

# Complete Genome Sequence of the Nonylphenol-Degrading Bacterium *Sphingobium cloacae* JCM 10874<sup>T</sup>

Mina Ootsuka,<sup>a,b</sup> Tomoyasu Nishizawa,<sup>a,b</sup> Hiroyuki Ohta<sup>a,b</sup>

United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, Fuchu-shi, Tokyo, Japan<sup>a</sup>; Ibaraki University College of Agriculture, Amimachi, Ibaraki, Japan<sup>b</sup>

***Sphingobium cloacae* JCM 10874<sup>T</sup> can degrade phenolic endocrine-disrupting chemicals, nonylphenol, and octylphenol. Here, we report the complete genome sequence of the JCM 10874<sup>T</sup> strain.**

Received 10 October 2016 Accepted 14 October 2016 Published 8 December 2016

**Citation** Ootsuka M, Nishizawa T, Ohta H. 2016. Complete genome sequence of the nonylphenol-degrading bacterium *Sphingobium cloacae* JCM 10874<sup>T</sup>. *Genome Announc* 4(6):e01358-16. doi:10.1128/genomeA.01358-16.

**Copyright** © 2016 Ootsuka et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Hiroyuki Ohta, [hiroyuki.ohita.1494@vc.ibaraki.ac.jp](mailto:hiroyuki.ohita.1494@vc.ibaraki.ac.jp).

Environmentally persistent alkylphenols, such as 4-nonylphenol (NP), are known to be estrogenic in animals (1). The chemicals are breakdown products of microbial degradation of man-made nonionic surfactants, namely, nonylphenol polyethoxylates (2). Tanghe et al. published the first report regarding the isolation of a bacterium able to degrade NP in 1999 (3). Following that study, two NP-degrading bacteria were isolated from sewage treatment plant wastewater and river sediment, which were later described as new species, *Sphingomonas* (*Sphingobium*) *cloacae* and *Sphingobium amiense* (4, 5). Further analysis of biodegradation activity revealed that *S. cloacae* S-3<sup>T</sup> (JCM 10874<sup>T</sup>) displays a catabolic activity specific to branched alkylphenols, while *S. amiense* YT<sup>T</sup> is a versatile organism capable of utilizing a wide range of phenolic compounds (6). Here, we investigated the genome of *S. cloacae* JCM 10874<sup>T</sup> to obtain important information regarding the catabolic genes of NP-degrading *Sphingomonas* (*Sphingobium*) species.

Genomic DNA was isolated from *S. cloacae* JCM 10874<sup>T</sup> and sequenced using PacBio RSII instruments. Reads were assembled using FALCON version 0.2.1 to produce seven contigs. The genome sequence was annotated using the Microbial Genome Annotation Pipeline (<http://www.migap.org>) and Rapid Annotations using Subsystems Technology version 2.0 (7), followed by manual annotation with the NCBI-nr databases using the BLASTP program (8). tRNAs were predicted using tRNAscan-SE (9).

The genome of *S. cloacae* JCM 10874<sup>T</sup> consists of 3,767,292 bp in one circular chromosome (SCLO1), with a coverage of 322-fold and 64.6% G+C content, 3,293 coding sequences (CDSs), six rRNA operons, and 49 tRNA genes. There are also five circular plasmids. The plasmids are pSCLO2 (sequence coverage, 301-fold; total nucleotide sequences, 375,493 bp; G+C content, 64.9%; total number of CDSs, 334), pSCLO3 (sequence coverage, 340-fold; total nucleotide sequences, 151,712 bp; G+C content, 62.8%; total number of CDSs, 137), pSCLO4 (sequence coverage, 225-fold; total nucleotide sequences, 108,910 bp; G+C content, 63.7%; total number of CDSs, 92), pSCLO5 (sequence coverage, 85-fold; total nucleotide sequences, 57,701 bp; G+C

content, 63.5%; total number of CDSs, 53), pSCLO7 (sequence coverage, 32-fold; total nucleotide sequences, 33,768 bp; G+C content, 62.9%; total number of CDSs, 33), and linear pSCLO6 (sequence coverage, 99-fold; total nucleotide sequences, 52,690 bp; G+C content, 62.4%; total number of CDSs, 51).

Two genes responsible for alkylphenol oxygenation at the initial step of degradation were detected: one copy of the octylphenol 4-monoxygenase gene (*opdA*) was present in pSCLO3, and one copy of the nonylphenol monoxygenase gene (*nmoA*) was present in pSCLO4. *opdA* shows an amino acid sequence identity of 99.1% with *OpdA* of *Sphingomonas* sp. TTNP3 (3, 10, 11), and *nmoA* shows 100.0% identity with *NmoA* of *Sphingomonas* sp. NP5 (12). Monoxygenase and dioxygenase genes related to cyclic hydrocarbon (cyclopentanone and hydroxyquinol) degradation were also found in the circular plasmids. A gene cluster (7,939 bp) that encodes a hydroquinone catabolic pathway, *hqdRAB-orf1-orf2-hqdCDEF*, was found in the linear plasmid. Amino acid sequence identities were 99.4% (*HqdA*) and 100% (*HqdR*, *HqdB*, *HqdC*, *HqdD*, *HqdE*, and *HqdF*) between *S. cloacae* JCM 10874<sup>T</sup> and the well-characterized *Sphingomonas* sp. strain TTNP3 (3, 13, 14). These results suggest that the degradation of alkylphenols is conducted by the concerted action of different plasmid systems in the bacterium.

**Accession number(s).** The genome sequence of JCM 10874 was deposited in the DDBJ/EMBL/GenBank databases under the accession numbers AP017655 to AP017661.

## ACKNOWLEDGMENT

This work was supported in part by a donation from Yuuji Ushiba.

## REFERENCES

- Kloas W, Lutz I, Einspanier R. 1999. Amphibians as a model to study endocrine disruptors: II. Estrogenic activity of environmental chemicals *in vitro* and *in vivo*. *Sci Total Environ* 225:59–68. [http://dx.doi.org/10.1016/S0048-9697\(99\)80017-5](http://dx.doi.org/10.1016/S0048-9697(99)80017-5).
- White R, Jobling S, Hoare SA, Sumpter JP, Parker MG. 1994. Environmentally persistent alkylphenolic compounds are estrogenic. *Endocrinology* 135:175–182. <http://dx.doi.org/10.1210/endo.135.1.8013351>.
- Tanghe T, Dhooge W, Verstraete W. 1999. Isolation of a bacterial strain

- able to degrade branched nonylphenol. *Appl Environ Microbiol* 65: 746–751.
4. Fujii K, Urano N, Ushio H, Satomi M, Kimura S. 2001. *Sphingomonas cloacae* sp. nov., a nonylphenol-degrading bacterium isolated from wastewater of a sewage-treatment plant in Tokyo. *Int J Syst Evol Microbiol* 51:603–610. <http://dx.doi.org/10.1099/00207713-51-2-603>.
  5. Ushiba Y, Takahara Y, Ohta H. 2003. *Sphingobium amiense* sp. nov., a novel nonylphenol-degrading bacterium isolated from a river sediment. *Int J Syst Evol Microbiol* 53:2045–2048. <http://dx.doi.org/10.1099/ijs.0.02581-0>.
  6. Ikunaga Y, Miyakawa S, Hasegawa M, Kasahara Y, Kodama O, Ohta H. 2004. Degradation profiles of Branched nonylphenol isomers by *Sphingobium amiense* and *Sphingomonas cloacae*. *Soil Sci Plant Nutr* 50:871–875. <http://dx.doi.org/10.1080/00380768.2004.10408548>.
  7. 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* 9:1471–1475. <http://dx.doi.org/10.1186/1471-2164-9-75>.
  8. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25:3389–3402. <http://dx.doi.org/10.1093/nar/25.17.3389>.
  9. Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res* 25: 955–964. <http://dx.doi.org/10.1093/nar/25.5.0955>.
  10. Porter AW, Hay AG. 2007. Identification of *opdA*, a gene involved in biodegradation of the endocrine disrupter octylphenol. *Appl Environ Microbiol* 73:7373–7379. <http://dx.doi.org/10.1128/AEM.01478-07>.
  11. Porter AW, Campbell BR, Kolvenbach BA, Corvini PF, Benndorf D, Rivera-Cancel G, Hay AG. 2012. Identification of the flavin monooxygenase responsible for *ipso* substitution of alkyl and alkoxyphenols in *Sphingomonas* sp. TTNP3 and *Sphingobium xenophagum* Bayram. *Appl Microbiol Biotechnol* 94:261–272. <http://dx.doi.org/10.1007/s00253-011-3621-8>.
  12. Takeo M, Maeda Y, Maeda J, Nishiyama N, Kitamura C, Kato D, Negoro S. 2012. Two identical nonylphenol monooxygenase genes linked to IS6100 and some putative insertion sequence elements in *Sphingomonas* sp. NP5. *Microbiology* 158:1796–1807. <http://dx.doi.org/10.1099/mic.0.055335-0>.
  13. Kolvenbach BA, Lenz M, Benndorf D, Rapp E, Fousek J, Vlcek C, Schäffer A, Gabriel FL, Kohler HP, Corvini PF. 2011. Purification and characterization of hydroquinone dioxygenase from *Sphingomonas* sp. strain TTNP3. *AMB Express* 1:8. <http://dx.doi.org/10.1186/2191-0855-1-8>.
  14. Kolvenbach BA, Dobrowinski H, Fousek J, Vlcek C, Schäffer A, Gabriel FL, Kohler HP, Corvini PF. 2012. An unexpected gene cluster for downstream degradation of alkylphenols in *Sphingomonas* sp. strain TTNP3. *Appl Microbiol Biotechnol* 93:1315–1324. <http://dx.doi.org/10.1007/s00253-011-3451-8>.