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Biodegradation of additive-free polypropylene by bacterial consortia enriched from the ocean and from the gut of *Tenebrio molitor* larvae

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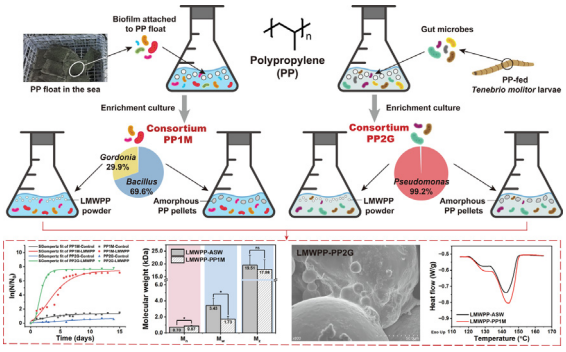
HIGHLIGHTS

- Two different bacterial consortia were enriched from the ocean and mealworm guts.
- Both consortia utilized each of two different additive-free PP for their growth.
- HT-GPC, SEM, ATR-FTIR and DSC analyses showed PP depolymerization and biodegradation.
- Preferences occurred in PP degradation by two consortia from different environments.

GRAPHICAL ABSTRACT



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ABSTRACT

Accumulation of highly recalcitrant PP wastes has caused a serious environmental pollution. We evaluated the biodegradation of two types of additive-free PP polymers by microbial degraders from different environments. Two bacterial consortia, designated as PP1M and PP2G, were enriched from the ocean and from the guts of *Tenebrio molitor* larvae. Both consortia were able to utilize each of two different additive-free PP plastics with relatively low molecular weights (low molecular weight PP powder and amorphous PP pellets) as the sole carbon source for growth. After a 30-day incubation, several plastic characterization methods, including high-temperature gel permeation chromatography, scanning electron microscopy, Fourier transform infrared spectroscopy, and differential scanning calorimetry, were used to characterize the PP samples. The bio-treated PP powder was covered with tight biofilms and extracellular secretions with significantly increased hydroxyl and carbonyl groups and slightly decreased methyl groups. This suggested that degradation and oxidation had occurred. The altered molecular weights and the increased melting enthalpy and average crystallinity of the bio-treated PP samples all suggested that both consortia preferred to depolymerize and degrade the fractions with molecular weights of ≤34 kDa and the amorphous phase fractions of the two types of PP. Furthermore, low molecular weight PP powder was more susceptible to bacterial degradation compared to amorphous PP pellets. This study provides a unique example of different types of additive-free PP degradation by different culturable bacteria from the ocean and insect guts as well as a feasibility of PP waste removal in different environments.

Abbreviations: ASW, artificial seawater basal medium; ATR-FTIR, attenuated total reflection Fourier transform infrared spectroscopy; DSC, differential scanning calorimetry; (HT-) GPC, (high-temperature) gel permeation chromatography; LB, lysogeny broth; LCFBM, liquid carbon free basal medium; LMWPP, low molecular weight polypropylene; PE, polyethylene; PP, polypropylene; PS, polystyrene; PVC, polyvinyl chloride; SDS, sodium dodecyl sulfate; SEM, scanning electron microscopy.

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1. Introduction

Petroleum-based plastics are polymers of olefin or ester monomers. They are industrially produced in large quantities and widely used worldwide. The global plastics production was 390.7 million tons in 2021 (PlasticsEurope, 2022). Polypropylene (PP), one of six widely used plastics, accounted for 19.3 % of global production and was the largest single plastic product in the market (PlasticsEurope, 2022). PP is widely used for food packaging, sweet and snack wrappers, and hinged caps (PlasticsEurope, 2022). The mass production and wide application of PP have resulted in PP waste accumulation on land and in the ocean. This waste has caused serious environmental pollution (Geyer et al., 2017). PP is a polyolefin plastic, with chiral carbon atoms on the main chain with different degrees of crystallinity and different molecular weight distribution indices. Therefore, there are several different types of PP (Huang et al., 2020; Natta and Corradini, 1960; Yang et al., 2021). Because of its high molecular weight and hydrophobicity, as well as the presence of side chain methyl groups, PP is resistant to degradation, and it was once considered to be a "non-biodegradable polymer" (Zheng et al., 2005). However, there are many microorganisms involved in the removal of environmental xenobiotics (Guieysse and Wuertz, 2012). Some of these microorganisms may also be able to degrade plastic wastes (Grima et al., 2000; Mohanan et al., 2020).

Several studies have reported the degradation of PP by microbes. An isotactic PP (with methyl group arranged on one side of the carbon chain) film was suggested to be degraded by an uncharacterized microbial consortium from the soil during a 5-month incubation, and possible metabolites were detected using gas chromatography–mass spectrometry (Cacciari et al., 1993). High molecular weight PP was also suggested to be degraded by *Stenotrophomonas panacihumi* PA3-2, a mesophilic bacterium isolated from municipal solid waste landfill soil, during a 90-day incubation with a final amount of degradation being equal to 12.7 ± 0.97 % (Jeon and Kim, 2016). Microbial degradation of PP has also been documented following thermal or short ultraviolet (UV) pretreatment and by co-degradation of PP with other carbon sources. Thermal and UV pretreated PP films were able to be degraded by a consortium containing four soil strains of *Pseudomonas* and *Bacillus* with gravimetric weight loss of 2.5 % after 12 months (Arkatkar et al., 2010), or by a combination of *Bacillus flexus* and *Pseudomonas azotoformans* with 1.95 % gravimetric weight loss and 22.7 % thermogravimetric weight loss after 12 months (Aravinthan et al., 2016). UV pretreated pro-oxidant blended PP was degraded by two independent fungi with 18.8 % and 9.42 % gravimetric weight loss and 86.3 % and 84.2 % thermogravimetric weight loss at 400 °C after 12 months, respectively (Jeyakumar et al., 2013). UV pre-treated low-density polyethylene (PE) and PP granules were co-degraded by a consortium of *Enterobacter* and *Pseudomonas* for 160 days (Skariyachan et al., 2021) or by isolates of *Bacillus* from river sediments (Nanthini Devi et al., 2021). Five independent strains of *Bacillus* isolated from digested municipal solid waste were all able to co-metabolize PP and poly-L-lactide (Jain et al., 2018). A thermophilic consortium of *Brevibacillus* sp. and *Aneurinibacillus* sp. from landfills could degrade a mixture of low- and high-density PE and PP films and pellets after 140 days (Skariyachan et al., 2018). In addition, similar to two common polyolefin plastics, PE (Yang et al., 2014) and polystyrene (PS) (Yang et al., 2014; Yang et al., 2020; Yang et al., 2015), PP was also reported to be metabolized by mealworms depending on their gut microbes (Yang et al., 2021). However, no cultivable microbial strain or consortium capable of degrading PP without pre-treatment has been isolated from mealworm guts to date.

PP degradation in marine environments has also been studied. Commercial PP sheets were immersed in ocean waters, and they lost 0.5 % of their weight after 6 months (Sudhakar et al., 2007). Isolates of *Bacillus* and *Rhodococcus* from mangrove sediments caused 4.0 % and 6.4 % weight loss of PP granules, respectively, after 40 days (Auta et al., 2018). Thus, the microbes that occur in marine environments may include potential plastic degraders.

Although previous reports indicated microbial degradation of PP, most of these degradations followed a pretreatment or involved co-degradation with other substrates. Also, there are several types of additive-free PP (Huang et al., 2020; Natta and Corradini, 1960; Yang et al., 2021), and their molecular weight and crystallinity properties can affect biodegradation efficiency (Potrykus et al., 2021). The differences in the degradation of different types of PP plastics, without pretreatment, have not been reported, particularly by bacteria occurring in different environments.

Here, we report the biodegradation of two types of additive-free PP plastics (low molecular weight PP (LMWPP) powder and amorphous PP pellets) by a consortium enriched from the sea and by a consortium enriched from the mealworm gut. The results increase our understanding of the differences among various types of additive-free PP degradation by cultivable bacteria from different environments and provide insight into the bacterial biodegradation of PP. The results also enrich bacterial resources for PP degradation and provide an example of the different types of PP degradation enabled by microbes from different environments.

2. Materials and methods

2.1. Materials and medium

LMWPP (CAS No.:9003-07-0) powder [number-average molecular weight (M_n): 415 Da, weight-average molecular weight (M_w): 1770 Da, and size-average molecular weight (M_z): 10,950 Da; the diameter distribution of powder ranges from 20 to 500 μm] was purchased from Macklin Co. (Shanghai, China). Amorphous PP pellets (CAS No.: 9003-07-0, M_n = 840 Da, M_w = 33,820 Da, M_z = 167,440 Da, average diameter = 4 mm, density = 0.9 g/mL, at 25 °C) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). PP powder (injection molding grade) was purchased from Maoming Petro-Chemical Shihua Co., Ltd. (Maoming, Guangdong Province, China). PP particles (black cylindrical particles, average diameter = 3.5 mm) were purchased from Yixin Plastic-producing Co., Ltd. (Taizhou, Zhejiang Province, China). All of the molecular weight data in this study were obtained by high-temperature gel permeation chromatography (HT-GPC). PP powder or pellets were washed twice with 2 % sodium dodecyl sulfate (SDS) (w/v), 75 % ethanol (v/v), and sterile deionized water, in turn for sterilization and then dried on a sterilized clean bench at room temperature. Both PP-degrading consortia were cultured aerobically in artificial seawater basal medium (ASW) (Ley et al., 2023), liquid carbon free basal medium (LCFBM) (International, 1996), or lysogeny broth (LB) medium (Bertani, 1951) at 30 °C.

2.2. Enrichment of cultivable PP degrading consortia

The PP black cylindrical particles were deployed in the Yellow Sea off Mai Island (36°3′13.3″ N, 120°25′31.5″ E) for 10 months as described previously (Xu et al., 2019). Fifty PP black cylindrical particles floating in the sea were put into 10 mL ASW and oscillated through the vortex for 10 min to obtain a bacterial suspension. Then, the suspension was inoculated in ASW (1 %, w/v) and incubated with PP powder (injection molding grade) (1 %, w/v) at 180 rpm and 30 °C for an enrichment. After a 30-day incubation, 1 mL of the culture was inoculated in 100 mL ASW with sterilized PP powder (injection molding grade) for another 30-day incubation for a further enrichment. The enrichment cultivation was repeated 10 times. Since PP is the predominant carbon source, this process could possibly lead to a population of PP-degrading bacteria. Finally, a marine-derived potential PP-degrading consortium was obtained and designated as PP1M.

Tenebrio molitor larvae (Coleoptera: Tenebrionidae) (sourced from Shandong Sishui-Limin Insect Breeding Plant, China) were fed with PP powder (injection molding grade) by spraying water from time to time to maintain the humidity for 14 days. The midgut suspensions of *Tenebrio molitor* larvae were collected as described previously (Yin et al., 2020) and added to a 250 mL Erlenmeyer flask containing 100 mL LCFBM on a rotary shaker (180 rpm) at 30 °C for a 30-day enrichment cultivation, with sterilized 1 % (w/v) PP powder (injection molding grade) as the sole carbon source. This enrichment cultivation was also repeated 10 times. Then, a

gut-derived potential PP-degrading consortium was obtained and designated as PP2G.

2.3. Sequencing of the V3-V4 region of 16S rRNA gene amplicons

Sequencing of the V3-V4 region of 16S rRNA gene amplicons was performed to characterize the community structure and bacterial diversity of both consortia. After 30-day incubation with PP samples, cells of both consortia were collected by centrifugation at 8000 × *g* for 10 min and then freeze-dried with liquid nitrogen. The V3-V4 regions of the 16S rRNA genes were PCR-amplified and sequenced by the Tsingke Company (Shanghai, China).

2.4. Determination of both consortia grown on PP plastics

The PP1M and PP2G consortia were both cultivated in 5 mL LB medium at 30 °C overnight. After the optical density at 600 nm (OD_{600}) reached approximately 0.6, the cells of consortia PP1M and PP2G were collected and washed twice with ASW and LCFBM, respectively. Then, the cells of the two consortia were re-suspended in an equal volume of their corresponding media. Cells of consortium PP1M were inoculated (1 ‰, v/v) in 100 mL ASW with 1 % (w/v) LMWPP powder or amorphous PP pellets (both being sterilized as aforementioned) as the sole carbon source and incubated at 180 rpm and 30 °C for 15 days or 45 days, respectively. Cells of consortium PP2G were inoculated in 100 mL LCFBM and incubated similarly. The increased biomass was monitored based on OD_{600} values using a spectrometer (Lambda 25 UV/VIS, PerkinElmer, USA). Two consortia inoculated in two basic media without PP plastics were used as negative controls. All experiments were conducted in triplicate. The growth curve was plotted based on the logarithm of the number of organisms plotted against time (Zwietering et al., 1990).

2.5. Biotreatment of PP plastic by consortia PP1M and PP2G

Consortia PP1M and PP2G were both cultivated in 100 mL LB medium at 30 °C overnight. After the OD_{600} values reached approximately 0.6, the cells of consortia PP1M and PP2G were collected and washed twice with ASW and LCFBM media, respectively. Then, the cells were re-suspended in an equal volume of their corresponding media. Each suspension was evenly divided into two parts and then sterilized LMWPP powder and amorphous PP pellets (1 %, w/v) were added to one part each. All of the above cultures were incubated at 180 rpm and 30 °C for 30 days. After incubation, bacterial cells were separated by centrifugation at 8000 × *g* for 10 min. The residual PP powder and pellets in the supernatant were soaked in 2 % SDS (w/v) overnight, and washed with 75 % ethanol (v/v) and sterile deionized water three times in turn to remove all the attachments on the PP surface. Then they were collected using a 0.45-μm filter membrane and dried before analysis. The two types of PP plastics without inoculation served as negative controls.

2.6. Characterization of molecular weight changes of PP polymer by HT-GPC

The bio-treated residual LMWPP powder (10 mg) and amorphous PP pellets (30 mg) (both being thoroughly washed and dried without attachments and H_2O) were dissolved in 5 mL and 15 mL *o*-dichlorobenzene, respectively. The sample concentration was about 2 mg/mL, and the solution was filtered through a 0.22-μm syringe filter prior to the injection. Then, the molecular weight change of bio-treated PP samples was analyzed by HT-GPC (HLC-8321GPC/HT, Japan) as described previously (Yin et al., 2020). The two types of PP plastics without inoculation served as the negative controls.

2.7. Morphological observation by scanning electron microscopy (SEM)

The residual LMWPP powder incubated separately with both consortia for 30 days was removed directly and dried at room temperature. Next, the air-dried PP powder was coated with aurum (Au) using a vacuum sputter (Leica, SCD050, German) for a sputtering time of 120 s before scanning electron microscopy (SEM) analysis. Then, the Au-coated PP powder was used for observation of the biofilm formed by both consortia, and the deterioration of the PP powder surface was observed via SEM (HITACHI, S3400II, Japan) as previously described (Yin et al., 2020; Yue et al., 2021) with modifications. The LMWPP powder without inoculation served as the negative control.

2.8. Chemical characterization of PP surface by attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy

Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopic analysis was used to detect the content of various functional groups in a compound, and it was performed on a Nicolet 6700 FTIR spectrometer (Thermo Fisher Scientific, USA) in this study. After 30 days of incubation, the chemical changes on the surface of the dried LMWPP powder and amorphous PP pellet samples (both being thoroughly washed and dried without attachments and H_2O) were detected by ATR-FTIR as previously described (Yin et al., 2020) with modifications. The analysis was performed by accumulating 64 scans with a scan range of 4000–500 cm^{-1} using OMNIC software (Version 8.2.387). The spectroscopic data focus was the carbonyl, hydroxyl, and methyl groups. The carbonyl index (*CI*) and hydroxyl index (*HI*) were calculated as previously described (Potrykus et al., 2021). The methyl group index (*MI*) was also calculated using spectroscopic data as previously described (Arkatkar et al., 2009). These parameters were able to assess the degree of oxidation of the two residual PP samples treated by both consortia.

2.9. Characterization of the crystalline properties of PP plastics by differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) analysis was performed using a DSC2A-01130 analyzer (TA Instruments, USA) under a nitrogen atmosphere at a heating rate of 10 °C/min. After the 30-day incubation by both consortia, residual LMWPP powder and amorphous PP pellets were collected, washed, and dried as aforementioned (both being without attachments and H_2O). Then, the samples (5–10 mg each) were loaded into an aluminum pan and placed at an appropriate position in the DSC instrument. After an equilibration phase at 20 °C, the samples were first heated to 230 °C, cooled to 20 °C, and then reheated to 230 °C. The second heating scan was used for subsequent analyses. The percentage crystallinity was determined using the ratio of the melting enthalpy (ΔH_m) of the test sample to that of 100 % crystalline PP (207 J/g) (Arkatkar et al., 2009; Paukkeri and Lehtinen, 1993; Potrykus et al., 2021). The ΔH_m of the test sample was obtained from the area under the melting peak of the second heating curve (Jain et al., 2018).

3. Results and discussion

3.1. Enrichment and diversity of two cultivable PP degrading consortia

After 10 cycles of enrichment with PP powder (injection molding grade) as the sole carbon source, a marine-derived consortium designated as PP1M and a mealworm gut-derived consortium designated as PP2G were enriched for further studies.

According to relative abundance analysis based on the V3-V4 region of 16S rRNA gene amplicons of consortia PP1M and PP2G (SRA accession numbers: SRR22282151 and SRR22282152, respectively), consortium PP1M mainly consisted of strains belonging to eight genera (*Bacillus* (about 69.6 %), *Gordonia* (about 29.9 %), *Pseudomonas*, *Caldicoprobacter*, *Ignatzschineria*, *Escherichia_Shigella*, *Tepidimicrobium*, *Keratinibaculum*) and others in nine families (Bacillaceae (about 69.6 %), Nocardiaceae (about 29.9 %), Caldicoprobacteraceae, Family_XI, Pseudomonadaceae, Wohlfahrtiimonadaceae, Enterobacteriaceae, Lachnospiraceae, Bacteroidaceae) of four phyla (Firmicutes (about 69.9 %), Actinobacteriota

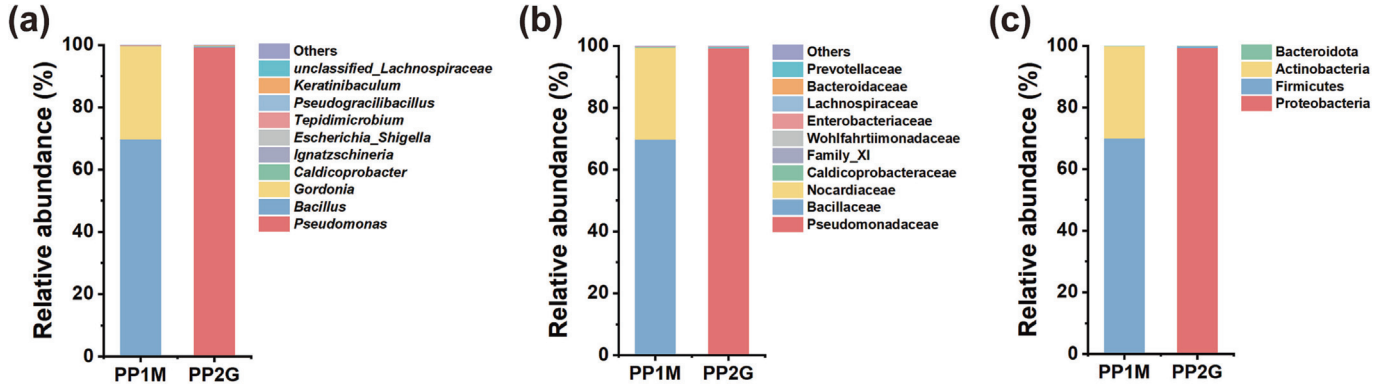

Fig. 1. Diversity of PP degrading bacterial consortia PP1M and PP2G at genus (a), family (b), and phylum (c) levels. The relative abundance analysis was based on the V3–V4 region of 16S rRNA gene amplicons of both consortia.

(about 29.9 %), Proteobacteria, and Bacteroidota). In contrast, consortium PP2G mostly consisted of eight genera (*Pseudomonas* (about 99.2 %), *Bacillus* (about 0.3 %), *Caldicoprobacter*, *Ignatzschineria*, *Pseudogracilibacillus*, *Escherichia_Shigella*, *Tepidimicrobium*, *Keratinibaculum*) and others in nine families (Pseudomonadaceae (about 99.2 %), Bacillaceae (about 0.3 %), Caldicoprobacteraceae, Family_XI, Wohlfahrtiimonadaceae, Enterobacteriaceae, Lachnospiraceae, Bacteroidaceae, Prevotellaceae) of four phyla (Proteobacteria (about 99.4 %), Firmicutes (about 0.6 %), Actinobacteriota, and Bacteroidota) (Fig. 1).

It was evident that consortia PP1M and PP2G enriched from the sea and mealworm guts contained different community structures. Probably, strains from the highly abundant genera listed above could play key roles in PP degradation in each consortium. However, we cannot exclude the possibility that strains from other genera with lower abundance might also be involved in degrading PP plastic. In addition, the microbial structure compositions of the two consortia were completely different. This suggests that microorganisms from different genera drive the plastic degradation in different environments. This may be the result of evolution of bacteria in different environments as an adaptive response to PP contamination.

3.2. Growth of the consortia PP1M and PP2G on two types of additive-free PP plastics

To explore the growth of both consortia on two types of additive-free PP plastics, consortium PP1M was cultured in ASW, and consortium PP2G was cultured in LCFBM. LMWPP powder and amorphous PP pellets were used as the sole carbon source. Growth data of both consortia were presented in semi-logarithmic plots fitted with the Gompertz model (Zwietering et al., 1990) (Fig. 2), and the maximum specific growth rates (μ_{max}) of both consortia were shown in Table 1.

As shown in Fig. 2, both consortia were able to utilize LMWPP powder (Fig. 2a) and amorphous PP pellets (Fig. 2b) as the sole carbon source and energy for their growth, while the negative controls virtually had no growth. In particular, when LMWPP powder was used as the substrate, consortium PP2G reached the logarithmic growth phase more rapidly than consortium PP1M (Fig. 2a), and the μ_{max} of consortium PP2G (4.0771) was much higher than that of consortium PP1M (1.1760) (Table 1), but both consortia eventually had similar increases in biomass (Fig. 2a). Similarly, when amorphous PP pellets were used as the substrate, consortium PP2G also reached the logarithmic growth phase faster than consortium PP1M (Fig. 2b) and had a higher μ_{max} (0.3185) compared to that of consortium PP1M (0.1938) (Table 1). However, the final biomass of consortium PP2G was only about half of that of consortium PP1M (Fig. 2b). LMWPP powder appeared to be more suitable for bacterial growth with more biomass increase compared to amorphous PP pellets (Fig. 2). This might be due to the smaller particle size and larger specific surface area of LMWPP powder compared to the amorphous PP pellets. These characteristics were beneficial to the attachment and growth of bacteria, as suggested previously (Zhang et al., 2021).

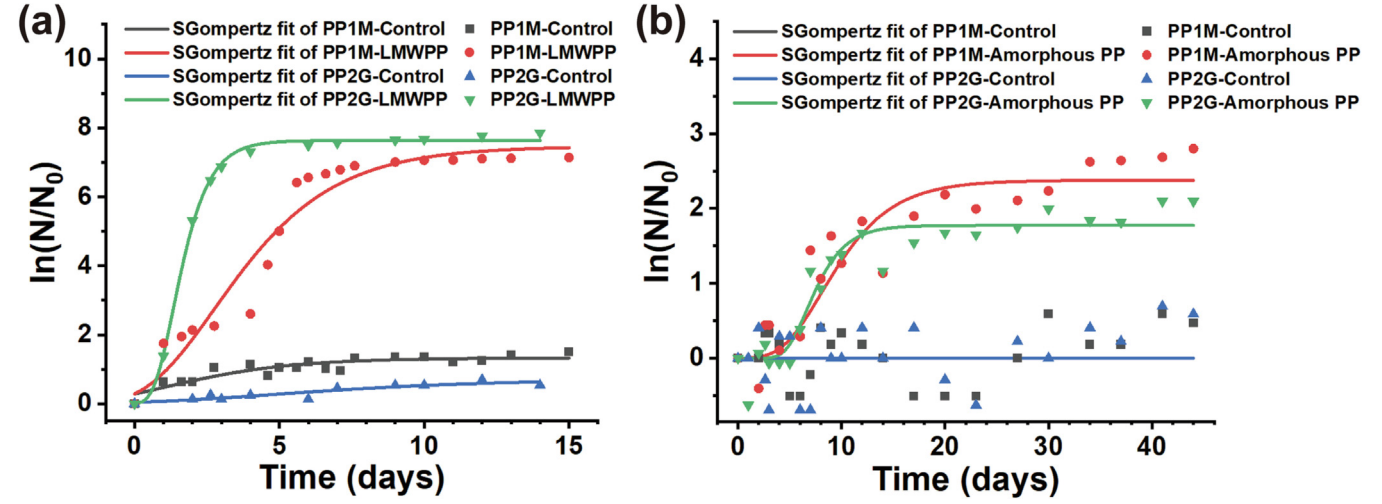

Fig. 2. Growth curves of consortia PP1M and PP2G on LMWPP powder (a) and amorphous PP pellets (b). N_0, initial number of cells; N, number of cells. The growth curves were fitted by Gompertz equation with Origin software (version 2022b 9.95.17), and all points represent the mean values of three parallel experiments (n = 3). The two fitted curves of consortia PP1M and PP2G without carbon sources were overlapped (b). The maximum specific growth rates (μ_{max}) were also calculated and listed in Table 1.

Table 1
Maximum specific growth rates of the growth curve fitted using the Gompertz model.

Consortia	Substrates	Maximum specific growth rates (μ_{max})
PP1M	Control[a]	0.1960
	LMWPP	1.1760
	Control	0.0005
	Amorphous PP	0.1938
PP2G	Control	0.0592
	LMWPP	4.0771
	Control	0.0002
	Amorphous PP	0.3185

[a] Control: Without carbon sources.

3.3. Molecular weight changes of PP treated by bacterial consortia

Characterization of molecular weight is essential for evaluating depolymerization and degradation of polymers (Eubeler et al., 2009). Most studies on biodegradation of plastics such as PE (Yamada-Onodera et al., 2001), PS (Tian et al., 2017; Wu and Criddle, 2021; Yang et al., 2015), and polyvinyl chloride (PVC) (Peng et al., 2020a; Xu et al., 2023) have used this analysis. Thus, HT-GPC was introduced to analyze the possible changes in the molecular weight of PP samples treated by both consortia in this study. In general, three parameters, M_n represents the lower molecular weight portion of the polymer, M_w represents the middle molecular weight fraction, and M_z represents the higher molecular weight fraction (Wu and Criddle, 2021). Here, M_n, M_w, and M_z values of bio-treated PP samples and negative controls (without inoculation) were shown in Fig. 3.

For LMWPP powder treated by consortium PP1M, compared with the negative controls, the M_n value increased by 24.3 % (from 0.70 ± 0.02 kDa to 0.87 ± 0.07 kDa), but the M_w and M_z values decreased by 49.6 % (from 3.43 ± 0.86 kDa to 1.73 ± 0.34 kDa) and 8.5 % (from 19.51 ± 3.87 kDa to 17.86 ± 3.74 kDa), respectively (Fig. 3a). Consortium PP2G exhibited a similar ability to degrade LMWPP powder. The M_n value increased by 22.5 %, and the M_w and M_z values decreased by 33.1 % and 27.7 % respectively (Fig. 3b). In short, after the bio-treatment by both consortia, the M_n values increased (Fig. 3a and b, pink highlighted), but the M_w and M_z values both decreased (Fig. 3a and b, blue highlighted). This suggests that the higher molecular weight portions (about 1.73–22.34 kDa) of the LMWPP powder were degraded into shorter chains, leading to decreased M_w and M_z, and the accumulation of newly generated smaller molecules (about 0.7–0.9 kDa) with short chains resulted in the increase of M_n. These results suggested preference for the limited extent depolymerization of PP by both consortia with higher molecular portions (approximately from 0.9 to 22.34 kDa) being degraded at higher rates than lower molecular portions (less than approximately 0.9 kDa). This phenomenon was similar to the limited extent depolymerization seen in previous descriptions of PP, PE, and PS biodegradation (Peng et al., 2020b; Yang et al., 2021; Yin et al., 2020).

For amorphous PP pellets treated by consortium PP1M, the M_n value decreased by 16.6 % (from 4.34 ± 0.16 kDa to 3.62 ± 0.29 kDa), while the M_w and M_z values increased by 0.9 % (from 34.58 ± 0.48 kDa to 34.90 ± 0.31 kDa) and 7.0 % (from 152.09 ± 6.14 kDa to 162.70 ± 2.17 kDa), respectively (Fig. 3c). However, for amorphous PP pellets treated by consortium PP2G, the M_n and M_w values decreased by 25.2 % and 1.4 % respectively, but the M_z value increased by 13.6 % (Fig. 3d). In short, for the amorphous PP pellets treated by both consortia, the M_n value decreased (Fig. 3c and d, blue highlighted), the M_w value changed little (Fig. 3c and d, green highlighted), whereas the M_z value increased (Fig. 3c and d, orange highlighted). These data show that microbes from the ocean and from mealworm guts had difficulty degrading higher

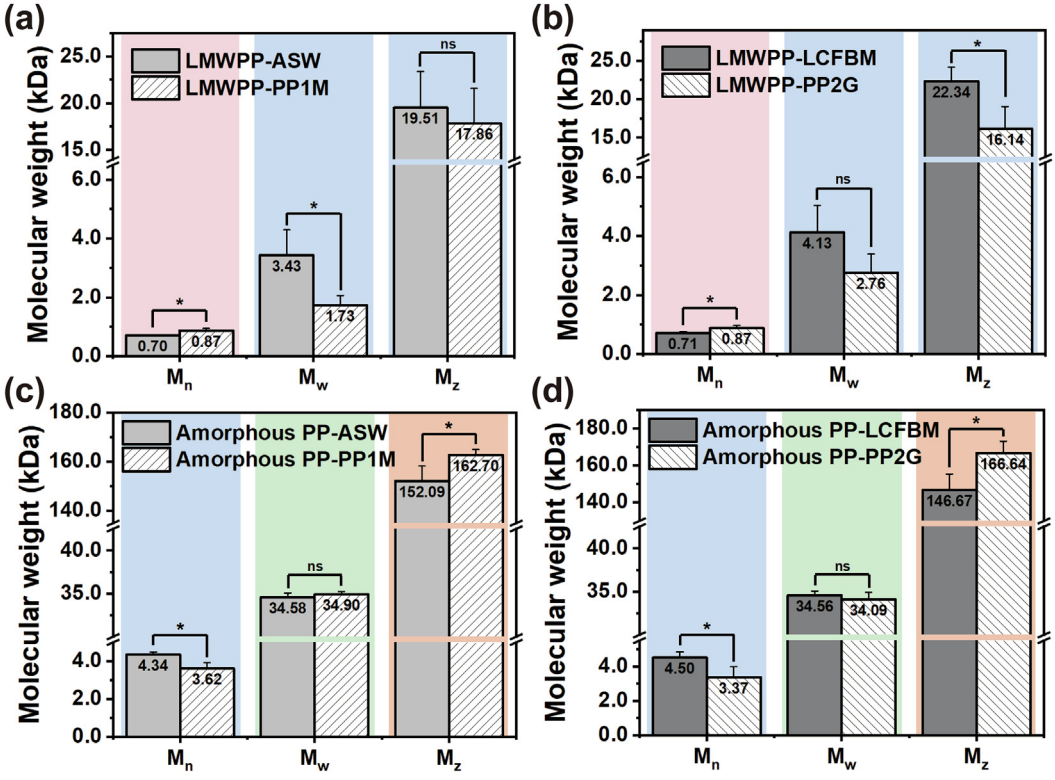

Fig. 3. Characterization of PP plastics degradation by HT-GPC. PP samples without inoculation in ASW or LCFBM served as negative controls. Bar diagrams show molecular weight changes in values of M_n, M_w, and M_z (All values represent mean ± SD, n = 3. Significance (Student's *t*-tests) $p < 0.05$ indicated by *, and no statistical significance indicated by ns.) The upper panel of bar diagrams shows the molecular weight changes in M_n, M_w, and M_z values of LMWPP powder treated by consortium PP1M (a) or consortium PP2G (b). The low panel of bar diagrams shows the molecular weight changes in M_n, M_w, and M_z values of amorphous PP pellets treated by consortium PP1M (c) or consortium PP2G (d). Pink highlighted: the molecular weight increased in LMWPP powder; blue highlighted: the molecular weight decreased in LMWPP powder or amorphous PP pellets; green highlighted: the molecular weight hardly changed in amorphous PP pellets; orange highlighted: the molecular weight increased in amorphous PP pellets.

molecular weight fractions (more than approximately 34 kDa) of amorphous PP pellets, and the depolymerization range of consortium PP2G was only slightly larger than that of consortium PP1M. Lower molecular weight fractions were degraded, leading to decreased M_n values, while high molecular weight fractions remained in the residual amorphous PP pellets, resulting in increased M_z values.

These results suggest that microbes from the ocean and from mealworm guts both tended to degrade PP fractions with molecular weights ranging from 0.9 kDa to 34 kDa. The data also indicate that molecular weight, a unique property of plastics, is a key factor affecting biodegradation efficiency as previously suggested (Potrykus et al., 2021). However, commercial PP products typically exhibit molecular weights greatly exceeding 34 kDa, with a common range of 100–600 kDa (Karger-Kocsis and Bárány, 2019; Mark, 2009), and also contain additives. In future research, commercial, high molecular weight PP polymers will be used to evaluate PP biodegradation. Furthermore, a microbial consortium consisting of plasticizer-degrading bacteria (Patil et al., 2006; Ren et al., 2018; Wright et al., 2020) together with PP-degrading bacteria from this study probably will be beneficial for effectively mitigating PP product pollution compared to PP-degraders alone.

3.4. Changes in topographical properties of bio-treated PP surfaces

Colonization of both consortia attached to the surface of LMWPP powder was observed via SEM (Fig. 4). Both negative controls, without inoculation, retained their original topographical features (Fig. 4a, b, e, and f). There were only tiny pits of LMWPP powders themselves on the surfaces. A few impurities might have been salt crystals from the media, and no colony or extracellular secretion was observed on the surfaces (Fig. 4a, b, e, and f). However, the surfaces of bio-treated LMWPP powder were fully covered with tight biofilms of consortium PP1M (Fig. 4c and d) or PP2G (Fig. 4g and h) and their extracellular secretion. The tight biofilm and the extracellular secretion resulted in the tiny pits of the PP powder themselves being completely filled and invisible. Two or more LMWPP powders were joined together due to the tight biofilms formed by consortium PP2G (Fig. 4g and h), and the filamentous secretions were clearly present at the edge of the coupling points (Fig. 4g and h). Unfortunately, due to the large particle size and the irregular shape, the sputter coating on amorphous PP pellets was poor, resulting in failing to obtain SEM images of these PP samples.

Biofilm formation was necessary for bacteria to colonize hydrophobic polyolefins (Yin et al., 2020), and this was useful for microorganisms to erode and degrade plastics (Mohanrasu et al., 2018). In this study, the cell appendages secreted by strains in both consortia may be used in their interactions. Therefore, the tight biofilms confirmed by both consortia and their extracellular secretions on the surfaces of PP samples indicated good growth of both consortia. These results were consistent with the growth of the consortia shown in Fig. 2 and suggested the degradation of LMWPP powder by each consortium.

3.5. Changes in chemical properties of bio-treated PP surface

To characterize the chemical modification of the PP sample surface treated by both consortia, the PP samples, with and without bio-treatment, were analyzed using ATR-FTIR. The values of carbonyl (—C═O), hydroxyl (—O—H), and methyl group (—CH_3) indexes revealed the degree of oxidation of the PP sample surface, and these values are shown in Table 2. For the LMWPP powder treated by consortium PP1M, compared with the negative control, the *CI* and *HI* values increased by 88.8 % and 100.5 %, respectively. However, the *MI* value decreased by 12.8 %. Increased *CI* and *HI* values (86.8 % and 73.0 %) and decreased *MI* value (21.8 %) were also observed when LMWPP powder was treated by consortium PP2G. When amorphous PP pellets were treated by consortium PP1M, the *CI* value increased by 24.4 %, the *HI* value increased by 39.3 %, and the *MI* value decreased by 0.98 %. The *CI* value of amorphous PP pellets treated by consortium PP2G increased by 48.3 %, the *HI* value increased by 59.9 %, and the *MI* value decreased by 1.53 % (Table 2). In summary, the surface oxidation degrees of the LMWPP powder by each consortium were similar, and they were higher than those produced by either consortium on amorphous PP pellets. However, the surface oxidation

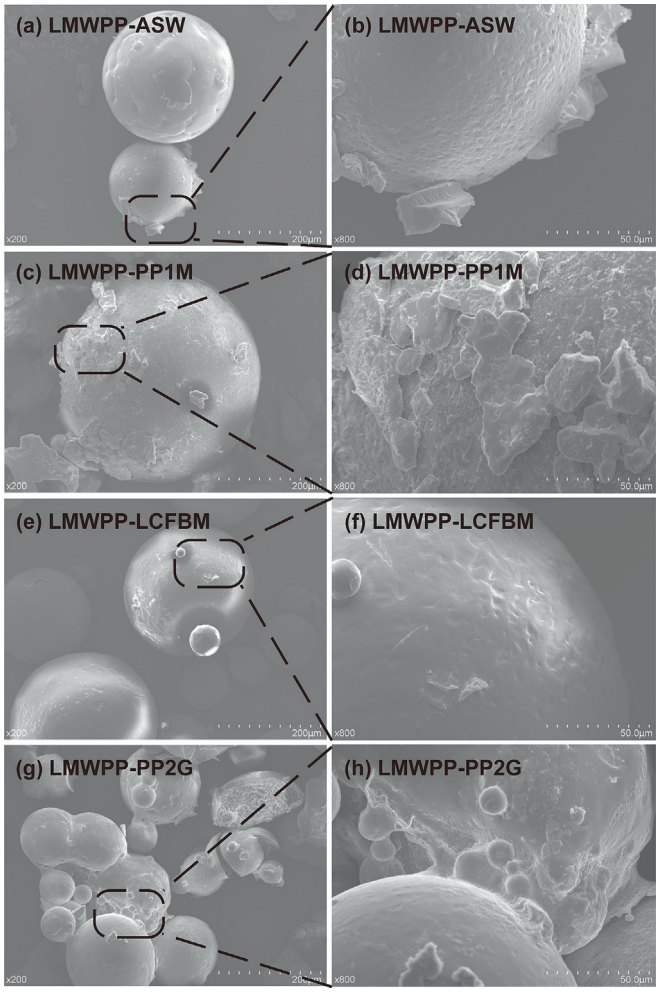

Fig. 4. Scanning electron microscopy (SEM) images of the bio-treated LMWPP powder. The whole images at 800× magnification (b, d, f, and h) represent the parts in the dashed box at 200× magnification (a, c, e, and g), respectively. LMWPP-ASW or LMWPP-LCFBM: LMWPP powder without inoculation in ASW or in LCFBM (negative control); LMWPP-PP1M or LMWPP-PP2G: LMWPP powder treated by consortium PP1M in ASW or by consortium PP2G in LCFBM.

Table 2
Chemical properties of LMWPP powder and amorphous PP pellets with or without the bio-treatment.

PP type	Treatment[a]	Carbonyl index (*CI*)	Hydroxyl index (*HI*)	Methyl group index (*MI*)
LMWPP	NC-ASW	0.233 ± 0.076	1.220 ± 0.501	0.685 ± 0.082
	PP1M-ASW	0.440 ± 0.078*	2.447 ± 0.232*	0.597 ± 0.029
	NC-LCFBM	0.237 ± 0.082	1.091 ± 0.351	0.686 ± 0.006
	PP2G-LCFBM	0.442 ± 0.153	1.888 ± 0.440	0.536 ± 0.097
Amorphous PP	NC-ASW	0.206 ± 0.020	0.653 ± 0.081	1.361 ± 0.096
	PP1M-ASW	0.256 ± 0.028	0.910 ± 0.095*	1.348 ± 0.099
	LCFBM	0.199 ± 0.050	0.748 ± 0.306	1.368 ± 0.001
	PP2G-LCFBM	0.295 ± 0.009*	1.197 ± 0.092	1.347 ± 0.001*

[a] NC-ASW: PP samples without inoculation in ASW served as the negative control; PP1M-ASW: PP samples treated by consortium PP1M in ASW; NC-LCFBM: PP samples without inoculation in LCFBM served as the negative control; PP2G-LCFBM: PP samples treated by consortium PP2G in LCFBM.

* Indicates significant difference from control (p value < 0.05).

degrees of amorphous PP pellets by the gut consortium PP2G were higher than those of amorphous PP pellets by the marine consortium PP1M.

The appearance of —O—H and —C═O groups is often a sign of oxidation of polymers, and this had also been used to confirm the oxidation and degradation of plastics such as PP, PE, PS, and PVC (Cacciari et al., 1993; Peng et al., 2020a; Peng et al., 2020b; Tian et al., 2017; Yin et al., 2020). In this study, the higher values of *CI* and *HI* indicated the increased absorption of carbonyl and hydroxyl groups in two types of bio-treated PP samples. This demonstrated the occurrence of oxidation on the PP surfaces after the bio-treatment, similar to a previous case of the natural degradation of PP over 5 years (Potrykus et al., 2021). The decreased *MI* values also suggested that oxidation took place at the main position of the carbon skeleton chain, resembling previously reported PP degradation with pretreatment (Arkatkar et al., 2009). These data confirm that both types of additive-free PP plastics could be oxidized and degraded by either consortium PP1M or PP2G. The higher surface oxidation degrees of the bio-treated LMWPP powder might also be due to the smaller particle size and larger specific surface area of LMWPP powder compared to amorphous PP pellets. This is consistent with the better bacterial growth on LMWPP powder than on amorphous PP pellets (Fig. 2).

3.6. Changes in the crystalline properties of bio-treated PP

In addition to changes in molecular weight and surface properties, biodegradation could also cause changes in the average crystallinity of PP (Jain et al., 2018; Potrykus et al., 2021). Here, DSC was used to detect the melting enthalpy (ΔH_m) of PP to calculate the average crystallinity value. The melting peaks from the second heating process of DSC curves were shown in Fig. 5. The ΔH_m values and the average crystallinity (%) were shown in Table 3. The areas of all melting peaks of bio-treated additive-free PP samples were larger than those of the negative controls (Fig. 5). After bio-treatment, the ΔH_m values of both LMWPP powder (from 17.592 J/g to 23.966 J/g by consortium PP1M, and from 26.585 J/g to 29.317 J/g by consortium PP2G) and amorphous PP pellets (from 24.809 J/g to 25.263 J/g by consortium PP1M, and from 23.964 J/g to 25.044 J/g by consortium PP2G) increased (Table 3). Following treatment by both consortia, the average crystallinity of LMWPP powder increased (from 8.50 % to 11.58 % by consortium PP1M, and from 12.84 % to 14.16 % by consortium PP2G). And the average crystallinity of amorphous PP pellets also increased (from 11.99 % to 12.20 % by consortium PP1M, and from 11.58 % to 12.10 % by consortium PP2G) compared to the negative controls (Table 3).

Table 3
Crystalline properties of LMWPP powder and amorphous PP pellets with or without the bio-treatment.

PP type	Treatment[a]	Melting enthalpy (ΔH_m, J/g)	Crystallinity (%)
LMWPP	NC-ASW	17.592	8.50
	PP1M-ASW	23.966	11.58
	NC-LCFBM	26.585	12.84
	PP2G-LCFBM	29.317	14.16
Amorphous PP	NC-ASW	24.809	11.99
	PP1M-ASW	25.263	12.20
	NC-LCFBM	23.964	11.58
	PP2G-LCFBM	25.044	12.10

[a] NC-ASW: PP samples without inoculation in ASW; PP1M-ASW: PP samples treated by consortium PP1M in ASW; NC-LCFBM: PP samples without inoculation in LCFBM; PP2G-LCFBM: PP samples treated by consortium PP2G in LCFBM.

High crystallinity means that the polymer chains are arranged tightly and regularly, which is unfavourable for plastic biodegradation (Inderthal et al., 2021). In contrast, low crystallinity indicates that the polymer chains are disordered and loose, which is more conducive to the acquisition and degradation of plastics by microorganisms (Inderthal et al., 2021). Crystallinity, a property of plastics, is an important factor affecting their

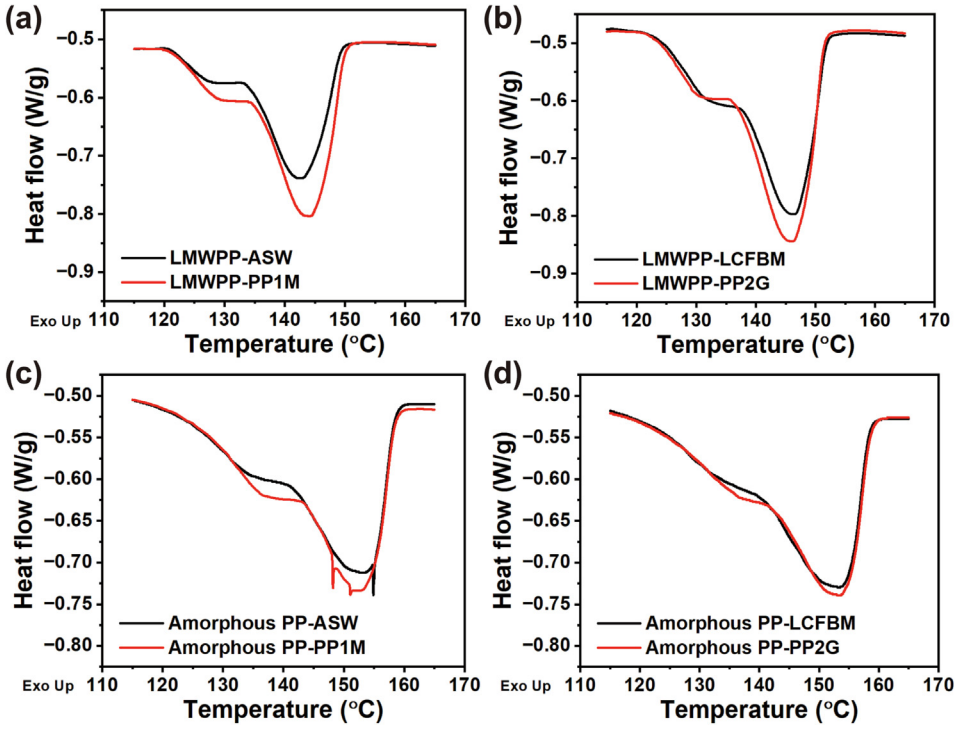

Fig. 5. DSC melting peak curves from the second heating process of two types of PP plastics. a: LMWPP powder with and without the treatment by consortium PP1M; b: LMWPP powder with and without the treatment by consortium PP2G; c: amorphous PP pellets with and without the treatment by consortium PP1M; d: amorphous PP pellets with and without the treatment by consortium PP2G. LMWPP-ASW or Amorphous PP-ASW: LMWPP powder or amorphous PP pellets without inoculation in ASW; LMWPP-PP1M or Amorphous PP-PP1M: LMWPP powder or amorphous PP pellets treated by consortium PP1M in ASW; LMWPP-LCFBM or Amorphous PP-LCFBM: LMWPP powder or amorphous PP pellets without inoculation in LCFBM; LMWPP-PP2G or Amorphous PP-PP2G: LMWPP powder or amorphous PP pellets treated by consortium PP2G in LCFBM.

biodegradation susceptibility (Potrykus et al., 2021). Thus, the amorphous phase fractions of a polymer with low crystallinity are prone to be attached to and utilized by microbes (Arkatkar et al., 2009). The loss of amorphous phase fractions can increase the average crystallinity of the residual polymer (Potrykus et al., 2021). Here, the increased average crystallinity of bio-treated additive-free PP samples sufficiently indicated the degradation of PP by consortium PP1M or PP2G.

4. Conclusion

In this study, two bacterial consortia PP1M and PP2G have been successfully enriched from the sea and from the guts of PP-feeding *Tenebrio molitor* larvae, respectively. Both consortia were able to utilize LMWPP powder or amorphous PP pellets, two types of additive-free PP with relatively low molecular weights, as the sole carbon source for their growth. Additionally, both consortia were capable of degrading PP polymer. After a 30-day incubation with each consortium, SEM analysis showed that the surface of the bio-treated LMWPP powder was covered with a dense biofilm and some extracellular secretions. The significantly increased *CI* and *HI* values and slightly decreased *MI* values from FTIR data revealed the occurrence of oxidation on the surface of the two bio-treated additive-free PP polymers compared to the negative controls. In addition, the surface oxidation degrees of the LMWPP powder (with the smaller particle size and larger specific surface area) by both consortia were similar, and they were significantly higher than those of the amorphous PP pellets. HT-GPC analysis indicated that these two types of PP were depolymerized, to a limited extent, by both consortia, and the portions with molecular weights of ≤ 34 kDa had greater degradation. The increased melting enthalpy and average crystallinity of the bio-treated PP samples also indicated the degradation of the amorphous phase fractions of PP polymer. All the evidence demonstrated that consortia PP1M and PP2G could depolymerize and degrade additive-free PP polymers with similar characteristics. In particular, the medium molecular weight portions and amorphous phase fractions of PP polymer appear to be more easily degraded. A smaller sample size and a larger specific surface area of PP polymer are also more conducive to bacterial degradation. This study provides an example of PP degradation by different culturable bacteria from seawater and from the gut of a stored product insect pest.

CRediT authorship contribution statement

Zhuo-Ning Xian: Writing - Original Draft, Conceptualization, Methodology, Visualization, Investigation. **Chao-Fan Yin**: Formal analysis, Methodology. **Li Zheng**: Reviewing & editing. **Ning-Yi Zhou**: Reviewing & editing. **Ying Xu**: Funding acquisition, Project administration, Supervision, Reviewing & editing.

Data availability

The V3-V4 regions of 16S rRNA gene datasets for consortia PP1M and PP2G from this work were deposited in the Sequence Read Archive (SRA) database under SRA accession numbers SRR22282151 and SRR22282152, respectively.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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