The Journal of Microbiology, June 2001, p.139-141 Vol. 39, No. 2
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NOTE

Isolation of a Medium Chain Length Polyhydroxyalkanoic Acids Degrading Bacterium, *Janthinobacterium lividum*

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(Received April 13, 2001 / Accepted May 21, 2001)

Medium-chain length polyhydroxyalkanoic acids (MCL-PHAs) degrading bacterium was isolated from the soil. The bacterium was identified as *Janthinobacterium lividum* by its biochemical properties, cell membrane fatty acids composition, and 16S rDNA sequence analysis. The bacterium showed a similarity of 0.911 with *J. lividum* according to the cell membrane fatty acids analysis and a similarity of 97% in the 16S rDNA sequence analysis. Culture supernatant of the bacterium showed the highest depolymerase activity toward polyhydroxynonanoic acid (PHN) that did not degrade the poly-β-hydroxybutyric acid (PHB). The esterase activity was also detected with *p*-nitrophenyl (PNP) esters of fatty acids such as PNP-octanoic acid, PNP-dodecanoic acid, PNP-decanoic acid, and PNP-hexanoic acid.

Key words: depolymerase, esterase, *Janthinobacterium lividum,* MCL-PHAs

Polyhydroxyalkanoic acids (PHAs) are known as intracellular storage macromolecules, which are accumulated as discrete granules by bacteria (1, 9). Because of their thermoplastic properties and biodegradabilities, these microbial polyesters are of biotechnological interest. PHAs are composed of linear hydroxyalkanoic acids with short (3 to 5 carbon atoms, SCL-PHAs) or medium (6 to 14 carbon atoms, MCL-PHAs) chain lengths and the best known, SCL-PHA, polyhydroxybutyric acid (PHB), is accumulated by bacteria (4, 9). PHA-degrading microbes are widely distributed and have been isolated from various environments (2, 5). However, the majority of these organisms are restricted to the degradation of PHB and other SCL-PHAs such as copolymers of hydroxybutyric acid and hydroxyvaleric acid, poly (3HB-*co*-3HV) (1, 3, 9). Only a few microbes have been reported as MCL-PHAs degraders (6, 11, 12). Recently, numerous extracellular MCL-PHA depolymerases have been purified and characterized from bacteria (7, 8, 10). In the present report, we describe the isolation and characterization of another bacterium with a capability of growing with polyhydroxynonanoic acid (PHN) as the sole source of carbon and energy.

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The soil suspension was inoculated into mineral salts medium containing 0.1% PHN as the sole carbon source and enriched three times. The composition of mineral salts

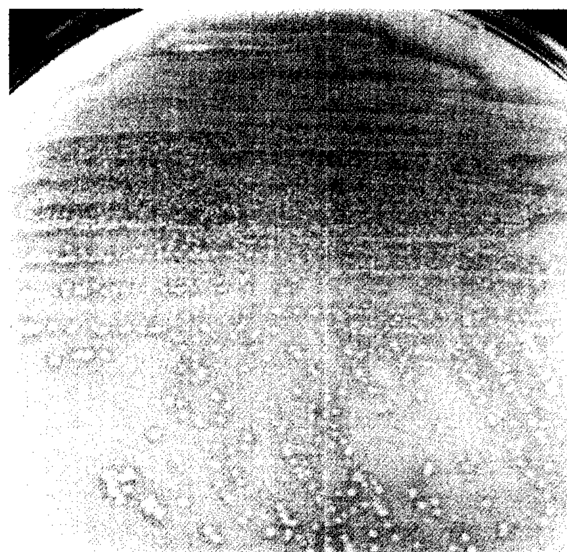

Fig. 1. PHN depolymerase activity of isolate KS02. The bacterium was grown on a mineral salts medium containing PHN as the sole carbon source at 30°C for 24 h.

Table 1. Morphological and physiological characteristics of isolate KS02

Characteristics	Results
Shape	Rod
Gram staining	Negative
Spore formation	-
Nitrate reduction	-
Indole production	+
Arginine dihydrolase	+
Urease	-
β-Glucosidase	+
Gelatin hydrolysis	-
β-Galactosidase	-
Oxidase	+
Utilization of Glucose, Mannose, Mannitol, Malate, Citrate, Phenyl acetate	+

medium was as follows; 2 g/l NH_4Cl, 1 g/l KH_2PO_4, 0.5 g/l $MgSO_4 \cdot 7H_2O$, 0.5 g/l KCl, 0.5 g/l $CaCl_2 \cdot 2H_2O$, 5 mg/l $FeSO_4 \cdot 7H_2O$, 5 mg/l $ZnSO_4 \cdot 7H_2O$, 5 mg/l $CuSO_4 \cdot 5H_2O$, and 0.5 g/l yeast extract. Clear zone-forming colonies were selected as PHN degrading bacteria and one of these isolates, KS02, was used for further experiments. Fig. 1 shows a clear zone that was formed by KS02 in the solid medium.

The KS02 strain is rod-shaped with rounded ends, non-sporulating, and a Gram negative bacterium. The morphological, cultural, and biochemical characteristics of KS02 are shown in Table 1, indicating that KS02 has similar characteristics to the *Janthinobacterium* group according to Bergey's Manual of Systematic Bacteriology. The cell

Table 2. Cell membrane fatty acid composition of isolate KS02

Fatty acid	Composition (%)
10:0 3OH	3.97
12:0	4.12
12:0 2OH	2.01
16:1 w7c/15 iso 2OH	47.36
16:0	33.59
17:0 cyclo	1.22
18:1 w7c/w9t/w12t	7.74

membrane fatty acids composition of the isolate was also analyzed using gas chromatography and it was compared with the data of Microbial Identification System (Table 2). The major fatty acids were 16:1 w7c/15 iso 2OH (47.36%), 16:0 (33.59%), and 18:1 w7c/w9t/w12t (7.74%). The fatty acid composition of KS02 showed the highest similarity to those of *Janthinobacterium*, especially *J. lividum* with a score of 0.911. To confirm these results, 16S rDNA sequences were analyzed. Amplification of 16S rDNA with universal primers 27F and 1492R resulted in an approximately 1.5 kbp fragment. The PCR product was extracted using the DNA PrepMate™ kit (Bioneer) and sequenced with Automated DNA Sequencer (ABI PRISM™ 377, Perkin Elmer). Homologous sequences were found by BLAST Search (http://www.nlm.ncbi.nih.gov/). The resulting DNA sequence showed a 97% (607/620) identity to that of *J. lividum*. The sequence has been deposited with the GenBank under the accession number of AF326087. The tree of 16S rDNA that was constructed by Neighbor-Joining algorithm is shown in Fig. 2. The

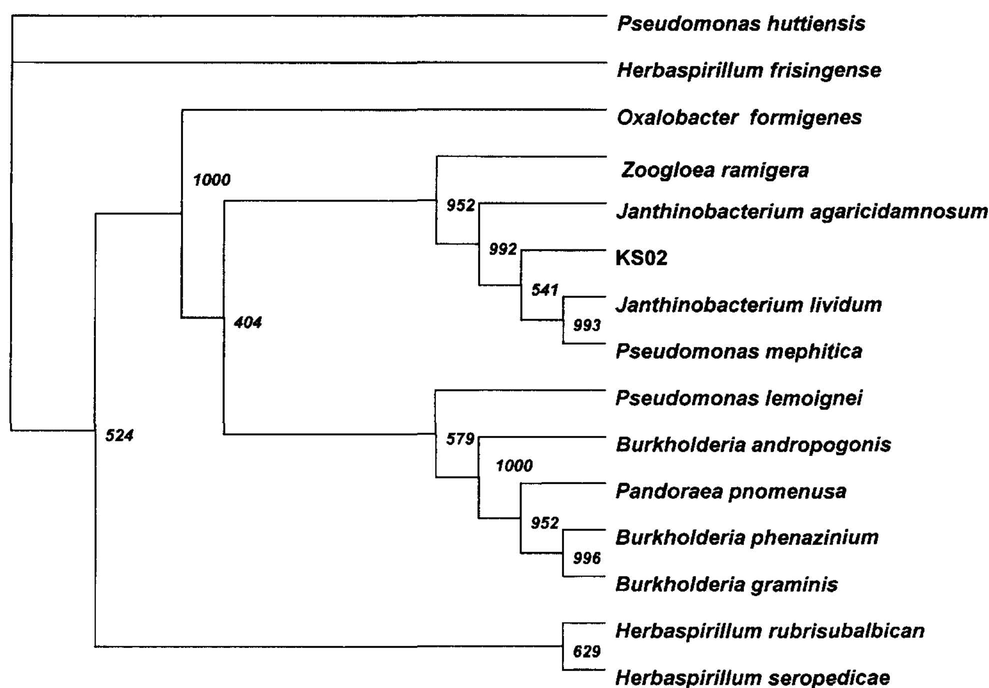

Fig. 2. Phylogenetic tree of an isolate, KS02 based on the 16S rDNA gene. The tree was constructed using the DNADIST and neighbour-joining method. Bootstrap values were calculated from 1000 replications.

Table 3. Degradation activity of polyhydroxyalkanoic acids and *p*-nitrophenyl esters by the culture supernatant of isolate KS02

Substrate	Hydrolyzing activity (U/ml)	Relative activity (%)
PHAs		
Butyric acid	-	0
Heptanoic acid	0.00160	28.2
Octanoic acid	0.00444	78.2
Nonanoic acd	0.00568	100
PNP-esters		
Butyric acid	0.00006	1.4
Hexanoic acid	0.00144	33.0
Octanoic acid	0.00436	100
Decanoic acid	0.00160	36.7
Dodecanoic acid	0.00184	42.2

isolate KS02 is phylogenetically related to *J. lividum, Pseudomonas mephitica,* and *J. agaricidamnosum.* According to these results, we have identified the isolate as *J. lividum,* beta subdivision of Proteobacteria, *Burkholderia* group.

PHA depolymerase activity was determined by measuring the changes in turbidity of the PHA suspension at 650 nm. One unit of enzyme activity was defined as the amount of enzyme capable of decreasing A_{650} by 1 unit per min. Esterase activity was assayed with *p*-nitrophenyl (PNP) derivatives as substrates. One unit of esterase activity was defined as the hydrolysis of 1 μmol of PNP derivatives in 1 min. The supernatant of isolate KS02 showed a specificity for the hydrolysis of PHN, polyhydroxyoctanoic acid (PHO), and polyhydroxyheptanoic acid (PHHp), in decreasing order, and no hydrolytic activity was detected with PHB. Esterase activity with *p*-nitrophenyl esters of fatty acids, PNP-butyric acid (0.00006 U/ml), PNP-hexanoic acid (0.00144 U/ml), PNP-octanoic acid (0.00436 U/ml), PNP-decanoic acid (0.00160 U/ml), and PNP-dodecanoic acid (0.00184 U/ml) were measured (Table 3). However, the hydrolytic and esterase activities were significantly lower than those of *Xanthomonas* sp. JS02 (7) and *Pseudomonas* sp. RY-1 (8). The substrate specificity was also different from that of *Xanthomonas* JS02, which showed a maximum activity with PNP-hexanoic acid (7). It could be concluded that the isolate KS02 may produce another type of MCL-PHA depolymerase. The purification and characterization of the enzyme remain to be studied.

Acknowledgment

This work was supported by a research grant from the Korea Science and Engineering Foundation (Grant No. 1999-2-20200-006-4).

References

1. Anderson, A.J. and E.A. Dawes. 1990. Occurrence, metabolism, metabolic role, and industrial use of bacterial polyhydroxyalkanoates. *Microbiol. Rev.* 54, 450-472.
2. Budwill, K.P., M. Fedorak, and W.J. Page. 1992. Methanogenic degradation of poly (3-hydroxyalkanoates). *Appl. Environ. Microbiol.* 58, 1398-1401.
3. Foster, L.J.R., S.J. Zervas, R.W. Lenz, and R.C. Fuller. 1995. The biodegradation of poly-3-hydroxyalkanoates, PHAs, with long alkyl substituents by *Pseudomonas maculicola. Biodegradation* 6, 67-73.
4. Forsyth, W.G.C., A.C. Hayward, and R.B. Roberts. 1958. Occurrence of poly-b-hydroxybutyric acid in aerobic gram-negative bacteria. *Nature* 182, 800-801.
5. Jendrossek, D., I. Knoke, R.H. Habibian, A. Steinbuchel, and H.G. Schlegel. 1993. Degradation of poly (3-hydroxybutyrate), PHB, by bacteria and purification of a novel PHB depolymerase of *Comamonas* sp. *J. Environ. Polym. Degrad.* 1, 53-63.
6. Ju, H.S., J. Kim, and H. Kim. 1998. Isolation of an aromatic polyhydroxyalkanoate-degrading bacteirum. *J. Microbiol. Biotechnol.* 8, 540-542.
7. Kim, H., H.S. Ju, and J. Kim. 2000. Characterization of an extracellular poly (3-hydroxy-5-phenylvalerate) depolymerase from *Xanthomonas* sp. JS02. *Appl. Microbiol. Biotechnol.* 53, 323-327.
8. Kim, H.M., K.E. Ryu, K.S. Bae, and Y.H. Rhee. 2000. Purification and characterization of extracellular medium-chain length polyhydroxyalkanoate depolymerase from *Pseudomonas* sp. RY-1. *J. Biosci. Bioeng.* 89, 196-198.
9. Madison, L.L. and G.W. Huisman. 1999. Metabolic engineering of poly(3-hydroxyalkanoates): from DNA to plastic. *Microbiol. Mol. Biol. Rev.* 63, 21-53.
10. Muller, B. and D. Jendrossek. 1993. Purification and properties of poly (3-hydroxyvaleric acid) depolymerase from *Pseudomonas lemoignei. Appl. Microbiol. Biotechnol.* 38, 487-492.
11. Schirmer, A., D. Jendrossek, and H.G. Schlegel. 1993. Degradation of poly (3-hydroxyoctanoic acid) [P(3OH)] by bacteria: purification and properties of a P(3OH) depolymerase from *Pseudomonas fluorescens* GK13. *Appl. Environ. Microbiol.* 59, 1220-1227.
12. Schirmer, A. and D. Jendrossek. 1994. Molecular characterization of the extracellular poly (3-hydroxyoctanoic acid) [P(3OH)] depolymerase gene of *Pseudomonas fluorescens* GK13 and of its gene product. *J. Bacteriol.* 176, 7056-7073.