Degradation of Poly(L-lactide) by a Mesophilic Bacterium

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Received 12 December 2007; accepted 6 September 2009
DOI 10.1002/app.31950
Published online 2 March 2010 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: A Gram negative, rod-shaped mesophilic bacterium active for poly(L-lactide) (PLA) degradation was isolated through the enrichment culture and clear-zone method. The isolated strain was identified to be *Bordetella petrii* PLA-3 on the basis of 16S rDNA gene sequence analysis. *B. petrii* PLA-3 was active not only for the degradation of low-molecular-weight PLA but also for the degradation of high-molecular-weight PLA. The strain seemed to attack the crystalline part of PLA as well as the amorphous region. The PLA film incubated in compost inoculated with the isolated strain lost its weight more notably and exhibited a lower molecular weight than that incubated in the sterilized compost without living microorganisms. Moreover, the profile of the cumulative amount of CO_2 after 20 days of burial in the sterilized compost and subsequent inoculation of the isolated strain into compost was nearly the same as that of CO_2 evolved from PLA buried in compost with the isolated strain at the very beginning when the time was shifted by 20 days. This indicated that not only the abiotic hydrolysis but also the microbial enzymes of the strain contributed to the initial chain cleavage of PLA molecules and resolved the doubt that PLA molecules should be initially cleaved into very low-molecular-weight substances by abiotic hydrolysis to be subsequently absorbed into and biodegraded by microorganisms. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 117: 67–74, 2010

Key words: biodegradable; degradation; polyesters; renewable resources; strain

INTRODUCTION

Petroleum-based synthetic plastics have been widely used because of their excellent physical properties and processability, together with their competitive price. However, the recalcitrant characteristics of plastics against degradation has provoked serious concerns with regard to environmental pollution.[1–5] Bioplastics can play a compensating role, together with recycling techniques, to reduce the accumulation of used plastics discarded in the environment.

Poly(L-lactide) (PLA) has drawn a lot of attention because of its biodegradability and biocompatibility. PLA can be produced from renewable resources, which is another important merit of PLA when one takes into consideration the depletion of fossil resources and global warming.[5–9] PLA has been used for niche applications such as sutures, bone fixation and drug-delivery systems.[6,7,10] However, the recent development of fermentation and polymer technologies makes PLA ready to replace many general-purpose plastics.

PLA-degrading microorganisms are not ubiquitous at all, and so far only a limited number of microorganisms have been reported to degrade PLA. Most of them are actinomycetes, such as *Amycolatopsis* spp. and *Saccharothrix* spp.[1,6,7] Jarerat et al.[8] revealed PLA-degrading microorganisms belonging to *Pseudonocardiaceae* spp., including *Amycolatopsis* spp., *Saccharothrix* spp., *Lentzea* spp., *Kibdelosporangium* spp., and *Streptoalloteichus* spp., through the examination of their phylogenetic position. *Fusarium moniliforme* and *Penicillium roqueforti* were also reported as PLA degraders.[11] PLA-degrading bacteria are much fewer in number compared to actinomycetes, and thereby, only four thermophilic bacteria, namely, *Geobacillus thermocatenulatus*,[9] *Bacillus smithii*,[3] *Bacillus licheniformis*,[12] and *Bacillus brevis*,[2] and one mesophilic bacterium, namely, *Paenibacillus amylolyticus*,[10] have been reported as PLA degraders so far.

The biodegradation of PLA will be an important topic in a near future because the demand of PLA is expected to increase enormously and because the degradation of PLA buried in soil is extremely slow because of the limited number of PLA degraders.

In this study, the isolation of mesophilic bacteria was attempted. Mesophilic bacteria are more useful than thermophilic ones because the degradation of PLA takes place at ambient conditions when it is buried in soil or in reclamation sites. The effects of the molecular weight and crystallinity on the biodegradation rate were also investigated. Changes in the mechanical properties were examined as a function of the biodegradation time in the presence and in the absence of the isolated strain.

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EXPERIMENTAL

Materials

PLAs with weight-average molecular weights (M_w's) of 5000, 11,000, and 34,000 were synthesized, whereas PLA with a M_w of 256,000 g/mol was obtained in pellet form from NatureWorks (Minneapolis, MN).

The synthesis of PLA was carried out as follows: L-lactic acid (200 mL) was added to a 500-mL, three-necked reactor. To remove water, the reactor was immersed in an oil bath at 100°C with a nitrogen flow for 1 h. The reactor was heated to 180°C under mechanical stirring at 300 rpm. Esterification was proceeded by the addition of titanium(IV) butoxide (0.08 mL) as a catalyst for 3 h, and then, we evacuated the reactor by reducing the pressure to 1 Torr step by step. The polycondensation reaction was followed at 1 Torr and 180°C for different reaction times. The PLA produced from the condensation polymerization of L-lactic acid was in the melt state. Because the glass reactor was not equipped with an extruder, instead of extruding the PLA melt, we dissolved the PLA lump in chloroform, and then, the solution was poured into a large excess of methanol. The resulting precipitated PLA was dried, ground into powder, and washed with methanol several times to remove the residual L-lactic acid monomer or oligomer. The polycondensation reaction for 8, 22, and 40 h yielded PLAs with M_w's of 5000, 11,000, and 34,000 (P5000, P11000, and P34000), respectively.

The molecular weight of the PLAs was determined with a Waters 410 detector gel permeation chromatograph (Milford, MA) with tetrahydrofuran as the solvent and with polystyrene as the standard. A 0.3–0.4% (w/v) solution was used at a flow rate of 1.0 mL/min. A commercially available PLA was obtained from NatureWorks with M_w and number-average molecular weight (M_n) values of 256,000 and 155,000 g/mol, respectively.

Culture medium

The composition of the enrichment medium for cultivation was as follows: $[(NH_4)_2SO_4]$ = 4 g/L, $[K_2HPO_4]$ = 2 g/L, $[KH_2PO_4]$ = 1 g/L, $[MgSO_4{\cdot}7H_2O]$ = 0.5, and [yeast extract] = 0.06 g/L. The final pH was adjusted to 7.0.[2,9]

The composition of the basal medium was as follows: [PLA powder] = 1 g/L, $[K_2HPO_4]$ = 2.34 g/L, $[KH_2PO_4]$ = 1.33 g/L, $[MgSO_4{\cdot}7H_2O]$ = 0.2, $[(NH_4)_2SO_4]$ = 1, [NaCl] = 0.5, and [yeast extract] = 0.06 (pH 7.0). The basal medium was supplemented with 1 mL of a trace element solution containing 11.9 mg of $CoCl_2$, 11.8 mg of $NiCl_2$, 6.3 mg of $CrCl_2$, 15.7 mg of $CuSO_4$, 0.97 g of $FeCl_3$, 0.78 g of $CaCl_2$, and 10.0 mg of $MnCl_2$ per liter of distilled deionized water.

The preparation of emulsified PLA agar plate for screening of PLA-degrading bacteria was as follows: emulsions were prepared with a detergent (Plysurf A210G). PLA (1 g) was dissolved in 40 mL of dichloromethane. The PLA solution was poured into 1000 mL of a basal medium containing 0.1 g of the detergent and blended with an ultrasonicator (280 W, 90 min). After emulsification, dichloromethane was removed by devolatilization at 80°C for 3 h.[4]

For a solid medium with the emulsified polymer, agar (15 g/L) was added to the basal medium.[4,10]

Isolation of the PLA-degrading microorganisms

The 60 soil samples, collected from various sites in Korea, were used to screen PLA-degrading bacteria. The soil sample (10 g) and distilled water (100 mL) were mixed for 10 min; then, the suspension solution was allowed to settle for 30 min. The supernatant was inoculated (0.5 mL) into the enrichment medium (10 mL).[13] About 50 mg of PLA was added to the enrichment medium and incubated at 37°C in a rotary shaker (120 rpm , Wisd, Seoul, Korea). We made subcultures four times by taking 0.5 mL of the original culture and inoculating it into fresh medium. The enriched culture broth was spread onto emulsified PLA (1 g/L) agar plates. PLA-degrading microorganisms were isolated from the colonies forming a clear zone on the agar plate with emulsified PLA. The isolates were stored at −80°C.[10]

Identification of the PLA-degrading strain

The morphology of the PLA degrading microorganism was examined with a JEOL (Tokyo, Japan) model JSM-5600LV scanning electron microscopy (SEM) microscope. Before the analysis, the sample was coated with gold to protect the sample morphology against the electron beam.

16S rDNA was amplified by with the universal primers forward distal1 (fD1) and reverse proximal 2 (rP2).[14] Then nucleotide sequences were determined with an Applied Biosystems 3100 sequencer (Foster, CA). The sequences were aligned together with those of representative members of selected genera with the CLUSTAL W program (Heidelberg, Germany).[15] The evolutionary tree for the data sets was inferred from the neighbor-joining method of Saitou and Nei[16] with MEGA version 3.1.[17] The stability of the relationships was assessed by bootstrap analyses of the neighbor-joining data on the basis of 1000 trials.

Biodegradation test of the PLAs with different molecular weights

The compost was prepared according to our previous article.[12] The biodegradation tests in compost with the laboratory-scale reactor were conducted according to KS M3100-1 : 2002 and MOD ISO 14855 : 1999.[18] The air flow rate was controlled at 40 mL/min. The mature compost was sterilized at 121°C for 30 min and dried at 105°C for 24 h. After sterilization, the compost (10 g) was added to sterilized distilled water (100 mL) and mixed vigorously. The supernatant was spread onto plate count agar, nutrient agar, actinomycete isolation agar, and Sabouraud dextrose agar and incubated at 37°C for 3 days. Neither bacteria nor actinomycetes nor fungi grew on the agar plates; this indicated that the compost was completely sterilized.

A mixture of the mature compost (200 g, wet weight) inoculated with 10^9 cfu/g of the isolated strain and the PLA sample as a test material (5% on dry basis) was introduced and incubated at 37°C. The moisture content was controlled at 65%. CO_2 produced from the reactor was absorbed by 0.4*N* potassium hydroxide and 2*N* barium chloride solutions, and the amount was determined by titration with 0.2*N* hydrochloric acid.

Biodegradation test of the PLAs with different crystallinities

PLA films with crystallinities of 0, 18, and 42% were prepared, and biodegradation tests were performed at 30°C in the sterilized compost inoculated with the isolated strain. The biodegradation tests were carried out at a temperature as low as possible, and 30°C was chosen rather than 37°C to minimize the crystallization, if any, during the tests. A PerkinElmer DSC 7 instrument (Waltham, MA) was used to measure the heat of fusion of the PLA samples. The crystallinity was determined with the heat of fusion of a perfect PLA crystal assumed to be 93 J/g.[19] The temperature and energy readings were calibrated with indium at each cooling rate used. The sample weight was kept at 5.5 ± 0.1 mg.

Burial degradation test of the PLA films

Dumbbell-shaped PLA specimens 0.2 mm thick were buried at 37°C in the sterilized compost and in the compost inoculated with the isolated strain after sterilization, and then, the weight loss and decrease in the molecular weight were monitored as a function of incubation time. The surface morphology of the PLA films after the incubation was examined with a scanning electron microscope (JEOL model JSM-5600LV). The tensile properties were measured with a universal testing machine (Instron 4665 ultimate tensile testing machine, Norwood, MA) at 20°C and under 30% humidity. The crosshead speed was set at 10 mm/min. At least 10 specimens were tested, and the results were averaged.

RESULTS AND DISCUSSION

It is well known that the biodegradation of PLA takes place extremely slowly because of the limited number of microorganisms capable of PLA degradation in nature. Moreover, most of the PLA degrading microorganisms reported so far in the literature have been thermophilic and, thus, have only been active at relatively high temperatures.

Because PLA molecules can be cleaved by abiotic hydrolysis and by microbial action, there has been some doubt whether PLA should be first fragmented into very low-molecular-weight substances by abiotic hydrolysis to be absorbed and biodegraded subsequently by microorganisms. With thermophilic microorganisms, the contribution of microbial action to the PLA chain scission cannot be allotted out and evaluated unambiguously because, aside from the microbial enzymatic hydrolysis of PLA, abiotic hydrolysis proceeds very quickly at the incubation temperature in the case of thermophilic microorganisms.

The isolation of mesophilic bacteria is very interesting, not only because the degradation of discarded PLA in the natural environment proceeds at ambient conditions but also the lower incubation temperature for mesophilic bacteria compared to that of thermophilic ones enables clearer differentiation of the microbial action on the PLA degradation from the contribution of the abiotic hydrolysis by reduction of the abiotic hydrolysis rate of PLA.

It is extremely rare to isolate mesophilic bacteria capable of PLA biodegradation. However, fortunately enough, we succeeded in isolating a mesophilic bacterium active for PLA biodegradation. Taking advantage of the mesophilic nature of the strain, we carried out biodegradation of PLA in the presence and in the absence of the isolated mesophilic bacterium to clarify doubt about whether PLA should be first fragmented into very low-molecular-weight substances by abiotic hydrolysis to be subsequently biodegraded by the microorganism.

Isolation of the PLA-degrading mesophilic bacterium

A mesophilic bacterium able to degrade PLA was isolated from the soil samples collected from 60 different locations in Korea. The isolated strain was a Gram-negative, rod-shaped bacterium whose SEM image is exhibited in Figure 1. Analysis of the 16S rDNA gene sequence indicated that the isolated

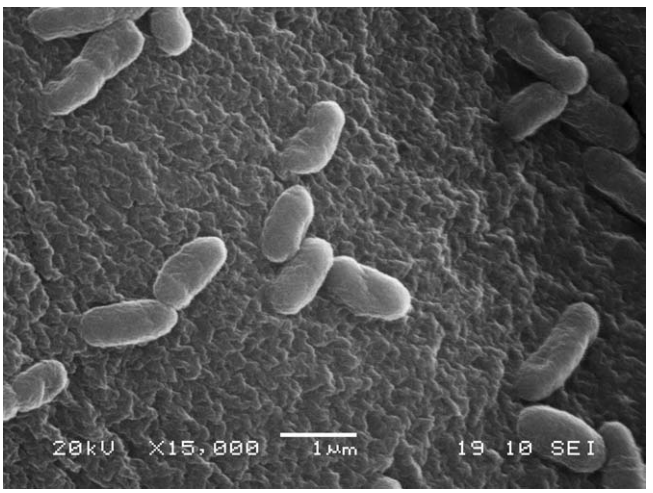

Figure 1 SEM micrograph of the isolated PLA degrading strain (15,000×).

strain possessed 92% similarity with *Bordetella petrii*. The phylogenetic tree was drawn up, as shown in Figure 2, on the basis of the 16S rDNA coding gene sequence analysis and the nearest relatives. Hence, this strain was identified to be *B. petrii* PLA-3, and the nucleotide sequence of the 16S rDNA gene was deposited in the GenBank nucleotide sequence database under accession number EF442019.

B. petrii has so far been known to be active for the degradation of aromatic compounds. According to Wang et al.,[20] *B. petrii*, isolated from a soil contaminated with chlorinated benzenes, mineralized 1,2,4-trichlorobenzene. Bianchi et al.[21] reported that the same bacterium, isolated from industrial composts, was capable of the bioremediation of toluene and naphthalene.

Microorganisms capable of PLA degradation are not ubiquitous at all, and thus, only a limited number of bacteria have been isolated. That is, only *Bacillus brevis*,[2] *Geobacillus thermocatenulatus*,[9] *Bacillus smithii*,[3] *Bacillus licheniformis*,[12] and *Paenibacillus amylolyticus*[10] have been reported to be able to degrade PLA. Most of the isolated bacteria are thermophilic in nature, and thereby, only the last strain is a mesophilic bacterium, and the first four strains are thermophilic ones. Mesophilic bacteria are more useful than thermophilic ones for PLA degradation in the natural environment because the former ones should mostly be in charge of the degradation of PLA buried in reclamation sites and in other soils. Therefore, the isolation of mesophilic bacteria capable of PLA degradation is valuable from industrial and social points of view and from an academic one.

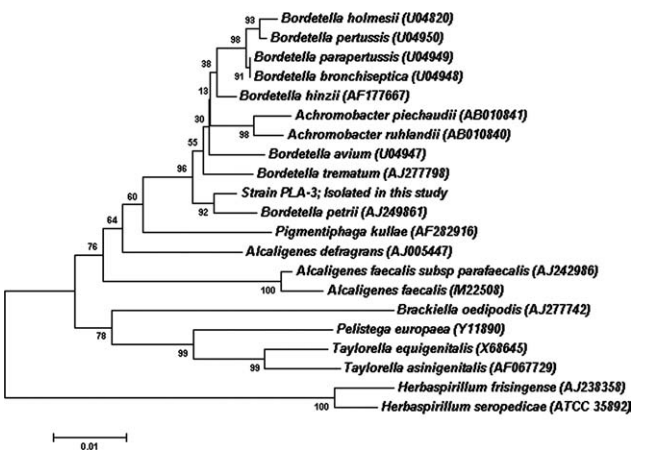

Figure 2 Phylogenetic position of the isolated PLA-degrading bacterium.

The PLA-degrading strains isolated so far belonged to *Bacillus* spp., whereas the strain isolated and identified in this study, *B. petrii* PLA-3, appertained to *Burkholderiales* spp. Therefore, *B. petrii* PLA-3 can be said to be a new strain possessing PLA degradation activity under ambient conditions because it has little phylogenetic similarity with the other PLA-degrading bacteria reported in the previous literatures.

Biodegradation of PLAs with different molecular weights

The activity of the isolated strain for PLA degradation was assessed in compost under controlled conditions by measurement of the net amount of CO_2 evolved from compost loaded with PLAs of different molecular weights, as disclosed in Figure 3. At 37°C, the isolated strain degraded not only low-molecular-weight PLA but also PLA with a M_w as high as

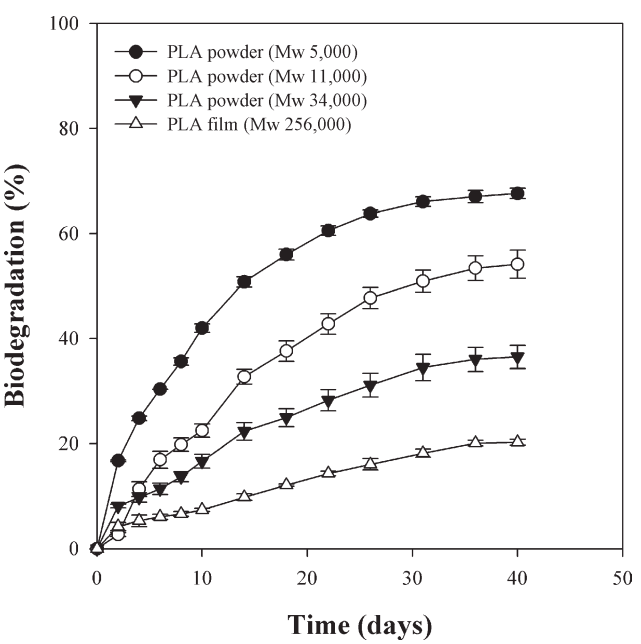

Figure 3 Biodegradation of PLAs at 37°C in controlled compost inoculated with the isolated bacterium after sterilization as a function of the molecular weight of PLA: M_w = (●) 5000, (○) 11,000, (▼) 34,000, and (▽) 256,000.

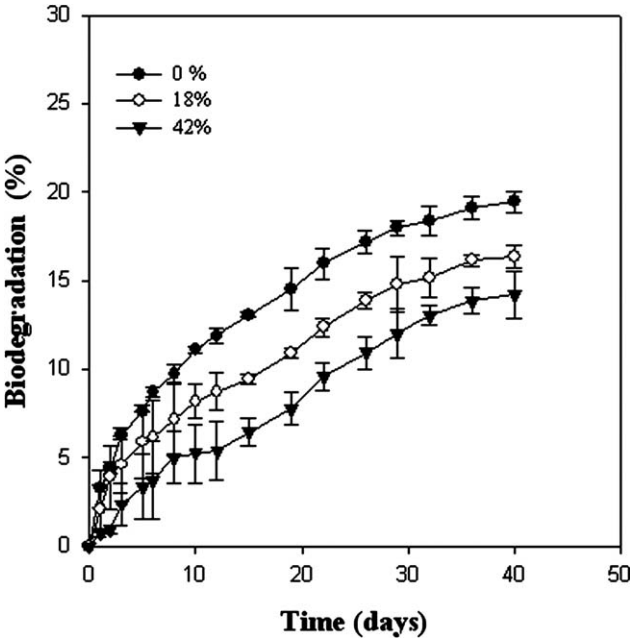

Figure 4 Biodegradation of PLAs with different crystallinities at 30°C in compost inoculated with the isolated bacterium after sterilization: initial crystallinity of PLA = (●) 0, (○) 18, and (▼) 42%.

256,000. After 40 days of biodegradation in compost at 37°C, 68, 54, and 37% of the carbons in the PLAs were mineralized into CO_2 for P5000, P11000, and P34000, respectively. In the same context, 19% of the carbons in the PLA with a M_w of 256,000 were converted into CO_2 during the same period of time.

The PLAs with M_w's of 5000, 11,000, and 34,000 were powdery in shape, whereas the PLA with a M_w of 256,000 was in film shape about 0.2 mm thick. All the samples could not be prepared as films because the former three PLAs were too brittle to maintain a film shape. However, all four kinds of PLA samples equally possessed 0% crystallinity.

Yang et al.[22] investigated the dependence of the biodegradation rate of PLA on the sample size by performing biodegradation tests in compost at 58°C. After 45 days of testing, 53 and 40% of the PLAs were biodegraded when the PLAs were in powder and film shapes, respectively.

The profile of the accumulated amount of CO_2 usually exhibits an initial induction period during the biodegradation test of PLA in compost, and a sigmoidally shaped profile is observed. In contrast, the CO_2 profile in Figure 3 demonstrates a very short induction period. This is because *B. petrii* PLA-3 was accustomed to the PLA degradation, and the corresponding enzyme was already induced in the cells of the strain. Further studies are needed for the identification of the PLA degradation enzymes to compare them with those of other mesophilic bacteria, such as *Paenibacillus amylolyticus*.[23]

The biodegradation rate decreased as the M_w of PLA increased from 5000 to 34,000, even though all of these PLA samples were powdery in shape; this indicated that the higher the molecular weight of PLA was, the slower the biodegradation rate was. Karjomaa et al.[24] explored the effect of molecular weight on the biodegradation rate of PLA and observed that the biodegradation rate decreased as M_n increased from 260 to 2880. Teeraphatpornchai et al.[10] investigated the PLA degradation activity of *Paenibacillus amylolyticus* as dependent on the molecular weight of the polymer by measuring the clear-zone size on an agar plate emulsified with PLA. The clear zone was larger than 5 mm when the M_w's of the PLAs were 5000 and 10,000. However, the clear-zone diameter decreased to 3–5 mm when the M_w's of the PLAs were 15,000 and 20,000.

Biodegradation of the PLAs with different crystallinities

PLA with a M_w of 256,000 was heated to 200°C and then annealed under different conditions to prepare PLAs with different crystallinities. Figure 4 shows the biodegradation behavior of the PLAs at 30°C in compost inoculated with *B. petrii* PLA-3 after sterilization. Because the crystallization of polymers takes place extremely slowly at temperatures lower than their glass-transition temperature, the initial crystallinity of PLA is believed to be almost preserved at 30°C (the glass-transition temperature of PLA is 55°C). PLAs with initial crystallinities of 0, 18, and

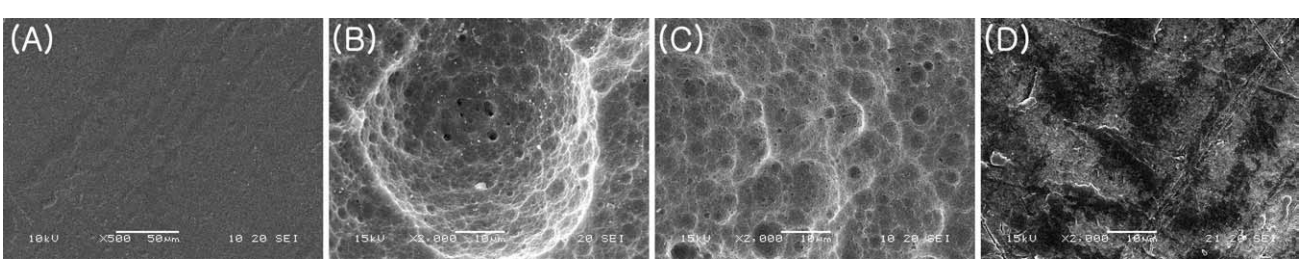

Figure 5 SEM micrographs of PLA films (M_w = 256,000) after 40 days of degradation at 30°C in compost inoculated with the isolated bacterium after sterilization (2000×): (A) before incubation and with initial crystallinities of (B) 0, (C) 18, and (D) 42%.

TABLE I
Crystallinity and Molecular Weight of PLA Before and After Incubation at 30°C in Compost

Initial crystallinity (%)[a]	Final crystallinity (%)[a]	M_w ($\times 10^4$)	M_n ($\times 10^4$)
—	—	25.6[b]	15.5[b]
0[c]	0[c]	12.1[c]	6.4[c]
18[c]	20[c]	17.9[c]	10.6[c]
42[c]	43[c]	19.2[c]	11.5[c]
18[d]	19[d]	23.0[d]	13.9[d]

[a] The crystallinity was determined from the DSC thermogram with a heat of fusion of the perfect PLA crystal assumed to be 93 J/g.[23]
[b] Virgin PLA sample.
[c] Degradation for 40 days in compost inoculated with *B. petrii* PLA-3.
[d] Degradation for 20 days in sterilized compost followed by degradation for another 20 days after inoculation with *B. petrii* PLA-3.

42% showed 19, 16, and 14% biodegradability, respectively, after 40 days of biodegradation; this confirmed that the lower the crystallinity was, the faster the biodegradation rate was. Moreover, the PLA degradation activity of *B. petrii* PLA-3 at 30°C was almost the same as that at 37°C, as compared in Figures 3 and 4 for the biodegradation of the PLAs with 0% crystallinity at the respective temperatures.

Figure 5 exhibits SEM images of the PLA samples. The PLA film with an initial crystallinity of 0% was eroded most significantly, whereas only a slight trace of erosion was observed on the surface of the same film but with an initial crystallinity of 42%.

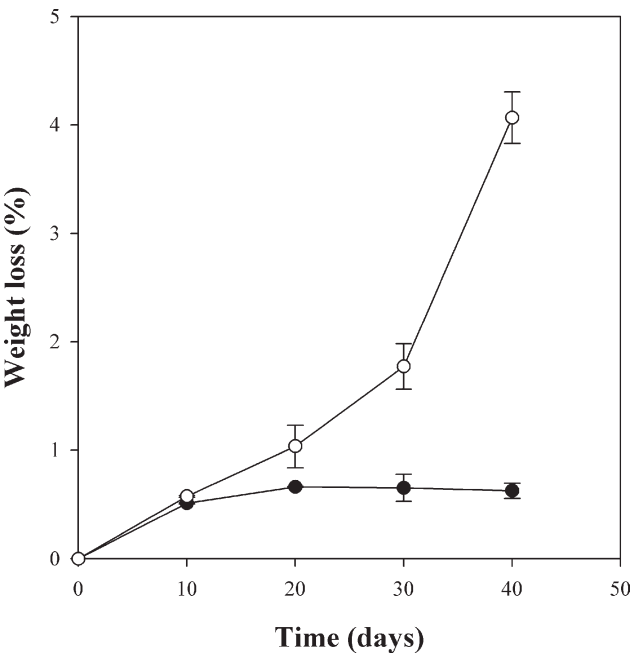

Figure 6 Percentage of weight loss in PLA films (M_w = 256,000, initial crystallinity = 0%) (○) buried at 37°C in compost inoculated with the isolated strain after the sterilization and (●) buried in sterilized compost.

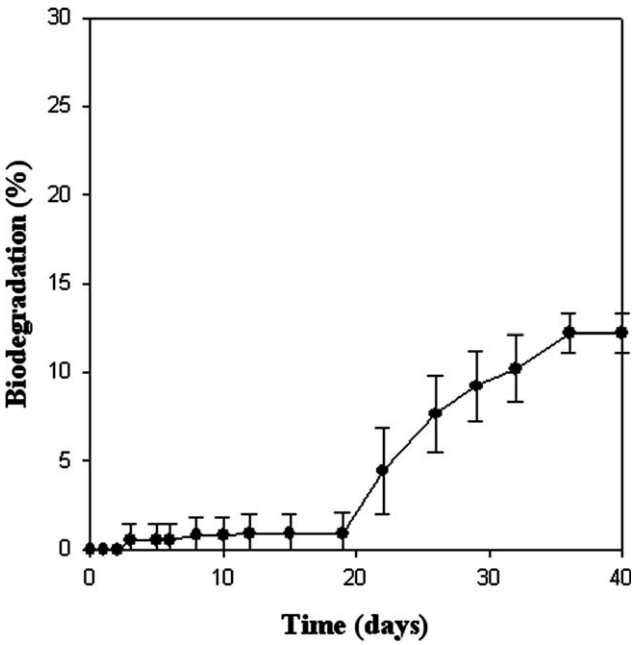

Figure 7 Biodegradation of PLA with an initial crystallinity of 18% at 30°C. The PLA sample was buried in sterilized compost for 20 days and then 10^9 cfu/g of *B. petrii* PLA-3 was inoculated into compost.

Table I summarizes the crystallinity and molecular weight of the PLA samples before and after biodegradation. When the initial crystallinity was 0%, it was preserved to be 0% even after 40 days of biodegradation; this confirmed the extremely slow crystallization rate of PLA under the test conditions. In line with the dependence of the biodegradation rate on the crystallinity of PLA on the basis of the cumulative amount of CO_2, the decrease in the molecular weight of PLA became faster as the PLA crystallinity decreased.

It is commonly accepted that the degradation of polymers occurs preferentially in the amorphous region rather than in the crystalline one. However, according to the results in Table I, the biodegradation did not result in a notable increase in the crystallinity; this indicates that the loosely stacked PLA crystallites gave way to degradation as did the amorphous PLA.

Degradation of the PLA films

PLA molecules should be cleaved into small molecules to be assimilated into the microbial cells. Therefore, abiotic hydrolysis of PLA is believed to precede the mineralization of the carbonaceous small-molecular-weight hydrolysis products into CO_2 by microorganisms during biodegradation. It has not been clarified whether the initial cleavage of PLA molecules is entirely due to abiotic hydrolysis or due also to microbial enzymes secreted out to

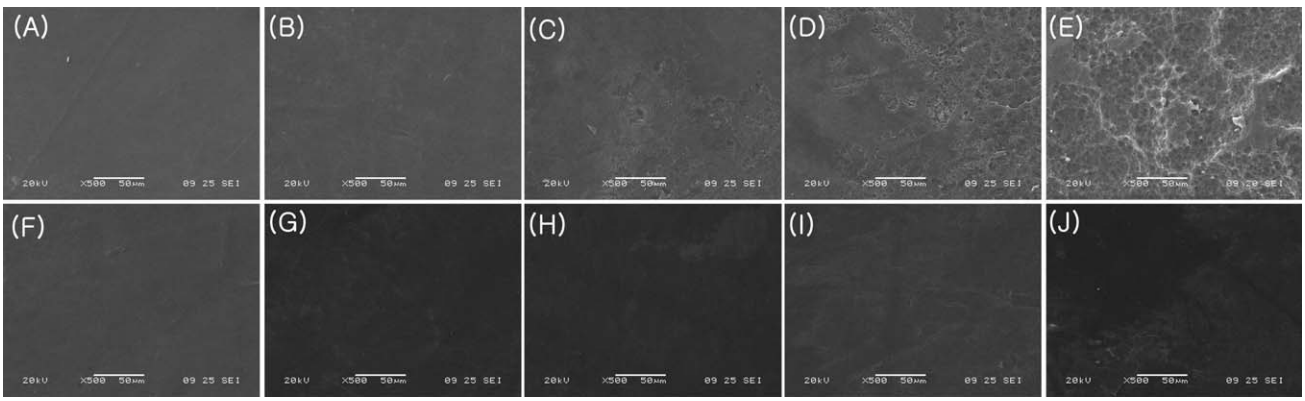

Figure 8 SEM micrographs of PLA films (M_w = 256,000, initial crystallinity = 0%) (A–E) buried at 37°C in compost inoculated with the isolated strain after sterilization and (F–J) buried in the sterilized compost (500×). Incubation time = (A,F) 0, (B,G) 10, (C,H) 20, (D,I) 30, and (E,J) 40 days.

further enhance the chain cleavage rate. To distinguish the contribution of the abiotic hydrolysis on the PLA chain scission from the enzymatic action, the weight loss of the PLA film samples (M_w of PLA = 256,000, initial crystallinity = 0%) buried at 37°C in the sterilized compost and in the compost inoculated with *B. petrii* PLA-3 after sterilization was monitored as a function of time, as shown in Figure 6. The weight loss was 0.5% after 40 days of incubation in the sterilized compost, whereas it was 4% in compost inoculated with the strain after sterilization.

The contribution of the abiotic hydrolysis of PLA to the biodegradability could also be guessed from the results shown in Figure 7. The PLA samples used for the experiments for Figure 7 had 18% initial crystallinity. The amount of CO_2 evolved for 20 days was almost negligible when the sample was buried in the sterilized compost. Moreover, the profile of the cumulative amount of CO_2 after 20 days of burial in the sterilized compost and the subsequent inoculation of 10^9 cfu/g of the isolated strain into compost was similar to that of PLA with the same initial crystallinity (18%) but buried in the sterilized compost inoculated with the isolated strain at the very beginning, as demonstrated in Figure 4, when the time was shifted by 20 days.

Figure 8 compares the morphology of the surface of the PLA films (M_w of PLA = 256,000, initial crystallinity = 0%) buried at 37°C for 40 days in the sterilized compost and in compost inoculated with *B. petrii* PLA-3 after sterilization. The surface of the latter PLA film exhibited a rough and somewhat eroded morphology, whereas the former PLA film preserved the initial surface morphology almost invariably.

The deterioration of tensile strength and elongation at break occurred faster in the PLA film buried in the compost inoculated with *B. petrii* PLA-3 after sterilization in comparison to that in the PLA film buried in the sterilized compost, as summarized in Table II. This was more evidence of the faster degradation of PLA in the presence of the PLA degrader.

CONCLUSIONS

A new PLA-degrading mesophilic bacterium was isolated through the enrichment culture and clear-zone method. The isolated strain was a Gram-negative,

TABLE II
Molecular Weight and Tensile Properties of the PLA Film with an Initial Crystallinity of 18% Buried at 37°C in Compost Inoculated with the Isolated Strain After Sterilization in Comparison to Those of the PLA Film Buried in Sterilized Compost

Compost type	Incubation time (days)	M_w ($\times 10^4$)	M_n ($\times 10^4$)	Elongation at break (%)	Tensile strength (MPa)
Inoculated with *B. petrii* PLA-3	0	25.6	15.5	3.80	48.1
	10	24.0	15.0	3.58	51.5
	20	23.0	13.9	2.37	39.1
	30	21.5	12.0	2.85	38.5
	40	19.0	10.5	1.44	28.9
Sterilized	0	25.6	15.5	3.80	48.1
	10	25.0	15.5	3.28	47.4
	20	24.4	14.2	3.23	45.1
	30	23.3	13.5	2.97	45.3
	40	23.0	11.0	2.58	41.1

rod-shaped one, and it was identified to be *B. petrii* PLA-3 on the basis of 16S rDNA gene sequence analysis. The isolated strain was active not only for the degradation of low-molecular-weight PLA but also for the degradation of a commercially available high-molecular-weight PLA. The biodegradation rate decreased with increasing crystallinity of PLA. However, not only the amorphous region but also the crystalline part of PLA was attacked by the microbial enzymes. The PLA film incubated for 40 days in the sterilized compost lost 0.5% of its initial weight, whereas that incubated in compost inoculated with the isolated strain after sterilization showed a 4% weight loss. Moreover, M_w of the latter PLA decreased more than that of the former PLA. The amount of CO_2 evolved for 20 days was almost negligible when the PLA samples were buried in the sterilized compost, whereas the profile of the net amount of CO_2, as a result of inoculation thereafter of the isolated strain into the compost, was similar to that of PLA buried in the sterilized compost inoculated with the isolated strain from the very beginning. These results clearly demonstrate that the PLA chains were cleaved by the microbial enzymes aside from the abiotic hydrolysis. The action of the microbial enzymes on the PLA degradation lowered the tensile strength and elongation at break of the PLA film more rapidly than the action of the abiotic hydrolysis alone.

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