



Complete Genome Sequence of *Sphingobium barthaii* KK22, a High-Molecular-Weight Polycyclic Aromatic Hydrocarbon-Degrading Soil Bacterium

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ABSTRACT *Sphingobium barthaii* KK22^T is a high-molecular-weight polycyclic aromatic hydrocarbon-degrading soil bacterium that has been investigated in biotransformation, microbial ecology, and DNA damage studies. The complete genome sequence of *S. barthaii* revealed four closed circular sequences, including two chromosomes, a megaplasmid, and a smaller plasmid, by hybrid assembly using short- and long-read sequencing technologies.

The Gram-negative alphaproteobacterium *Sphingobium barthaii* KK22^T has been investigated (i) for its capabilities to biotransform high-molecular-weight (HMW) polycyclic aromatic hydrocarbons (PAHs), (ii) for its major role in a hydrocarbon-degrading bacterial consortium, and (iii) as a model organism in DNA damage studies (1–7). It was isolated from a diesel fuel-grown bacterial consortium that originated from cattle pasture soil from the Gulf region of Texas (1, 8, 9). Some sphingomonads may possess a second chromosome in addition to multiple plasmids, including large (>100-kbp) plasmids or “megaplasmids,” that are potentially responsible for the catabolic flexibility of this bacterial group (10). The draft genome sequence of *S. barthaii* was announced in 2013 (11); however, functional gene distributions on the chromosomes and plasmids were not able to be determined. Now, the new information presented here shall not only provide clear insights into the metabolic capabilities of *S. barthaii* but also support its potential biotechnological applications in bioremediation and green chemistry (12).

Here, a hybrid assembly using short-read (DNBSEQ-G400; MGI Tech, Shenzhen, China) and long-read (GridION X5; Oxford Nanopore Technologies, Oxford, UK) sequencing technologies was employed to close the chromosomal and plasmid sequences of *S. barthaii*. *S. barthaii* that had been grown for 4 days on 300 mg liter⁻¹ phenanthrene in Stanier’s basal medium at 30°C with rotary shaking at 150 rpm in the dark (7) was subjected to genomic DNA extraction using the DNeasy PowerWater DNA isolation kit (Qiagen, Hilden, Germany). For the DNBseq analysis, genomic DNA was sheared to average 400-bp fragments with an S2 device (Covaris, Woburn, MA, USA), and the DNBseq library was prepared using the MGIEasy universal DNA library preparation kit (MGI Tech) according to the manufacturer’s instructions. A total of 20,576,138 reads with a 150-bp paired-end read length were sequenced with the DNBSEQ-G400 platform and were subjected to trimming and quality filtering using Cutadapt (v. 2.7), SeqKit (v. 0.11.0), and Sickle (v. 1.33). For the GridION analysis, 1,000 ng of genomic DNA was barcoded using native barcoding expansion (Oxford Nanopore Technologies), and the library was prepared using a ligation sequencing kit (SQK-LSK109; Oxford Nanopore Technologies). A total of 457,744 reads with an average length of 6,451.8 bp and an N_{50} value of 10,186 bp were generated by the GridION platform using R9.4.1 flow cells and Guppy (v. 4.0.11) for live base calling. The raw reads were subjected to trimming and quality filtering using Porechop (v. 0.2.3) and FilTlong (v. 0.2.0; minimum length of 1,000 bp), and

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error correction was performed using Canu (v. 2.0). All software was used with default settings unless otherwise specified.

After the trimming and quality filtering of the raw reads, *de novo* hybrid assembly was performed using Unicycler (v. 0.4.7) (13), and the assembly was validated with Bandage (v. 0.8.1). The complete genome sequence thus determined (4,984,172 bp, with 787.5× coverage) consisted of two circular chromosomes (3,389,351 and 1,187,212 bp), a circular megaplasmid (387,592 bp), and a circular small plasmid (20,017 bp) with an overall G+C content of 64.7%. Gene annotation by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (v. 4.13) identified 4,623 coding sequences (CDSs), 9 rRNAs, 54 tRNAs, and 1 transfer-messenger RNA. Among the CDSs, 3,220 (69.7%) and 989 (21.4%) sequences were identified on the large and small chromosomes, respectively, while 391 (8.5%) and 23 (0.5%) sequences were identified on the megaplasmid and the small plasmid, respectively.

Notably, it was found that all seven sets of aromatic ring-hydroxylating dioxygenase (ARHD) genes encoded in the *S. barthaii* genome (2) were located on the megaplasmid, with six sets being tightly clustered. This ARHD gene cluster is shared among some aromatic hydrocarbon-degrading sphingomonads and is considered to be responsible for biotransformation of various xenobiotic aromatic hydrocarbons (14, 15). However, due to the incompleteness of the sphingomonad genomes previously sequenced, there is only one *Sphingobium* genome (*S. yanoikuyae* SJTF8) available in public databases for which the ARHD gene cluster was reported, also located on its megaplasmid. The genome presented here is the second report of a *Sphingobium* megaplasmid that carries the ARHD gene cluster, and these results support the hypothesis that transfer of these plasmids in sphingomonads is of major importance in regard to biodegradation of aromatic hydrocarbons including HMW PAHs (10).

Data availability. The complete chromosome and plasmid sequences of *S. barthaii* KK22 were deposited in NCBI GenBank under the accession numbers [CP060035](#), [CP060036](#), [CP060037](#), and [CP060038](#) and in the Integrated Microbial Genome and Microbiomes (IMG/MER) database (Joint Genome Institute, IMG accession number [2883162986](#)). The raw sequences are available from SRA accession numbers [SRR12950076](#) and [SRR12950077](#) under BioProject number [PRJNA655586](#) and BioSample number [SAMN16591971](#).

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REFERENCES

- Mori JF, Kanaly RA. 2020. Multispecies diesel fuel biodegradation and niche formation are ignited by pioneer hydrocarbon-utilizing proteobacteria in a soil bacterial consortium. *Appl Environ Microbiol* 87:e02268-20. <https://doi.org/10.1128/AEM.02268-20>.
- Maeda AH, Nishi S, Hatada Y, Ohta Y, Misaka K, Kunihiro M, Mori JF, Kanaly RA. 2020. Chemical and genomic analyses of polycyclic aromatic hydrocarbon biodegradation in *Sphingobium barthaii* KK22 reveals divergent pathways in soil sphingomonads. *Int Biodeterior Biodegradation* 151:104993. <https://doi.org/10.1016/j.ibiod.2020.104993>.
- Kanaly RA, Micheletto R, Matsuda T, Utsuno Y, Ozeki Y, Hamamura N. 2015. Application of DNA adductomics to soil bacterium *Sphingobium* sp. strain KK22. *Microbiologyopen* 4:841–856. <https://doi.org/10.1002/mbo3.283>.
- Maeda AH, Kunihiro M, Ozeki Y, Nogi Y, Kanaly RA. 2015. *Sphingobium barthaii* sp. nov., a high molecular weight polycyclic aromatic hydrocarbon-degrading bacterium isolated from cattle pasture soil. *Int J Syst Evol Microbiol* 65:2919–2924. <https://doi.org/10.1099/ijs.0.000356>.
- Maeda AH, Nishi S, Hatada Y, Ozeki Y, Kanaly RA. 2014. Biotransformation of the high-molecular weight polycyclic aromatic hydrocarbon (PAH) benzo[k]fluoranthene by *Sphingobium* sp. strain KK22 and identification of new products of non-alternant PAH biodegradation by liquid chromatography electrospray ionization tandem mass spectrometry. *Microb Biotechnol* 7:114–129. <https://doi.org/10.1111/1751-7915.12102>.
- Kanaly RA, Hamamura N. 2013. 9,10-Phenanthrene-dione biodegradation by a soil bacterium and identification of transformation products by LC/ESI-MS/MS. *Chemosphere* 92:1442–1449. <https://doi.org/10.1016/j.chemosphere.2013.03.054>.
- Kunihiro M, Ozeki Y, Nogi Y, Hamamura N, Kanaly RA. 2013. Benz[a]anthracene biotransformation and production of ring fission products by *Sphingobium* sp. strain KK22. *Appl Environ Microbiol* 79:4410–4420. <https://doi.org/10.1128/AEM.01129-13>.
- Kanaly RA, Watanabe K. 2004. Multiple mechanisms contribute to the biodegradation of benzo[a]pyrene by petroleum-derived multicomponent nonaqueous-phase liquids. *Environ Toxicol Chem* 23:850–856. <https://doi.org/10.1897/03-191>.
- Kanaly RA, Bartha R. 1999. Cometabolic mineralization of benzo[a]pyrene caused by hydrocarbon additions to soil. *Environ Toxicol Chem* 18:2186–2190. <https://doi.org/10.1002/etc.5620181010>.
- Stolz A. 2014. Degradative plasmids from sphingomonads. *FEMS Microbiol Lett* 350:9–19. <https://doi.org/10.1111/1574-6968.12283>.
- Maeda AH, Nishi S, Ozeki Y, Ohta Y, Hatada Y, Kanaly RA. 2013. Draft genome sequence of *Sphingobium* sp. strain KK22, a high-molecular-weight

- polycyclic aromatic hydrocarbon-degrading bacterium isolated from cattle pasture soil. *Genome Announc* 1:e00911-13. <https://doi.org/10.1128/genomeA.00911-13>.
12. Gibson DT, Parales RE. 2000. Aromatic hydrocarbon dioxygenases in environmental biotechnology. *Curr Opin Biotechnol* 11:236–243. [https://doi.org/10.1016/S0958-1669\(00\)00090-2](https://doi.org/10.1016/S0958-1669(00)00090-2).
 13. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 13:e1005595. <https://doi.org/10.1371/journal.pcbi.1005595>.
 14. Khara P, Roy M, Chakraborty J, Ghosal D, Dutta TK. 2014. Functional characterization of diverse ring-hydroxylating oxygenases and induction of complex aromatic catabolic gene clusters in *Sphingobium* sp. *PNB. FEBS Open Bio* 4:290–300. <https://doi.org/10.1016/j.fob.2014.03.001>.
 15. Zhao Q, Yue S, Bilal M, Hu H, Wang W, Zhang X. 2017. Comparative genomic analysis of 26 *Sphingomonas* and *Sphingobium* strains: dissemination of bioremediation capabilities, biodegradation potential and horizontal gene transfer. *Sci Total Environ* 609:1238–1247. <https://doi.org/10.1016/j.scitotenv.2017.07.249>.