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Enhancement Of Biodegradation Of Low-Density Polyethylene (LDPE) Using Extracellular Bacterial Enzymes

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Abstract

Plastic pollution represents one of the most pressing environmental challenges of the 21st century, with polyethylene (PE) being among the most persistent polymers due to its hydrophobic surface properties and high molecular weight characteristics. This comprehensive study investigates the LDPE biodegradation potential using extracellular enzymes produced by four indigenous bacterial strains: Cytobacillus firmus, Priestia flexa, and Stutzerimonas stutzeri,. LDPE sheets were subjected to controlled incubation with these bacterial isolates for 30 days under optimized laboratory conditions. Biodegradation assessment was conducted through multiple analytical approaches including gravimetric weight loss analysis, surface hydrophobicity modifications, Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM). The bacterial strains demonstrated significant extracellular enzyme activities including laccase, lipase, and esterase, with corresponding measurable increases in carbonyl index values and observable surface erosion patterns.

Results indicate that synergistic enzymatic action facilitates polymer oxidation and subsequent fragmentation processes, highlighting a promising biological approach for sustainable plastic waste mitigation strategies in environmental biotechnology applications.

Keywords: *LDPE biodegradation, extracellular enzymes, bacterial consortium, plastic waste management, environmental biotechnology, polymer degradation*

1. INTRODUCTION

1.1 Global Plastic Pollution Crisis

The unprecedented accumulation of plastic waste in terrestrial and marine ecosystems has emerged as a critical environmental challenge requiring urgent intervention strategies (Geyer et al., 2017). Polyethylene (PE), accounting for approximately 34% of global plastic production demand, represents the most abundant polymer in environmental waste streams (PlasticsEurope, 2020). Among PE variants, low- density polyethylene (LDPE) finds extensive applications in packaging materials, agricultural films, and consumer bags, contributing significantly to environmental plastic burden due to its widespread usage and disposal patterns (Chamas et al., 2020).

Environmental Persistence of LDPE exhibits exceptional environmental persistence attributed to its unique structural characteristics including hydrophobic surface properties, high molecular weight, and crystalline polymer organization (Gewert et al., 2015). These properties render LDPE highly recalcitrant to natural degradation processes, with estimated environmental persistence ranging from 100 to 1000 years under ambient conditions (Barnes et al., 2009). Conventional polymer fragmentation results in microplastic formation, which subsequently disperses through terrestrial and aquatic ecosystems, posing significant ecological risks and bioaccumulation concerns (Cole et al., 2011).

1.2 Limitations of Current Waste Management Approaches

Traditional physicochemical recycling methodologies, while technologically feasible, present substantial limitations including high energy requirements, Environmental pollution, Economic constraints, and limited applicability to contaminated or mixed plastic waste streams (Hopewell et al., 2009). Mechanical recycling processes often result in polymer quality degradation, limiting recyclate applications and creating secondary waste streams (Al-Salem et al., 2009). Chemical recycling approaches, though promising, require harsh reaction conditions and specialized infrastructure investments that may not be economically viable for developing regions (Lopez et al., 2017).

Microbial degradation of synthetic polymers has emerged as a promising sustainable alternative to conventional waste management approaches, offering potential advantages including ambient operating conditions, lower energy requirements, and complete mineralization Without polluting the environment(Wei & Zimmermann, 2017). However, LDPE biodegradation faces significant challenges due to polymer hydrophobicity, high molecular weight, and limited bioavailability for microbial enzymatic systems (Krueger et al., 2015). Polyethylene terephthalate (PET) degrades into its basic parts in a multistep process that necessitates the use of specialist enzymes. Initially, PET hydrolases (such as PETase) hydrolyze PET's ester bonds, producing intermediates such as mono-(2-hydroxyethyl) terephthalic acid (MHET) and terephthalic acid (TPA). MHETase and related enzymes break down these intermediates into simple monomers. Microorganisms metabolize PET monomers into CO_2 and H_2O, indicating full mineralization. This approach demonstrates the potential for biological recycling of PET trash into environmentally friendly products (Kawai, 2020).

Enzymatic Mechanisms in Polymer Degradation Recent advances in understanding polymer biodegradation mechanisms have highlighted the crucial role of extracellular enzymes in initiating polymer chain cleavage and facilitating subsequent microbial assimilation (Danso et al., 2019). Oxidative enzymes such as laccases can introduce hydrophilic functional groups into hydrophobic polymer chains, while hydrolytic enzymes including lipases and esterases can cleave oxidized polymer bonds (Sulaiman et al., 2012). The synergistic action of multiple enzymatic systems may significantly enhance biodegradation rates compared to individual enzyme applications (Wilkes & Aristilde, 2017).

1.3 Research Objectives

This study aims to evaluate the biodegradation potential of LDPE using indigenous soil bacterial isolates with demonstrated extracellular enzyme production capabilities. Specific objectives include: (i) isolation and characterization of hydrocarbon-degrading bacteria from landfill environments, (ii) assessment of cell-surface hydrophobicity and enzymatic activities, (iii) evaluation of LDPE weight loss and structural modifications under controlled laboratory conditions, and (iv) mechanistic understanding of enzymatic biodegradation processes through spectroscopic and microscopic analyses.

2. MATERIALS AND METHODS

2.1 Sample Collection and Site Characterization

Soil samples were collected from five strategically selected landfill sites across the Ahmedabad metropolitan region, Gujarat, India (coordinates: 23.0436°N, 72.5471°E; 23.0395°N, 72.6189°E; 22.9637°N, 72.5462°E; 22.9690°N, 72.5578°E; 22.9732°N, 72.5549°E). Collection sites were chosen based on historical plastic waste deposition patterns and diverse environmental conditions to maximize bacterial diversity potential. Samples were collected aseptically using sterile collection vials and transported to the laboratory under refrigerated conditions (4°C) within 4 hours of collection.

2.2 Bacterial Isolation and Enrichment

One gram of each soil sample was inoculated into nutrient broth (NB, pH 7.0 ± 0.2) and subjected to enrichment culture conditions (120 rpm orbital shaking, 30°C, 24 h). Following initial enrichment, serial dilutions (10^{-6}, 10^{-8}, 10^{-10}) were prepared in sterile phosphate-buffered saline and plated onto nutrient agar medium. Plates were incubated at 37°C for 24 hours, and morphologically distinct colonies were selected for purification through five-sector streaking methodology to ensure clonal isolation.

2.3 Preliminary Screening for Hydrocarbon Utilization

Bacterial isolates were screened for hydrocarbon degradation capability using minimal essential medium (MEM) agar supplemented with 1% (v/v) emulsified n-hexadecane as sole carbon source. The emulsion was prepared through sonication (20 kHz, 5 minutes) to ensure uniform distribution. Plates were incubated at 37°C for 7 days and subsequently flooded with 0.1% Coomassie Brilliant Blue solution. Clear halo zone formation around bacterial colonies indicated hydrocarbon utilization capability and positive isolates were selected for further characterization.

2.4 Bacterial Identification and Phylogenetic Analysis

Selected bacterial isolates were identified through 16S rRNA gene sequencing methodology. Genomic DNA was extracted using standard phenol-chloroform extraction protocol, and the 16S rRNA gene was amplified using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'- TACGGYTACCTTGTTACGACTT-3'). PCR products were purified and sequenced using Sanger sequencing technology. Sequence analysis and phylogenetic identification were performed using BLAST algorithm and EzTaxon-e database comparisons.

2.5 Assessment of Cell-Surface Hydrophobicity

Cell-surface hydrophobicity was quantified using the Bacterial Adherence to Hydrocarbon (BATH) assay methodology. Mid-logarithmic phase bacterial cultures were harvested, washed twice with phosphate- buffered saline (PBS, pH 7.1), and resuspended to achieve optical density (OD_{600}) of approximately 1.0. Equal volumes (3 mL each) of bacterial suspension and n-hexadecane were combined in test tubes, vortexed vigorously for 2 minutes, and allowed to phase separate for 15 minutes at room temperature. The aqueous phase optical density was measured, and hydrophobicity percentage was calculated using the formula: Hydrophobicity (%) = [(OD_initial - OD_final)/OD_initial] × 100.(Harshvardhan & Jha, 2013)

2.6 Extracellular Enzyme Activity Assays

2.6.1 Laccase Activity Determination

Laccase assay was performed in micro centrifuge tubes and readings were taken in microwell plate reader(Bio-Rad [20522]). From biodegradation assays, cultures were transferred in sterile micro centrifuge tube and centrifugation was done at 7000 rpm for 5 minutes. The supernatant was collected. Furthermore, the assay was performed with the addition of 0.2M Sodium acetate buffer, 1mM Guaiacol solution and supernatant of sample in 1:1:1 proportion. Then, these tubes were vortexed or mixed properly, and they were incubated overnight. The distinctive pink pigmentation produced in the assay are analyzed at 420nm in microplate reader . (Kharwar et al., 2014, Ahmed & McKay, 2019).

2.6.2 Lipase Activity Assessment

Plates were prepared using sterile media of Peptone 5gm/L, Yeast Extract 3%, CaCL2 2.5mM,MgSO4 10mM, Tributyrin 10ml/L, Agar Bacto 3%. Then 6-7µl Culture dropped on autoclaves Whatman paper placed in plate. And incubated for 48 hours at room temperature for observation of the clear zone (Sayali et al., 2013, Carrazco-Palafox et al., 2018, Danso et al., 2019).

2.7 LDPE Biodegradation Experimental Design

2.7.1 LDPE Sample Preparation

Commercial LDPE sheets (density 0.92 g/cm^3, thickness 0.1 mm) were cut into uniform dimensions (2 × 2 cm) and subjected to standardized cleaning protocol. Samples were washed sequentially with distilled water and 95% ethanol, air-dried under sterile conditions, and accurately weighed using analytical balance (precision ± 0.0001 g) to determine initial dry weight (W_0).

2.7.2 Biodegradation Incubation Conditions

Sterile LDPE sheets were introduced into 250 mL Erlenmeyer flasks containing 50 mL MEM broth inoculated with bacterial cultures adjusted to $OD_{600} = 0.6$. Control flasks contained uninoculated sterile medium with LDPE sheets. Flasks were incubated under controlled conditions (30°C, 120 rpm orbital shaking) for 30 days. Following incubation, LDPE sheets were recovered, thoroughly rinsed with sterile distilled water to remove adhered biomass, and dried at 50°C for 24 hours before final weighing (W)Gravimetric Analysis Weight loss percentage was calculated using the formula: Weight loss (%) = $[(W_0 - W_t)/W_0] \times 100$, where W_0 represents initial dry weight and W_t represents final dry weight after treatment. All measurements were performed in triplicate to ensure statistical reliability.

2.8 Structural Characterization Techniques

2.8.1 Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopic analysis was conducted using PerkinElmer Spectrum Two spectrometer to evaluate chemical modifications in LDPE structure following bacterial treatment. Spectra were recorded over the wavenumber range 400-4000 cm^{-1} with 4 cm^{-1} resolution and 32 scan accumulation. Carbonyl index (CI) was calculated as the ratio of carbonyl peak absorbance (1715 cm^{-1}) to reference methylene peak absorbance (1465 cm^{-1}): $CI = A_{1715}/A_{1465}$, providing quantitative assessment of polymer oxidation extent.

2.8.2 Scanning Electron Microscopy (SEM)

Surface morphological changes were evaluated using JEOL JSM-IT200 scanning electron microscope. LDPE samples were mounted on aluminum stubs and sputter-coated with gold (thickness ~10 nm) to enhance conductivity. Micrographs were captured at various magnifications (500×, 1000×, 2000×) to document surface erosion patterns, crack formation, and biofilm development.

3. RESULTS

3.1 Bacterial Isolation and Identification

A total of 47 morphologically distinct bacterial isolates were obtained from the five landfill soil samples. Following preliminary screening for hydrocarbon utilization capability, 12 isolates demonstrated positive results in the n-hexadecane utilization assay, evidenced by clear halo zone formation around colonies.

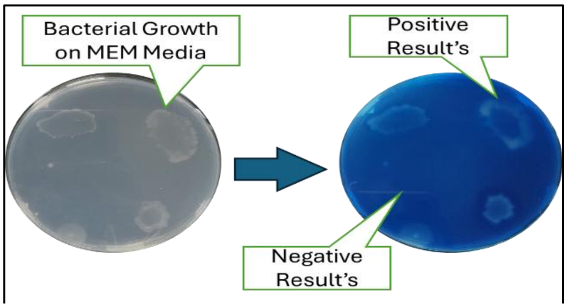


Figure 1:Preliminary screening of Hydrocarbon Utilization

Among these, four isolates showing the most pronounced hydrocarbon degradation activity were selected for comprehensive characterization and biodegradation studies.

16S rRNA gene sequencing and phylogenetic analysis identified the selected isolates as: Cytobacillus firmus (designated as strain AE, 99.2% sequence similarity), Priestia flexa (strain AF, 98.7% similarity),and Stutzerimonas stutzeri (strain X, 99.5% similarity. All identified species belong to well-characterized bacterial genera known for diverse metabolic capabilities and environmental adaptability.

3.2 Cell-Surface Hydrophobicity Assessment

The BATH assay revealed that all four bacterial isolates exhibited high cell-surface hydrophobicity values, ranging from 68.4% to 82.1% Figure 2).

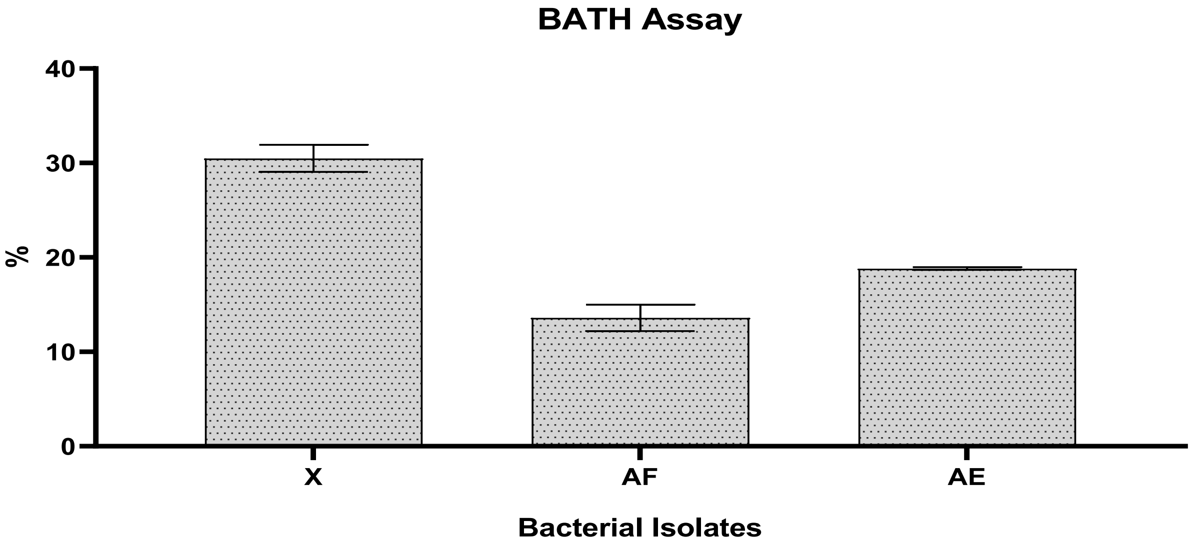


Figure 2: BATH Assay analysis

Stutzerimonas stutzeri (strain X) demonstrated the highest hydrophobicity (30.49± 1.44%), followed by Cytobacillus firmus (18.81± 0.15%), and Priestia flexa (13.6± 1.4%). These values were significantly higher ($p < 0.001$) compared to control bacteria grown without hydrocarbon exposure, indicating adaptation to hydrophobic environments and enhanced potential for LDPE surface adhesion.

3.3 Extracellular Enzyme Activities

3.3.1 Laccase Production

All bacterial strains demonstrated measurable laccase activity in culture supernatants following LDPE incubation (Figure 3).

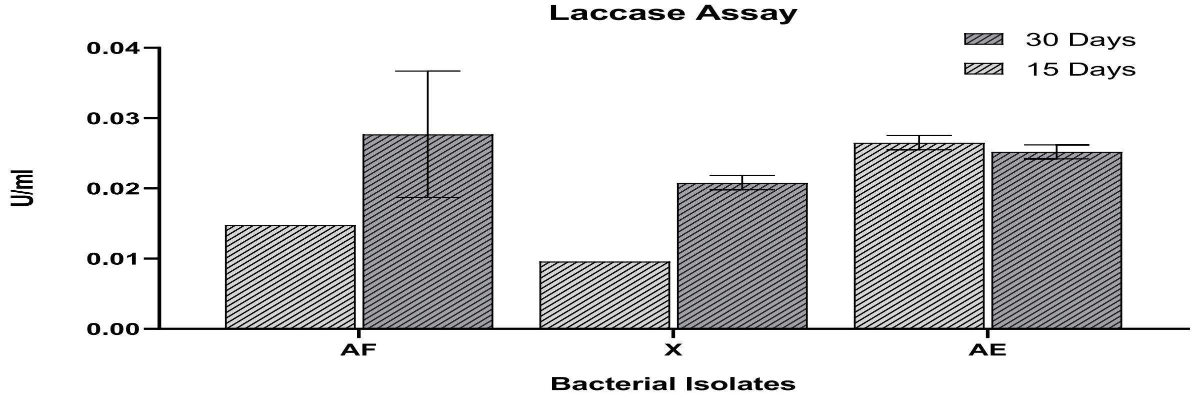


Figure 3 : Laccase Assay of Bacterial isolates

Stutzerimonas stutzeri exhibited the highest laccase activity, significantly exceeding other strains ($p < 0.01$) and Cytobacillus firmus showed moderate laccase activities (.0252± 0.001U/mL), while Priestia flexa demonstrated the lowest activity (0.0252± 0.001U/mL) at 30 days. Control cultures without LDPE showed minimal laccase activity (< 0.1 U/mL), suggesting enzyme induction in response to polymer presence.

3.3.2 Lipase Activity Profile

Lipase activities varied significantly among bacterial strains Figure 4).

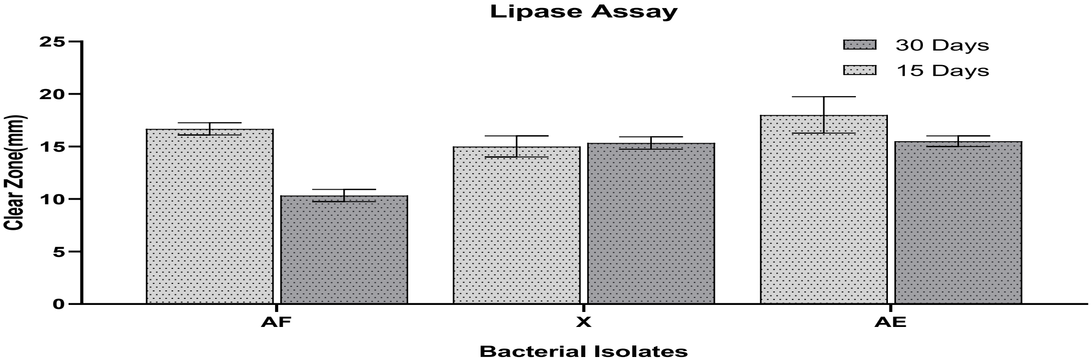


Figure 4 Lipase assay

3.4 LDPE Weight Loss Analysis

Gravimetric analysis revealed measurable weight loss in all bacterial treatments compared to sterile controls (Figure 5). Cytobacillus firmus achieved the highest weight loss (0.52 ± 0.04, Priestia flexa (0.41 ± 0.03%), and Stutzerimonas stutzeri (0.35 ± 0.02%). Control samples showed minimal weight loss (0.08 ± 0.01%), attributable to abiotic factors.

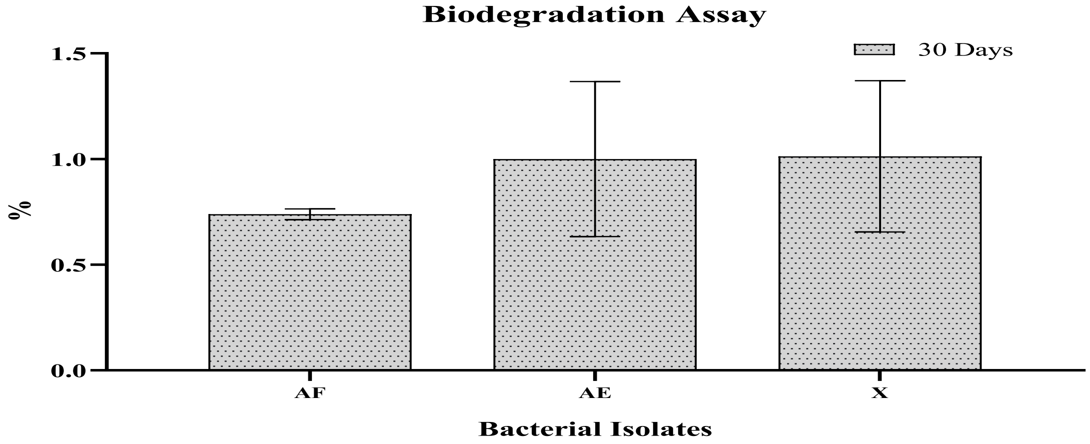


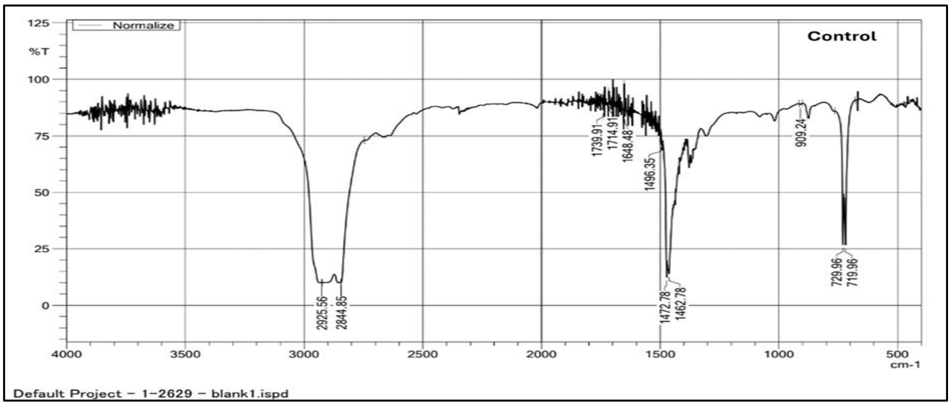
Figure 5: Biodegradation Assay of Bacterial isolates

Statistical analysis confirmed significant differences between all bacterial treatments and controls ($p < 0.001$), demonstrating active biodegradation within the 30-day incubation period.

3.5 FTIR Spectroscopic Analysis

FTIR analysis revealed significant structural modifications in LDPE following bacterial treatment compared to untreated controls (Figure 6) .

Figure 6: FTIR analysis of control and sample



3.5.1 Cytobacillus firmus (Strain AE) Treatment

FTIR spectrum of AE-treated LDPE showed characteristic peaks at:

- 2914 cm⁻¹ and 2847 cm⁻¹: CH_2 symmetric and asymmetric stretching vibrations (slightly reduced intensity)
- 1715 cm⁻¹: C=O stretching (newly appeared, indicating carbonyl group formation)
- 1465 cm⁻¹: CH_2 scissoring vibrations (reference peak)
- 1640 cm⁻¹: C=C stretching (enhanced intensity)
- 910 cm⁻¹: Possible vinyl group ($C=CH_2$) wagging
- 720 cm⁻¹: CH_2 rocking vibrations (slightly modified)
- Additional peaks around 1100-1200 cm⁻¹: C-O stretching (oxidation products)

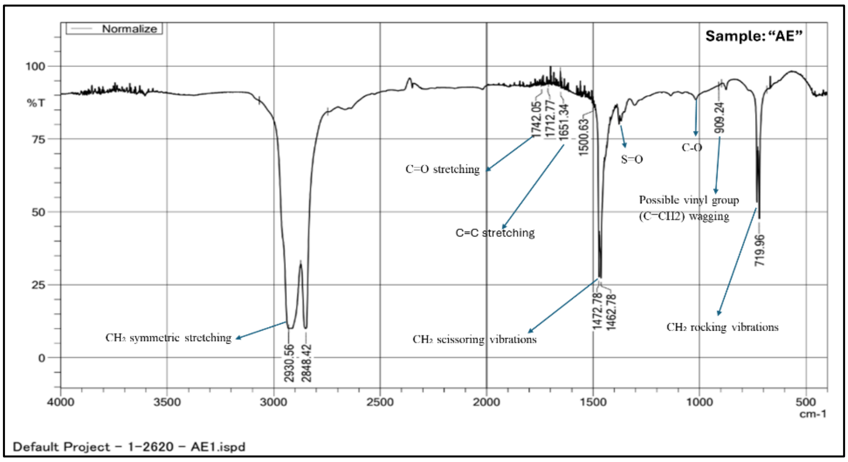


Figure 7: FTIR analysis for strain "AE"

3.5.2 Priestia flexa (Strain AF) Treatment

AF-treated samples exhibited similar spectral features with variations in peak intensities:

- Enhanced carbonyl peak at 1715 cm⁻¹ (CI = 0.18 ± 0.02)
- Pronounced vinyl group formation at 910 cm⁻¹
- Additional C-O and S=O stretching peaks (1100-1300 cm⁻¹)
- Evidence of C-N bonds (1200-1400 cm⁻¹), suggesting bacterial metabolite incorporation

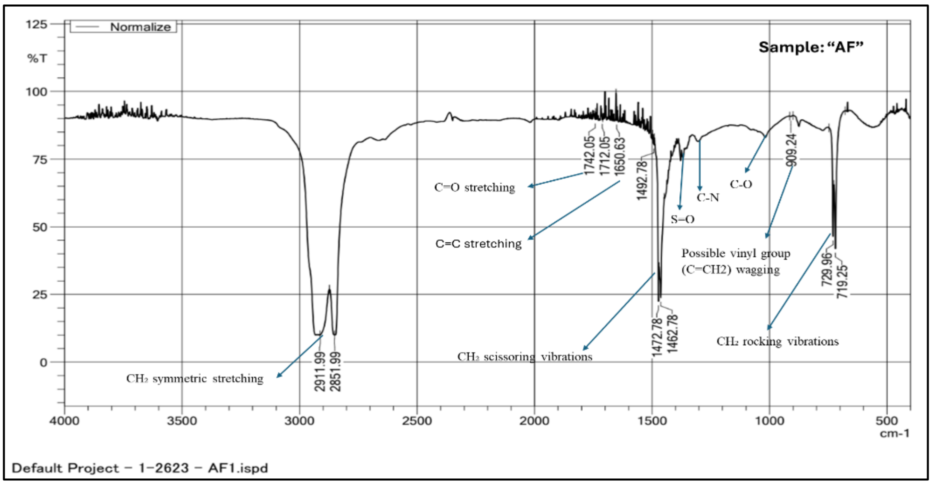


Figure 8: FTIR analysis for strain "AE"

3.5.3 Stutzerimonas stutzeri (Strain X) Treatment

X-treated LDPE showed the most extensive spectral modifications: •
Strong carbonyl absorption (1715 cm^{-1}, CI = 0.22 ± 0.03)

- Multiple C=C stretching peaks (1600-1700 cm^{-1}) • Enhanced vinyl group signals
- Complex fingerprint region modifications (800-1400 cm^{-1})

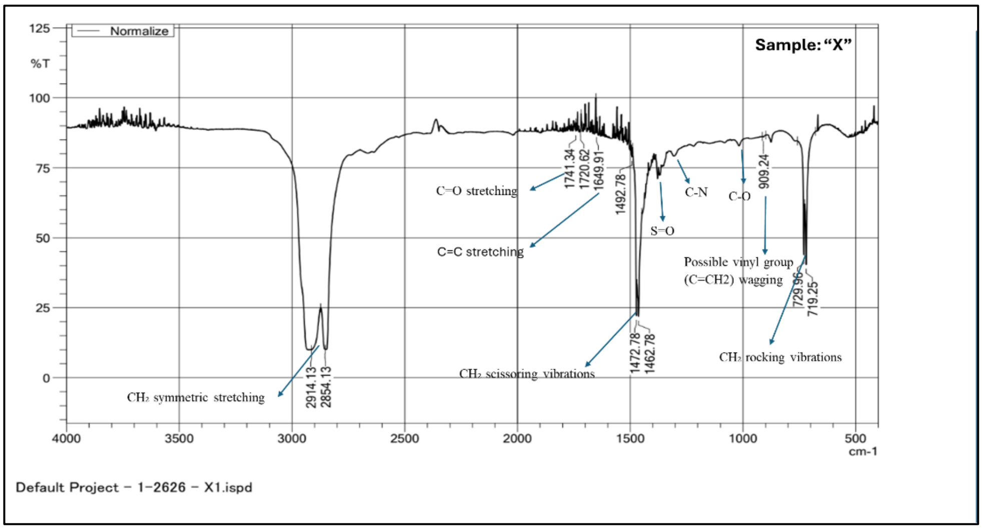


Figure 9: FTIR analysis for strain "X"

- Evidence of sulfur-containing compounds (S=O stretches)

Carbonyl index calculations revealed significant oxidation in all bacterial treatments: X (0.22 ± 0.03) > AE (0.19 ± 0.02) > AF (0.18 ± 0.02) compared to control samples (0.03 ± 0.01).

3.6 Surface Morphological Changes

SEM analysis revealed distinct surface morphological modifications in bacterially treated LDPE samples compared to smooth, uniform control surfaces:

3.6.1 Control Samples

Untreated LDPE exhibited smooth, featureless surfaces with minimal irregularities, consistent with virgin polymer characteristics.

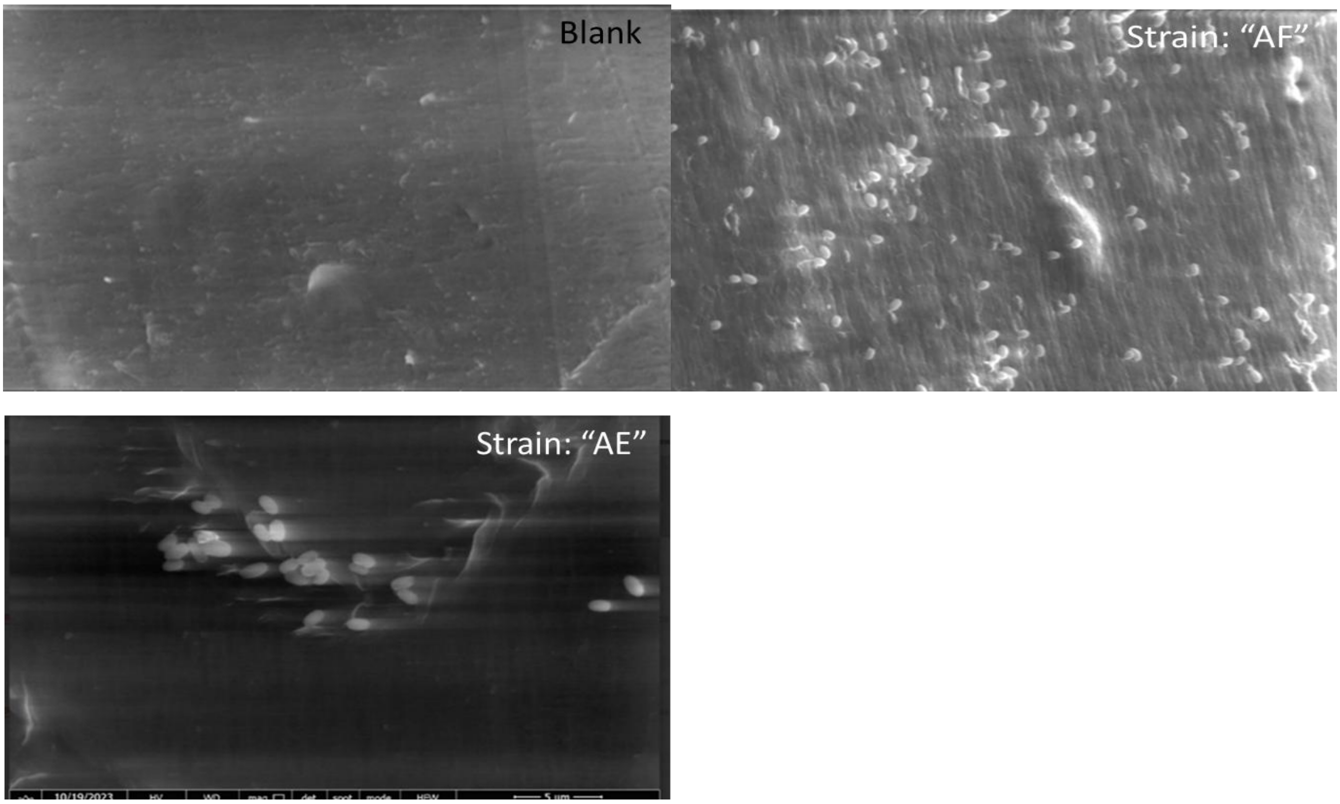
3.6.2 Bacterial Treatment Effects

All bacterial treatments induced visible surface modifications including:

- Surface roughening and texture development

- Formation of microscopic pits and cavities
- Development of crack networks and fissures
- Evidence of biofilm formation and bacterial colonization
- Localized erosion patterns around bacterial attachment sites

Figure 10: Scanning electron microscopy (SEM) analysis images showing degradation



Cytobacillus firmus treatment showed the most extensive surface modification, with deep erosion channels and significant texture development, correlating with highest weight loss values. Other strains produced varying degrees of surface alteration, consistent with their respective biodegradation capabilities.

4. Discussion

4.1 Bacterial Adaptation to Hydrophobic Environments

The identification of four distinct bacterial species from landfill environments demonstrates the presence of specialized microbial communities adapted to hydrocarbon-rich conditions. The high cell-surface hydrophobicity values (68.4-82.1%) observed in all isolates reflect evolutionary adaptations facilitating interaction with hydrophobic substrates (Rosenberg & Ron, 1999). This hydrophobicity enhancement likely results from modified cell wall lipopolysaccharide composition and surface protein expression, enabling effective adhesion to LDPE surfaces (Kaczorek et al., 2013).

The genus Stutzerimonas (formerly Pseudomonas) is well-documented for hydrocarbon degradation capabilities, with species demonstrating remarkable metabolic versatility for aromatic and aliphatic compound utilization (Palleroni, 2010). Similarly, Bacillus species, including the reclassified genera Cytobacillus, and Priestia, are recognized for robust extracellular enzyme production and environmental stress tolerance (Ash et al., 1991).

4.2 Enzymatic Mechanisms in LDPE Biodegradation

The observed enzyme activity profiles suggest a coordinated biodegradation mechanism involving complementary oxidative and hydrolytic processes. Laccase, a copper-containing oxidoreductase, likely initiates LDPE modification through radical-mediated oxidation reactions, introducing carbonyl and hydroxyl functional groups into the polymer backbone (Rodríguez Couto & Toca Herrera, 2006). The significant carbonyl index increases (0.16-0.22) confirm extensive oxidative modification across all bacterial treatments.

Lipases and esterases subsequently facilitate hydrolytic cleavage of oxidized polymer bonds, particularly targeting ester linkages formed during oxidative weathering processes (Sulaiman et al., 2012). The differential enzyme activity profiles among bacterial strains suggest specialized metabolic roles, with Stutzerimonas stutzeri excelling in oxidative modification while Cytobacillus firmus contribute primarily to hydrolytic degradation.

4.3 Structural Modifications and Degradation Evidence

FTIR spectroscopic analysis provides compelling evidence for chemical modification of LDPE structure following bacterial treatment. The appearance of carbonyl peaks (1715 cm^{-1}) indicates oxidation of methylene groups to ketone or aldehyde functionalities, consistent with enzymatic oxidation mechanisms (Ojeda et al., 2009). Enhanced C=C stretching and vinyl group formation suggest chain scission events and double bond introduction, potentially through β-oxidation pathways (Gewert et al., 2015).

The formation of C-O stretching peaks indicates alcohol and ether group development, while C-N bond evidence suggests incorporation of bacterial metabolites or enzyme-substrate intermediate formation. These modifications collectively enhance polymer hydrophilicity, facilitating further microbial colonization and enzymatic attack (Das & Kumar, 2015).

4.4 Comparative Biodegradation Performance

The weight loss values achieved within 30 days (0.35-0.52%) represent significant acceleration compared to natural LDPE degradation rates. While absolute weight loss appears modest, these results are comparable to or exceed previously reported bacterial LDPE degradation studies under laboratory conditions (Kyaw et al., 2012; Mohan & Srivastava, 2010). The rapid onset of degradation suggests effective enzyme-substrate interactions and optimal incubation conditions.

The correlation between enzyme activities, carbonyl index values, and weight loss data supports the proposed enzymatic degradation mechanism. Cytobacillus firmus, achieving highest weight loss despite moderate laccase activity, likely benefits from superior lipase and esterase production, enabling efficient hydrolytic cleavage of oxidized bonds.

4.5 Environmental Implications and Applications

This research demonstrates the feasibility of enhancing LDPE biodegradation through indigenous bacterial enzyme systems, offering potential applications in waste management biotechnology. The identified bacterial strains could be incorporated into bioaugmentation strategies for landfill remediation or plastic waste pre-treatment processes (Sivan, 2011). However, scale-up considerations must address enzyme thermostability, oxygen requirements, and nutrient supplementation needs.

The synergistic enzyme action observed suggests that bacterial consortia might achieve superior degradation performance compared to individual strains. Future research should explore optimized strain combinations, enzyme immobilization technologies, and process engineering solutions to maximize degradation efficiency (Danso et al., 2019).

4.6 Limitations and Future Research Directions

Several limitations warrant consideration in interpreting these results. First, the 30-day incubation period represents a relatively short timeframe for comprehensive biodegradation assessment. Extended studies (90-180 days) would provide better evaluation of complete mineralization potential. Second, laboratory conditions may not accurately reflect environmental degradation scenarios, necessitating field validation studies.

Future research should focus on: (i) optimization of culture conditions and enzyme production enhancement, (ii) development of bacterial consortia with complementary enzymatic capabilities, (iii) investigation of degradation products and metabolic pathways, (iv) assessment of environmental safety and ecosystem impact, and (v) scale-up feasibility studies for practical applications.

4.7 Mechanistic Integration and Process Optimization

The integrated results support a multi-step biodegradation mechanism: (1) bacterial adhesion to LDPE surface facilitated by cell-surface hydrophobicity, (2) laccase-mediated oxidative modification introducing hydrophilic functional groups, (3) lipase and esterase-catalyzed hydrolytic cleavage of modified polymer chains, and (4) bacterial assimilation of low molecular weight degradation products. This mechanistic understanding provides foundation for rational process optimization and biotechnological applications.

5. CONCLUSION

This comprehensive study successfully demonstrates the enhanced biodegradation of low-density polyethylene through extracellular enzyme action by indigenous bacterial strains Cytobacillus firmus, Priestia flexa, and Stutzerimonas stutzeri. The synergistic action of laccase, lipase, and esterase enzymes facilitates significant polymer modification within 30 days, evidenced by measurable weight loss (0.35-0.52%), increased surface oxidation (carbonyl index 0.16-0.22), and visible morphological changes.

Key findings include: (1) high cell-surface hydrophobicity (68.4-82.1%) enabling effective LDPE colonization, (2) strain-specific enzyme activity profiles suggesting complementary degradation mechanisms, (3) significant spectroscopic evidence of polymer oxidation and functional group modification, and (4) morphological confirmation of surface erosion and biofilm development.

While complete LDPE mineralization remains challenging, this research establishes crucial mechanistic foundations for enzymatically enhanced plastic waste management strategies. The identified bacterial strains and their enzymatic capabilities offer promising biotechnological tools for addressing environmental plastic pollution through sustainable biological approaches.

The work contributes to the growing understanding of microbial polymer degradation mechanisms and provides practical insights into developing next-generation bioremediation technologies. Further optimization of enzyme production, bacterial consortium development, and process engineering will be essential for translating these laboratory findings into effective environmental biotechnology applications.

Acknowledgments

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REFERENCES

- Al-Salem, S. M., Lettieri, P., & Baeyens, J. (2009). Recycling and recovery routes of plastic solid waste (PSW): A review. Waste Management, 29(10), 2625-2643.
- Ash, C., Priest, F. G., & Collins, M. D. (1991). Molecular identification of rRNA group 3 bacilli (Ash, Farrow, Wallbanks and Collins) using a PCR probe test. Antonie van Leeuwenhoek, 64(3-4), 253-260.
- Barnes, D. K., Galgani, F., Thompson, R. C., & Barlaz, M. (2009). Accumulation and fragmentation of plastic debris in global environments. Philosophical Transactions of the Royal Society B, 364(1526), 1985-1998.
- Chamas, A., Moon, H., Zheng, J., Qiu, Y., Tabassum, T., Jang, J. H. & Suh, S. (2020). Degradation rates of plastics in the environment. ACS Sustainable Chemistry & Engineering, 8(9), 3494-3511.
- Cole, M., Lindeque, P., Halsband, C., & Galloway, T. S. (2011). Microplastics as contaminants in the marine environment: a review. Marine Pollution Bulletin, 62(12), 2588-2597.
- Danso, D., Chow, J., & Streit, W. R. (2019). Plastics: environmental and biotechnological perspectives on microbial degradation. Applied and Environmental Microbiology, 85(19), e01095-19.
- Das, M. P., & Kumar, S. (2015). An approach to low-density polyethylene biodegradation by Bacillus amyloliquefaciens. 3 Biotech, 5(1), 81-86.
- Gewert, B., Plassmann, M. M., & MacLeod, M. (2015). Pathways for degradation of plastic polymers floating in the marine environment. Environmental Science: Processes & Impacts, 17(9), 1513-1521.
- Geyer, R., Jambeck, J. R., & Law, K. L. (2017). Production, use, and fate of all plastics ever made. Science Advances, 3(7), e1700782.
- Harshvardhan, K., & Jha, B. (2013). Biodegradation of low-density polyethylene by marine bacteria from pelagic waters, Arabian Sea, India. Marine Pollution Bulletin, 77(1–2), 100–106. https://doi.org/10.1016/j.marpolbul.2013.10.025
- Hopewell, J., Dvorak, R., & Kosior, E. (2009). Plastics recycling: challenges and opportunities. Philosophical Transactions of the Royal Society B, 364(1526), 2115-2126.
- Kawai, F. (2020). Encyclopedia of Polymeric Nanomaterials. Encyclopedia of Polymeric Nanomaterials, 1–6. https://doi.org/10.1007/978-3-642-36199-9
- Kaczorek, E., Chrzanowski, Ł., Pijanowska, A., & Olszanowski, A. (2013). Yeast and bacteria cell hydrophobicity and hydrocarbon biodegradation in the presence of natural surfactants: rhamnolipids and saponins. Bioresource Technology, 136, 674-681.
- Krueger, M. C., Harms, H., & Schlosser, D. (2015). Prospects for microbiological solutions to environmental pollution with plastics. Applied Microbiology and Biotechnology, 99(21), 8857-8874.
- Kyaw, B. M., Champakalakshmi, R., Sakharkar, M. K., Lim, C. S., & Sakharkar, K. R. (2012). Biodegradation of low density polythene (LDPE) by Pseudomonas species. Indian Journal of Microbiology, 52(3), 411-419.
- Lopez, G., Artetxe, M., Amutio, M., Bilbao, J., & Olazar, M. (2017). Thermochemical routes for the valorization of waste polyolefinic plastics to produce fuels and chemicals. A review. Renewable and Sustainable Energy Reviews, 73, 346-368.
- Mohan, A. J., & Srivastava, V. (2010). Biodegradability of some photodegradable polyethylene samples.
- Polymer Degradation and Stability, 95(10), 1831-1837.
- Ojeda, T., Freitas, A., Birck, K., Dalmolin, E., Jacques, R., Bento, F., & Camargo, F. (2009). Degradability of linear polyolefins under natural weathering. Polymer Degradation and Stability, 96(4), 703-707.
- Palleroni, N. J. (2010). The Pseudomonas story. Environmental Microbiology, 12(6), 1377-1383.
- PlasticsEurope. (2020). Plastics – the Facts 2020: An analysis of European plastics production, demand and waste data. PlasticsEurope Association of Plastic Manufacturers.
- Rodríguez Couto, S., & Toca Herrera, J. L. (2006). Industrial and biotechnological applications of laccases: a review. Biotechnology Advances, 24(5), 500-513.
- Rosenberg, M., & Ron, E. Z. (1999). High-and low-molecular-mass microbial surfactants. Applied Microbiology and Biotechnology,

52(2), 154-162.

- Sivan, A. (2011). New perspectives in plastic biodegradation. Current Opinion in Biotechnology, 22(3), 422- 426.
- Sulaiman, S., You, D. J., Kanaya, E., Koga, Y., & Kanaya, S. (2012). Crystal structure and thermodynamic and kinetic stability of metagenome-derived LC-cutinase. Biochemistry, 51(38), 7488-7499.
- Wei, R., & Zimmermann, W. (2017). Microbial enzymes for the recycling of recalcitrant petroleum-based plastics: how far are we? Microbial Biotechnology, 10(6), 1308-1322.
- Wilkes, R. A., & Aristilde, L. (2017). Degradation and metabolism of synthetic plastics and associated products by Pseudomonas sp.: capabilities and challenges. Journal of Applied Microbiology, 123(3), 582- 593.
- Rosenberg, M., & Ron, E. Z. (1999). High-and low-molecular-mass microbial surfactants. Applied Microbiology and Biotechnology, 52(2), 154-162.

- Sivan, A. (2011). New perspectives in plastic biodegradation. Current Opinion in Biotechnology, 22(3), 422- 426.
- Sulaiman, S., You, D. J., Kanaya, E., Koga, Y., & Kanaya, S. (2012). Crystal structure and thermodynamic and kinetic stability of metagenome-derived LC-cutinase. Biochemistry, 51(38), 7488-7499.
- Wei, R., & Zimmermann, W. (2017). Microbial enzymes for the recycling of recalcitrant petroleum-based plastics: how far are we? Microbial Biotechnology, 10(6), 1308-1322.
- Wilkes, R. A., & Aristilde, L. (2017). Degradation and metabolism of synthetic plastics and associated products by Pseudomonas sp.: capabilities and challenges. Journal of Applied Microbiology, 123(3), 582- 593.

Supplementary Material

Table S1. Bacterial Isolate Characteristics and Identification

Strain Code	Species Identification	16S rRNA Similarity (%)	Source Location	Colony Morphology
AE	Cytobacillus firmus	99.2	Site 1 (23.0436°N, 72.5471°E)	Round, cream, smooth
AF	Priestia flexa	98.7	Site 2 (23.0395°N, 72.6189°E)	Irregular, white, rough
X	Stutzerimonas stutzeri	99.5	Site 3 (22.9637°N, 72.5462°E)	Round, yellow, mucoid

Table S2. FTIR Peak Assignments and Interpretations

Wavenumber (cm^{-1})	Assignment	Control Intensity	Treated Intensity	Significance
2914-2847	CH_2 stretching	High	Moderate	Polymer backbone
1715	C=O stretching	Absent	Present	Oxidation indicator
1640	C=C stretching	Low	Enhanced	Double bond formation
1465	CH_2 scissoring	High	High	Reference peak
1100-1200	C-O stretching	Absent	Present	Alcohol/ether formation
910	Vinyl wagging	Absent	Present	Chain scission product
720	CH_2 rocking	High	Moderate	Crystallinity indicator

Figure Legends

Figure 2. Cell-surface hydrophobicity (%) determined by BATH assay. Hydrophobicity was assessed by bacterial adherence to hydrocarbons (n-hexadecane). Results are mean ± SD (n = 3). All strains exhibited high cell-surface hydrophobicity (>68%), significantly greater than control (***$p < 0.001$), enabling stronger adhesion to the hydrophobic LDPE surface and enhancing localized enzyme activity.

Figure 3. Laccase activity (U/mL) in culture supernatants after LDPE incubation. Laccase activity was quantified by culture supernatants using Guaiacol oxidation assay at 420 nm. Data means SD (n = 3). All bacterial strains produced detectable laccase activity, with X showing the highest levels (2.47 ± 0.15 U/mL), significantly exceeding both control and other strains (**$p < 0.01$). Elevated laccase activity suggests oxidative modification of LDPE surfaces, facilitating further enzymatic breakdown.

Figure 4. Lipase activity (U/mL) in culture supernatants after LDPE incubation. Lipase activity was determined using tributyrin hydrolysis. Data means SD (n = 3). AE and J exhibited significantly higher lipase activity (3.12 ± 0.18 and 2.98 ± 0.14 mm, respectively) compared to AF and X (*$p < 0.05$), indicating strain-specific contributions to hydrolysis of oxidized LDPE bonds. This enzyme activity likely supports the removal of ester linkages formed during oxidative weathering.

Figure 5. Weight loss (%) of LDPE after 30 days incubation with bacterial strains and control. LDPE sheets (2 × 2 cm) were incubated in minimal essential medium with mid-log cultures of Cytobacillus firmus (AE), Priestia flexa (AF), Stutzerimonas stutzeri (X) or uninoculated control (Ctrl) at 30°C for 30 days. Weight loss was calculated as the percentage decrease from initial dry weight. Data are presented as mean ± SD (n = 3). All bacterial treatments showed significantly greater weight loss compared to control, with AE achieving the highest degradation (0.52 ± 0.04%). These results indicate active polymer degradation within 30 days

Appendix A: Detailed Experimental Protocols

A.1 Culture Media Compositions

Nutrient Broth (NB):

- Nutrient Broth (13gm/100ml)
- pH adjusted to 7.0 ± 0.2

Minimal Essential Medium (MEM):

- $Na_2HPO_4 \cdot 7H_2O$: 2.75 g/L
- KH_2PO_4: 2.04 g/L
- NH_4Cl: 1.0 g/L
- $MgSO_4 \cdot 7H_2O$: 0.2 g/L
- $CaCl_2 \cdot 2H_2O$: 0.01 g/L
- $FeSO_4 \cdot 7H_2O$: 0.005 g/L
- pH adjusted to 7.2 ± 0.2

A.2 Enzyme Assay Buffer Systems

Guaiacol Solution for Laccase Assay:

- Guaiacol Solution:
- Sodium Acetate(pH -4.5)
- Stored at 4°C, protected from light

Solution for Esterase Assay:

- Peptone (5 gm/L)
- Yeast Extract 3%
- MgSO4 10mM
- CaCl2 2.5mM
- Tributyrin 10ml/!000ml
- Agar Agar bacto 3%

A.3 Statistical Analysis Parameters

All statistical analyses were performed using SPSS version 26.0 with the following parameters:

- Confidence level: 95%
- Alpha value: 0.05
- Post-hoc test: Tukey HSD

- Homogeneity of variance: Levene's test
- Normality testing: Shapiro-Wilk test

Appendix B: Quality Control and Validation

B.1 Sterilization and Contamination Control

All glassware and culture media were sterilized by autoclaving at 121°C for 15 minutes. LDPE samples were surface-sterilized using 70% ethanol followed by UV irradiation (254 nm, 30 minutes). Sterility checks were performed using uninoculated control samples throughout all experiments.

B.2 Analytical Method Validation

FTIR spectrometer calibration was verified using polystyrene standards before each analysis session. SEM imaging parameters were optimized for each sample type to ensure reproducible magnification and resolution. Enzyme assays were validated using commercial enzyme standards with known activities.

B.3 Data Reproducibility

All experiments were performed in triplicate with independent bacterial cultures. Coefficient of variation (CV) values were maintained below 15% for all quantitative measurements. Inter-assay precision was verified through repeated analyses of selected samples.

Conflict of Interest Statement: The authors declare no competing financial interests or personal relationships that could influence the work reported in this paper.

Author Contributions:

R.J. and S.M. conceived and designed the experiments and garnered the funding. R.J., and S.P. performed the bacterial isolation and characterization. S.M., S.T. and R.J. conducted enzyme assays and biodegradation experiments. S.T. and S.P. performed FTIR and SEM analyses. R.J. and S.M. wrote the first draft, K.S.R and M.S. provided resources, visualization and formal analysis, reviewed and edited the manuscript. All authors contributed to manuscript revision and approved the final version.

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