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Biodegradation of polyethylene mulching films by a co-culture of *Acinetobacter* sp. strain NyZ450 and *Bacillus* sp. strain NyZ451 isolated from *Tenebrio molitor* larvae

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ABSTRACT

Microbial degradation of polyethylene (PE) mulching films has attracted much attention, but to date its relatively rapid biodegradation was limited. Here, a consortium of two bacterial isolates, *Acinetobacter* sp. strain NyZ450 and *Bacillus* sp. strain NyZ451 from the gut of PE-feeding *Tenebrio molitor* larvae (mealworms), was suggested to utilize PE, although each strain had no such ability alone. This consortium removed approximately 18% of PE film after 30 days at 23 °C. High-temperature gel permeation chromatography analysis indicated that the number-, weight-, and size-averaged molecular weights of PE were decreased by 14%, 24% and 21% from their respective initial values of 27,494 Da, 179,374 Da and 697,487 Da after a 20-day incubation with this consortium. Under scanning electron microscope, a biofilm formed by these strains was also observed on the film's surface. Fourier transform infrared spectroscopy analysis indicated that the films incubated with these two strains or either of them were all oxidized compared with the control without inoculation. Plate colony counting and electron microscopic observation showed that strain NyZ450 was predominant in the co-culture. This study enriched microbial resources for PE degradation and provided an example for the interaction of two bacterial strains for enhanced PE degradation.

1. Introduction

Hundreds of millions of tons plastics were produced every year (PlasticsEurope, 2018). Because of the nature of high molecular weight, highly stable covalent bond and highly hydrophobic, petroleum-based plastics especially "C–C" inert structural backbone plastics are recalcitrant to be broken down in the environment (Peixoto et al., 2017; Wei and Zimmermann, 2017). More than eight billion tons of plastics exist on the Earth and more than half of them were discarded in the environment (Geyer et al., 2017). Polyethylene (PE, $(CH_2–CH_2)_n$) is a typical representative of recalcitrant "C–C" backbone plastics. Based on the differences in the density, degree of branching and availability of functional groups on the surface, the most common PE types are low-density polyethylene (LDPE), high-density polyethylene (HDPE), linear low-density polyethylene (LLDPE), low molecular weight polyethylene (LMWPE) and cross-linked polyethylene (XLPE). It is well known that PE is one of the most widely used petroleum-based plastics all over the world. PE-based mulching films which were composed of LDPE and LLDPE have been heavily used in agriculture (Hochmuth et al., 2015; Lamont, 2017). But dumped mulching films were very difficult to be recycled, resulting in severe crop failures and environmental problems (Kasirajan and Ngouajio, 2012). One of the promising ways to make the PE degradable and recyclable may be through microbial versatile activities, which have been proved to be one of productive methods to get rid of xenobiotics in the past (Raddadi and Fava, 2019).

A previous report indicated that only 0.2%–0.5% PE film was removed after 10 years in landfills by ^{14}C labeling and tracking the release of $^{14}CO_2$ (Albertsson and Karlsson, 1988). The degradability of PE basically depends on its molecular weight, crystallinity and pretreatments. The molecular weight of PE polymers is measured using the number-, weight-, and size-averaged molecular weights (Mn, Mw and Mz). Generally, the higher the molecular weight and crystallinity of PE, the harder it is to be biodegraded. Different from HDPE, LDPE and LLDPE exhibit a lower crystallinity because of having more branch structures, making them less recalcitrant to biodegradation (Fontanella et al., 2010). On the other hand, the pretreatment is also an effective way to accelerate PE degradation. For example, the addition of photosensitizers in PE accelerated its

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disintegration in the environment (Albertsson and Karlsson, 1988). The degradation of PE can also be accelerated to some extent by the pre-treatments with UV light, heat and mechanical shear (Albertsson and Karlsson, 1990).

In previous studies, some insects such as yellow mealworms (*Tenebrio molitor* larvae) (Brandon et al., 2018; Yang et al., 2015a, b; Yang et al., 2020), superworms (*Zophobas atratus* larvae) (Peng et al., 2020), Indian meal moths (*Plodia interpunctella*) (Yang et al., 2014) and greater wax moths (*Galleria mellonella*) (Bombelli et al., 2017), and soil animal such as land snails (*Achatina fulica*) (Song et al., 2020) were reported for their special nature of eating plastics including polystyrene and polyethylene, and their gut microbes were demonstrated to be essential for plastics digestion. So far, surprisingly large numbers of bacterial genera from the soil (Peixoto et al., 2017) or the gut of insect (Yang et al., 2014) have been reported as possible PE degraders. Nevertheless, the PE catabolic abilities from reported PE-degrading bacterial isolates were all extremely weak. Discarded PE mulch films are a major source of plastics pollution in soil, but reports on the relatively rapid biodegradation of PE mulching films are still limited. For instance, *Bacillus aryabhattai* 5–3 only removed 3.85% of PE mulching films after a 30-day incubation (Hou et al., 2019). *Pseudomonas* sp. AKS2, a soil bacterial isolate, was able to remove 5% 20-μm thick LDPE carrier bags from the market after a 45-day incubation (Tribedi and Sil, 2013). As to the gut bacteria, *Enterobacter asburiae* YT1 and *Bacillus* sp. YP1 could individually remove approximately 6.1% and 10.7% of LDPE films (Mw = 88,200 Da) after 60 days (Yang et al., 2014), *Rhodococcus ruber* C208 removed 4% films of branched LDPE (Mw = 191,000 Da) containing UV photosensitizer pretreated with UV irradiation after 30 days (Orr et al., 2004), and *Pseudomonas* sp. E4 removed 4.9–28.6% LMWPE films (Mw = 1700–23, 700 Da) after 80 days (Yoon et al., 2012). However, there are limited reports on the relatively rapid biodegradation of agricultural PE-based mulching film by microbes. The reports on biodegradation mechanism of C–C backbone plastics such as PE, especially those on the related genes and enzymes, are extremely scarce. It was reported that some lignin degrading enzymes such as laccase were active on PE plastics with low activities (Santo et al., 2013). Another example is that an alkane hydroxylase from *Pseudomonas* sp. E4 expressed in *E. coli* BL21 showed activity for low molecular weight polyethylene in compost (Yoon et al., 2012).

In addition, the occurrence of microbial interaction cannot be neglected in the natural environment or the gut of insect (Sakazawa et al., 1981; Scherlach and Hertweck, 2018). Considering that the plastic nature of high molecular weight, high hydrophobicity and inert covalent bond, the limited catabolic ability of reported single degraders has restricted their PE degrading efficiency. The utilization by microbial consortia has shown an improved performance in the biodegradation for PE plastics (Esmaeili et al., 2013; Mukherjee et al., 2016; Roy et al., 2008). Therefore, characterization of the interaction among different bacterial strains is an important process during the plastic degradation by bacterial consortia. This study reports the biodegradation of PE mulching films by the combination of two bacterial isolates from mealworm guts and demonstrates their relationship between the two strains for PE biodegradation. This will fill a gap in our understanding of the PE mulching film degradation by a bacterial consortium and also enrich bacterial resources for plastic degradation.

2. Materials and methods

2.1. LDPE particles and PE mulching film, bacterial strains and medium

LDPE particles (the number-, weight- and size-averaged molecular weights (Mn, Mw and Mz) were 9,285 Da, 68,220 Da and 348,240 Da, respectively) with no additives were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). PE mulching film was sourced from commercially available products. The property of the obtained PE mulching film was characterized by attenuated total reflection fourier transformed infrared (ATR-FTIR, Nicolet 6700, Thermo Scientific, USA) and high-temperature gel permeation chromatography (HT-GPC, HLC-8321GPC/HT, TOSOH, Japan). The obtained commercial PE mulching films were considered to contain mainly LDPE and LLDPE based on the infrared signals corresponding to the functional groups of PE by ATR-FTIR, and no other peaks than the one of PE were observed by HT-GPC. The values of Mn, Mw and Mz of PE mulching films were 27,494 Da, 179,374 Da and 697,487 Da respectively. Prior to serving as the carbon source, PE particles and mulching films (pieces with the size of approximately 6 cm × 6 cm, and the average weight of each piece being approximately 40 mg) were washed with 2% (w/v) SDS, sterile water, and absolute ethanol in turn, and then dried in the sterilized clean bench. PE-degrading strains were isolated and cultured aerobically in lysogeny broth (LB) at 30 °C or liquid carbon free basal medium (LCFBM) (Yang et al., 2014) at 23 °C with PE as the sole carbon source. Carbon free basal agar medium (CFBAM) and LB agar medium were all prepared by adding 1.5% (w/v) agar.

2.2. Enrichment and isolation of bacterial strains for PE degradation

Tenebrio molitor larvae (sourced from Shandong Sishui-Limin Insect Breeding Plant, China) were fed with PE mulching films by spraying water from time to time to maintain the humidity for two weeks. A group of 15 *Tenebrio molitor* larvae was collected to prepare the gut suspension. *Tenebrio molitor* larvae were immersed in 75% (v/v) ethanol for 1 min and then washed twice with sterile water. The whole guts of 15 *Tenebrio molitor* larvae were extracted and pooled into a 10 mL centrifuge tube containing 5 mL saline solution. After vibrating by vortex for 5 min, the gut tissue was carefully removed and the remaining gut suspension was moved into a 250 mL Erlenmeyer flask containing 100 mL LCFBM for enrichment cultivation, with a piece of PE mulching film (approximately 6 cm × 6 cm) as the carbon source. Since PE is the predominant carbon source, this process would theoretically lead to a sufficient population of possible PE-degrading bacteria. The mixture was then incubated at 23 °C with shaking at 180 rpm for 10 days, before the culture was serially diluted and spread on LB agar plate to form single colonies. After a 24-h incubation, emerged single colonies were picked and the pure culture was obtained by repeatedly plate streaking. The obtained pure bacterial cultures were then further verified for PE degradation capacity in PE-containing LCFBM. Two strains obtained in this way were designated strains NyZ450 and NyZ451, respectively. The almost full-length sequence of 16S rRNA gene of aforementioned stains was amplified using the 27F and 1492R universal primers (Lane, 1991). The obtained 16S rRNA genes were sequenced and aligned with those of the organisms in the database of National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST).

2.3. Antibiotic resistance profile assay

To assay the antibiotics resistance profile of isolates, strains NyZ450 and NyZ451 were grown on LB agar plate added with different antibiotics, including kanamycin (50 μg mL^{-1}), ampicillin (100 μg mL^{-1}), chloramphenicol (12.5 μg mL^{-1}), erythromycin (20 μg mL^{-1}), gentamicin (20 μg mL^{-1}), tetracycline (20 μg mL^{-1}), streptomycin (100 μg mL^{-1}), and spectinomycin (100 μg mL^{-1}).

2.4. Measurement of bacterial growth on pure PE particles

Seeding cells of strains NyZ450 and NyZ451 were prepared by separately growing them in liquid LB at 30 °C overnight to an optical density of 600 nm (OD_{600}) of approximately 0.8, and the cells of two strains were washed twice with LCFBM and then suspended in an equal volume of LCFBM, respectively. Strains NyZ450 (1%, v/v), NyZ451 (1%, v/v) and NyZ450 together with NyZ451 (1:1, 0.5% plus 0.5%, v/v) were separately inoculated in 100 mL LCFBM with 0.5% (w/v) PE particles (sterilized as described above) on a rotary shaker (180 rpm) at 23 °C.

Above groups inoculated into the LCFBM without PE particles were used as the negative controls. The bacterial growth was measured at an optical density of 600 nm by a spectrometer (Lambda 25 UV/VIS, PerkinElmer).

2.5. Weight loss of PE mulching film treated by isolated strains

Four pieces of sterilized PE mulching films were measured for their initial dry weights, respectively. Seeding cells of strains NyZ450 and NyZ451 were prepared and resuspended in LCFBM as aforementioned. And 1 mL of the bacterial suspension of strains NyZ450 and NyZ451 individually, and a consortium of two strains (1:1) was separately grown in 10 mL LCFBM with a piece of sterilized PE mulching film (approximately 4g L^{-1}) on a rotary shaker (180 rpm) at 23 °C. After a 30-day incubation, the residual PE mulching films were collected and washed to remove the cells on the surface using 2% (w/v) SDS solution and sterile water, and then dried at room temperature. The residual PE dry weight was measured after being completely dried. The PE-degrading ability was expressed as [(initial PE dry weight - residual PE dry weight)/initial PE dry weight x 100%]. Such experiments were repeated three times.

2.6. High-temperature gel permeation chromatography (HT-GPC) characterization of molecular weight change of PE mulching film treated by isolated strains

The mixture of equal volume of LCFBM and LB was used as a medium. Strains NyZ450 and NyZ451 were cultured in LB medium overnight (OD_{600} = 0.6) and then washed and suspended into the aforementioned mixed medium. Strains NyZ450, NyZ451 and the consortium of the two strains were separately inoculated in 40 mL medium with sterilized PE mulching films (100 mg, approximately 2.5 g L^{-1}). The same system without bacteria served as the negative control. After a 20-day incubation, the PE mulching films were collected and washed adequately with 2% SDS and sterile water before being dissolved in 1, 2-dichlorobenzene (0.2%, w/v). The samples were analyzed to obtain their molecular weights using HT-GPC (EcoSEC HLC-8321GPC/HT, Japan) calibrated with monodisperse polystyrene standards.

2.7. Characterization of biofilm formation using scanning electron microscopy

To further test their capability of forming biofilms by these two strains on PE mulching film surface, the PE films treated with them were characterized by scanning electron microscopy (SEM). The consortium of strains NyZ450 and NyZ451 (1:1) was spread across a CFBAM plate, and then covered with a piece of sterilized PE mulching film. A piece of sterilized PE mulching film covering on a CFBAM plate without inoculation served as the negative control. The above two plates were cultured at 30 °C. After 20 days, the PE mulching films were peeled off carefully and air-dried adequately before being coated with Au for SEM observation. The biofilm formed on PE films surface was observed by field emission scanning electron microscope (FESEM, Sirion 200, FEI, USA) operating at an electron beam intensity of 5 kV. The morphological characteristics of single strains were also observed using SEM as described above.

2.8. Attenuated total reflection fourier transformed infrared (ATR-FTIR) spectroscopy of PE mulching film

To characterize the surface chemical modification of PE mulching films treated with strains NyZ450 and NyZ451, the treated PE films were analyzed using ATR-FTIR. Strains NyZ450 and NyZ451, and the consortium were individually spread across CFBAM plate and then covered with a piece of sterilized PE mulching film. A piece of sterilized PE mulching film covering on a CFBAM plate without inoculation served as the negative control. All plates were cultured at 30 °C. After 12 days, the PE mulching films were collected and washed as described above and dried at room temperature. Then the chemical changes of PE mulching films were characterized by ATR-FTIR (Nicolet 6700, Thermo Scientific, USA) with a scan range of 4000–400 cm^{-1} using OMNIC software.

2.9. Colony counting of mixed bacterial strains

To characterize the growth of the consortium on PE mulching films, the two stains were cultivated until the log phase (OD_{600} = 0.6), respectively, and equal number of cells were then mixed and inoculated in LCFBM with a piece of PE mulching film. The mixed cultures were sampled every day, and diluted properly before being spread on LB plates with suitable antibiotics according to their antibiotic resistance profiles. After 24 h, the numbers of forming colonies were counted to describe the growth of the two strains on PE mulching films in the mixed culture.

3. Results

3.1. Isolation and classification of PE-degrading bacterial strains

The gut suspensions of PE-feeding *Tenebrio molitor* larvae were used for the enrichment of PE-degrading bacterial strains. After repeated enrichments, two bacterial strains with different morphological characteristics on LB agar plates were chosen for further studies. They were designated *Acinetobacter* sp. strain NyZ450 (GenBank accession number MT459299 for its 16S rRNA gene) and *Bacillus* sp. strain NyZ451 (MT459300), respectively, according to the phylogenetic analysis of their 16S rRNA genes.

3.2. Growth of a consortium of strains NyZ450 and NyZ451 with PE particles

Strains NyZ450 and NyZ451 were individually or together cultured in LCFBM with pure PE particles. The growth curves shown in Fig. 1 indicated that the consortium was able to grow on PE particles after a 30-day incubation, whereas each of them had no ability to utilize PE for its growth individually. Neither a single strain nor the consortium was able to grow in a medium without PE. These results indicated that the two isolates were both indispensable when they grew on PE.

3.3. Weight loss of PE mulching film treated by a consortium of strains NyZ450 and NyZ451

To assess the weight loss of PE mulching film treated by the bacterial consortium, strains NyZ450 and NyZ451 were cultured individually or together in 10 mL of LCFBM with approximately the same weight of PE mulching films (approximately 4g L^{-1}). A piece of PE film immersed into LCFBM without inoculation served as the negative control. The changes in dry weight of PE films were measured after a 30-day inoculation. As shown in Fig. 2a, the co-culture of strains NyZ450 and NyZ451 could remove approximately 18% of PE mulching films, but neither of them exhibited this ability alone. This result was consistent with the aforementioned results of the bacterial growth on PE. And the aberrant negative reduction mainly resulted from inadequate drying or tightly bound biofilm residue on PE film.

HT-GPC was used to analyze the changes in the molecular weight of PE mulching films being treated for 20 days by the strains. As shown in Fig. 2b, comparing to the negative control (its Mn, Mw and Mz were 27,494 Da, 179,374 Da and 697,487 Da, respectively), the molecular weight of the PE mulching film treated by strain NyZ450 decreased to some extent (Mn = 24,708 Da, 10% decrease; Mw = 161,247 Da, 10% decrease; Mz = 604,499 Da, 13% decrease) but that by strain NyZ451 increased (Mn = 29,001 Da, 5% increase; Mw = 193,118 Da, 7% increase; Mz = 916,555 Da, 36% increase). Particularly, the molecular weight of PE

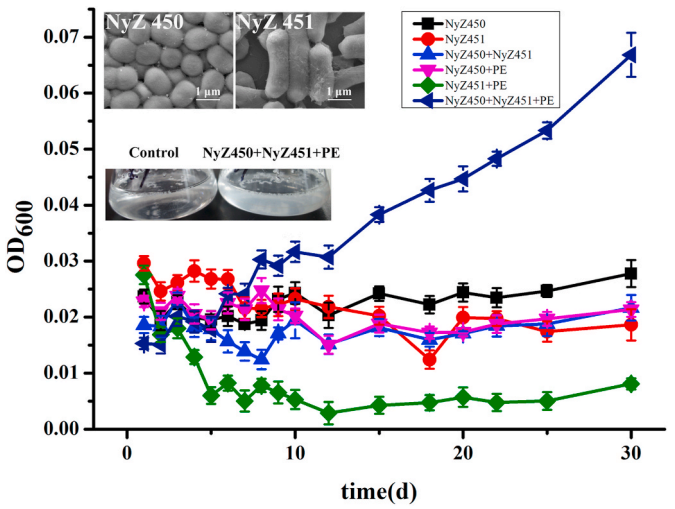

Fig. 1. Growth curves of strains grown on PE. 0.5 g pure PE particles (the number-, weight- and size-averaged molecular weights (Mn, Mw and Mz) were 9,285 Da, 68,220 Da and 348,240 Da based on analysis using HT-GPC) were placed into 100 mL LCFBM served as the sole carbon source. *Acinetobacter* sp. strain NyZ450 and *Bacillus* sp. strain NyZ451 were prepared by culturing them with LB separately overnight and then washed adequately with LCFBM. All strains were cultured at 23 °C, 180 rpm with inoculation individually (1%, v/v) or together (1:1, 1%, v/v). Samples were collected in turn to measure OD_{600} by a spectrometer as follows: ■ (black), strain NyZ450 without carbon source; ● (red), strain NyZ451 without carbon source; ▲ (lake blue), strains NyZ450 and NyZ451 together without carbon source; ▼ (pink), strain NyZ450 with PE particles; ◆ (green), strain NyZ451 with PE particles; ◂ (dark blue), strains NyZ450 and NyZ451 together with PE particles. Only the consortium was able to grow on PE. All experiments were repeated three times. Error bars indicated standard deviation (n = 3). The two inserted optical photos show the morphological characteristics of strains NyZ450 and NyZ451 observed by SEM (upper), and the growth of the consortium on PE and the control (with PE but without strains) in flask (below). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

mulching films treated by the consortium obviously decreased (Mn = 23,583 Da, 14% decrease; Mw = 136,883 Da, 24% decrease; Mz = 501,256 Da, 21% decrease), compared to the aforementioned data obtained from the treatments by either of two strains. The HT-GPC analysis indicated that strain NyZ450 and the co-culture conducted broad depolymerization (decrease in both Mn and Mw) whereas strain NyZ451 conducted limited extent depolymerization (increase in both Mn and Mw).

3.4. Biofilm formation on the surface of PE mulching films

Biofilm formation is a useful strategy for bacteria to colonize on the hydrophobic PE mulching films. The colonization of the consortium on PE films was characterized by SEM after 20 days. The observation at scales of 1 μm (Fig. 3a), 2 μm (Fig. 3b) and 5 μm (Fig. 3c) shows that the two bacterial isolates grew on the PE surface and formed a tight biofilm. The erosions and cracks on the PE film surfaces occurred after being treated with the consortium, and the cell appendages secreted by the two bacteria were used in the interaction between them. As to the negative control without the consortium (the inserted pictures), no colonies were present on the film, and the dense structure on its surface remained unchanged.

3.5. Surface chemical oxidation by PE-utilizing strains

ATR-FTIR analysis was used to test the chemical oxidation on the surface of PE mulching films treated by strains. The PE samples were treated with the consortium, strain NyZ450 or strain NyZ451 for 12 days. Compared to the negative controls without inoculation, all the treated samples were featured by two new peaks corresponding to those of the O–H bond (3,350 cm^{-1}) (Fig. 4a) and the –C═C- bond (1,650 cm^{-1}) (Fig. 4b), indicating the occurrence of oxidation on PE surfaces. The results suggested that both strains have the capability of oxidizing PE mulching films. However, the spectra of the co-culture showed evidently higher intensities than that of the single cultures, and this observation indicated a higher oxidization capacity with the two strains together.

3.6. Cell number changes of the isolates growing on PE medium together

Strains NyZ450 and NyZ451 were both resistant to kanamycin (50 μg mL^{-1}), ampicillin (100 μg mL^{-1}), erythromycin (20 μg mL^{-1}), spectinomycin (100 μg mL^{-1}), streptomycin (100 μg mL^{-1}) and gentamicin (20 μg mL^{-1}). In addition, strain NyZ450 was sensitive to tetracycline (20 μg mL^{-1}) and resistant to chloramphenicol (12.5 μg mL^{-1}), whereas strain NyZ451 was sensitive to chloramphenicol (12.5 μg mL^{-1}) and resistant to tetracycline (20 μg mL^{-1}). The difference in the profile made it possible for distinguishing them from the co-culture grown on

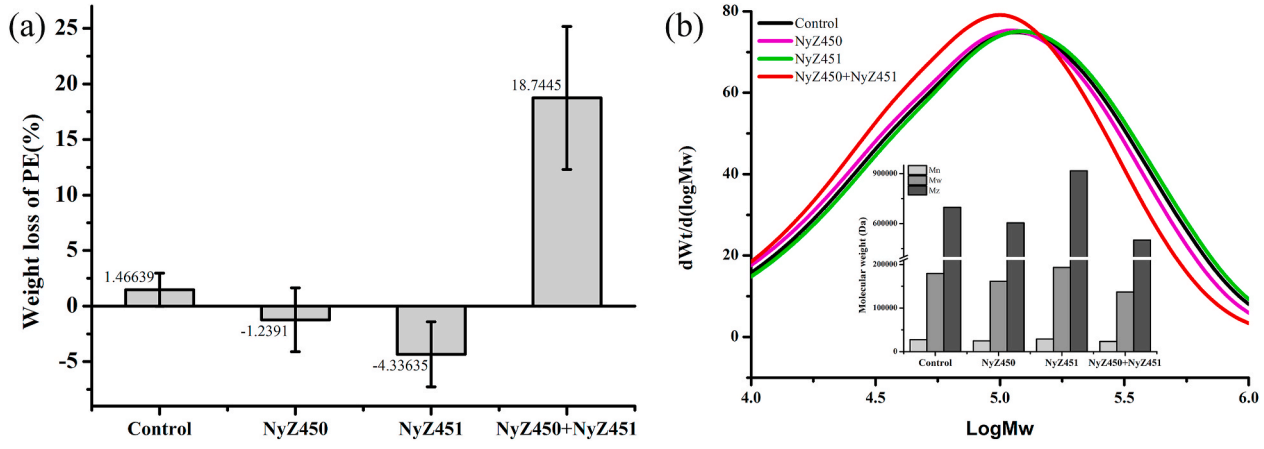

Fig. 2. Weight loss and molecular weight change of PE mulching films treated by two isolates. The culture was incubated in LCFBM at 23 °C with shaking at 180 rpm. Commercial PE mulching films were cut into approximately 6 cm × 6 cm (about 40 mg) and sterilized with ethanol. (a) PE mulching films were inoculated with strain NyZ450 and strain NyZ451 individually (1/10, v/v) and together (1:1, 1/10, v/v) for 30 days. The same PE mulching film without inoculation served as a negative control. All experiments were repeated three times. Error bars indicated standard deviation (n = 3). (b) The curves show a shift in molecular weight distribution of PE mulching films and the inserted figure shows molecular weight changes in Mn, Mw and Mz, compared to the control (without inoculation).

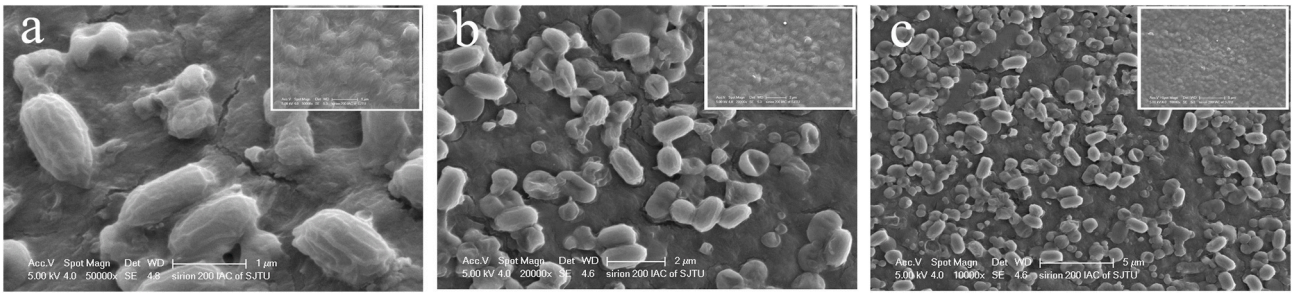

Fig. 3. Biofilm formation on the treated PE mulching films. The consortium containing strains NyZ450 and NyZ451 was coated on CFBAM plate and then covered with sterilized PE mulching films. The same PE mulching film without inoculation served as a negative control. After a 20-day incubation at 30 °C, the physical surface topography of the PE mulching films inoculated with or without the consortium were observed with SEM at scale of 1 μm (a), 2 μm (b) and 5 μm (c). The inserted are the negative controls. The consortium formed a tight biofilm on the PE surface and caused cracks on the PE surface.

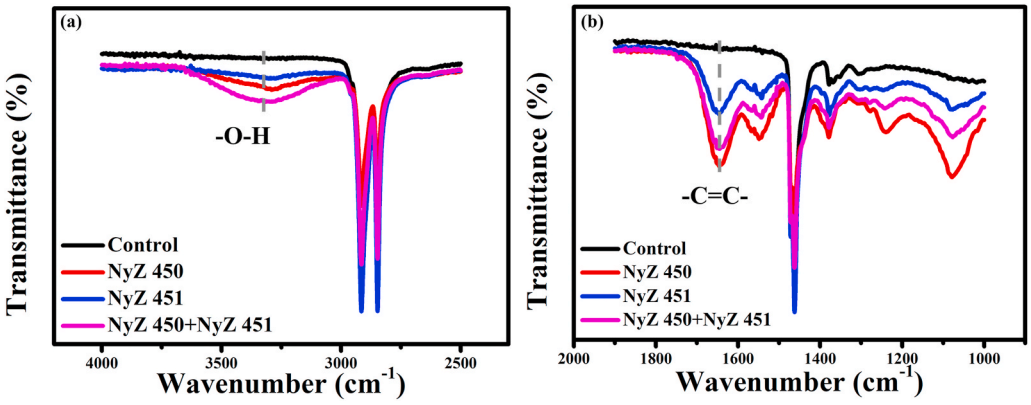

Fig. 4. Attenuated total reflection fourier transform infrared spectroscopy (ATR-FTIR) analysis of PE mulching films. PE mulching films samples were treated with strain NyZ450 and strain NyZ451 individually or together on CFBAM at 30 °C for 12 days. The PE samples were washed adequately with SDS solution, sterile water and then dried adequately prior to ATR-FTIR observation. The same PE mulching film without inoculation served as a negative control. The additional peaks corresponding to O–H bonds (a) and –C⁼C- bonds (b) indicate surface oxidation of PE, while the negative controls do not have the corresponding peaks.

PE mulching films when plate colony counting was used. In the consortium, the biomass of strain NyZ450 increased over time and was dominant with approximately 10^8 CFU mL^{-1} after 5 days, and the biomass of strain NyZ451 was approximately 10^5 CFU mL^{-1} and remained largely unchanged, which was in accord with the results of HT-GPC analysis (Fig. 2b) and SEM observation (Fig. 3c).

4. Discussion

Despite the discarded PE mulching films being a major source of plastics pollution in soil, reports on the relatively rapid biodegradation of PE mulching films were still limited. Furthermore, although many bacterial isolates from the soil (Peixoto et al., 2017) or the gut of insects (Yang et al., 2014) were reported to be PE degraders, the PE catabolic abilities of the pure cultures were all extremely weak and need to be further improved.

In this study, the degradation of PE mulching films was accomplished by a consortium containing two strains, with either of them being indispensable. Comparing with the treatment by strain NyZ450 or NyZ451 alone, the one by the consortium of two strains caused a significant decrease in the molecular weight of PE (14%, 24%, and 21% reduction in Mn, Mw, and Mz, respectively). These results indicated a significant improvement in the PE degradation by the collaboration of two strains. GPC results as well as ATR-FTIR analysis showed either single culture could attack PE mulching films. However, the capacity of either strain was likely limited and could not support their growth. It is generally recognized that there are two problems needed to be overcome for bacteria on PE biodegradation. Firstly, PE, a typical representative of "C–C" bond plastics, was difficult to be assimilated due to lack of hydrolytic functional groups (Krueger et al., 2015; Restrepo-Flórez et al., 2014). For further scission of PE backbone, activation of inert "C–C" bond by hydroxylation or carboxylation is essential but difficult for a single strain to fulfil this task. Secondly, there was a scarce opportunity for strains to physically approach and even colonize on extremely hydrophobic PE. Many studies on biodegradation of "C–C" backbone plastics therefore depended on various pretreatments such as peroxidation and preheating to make the inert structure more bioavailable and easier to be degraded (Jeyakumar et al., 2013; Motta et al., 2009; Ojeda et al., 2009). Thus PE degradation by a bacterial consortium probably is an alternative solution to this existing hurdle.

Despite that strain NyZ450 was the major player in the co-culture grown on PE and the biomass of strain NyZ451 had no apparent changes, it is clear to us that only the combination of strains NyZ450 and NyZ451 possessed the ability to degrade PE mulching films. Therefore, it probably suggested that strain NyZ450 was responsible for PE breakdown and utilized PE mulching films predominately as the carbon source, whereas strain NyZ451 played an auxiliary role with a relatively limited ability to break chemical bonds of PE. Given strains NyZ450 and NyZ451 being both vital during their growth on PE, the cooperation between these two strains may have complementary catabolic pathways or exhibit enhanced capability of activating recalcitrant substrate. This is similar to, in a way, a previous report on polyvinyl alcohol degradation by a cooperation of two bacterial strains in which one strain produced a unique growth stimulant for the other one. (Sakazawa et al., 1981). Thus this cooperation could have also happened in the interaction of strains NyZ450 and NyZ451 in this study, considering the

prospective complex of PE degradation pathway and significant difference in biomass for each strain in the co-culture. Previously it was reported that *Bacillus* spp. served as a genus of well-known biosurfactant producers, and biosurfactants were of benefit to the natural or induced bioremediation by improving the xenobiotic's hydrophilicity (Ławniczak et al., 2013). Since strain NyZ451 is in the genus *Bacillus* and plays an essential but not predominant role in PE degradation by the co-culture, strain NyZ451 maybe provide biosurfactants during PE degradation to increase the bioavailability of PE. The improved hydrophilicity may then lead to the slight increase of molecular weight of PE. However, solid evidences are surely required to confirm this possibility.

Given the fact that both strains NyZ450 and NyZ451 could oxidize PE surface by ATR-FTIR observation, it can be proposed that during the PE degradation by this consortium, two strains were involved in several common catabolic steps, such as the oxidation. It is generally accepted that the oxidation of PE mulching films was the key premier step for PE biodegradation, the power to degrade inert PE mulching films may been enhanced by multiple steps of the same oxidation. Thus it is likely that the duplicated oxidation functions of these two strains were beneficial to PE degradation.

5. Conclusions

In this study, a couple of PE-degrading bacteria *Acinetobacter* sp. strain NyZ450 and *Bacillus* sp. strain NyZ451 were isolated from the gut of PE-feeding *Tenebrio molitor* larvae. The co-culture of strains NyZ450 and NyZ451 was capable of growing with PE as the sole carbon source and removing about 18% of PE mass after a 30-day incubation. The consortium of two strains caused an obvious decrease in molecular weights of PE mulching films (14%, 24% and 21% reduction in Mn, Mw and Mz, respectively), and formed a tight biofilm on the surface of PE mulching film. The observation of –C═C- stretching and -O-H stretching by ATR-FTIR indicated the occurrence of PE oxidation after being inoculated with the bacterial consortium. Bacterial strains NyZ450 and NyZ451 likely constituted the integrated strategy for PE catabolism, and strain NyZ450 probably dominated the construction and development of the consortium.

Ethical approval

This article does not include any studies of human participants or animals by the authors of this investigation.

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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