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Biodegradation and Mineralization of Polystyrene by Plastic-Eating Mealworms: Part 2. Role of Gut Microorganisms

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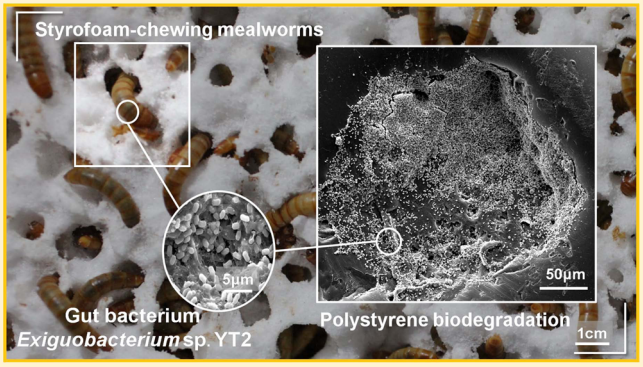
Supporting Information

ABSTRACT: The role of gut bacteria of mealworms (the larvae of *Tenebrio molitor* Linnaeus) in polystyrene (PS) degradation was investigated. Gentamicin was the most effective inhibitor of gut bacteria among six antibiotics tested. Gut bacterial activities were essentially suppressed by feeding gentamicin food (30 mg/g) for 10 days. Gentamicin-feeding mealworms lost the ability to depolymerize PS and mineralize PS into CO_2, as determined by characterizing worm fecula and feeding with ^{13}C-labeled PS. A PS-degrading bacterial strain was isolated from the guts of the mealworms, *Exiguobacterium* sp. strain YT2, which could form biofilm on PS film over a 28 day incubation period and made obvious pits and cavities (0.2−0.3 mm in width) on PS film surfaces associated with decreases in hydrophobicity and the formation of C−O polar groups. A suspension culture of strain YT2 (10^8 cells/mL) was able to degrade 7.4 ± 0.4% of the PS pieces (2500 mg/L) over a 60 day incubation period. The molecular weight of the residual PS pieces was lower, and the release of water-soluble daughter products was detected. The results indicated the essential role of gut bacteria in PS biodegradation and mineralization, confirmed the presence of PS-degrading gut bacteria, and demonstrated the biodegradation of PS by mealworms.



INTRODUCTION

Global production of petroleum-based plastic has grown 200-fold from 1.5 million tons in 1950 to 299 million tons in 2013.[1,2] Tremendous consumption of plastic has produced large amounts of plastic waste, which has aroused global environmental concern.[2−7] Polystyrene (PS), which is a common petroleum-based plastic with Styrofoam (expanded PS foam) as a major product, showed an annual global production of approximately 21 million tons in 2013.[1] Although styrene monomers[8,9] and oligomers[10] are susceptible to biodegradation, PS is generally considered non-biodegradable as a result of its high molecular weight and highly stable structure.[11] Using a ^{14}C-labled PS tracer, previous investigations have shown that the rate of PS biodegradation in different microbial consortia, such as soil, sewage sludge, decaying garbage, or manure, ranged from 0.01 to less than 3% within 4 months.[12−14] A few bacteria isolated from soil were found to be capable of colonizing PS film surfaces,[15,16] and researchers have claimed success in the isolation of PS-degrading microorganisms, such as *Rhodococcus ruber*.[15] However, no convincing evidence has been provided that these isolates were deposited in any culture collection center or changed the physical and chemical properties of PS.

Some mandibulate insects and stored-product insect pests found in storehouses and kitchens are able to chew and eat plastic packages of grain.[17−21] In our companion paper (10.1021/acs.est.5b02661), we reported that the larvae of *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae), which are commonly known as mealworms, are able to chew and eat Styrofoam. Analyses of fecula egested showed that the cleavage of long-chain PS molecules and the formation of low-molecular-weight metabolites occurred in the gut. On the basis of the carbon balance of the ingested Styrofoam and ^{13}C-tracer tests, the ingested PS was mainly mineralized to CO_2

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(47.7%), with a small fraction incorporated into lipid biomass (10.1021/acs.est.5b02661).

The midgut lumen of the mealworm harbors a variety of microorganisms that play an important role in the digestion of refractory food.[22] The number of cultivable microorganisms in the gut of each mealworm is approximately $5-6 \times 10^5$ colony-forming units (CFU). Gut microbial populations are influenced by food. Antibiotics, such as nystatin and ampicillin, can suppress gut microbiota.[22] Thus, if gut microbiota are essential for PS biodegradation, the suppression of gut microbiota by antibiotics will negatively impact PS degradation. The isolation of PS-degrading bacteria from the gut of the mealworm is another approach to establish the essential role of microbiota in PS degradation. As described elsewhere, we recently isolated two polyethylene (PE)-degrading bacterial strains, *Bacillus* sp. YP1 and *Enterobacter asburiae* YT1, from the PE plastic-eating waxworm gut and identified the presence of PE-degrading microorganisms in this environment.[20,21]

In this study, we attempted to determine whether antibiotic suppression of gut microorganisms would impair the capability of the mealworm for PS biodegradation and mineralization and to isolate PS-degrading bacterial strains from the mealworm gut to understand the role of mealworm gut microbiota in PS biodegradation.

MATERIALS AND METHODS

Test Materials and Medium. Detailed information regarding test materials is described in our companion paper (10.1021/acs.est.5b02661). Styrofoam feedstock was obtained from SINOPEC Beijing Yanshan Company, Beijing, China. Both α ^{13}C-labeled and β ^{13}C-labeled PS were purchased from Sigma-Aldrich, St. Louis, MO. To prepare PS film for microbial degradation, a Styrofoam sample was dissolved in xylene solvent (0.03 g/mL). The solution was then spread on a glass plate. After 5 h, the formed films were taken off the glass plate and fixed in a hood at room temperature for 3 days. The films were then rinsed with methanol solvent, followed by deionized water, and dried again prior to use. The thickness of the films prepared was approximately 0.02 mm. According to a gas chromatographic (GC) analysis, no residual xylene was detected in the PS film. The PS film was cut into 50 × 50 mm square sheets for the growth of bacterial biofilm on an agar plate and small 3 × 3 mm pieces for the growth of bacterial suspension in a liquid medium.

Mealworms (the larvae of *T. molitor* Linnaeus) were purchased from Daxing Insect Breeding Plant, Beijing, China.

Liquid carbon-free basal medium (LCFBM) was prepared with deionized water and contained (per 1000 mL) 0.7 g of KH_2PO_4, 0.7 g of K_2HPO_4, 0.7 g of $MgSO_4 \cdot 7H_2O$, 1.0 g of NH_4NO_3, 0.005 g of NaCl, 0.002 g of $FeSO_4 \cdot 7H_2O$, 0.002 g of $ZnSO_4 \cdot 7H_2O$, and 0.001 g of $MnSO_4 \cdot H_2O$ according to the ASTM standard for determining the resistance of plastics to bacteria (ASTM G22-76).[23] LCFBM was used for the enrichment of PS-degrading microorganisms and characterization of the degradation of PS films. Carbon-free basal agar medium (CFBAM) was prepared by adding 15 g of agar to 1000 mL of LCFBM. The Luria-Bertaini (LB) medium was prepared with deionized water containing (per 1000 mL) 10 g of bacteriological tryptone, 5 g of yeast extract, 10 g of NaCl, and 15 g of agar. The liquid nutrient broth (NB) medium was prepared by dissolving 3 g of beef extract, 10 g of bacteriological tryptone, and 5 g of NaCl in 1 L of deionized water. The pH of the NB medium was adjusted to approximately 7.0. Saline water (SW) was prepared by dissolving 8.5 g of NaCl (0.85%, w/v) with 1 L of deionized water, and the pH was adjusted to 7.2. All media were sterilized by autoclaving at 121 °C for 30 min.

Antibiotic Suppression Treatment Assay. The morphology of the microorganisms in the midgut contents of mealworms was observed by an environment scanning electron microscope (ESEM, Quanta FEG250, FEI Company, Hillsboro, OR).

A group of 15 mealworms fed with Styrofoam as a sole diet for 2 weeks was collected. The surfaces of the worms were sterilized by immersion in 75% ethanol for 1 min and then rinsed 2 times with sterile SW. Next, their guts were drawn out and pooled in a 10 mL centrifuge tube containing 5 mL of SW. After being shaken on a vortex mixer for 5 min, the gut tissues were carefully removed with a pipet. The gut suspension was prepared for a subsequent antibiotic screening test.

Six different antibiotics, including ampicillin, chloramphenicol, erythromycin, gentamicin, tetracycline, and vancomycin, were screened for their ability to inhibit mealworm gut bacteria based on the inhibition halos test (Figure S1 of the Supporting Information) described in the literature.[24] The gut suspension (100 μL) was inoculated and spread across a LB medium plate. Subsequently, the discs of six antibiotics (30 μg of antibiotics per disc) were placed on the surface of the inoculated plates. Discs containing no antibiotic served as controls. The plates were incubated in triplicate for 24 h at 30 °C, and then the inhibition halos were measured. The antibiotic generating the largest inhibition halo was selected to prepare the antibiotic diet for suppressing gut bacteria.

Three groups of mealworms (400 in each group) were fed with the selected antibiotic diet (30 mg/g of bran food) for 10 days, whereas the other three control groups were fed with normal bran without antibiotic. At 0, 3, 7, and 10 days, 15 mealworms were randomly collected from each group to prepare a gut suspension, as described above. The suppression of gut bacteria was assessed on the basis of the results of the bacterial numbers estimated by the series dilution method of plate counting.[24] After bacterial number counting on day 10, the remaining antibiotic-treated mealworms were subsequently fed with Styrofoam and ^{13}C-labeled PS according to the previous procedure and their fecula was collected for physical and chemical characterization. CO_2 released was collected for a stable isotopic test (10.1021/acs.est.5b02661).

Isolation of PS-Degrading Microorganisms. A group of 50 mealworms fed with Styrofoam as a sole diet for 2 weeks was collected to prepare a gut cell suspension as an inoculum of PS-degrading bacterial enrichment, as described above. This suspension was transferred to a 250 mL Erlenmeyer flask that contained 1 g of small PS pieces and 80 mL of LCFBM. This flask was incubated on a rotary shaker (120 rpm) at ambient temperature. After 60 days, the residual PS pieces were removed, and the enrichment was spread across plates with LB agar. After incubation for 24 h at ambient temperature (22–24 °C), the colonies were picked and spread to other plates with fresh LB agar medium, where they were kept until pure colonies of isolates were obtained on the basis of observations of the morphologies of colonies formed on the same plate and microscopic examinations of cell morphology.

Preliminary Screening of Isolates for PS-Degrading Ability. The bacterial isolates were grown in the NB medium for 12 h. The cells were then collected via centrifugation (10 000 rpm) and rinsed with SW to remove residual medium.

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This procedure was repeated 3 times. Next, the collected cells were resuspended in SW to obtain a cell suspension with a concentration of approximately 10^8 cells per mL. The melted sterile CFBAM (15 mL) was poured into a glass Petri dish (90 mm diameter) and cooled to ambient temperature. The cell suspension (0.5 mL) was spread homogeneously across the surface of the CFBAM plate, which was subsequently covered with a PS film (50 × 50 mm). In the uninoculated controls, the PS films were added without the inoculation of the cell suspension. CFBAM plates inoculated with the cell suspension without the added PS film were used as controls to determine whether the isolates could grow on agar alone. All plates were incubated at ambient temperature (22−24 °C) and 85% relative humidity for 28 days. Three plates were prepared for each isolated strain. Growth on PS was preliminarily determined by observing the formation of a biofilm and counting the number of the isolate grown on the PS film surface after 28 days. The numbers of bacteria that had grown on the PS film surface were counted using the previously described procedure.[20] The isolate, which had a high cell number on the PS film surface, was targeted for further characterization.

Fluorescence Microscope and Scanning Electron Microscope (SEM) Observation of Biofilm. The biofilm of the isolated strain on the PS film sheet was examined using a SEM (Quanta FEG250, FEI Company, Hillsboro, OR).[20] The viability of the bacterial cells was characterized *in situ* using a fluorescence microscope (BX51, Olympus, Tokyo, Japan) after staining with the LIVE/DEAD *Bac*Light Bacterial Viability Kit.[20,25,26]

Analysis of Surface Properties of PS Film. To examine the changes in the physicochemical properties of PS film, the biofilm on the PS film sheet on the CFBAM plate was completely removed by mixing the biofilm with 2% (w/v) aqueous sodium dodecyl sulfate (SDS) for 4 h and then rinsing it with deionized water according to the washing procedure reported by Sivan et al.[26] Following treatment, no cells were observed on the surface of the PS film sheet. The PS film sheets in the uninoculated control were also treated using the same procedure. Afterward, the change in the hydrophobicity of the PS film sheet surface was determined by measuring the water contact angle (WCA) using a contact angle measuring device (OCA40, DataPhysics, Filderstadt, Germany). Surface chemical components were investigated using X-ray photoelectron spectroscopy (XPS, Thermo Scientific, Waltham, MA). During the XPS spectra analysis, scanning was carried out over a broadband energy range (0−1200 eV) at an electron takeoff angle of 90° from the sample areas less than 1 mm in diameter. The overlapping peaks in the C 1s region were resolved into their individual components using a peak-fitting program (XPSPEAK, version 3.0).

Biodegradation Assays. PS biodegradation by the isolated bacterial strain was characterized by weight loss and molecular weight shifts after 60 days of incubation in the LCFBM. The cell suspension of strain YT2 was prepared by resuspending the collected cells grown on the NB medium with SW, as described above. PS pieces (100 mg) and cell suspension (10 mL) were added to a 150 mL Erlenmeyer flask with 40 mL of LCFBM. The inoculated cell concentration was approximately 10^8 cells per mL. A flask without inoculation served as the uninoculated control. Both flasks were incubated on a rotary shaker (120 rpm) at ambient temperature. At the end of a 60 day incubation period, the residual PS pieces were collected, washed according to the previously described procedure of Sivan et al., and dried at ambient temperature for the measurement of residual weight.[20,26]

The washed residual PS pieces were randomly sampled for a molecular weight distribution analysis using gel permeation chromatography (GPC, V2000, Waters, Milford, MA). To determine the daughter products of PS degradation, the liquid culture of strain YT2 harvested was centrifuged at 10 000 rpm for 15 min and the supernatant was filtered via a 0.22 μm membrane filter. The filtrate was freeze-dried and then redissolved in 0.1 mL of dichloromethane. The water-soluble daughter products were identified by a gas chromatograph/mass spectrometer (GC/MS, Agilent 5975, Palo Alto, CA) equipped with a DB-5 capillary column. The oven temperature was first held at 50 °C for 1 min, then increased to 250 °C at 10 °C/min, and held at 250 °C for 5 min.

Sequencing and Phylogenetic Analysis. The genomic DNA needed for 16S rDNA amplification of the isolated strains was extracted from cells grown in the late log phase using a conventional proteinase K treatment and phenol−chloroform extraction. Amplification of the 5′ end of the gene was performed with the universal primers 8-F (5-AGAGTTTGA-TYMTGGCTCAG-3′) and 1942-R (5′-GGTTACCTTGT-TACGACTT-3′). The obtained sequences were aligned with organisms present in the GenBank database using the Basic Local Alignment Search Tool (BLAST) created by the National Center for Biotechnology Information, Bethesda, MD. The sequence of our isolated strain was deposited in GenBank.

RESULTS AND DISCUSSION

Effect of Antibiotics on the Activity of Gut Bacteria. A great number of bacterial cells with various morphotypes (cocci and short and long rods) inhabited the midgut content of the Styrofoam-eating mealworm, as observed by ESEM (Figure 1a). This observation indicated that the midgut of the mealworm did harbor a diversity of microorganisms.[22]

The results of the antibiotic screening test indicated that 3 of the 6 tested antibiotics were able to inhibit the growth of gut bacteria in the LB medium with the formation of clear inhibition halos (Figure S1 of the Supporting Information). The effective antibiotics were ampicillin, gentamicin, and tetracycline. Among these antibiotics, gentamicin showed the best ability to inhibit the growth of gut bacteria, with clearer and broader halos (Figure S1 of the Supporting Information). Gentamicin is well-known as a bactericidal antibiotic that works by irreversibly binding the 30S subunit of the bacterial ribosome, interrupting protein synthesis. Consequently, gentamicin was selected for the suppression of mealworm gut bacteria.

When mealworms were fed with gentamicin-containing bran (30 mg/g of bran), gut bacteria were significantly suppressed on the basis of the estimation of gut bacterial numbers by the series dilution method of plate counting (Figure 1b). After 10 days, there were no viable bacterial colonies in the LB medium plate inoculated with gut suspension, indicating that the level of gut bacteria was too low to grow in the LB medium.

Subsequently, these gentamicin-treated mealworms were tested for their PS-degrading capability. The gentamicin-treated and control (untreated) mealworms were fed with Styrofoam, and the molecular weights of their fecula were analyzed. In comparison to the molecular weight of the feedstock Styrofoam (M_n = 40 430, and M_w = 124 200), the M_n (32 260) and M_w (98 330) of the fecula of the control mealworms without

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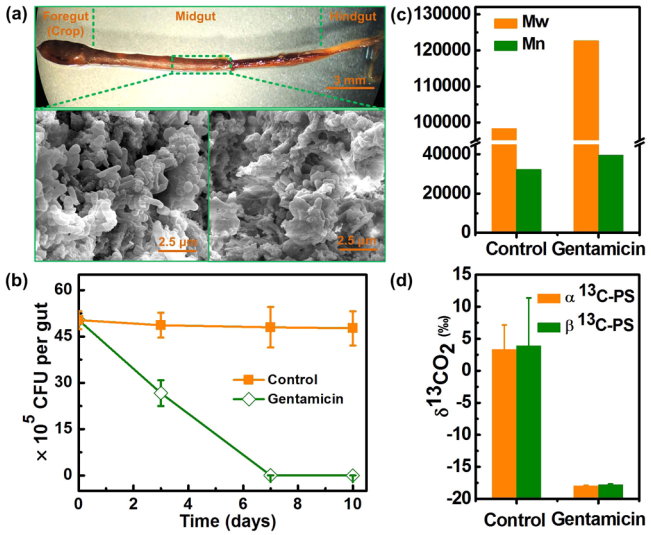

Figure 1. Contribution of gut bacteria to Styrofoam degradation. (a) Optical photographs of gut structure and ESEM images of gut microorganisms that grew in the midgut of Styrofoam-eating mealworms. (b) Number of total visible bacteria extracted from mealworms fed with gentamicin decreased over a 10 day incubation period. Gentamicin at 30 mg was mixed with 1 g of bran as antibiotic food, whereas antibiotic-free bran served as the control. (c) Weight-average molecular weight (M_w) and number-average molecular weight (M_n) of ingested Styrofoam after passage through the gut of gentamicin-treated mealworms or untreated control mealworms. (d) δ ^{13}C values of CO_2 were produced by gentamicin-treated mealworms or untreated control mealworms fed with α ^{13}C- and β ^{13}C-labeled PS after 16 days [t test; $p < 0.001$; mean ± standard deviation (SD); $n = 3$ groups for each condition; 40 mealworms as one group].

gentamicin treatment (Figure 1c) dropped significantly by 8170 and 25 870, respectively. However, the fecula of the gentamicin-treated mealworms had a M_n of 39 620 and a M_w of 122 650 (Figure 1c), which were decreased insignificantly by only 810 and 1550, respectively. This result indicated that the suppression of gut microbiota by gentamicin impaired the ability of the mealworms to depolymerize PS.

On day 10, the gentamicin-treated mealworms were also fed with jelly food containing $\alpha^{13}C$- or $\beta^{13}C$-labled PS for 16 days. CO_2 in the off air was collected for the analysis of ^{13}C. The results showed that the gentamicin-treated worms did not produce ^{13}C-enriched CO_2, whereas the untreated control did (t test; $p < 0.001$; Figure 1d). This result indicated that the suppression of gut bacteria impaired the ability of the mealworms to mineralize PS.

Preliminary Screening of PS-Degrading Isolates. A total of 13 bacterial cultures were isolated by picking up colonies formed from the enrichment of PS-eating mealworm guts (Table S1 of the Supporting Information). Afterward, we characterized biofilm formation on the PS film sheets to screen the culture for potential PS biodegradation because the formation of a biofilm enables microorganisms to use efficiently non-soluble substrates, as described previously for the characterization of PE-degrading bacterial strains.[20,25,26] Accordingly, screenings for potential PS-degrading bacteria among these isolated cultures were performed in terms of the number of the cells in the biofilm colonized on the PS film in the CFBAM plates (Figure S2 of the Supporting Information).

Among the 13 isolates, one non-spore forming Gram-positive bacterium was the most abundant ($9.3 \pm 0.3 \times 10^8$ CFU/cm^2) when grown as a biofilm on the PS film sheet (Figure S2 of the Supporting Information). This strain was taxonomically identified as *Exiguobacterium* sp. strain YT2 based on its 16S rRNA sequence (Figure S3 of the Supporting Information) and, consequently, was selected as a candidate of potential PS degraders for further study. The sequence of strain YT2 has been deposited in GenBank under reference KP731587. The *Exiguobacterium* sp. strain YT2 has been deposited at the China General Microbiological Culture Collection Center (CGMCCC 10521).

Formation of Biofilm on the PS Film Surface by Isolated Bacterial Strain YT2. On the CFBAM plates (insets of panels a and b of Figure 2), bacterial strain YT2 could grow

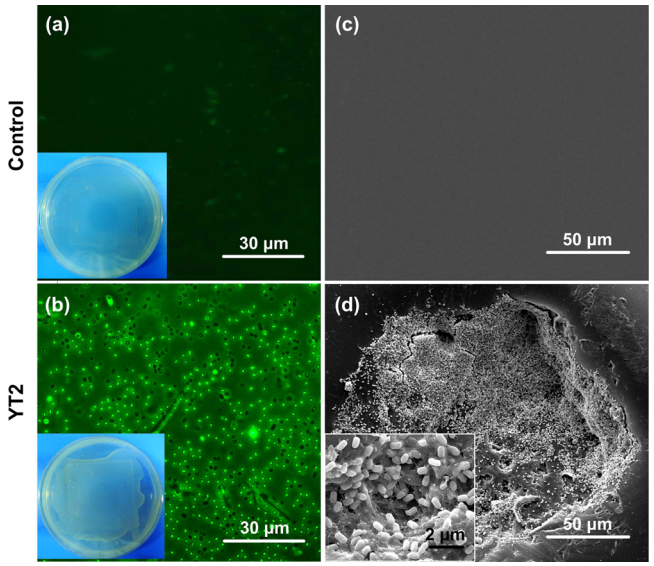

Figure 2. Biofilm formation and deterioration of PS film surface topography after a 28 day incubation with strain YT2. (a and b) Fluorescent microscopic images of biofilm show the presence of active cells after a 28 day incubation. Live cells are green, and dead cells are red. The PS sheets were covered in CFBAM. In the insets of panels a and b, the formation of colonies on the PS film sheets on CFBAM plates was observed but no colonies were present on the uninoculated control. (c and d) SEM observations of the physical surface topography of the uninoculated control versus the PS film incubated with strain YT2 after 28 days.

on the PS film by forming opaque colonies visible to the naked eye. No visible colony occurred on the uninoculated control CFBAM plates with a PS film without inoculation of strain YT2, and no visible colony appeared on the surfaces of the control CFBAM plates inoculated with strain YT2 cells without a PS film. This observation indicated that strain YT2 did grow on the PS film but did not grow on the agar medium. The viability of cells in the biofilms grown on the PS film was further examined using a fluorescence microscope after staining with the LIVE/DEAD *Bac*Light Bacterial Viability Kit.[20,25,26] After a 28 day incubation, live cells with green color dominated the biofilm, whereas only a limited number of dead cells (red color) were observed under fluorescent light (panels a and b of Figure 2). The predominance of live cells in the biofilm indicated that these cells may be capable of receiving sufficient metabolized substrates from the PS film for growth via degradation of the PS film.

Changes in Physicochemical Surface Properties. SEM observation (panels c and d of Figure 2) showed that the

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biofilm of strain YT2 generated obvious surface deterioration, with the formation of pits and cavities on the surface of the PS film. The surface of the uninoculated control was smooth and did not have any defects. The typical cavities on the surface of the PS film had a maximum width of approximately 200 × 200 μm (Figure 2d). This result indicated that strain YT2 is capable of degrading the PS film and damaging the PS physical integrity.

The water contact angle (WCA) was used to analyze changes in surface hydrophobicity. After the formed biofilm was completely removed from the PS film samples, the WCA of the surface of the PS film inoculated with strain YT2 was 80.8 ± 3.0° (n = 5), which was much lower than the WCA of the uninoculated control (95.8° ± 1.6°; n = 5; Figure 3a). This

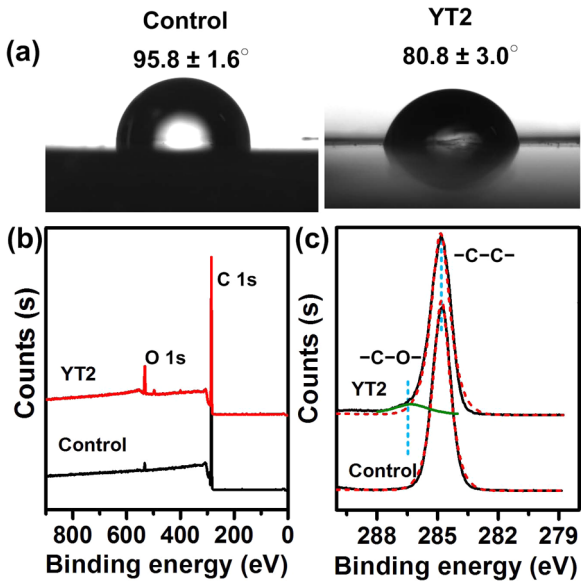

Figure 3. Surface chemical analysis of the PS samples of the uninoculated control and the samples inoculated with strain YT2 after a 28 day incubation. (a) WCAs of the PS films inoculated with the strain YT2 decreased in comparison to the WCAs of the uninoculated control, indicating a decrease in surface hydrophobicity. (b) XPS scanning and (c) C 1s spectra of the uninoculated control and the residual PS films inoculated with strain YT2.

result indicated that the formation of the biofilm by strain YT2 also decreased the hydrophobicity of the tested PS samples. This decline in hydrophobicity would reduce resistance to subsequent degradation by bacterial cells. A similar observation was reported previously in characterizing two PE-degrading bacterial strains.[20]

X-ray photoelectron spectroscopy (XPS) was used to analyze changes in surface chemical components and functional groups. Figure 3b shows the XPS scanning spectra (0−900 eV) for the PS film incubated with strain YT2 versus the uninoculated control. In the uninoculated control, only surface carbon (284.8 eV) and a limited amount of oxygen (532.3 eV) were observed. The spectrum of the PS sample with strain YT2 showed that the amount of oxygen increased significantly, whereas the amount of carbon appeared constant. A comparison of the XPS spectra of C 1s on the PS film surface inoculated with strain YT2 versus the uninoculated control (Figure 3c) demonstrated that the peak-fitting result of C 1s for the uninoculated control showed only one peak at 284.8 eV, which was assigned to a −C−C− group. For the strain YT2-incubated samples, in addition to the peak at 284.8 eV, another peak appeared at 286.5 eV and was assigned to the −C−O− group, implying oxidation to alcohol- and carboxylic-acid-like compounds.[27] The O/C ratios of the strain YT2-incubated samples were remarkably higher than the O/C ratios of the uninoculated controls (0.10 versus 0.02). The uninoculated controls showed 100% relative abundances of −C−C− group peaks and did not have any −C−O− group peaks. However, the relative abundances of the −C−C− and −C−O− group peaks of the strain YT2-incubated samples were 91 and 9%, respectively. These results indicated that strain YT2 was capable of attacking or oxidizing the PS structure to produce more polar derivatives.

Weight Loss and the Molecular Weight Decrease of the PS Samples by Strain YT2. Degradation efficiency can be directly measured by the weight loss of a sample. The weight loss of the PS samples inoculated with strain YT2 is presented in Figure 4a. After a 60 day incubation with strain YT2 in

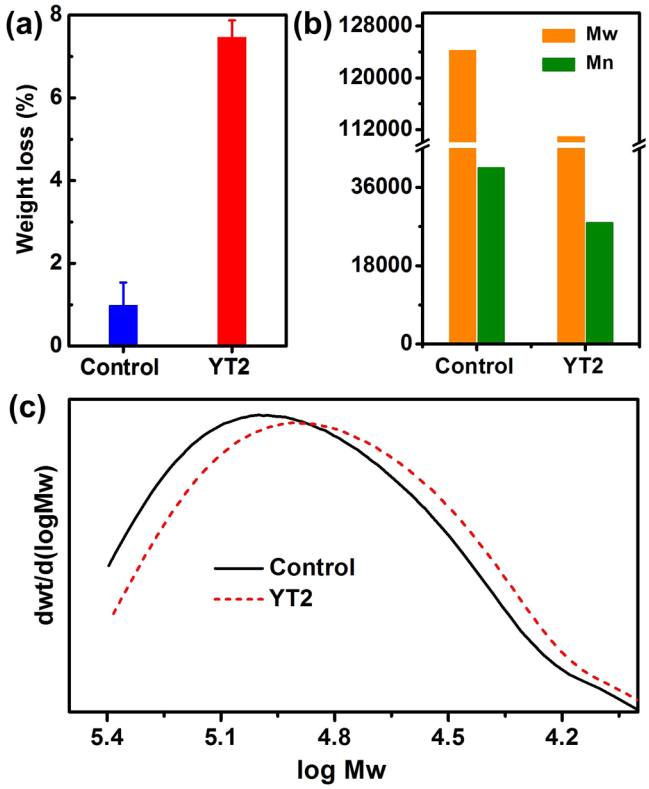

Figure 4. Characterization of PS biodegradation by strain YT2 in LCFBM compared to the control (uninoculated medium) after a 60 day incubation (mean value ± SD; n = 3). (a) Change in the dry weight of the PS pieces. (b) Molecular weight (M_w/M_n) and (c) MWD shift of the residues inoculated with strain YT2 versus the uninoculated control.

LCFBM, the weight loss of the PS pieces was 7.4 ± 0.4%, which was much higher than the 0.8% weight loss elicited by *R. ruber* C208 over a 56 day period as reported by Mor et al.[15]

The molecular weight and molecular weight distribution (MWD) of the PS samples after a 60 day incubation were determined using GPC. The molecular weights (M_n/M_w) of the strain YT2-incubated PS sample versus the uninoculated control were 37 480/110 070 and 40 430/124 200, respectively, which represented a ∼7−11% reduction from the control PS (Figure 4b). The MWDs of the PS samples incubated with the strain YT2 showed a clear negative trend compared to the

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uninoculated control PS (Figure 4c). The decrease in MWD suggested that cleavage/depolymerization of the PS long-chain structure occurred and that lower molecular weight fragments were formed in the presence of strain YT2. In addition, the analysis of samples extracted from LCFBM using GC/MS indicated that more than 40 peaks were found in the medium with strain YT2 but that no peaks were found in the control (Figure S4 and Table S2 of the Supporting Information).

In summary, the weight loss and molecular weight decrease of the PS samples supported the conclusion that strain YT2, which was isolated from the mealworm gut, was capable of degrading PS.

Implications. Our antibiotic test results confirmed that antibiotic suppression of gut bacteria impaired the ability of the mealworm to depolymerize long-chain PS molecules and further mineralize the metabolites to CO_2. This study is the first to report the presence of PS-degrading bacteria in the guts of mealworms. We enriched a mixed culture from Styrofoam-eating mealworm gut content using PS pieces as the sole carbon source and isolated 13 pure bacterial cultures. Among them, one isolated bacterial *Exiguobacterium* sp. strain YT2 was selected, and PS degradation by strain YT2 was confirmed by not only bacterial growth on the PS film, which causes changes in surface topography, decreases in hydrophobicity, and the formation of carbonyl groups, but also the measurement of weight loss and the identification of the loss of molecular weight and the release of water-soluble daughter products. By combination of the results of antibiotic suppression and microbial culture-dependent isolation approaches, this study has provided evidence that mealworm gut microbiota play an essential role in PS biodegradation in the gut. In this study, a LB agar medium was used for the isolation of PE- and PS-degrading bacteria. As a result of the high NaCl content (10 g/L), this medium was a selective medium. Further research is needed to test or develop other media for optimal isolation of PE- and PS-degrading bacteria.

On the basis of the short retention time of gut contents (<24 h) and up to 47.7% carbon conversion into CO_2 by the mealworms (10.1021/acs.est.5b02661), the PS degradation efficiency (e.g., 7.4% over 60 days) demonstrated that the isolated bacterial strain YT2 outside the living host appears to show much poorer PS degradation efficiency than that demonstrated in the gut system. The PS degradation by strain YT2 outside the worm gut could be limited by unknown factors because less energy was generated for cell growth. Similar low apparent yield coefficients were observed when PE-degrading *E. asburiae* YT1 and *Baccilus* sp. YP1 grew on PE films with 0.82 and 0.66 g of cells/g of PE, respectively.[20]

We could expect that PS degradation in mealworm guts is analogous to microbial degradation of lignocelluloses in ruminating mammals and wood in termites for the mutual benefits of the metabolism of microbial consortia and host. The gut of the mealworm can be considered an efficient bioreactor. Physicochemical "treatments" (by chewing, ingesting, mixing with gut contents, etc.) together with the activity of enzymes secreted by the worm are also possibly critical for the success of rapid PS degradation in this bioreactor. More research will be conducted to fully understand the synergic actions between worm digestion and microbial metabolism and to better understand the enzymatic systems involved in the biodegradation of PS as well as other plastics, such as PE, which could be helpful in the development of promising remediation approaches for plastic wastes.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b02663.

Results of different antibiotics on gut bacterial growth (Figure S1), changes in bacterial cell numbers on the PS film incubated with 13 different isolated bacterial cultures (Figure S2), 16S RNA gene-based neighbor-joining phylogenetic tree of strain YT2 (Figure S3), GC of the extract from the culture inoculated with strain YT2 and the uninoculated control after 60 days (Figure S4), a total of 13 bacterial strains isolated from the PS enrichment of gut microbes (Table S1), and GC/MS results of water-soluble products from PS pieces in the presence and absence of strain YT2 (Table S2) (PDF)

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Notes

The authors declare no competing financial interest.

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