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

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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.[1] 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.[2]

The production of 1 ton of terephthalic acid typically generates 3–10 tons of wastewater containing high concentrations of terephthalic acid.[3-5] 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,[6-11] thereby posing a serious threat to human health. Recent research has shown that terephthalic acid can be degraded by physical and chemical methods,[12-19] but these methods are complex and expensive. Microbial degradation has become a popular alternative because it is environmentally friendly.[3,20] 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[21] 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.[22] 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.[23] 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 KH_2PO_4 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 $MgSO_4$, 3 g/L KH_2PO_4, 1 g/L NH_4Cl, 0.5 g/L NaCl, 7 g/L Na_2HPO_4 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).

Extraction, amplification and analysis of genomic DNA

A 2-mL bacterial suspension was centrifuged at 12 000 rpm for 1 min, and the supernatant fluid was abandoned before 750 μL GTE (glucose 50 mmol/L, Tris-HCl 25 mmol/L, EDTA 10 mmol/L) was added. After stirring and mixing, 7.5 μL lysozyme (100 mg/mL) was added. The sample was then incubated for 30 min at 37 °C before 7.5 μL proteinase (20 mg/mL) was added. After mixing, the sample was then incubated for a further 1 h at 55 °C. During the incubation, the tubes were lightly shaken every 10 min. After incubation, 750 μL phenol-chloroform was added. The samples were then centrifuged at 12 000 rpm for 5 min and the supernatant was collected. Genomic DNA was extracted and purified using chloroform-isoamyl alcohol. The aqueous phase was transferred to a new tube, 50 μL natrium aceticum (3 mol/L, pH 5.2) and 100 μL ethanol were added, and the tube was kept static for 30 min at -20 °C. The pellet was obtained by centrifugation at 12 000 rpm for 10 min at 4 °C and the sample was washed twice with ethanol. After ethanol was removed, the pellet was stored at 4 °C.

The 16S rDNA was amplified using the universal primer pairs of 1492r (5'-GGTTACCTTGTTACGACTT 3') / 27f (5'-AGAG TTTG ATCC TGGC TCAG3'). The reaction mixture for the polymerase chain reaction (PCR) amplification was prepared in a total volume of 50 μL with 5.0 μL 100×T PCR buffer, 3.2 μL dNTP (2 mmol/L), 2.5 U Taq DNA polymerase (Shanghai Sangon, China) and 10 μL of each primer (10 μmol/L). The amplifications were performed in a ThermoHybaid PCR Sprint Thermal Cycler (Thermo Electron, Waltham, MA, USA). The PCR reaction cycles were as follows: 5 min at 94 °C for initial denaturation, 1 min at 94 °C for denaturation, 1 min at 55 °C for annealing of 16S rDNA, 3 min at 72 °C for extension with a total of 30 cycles of amplification and 10 min of final extension. The 16S rDNA was purified using an UltraClean PCR Clean-up kit and sequenced by Sangon Biotech Co. Ltd. (Shanghai, China). The 16S rDNA sequence analysis was performed with MEGA.4.0 software using the neighbour-joining method.

Preparation of resting cells

The strain identified as *Arthrobacter* sp.0574 by gene sequencing of 16S rDNA was grown in medium containing 3 g/L KH_2PO_4, 0.5 g/L NaCl, 1 g/L terephthalic acid, 0.25 g/L $MgSO_4$, 7 g/L Na_2HPO_4 and 1.4 g/L urea at a pH of 7.3, incubated at a shaking speed of 150 rpm at 30 °C for 48 h. Cells were harvested by centrifugation at 8000 rpm for 10 min at 4 °C, and washed with 0.05 mol/L Tris-HCl (pH 7.0) twice.

Effects of pH, temperature, shaking speed and concentration

Terephthalic acid biodegradation by *Arthrobacter* sp.0574 resting cells was carried out using a terephthalic acid concentration of 1 g/L, under a range of temperatures (20–40 °C), pH (5.0–10) and shaking speeds (50–200 rpm). Different concentrations of terephthalic acid from 3 g/L to 10 g/L at a pH of 7.0 were prepared. The resting cell concentrations in these experiments were about 1 g(dry weight)/L, and the experiment was conducted with a shaking speed of 150 rpm at a temperature of 30 °C.

Analysis

Biomass concentration was determined using optical density at a wavelength of 660 nm. The measurement of terephthalic acid concentration was carried out as described previously.[24]

Results and discussion

Isolation of bacterial strain

A pure strain isolated from the sludge samples was identified as *Arthrobacter* sp.0574 according to its physiological and biochemical characteristics and 16S rDNA gene sequencing. *Arthrobacter* sp.0574 is a novel strain identified for the degradation of terephthalic acid, since Karegoudar and Pujar's[21] identification of a *Bacillus* species for the biodegradation of terephthalic acid. The physiological and biochemical characteristics of *Arthrobacter* sp.0574 are shown in Table 1 and its morphology is shown in Figure 1.

Table 1: Characteristics of *Arthrobacter* sp.0574

Identifying characteristic	Result	Identifying characteristic	Result
Colonial morphology	Rounded with a burnished, moistened, yellow appearance	Gelatinase	Positive
Gram stain	Positive	Methyl red	Negative
Cell shape	Rod-shaped	Nitrate reduction	Negative
Aerobism	Positive	Urea	Positive
Cell size	0.4–0.5 μm wide 0.7–0.8 μm long	Maltose	Positive
Dynamic experiment	Non-motile	Chestnut sugar	Negative
Haemolysis	Negative	Fructose	Negative
Oxidase	Negative	Lactose	Negative
Catalase	Positive	Xylose	Negative
Amylolytic enzyme	Negative	Mannose	Negative

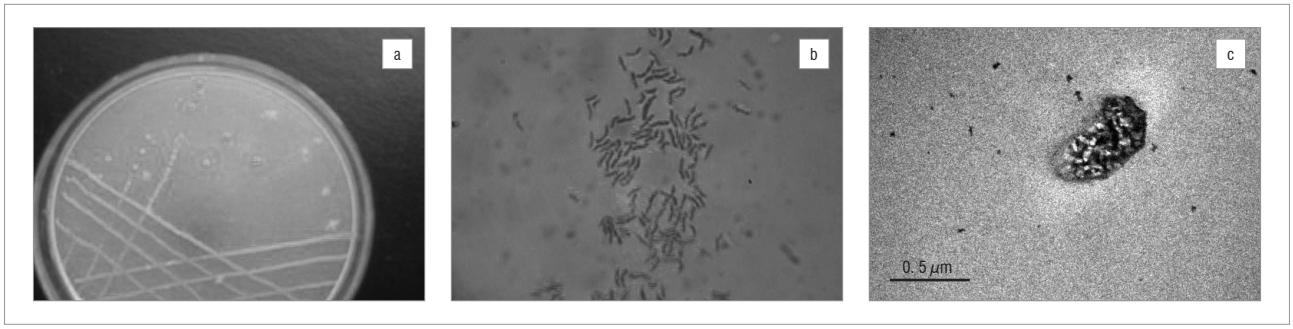


Figure 1: The morphology of *Arthrobacter* sp.0574. (a) Colonial morphology, (b) a light micrograph of *Arthrobacter* sp.0574 (1000×) and (c) an electron micrograph of *Arthrobacter* sp.0574.

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).

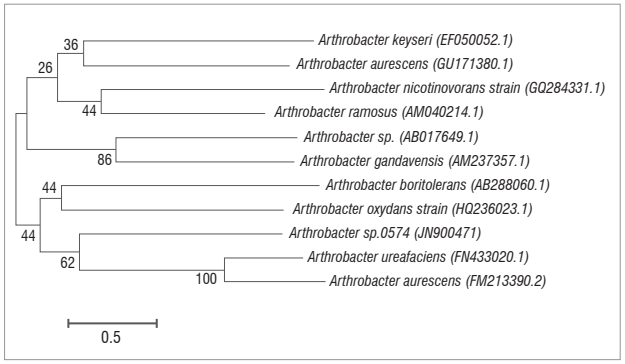
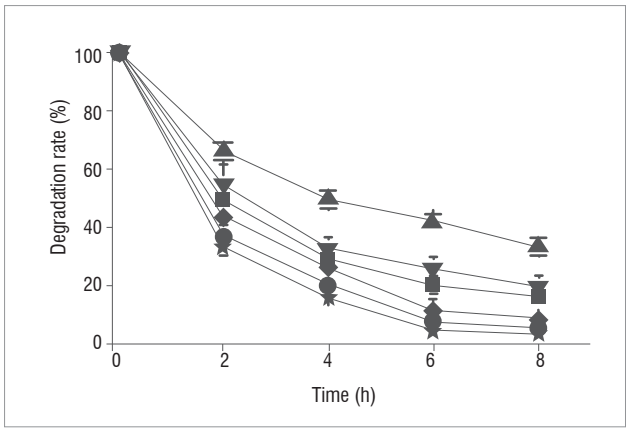


Figure 2: A neighbour-joining analysis tree of the strain *Arthrobacter* sp.0574 16S rDNA.

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.



pH 5 (▼), pH 6 (◆) , pH 7 (★), pH 8 (●), pH 9 (■), pH 10 (▲)

Figure 3: Effect of pH on the degradation of terephthalic acid by *Arthrobacter* sp.0574.

Degradation reached a maximum value of 95% at 30 °C. Higher and lower temperatures decreased the degradation rate, as shown in Figure 4.

As *Arthrobacter* sp.0574 is an aerobic bacterial strain, we determined the effect of shaking speed on degradation rate. Figure 5 shows that when the shaking speed ranged from 70 rpm to 200 rpm, the degradation was greater than 70%. The optimal shaking speed was found to be 150 rpm, which was adopted for all further experiments.

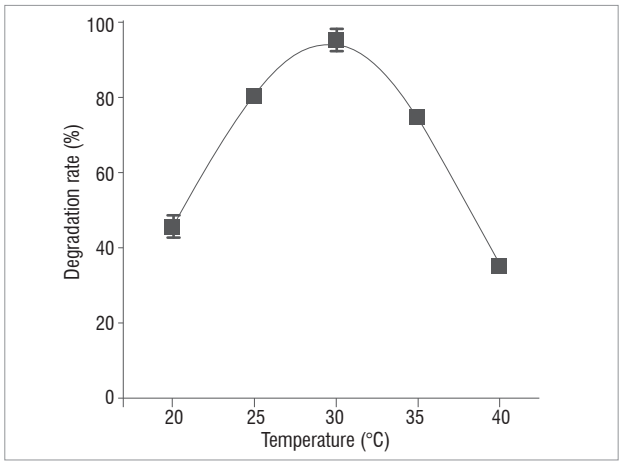


Figure 4: Effect of temperature on the degradation of terephthalic acid by *Arthrobacter* sp.0574.

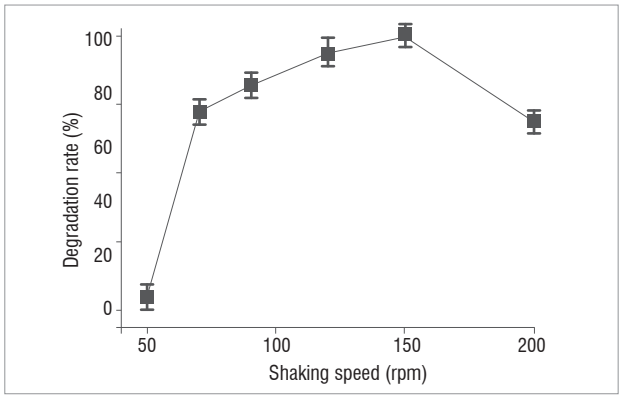
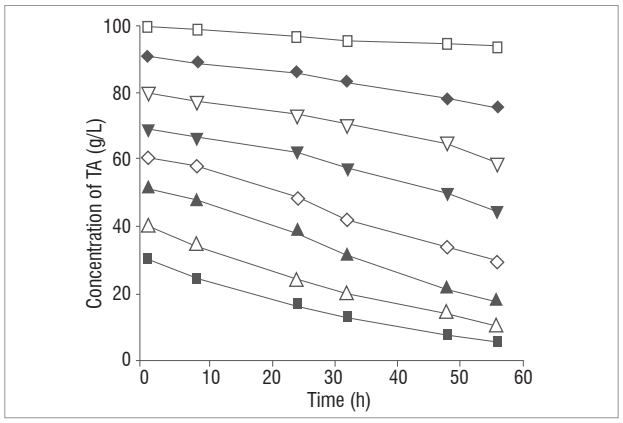


Figure 5: Effect of shaking speed on the degradation of terephthalic acid by *Arthrobacter* sp.0574.

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.



3 g/L (■), 4 g/L (△), 5 g/L (▲), 6 g/L (◇), 7 g/L (▼), 8 g/L (▽), 9 g/L (◆), 10 g/L (□)

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

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.

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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.

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