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Unique *Raoultella* species isolated from petroleum contaminated soil degrades polystyrene and polyethylene

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ABSTRACT

Polyolefin plastics, such as polyethylene (PE) and polystyrene (PS), are the most widely used synthetic plastics in our daily life. However, the chemical structure of polyolefin plastics is composed of carbon-carbon (C-C) bonds, which is extremely stable and makes polyolefin plastics recalcitrant to degradation. The growing accumulation of plastic waste has caused serious environmental pollution and has become a global environmental concern. In this study, we isolated a unique *Raoultella* sp. DY2415 strain from petroleum-contaminated soil that can degrade PE and PS film. After 60 d of incubation with strain DY2415, the weight of the UV-irradiated PE (UVPE) film and PS film decreased by 8% and 2%, respectively. Apparent microbial colonization and holes on the surface of the films were observed by scanning electron microscopy (SEM). Furthermore, the Fourier transform infrared spectrometer (FTIR) results showed that new oxygen-containing functional groups such as -OH and -C═O were introduced into the polyolefin molecular structure. Potential enzymes that may be involved in the biodegradation of polyolefin plastics were analyzed. These results demonstrate that *Raoultella* sp. DY2415 has the ability to degrade polyolefin plastics and provide a basis for further investigating the biodegradation mechanism.

1. Introduction

Synthetic plastics are widely used in our daily life owing to their excellent properties, such as durability, low manufacturing costs, and versatility (Kurniawan et al., 2021; Millican and Agarwal, 2021). While plastic-based medical equipment played an important role in preventing the spread of virus during COVID-19 pandemic, it led to a considerable increase in plastic waste (Horton, 2022). The polyolefin plastics, such as polyethylene (PE) and polystyrene (PS), account for 77% of the total market (Danso et al., 2019; Krueger et al., 2015). However, only 9–12% of plastic wastes are recycled or incinerated. The rest are accumulated in landfills or is released into the marine environment with a total of 4.9 billion tonnes, and the amount is increasing annually (Geyer et al., 2017). These plastic wastes require quite a long time to naturally decompose, causing serious environmental pollution. The existing disposal methods for the plastic wastes are still unsatisfactory. For example, burning the collected plastic wastes results in a considerable amount of carbon dioxide emissions, which may contribute to global warming and climate change (Jeon et al., 2021). Chemical catalytic oxidation is economically uncompetitive and has the risk of secondary environmental pollution. Alternatively, the biodegradation of plastic wastes has been proven to be low-carbon and environmental-friendly, and is gaining increasing attention (Krueger et al., 2015; Zhang, N. et al., 2022).

Biodegradation of plastics can be defined as the depolymerization of polymers into small molecules, such as carbon dioxide or short-chain organic substrates, by biological processes. The polyester-based plastics, such as polyethylene terephthalate (PET) and polyurethane (PU), have been reported to be effectively biodegraded by certain microorganisms (Liu et al., 2021; Liu et al., 2022). Even some excellent enzymes, such as *Is*PETase, the leaf-branch compost cutinase (LCC), and their variants, have been identified as the critical components for breaking polyester plastics (Hou et al., 2022; Yoshida et al., 2016). However, efficient biodegradation of polyolefin plastic has not yet been achieved. Polyolefin plastics comprise carbon-carbon (C-C) bonds and variable side chain groups. C-C bond is difficult to be broken due to several thermodynamic and kinetic constraints, including high bond dissociation energies (BDEs) of ~90 kcal mol^{-1}, steric inaccessibility arising from the surrounding C-H bonds, and unfavorable orbital orientations for cleavage (Yan B, 2021). These inherent chemical properties

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contribute to the high stability of polyolefin plastics, making them resistant to biodegradation. Nevertheless, some specific microorganisms have been found to possess the potential to reduce the chemical inertness of polyolefin plastics (Das and Mukherjee, 2007; Zhang, N. et al., 2022). Basically, the extracellular enzymes secreted by the microorganisms firstly adsorb to the surface of the polymer, followed by hydro-peroxidation of the nonpolar bonds and then hydrolysis of the polar functional groups within the polymers. The resulting short-chain molecules can then be assimilated by the microorganisms (Mohanan et al., 2020).

In recent years, microorganisms isolated from the oceans, landfills, plastic-contaminated soils, and even the worms' guts have been reported to degrade polyolefin plastics. The marine fungus *Alternaria alternata* FB1 can efficiently degrade PE film with a 95% molecular weight decrease in 120 d (Gao et al., 2022). The bacterial strains *Pseudomonas knackmussii* N1–2, and *Pseudomonas aeruginosa* RD1–3 isolated from plastic-contaminated soil were reported to cause 5.95% and 3.62% weight loss of PE mulch within 8 weeks, respectively (Hou et al., 2022). *Acinetobacter pitti* IRN19 isolated from plastic-dump soil was shown to degrade PE film with a 26.8% weight loss within 4 weeks (Montazer et al., 2018). Gut microbes from insect larvae, such as *Klebsiella sp*. EMBL-1, *Enterobacter asburiae* YT1, and *Bacillus* sp. YP1, have been proven to degrade various polyolefin plastics, including PE, PS, and polyvinyl chloride (PVC) (Yang et al., 2014). The discovery of these microorganisms brings hope to the biodegradation of polyolefin plastic wastes, but more efficient microorganisms remain to be discovered.

Petroleum-contaminated soils are rich in alkane compounds, which are chemically similar to polyolefin plastics. Hence, it is an important source for screening polyolefin plastics degrading microorganisms. Many hydrocarbon-degrading microorganisms have been isolated from petroleum-contaminated soils, such as *Pseudomonas* sp. PP3, *Pseudomonas* sp. PP4 (Muthukumar et al., 2022), *Enterobacter* sp. MX1 (Zhang et al., 2022), *Pseudomonas aeruginosa* DN1 (He et al., 2017) and *Bacillus subtilis* DM-04 (Das and Mukherjee, 2007). However, the potential of microorganisms from petroleum-contaminated soils for the degradation of polyolefin plastics was less explored (Jin and Kim, 2017; Roberts et al., 2020). Therefore, the objective of this study was to isolate and characterize polyolefin plastic degrading microorganisms from petroleum-contaminated soils. To better understand the biodegradation of polyolefin plastics by the isolated microorganism, whole-genome sequencing analysis was performed, and candidate enzymes that may be associated with the degradation of polyolefin plastics were predicted. These findings could expand the microbial resources for the biodegradation of polyolefin plastics, and provide the groundwork for further investigating the potential mechanisms driving the biodegradation of polyolefin plastics.

2. Materials and methods

2.1. Materials

The paraffin liquid (CAS: 8012–95–1) used in this study was purchased from Macklin (Shanghai, China). The main component is a mixture of liquid hydrocarbons (C16 to C24), including saturated naphthenic hydrocarbon and saturated alkane. The commercial PE film (ET311201, 0.1 mm in thickness, additive free) and PS film (ST311050, 0.05 mm in thickness, additive free) were purchased from the Goodfellow Company (UK) and cut into 3 cm × 3 cm square sheets for microbial incubation experiments. The homemade PS films were prepared by dissolving 150 mg of PS powder (purchased from BASF, Germany) (additive free) into 6 mL tetrahydrofuran and then poured into a glass Petri dish (Kim et al., 2021). The glass Petri dish was covered with aluminum foil and placed in an oven at 50 ℃. After tetrahydrofuran volatilization, the homemade PS film was formed and removed from the glass Petri dish and cut into small pieces (5 mm × 10 mm). In this study, homemade PS films were only used for preliminary screening experiment, and the other experiments were performed with commercial PS films. The UVPE films were prepared by exposing the PE film to a UVB lamp (λ = 313 nm) for 20 d and cutting it into 2 cm × 2 cm squares sheets for microbial incubation experiments. All of the plastic films used in this study were washed with 1% SDS, sterilized water, and 75% ethanol, then dried on the laminar-flow clean bench (Zhang et al., 2020). The plastic films were weighted using the electronic analytical balance (METTLER TOLEDO AB104-S) for further experiments.

2.2. Screening and isolation of the liquid paraffin degrading microorganisms

The petroleum-contaminated soil samples were collected from the oil field in Dongying, China, and kept at 4 ℃ until used for the screening. The initial microorganisms screening was carried out in a medium with liquid paraffin as the sole carbon source. Samples were mixed with sterile water and inoculated into 100 mL mineral medium (1 g/L $(NH_4)_2SO_4$, 0.5 g/L KH_2PO_4, 1.5 g/L Na_2HPO_4, 1 g/L NaCl, 0.2 g/L $MgSO_4{\cdot}7\ H_2O$) in 300 mL shake flask with 2% paraffin liquid. Considering the influence of dissolved oxygen, the cultivation was carried out at 30 ℃ by shaking (100 rpm) or resting. The cultures were transferred into fresh paraffin liquid mineral medium every 7 d. The experiment was performed once. After three weeks of cultivation, the microorganisms were collected by centrifugation at 4 ℃ and 4000 rpm for 10 min and sent to GENEWIZ (Suzhou, China) for amplicon sequencing of the 16 S rRNA.

In addition, the cultures were diluted to 10^{-4} and spread on the LB plate (10 g/L peptones, 10 g/L NaCl, 5 g/L yeast, and 2% agar) for isolating pure strains that degrade liquid paraffin. After 12 h incubation at 37 ℃, diverse bacterial colonies were picked and streaked on the LB plate for further polyolefin degradation experiment.

2.3. 16 S rRNA gene sequencing

The genomic DNA of the microbial culture was extracted as per manufacturer's instructions using the genome extraction kit (TIANGEN BIOTECH, China). Then the 16 S rRNA gene was amplified by the universal primers: 27 F (5′-AGAGTTTGATCMTGGCTCAG-3′), 1492 R (5′-TACGGYTACCTTGTTACGACTT-3′) using the Phanta Max Super-Fidelity DNA Polymerase (Vazyme, China) in a 50 μL final volume (Su et al., 2021). The PCR procedure was as follows: initial denaturation at 95 °C for 3 min, 30 cycles of denaturation at 95 °C for 10 s, annealing at 56 °C for 15 s, and extension at 72 °C for 1.5 min, and a final extension at 72 °C for 10 min. Then, the PCR products were sequenced (Tsingke Biotech Co. Ltd, China) and the sequence reads were used as queries to search against the NCBI database. The evolutionary history was assessed using the Maximum Likelihood method with 1000 bootstrap replicates based on the Kimura 2-parameter model. The phylogenetic tree was constructed using MEGA7 software.

2.4. Biodegradation of PS and PE by bacteria isolated from liquid paraffin enrichment

The isolated liquid paraffin degrading strains were first tested for the ability to degrade homemade PS film and then commercial PS film. The bacterial isolates were grown in 5 mL of LB medium overnight, pelleted by centrifugation at 7000 rpm for 5 min, and washed 3 times with mineral medium to completely remove any residual nutrients from the LB medium. The bacterial cells were then cultivated in a 24-well plate at 37 ℃ using mineral medium supplemented with the commercial PS films and homemade PS films as substrates (5 mm × 10 mm). To further verify the ability to degrade PS, the *Raoultella* sp. DY2415 was cultivated in the nutritious medium LB and supplemented with PS films for 4 d at 37 ℃. The experiment was repeated twice. After cultivation, the PS films were collected and washed with 1% SDS, sterilized water, and 75% ethanol, then dried on the laminar-flow clean bench. Finally, the dried

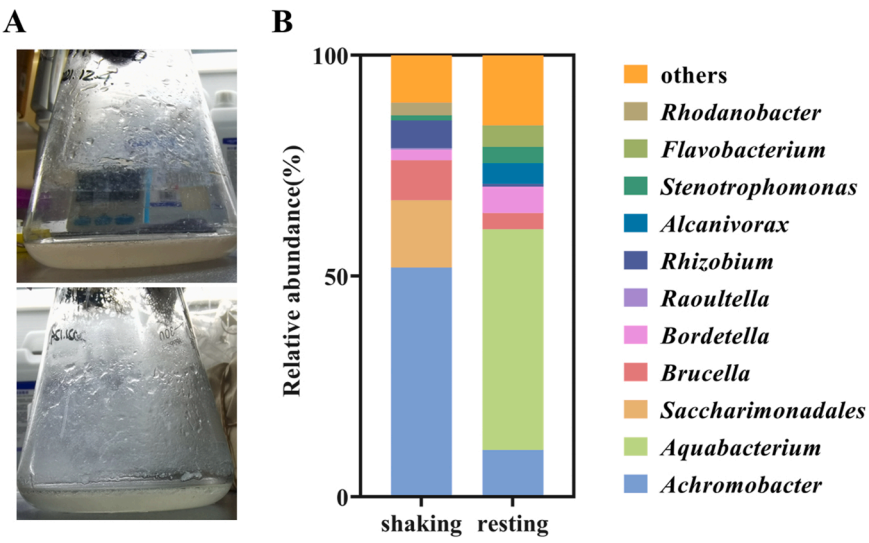

Fig. 1. Preliminary screening of polyolefin plastics degrading microorganisms using paraffin liquid as the sole carbon source. (A) Obvious microbial growth was found by shaking (Top) or resting (Down) incubation of petroleum-contaminated soil sample with 2% liquid paraffin as the only carbon source at 30 ℃. Microbial growth was reflected by the apparent turbidity of the medium. (B) Microbial composition of the culture after shaking and resting incubation. The sample was cultured in mineral medium with 2% liquid para as the only carbon source at 30 ℃ for three weeks.

PS film was weighed and performed for SEM observation.

To further validate the degradation of polyolefin plastics, the isolated *Raoultella* sp. DY2415 was respectively incubated with PE and UVPE films. Before the cultivation, the plastic film was cut into small pieces (2 cm × 3 cm), weight, and recorded. Then the film was disinfected, dried, and finally put into the minimal medium. The bacteria were cultivated in LB medium for 12 h and then washed with minimal medium three times to remove the residual nutrients. After the last washing, the bacteria cell pellets were suspended and inoculated into the 300 mL flasks containing 100 mL mineral medium supplemented with the sterilized plastic film which was weighed before. After cultivation at 37 ℃, 220 rpm for 2 months, weight loss, FTIR, and SEM analysis of the PE and UVPE films were performed to verify the degradation. Due to the long time of the cultivation, the experiment was performed one time.

2.5. Weight loss analysis

After incubation, the plastic film was collected and washed with 1% SDS buffer, 75% ethanol, and sterilized water. After air drying, the films were weighed using the analytical balance (METTLER TOLEDO AB104-S) with a resolution of 0.1 mg. The weight loss (%) was calculated using the formulae below;

$$Weight\quad loss\quad (\%) = [(Iw - Fw) \div Iw] \times 100$$

Iw = Initial weight of the film before degradation; Fw = Final weight of the film after degradation.

2.6. FTIR analysis

The surface chemical group change of the PE and PS films was analyzed by FTIR. Briefly, after microbial incubation, the biofilm attached to the plastic was completely removed by 1% SDS, sterilized water, and 75% ethanol (Zhang et al., 2020). After air drying, the clean plastic films were analyzed using a Nicolet 6700 (Waltham, USA) spectrometer over the wavelength range of 400–4500 cm^{-1} at a resolution of 1 cm^{-1} in ATR mode. Thirty-two scans were taken for each spectrum.

2.7. SEM analysis

The plastic films were soaked in 2.5% glutaraldehyde for 3 h at 4 ℃ and dehydrated with 30–100% graded ethanol for 15 min, then critical-point-dried with CO_2 using EM CPD300 Critical Point Dryer (Leica, Germany). Dried specimens were sputter-coated for 4 min with gold and platinum (10 nm) to increase the surface conductivity for analysis by the Quanta 250 FEG SEM (FEI, USA) operating at an accelerating voltage of 5 kV.

2.8. Gas chromatography-mass spectrometry (GC-MS) analysis

The products of PE biodegradation were detected by GC-MS. Briefly, after 60 d incubation of strain *Raoultella* sp. DY2415 in the mineral medium supplemented with PE, the corresponding cell suspension was centrifuged (12,000 g, 20 min, 4 ℃) to collect the supernatant. The supernatant was freeze-dried and re-dissolved in 1 mL dichloromethane, then 2 μL filtered supernatant was used for GC-MS analysis performed on QP2010 SE GC-MS system (Shimadzu, Japan) equipped with an Rtx-5MS (30 m long, 0.25 mm internal diameter and 0.25 μm thickness). The injection port was set at 280 ℃. During operation, the column temperature was held for 2 min at 50 ℃, then raised to 280 ℃, and finally, held for 15 min at 280 ℃. The flow rate was 1 mL/min. Helium was used as a carrier gas. Ions/fragments were monitored in scanning mode through 30–450 Amu.

2.9. Genomic analysis

The bacterium was cultured in the LB medium until the logarithmic growth period (OD_{600} = 0.5–0.6), then washed with 1 × PBS buffer 3 times. The cells were collected for total DNA extraction with a DNeasy Blood and Tissue Kit (Qiagen, Germany) according to the manufacturer's instructions. Genomic sequencing was performed by BGI (Qingdao, China) using Oxford Nanopore. The experiment pipeline mainly included sample quality testing, library construction, and library sequencing. Sequencing libraries were generated using Ligation Sequencing Kit (Oxford Nanopore Technologies). The content of bioinformatics analysis included data filtering, assembly, genomic component analysis, analysis on gene function. The gene functions were annotated by five databases, including GO (Harris et al., 2004), KEGG (Kanehisa et al., 2004), COG (Galperin et al., 2015), NR (Saier et al., 2014) and Swiss-prot (Bairoch. And Apweiler, 2000). The calculation of average nucleotide identity (ANI) was performed on http://enve-omics.ce.gatech.edu/ani/index.

Table 1
The isolated strains from petroleum-contaminated soil.

Strain number*	Genus	Degrading homemade PS film	Degrading commercial PS film
DY2403, DY2416, DY2417, DY2432	*Stenotrophomonas*	+	-
DY2401, DY2402, DY2404, DY2405, DY2412, DY2413, DY2421, DY2422, DY2424, DY2428, DY2430, DY2607, DY2608, DY2614, DY2619, DY2620, DY2423	*Pseudomonas*	-	-
DY2602	*Achromobacter*	+	-
DY2408	*Klebsiella*	-	-
DY2409, DY2624, DY2625, DY2419, DY2611, DY2621, DY2622	*Enterobacter*	+	-
DY2414, DY2415, DY2605, DY2613	*Raoultella*	+	+
DY2417, DY2616, DY2628,	*Bacillus*	-	-
DY2603	*Rhizobium*	-	-

* Strains isolated from two microbial consortiums of petrol-contaminated soil.

3. Results and discussion

3.1. Screening and isolation of the polyolefin degrading microorganisms

Liquid paraffin is a mixture of long-chain alkanes with structural similarity to polyolefin plastics (Delegan et al., 2019; Jeon and Kim, 2015). Therefore, it is chosen as a suitable substrate for the preliminary screening of polyolefin plastics degrading microorganisms. The petroleum-contaminated soil samples were collected from the oil fields in Dongying (Shandong, China), and then mixed with sterile water. The supernatant was inoculated into the mineral medium with 2% paraffin liquid as the sole carbon source for preliminary enrichment and screening. After three weeks of incubation, the medium was significantly turbid, both by shaking and resting culture. However, an obvious difference was observed in the color change of the cultures. The shaking group turned red, while the stationary culture remained faintly yellow (Fig. 1 A). We speculated that different microbial species were enriched from the same sample under different culture conditions.

To determine the microbial composition and abundance, microorganisms were collected from shaking and resting culture for amplicon sequencing. The results revealed high bacterial diversity and richness in both shaking and resting cultures. Bacteria belonging to 6 phyla, 7 classes, 27 orders, 38 families, 63 genera, and 91 OTUs were detected based on 42,736 sequences within the shaking culture. As for resting culture, there were 95 OTUs belonging to 9 phyla, 10 classes, 29 orders, 42 families, and 62 genera. There were 12 microorganisms with an abundance of more than 1% in the resting culture, of which *Aquabacterium* and *Achromobacter* accounted for the highest proportion, with percentages of 49.9% and 11.0%, respectively (Fig. 1B). Correspondingly, 10 microorganisms with an abundance of more than 1% were present in the shaking culture, of which the percentage of *Aquabacterium* decreased from 49.9% to 0.01%, while the percentage of *Achromobacter* increased from 11.0% to 51.9% (Fig. 1B). These results indicated that the microbial consortiums from the samples adapted to different culture methods for effective assimilation of the liquid paraffin. And provided valuable microbial resources for screening polyolefin-degrading microorganisms.

In order to obtain pure strain that can degrade polyolefin plastics, we diluted the culture and spread on LB agar plate. Then different colonies were selected for further 16 S sRNA gene sequencing and the sequence reads were used as query to search against the NCBI database. After several rounds of purification by plate streaking, 39 colonies were isolated and belonged to eight genera, including *Stenotrophomonas* (4 strains), *Pseudomonas* (18 strains), *Achromobacter* (1 strain), *Klebsiella* (1 strain), *Enterobacter* (7 strains), *Raoultella* (4 strains), *Bacillus* (3 strains) and *Rhizobium* (one strain) (Table 1). In order to further verify the degradation of polyolefin plastics by of the 8 genera. We chose homemade PS films as the substrates for preliminary screening, and the commercial PS films as substrates for the final screening. The homemade PS films were formed using a solvent evaporation method, whereas the commercial PS films were formed using casting method. SEM observation showed that the surface of the commercial PS film was smooth, whereas the homemade PS film showed many holes on the surface which may made it easier for bacteria to colonize (Fig. S1). After one week of incubation in the 24-well plate using mineral medium, possible growth on the surface of the homemade PS film was observed among certain microorganisms (Fig. S2, red circle), including the genera of *Stenotrophomonas, Achromobacter, Enterobacter,* and *Raoultella*. It has been reported that *Stenotrophomonas* sp. and *Achromobacter* sp. isolated from waste dump sites, drilling fluid, or the oil sludge showed varying degrees of degradation towards multiple polyolefin plastics, including LDPE and polyvinyl alcohol (PVA) (Dey et al., 2020; Maleki Rad et al., 2022; Ullah et al., 2018). In addition, *Enterobacter* is also a class of microorganisms that are known for polyolefin degrading activity, e.g. *Enterobacter* sp. D1 isolated from the guts of wax moth *Galleria mellonella* (Ren et al., 2019), and *Enterobacter cloacae* AKS7 isolated from LDPE waste soil (Sarker et al., 2021). *Raoultella* has not been reported to have the activity of degrading polyolefin plastics. However, it is worth noting that in our results only strains from the *Raoultella* genus caused the sharp edges of commercial PS film to be rounded and smooth (Fig. S2, red arrow),

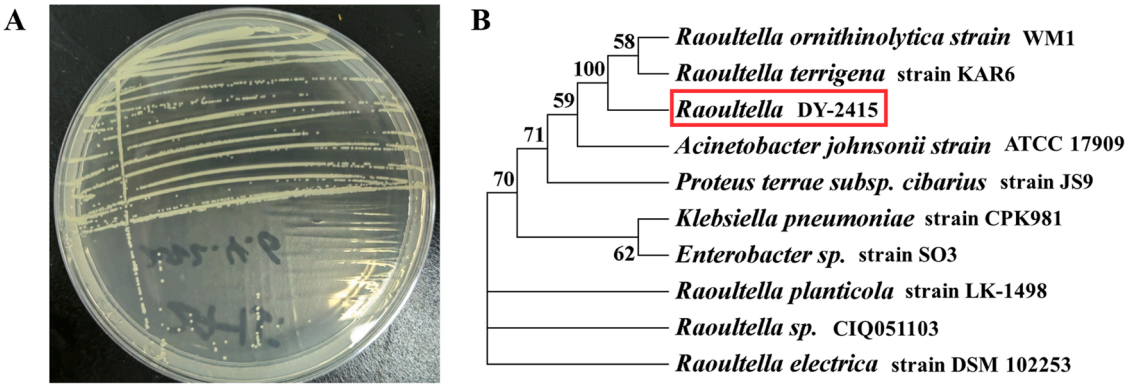

Fig. 2. Characterization of *Raoultella* sp. DY2415. (A) The morphology of *Raoultella* sp. DY2415 on the LB agar plate after cultivated at 37 ℃ for 12 h. (B) Phylogenetic tree analysis of *Raoultella* sp. DY2415 based on 16 S rRNA gene. The tree was constructed by maximum likelihood method.

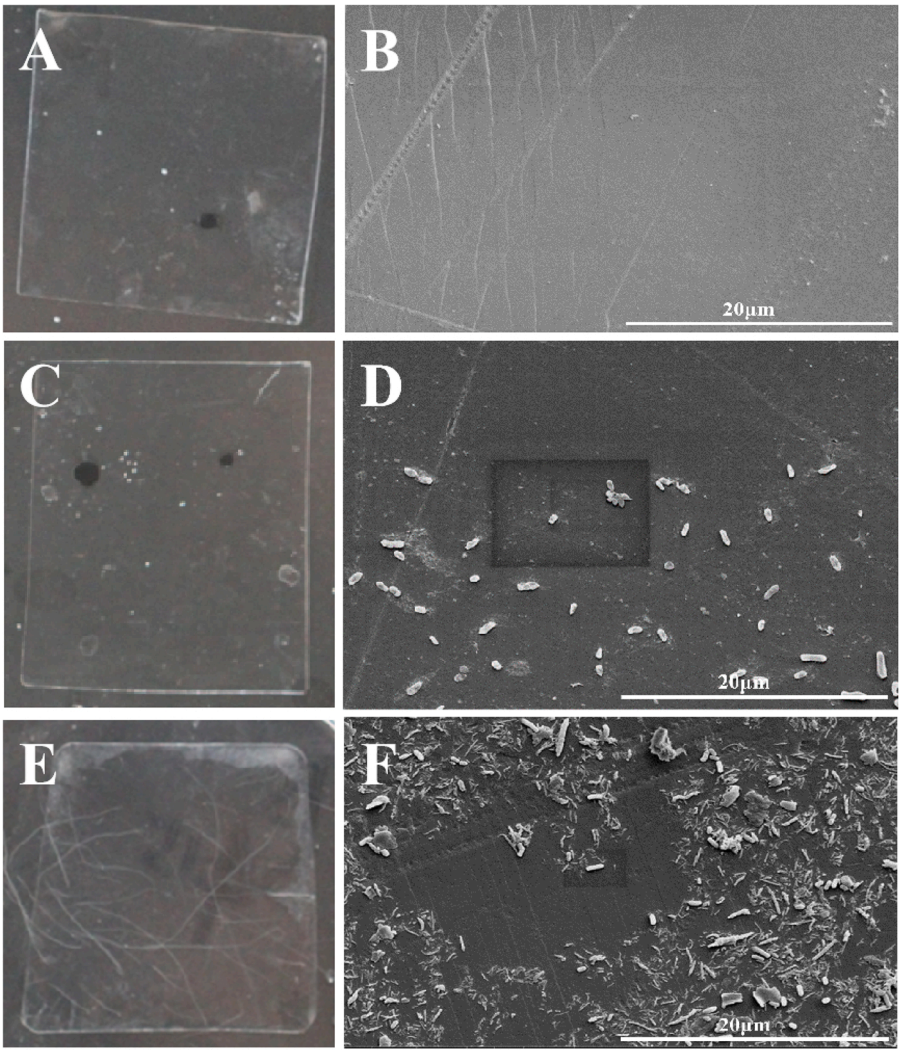

Fig. 3. The *Raoultella* sp. DY2415 strain degrades PS films. Morphology of PS films before (A) and after (E) incubation with *Raoultella* sp. DY2415 for 4 d. SEM observation of the PS films before (B) and after (F) incubation with *Raoultella* sp. DY2415 for 4 d. Morphology of PS films incubated with E. coli after 4 d (C). SEM observation of the PS films incubation with *E. coli* DH5α after 4 d (D). The commercial PS films used were ST311050 (0.05 mm in thickness).

revealing a more noticeable biodegradation potential for polyolefin plastics. The holes on the surface of homemade PS film may be the reason why homemade PS film is easier to degrade than commercial PS film. It also explained that *Stenotrophomonas*, *Achromobacter* and *Enterobacter* only showed the degradation of homemade PS films.

Raoultella is a gram-negative bacterium and belongs to the *Enterobacteriaceae* family (Hajjar et al., 2020). At the previous screening of liquid paraffin degrading microorganisms, the *Raoultella* only grow under shaking condition with a relative abundance of 0.3%. The morphology of the isolated strain (named *Raoultella* sp. DY2415) on the LB agar plate was pale-yellow, round, smooth, and moist (Fig. 2A). It has been reported that *R. ornithinolytica* OKOH-1 and *R. ornithinolytica* strain S1 serve an important role in the pretreatment of lignocellulosic biomass (Falade et al., 2017). Lignin is the second most abundant natural polymer on the earth. It is linked by myriad C-O and C-C bonds, similar to polyolefin plastics (Chen et al., 2020). Lignin and the polyolefin plastics both consist of C-C bonds means that some lignin degrading enzymes, especially oxidoreductases, may degrade polyolefin plastics. Laccase are reported to degrade both PE and lignocellulose (Inderthal et al., 2021). It has also been reported that ligninolytic fungi can cleave water-soluble PS model compounds and could oxidize the surface of PS itself (Inderthal et al., 2021). In addition, DY2415 is evolutionary close to *Acinetobacter* and *Enterobacter* (Fig. 2B), which were reported to have the capacity to degrade PE and PS (Montazer et al., 2018; Sarker et al., 2021). These further imply that *Raoultella* sp. DY2415 has significant potential to degrade polyolefin plastics.

3.2. Biodegradation of PS by the Raoultella sp. DY2415 strain

LB medium consists of nutrients that allow better growth of microorganisms, producing more enzymes. Therefore, after culturing with the minimal medium, LB medium was used to culture *Raoultella* sp. DY2415 and evaluate for the degradation of PS film. We found that the supplement of PS film into the LB medium significantly enhanced the cell growth of DY2415. After 4 d of incubation, the OD_{600} of cells containing PS films was 7.4, which was twice higher than the control (Fig. S3). This suggests that *Raoultella* sp. DY2415 is likely to degrade the PS films to produce small molecules, and these compounds can be assimilated for cell growth.

To prove this hypothesis, the PS films were collected after 4 d of incubation and analyzed for morphology and weight loss (Fig. 3). We found that the corners of the PS films incubated with *Raoultella* sp. DY2415 became round compared with the control (Fig. 3C), which was incubated with *Escherichia coli* DH5α. This phenomenon was similar to that of PET and PE films incubated with the microbial consortium CAS6, which lost the sharp morphology of the films and proved to be

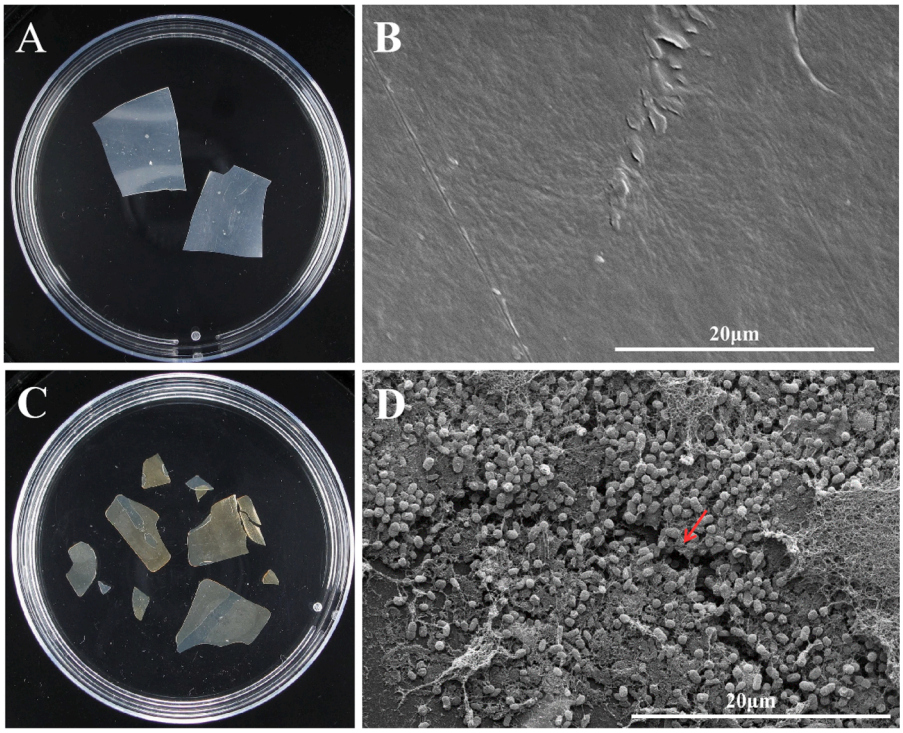

Fig. 4. The degradation of UV-irradiated film by *Raoultella* sp. DY2415. Morphology of UVPE films before (A) and after (C) incubation with *Raoultella* sp. DY2415 for 60 d. SEM observation of the UVPE film before (B) and after (D) incubation with *Raoultella* sp. DY2415 for 60 d. UVPE films used were PE films ET311201 (0.1 mm in thickness) irradiated for 20 d. The red arrow points to "the clear cleavage".

biodegraded (Gao and Sun, 2021). The weight of the PS film decreased from 20.2 mg to 19.8 mg, indicating a 2% weight loss. Furthermore, compared with the control, SEM images of *Raoultella* sp. DY2415 showed a large number of bacteria adhered to the surface of the PS film (Fig. 3F), while the edges of the PS film were folded up and became rough (Fig. S4). Although the 2% weight loss of the PS films was difficult to directly support a significant increase in cell biomass, the small molecules generated by the biodegradation of PS were likely to stimulate cell growth using nutrition from the LB medium and generate more cells for further degradation.

3.3. Biodegradation of PE by the Raoultella sp. DY2415 strain

In addition to PS, PE is also a class of widely used polyolefin plastics (Bardaji et al., 2020). To determine the biodegradation of PE, *Raoultella* sp. DY2415 was incubated with the PE and UVPE films. After 60 d of incubation, the surface of the PE film appeared with some small pits and holes (Fig. S5), while the morphological changes on the surface of the PE film after UV irradiation were even more obvious. The UVPE film became fragmented, and the surface turned yellow (Fig. 4C). The weight of UVPE film decreased from 61.4 mg to 56.9 mg, indicating an 8% weight loss in 60 d.

SEM image showed obvious colonization of the *Raoultella* sp. DY2415 strain on the surface of UVPE films (Fig. 4D). It can generate some substances similar to the biofilm which have been considered to play an important role in PE degradation (Chattopadhyay, 2022; Morohoshi et al., 2018). Of note, the surface of UVPE films formed a clear cleavage after incubation with DY2415 for 60 d, while the initial UVPE film was smooth (Fig. 4). The results illustrated that *Raoultella* sp. DY2415 can degrade PE as well as UVPE films, and the UV irradiation of PE film could be better degraded by *Raoultella* sp. DY2415. It has been reported that UV irradiation can partly oxidize polyolefin plastics, and introduce new oxygen-containing functional groups, such as the ketone group and hydroxyl group (Tribedi and Dey, 2017). Thus, UVPE

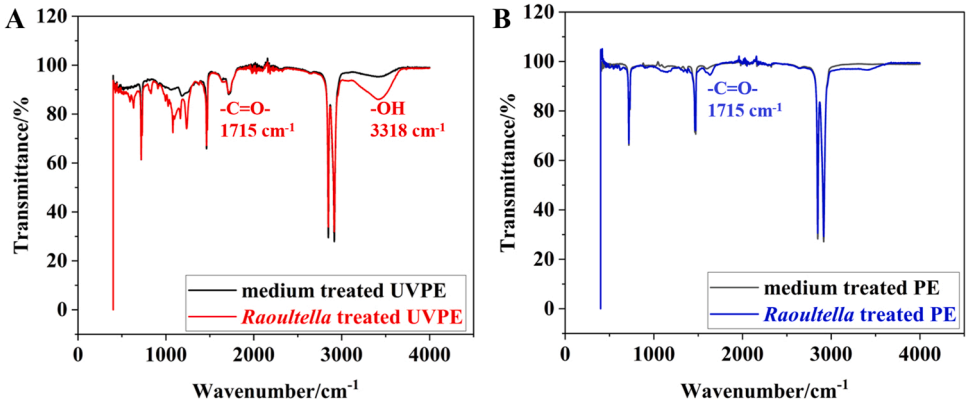

Fig. 5. FTIR analysis of the UVPE (A) and PE (B) films incubated with *Raoultella* sp. DY2415 in the mineral medium for 60 d. The peaks at 1715 cm^{-1} correspond to the formation of carbonyl groups. The peaks at 3318 cm^{-1} correspond to the formation of hydroxyl groups.

Table 2
Carbonyl index and hydroxyl index from FTIR.

	PE	PE-*Raoultella*[a]	UVPE	UVPE-*Raoultella*[b]
Carbonyl Index	0.036	0.043	0.408	0.458
Hydroxyl Index	0.036	0.107	0.158	0.408

[a] The PE film incubated with *Raoultella* sp. DY2415 for 60 days in the mineral medium.
[b] The UVPE film incubated with *Raoultella* sp. DY2415 for 60 days in the mineral medium.

probably provides more attackable groups for *Raoultella* sp. DY2415, enabling it to be more susceptible to colonization and degradation.

To further analyze the change in chemical groups, FTIR analysis was performed on the PE films. We found that the pretreatment of PE with UV radiation produced a distinct peak at 1715 cm^{-1} (Fig. 5A), indicating the appearance of a carbonyl bond (-C═O-) (Sandt et al., 2021). After incubation with *Raoultella* sp. DY2415 for 60 d, a more obvious peak appeared at 3318 cm^{-1}, which was attributed to the hydroxyl group (Bombelli et al., 2017). This FTIR data suggested that UV pretreatment can partially oxidize PE films and facilitate the further biodegradation of PE by *Raoultella* sp. DY2415 to produce more oxygen-containing functional groups. Our FTIR result was similar to the previously reported spectra of the PE film treated with the fungus *Alternaria alternata* FB1 (Gao et al., 2022). They all have absorption peaks at 1715 cm^{-1} and 3318 cm^{-1}, respectively, representing carbonyl bonds and hydroxyl groups. Furthermore, new absorption peaks appear between 1000 cm^{-1} and 1200 cm^{-1}, which may suggest some new groups have been added to the PE chain. In addition, the carbonyl index (CI) and hydroxyl index (HI) were significant factors that can be used to assess the degradation of the polymer (Campanale et al., 2023; Syranidou et al., 2023). CI was defined as the ratio between the intensity of carbonyl groups (peaks at 1715 cm^{-1}) and the methylene groups (peaks at 1472 cm^{-1}). HI was calculated from the ratio of the peak at 3318 cm^{-1} against the peak at 1472 cm^{-1}. CI and HI of the UVPE film treated by *Raoultella* sp. DY2415 was 1.1-fold, 2.6-fold than that of the control, respectively (Table 2). Whereas for PE films without UV irradiation, *Raoultella* sp. DY2415 incubation still resulted in peaks at 1715 cm^{-1} and 3318 cm^{-1} on the FTIR spectrum, albeit faintly (Fig. 5B), indicating the formation of carbonyl groups and hydroxyl groups. CI and HI of the PE film treated by *Raoultella* sp. DY2415 was 1.2-fold, 3.0-fold than that of the control, respectively (Table 2). The appearance of carbonyl groups was reported to be a sign of PE degradation (Cai et al., 2018).

Though the weight loss of PE film was slight and difficult to detect, we detected some small molecules released from the medium, including "2,2-dimethyl-1-octanol", "dodecane,2,6,11-trimethyl", "decane,2,3,5, 8-tetramethyl", "2-ethyl-2-methyl-tridecanol", "nonadecane" and "methoxy acetic acid, 4-tridecyl ester" (Fig. S6). The formation of branched hydrocarbon compounds is uncommon and we speculate that they may derive from the original branching structure of LDPE. In addition, the mechanism of polyolefin plastic biodegradation is complex and may cause carbon rearrangement. These results showed obvious structural changes in the PE and UVPE films treated by *Raoultella* sp. DY2415 and provided evidence of biodegradation. The area of the hydroxyl absorption peak of UVPE was significantly greater than that of PE, indicating that the biodegradation of UVPE film was more significant than the original PE film. The differences in chemical structure between PE and PS lead to varying biodegradation properties of *Raoultella* sp. DY2415 to them (Fig. S7). The weight loss of the PS film after 4 d of incubation was more noticeable than that of the PE film after 60 d of incubation, which appeared to indicate that *Raoultella* sp. DY2415 tend to degrade PS. However, the biodegradation mechanism of polyolefin plastics is complex, and more enzymatic studies are needed in the subsequent work to comprehensively analyze the properties of *Raoultella* sp. DY2415 to degrade different polyolefin plastics.

Since UV radiation can promote the biodegradation of PE by *Raoultella* sp. DY2415, we extended the UV pre-treatment period of the PE films to 40 d and made them fragmented. The fragmented UVPE films (f-UVPE) were incubated with the *Raoultella* sp. DY2415 strain in the mineral medium for 7 d. The results showed that fragmented UVPE could be more rapidly degraded by *Raoultella* sp. DY2415 and assimilated for cell growth (Fig. 6 A). The OD_{600} increased from 0.2 to 0.5 within 7 d (4.29% increase per day) in mineral medium with the f-UVPE film as the sole carbon source, which was considered a significant biomass increment in the case of PE degradation (Fig. 6B). In the previous study, after 8-weeks of incubation with the PE film, the ΔOD_{600} values of RD1–3 strain only increased by 0.18% per day (Hou et al., 2022). The OD_{600} of the marine strain H-255 incubated with PE film increased by 4% per day over the 5 d of incubation (Khandare et al., 2021). Our results showed that *Raoultella* sp. DY2415 strain can degrade f-UVPE films and assimilate the produced small molecules for cell growth.

To explore the biodegradation mechanisms of polyolefin plastics, the complete genome of *Raoultella* sp. DY2415 was sequenced based on the Nanopore third-generation sequencing (Bioproject accession: PRJNA898983). *Raoultella* sp. DY2415 consists of a 5,565,348 bp circular chromosome with a GC content of 56.81%, a 152,330 bp plasmid with a GC content of 51.15%, and 5,456 open reading frames (ORFs). Average nucleotide identity (ANI) analysis showed that the genome sequence of *Raoultella* sp. DY2415 shared 99.58% identity with its closest relative, *Raoultella ornithinolytica* strain 23 (Bioproject accession: PRJNA604100).

Functional annotation of the predicted protein-coding genes was performed based on homology-based search using several well-known databases, i.e., GO (Harris et al., 2004), KEGG (Kanehisa et al., 2004), COG (Galperin et al., 2015), NR (Saier et al., 2014) and Swiss-prot (Bairoch. and Apweiler, 2000). The COG analysis revealed up to 621 genes involved in carbohydrate transport and metabolism (Fig. S8), accounting for 11.38% of all predicted protein-coding genes. There are a

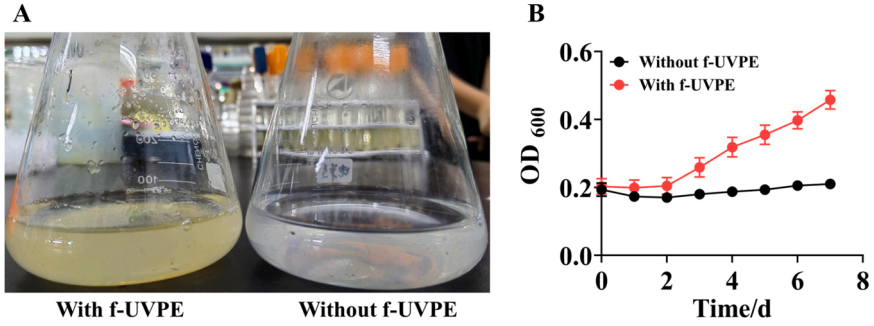

Fig. 6. (A) *Raoultella* sp. DY2415 grown in the mineral medium supplemented with fragmented UVPE film at 37 ℃ for 7 d. (B) Growth curves of *Raoultella* sp. DY2415 in the mineral medium with fragmented UVPE films at 37 ℃ for 7 d. Error bars represent the standard deviation of three independent experiments. 3.4. Exploring the potential polyolefin-degrading enzymes.

Table 3
The *Raoultella* sp. DY2415 genes with auxiliary activities (AAs) annotated in Carbohydrate-Active Enzymes Database (CAZy).

Gene ID	Enzyme	EC number
3944 1790 1766 3944	cellobiose dehydrogenase	EC 1.1.99.18
3944 1790 1766 3944	glucose 1-oxidase	EC 1.1.3.4
3944 1790 1766 3944	aryl alcohol oxidase	EC 1.1.3.7
3944 1790 1766 3944	alcohol oxidase	EC 1.1.3.13
3944 1766 3944	pyranose oxidase	EC 1.1.3.10
1790 1766 3944	glucose 1-dehydrogenase (FAD, quinone)	EC 1.1.5.9
1790 1766 3944	pyranose dehydrogenase	EC 1.1.99.29
1790	ecdysone oxidase	EC 1.1.3.16
1790 1766	oligosaccharide dehydrogenase (FAD)	EC 1.1.5.-
3422	1,4-benzoquinone reductase	EC 1.6.5.6

series of COG2814 (major facilitator Superfamily), COG1472 (hydrolase family 3), and COG4993 (dehydrogenase) genes involved in the degradation of xenobiotics. COG2814 contains the secondary active transporter (Chaudhary et al., 2016). It can transport various substrates, including carbohydrates, irons, and other small molecules. In the Carbohydrate-Active Enzymes Database (CAZy), *Raoultella* sp. DY2415 contained 299 genes distributed unequally among glycoside hydrolases (GHs, 50.8%), glycosyl transferases (GTs, 28.1%), polysaccharide lyases (PLs, 4.7%) carbohydrate esterases (CEs, 3.7%), auxiliary activities (AAs, 2.7%) and carbohydrate-binding modules (CBMs, 10%). The AAs genes encoded enzymes with different activities listed in Table 3 which are associated with oxidation reactions and may take part in the degradation of polyolefin plastics.

Furthermore, KEGG metabolic pathway and network analysis indicated that *Raoultella* sp. DY2415 encoded 91 genes for the metabolism and biodegradation of xenobiotics involving 18 classes of compounds, such as styrene, and benzoate (Fig. S9, Table S1). The abundant xenobiotics metabolism and biodegradation gene clusters were likely to contribute to the degradation of polyolefins by *Raoultella* sp. DY2415 (Table S1). Our results are similar to the previously reported genomic analysis of PE mulch-degrading bacteria (Hou et al., 2022), suggesting the presence of polyolefin biodegradation pathways in *Raoultella* sp. DY2415.

Laccase (Sowmya et al., 2014), peroxidase (Mukherjee and Kundu, 2014), alkane hydroxylase (Sulaiman et al., 2012), and other representative enzymes have been reported to be involved in polyolefin degradation. We identified 51 genes encoding dioxygenases, monooxygenases, hydroxylases, peroxidases, and laccase in the genome of *Raoultella* sp. DY2415 (Table S2). In addition, we also analyzed the genes that were homologous to those enzymes known to be involved in PE degradation. We found that strain DY2415 had some several candidate plastic-degrading genes, annotated as laccase, manganese peroxidase and Rubredoxin (Table 3, Table S3). In particular, the 4450 gene encoded a protein with 38.6% similarity to the previously reported polyolefin degrading related polyphenol oxidase from *Rhodococcus ruber* (Fujisawa et al., 2001; Santo et al., 2013). And the proteins encoded by 1123, and 1124 genes are similar to the polyolefin degrading related rubredoxin reductase from *Pseudomonas putida* (Jeon and Kim, 2016). These potential polyolefin-degrading enzymes may play an important role in terminal oxidation and subterminal hydroxylation of polyolefin plastics (Zhang, N. et al., 2022). After oxidation by oxidoreductases, ester bonds may be produced in PE (Yeom et al., 2022). Therefore, hydrolase, especially esterase/lipase, may also be involved in the subsequent degradation. We found 7 potential genes encoding hydrolase in the genome of *Raoultella* sp. DY2415 (Table S2). They may participate in the subsequent hydrolysis steps of oxygenated polyolefin plastics. Certainly, further studies are needed to confirm the activity of these enzymes and their roles in polyolefin degradation. Table 4.

3.4. Study limitations

While this study isolated and characterized a novel *Raoultella* species that can degrade polyolefin plastics, some limitations remain. The biodegradation efficiency of *Raoultella* sp. DY2415 on polyolefin plastics is far from adequate for practical applications. Furthermore, the mechanism of plastic biodegradation by *Raoultella* sp. DY2415 is still unclear, and the role of candidate enzymes that may be relevant to polyolefin plastic degradation still needs further experimental verification.

4. Conclusions

In this study, we isolated and identified a strain named *Raoultella* sp. DY2415, which could degrade UVPE, PE, and PS film. It is the first strain in *Raoultella* genus that has been reported to have the ability to degrade polyolefin plastics. Through SEM, FTIR, and GC-MS analysis, we verified the typical degradation, including colonization, scission as well as the destruction of UVPE and PS film after being treated with *Raoultella* sp. DY2415 and found new functional groups on the surface of the plastic film. In addition, 8% weight loss of UVPE film was observed after incubation with *Raoultella* sp. DY2415 for 60 d. Genomic analysis revealed several candidate enzymes that may be relevant to polyolefin degradation. This work expands the microbial resources for degrading polyolefins and provides the foundation for further investigating the biodegradation mechanism of C-C backbone plastics.

Ethics approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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Table 4
Homologous genes in *Raoultella* sp. DY2415 genome to the reported PE degrading enzymes.

Enzyme	Source	Degradation effect	Homogenous gene in strain DY2415*	Reference
Laccase	*Rhodococcus ruber*	Reduction of 20% average molecular weight (Mw) and 15% average molecular number (Mn) of LDPE film	4450	(Santo et al., 2013)
Manganese peroxidase	*Penicillium simplicissimum*	0.3% weight loss of PE film	3159	(Sowmya et al., 2014)
Rubredoxin reductase	*Pseudomonas putida*	Production of CO_2 and biomass due to LMWPE degradation	1123, 1124	(Jeon and Kim, 2016)

* The genes in *Raoultella* sp. DY2415 genome with amino acid sequence similarity greater than 30% to the reported PE degrading enzymes.

CRediT authorship contribution statement

Yingbo Yuan: Investigation, Experiment design, Data analysis; Writing – original draft. **Pan Liu:** Investigation; Writing – review & editing. **Junling Bian:** Writing – review & editing. **Yi Zheng:** Writing – review & editing. **Qingbin Li:** Writing – review & editing. **Quanfeng Liang:** Writing – review & editing. **Tianyuan Su:** Research ideation; Experiment design; Manuscript revision. **Longyang Dian:** Research ideation; Experiment design; Manuscript revision. **Qingsheng Qi:** Research ideation; Experiment design; Manuscript revision.

Declaration of Competing Interest

The authors declare no competing financial interests.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2023.115232.

References

Bairoch, A., Apweiler, R., 2000. The SWISS-PROT protein sequence database and its supplement TrEMBL in 2000. Nucleic Acids Res. 28, 45–48. https://doi.org/10.1093/nar/28.1.45.

Bardaji, D.K.R., Moretto, J.A.S., Furlan, J.P.R., Stehling, E.G., 2020. A mini-review: current advances in polyethylene biodegradation. World J. Microbiol Biotechnol. 36 (2), 32–42. https://doi.org/10.1007/s11274-020-2808-5.

Bombelli, P., Howe, C.J., Bertocchini, F., 2017. Polyethylene bio-degradation by caterpillars of the wax moth Galleria mellonella. Curr. Biol. 27 (8), 292–293. https://doi.org/10.1016/j.cub.2017.02.060.

Cai, L., Wang, J., Peng, J., Wu, Z., Tan, X., 2018. Observation of the degradation of three types of plastic pellets exposed to UV irradiation in three different environments. Sci. Total Environ. 628–629, 740–747. https://doi.org/10.1016/j.scitotenv.2018.02.079.

Campanale, C., Savino, I., Massarelli, C., Uricchio, V.F., 2023. Fourier transform infrared spectroscopy to assess the degree of alteration of artificially aged and environmentally weathered microplastics. Polym. (Basel) 15 (4). https://doi.org/10.3390/polym15040911.

Chattopadhyay, I., 2022. Role of microbiome and biofilm in environmental plastic degradation. Biocatal. Agric. Biotechnol. 39. https://doi.org/10.1016/j.bcab.2021.102263.

Chaudhary, N., Gonzalez, E., Chang, S.H., Geng, F., Rafii, S., Altorki, N.K., McGraw, T.E., 2016. Adenovirus Protein E4-ORF1 activation of PI3 kinase reveals differential regulation of downstream effector pathways in adipocytes. Cell Rep. 17 (12), 3305–3318. https://doi.org/10.1016/j.celrep.2016.11.082.

Chen, C.-C., Dai, L., Ma, L., Guo, R.-T., 2020. Enzymatic degradation of plant biomass and synthetic polymers. Nat. Rev. Chem. 4 (3), 114–126. https://doi.org/10.1038/s41570-020-0163-6.

Danso, D., Chow, J., Streit, W.R., 2019. Plastics: environmental and biotechnological perspectives on microbial degradation. Appl. Environ. Microbiol 85 (19). https://doi.org/10.1128/AEM.01095-19.

Das, K., Mukherjee, A.K., 2007. Crude petroleum-oil biodegradation efficiency of *Bacillus subtilis* and *Pseudomonas aeruginosa* strains isolated from a petroleum-oil contaminated soil from North-East India. Bioresour. Technol. 98 (7), 1339–1345. https://doi.org/10.1016/j.biortech.2006.05.032.

Delegan, Y.A., Valentovich, L.N., Shafieva, S.M., Ganbarov, K.G., Filonov, A.E., Vainstein, M.B., 2019. Characterization and genomic analysis of highly efficient thermotolerant oil-degrading bacterium *Gordonia* sp. 1D. Folia Microbiol (Praha) 64 (1), 41–48. https://doi.org/10.1007/s12223-018-0623-2.

Dey, A.S., Bose, H., Mohapatra, B., Sar, P., 2020. Biodegradation of unpretreated low-density polyethylene (LDPE) by *Stenotrophomonas* sp. and *Achromobacter* sp., isolated from waste dumpsite and drilling fluid. Front Microbiol 11, 603210. https://doi.org/10.3389/fmicb.2020.603210.

Falade, A.O., Eyisi, O.A.L., Mabinya, L.V., Nwodo, U.U., Okoh, A.I., 2017. Peroxidase production and ligninolytic potentials of fresh water bacteria *Raoultella ornithinolytica* and *Ensifer adhaerens*. Biotechnol. Rep. (Amst.) 16, 12–17. https://doi.org/10.1016/j.btre.2017.10.001.

Fujisawa, M., Hirai, H., Nishida, T., 2001. Degradation of polyethylene and nylon-66 by the laccase-mediator system. J. ofPolymers Environ. 9, 103–108. https://doi.org/10.1023/A:1020472426516.

Galperin, M.Y., Makarova, K.S., Wolf, Y.I., Koonin, E.V., 2015. Expanded microbial genome coverage and improved protein family annotation in the COG database. Nucleic Acids Res 43(Database Issue) D261–D269. https://doi.org/10.1093/nar/gku1223.

Gao, R., Sun, C., 2021. A marine bacterial community capable of degrading poly (ethylene terephthalate) and polyethylene. J. Hazard Mater. 416, 125928 https://doi.org/10.1016/j.jhazmat.2021.125928.

Gao, R., Liu, R., Sun, C., 2022. A marine fungus *Alternaria alternata* FB1 efficiently degrades polyethylene. J. Hazard Mater. 431, 128617 https://doi.org/10.1016/j.jhazmat.2022.128617.

Geyer, R., Jambeck, J.R., Law, K.L., 2017. Production, use, and fate of all plastics ever made. Sci. Adv. 3 (7) https://doi.org/ARTNe1700782.

Hajjar, R., Ambaraghassi, G., Sebajang, H., Schwenter, F., Su, S.H., 2020. *Raoultella ornithinolytica*: emergence and resistance. Infect. Drug Resist 13, 1091–1104. https://doi.org/10.2147/IDR.S191387.

Harris, M.A., et al., 2004. The gene ontology (GO) database and informatics resource. Nucleic Acids Res 32(Database Issue) D258–D261. https://doi.org/10.1093/nar/gkh036.

He, C., Dong, W., Li, J., Li, Y., Huang, C., Ma, Y., 2017. Characterization of rhamnolipid biosurfactants produced by recombinant *Pseudomonas aeruginosa* strain DAB with removal of crude oil. Biotechnol. Lett. 39 (9), 1381–1388. https://doi.org/10.1007/s10529-017-2370-x.

Horton, A.A., 2022. Plastic pollution: when do we know enough? J. Hazard Mater. 422, 126885 https://doi.org/10.1016/j.jhazmat.2021.126885.

Hou, L., Xi, J., Liu, J., Wang, P., Xu, T., Liu, T., Qu, W., Lin, Y.B., 2022. Biodegradability of polyethylene mulching film by two *Pseudomonas* bacteria and their potential degradation mechanism. Chemosphere 286 (Pt 3), 131758. https://doi.org/10.1016/j.chemosphere.2021.131758.

Inderthal, H., Tai, S.L., Harrison, S.T.L., 2021. Non-hydrolyzable plastics - an interdisciplinary look at plastic bio-oxidation. Trends Biotechnol. 39 (1), 12–23. https://doi.org/10.1016/j.tibtech.2020.05.004.

Jeon, H.J., Kim, M.N., 2015. Functional analysis of alkane hydroxylase system derived from *Pseudomonas aeruginosa* E7 for low molecular weight polyethylene biodegradation. Int. Biodeterior. Biodegrad. 103, 141–146. https://doi.org/10.1016/j.ibiod.2015.04.024.

Jeon, H.J., Kim, M.N., 2016. Comparison of the functional characterization between alkane monooxygenases for low-molecular-weight polyethylene biodegradation. Int. Biodeterior. Biodegrad. 114, 202–208. https://doi.org/10.1016/j.ibiod.2016.06.012.

Jeon, J.M., Park, S.J., Choi, T.R., Park, J.H., Yang, Y.H., Yoon, J.J., 2021. Biodegradation of polyethylene and polypropylene by *Lysinibacillus* species JJY0216 isolated from soil grove. Polym. Degrad. Stabil. 191 https://doi.org/ARTN109662.

Jin, C.E., Kim, M.N., 2017. Change of bacterial community in oil-polluted soil after enrichment cultivation with low-molecular-weight polyethylene. Int. Biodeterior. Biodegrad. 118, 27–33. https://doi.org/10.1016/j.ibiod.2017.01.020.

Kanehisa, M., Goto, S., Kawashima, S., Okuno, Y., Hattori, M., 2004. The KEGG resource for deciphering the genome. Nucleic Acids Res 32(Database Issue) D277–D280. https://doi.org/10.1093/nar/gkh063.

Khandare, S.D., Chaudhary, D.R., Jha, B., 2021. Marine bacterial biodegradation of low-density polyethylene (LDPE) plastic. Biodegradation 32 (2), 127–143. https://doi.org/10.1007/s10532-021-09927-0.

Kim, H.W., Jo, J.H., Kim, Y.B., Le, T.K., Cho, C.W., Yun, C.H., Chi, W.S., Yeom, S.J., 2021. Biodegradation of polystyrene by bacteria from the soil in common environments. J. Hazard Mater. 416, 126239 https://doi.org/10.1016/j.jhazmat.2021.126239.

Krueger, M.C., Harms, H., Schlosser, D., 2015. Prospects for microbiological solutions to environmental pollution with plastics. Appl. Microbiol Biotechnol. 99 (21), 8857–8874. https://doi.org/10.1007/s00253-015-6879-4.

Kurniawan, S.B., Abdullah, S.R.S., Imron, M.F., Ismail, N.I., 2021. Current state of marine plastic pollution and its technology for more eminent evidence: A review. J. Clean. Prod. 278. https://doi.org/10.1016/j.jclepro.2020.123537.

Liu, P., Zhang, T., Zheng, Y., Li, Q., Su, T., Qi, Q., 2021. Potential one-step strategy for PET degradation and PHB biosynthesis through co-cultivation of two engineered microorganisms. Eng. Microbiol. 1, 100003 https://doi.org/10.1016/j.engmic.2021.100003.

Liu, P., Zheng, Y., Yuan, Y., Zhang, T., Li, Q., Liang, Q., Su, T., Qi, Q., 2022. Valorization of polyethylene terephthalate to muconic acid by engineering *Pseudomonas Putida*. Int J. Mol. Sci. 23 (19) https://doi.org/10.3390/ijms231910997.

Maleki Rad, M., Moghimi, H., Azin, E., 2022. Biodegradation of thermo-oxidative pretreated low-density polyethylene (LDPE) and polyvinyl chloride (PVC) microplastics by *Achromobacter denitrificans* Ebl13. Mar. Pollut. Bull. 181, 113830 https://doi.org/10.1016/j.marpolbul.2022.113830.

Millican, J.M., Agarwal, S., 2021. Plastic pollution: a material problem. Macromolecules 54 (10), 4455–4469. https://doi.org/10.1021/acs.macromol.0c02814.

Mohanan, N., Montazer, Z., Sharma, P.K., Levin, D.B., 2020. Microbial and enzymatic degradation of synthetic plastics. Front Microbiol 11, 580709. https://doi.org/10.3389/fmicb.2020.580709.

Montazer, Z., Habibi-Najafi, M.B., Mohebbi, M., Oromiehei, A., 2018. Microbial degradation of UV-pretreated low-density polyethylene films by novel polyethylene-degrading bacteria isolated from plastic-dump soil. J. Polym. Environ. 26 (9), 3613–3625. https://doi.org/10.1007/s10924-018-1245-0.

Morohoshi, T., Oi, T., Aiso, H., Suzuki, T., Okura, T., Sato, S., 2018. Biofilm formation and degradation of commercially available biodegradable plastic films by bacterial consortiums in freshwater environments. Microbes Environ. 33 (3), 332–335. https://doi.org/10.1264/jsme2.ME18033.

Mukherjee, S., Kundu, P.P., 2014. Alkaline fungal degradation of oxidized polyethylene in black liquor: Studies on the effect of lignin peroxidases and manganese peroxidases. Journal of Applied Polymer Science 131(17), n/a-n/a. https://doi.org/10.1002/app.40738.

Muthukumar, B., Parthipan, P., AlSalhi, M.S., Prabhu, N.S., Rao, T.N., Devanesan, S., Maruthamuthu, M.K., Rajasekar, A., 2022. Characterization of bacterial community in oil-contaminated soil and its biodegradation efficiency of high molecular weight (>C40) hydrocarbon. Chemosphere 289, 133168. https://doi.org/10.1016/j.chemosphere.2021.133168.

Ren, L., Men, L., Zhang, Z., Guan, F., Tian, J., Wang, B., Wang, J., Zhang, Y., Zhang, W., 2019. Biodegradation of Polyethylene by *Enterobacter* sp. D1 from the Guts of Wax Moth Galleria mellonella. Int J. Environ. Res Public Health 16 (11). https://doi.org/10.3390/ijerph16111941.

Roberts, C., Edwards, S., Vague, M., Leon-Zayas, R., Scheffer, H., Chan, G., Swartz, N.A., Mellies, J.L., 2020. Environmental consortium containing pseudomonas and bacillus species synergistically degrades polyethylene terephthalate plastic. mSphere 5 (6). https://doi.org/10.1128/mSphere.01151-20.

Saier ., M.H., Jr, Reddy, V.S., Tamang, D.G., Vastermark, A., 2014. The transporter classification database. Nucleic Acids Res 42(Database Issue) 251–258. https://doi.org/10.1093/nar/gkt1097.

Sandt, C., Waeytens, J., Deniset-Besseau, A., Nielsen-Leroux, C., Rejasse, A., 2021. Use and misuse of FTIR spectroscopy for studying the bio-oxidation of plastics. Spectrochim. Acta A Mol. Biomol. Spectrosc. 258, 119841 https://doi.org/10.1016/j.saa.2021.119841.

Santo, M., Weitsman, R., Sivan, A., 2013. The role of the copper-binding enzyme – laccase – in the biodegradation of polyethylene by the actinomycete *Rhodococcus ruber*. Int. Biodeterior. Biodegrad. 84, 204–210. https://doi.org/10.1016/j.ibiod.2012.03.001.

Sarker, R.K., Chakraborty, P., Sarkar, S., Ghosh, M.M., Tribedi, P., 2021. Bioaugmentation of *Enterobacter* cloacae AKS7 causes an enhanced degradation of low-density polyethylene (LDPE) in soil: a promising approach for the sustainable management of LDPE waste. Arch. Microbiol 204 (1), 74. https://doi.org/10.1007/s00203-021-02645-4.

Sowmya, H.V., Ramalingappa, Krishnappa, M., Thippeswamy, B., 2014. Degradation of polyethylene by *Penicillium simplicissimum* isolated from local dumpsite of Shivamogga district. Environ., Dev. Sustain. 17 (4), 731–745. https://doi.org/10.1007/s10668-014-9571-4.

Su, T., Guo, Q., Zheng, Y., Chang, Y., Gu, F., Lu, X., Qi, Q., 2021. Base editing-coupled survival screening enabled high-sensitive analysis of PAM compatibility and finding of the new possible off-target. iScience 24 (7), 102769. https://doi.org/10.1016/j.isci.2021.102769.

Sulaiman, S., Yamato, S., Kanaya, E., Kim, J.J., Koga, Y., Takano, K., Kanaya, S., 2012. Isolation of a novel cutinase homolog with polyethylene terephthalate-degrading activity from leaf-branch compost by using a metagenomic approach. Appl. Environ. Microbiol 78 (5), 1556–1562. https://doi.org/10.1128/AEM.06725-11.

Syranidou, E., Karkanorachaki, K., Barouta, D., Papadaki, E., Moschovas, D., Avgeropoulos, A., Kalogerakis, N., 2023. Relationship between the carbonyl index (CI) and fragmentation of polyolefin plastics during aging. Environ. Sci. Technol. 57 (21), 8130–8138. https://doi.org/10.1021/acs.est.3c01430.

Tribedi, P., Dey, S., 2017. Pre-oxidation of low-density polyethylene (LDPE) by ultraviolet light (UV) promotes enhanced degradation of LDPE in soil. Environ. Monit. Assess. 189 (12), 624. https://doi.org/10.1007/s10661-017-6351-2.

Ullah, M., Weng, C.H., Li, H., Sun, S.W., Zhang, H., Song, A.H., Zhu, H., 2018. Degradation of polyvinyl alcohol by a novel bacterial strain Stenotrophomonas sp. SA21, 2056-SA2061 Environ. Technol. 39 (16). https://doi.org/10.1080/09593330.2017.1349189.

Yan B, S.C., Beckham, G.T., Chen, E.Y.-X., Román-Leshkov, Y., 2021. Breaking C-C bonds via electrochemically mediated hydrogen atom transfer reactions. ChemRxiv. https://doi.org/10.26434/chemrxiv.14525619.v1.

Yang, J., Yang, Y., Wu, W.M., Zhao, J., Jiang, L., 2014. Evidence of polyethylene biodegradation by bacterial strains from the guts of plastic-eating waxworms. Environ. Sci. Technol. 48 (23), 13776–13784. https://doi.org/10.1021/es504038a.

Yeom, S.J., Le, T.K., Yun, C.H., 2022. P450-driven plastic-degrading synthetic bacteria. Trends Biotechnol. 40 (2), 166–179. https://doi.org/10.1016/j.tibtech.2021.06.003.

Yoshida, S., Hiraga, K., Takehana, T., Taniguchi, I., Yamaji, H., Maeda, Y., Toyohara, K., Miyamoto, K., Kimura, Y., Oda, K., 2016. A bacterium that degrades and assimilates poly(ethylene terephthalate. Science 351 (6278), 1196–1199. https://doi.org/10.1126/science.aad6359.

Zhang, J., Gao, D., Li, Q., Zhao, Y., Li, L., Lin, H., Bi, Q., Zhao, Y., 2020. Biodegradation of polyethylene microplastic particles by the fungus *Aspergillus flavus* from the guts of wax moth Galleria mellonella. Sci. Total Environ. 704, 135931 https://doi.org/10.1016/j.scitotenv.2019.135931.

Zhang, N., Ding, M., Yuan, Y., 2022. Current advances in biodegradation of polyolefins. Microorganisms 10 (8), 1537. https://doi.org/10.3390/microorganisms10081537.

Zhang, P., You, Z., Chen, T., Zhao, L., Zhu, J., Shi, W., Meng, Q., Sun, Y., 2022. Study on the breeding and characterization of high-efficiency oil-degrading bacteria by mutagenesis. Water 14 (16), 2544. https://doi.org/10.3390/w14162544.