KOR. JOUR. MICROBIOL April, 1994 p. 172~175 Vol. 32, No. 2

Biodegradation of Saturated Hydrocarbons by *Xanthomonas campestris* M12

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***Xanthomonas campestris* M12 carrying OCT plasmid which could dissimilate octane was able to utilize n-alkanes of eight to sixteen carbon atoms via the capacity of this plasmid. M12 strain could utilize terminal oxidation products of these primary alkanes, alcohols, aldehydes and fatty acids but not hexanoic acid, adipic acid, pimelic acid and heptanal. This strain also biodegraded n-alkanes by monoterminal or diterminal oxidation of straight-chain fatty acids, and branched-chain alkane.**

KEY WORDS □ saturated hydrocarbon, biodegradation, *Xanthomonas campestris*

n-Alkanes in crude oil were readily oxidized by several strains of *Pseudomonas* via initial oxidation of a terminal methyl group, $R\text{-}CH_3 \rightarrow R\text{-}CH_2OH \rightarrow R\text{-}CHO \rightarrow R\text{-}COOH$, followed by β-oxidation (6, 9).

P. putida strains utilized n-alkanes of 6 to 10 carbon atoms by virtue of the transmissible OCT plasmid, which was able to dissimilate octane via octanol, octanaldehyde, and octanoic acid. *P. putida* strains hydroxylated these alkanes to the corresponding primary alcohols (1, 9).

This dissimilative pathway has been established by adaptation experiment and chromatographic analysis of the products of alkane oxidation. The result of mutant analysis showed that the physiological role of whole-cell alkane hydroxylation complex is to initiate the monoterminal oxidation of alkane chains (13). *P. putida* degraded n-alkanes by means of monoterminal and diterminal oxidation of straight-chain fatty acid (9). However, alkyl-branched alkanes were generally less susceptible to biodegradation (7, 11).

We have been studying *X. campetris* M12 carrying OCT plasmid that was able to carry out the oxidation of straight-chain alkanes such as octane (2). This paper reports the results of physiological experiments with alkane-utilizing *X. campestris* M 12 strain. We elucidate the nature of OCT plasmid-coded utilization of alkanes, degradative pathway on the basis of utilization of the oxidative products of alkanes, and the monoterminal or diterminal oxidation of n-alkanes.

MATERIALS AND METHODS

Bacterial strain

Xanthomonas campestris M12 was isolated from sea of water Masan Harbor in Korea by alkane enrichment culture (2). *X. campestris* M12-0 strain was constructed according to the curing experiment of plasmid from M12 strain.

Media

PA salts was the minimal salts solution of Robinson (10). Complete medium (TYE) contained (per liter): 10 g of tryptone, 5 g of yeast extract, and 5 g of NaCl. Agar was added at 1.5% to prepare solid medium.

Chemicals

Alkanes, aliphatic alcohols, aldehydes, and fatty acids were generally purchased from Sigma Chemical Co. and Fluka Chemical Co. with a purity of over 98% except for 1,6-hexanediol (97%) and undecanoic acid (97%).

Growth tests

All growth tests were performed on solid PA salts medium. Soluble substrates were added at the concentrations in the tables (Tables 3 and 4). Growth with hydrocarbons was determined after replica-plating dense patches of bacterial growth from TYE agar. The hydrocarbon substrate was added by placing a few drops on a piece of filter paper in the lid of the petridish, and plates were incubated for 48 hr in the sealed tins at 32℃. Due to the toxicity of high concentration of aliphatic alcohols, aldehydes and fatty acids, we limited the substrate concentration to approximately 0.02~0.05 m*l* in the lid of a petridish (9).

Curing expreiment

Plasmid curing with mitomycin C was carried

Table 1. *Physiological and biochemical characteristics of X. campestris M12.*

Gram staining	−	Arginine decarboxylase production	−
Shape	rod	Ornithine decarboxylase production	−
Mobility	−	Urease production	−
Oxidase production	−	Carbohydrate utilization	
Catalase production	+	Glucose	+
Growth on nutrient agar	poor	Sucrose	+
Indole production	−	Salicine	+
Methyl red	+	Arabinose	+
Voges-Proskauer production	−	Sorbitol	−
Citrate utilization	−	Dulcitol	−
Esculine utilization	+	Rhamnose	−
Lysine decarboxylase production	−	Adonitol	−

+, positive; −, negative.

Table 2. *Utilization of alkane components by X. campestris M12 and M12-O.*

Carbon Sources	M12[a]	M12-O[b]
Hexane (C_6)	−	−
Heptane (C_7)	−	−
Octane (C_8)	+	−
Nonane (C_9)	+	−
Decane (C_{10})	+	−
Undecane (C_{11})	++	−
Dodecane (C_{12})	++	−
Tridecane (C_{13})	++	−
Tetradecane (C_{14})	++	−
Pentadecane (C_{15})	++	−
Hexadecane (C_{16})	++	−

++, well growth; +, poor growth; −, no growth.
[a] *X. campestris* carrying OCT plasmid.
[b] M12 cured of OCT plasmid.

out by the method of Chakrabarty *et al.* (1).

RESULTS AND DISCUSSION

Among crude oil degradative eight isolates, specially orange-colored isolate M12 was found to be high utilization of octane or hexadecane in n-alkanes. Thus the results of identification of the isolate by Bergey's Manual of Determinative Bacteriology named it *Xanthomonas campestris* M 12, referring to *X. campestris* ATCC8718 (see Table 1).

Table 2 shows that *X. campestris* M12 was able to grow on n-alkanes of eight (C_8) to sixteen (C_{16}) carbon atoms but not on shorter n-alkanes such as hexane (C_6) or heptane (C_7) by virtue of OCT plasmid.

As the comparative results with M12 strain, strain M12-0 which did not carry the plasmid was made by curing approach (Fig. 1). As expected,

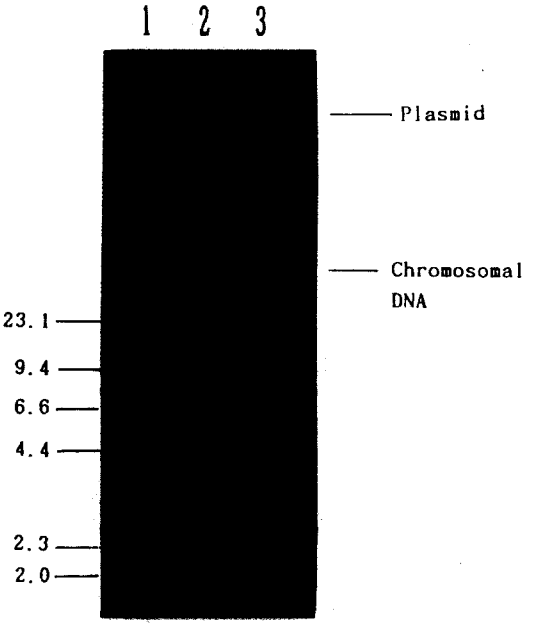

Fig. 1. *Plasmid patterns of X. campestris M12 and the cured strain (M12-O).*
lane 1, λ/*Hin*dIII, lane 2, M12-O (OCT); lane 3, M12 (OCT$^+$).

the M12-0 was not able to grow on any kinds of n-alkanes. This result in this experiment seemed that the plasmid is inevitable for the strain to utilize these substrates. Thus this plasmid, which specifies a degradative pathway that converts n-alkanes to simple metabolites, has been found as n-alkane biodegradative plasmid.

However, the strain carrying this plasmid grown on n-alkanes of 8 to 16 carbon atoms is quite different from that of *P. putida* strain which was able to grow on those of 6 to 10 carbon atoms (9). This difference could be due to the different

Table 3. *Growth of X. campestris M12 on alkane oxidation products.*

Carbon sources	*X. campestris* M12
Hexane (C_6)	+
1-Hexanal	+
Hexanol	+
Hexanoic acid	+
1,6-Hexanediol	+
Adipic acid	−
2-Hexanone	+
Heptane (C_7)	+
Heptanol	+
Heptanal	−
Heptanoic acid	+
Pimelic acid	−
2-Heptanone	−
Octane (C_8)	+
1-Octanol	+
Octanal	+
Octanoic acid	+
2-Octanone	−
Nonane (C_9)	+
1-Nonanol	+
Nonanal	+
Nonanoic acid	+

+, growth; −, no growth.
All substrates were added in the vapor phase except each 2 mM of 1,6-hexanediol, adipic acid, and pimelic acid which were incorporated directly in the PA salts agar.

plasmid specificity.

Some strains of *Pseudomonas* were able to utilize alkanes as the sole carbon and energy source. This initial pathway has been established by various experiments (5, 9, 12). Even if we did not examine the alkane hydroxylase system of n-alkane by *X. campestris* M12, specially alkane hydroxylase and NAD-dependent alkane dehydrogenase activity in the initial biodegradation of n-alkane by *P. putida* (4) or *P. maltophilia* N246 (3) was coded for by OCT plasmid. By the terminal oxidation pathways, one or both of the terminal methyl group are oxidized to the corresponding fatty acids, which was classified as the mono- and diterminal pathway, respectively.

In contrast to the exact mechanism for the oxidation of an alkane to the corresponding alcohol, the oxidative pathway from the primary alcohol via the aldehyde to the 1-monocarboxylic acid has been reviewed by Klug and Markovetz (6).

Therefore, in order to clarify the n-alkane degradative pathway, it was investigated that *X. campestris* M12 was able to grow on n-alkane products. Table 3 shows that *X. campestris* M12 was also able to utilize the primary alcohols, aldehydes, and monocarboxylic acids derived from hexane, heptane, octane, and nonane, as well as 1,6-hexanediol but not hexanoic acid, adipic acid, heptanal, heptanoic acid, pimelic acid, 2-hepanone or 2-octanone. It is assumed that lack of growth on these alkane products may be due to difficulties in uptake of these substrates or toxic materials to the bacterial growth (9).

Table 4. *Growth of X. campestris M12 on monocarboxylic acid and dicarboxylic acids and terminal branched dimethyloctane.*

Carbon sources	*X. campestris* M12
Monocarboxylic acid	
Octanoic acid	+
Heptanoic acid	+
Hexanoic acid	+
Palmitic acid	+
Butyric acid	+
Acetic acid	+
Dicarboxylic acid	
Glutaric acid	+
Succinic acid	+
Malonic acid	+
Glyoxylate	−
Glyoxylate + acetate	+
Glyoxylate + octanoic acid	+
Glyoxylate + octane	+
2,6-Dimethyloctane	++

++, well growth; +, growth; −, no growth.

Further oxidation of monocarboxylic and dicarboxylic acids of fatty acids is investigated by Nieder and Shapiro (9). Table 4 shows that *X. campestris* M12 was also able to utilize monocarboxylic and dicarboxylic acids in fatty acids.

The nature of metabolic pathways in the microbial metabolism of branched alkanes has been primarily deduced from the isolation and characterization of products due to alkane oxidation (7). Schaeffer *et al.* (11) reported that,in the case of peudomonads containing OCT plasmid, whole-cell oxidation of n-octane was poorly induced by terminally branched dimethyloctanes.

However, in this experiment, Table 4 shows that *X. campestris* M12 carrying the plasmid could easily utilize 2,6-dimethyloctane, branched alkane, as a sole carbon source.

ACKNOWLEDGEMENTS

This work was supported by Korean Science and Engineering Foundation research fund for SRC (Research Center for Molecular Microbiology, Seoul National University).

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(Received February 6, 1994)
(Accepted February 24, 1994)

초 록: *Xanthomonas campestris* M12에 의한 포화 탄화수소의 생분해

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Xanthomonas campestris M12는 octane을 분해할 수 있는 OCT 플라스미드를 갖고 있으며, 이 플라스미드의 분해 능력으로 C_8에서 C_{16}까지의 n-alkane을 이용할 수 있었다. *X. campestris* M12 균주는 n-alkane을 이용하여 alcohol, aldehyde, fatty acid를 거쳐서 분해할 수 있으나 hexanoic acid, adipic acid, pimelic acid 그리고 heptanal은 이용할 수 없었다. 또한 이 균주는 straight fatty acids의 monoterminal oxidation과 diterminal oxidation에 의하여 n-alkane을 분해할 수 있으며, branched-chain alkane도 이용할 수 있었다.