Degradation of terephthalic acid by a newly isolated strain of *Arthrobacter* sp.0574

Terephthalic acid is an important industrial chemical but its production typically generates 3–10 tons of wastewater, which is a significant source of pollution. Although recent research has shown that terephthalic acid can be degraded by physical and chemical methods, these methods are complex and expensive. Microbial degradation of terephthalic acid is a popular alternative because it is environmentally friendly. We isolated a Gram-positive strain capable of growing aerobically on terephthalic acid as the sole carbon and energy source. It was identified as *Arthrobacter* sp. by 16S rDNA sequencing and its physiological and biochemical characteristics. For terephthalic acid degradation, the optimal temperature of the resting cells was 30 °C, optimal shaking speed was 150 rpm, the most suitable pH was 7.0, and the ability to degrade terephthalic acid was inhibited by concentrations of terephthalic acid above 10 g/L.

**Introduction**

Terephthalic acid is an important industrial chemical for the syntheses of plastics, dyes, pesticides and chemical fibres which are widely used in our daily life. Because of its extensive applications, large-scale production and chemical characteristics, this refractory organic material and its associated class of organics have become ubiquitous environmental pollutants, and they have been found in sediments, natural waters, soils and aquatic organisms. The production of 1 ton of terephthalic acid typically generates 3–10 tons of wastewater containing high concentrations of terephthalic acid. This wastewater is thus a source of significant pollution. Terephthalic acid is known to inhibit microbial growth, cause bladder cancer, and impair renal, liver and testicular function, thereby posing a serious threat to human health. Recent research has shown that terephthalic acid can be degraded by physical and chemical methods, but these methods are complex and expensive. Microbial degradation has become a popular alternative because it is environmentally friendly. Because the effectiveness of microbial degradation depends on the activity of the selected microorganism, research has focused mainly on the screening of microorganisms with a strong ability to degrade terephthalic acid. Karegoudar and Pujar reported on the degradation of terephthalic acid by a *Bacillus* species. The genus *Pseudomonas* sp. has also recently received much attention because of its ability to degrade terephthalic acid. In our laboratory, we have recently shown that a new isolate identified as *Arthrobacter* sp. has a strong ability to degrade terephthalic acid.

The use of resting cells for degradation rather than the isolated enzyme is generally preferred because enzyme purification is expensive and the cells offer protection to the enzymes from the harsh environment of the degradation process. In this paper, we describe degradation of terephthalic acid by a newly isolated strain – *Arthrobacter* sp.0574. We also attempt to determine the optimal conditions for efficient degradation.

**Materials and methods**

**Reagents**

Terephthalic acid with a purity greater than 99% was purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). Other chemicals used were of analytical grade.

**Media and culture conditions**

The enrichment media used in this study contained 5 g/L terephthalic acid, 3 g/L beef extract, 10 g/L peptone, 3 g/L KH₂PO₄, and 5 g/L NaCl, and had a pH of 7.3. The screening media contained 1 g/L terephthalic acid, 0.25 g/L MgSO₄, 3 g/L KH₂PO₄, 1 g/L NH₄Cl, 0.5 g/L NaCl, 7 g/L Na₂HPO₄, and 15 g/L agar, and had a pH of 7.3. Both media were autoclaved at 121 °C for 21 min, and the strain was cultured aerobically at 30 °C.

**Screening and identification of bacterial strain**

Sludge samples were collected from the Ningbo Lihe Chemical Fibre company in Ningbo (Zhejiang Province of China) that produces chemical fibres. After 7 days of enrichment and screening, the most efficient strain was chosen for the study.

**Observation of morphology**

Morphology of this bacterial strain was observed with a light microscope (LEICA DMLB, LEICA Microsystems AG, Wetzlar, Germany) and a transmission electron microscope (JEM-2100, JEOL Ltd., Japan).
Biodegradation of terephthalic acid

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Table 1: Characteristics of Arthrobacter sp.0574

<table>
<thead>
<tr>
<th>Identifying characteristic</th>
<th>Result</th>
<th>Identifying characteristic</th>
<th>Result</th>
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</thead>
<tbody>
<tr>
<td>Colonial morphology</td>
<td>Rounded with a burnished, moistened, yellow appearance</td>
<td>Gelatinase</td>
<td>Positive</td>
</tr>
<tr>
<td>Gram stain</td>
<td>Positive</td>
<td>Methyl red</td>
<td>Negative</td>
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<tr>
<td>Cell shape</td>
<td>Rod-shaped</td>
<td>Nitrate reduction</td>
<td>Negative</td>
</tr>
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<td>Aerobism</td>
<td>Positive</td>
<td>Urea</td>
<td>Positive</td>
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<tr>
<td>Cell size</td>
<td>0.4–0.5 µm wide 0.7–0.8 µm long</td>
<td>Maltose</td>
<td>Positive</td>
</tr>
<tr>
<td>Dynamic experiment</td>
<td>Non-motile</td>
<td>Chestnut sugar</td>
<td>Negative</td>
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<td>Haemolysis</td>
<td>Negative</td>
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<td>Amylolytic enzyme</td>
<td>Negative</td>
<td>Mannose</td>
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Figure 1: The morphology of Arthrobacter sp.0574. (a) Colonial morphology, (b) a light micrograph of Arthrobacter sp.0574 (1000×) and (c) an electron micrograph of Arthrobacter sp.0574.

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16S rDNA analysis

A 1383-bp sequence was amplified from the genomic DNA and sequenced by Shanghai Sangon (China). The sequence was submitted to GenBank (accession number JN900471). As shown in Figure 2, two strains – AB288060.1 and HQ236023.1 – were relatively related to strain Arthrobacter sp.0574 (supported by 44% bootstrapping). Strains FN433020.1 and FM213390.2 were on the 62%-bootstrap-supported branch with Arthrobacter sp.0574 (JN900471).

Effects of pH, temperature, shaking speed and concentration

The effects of six pH values, five temperatures, six shaking speeds and eight initial terephthalic acid concentrations were investigated.

A comparison of the degradation of terephthalic acid at various pH is presented in Figure 3. It can be seen that the optimal pH of resting cells for degradation was 7.0. At a pH of 5 and a pH of 10, the degradation rate was lowest, indicating that the resting cells seem to tolerate a neutral environment and the catalysing enzyme is inhibited by both alkaline and acidic environments.

Increasing the initial terephthalic acid concentration decreased the degradation extent, indicating that the ability of the resting cells to degrade the terephthalic acid was reduced. When the initial terephthalic acid concentration was between 3 g/L and 7 g/L, the terephthalic acid concentration decreased after degradation for 58 h, whereas when the initial terephthalic acid concentration was between 8 g/L and 9 g/L, the terephthalic acid concentration changed little after the degradation period. An initial terephthalic acid concentration of 10 g/L remained largely unchanged after the degradation period of 58 h. Therefore a terephthalic acid concentration greater than 10 g/L inhibits the cells’ ability to degrade terephthalic acid.

![Figure 2: A neighbour-joining analysis tree of the strain Arthrobacter sp.0574 16S rDNA.](image)

![Figure 3: Effect of pH on the degradation of terephthalic acid by Arthrobacter sp.0574.](image)

![Figure 4: Effect of temperature on the degradation of terephthalic acid by Arthrobacter sp.0574.](image)

![Figure 5: Effect of shaking speed on the degradation of terephthalic acid by Arthrobacter sp.0574.](image)

![Figure 6: Time course of terephthalic acid (TA) degradation by Arthrobacter sp.0574 for different initial concentrations of TA.](image)
Conclusion

We initially identified an Arthrobacter sp. as a novel bacterial strain for the degradation of terephthalic acid according to its morphological, physiological and biochemical characteristics and 16S rDNA sequence analysis. The effects of environmental factors (temperature, pH and shaking speed) on terephthalic acid degradation were investigated and the optimal conditions for degradation were found to be a temperature of 30 °C, a pH of 7.0 and a shaking speed of 150 rpm. Degradation was inhibited if the initial terephthalic acid concentration was above 10 g/L. To further develop and utilise this method of degradation, the mechanisms, metabolic pathways, intermediate product types, and toxicity and accumulation mechanisms of the degradation process need to be determined.

Acknowledgements

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Authors’ contributions

W.Z.-J. and Z.J. were the project leaders. Z.Y.-M. and S.Y.-Q. were responsible for the experimental and project design, and performed most of the experiments. Z.Y.-M. and S.Y.-Q. wrote the manuscript.

References