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Isolation of Polyacrylamide-degrading Microorganisms from Soil

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Abstract Two polyacrylamide-degrading bacterial strains, No. 2 and No. 11, were isolated from soil, and identified as *Bacillus sphaericus* No.2 and *Acinetobacter* sp. No. 11, respectively. Both strains grew on medium containing polyacrylamide as sole carbon and nitrogen sources. *B. sphaericus* No. 2 and *A.* sp. No. 11 reduced by 16% and 19% of the initial polyacrylamide concentration, respectively. Optimal pH and temperature in growth of *Acinetobacter* sp. No. 11 were 8.0 and 37°C, respectively. After 14-day cultivation of *A.* sp. No. 11, the average molecular weight of polyacrylamide has been shifted from 2.3×10^6 to 0.5×10^6.

Keywords: polyacrylamide, microbial degradation, biodegradation, bioremediation

Environmental pollution increases with the progress of chemical industry in all over the world. Various kinds of plastic have been produced and a large quantity of plastic has being discarded into the environment. Many of them are decomposed at a very low rate in the environment by microorganisms because of their non-naturally complex structure. Some water-soluble polymers such as polyethylene glycol [1], polyvinyl alcohol [2] and polyacrylate [3,4] have been reported to be degraded by microorganisms. Polyacrylamide (PAM) is widely used as a strengthener for pulp fiber. Large amount of waste paper containing PAM has been discharged into the environment and it is required to remove the remaining PAM from the environment. Nakayama and Kinoshita have already isolated PAM-degrading bacteria, *Enterobacter agglomerans* A and *Azomonas macrocytogenes* B, from soil [5]. In the communication we report other kinds of PAM-degrading bacteria isolated from soil and their characteristics.

The isolation for PAM-degrading microorganisms was carried out at 30°C by conventional enrichment culture technique using soil samples. The composition of the medium containing PAM (PAM-medium) was 1% PAM, 0.26% K_2HPO_4, 0.1% NaH_2PO_4, 0.02% $MgSO_4 \cdot 7H_2O$, 0.001% $FeCl_3$, and 0.005% $CaCl_2$. PAM was purchased as 10% solution (viscosity 30-70 p) from Wako Pure Chem. Ind. Ltd., Osaka, Japan. Initial pH was adjusted to 7.2. One gram of soil sample was mixed with 30 mL of the PAM-medium. We added 0.1% of yeast extract into the first cultivation of sample. After aerobic cultivation at 30°C for several days, an aliquot of the culture broth was inoculated into 30 mL of fresh medium in a 100 mL conical flask, and cultivated for several days. Then an aliquot of the culture broth was streaked on an agar plate containing the nutrient broth (NB, Difco, Detroit, MI, USA) supplemented with 1.5% agar, the plates were incubated for several days at 30°C and a single colony was isolated. These procedures were repeated for the isolation of the microorganism.

Two kinds of strain, which could grow in the PAM medium, were isolated from soil samples. The cells of strain No. 2, isolated from Minatogaoka Park, Tokyo, Japan, were gram-positive, rod shaped (5 by 2 μm) and motile with flagella, and formed thin yellow, smooth colonies and spores. Strain No. 2 could not grow under anaerobic condition. The biochemical properties are shown in Table 1, and strain No.2 was identified as *Bacillus sphaericus* according to Bergey's manual of systematic bacteriology [6]. The cells of strain No. 11, isolated from Kanayamagawa River, Yamanashi, Japan, were gram-negative, rod shaped (2 by 1 μm) and formed thin yellow, smooth colonies. Strain No. 11 could not grow under anaerobic condition. The biochemical properties are shown in Table 2, and strain No. 11 was identified as *Acinetobacter* species according to Bergey's manual of systematic bacteriology [7]. However the strain No. 11 did not belong to any genus in *Acinetobacter* species, so we decided to call the strain as *Acinetobacter* sp. No. 11.

The time course of PAM degradation of *B. sphaericus* No.2 was shown in Fig. 1(A). The growth of strains was measured based on the optical density at 660 nm. PAM concentration was determined by total organic carbon (TOC) analyzer (Sumigraph NCH-21, Shimadzu, Kyoto, Japan) by using the supernatant of culture broth. After cultivation for 14 d, OD reached 0.32 and PAM concentration decreased by 16% of initial PAM concentration. The time course of PAM degradation of *A.* sp. No.11 was shown in Fig. 1(B). After cultivation for 14 d, OD reached 0.35 and PAM concentration decreased by 19% of initial PAM concentration. Based on the results of these experiments, *A.* sp. No. 11 was slightly superior to *B. sphaericus* No. 2 in PAM degradation. Therefore further experiments were carried out by using *A.* sp. No. 11.

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Table 1. Taxonomical characteristics of PAM-degrading bacterial strain No. 2

Morphology	Physiology
Motility: +	Catalase: +
Gram-staining: +	Oxydase: –
Shape: rod	Reduction of nitrate: +
Size: 5 × 2 μm	VP test: –
Sporulation: +	Casein decomposition: –
	Gelatin decomposition: –
Culture	Starch decomposition: –
Slant: thin yellow, opac, smooth	DNA decomposition: –
Growth temperature: 15-37°C	Urea decomposition: –
Growth pH: 5.0-10.5	L-Tyrosin decomposition: –
Aerobic	Utilization of cirate: –
	Indole production: –
	Hydrogen sulfite production: –
	Assimilation +:
	Assimilation –: D-glucose, L-arabinose, D-fructose, D-galactose, maltose, lactose, sucrose, trehalose, glycerol, D-mannitol, D-sorbitol, L-sorbose, D-mannose, L-rhamnose, *myo*-inositol, adonitol

Table 2. Taxonomical characteristics of PAM-degrading bacteria strain No. 11

Morphology	Physiology
Motility: –	Catalase: –
Gram-staining: –	Oxydase: –
Shape: rod	Reduction of nitrate: –
Size: 2 × 1 μm	VP test: –
	Casein decomposition: –
Culture	Gelatin decomposition: –
Slant: thin yellow, opac, smooth	Starch decomposition: –
Growth temperature: 15-37°C	DNA decomposition: +
Growth pH: 5.0-9.5	Urea decomposition: +
Aerobic	L-Tyrosin decomposition: +
	Utilization of cirate: –
	Indole production: –
	Hydrogen sulfite production: –
	Assimilation +: D-glucose, L-arabinose, D-fructose, D-galactose, D-xylose, glycerol, D-mannose
	Assimilation –: maltose, lactose, sucrose, trehalose, D-mannitol, D-sorbitol, L-sorbose, L-rhamnose, *myo*-inositol, adonitol

The optimum pH for cultivation of *A.* sp. No. 11 was around 8.0 in the PAM medium and around 7.0 in NB (Fig. 2). In the experimental range, the best temperature for the growth of *A.* sp. No. 11 was 37°C (Fig. 3).

The molecular weight of PAM was calibrated by Toyopearl HW-65 column chromatography using the culture supernatant. The average molecular weight of PAM, MW_{AVE}, was calculated by the following equation,

$$MW_{AVE} = \frac{\sum_j x_j MW_j}{\sum_j x_j}$$

where x_j and MW_j are the ratio of fraction j and the molecular weight corresponding to fraction j, respectively.

After 14-d cultivation of *A.* sp. No. 11, the average molecular weight of PAM in the culture supernatant has been sifted from 2.3×10^6 to 0.5×10^6 as shown in Fig. 4. Fig. 5 shows changes of average molecular weight of PAM by the cultivation of *A.* sp. No. 11. The average molecular weight of PAM reached 0.5×10^6 after first 5-day cultivation and then hardly changed until the culture ended. However, *A.* sp. No. 11 continued to grow by 10 day. The experimental results by ^{1}H-NMR revealed that the amide part of PAM was not utilized as the carbon source, but also the main chain was degraded [5]. The decomposition mechanism of PAM might be that at first large molecular size of PAM was cleaved and become to smaller ones, and then the main chain of PAM was utilized as a carbon source.

The growth of PAM-degrading microorganism, *E.*

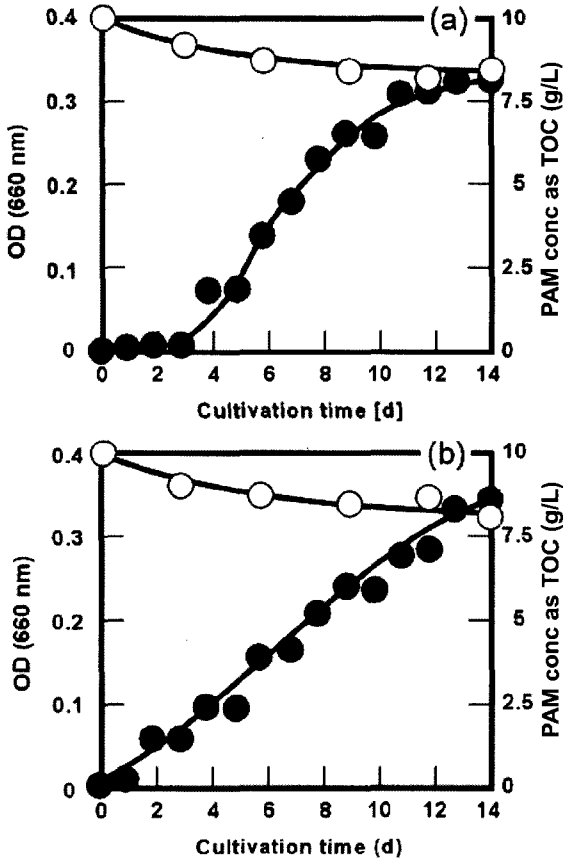

Fig. 1. Time courses of PAM-degradation by *B. sphaericus* No.2 (a) and *A.* sp. No. 11 (b). Cells were grown at 30°C in PAM-medium under aerobic condition. Closed and open circles show OD at 660 nm and residual PAM concentration, respectively.

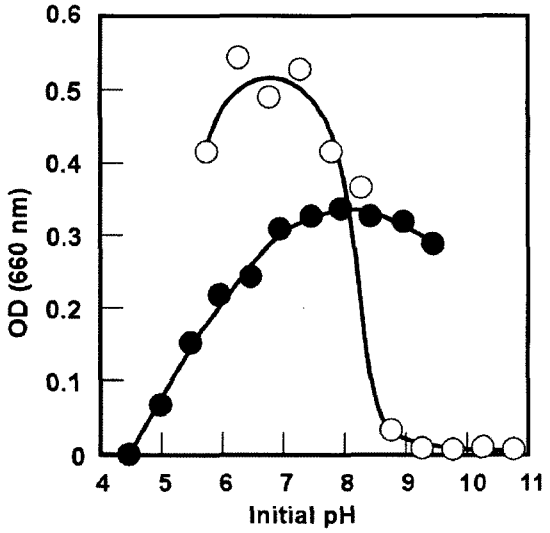

Fig. 2. Effect of pH on the growth of *A.* sp. No. 11 in NB (open circle) and the PAM medium (closed circle) after cultivation at 30°C for 10 days.

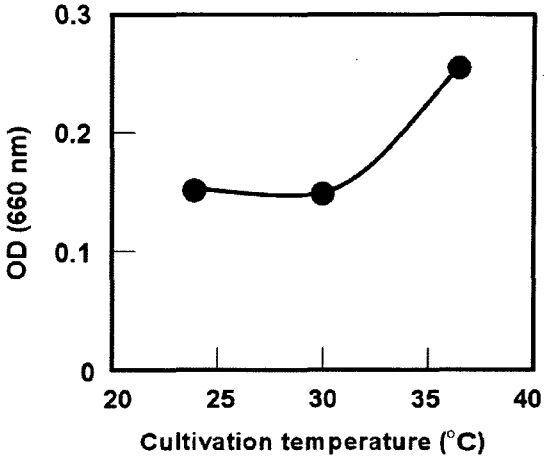

Fig. 3. Effect of temperature on the growth of *A.* sp. No. 11 in PAM medium after cultivation for 5 days.

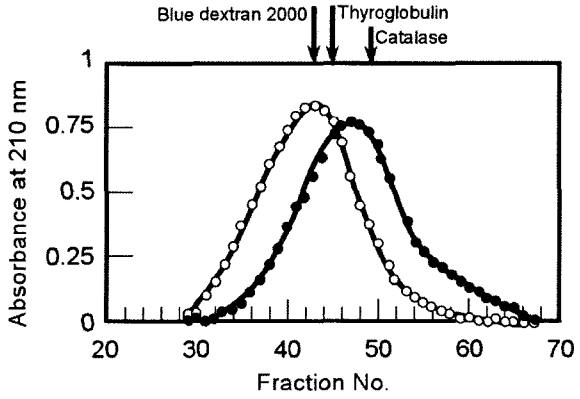

Fig. 4. Comparison of molecular weight distribution of PAM before cultivation (closed circle) and after 14-day cultivation of *A.* sp. No. 11 (open circle). The supernatant of broth was gel filtrated by Toyopearl HW-65 (column length ø 2.5 mm × 860 mm) in 0.2 M NaCl with detection absorbance wavelength set at 210 nm with Blue-dextran 2000 (MW = 2,000,000), Thyrogloburin (MW = 669,000), and Catalase (MW = 232,000) as molecular markers.

agglomerans A, attained to its maximum level (OD at 660 nm = 0.8) at 27-h cultivation [5]. The growth rate and the maximum growth of PAM-degrading microorganism, *E. agglomerans* A, were rather greater than those of *A.* sp. No. 11. It is indicated that in all cases the growth was delayed a few days more and the maximum growth of the *E. agglomerans* A was almost half that observed using the PAM synthesized chemically in their laboratory [5]. This may mean that the difference of growth between *E. agglomerans* A and *A.* sp. No. 11 ascribe to the difference of PAM's origin.

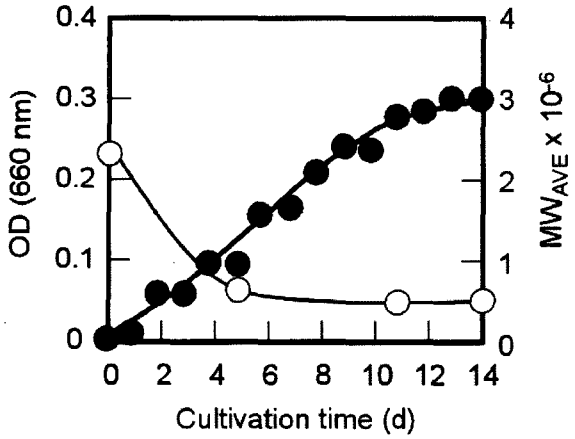

Fig. 5. Changes of average molecular weight of PAM by *A. sp.* No. 11 degradation. Culture conditions are the same as shown in Fig. 1. Symbols: open circle, average molecular weight of PAM; closed circle, OD at 660 nm.

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