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GENOME SEQUENCES

Draft Genome Sequences of Two *Sphingobium* Species Associated with Hexachlorocyclohexane (HCH) Degradation Isolated from an HCH-Contaminated Soil

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ABSTRACT The draft genome sequences of two *Sphingobium* strains that are hexachlorocyclohexane (HCH) degraders are presented. The strains were isolated from HCH-contaminated soil in Kitengela, Kenya. Both genomes possess the *lin* genes responsible for HCH degradation and gene clusters for degradation of other xenobiotic compounds.

Bioremediation of biodegradable lindane (γ-hexachlorocyclohexane [HCH]) is a viable detoxification strategy for maintaining environmental health (1, 2). Several microorganisms can degrade HCH isomers (3, 4). *Sphingomonadaceae* seem to play a central role in the complete mineralization of HCH, with the catabolic genes initially identified in *Sphingobium japonicum* UT26 (5).

Here, two *Sphingobium* species (*Sphingobium* sp. strains S6 and S8) were isolated from HCH-contaminated soil collected from an obsolete former pesticide store in Kitengela, Kenya (01.49 S, 37.048 E). For bacterial isolation, we used a minimum salt medium (MSM) (6) spiked with 100 μg/mL γ-HCH. Pure colonies were obtained by spreading serial dilutions (10^{-3} to 10^{-6}) of the enrichment cultures onto 1:10 diluted Luria-Bertani (LB) agar plates supplemented with 100 μg/mL γ-HCH, followed by incubation at 30°C for 72 h. The HCH degradation capacity was assessed by dechlorinase assay according to the method of Phillips et al. (7) and in the liquid medium following Boltner et al. (8).

Genomic DNA was extracted from 48-h LB cultures grown at 30°C using a Wizard genomic DNA purification kit (Promega, USA). DNA was quantified using a Qubit fluorometer (Thermo Fisher Scientific, USA). According to the manufacturer's instructions, a NEBNext Ultra II FS DNA library kit (New England Biolabs, USA) was used to prepare a paired-end 300-bp library for genome sequencing on an Illumina MiSeq platform. We used Sickle v1.33 (9) with a Phred quality score of >30 for sequence trimming. *De novo* sequence assembly was performed using SPAdes v3.15.2 (10), while CheckM v1.0.18 and RefineM v0.0.25 (11) were used for quality checking and to provide completeness and contamination information, respectively. The genomes were annotated using PROKKA v1.14.5 (12) and the Rapid Annotation using Subsystems Technology toolkit (RASTtk) v2.0 (13). Unless otherwise stated, default parameter settings were applied for all software used.

The draft genomes of *Sphingobium* sp. strains S6 and S8 had 42 and 44 contigs, with total lengths of 4,173,956 bp and 4,170,555 bp, respectively. Both genomes showed 99.2% completeness and 2.06% contamination. The GC contents of *Sphingobium* sp. strains S6 and S8 were 62.4% and 62.53%, respectively. The annotations are summarized in Table 1. Previous reports show that HCH-degrading sphingomonads share the same degradation pathway that requires genes *linA* through *linF* (3, 8). Analysis of the draft genomes of the *Sphingobium* species described in this study revealed the presence of

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TABLE 1 Summary of the draft whole-genome sequences of HCH-degrading *Sphingobium* strains isolated from an HCH-contaminated site in Kitengela, Kenya

Strain	GenBank accession no.	No. of contigs	N_{50} (bp)	Avg coverage (×)	Genome size (bp)	%GC	No. of rRNAs	No. of tRNAs	No. of tmRNAs[a]	No. of coding sequences	Transposon families	Closest phylogenetic neighbors (%ANI)[b]
Sphingobium sp. strain S6	CAJHOG000000000	44	538,820	48.72	4,173,956	62.40	3	49	1	4,015	IS*21* (*n* = 2), ISNCY (*n* = 1), IS*3* (*n* = 2), IS*5* (*n* = 1), IS*630* (*n* = 1), IS*256* (*n* = 1), IS*481* (*n* = 1)	*Sphingobium* sp. strain S8 (99.96), *Sphingobium indicum* B90A (85.93) (16), *Sphingobium japonicum* UT26S (85.51) (17), *Sphingobium quisquiliarum* P25 (83.88) (18), *Sphingobium chlorophenolicum* L-1 (83.65) (19)
Sphingobium sp. strain S8	CAJHOH000000000	48	454,689	36.73	4,170,555	62.53	3	47	1	4,039	IS*21* (*n* = 2), IS*5* (*n* = 1), IS*630* (*n* = 1), IS*256* (*n* = 1), IS*481* (*n* = 1)	*Sphingobium* sp. strain S6 (99.96), *Sphingobium indicum* B90A (85.76) (16), *Sphingobium japonicum* UT26S (85.41) (17), *Sphingobium quisquiliarum* P25 (83.85) (18), *Sphingobium chlorophenolicum* L-1 (83.57) (19)

[a] tmRNAs, transfer-messenger RNAs.

[b] Phylogenetically related neighbors were computed based on average nucleotide identity (ANI) [20] analysis.

one copy each of *linA*, *linB*, *linC*, *linD*, *linE*, and *linF*, and two copies each of *linG*, *linH*, *linJ*, and *linX* per genome. Annotation using RASTtk showed gene clusters for the potential degradation of xenobiotic compounds such as 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane (DDT); 1,4-dichlorobenzene; tetrachloroethene; 2,4-dichlorobenzoate; fluorobenzoate; benzoate; toluene; and xylene. In addition, the genetic potential for the production of carotenoids was predicted using antiSMASH v6.0.0 (14) in both strains.

The availability of the genome sequences of the two *Sphingobium* species may be instrumental in promoting HCH degradation by mixed (multidomain) microbial communities such as fungal bacterial associations (15).

Data availability. These whole-genome shotgun projects have been deposited at ENA/DDBJ/GenBank under accession numbers CAJHOG000000000 and CAJHOH000000000 for *Sphingobium* sp. strains S6 and S8, respectively. The versions described in this paper are CAJHOG000000000.1 and CAJHOH000000000.1 for *Sphingobium* sp. strains S6 and S8, respectively. The raw data are available at ENA under SRA accession numbers ERR4392070 and ERR4392071. All project data are available under BioProject accession number PRJEB39494.

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REFERENCES

1. Girish K, Kunhi AAM. 2013. Microbial degradation of gamma-hexachlorocyclohexane (lindane). Afr J Microbiol Res 7:1635–1643. https://doi.org/10.5897/AJMRx12.001.
2. Pant G, Mistry SK, Sibi G. 2013. Isolation, identification and characterization of p, p′-DDT degrading bacteria from soil. J Env Sci Technol 6:130–137. https://doi.org/10.3923/jest.2013.130.137.
3. Lal R, Pandey G, Sharma P, Kumari K, Malhotra S, Pandey R, Raina V, Kohler H-PE, Holliger C, Jackson C, Oakeshott JG. 2010. Biochemistry of microbial degradation of hexachlorocyclohexane and prospects for bioremediation. Microbiol Mol Biol Rev 74:58–80. https://doi.org/10.1128/MMBR.00029-09.
4. Bumpus JA, Tien M, Wright D, Aust SD. 1985. Oxidation of persistent environmental pollutants by a white rot fungus. Science 228:1434–1436. https://doi.org/10.1126/science.3925550.
5. Nagata Y, Endo R, Ito M, Ohtsubo Y, Tsuda M. 2007. Aerobic degradation of lindane (γ-hexachlorocyclohexane) in bacteria and its biochemical and molecular basis. Appl Microbiol Biotechnol 76:741–752. https://doi.org/10.1007/s00253-007-1066-x.
6. Senoo K, Wada H. 1989. Isolation and identification of an aerobic γ-HCH-decomposing bacterium from soil. Soil Sci Plant Nutr 35:79–87. https://doi.org/10.1080/00380768.1989.10434739.
7. Phillips TM, Seech AG, Lee H, Trevors JT. 2001. Colorimetric assay for Lindane dechlorination by bacteria. J Microbiol Methods 47:181–188. https://doi.org/10.1016/S0167-7012(01)00299-8.
8. Boltner D, Moreno-Morillas S, Ramos J-L. 2005. 16S rDNA phylogeny and distribution of lin genes in novel hexachlorocyclohexane-degrading Sphingomonas strains. Environ Microbiol 7:1329–1338. https://doi.org/10.1111/j.1462-5822.2005.00820.x.
9. Joshi N, Fass JN. 2011. Sickle—a sliding-window, adaptive, quality-based trimming tool for FastQ files (version 1.33). https://github.com/najoshi/sickle.
10. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin 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. https://doi.org/10.1089/cmb.2012.0021.
11. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res 25:1043–1055. https://doi.org/10.1101/gr.186072.114.
12. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153.
13. Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Thomason JA, III, Stevens R, Vonstein V, Wattam AR, Xia F. 2015. RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci Rep 5:8365. https://doi.org/10.1038/srep08365.
14. Blin K, Shaw S, Steinke K, Villebro R, Ziemert N, Lee SY, Medema MH, Weber T. 2019. antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. Nucleic Acids Res 47:W81–W87. https://doi.org/10.1093/nar/gkz310.
15. Espinosa-Ortiz EJ, Rene ER, Gerlach R. 29 Jul 2021. Potential use of fungal-bacterial co-cultures for the removal of organic pollutants. Crit Rev Biotechnol https://doi.org/10.1080/07388551.2021.1940831.
16. 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. https://doi.org/10.1128/JB.00901-12.
17. Nagata Y, Ohtsubo Y, Endo R, Ichikawa N, Ankai A, Oguchi A, Fukui S, Fujita N, Tsuda M. 2010. Complete genome sequence of the representative γ-hexachlorocyclohexane-degrading bacterium Sphingobium japonicum UT26. J Bacteriol 192:5852–5853. https://doi.org/10.1128/JB.00961-10.
18. Kumar Singh A, Sangwan N, Sharma A, Gupta V, Khurana JP, Lal R. 2013. Draft genome sequence of Sphingobium quisquiliarum strain P25^T, a novel hexachlorocyclohexane (HCH)-degrading bacterium isolated from an HCH dumpsite. Genome Announc 1:e00717-13. https://doi.org/10.1128/genomeA.00717-13.
19. Copley SD, Rokicki J, Turner P, Daligault H, Nolan M, Land M. 2012. The whole genome sequence of Sphingobium chlorophenolicum L-1: insights into the evolution of the pentachlorophenol degradation pathway. Genome Biol Evol 4:184–198. https://doi.org/10.1093/gbe/evr137.
20. Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM. 2007. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 57:81–91. https://doi.org/10.1099/ijs.0.64483-0.