



## Biodegradation of polystyrene by bacteria isolated from the yellow mealworm (*Tenebrio molitor*) gut

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### ARTICLE INFO

Editor: P. Fernández-Ibáñez

#### Keywords:

*Tenebrio molitor*  
Polystyrene  
Biodegradation  
*Klebsiella*  
*Cellulosimicrobium*

### ABSTRACT

Polystyrene (PS) is commonly used in human production and life because it is chemically stable and easy to produce and process. However, PS is difficult to degrade naturally, which leads to environmental pollution and threats to human and animal health. In this study, two bacterial strains known to degrade PS, *Klebsiella* sp. WJ2020 and *Cellulosimicrobium* sp. WJ2025 were isolated from *Tenebrio molitor* intestines. Both strains could grow with PS as their sole carbon source and caused weight loss of 4.35% and 6.93% to the PS films over a 60 day-incubation, respectively. The number-average molecular weights of the PS after incubation with the strains also decreased by 4.85% and 10.48%, respectively. The surface of the PS films had a significant lamellar etching after microbial action. Moreover, WJ2020 and WJ2025 imparted more oxygen to the PS surface and formed additional hydroxyl groups, which led to a decrease in the hydrophobicity of the PS film surface. The roughness of the degraded PS films was increased compared to the PS films without bacterial treatment. Results from this study provide a potential solution for the natural biodegradation of PS while adding to the scientific knowledge of the function of the gut microorganisms of the yellow mealworm.

### 1. Introduction

Plastic is an important organic-synthetic polymer with a wide range of applications. The most widely produced and used plastics worldwide today include polyethylene (PE), polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), and polyurethane (PUR) [1]. With the growth of the plastics industry and the widespread use of disposable plastic products, the environmental pollution, including water and soil pollution caused by plastic waste, has become increasingly serious [2,3]. Plastic pollution is a serious threat to the global ecological environment [4], and the presence of microplastics acquired through the food chain has been detected in the human and animal body [5–7]. This poses an unprecedented threat to human survival and health [8].

Polystyrene is a polymer synthesized from styrene monomers by a free radical polycondensation reaction [9]. The widespread use of PS has led to the generation of large amounts of waste, especially expanded polystyrene (EPS) waste. Traditional disposal methods for waste PS and other plastics include landfill, incineration and recycling [10]. More than 90% of these waste plastics are disposed of by landfilling or

incineration, with only about 9% being recycled [11]. Although landfills and incineration plants are easy to operate, they are a potentially harmful source of environmental pollution. The use of biodegradation technology to treat plastic waste is considered being the most promising and environmentally friendly treatment method. However, waste PS is difficult to degrade in the natural environment because of its linear carbon backbone and alternating backbone atoms attached to phenyl moieties [12]. In recent years, several reports have shown that some insect larvae, such as *Tenebrio molitor*, *Tenebrio obscurus*, and *Zophobas atratus*, can ingest PS and participate in its degradation [13–16] and using these insect larvae to treat plastic waste, including PS, is easy, low cost and environmentally friendly.

Some researchers have identified microorganisms with PS degradation ability from yellow mealworm (*T. molitor*) larvae [17]. Characterization of the degraded PS showed that microorganisms are involved in PS degradation and may even play a dominant role in the degradation process. Lou et al. analyzed the molecular weight of residual PS and low-density polyethylene (LDPE) after feeding it to yellow mealworms, to demonstrate bacterial depolymerization and biodegradation. Significant changes also occurred in the intestinal microbial community of the

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yellow mealworms after feeding on plastics. Changes in the gut microbiome because of dietary shifts suggest that multiple plastic-degrading bacteria may be present in the gut of yellow mealworms [18].

Microorganisms capable of degrading PS have also been isolated from the intestines of insect larvae. Yang et al. isolated a PS-degrading strain, *Exiguobacterium* sp. YT2, from the intestine of yellow mealworms, and it degraded PS by up to  $7.4\% \pm 0.4\%$  during 60 days of incubation [19]. Woo et al. reported that the biodegradation of PS by *Serratia* sp. WSW, isolated from the intestinal flora of *Plesiophthalmus davidi*, formed biofilms and cavities in PS films within 20 days, but the degradation was less pronounced than that of the intact intestinal flora [20]. *Pseudomonas aeruginosa* DSM 50071, isolated from the intestine of the superworm (*Zophobas atratus*) also could degrade PS, and molecular analysis showed that the gene expression level of serine hydrolase was greatly increased during PS degradation [13]. In a previous study, a PS-degrading strain *Massilia* sp. FS1903 was isolated from the intestine of the greater wax moth larvae (*Galleria mellonella*), and this strain degraded PS by nearly 13% after 30 days of incubation [21].

Here, two previously unreported PS-degrading bacterial strains were successfully isolated from the intestines of PS-ingesting yellow mealworms. To determine the PS degrading ability of the strains, the physical and chemical changes in the degraded PS films were investigated with scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS), water contact angle (WCA), attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR), and gel permeation chromatography (GPC). This work will help to identify and understand PS-degrading strains and provide solutions for the future development of biodegradation technologies for difficult-to-degrade synthetic polymers.

## 2. Materials and methods

### 2.1. Experimental materials

*Tenebrio molitor* larvae were purchased from Tianjin Huiyude Biotechnology Co. (Tianjin, China). EPS foam with a PS purity of over 98% was obtained from Nannan Building Materials Co. (Zhejiang, China). The number-average molecular weight (Mn) and weight-average molecular weight (Mw) of EPS were 85,000 and 270,000 Da, respectively, as measured by gel permeation chromatography (GPC). The PS film for microbial degradation was prepared from the EPS according to the methodology of Yang et al. with minor modifications [19]. When EPS was fully dissolved in dichloromethane, three times the volume of anhydrous methanol was added and the PS precipitate was collected to remove the additives from the EPS. The collected PS precipitate was washed, dried and re-dissolved in dichloromethane (0.06 g/mL), then 10 mL of the solution was placed in a 9 cm diameter glass petri dish. Once the dichloromethane evaporated completely, the resulting film was collected after three days at room temperature. The film was then rinsed with de-ionized water and dried before use. The thickness of the prepared film was approximately 0.03 mm.

The mineral salt medium (MSM, pH 7.2) contained 4.54 g/L  $\text{KH}_2\text{PO}_4$  (Mw, 174.2), 11.94 g/L  $\text{Na}_2\text{HPO}_4 \cdot 12 \text{H}_2\text{O}$  (Mw, 358.1), 1.0 g/L  $\text{NH}_4\text{Cl}$  (Mw, 53.5), 0.5 g/L  $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$  (Mw, 246.47), 5 mg/L  $\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$  (Mw, 147.0), 2 mg/L  $\text{FeSO}_4$  (Mw, 151.9), 1 mg/L  $\text{MnSO}_4$  (Mw, 151.0), and 2 mg/L  $\text{ZnSO}_4$  (Mw, 161.5). All chemicals used were of analytical grade.

### 2.2. Larval feeding and isolation of intestinal PS-degrading bacterial strains

For the feeding experiment, the experimental larval group was fed only EPS, while the control group was fed wheat bran. Yellow mealworms ( $n = 200$ ) were starved for 36 h and then placed in breathable feeding boxes made of PP and fed small pieces of EPS or wheat bran at room temperature for 28 days, during which they were rehydrated every seven days. All larval molts and dead worms were periodically picked

out during the experiment to prevent them from supplementing their EPS diet with cannibalism. After 28 days of feeding, 30 well-grown mealworms were removed and their intestines were dissected and crushed under aseptic conditions. The crushed intestine was added to a 1.5 mL centrifuge tube containing 0.5 mL of sterilized water and shaken thoroughly for 5 min to create a cell suspension. About 0.3 mL of the cell suspension was inoculated into 100 mL MSM containing PS film and incubated at 30 °C with shaking (150 r/min). After two months, the above culture was diluted and spread on Luria-Bertani (LB) agar plates and incubated at 30 °C for 24 h. Bacterial colonies with different morphological characteristics were selected for further delineation and isolation until pure colonies were obtained. Selected pure colonies were cultured in LB liquid medium for 18–20 h and the cells were collected by centrifugation and washed with sterile saline. The bacterial cells were diluted and evenly spread on MSM agar plates and covered with sterile PS film (1 cm × 3 cm) and incubated at 30 °C [21]. Colony growth was observed periodically to determine the potential PS degradation ability of the strains.

### 2.3. Bacterial identification

Genomic DNA was extracted from logarithmic growth stage colonies using the TIANamp Bacteria DNA Kit (Tiangen Biotech, Beijing, China) for 16 S rDNA amplification and identification. Then genomic DNA was amplified by polymerase chain reaction using the universal primers 27-F (5'-AGAGTTTGATCTGGCTCAG-3') and 1492-R (5'-GGTTACCTTGT-TACGACTT-3') [22]. The sequences obtained were compared to those of known organisms in the EzBioCloud Database (<https://www.ezbiocloud.net/>). A phylogenetic tree was constructed in MEGA 7.0 using the neighbor-joining method with 1000 bootstrap replicates [23]. The assay of microbial physiological and biochemical characteristics was carried out according to Bergey's Manual of Determinative Bacteriology (Ninth Edition) [24].

### 2.4. Analysis of PS film surface with or without degradation

The selected potential PS-degrading strains were inoculated on MSM plates with PS films and cultivated at 30 °C for 60 days. Before being analyzed for characterization, PS films were soaked in 2% (w/v) sodium dodecyl sulfate (SDS) solution and shaken for 4 h, and then washed with deionized water to remove the biofilm from the PS film surface [25].

#### 2.4.1. SEM

Microscopic morphological changes on the surface of PS films were observed using the Hitachi SU8010 SEM at an acceleration voltage of 20 kV. To verify the changes in the elemental composition of the PS film's surface during degradation, the carbon and oxygen elemental composition on the PS film's surface was observed using an energy dispersive spectroscopy (EDS) module connected to the scanning electron microscope [26].

#### 2.4.2. XPS

The elemental distribution on the PS film's surface was analyzed using the Thermo Fisher ESCALAB Xi+ XPS, to verify the degradation behavior of the potential PS-degrading strains. The PS films (0.5 cm × 0.5 cm) were immobilized and fixed on carbon ribbon and assayed in the energy range of 2 +P-300 eV, C1s.

#### 2.4.3. WCA

Changes in hydrophilicity of the PS film's surface were assessed using the KRUESS DSA100 WCA. The contact angle test was performed under static conditions at room temperature. The WCA results were taken as the average value of five measurements.

#### 2.4.4. ATR- FTIR

Analysis of the PS films was carried out in attenuated total reflection

(ATR) mode using the Agilent Cary 660 Fourier transform infrared (FTIR) spectrometer. The samples were scanned at a frequency range of 4000 to 400  $\text{cm}^{-1}$ .

#### 2.4.5. GPC

To verify whether the PS films underwent depolymerization after microbial treatment, the weight-average molecular weight ( $M_w$ ), and number-average molecular weight ( $M_n$ ) of the PS films were determined using GPC after 60 days of co-culture with the microorganisms. The GPC was performed using a Waters 1515 Isocratic HPLC Pump with a Waters 1515 refractive index detector with a temperature controller. The samples were fully dissolved in chloroform to obtain a 1.5 mg/mL solution. Tests were performed using 10  $\mu\text{L}$  filtrate, filtered through a 0.22  $\mu\text{m}$  filter membrane. Waters Styragel HT 3 (7.8  $\times$  300 mm) and HT4 columns (7.8  $\times$  300 mm) were used in tandem and different molecular weights of PS were used as standards. The pore size of the columns was 10  $\mu\text{m}$ ; the column temperature was 35  $^{\circ}\text{C}$ ; the column pressure was 1600 psi; the mobile phase was chloroform and the flow velocity was 0.8 mL/min.

#### 2.5. Weight loss of polystyrene films degraded by polystyrene-degrading bacterial strains

Polystyrene films (1 cm  $\times$  3 cm) were soaked in 75% ethanol (v/v) for 2 h and then irradiated front and back under a 30 w UV lamp for 30 min [27]. The selected bacterial strains were inoculated in LB medium (pH 7.2) for 18 h at 30  $^{\circ}\text{C}$  with shaking. At the end of the incubation, the strains were collected by centrifugation and bacterial suspensions ( $\text{OD}_{600 \text{ nm}} = 1.0$ ) were prepared using sterile MSM. Approximately 1% (v/v) of the bacterial suspension was inoculated in MSM medium containing the above-mentioned PS films for 60 days at 30  $^{\circ}\text{C}$  with shaking. After 60 days, the films were removed and soaked in 2% SDS (w/v) for 4 h and washed with deionized water. The films were subsequently dried and weighed to calculate the weight loss due to degradation.

#### 2.6. Statistical analysis

All experiments were performed in triplicate. The data are expressed as mean  $\pm$  standard deviation and the statistical analysis was carried out using Duncan's multiple range test or one-way ANOVA with a significance level set at 5% ( $P < 0.05$ ) by SPSS26.

### 3. Results

#### 3.1. Analysis of *T. molitor* feeding on EPS

The larvae could feed on EPS provided and gnawed many gaps in the EPS. Parts of EPS were even chewed into powder (Fig. 1). However, compared with the control group, the larvae did not feed as well on EPS as on wheat bran. On the edge of the container where the larvae are fed, EPS powder and debris collect, and this grew with time, which was also reported in other studies [28]. No significant mortality of larvae was observed during the experiment, suggesting that larvae can survive on EPS as their only food source.

#### 3.2. Screening of PS-degrading strains

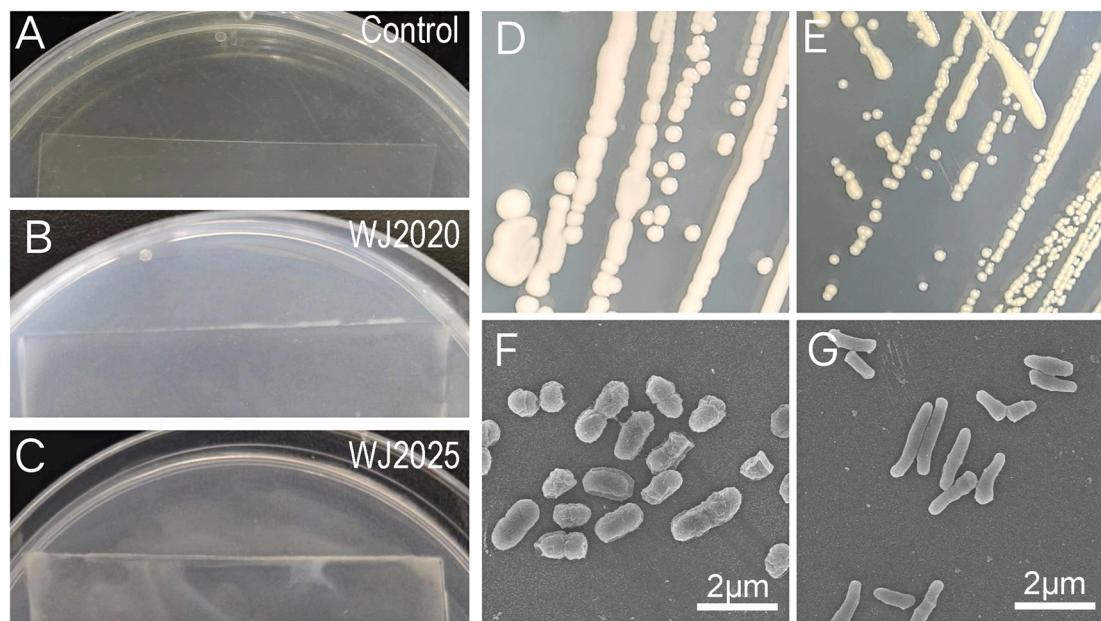
After 60 days in the enrichment culture, the MSM containing the crushed intestinal suspension became turbid. After culture and purification, two single colonies of PS-degrading bacterial strains (named WJ2020 and WJ2025) grew on the edges of the PS film compared to the plates without inoculation (Figs. 2A, 2B, and 2C). It was tentatively determined that WJ2020 and WJ2025 could grow on PS as their sole carbon source. When grown on LB medium, WJ2020 colonies were round, moist, and grayish white (Fig. 2D), while the cells were about 1  $\mu\text{m}$ -2  $\mu\text{m}$  in length and 0.8–0.9  $\mu\text{m}$  in width, rounded at the ends, and meristematic from the center (Fig. 2F). The WJ2025 colonies were round, moist, smooth and lemon yellow (Fig. 2E) and the cells were about 1.5  $\mu\text{m}$ -2.5  $\mu\text{m}$  in length and 0.4–0.5  $\mu\text{m}$  in width, the cells were irregular rod-shaped, and meristematic from the center (Fig. 2G).

#### 3.3. Identification of PS-degrading strains

Two phylogenetic trees were constructed, based on 16 S rRNA sequencing results, to determine the taxonomic relationships of the two PE digesting strains (Fig. 3). Phylogenetic analyses revealed that WJ2020 (GenBank accession no. OP686687.1) clustered with members of the genus *Klebsiella* and was 99% similar to *Klebsiella aerogenes* NBRC13534 (GenBank accession no. NR 113614.1), while WJ2025 (GenBank accession no. OP686575.1) clustered with members of the genus *Cellulosimicrobium* and was 99% similar to *Cellulosimicrobium funkei* W6122 (GenBank accession no. NR 042937.1). Therefore, it was determined that WJ2020 belongs to the genus *Klebsiella*, while WJ2025 belongs to the genus *Cellulosimicrobium*. The physiological and biochemical characteristics of the two strains are described in Table S1.



**Fig. 1.** Yellow mealworm (*Tenebrio molitor*) larvae feeding on EPS. The red circles indicate holes left by yellow mealworms feeding. An arrow in the upper right points to the hole left by the yellow mealworm's feeding. An arrow in the lower right points to the feeding yellow mealworm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



**Fig. 2.** Growth of WJ2020 and WJ2025 on MSM with PS film and LB medium. A. MSM covered with PS film without inoculation; B. WJ2020 grows on MSM covered with PS film; C. WJ2025 grows on MSM covered with PS film; D. WJ2020 colonies on LB medium; E. WJ2025 colonies on LB medium; F. SEM of WJ2020; G. SEM of WJ2025.

#### 3.4. Analysis of PS film after biodegradation

The degradation of the PS films by the bacteria as observed by SEM is shown in Fig. 4. The surface of the PS film without microbial digestion was smooth and flat (Fig. 4A), while the bacterial strains WJ2020 and WJ2025 damaged the PS film after 30 days of incubation (Fig. 4B and Fig. 4C), as the surface of the PS film was scratched and rough. The maximum pore size of the cracks and holes that appeared reached 3  $\mu\text{m}$ .

The PS films that were thoroughly cleaned after incubation with PS-degrading strains were analyzed using SEM with EDS (Fig. 5). There was no significant difference in the number of carbon atoms on the surface between the control and the microbial incubated samples. On the contrary, oxygen atoms were detected on the surface of PS films after microbial incubation, indicating that PS degradation-related enzymes secreted by WJ2020 or WJ2025 had promoted oxidation as part of degradation. This oxidation did not happen in the control [13].

Results from the XPS analyses are shown in Fig. 6. The C1s peak at 285 eV for the control sample was stronger, while the O1s peak at 533 eV was faint. The PS films, after incubation with WJ2020 or WJ2025, still had a significant C1s peak at 285 eV, while the O1s peaks at 533 eV were stronger, indicating an increase in oxygen content. Compared to the control sample, the C1s peak spectra of the PS films co-cultured with the bacterial strains for 60 days showed two new weak peaks at 286.5 eV and 288.2 eV, attributed to -C-O- and -C=O-, respectively, suggesting that oxidation occurred on the PS film surface [13,19,29,30]. In agreement with the results of the EDS analysis, both WJ2020 and WJ2025 could immobilize oxygen on the surface of the PS film.

As shown in Fig. 7, the WCA of the control PS film was  $93.19 \pm 0.52^\circ$ , while the WCA of the PS film incubated with WJ2020 and WJ2025 was  $70.53 \pm 9.07^\circ$  and  $81.54 \pm 4.14^\circ$ , respectively.

Fig. 8 shows the results of the ATR-FTIR analyses. The peaks of the PS at  $3026\text{ cm}^{-1}$  and  $1598\text{ cm}^{-1}$  represent the telescopic vibration and the C=C vinyl of the aromatic C-H, respectively [31]. The peak at  $1492\text{ cm}^{-1}$  represents the deformed vibration of the benzene ring, and the peak at  $750\text{ cm}^{-1}$  is the deformed vibration of the benzene derivative. The peak at  $2920\text{ cm}^{-1}$  is related to the antisymmetric telescopic vibration of -CH<sub>2</sub> [32]. The microbial-treated PS films showed a weak

hydroxyl peak at  $3200$  to  $3600\text{ cm}^{-1}$  because of the stretching vibration of the O-H in the alcohols and phenols. A newly appeared absorption peak at  $1643\text{ cm}^{-1}$  was observed, possibly as a result of the C=O stretching vibration or the C=C stretching vibration.

#### 3.5. Weight loss and molecular weight change of PS films after biodegradation

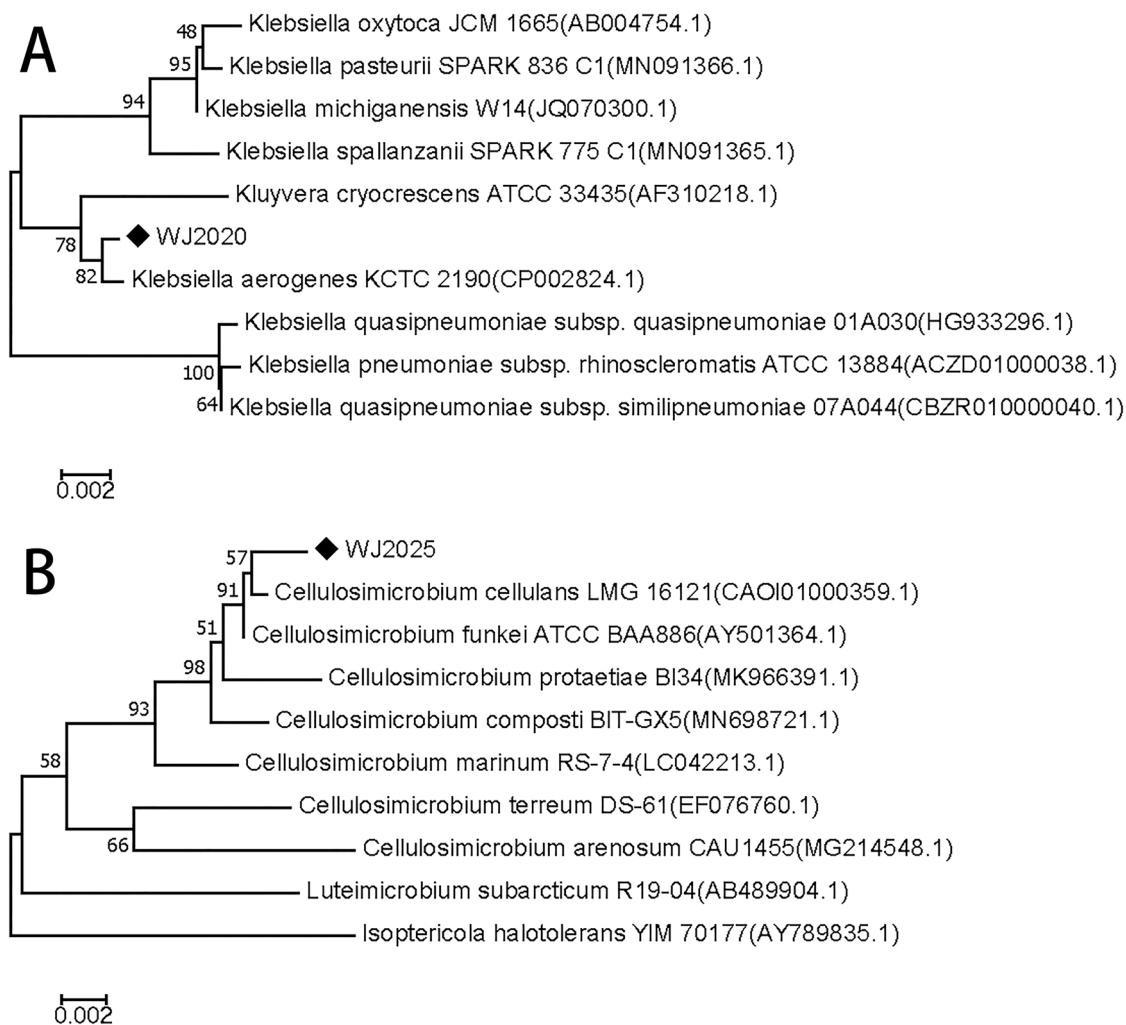
The weight loss of the PS films after co-cultivation with strains WJ2020 and WJ2025 at  $30^\circ\text{C}$  for 60 days is shown in Fig. 9A. The undegraded PS films showed some weight loss, while the weight loss of the PS films incubated with WJ2020 and WJ2025 was higher at 4.35% and 6.93%, respectively. It is noteworthy that the degradation effect of the mixed strains was lower than that of single strains. In general, mixed strains tend to degrade plastics better than single strains. Here, the mixed strains did not show higher weight loss than the single strains, possibly due to an antagonistic effect between WJ2020 and WJ2025, although they were both isolated from yellow mealworm gut. The existence of this antagonistic effect needs to be verified further. The GPC results showed that the molecular weights of the PS films after the action of WJ2020 and WJ2025 changed (Fig. 9B), where Mn decreased in both experimental cases by 4.85% and 10.48%, respectively.

## 4. Discussion

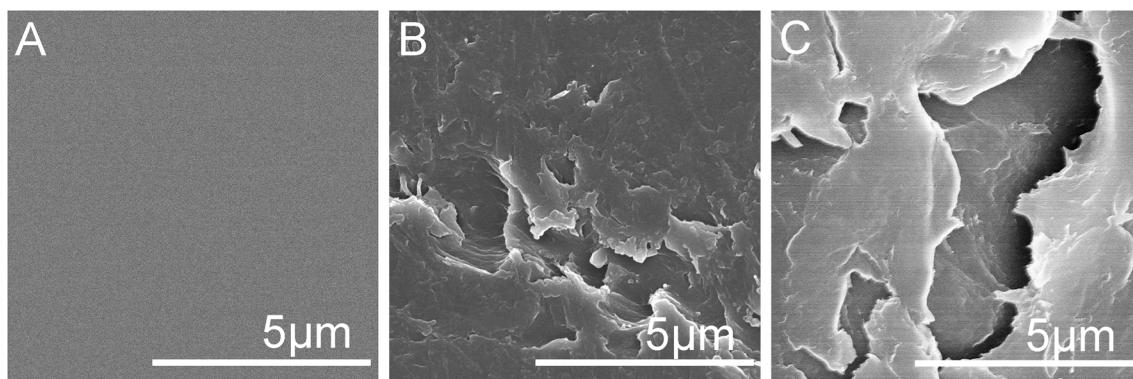
### 4.1. Yellow mealworms feeding on PS and PS-degrading strains isolated from their guts

According to previous studies, larvae of Coleoptera and Lepidoptera have the habit of feeding on EPS [33], where microorganisms in the insect's gut play a crucial role in the digestion of PS [17,34]. Yellow mealworms and barley worms are most frequently reported as feeding on EPS. Although yellow mealworms can feed on EPS, it does not provide the necessary nutrients for growth [15]. It was also hypothesized that feeding on EPS as a sole food source would be harmful to the larvae over a short period.

Biodegradation occurs in environments where various microorganisms are present and the biodegradation of materials is closely related to



**Fig. 3.** Neighbor-joining phylogenetic tree of bacterial isolates WJ2020 (A) and WJ2025 (B) sequences based on the 16 S rDNA gene.

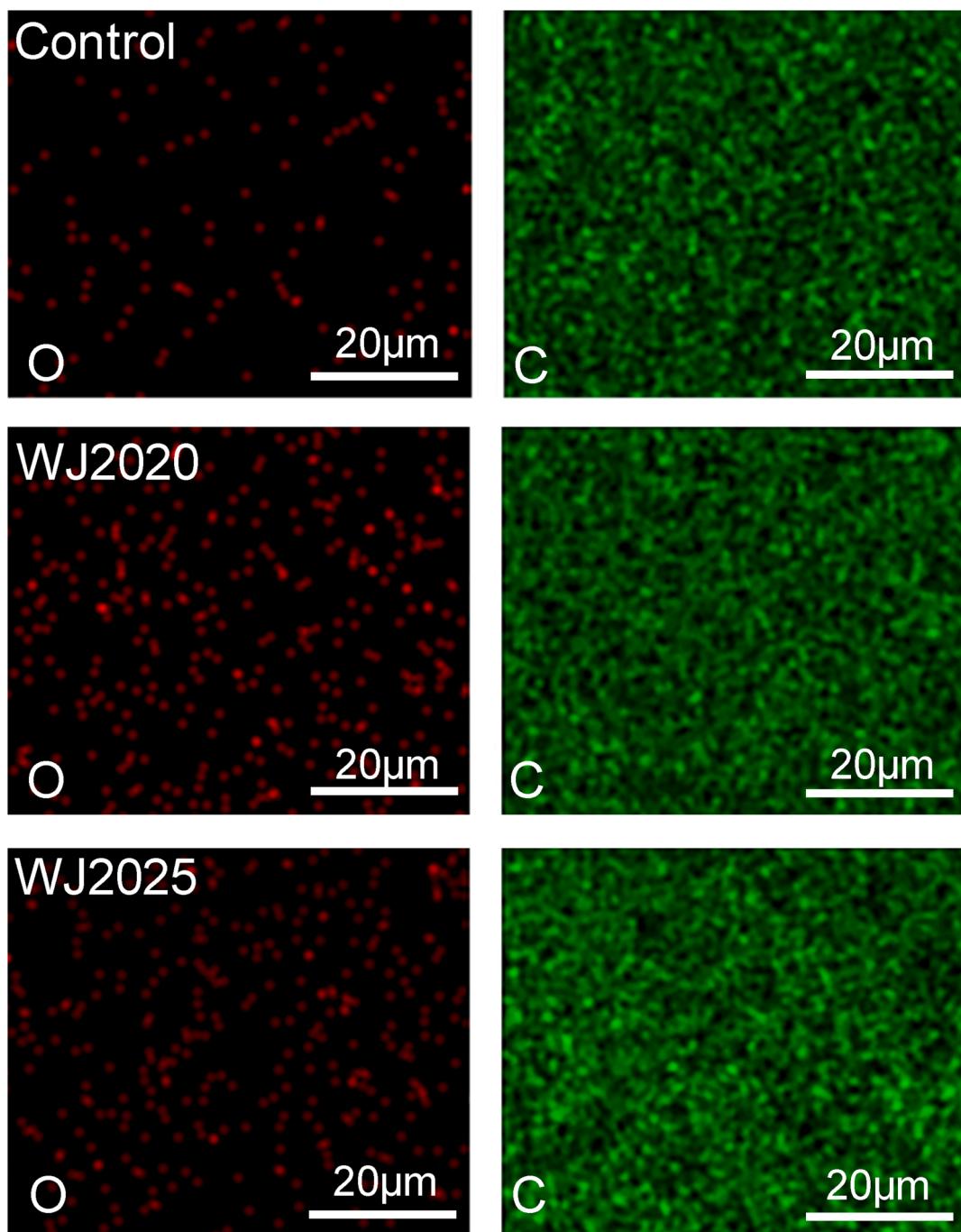


**Fig. 4.** SEM of PS films incubated with PS-degrading bacterial strains for 30 days. A. without incubation; B. incubated with WJ2020; C. incubated with WJ2020.

the specific conditions in the environment where this process takes place. In the present study, two PS-degrading bacterial strains were identified and tested, *Klebsiella* sp. WJ2020 and *Cellulosimicrobium* sp. WJ2025, isolated from yellow mealworm intestines. In previous studies, bacteria from the *Klebsiella* genus were found to degrade PS [35], while bacteria from the *Cellulosimicrobium* genus were found to degrade petroleum hydrocarbons [36]. This provides the reasonable assumption that strains WJ2020 and WJ2025 should be able to degrade PS.

#### 4.2. Physicochemical changes of PS films after microbial degradation

During the degradation process, microorganisms attach to plastic and form biofilms, which secrete degrading enzymes. These degrading enzymes further break down the plastic and use the polymer as a substrate for growth [37]. In a previous study, the gene expression of serine hydrolase in *Pseudomonas* sp. DSM 50071 was significantly increased during the degradation of PS and enzyme inhibition studies, further confirming the enzyme-mediated PS biodegradation [13]. Whether the



**Fig. 5.** Changes in the atomic composition of PS film incubated with bacterial strains WJ2020 and WJ2025. Note: “C” represents carbon and “O” represents oxygen.

degradation of PS by WJ2020 and WJ2025 was influenced by serine hydrolases needs to be further verified. In this study, it was obvious from the SEM analysis that WJ2020 and WJ2025 could damage the surface of the PS films, leaving it rough and pitted, most likely due to some enzyme secreted by the strains. The surface roughness of the support medium provides sufficient attachment sites for microorganisms and also protects them from the shear forces generated by hydrodynamics, which contribute to the further degradation of the films by bacteria [38]. The hydrophobicity of the PS film surface is a key factor in determining microbial colonization and biodegradability [39]. A change in WCA indicates that the surface properties of PS films were changed due to the influence of the microorganisms, which reduced the hydrophobicity of the PS film surface. The change in surface hydrophilicity is most likely caused by the bacteria creating voids on the film surface [20].

Undeniably, the occurrence of oxidation on the PS film surface is also evidence of PS degradation [20,33]. The XPS results confirmed the increase in oxygen with the appearance of carbon-oxygen single and carbon-oxygen double bonds on the PS surface [40]. The appearance of hydroxyl groups in the PS films after degradation as detected by FTIR, and further validates this finding. The presence of alcohols (C-OH) provides evidence for oxidation during the co-culture of bacteria and films [12]. Both strain WJ2020 and strain WJ2025 can undergo oxidation on the surface of the PS films, which also confirmed the previous conjecture and is consistent with the results of the XPS analysis. Besides, the levels of Mn and the Mw of the PS after microbial treatment changed. Finally, in previous reports, the molecular weight of PS films degraded by *Acinetobacter* sp. AnTc-1 was reduced by 13–25%, suggesting that different bacterial strains can degrade PS to different degrees [26].

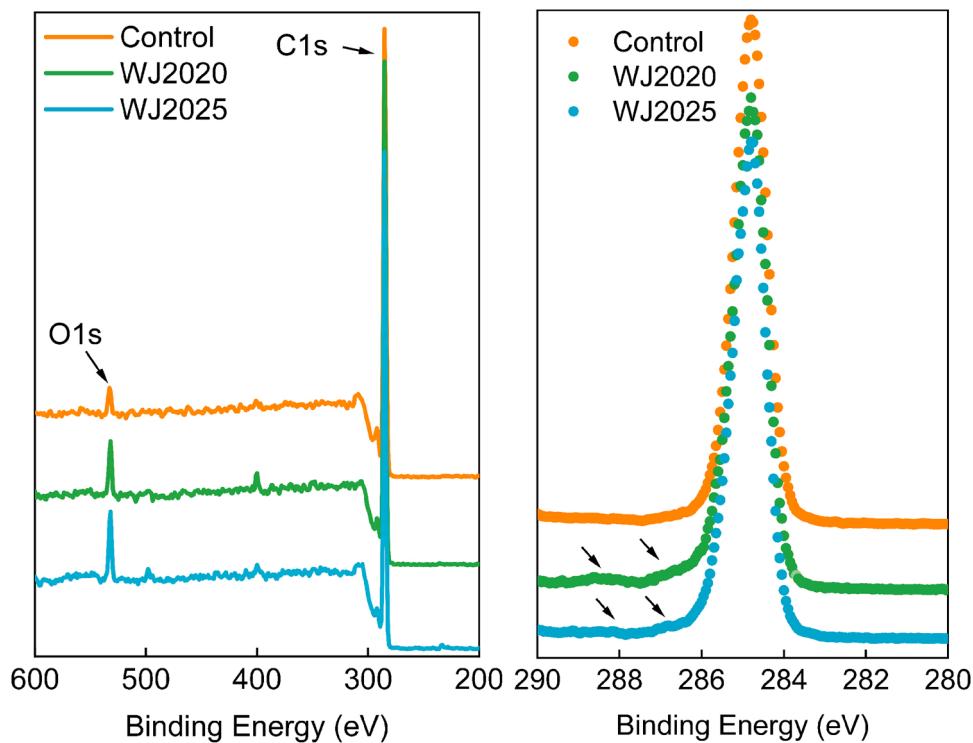


Fig. 6. XPS of PS films incubated with bacterial strains WJ2020 and WJ2025.



Fig. 7. WCA of PS film inoculated with PS-degrading bacterial strains. A. Control PS film without inoculation; B. PS film inoculated with WJ2020; C. PS film inoculated with WJ2025.

#### 4.3. PS weight loss and molecular weight changes induced by PS-degrading strains

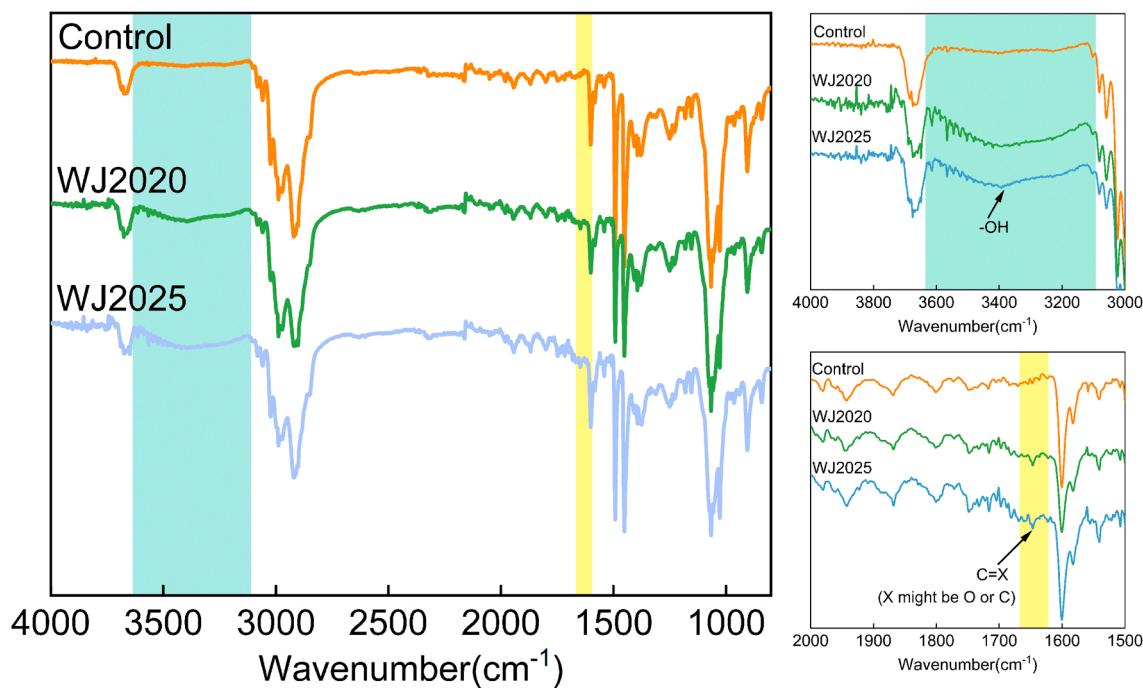
The most visual evidence of biodegradation of plastic still depends on the mass loss, where WJ2020 and WJ2025 were able to reduce the PS films' mass by 4.35% and 6.93%, respectively, over 60 days, which is much higher than the previously reported 0.8% caused by *Rhodococcus ruber* C208 over 56 days [41]. However, there are also some bacterial strains with a higher degradation capacity for PS than the strains in this study. The PS-degrading strain *Exiguobacterium* sp. YT2, isolated from the gut of mealworms, degraded PS by up to  $7.4 \pm 0.4\%$  after 60 days of incubation [19]. In a previous study, a PS-degrading strain, *Masilia* sp. FS1903, was isolated from the intestines of the greater wax moth larvae (*Galleria mellonella*) fed on EPS. The mass loss of PS films after 30 days of co-culture with this strain reached  $12.97 \pm 1.05\%$  [21]. It is possible that this difference is due to the different material sources and morphologies of the PS, and of course, microbial species differences also have an important influence. The PS degradation ability of the mixed strain was lower than that of the single strain, suggesting that there is some competition between the two strains in PS degradation. Although

both microorganisms were from the gut of the yellow mealworm, they did not synergize in PS degradation to obtain a higher degradation efficiency. The reason for this needs to be further investigated.

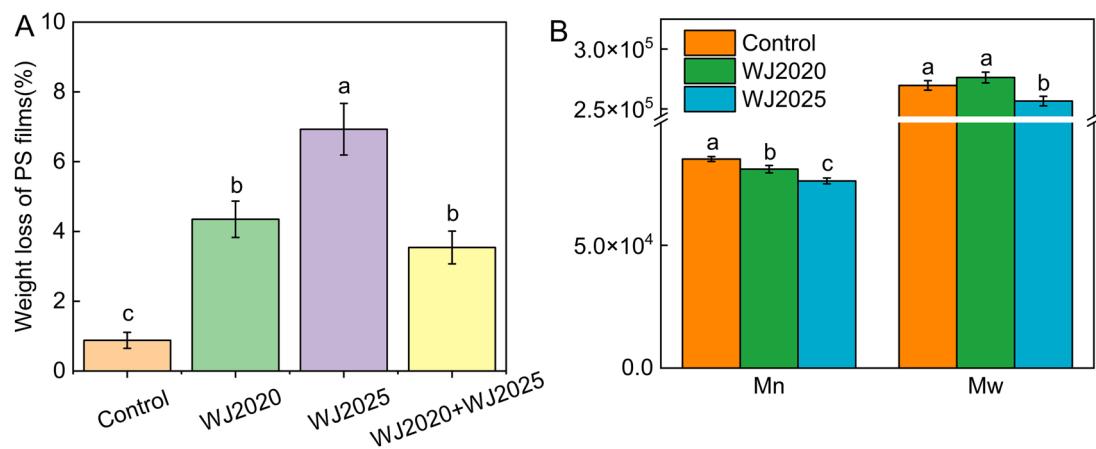
In general, the gut flora of insects is complex, and the mechanisms of microbial interactions are still unclear. While the presence of PS-degrading bacteria in insects may nurture a new era in the biodegradation of plastics, further studies are needed to refine understanding of the mechanism, synergistic pathways, and interactions of multiple microorganisms in the PS biodegradation process.

#### 5. Conclusion

In this study, two bacterial strains with PS degradation ability were isolated from *Tenebrio molitor* larval intestines, namely *Klebsiella* sp. WJ2020 and *Cellulosimicrobium* sp. WJ2025. Polystyrene films showed weight loss after 60 days of co-culture with WJ2020 and WJ2025, while SEM observation showed that erosion occurred on the surface of degraded PS films. The results showed that WJ2020 and WJ2025 could impart more oxygen to the PS surface, which resulted in a significant decrease in the hydrophobicity of the PS film surface. The use of



**Fig. 8.** ATR-FTIR of PS films co-incubated with PS-degrading bacterial strains.



**Fig. 9.** Change in weight loss (A) and average molecular weight (B) of PS films inoculated with PS-degrading bacterial strains (The values with different lowercase letters indicate significant differences).

microorganisms to degrade polymers has significant potential and value and more in-depth studies should start with the mining of degradation enzymes and degradation genes, and focus on the biodegradation of other non-degradable plastics.

#### CRediT authorship contribution statement

**Wang Zhanyong:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization. **Lin Wen:** Writing – original draft, Methodology, Investigation. **Su Tingting:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Yao Yu:** Methodology, Investigation, Formal analysis, Data curation.

#### Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Zhanyong Wang reports financial support was provided by National

Natural Science Foundation of China. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

No data was used for the research described in the article.

#### Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant No. 32270117) and the Talent Program of Shenyang Agricultural University (Grant No. 2021Y001).

#### Appendix A. Supporting information

Supplementary data associated with this article can be found in the

online version at doi:10.1016/j.jece.2024.112071.

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