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Biodegradation of poly(butylene succinate) by *Fusarium* sp. FS1301 and purification and characterization of poly(butylene succinate) depolymerase

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ABSTRACT

Fusarium sp. FS1301, a poly(butylene succinate) (PBS)-degrading strain, was isolated by screening oil-polluted soil. In this study, the biodegradation behavior of PBS films in the presence of *Fusarium* sp. FS1301 was investigated. Specifically, the characteristics of PBS films before and after degradation were analyzed. Differential scanning calorimetry and scanning electron microscopy results revealed that both the amorphous and crystalline regions of PBS were degraded by *Fusarium* sp. FS1301. PBS depolymerase was purified from the culture supernatant of liquid mineral medium with PBS emulsion. The molecular mass of the purified enzyme was determined to be 20 kDa by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The optimal pH and temperature for enzyme activity were 8.0 and 50 °C, respectively. PBS depolymerase maintained its activity from 20 °C to 50 °C and from pH 5.0 to 9.0. PBS depolymerase also significantly degraded poly(ε-caprolactone) but could not degrade poly(hydroxybutyrate) and poly(lactic acid). Na^+ and K^+ robustly promoted enzyme activity, whereas EDTA and β-mercaptoethanol significantly inhibited it. The main degradation products of PBS depolymerase were identified as 1, 4-succinate, succinate-butanediol, succinate-butanediol-succinate, and succinate-butanediol-succinate-butanediol by mass spectrometry. Furthermore, purified PBS depolymerase was found to be identical to cutinase from *Fusarium solani* by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

1. Introduction

Consumption of plastics synthesized using petroleum byproducts is continuously increasing, and most of such plastics cannot be degraded in the environment [1–3]. To solve this problem, biodegradable plastics are being promoted as environment-friendly substitutes. Understanding the mechanisms and optimal conditions for degradation of biodegradable plastics is, therefore, of great commercial and public health significance.

Biodegradable plastics can be degraded into H_2O and CO_2 in the environment; as such, these plastics do not produce “white pollution” and are environment-friendly [4–7]. Poly(butylene succinate) (PBS) is one of the most promising biodegradable aliphatic polyesters. Compared with common plastics, PBS not only has several good thermomechanical properties, but is also biodegradable and environmentally compatible [8,9]. For these reasons, PBS is widely used in the production of bottles, shopping bags, and agricultural materials [10,11].

Several studies on PBS degradability have reported the existence of PBS-degrading microorganisms. Ishii et al. found that the PBS-degrading fungus *Aspergillus fumigatus* degrades 80% of PBS after 30 days in soil [10]. Kitamoto et al. isolated two strains of *Pseudozyma antarctica* and reported that these strains degraded 60% and 20% of PBS on agar plates [12]. Uchida et al. cloned PBS depolymerase from *Acidovorax delafieldii* and found that PBS depolymerase showed similarities to lipases of *Streptomyces* and *Mollaxella* spp [13]. Maeda et al. purified a PBS-degrading enzyme from *Aspergillus oryzae* and identified the enzyme as cutinase, which degrades cutin, the waxy polymer covering the surfaces of plants [14]. These studies have shown that many microorganisms in the environment have the ability to degrade PBS. However, only a few studies have focused on the isolation of PBS-degrading strain and purification of PBS depolymerase. Therefore, isolation of PBS-degrading microorganisms, purification of the enzyme, and understanding of the degradation mechanism are of great importance.

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In the present study, *Fusarium* sp. FS1301, a PBS-degrading strain, was isolated and found to have high degradability of PBS. The degradation behaviors of *Fusarium* sp. FS1301 were characterized by analyzing PBS films before and after degradation. Specifically, PBS degradation rate was calculated and degraded PBS films were characterized. Furthermore, PBS depolymerase was purified and its molecular properties were documented.

2. Materials and methods

2.1. Chemicals and cultivation medium

PBS was obtained from Anqing He Xing Chemical Corp. Ltd. (Anqing, China). The number-average molecular weight of PBS was 150 000 to 210 000.

The cultivation medium was a mineral medium that contained PBS emulsion (0.1%, w/v) with or without agar (2%, w/v). PBS (0.1%, w/v) was emulsified by adding Plysurf A210G (0.01%, w/v) in 10 mM potassium phosphate buffer (pH 7.0) [15]. The mineral medium contained $MgSO_4 \cdot 7H_2O$ (0.5 g) NH_4Cl (1.0 g), $CaCl_2 \cdot 2H_2O$ (0.005 g), KH_2PO_4 (5.54 g), and $Na_2HPO_4 \cdot 12H_2O$ (11.94 g), pH 7.0, in 1000 mL deionized water. Unless otherwise stated, all chemicals used were of analytical grade.

2.2. Isolation and identification of PBS-degrading microorganisms

Oil-polluted soil samples from the LiaoHe Oil Field were transferred into liquid isolation medium cultivated at 30 °C and shaken (100 rpm) for 15 days. Then, a 1 mL culture of the solid isolation medium was cultivated at 30 °C for 5 days. Clear zone-forming colonies were purified via repeated transfer onto PBS mineral agar plates. The internal transcribed spacer sequences of the strains were sequenced by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China) and compared with those sequenced by the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov).

2.3. Degradation of PBS films by PBS-degrading microorganisms

Fusarium sp. FS1301 was cultivated in 250 mL flasks with 100 mL of liquid mineral medium containing PBS films (30 mm × 10 mm × 0.1 mm) as the sole carbon source and shaken (100 rpm) at 30 °C. After incubation for different durations, films were carefully collected and thoroughly washed with methanol and deionized water to remove any media components or mycelium that might be present on the surface of the films. Then, they were completely vacuum dried at 50 °C until a constant weight was obtained [16]. The surfaces of the predegraded and postdegraded PBS films were observed under a scanning electron microscope (XL30 ESEM FEG; FEI Co., Netherlands) at an accelerating voltage of 20 kV.

2.4. Characterization of poly(butylene succinate) (PBS) degraded films

The thermal behaviors of predegraded and postdegraded films were investigated by thermogravimetric analysis (TGA; Pyris-1, Perkin–Elmer, Waltham, MA, USA). All scans were carried out from 100 °C to 600 °C at a heating rate of 10 °C min^{-1} under nitrogen atmosphere. The thermal properties of the samples were analyzed using differential scanning calorimetry (DSC; Diamond, Perkin–Elmer USA). The predegraded and postdegraded films were tested by heating from 50 °C to 230 °C at a rate of 10 °C min^{-1} under nitrogen atmosphere.

2.5. PBS depolymerase activity

PBS depolymerase activity was tested as follows: 1 mL of the enzyme solution was added into 3 mL of PBS emulsion and incubated at 40 °C for 20 min. An ultraviolet spectrophotometer (2600 UV-VIS; UNICO, USA) was used to test the optical density ($OD_{630\ nm}$) before and after incubation. One unit of PBS-degrading enzyme activity was defined as a 0.001 OD decrease in absorbance per minute at 630 nm [17].

2.6. Purification of PBS-degrading enzymes

After cultivation of *Fusarium* sp. FS1301 in PBS-emulsified liquid medium at 30 °C for 5 days, the culture medium was centrifuged at 18 000 × *g* for 10 min. Five liters of cultivated suspension were concentrated via ultrafiltration using a membrane with a molecular weight cutoff of 10 kDa. The concentrated supernatant (total volume, 750 mL) was dialyzed against 20 mM potassium phosphate buffer (pH 7.0) and applied into a DEAE Sepharose Fast Flow column (1 cm × 20 cm) washed with the same buffer. A linear gradient of NaCl (0 M–1 M) was applied to wash the adsorbed proteins. The collected active fractions were dialyzed against potassium phosphate buffer (pH 7.0) and freeze dried. The freeze-dried enzyme powder was re-dissolved and applied into Sephadex G-100 (1.6 cm × 90 cm) columns. The collected active fractions were dialyzed against potassium phosphate buffer (pH 7.0). The purified PBS-degrading polymerase was combined and kept at −80 °C. All steps were performed at 4 °C.

The purified enzyme was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using a 12% gel. The gel was stained with Coomassie Brilliant Blue R-250 to locate the protein bands. A low molecular weight marker (Pharmacia Co., USA) was used as a protein standard. Protein concentrations were quantified using the BCA Protein Assay Kit (Walterson Biotechnology, Inc., China) [18].

2.7. Effects of pH and temperature on degradation activity of the purified enzyme

(1) Optimal pH and temperature: The optimal pH range of the purified enzyme was 4.0–10.0. The optimal temperature range of the enzyme was 30 °C–70 °C.
(2) Stability of enzyme activity at different pH: The purified enzyme was kept at different pH (4.0–10.0) for 24 h at 37 °C. The activity was tested under standard conditions.
(3) Thermostability of the enzyme: The purified enzyme was dissolved in potassium phosphate buffer (pH 7.0) and kept at 20 °C–80 °C for 1 h. The residual activity was tested under standard conditions. The standard conditions were as described in Section 2.5. The activity of the purified enzyme tested under standard conditions was considered to be 100%.

2.8. Activities of purified PBS depolymerase for various substrates

The purified enzyme was transferred into poly(lactic acid) (PLA) emulsion (0.1%, w/v), PHB emulsion (0.1%, w/v), and poly(ε-caprolactone) (PCL) emulsion (0.1%, w/v). The activity of the purified enzyme for PLA, PHB, and PCL was as described in Section 2.6.

2.9. Effects of metal ions and chemicals

Different metal ions (Na^+, K^+, Mg^{2+}, Ni^{2+}, Cu^{2+}, Fe^{2+}, and Zn^{2+}) and different chemicals (EDTA, β-mercaptoethanol, H_2O_2, and Tween 20) were added into PBS emulsion. The activity of the