash O, Gupta SK, Kumari K, Sharma P, Jit S, Verma M, Holliger C, Lal R. 2009. 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. <http://dx.doi.org/10.1111/j.1365-2672.2008.03982.x>.
2. Singh A, Lal R. 2009. *Sphingobium ummariense* sp. nov., a hexachlorocyclohexane (HCH)-degrading bacterium, isolated from HCH-contaminated soil. *Int. J. Syst. Evol. Microbiol.* 59:162–166. <http://dx.doi.org/10.1099/ijss.0.65712-0>.
 3. Dadhwal M, Jit S, Kumari H, Lal R. 2009. *Sphingobium chinhatense* sp. nov., a hexachlorocyclohexane (HCH) degrading bacterium isolated from an HCH dump site. *Int. J. Syst. Evol. Microbiol.* 59:3140–3144. <http://dx.doi.org/10.1099/ijss.0.005553-0>.
 4. Bala K, Sharma P, Lal R. 2010. *Sphingobium quisquiliarum* sp. nov., P25T a hexachlorocyclohexane (HCH) degrading bacterium isolated from HCH-contaminated soil. *Int. J. Syst. Evol. Microbiol.* 60:429–433. <http://dx.doi.org/10.1099/ijss.0.010868-0>.
 5. Sharma P, Verma M, Bala K, Nigam A, Lal R. 2010. *Sphingopyxis ummariensis* sp. nov., isolated from hexachlorocyclohexane dumpsite in north India. *Int. J. Syst. Evol. Microbiol.* 60:780–784. <http://dx.doi.org/10.1099/ijss.0.008805-0>.
 6. Garg N, Bala K, Lal R. 2012. *Sphingobium lucknowense* sp. nov., a hexachlorocyclohexane (HCH)-degrading bacterium isolated from HCH contaminated soil. *Int. J. Syst. Evol. Microbiol.* 62:618–623. <http://dx.doi.org/10.1099/ijss.0.028886-0>.
 7. 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. <http://dx.doi.org/10.1371/journal.pone.0046219>.
 8. Sangwan N, Verma H, Kumar R, Negi V, Lax S, Khurana P, Khurana JP, Gilbert JA, Lal R. 2014. Reconstructing an ancestral genotype of two hexachlorocyclohexane-degrading *Sphingobium* species using metagenomic sequence data. *ISME J* 8:398–408. <http://dx.doi.org/10.1038/ismej.2013.153>.
 9. Geueke B, Garg N, Ghosh S, Fleischmann T, Holliger C, Lal R, Kohler HP. 2013. Metabolomics of hexachlorocyclohexane (HCH) transformation: ratio of LinA to LinB determines metabolic fate of HCH isomers. *Environ. Microbiol.* 15:1040–1049. <http://dx.doi.org/10.1111/1462-2920.12009>.
 10. Kumari R, Subudhi S, Suar M, Dhingra G, Raina V, Dogra C, Lal S, van der Meer JR, Holliger C, Lal R. 2002. Cloning and characterization of *lin* genes responsible for the degradation of hexachlorocyclohexane isomers by *Sphingomonas paucimobilis* strain B90. *Appl. Environ. Microbiol.* 68: 6021–6028. <http://dx.doi.org/10.1128/AEM.68.12.6021-6028.2002>.
 11. Sharma P, Raina V, Kumari R, Malhotra S, Dogra D, Kumari H, Kohler H-PE, Buser HR, Holliger C, Lal R. 2006. Haloalkane dehalogenase *LinB* is responsible for β - and δ -hexachlorocyclohexane in *Sphingobium indicum* B90A. *Appl. Environ. Microbiol.* 72:5720–5727. <http://dx.doi.org/10.1128/AEM.00192-06>.
 12. Jit S, Dadhwal M, Kumari H, Jindal S, Kaur J, Lata P, Niharika N, Lal D, Garg N, Gupta SK, Sharma P, Bala K, Singh A, Vijgen J, Weber R, Lal R. 2011. Evaluation of hexachlorocyclohexane contamination from the last lindane production plant operating in India. *Environ. Sci. Pollut. Res. Int.* 18:586–597. <http://dx.doi.org/10.1007/s11356-010-0401-4>.
 13. 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. <http://dx.doi.org/10.1128/JB.00901-12>.
 14. Niharika N, Sangwan N, Ahmad S, Singh P, Khurana JP, Lal R. 2013. Draft genome sequence of *Sphingobium chinhatense* strain IP26T, isolated from a hexachlorocyclohexane dumpsite. *Genome Announc.* 1(4): e00680-13. <http://dx.doi.org/10.1128/genomeA.00680-13>.
 15. Kumar Singh A, Sangwan N, Sharma A, Gupta V, Khurana JP, Lal R. 2013. Draft genome sequence of *Sphingobium quisquiliarum* strain P25T, a novel hexachlorocyclohexane (HCH)-degrading bacterium isolated from an HCH dumpsite. *Genome Announc.* 1(5):e00717-13. <http://dx.doi.org/10.1128/genomeA.00717-13>.
 16. Mukherjee U, Kumar R, Mahato NK, Khurana JP, Lal R. 2013. Draft genome sequence of *Sphingobium* sp. strain HDIPO4, an avid degrader of hexachlorocyclohexane. *Genome Announc.* 1(5):e00749-13. <http://dx.doi.org/10.1128/genomeA.00749-13>.
 17. Kohli P, Dua A, Sangwan N, Oldach P, Khurana JP, Lal R. 2013. Draft genome sequence of *Sphingobium ummariense* strain RL-3, a hexachlorocyclohexane-degrading bacterium. *Genome Announc.* 1(6): e00956-13. <http://dx.doi.org/10.1128/genomeA.00956-13>.
 18. Kumar R, Dwivedi V, Negi V, Khurana JP, Lal R. 2013. Draft genome sequence of *Sphingobium lactosutens* strain DS20T, isolated from a hexachlorocyclohexane dumpsite. *Genome Announc.* 1(5):e00753-13. <http://dx.doi.org/10.1128/genomeA.00753-13>.
 19. Saxena A, Nayyar N, Sangwan N, Kumari R, Khurana JP, Lal R. 2013. Genome sequence of *Novosphingobium lindaniclasticum* LE124T, isolated from a hexachlorocyclohexane dumpsite. *Genome Announc.* 1(5): e00715-13. <http://dx.doi.org/10.1128/genomeA.00715-13>.
 20. Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. *Genome Res.* 18(5):821–829. <http://dx.doi.org/10.1101/gr.074492.107>.
 21. 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. <http://dx.doi.org/10.1101/gr.089532.108>.
 22. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotnik AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* 19:455–477. <http://dx.doi.org/10.1089/cmb.2012.0021>.
 23. Lin SH, Liao YC. 2013. CISA: contig integrator for sequence assembly of bacterial genomes. *PLoS One* 8:e60843. <http://dx.doi.org/10.1371/journal.pone.0060843>.
 24. Lagesen K, Hallin P, Rødland EA, Stærfeldt HH, Rognes T, Ussery DW. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res.* 35:3100–3108. <http://dx.doi.org/10.1093/nar/gkm160>.
 25. Laslett D, Canback B. 2004. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. *Nucleic Acids Res.* 32:11–16. <http://dx.doi.org/10.1093/nar/gkh152>.
 26. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formisano 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. <http://dx.doi.org/10.1186/1471-2164-9-75>.
 27. Moriya Y, Itoh M, Okuda S, Yoshizawa AC, Kanehisa M. 2007. KAAS: an automatic genome annotation and pathway reconstruction server. *Nucleic Acids Res.* 35:W182–W185. <http://dx.doi.org/10.1093/nar/gkm321>.
 28. Dupont CL, Rusch DB, Yooseph S, Lombardo MJ, Richter RA, Valas R, Novotny M, Yee-Greenbaum J, Selengut JD, Haft DH, Halpern AL, Lasken RS, Nealson K, Friedman R, Venter JC. 2012. Genomic insights to SAR86, an abundant and uncultivated marine bacterial lineage. *ISME J* 6:1186–1199. <http://dx.doi.org/10.1038/ismej.2011.189>.
 29. 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 prospects for bioremediation. *Microbiol. Mol. Biol. Rev.* 74:58–80. <http://dx.doi.org/10.1128/MMBR.00029-09>.
 30. Nagata Y, Natsui S, Endo R, Ohtsubo Y, Ichikawa N, Ankai A, Oguchi A, Fukui S, Fujita N, Tsuda M. 2011. Genomic organization and genomic structural rearrangements of *Sphingobium japonicum* UT26, an archetypal γ -hexachlorocyclohexane-degrading bacterium. *Enzyme Microb. Technol.* 49:499–508. <http://dx.doi.org/10.1016/j.enzmictec.2011.10.005>.
 31. Kaur J, Verma H, Tripathi C, Khurana JP, Lal R. 2013. Draft genome sequence of a hexachlorocyclohexane-degrading bacterium, *Sphingobium baderi* strain LL03T. *Genome Announc.* 1(5):e00751-13. <http://dx.doi.org/10.1128/genomeA.00751-13>.