Degradation of poly(butylene adipate-co-terephthalate) by Stenotrophomonas sp. YCJ1 isolated from farmland soil

Hao Jia¹, Min Zhang¹, Yunxuan Weng², Yao Zhao¹, Chengtao Li¹, Aqsa Kanwal¹

¹School of Environmental Science and Engineering, Shaanxi University of Science and Technology, Xi’an 170021, China
²Beijing Key Laboratory of Plastics Health and Safety Quality Evaluation Technology, Beijing Technology and Business University, Beijing 100048, China

A R T I C L E   I N F O
Article history:
Received 25 June 2020
Revised 2 October 2020
Accepted 3 October 2020
Available online 28 October 2020

Keywords:
Biodegradable
Poly(butylene adipate-co-terephthalate) (PBAT)
Stenotrophomonas sp.
Lipase
Degradation mechanism

A B S T R A C T
In recent years, poly (butylene adipate-co-terephthalate) (PBAT) has been widely used. However, PBAT-degrading bacteria have rarely been reported. PBAT-degrading bacteria were isolated from farmland soil and identified. The effects of growth factors on the degradation of PBAT and the lipase activity of PBAT-degrading bacteria were assessed. The degradation mechanism was analyzed using scanning electron microscopy, attenuated total reflection Fourier transform infrared spectroscopy, proton nuclear magnetic resonance, X-ray diffraction, and liquid chromatography-mass spectrometry. The results showed that Stenotrophomonas sp. YCJ1 had a significant degrading effect on PBAT. Under certain conditions, the strain could secrete 10.53 U/mL of lipase activity and degrade 10.14 wt.% of PBAT films. The strain secreted lipase to catalyze the degradation of the ester bonds in PBAT, resulting in the production of degradation products such as terephthalic acid, 1,4-butanediol, and adipic acid. Furthermore, the degradation products could participate in the metabolism of YCJ1 as carbon sources to facilitate complete degradation of PBAT, indicating that the strain has potential value for the bioremediation of PBAT in the environment.

© 2020 The Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. Published by Elsevier B.V.

Introduction

Synthetic polymers have been widely used in production and life activities because of their good performance. However, large quantities of synthetic polymers that are difficult to degrade exist in the environment, thereby causing serious “white pollution” (Marcus, 2018). Additionally, synthetic polymers can be broken down into particles (particle size < 5 mm) in the environment via exposure to sunlight to form microplastics, which are widely distributed in rivers, oceans, and other water bodies (Falco et al., 2017; Lacerda et al., 2019). Microplastics enter the human body through the food chain and can cause immeasurable harm to the human body. These phenomena have prompted researchers to devote their attention to biodegradable polymers (Gall and Thompson, 2015; Wright and Kelly, 2017).

Poly(butylene adipate-co-terephthalate) (PBAT) is a biodegradable polymer composed of an aromatic polyester.
It is widely used in food, medical, textile, agricultural and other industries because of its good mechanical behavior, and it can be degraded by microorganisms in the environment (Fukushima et al., 2012; Phongam et al., 2015). The biodegradation of polyester polymers in the environment mainly occurs through the production of lipase by degrading bacteria, and lipase hydrolysis cleaves the ester bonds of the polymers to degrade them into small molecules (Biundo et al., 2016; Kasuya et al., 2009). However, according to current research, the degradation products of PBAT, e.g., terephthalic acid and adipic acid, can change the pH of the environment and community structure of environmental microorganisms, as well as cause physiological toxicity in microorganisms and plants (Kim et al., 2001; Pang et al., 2015; Varaprasad et al., 2017). Currently, there are few types of PBAT-degrading microorganisms, mainly including Sphingopyxis ginsengisoli, Bacillus pumilus, Pseudomonas pseudoalcaligenes, Cryptococcus, and Trichoderma asperellum (Aarthy et al., 2018; Huo et al., 2017; Muroi et al., 2017; Silva et al., 2001; Wallace et al., 2017).

These bacteria have some disadvantages such as low degradation rate, and reports on whether the degrading bacteria can degrade or metabolize PBAT degradation products are scarce. Therefore, the identification of new lipase-producing and PBAT-degrading bacteria has become a top priority to provide a theoretical basis and application prospect for the bioremediation of PBAT in the environment (Richardson et al., 2016).

In this study, PBAT-degrading bacteria (Stenotrophomonas sp. YCJ1) were isolated and identified from farmland soil. The taxonomic status of Stenotrophomonas sp. is belonging to Proteobacteria, Gammaproteobacteria, Xanthomonadales, Xanthomonadaceae, Stenotrophomonas. It is a Gram-negative bacterium. The colonies were yellow, round, raised, opaque, neat, and sticky. It did not require high nutrition and grew at 20–45°C. It has been widely found in natural environments such as soil and water. Its degradation characteristics and lipase activity were studied. By analyzing the morphology and structure of PBAT before and after degradation and analyzing the composition of the degradation liquid via liquid chromatography-mass spectrometry (LC/MS), the degradation mechanism of YCJ1 for PBAT was deduced, providing technical support for the highly efficient degradation of PBAT in the environment.

1. Materials and methods

1.1. Soil collection and sample preparation

Soil was collected from apple farms in Yanxian Town, Shaanxi Province, China (108.71 E, 34.33 N). We used a sterile sampler to randomly collect soil from the depth of 20 cm in different locations. We thoroughly mixed the soil, passed it through a 60-mesh sieve, placed it in a vacuum tube and stored it at 4°C. We used a tape-casting method (Pal et al., 2016) to prepare a 10 mm × 10 mm × 0.05 mm PBAT film for the degradation test, and the PBAT (number-average molar mass of 1.0 × 10^5) was purchased from Zhubai Wantong Chemical Co., Ltd. (Guangdong, China).

1.2. Medium

A Murashige and Skoog medium (SM) (pH 7.2–7.4) was used containing the following components (g/L): KH₂PO₄, 1.00; Na₂HPO₄, 1.50; NH₄Cl, 2.00; CaCl₂•2H₂O, 0.10; KCl, 0.15; MgSO₄•7H₂O, 0.20; FeSO₄•7H₂O, 0.01; ZnSO₄•7H₂O, 0.01; MnSO₄•H₂O, 0.001; and agar-agar, 20.00. LB medium (g/L): tryptone, 10.00; yeast extract, 5.00; NaCl, 10.00; pH 7.2–7.4. The screening medium (g/L) contained the following: SM medium in which PBAT is the sole carbon source. Its preparation method was as follows: 0.10 g of PBAT was dissolved in 10 mL of chloroform. After it completely dissolved, 100 mL of the SM medium and 0.5 mL of Tween 80 were added as an emulsifier for phacoemulsification.

1.3. Isolation and identification of PBAT-degrading bacteria

The soil was configured as a bacterial suspension, and the supernatant was inoculated into the screening medium using the serial dilution-plating method at 30°C and 130 r/min. After the screening medium became turbid, the bacterial solution was added to the screening medium plate and incubated at 30°C. A single colony was selected from the plate for inoculation on the screening medium until a single strain (YCJ1) was obtained. Preparation of inoculum: YCJ1 was inoculated into 100 mL of LB medium and cultured for 12 hr. on a shaker at 37°C and 130 r/min.

Morphological observation: The YCJ1 was observed on the screening medium via scanning electronic microscopy (SEM, FEI Q45, FEI) (Wang et al., 2018). Physiological and biochemical experiments (Dong and Cai, 2001): YCJ1 was subjected to Methyl Red, Voges–Proskauer, oil hydrolysis tests and so on. 16S rRNA gene sequence analysis: The 16S rRNA gene universal primers 27F and 1492R were used, and total DNA was used as a template for PCR amplification. The reaction system consisted of 20.0 μL of 2 × Taq PCR Master Mix, 2.0 μL of 27F/1492R (10.0 μmol/L) primers, 3.0 μL of total DNA, and 23.0 μL of ddH₂O. After the PCR reaction, the amplified product was sent to Biotechnology Engineering Co., Ltd (Shanghai, China) for sequencing. The sequencing result was submitted to the GenBank database, and BLAST was performed using the NCBI sequence database. The phylogenetic tree was constructed using MEGA 7.0 by the neighbor-joining method.

1.4. Utilization of PBAT degradation products

To consider the utilization of the degradation products of PBAT by YCJ1, 100 mL of SM medium and a PBAT film were added in a 250 mL Erlenmeyer flask. Then, 2 wt.% terephthalic acid, 1,4-butanediol, adipic acid, glucose and starch were added to each Erlenmeyer flask at pH 7.2. Next, 1 mL of the inoculum was inoculated into the Erlenmeyer flask and placed on a shaker at 30°C for 5 days. The degradation rate of the PBAT film was measured using the weight loss method. The degradation rate was calculated by Eq. (1):

\[ r = \frac{(W_0 - W_t)}{W_0} \times 100\% \] (1)
where, \( r \) (wt.\%) is the degradation rate of PBAT, \( W_0 \) (g) is the weight of the PBAT film before degradation, and \( W_1 \) (g) is its weight after degradation.

The lipase activity of YCJ1 was measured by the p-nitrophenol (p-NP) method (Biundo et al., 2016) to determine the utilization of the degradation products of PBAT by YCJ1. The specific operation method was as follows: 4 mL substrate solution \( (V(A): V(B) = 1:9) \) was put into an Erlenmeyer flask, and 200 \( \mu L \) of crude enzyme solution was added, mixed well and reacted at 40°C and 150 r/min 10 min. With p-NP as the standard and the pre-inactivated crude enzyme solution as the blank control, the OD value was measured at 410 nm. All experiments were performed in triplicate. In addition, 30 mg of p-NP was dissolved in 10 mL of isopropanol (A). Tris-HCl buffer with a volume fraction of 0.1% gum arabic and a volume fraction of 0.4% Triton X-100 was adjusted to pH 8.0 (B). One unit of lipase activity (U) was defined as the amount of enzyme that releases 1 \( \mu G \) of p-NP per min.

1.5 Degradation and lipase-producing characteristics of YCJ1

To each 250 mL Erlenmeyer flask, 100 mL of SM medium, 2 wt.% 1,4-butanediol, and a PBAT film were sequentially added. Based on this, the single-factor experiment method was used to study its degradation and enzyme production characteristics. First, the pH was sequentially changed to 6.3, 6.6, 6.9, 7.2, 7.5, and 7.8. Then, 1 mL of the inoculum was inoculated into Erlenmeyer flasks and placed on a shaker at 30°C for 10 r/min for 5 days. Second, at pH 7.5, the culture temperature was changed to 27°C, 32°C, 37°C, 42°C, and 47°C. Next, 1 mL of the inoculum was inoculated into Erlenmeyer flasks and placed on a shaker at 130 r/min for 5 days. Lastly, at pH 7.5 and 37°C, the inoculum amount was changed to 0.5 mL, 1.0 mL, 1.5 mL, 2.0 mL, 2.5 mL, and 3.0 mL. Then, these Erlenmeyer flasks were placed on a shaker at 130 r/min for 5 days. The lipase activity of YCJ1 and degradation rate of the PBAT film were tested to study the effects of the culture conditions.

1.6 PBAT degradation by YCJ1

The PBAT degradation experiment was performed under optimal conditions: 100 mL of SM medium, 2 wt.% of 1,4-butanediol, 1.5 mL of inoculum, and a PBAT film were added into a 250 mL Erlenmeyer flask, which was placed on a shaker at pH 7.5 and 37°C for 5 days. The weight loss of PBAT film, lipase activity, OD200, and pH in the system were tested every 12 hr. The morphology and structure of the PBAT film and the degradation solution before and after degradation were analyzed as follows.

1.7 Scanning electron microscopy (SEM) of PBAT films

The PBAT films before and after degradation were vacuum-dried at 60°C for 24 hr. and then subjected to gold spray treatment. The surface topography of PBAT films were observed using an SEM (Q45, FEI, America).

1.8 Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) of PBAT films

The PBAT films before and after degradation were repeatedly washed with deionized water to remove impurities on the surface, and dried under vacuum at 60°C for 24 hr. Furthermore, PBAT films were tested using ATR-FTIR (VECTOR-22, Bruker, Germany) in the region from 4000 to 800 cm\(^{-1}\) to analyze the structural changes of PBAT.

1.9 Proton nuclear magnetic resonance (\(^1\)H NMR) of PBAT films

To further analyze the structural changes of the PBAT films after degradation, we analyzed the PBAT films by \(^1\)H NMR (DPX 400, Bruker, Germany). The PBAT films also were tested using NMR operated at 400 MHz. All samples were dissolved in a CDCl\(_3\) solution and scanned 64 times each.

1.10 X-ray diffraction (XRD) of PBAT films

The PBAT films before and after degradation were tested using XRD (D/max 2200 PC, Rigaku, Japan), and the power source was a CuK\(\alpha\) target with a scanning range of 10°-50°.

1.11 Liquid chromatography coupled to mass spectrometry (LC/MS) of degradation solution

To study the degradation of PBAT, we analyzed the products in the degradation solution by LC/MS. The degradation solution was filtered before and after degradation through a 0.2 \( \mu m \) filter (poly(ether sulfone), PBS) to remove impurities and tested using high-performance LC system (Agilent 1100, Agilent, America) with a TSQ Quantum Ultra triple quadrupole mass spectrometer (Thermo TSQ, Thermo Scientific, America). Degradation products in the degradation solution were separated on an Agilent TC-C(18) column at 30°C, using a mixture of methanol/water as the mobile phase at a flow rate of 0.2 mL/min. An electrospray ionization source (ESI) was applied and operated in the negative mode; the ion source temperature was 300°C. The flow rate of dry gas was 9 L/min and the atomizing gas pressure was 2.76 \( \times 10^5 \) kPa. The m/z range was 60–1100.

1.12 Nucleotide sequence accession numbers

The 16S rRNA sequence of Stenotrophomonas sp. YCJ1 was deposited in the GenBank database under accession number MK989996. It was also deposited at the China General Microbiological Culture Collection Center (CGMCC) under deposit number CGMCC No. 18059.

2. Results

2.1 Isolation and identification of YCJ1

A novel bacterial strain, YCJ1, which could use PBAT as the sole carbon source for growth, was isolated from farmland...
soil. On the screening medium, colonies of YCJ1 were orange-red with smooth surfaces and flat edges (Fig. 1a). Based on the SEM image (Fig. 1b) and physicochemical tests (Fig. 1c), this strain was found to be a Gram-negative bacillus that secretes lipase, which could degrade aliphatic-aromatic copolymers. The strain was sequenced using the NCBI online BLAST tool, and the results showed that the 16S rRNA gene sequence of the strain was up to 99.3% identical to that of Stenotrophomonas sp. The results of phylogenetic tree revealed that the strain was similar to Stenotrophomonas pavanii Ramos (LDJN01000038) (Appendix A Fig. S1). On the basis of the above results, YCJ1 was initially considered to belong to Stenotrophomonas sp.

2.2. Utilization of PBAT degradation products by YCJ1

Compared with the CK (without additional carbon sources) group (Fig. 2a), the PBAT degradation rate of YCJ1 was 1.82 wt.% within 5 days, and the strain could produce 0.30 U/mL lipase. On addition of PBAT degradation products such as terephthalic acid, adipic acid, and 1,4-butanediol, the PBAT degradation rate by YCJ1 increased to 5.44 wt.%, 5.33 wt.%, and 7.18 wt.%, respectively, and the lipase activity increased to 2.64, 2.85, and 3.64 U/mL, respectively. At the same time, its OD600 increased from 0.325 to 1.562, 1.325, and 1.785, respectively. The results showed that YCJ1 can efficiently utilize PBAT degradation products as growth substrates within 5 days to increase its biomass and enhance its lipase activity.

2.3. Degradation and lipase-producing characteristics of YCJ1

pH had a considerable influence on the PBAT degradation rate and lipase activity of the strain (Fig. 2b). When the pH was 6.3, the PBAT degradation rate was 4.08 wt.% and the lipase activity was 3.18 U/mL. As pH increased, the PBAT degradation rate and lipase activity initially increased before subsequently decreasing. At pH 7.5, the degradation rate and enzyme activity were the highest (7.75 wt.% and 5.17 U/mL, respectively). The effects of temperature (Fig. 2c) and inoculum amount (Fig. 2d) on YCJ1 were similar to those of pH. This phenomenon was related to the optimal culture conditions of YCJ1 and its lipase. In summary, when 2 wt.% 1,4-butanediol was added to 100 mL of SM medium and the pH, temperature, and inoculum amount were adjusted to 7.5, 37°C, and 1.5 mL, respectively, the PBAT degradation rate and lipase activity of YCJ1 peaked at 10.14 wt.% and 10.53 U/mL, respectively.

2.4. Degradation characteristics of YCJ1

Between 0 and 48 hr. (Fig. 3a), the growth of YCJ1 (OD600) increased rapidly and slowly stabilized, and the lipase activity also stabilized. PBAT films were slowly degraded and degradation products (such as adipic acid, terephthalic acid) were released, causing the system’s pH to gradually decrease. Between 48 and 72 hr., as the degradation rate of PBAT accelerated, the pH, lipase activity, and OD600 in the system began descending, which was due to the inhibition of microorganisms at low pH. After 72 hr., the pH, lipase activity, and OD600 gradually increased in the system, which was due to the metabolism of degradation products by YCJ1. This process further confirmed that YCJ1 could utilize the degradation products.

2.5. SEM of PBAT films

Compared with the SEM image of PBAT before degradation (Fig. 3b), the surface of the PBAT films became rough after degradation, and there were obvious erosions and voids. Many strains and their secretions adhered to the films, indicating that the adhesion became stronger (Peixoto et al., 2016), which facilitated contact between YCJ1 and PBAT.

2.6. ATR-FTIR of PBAT films

Before degradation, the absorption peaks of the C–H asymmetric and symmetric stretching vibrations were found at 2964 cm⁻¹ and 2871 cm⁻¹, respectively, and the -C = O stretching vibration was found at 1715 cm⁻¹ (Fig. 3c). After degradation, the absorption peak of the C–H stretching vibration was significantly weakened. In addition, the absorption peak of the -C = O stretching vibration was weakened and the free carbonyl peak appeared at 1758 cm⁻¹. This was due to the cleavage of the ester bond between butanediol and terephthalic acid or adipic acid in the PBAT films. It could be seen that under the degradation of PBAT-degrading bacteria and the catalysis by its lipase, the structure of the PBAT films was broken.
2.7. 2.7 XRD of PBAT films

The diffraction peaks of (010), (111), and (100) reflections, observed at 2θ of 17.22’, 20.85’, and 23.23’, respectively, are the characteristic peaks of PBAT (Gan et al., 2004). The crystalline form of PBAT did not change significantly before and after degradation (Fig. 3d). After degradation, the intensity of the (010) and (100) peaks were enhanced and the intensity of the (111) peak decreased. First, the strain YCJ1 became attached to crystal faces on which attachment was easy (different crystal faces have different ability to attach bacteria) to degrade the non-crystalline region (Gan et al., 2005). Furthermore, the molecular chains in the crystalline region of the PBAT films were tightly arranged and regular, making them difficult to degrade by the strain YCJ1 and its lipase. Therefore, the non-crystalline region of the PBAT films was mostly degraded (Qu et al., 2017). Then, under the influence of the strain YCJ1, lipase and the crystal structure, the high-energy and unstable degradation region of PBAT films spontaneously recrystallized into a low-energy and stable degradation region (Zhang et al., 2012). In this process, the crystal surfaces with more bacteria attached were hindered by the bacteria and its enzymes, resulting in less spontaneous crystallization. On the contrary, there was more spontaneous crystallization on the crystal surfaces with less or no bacteria attached. Therefore, after degradation, the intensities of the peaks corresponding to different crystal faces increased and decreased to varying degrees.

2.8. 1H NMR of PBAT films

In Fig. 4, the signals at 4.408 and 4.178 mg/L corresponded to h and k, respectively, for the hydrogen atom on two methylene groups connected to an oxygen atom in 1,4-butanediol in the PBAT films. After YCJ1 degraded the PBAT films, the peak shapes were markedly weakened and the peak intensities were reduced. At the same time, other peaks for the PBAT films remained unchanged. This was because the lipase produced by YCJ1 could recognize and attack the ester bonds in the PBAT films to catalytically degrade PBAT (Aarthy et al., 2018; Nakajima-Kambe et al., 2009).

2.9. LC/MS of degradation solution

After degradation of PBAT, various degradation products such as oligomers and monomers appeared in the degradation solution (Appendix A Fig. S2), and they could be represented by a combination of monomers such as terephthalic acid (T), adipic acid (A), and 1,4-butanediol (B). After estimating and predicting the structure of the degradation products (Table 1) according to the method of (Shah et al., 2013), we could infer that the degradation of PBAT was caused by the breaking of the ester...
Fig. 3 – Degradation characteristics of PBAT films by YCJ1. (a) Changes in degradation rate of PBAT films, lipase, pH and OD600 during PBAT degradation, (b) SEM image of YCJ1, (c) ATR-FTIR spectrum of PBAT film, (d) XRD of PBAT films.

Fig. 4 – 1H NMR spectrum of PBAT films.
Table 1 – Degradation products identified by LC/MS during PBAT biodegradation.

<table>
<thead>
<tr>
<th>Number</th>
<th>Mass-to-change ratio (m/z)</th>
<th>Formula</th>
<th>Proposed structure: monomers</th>
<th>Proposed structure: oligomers</th>
<th>Representation</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>166.16</td>
<td>C₈H₄O₄</td>
<td>T</td>
<td></td>
<td></td>
<td>(1)</td>
</tr>
<tr>
<td>2</td>
<td>145.90</td>
<td>C₈H₁₀O₄</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>90.23</td>
<td>C₆H₁₀O₂</td>
<td>B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>238.85</td>
<td>C₁₂H₁₀O₃</td>
<td>TB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>218.09</td>
<td>C₁₀H₁₀O₃</td>
<td>AB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>310.28</td>
<td>C₁₀H₁₂O₄</td>
<td>BTB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>366.22</td>
<td>C₁₀H₁₂O₆</td>
<td>ABT</td>
<td></td>
<td></td>
<td>(2)</td>
</tr>
<tr>
<td>8</td>
<td>489.29</td>
<td>C₁₂H₁₂O₁₀</td>
<td>BABBAB</td>
<td>All oligomers are formed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>512.64</td>
<td>C₁₀H₉O₁₀</td>
<td>BABTB</td>
<td>by the</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>710.96</td>
<td>C₁₀H₉O₁₄</td>
<td>BABABTB</td>
<td>dehydration–condensation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>766.21</td>
<td>C₁₀H₉O₁₆</td>
<td>TBABABA</td>
<td>reaction of monomers.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>818.28</td>
<td>C₁₀H₉O₁₇</td>
<td>BABABABA</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

bonds inside the material. This further confirmed that the lipase could recognize the ester bonds in PBAT and hydrolyze PBAT into oligomers and monomers of different sizes, which was consistent with the results of ATR-FTIR and ¹H NMR analyses.

3. Discussion

In this study, a novel PBAT-degrading bacterium YCJ1 was isolated from farmland soil and identified as Stenotrophomonas. Previous studies had shown that Stenotrophomonas could degrade organic pollutants such as polyethylene (Mehmood et al., 2016; Peixoto et al., 2016), chlorosulfuron-ethyl (Zhang et al., 2016), chlorothalonil (Zhang et al., 2017), polycyclic aromatic hydrocarbons (Kuppusamy et al., 2016), and endosulfan (Ozdal et al., 2016). The degradation of PBAT by Stenotrophomonas was reported for the first time.

Furthermore, YCJ1 could degrade 10.14 wt.% (504 μg/(day·cm²)) of PBAT in 5 days, as calculated by the methods in (Muroi et al., 2017). Compared with the currently reported PBAT-degrading bacteria (Huo et al., 2017; Muroi et al., 2017; Xu et al., 2018), YCJ1 had a higher ability to degrade PBAT. However, this bacterium was more sensitive to environmental factors such as temperature and pH. Huo et al. (2017) reported that the degradation rate of a PBAT-degrading bacterium (Sphingopyxis ginsengisoli) in a liquid culture medium in 60 days was 0.92 wt.%. Muroi et al. (2017) reported that the degradation rate of PBAT by Bacillus pumilus at 30°C to 40°C was 12.2 μg/(day·cm²).

Through the degradation characteristics (Figs. 2 and 3), we could prove that YCJ1 could use the degradation products of PBAT as a nutrient source, which could considerably reduce the inhibitory effect of degradation products such as terephthalic acid on PBAT degradation. Thus, the degradation of PBAT was accelerated under chemical equilibrium (Yin et al., 2017), which could reduce the physiological toxicity of degradation products for the microorganisms and plants in the environment (Pang et al., 2015; Varaprasad et al., 2017).

In order to study why the degradation rate of PBAT by YCJ1 was very high, we analyzed the degradation products. From SEM images, the YCJ1 could colonize on the surface of the PBAT films and the YCJ1 were prone to collect in rough places on PBAT films (Bubpachat et al., 2018). Then the YCJ1 secreted lipase to quickly degrade it and form small holes. From the ATR-FTIR, ¹H NMR spectrum and LC/MS analyses, we could clearly see that the ester bonds in PBAT films were catalyzed by lipase from YCJ1 into multiple fragments (Judging by the ab-
At the free ester bonds and fragments of degradation products.

According to the above studies, the degradation pathway of PBAT could be analyzed. Apparently, the PBAT degradation pathway related to YCJ1 proceeded via the following steps (Fig. 5). First, the strain secreted PBAT-degrading lipase, which recognized the ester bond in PBAT and catalyzed its hydrolysis into oligomers and monomers of different sizes (Kasuya et al., 2009; Bubpatch et al., 2018). The oligomers were catalytically degraded into monomers such as terephthalic acid, adipic acid, and 1,4-butylen via repeated lipase-mediated degradation. Furthermore, the monomers could be transported by the transport protein of YCJ1 to the bacterial cells. After undergoing a series of gradual degradation reactions, these products finally participated in the tricarboxylic acid cycle, through which the monomers were rapidly metabolized into water and carbon dioxide (Wilkes and Aristilde, 2017). Thus, the degradation of PBAT was accelerated in a chemical equilibrium, which could reduce the physiological toxicity of the degradation products to microorganisms and plants in the environment. This indicated that YCJ1 has good application value in the bioremediation of PBAT in the environment.

Conclusions

We have reported for the first time that Stenotrophomonas sp., which could secrete lipase to catalyze the cleavage of ester bonds in the material and use the degradation products as carbon sources, could degrade PBAT. The degradation rate of PBAT by YCJ1 was better than those of previously studied bacteria. Stenotrophomonas sp. YCJ1 could be used in many fields such as environmental restoration.

Declaration of Competing Interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropri-ately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled, “Degradation of poly(butylene adipate-co-terephthalate) by Stenotrophomonas sp. YCJ1 isolated from farmland soil”.

Acknowledgments

This work was supported by the Research Fund at the Shaanxi Provincial Science and Technology Department of China (No. 2018SF-375) and Beijing Key Laboratory of Plastics Health and Safety Quality Evaluation Technology, Beijing Technology and Business University (No. QTETJP2018 004).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jes.2020.10.001.

REFERENCES


