Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Biotechnology 115 (2005) 129–136
JOURNAL OF Biotechnology
www.elsevier.com/locate/jbiotec

Isolation and characterization of *Rhodococcus* sp. strains TMP2 and T12 that degrade 2,6,10,14-tetramethylpentadecane (pristane) at moderately low temperatures

Namio Kunihiro, Mitsuru Haruki[1], Kazufumi Takano, Masaaki Morikawa*, Shigenori Kanaya

Department of Material and Life Science, Graduate School of Engineering, Osaka University, Suita, Osaka 565-0871, Japan

Received 29 March 2004; received in revised form 14 July 2004; accepted 30 July 2004

Abstract

Branched alkanes including 2,6,10,14-tetramethylpentadecane (pristane) are more resistant to biological degradation than straight-chain alkanes especially under low-temperature conditions, such as 10 °C. Two bacterial strains, TMP2 and T12, that are capable of degrading pristane at 10 °C were isolated and characterized. Both strains grew optimally at 30 °C and were identified as *Rhodococcus* sp. based on the 16S rRNA gene sequences. Strain T12 degraded comparable amounts of pristane in a range of temperatures from 10 to 30 °C and strain TMP2 degraded pristane similarly at 10 and 20 °C but did not degrade it at 30 °C. These data suggest that the strains have adapted their pristane degradation system to moderately low-temperature conditions.

Keywords: Pristane; Degradation; Low-temperature conditions; *Rhodococcus*

1. Introduction

Biodegradation of environmental pollutants has been anticipated to be one of the sustainable technologies with low impact on the earth (Leahy and Colwel, 1990). Many kinds of mesophilic bacteria that can degrade toxic hydrocarbons have been isolated and characterized. However, most of their

* Corresponding author. Present address: Division of Bioscience, Graduate School of Environmental Earth Science, Hokkaido University, Sapporo, Hokkaido 060-0810, Japan. Tel.: +81 11 706 2253; fax: +81 11 706 2253.
E-mail address: morikawa@ees.hokudai.ac.jp (M. Morikawa).
[1] Present address: Department of Materials Chemistry and Engineering, College of Engineering, Nihon University, Koriyama, Fukushima 963-8642, Japan.

0168-1656/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jbiotec.2004.07.018

metabolic activities significantly drop at the temperatures below 20 °C (Atlas, 1981). Under these moderately low-temperature conditions (<20 °C), hydrocarbons are less volatile and become more insoluble so that they are resistant to biodegradation. Thus, these pollutants will remain undegraded in the environment under low-temperature rather than medium- or high-temperature conditions (Atlas, 1981; Kato et al., 2001). Although there are a few reports on psychrophilic and psychrotrophic alkane-degrading bacteria (Whyte et al., 1998; Margesin and Schinner, 1998; Rapp and Gabriel-Jurgens, 2003), knowledge is yet limited due to the small number of collections of such bacteria.

It is known that branched alkanes are more resistant to biological degradation than straight-chain alkanes. Such a resistance is affected by the position, size, and stereochemistry of the branches (Cox et al., 1974; Cantwell et al., 1978; Schaeffer et al., 1979). In general, they are resistant to degradation when branches are located near or at the end of the molecules because most degradation starts externally by terminal oxidation (Lal and Khanna, 1996; Huu et al., 1999; Jenisch-Anton et al., 1999; Berekaa and Steinbuchel, 2000). 2,6,10,14-Tetramethylpentadecane (pristane), is a model compound of multiply branched alkanes, which is ubiquitously present in crude oils, soils, and marine sediments, suggesting its long half-life in nature (Volkman and Maxwell, 1986). Above context prompted us to isolate and analyze bacteria that can degrade pristane at 10 °C.

2. Materials and methods

2.1. Culture media and isolation of bacteria

Pristane-degrading bacteria were isolated in minimal medium BM2 with pristane as a sole carbon source. For the preparation of BM2, 1 l of the basal salts was autoclaved, cooled and supplemented with 1 ml each of filtrated vitamin mixture and trace elements. Basal salts contain per liter: 1 g of $(NH_4)_2SO_4$, 1 g of K_2HPO_4, 0.2 g of $MgCl_2$, 0.04 g of $MgSO_4$, 0.02 g of KCl, and 0.02 g of $CaCl_2$ (pH 7.2). Vitamin mixture contains per liter: 0.6 mg each of biotin, and folic acid; 59 mg each of inositol, *p*-aminobenzoic acid, and riboflavin; and 120 mg each of calcium-pantothenate, niacin, pyridoxine-HCl, and thiamine-HCl. Trace elements contains 1 mg of $CoCl_2 \cdot 6H_2O$, 3 mg of $CuSO_4 \cdot 5H_2O$, 7 mg of $Na_2MoO_4 \cdot 2H_2O$, 15 mg of H_3BO_3, 17 mg of $MnSO_4 \cdot H_2O$, 29 mg of $ZnSO_4 \cdot 7H_2O$, 43 mg of $FeSO_4 \cdot 7H_2O$, and 2 g of EDTA. Pristane was added to BM2 at 0.25% (v/v).

About a 100 mg each of soil sample was inoculated to 4 ml of BM2 supplemented with 0.25% (v/v) pristane in 20 ml vials (Maruemu, Tokyo, Japan) with butyl rubber stoppers and incubated at 10 or 20 °C for 2 weeks. Then the cultures were transferred to new media at 100 times dilution rate and further incubated for another 2 weeks. The cultures were serially diluted and streaked onto BM2 solid media containing 1.5% of agar. After spreading 20 μl of pristane onto the plates, they were incubated for 1 week.

Because the two strains chosen for further analyses grew better in L-broth than BM2 supplemented with pristane, cell growth was examined at various temperatures in L-broth. L-broth contains per liter 5 g of yeast extract, 10 g of Bacto-tryptone (Difco, Sparks, MD), and 5 g of NaCl (pH 7.2).

2.2. Cloning and sequencing of 16S rRNA gene

Genomic DNA were prepared from colonies by using Insta-Gene matrix (Bio-Rad, Hercules, CA) and used for PCR. The 16S rRNA gene locus was amplified by PCR with EX Taq DNA polymerase (Takara Bio, Kyoto, Japan) and a GeneAmp PCR System 2400 (Perkin-Elmer, Foster City, CA). PCR primers were A-160 (5′-GGCGGACGGCTCAGTAACACG-3′) and A-1500 (5′-GTGACGGGCGGTGTGTGCAAGG-3′). PCR was started with an initial denaturation at 94 °C for 5 min, followed by 40 cycles of 94 °C for 1 min, 45 °C for 1 min, and 72 °C for 1.5 min, and the last elongation step was done at 72 °C for 10 min. The PCR amplified 16S rRNA gene fragments (about 1.3 kb) were purified by agarose gel electrophoresis, ligated into pCR2.1 vector (Invitrogen, Groningen, The Netherlands) and used for the transformation of *E. coli* DH5α. Nucleotide sequences of the 16S rRNA genes were determined by ABI PRISM 310 genetic analyzer (Perkin-Elmer). The BLASTN program (http://www.ncbi.nlm.nih.gov/BLAST/, NCBI, Bethesda, MD) was used for gene homology search with the standard program default. The

nucleotide sequences of the 16S rRNA genes from strains TMP2 and T12 have been deposited in the EMBL/GenBank/DDBJ databases under accession numbers AB108557 and AB108558, respectively.

2.3. Degradation of n-alkanes, pentadecane, and pristane

Each bacterial strain was precultured in L-broth at 30 °C for 2 days. Cells were collected by centrifugation at 12 000 × *g* for 5 min (4 °C) and washed with 0.85% NaCl. Finally, cell suspension was adjusted to $OD_{600} = 4$ and used for further experiments.

Degradation tests were performed in 20 ml vials containing, 3.9 ml of BM2, 100 μl of cells suspension (final $OD_{600} = 0.1$), and 10 μl (0.25%) of *n*-alkanes (Standard gas oil #S0434, Tokyo Kasei, Tokyo, Japan), pentadecane, or pristane (Tokyo Kasei). Standard gas oil is mainly composed of *n*-alkanes with a range of carbon numbers from 9 to 24 (C9–C24). For the degradation tests of mixed substrate, 5 μl (0.125%) each of pentadecane and pristane was used for substrate. The vials were shaken at 10, 20, and 30 °C with a rotation speed of 120 min^{-1}. Cell suspension was replaced by 0.85% NaCl in negative control vials and the amounts of substrate decreased in this experiment were subtracted from all data at each sampling time. These difference values are defined as the amount degraded. After a degradation period, remaining hydrocarbons were extracted by hexane containing biphenyl (Wako, Tokyo, Japan) at 20 ppm (w/v) as an internal standard. The amount of each hydrocarbon fraction was quantified by the gas chromatography (GC/FID). GC/FID was done by HP6890 (Hewlett-Packard, Fort Collins, CO) attached with a non-polar capillary column HP-1 (0.2 mm in diameter by 30 m in length with 0.25 μm film thickness, Hewlett-Packard). Helium was used as a carrier gas at a flow rate of 1.0 ml/min. Temperature program were as follows: an initial oven temperature was kept at 80 °C for 2 min, then linearly increased at a rate of 10 °C/min up to 300 °C and kept for 10 min. Temperatures of the injector and detector were both maintained at 300 °C. GC/MS analysis was done by JEOL JMS-DX303 mass spectrometer (JEOL, Tokyo, Japan) at 70 eV.

3. Results and discussion

3.1. Isolation, identification and growth of bacteria

After repetitive transfer of the samples to new media, 14 bacterial strains grew and formed colonies at 20 °C on the BM2-agar plate overlaid with pristane as a sole carbon source. When the culture extracts were analyzed by GC/FID, strains TMP2 and T12 showed higher pristane degradation abilities than others and used for further analyses. Strain TMP2 was isolated from the soil at a rice field in Japan and strain T12 was isolated from the soil at a dumping ground in India. No pristine-degrading bacteria were obtained from cold places such as frozen oil sands at Ft. McMurray, Alberta, Canada. In order to identify these strains, the 16S rRNA genes were amplified by PCR using genomic DNA of these strains as a template. Nucleotide sequences of the DNA fragment encoding 90% of the 16S rRNA gene clearly demonstrated that both strains TMP2 and T12 belong to genus *Rhodococcus*, with the highest identity of 99.8 and 99.7% to *Rhodococcus erythropolis*, respectively. They also share 99.8 and 99.1% identity with *Rhodococcus* sp. 7/1 which was isolated from Antarctica as an alkane degrader (Bej et al., 2000).

Although the enrichment culture was prepared at 10 and 20 °C, the isolated strains TMP2 and T12 had the same optimum growth temperature of 30 °C, at which their specific growth rates, μ, were 0.12 and 0.11 h^{-1} in a shaking flask containing L-broth, respectively. Strain TMP2 grew at 10 °C with $\mu = 0.047\ h^{-1}$ and 20 °C with 0.053 h^{-1} and strain T12 grew at 10 °C with $\mu = 0.023\ h^{-1}$ and 20 °C with 0.033 h^{-1} in L-broth. Strains TMP2 and T12 grew at 10 °C with a lag-time of 40 and 70 h, respectively. Strain TMP2 could grow at 37 °C ($\mu = 0.037\ h^{-1}$), whereas strain T12 only poorly grew at 37 °C ($\mu = 0.0015\ h^{-1}$).

3.2. Degradation of pristane, n-alkanes, and pentadecane

Degradation of pristane by the strains was tested at 10, 20, and 30 °C (Fig. 1a). It is clearly shown that the degradation rates of pristane by strain TMP2 are higher at 10 and 20 °C than at 30 °C. No pristane degradation was observed for strain TMP2 at 30 °C. Strain T12 degraded comparable amounts of pristane

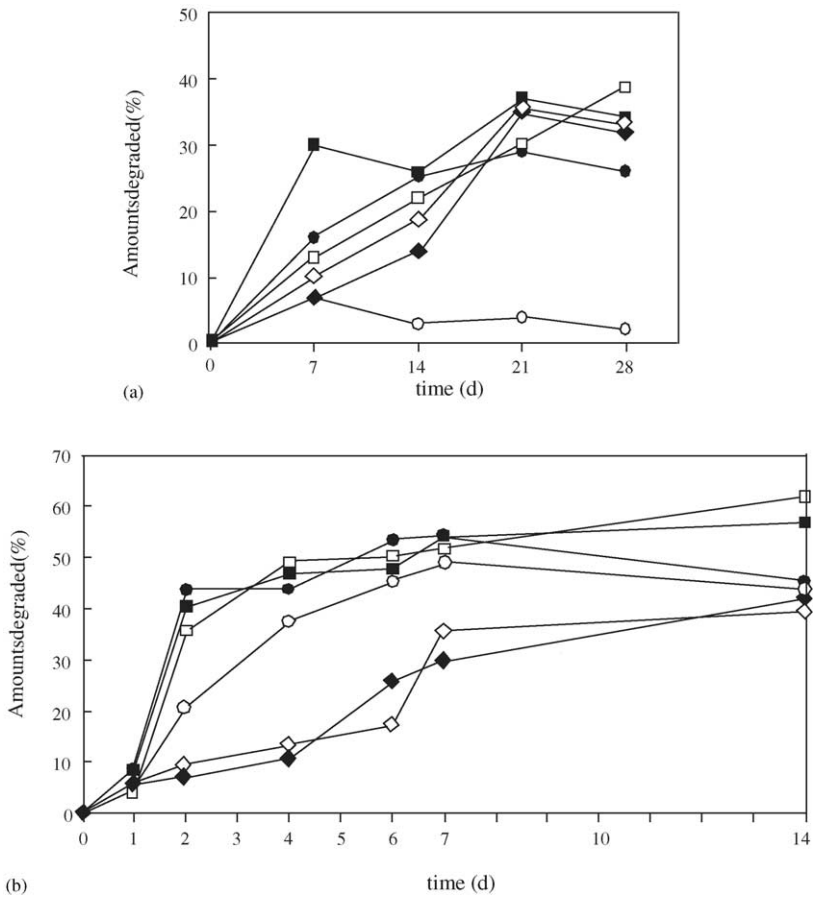

Fig. 1. Degradation of pristane (a) and *n*-alkanes (b) by strains TMP2 and T12. Symbols are as follows: TMP2: 10 °C (◊); 20 °C (□); 30 °C (○); T12: 10 °C (♦); 20 °C (■); 30 °C (●). Every data point is the average of two independent experiments.

in a range of temperatures from 10 to 30 °C. These data suggest that strains TMP2 and T12 have adapted their pristane degradation systems to low-temperature conditions.

It has been reported that *Brevibacterium erythrogenes* degrades both normal and branched alkanes (Pirnik et al., 1974). Therefore, degradation of *n*-alkanes by the strains was tested at the same conditions as those for pristane degradation (Fig. 1b). Both strains degraded *n*-alkanes (C9–C22) much faster than pristane at any temperature examined, supporting general understanding that pristane is more resistant than *n*-alkanes to biological degradation. Both strains did not degrade *n*-alkanes of C23 and C24. It was also found that strains TMP2 and T12 degraded *n*-alkanes with faster rate at 20 and 30 °C than at 10 °C like many other mesophilic *Rhodococcus* spp. (Koike et al., 1999; Sharma and Pant, 2000). The different temperature preference for degradation of pristane and *n*-alkanes in strain TMP2 suggests that different enzymes are involved in *n*-alkane and pristane degradations in this strain. It is less probable that an enzyme shows different substrate specificity under different temperature conditions. Because strain TMP2 grows normally and even the fastest at 30 °C, it is also less plausible that the membrane permeability was changed under this temperature condition. In order to test whether degradation pathways of pristane and *n*-alkanes are independent or not in these strains, degradation yields of pristane, pentadecane, and their mixture were compared at 20 °C.

When 0.125% (v/v) pristane alone was used as a substrate, strains TMP2 and T12 degraded it at the extents of 15 and 40% in 1 week at 20 °C, respectively. When 0.125% (v/v) pentadecane alone was used as a substrate, their degradation was 66 and 67%. When a mixture of equal volume, 0.125% (v/v) each, of pristane and pentadecane was used as a substrate, significant reduction in the degradation of pristane was observed for both strains. Strains TMP2 and T12 degraded only 2% (13% in 2 weeks) and 1% (9% in 2 weeks) of pristane, whereas they degraded 47 and 53% of pentadecane in 1 week, respectively. This result suggests that the degradation pathways of pristane and pentadecane are related at the level of either gene regulation, such as a catabolite repression, or common substrate specificity of enzymes in the pathway, such as a competitive inhibition. Although both the strains grew on these hydrocarbons, they did not completely degrade pristane, pentadecane, and *n*-alkanes. This is probably due to the system that cultivation was done in a securely sealed vial bottles with limited amounts of oxygen and/or nutrients.

3.3. Pristane degradation pathway of strain TMP2

Degradation of multiply branched alkanes, such as pristane and squalene, has been reported for strains in the genera *Brevibacterium*, *Corynebacterium*, *Mycobacterium*, *Nocardia*, and *Moraxella*. Pristanol, pristanic acid, and squalene dioic acid were identified as degradation intermediates (McKenna and Kallio, 1971; Cox et al., 1974; Nakajima et al., 1974; Pirnik et al., 1974; Berekaa and Steinbuchel, 2000). They all seem to adopt terminal oxidation pathways like that for *n*-alkane by alkane hydroxylase and cytochrome P450 monooxygenase (Kok et al., 1989; Scheller et al., 1998). More recently, a psychrophilic *Rhodococcus* sp. has been reported for degradation of *n*-alkanes, pristane, and chlorinated benzenes (Rapp and Gabriel-Jurgens, 2003). However, degradation pathway of pristane has not been examined. In this experiment possible degradation metabolites were observed only for strain TMP2 at 10 and 20 °C. There are three candidate peaks detected whose area was increased as that of pristane decreased (Fig. 2a). The fraction eluted at 9.9 min (SC# 1194) and 14.2 min (SC# 1708) are an internal standard, biphenyl, and remaining substrate pristane, respectively. The most major metabolite that is eluted at 17.1 min (SC# 2056) is suggested to be 1-pristene (MW = 226, Fig. 2c). This assignment was supported by observations that a series of fragment peaks are two mass units less each than those of pristane. The second major products are eluted at 16.3 min (SC# 1965–70). This peak was found to be a mixture of two compounds that are not separated by GC/FID. Similar fragment peaks distribution suggests that the compound at SC# 1970 shares common basic structure with SC# 2056 with shorter carbon chain (data not shown). Further analytical data including chemical modification experiments are necessary to conclude that the compound (SC# 2056) is 1-pristene. It is informative that an oxidative pathway involving the *cis*-desaturation step has been suggested for alkane utilization by *Rhodococcus* sp. strain KSM-B-3M (Koike et al., 1999). The strain introduced a double bond to hexadecane mainly at the ninth carbon from the terminal methyl groups. Recently, the functional and structural similarity between alkane monooxygenase and desaturase has also been reported (Shanklin and Whittle, 2003).

3.4. Degradation of aromatic hydrocarbons

Recently, *Rhodococcus* sp. MS11 has been reported to degrade versatile hydrocarbons, such as polychlorinated benzene, benzoic acid, phenol, and straight and branched alkanes (Rapp and Gabriel-Jurgens, 2003). Then, we tested several aromatic hydrocarbons for degradation ability of the strains. It was found that both TMP2 and T12 neither grew nor degraded aromatic hydrocarbons, such as 1,2,4-trichlorobenzene, benzoic acid, biphenyl, and dibenzothiophene.

3.5. Biosurfactant production

Microorganisms are able to take up and assimilate water-immiscible compounds such as hydrocarbons by adhering cells to the substrate or lowering the interface tension by biosurfactant production (Huy et al., 1999). Some *Rhodococcus* bacteria, including *R. erythropolis*, produce glycolipid-type or trahaloselipid-type biosurfactants and they play an important role for the metabolisms of hydrocarbons (Lang and Philp, 1998; Rapp and Gabriel-Jurgens, 2003). We previously constructed a convenient assay system for biosurfactant production by utilizing oil-displacement activity

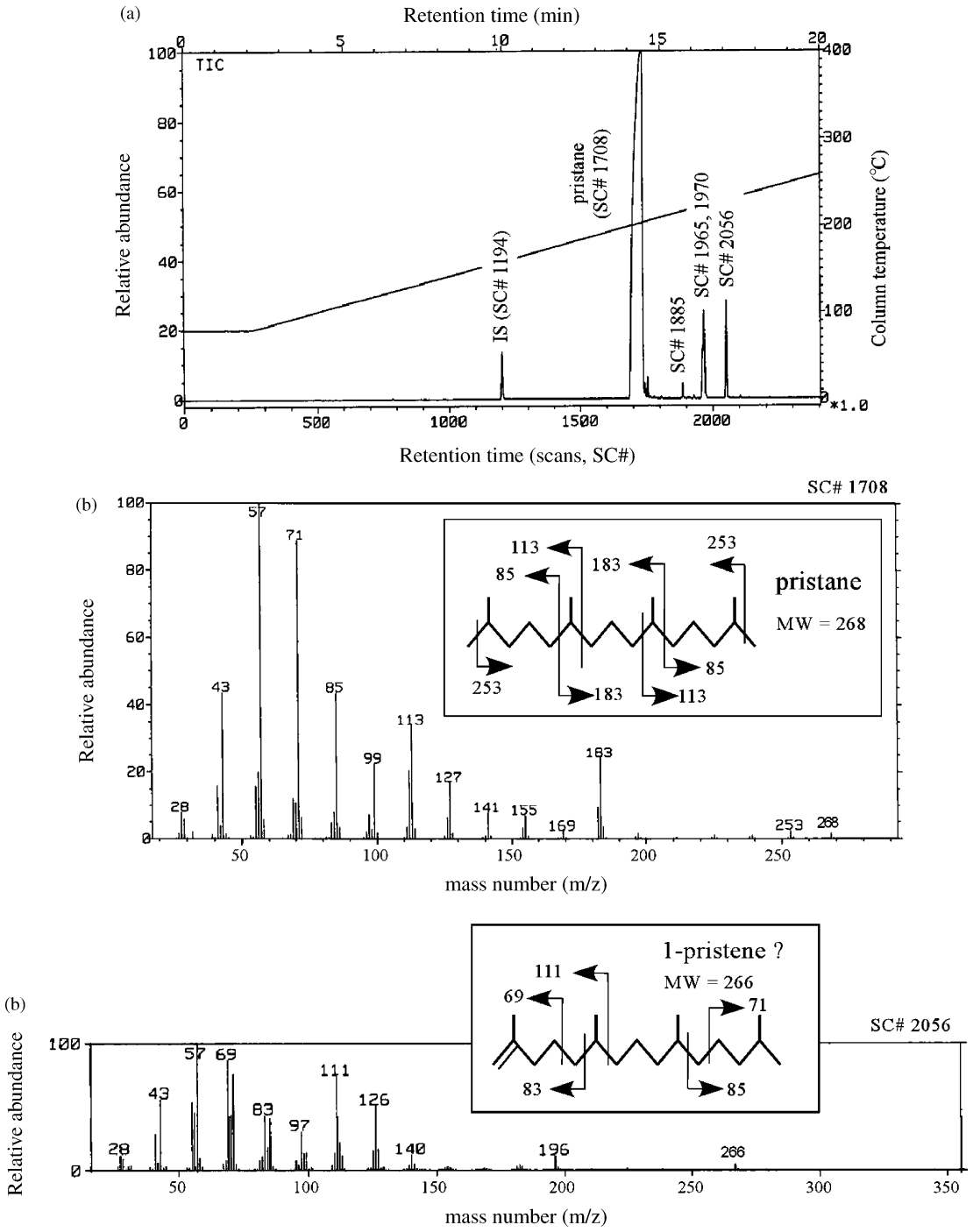

Fig. 2. GC/MS analyses of pristane degradation metabolites by strain TMP2 at 20 °C. Hexane-extractable fraction was directly analyzed by GC/MS. Results are almost identical at 20 and 10 °C, and therefore the results obtained at 10 °C are not shown. (a) Total ion chromatogram, (b) mass spectrum of pristane (SC# 1708), and (c) mass spectrum of a major metabolite (SC# 2056). IS indicates position of biphenyl used as an internal standard.

(Morikawa et al., 1993). It was found that neither strain TMP2 nor strain T12 showed oil-displacement activity in the spent medium, suggesting that they do not produce biosurfactants extracellularly. The difference in the cell surface structure between the new isolates and other biosurfactant-producing *Rhodococcus* spp. is of great interest.

4. Conclusions

Here, we isolated mesophilic *Rhodococcus* sp. strains TMP2 and T12 that are capable of degrading branched alkane, pristane, at 10 °C. Although the cell growth and the degradation rates of *n*-alkanes were maximal at 30 °C, the strains showed comparable or even higher degradability of pristane at temperatures 10 and 20 °C than at 30 °C. They should be useful for biodegradation of pristane and *n*-alkanes under temperature conditions lower than 30 °C.

An alkane-degrading psychrotrophic *Rhodococcus* sp. Q15 has also been reported (Whyte et al., 1998). Although the strain was capable of degrading short-chain *n*-alkanes even at 0 °C, it did not degrade branched alkanes, such as phytane and pristane, in diesel oil. Unfortunately, its degradation ability for branched alkanes has not been tested in a pure substrate system. Recently, a psychrophilic *Rhodococcus* sp. strain MS11 was reported for degradation of versatile hydrocarbons including pristane (Rapp and Gabriel-Jurgens, 2003). The strain degraded significant amounts of polychlorinated benzenes and also produced trehaloselipid-type biosurfactants. Because strains TMP2 and T12 that were isolated in this study did not degrade aromatic hydrocarbons and did not produce biosurfactants, the isolated strains are in a different group from strain MS11.

Further understanding and improvement of the metabolic activities in these strains would contribute to expand the application field of on-site bioremediation technology.

Acknowledgment

This work was supported by a grant from Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN).

References

Atlas, R.M., 1981. Microbial degradation of petroleum hydrocarbons: an environmental perspective. Microbiol. Rev. 45, 180–209.

Bej, A.K., Saul, D., Aislabie, J., 2000. Cold tolerant alkane-degrading *Rhodoccocus* species from Antarctica. Polar Biol. 23, 100–105.

Berekaa, M.M., Steinbuchel, A., 2000. Microbial degradation of the multiply branched alkane 2,6,10,15,19,23-hexamethyltetracosane (squalene) by *Mycobacterium fortuitum* and *Mycobacterium ratisbonense*. Appl. Environ. Microbiol. 66, 4462–4467.

Cantwell, S.G., Lau, E.P., Watt, D.S., Fall, R.R., 1978. Biodegradation of acyclic isoprenoids by *Pseudomonas* species. J. Bacteriol. 135, 324–333.

Cox, R.E., Maxwell, J.R., Ackman, R.G., Hooper, S.N., 1974. Stereochemical studies of acyclic isoprenoid compounds. IV. Microbial oxidation of 2,6,10,14-tetramethylpentadecane (pristane). Biochim. Biophys. Acta 360, 166–173.

Huu, N.B., Denner, E.B., Ha, D.T., Wanner, G., Stan-Lotter, H., 1999. *Marinobacter aquaeolei* sp. nov., a halophilic bacterium isolated from a Vietnamese oil-producing well. Int. J. Syst. Bacteriol. 49, 367–375.

Huy, N.Q., Jin, S., Amada, K., Haruki, M., Huu, N.B., Hang, D.T., Ha, D.T.C., Imanaka, T., Morikawa, M., Kanaya, S., 1999. Characterization of petroleum-degrading bacteria from oil-contaminated sites in Vietnam. J. Biosci. Bioeng. 88, 100–102.

Jenisch-Anton, A., Mikolajczak, A., Rabenstein, A., Klindworth, J., Fischer, U., Michaelis, W., 1999. Biodegradation of a high molecular weight aliphatic ether: indications of an unusual biodegradation pathway. Biodegradation 10, 383–392.

Kato, T., Haruki, M., Imanaka, T., Morikawa, M., Kanaya, S., 2001. Isolation and characterization of long-chain-alkane degrading *Bacillus thermoleovorans* from deep subterranean petroleum reservoirs. J. Biosci. Bioeng. 91, 64–70.

Koike, K., Ara, K., Adachi, S., Takigawa, H., Mori, H., Inoue, S., Kimura, Y., Ito, S., 1999. Regiospecific internal desaturation of aliphatic compounds by a mutant *Rhodococcus* strain. Appl. Environ. Microbiol. 65, 5636–5638.

Kok, M., Oldenhuis, R., van der Linden, M.P., Raatjes, P., Kingma, J., van Lelyveld, P.H., Witholt, B., 1989. The *Pseudomonas oleovorans* alkane hydroxylase gene: sequence and expression. J. Biol. Chem. 264, 5435–5441.

Lal, B., Khanna, S., 1996. Degradation of crude oil by *Acinetobacter calcoaceticus* and *Alcaligenes odorans*. J. Appl. Bacteriol. 81, 355–362.

Lang, S., Philp, J.C., 1998. Surface-active lipids in rhodococci. Antonie Van Leeuwenhoek 74, 59–70.

Leahy, J.G., Colwel, R.R., 1990. Microbial degradation of hydrocarbon in the environment. Microbiol. Rev. 54, 305–315.

Margesin, R., Schinner, F., 1998. Low-temperature bioremediation of a waste water contaminated with anionic surfactants and fuel oil. Appl. Microbiol. Biotechnol. 49, 482–486.

McKenna, E.J., Kallio, R.E., 1971. Microbial metabolism of the isoprenoid alkane pristane. Proc. Natl. Acad. Sci. U.S.A. 68, 1552–1554.

Morikawa, M., Daido, H., Takao, T., Murata, S., Shimonishi, Y., Imanaka, T., 1993. A new lipopeptide biosurfactant produced by *Arthrobacter* sp. MIS38. J. Bacteriol. 175, 6459–6466.

Nakajima, K., Sato, A., Misono, T., Iida, T., Nagayasu, K., 1974. Microbial oxidation of isoprenoid alkane pristane. Agr. Biol. Chem. 38, 1859–1865.

Pirnik, M.P., Atlas, R.M., Bartha, R., 1974. Hydrocarbon metabolism by *Brevibacterium erythrogenes*: normal and branched alkanes. J. Bacteriol. 119, 868–878.

Rapp, P., Gabriel-Jurgens, L.H., 2003. Degradation of alkanes and highly chlorinated benzenes, and production of biosurfactants, by a psychrophilic *Rhodococcus* sp. and genetic characterization of its chlorobenzene dioxygenase. Microbilogy 149, 2879–2890.

Schaeffer, T.L., Cantwell, S.G., Brown, J.L., Watt, D.S., Fall, R.R., 1979. Microbial growth on hydrocarbons: terminal branching inhibits biodegradation. Appl. Environ. Microbiol. 38, 742–746.

Scheller, U., Zimmer, T., Becher, D., Schauer, F., Schunck, W.H., 1998. Oxydation cascade in conversion of *n*-alkanes to alpha, omega-dioic acids catalyzed by cytochrome P450 52A3. J. Biol. Chem. 273, 32528–32534.

Shanklin, J., Whittle, E., 2003. Evidence linking the *Pseudomonas oleovorans* alkane omega-hydroxylase, an integral membrane di-iron enzyme, and the fatty acid desaturase family. FEBS Lett. 545, 188–192.

Sharma, S.L., Pant, A., 2000. Biodegradation and conversion of alkanes and crude oil by a marine *Rhodococcus* sp. Biodegradation 11, 289–294.

Volkman, J.K., Maxwell, J.R., 1986. Acyclic isoprenoids as biological markers. In: Johns, R.B. (Ed.), Biological Markers in the Sedimentary Record. Elsevier, Amsterdam, The Netherlands, pp. 1–46.

Whyte, L.G., Hawari, J., Zhou, E., Bourbonniere, L., Inniss, W.E., Greer, C.W., 1998. Biodegradation of variable-chain-length alkanes at low-temperatures by a psychrotrophic *Rhodococcus* sp. Appl. Environ. Microbiol. 64, 2578–2584.