Microbial Degradation of Disodium Terephthalate by Alkaliphilic Dietzia sp. Strain GS-1

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An alkaliphilic Dietzia sp., strain GS-1, which degraded disodium terephthalate (DT), was isolated from soil. Strain GS-1 degraded 19.3 mM of DT in 168 h at pH 10. The maximum degradation velocity was 0.46 mmol/h. The resting cells efficiently degraded 28.7 mmol of DT in 51 h at 28°C and pH 10. The degradation velocity was 0.41 mmol/(h·g-wet cell).

Key words: alkaliphilic bacterium; Dietzia sp.; disodium terephthalate; microbial degradation; resting cell reaction

At present, large quantities of polyester textile are manufactured and widely used in the world. The polyester textile consists of poly(ethylene terephthalate) (PET) fiber. To soften the touch of the textile, it is treated with a high concentration of sodium hydroxide, namely by alkaline hydrolysis (alkali loss process). This treatment results in 20% (w/w) loss of the textile and elution of appreciable amounts of DT and ethylene glycol. The wastewater from the industry manufacturing the textile is an alkaline solution (pH 12–14) and contains DT and ethylene glycol in large amounts. DT in the wastewater is precipitated as terephthalic acid by the addition of acid, and collected and then burned. This treatment is costly, and generates CO₂ by burning of the terephthalic acid. DT should be used for production of effective material or can be used as substrate for microbial conversion.

We thus embarked on a research project attempting to use DT effectively by biological methods. So far, there are some reports of aerobic¹⁻⁴ and anaerobic⁵,⁶ microbial degradation of DT under neutral conditions. In contrast, none have been reported for microbial degradation of DT under alkaline conditions. We first focused our work on the microbial degradation of DT under alkaline conditions. We report here aerobic degradation of DT by an alkaliphilic bacterium, Dietzia sp. strain GS-1.

All chemicals used are the highest grade. The microorganism was isolated from soil by an enrichment technique. The D-medium used contained 2 g of (NH₄)₂HPO₄, 2 g of K₂HPO₄, 1 g of Na₂HPO₄, 0.2 g of MgSO₄·7H₂O, 4.2 g (20 mm) of DT, and 10 g of Na₂CO₃ per liter of distilled water. The D-medium was sterilized by autoclaving. A Na₂CO₃ solution (100 g/l) was sterilized separately and aseptically added to the sterilized D-medium. The pH of the D-medium was 10. The enrichment was done at 28°C in a 18 mm diameter × 180 mm test tube containing 5 ml of the D-medium on a reciprocal shaker (170 strokes/min). Colonies were isolated from the successful enrichment cultures by spreading and incubation at 28°C on agar plates containing the D-medium. The isolated colonies were confirmed to grow in the liquid D-medium.

Four strains degrading DT were isolated from 3 samples of 46 soil samples by the enrichment cultivation. Among the isolates, a strain grew well in the alkaline D-medium containing DT as carbon and energy sources. The isolated strain was designated as GS-1. Strain GS-1 was further characterized. Strain GS-1 was identified by NCIMB Japan (Shizuoka, Japan). Strain GS-1 was irregular rod-shaped, and its V-form was also observed. Table 1 shows the characteristics of strain GS-1. Strain GS-1 was an aerobic, Gram-positive, and catalase-positive coryneform bacterium. On the basis of the partial nucleotide sequence (501 bp) of the 16S rRNA gene, strain GS-1 was identified as a Dietzia sp. Moreover, the nucleotide sequence of strain GS-1 showed 98.6% identity with that of Dietzia maris (BLAST accession no. Y18883.1). To date, a large number of alkaliphiles have been isolated, and a number of alkaliphilic Bacilli are well known.⁷ However, it was difficult to isolate a DT-degrading alkaliphilic microorganism, because of the alkaline medium and limitation of car-

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Abbreviations: DT, disodium terephthalate; PET, poly(ethylene terephthalate)
Table 1. Characteristics of Strain GS-1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>Gram stain</td>
<td>+</td>
</tr>
<tr>
<td>Morphology</td>
<td>irregular rod*</td>
</tr>
<tr>
<td>Size</td>
<td>1–1.5 × 2 μm</td>
</tr>
<tr>
<td>Motility</td>
<td>–</td>
</tr>
<tr>
<td>Spores</td>
<td>–</td>
</tr>
<tr>
<td>Requirement of free oxygen</td>
<td>aerobic</td>
</tr>
<tr>
<td>Oxidase activity</td>
<td>–</td>
</tr>
<tr>
<td>Catalase activity</td>
<td>+</td>
</tr>
<tr>
<td>Anaerobic fermentation of glucose</td>
<td>–</td>
</tr>
<tr>
<td>Colony color</td>
<td>orange</td>
</tr>
</tbody>
</table>

+, positive; –, negative.
*Some of the cells were V-form.

The experiment of DT degradation with strain GS-1 was done as follows. A loopful of strain GS-1 culture was inoculated into the test tube containing 5 ml of the D-medium and cultivated for 3 days at 28°C on the reciprocal shaker. One ml of the culture was transferred into a 500-ml baffled-walled flask containing 100 ml of the D-medium and incubated at 28°C on a rotary shaker (166 rpm). Five ml of the culture was withdrawn for measurement of the cell growth, pH, and DT concentration. The cell growth was monitored by measuring the optical density at 660 nm (OD660). The OD660 was measured with a Lambda Bio 20 spectrometer operated by a UV Winlab (Perkin-Elmer Corp., Norwalk, USA). The culture was centrifuged at 8000 × g and 4°C. The pH of the supernatant was then measured with a pH BOYP2 (Shidengen Electric MFG. Co., Ltd., Tokyo, Japan). Subsequently, samples of the supernatant were diluted with distilled water. Thereafter, the remaining substrates were measured by direct UV-analysis at λmax (240 nm) of DT with the spectrometer.

As shown in Fig. 1, strain GS-1 had a 50-h lag period before growth started. At 72 h after the cultivation, exponential growth occurred and the cell growth was completed at 168 h. The doubling time was 6.4 h. On the other hand, terephthalate-degrading Bacillus sp. had no lag phase. The growth of strain GS-1 seemed to be slower than the known terephthalate-degrading bacterium, general microorganisms, or alkaliphiles. Strain GS-1 degraded 19.3 mM (96.5%) of DT from an initial concentration of 20 mM DT in 168 h with the growth. The greatest degradation occurred between 72 and 100 h. The maximum degradation velocity was 0.46 mmol/h. No change of pH was observed in the D-medium during growth. Kargoule and Pujar reported that a terephthalate-degrading Bacillus sp. degraded 0.2% (about 10 mM) of terephthalate in 24 h. Thus, the degradation rate of strain GS-1 between 72 and 100 h was almost equivalent to that of the terephthalate-degrading Bacillus sp.

The experiment of DT degradation with the resting cells of strain GS-1 was done as follows. Strain GS-1 was cultivated for 7 days at 28°C in the flasks containing 100 ml of the medium on the rotary shaker. The cells were harvested from the 500 ml cultures by centrifugation at 18780 × g for 5 min at 4°C and then washed 2 times with a 0.05 M glycine-NaOH buffer, pH 10.0. The cell paste (1.36 g) was obtained and suspended in 10 ml of the same buffer. The cell suspension was added to 90 ml of 10 g/l DT in the same buffer. The reaction mixture was shaken at 28°C on the rotary shaker (166 rpm) in a 500-ml flask. The estimation for the resting cell reaction was also done by the same method described above. When the reaction ended, the cells were collected by centrifugation and washed twice with the same buffer. Once again the resting cell reaction was done as described above.

Figure 2 shows that the resting cells of strain GS-1 efficiently degraded 28.7 mM (67.1%) of DT from the initial concentration of 42.8 mM DT in 51 h at pH 10.0. The degradation velocity was 0.41 mmol/h (g wet cell). The reaction ended at 191 h. The second reaction using the cells recovered after the first resting cell reaction degraded DT again (data not shown), however, the degradation activity decreased to about 30%. Thus, the ending of the first resting cell reaction suggests inhibition by the reaction products rather than inactivation of enzymes for DT metabolism.

Maeda et al. reported aromatic compound-degrading alkalitrophic bacteria and that alkalitrophic Dietzia sp. used benzoate on the minimal medium (an alkaline C-medium, pH 9.0). We report here for the first time the isolation of DT-degrading alkaliphilic Dietzia sp. and aerobic microbial degradation of DT under alkaline conditions. To use DT effectively, fur-
ther work is in progress to isolate enzymes concerned in DT metabolism and to identify intermediates of DT degradation. Engelhardt et al. demonstrated that Nocardia sp. DSM 43251 produced protocatechuc acid as an intermediate metabolite. Schläfi et al. described terephthalate 1,2-dioxygenase in Comamonas testosteroni T-2 as converting terephthalate to (1R,2S)-diethyl-3,5-cyclohexadiene-1,4-dicarboxylic acid. Oomori et al. reported production of 4,5-dihydro-4,5-dihydroxyxylate from phthalate by a mutant strain of Pseudomonas testosteroni M4-1 and described the compound as having important properties as a monomer for functional polymers. Ikeda et al. and Hudlicky et al. described enzymatic and microbial dihydroxylation of aromatics and dioxygenases as being very important to the preparation of useful synthetic intermediates. We hence expect that an enzyme concerned in DT metabolism, such as dioxygenase, or strain GS-1 having the enzyme, can be used as a biocatalyst in the preparation of useful synthetic intermediates, and that effective use of DT would result in a novel recycling of PET products.

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