**Complete Genome Sequence of Bisphenol A-Degrading Bacterium *Sphingobium* sp. Strain A3, Isolated from Contaminated Soil**

Ji Young Jung, Hye Kyeoung Kang, Dae Won Jeong, Hyun Mi Jin, Byung-Gon Ryu, Bok Yeon Jo, Eu Jin Chung, Sang-Soo Han

Microbial Research Department, Nakdonggang National Institute of Biological Resources, Sangju-si, Gyeongsangbuk-do, South Korea

**Abstract**

This study reports the complete genome sequence of bisphenol A-degrading bacterium *Sphingobium* sp. strain A3, which was isolated from a contaminated soil sample from the site of a factory fire in South Korea. The genome consists of a 6.53-Mbp chromosome and eight plasmid contigs (532,947 bp), with 6,406 protein-coding sequences and a GC content of 63.82%.

Bisphenol A (BPA) is used in plastic bottles and food packaging (1, 2). BPA mimics the structure and function of the hormone estrogen, and it can interfere with normal bodily processes (3). Many studies have suggested that bacteria could provide a promising strategy for xenobiotic cleanup and bioremediation (4, 5). BPA-degrading bacteria, such as *Achromobacter* (6), *Pseudomonas* (7), *Bacillus* (8), and *Sphingomonas* (9), have been isolated. Although the isolation of novel bacteria led to proposed metabolic pathways of BPA degradation using intermediates detected during the degradation process, the genetic mechanisms of BPA degradation are not yet understood (10, 11).

The BPA-degrading bacterium *Sphingobium* sp. strain A3 was isolated from contaminated soil, and the whole genome was sequenced to understand its metabolic capacity and functional potential. Contaminated soil samples were collected at the site of a factory fire (35°18′55.1″N, 128°45′41.0″E) in the Gyeongsangnam-do province (South Korea). Enrichment cultures were conducted aerobically at 30°C for 2 weeks, with shaking, using contaminated soil in mineral salt medium (MSM) with 500 ppm BPA as the sole carbon source.

For Illumina sequencing, genomic DNA (100 ng) was isolated from the A3 strain using Maxwell 16 DNA purification kits (Promega, Madison, WI, USA). The genomic DNA was sequenced at Macrogen, Inc. (South Korea), using a combination of the PacBio RS II single-molecule real-time (SMRT) (13) sequencing platform, with a 20-kb SMRTbell template library, and the Illumina HiSeq X Ten sequencing platform (2 \times 151 bp). For PacBio sequencing, genomic DNA (8 \mu g) was sheared to approximately 20 kb with a g-TUBE (Covaris) and purified using AMPurePB beads (Beckman Coulter), and the sequencing library was prepared using the SMRTbell template preparation kit v1.0 (PacBio). For Illumina sequencing, genomic DNA (100 ng) was used for library preparation.
was sheared using an LE220 focused ultrasonicator (Covaris) and the sequencing library (350-bp insert size) was generated using a TruSeq Nano DNA library preparation kit. A total of 94,869 PacBio subreads (mean subread length, 6,496 bp; N50, 10,298 bp) were generated for preassembly and de novo assembly using FALCON-integrate software v2.1.4. A total of 12,165,610 raw Illumina paired-end reads (1.83 Gbp) were generated, and 5,664,022 clean reads (0.85 Gbp), in which 90% of the bases in each read had a Phred score of 30, were used for error correction with Pilon v1.21 for the final genome assembly. UGENE v1.32.0 was used to construct a self-dotplot to check the circularity of contigs. Overlapping ends were trimmed out. The genome was then annotated using NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v4.12 (14). Default parameters were used for all software unless otherwise specified.

The final genome, assembly, and annotation statistics are shown in Table 1. The A3 genome was closely related to two strains, Sphingobium yanoikuyae ATCC 51230 (GenBank accession number NZ_CP023741.1) and Sphingobium scionense DSM 19371 (GenBank accession number JACIEU000000000.1), based on 16S rRNA sequence identity (100% and 99.1%, respectively), average nucleotide identity (95.79% and 92.66%, respectively), and digital DNA-DNA hybridization (68.0% and 50.6%, respectively) (15, 16).

Although there is little information available on the enzymes and genes that are involved in the BPA degradation pathway, a few studies suggest that cytochrome P450 monooxygenase (17), laccase (18), lignin peroxidase (19), and manganese peroxidase (20) can degrade BPA. In the A3 genome, 10 P450 cytochromes were found in the chromosome and plasmid, and they showed high levels of similarity to previously reported P450 cytochromes of Sphingobium sp. strain YL23 (10, 21). Further in-depth biochemical and genomic analyses are needed to better understand BPA degradation pathways.

**Data availability.** The genome sequences and raw sequencing reads for the A3 strain were deposited under GenBank accession numbers CP060122, CP060123, CP060124, CP060125, CP060126, CP060127, CP060128, CP060129, and CP060130, BioProject accession number PRJNA649365, BioSample accession number SAMN15665156, and SRA accession numbers SRR12349700 and SRR12349701.

**ACKNOWLEDGMENT**

This work was carried out with support from a Nakdonggang National Institute of Biological Resources grant (project NNIBR202002104) funded by the Ministry of Environment, South Korea.

**REFERENCES**

1. Baker ME, Chandsawangbhuwana C. 2012. 3D models of MBP, a biologically active metabolite of bisphenol A, in human estrogen receptor α and estrogen receptor β. PLoS One 7:e46078. https://doi.org/10.1371/journal.pone.0046078.

2. Umar M, Roddick F, Fan L, Aziz HA. 2013. Application of ozone for the removal of bisphenol A from water and wastewater: a review. Chemosphere 90:2197–2207. https://doi.org/10.1016/j.chemosphere.2012.09.090.
3. Gao H, Yang B-J, Li N, Feng L-M, Shi X-Y, Zhao W-H, Liu S-J. 2015. Bisphenol A and hormone-associated cancers: current progress and perspectives. Medicine (Baltimore) 94:e211. https://doi.org/10.1097/MD.0000000000000211.

4. Ławniczak Ł, Woźniak-Karczewska M, Loibner AP, Heipieper HJ, Chrzanowski Ł. 2020. Microbial degradation of hydrocarbons: basic principles for bioremediation: a review. Molecules 25:856. https://doi.org/10.3390/molecules25040856.

5. Singh K, Chandra S. 2014. Treatment of petroleum hydrocarbon polluted environment through bioremediation: a review. Pak J Biol Sci 17:1–8. https://doi.org/10.3923/pjbs.2014.1.8.

6. Zhang C, Zeng G, Yuan L, Yu J, Li J, Huang G, Xi B, Liu H. 2007. Aerobic degradation of bisphenol A by Achromobacter xylosoxidans strain B-16 isolated from compost leachate of municipal solid waste. Chemosphere 68:181–190. https://doi.org/10.1016/j.chemosphere.2006.12.012.

7. Eltoukhy A, Jia Y, Nahurira R, Abo-Kadoum MA, Khokhar I, Wang J, Yan Y. 2020. Biodegradation of endocrine disruptor bisphenol A by Pseudomonas putida strain YC-AE1 isolated from polluted soil, Guangdong, China. BMC Microbiol 20:1. https://doi.org/10.1186/s12866-020-1699-9.

8. Suyamud B, Inthorn D, Panyapinyopol B, Thiravetyan P. 2018. Biodegradation of bisphenol A by a newly isolated Bacillus megaterium strain ISO-2 from a polycarbonate industrial wastewater. Water Air Soil Pollut 229:348. https://doi.org/10.1007/s11270-018-3983-y.

9. Fujiwara H, Soda S, Fujita M, Ike M. 2016. Kinetics of bisphenol A degradation by Sphingomonas paucimobilis FJ-4. J Biosci Bioeng 122:341–344. https://doi.org/10.1016/j.jbiosc.2016.02.015.

10. Hu A, Lv M, Yu C-P. 2013. Draft genome sequence of the bisphenol A-degrading bacterium Sphingobium sp. strain YL23. Genome Announc 1:e00549-13. https://doi.org/10.1128/genomeA.00549-13.

11. Im J, Loffler FE. 2016. Fate of bisphenol A in terrestrial and aquatic environments. Environ Sci Technol 50:8403–8416. https://doi.org/10.1021/acs.est.6b00877.

12. Vijayalakshmi V, Senthilkumar P, Mophin-Kani K, Sivamani S, Sivarajasekar N, Vasantharaj S. 2018. Bio-degradation of bisphenol A by Pseudomonas aeruginosa PA1 isolated from effluent of thermal paper industry: kinetic modeling and process optimization. J Radiat Res Appl Sci 11:56–65. https://doi.org/10.1016/j.jrras.2017.08.003.

13. Chin C-S, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat Methods 10:563–569. https://doi.org/10.1038/nmeth.2474.

14. Tatusova T, DiCuccio M, Badretdin A, Chetverin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569.

15. Yoon SH, Ha SM, Lim J, Kwon S, Chun J. 2017. A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie Van Leeuwenhoek 110:1281–1286. https://doi.org/10.1007/s10482-017-0844-4.

16. Meer-Klothoff JP, Göker M. 2019. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 10:2182. https://doi.org/10.1038/s41467-019-10210-3.

17. Jia Y, Eltoukhy A, Wang J, Li X, Hlaing TS, Aung MM, Nwe MT, Lamraoui I, Yan Y. 2020. Biodegradation of bisphenol A by Sphingobium sp. YC-JY1 and the essential role of cytochrome P450 monooxygenase. Int J Mol Sci 21:3588. https://doi.org/10.3390/ijms21103588.

18. Daâssi D, Prieto A, Zouari-Mechichi H, Martínez MJ, Nasri M, Mechichi T. 2016. Degradation of bisphenol A by different fungal laccases and identification of its degradation products. Int Biodeterior Biodegradation 110:181–188. https://doi.org/10.1016/j.biodev.2016.03.017.

19. Gassara F, Brar SK, Verma M, Tyagi RD. 2013. Bisphenol A degradation in water by ligninolytic enzymes. Chemosphere 92:1356–1360. https://doi.org/10.1016/j.chemosphere.2013.02.071.

20. Hirano T, Honda Y, Watanabe T, Kuwahara M. 2000. Degradation of bisphenol A by the lignin-degrading enzyme, manganese peroxidase, produced by the white-rot basidiomycete, Pleurotus ostreatus. Biosci Biotechnol Biochem 64:1958–1962. https://doi.org/10.1271/bbb.64.1958.

21. Sasaki M, Tsuchido T, Matsumura Y. 2008. Molecular cloning and characterization of cytochrome P450 and ferredoxin genes involved in bisphenol A degradation in Sphingomonas bisphenolicum strain A01. J Appl Microbiol 105:1158–1169. https://doi.org/10.1111/j.1365-2672.2008.03843.x.