Isolation and characterization of a bacterium which utilizes polyester polyurethane as a sole carbon and nitrogen source

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Abstract

Various soil samples were screened for the presence of microorganisms which have the ability to degrade polyurethane compounds. Two strains with good polyurethane degrading activity were isolated. The more active strain was tentatively identified as Comamonas acidovorans. This strain could utilize polyester-type polyurethanes but not the polyether-type polyurethanes as sole carbon and nitrogen sources. Adipic acid and diethylene glycol were probably the main degradation products when polyurethane was supplied as a sole carbon and nitrogen source. When ammonium nitrate was used as nitrogen source, only diethylene glycol was detected after growth on polyurethane.

Keywords: Polyurethane; Biodegradation; Comamonas acidovorans

1. Introduction

Polyurethane (PUR) is a synthetic polymer widely used as a raw material for various industries. PURs, like some other plastics, are known to resist biodegradation. Darby and Kaplan [1] first reported the degradation of PURs by a fungus and found that the polyester-type PURs were more degradable than the polyether-type PURs. Since then, a number of fungi have been isolated and characterized in terms of their ability to degrade polyester polyurethane [2–8]. Although a number of workers have reported the isolation of polyester-type PUR degrading fungi, only a few reports on bacteria that degrade this material have been published [9,10]. In all these cases, however, the microorganisms could degrade polyester-type PUR only if provided with additional utilisable nutrients and could not utilize them as sole carbon source. In this paper, we report the isolation of a bacterium which utilizes polyester-type PUR as a sole carbon and nitrogen source.

2. Materials and methods

2.1. Materials

All poly(diethylene glycol adipate)s were kindly supplied by Suzuki Motor Co. (Japan). Poly(di-
Polyethylene glycols were purchased from Aldrich Chem. Inc. (USA), and 2,4-Tolylene diisocyanate, from Wako Pure Chem. Co. (Japan).

2.2. Synthesis of PURs

PURs were synthesized by reacting poly(propylene glycol)s or poly(diethylene glycol adipate)s with 2,4-tolylene diisocyanate under anhydrous condition as described by Darby and Kaplan [1]. The properties of the synthesized PURs are shown in Table 1.

2.3. Media

The composition of the basal medium was as follows (mg/l): KH₂PO₄, 2,000; K₂HPO₄, 7,000; NH₄NO₃, 1000; MgSO₄·7H₂O, 100; ZnSO₄·7H₂O, 1; CuSO₄·7H₂O, 0.1; FeSO₄·7H₂O, 10; MnSO₄·4·6H₂O, 2; the final pH was 7.2. Ammonium nitrate was omitted when PUR was supplied as a sole carbon and nitrogen source.

2.4. Enrichment of PUR degrading culture

Many soil samples collected in various parts of Tsukuba City in Japan were used to screen for PUR utilizing bacteria. The test soil (0.2 g) was added to a test tube (24-mm diameter) containing 5 ml of basal medium. A cube-shaped PUR (about 50 mg) was added to the medium and incubated at 30°C with reciprocally shaking (120 oscillations/min). Subculturing, in the same medium, was carried out every week. Each transfer involved the addition of 0.1 ml of the original culture to fresh medium. Single colony isolation was done using 1/10-dilutions and nutrient broth agar plates. Each isolate was tested for PUR degradation activity as described below. Isolates which showed PUR degradation activity were stored in 20% (v/v) glycerol at −35°C.

2.5. Identification methods

Strain TB-35 was characterized and identified according to Bergey’s Manual of Systematic Bacteri-

Table 1
Utilization of various PURs and polyester/polyethers as sole carbon sources by strain TB-35

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Mol. mass</th>
<th>-OH Number</th>
<th>Components of PUR</th>
<th>Growth (OD₆₅₀)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyester</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDA A</td>
<td>554</td>
<td>4.0</td>
<td>2,4-TDI</td>
<td>2.98</td>
</tr>
<tr>
<td>PDA B</td>
<td>935</td>
<td>2.0</td>
<td>2,4-TDI</td>
<td>3.29</td>
</tr>
<tr>
<td>PDA C</td>
<td>2,500</td>
<td>2.7</td>
<td>2,4-TDI</td>
<td>3.64</td>
</tr>
<tr>
<td>PDA D</td>
<td>2,650</td>
<td>2.4</td>
<td>2,4-TDI</td>
<td>2.48</td>
</tr>
<tr>
<td>Polyether</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPG A</td>
<td>3,000</td>
<td>3.0</td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>PPG B</td>
<td>2,000</td>
<td>3.0</td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>PPG C</td>
<td>1,000</td>
<td>3.0</td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>Polyester-type PUR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PUR A</td>
<td>PDA A</td>
<td>2,4-TDI</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
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<td>0.14</td>
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<td>2,4-TDI</td>
<td>2.48</td>
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</tr>
<tr>
<td>PUR D</td>
<td>PDA D</td>
<td>2,4-TDI</td>
<td>2.03</td>
<td></td>
</tr>
<tr>
<td>Polyester-type PUR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PUR E</td>
<td>PPG A</td>
<td>2,4-TDI</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>PUR F</td>
<td>PPG B</td>
<td>2,4-TDI</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>PUR G</td>
<td>PPG C</td>
<td>2,4-TDI</td>
<td>0.03</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: PDA, poly(diethylene glycol adipate); PPG, Poly(propylene glycol); 2,4-TDI, 2,4-tolylene diisocyanate.

* Average number of free -OH residues of diol. During diol synthesis, a small amount of trimethylolpropane (polysters) or glycerol (polyethers) were added into the reaction mixture to control the -OH number in the various syntheses.
ology [11] and the report of Komagata et al. [12]. Motility was examined by the hanging drop method. Mol% of G + C content of DNA from strain TB-35 was estimated with a DNA-GC kit (Yamasa Shouyu, Tokyo).

2.6. Degradation assay

Unless otherwise indicated, PUR C (Table) was used for the degradation experiments. A cube-shaped PUR (about 50 mg) was added to a test tube (24-mm diameter) containing 5 ml of the basal medium. After inoculation, tubes were incubated for 7 days at 30°C with reciprocally shaking (120 oscillations/min). Experiments were done in triplicate.

PUR degradation was monitored by measuring the weight of PUR before and after incubation. PUR cubes were washed twice with distilled water, dried overnight at 80°C and weighed.

2.7. Analytical methods

The PUR breakdown products were analyzed by high performance liquid chromatography (HPLC) equipped with a refractive index (RI) detector. The conditions for HPLC were previously described [13]. Growth of strain TB-35 was estimated by measuring the optical density of cultures at 580 nm with a spectrophotometer (model 100-50; Hitachi, Japan).

3. Results

3.1. Screening of PUR degrading microorganisms

For screening PUR degrading microorganisms, a polyester-type polyurethane (PUR C) and a polyether-type polyurethane (PUR E) were used (Table 1). Two of the 80 soil samples tested caused a decrease in weight of the PUR after 14 days of cultivation. Two strains of PUR degrading bacteria were isolated from these active cultures. Based on degradation activity, strain TB-35 was selected as the better strain. Degradation of polyester-type PUR (PUR E) was not observed.

3.2. Taxonomical studies of strain TB-35

Physiological properties of strain TB-35 were as follows: cell morphology, rods; Gram stain, negative; respiration, aerobic; motility, positive; oxidase, positive; catalase, positive; nitrate reduction, positive; indole production, negative; arginine dihydrolase, negative; urease, negative; aesculin hydrolysis, negative; gelatin hydrolysis, negative; β-galactosidase, negative; acid from fructose O-F, positive; Tween 80 hydrolysis, positive. The strain could grow on mannitol, gluconate, adipate, malate, citrate, and acetate, but did not grow on glucose, arabinose, mannose, maltose, caprate, phenylacetate, and N-acetylglucosamine. Cellular quinone type and mol% of G + C content of DNA from the strain were ubiquinone (Q-8) and 68.8%, respectively. From these characteristics, strain TB-35 was tentatively identified as Comamonas acidovorans [11,12].

3.3. Time course of PUR degradation

Time courses of PUR degradation by strain TB-35 were examined. PUR (about 50 mg) was degraded completely within 7 days during the logarithmic phase of growth when PUR was supplied as a sole carbon source. Alternatively, when PUR was supplied as a sole carbon and nitrogen source, degradation was slow and only about 48% of the added PUR was degraded. PUR degradation of uninoculated controls was negligible (< 1 mg) after 7-days incubation. Strain TB-35 could not grow on the basal medium without PUR.

3.4. Analysis of water soluble PUR breakdown products

Metabolites resulting from the degradation of PUR by strain TB-35 were studied. In the case of PUR supplied as a sole carbon source, one large peak and one small peak were detected by HPLC analysis. The retention time of these peaks corresponded to those of authentic diethylene glycol and trimethylolpropane, respectively. On the other hand, when PUR was supplied as a sole carbon and nitrogen source, an additional peak whose retention time corresponded to that of authentic adipic acid was detected. No other peaks were detected by HPLC analysis. These peaks were not found in uninoculated controls containing PUR.
3.5. Utilization of various PURs and polyesters/polyethers as sole carbon sources

The utilization of various PURs, polyesters and polyethers by strain TB-35 were examined (Table 1). Strain TB-35 could use all polydiethylene glycol adipates (50 mg) as sole carbon source. In this case, this strain required CaCO₃ (1%) to maintain a neutral pH because the pH dropped rapidly to < pH 4.0. In case of polyester-type PURs, TB-35 grew only on PURs with long-chain polyester segments and degraded them completely. TB-35 could use neither polypropylene glycol nor polyether-type polyurethane.

4. Discussion

In all previously reported cases [1-10], however, microorganisms could degrade polyester-type polyurethane only if provides with additional utilizable nutrients. In contrast, strain TB-35 was considered to be able to utilize polyester-type PUR as both sole carbon and nitrogen sources. Analysis of water soluble PUR breakdown products revealed diethylene glycol and adipic acid as probably the main metabolites. These observations implied that an esterase-like enzyme play at least some role in PUR degradation by TB-35, since these metabolites were considered to be derived from the hydrolysis of the polyester segment of the PUR. We could not find any metabolites derived from the diisocyanate segment. It is still unknown whether these are insoluble in water or were completely degraded to CO₂ by strain TB-35. Further work is needed to determine the PUR degradation pathways by TB-35.

Acknowledgements

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References