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Isolation of a Marine Bacterium Capable of Biodegrading Poly(butylene succinate)

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Abstract

We developed a poly(butylene succinate) (PBS) indicator plate and isolated a marine bacterial colony capable of biodegrading PBS based on the appearance of a clear zone. Growth of the PBS-2 isolate was observed over 4 days of culture at 37°C in PBS-tryptone basal liquid medium, but not in PBS-deprived control medium. The PBS-2 isolate was named *Paenibacillus* sp. PBS-2 based on 16S rDNA gene sequencing. The PBS-biodegrading marine bacterium isolated in this study will contribute to the effective management of PBS waste problems in marine environments.

Key words: PBS indicator plate, Growth kinetics, 16S rDNA gene, PBS waste problems

Introduction

The ocean contains a vital stock of resources for future human generations, but the present environmental health of our coastal seas is being contaminated by marine litter. At present, many thousands of tons of derelict fishing gear and ropes are dumped into the water annually. The most hazardous materials to marine ecology are the synthetic nondegradable polymers used in fishing gear, fishing nets, ropes, and buoys; such items cause serious environmental pollution. Therefore, an urgent need exists to manufacture environmentally friendly fishing gear and aquaculture apparatuses from biodegradable materials that require no processing to make them safe for the environment.

To this end, we studied the characteristics of poly(butylene succinate), or PBS, a dicarboxylic acid that consists of succinic acid and glycerol produced from 1,4-butylenediol, itself obtained as an intermediate in esterification and condensation reactions. PBS not only has excellent intensity/strength, high crystallization, and a low rate of elongation/extension, it is also biodegradable (Fujimaki, 1998). Thus, PBS is currently widely used in vacuum casting, rope sheets, blow molding, promulgation, and net shooting for fishing apparatuses (Mohanty et al., 2000).

Based on the widespread use of PBS in the manufacture of fishing equipment, investigations on the biodegradation of PBS by microorganisms are necessary. To date, several studies have reported on PBS biodegradation by microorganisms, including *Bacillus* (Tansengco and Tokiwa, 1998); *Thermomonospora fusca* (Kleeberg et al., 1998); *Acidovorax delafieldii* (Uchida et al., 2000); *Actinomycetes* (Sakai et al., 2002; Tokiwa and Jarerat, 2003); *Bacillus pumilus* (Hayase et al., 2004); *Aspergillus oryzae* (Maeda et al., 2005); *Cryptococcus* sp. (Masaki et al., 2005); and *Aspergillus versicolor*, *Penicillium*, *Bacillus*, and *Thermopolyspora* (Zhao et al., 2005). The microbes used in these studies originated mainly from soil or compost samples, or were taken from established culture collections. In the present study, we isolated and identified an environmentally friendly PBS-degradative marine bacterium from marine sand samples. We also confirmed the PBS utiliza-

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tion kinetics of the microbe.

Materials and Methods

Polymer sample and chemicals

PBS (448028; Sigma, St. Louis, MO, USA) as a carbon source, tryptone (0123-17-3; Difco, Detroit, MI, USA), and yeast extract (212750; Difco) were used. All other chemicals were of analytical grade for biochemical use.

Marine sand samples, culture, and data analysis

Marine sand samples were collected from March to October 2009, in Gi-jang, Busan, Korea. All samples were suspended in distilled water, and the water and sand layers were separated by sedimentation. Next, the upper water layer was spread on PBS-tryptone basal agar plates and incubated at 20°C and 37°C, respectively. The bacterial isolate was cultured in PBS-tryptone basal liquid medium at 37°C with constant shaking at 200 rpm. Samples were taken at regular intervals to detect growth (measurement of the OD_{600}). The results were evaluated by analysis of variance carried out on triplicate data from each treatment.

Identification by 16S rDNA sequencing

Total genomic DNA was extracted from the isolate and 16S rDNA gene sequencing was carried out using the following universal primers: forward 9F (5′-GAG TTT GAT CCT GGC TCA G-3′) and reverse 1542R (5′-AGA AAG GAG GTG ATC CAG CC-3′) (Yoon et al., 1998). Sequence analysis and database similarity searches were done using the server at the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/BLAST/). A phylogenetic tree was constructed using the EzTaxon server (http://www.eztaxon.org/) (Chun et al., 2007).

Results and Discussion

To develop a PBS indicator plate, PBS powder (2 g/L) was used as a carbon source, and tryptone (100 mg/L) was used instead of yeast extract (100 mg/L), which was used in the basal medium, as described by Tansengco and Tokiwa (1998) (Table 1). The above emulsified medium (pH 7.2) was used as liquid medium, and agar (20 g) was added to produce a solid medium. The media were sterilized by autoclaving for 15 min at 121°C. We named the medium PBS-tryptone basal liquid or agar medium; the PBS-deprived medium was named tryptone basal liquid medium or PBS-deprived control medium. PBS-tryptone basal agar plates were used as indicator plates to screen for PBS-biodegradative bacterial colonies by the appearance of a clear zone, which was not observed on the PBS-yeast extract basal agar plates (see below).

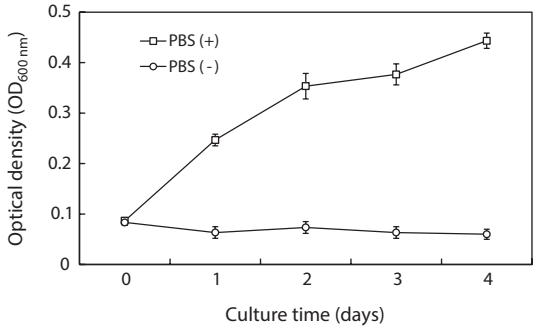

Fig. 1. Survival growth curves of the poly(butylene succinate) (PBS)-biodegrading bacterial strain. PBS-2 was cultured in PBS-tryptone basal liquid medium or PBS-deprived control liquid medium at 37°C for 4 days with constant shaking at 200 rpm. Samples were taken at regular intervals and diluted tenfold with the respective medium to assay growth (measurement of the OD_{600}). The results were evaluated by analysis of variance carried out on triplicate data from each treatment.

To isolate PBS-degrading strains, the prepared upper water layers of marine sand samples were spread on PBS-tryptone basal agar plates and incubated at 20°C and 37°C, respectively. After 11 days at 37°C, we observed a weak clear zone around the PBS-2 colony (data not shown). However, the strain did not show PBS-degrading activity at 20°C. The PBS-2 colony did not produce a clear zone on the PBS-yeast extract basal agar plates at 37°C.

To determine the growth kinetics, PBS-2 was inoculated into 2 mL of PBS-tryptone basal liquid medium and incubated overnight at 37°C with constant shaking at 200 rpm. The overnight culture was then inoculated (1%) into 50 mL of fresh

Table 1. Ingredient of the tryptone basal medium in the presence or absence of PBS as carbone source

Ingredient (/L)	PBS-tryptone basal medium	PBS-deprived tryptone basal medium
PBS, 2 g	+	-
Tryptone, 100 mg	+	+
$(NH_4)_2SO_4$, 1 g	+	+
$MgSO_47H_2O$, 200 mg	+	+
NaCl, 100 mg	+	+
$CaCl_22H_2O$, 20 mg	+	+
$FeSO_47H_2O$, 10 mg	+	+
$Na_2MoO_42H_2O$, 0.5 mg	+	+
$Na_2WO_42H_2O$, 0.5 mg	+	+
$MnSO_4$, 0.5 mg	+	+
K_2HPO_4, 1.6 g	+	+
KH_2PO_4, 200 mg	+	+

PBS, poly(butylene succinate).
+, positive; -, negative.

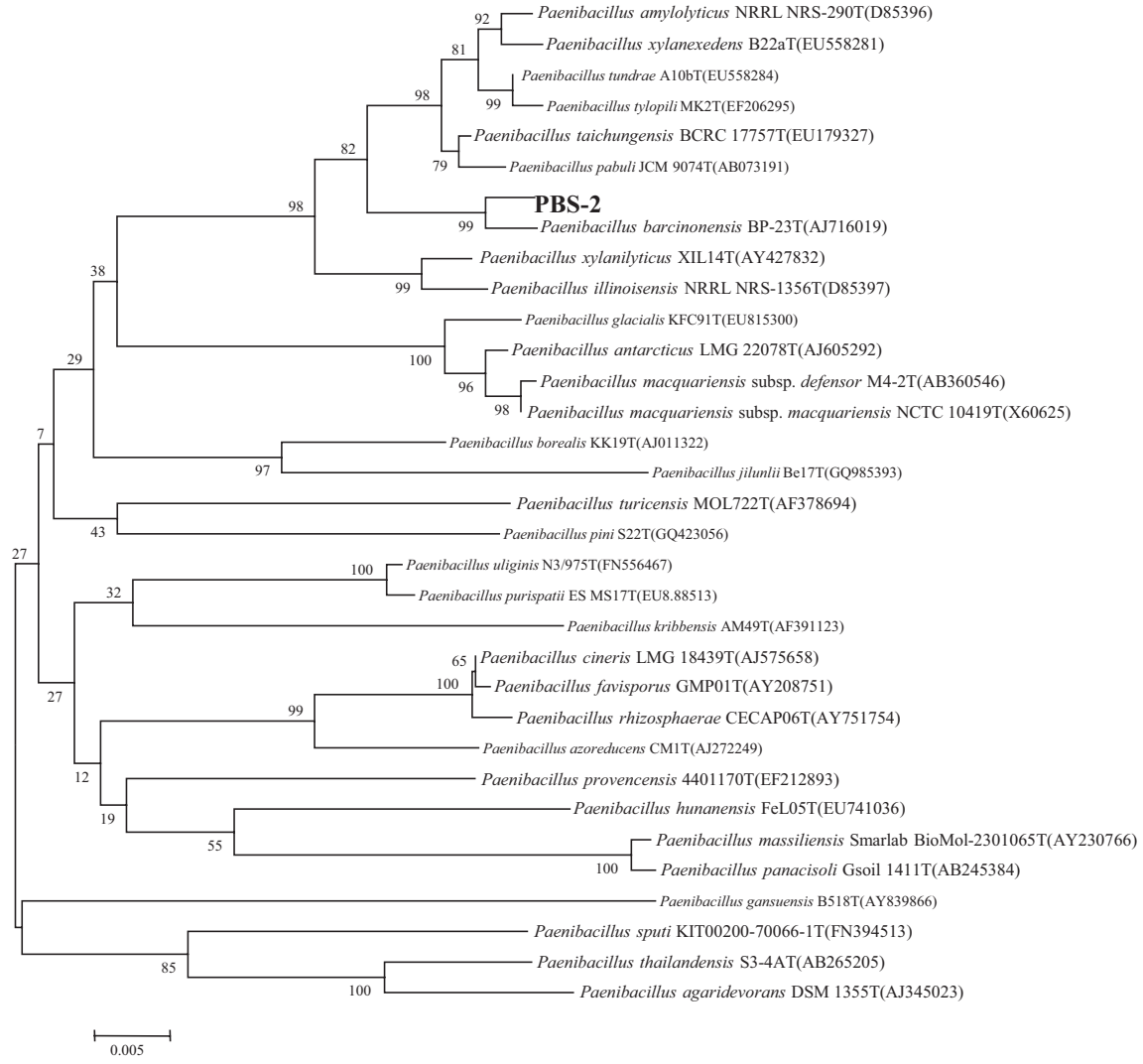

Fig. 2. Phylogenetic tree of the poly(butylene succinate) (PBS)-biodegrading bacterial strain. The 16S rDNA gene sequence of PBS-2 was deposited in the NCBI database under GenBank accession number JF502464. The branching patterns were generated by the neighbor-joining method on the basis of 16S rRNA gene homology, showing the positions of *Paenibacillus* sp. PBS-2 and related taxa. Bootstrap values (expressed as percentages of 1000 replications) of >50% are shown at the branch points. Scale bar = 0.005 substitutions per nucleotide position.

PBS-tryptone basal liquid medium or PBS-deprived control liquid medium and incubated at 37°C with constant shaking at 200 rpm. The medium was diluted tenfold with PBS-tryptone basal liquid medium and PBS-deprived control liquid medium, respectively, and the cell density was determined by measuring the OD_{600} at 24-h intervals (Fig. 1).

The data suggest that the growth of PBS-2 at 37°C reached the log phase after 1 day; the log phase continued up to the 4-day limit of observation, but the OD value did not increase in the PBS-deprived control medium (Fig. 1). The 4-day growth of PBS-2 in the PBS-tryptone basal liquid medium suggests that the degraded PBS carbon source was available for all 4 days of observation.

Next, to identify the PBS-2 strain, total genomic DNA was extracted from the PBS-2 isolate and 16S rDNA gene sequencing was carried out as described previously (Yoon et al., 1998). PBS-2 was 99.0%, 97.7%, and 97.5% similar to *Paenibacillus barcinonensis*, *Paenibacillus taichungensis*, and *Paenibacillus amylolyticus*, respectively; thus, we named it *Paenibacillus* sp. PBS-2. The PBS-2 sequence has been deposited in the NCBI database under GenBank accession number JF502464. A phylogenetic tree was generated using the PBS-2 16S rRNA sequence as described previously (Fig. 2) (Chun et al., 2007).

The PBS-tryptone basal agar plates developed in this study were useful for the screening and isolation of bacteria capable of biodegrading PBS; in comparison, no clear zone was produced on PBS-yeast extract basal agar plates. The growth kinetics in both fresh PBS-tryptone basal liquid medium and

PBS-deprived control medium showed clear evidence of PBS degradation by PBS-2. Therefore, the isolated *Paenibacillus* sp. PBS-2 from marine sand samples may be a useful marine PBS-biodegrading bacterium. Environmentally friendly marine bacteria capable of biodegrading PBS together with the recycling of PBS materials will play an important role in the effective management of PBS waste problems in marine environments.

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