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Indigenous *Bacillus* biodiversity from plastic-contaminated soil and its potential for low-density polyethylene (LDPE) biodegradation

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Abstract. *Irawati W, Lie ST, Fideli GD, Pongajow BYC, Jo J. 2026. Indigenous* Bacillus *biodiversity from plastic-contaminated soil and its potential for low-density polyethylene (LDPE) biodegradation. Biodiversitas 27 (5): d270528. https://doi.org/10.13057/biodiv/d270528.* Increasing plastic use has led to persistent accumulation of low-density polyethylene (LDPE) in terrestrial environments. This study isolated and characterized indigenous *Bacillus*-like bacteria from plastic-contaminated soil in the public parking area of Universitas Pelita Harapan and evaluated their capacity to induce early-stage LDPE modification. Soil suspensions were plated on King's B agar supplemented with polyethylene glycol (PEG), yielding eight morphologically distinct isolates. LDPE strips were incubated for 30 days in minimal salt medium (MSM) inoculated with each isolate, with LDPE as the principal experimental substrate and no additional organic carbon intentionally supplied. Surface changes were assessed by scanning electron microscopy (SEM), chemical changes by Fourier-transform infrared (FTIR) spectroscopy and carbonyl index (CI) calculation, and taxonomic identity by 16S rRNA gene sequencing. All eight isolates were Gram-positive, catalase-positive, endospore-forming rods, and 16S analysis assigned them to *Bacillus velezensis*, *Bacillus altitudinis*, *Bacillus licheniformis*, *Bacillus subtilis*, and *Bacillus paralicheniformis*. SEM revealed isolate-specific colonization-associated surface changes, ranging from roughening and small pits to deep cracks and perforation, with A.4.I.1 showing the most extensive damage. FTIR spectra indicated oxidation-related changes, including intensified carbonyl and C-O bands; screening-level CI values ranged from 0.190 to 0.346, again highest for A.4.I.1. These findings suggest that plastic-impacted urban soil can serve as a reservoir of LDPE-associated *Bacillus* isolates and identify *B. velezensis* A.4.I.1 as a priority candidate for follow-up biodegradation studies. Because FTIR/CI observations were exploratory and no mass-loss or mineralization endpoints were measured, the results should be interpreted as evidence of biodeterioration and early oxidative modification rather than complete LDPE biodegradation.

Keywords: *Bacillus* diversity, biodeterioration, indigenous bacteria, LDPE, urban soil

INTRODUCTION

Plastic pollution has become a persistent environmental problem because synthetic polymers are widely used, durable, and often poorly recovered after disposal. Since the beginning of large-scale plastic production, global plastic accumulation has increased rapidly, while recycling and waste-management systems have not expanded at the same rate (Geyer et al. 2017; Narancic and O'Connor 2019). Mismanaged plastic waste can accumulate in landfills, soils, rivers, and coastal environments, where it undergoes slow weathering and fragmentation into smaller particles (Restrepo-Flórez et al. 2014; Jambeck et al. 2015). In densely populated urban areas, plastic-contaminated soil may therefore represent both a pollution reservoir and a selective habitat for microorganisms adapted to anthropogenic stress.

Low-density polyethylene (LDPE) is one of the most common plastic polymers used in packaging films, carrier bags, and agricultural covers. Its long saturated carbon backbone, branching structure, hydrophobicity, and chemical resistance make LDPE highly resistant to abiotic degradation and microbial attack (Restrepo-Flórez et al. 2014; Ghatge et al. 2020). Although reduction, reuse, and recycling remain essential strategies, their limitations have encouraged interest in microbial and enzymatic approaches as complementary tools for plastic-waste management (Narancic and O'Connor 2019; Mohanan et al. 2020).

Polyethylene biodegradation is generally considered a stepwise process involving surface oxidation, microbial attachment, biofilm formation, polymer-chain scission, assimilation of smaller fragments, and eventual mineralization (Albertsson et al. 1987; Ru et al. 2020; Cai et al. 2023). However, complete biodegradation is difficult to demonstrate, especially in short-term laboratory studies. Therefore, microscopy and spectroscopy are often used as screening tools to detect early biodeterioration, such as surface roughening, pits, cracks, and formation of oxygen-containing functional groups (Sudhakar et al. 2008; Urbanek et al. 2018). These changes indicate polymer modification but should be distinguished from definitive evidence of mineralization.

Among bacteria reported in polyethylene studies, members of *Bacillus* Cohn 1872 are particularly relevant because they are widespread in soil, produce resistant endospores, tolerate environmental stress, and can form

attached communities on hydrophobic surfaces. Several studies have reported LDPE surface erosion and Fourier-transform infrared spectroscopy (FTIR)-detectable chemical changes after incubation with *Bacillus* isolates (Auta et al. 2017; Yao et al. 2022; Jayan et al. 2023; Jebashalomi et al. 2024). Indigenous bacteria from plastic-impacted environments are especially valuable for screening because they may already be acclimatized to local polymer-associated conditions and other environmental stressors (Mohanan et al. 2020; Khampratueng et al. 2024).

Public parking areas are urban microhabitats that may receive plastic fragments, dust, fuel residues, and fluctuating temperature and moisture exposure. These conditions may favor resilient, surface-colonizing bacteria, but such sites remain relatively understudied as sources of LDPE-associated microorganisms. Therefore, this study isolated indigenous *Bacillus*-like bacteria from plastic-contaminated soil in a university parking area in Tangerang, Indonesia, and evaluated their ability to induce early-stage LDPE modification. Surface alteration was assessed using scanning electron microscopy (SEM), chemical changes were examined by FTIR and carbonyl index analysis, and bacterial identity was determined by 16S rRNA gene sequencing. This study provides screening-level evidence of LDPE biodeterioration by cultivable *Bacillus* isolates from an urban plastic-impacted microhabitat.

MATERIALS AND METHODS

Soil sample extraction

Soil samples were collected from two plastic-contaminated sampling points within the public parking area of Universitas Pelita Harapan, Tangerang, Indonesia, using a purposive sampling approach targeting visible plastic-contaminated soil. At each sampling point, approximately 100 g of topsoil from 0-10 cm depth was aseptically collected using sterile spatulas and transferred into sterile ziplock bags. The samples were transported to the laboratory in an insulated container and stored at 4°C until processing. Two soil samples were transported to the laboratory in an insulated container and stored at 4°C until processing. During sampling, tools and containers were sterilized with 70% ethanol between locations to minimize cross-contamination. Unused sterile nutrient agar plates were incubated alongside working plates as negative controls for airborne contamination. Because the study was designed as exploratory isolation rather than formal biodiversity estimation, the two samples were used to recover representative cultivable *Bacillus* from a defined plastic-impacted microhabitat, and diversity inferences are limited to the isolates recovered from this site.

Procedures

Bacteria isolation and purification

Bacterial isolation was carried out using the spread-plating method. Briefly, 10 g of soil from each sample was suspended in 90 mL of sterile distilled water and shaken at 150 rpm for 30 min at 37°C to facilitate release and recovery of mesophilic heterotrophic bacteria, including *Bacillus*-like isolates, from the soil matrix. Serial ten-fold dilutions were prepared up to 10^{-6}, and 0.1 mL aliquots from appropriate dilutions were spread on King's B agar supplemented with 2% (w/v) polyethylene glycol (PEG). Plates were incubated aerobically at 37°C for 48 h. The temperature of 37°C was used as it supports the growth of mesophilic heterotrophic bacteria commonly found in soil samples. Morphologically distinct colonies were recorded, labeled, and purified by repeated streaking. King's B agar served as a nutritionally supportive medium for recovery of heterotrophic soil bacteria, whereas PEG was included only as an enrichment component to favor isolates tolerant of polymer-like substrates; its presence was not taken as evidence of LDPE-degrading ability. Pure isolates were maintained on nutrient agar slants at 4°C for short-term storage and as glycerol stocks at -20°C for long-term preservation.

Identification of cell morphology and colony

Colony morphology of purified isolates was recorded (shape, pigmentation, elevation, optical characteristics, and margin). Cellular morphology and Gram reaction were determined using Gram staining. Endospore formation was assessed using the Schaeffer-Fulton method, and catalase activity was tested by adding 3% H_2O_2 to fresh colonies and observing bubble formation. These phenotypic traits were used as preliminary criteria to classify isolates as *Bacillus*-like (Gram-positive, rod-shaped, endospore-forming) before molecular identification.

Preparation of minimal salt medium

Minimal salt medium (MSM) was prepared according to Ali et al. (2023) method with minor modifications. Each liter of MSM contained: K_2HPO_4 1.73 g, KH_2PO_4 0.68 g, $MgSO_4 \cdot 7H_2O$ 0.10 g, NaCl 4.00 g, $FeSO_4 \cdot 7H_2O$ 0.03 g, NH_4NO_3 1.00 g, and $CaCl_2 \cdot 2H_2O$ 0.02 g. The pH was adjusted to 7.0±0.2 with 1 M NaOH or HCl before sterilization at 121°C for 15 min. The medium did not contain an additional organic carbon source, so LDPE acted as the principal experimental substrate during the degradation assay.

Preparation of LDPE films

The plastic used in this study was low-density polyethylene (LDPE), cut into strips measuring 5 × 1 cm. To remove surface additives and contaminants, strips were washed with detergent, rinsed thoroughly with tap water and then with distilled water, and air-dried. For sterilization, strips were immersed in 70% ethanol for 30 min, rinsed in sterile distilled water, and dried under UV light in a laminar airflow cabinet for 24 h.

Preparation and standardization of bacterial inoculum

For the degradation assay, each isolate was first grown in a rich broth medium at 37°C with shaking (150-180 rpm) until the culture reached an optical density at 600 nm (OD_{600}) of approximately 0.6, measured using a spectrophotometer. At this point, an equal volume of culture was used for inoculation so that isolates were introduced at a comparable OD-based cell density. No separate growth-

curve experiment was performed to convert OD_{600} values into colony-forming units; OD_{600} was used operationally as a mid-exponential-phase reference for inoculation across isolates. Because no isolate-specific OD_{600}-CFU calibration was established, inoculum equivalence should be regarded as operational rather than absolute.

LDPE degradation assay in MSM

Sterile LDPE strips were placed into 250 mL Erlenmeyer flasks containing 100 mL of MSM. Each flask received one LDPE strip and 1% (v/v) of the standardized bacterial inoculum. For each isolate, three independent flasks (biological replicates) were prepared. Flasks were incubated at 37°C for 30 days under orbital shaking at 120-150 rpm to maintain aeration. An abiotic control was included, consisting of a sterile LDPE strip incubated in sterile, uninoculated MSM without bacterial inoculum. The abiotic control was maintained under the same experimental conditions as the inoculated flasks, including the same medium volume, flask type, temperature, shaking speed, and incubation period, to distinguish abiotic changes from bacteria-associated effects. Throughout incubation, no additional carbon source was supplied; therefore, LDPE served as the sole carbon and energy source for bacterial growth. At the end of the incubation period, LDPE strips were gently removed, washed with sterile distilled water to remove loosely attached cells and medium residues, and air-dried at room temperature. Dried strips were then either mounted for SEM analysis or stored in desiccators before FTIR analysis.

Scanning electron microscopy analysis

Changes in LDPE surface morphology after incubation were examined by scanning electron microscopy (SEM). LDPE strips from each treatment and from the abiotic control were fixed on aluminum stubs using conductive carbon tape and sputter-coated with a thin layer of gold to improve conductivity. Samples were analyzed at the Advanced Microscopy Laboratory, Universitas Prasetiya Mulya (Collaborative STEM Laboratories). Imaging was performed at 5-15 kV accelerating voltage and ×1,000 to ×5,000 magnification to visualize cracks, pits, biofilm structures, and other surface features.

Fourier transform infrared (FTIR) analysis

Chemical changes in LDPE were evaluated using Fourier transform infrared (FTIR) spectroscopy. After 30 days of incubation, LDPE strips from each bacterial treatment and from the abiotic control were analysed at PT Nanotech Indonesia Global using a Nicolet iS10 FTIR spectrometer in the mid-infrared range (4000-400 cm^{-1}). Spectra were acquired under the service provider's standard operating conditions. The extent of LDPE oxidation was quantified using the carbonyl index (CI), a semi-quantitative indicator calculated from baseline-corrected spectra. For each spectrum, CI was calculated as the ratio of absorbance at the carbonyl band (A_{1652}, C=O stretching) to the absorbance of the reference methylene band (A_{1461}, CH_2 bending):

$$CI = \frac{A_{1652}}{A_{1461}}$$

An increase in CI relative to the abiotic control was interpreted as evidence of increased LDPE oxidative modification, not as stand-alone proof of complete biodegradation.

Analysis 16S rRna gene sequencing

Molecular identification of bacterial isolates was performed by 16S rRNA gene sequencing at PT Genetika Science Indonesia. Genomic DNA was extracted from overnight cultures using a commercial kit. The 16S rRNA gene was amplified by PCR using universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) under standard cycling conditions. Amplicons were purified and sequenced in both directions. Consensus sequences were assembled and edited, then compared against the NCBI database using BLASTn to obtain the closest relatives and percentage similarity. Multiple sequence alignment of isolates and reference *Bacillus* sequences was conducted using Clustal Omega. A neighbour-joining phylogenetic tree was constructed in MEGA X using the Tamura-Nei substitution model with 1,000 bootstrap replicates. *Escherichia coli* JCM 1649 was used as an outgroup. The final tree was visualized and annotated using iTOL.

Data analysis

FTIR measurements were obtained once for the abiotic control and once for each bacterial treatment (n = 1 per condition). Accordingly, CI values are reported as single observed values for exploratory comparison only, and no inferential statistical analysis was applied. Interpretation focuses on concordance between CI, qualitative FTIR band changes, and SEM observations of surface alteration.

RESULTS AND DISCUSSION

Isolation and characterization of bacterial isolates

Soil collected from plastic-contaminated spots in the public parking area of Universitas Pelita Harapan yielded multiple colony types on King's B agar supplemented with 2% PEG (Figure 1). Eight morphologically distinct colonies were purified by repeated streaking and designated A.4.I.1, A.4.I.2, A.4.I.5, A.4.I.6, A.4.I.7, A.4.I.8, A.4.I.9, and A.4.I.10 (Figure 2). Each colony formed was isolated and further purified to get individual bacterial colonies. This process resulted in 8 different colonies that can be seen in Figure 2. All isolates that displayed distinct colony and cellular morphologies were further determined through Gram staining, catalase testing, and endospore staining (Table 1).

The observed variation in colony architecture suggests that even within a single urban microhabitat, cultivable *Bacillus* isolates display differentiated surface-growth phenotypes that may influence attachment and colonization of polymer surfaces. Results showed that most isolates formed wrinkled colonies with undulate margins and white

pigmentation, while A.4.I.1 displayed a root-like colony form and A.4.I.9 produced circular colonies with entire margins. All eight isolates were Gram-positive, catalase-positive, and endospore-forming rods. Molecular identification based on 16S rRNA gene sequencing assigned the isolates to *Bacillus velezensis*, *Bacillus altitudinis*, *Bacillus licheniformis*, *Bacillus paralicheniformis*, and *Bacillus subtilis*.

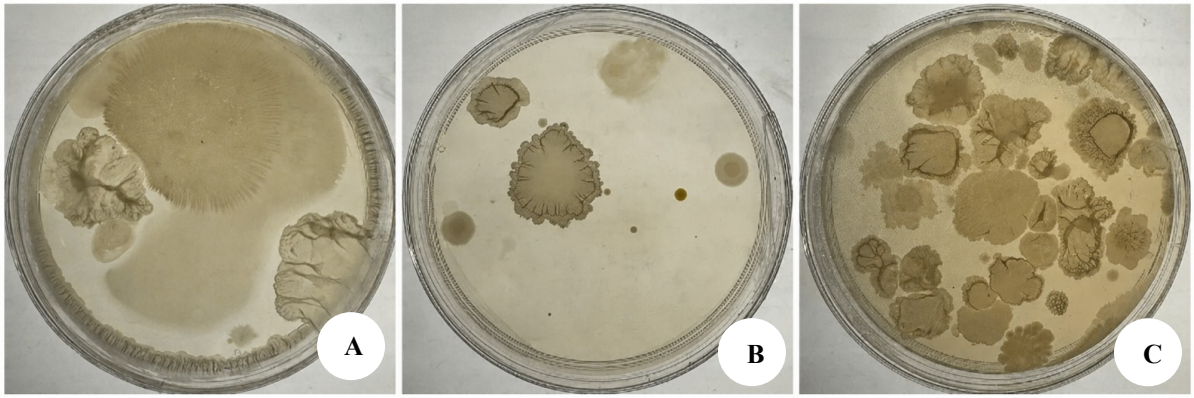

Figure 1. Morphological diversity of bacterial colonies isolated from plastic waste samples. A. Petri dish 1, B. Petri dish 2, C. Petri dish 3

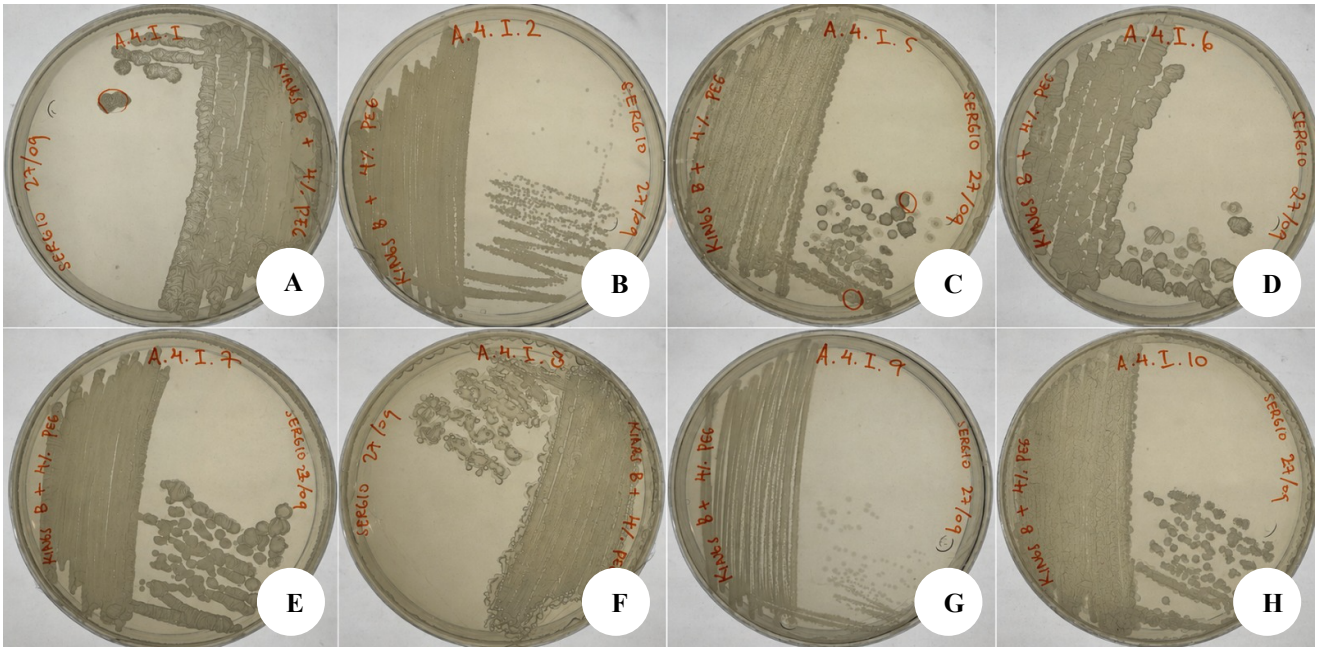

Figure 2. Purification of isolates. A. A.4.I.1, B. A.4.I.2, C. A.4.I.5, D. A.4.I.6, E. A.4.I.7, F. A.4.I.8, G. A.4.I.9, H. A.4.I.10

Table 1. Characterization of bacterial isolates based on colony morphology, Gram reaction, catalase activity, and endospore formation

Isolates code	Colony shape	Margin	Elevation	Optical characteristics	Color	Gram stain	Catalase test	Endospore stain	Cell shape
A.4.I.1	Root-like	Lobate	Pulvinate	Opaque	White	+	+	+	Rod-shaped
A.4.I.2	Wrinkled	Undulate	Flat	Opaque	White	+	+	+	Rod-shaped
A.4.I.5	Wrinkled	Undulate	Pulvinate	Opaque	White	+	+	+	Rod-shaped
A.4.I.6	Wrinkled	Undulate	Pulvinate	Opaque	White	+	+	+	Rod-shaped
A.4.I.7	Wrinkled	Undulate	Flat	Opaque	White	+	+	+	Rod-shaped
A.4.I.8	Wrinkled	Undulate	Flat	Opaque	White	+	+	+	Rod-shaped
A.4.I.9	Circular	Entire	Flat	Opaque	White	+	+	+	Rod-shaped
A.4.I.10	Wrinkled	Undulate	Undulate	Opaque	White	+	+	+	Rod-shaped

Plastic degradation assay

Plastic degradation assay in minimal salt medium (MSM) supplemented with LDPE strips showed that all treatments appeared visually clear at day 0, with no observable turbidity or color change (Figure 3). After 14 days of incubation, inoculated flasks exhibited visible turbidity and changes in medium coloration (Figure 4). In contrast, the control flasks remained clear without noticeable turbidity or color change throughout the incubation period.

Scanning electron microscopy (SEM) analysis of LDPE plastic degraded by bacterial isolates

SEM imaging showed clear differences between LDPE strips incubated with bacterial isolates and the abiotic control. The control LDPE retained a smooth, relatively homogeneous surface with no visible cracks, pits, or deposits after 30 days (Figure 5). Descriptively, A.4.I.1 showed the strongest surface alteration, A.4.I.5 and A.4.I.10 showed intermediate-to-strong erosion and cracking, A.4.I.2, A.4.I.7, A.4.I.8, and A.4.I.9 showed moderate roughening and deposits, and A.4.I.6 showed the mildest effect.

In contrast, inoculated LDPE strips exhibited isolate-specific surface alterations after 30 days (Figure 6). The most pronounced physical disruption was observed for A.4.I.1 (Figure 6.A), including large holes, deep cracks, and surface roughening. A.4.I.5 (Figure 6.C) and A.4.I.10 (Figure 6.H) showed visible erosion with irregular and cracked regions. For A.4.I.2 (Figure 6.B), A.4.I.7 (Figure 6.E), A.4.I.8 (Figure 6.F), and A.4.I.9 (Figure 6.G), LDPE surfaces generally showed roughened textures, scattered small pits, and attached cell-like or extracellular polymeric substance (EPS)-like material. A.4.I.6 (Figure 6.D) exhibited only slight roughening with few discrete defects.

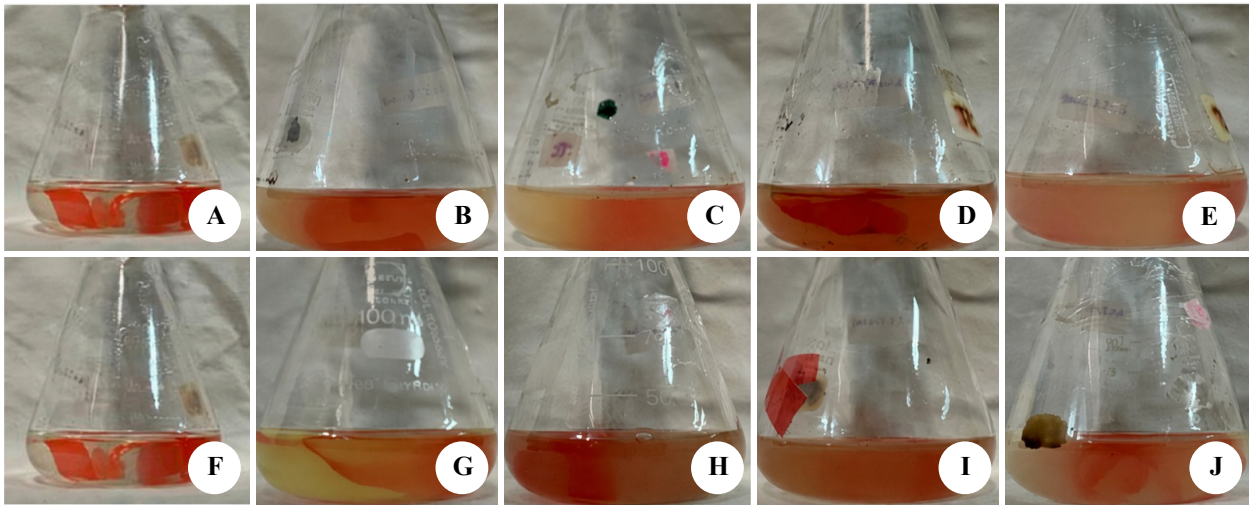

Figure 3. Plastic degradation assay at day 0. A. Control, B. A.4.I.1, C. A.4.I.2, D. A.4.I.5, E. A.4.I.6, F. Control, G. A.4.I.7, H. A.4.I.8; I. A.4.I.9, J. A.4.I.10

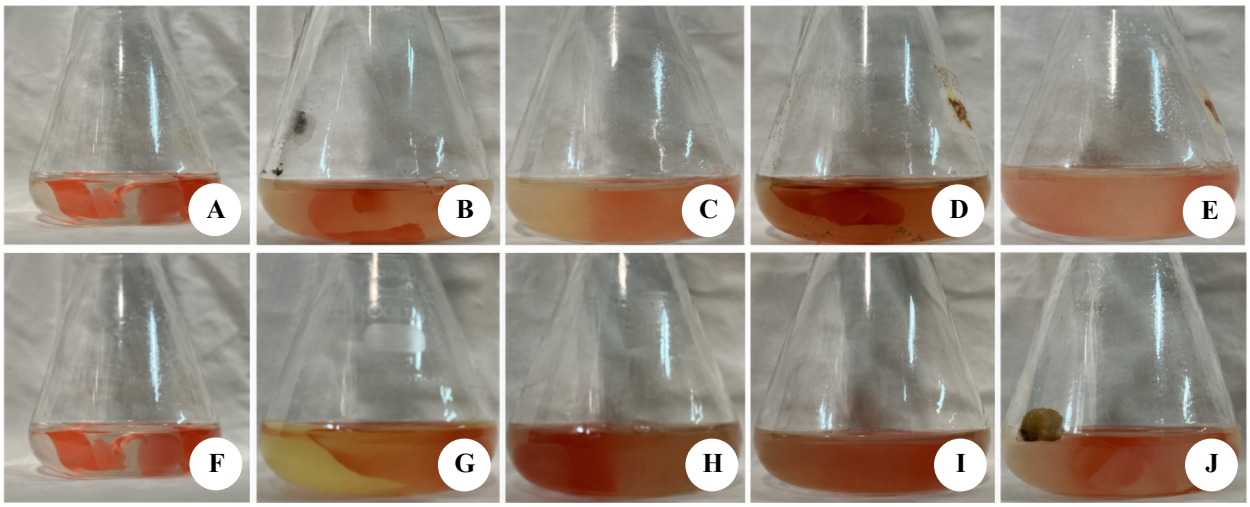

Figure 4. Plastic degradation assay at day 14. A. Control, B. A.4.I.1, C. A.4.I.2, D. A.4.I.5, E. A.4.I.6, F. Control, G. A.4.I.7, H. A.4.I.8, I. A.4.I.9, J. A.4.I.10

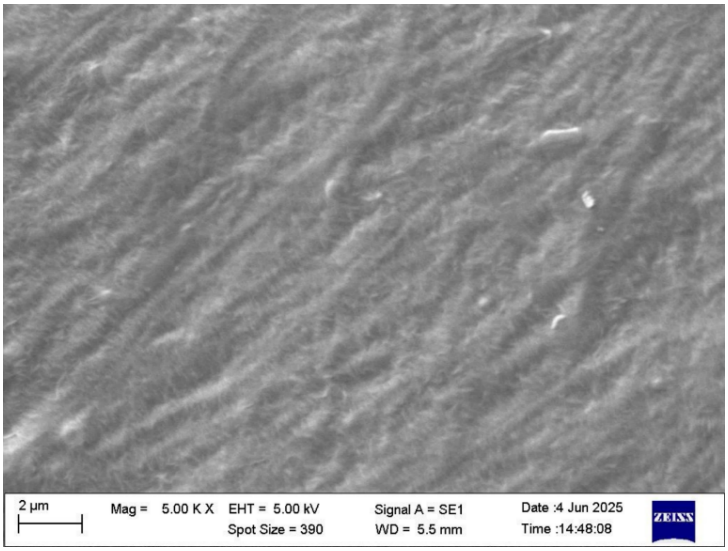

Figure 5. Control LDPE film after 30 days of incubation in sterile MSM

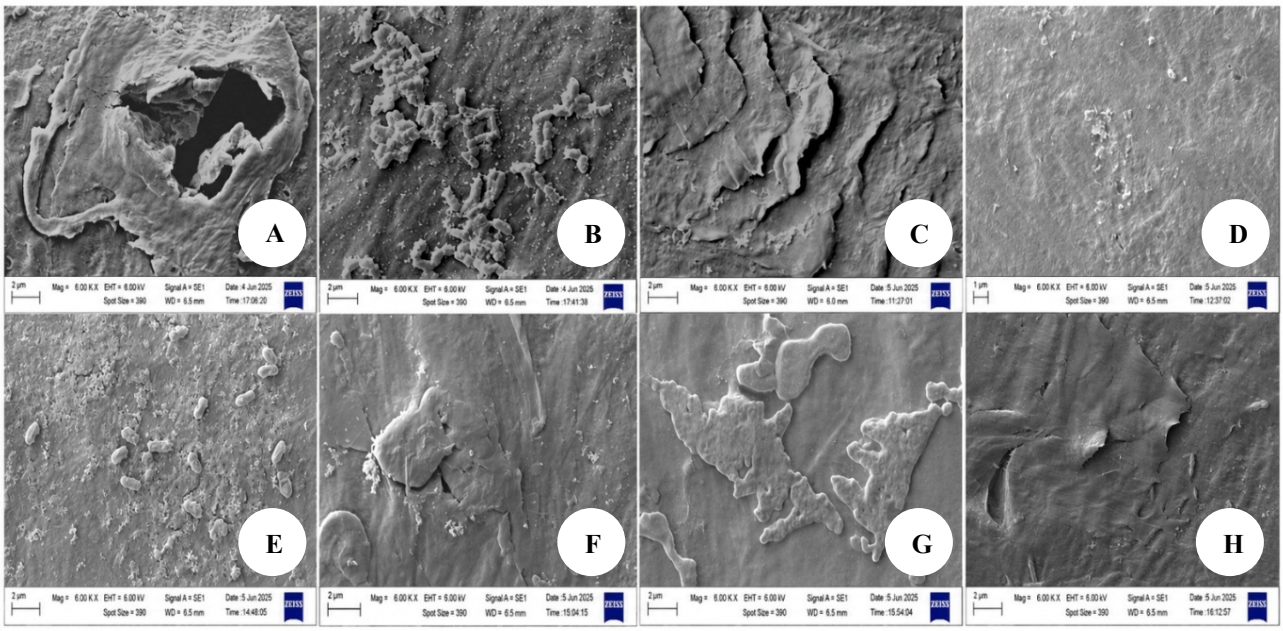

Figure 6. SEM images of LDPE films after 30 days of incubation with bacterial isolates. A. A.4.I.1, B. A.4.I.2, C. A.4.I.5, D. A.4.I.6, E. A.4.I.7, F. A.4.I.8, G. A.4.I.9, H. A.4.I.10. Key observed features: A.4.I.1, holes and deep cracks; A.4.I.2, roughening and attached material; A.4.I.5, erosion and cracking; A.4.I.6, slight roughening; A.4.I.7, pits and attached material; A.4.I.8, pits and attached material; A.4.I.9, EPS-like deposits; A.4.I.10, erosion and cracking

Fourier transform infrared spectroscopy (FTIR) analysis of LDPE plastic degraded by bacterial isolates

FTIR spectra of the abiotic control showed the characteristic LDPE profile, including major C-H stretching bands near 2915 and 2847 cm^{-1} and CH_2 bending bands near 1461-1472 cm^{-1} (Figure 7). After 30 days of incubation, inoculated films showed additional or intensified bands around 1652 cm^{-1} and 1040 cm^{-1} relative to the control, consistent with oxidation-related modification of the LDPE surface. Because FTIR spectra and CI values were obtained once per treatment, these differences are interpreted descriptively as screening-level observations rather than statistically ranked effects.

After 30 days of incubation with bacterial isolates, additional bands were observed or became more pronounced relative to the control, including around 1652 cm^{-1}, 1040 cm^{-1}, and 1537 cm^{-1} (Figure 7).

To summarize the 1652 cm^{-1} change across isolates, a carbonyl index (CI) was calculated as the ratio of absorbance at 1652 cm^{-1} to absorbance at 1461 cm^{-1} (Table 2). CI values ranged from 0.190 to 0.346 across isolates. The highest CI was observed for A.4.I.1 (0.346), followed by A.4.I.2 (0.246) and A.4.I.7 (0.243), while A.4.I.5 (0.190) was the lowest.

Isolates identification by 16S rRNA gene sequencing

The neighbour-joining tree recovered species-level *Bacillus* groupings that were broadly consistent with the BLASTn assignments. Isolates A.4.I.1 and A.4.I.6 clustered with *B. velezensis*, A.4.I.2 clustered with *B. altitudinis*, and A.4.I.7 clustered with *B. subtilis* (Figure 8). Isolates A.4.I.5, A.4.I.8, and A.4.I.9 were assigned to *B. licheniformis* based on BLASTn similarity, whereas A.4.I.10 was assigned to *B. paralicheniformis* (Table 3). Because *B. licheniformis* and *B. paralicheniformis* are closely related taxa, partial 16S rRNA gene sequences may provide limited resolution between them. Therefore, the phylogenetic result was interpreted together with BLASTn similarity values, and the species names are presented as the closest taxonomic assignments based on the available 16S rRNA sequence data.

Discussion

The appearance of turbidity and color changes in inoculated flasks after 14 days indicates microbial growth under nutrient-limited conditions, suggesting that the bacterial isolate was able to utilize LDPE as a carbon source. The absence of similar changes in control flasks confirms that abiotic degradation of LDPE was negligible, and that the observed effects were primarily due to microbial activity. The ability of bacterial isolates to grow in MSM containing LDPE reflects their potential to utilize polymer-derived compounds. During this process, microorganisms may promote polymer oxidation, surface biodeterioration, and fragmentation into lower-molecular-weight compounds that can subsequently enter microbial metabolic pathways (Ghatge et al. 2020; Cai et al. 2023; Zhang et al. 2023).

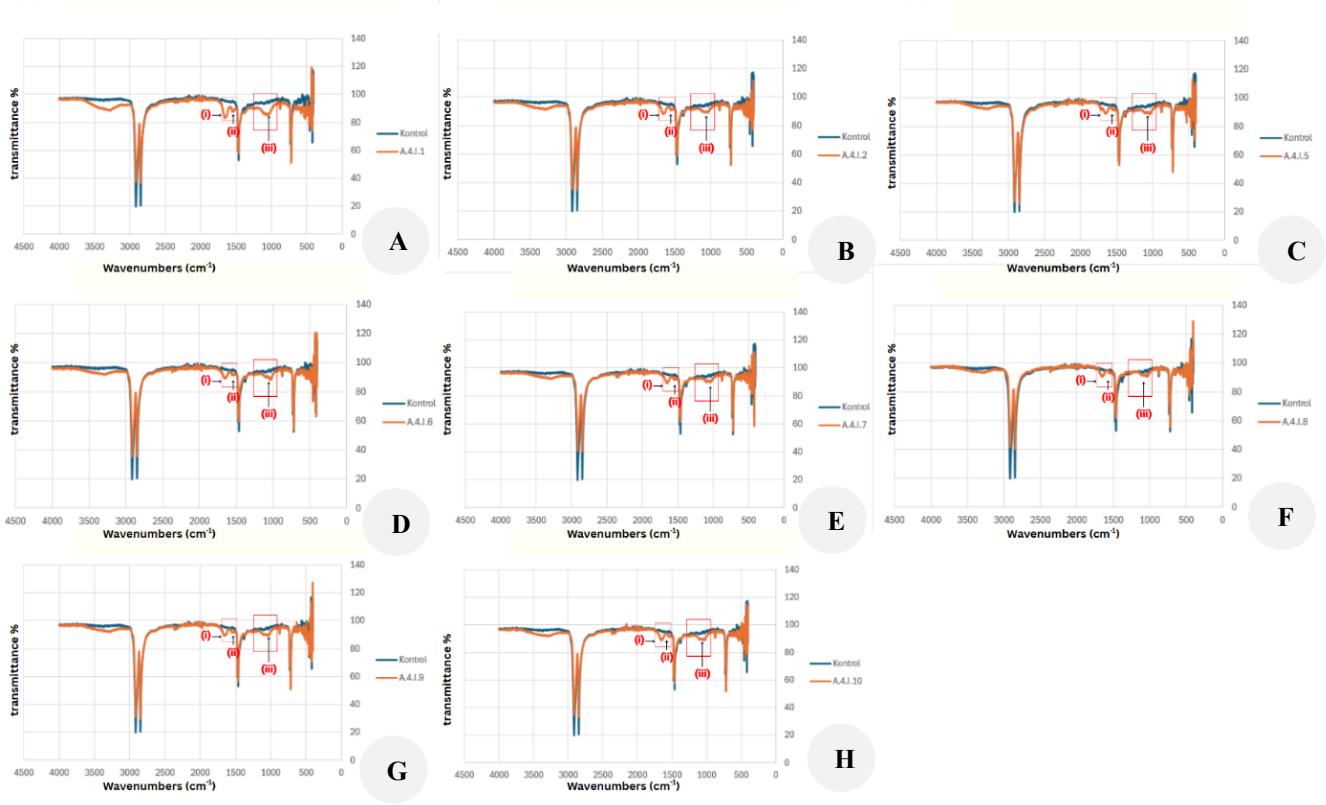

Figure 7. FTIR spectra of LDPE films after 30 days of incubation with bacterial isolates. A. A.4.I.1, B. A.4.I.2, C. A.4.I.5, D. A.4.I.6, E. A.4.I.7, F. A.4.I.8, G. A.4.I.9, H. A.4.I.10. Highlighted regions: (i) 1650-1750 cm^{-1}; (ii) ~1040 cm^{-1}; (iii) ~1537 cm^{-1}

Table 2. Absorbance values used for carbonyl index calculation (CI = A1652/A1461) after 30 days incubation

Isolates code	C=O (%Transmission) 1652 cm^{-1}	Reference Peak (%Transmission) 1461 cm^{-1}	C=O (Absorbance)	Reference peak (Absorbance)	Carbonyl index (CI)
A.4.I.1	82.74	57.79	0.082	0.238	0.346
A.4.I.2	87.83	58.98	0.056	0.229	0.246
A.4.I.5	88.73	53.29	0.052	0.273	0.190
A.4.I.6	89.02	59.57	0.051	0.225	0.225
A.4.I.7	88.83	61.47	0.051	0.211	0.243
A.4.I.8	90.27	61.80	0.044	0.209	0.213
A.4.I.9	89.15	56.26	0.050	0.250	0.200
A.4.I.10	88.55	57.58	0.053	0.240	0.220

Note: FTIR spectra (and CI values) were obtained once per treatment; therefore, CI values are reported as indicative rather than statistically compared across isolates

Table 3. Identification of bacterial isolates based on 16S rRNA gene sequences

Isolates code	Accession number	Length (bp)	Species most related	Sequence similarity (%)
A.4.I.1	PX484458	1417	*Bacillus velezensis* Ruiz-García et al. 2005	100
A.4.I.2	PX484456	1404	*Bacillus altitudinis* Shivaji et al. 2006	100
A.4.I.5	PX484460	1419	*Bacillus licheniformis* (Weigmann, 1898) Chester, 1901	99.93
A.4.I.6	PX484459	1413	*Bacillus velezensis* Ruiz-García et al. 2005	99.93
A.4.I.7	PX484457	1403	*Bacillus subtilis* (Ehrenberg 1835) Cohn 1872	99.93
A.4.I.8	PX484463	1083	*Bacillus licheniformis* (Weigmann, 1898) Chester, 1901	100
A.4.I.9	PX484464	1083	*Bacillus licheniformis* (Weigmann, 1898) Chester, 1901	100
A.4.I.10	PX484461	1420	*Bacillus paralicheniformis* Dunlap et al. 2015	99.58

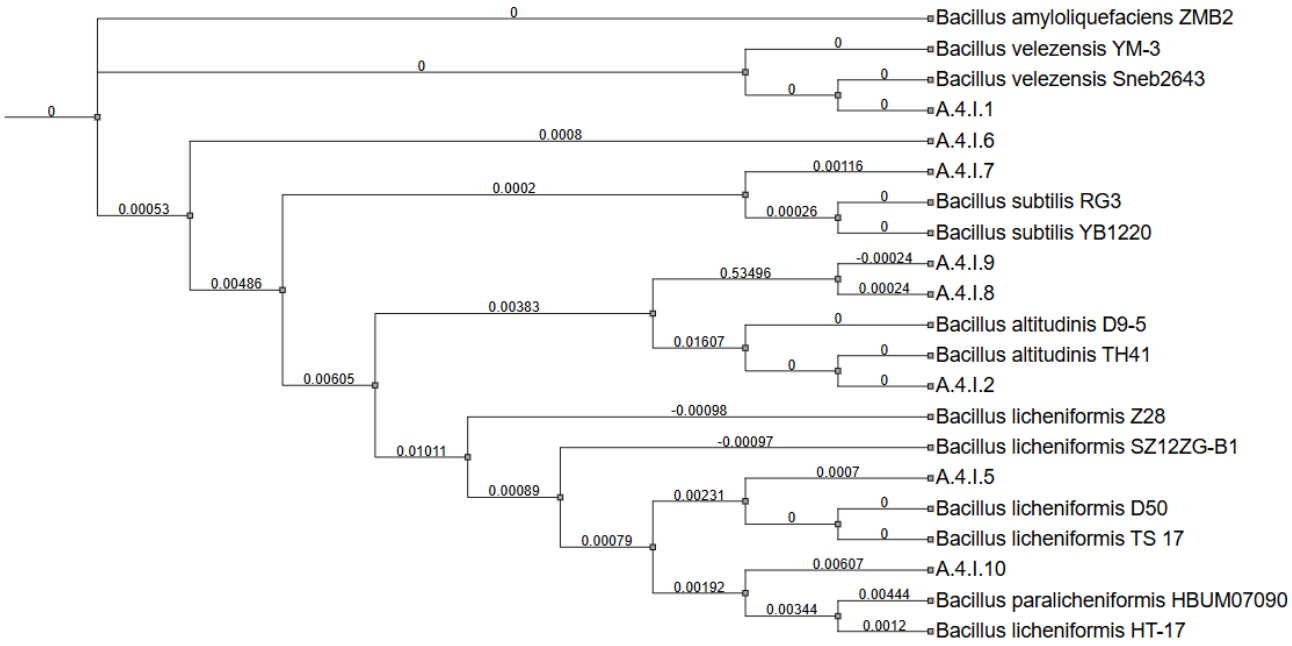

Figure 8. Neighbor-joining phylogenetic tree based on partial 16S rRNA gene sequences of isolates A.4.I.1, A.4.I.2, A.4.I.5, A.4.I.6, A.4.I.7, A.4.I.8, A.4.I.9, and A.4.I.10 and closely related *Bacillus* reference strains retrieved from GenBank. The tree was constructed in MEGA X using the Tamura-Nei model; bootstrap values (1000 replicates) are shown at the nodes (values <50% not shown). *Escherichia coli* JCM 1649 was used as the outgroup. Scale bar indicates substitutions per nucleotide position

The indigenous bacterial isolates recovered from plastic-contaminated soil in the public parking area of Universitas Pelita Harapan showed isolate-dependent ability to alter untreated low-density polyethylene (LDPE) under carbon-limited conditions. Further support for LDPE degradation is provided by FTIR analysis, which showed changes in functional groups such as the appearance or increase of hydroxyl (-OH), carbonyl (C=O), and C-O bonds. These functional groups are commonly associated with oxidative biodegradation of polyethylene (Khampratueng et al. 2024). Such chemical modifications indicate structural alterations in the polymer chain and suggest microbial involvement in the degradation process. Similar findings have been reported in recent studies, where bacterial isolates induced changes in LDPE chemical structure detected by FTIR, including reduction of C-H bonds and formation of oxygen-containing functional groups (Widiyanti et al. 2025). These transformations are indicative of depolymerization and oxidation processes during biodegradation.

Across treatments, SEM revealed surface roughening, pitting, cracking, and perforation, while FTIR indicated oxidation-related changes, particularly in the carbonyl and C-O regions. Taken together, these data support biodeterioration and early-stage oxidative modification rather than complete biodegradation, because polymer mass loss, crystallinity change, molecular-weight reduction, and end-product formation were not measured.

The SEM observations provide the clearest direct evidence of biodeterioration. Among the eight isolates, A.4.I.1 produced the strongest visible disruption, followed by A.4.I.5 and A.4.I.10, whereas A.4.I.6 showed only mild roughening. The remaining isolates caused intermediate roughening, small pits, or surface-associated deposits. Similar

morphologies have been reported during early bacterial attack on untreated LDPE, in which attachment and biofilm formation precede more pronounced erosion and crack development (Harshvardhan and Jha 2013; Dey et al. 2020; Yao et al. 2022). The observation of deposits and roughened regions in several treatments is therefore consistent with colonization-associated surface conditioning rather than with deep polymer mineralization (Budhiraja et al. 2025).

FTIR results were congruent with the SEM observations. Inoculated films showed new or intensified bands around 1652 cm^{-1} and 1040 cm^{-1} relative to the abiotic control, indicating oxidation-related surface modification. The carbonyl index ranged from 0.190 to 0.346, with the value for A.4.I.1 being the highest value. These CI values were best interpreted descriptively because FTIR was performed once per treatment. CI is sensitive to baseline correction, band selection, film thickness, and surface heterogeneity (Dey et al. 2020; Nguyen et al. 2024; Kong et al. 2025). Even so, the pattern is consistent with modest oxidation under untreated-film and short-incubation conditions. Stronger oxidation is often reported when LDPE is pretreated or when incubations are longer and more favorable to biofilm development (Auta et al. 2017; Ji et al. 2024; Afify et al. 2025; Soni et al. 2025).

The CI values observed in this study are consistent with modest oxidation under untreated-film and short-incubation conditions. In contrast, stronger oxidation and more pronounced physical deterioration have often been reported in studies using pretreated LDPE (for example, UV, plasma, or irradiation treatment) or longer incubation periods under conditions that promote biofilm development (Auta et al. 2017; Ji et al. 2024; Afify et al. 2025; Kong et al. 2025). Thus, the present results support the occurrence of early-stage oxidative modification of LDPE by the tested isolates, but they did not by themselves justify a claim of complete biodegradation. Additional endpoints, such as mass loss, molecular-weight reduction, crystallinity changes, enzyme activity, or identification of metabolic intermediates, would be needed to confirm further degradation (Jeon and Kim 2015; Suzuki et al. 2025; Kong et al. 2025).

A plausible interpretation is biofilm-mediated surface oxidation followed by limited chain scission. *Bacillus* cells may first colonize the hydrophobic LDPE surface, form localized biofilms, and create a microenvironment in which extracellular enzymes, surfactants, and reactive metabolites promote oxidation of the polymer surface. The introduction of oxygen-containing groups can increase surface polarity and make the material more susceptible to subsequent fragmentation and uptake of smaller molecules. This conceptual pathway is consistent with literature on polyethylene biodeterioration and with the surface-associated material observed here, but it remains a hypothesis in the present study because enzyme activities and metabolic intermediates were not directly measured (Jeon and Kim 2015; Tarafdar et al. 2021; Wang et al. 2023; Suzuki et al. 2025).

This study remains to be explored in several respects. Only two soil samples were collected from one parking-area microhabitat, the isolation strategy targeted cultivable Bacillus-like bacteria rather than full community diversity, inoculum equivalence was operationally standardized by OD_{600} rather than CFU calibration, and FTIR/CI measurements were not replicated for inferential analysis. In addition, the abiotic control alone did not fully separate metabolic effects from attached residue effects, and growth in LDPE-containing MSM cannot exclude small contributions from inoculum carryover. Future work should therefore expand spatial sampling, include replicated analytical endpoints, verify public sequence accessions, and combine SEM/FTIR with gravimetric mass loss, crystallinity analysis, tensile testing, CO_2 evolution, and direct assays of biofilm formation and enzyme activity. Within the current dataset, however, *B. velezensis* A.4.I.1 remains the strongest candidate for further study.

Morphological tests and 16S rRNA sequencing placed all isolates within Bacillus, including *B. velezensis, B. altitudinis*, *B. licheniformis*, *B. paralicheniformis*, and *B. subtilis*. These findings indicate that the isolates are dominated by resilient taxa that can persist under stressed environmental conditions. Their repeated recovery from this plastic-impacted urban microhabitat suggests possible habitat filtering under anthropogenic stress, where exposure to plastic fragments, dust, fuel residues, and fluctuating temperature and moisture may favor organisms capable of survival and surface attachment.

Bacillus spp. is widely reported from stressed terrestrial habitats due to their ability to form endospores, metabolic flexibility, and strong surface-colonization capacity (Auta et al. 2017; Atanasova et al. 2021; Tarafdar et al. 2021). In this context, the present study contributes to biodiversity knowledge by documenting cultivable *Bacillus* diversity associated with a specific urban plastic-related niche.

From an ecological and applied perspective, the finding that multiple *Bacillus* isolates from an urban soil microhabitat were able to induce oxidative changes on untreated LDPE suggests that plastic-impacted soils may serve as reservoirs of microorganisms with plastic-associated metabolic potential. At the same time, these results indicate that biodegradation performance may be improved by selecting top-performing isolates such as A.4.I.1, evaluating defined microbial consortia, and combining biological treatment with suitable pretreatment strategies in future studies.

In conclusion, this exploratory study isolated eight *Bacillus* spp. from plastic-contaminated urban soil and showed that they can induce isolate-dependent LDPE surface deterioration and oxidative modification under carbon-limited conditions. The strongest combined SEM and FTIR screening signal was produced by *B. velezensis* A.4.I.1, which should be prioritized for follow-up study. These findings support the view that plastic-impacted urban soils can serve as reservoirs of *Bacillus* with plastic-associated functional traits. Because the evidence is based on short-term SEM observations and single-measurement FTIR/CI data without mass-loss or mineralization endpoints, the results should be interpreted as screening-level biodeterioration rather than definitive proof of complete LDPE biodegradation.

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