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Growth and Biodegradability of Facultative Psychrophilic SDBS-degrading *Pseudomonas* spp.

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Facultative Psychrophilic *Pseudomonas* spp.의 생장 및 SDBS분해능에 대하여

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ABSTRACT

Facultative psychrophilic bacteria utilizing SDBS (Sodium Dodecyl Benzene Sulfonate) as their carbon source were isolated in the Han River.

All of these isolated facultative psychrophilic bacteria were identified as *Pseudomonas* spp.

The growth and biodegradation rates of *Ps. fluorescens* LP6, *Ps. fluorescens* LS6 and *Ps. putida* LC1 among 8 identified facultative psychrophilic bacteria were investigated with spectrophotometer.

The specific growth rates of these three facultative psychrophilic bacteria at 25°C were higher than those at any other temperatures. However, the final cell yields were the highest for cells grown at 5°C.

The biodegradation of SDBS by *Ps. fluorescens* LP6 was started at the stationary phase of cells. The biodegradation rate of SDBS by *Ps. fluorescens* LP6 was the highest when the cells were cultured at 25°C.

INTRODUCTION

Alkyl benzene sulfonate (ABS) constitutes the major class of detergent used for household purposes, therefore play the greatest part in water pollution problems in the Han River.

The ABS which has been in use for the past decade or more, is derived from tetrapropylene, a product of the petroleum industry and is a mixture of several hundred isomers and homologs with highly branched alkyl groups ranging from ten to fifteen carbon atoms with an average of twelve (Swisher, 1964).

For more than 20 years many reports were published about the biodegradation of alkyl benzene sulfonate and sodium dedecyl benzene sulfonate with analytical methods.

Recetnly Huddleston and Nielsen (1979) showed that the biodegradation pathway consists of many steps and that the loss of surface activity occurs early in the sequence (Huddleston *et al.*, 1963).

Fredricks showed that the most rapid growth of fresh isolates from soil samples occurred with the normal paraffins C_8 -C_{18} but growth on normal C_5 -C_8 hydrocarbons was much less rapid. This may be explained by the known toxicity of the lower alkanes to bacteria because

of their lipid solvent properties.

Swisher (1964) and Kaelble (1964) have shown that gas chromatography provides a very effective analytical method for determining intermediates in and degradation in such systems.

Frazee *et al.* (1964) have reported the infrared determination of alkyl branching in detergent ABS.

River Die-away technique was used in order to study the detergent biodegradability by Setzkorn *et al.* (1964).

Cain (1972) and Willetts (1972, 1974) have shown the enzymatic studies on the microbial degradation of alkyl benzene sulfonates with short-chain length, however, those on the degradation of alkyl benzene sulfonates with long-chain length have not occurred sufficiently.

There have been numerous reports on microorganisms which can grow in various environmental temperatures (Baig *et al*, 1969; Helen *et al*, 1976; Maxwell, 1967; Srivastava, 1979). Protein synthesis (Krajewska, 1967; Malcolm, 1968), ribonucleic acid synthesis (Malcolm, 1968), ribosome stability (Pace, 1967), in psychrophile and membrane lipid, cytochrome composition of psychrophile (Watson *et al*, 1976; Arthur, 1976) were also observed. However , whether the household-detergents were degraded biologically in ecosystems didn't have been studied yet.

In this reports, the growth and biodegradation rates of facultative psychrophilic bacteria in the Han River were investigated with spectrophotometer.

MATERIALS AND METHODS

1. Isolation of bacterial strains

The facultative psychrophile utilizing Sodium Dodecyl Benzene Sulfonate (SDBS) were isolated in the Han River by repetition of plating on salt medium (Table 1).

Approximately 0.1ml of water samples were pipetted on minimal salt medium containing SDBS as carbon source. These plates were incubated at 4°C until colonies were seen.

Table 1. Minimal Salt Medium

$(NH_4)_2\ SO_4$	2.0 g
KH_2PO_4	2.0 g
Na_2HPO_4	3.0 g
$Mg\ SO_7\ 7\ H_2O$	0.01 g
$Fe\ Cl_3$	0.01 g
D.W.	1,000ml
Agar (if necessary)	15 g

pH was adjusted to 7.2

Stock cultures were maintained on nutrient agar slants containing 100 ppm SDBS, stored in the refrigerator and transferred biweekly.

2. Indentification of facultative psychrophilic bacteria

General characteristics of eight isolated microorganisms were examined according to the methods in "Biochemical test for identification of medical bacteria", "Guide to the identification of The Genera of Bacteria", "Methods in Microbiology" and other papers (Pichett *et al*, 1969).

3. Preparation of Inoculum

One loopful of precultured strains were inoculated into the 250ml flask containing 50ml nutrient broth added 100ppm SDBS and incubated aerobically at 25°C for 12 hours in New Brunswick ecolagen shaker (200rpm). The cells were harvested, washed three times, suspended in minimal salt solution, and inoculated into the minimal salt media containing 100ppm SDBS.

4. Measurement of Growth

The growth in minimal salt medium containing SDBS as carbon source was determined by turbidity at 420nm with Gilford Spectrophotometer.

5. Quantitative analysis of SDBS

1) Materials

SDBS was obtained from Kokusan Chemical Work, Ltd. Tokyo, Japan. Methylene blue and

chloroform were the products of E. Merck Darmstadt.

2) Stock solution

An aqueous solution of 0.5% methylene blue was prepared in dark bottle. About one twentieth volume of chloroform was underlaid and stored at room temperature.

3) Procedures

SDBS was estimated using the Hayaishi method (Hayaishi, 1975). The absorbance of chloroform phase at 655 nm was measured with Gilford Spectrophotometer. The blank contained no SDBS.

RESULTS AND DISCUSSIONS

1. Identification of isolated strains

Eight isolated strains were identified according to Bergy's Manual of Determinative Bacteriology (eight edition) and Pickett's paper (Pickett *et al*, 1969). The morphological and physioloical characteristics of eight isolated strains were described in Table 2. The utilization of various carbon sources was shown in Table 3.

Table 2. General characteristics of facultative psychrophilic SDBS degrading bacteria

	AP9	LP7	LS6	AP14	LC1	AP22	LS4	LP6
Gram staining	–	–	–	–	–	–	–	–
Form	rod	rod	rod	rod	rod	rod	rod	rod
Growth at 42°C	–	–	–	–	–	–	–	–
Growth at 5°C	+	+	+	+	+	+	+	+
Growth at -5°C	–	–	–	–	+	–	+	+
Oxidase	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+
Motility	+	+	+	+	+	+	+	+
MacConkey	+	+	+	+	+	+	+	+
Citrate	+	–	+	+	+	+	+	+
OF/O	+	+	+	+	+	+	+	+
OF/F	–	–	–	–	–	–	–	–
NO_2	+	–	+	+	–	+	+	+
N_2	–	–	–	–	–	–	+	+
H_2S	–	–	–	–	–	–	–	–
Starch hydrolysis	–	–	–	–	–	–	–	–
Gelatin hydrolysis	+	–	–	+	–	–	+	+
Arginine dihydrolase	+	+	+	+	+	+	+	+
Lysine decarboxylase	–	–	–	–	–	–	–	–
Ornithine decarboxylase	–	–	–	–	–	–	–	–
Tryptophane deaminase	–	–	–	–	–	–	–	–
Urea	–	–	–	–	–	–	–	–
Indol	–	–	–	–	–	–	–	–
MR	–	–	–	–	±	–	–	–
VP	–	–	–	–	–	–	–	–

All of the strains were gram-negative, rod-form, aerobic, motile, catalase-positive, oxidase-positive, and starch hydrolysis-negative. All of

the strains grew at 5°C on nutrient agar containing 100ppm SDBS but didn't grow at 42°C *Ps. fluorescens* LP6, *Ps. putida* LC1, and *Pseudomonas* sp. LS4 grew at -5°C on that media.

As shown in Table 2 and 3, four strains belonged to *Ps. fluorescens* and two strains *Ps. putida* (Table 4).

Table 3. Carbohydrate utilization of facultative Psychrophilic SDBS degrading bacteria

	AP9	LP7	LS6	AP14	LC1	AP22	LS4	LP6
Glucose	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+
Mannose	–	–	–	–	–	–	–	–
Lactose	–	–	–	–	–	–	–	–
Inositol	–	–	–	–	–	–	–	–
Fructose	–	–	–	–	–	–	–	–
Sorbitol	–	–	–	–	–	–	–	–
Adonitol	–	–	–	–	–	–	–	–
Rhamnose	–	–	–	–	–	–	–	–
Cellobiose	+	+	+	+	+	–	–	+
Melibiose	+	+	+	+	+	–	–	+
Raffinose	–	–	–	–	–	–	–	–
Sucrose	–	–	–	–	–	–	–	–
Arabinose	+	–	–	–	–	–	–	+

Table 4. Identification of facultative psychrophilic SDBS degrading bacteria

Strain	Identification
AP9	*Pseudomonas fluorescens*
LP7	*Pseudomonas putida*
LS6	*Pseudomonas fluorescens*
AP14	*Pseudomonas fluorescens*
LC1	*Pseudomonas putida*
AP22	*Pseudomonas* sp.
LS4	*Pseudomonas* sp.
LP6	*Pseudomonas fluorescens*

2. The growth of facultative psychrophilic bacteria

Prepared 4-5 x 10^7 cells/ml of *Ps. fluourescens* LP6 and LS6 and *Ps. putida* LC1 were inoculated into the 50ml minimal salt medium containing 100ppm SDBS and 0.03% yeast extract and incubated at 5°C, 15°C, 25°C, and 35°C, with sufficient aeration and agitation. In each regular interval, 5ml of culture broth was sampled and the turbidity was measured at 420nm. The specific growth rates of these three facultative psychrophilic bacteria at 25°C was higher and at 35°C lower than those at any other temperatures, respectively. However, when *Ps. fluorescens* LP6 was cultured at 5, 15, 25°C, the cells reached stationary phase after 24h, 12h, 8h, and the OD at the statnioary phase was 0.625, 0.531, 0.527 (Fig. 1). The growth pattern of *Ps. putida* LC1 was similar to that of *Ps. fluorescens* LP6 (Fig. 2). When *Ps. fluorescens* LS6 was cultured

Table 5. Specific growth rates of facultative psychrophilic bacteria at different temperature

Strain	Specific growth rate at (h^{-1})			
	5°C	15°C	25°C	35°C
Ps. fluorescens LS6	0.097	0.177	0.387	0.062
Ps. fluorescens LP6	0.114	0.115	0.436	0.078
Ps. putida LC1	0.099	0.145	0.228	0.076

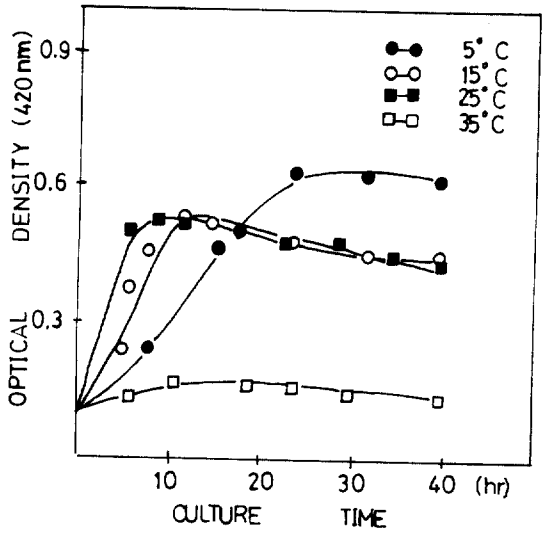

Fig. 1 Growth of *Ps. fluorescens* LP6 in minimal salt media containing 100 ppm SDBS and 0.03 % yeast extract. initial inoculum size; 4-5 x 10^7 cells/ml

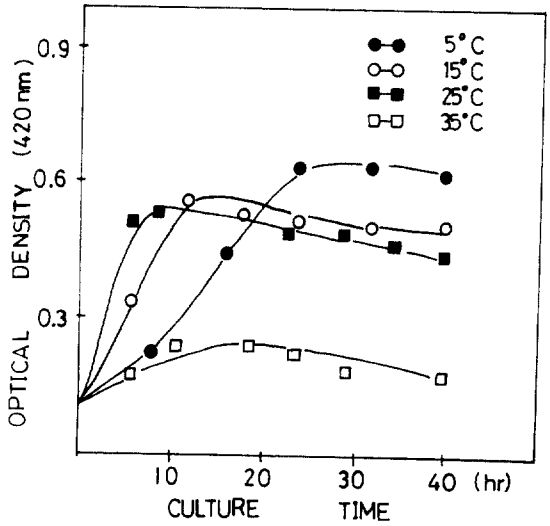

Fig. 2 Growth of *Ps. putida* LC1 in minimal salt media containing 100 ppm SDBS and 0.03 % yeast extract. initial inoculum size; 4-5 x 10^7 cells/ml.

at 5, 15, 25°C, the OD range at the stationary phase was 0.545 to 0.585 and there was a long lag-period at 5°C (Fig. 3). In all these three cases, the growth at 35°C was not nearly occured.

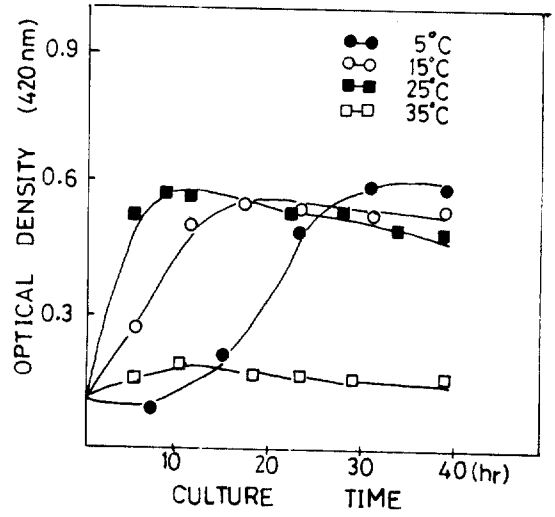

Fig. 3 Growth of *Ps. fluorescens* LS6 in minimal salt media containing 100 ppm SDBS and 0.03 % yeast extract. initial inoculum size; 4-5 x 10^7 cells/ml.

3. Biodegradation of SDBS

When 4-5X10^7 cells/ml of *Os. fluorescens* LP6, LP6, and *Ps. putida* LC1 were inoculated, the growth and the extent of biodegradation of SDBS were shown in figure 4. The biodegradation was started at the stationary phase of cells. After 24 hour-culture, SDBS degradation by *Ps. fluorescens* LP6, LS6, and *Ps. putida* LC1 were 49%, 47%, 43%, respectively. When *Ps.* fluorescens LP6 were cultured at 25°C, the biodegra-

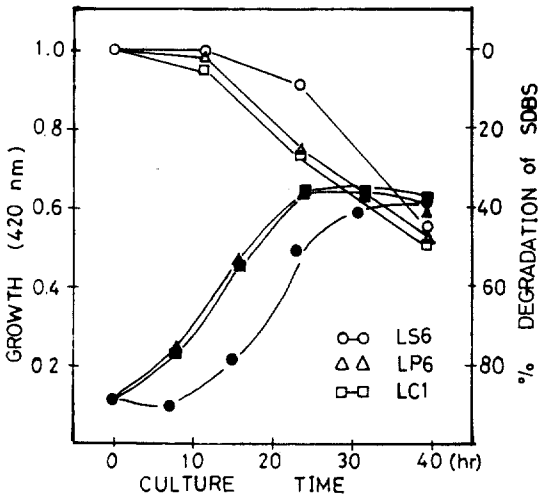

Fig. 4 The relationship between growth and degradation of three strains in the minimal salt media containing 100 ppm SDBS and 0.03 % yeast extract. Culture temperature, 15°C; initial inoculum size; 4.3 x 10^7 cells/ml. % degradation = utilized SDBS/initial SDBS x 100; opened, growth; closed, % degradation of SDBS

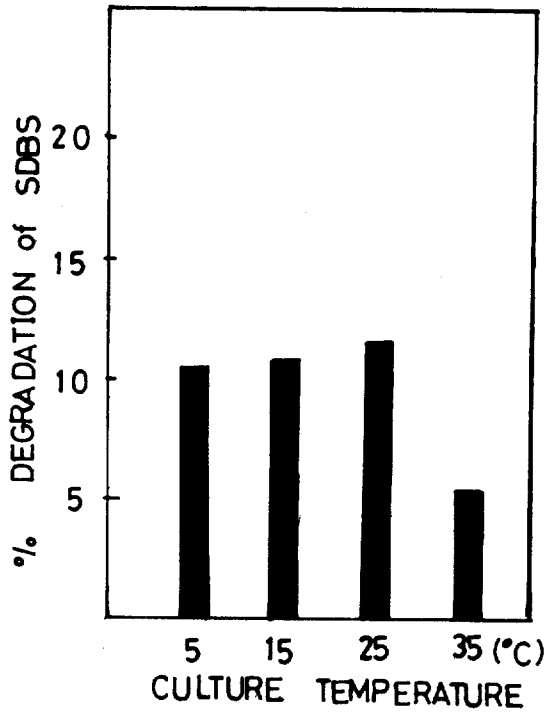

Fig. 5 Effect of temperature on the degradarion of SDBS by facultative psychrophilic *Ps. fluorescens* LP6. Culture time, 24 hours; initial inoculum size, 9 x 10^7 cells/ml. % degradation = utilized SDBS / initial SDBS x 100.

dation rates of SDBS was the highest. Even at 5°C, about 10% of SDBS was degraded by this strain. But at 35°C, 6.2% of SDBS was degraded (Fig. 5).

Many studies on the psychrophilic bacteria have been done in arctic zone and subarcitc zone. However, there are few reports on those in temperate zone. Monthly variation of temperature in Han River ranged from 0.3°C to 27°C (Hong's unpublished data). Even in this temperate zone, facultative psychrophilic bacteria were found. During the winter, these bacteria could play a role in self-purification of water pollution.

적 요

한강 수계에서 SDBS (Sodium Dodecyl Benzene Sulfonate)를 유일한 탄소원으로 가지는 최소배지에서 서란 120 균주중 4℃에서 생장이 좋은 8균주를 선택하여 동정하였으며, 이들의 온도에 따른 생장과 분해능을 흡광도 분석기를 이용하여 측정하였다.

동정 결과, *Ps. fluorescens* 가 4균주, *Ps. putida* 가 2균주 이었다. 이들중 *Ps. fluorescens* LP6, *Ps. putida* LCI, Ps. fluorescens LS6의 비생장율은 모두 25℃에서 가장 높은 값을 나타냈으나, 정체기에서의 균체량은 5℃에서 가장 높았다. 따라서 이들 3균주는 faculfative psychrophilic bacteria로 사료된다. 한편 SDBS의 분해는 세포가 정체기에 들어가면서 시작되었으며, 분해능은 25℃에서 가장 좋았다.

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