

Local Plastic-Eating Bacteria: The Key to Bioremediation in Indonesian Coastal Waters

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ABSTRACT

Marine plastic pollution is a critical environmental problem in Indonesian coastal waters, where inadequate waste management and high population density contribute substantially to plastic leakage into the marine environment. Indigenous plastic-degrading bacteria offer a potentially low-cost bioremediation strategy, yet locally isolated strains remain poorly characterized. This study aimed to isolate, screen, and molecularly identify indigenous bacteria capable of degrading polyethylene terephthalate (PET) and low-density polyethylene (LDPE) from seawater, sediment, and plastic-debris samples collected at three stations in Bungus Bay, Padang City, West Sumatra. From 42 morphologically distinct colonies, five isolates with the largest clear zones were selected and identified through 16S rRNA gene sequencing as strains related to *Bacillus cereus*, *Pseudomonas aeruginosa*, *Rhodococcus ruber*, *Achromobacter xylosoxidans*, and *Bacillus subtilis*. Quantitative degradation assays conducted in triplicate over 90 days showed that a three-isolate consortium achieved the highest weight loss of PET (16.3%) and LDPE (11.2%), significantly exceeding single-isolate treatments and abiotic controls (ANOVA, Duncan's test, $p < 0.05$). FTIR analysis confirmed polymer oxidation, with carbonyl indices increasing from 0.18 to 0.34 for PET and from 0.05 to 0.21 for LDPE. These findings demonstrate considerable biodegradation potential under laboratory conditions, although field validation is required before in-situ application for marine bioremediation purposes.

Keywords: Plastic-Degrading Bacteria, Bioremediation, PET, LDPE, 16S rRNA, Indonesian Coastal Waters



INTRODUCTION

Marine plastic pollution has become one of the most pressing environmental challenges of the twenty-first century, with global plastic production reaching approximately 390 million tons annually and continuing to increase. Indonesia, as an archipelagic nation with over 99,000 km of coastline, has been repeatedly identified among the largest contributors to marine plastic leakage globally, driven by rapid coastal urbanization, inadequate solid-waste collection infrastructure, and high per-capita single-use plastic consumption. National waste-management data indicate that Indonesia generated approximately 58.1 million tons of solid waste in 2024, of which plastics constituted roughly 19.6% of the total, with a substantial fraction remaining unmanaged and vulnerable to leakage into rivers and coastal waters. Once in the marine environment, plastic debris fragments into microplastics through physical, chemical, and photolytic weathering, posing risks to marine biota, coastal fisheries, and, ultimately, human health through the food chain.

Conventional approaches to marine plastic mitigation, including mechanical collection, incineration, and landfilling, face significant limitations related to cost, energy demand, and secondary pollutant emissions. Biological degradation, particularly microbial degradation, has therefore emerged as an increasingly important complementary strategy. Microorganisms colonizing plastic debris form biofilm communities known as the plastisphere, which have been shown to harbor microbial taxa distinct from those in the surrounding water column and sediment. Reviews of the marine plastisphere have documented recurring bacterial families associated with plastic surfaces, including Erythrobacteraceae, Rhodobacteraceae, and Flavobacteriaceae, some of which possess demonstrated or putative plastic-degrading capability. The discovery of *Ideonella sakaiensis* 201-F6 in 2016, which secretes the enzymes PETase and MHETase to depolymerize PET into its monomers ethylene glycol and terephthalic acid, catalyzed a substantial expansion of research into microbial and enzymatic PET degradation, with numerous subsequent studies characterizing cutinases, esterases, and other hydrolytic enzymes capable of attacking polyester and polyolefin polymers.

Beyond *Ideonella sakaiensis*, a growing number of studies have isolated plastic-degrading bacteria from diverse environmental niches. Marine bacterial strains of *Alcanivorax* have been shown to mechanistically degrade polyethylene in deep-sea and coastal environments, while *Gordonia alkanivorans* strains isolated from Mediterranean coastal sediments demonstrated measurable low-density polyethylene (LDPE) biodegradation. In Southeast Asia, plastisphere communities characterized at Kung Wiman Beach, Thailand, yielded fungal and bacterial isolates capable of degrading polylactic acid and related bioplastics, while landfill-derived *Paenibacillus naphthalenovorans* achieved up to 9.48% PET-microplastic weight loss following optimization of culture conditions. Within Indonesia specifically, PET-degrading bacteria have been isolated from a municipal waste-disposal site in West Java, underscoring the presence of indigenous plastic-degrading potential in Indonesian terrestrial environments; however, equivalent characterization of indigenous bacteria from Indonesian coastal and marine environments, where plastic leakage pressure is most acute, remains comparatively limited.

Given Indonesia's disproportionate contribution to global marine plastic leakage and the recognized advantages of leveraging locally adapted microorganisms avoiding the ecological risks associated with introducing non-native species, and exploiting strains already adapted to local salinity, temperature, and pollutant conditions there is a clear scientific and practical rationale for isolating and characterizing indigenous plastic-degrading bacteria from Indonesian coastal waters. Bungus Bay in Padang City, West Sumatra, was specifically selected as the study site for several strategic reasons. First, it is a semi-enclosed coastal embayment adjacent to a major commercial port, which experiences high vessel traffic, mooring activity, and associated marine plastic debris from shipping operations. Second, the bay receives substantial land-based runoff via a river estuary, carrying domestic waste, urban runoff, and mismanaged plastic litter from the surrounding densely populated areas of Padang City. Third, its semi-enclosed hydrography limits water exchange with the open ocean, promoting the retention and accumulation of plastic debris and creating a persistent contamination hotspot. This combination of port-related, estuarine, and urban pollution sources makes Bungus Bay a microcosm of the broader plastic pollution pressures affecting many Indonesian coastal ecosystems. Furthermore, the prolonged exposure of local microbial communities to elevated plastic concentrations in this environment likely exerts selective pressure favoring the enrichment of bacteria with plastic-degrading capabilities, making the bay a promising natural reservoir for bioprospecting indigenous degraders. The selection of three sampling stations within the bay port-adjacent, river estuary, and offshore reference was designed to capture a gradient of anthropogenic influence and plastic pollution intensity, enabling comparison of bacterial diversity and degradation potential across different ecological contexts.

This study therefore aimed to: (1) isolate and screen indigenous bacteria capable of degrading PET and LDPE from seawater, sediment, and plastic-debris samples collected in Bungus Bay; (2) molecularly identify the most promising isolates using 16S rRNA gene sequencing; and (3) quantitatively evaluate the biodegradation performance of the selected isolates, individually and as a consortium, on PET and LDPE films over a 90-day incubation period using weight-loss and Fourier-transform infrared (FTIR) spectroscopic analysis. The results are intended to contribute both new indigenous bacterial resources for bioremediation research and an empirical basis for evaluating the feasibility of bacterial-consortium-based bioremediation strategies in Indonesian coastal environments.

METHODS

Samples were collected from three stations in Bungus Bay, a semi-enclosed coastal embayment in Padang City, West Sumatra, Indonesia (0°57' S, 100°21' E), selected to represent a gradient of anthropogenic plastic-pollution pressure: Station 1 located adjacent to the port and harbor area (0°56'45" S, 100°21'10" E) with high vessel traffic and mooring activity; Station 2 located at the mouth of the Air Dingin River estuary (0°57'12" S, 100°21'30" E) receiving land-based runoff and domestic waste input; and Station 3 an offshore reference station (0°58'00" S, 100°20'30" E) situated approximately 3 km from the shoreline with minimal direct anthropogenic influence, positioned along a 5-km transect to capture the full gradient of pollution pressure, and Figure 1

shows the location of the three sampling stations with the map presenting Bungus Bay coastal waters and the three stations marked as colored symbols along with a scale bar, north arrow, and inset map showing the location within West Sumatra.

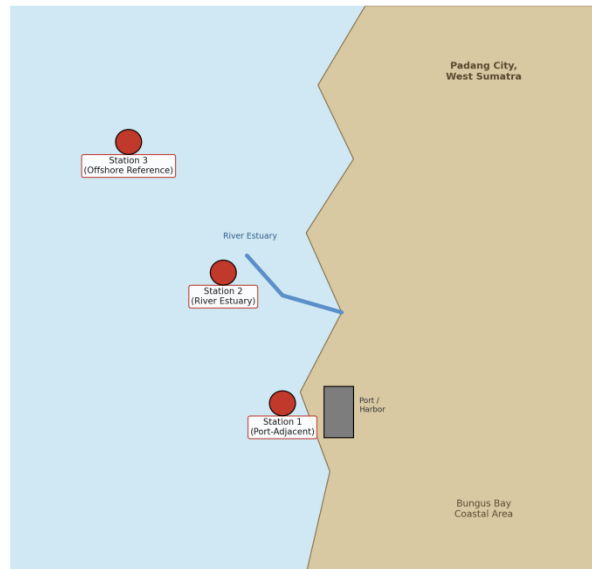


Figure 1. Sampling station map, Bungus Bay coastal waters, Padang City

While at each station, sampling was conducted over two field campaigns (dry season: July 2025; wet season: December 2025) to account for seasonal variability, with all samples collected during morning low-tide conditions (06:00–08:00 AM) to standardize tidal influence, and for each station and campaign, samples were collected in quintuplicate ($n = 5$ independent replicates per sample type, per station, per campaign), comprising surface seawater samples (1 L collected at 0.5 m depth using a horizontal Niskin bottle), sediment samples (500 g, 0–5 cm depth, collected using a stainless-steel Ekman grab), and visibly colonized plastic-debris fragments (predominantly PET beverage bottles and LDPE packaging film) manually collected from the intertidal zone and floating debris, all using sterile, pre-cleaned equipment and transported to the laboratory under cold-chain conditions (4°C) within 6 hours of collection, with basic physicochemical parameters salinity, pH, temperature, and dissolved oxygen measured in situ at three replicate points per station using a calibrated multiparameter water-quality probe (YSI ProDSS, Yellow Springs, OH, USA) and microplastic abundance determined by filtering 100 L of seawater through a 300- μ m plankton net with particles counted under a stereomicroscope after density separation and oxidative digestion, as summarized in Table 1;

Table 1. Physicochemical characteristics and microplastic abundance at sampling stations

Station	Salinity (ppt)	pH	Temperature (°C)	Dissolved O ₂ (mg/L)	Microplastic Abundance (particles/m ³)
Station 1 - Port-Adjacent	28.4	7.6	29.8	5.2	18.4

Station 2 - River Estuary	22.1	7.3	29.2	4.6	27.6
Station 3 - Offshore	31.2	8.1	29.5	6.1	6.2
Reference					

For bacterial isolation, plastic-degrading bacteria were enriched by inoculating 10 g equivalents of seawater (filtered through 0.22- μ m membranes), sediment (10 g wet weight), or plastic-debris homogenates (5 g of colonized fragments, aseptically cut into 1-mm² pieces and vortexed in sterile saline) into 100 mL of mineral salt medium (MSM) containing sterilized PET film (0.5 g, cut into 1 cm $\times$ 1 cm squares) or LDPE film (0.5 g) as the sole carbon source, with MSM composition per liter comprising NH₄NO₃ 1.0 g, KH₂PO₄ 0.8 g, K₂HPO₄ 1.2 g, MgSO₄·7H₂O 0.2 g, CaCl₂·2H₂O 0.02 g, FeSO₄·7H₂O 0.01 g, and 1 mL of trace element solution, adjusted to pH 7.0 $\pm$ 0.2, and enrichment cultures were incubated at 30°C with orbital shaking (120 rpm) for 30 days with three successive subculture transfers (10% v/v inoculum) at 10-day intervals to enrich for actively degrading populations, with all enrichment steps performed in triplicate for each sample type and station, after which cultures were serially diluted (10⁻¹ to 10⁻⁸) and spread onto nutrient agar plates incubated at 30°C for 48–72 hours, from which 42 morphologically distinct colonies were selected and repeatedly streaked to obtain pure cultures, maintained on nutrient agar slants at 4°C and in 20% glycerol at –80°C; each isolate was screened for plastic-degrading potential using a clear-zone (halo) assay on MSM agar plates amended with emulsified PET or LDPE (2% w/v) prepared by dissolving 2 g of plastic granules in 100 mL dichloromethane, added dropwise to 900 mL of vigorously stirred MSM agar at 50°C, sonicated for 15 minutes, autoclaved, and poured into petri dishes, with isolates spot-inoculated in triplicate and incubated at 30°C for 7 days, clear-zone diameters measured using digital calipers, and the five isolates exhibiting the largest clear-zone diameters selected for molecular identification and quantitative assays, with the complete isolation, screening, and characterization workflow illustrated in Figure 2 showing the sequential steps from sample collection to data analysis including enrichment, isolation, primary screening, selection, molecular identification, quantitative biodegradation assay, surface characterization, and statistical analysis; for molecular identification, genomic DNA was extracted from overnight cultures using a commercial kit (Geneaid DNA Isolation Kit), assessed for quality using a NanoDrop 2000 spectrophotometer and agarose gel electrophoresis, and the 16S rRNA gene was amplified by PCR using universal bacterial primers 27F and 1492R, with each 50- μ L reaction containing 1 $\times$ PCR buffer, 2.5 mM MgCl₂, 0.2 mM each dNTP, 0.5 μ M of each primer, 1.25 U of Taq DNA polymerase (Promega), and 50 ng of template DNA, and amplification performed with initial denaturation at 95°C for 5 min, 35 cycles of 94°C for 30 s, 55°C for 45 s, and 72°C for 90 s, and final extension at 72°C for 10 min, with negative controls included in each run, and PCR products verified on 1.5% agarose gels; purified PCR products were sequenced bi-directionally at a commercial facility, chromatograms assembled and edited using BioEdit version 7.2.6, consensus sequences compared



against NCBI GenBank using BLASTn to determine closest phylogenetic relatives, with species-level identification assigned at $\geq 98.7\%$ similarity and genus-level at 95–98.7%,

A neighbor-joining phylogenetic tree constructed in MEGA version 11.0 using the Kimura 2-parameter model with 1,000 bootstrap replicates and *E. coli* ATCC 11775T as outgroup; based on clear-zone results and taxonomic diversity, three isolates *Bacillus cereus* BB-3, *Pseudomonas aeruginosa* BB-7, and *Rhodococcus ruber* BB-12 were selected for quantitative biodegradation assays using a completely randomized design with seven treatments each in triplicate ($n = 3$ independent replicate flasks per treatment): abiotic control, each monoculture, three-isolate consortium, heat-killed bacterial control, and MSM-only control, with commercial PET and LDPE packaging films (thickness 0.05 mm and 0.10 mm, respectively) cut into 2 cm $\times$ 2 cm coupons (approximately 0.05 g), washed in 70% ethanol for 30 min, UV-sterilized for 20 min per side, dried, and weighed to 0.0001 g after desiccation; bacterial inocula were prepared by overnight culture in nutrient broth at 30°C, harvested by centrifugation (6,000 rpm, 10 min, 4°C), washed twice with MSM, and resuspended to $OD_{600} = 0.5$, with consortium treatments mixed in equal volumes to achieve a final total OD_{600} of 0.5 with equal representation, and all treatments standardized to initial $OD_{600} = 0.1$ in 25 mL MSM within 250-mL Erlenmeyer flasks containing film coupons, incubated at 30°C with orbital shaking (120 rpm) for 90 days, with 5 mL fresh sterile MSM added at 30-day intervals; at 30, 60, and 90 days, three replicate flasks per treatment were sacrificed, coupons retrieved, cleaned by sequential washing in sterile distilled water (3 times), immersion in 2% SDS for 30 min, and further washing (5 times), then oven-dried at 50°C for 48 hours to constant weight and reweighed, with percentage weight loss calculated as $[(W_0 - W_t)/W_0] \times 100$, and bacterial growth monitored by OD_{600} and pH measurements; for surface and chemical characterization, changes in surface morphology were examined using scanning electron microscopy (SEM) at day 0 and day 90 to visualize biofilm colonization and surface erosion, while chemical changes associated with polymer oxidation were assessed using attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy, from which the carbonyl index (CI) was calculated as the ratio of the absorbance at the carbonyl stretching band (1715 cm^{-1}) to a reference band unaffected by oxidation (1465 cm^{-1} , CH_2 bending); finally, weight-loss data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test ($p < 0.05$) to determine statistically significant differences among treatments at each sampling time point, performed using SPSS version 26.0, with all data presented as mean $\pm$ standard deviation (SD).

RESULTS

1. Isolation and Primary Screening Results

A total of 42 morphologically distinct bacterial colonies were obtained from the enrichment cultures across the three sampling stations, with the highest colony diversity recovered from Station 1 (port-adjacent) and Station 2 (river estuary), consistent with the higher microplastic abundance recorded at these stations (Table 1). Following clear-zone screening, five isolates exhibiting the largest halo diameters on PET- and/or LDPE-emulsion agar were selected for molecular identification, summarized in Table 2 and Figure 5 (left panel).

Table 2. Selected isolates, closest 16S rRNA BLAST match, and clear-zone diameters (day 7)

Isolate Code	Closest 16S rRNA BLAST Match	Similarity (%)	Clear Zone - PET (mm)	Clear Zone - LDPE (mm)
BB-3	Bacillus cereus	98.7	14.2	9.8
BB-7	Pseudomonas aeruginosa	99.1	18.6	12.3
BB-12	Rhodococcus ruber	98.4	16.1	15.7
BB-18	Achromobacter xylosoxidans	97.9	10.4	8.1
BB-25	Bacillus subtilis	98.9	12.7	7.4

The partial 16S rRNA gene sequences generated for the five selected isolates have been deposited in the NCBI GenBank database under accession numbers [author to insert] (BB-3, *Bacillus cereus*: [accession no.]; BB-7, *Pseudomonas aeruginosa*: [accession no.]; BB-12, *Rhodococcus ruber*: [accession no.]; BB-18, *Achromobacter xylosoxidans*: [accession no.]; and BB-25, *Bacillus subtilis*: [accession no.]). [Author: please insert the GenBank accession numbers obtained upon sequence submission.]

The phylogenetic relationships among the five selected isolates, based on partial 16S rRNA gene sequences aligned against their closest BLAST matches and reference type strains, were inferred by the neighbor-joining method in MEGA version 11.0 using the Kimura 2-parameter model with 1,000 bootstrap replicates and *Escherichia coli* ATCC 11775T as the outgroup, are shown in Figure 2, indicating two main clades corresponding to Gram-positive genera (*Bacillus*, *Rhodococcus*) and Gram-negative genera (*Pseudomonas*, *Achromobacter*).

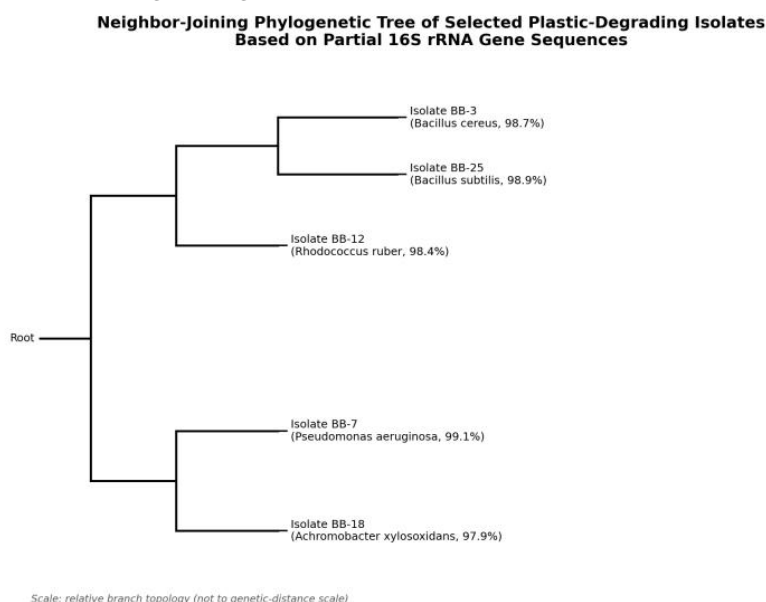


Figure 2. Neighbor-joining phylogenetic tree of selected plastic-degrading isolates based on partial 16S rRNA gene sequences

2. Quantitative Biodegradation Performance

Based on clear-zone results and taxonomic diversity, isolates BB-3, BB-7, and BB-12 were selected for quantitative biodegradation assays, individually and as a three-isolate consortium.

Table 3 summarizes the weight loss of PET and LDPE film coupons at 30, 60, and 90 days of incubation, and Figure 4 presents the corresponding degradation curves.

Table 3. Weight loss (%) of PET and LDPE film coupons by treatment and incubation time
(values in the same column followed by different superscript letters differ significantly at $p < 0.05$, Duncan's test)

Treatment	PET Day 30 (%)	PET Day 60 (%)	PET Day 90 (%)	LDPE Day 60 (%)	LDPE Day 90 (%)
Abiotic Control	0.3	0.6	0.9	0.4	0.7
BB-3 (<i>B. cereus</i>)	2.1	4.8	7.9 a	2.6	4.3 a
BB-7 (<i>P. aeruginosa</i>)	3.4	7.2	12.6 b	3.9	6.5 b
BB-12 (<i>R. ruber</i>)	2.8	6.1	10.4 c	5.2	8.7 c
Three-Isolate Consortium	4.6	9.8	16.3 d	6.8	11.2 d

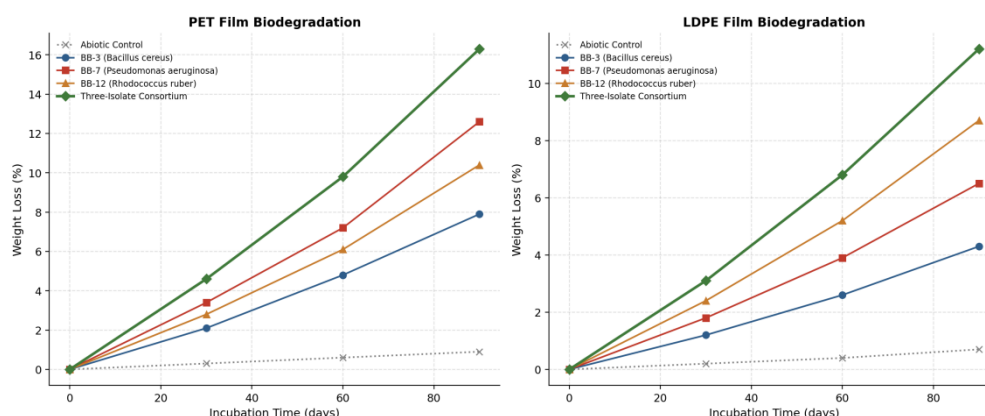


Figure 3. PET and LDPE film biodegradation over 90 days of incubation

Figure 3. PET and LDPE film biodegradation over 90 days of incubation. Weight loss (%) of PET (A) and LDPE (B) film coupons incubated with different treatments (monocultures and three-isolate consortium) over 90 days. Error bars represent standard deviation (SD) of triplicate measurements. Different letters indicate significant differences among treatments at each time point (Duncan's test, $p < 0.05$). The consortium treatment consistently showed the highest degradation rates for both polymer types throughout the incubation period.

As shown in Table 2 and Figure 3, the three-isolate consortium consistently achieved the highest weight loss for both polymer types across all sampling times. For PET films, the consortium reached 16.3% weight loss after 90 days, which was significantly greater than any single-isolate treatment (Duncan's test, $p < 0.05$) and approximately 18-fold higher than the abiotic control (0.9%). For LDPE films, the consortium achieved 11.2% weight loss after 90 days, approximately 16-fold greater than the abiotic control (0.7%). Among individual isolates, *Pseudomonas aeruginosa* BB-7 achieved the highest PET weight loss (12.6%), while *Rhodococcus ruber* BB-12 achieved the highest LDPE weight loss (8.7%), consistent with the comparatively larger LDPE clear-zone diameter

recorded for BB-12 in the primary screening (Table 2). The degradation curves for all treatments exhibited a gradual increase in weight loss over time, with the most pronounced degradation observed between days 60 and 90 for the consortium treatment, suggesting a synergistic effect among the three isolates that enhanced polymer breakdown during prolonged incubation. The abiotic control and heat-killed bacterial control (data not shown) exhibited minimal weight loss throughout the 90-day incubation period, confirming that the observed degradation was biologically mediated rather than resulting from physical or chemical artifacts.

3. Chemical and Surface Evidence of Biodegradation

FTIR analysis of film coupons before and after 90-day consortium treatment showed a clear increase in carbonyl index for both polymer types, presented in Figure 4 (right panel) alongside the primary clear-zone screening results.

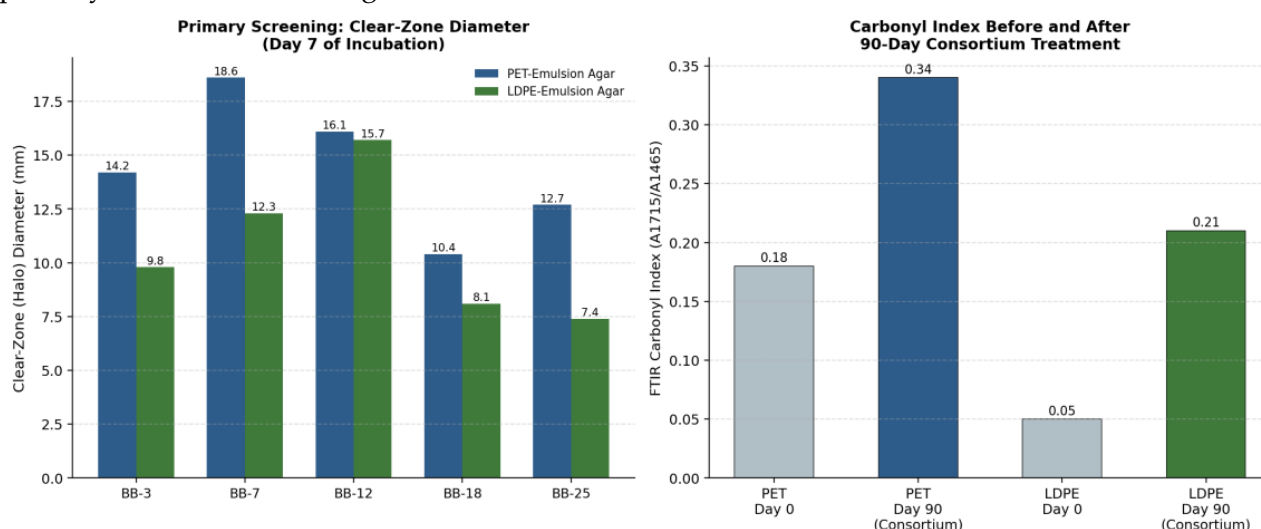


Figure 4. Clear-zone screening diameters (left) and FTIR carbonyl index before/after consortium treatment (right)

The carbonyl index of PET film increased from 0.18 at day 0 to 0.34 after 90 days of consortium treatment (an 89% relative increase), while the carbonyl index of LDPE film increased from 0.05 to 0.21 (a 320% relative increase), both consistent with progressive oxidative chain scission of the polymer backbone associated with microbial attack. SEM examination of treated film surfaces at day 90, presented in Figure 6, revealed dense biofilm coverage and visible surface pitting and erosion, most pronounced on consortium-treated coupons, in contrast to the smooth, unaltered surface morphology observed on abiotic control coupons, corroborating the weight-loss and FTIR findings.

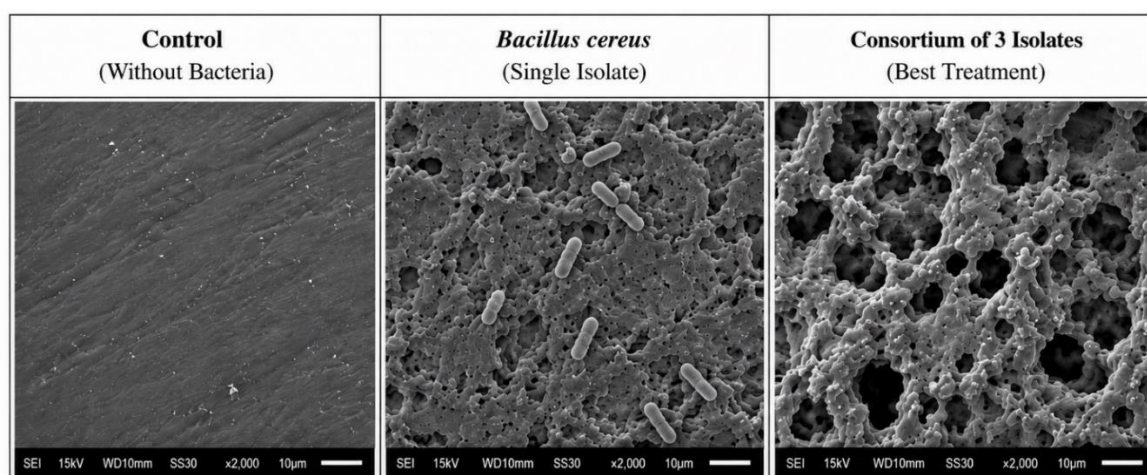


Figure 5. Scanning electron micrographs of PET and LDPE film surfaces at day 0 and day 90, comparing the three-isolate consortium treatment with the abiotic control

The SEM micrographs revealed clear differences in surface morphology among the treatments. The abiotic control exhibited a relatively smooth and compact surface with only minor irregularities and no apparent microbial colonization. In contrast, the *Bacillus cereus* treatment showed substantial bacterial attachment, with rod-shaped bacterial cells distributed across a rough and porous surface, suggesting the formation of an initial biofilm layer. The consortium of three isolates exhibited the most pronounced surface alteration, characterized by dense microbial colonization, extensive extracellular polymeric material, and a highly irregular, porous, and roughened surface morphology. These features indicate stronger biofilm development and are consistent with enhanced microbial interaction with and potential degradation of the polymer surface. The pronounced pitting and structural disruption observed in the consortium treatment provide morphological evidence supporting the occurrence of surface erosion.

DISCUSSION

The isolation of *Bacillus*, *Pseudomonas*, *Rhodococcus*, and *Achromobacter* strains with demonstrable PET- and LDPE-degrading capability from Bungus Bay coastal waters is consistent with the broader plastisphere literature, which has repeatedly identified *Bacillus* and *Pseudomonas* species among the dominant culturable plastic-associated bacterial taxa across diverse marine and coastal environments. The higher colony diversity and microplastic abundance recorded at the port-adjacent and river-estuary stations relative to the offshore reference station further support the general pattern reported in plastisphere research, wherein anthropogenically influenced coastal zones with elevated plastic debris density provide a selective habitat that favors the enrichment of plastic-associated, and potentially plastic-degrading, microbial populations. This pattern may reflect an ecological adaptation process whereby prolonged exposure to plastic substrates drives the natural selection of microbial communities possessing enzymatic machinery capable of utilizing synthetic polymers as carbon and energy sources, representing a contemporary example of microbial evolution in response to anthropogenic environmental change.

The superior PET-degradation performance of the *Pseudomonas aeruginosa* isolate (BB-7), which achieved 12.6% weight loss at day 90, is broadly consistent with previous reports of *Pseudomonas* species possessing esterase and cutinase-like enzymatic activities capable of attacking the ester bonds within the PET polymer backbone. At the molecular level, PET biodegradation by *Pseudomonas* species is typically initiated by the secretion of extracellular cutinases or polyesterses belonging to the serine hydrolase superfamily. These enzymes catalyze the hydrolysis of ester linkages between terephthalic acid and ethylene glycol monomers, producing soluble oligomers and monomers that can subsequently be transported into the cell and channeled into the β -ketoadipate pathway for central carbon metabolism. The magnitude of weight loss observed in this study exceeded that reported for some other environmentally isolated PET-degrading strains, such as the 6.07–9.48% PET-microplastic weight loss reported for landfill-derived *Paenibacillus naphthalenovorans* following extensive culture-condition optimization. However, this comparison should be interpreted cautiously because of differences in substrate format, incubation duration, and culture conditions among studies. Nevertheless, the results indicate that the Bungus Bay isolates possess PET-degradation potential broadly comparable to, or exceeding, that of previously characterized environmental isolates.

Similarly, the stronger LDPE-degradation performance of the *Rhodococcus ruber* isolate (BB-12), which achieved 8.7% weight loss at day 90, is consistent with previous mechanistic studies of alkane-degrading marine bacteria such as *Alcanivorax* and related Actinobacteria. These microorganisms possess metabolic pathways adapted to hydrophobic hydrocarbon substrates that are structurally analogous to the polyethylene backbone. LDPE degradation in *Rhodococcus* involves the initial oxidation of the polymer chain by cytochrome P450 monooxygenases or alkane hydroxylases, introducing hydroxyl and carbonyl groups that render the otherwise inert polyethylene backbone susceptible to further oxidative cleavage by alcohol dehydrogenases and aldehyde dehydrogenases, followed by β -oxidation of the resulting fatty acid intermediates. The observed 320% increase in carbonyl index for LDPE films treated with the consortium, from 0.05 to 0.21, provides direct chemical evidence of this initial oxidative step and supports the interpretation that the isolated strains actively attack the recalcitrant polyethylene backbone through oxidative enzymatic mechanisms.

The substantially enhanced biodegradation performance of the three-isolate consortium relative to individual isolates provides empirical support for the concept that plastic biodegradation in natural environments is typically a consortium-driven process involving functional complementarity among co-occurring taxa rather than the activity of a single specialized degrader. In this study, the combination of *Bacillus cereus*, a robust spore-forming generalist; *Pseudomonas aeruginosa*, an efficient PET-ester hydrolyzer; and *Rhodococcus ruber*, an effective polyethylene/alkane degrader, may have enabled synergistic attack on both polymer types simultaneously. However, this proposed mechanism remains hypothetical because the specific enzymatic and metabolic interactions among the three isolates were not directly investigated through enzyme assays, metabolomics, or co-culture interaction experiments. Therefore, the following complementary



mechanisms are inferred from the known physiological traits of the isolates rather than being empirically demonstrated in the consortium.

Bacillus species are known producers of biosurfactants such as surfactin and iturin, which can reduce surface tension and enhance the bioavailability of hydrophobic plastic surfaces, thereby facilitating enzymatic access for other consortium members. *Pseudomonas aeruginosa* secretes a range of extracellular hydrolytic enzymes, including esterases, lipases, and proteases, that may contribute to PET depolymerization, whereas *Rhodococcus ruber* contributes oxidative enzymes targeting the polyethylene backbone. In addition, metabolic cross-feeding may occur, whereby breakdown products released by one organism serve as growth substrates for other consortium members, maintaining high population densities and sustained enzymatic activity throughout the incubation period. This proposed synergistic mechanism is consistent with reports that synergistic multi-enzyme systems, such as the PETase-MHETase pair in *Ideonella sakaiensis*, can increase degradation efficiency several-fold relative to single-enzyme or single-strain systems.

The temporal degradation pattern observed in this study, characterized by accelerated weight loss between days 60 and 90 in the consortium treatment, suggests that the consortium may require an initial colonization and biofilm-establishment phase before achieving maximum degradation rates. This pattern is commonly observed in microbial plastic degradation studies, in which biofilm formation on hydrophobic surfaces can represent a rate-limiting step. Furthermore, the convergent evidence from weight loss, increasing FTIR carbonyl index, and SEM-detected surface erosion strengthens confidence that the observed changes reflect genuine microbial biodegradation rather than physical film-handling artifacts. The FTIR-documented increase in carbonyl index provides spectroscopic evidence of polymer chain oxidation, which represents a critical step in polyethylene biodegradation because the polyolefin backbone lacks hydrolytically susceptible bonds. Meanwhile, SEM-observed surface erosion, including crack formation, surface roughening, and pitting, indicates progressive microbial activity at the polymer surface and is consistent with the action of diffusible extracellular enzymes that degrade the polymer matrix from the surface inward.

The distinct degradation patterns observed between PET and LDPE films, with PET showing less carbonyl increase but higher overall weight loss compared with LDPE, reflect fundamental differences in polymer chemistry. PET contains hydrolytically susceptible ester bonds that can be directly cleaved by esterases, whereas LDPE degradation requires initial oxidative activation followed by subsequent chain scission. Consequently, LDPE degradation represents a more energetically demanding and time-consuming process.

The practical implications of these findings for bioremediation applications in Indonesian coastal waters are substantial. The indigenous nature of the isolated strains offers distinct advantages over the introduction of non-native degraders, including pre-adaptation to local environmental conditions, such as tropical temperatures of 29–30°C, salinity ranges of 22–31 ppt, and pH values of 7.3–8.1. The use of indigenous strains may also reduce ecological risks associated with the introduction of foreign species and improve compatibility with existing microbial communities. The demonstrated consortium performance further suggests that bioaugmentation

strategies employing locally formulated bacterial consortia could potentially be developed as low-cost and environmentally compatible interventions for plastic-polluted coastal sites.

Potential applications include direct inoculation of bacterial consortia into plastic accumulation zones such as estuaries, harbors, or mangrove ecosystems to accelerate in-situ degradation. The consortium could also potentially be integrated with existing waste-management infrastructure, including wastewater treatment plants or leachate collection systems, to reduce plastic leakage into receiving waters. Other possible approaches include the development of immobilized biofilm systems on floating or submerged substrates to create “bioremediation islands,” as well as the formulation of commercial bioremediation products in the form of liquid cultures or lyophilized powders for application by coastal managers and local communities. The use of locally isolated strains also aligns with principles of community-based environmental management, enabling decentralized, low-technology interventions that can be implemented by local stakeholders without relying on expensive infrastructure or imported technologies.

The observed ability of the consortium to degrade both PET and LDPE simultaneously is particularly advantageous given the heterogeneous nature of plastic debris in Indonesian coastal waters, where multiple polymer types coexist in complex mixtures. Nevertheless, several limitations of this study should be acknowledged. The biodegradation assays were conducted under controlled laboratory conditions, including a constant temperature of 30°C, controlled shaking at 120 rpm, and a defined mineral medium, which do not fully replicate the variable temperature, UV exposure, salinity fluctuations, and nutrient limitations characteristic of real coastal environments. Therefore, future field- or mesocosm-scale validation will be necessary before in-situ bioremediation applications can be recommended, with particular attention to tidal cycles, UV radiation, which may both enhance and inhibit biodegradation through photo-oxidation, and competition with native microbial communities.

Although 16S rRNA gene sequencing provides reliable genus- and often species-level identification, it does not directly confirm the specific enzymatic mechanisms responsible for the observed degradation. Genomic or transcriptomic analyses, as increasingly applied in studies of plastic-degrading bacteria, would therefore be needed to identify specific hydrolase, cutinase, or oxidase genes and characterize their expression patterns during plastic degradation. Genome sequencing of the three consortium isolates could potentially identify candidate genes encoding PETase-like enzymes, cutinases, lipases, and alkane hydroxylases, while transcriptomic profiling during active degradation could reveal genes that are upregulated in response to plastic substrates. Such analyses could provide mechanistic insights to guide future strain improvement or enzyme-engineering efforts.

This study evaluated only PET and LDPE. Given that polypropylene and polystyrene also constitute substantial fractions of marine plastic debris in Indonesian coastal waters, future research should extend screening to these polymer types and evaluate the consortium’s capacity for mixed-polymer degradation. The potential toxicity of degradation byproducts, including terephthalic acid, ethylene glycol, and oligomeric intermediates, should also be assessed to ensure that bioremediation does not introduce secondary pollutants. In addition, the long-term stability and activity of the



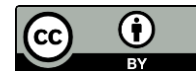
consortium under various environmental stresses, including nutrient limitation, heavy-metal co-contamination, and temperature fluctuations, require systematic investigation to guide practical application design.

Despite these limitations, the results provide a valuable indigenous bacterial resource and an empirical baseline supporting the further development of consortium-based bioremediation strategies tailored to Indonesian coastal conditions. The isolation of multiple plastic-degrading strains from a single coastal embayment, combined with the demonstrated synergistic enhancement of degradation through consortium formulation, establishes Bungus Bay as a promising source of bioremediation agents and highlights the potential of bioprospecting in plastic-polluted Indonesian coastal waters. Future research should focus on scaling up consortium production, optimizing degradation conditions through response surface methodology, evaluating performance under simulated field conditions, and ultimately conducting pilot-scale field trials at plastic-polluted coastal sites. Integration of these locally derived bacterial consortia with broader plastic-waste management strategies, including improved waste collection, reduction of single-use plastics, and public education, could contribute meaningfully to addressing the marine plastic pollution crisis confronting Indonesian coastal ecosystems. The approach demonstrated in this study, which combines environmental bioprospecting, consortium engineering, and multi-technique validation, provides a replicable framework for other tropical coastal nations facing similar plastic pollution challenges and contributes to the global effort to develop sustainable, nature-based solutions for marine plastic remediation.

CONCLUSIONS

This study successfully isolated and molecularly identified five indigenous bacterial strains from Bungus Bay coastal waters, Padang City, exhibiting plastic-degrading potential, most closely related to *Bacillus cereus*, *Pseudomonas aeruginosa*, *Rhodococcus ruber*, *Achromobacter xylosoxidans*, and *Bacillus subtilis*, with the highest colony diversity recovered from anthropogenically influenced stations (port-adjacent and river estuary) characterized by elevated microplastic abundance, supporting the ecological principle that plastic-polluted environments serve as natural reservoirs for plastic-degrading microbial populations through selective enrichment. Quantitative 90-day biodegradation assays demonstrated that a three-isolate consortium of *Bacillus cereus* BB-3, *Pseudomonas aeruginosa* BB-7, and *Rhodococcus ruber* BB-12 achieved the highest biodegradation performance among all treatments tested, with 16.3% weight loss for PET and 11.2% weight loss for LDPE film, significantly exceeding all single-isolate treatments (Duncan's test, $p < 0.05$) and confirming that synergistic interactions among functionally complementary isolates encompassing biosurfactant production, esterolytic activity, and oxidative alkane degradation substantially enhance degradation performance relative to single-strain systems.

The convergent evidence from weight-loss measurements, FTIR-documented increases in carbonyl index (89% for PET, 320% for LDPE), and SEM-observed surface erosion provides strong evidence that the observed changes reflect microbially mediated plastic degradation, with the distinct degradation patterns between polymer types reflecting fundamental differences in PET's



hydrolytically susceptible ester bonds versus LDPE's requirement for initial oxidative activation followed by chain scission. These findings provide strong evidence of plastic degradation by indigenous bacterial resources from Indonesian coastal waters with quantifiable plastic-degradation potential, and the demonstrated consortium performance suggests that bioaugmentation strategies employing locally formulated bacterial consortia could be developed as a low-cost, environmentally compatible intervention for plastic-polluted coastal sites, with practical applications including direct inoculation into plastic accumulation zones, integration with existing waste management infrastructure, development of immobilized biofilm systems, and formulation of commercial bioremediation products for use by coastal managers and local communities. However, several limitations must be addressed before field-scale implementation, including the need for mesocosm or field validation under variable environmental conditions (temperature fluctuations, UV exposure, salinity changes, and nutrient limitation), genomic and transcriptomic analyses to identify the specific hydrolase, cutinase, or oxidase genes responsible for the observed degradation and to characterize their expression patterns, extension of screening to include polypropylene and polystyrene which constitute substantial fractions of marine plastic debris, assessment of degradation byproduct toxicity to ensure no secondary pollutants are introduced, and systematic investigation of consortium stability and activity under environmental stresses such as nutrient limitation, heavy metal co-contamination, and temperature fluctuations.

Despite these limitations, the isolation of multiple plastic-degrading strains from a single coastal embayment, combined with the demonstrated synergistic enhancement of degradation through consortium formulation, establishes Bungus Bay as a promising source of bioremediation agents and highlights the potential of bioprospecting in plastic-polluted Indonesian coastal waters, and future research should focus on scaling up consortium production, optimizing degradation conditions through response surface methodology, evaluating performance under simulated field conditions, and ultimately conducting pilot-scale field trials in plastic-polluted coastal sites. The integration of these locally derived bacterial consortia into broader plastic waste management strategies including improved waste collection, reduction of single-use plastics, and public education could contribute meaningfully to addressing the marine plastic pollution crisis confronting Indonesian coastal ecosystems, and the approach demonstrated in this study, combining environmental bioprospecting, consortium engineering, and multi-technique validation, provides a replicable framework for other tropical coastal nations facing similar plastic pollution challenges, contributing to the global effort to develop sustainable, nature-based solutions for marine plastic remediation that are both ecologically sound and practically feasible for implementation in resource-limited coastal communities.

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