

Complete Genome Sequence of *Sphingobium* sp. Strain SYK-6, a Degrader of Lignin-Derived Biaryls and Monoaryls

Eiji Masai,^a Naofumi Kamimura,^a Daisuke Kasai,^a Akio Oguchi,^b Akiho Ankai,^b Shigehiro Fukui,^b Mikio Takahashi,^b Isao Yashiro,^b Hiroki Sasaki,^b Takeshi Harada,^b Sanae Nakamura,^b Yoko Katano,^b Sachiko Narita-Yamada,^b Hidekazu Nakazawa,^b Hirofumi Hara,^c Yoshihiro Katayama,^d Masao Fukuda,^a Shuji Yamazaki,^b and Nobuyuki Fujita^b

Department of Bioengineering, Nagaoka University of Technology, Nagaoka, Niigata,^a Biological Resource Center, National Institute of Technology and Evaluation, Shibuya-ku, Tokyo,^b Department of Biomedical Engineering, Okayama University of Science, Kita-ku, Okayama,^c and College of Bioresource Sciences, Nihon University, Fujisawa, Kanagawa,^d Japan

Sphingobium sp. strain SYK-6 is able to grow on an extensive variety of lignin-derived biaryls and monoaryls, and the catabolic genes for these compounds are useful for the production of industrially valuable metabolites from lignin. Here we report the complete nucleotide sequence of the SYK-6 genome which consists of the 4,199,332-bp-long chromosome and the 148,801-bp-long plasmid.

Lignin is a complex heteropolymer produced from hydroxycinnamyl alcohols through radical coupling (13). Due to the fact that lignin is the most abundant aromatic substance in nature, the potential for obtaining industrially valuable chemicals from lignin is exceptionally high (1, 12). Microbial catabolic functions degrading lignin-derived aromatics are crucial in establishing processes for effective utilization of lignin.

Sphingobium sp. strain SYK-6 (NBRC 103272) is a unique bacterium capable of utilizing various types of lignin-derived biaryls and monoaryls as the sole source of carbon and energy (11). In this strain, lignin-derived biaryls are degraded by a wide variety of specific enzymes to syringate and vanillate. Then, syringate is converted to 3-O-methylgallate, and vanillate is converted to protocatechuate. The resulting metabolites are further degraded through the multiple ring cleavage pathways (7, 8). A significant portion of the SYK-6 genes involved in the catabolism of ligninderived aromatics has been isolated and characterized thus far (11). In addition to our previous results, information on the whole-genome sequence of this bacterium will provide a more complete understanding of a bacterial lignin catabolic system.

DNA shotgun libraries with inserts of 1.5 and 5.0 kb in pUC118 and a fosmid library with inserts of 40 kb in pCC1FOS (Epicentre Biotechnologies, Madison, WI) were constructed. These clones were end sequenced using dye terminator chemistry on an ABI Prism 3730 sequencer, and the sequences of ca. 48,192 reads were assembled using the PHRED/PHRAP/CONSED software (3–5). Fosmid clones that link two contigs were selected and sequenced by primer walking to close any gaps. The prediction of open reading frames (ORFs) was performed using Glimmer 3 (2). Putative nontranslated genes were identified using the Rfam (6), tRNAscan-SE (10), and ARAGORN (9) programs.

The genome of SYK-6 consists of a circular chromosome (4,199,332 bp; 65.57% G+C; 3,913 ORFs) and a circular plasmid, pSLGP (148,801 bp; 64.40% G+C; 150 ORFs). The chromosome has two copies of rRNA operons and 50 tRNA genes. Comparisons between the genomes of SYK-6 and six other sphingomonad strains, *Sphingobium japonicum* UT26S, *Sphingomonas wittichii* RW1, *Sphingobium chlorophenolicum* L-1, *Novosphingobium aromaticivorans* DSM 12444, *Novosphingobium* sp. strain PP1Y, and *Sphingopyxis alaskensis* RB2256, revealed no synteny, but approx-

imately 48 to 57% of total ORFs in strain SYK-6 were orthologous to those of other strains. Interestingly, ca. 120 ORFs on pSLGP were almost identical to those on chromosome 1 of strain UT26S, suggesting a plasmid-mediated gene transfer between sphingomonads. Genes involved in the catabolism of lignin-derived aromatics are located on the chromosome and scattered throughout at least 10 different loci. A significantly higher number of major facilitator superfamily transporters were predicted for strains SYK-6, RW1, and PP1Y (66 to 70 transporters), whereas the remaining four strains were predicted to have between 31 and 46 transporters. This may reflect that SYK-6 requires a large number of transporters to take up an extensive variety of lignin-derived aromatics.

Nucleotide sequence accession numbers. The nucleotide sequences of the *Sphingobium* sp. strain SYK-6 chromosome and pSLGP were deposited in the DDBJ/EMBL/GenBank databases under accession numbers AP012222 and AP012223, respectively. The annotated genome sequence is also available in the DOGAN genome database (http://www.bio.nite.go.jp/dogan/Top).

ACKNOWLEDGMENTS

This work was supported by Management Expenses Grants from the government to the National Institute of Technology and Evaluation and to the Nagaoka University of Technology.

REFERENCES

- 1. Bugg TD, Ahmad M, Hardiman EM, Singh R. 2011. The emerging role for bacteria in lignin degradation and bio-product formation. Curr. Opin. Biotechnol. 22:394–400.
- Delcher AL, Bratke KA, Powers EC, Salzberg SL. 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. Bioinformatics 23:673–679.
- 3. Ewing B, Green P. 1998. Base-calling of automated sequencer traces using phred. II. Error probabilities. Genome Res. 8:186–194.
- 4. Ewing B, Hillier L, Wendl MC, Green P. 1998. Base-calling of automated

Received 26 October 2011 Accepted 28 October 2011 Address correspondence to Eiji Masai, emasai@vos.nagaokaut.ac.jp. Copyright © 2012, American Society for Microbiology. All Rights Reserved. doi:10.1128/JB.06254-11 sequencer traces using phred. I. Accuracy assessment. Genome Res. 8:175–185.

- 5. Gordon D, Abajian C, Green P. 1998. Consed: a graphical tool for sequence finishing. Genome Res. 8:195–202.
- 6. Griffiths-Jones S, et al. 2005. Rfam: annotating non-coding RNAs in complete genomes. Nucleic Acids Res. 33:D121–D124.
- Kamimura N, et al. 2010. Regulatory system of the protocatechuate 4,5-cleavage pathway genes essential for lignin downstream catabolism. J. Bacteriol. 192:3394–3405.
- Kasai D, Masai E, Miyauchi K, Katayama Y, Fukuda M. 2005. Characterization of the gallate dioxygenase gene: three distinct ring cleavage dioxygenases are involved in syringate degradation by *Sphingomonas paucimobilis* SYK-6. J. Bacteriol. 187:5067–5074.
- 9. Laslett D, Canback B. 2004. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. Nucleic Acids Res. 32:11–16.
- 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.
- 11. Masai E, Katayama Y, Fukuda M. 2007. Genetic and biochemical investigations on bacterial catabolic pathways for lignin-derived aromatic compounds. Biosci. Biotechnol. Biochem. 71:1–15.
- 12. Otsuka Y, et al. 2006. Efficient production of 2-pyrone 4,6-dicarboxylic acid as a novel polymer-based material from protocatechuate by microbial function. Appl. Microbiol. Biotechnol. 71:608–614.
- Ralph J, et al. 2004. Lignins: natural polymers from oxidative coupling of 4-hydroxyphenylpropanoids. Phytochem. Rev. 3:29–60.