ISSN: 1648-6897 / eISSN: 1822-4199

VILNIUS TECH
Vilnius Gediminas Technical University

JOURNAL of ENVIRONMENTAL ENGINEERING & LANDSCAPE MANAGEMENT

2024
Volume 32
Issue 2
Pages 85–92
https://doi.org/10.3846/jeelm.2024.20966

A POLYPROPYLENE-DEGRADING *PSYCHROBACILLUS* STRAIN ISOLATED FROM A LANDFILL

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Highlights:

- a novel function of *Psychrobacillus* sp.: polypropylene degradation;
- the weight loss of polypropylene particles is 9±0.40%;
- speculate the possible enzymes involved in the polypropylene degradation.

Article History:

- received 01 December 2022
- accepted 05 December 2023

Abstract. Polypropylene (PP) is one of the most widely used plastics around the world. However, PP is recalcitrant to degradation under natural conditions, and its accumulation is increasingly threatening the environment. The stain LICME-ZWZR-10 was isolated from a landfill using PP as its sole carbon source. It was found to share 99.50% genetic similarity with *Psychrobacillus* sp. AK 1817. Upon incubation with *Psychrobacillus* sp. LICME-ZWZR-10, PP particles developed a rough surface with depressions and cracks, which were discerned through scanning electron microscopy (SEM). At a moderate temperature of 20 °C, this strain successfully degraded PP particles with an average diameter of 850 μm, leading to a 9±0.40% reduction in particle weight over a span of 30 days. Fourier transform infrared spectroscopy (FTIR) released the emergence of carbonyl and ether-based functional groups on PP. Furthermore, genomic analysis unveiled the presence of a laccase-encoding gene in *Psychrobacillus* sp. LICME-ZWZR-10, suggesting its potential involvement in the biodegradation of PP.

Keywords: polypropylene, *Psychrobacillus* sp., biodegradation, genomic analysis.

Online supplementary material: Supporting information for this paper is available as online supplementary material at https://doi.org/10.3846/jeelm.2024.20966

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1. Introduction

Due to their extraordinary versatility and low price, plastics are widely used in all aspects of our daily life. The simple polyolefins polyethylene (PE) and polypropylene (PP) account for almost half of the plastics produced globally (Canopoli et al., 2020). However, they are comparatively difficult to be degraded because of the high molecular weight, strong hydrophobicity, high chemical bond energy and low bioavailability (Kalčíková, 2020; Lozano & Rillig, 2020). Thus, polyolefins can persist in the environment for decades or even hundreds of years, with possibly deleterious effects on the biosphere (Carbery et al., 2018; Kedzierski et al., 2020). Conventional plastic disposal methods include incineration and landfilling (Zhang et al., 2021), but these approaches generate large amounts of greenhouse gases or occupy large areas of land. Therefore, various advanced plastic treatment technologies have been developed, such as photodegradation (Delre et al., 2023), thermal degradation (Chen et al., 2023) and biodegradation (Thew et al., 2023). Biodegradation of plastics, which uses live cells or enzymes, is environmentally friendly (Zhang et al., 2021).

Since the early 1970s, researchers have been engaged in studying the biodegradation of polyolefins and found certain microorganisms, such as *Aspergillus variegatus*, *Penicillium xanthophyllum* and *Penicillium oxalicum*, which exhibit degradation effects on high-density polyethylene or low-density polyethylene (Zhang et al., 2022a). In addition, microbes such as *Bacillus*, *Pseudomonas*, *Staphylococcus*, and *Streptococcus* also showed the ability to degrade PE (HDPE and LDPE) (Zhang et al., 2022b).

PP is the second most abundant plastics, with the global demand of 87.35 million tons predicted in 2022 (Bai et al., 2019). PP is a typical plastic with a carbon-carbon skeleton (Ghatge et al., 2020). Its recalcitrance to biodegradation can be attributed to three main factor: 1) Its chemical structure is stable, with chemical groups that cannot easily be oxidized or hydrolyzed; 2) Its molecular size is too large to enter any cell, so extracellular enzymes are be involved in the early stage of PP degradation; 3) It is highly hydrophobic, which makes it difficult for cells and enzymes to contact and react with it, also contributing to the difficulty of PP biodegradation. A few bacterial isolates of *Bacillus subtilis*, *Bacillus flexus* and *Pseudomonas aeruginosa* isolated from the soil were reported to be able to utilize PP as the only source of carbon (Aravinthan et al., 2016; Wang et al., 2022). Microbiota have also been studied for their ability of PP degradation, which will shorten the lead time (Lim et al., 2022). Recently, several teams attempted to improve the biodegradation efficiency of PP by pretreatments, and UV irradiation or metal ion oxidants were able to facilitate the biodegradation of PP to some extent (Yasin et al., 2022). Although there a number of microorganisms were reported to degrade PP, there are much fewer PP degraders than species capable of degrading other plastics. Therefore, it is necessary to screen new microorganisms for efficient PP biodegradation.

Using PP as the sole carbon source, we isolated a bacteria strain from the soil of the Nanjing waste dump, which was designated as LICME-ZWZR-10. Further 16S rDNA sequencing confirmed its taxonomic classification as *Psychrobacillus* sp., a Gram-positive, rod-shaped bacterium that belongs to the *Bacillus* family. Colonies cultured on Luria-Bertani (LB) appeared round, pale white and yellow. The strain is adapted to proliferate in a relative cold environment, with an optimal growth temperature of 0–20 °C. It has been reported to be involved in the biotransformation of triterpenes and oil degradation (Chiang et al., 2017; Choi et al., 2020). After 30 days of incubation in the minimal medium with PP particles as the sole carbon source, *Psychrobacillus* sp. LICME-ZWZR-10 achieved a weight loss of PP particles equivalent to 9±0.40%. Additionally, we investigated the physical and chemical changes on the surface of PP degraded by *Psychrobacillus* sp. LICME-ZWZR-10. Furthermore, we examined the genome of *Bacillus* sp. FJAT-22090, which shares a 99.50% genetic similarity with LICME-ZWZR-10, to speculate on the potential enzymes involved in the PP degradation process.

2. Materials and methods

2.1. Isolation and identification of the PP-degrading strain

Soil samples were collected from the campus dump of Nanjing Normal University, and dissolved with sterile 0.9% NaCl, and placed into 250 mL Erlenmeyer flasks containing 100 mL minimum salts medium (MSM), with PP as the only carbon source, to screen microorganisms utilizing PP at 30 °C, 220 rpm for 15 days.

The minimal medium was prepared as follows: K_2HPO_4 0.5 g/L, KH_2PO_4 0.04 g/L, NaCl 0.1 g/L, $CaCl_2.2H_2O$ 0.002 g/L, $(NH_4)_2SO_4$ 0.2 g/L, $MgSO_4.7H_2O$ 0.02 g/L, $FeSO_4$ 0.001 g/L. PP particles without any additives (CAS 9003–07-0, white, d = 856.1 ± 69.7 μm, Mn = 10 000 ± 500 Da, ρ = 0.9 g·cm^{-3}) were purchased from MACKLIN (Shanghai), washed with 75% ethanol solution, and sterilized under the UV lamp (20 W) of an ultra-clean bench for 12 h.

After 30 days, some flasks became turbid, and the suspensions were spread on LB agar plates for culture at 30 °C for 24 h. Individual colonies were picked and passaged at LB agar plates three times to obtain pure colonies, which were further verified for their ability to utilize PP in MSM as the sole carbon source, resulting in strain LICME-ZWZR-10.

The 16S rRNA gene of the strain was amplified using the universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-AAGGAGGTGATCCAGCCCGCA-3′), and searched against the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST).

3. Degradation of PP by the isolated strain

3.1. Monitoring the bacteria growing in MSM with pure PP particles

Inoculum development was conducted in LB medium, after which the cells of LICME-ZWZR-10 were collected by centrifugation at 3075 g, washed with sterile 0.9% NaCl three times to remove residual LB medium, resuspended in the same buffer, and used to inoculate MSM with or without 5% (w/v) PP. MSM with PP but without the bacteria was also incubated under the same conditions as a negative control, and three replicates were included for all cultures. All the groups were incubated at 20 °C, 220 rpm for 30 days, during which the OD_{600} was measured every week using a UV-1800PC spectrophotometer (BioTek Corp., Shanghai).

3.2. Visualization of the PP surface by scanning electron microscopy and atomic force microscopy

To visualize the bacteria adhering to the PP surface, the PP particles treated with the PP-degrading strain were carefully washed with distilled water 3 times to remove the loosely attached bacteria, then soaked in 2.5% glutaraldehyde for 12 h at room temperature to fix the bacteria to the surface of PP, and gradually dehydrated with 50%, 70% and 100% ethanol. After drying in a 65 °C oven, all the samples were fixed onto copper plates and sputtered with gold at 15 mA using an automatic sputtering coater (JFC1600) to increase the conductivity, to enable observation by SEM (JSM-5610LV, JEOL, Japan) at a voltage of 3.0 kV. For the PP surfaces analysis, the PP particles incubated with or without the PP-degrading strain were

washed three times with 75% ethanol, soaked for 12 h in a 10% sodium dodecyl sulfate (SDS) solution to remove any bacteria cells, and washed three times with distilled water. After drying in a 65 °C oven, the changes of the PP surface were observed by AFM (Dimension Icon, Veeco, Billerica, MA, USA) at a scanning speed of 1.0 Hz, as well as by SEM as described above.

3.3. Weight loss of PP particles

The initial weight of PP particles was measured. After 30 days of cultivation, all the PP samples were collected by vacuum filtration through a polytetrafluoroethylene (PTFE, 0.22 μm) filter membrane. A small portion of samples adhering to the wall of the shaking flask was rinsed with distilled water and then filtered. The collected PP particles were cleaned according to method 2.2. After drying, the weight of the residual PP particles was measured, and the weight loss calculated using the formula:

$$\text{weight loss}(\%) = \frac{\text{initial PP weight} - \text{residual PP weight}}{\text{initial PP weight}} \times 100\%.$$

3.4. Analyzing the chemical groups on the PP surface by Fourier-Transform Infrared Spectroscopy

The PP particles cleaned as in 2.2 were analyzed by Fourier Transform Infrared Spectroscopy (FTIR) (Vertex 70, Bruck, Germany). The absorbance in the mid-infrared region of 4000–400 cm^{-1} was measured with a scanning resolution of 4 cm^{-1}. The background absorbance was measured and subtracted from the tested samples.

3.5. Gel permeation chromatography (GPC) analysis

Samples were dissolved in 5 ml of 1,2,4-Trichlorobenzene. The molecular weights were determined by PL-GPC220 at 140 °C using 1,2,4-Trichlorobenzene as mobile phase.

4. Genome analysis

In order to investigate the mechanism of PP degradation by *Psychrobacillus* sp. LICME-ZWZR-10, the genome of *Bacillus* sp. FJAT-22090, with a similarity of 99.5% to LICME-ZWZR-10 was analyzed. Sequence alignment was performed with the enzymes degrading polyolefin plastics and the proteins encoded by the genome of *Bacillus* sp. FJAT-22090 to speculate on the possible enzymes degrading PP in *Psychrobacillus* sp. LICME-ZWZR-10.

5. Statistical analyses

All the experiments were performed in triplicates and the results presented as a mean value with standard deviation (mean ± SD). The data were analyzed using analysis of variance, and the significance of differences between the means was assessed using student's *t*-test at a significance level of $p < 0.050$. Statistical analysis was conducted using SPSS statistics software version 27.0 (IBM Corp., USA).

6. Results and discussion

6.1. Screening and identification of PP-degrading bacteria

PP particles were sterilized by a UV lamp for 12 h. To exclude any potential influence of the UV treatment, we used FITR to compare the samples with and without UV treatment. The results showed that no discernible changes occurred following UV irradiation (Figure S1). As a result, we employed the UV-treated PP samples for bacterial screening.

When utilizing MSM with PP as the sole carbon source, the appearance of turbidity in one flask signified the presence of proliferating microorganisms. Subsequently, a bacterial strain was isolated and confirmed to utilize PP as the sole carbon source. According to the phylogenetic analysis of the 16S rRNA genes, this strain was classified as *Psychrobacillus* sp., sharing a remarkable genetic similarity of 99.50% with *Psychrobacillus* sp. AK 1817. Accordingly, this strain was designated as *Psychrobacillus* sp. LICME-ZWZR-10 (Figure 1) and has been deposited in the China Center for Type Culture Collection under the accession number CCTCC M 2021013. It is noteworthy that other members of the genus *Psychrobacillus* sp., such as *Psychrobacillus* sp. AK1817 (Pham et al., 2015) and *Psychrobacillus* sp. PL87 (Kato et al., 2019), have demonstrated the capacity to degrade petroleum and poly 3-hydroxybutyrate-co-3-hydroxyhexanoate, respectively. This suggests that certain characteristics of *Psychrobacillus* sp. may be beneficial for the decomposition of long-chain alkanes.

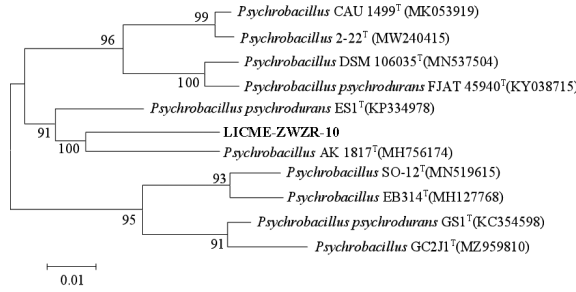

Figure 1. Neighbor-joining (NJ) phylogenetic tree based on 16S rRNA gene sequence showing the phylogenetic position of strain LICME-ZWZR-10 among closely related taxa

6.2. Proliferation of LICME-ZWZR-10 in MSM with PP as the sole source of carbon

When the LICME-ZWZR-10 strain was cultivated in the MSM with PP, the OD_{600} exhibited a continuous increase within 2 weeks, followed by a stabilization during the second to third week, and a notable decline at the 28th day

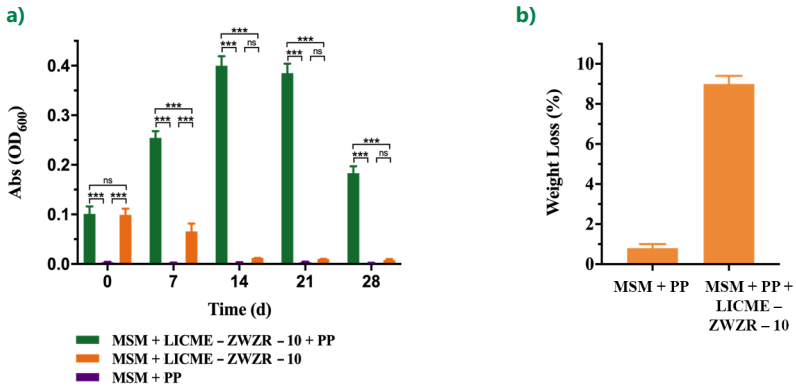

Figure 2. Characterization of PP particles incubated with LICME-ZWZR-10 for 30 days: a) The OD_{600} of bacterial culture with PP particles and statistical analysis of differences in OD_{600}; b) The weight loss percentage of PP particles incubated with or without LICME-ZWZR-10. All values represent mean ± SD (n = 3). Significance (Student's *t*-tests) is indicated by asterisks: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$; ns – no statistical significance

(Figure 2a). There was no increase in the OD_{600} of the sterile MSM with PP, or that of MSM inoculated with LICME-ZWZR-10 but without PP, indicating that increase in the OD_{600} of MSM with PP and LICME-ZWZR-10 was due to utilization of PP by the strain. In a previous report (Auta et al., 2018), the OD_{600} of *Bacillus* sp. strain 36 also showed a significant decline in the initial period of cultivation with PP as the sole carbon source. This may be due to the nutrient deficiency of MSM culture medium and the inability of microorganisms to fully adapt to the culture conditions. However, the growth curve of LICME-ZWZR-10 still differed to some extent from that of reported PP degrading bacteria (Auta et al., 2017; Habib et al., 2020; Jeon et al., 2021), which might be related to the characteristics of the bacterial species themselves, such as the substrate colonization pathways and their abilities to adapt to the environment (Ru et al., 2020). Indeed, the weight of the degraded PP decreased by 9±0.4% (Figure 2c). This weight loss can be directly attributed to the proliferation of LICME-ZWZR-10, as shown in Figure 2a. Compared with the previously reported PP-degrading bacteria, the degradation efficacy of LICME-ZWZR-10 was outstanding. It should be noted that various composting methods and intense pre-treatment can promote microbial degradation behavior (Jeon & Kim, 2016; Skariyachan et al., 2018). However, we used a simple MSM medium and there was no intense pre-treatment of PP in this study. The structure of PP includes crystalline and amorphous regions (Várdai et al., 2022). Biodegradation first occurs in the amorphous part (Canopoli et al., 2020). Thus, the partial weight loss might be attributed to the preferential consumption of the amorphous zones of PP, leaving the highly crystalline zones mostly intact.

6.3. Surface topography analysis of degraded PP particles by SEM and AFM

Surface morphological alterations on the PP particles were visualized by SEM and AFM. The biodegradation process of polymers can be divided into four steps: biodeterioration, depolymerization, bioassimilation, and mineralization (Lucas et al., 2008; Zhang et al., 2021). Among these stages, the initial formation of biofilms by microorganisms on the surface of polymers marks the onset of biodeterioration. As expected, the LICME-ZWZR-10 strain adhered to the PP surface, which was demonstrated through SEM imaging (Figure 3c). Since PP plastic is stable, PP particles were almost completely smooth under SEM after incubation in MSM without bacteria for one month (Figure 3a). By contrast, incubation with LICME-ZWZR-10 in MSM for 30 days made the PP particles rough with visible cracks (Figure 3b), as the distinct consequence of degradation. The surface of the plastic showed similar aging phenomena to those observed in previous reports (Canopoli et al., 2020), including cracking and peeling (Sawpan et al., 2019; Tang et al., 2019; Wróbel et al., 2023). The formation of cracks originates from voids, which are caused by changes in specific volume during the crystallization of PP (Craig et al., 2005; Nakatani et al., 2022). Microorganisms secrete a series of extracellular enzymes that act on the surface of the plastic

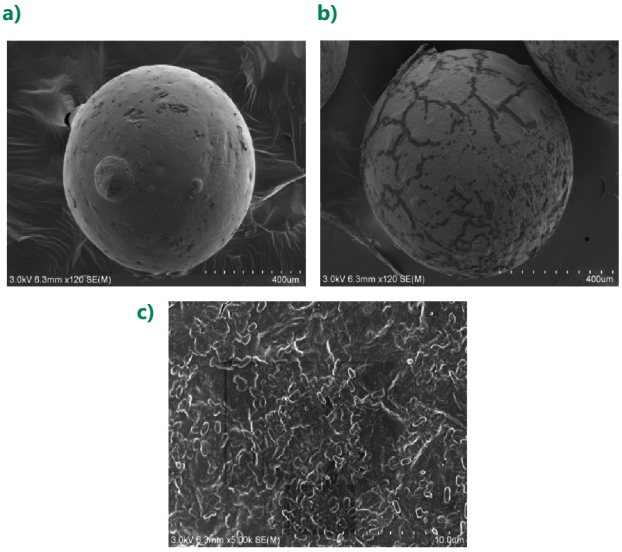

Figure 3. SEM images of PP incubated with or without LICME-ZWZR-10: a) The control group without LICME-ZWZR-10 bacteria; b) The experimental group with LICME-ZWZR-10 bacteria; c) Cells of strain LICME-ZWZR-10 on the PP surface after 30 days of cultivation

to increase its hydrophilicity and break down the polymer molecules into short chains, which then enter the cell to be metabolized and completely mineralized. The continued action of the enzymes and cells eventually resulted in erosion marks. In a recent study by Faiza et al. (2021), the surface of composites showed similar cracks and erosion after treatment with the plant-derived proteases papain and bromelain. At the same time, the AFM results showed that the surface of PP particles incubated with bacteria showed obvious depression (Figure S2b), which were not obserced in the control group (Figure S2a), consistent with the SEM results.

6.4. Formation of oxidized functional groups

To further characterize the degradation of PP by LICME-ZWZR-10, we analyzed the chemical groups of PP by using FTIR. While, pure PP only contains C-C and C-H bonds, biodegradation leads to reactions such as oxidation, reduction, esterification or hydrolysis, potentially leading to the formation of new chemical groups such as C-O, C=O or O-H (Canopoli et al., 2020; Ren et al., 2019). Indeed, the FTIR spectrum of PP treated with LICME-ZWZR-10 exhibited significant differences from that of untreated PP. New absorption peaks appeared at 1654 cm^{-1} and 1079 cm^{-1}, which were attributed to carbonyl (-C=O) and ether groups (-C-O-C-), respectively (Figure 4). The emergence of carbonyl groups is an important indicator of the initiation of degradation (Pires et al., 2019). The formation of CO groups within the main chain of PP enhances the hydrophilicity of the polymer, facilitating interactions with degrading microorganisms and enzymes (Rana et al., 2022). The presence of CO groups is in agreement with the results of previous studies on PP-degrading bacteria (Devi et al., 2021; Sun et al., 2023). The appearance of two new oxygen-containing groups indicates that PP was indeed oxidized, which was induced by the activities of LICME-ZWZR-10. Additionally, all the samples exhibited strong absorbance at 728 cm^{-1}, 843 cm^{-1} and 1155 cm^{-1}, which was attributed to the C-H bending vibration in the main chain of PP.

6.5. Gel permeation chromatography analysis of the molecular weight of degraded PP particles

To investigate if the molecular weight of PP also changed after degradation by LICME-ZWZR-10, the average molecular weight (Mn) and polydispersity index (PDI) values were measured by gel permeation chromatography (GPC). The Mn value increased from 841 to 1092 after 15 days of degradation (Table 1). This was consistent with the general degradation pattern of polyolefins, where the short molecular chains are degraded first, leading to an increase in overall molecular weight (Liu et al., 2022; Nakatani et al., 2022; Yang et al., 2022). The DPI value represents the degree of variance, and its increase indicates that there were more oligomers and monomers with various molecular weights in the early stage of degradation (Cho et al., 2022).

Table 1. Molecular weight of degraded PP analyzed by GPC

Samples	M_n	DPI
PP incubated in MSM without bacteria	841	3.08
PP incubated in MSM with LICME-ZWZR-10	1092	3.29

Note: M_n – number-average molecular weight; DPI – polydispersity index.

6.6. Evaluation of possible enzymes involved in PP degradation

There are few reports on the enzymes involved in PP degradation. To speculate on the possible enzymes of *Psychrobacillus* sp. LICME-ZWZR-10 responsible for PP degradation, the annotated genome of *Bacillus* sp. FJAT-22090 from NCBI was analyzed, as it shares a remarkable 99.5% genetic similarity with *Psychrobacillus* sp. LICME-ZWZR-10. The genome of *Bacillus* sp. FJAT-22090 contained 5.16576 Mb with a GC content of 37.15%. It was predicted to encode 4957 proteins, which included 51 oxidoreductases. The primary structure of PP closely resembles that of PE, both characterized by a typical C-C skeleton. Therefore, the enzymes reported to degrade PE were compared

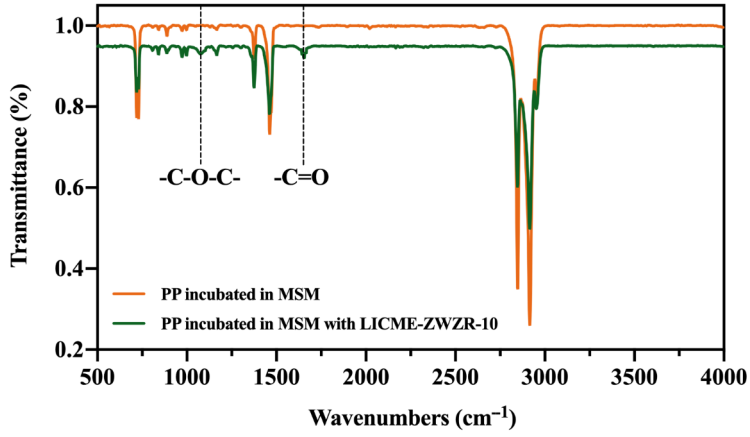

Figure 4. Distribution of carbonyl groups (-C=O, 1658 cm^{-1}) and ether groups (-C-O-C-, 1079 cm^{-1}) on the surface PP incubated with strain LICME-ZWZR-10 for 30 days

with candidate proteins encoded by the genome of *Bacillus* sp. FJAT-22090. Two enzymes are considered to potentially have similar function if the similarity of their amino acid sequence is above 24% (Guo et al., 2020). The comparison results showed that a laccase, with 27.43% similarity to the PE-degrading laccase from *Rhodococcus ruber* (Santo et al., 2013), might be critical for the degradation of PP by *Psychrobacillus* sp. LICME-ZWZR-10 (Table S1). Laccases are copper-containing polyphenol oxidases with a high oxidizing capacity for lignocellulose and other polymers. Notably, a laccase was reported to oxidize kenaf fibre resulting in a significant increase of its wetting and adhesion properties (Islam et al., 2013). Utilizing atmospheric dioxygen as the oxidizing agent, laccase initiates an attack on PE molecules, resulting in the generation of oxygen-containing functional groups such as carbonyl, hydroxyl and ether bonds (Kowalczyk et al., 2017; Ren et al., 2019). The four steps of biodegradation, fragmentation, assimilation and mineralization are accomplished through the action of multiple enzymes and biosurfactants secreted by microorganisms. The degradation process of PP by LICME-ZWZR-10 follows a similar pattern. LICME-ZWZR-10 is likely to secret biosurfactants, which assist in the adhesion and colonization of the bacteria on the PP surface, facilitating the formation of a biofilm (Figure 3c). Subsequently, oxygen-containing groups (Figure 4) are introduced through the catalytic action of the extracellular enzymes, which contributes to the decomposition of PP into smaller molecules. Finally, the smaller molecules can enter the bacterial cell to be metabolized and finally fully mineralized. However, the enzymes that take part in the degradation process need to be further studied in the future.

7. Conclusions

In this work, we screened the PP-degrading strain of *Psychrobacillus* sp. LICME-ZWZR-10 from a landfill. It showed the ability to degrade PP at a moderate temperature of 20 °C, and the weight loss of PP particles reached 9±0.40% after 30 days of incubation, which was outstanding compared to previous studies. Notably, the PP particles degraded in this study showed high weight loss without intense pretreatment which was accompanied by the appearance of clearly visible cracks and new oxygen-containing functional groups. Degradation of PP is a new function of *Psychrobacillus* sp., which were previously only known to degrade petroleum and poly 3-hydroxybutyrate-co-3-hydroxyhexanoate. Finally, we identified a laccase gene in the genome of a closely related strain, which may be responsible for PP degradation. As there are remarkably few reports on PP-degrading enzymes, the identification of the genes encoding PP-degrading enzymes in *Psychrobacillus* sp. is an important direction for our future research.

Author contributions

JDZ, YHL and PLW performed the experiments, LHZ, YHL and ZDZ analyzed the data, JDZ, YHL and LHZ wrote the paper, ZDZ, LHZ and YHL revised the paper, LHZ supervised the study.

Funding

This work was financially supported by the National Key Research and Development Program of China (2019YFA0706900), the Natural Science Fund for Colleges and Universities in Jiangsu Province (22KJB530008) and Natural Science Funds of Xinjiang for Distinguished Young Scholar (2022D01E19). The authors have no relevant financial or non-financial interests to disclose. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that they have no conflicts of interest related to the publication of this paper.

References

Aravinthan, A., Arkatkar, A., Juwarkar, A. A., & Doble, M. (2016). Synergistic growth of *Bacillus* and *Pseudomonas* and its degradation potential on pretreated polypropylene. *Preparative Biochemistry & Biotechnology*, *46*(2), 109–115. https://doi.org/10.1080/10826068.2014.985836

Auta, H. S., Emenike, C. U., & Fauziah, S. H. (2017). Screening of *Bacillus* strains isolated from mangrove ecosystems in Peninsular Malaysia for microplastic degradation. *Environmental Pollution*, *231*, 1552–1559. https://doi.org/10.1016/j.envpol.2017.09.043

Auta, H. S., Emenike, C. U., Jayanthi, B., & Fauziah, S. H. (2018). Growth kinetics and biodeterioration of polypropylene microplastics by *Bacillus* sp. and *Rhodococcus* sp. isolated from mangrove sediment. *Marine Pollution Bulletin*, *127*, 15–21. https://doi.org/10.1016/j.marpolbul.2017.11.036

Bai, B., Jin, H., Fan, C., Cao, C., Wei, W., & Cao, W. (2019). Experimental investigation on liquefaction of plastic waste to oil in supercritical water. *Waste Management*, *89*, 247–253. https://doi.org/10.1016/j.wasman.2019.04.017

Canopoli, L., Coulon, F., & Wagland, S. T. (2020). Degradation of excavated polyethylene and polypropylene waste from landfill. *Science of the Total Environment*, *698*, Article 134125. https://doi.org/10.1016/j.scitotenv.2019.134125

Carbery, M., O'Connor, W., & Palanisami, T. (2018). Trophic transfer of microplastics and mixed contaminants in the marine food web and implications for human health. *Environment International*, *115*, 400–409. https://doi.org/10.1016/j.envint.2018.03.007

Chen, W. H., Sun, S. C., Ocreto, J. B., Nguyen, T. B., Lam, S. S., Park, Y. K., & Dong, C. D. (2023). Thermodegradation characterization of microplastics: Dispersion effect and pyrolysis kinetics by artificial intelligence. *Chemical Engineering Journal*, *457*, Article 141285. https://doi.org/10.1016/j.cej.2023.141285

Chiang, C. M., Wang, T. Y., Ke, A. N., Chang, T. S., & Wu, J. Y. (2017). Biotransformation of ergostane triterpenoid antcin K

from *Antrodia cinnamomea* by soil-isolated *Psychrobacillus* sp. AK 1817. *Catalysts*, *7*(10), Article 299. https://doi.org/10.3390/catal7100299

Cho, J. Y., Park, S. L., Kim, S. H., Jung, H. J., Cho, D., Kim, B. C., Bhatia, S. K., Gurav, R., Park, S. H., Park, K., & Yang, Y. H. (2022). Novel Poly(butylene adipate-co-terephthalate)-degrading *Bacillus* sp. JY35 from wastewater sludge and its broad degradation of various bioplastics. *Waste Management*, *144*, 1–10. https://doi.org/10.1016/j.wasman.2022.03.003

Choi, J. Y., Kim, S. C., & Lee, P. C. (2020). Comparative genome analysis of *Psychrobacillus* strain PB01, isolated from an iceberg. *Journal of Microbiology and Biotechnology*, *30*(2), 237–243. https://doi.org/10.4014/jmb.1909.09008

Craig, I. H., White, J. R., & Kin, P. C. (2005). Crystallization and chemi-crystallization of recycled photo-degraded polypropylene. *Polymer*, *46*(2), 505–512. https://doi.org/10.1016/j.polymer.2004.11.019

Delre, A., Goudriaan, M., Morales, V. H., Vaksmaa, A., Ndhlovu, R. T., Baas, M., Keijzer, E., de Groot, T., Zeghal, E., Egger, M., Röckmann, T., & Niemann, H. (2023). Plastic photodegradation under simulated marine conditions. *Marine Pollution Bulletin*, *187*, Article 114544. https://doi.org/10.1016/j.marpolbul.2022.114544

Devi, K. N., Raju, P., Santhanam, P., Kumar, S. D., Krishnaveni, N., Roopavathy, J., & Perumal, P. (2021). Biodegradation of low-density polyethylene and polypropylene by microbes isolated from Vaigai River, Madurai, India. *Archives of Microbiology*, *203*(10), 6253–6265. https://doi.org/10.1007/s00203-021-02592-0

Faiza, W., Firouzi, A., Islam, M. R., Sumdani, G., & Taher, A. (2021). Degradation analysis of epoxy resin composites reinforced with bioprotein: Effects of hydrolysis using papain and bromelain. *Polymer Composites*, *42*(6), 2717–2727. https://doi.org/10.1002/pc.26007

Ghatge, S., Yang, Y., Ahn, J.-H., & Hur, H.-G. (2020). Biodegradation of polyethylene: A brief review. *Applied Biological Chemistry*, *63*(1), Article 27. https://doi.org/10.1186/s13765-020-00511-3

Guo, Y., Zhou, J., Tang, Y., Ma, Q., Zhang, J., Ji, C., & Zhao, L. (2020). Characterization and genome analysis of a zearalenone-degrading *Bacillus velezensis* strain ANSB01E. *Current Microbiology*, *77*(2), 273–278. https://doi.org/10.1007/s00284-019-01811-8

Habib, S., Iruthayam, A., Abd Shukor, M. Y., Alias, S. A., Smykla, J., & Yasid, N. A. (2020). Biodeterioration of untreated polypropylene microplastic particles by Antarctic bacteria. *Polymers*, *12*(11), Article 2616. https://doi.org/10.3390/polym12112616

Islam, M. R., Beg, M. D. H., & Gupta, A. (2013). Characterization of laccase-treated kenaf fibre reinforced recycled polypropylene composites. *Bioresources*, *8*(3), 3753–3770. https://doi.org/10.15376/biores.8.3.3753-3770

Jeon, H. J., & Kim, M. N. (2016). Isolation of mesophilic bacterium for biodegradation of polypropylene. *International Biodeterioration & Biodegradation*, *115*, 244–249. https://doi.org/10.1016/j.ibiod.2016.08.025

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. *Polymer Degradation and Stability*, *191*, Article 109662. https://doi.org/10.1016/j.polymdegradstab.2021.109662

Kalčíková, G. (2020). Aquatic vascular plants – A forgotten piece of nature in microplastic research. *Environmental Pollution*, *262*, Article 114354. https://doi.org/10.1016/j.envpol.2020.114354

Kato, C., Honma, A., Sato, S., Okura, T., Fukuda, R., & Nogi, Y. (2019). Poly 3-hydroxybutyrate-*co*-3-hydroxyhexanoate films can be degraded by the deep-sea microbes at high pressure and low temperature conditions. *High Pressure Research*, *39*(2), 248–257. https://doi.org/10.1080/08957959.2019.1584196

Kedzierski, M., Lechat, B., Sire, O., Le Maguer, G., Le Tilly, V., & Bruzaud, S. (2020). Microplastic contamination of packaged meat: Occurrence and associated risks. *Food Packaging and Shelf Life*, *24*, Article 100489. https://doi.org/10.1016/j.fpsl.2020.100489

Kowalczyk, A., Chyc, M., Ryszka, P., & Latowski, D. (2017). Erratum to: *Achromobacter xylosoxidans* as a new microorganism strain colonizing high-density polyethylene as a key step to its biodegradation. *Environmental Science and Pollution Research*, *24*(6), Article 5985. https://doi.org/10.1007/s11356-016-8267-8

Lim, B. K. H., & San Thian, E. (2022). Biodegradation of polymers in managing plastic waste – A review. *Science of the Total Environment*, *813*, Article 151880. https://doi.org/10.1016/j.scitotenv.2021.151880

Liu, X. R., Zhang, Y. M., Sun, Q. F., Liu, Z. H., Zhao, Y. L., Fan, A. L., & Su, H. J. (2022). Rapid colonization and biodegradation of untreated commercial polyethylene wrap by a new strain of *Bacillus velezensis* C5. *Journal of Environmental Management*, *301*, Article 113848. https://doi.org/10.1016/j.jenvman.2021.113848

Lozano, Y. M., & Rillig, M. C. (2020). Effects of microplastic fibers and drought on plant communities. *Environmental Science & Technology*, *54*(10), 6166–6173. https://doi.org/10.1021/acs.est.0c01051

Lucas, N., Bienaime, C., Belloy, C., Queneudec, M., Silvestre, F., & Nava-Saucedo, J. E. (2008). Polymer biodegradation: Mechanisms and estimation techniques. *Chemosphere, 73*(4), 429–442. https://doi.org/10.1016/j.chemosphere.2008.06.064

Nakatani, H., Ohshima, Y., Uchiyama, T., & Suguru, M. (2022). Degradation and fragmentation behavior of polypropylene and polystyrene in water. *Scientific Reports*, *12*(1), Article 18501. https://doi.org/10.1038/s41598-022-23435-y

Pham, V. H. T., Jeong, S. W., & Kim, J. (2015). *Psychrobacillus soli* sp. nov., capable of degrading oil, isolated from oil-contaminated soil. *International Journal of Systematic and Evolutionary Microbiology*, *65*, 3046–3052. https://doi.org/10.1099/ijs.0.000375

Pires, J. P., Miranda, G. M., de Souza, G. L., Fraga, F., Ramos, A. D., de Araujo, G. E., Ligabue, R. A., Azevedo, C. M. N., Lourega, R. V., & de Lima, J. E. A. (2019). Investigation of degradation of polypropylene in soil using an enzymatic additive. *Iranian Polymer Journal*, *28*(12), 1045–1055. https://doi.org/10.1007/s13726-019-00766-8

Rana, A. K., Thakur, M. K., Saini, A. K., Mokhta, S. K., Moradi, O., Rydzkowski, T., Alsanie, W. F., Wang, Q. L., Grammatikos, S., & Thakur, V. K. (2022). Recent developments in microbial degradation of polypropylene: Integrated approaches towards a sustainable environment. *Science of the Total Environment*, *826*, Article 154056. https://doi.org/10.1016/j.scitotenv.2022.154056

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*. *International Journal of Environmental Research and Public Health*, *16*(11), Article 1941. https://doi.org/10.3390/ijerph16111941

Ru, J., Huo, Y., & Yang, Y. (2020). Microbial degradation and valorization of plastic wastes. *Frontiers in Microbiology*, *11*, Article 442. https://doi.org/10.3389/fmicb.2020.00442

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. *International Biodeterioration & Biodegradation*, *84*, 204–210. https://doi.org/10.1016/j.ibiod.2012.03.001

Sawpan, M. A., Islam, M. R., Beg, M. D. H., & Pickering, K. (2019). Effect of accelerated weathering on physico-mechanical properties of polylactide bio-composites. *Journal of Polymers and the Environment*, *27*(5), 942–955. https://doi.org/10.1007/s10924-019-01405-2

Skariyachan, S., Patil, A. A., Shankar, A., Manjunath, M., Bachappanavar, N., & Kiran, S. (2018). Enhanced polymer degradation of polyethylene and polypropylene by novel thermophilic consortia of *Brevibacillus* sps. and *Aneurinibacillus* sp. screened from waste management landfills and sewage treatment plants. *Polymer Degradation and Stability*, *149*, 52–68. https://doi.org/10.1016/j.polymdegradstab.2018.01.018

Sun, Y., Zhang, Y., Hao, X., Zhang, X., Ma, Y., & Niu, Z. (2023). A novel marine bacterium *Exiguobacterium marinum* a-1 isolated from in situ plastisphere for degradation of additive–free polypropylene. *Environmental Pollution*, *336*, Article 122390. https://doi.org/10.1016/j.envpol.2023.122390

Tang, C. C., Chen, H. I., Brimblecombe, P., & Lee, C. L. (2019). Morphology and chemical properties of polypropylene pellets degraded in simulated terrestrial and marine environments. *Marine Pollution Bulletin*, *149*, Article 110626. https://doi.org/10.1016/j.marpolbul.2019.110626

Thew, X. E. C., Lo, S. C., Ramanan, R. N., Tey, B. T., Huy, N. D., & Wei, O. C. (2023). Enhancing plastic biodegradation process: Strategies and opportunities. *Critical Reviews in Biotechnology*, 1–18. https://doi.org/10.1080/07388551.2023.2170861

Várdai, R., Schäffer, A., Ferdinánd, M., Lummerstorfer, T., Jerabek, M., Gahleitner, M., Faludi, G., Móczó, J., & Pukánszky, B. (2022). Crystalline structure and reinforcement in hybrid PP composites. *Journal of Thermal Analysis and Calorimetry*, *147*(1), 145–154. https://doi.org/10.1007/s10973-021-11053-1

Wang, P. L., Zhao, J. D., Ruan, Y. Q., Cai, X. Y., Li, J., Zhang, L. H., & Huang, H. (2022). Degradation of Polypropylene by the *Pseudomonas aeruginosa* strains LICME WZH-4 and WGH-6. *Journal of Polymers and the Environment*, *30*(9), 3949–3958. https://doi.org/10.1007/s10924-022-02480-8

Wróbel, M., Szymańska, S., Kowalkowski, T., & Hrynkiewicz, K. (2023). Selection of microorganisms capable of polyethylene (PE) and polypropylene (PP) degradation. *Microbiological Research*, *267*, Article 127251. https://doi.org/10.1016/j.micres.2022.127251

Yang, S. S., Ding, M. Q., Ren, X. R., Zhang, Z. R., Li, M. X., Zhang, L. L., Pang, J. W., Chen, C. X., Zhao, L., Xing, D. F., Ren, N. Q., Ding, J., & Wu, W. M. (2022). Impacts of physical-chemical property of polyethylene on depolymerization and biodegradation in yellow and dark mealworms with high purity microplastics. *Science of the Total Environment*, *828*, Article 154458. https://doi.org/10.1016/j.scitotenv.2022.154458

Yasin, N. M., Akkermans, S., & Van Impe, J. F. M. (2022). Enhancing the biodegradation of (bio)plastic through pretreatments: A critical review. *Waste Management*, *150*, 1–12. https://doi.org/10.1016/j.wasman.2022.06.004

Zhang, F., Zhao, Y. T., Wang, D. D., Yan, M. Q., Zhang, J., Zhang, P. Y., Ding, T. G., Chen, L., & Chen, C. (2021). Current technologies for plastic waste treatment: A review. *Journal of Cleaner Production*, *282*, Article 124523. https://doi.org/10.1016/j.jclepro.2020.124523

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

Zhang, Y., Pedersen, J. N., Eser, B. E., & Guo, Z. (2022b). Biodegradation of polyethylene and polystyrene: From microbial deterioration to enzyme discovery. *Biotechnology Advances*, *60*, Article 107991. https://doi.org/10.1016/j.biotechadv.2022.107991