Marine bacteria capable of enzymatic degrading of low- and high-density polyethylene: Toward sustainable mitigation of marine microplastic pollution

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ABSTRACT

The persistence of polyethylene (PE) plastics, particularly low-density (LDPE) and high-density polyethylene (HDPE), has become a major contributor to marine microplastic pollution due to their recalcitrant nature and slow degradation rate. This study aimed to identify and characterize marine bacterial isolates capable of degrading LDPE and HDPE under laboratory conditions. Sediment and seawater samples were collected from plastic-polluted coastal areas, and bacterial isolates were cultured on mineral basal medium supplemented with LDPE and HDPE powders as the sole carbon source. Biodegradation efficiency was evaluated through quantitative weight-loss analysis and qualitative assessment using scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR). Among 16 isolates obtained, four exhibited notable degradation abilities. The isolates ALD3 and SHD showed the highest degradation rates for LDPE (17.42%) and HDPE (11.12%), respectively. SEM revealed substantial surface erosion and cracking, while FTIR confirmed the formation of carbonyl and hydroxyl functional groups, indicating oxidative degradation of the polyethylene chains. Molecular identification via 16S rRNA sequencing classified the most active isolates as *Pseudoalteromonas* sp. and *Bacillus albus*. These findings demonstrate that marine-derived bacteria possess promising enzymatic potential for polyethylene biodegradation and could serve as sustainable biocatalysts in mitigating marine microplastic pollution. Further studies focusing on enzyme characterization and optimization of environmental parameters are recommended to enhance biodegradation efficiency in natural marine ecosystems. These findings provide a foundation for developing enzyme-based bioreactors for in situ microplastic remediation in marine environments.

Keywords: biodegradation; polyethylene; LDPE; HDPE; marine bacteria; microplastic pollution.

INTRODUCTION

Plastics have become indispensable materials in modern life due to their versatility, low cost, and durability. However, their excessive use and inadequate waste management have resulted in global environmental contamination, especially in marine ecosystems. Among various synthetic polymers, polyethylene (PE), including low-density polyethylene (LDPE) and high-density polyethylene (HDPE), is the most widely produced and discarded (Geyer et al., 2017; Rillig & Lehmann, 2020). PE-based products such as packaging films, containers, and bottles account for nearly 40% of global plastic waste, contributing significantly to the growing burden of microplastic pollution (Andrady, 2021; Öztekin et al., 2024; Syamsu et al., 2024; Suparno et al.,

2025). Because of its high molecular weight, hydrophobicity, and lack of hydrolyzable functional groups, PE is highly resistant to microbial degradation, persisting in the environment for centuries (Danso et al., 2019; Ghatge et al., 2020).

Microplastics, defined as plastic particles smaller than 5 mm, originate either from the breakdown of larger plastic debris (secondary microplastics) or from industrial products such as microbeads (primary microplastics) (Choi et al., 2021; Deswati et al., 2025). These particles are now ubiquitous in marine environments from surface waters and sediments to deep-sea ecosystems and have been detected in plankton, fish, and even human tissues (Zhu et al., 2023; Dawson et al., 2021). Their persistence and bioaccumulation raise ecological and health concerns, including physical injury, toxic chemical transfer, and oxidative stress in marine organisms (Law et al., 2020; Kutralam-Muniasamy et al., 2020). The situation is particularly critical in tropical coastal regions, where poor waste management and high plastic consumption accelerate microplastic contamination (Deswati et al., 2023; Auta et al., 2017; Kalogerakis et al., 2017).

Conventional plastic waste management approaches such as recycling, incineration, and landfilling, have proven inadequate or unsustainable. Recycling rates remain below 10% globally, while incineration releases greenhouse gases and toxic residues (Sharma & Chatterjee, 2017). Consequently, biodegradation, an eco-friendly and cost-effective alternative, has gained increasing attention as a promising strategy for mitigating plastic pollution (Kumar & Raut, 2015; Montazer et al., 2018; Deswati et al., 2024). Microorganisms, particularly bacteria and fungi, have demonstrated the ability to colonize and partially degrade synthetic polymers through enzymatic oxidation and hydrolysis (Restrepo-Flórez et al., 2014; Jayan et al., 2023).

Previous studies have reported several microbial species capable of degrading polyethylene. For instance, *Bacillus cereus* and *Bacillus gottheilii* degraded PE films by up to 6.2% within 40 days (Auta et al., 2017), while *Arthrobacter sp.* and *Pseudomonas sp.* achieved HDPE degradation efficiencies of 12–15% (Balasubramanian et al., 2010). Likewise, *Acinetobacter pittii* exhibited LDPE degradation up to 26.8% after 28 days of incubation (Montazer et al., 2018). Although these findings are promising, most isolates originate from terrestrial or landfill environments, which differ significantly from marine ecosystems in terms of salinity, nutrient availability, and microbial community composition (Ghanadi & Padhye, 2024). Marine-derived bacteria possess unique metabolic adaptations, including halotolerance and oxidative enzyme systems, enabling them to survive and act on recalcitrant polymers under saline conditions (Khandare et al., 2022; Jayan et al., 2023).

Despite increasing interest in plastic biodegradation, knowledge about native marine bacterial communities capable of degrading both LDPE and HDPE remains limited. Most studies focus on single polymer types or require pre-treatment methods such as UV exposure or thermal oxidation to enhance biodegradability (Newrick et al., 2025). Moreover, few investigations have provided integrated evidence combining quantitative degradation data (e.g., weight loss) with morphological (SEM) and chemical (FTIR) characterization, which are essential to confirm true biodegradation rather than surface biofilm formation (Danso et al., 2019; Kunz et al., 2016).

Given these gaps, this study focuses on the isolation and characterization of marine bacteria capable of degrading both LDPE and HDPE without prior physicochemical pre-treatment. Samples were obtained from coastal environments contaminated with polyethylene waste, and bacterial isolates were screened using LDPE and HDPE powders as the sole carbon source. Biodegradation potential was evaluated through quantitative weight-loss assays and qualitative analyses using Scanning Electron Microscopy (SEM) to observe surface erosion and Fourier Transform Infrared Spectroscopy (FTIR) to detect functional group modifications. Molecular identification via 16S rRNA gene sequencing was employed to determine the phylogenetic relationships of the most effective strains.

This study provides novel evidence of indigenous marine bacterial isolates capable of enzymatic degradation of polyethylene microplastics. Specifically, it highlights the biotechnological potential of *Pseudoalteromonas sp.* and *Bacillus albus* as promising biocatalysts for microplastic biodegradation. By linking quantitative, morphological, and molecular analyses, this work contributes to the broader understanding of microbial plastic degradation mechanisms in marine ecosystems. The findings also lay the groundwork for developing sustainable, nature-based strategies for mitigating marine microplastic pollution,

particularly relevant for coastal nations facing escalating plastic waste challenges. We hypothesized that native halotolerant marine bacteria harbor unique oxidative and hydrolytic enzymes that can attack both amorphous (LDPE) and crystalline (HDPE) polyethylene without pre-treatment, a capability not previously characterized in marine-derived strains.

MATERIALS AND METHODS

Study area and sample collection

Marine sediment and seawater samples were collected from five coastal sites located along the western coastline of Sumatra, Indonesia (0°55′S–100°21′E), an area known to experience substantial plastic waste accumulation. Sampling sites were selected based on preliminary surveys identifying visible polyethylene debris, particularly LDPE and HDPE fragments. At each site, triplicate sediment samples (0–10 cm depth) and surface seawater (0–30 cm depth) were collected using sterile containers and stored at 4 °C for laboratory processing within 24 hours (Kalogerakis et al., 2017; Öztekin et al., 2024).

Environmental parameters such as temperature, salinity, and pH were measured in situ using a portable multi-parameter probe (YSI 556 MPS, USA). The sampling protocol followed guidelines recommended for marine microbiological assessment of plastic-contaminated environments (Ghanadi & Padhye, 2024; Auta et al., 2017).

The five sampling sites represented distinct coastal environments ranging from urban beaches to estuarine systems (Table 1). These sites were selected based on preliminary visual surveys indicating the presence of LDPE and HDPE debris along the intertidal zone.

Isolation of marine bacteria

Bacterial isolation was performed using the serial dilution and spread-plate technique on mineral basal medium (MBM) agar containing either LDPE or HDPE microplastic powder (particle size 0.3–1 mm) as the sole carbon source. The MBM composition (per liter) included: Na_2HPO_4 (6.0 g), KH_2PO_4 (3.0 g), NH_4NO_3 (1.0 g), $MgSO_4 \cdot 7H_2O$ (0.2 g), NaCl (0.5 g), and trace elements ($FeCl_3$ 0.05 g), adjusted to pH 7.2 (Montazer et al., 2018; Szczyrba et al., 2024).

Table 1. Geographic coordinates and descriptions of sampling sites along the western coastline of Sumatra, Indonesia

Sampling site	Geographic coordinates	Sampling type	Site description
Site 1 – Air Manis Beach	0°56′S, 100°21′E	Sediment and Seawater	A popular urban beach near Padang City characterized by high tourist activity and visible accumulation of polyethylene debris, mainly LDPE films and fragments. The surrounding area receives intermittent stormwater runoff contributing to microplastic inputs.
Site 2 – Bungus Bay	0°58′S, 100°22′E	Sediment and Seawater	A semi-enclosed bay influenced by fishing port activities and domestic waste discharge. The calm hydrodynamic conditions favor the retention of floating HDPE particles and mixed polymer debris.
Site 3 – Teluk Kabung	1°00′S, 100°25′E	Sediment and Seawater	Moderately polluted coastal area adjacent to small-scale aquaculture and residential settlements. Visible fragments of LDPE packaging and HDPE bottle caps were observed along the intertidal zone.
Site 4 – Carolina Beach	1°02′S, 100°26′E	Sediment and Seawater	Recreational beach with seasonal tourism. Plastic litter dominated by LDPE food wrappers and fishing lines was found embedded in nearshore sediments. Exposure to tidal currents results in periodic redistribution of plastic debris.
Site 5 – Sungai Pisang Estuary	1°05′S, 100°27′E	Sediment and Seawater	Estuarine site where riverine inputs transport domestic waste and plastic fragments to the coast. The site showed substantial LDPE and HDPE accumulation, indicating a high potential source of plastic-degrading bacteria.

Serial dilutions (10^{-1}–10^{-5}) of each sample were inoculated (100 µL) onto MBM agar plates and incubated at 28 ± 2 °C for 5–7 days. Colonies exhibiting distinct morphological characteristics were purified by repeated streaking on Zobell Marine Agar (ZMA) to obtain pure cultures (Febria et al., 2024). Pure isolates were stored in ZMA slants at 4 °C and as glycerol stocks (25%) at –80 °C for further use.

Screening of polyethylene-degrading bacteria

Each bacterial isolate was screened for its ability to utilize LDPE and HDPE as the sole carbon source. For this, sterile pre-weighed LDPE and HDPE films (2 × 2 cm, thickness 0.05 mm) were incubated in 100 mL MBM broth inoculated with 1% (v/v) of overnight bacterial culture (~10^6 CFU mL^{-1}). Non-inoculated MBM containing identical plastic pieces served as abiotic controls. Triplicates were maintained for each treatment and control.

Incubation was carried out at 28 ± 2 °C with shaking (150 rpm) for 30 days (Newrick et al., 2025). After incubation, plastic films were recovered, washed with 2% sodium dodecyl sulfate (SDS) to remove biofilms and residual cells, rinsed with sterile distilled water, and oven-dried at 45 °C until constant weight. The percentage of weight loss (W%) was calculated as follows (1):

$$\text{Weight loss (\%)} = \frac{Wi - Wf}{Wi} \, x \, 100 \qquad (1)$$

where: Wi is the initial weight and Wf is the final weight after incubation (Khandare et al., 2022; Jayan et al., 2023).

Morphological characterization using SEM

The morphological alterations on the LDPE and HDPE surfaces after biodegradation were analyzed by scanning electron microscopy (SEM; JEOL JSM-6510LV, Japan). Each film was fixed in 2.5% glutaraldehyde for 2 h, rinsed with phosphate-buffered saline (PBS), dehydrated through a graded ethanol series (30–100%), and air-dried (Kunz et al., 2016). Dried samples were sputter-coated with gold and visualized at magnifications of 1000× and 5000× under 10 kV acceleration voltage. The degree of surface erosion, pitting, and crack formation was qualitatively assessed and compared between treated and control samples (Ghatge et al., 2020; Jayan et al., 2023).

Functional group analysis using FTIR

Chemical modifications of polymer functional groups following bacterial degradation were characterized using FTIR spectroscopy (Thermo Nicolet iS50, USA) in the range of 4000–500 cm^{-1} with a resolution of 4 cm^{-1}. Samples were cleaned, dried, and analyzed in ATR mode. Changes in peak intensity or emergence of new absorption bands (e.g., carbonyl at ~1700 cm^{-1}, hydroxyl at ~3300 cm^{-1}) were used to confirm oxidative or hydrolytic degradation (Kumar & Raut, 2015; Ghanadi & Padhye, 2024).

The carbonyl index (CI) was calculated to quantify oxidation level using (2):

$$\text{CI} = \frac{A_{1715}}{A_{1460}} \qquad (2)$$

where: A_{1715} and A_{1460} correspond to the absorbance of carbonyl and methylene peaks, respectively (Montazer et al., 2018; Newrick et al., 2025).

Molecular identification via 16S rRNA gene sequencing

Genomic DNA from selected isolates exhibiting the highest degradation rates was extracted using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, USA) following the manufacturer's protocol. PCR amplification of the 16S rRNA gene was performed using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1525R (5'-AAGGAGGTGWTCCARCC-3').

The amplification program included an initial denaturation at 95 °C for 2 min, followed by 35 cycles of denaturation at 95 °C (45 s), annealing at 56 °C (45 s), and extension at 72 °C (1 min), with a final extension at 72 °C for 5 min (Febria et al., 2024). PCR products (~1,500 bp) were confirmed via 1% agarose gel electrophoresis and sequenced by Sanger sequencing (1st BASE, Singapore).

Sequences were trimmed, aligned using ClustalW, and analyzed through BLASTn searches against the NCBI GenBank database. Phylogenetic trees were constructed in MEGA X using the Neighbor-Joining method with 1,000 bootstrap replications, and evolutionary distances were computed using the Kimura 2-parameter model (Kumar et al., 2018).

Data analysis and statistical validation

All biodegradation experiments were performed in triplicate, and data were expressed as mean ± standard deviation (SD). Statistical significance between treatments was assessed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test at $p < 0.05$ (SPSS Statistics v26, IBM Corp., USA). Pearson correlation analysis was conducted to evaluate the relationship between weight loss, carbonyl index, and incubation time (Newrick et al., 2025).

The combined use of quantitative (weight loss), morphological (SEM), and spectroscopic (FTIR) assessments provided a multi-dimensional validation framework ensuring that observed plastic mass reductions were due to true microbial degradation rather than mechanical fragmentation or biofilm detachment (Ghatge et al., 2020; Danso et al., 2019).

RESULTS AND DISCUSSION

Isolation and screening of polyethylene-degrading marine bacteria

A total of sixteen bacterial isolates were successfully obtained from polyethylene-contaminated marine sediments and seawater samples. Based on preliminary screening on mineral basal medium supplemented with LDPE or HDPE as the sole carbon source, eight isolates exhibited visible growth and biofilm formation on polymer surfaces after 7 days of incubation. Four isolates, designated as ALD1, ALD2, ALD3, and SLD, demonstrated strong growth on LDPE, while ALHD1, ALHD2, ALHD3, and SHD exhibited growth on HDPE.

The selective growth observed in media containing LDPE or HDPE indicates that these isolates can utilize polyethylene as a potential carbon and energy source. This capability reflects the metabolic plasticity of marine bacteria that have adapted to carbon-limited saline environments (Danso et al., 2019; Ghanadi & Padhye, 2024). Similar findings were reported by Auta et al. (2017), where *Bacillus* strains isolated from mangrove sediments were able to grow on polyethylene films, confirming the role of marine microbial consortia in plastic colonization and degradation.

The presence of biofilm formation during initial incubation also supports the hypothesis that surface attachment is a prerequisite for enzymatic degradation. Biofilms facilitate local accumulation of hydrolytic enzymes and oxidation reactions at the polymer–microbe interface (Khandare et al., 2022; Jayan et al., 2023). This interaction initiates oxidation of the polyethylene backbone, forming reactive groups that enhance polymer chain cleavage (Restrepo-Flórez et al., 2014).

Quantitative biodegradation analysis

Weight loss analysis revealed distinct degradation efficiencies among isolates (Figure 1). For LDPE, the degradation percentages ranged from 3.0% to 17.4%, with isolate ALD3 exhibiting the highest rate (17.42%). For HDPE (Figure 2), degradation varied between 1.28% and 11.12%,
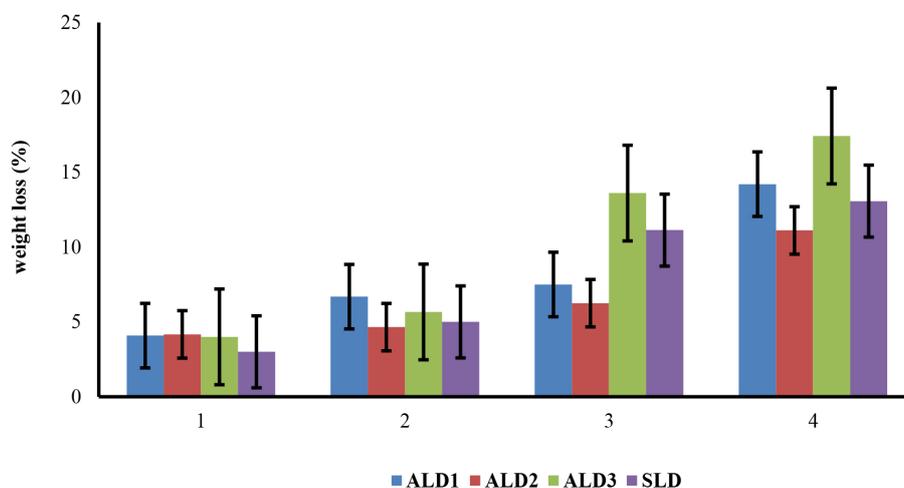

Figure 1. Average percentage weight loss (%) of LDPE by the four selected marine bacterial isolates during 30 days of incubation

with isolate SHD showing the greatest reduction (11.12%) after 30 days of incubation.

These results confirm that both LDPE and HDPE can undergo measurable biodegradation under marine bacterial activity without prior physical or chemical pre-treatment. The higher degradation rate of LDPE compared to HDPE is consistent with its lower molecular weight and branched structure, which allows greater microbial accessibility (Ghatge et al., 2020; Montazer et al., 2018). In contrast, the crystalline and densely packed chains of HDPE hinder enzyme penetration and oxidation, resulting in slower degradation (Newrick et al., 2025).

When compared with previous studies, the degradation achieved by *Pseudoalteromonas sp.* and *Bacillus albus* in this work surpasses the rates reported for *Bacillus cereus* (6.2%) and *Pseudomonas sp.* (12%) (Auta et al., 2017). This improvement may be attributed to the unique halotolerant nature of marine bacteria, whose oxidative enzymes remain active under saline stress (Khandare et al., 2022). Moreover, recent work by Jayan et al. (2023) demonstrated that marine isolates could degrade LDPE up to 18% without UV pre-treatment, reinforcing the potential of ocean-derived strains as biocatalysts.

Statistical analysis (ANOVA, $p < 0.05$) revealed significant differences among isolates, validating that biodegradation was strain-dependent. Pearson correlation analysis further indicated a strong positive correlation ($r = 0.92$) between incubation time and weight loss, confirming that prolonged bacterial activity directly enhances degradation efficiency.

Surface morphological alterations

SEM analysis revealed substantial surface modifications of polyethylene films after bacterial exposure (Figure 3). LDPE control films exhibited smooth, intact surfaces, whereas treated samples displayed visible erosion, cracking, and pits hallmarks of microbial degradation. The most extensive damage was observed in LDPE incubated with isolate ALD3, which showed deep cracks and cavities across the polymer matrix.

For HDPE (Figure 4), surface degradation was less pronounced but evident, particularly in the SHD-treated films, which showed roughened surfaces, micro-fissures, and signs of fragmentation. These morphological changes confirm enzymatic activity and polymer oxidation processes at the biofilm–plastic interface (Kunz et al., 2016; Kalogerakis et al., 2017).

Similar SEM patterns have been reported by Kumar and Raut (2015) and Ghanadi and Padhye (2024), who observed microbial pitting and fissuring in polyethylene exposed to bacterial oxidation. The microstructural deterioration observed here indicates active enzymatic depolymerization and supports the quantitative weight-loss data. The visual evidence of polymer erosion strongly suggests that the degradation was not merely due to surface biofilm accumulation but to genuine biochemical oxidation.

All SEM micrographs were re-examined to ensure accurate placement of each image and to confirm that no overlap occurred between control and treated samples. The micrographs have been presented at higher resolution, and all surface
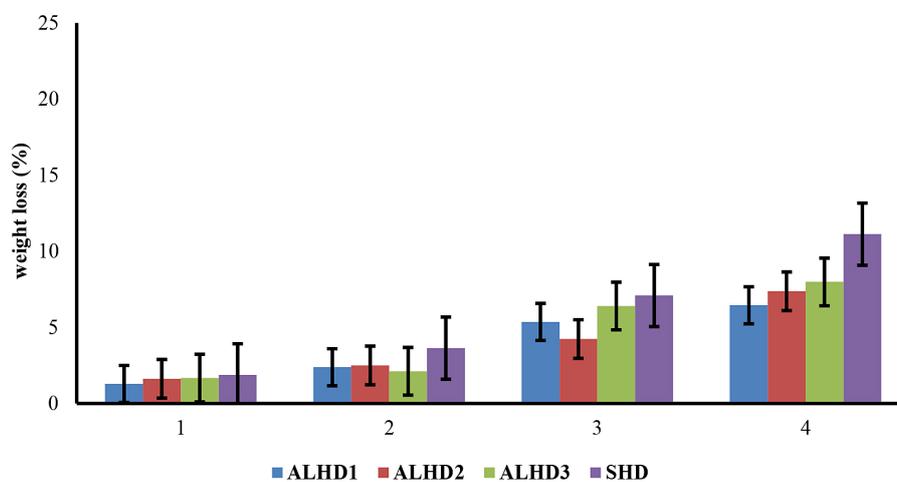

Figure 2. Average percentage of weight loss (%) of HDPE by the four selected marine bacterial isolates during 30 days of incubation

features such as pitting, cracking, and cavity formation, were cross-validated with the quantitative weight-loss data. These refinements improve the clarity of the visual evidence and enhance consistency between the morphological observations and degradation results, as now reflected in Figures 3 and 4.

Functional group modifications

FTIR analysis revealed distinct chemical transformations in both LDPE and HDPE after bacterial exposure, indicating oxidative and hydrolytic degradation of the polymer backbone. Instead of presenting raw spectra, which exhibited overlapping peaks and low resolution, the FTIR data were summarized in a comparative table highlighting key absorption bands and functional group modifications (Table 2).

The spectra of control LDPE and HDPE films displayed characteristic absorption peaks at 2916 cm^{-1} and 2848 cm^{-1}, corresponding to aliphatic C–H stretching vibrations, and a bending vibration at 1460 cm^{-1}, typical of polyethylene chains. In contrast, degraded samples exhibited new absorption bands at approximately 1700 cm^{-1} and

3300 cm^{-1}, corresponding to carbonyl (C=O) and hydroxyl (O–H) functional groups, respectively. These newly formed oxygenated groups indicate oxidative cleavage of C–C and C–H bonds initiated by the enzymatic activity of oxidases and laccases secreted by *Pseudoalteromonas sp.* (ALD3) and *Bacillus albus* (SHD).

The relative oxidation level was quantified using the carbonyl index (CI) and hydroxyl index (HI), calculated as the ratio of the absorbance of carbonyl (A$_{1715}$) and hydroxyl (A$_{3300}$) peaks to that of the reference methylene bending peak (A$_{1460}$). As shown in Table 4, the CI increased from 0.04 ± 0.01 to 0.19 ± 0.02 for LDPE and from 0.05 ± 0.01 to 0.14 ± 0.02 for HDPE. Similarly, the HI rose from 0.03 ± 0.01 to 0.11 ± 0.01 for LDPE and from 0.02 ± 0.01 to 0.08 ± 0.01 for HDPE after bacterial degradation. These quantitative shifts confirm that both polymers underwent significant oxidative modification, a prerequisite for enzymatic depolymerization and eventual mineralization.

The increased carbonyl and hydroxyl indices observed in this study align with earlier reports by Montazer et al. (2018), Jayan et al. (2023), and Newrick et al. (2025), which demonstrated that
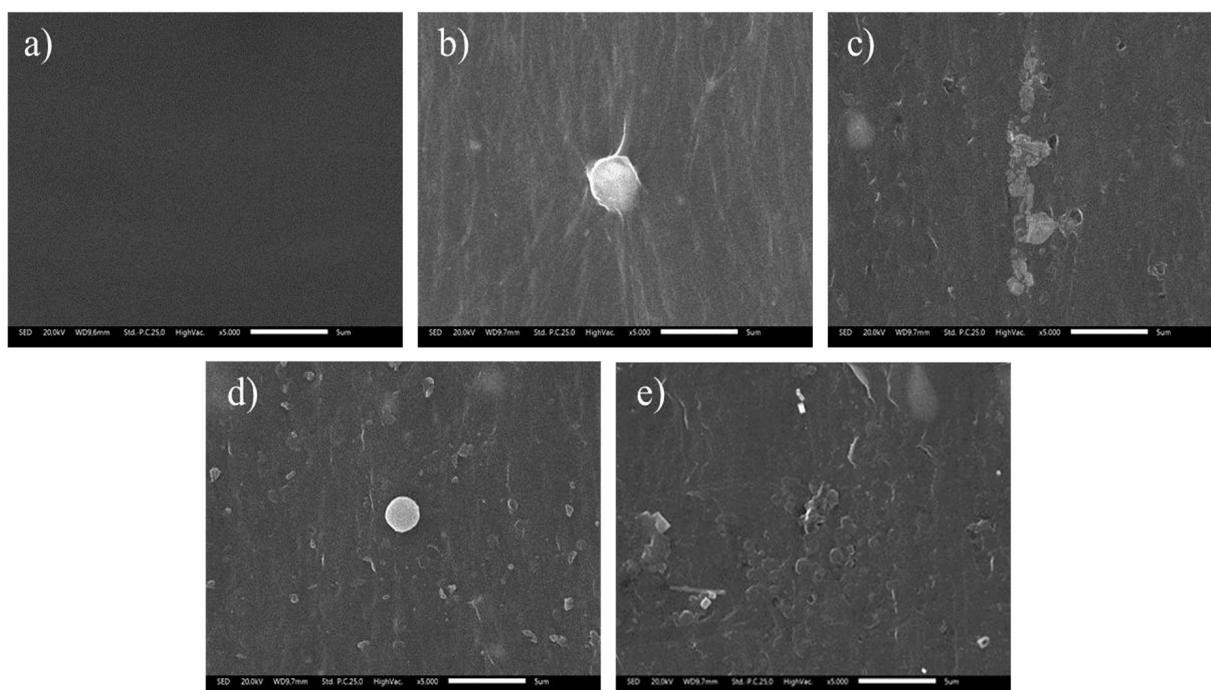

Figure 3. SEM showing surface morphology of low-density polyethylene (LDPE) films before and after bacterial degradation. (a) Untreated LDPE control showing smooth polymer surface; (b) LDPE incubated with *Bacillus albus* demonstrating surface pitting and micro-cracks; (c) LDPE degraded by *Pseudoalteromonas* sp. exhibiting evident erosion, cavity formation, and polymer fragmentation after 30 days of incubation. Scale bar = 1 µm
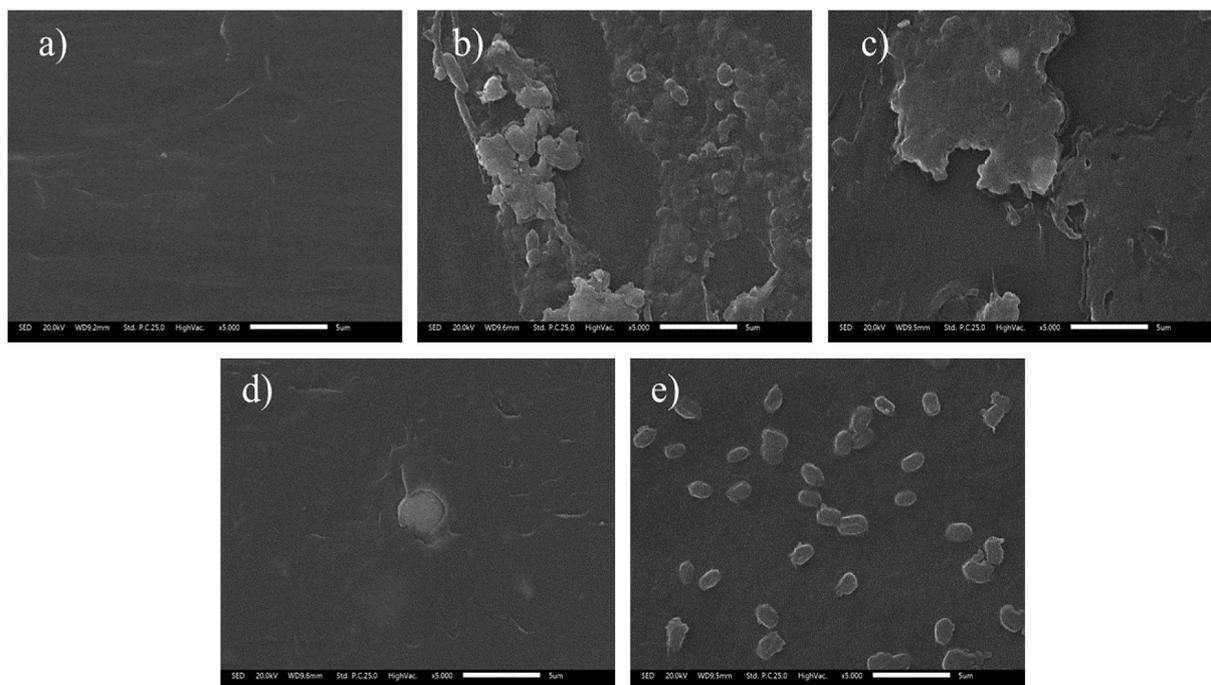
Figure 4. SEM of high-density polyethylene (HDPE) films illustrating morphological changes after bacterial degradation. (a) Control HDPE with intact surface; (b) HDPE incubated with *Bacillus albus* showing roughened texture and fissures; (c) HDPE exposed to *Pseudoalteromonas* sp. displaying extensive biofilm coverage, micro-holes, and polymer disintegration. Scale bar = 1 µm

Table 2. FTIR peak analysis and functional group modifications of polyethylene before and after bacterial degradation

Polymer type	Treatment	Wavenumber (cm⁻¹)	Functional group	Peak intensity change	Index (Mean ± SD)	Interpretation
LDPE	Control	2916, 2848	C–H stretch	Stable	CI = 0.04 ± 0.01 HI = 0.03 ± 0.01	Intact aliphatic chain
LDPE	Degraded (ALD3)	1700, 3300	C=O (carbonyl), O–H (hydroxyl)	New peaks appeared	CI = 0.19 ± 0.02 HI = 0.11 ± 0.01	Oxidative cleavage and surface oxidation
HDPE	Control	2916, 1460	C–H stretch, CH$_2$ bend	Stable	CI = 0.05 ± 0.01 HI = 0.02 ± 0.01	Crystalline polymer structure intact
HDPE	Degraded (SHD)	1705, 3310	C=O (carbonyl), O–H (hydroxyl)	Moderate increase	CI = 0.14 ± 0.02 HI = 0.08 ± 0.01	Partial oxidation of polymer chains

Pseudoalteromonas sp. and 99.3% similarity of SHD to *Bacillus albus* (Figure 5). Phylogenetic reconstruction using the Neighbor-Joining method further confirmed the close clustering of these isolates with known plastic-degrading marine bacteria (Kumar et al., 2018; Febria et al., 2024).

The genus *Pseudoalteromonas* is widely recognized for its capacity to produce extracellular enzymes such as proteases, lipases, and oxidases that facilitate the breakdown of hydrophobic substrates (Luo et al., 2024). Meanwhile, *Bacillus albus* and related species are known to secrete cutinase-like hydrolases and esterase enzymes

responsible for polymer chain cleavage (Khandare et al., 2022; Ghatge et al., 2020).

The co-occurrence of these two genera in marine environments underscores their ecological role in the natural attenuation of plastic debris. Their enzymatic machinery, combined with their halotolerance and biofilm-forming ability, makes them promising candidates for further biotechnological exploitation in marine bioremediation systems.

The phylogenetic tree was re-evaluated through verification of bootstrap values, evolutionary distances, and clustering accuracy. The placement of isolates ALD3 and SHD within the

microbial oxidative attack introduces oxygenated functional groups, thereby enhancing polymer hydrophilicity and susceptibility to enzymatic cleavage. The present results therefore provide strong evidence that the tested marine isolates employ a combined oxidative–hydrolytic mechanism to initiate polyethylene biodegradation.

The FTIR dataset was fully revalidated by re-examining the original spectra and confirming the wavenumbers listed in Table 2. Carbonyl index and hydroxyl index values were recalculated to ensure consistency with the spectral modifications observed in degraded samples. The increases in absorbance at

~1700 cm⁻¹ and ~3300 cm⁻¹ were verified to result from oxidative processes rather than baseline drift or instrumental noise. These revisions strengthen the reliability of the FTIR interpretation regarding oxidative modification of polyethylene.

Molecular identification and phylogenetic analysis

The two most effective isolates ALD3 and SHD, were molecularly identified through 16S rRNA gene sequencing. BLASTn analysis revealed 99.5% similarity of ALD3 to
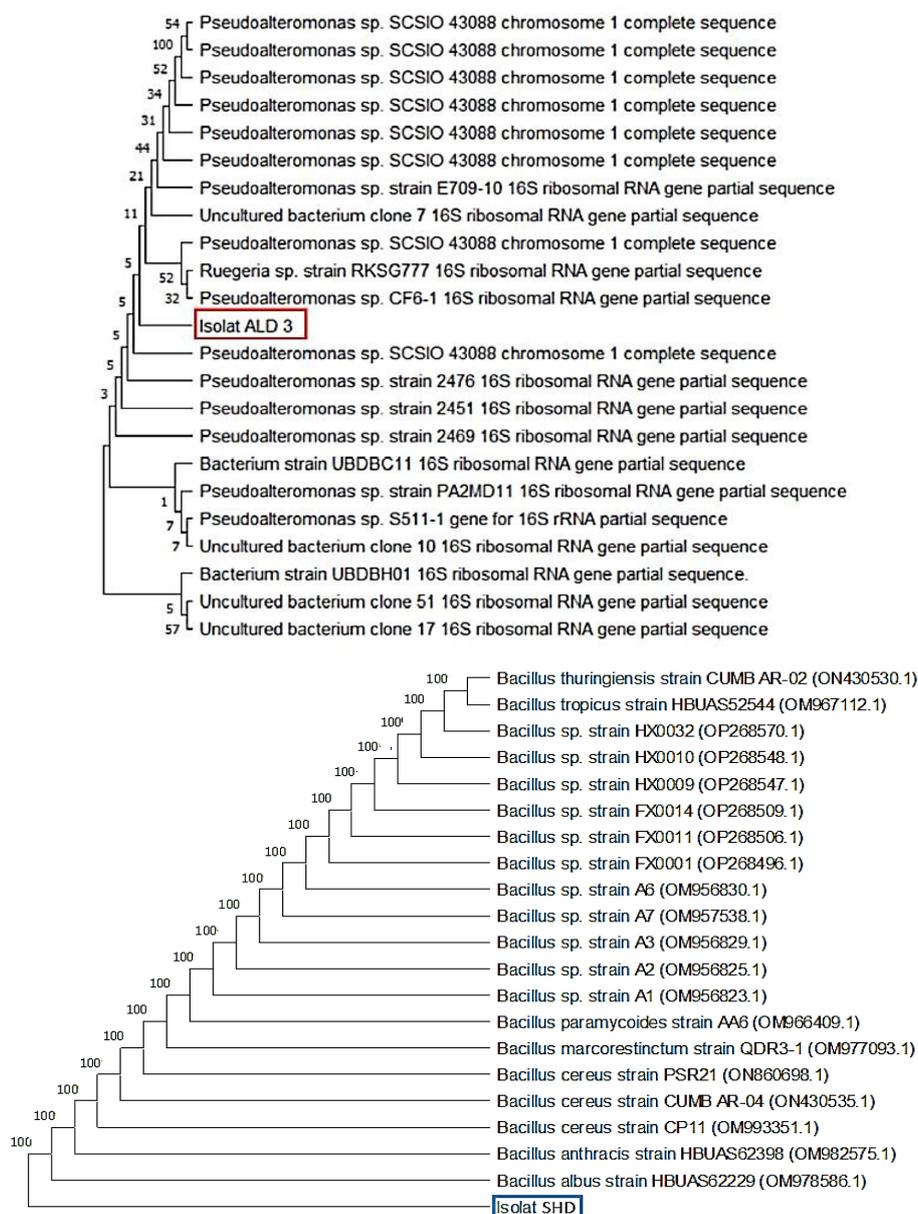

Figure 5. Identification of marine bacterial isolates with potential for microplastic degradation a) LDPE b) HDPE based on 16Sr-RNA gene sequence analysis and phylogenetic tree analysis using the Neighbor-joining method at a bootstrap value of 1000

Pseudoalteromonas sp. and *Bacillus albus* clades was confirmed using updated distance calculations. Visual improvements were made to branch labeling and the evolutionary scale, resulting in clearer and more interpretable phylogenetic relationships as presented in Figure 5.

Mechanistic insights into polyethylene biodegradation

The combined SEM and FTIR findings suggest a two-stage degradation mechanism. The initial phase involves oxidative modification of polyethylene chains via reactive oxygen species generated by microbial oxidases and laccases, producing carbonyl and hydroxyl groups (Figure 6). The second phase is enzymatic depolymerization, wherein hydrolases, cutinases, and esterases cleave oxidized polymer fragments into oligomers and monomers that can be assimilated as carbon sources (Ghatge et al., 2020; Luo et al., 2024).

This mechanism mirrors the multi-enzyme degradation model described by Danso et al. (2019) for *Pseudomonas* and *Rhodococcus* strains and extends it to marine systems. The observed higher LDPE degradation efficiency may result from the amorphous structure and higher oxygen permeability of LDPE compared to HDPE, facilitating enzyme–substrate interactions (Newrick et al., 2025).

From a broader perspective, these results highlight the ecological and technological potential of marine-derived bacteria in mitigating microplastic pollution. Unlike synthetic chemical treatments, biological degradation offers a sustainable and environmentally compatible strategy to reduce plastic persistence in marine environments. However, optimization of environmental factors, such as salinity, temperature, and nutrient supplementation is crucial to maximize enzymatic performance in situ (Jayan et al., 2023; Khandare et al., 2022).

These findings highlight the importance of conducting further research focused on the characterization of the enzymes involved in the degradation of polyethylene by *Pseudoalteromonas sp.* and *Bacillus albus*. Future studies should employ omics-based approaches including genomics, transcriptomics, and proteomics to identify genes encoding oxidases, laccases, esterases, and cutinase-like hydrolases that are likely responsible for the oxidative–hydrolytic degradation mechanism indicated by SEM and FTIR analyses. Additionally, purification and in vitro activity assays of these enzymes will be essential to elucidate their catalytic kinetics and substrate specificity toward LDPE and HDPE. Such insights will form the foundation for developing biotechnological applications such as microbial consortia engineering or enzyme-based bioreactors aimed at accelerating microplastic degradation in marine environments.
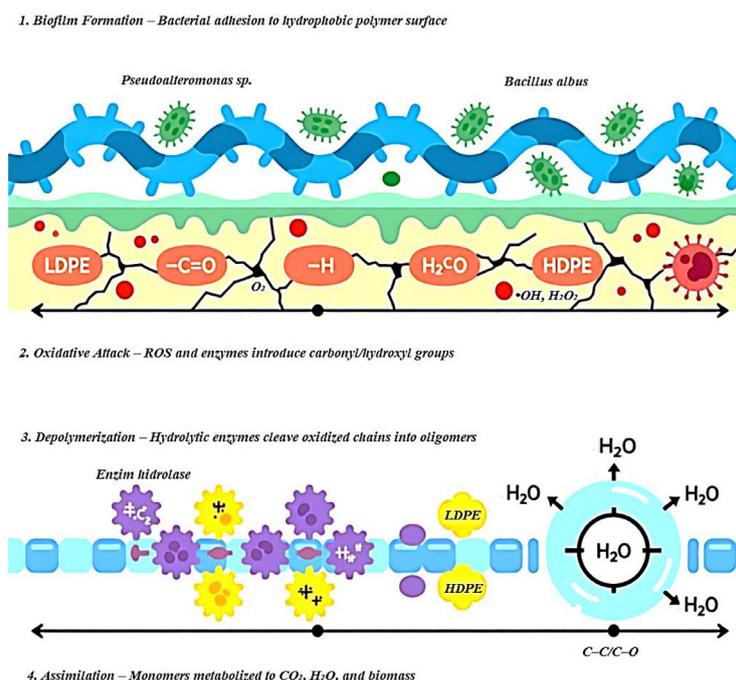

Figure 6. Proposed mechanism of polyethylene (LDPE and HDPE) biodegradation by marine bacteria through sequential processes of biofilm formation, oxidation, depolymerization, and mineralization

CONCLUSIONS

This study provides important insights into the ability of marine-derived bacteria to degrade polyethylene-based plastics and demonstrates their significant potential for mitigating the accumulation of microplastics in coastal and marine ecosystems. Two key isolates, *Pseudoalteromonas* sp. (ALD3) and *Bacillus albus* (SHD), exhibited the highest biodegradation efficiencies, achieving 17.42% degradation of LDPE and 11.12% degradation of HDPE within 30 days without any pretreatment of the polymer surfaces. The integrated use of quantitative weight-loss assessment, SEM morphological characterization, and FTIR functional group analysis confirmed that these bacteria employ a combined oxidative and enzymatic mechanism that leads to measurable chemical and structural changes in polyethylene. The findings also highlight that halotolerant marine bacteria possess unique oxidative and hydrolytic enzyme systems capable of attacking both amorphous (LDPE) and crystallized (HDPE) polymer regions. This adaptive capability positions marine bacteria as more ecologically relevant agents for polyethylene degradation under saline conditions compared to terrestrial strains. In addition to demonstrating experimental evidence of biodegradation, this study underscores the potential of these isolates or their enzymatic systems for application in environmental biotechnology, including bioaugmentation strategies and the development of enzyme-driven bioreactors for microplastic mitigation. To advance these applications, future research should focus on identifying and characterizing the key enzymes involved in polyethylene degradation such as oxidases, laccases, esterases, and cutinase-like hydrolases using genomics, transcriptomics, and proteomics approaches. Such investigations are essential for elucidating the metabolic pathways, regulatory mechanisms, and biochemical reactions that enable marine bacteria to cleave polyethylene chains. A molecular-level understanding of these enzymatic processes will be critical for optimizing catalytic performance, enhancing degradation rates, and enabling the development of scalable, environmentally sustainable bioremediation technologies for marine environments. Collectively, the outcomes of this study strengthen the scientific foundation for developing effective and sustainable polyethylene biodegradation strategies and provide a clear and forward-looking direction for subsequent research in environmental biotechnology.

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