

Biodegradation of polyethylene film by the *Bacillus* sp. PELW2042 from the guts of *Tenebrio molitor* (Mealworm Larvae)

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ARTICLE INFO

Keywords:

Polyethylene
Biodegradation
Bacillus
Tenebrio molitor
Gut microbiome

ABSTRACT

The chemical molecular structure of polyethylene (PE) is very stable and extremely difficult to degrade in the environment, thus posing a threat to the environment. In this study, a high-density polyethylene (HDPE)-degrading *Bacillus* strain PELW2042, was isolated from the gut contents of *Tenebrio molitor* larvae (yellow mealworms). Results showed that after 42 days of incubation with PELW2042, a large number of pits and cracks were observed on the surface of HDPE film by scanning electron microscopy (SEM). The Fourier Transform-Infrared Spectroscopy (FT-IR) detected absorption peaks near 1700 cm^{-1} and 1249 cm^{-1} , indicating the formation of carbonyl groups (C=O) and ether groups (C-O-C); the X-ray photoelectron spectroscopy (XPS) spectra further demonstrated the generation of new oxygen-containing functional groups. X-Ray Diffraction (XRD) and differential scanning calorimetry (DSC) analyses revealed a $21.63 \pm 0.18\%$ decrease in the relative crystallinity of the HDPE film, indicating that the structure of the HDPE changes. Finally, it was also shown that the weight loss of HDPE film was as high as $17.36 \pm 0.56\%$. After 42 days of treatment by strain PELW 2042, the molecular weights of the experimental group samples were significantly decreased by $23.31 \pm 1.25\%$ and $30.07 \pm 1.37\%$ for Mw (121700 Da) and Mn (50700 Da), respectively, compared to those of the untreated samples (Mw=158700 Da and Mn=72500 Da). All these results indicate that *Bacillus* sp. PELW2042 has a high ability to degrade HDPE. This provides a source of strains to accelerate the biodegradation of waste polyethylene plastics.

1. Introduction

Plastic products are widely used because of their outstanding features such as light weight, durability, versatility and low cost. Plastics have been widely produced and used since 1950, with global production already reaching 390.7 million tons per year by 2021, and potentially 800 million and 1600 million tons by 2035 and 2050, respectively [1–3]. However, only 14 % of these are recycled, which means that as much as 86 % of the waste was discarded in landfills or released into the environment due to the mismanagement in various ways [4,5]. As global demand for plastics continues to grow, there should be immediate and fundamental changes in key policies, consumption behaviour and waste management practices for plastic products, otherwise the trend of plastic emissions into the open environment is unlikely to decrease by 2030 [6]. It has been shown that once in the environment, plastics undergo physical, chemical and biological ageing processes under the action of

nature. This causes them to break down into smaller pieces, i.e. microplastics. Microplastics are usually defined as plastic fibers, particles or films with particle size less than 5 mm [7]. Microplastics have been detected worldwide in air [8,9], ocean [10–13], soil [14,15], sediment [16], and surface water [17,18]. In addition, the high ability of microplastics to absorb organic contaminants from the surrounding environment results in the easy transfer of hazardous chemicals through the food web to various wildlife and humans via plastic waste [19].

Among the various plastic wastes, polyethylene is of particular concern. PE is often used to produce agricultural films, toys, bags and shampoo bottles, and accounts for 64 % of total global consumption of synthetic polymers [20]. In recent years, a lot of microorganisms with the capable of biodegrading PE have been screened from natural environments, such as soil, ocean, waste disposal stations, specific media, etc [21–24]. In 2018, Muonja et al. [25] isolated a number of PE-degrading bacteria from the Dandora landfill. 16SrDNA sequence analysis showed

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that these bacteria belonged to the genus *Pseudomonas bacteriophage*. After 16 weeks of incubation with low-density polyethylene (LDPE) as the sole carbon source, FTIR analysis showed the appearance of new functional groups due to hydrocarbon degradation. In 2020, Maroof et al. [26] screened six strains of bacteria with potential biodegradation activity from a waste treatment plant in Pakistan. After 90 d of incubation with the additive-free LDPE film as the sole carbon source, a slight surface breakdown of the LDPE film was observed by using SEM, and the FTIR spectrum showed the formation of a typical carbonyl peak. Nevertheless, the degree and rate of microbial degradation of polyethylene plastics is significantly lower than that of biodegradable plastics, such as polylactic acid (PLA) [27]. Therefore, new screened microorganisms that can efficiently biodegrade PE have become a hot spot of global attention.

The biodegradation of plastics by yellow mealworm larvae has attracted much attention in recent years. This provides a new development idea for the biodegradation of plastics. A PS-degrading bacterial strain, *Exiguobacterium* sp. strain YT2, was isolated from the intestine of the mealworm by Yang et al. [28]. The results indicated that intestinal bacteria of the mealworm play an important role in PS biodegradation and mineralization. In addition, Brandon et al. found that yellow mealworms could convert the ingested PE to CO₂ with a conversion rate of $42.0 \pm 1.4\%$ within 32 days. Analysis of the gut microbiome by high-throughput sequencing showed that *Citrobacter* sp. and *Kosakonia* sp. were associated with the biodegradation of PE [29]. Yang et al. [30] found that although yellow mealworms were able to biodegrade PS and LDPE, the degradation of PS was higher than that of LDPE. Finally, the gut microbial community analysis showed a significant shift in gut flora towards communities associated with PS and LDPE biodegradation under different feeding conditions. These results suggest that the degradation of plastics by yellow mealworm larvae is a non-specific one. Several cultures of PE-degrading bacteria have been isolated from plastic-degrading yellow mealworm larvae [31,32]. Yang et al. [31] isolated two biodegradable PE strains, namely *Enterobacter asburiae* YT1 and *Bacillus* sp. YP1, from the intestine of *plodia interpunctella* larvae (Indian meal moth). The results showed that suspension cultures of YT1 and YP1 (10^8 cells/ml) were able to degrade approximately $6.1 \pm 0.3\%$ and $10.7 \pm 0.2\%$ of the PE film (100 mg), respectively, over a 60-day culture period. Yin et al. [32] isolated two PE-degrading bacteria, *Acinetobacter* sp. strain NyZ450 and *Bacillus* sp. strain NyZ451, from the intestine of yellow mealworm larvae. Results showed that the two strains co-cultured had the ability to biodegrade PE, but each strain alone did not have such ability. This work a paradigm for the enhanced degradation of polyethylene by the interaction of two bacteria. These results suggest that the gut of insects may be a potential resource for screening PE-degrading microorganisms.

In this work, using HDPE film as the only carbon source, a *bacillus* strain was isolated from the intestine of the mealworm. The degradability and properties of the strain was also evaluated based on mass loss, changes in surface physicochemical properties, crystallinity and molecular weight of the HDPE films during the limited incubation period. Our results confirm the biodegradation of HDPE by intestinal bacteria isolated from yellow mealworms, which is also considered as a good source of plastic degrading microorganisms.

2. Material and methods

2.1. Plastic materials

High purity HDPE film (Lanzhou Petrochemical Co. HDPE) [weight-averaged molecular weight (Mw): 158700 ± 980 Da, and number-averaged molecular weight (Mn): 72500 ± 590 Da] was used as a feed for the mealworms. HDPE film is cut into 3×8 pieces and cleaned with a stream of air to remove any residues.

Traditional polyethylene film sterilisation method: cut the film into $1 \text{ cm} \times 5 \text{ cm}$ strips, soak in 99.7 % anhydrous ethanol for 0.5 h, rinse 3

times with sterile water, blot the surface with sterile filter paper, air dry on the ultra-clean table and irradiate with Ultraviolet light (UV) light for 15 min

2.2. Mealworm maintenance

Mealworms, larvae of *Tenebrio molitor* (average weight: 65–75 mg/worm), were purchased online from insect breeding plants in Shenzhen China. Prior to arrival, the mealworms were fed bran; after arrival, they were subject to a 48 h starvation period before initiating tests with the experimental diet of HDPE. Mealworms (~300 per experimental condition) were bred in iron box (volume: 200 ml) and kept in incubators maintained at 25 °C and 70 % humidity [33,34]. The HDPE-fed mealworms were initially given 2.6 g of HDPE. Mealworms were kept on the experimental diet for ~3 weeks to acclimate to the experimental diet prior to use in any experiments. These mealworms were cultured for one month and then used in subsequent experiments.

2.3. Culture medium

The inorganic salt medium was prepared by dissolving K₂HPO₄ 0.7 g, KH₂PO₄ 0.7 g, MgSO₄·7H₂O 0.7 g, NH₄NO₃ 1.0 g, NaCl 0.005 g, FeSO₄·7H₂O 0.002 g, ZnSO₄·7H₂O 0.002 g, MnSO₄·H₂O 0.001 g in 1 L of deionized water [35]. The Luria-Bertani (LB) medium was prepared by dissolving 10 g NaCl, 10 g tryptone, and 5 g yeast extract in 1 L deionized water [35]. 15 g agar was added to prepare the LB agar medium. 0.9 g NaCl was dissolved in 100 ml deionized water to prepare saline. All media buffers and solutions were subjected to high-pressure steam sterilization (121 °C, 103.4 kPa, 20 min).

2.4. Isolation of the PE-degrading strains

Larvae (~300) were fed with HDPE film for 21 days, and then 10 larvae were collected. After sterilization, the larvae were dissected and then the intestinal tissue was placed into a 1.5 ml centrifuge tube containing 1 ml of normal saline, and then shaken on a vortex mixer for 5 min. A pure intestinal cell suspension was obtained and used as a bacterial inoculum to enrich HDPE-degrading bacteria. The cell suspension (1 ml) was distributed evenly on the surface of a MSM agar plate, which was then covered with HDPE film. Two control groups were set up. One was only inoculated with bacterial culture, while the other was only covered with HDPE film. After culturing for 72 h at 35 °C, colonies were picked and then spread on fresh LB agar plates until a pure colony was finally obtained according to the standard methodology of bacterial isolation [36]. The single colonies obtained by scribing on solid medium were stored at 4 °C for backup.

2.5. Phylogenetic analysis of PE-degrading strains

The 16SrRNA gene sequence of the strain was amplified and sequenced, and the DNA of individual strains was extracted using the kit (SK8255 Bioengineering (Shanghai) Co.), and the bacterial universal primers 27F and 1492R were used as amplification primers for PCR amplification.

16SrDNA sequence primer 27F: 5'- CAGAGTTGATCCTGGCT -3'; 1492R: 5'-GGTTACCTTGTACCTT-3'.

The amplified products were detected by electrophoresis, and sequenced by Bioengineering (Shanghai) Co. The obtained sequences were analyzed by BLAST with the existing sequences in the NCBI database, and strains with similar homology were selected to construct a phylogenetic tree using MEGA 6 software.

2.6. Biodegradation assays

Under aseptic conditions, the above-isolated strains were inoculated into a liquid inorganic salt medium containing HDPE film pieces at a

speed of 150 r/min, and cultured in a shaker flask at 35 °C. The polyethylene films were removed from the co-cultures after 42 d of treatment with the inoculated strains. The polyethylene was immersed in a 2 % (v/v) sodium dodecyl sulphate (SDS) solution for 4 h, then washed several times with sterile water to remove biofilm from the surface of the film, dried at 40 °C and used for subsequent characterization tests.

2.6.1. Weight loss

Measurement of weight loss is a commonly used and standardized method for the assessment of plastic biodegradation. Weigh the film on a 10,000 ppm balance according to the formula below to calculate the weight loss of the film during incubation. The above experiments were done in 3 parallel for each group to minimize the error.

$$\text{Weight loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100\%$$

2.6.2. Contact angle

The membranes were washed and dried naturally, and the static water contact angle was measured with a water contact angle meter (JC2000D1; China) to analyze the change in hydrophobicity of the membrane surface. Each sample was placed on a horizontal platform, and a small amount of water was added dropwise to the sample membrane. The above experiments were done in 3 parallel for each group to minimize the error.

2.6.3. Changes in surface micromorphology and chemical functional groups of HDPE films

A scanning electron microscope was used to examine the surface topography and characterization of the HDPE sheet before and after biodegradation (SEM, Philips-X LP30, FEI company, OR, USA). HDPE sheets were removed from the medium and dried for 24 h. The HDPE sheets were vapor-fixed in a sealed container at 25 °C for two days. The samples were gold-coated using BAL-TEC-SCDOOS and SEM examined.

Infrared absorption spectra were recorded with a Satellite 5000 infrared spectrophotometer (FTIR, Thermo, USA), using air as a reference, with a spectral sweep in the range of 400–4000 cm⁻¹. Sample vibration spectra were acquired using the attenuated total reflection (ATR) mode of FTIR with a resolution of 4 cm⁻¹.

To investigate degradation and determine the variations of functional groups on the surface of the HDPE film, XPS (Thermo Scientific K-Alpha, Thermo Fisher, United States) was used to measure the binding energy. The HDPE film (5 × 5 mm) was fixed on a carbon ribbon and measured within the energy range of 2+ P–300 eV, C1s.

2.6.4. Thermal properties DSC

The thermal behavior was investigated using TGA-DTA-DSC (Perkin Elmer Diamond Simultaneous) under nitrogen atmosphere in the temperature range of 50–500 °C at a constant heating rate of 10 °C/min [37].

2.6.5. XRD

XRD measurements were performed to determine the crystal structure on a Bruker D8 Advance diffractometer operating at 40 kV and 40 mA, using Cu-Kα radiation ($\lambda = 1.5406 \text{ \AA}$) and diffracted beam monochromator, using a step scan mode with the step size of 0.075° (2θ) and scan rate of 1°/min [38].

2.6.6. HT-GPC

Number-average molecular weight (Mn), weight-average molecular weight (Mw) and molecular weight distribution (MWD) were determined by high temperature-gel permeation chromatography [39]. HDPE samples were extracted by 1,2,4-trichlorobenzene and injected into (Injection volume: 200 μL) a HT-GPC operating at a 1,2,4-trichlorobenzene eluent flow rate of 1.0 ml/min and temperature of 150 °C (HT-GPC 120, Agilent, Japan).

2.7. Statistical analysis

Statistical ANOVAs were performed using SPSS 20.0 (SPSS Inc., Chicago, United States) to evaluate the differences in contact angle changes and weight loss produced by bacteria. Pairwise comparisons were analyzed with the student's t-test, as all data were normally distributed. All error values are reported as the mean value ± standard deviation.

3. Results and discussion

3.1. Feeding behavior of mealworms on HDPE Film

After one week of feeding yellow mealworms with HDPE film, holes had appeared on the surface of the film (Fig. 1b). After one month of feeding, the weight loss of the HDPE film reached 32 % (Fig. 1c). One month after consuming the HDPE film, the yellow mealworms grew well and increased their average weight. The average consumption of HDPE film by larvae after one month was calculated to be 90.28 mg/100 larvae-d based on the data in Fig. 1c. The survival rate after one month was 57.26 ± 2.72 % after counting the surviving mealworms. The above results indicate that HDPE films are not-harmful to yellow mealworms during the short term.

3.2. Isolation and screening of HDPE-degrading microorganisms

After one week of incubation, numerous strains of bacteria grew around the edges of the HDPE film in the experimental group (Fig. 2b), while no microorganisms were found in the control group (Fig. 2a). The results showed that bacterial colonies around HDPE are derived from the gut of the yellow mealworm and have the ability to degrade HDPE. After culturing and purification, a pure colony was finally obtained, which was named as PELW2042. The Gram stain result of strain PELW2042 was purple, indicating that it is a Gram-positive bacterium (Fig. S1). Through the study of its optimal growth conditions in LB medium, the results showed that the optimal growth temperature of strain PELW2042 were 35 °C and the optimal growth pH were 7 (Fig. S2).

When cultivated on solid LB medium, the colonies of strain PELW2042 were round with clear edges and a slightly yellowish color (Fig. 2c). The shape of the bacterial cells examined by using SEM. The results showed that PELW2042 cells were an elliptical rod shape with the length of approximately 1.7 μm and the diameter of 0.5 μm (Fig. 2d). After Calmodulin (CaM) gene sequence analysis, the strain PELW2042 was identified as *Bacillus* as the 100 % evolutionary homologous with *Bacillus* by the Neighbor-Joining method (Fig. 3), and was thus named as *Bacillus* sp. PELW2042 (GenBank: OQ547295). In previous studies, different species of *Bacillus* have been used to degrade a variety of different plastic wastes. *Bacillus* strain 27 screened from mangrove sediments showed 4.0 % degradation of PP microplastics [40]. In addition, *Bacillus* strain YP1 (extracted from the intestine of Waxworms) can degrade PE [31]. The results showed that the bacterium YP1 was able to degrade 10.7 % of PE within 60 days of incubation.

3.3. Surface morphology of HDPE

As shown in Fig. 4, the untreated HDPE film had a smooth surface with no cracks (Fig. 4a), while the HDPE film treated with strain PELW2042 for 20 days had visible cracks on the surface. Furthermore, it was also observed that the strain adhered to the surface of the HDPE film forming a biofilm (Fig. 4b). The formation of biofilms indicates that bacteria was able to use insoluble materials efficiently (Include PE) [41]. After 42 days of co-culture, significant pits and cavities were observed on the surface of the HDPE film with a maximum width of about 20 μm (Fig. 4c-d). The formation of cavities and pits reflected the activity of the biofilm produced by strain PELW2042 on the HDPE membrane surface.

Sowmya et al. [42] concluded that SEM analysis of plastic

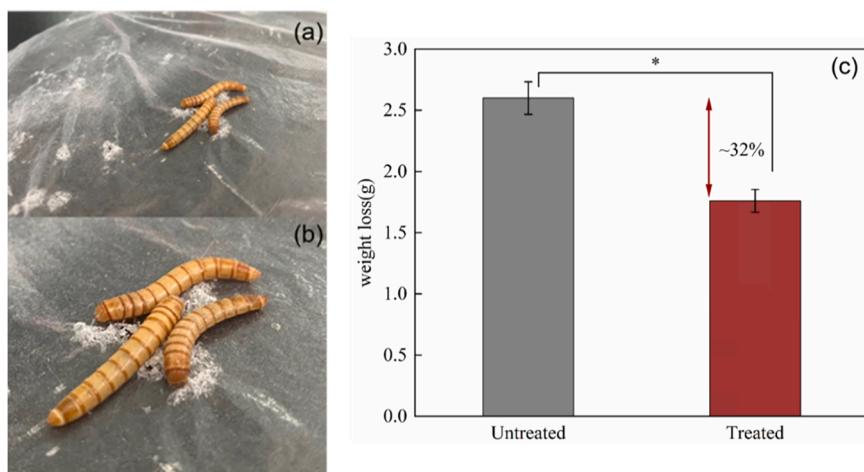


Fig. 1. Biodegradation of HDPE by *Tenebrio molitor* (a) HDPE film after exposure to ~300 *Tenebrio molitor* for one week; (b) Magnification of the area indicated in a; (c) Mass loss of HDPE film after exposure to ~300 *Tenebrio molitor* for one month, showing a reduction of ~32 % from the original mass (The mass loss percentage of HDPE was significantly increased ($P < 0.05$) after feeding.).

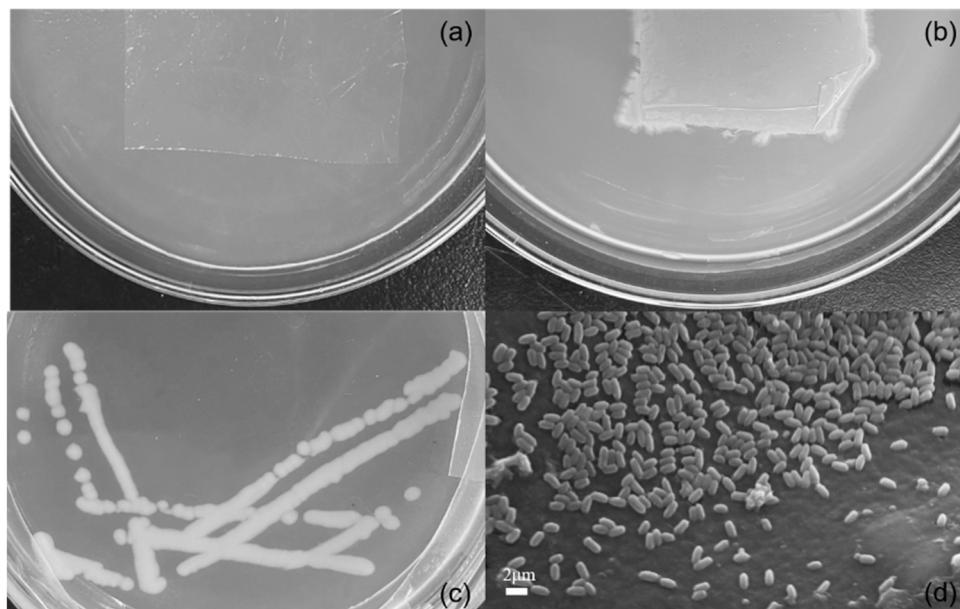


Fig. 2. (a) the control (b) HDPE degrading strain (c) Colonies of PELW2042 on LB agar plates (d) SEM of PELW2042.

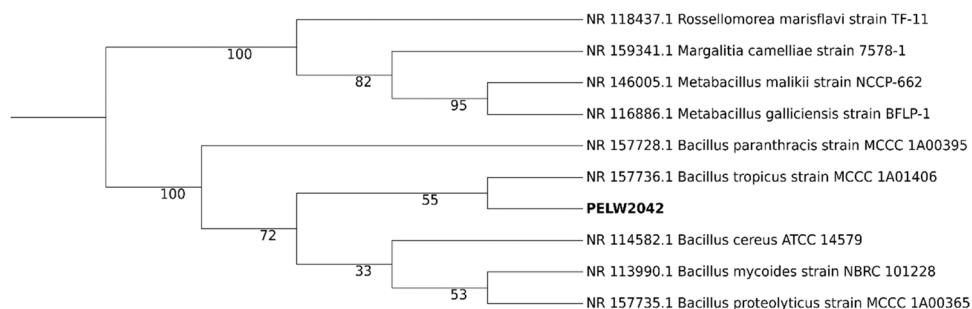


Fig. 3. Neighbor-joining phylogenetic tree of strain PELW2042 based on the 16S rRNA gene.

degradation by bacterial isolates is a direct method to confirm the evidence of pores, cavities and damage of biodegradation. Previous studies on PE degradation bacteria have also shown that the PE film surface produces breakage [43,44]. In contrast, the cavities caused by the strain

PELW2042 exhibited a larger pore size. This indicates that the strain PELW2042 screened from the intestine of mealworm has high activity in biodegradation of PE.

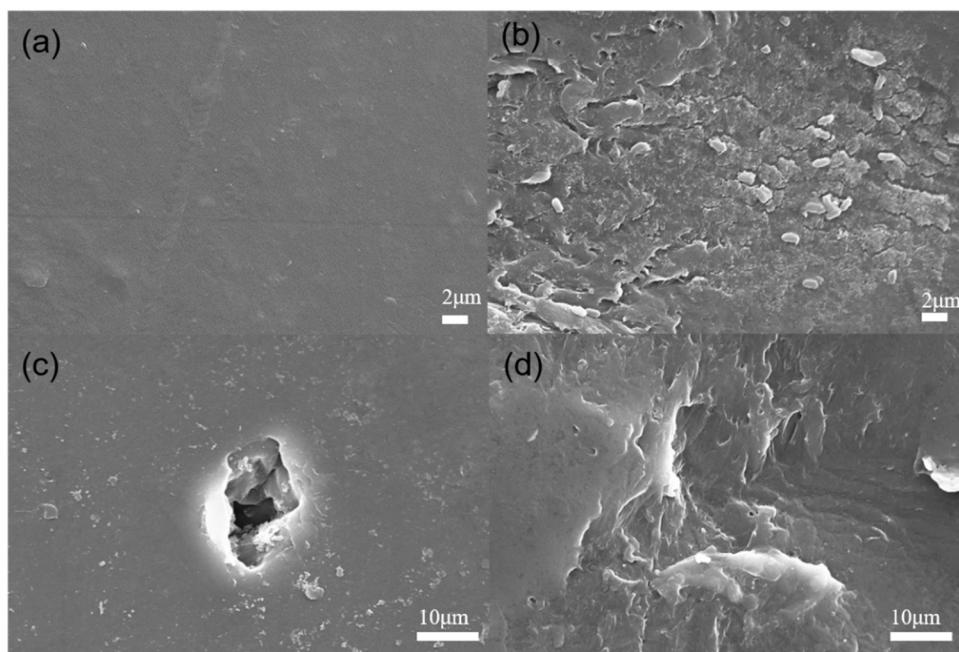


Fig. 4. (a) Blank control; (b) Biofilm formation with cracks after 10 days of co-culture with strain PELW2042; (c) Showed the formation of HDPE surface cavities after 42 days of co-culture; (d) Co-culture with strain PELW2042 for 42 days showed the formation of a huge breakage.

3.4. Hydrophobic/hydrophilic

The size of the contact angle reflects the degree of hydrophilicity or hydrophobicity of the HDPE film surface. Change in surface hydrophobicity of plastic material is an important indication of biodegradation because the degree of hydrophobicity on the HDPE film surface determines the degree of microbial colonization of the polymer matrix. In general, it is believed that more hydrophilic surfaces are more likely to be colonized by microorganisms. As shown in Fig. 5, the contact angle of the HDPE surface was reduced from 86.37° to 74.29° by 14.0 % of treatment with PELW2042. The reduction in contact angle indicates that the HDPE film surface becomes more hydrophilic, which makes it less difficult for microorganisms to adhere to and colonize the surface.

PE has a special environmental durability that makes it extremely difficult to degrade in the natural environment. The resistance of PE to microbial degradation may be due to its physicochemical characteristics, including high molecular weight (Mw), polymer structure without functional groups, and hydrophobicity [45]. Among other things, the high hydrophobicity of PE results in microorganisms or extracellularly secreted enzymes not being able to interact directly with the PE surface, which in turn prevents biodegradation from taking place [46]. Studies have showed that bacteria lower the surface tension of plastics by secreting surfactants, which helps bacteria adhere to the surface of PE films [47]. Vimala et al. [48] also found that surfactants isolated from *Bacillus subtilis* could improve the efficiency of PE degradation by

Bacillus subtilis. This suggests that the high degradation rate of HDPE by strain PELW2042 may be partly due to its secreted surfactant.

3.5. Changes in chemical structure of HDPE

As a useful tool for identifying new functional group changes, infrared spectroscopy is used to analyze the effect of the biodegradation on the HDPE film surface. Fig. 6 showed that there is a significant difference in the spectrograms of HDPE treated with PELW2042 compared to the untreated HDPE. Obviously, spectrum showed an absorption peak at 1249 cm^{-1} and $1665\text{--}1710\text{ cm}^{-1}$ corresponding to ether groups (-C-O-C-) and carbonyl groups (-C=O) respectively (Fig. 6). The formation of carbonyl groups (-C=O) is considered to be the starting step in the biodegradation of plastics [49].

XPS can also be used to analyze changes in surface chemical composition and functional groups. Fig. 7 showed a comparison of XPS scanning spectra of HDPE films from the untreated and inoculated treated groups. The results showed that the untreated HDPE films had only a peak at 284.8 eV representing surface carbon (Fig. 7a), while the

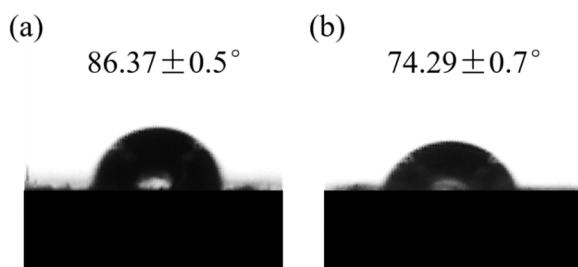


Fig. 5. WCA of HDPE film without (a) or with inoculation by strain PELW2042 (b).

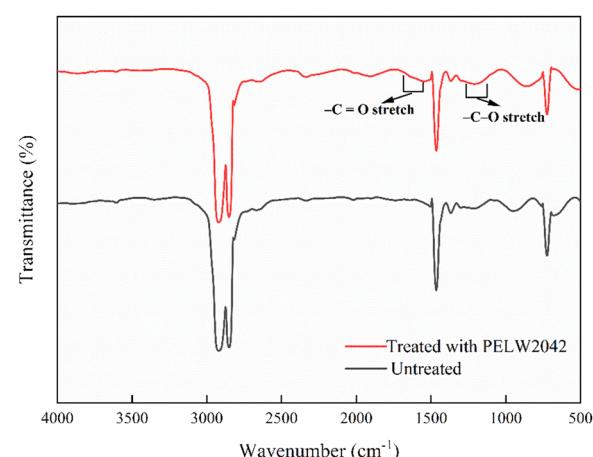


Fig. 6. ATR-FTIR spectra of untreated and strain PELW2042-treated HDPE.

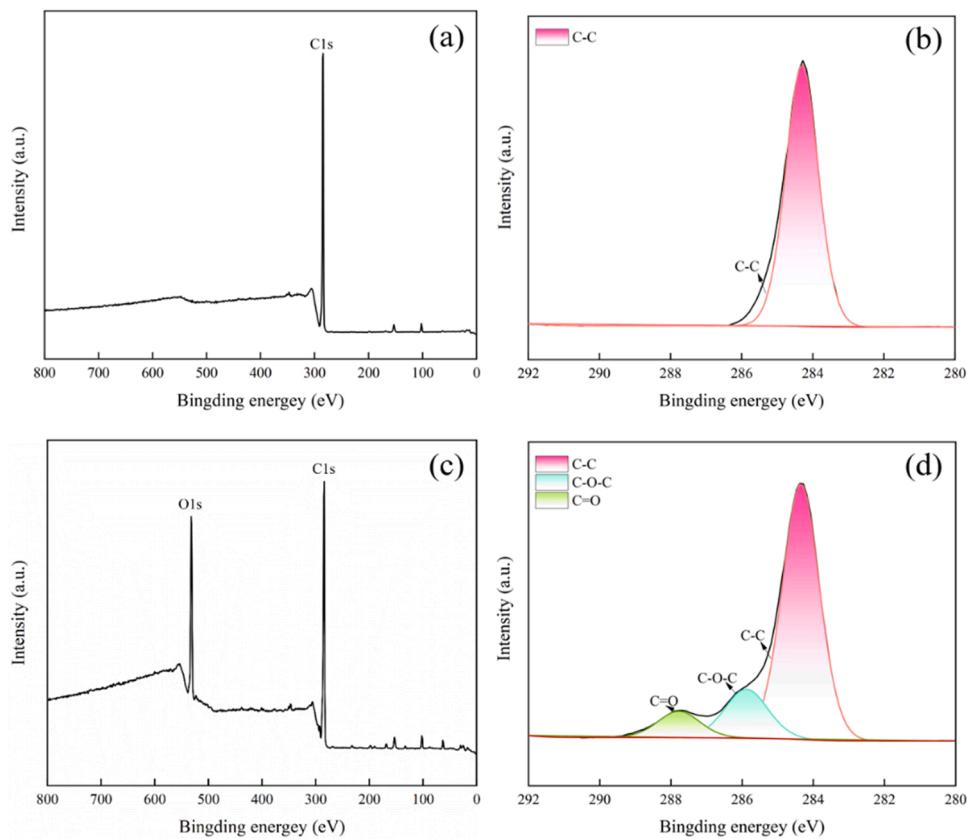


Fig. 7. (a) Total XPS spectra before strain PELW2042 treatment; (b) C1s spectrum before strain PELW2042 treatment; (c) Total XPS spectra after 42 days of strain PELW2042 treatment; (d) C1s spectrum after 42 days of strain PELW2042 treatment.

surface of HDPE films treated with PELW2042 showed another significant peak at 532.3 eV, which indicates increased surface oxygen content in addition to 284.8 eV (Fig. 7c). The C1 peaks of the treated membranes could be fitted as 284.8 eV (C–C), 285.9 eV (C–O–C) and 287.8 eV (–C=O) (Fig. 7d). This result was consistent with the above FTIR analysis, and further confirms the formation of new functional groups on the PE surface.

According to previous studies [50–52], the presence of carbonyl and ether groups provided evidence of biodegradation of HDPE and ultimately promotes the biodegradation of PE. The formation of oxygen-containing groups was the result of the action of some hydroxylating enzymes, such as AlkB or P450. Hydroxylases perform initial hydroxylation at the PE terminal ω -carbon, followed by the formation of the corresponding ketone via primary alcohol dehydrogenase (Adh) and

finally conversion to carboxyl groups to produce alkanoic acids [53].

3.6. XRD\DSC

XRD analysis is one of the most used methods for the primary characterization of material properties. As showed in Fig. 8a, peak difference method and simulation calculation showed that the crystallinity of HDPE film decreased from 45.14 % to 23.51 % after 42 days of co-culture of strain PELW2042 with HDPE film. The decrease in crystallinity indicates a change was generated in the molecular structure of the polyethylene polymer. During the biodegradation, the amorphous part of the polyethylene sample is usually attacked by microorganisms. As a result, biodegradation leads to a decrease in the total melt peak of polyethylene samples. As showed in Fig. 8b, the melting point peak was

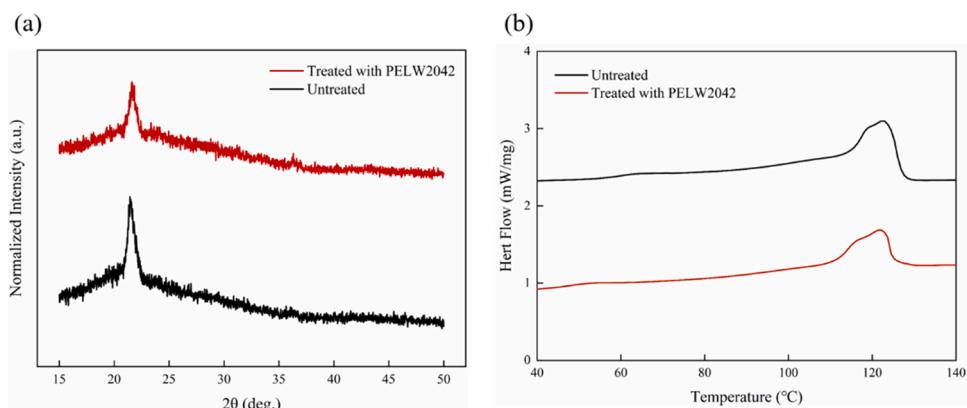


Fig. 8. (a) XRD analysis of untreated and PELW2042-treated HDPE; (b) DSC analysis of untreated and PELW2042-treated HDPE.

reduced in the experimental group compared to the untreated group. As mentioned above, polyethylene undergoes self-oxidation, which de-polymerizes the amorphous region and decreases the percentage of crystallinity with the participation of the strain during the degradation process. At the same time, the decrease of crystallinity can increase the content of hydrophilic groups in the polymer, this promotes easier diffusion of water into the sample. On the other hand, the penetration of water molecules into PE also accelerates the microbial degradation rate of PE.

3.7. Weight loss and molecular weight decrease of HDPE samples

The weight loss is an important monitor index for biodegradation of polymer, which includes mass loss and molecular weights loss (Mw loss and Mn loss). After 42 days of co-culture in strain PELW2042, the mass loss of HDPE film was up to $17.36 \pm 0.56\%$. In addition, strain PELW2042 performed well in terms of molecular weight variation compared to the previously reported work. After 42 days of treatment by strain PELW2042, the molecular weights of the experimental group samples were significantly decreased about $23.31 \pm 1.25\%$ and $30.07 \pm 1.37\%$ for Mw (121700 Da) and Mn (50700 Da), respectively, compared to those of the untreated samples (Mw=158700 Da and Mn=72500 Da). From the Mw distribution curve, the PE treated with PELW2042 showed a significant decreased trend, which indicated that the long chain structure of HDPE was depolymerized and formed lower molecular weight fragments in the presence of strain PELW2042. It proved that the strain effectively metabolized and biodegraded the HDPE film. The weight loss was mainly ascribed to the consumption of polyethylene as the sole carbon source. At the same time, the consumption of polyethylene by the strain will cause the long chain of polyethylene to break. According to the GPC data, the fracture occurs at the end of the long molecular chain of polyethylene, which narrows the molecular weight distribution of polyethylene and degrades the number average molecular weight. As a result, with the extension of the degradation time, the oxidation level of polyethylene and the weight loss rate of polyethylene gradually increases, while the chemical inertness and the molecular weight gradually decreases, as well as the molecular weight distribution.

Most PE-degrading microorganisms were isolated from soil, waste treatment plants and seawater samples [54–56]. Compared to these strains, strain PELW2042 produced a higher weight loss in a shorter period of time. However, such weight loss is very low compared to the direct consumption of PE by insect larvae [30]. This phenomenon suggests that insect hosts may play a significant role in the biodegradation of plastics. Brandon et al. [57] showed that insect larvae secrete emulsifiers to mediate plastic biodegradation. These results suggest that

insect larvae and their gut microbiome could accelerate the biodegradation of plastics through synergistic effects. Therefore, the specific mechanism of this synergistic effect needs further study to provide a theoretical basis for the biodegradation of waste plastics. (Figs. 9 and 10).

4. Conclusions

In this study, a strain of *Bacillus* sp. with the ability to biodegrade HDPE was isolated from the gut of mealworm. The degradation capacity of this strain, PELW2042 (GenBank: OQ547295), was comparable or better than any other bacteria previously identified. The degradability of the strains was confirmed by not only the weight loss and molecular weight reduction, but also the changes in surface morphology, reduction in hydrophobicity, formation of carbonyl groups and reduction in crystallinity. Future study also needed to confirm the performance of biodegradation of LDPE and LLDPE with different molecular sizes and feasibility of biodegradation of other plastic polymers e.g., PP, PVC and PS by this strain. The discovery of PE-degrading bacteria in the intestine of yellow mealworms and their degradation properties provide potential solutions for further research on microbial degradation of waste plastics.

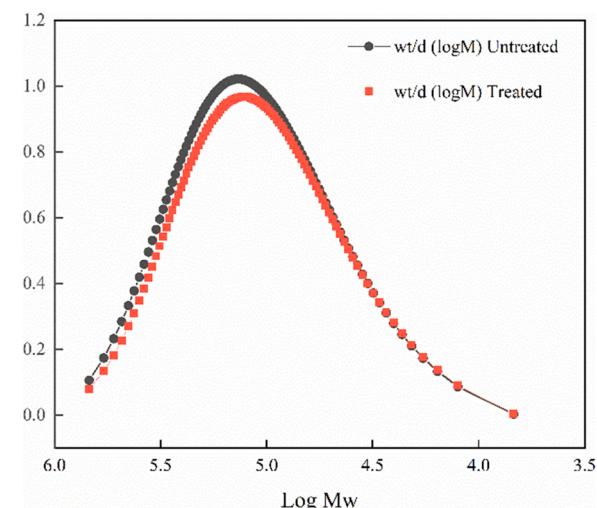


Fig. 10. The Mw distribution curve of HDPE after 42 days of incubation with strain PELW2042.

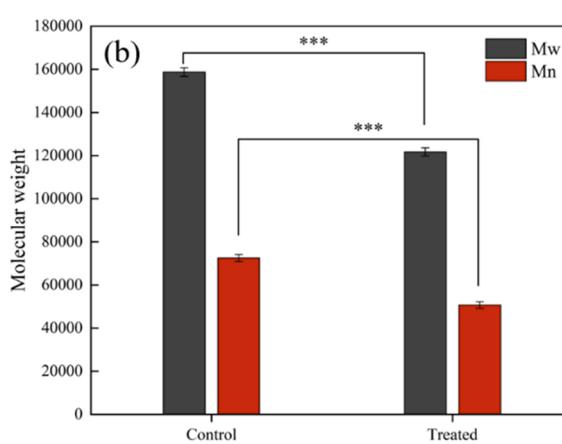
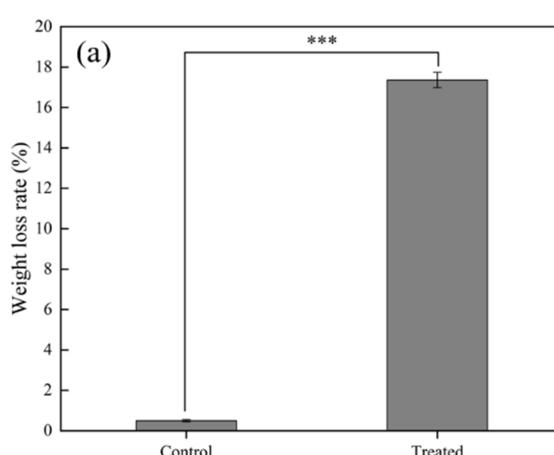


Fig. 9. The weight loss rate ($P < 0.0001$) (a) and Molecular weight (Mn and Mw) changes (b) of HDPE after 42 days of incubation with strain PELW2042 (The Mw and Mn after degradation were significantly decreased ($P < 0.0001$) compared to that of incubated before.).

CRediT authorship contribution statement

Hong Zhang: Conceptualization, Funding acquisition, Writing-review & editing, Supervision. **Qiang Liu:** Investigation, Formal analysis, Validation, Writing-original draft. **Hui Wu:** Conceptualization, Resources, Validation, Writing-review & editing. **Wenxiao Sun:** Investigation, Validation, Writing-original draft. **Fan Yang:** Writing-review & editing. **Yuhao Ma:** Methodology, Writing-review & editing. **Yanjiao Qi:** Writing-review & editing.

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.

Acknowledgments

This work is supported by the "Star of Innovation" program for graduate students of the Gansu Provincial Education Department of China (2022CXZX-204). The Science and Technology Plan of the Gansu Province of China (20YF8GA044). This work was supported by Gansu Provincial Department of Agriculture and Rural Affairs Project "Research on Functional Modification of Recycled Waste Mulch as Highway Asphalt Additive and its Engineering Demonstration" China (2022620005001932).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.procbio.2023.04.006](https://doi.org/10.1016/j.procbio.2023.04.006).

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