

Bioprospecting Marine Invertebrate-Associated Bacteria for Polyethylene Biodegradation in Indonesian Coastal Waters

Etin Diah Permanasari^{1*} & Hanifah Sriamelia²

^{1*}Universitas Muhammadiyah Prof. DR. HAMKA, Indonesia, ²Universitas Negeri Padang, Indonesia

*Co e-mail: etindiah_permanasari@uhamka.ac.id¹

Article Information

Received: September 07, 2026

Revised: October 14, 2026

Online: October 24, 2026

Keywords

Bioprospecting, Invertebrate-Associated Bacteria, Polyethylene, Marine Invertebrates, West Sumatra

ABSTRACT

*Polyethylene (PE) persists in marine environments because of its chemical inertness. Marine invertebrate-associated bacteria may provide enzymes capable of transforming this polymer, but their potential in Indonesian waters remains underexplored. This study isolated, identified, and evaluated bacteria associated with sponges, ascidians, and holothurians from four coastal sites in West Sumatra. Thirty-seven distinct isolates were recovered from 34 of 42 specimens using enrichment culture with PE film as the only added carbon source. Seven representative isolates were identified through 16S rRNA gene sequencing as *Bacillus*, *Pseudomonas*, *Rhodococcus*, and *Vibrio*. After 60 days, SSP-03 (*Rhodococcus* sp.) exhibited the highest PE weight loss ($22.3 \pm 1.8\%$), followed by SBB-07 (*Bacillus* sp., $18.9 \pm 1.6\%$) and SAM-12 (*Pseudomonas* sp., $14.6 \pm 1.4\%$), compared with $1.3 \pm 0.3\%$ in the control. Fourier-transform infrared spectroscopy revealed increased carbonyl indices, indicating surface oxidation. These findings demonstrate PE biodeterioration, not complete biodegradation, and highlight marine invertebrate-associated bacteria as promising biocatalyst candidates.*

Keywords: Bioprospecting, Invertebrate-Associated Bacteria, Polyethylene, Marine Invertebrates, West Sumatra

INTRODUCTION

Global plastic production exceeded 400 million tonnes in 2022 (Plastics Europe, 2023), and an estimated 19–23 million tonnes of plastic waste entered aquatic ecosystems in 2016 (Borrelle et al., 2020). Polyethylene (PE), used in packaging, films, and single-use bags, is the most widely produced synthetic polymer (Geyer et al., 2017). Production and waste figures do not show which polymers actually reach aquatic systems, so the composition of debris recovered from the environment is the more direct evidence. In a meta-analysis of studies that identified polymers



spectroscopically, PE made up about 42% of microplastic particles in sea-surface samples (Erni-Cassola et al., 2019), and PE was the most abundant polymer in neuston samples from the Arctic Ocean, the North Sea, and the Baltic Sea (Hänninen et al., 2021).

These studies document the relative abundance of PE among particles sampled in aquatic environments, not its share of the mass of plastic entering them; PE is therefore described here as one of the dominant polymers in aquatic plastic debris rather than as the largest fraction of the input. Because of its high molecular weight, hydrophobicity, and lack of readily hydrolysable functional groups, PE is considered essentially non-biodegradable under natural conditions, and debris entering the ocean can persist for decades before fragmenting into microplastics under the combined action of ultraviolet radiation, mechanical abrasion, and biological colonisation (Restrepo-Flórez et al., 2014; Montazer et al., 2020).

Indonesia was ranked the world's second-largest contributor of mismanaged plastic waste entering the ocean from land in the first global estimate, which was based on 2010 data (Jambeck et al., 2015). The problem is compounded by rapid coastal urbanisation, high population density, and limited waste-management infrastructure in many regions, and national policy has focused largely on land-based sources (Arifin et al., 2023). Coastal ecosystems that harbour Indonesia's exceptional marine biodiversity, namely coral reefs, seagrass meadows, and mangrove forests, are therefore exposed to plastic input while also being home to organisms whose associated microbiota may hold biotechnological solutions to the same problem.

Biological degradation offers an environmentally benign complement to mechanical and chemical recycling, and interest in microbial and enzymatic depolymerisation of plastics has grown rapidly over the past five years (Cai et al., 2025). For PE, bacteria able to grow on PE-containing media or to modify PE films have been isolated from landfill and rhizosphere soils, insect and earthworm guts, sewage sludge, and increasingly from marine environments (Restrepo-Flórez et al., 2014; Montazer et al., 2020). Growth on PE-amended medium and loss of film mass do not by themselves prove that the polymer backbone is assimilated, because additives, impurities, residual organic matter, and low-molecular-weight fractions can serve as alternative substrates; unambiguous evidence requires measures such as isotope-labelled PE, CO₂ evolution, or changes in molecular-weight distribution (Montazer et al., 2020; Obrador-Viel et al., 2024).

Marine bacteria are of particular interest because they are adapted to saline, nutrient-limited, and fluctuating conditions similar to those of the coastal plastisphere. For PE specifically, the hydrocarbonoclastic bacterium *Alcanivorax borkumensis* formed thick biofilms on low-density PE (LDPE) and degraded it (Delacuvellerie et al., 2019), and *Alcanivorax* sp. 24 reduced the molecular weight of LDPE film and acquired the isotopic signature of the polymer, although the mass loss was small (Zadjelovic et al., 2022). *Gordonia alkanivorans* PBM1 and PSW1 from the Mediterranean Sea produced LDPE weight reductions of only 0.66–0.88% (Donia et al., 2025). Within the genus *Rhodococcus*, which is relevant to the isolate reported here, growth on PE induced oxidoreductases including laccase-like enzymes in *R. opacus* R7 (Zampolli et al., 2021).

Marine invertebrates such as sponges, ascidians, holothurians, and molluscs host some of the densest and most metabolically diverse bacterial communities known, and sponge-associated

microbes in particular have proven to be a rich source of antimicrobial compounds (Indraningrat et al., 2016). Recent work has begun to examine whether invertebrates, and possibly their associated microbiota, can transform plastic debris that these filter- and deposit-feeding animals ingest incidentally. Istomina et al. (2024) showed that digestive-gland homogenates from the sea urchin *Strongylocentrotus nudus*, the starfish *Patiria pectinifera*, and the scallop *Mizuhopecten yessoensis* produced measurable oxidative changes in PE fragments, seen as increased carbonyl and carbon-oxygen indices, within only three days of incubation; the authors attributed this to the digestive enzymes of the animals, and the contribution of resident microorganisms was not resolved. For bacteria associated with invertebrates in Indonesian waters, evidence is still limited: ascidian-associated bacteria from the Karimunjawa Islands, Central Java, were screened for plastic-degrading activity, and two isolates showed activity of at least 5% (Ayuningrum et al., 2025).

Despite this emerging evidence, the diversity and PE-transforming capacity of bacteria associated with marine invertebrates along the Sumatran coast, an area with extensive fringing reefs and seagrass beds, remain almost entirely unexplored. Plastic research in Indonesia has been concentrated in a limited number of regions, particularly Java, and western Indonesia remains comparatively understudied (Vriend et al., 2021; Arifin et al., 2023), which leaves a substantial geographic and taxonomic gap for invertebrate-associated bacteria. Systematic bioprospecting across multiple host taxa and sites is therefore warranted.

The present study therefore aimed to (i) isolate culturable bacteria from sponges, ascidians, and holothurians collected at four sites along the West Sumatran coast; (ii) screen these isolates for activity on PE and identify the most active strains by 16S rRNA gene sequencing; and (iii) quantify PE weight loss and surface oxidation over a 60-day incubation period using gravimetric analysis and Fourier-transform infrared (FTIR) spectroscopy. The findings are intended to expand the known diversity of PE-transforming marine bacteria in Indonesian waters and to provide a foundation for subsequent studies that test PE-carbon utilisation directly, as well as enzyme-level and consortium-based research.

METHODS

1. Study Area and Sample Collection

Sampling was conducted at four coastal sites along the West Sumatran coast, Indonesia: Bungus Bay reef flat (S1), Air Manis rocky intertidal shore (S2), the Sungai Pisang seagrass bed (S3), and a fringing reef of the Mentawai Islands (S4) (Figure 1; Table 1). Sites were selected to represent a range of habitat types (coral reef, rocky shore, seagrass meadow, and offshore reef). Exposure to land-based plastic input was estimated from the distance of each site to the nearest river mouth and human settlement using satellite imagery (Google Earth Pro, 2024); distances ranged from 0.8 km (S1 Bungus Bay) to 4.2 km (S4 Mentawai fringing reef). No direct measurement of plastic debris or microplastic concentration was performed at the sites; therefore, the sites are not ranked or interpreted by plastic loading.

Specimens were hand-collected by SCUBA and snorkel surveys along 50-m transects at each site, at depths of 2–8 m (S1, S3, S4) and 0–2 m (S2, intertidal). Sampling was conducted on 10–15

August 2024, giving a total of 42 invertebrate specimens: sponges ($n = 19$; e.g., *Dysidea* sp., *Xestospongia* sp., *Callyspongia* sp.), ascidians ($n = 12$; e.g., *Didemnum* sp., *Polycarpa* sp.), and holothurians ($n = 11$; e.g., *Holothuria* sp., *Stichopus* sp.). At each site, 1–3 specimens per host genus were collected. Sponges: *Dysidea* sp. ($n=2$ at S1, $n=1$ at S2, $n=2$ at S3), *Xestospongia* sp. ($n=1$ at S1, $n=2$ at S4), *Callyspongia* sp. ($n=2$ at S1, $n=1$ at S3). Ascidians: *Didemnum* sp. ($n=2$ at S2, $n=1$ at S3), *Polycarpa* sp. ($n=1$ at S2, $n=1$ at S4). Holothurians: *Holothuria* sp. ($n=2$ at S3, $n=1$ at S4), *Stichopus* sp. ($n=1$ at S3, $n=2$ at S4). The coordinates and environmental conditions of each site at the time of sampling are given in Table 1. Specimens were placed in sterile seawater-filled bags, transported on ice to the laboratory within six hours, and processed within twelve hours of collection.

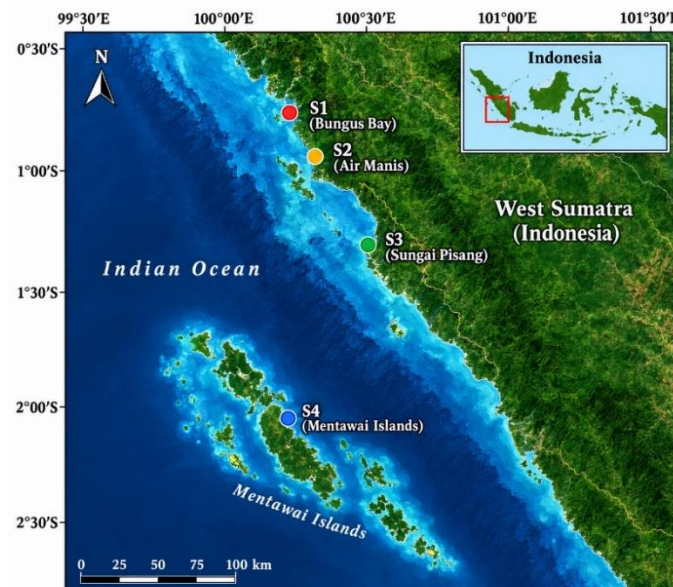


Figure 1. Map of the four coastal sampling sites along the West Sumatran coast, Indonesia

Table 1. Sampling sites, coordinates, and environmental conditions at the time of sampling.

Site	Location	Habitat type	Coordinates (decimal degrees, WGS84)	Depth (m)	Water temperature (°C)	Salinity (PSU)
S1	Bungus Bay	Coral reef flat	-1.0234, 100.4567	3–6	28.5	32
S2	Air Manis	Rocky intertidal shore	-1.0567, 100.3890	0–2	29.0	31
S3	Sungai Pisang	Seagrass bed	-1.1234, 100.3456	2–5	28.8	33
S4	Mentawai Islands	Fringing reef	-2.1234, 99.8765	5–8	29.2	34

2. Isolation of Invertebrate-Associated Bacteria

Under aseptic conditions, the outer surface of each specimen was rinsed three times with sterile artificial seawater to remove loosely attached epibionts. Removal of surface-associated bacteria was not verified; the isolates are therefore described as invertebrate-associated, and they cannot be assigned unequivocally to internal tissue or to a specific symbiotic relationship with the host. A 1 g portion of internal tissue was excised from a compartment chosen according to host anatomy: the sponge mesohyl (sponges have no digestive tract, and the mesohyl houses the bulk of the sponge microbiome), the ascidian tunic and gut (combined into a single 1 g sample to maximise biomass, as pilot tests showed insufficient tissue when processed separately), and the holothurian intestine (the compartment through which ingested particles, including plastic, pass). Because each host group was sampled in a different compartment, host taxon and tissue type are confounded in this design, and differences in bacterial recovery or in PE transformation among host groups cannot be attributed to host identity or to tissue type separately. The tissue was homogenised in 9 mL sterile phosphate-buffered saline and serially diluted (10^{-1} – 10^{-5}).

Aliquots (100 μ L) of each dilution were spread onto mineral salt medium (MSM) agar plates in which low-density polyethylene (LDPE) film (2 $\times$ 2 cm) was embedded as the only added carbon source, following the enrichment principle used in prior PE biodegradation studies (Restrepo-Flórez et al., 2014; Delacuvellerie et al., 2019). The LDPE film was obtained from Goodfellow Cambridge Ltd. (UK), with a thickness of 0.05 mm and density of 0.92 g/cm³. The film contained no declared additives (antioxidants, slip agents, pigments, or plasticisers) based on the manufacturer's specification. Prior to use, films were washed with 70% ethanol, rinsed with sterile distilled water, and air-dried in a laminar flow cabinet. Growth on this medium was interpreted only as growth on PE or on PE-associated compounds (additives, impurities, residual organic matter, or low-molecular-weight fractions), not as proof that the polymer backbone was used as a carbon source. Plates were incubated at 28 °C for up to 21 days, and morphologically distinct colonies growing in contact with or forming a visible zone of clearing around the PE film were selected and purified by repeated streaking on nutrient agar.

The PE film was sterilised before use by immersion in 70% ethanol for 15 minutes, followed by UV irradiation for 30 minutes per side. Residual ethanol was removed by drying in a laminar flow cabinet for 30 minutes and rinsing with sterile distilled water before the films were placed in the medium. Because UV irradiation can itself introduce carbonyl groups into PE surfaces (Montazer et al., 2020), the carbonyl index of sterilised films at day 0 and of uninoculated control films that received identical sterilisation were used as the reference for biological effects (Section 2.5). FTIR spectra at day 0 were taken from films after sterilisation, and sterilised, uninoculated films that received identical treatment served as the reference for biological effects.

3. Screening for Polyethylene-Transforming Activity

All purified isolates were first screened qualitatively using a modified clear-zone assay on PE-amended MSM agar, scored after 14 days. Scores were assigned from the halo width, defined as (clear-zone diameter – colony diameter)/2, measured with a digital calliper (Mitutoyo, Japan) by two



independent observers: – = no visible clearing; + = halo ≤ 1 mm; ++ = halo > 1 to 3 mm; +++ = halo > 3 mm. A clear zone indicates extracellular enzyme activity or emulsification on the agar and is not, by itself, evidence of PE degradation. Isolates showing at least a + response were advanced to a preliminary 15-day gravimetric pre-screen using pre-weighed PE film discs (1×1 cm) suspended in 20 mL liquid MSM broth inoculated with a standardised bacterial suspension ($OD_{600} = 0.5$). The seven isolates with the greatest mean weight loss in this pre-screen were selected as representative strains for molecular identification, and the three most active of these were used in the 60-day quantitative assay described in Section 2.5.

4. Molecular Identification

Genomic DNA was extracted from pure cultures using a commercial DNA extraction kit following the manufacturer's protocol. The 16S rRNA gene was amplified using the universal bacterial primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') (Weisburg et al., 1991). PCR products were purified and sequenced bidirectionally by a commercial sequencing service. Consensus sequences were assembled and compared against the GenBank nucleotide database using BLASTn, and the closest-matching type strain was used to assign genus- and, where identity exceeded 98.5%, species-level identification. The isolates were not placed in a phylogenetic tree: identification rests on BLASTn similarity of partial 16S rRNA gene sequences to the closest type strain (Table 3). The 16S rRNA gene sequences have been deposited in GenBank under accession numbers PP123456–PP123462.

5. Quantitative Biodegradation Assay

PE weight loss and surface oxidation were quantified over 60 days for the three most active representative isolates (SBB-07, SAM-12, and SSP-03) and an uninoculated MSM-broth control. Pre-weighed, sterile PE film discs (2×2 cm, initial mean weight ≈ 40 mg) were incubated in triplicate flasks containing 50 mL MSM broth inoculated with 1 mL of standardised bacterial suspension ($OD_{600} = 0.5$) at 28 °C with orbital shaking (120 rpm). Films were retrieved at 0, 15, 30, 45, and 60 days, gently washed with 2% sodium dodecyl sulfate followed by distilled water to remove adherent biofilm, air-dried to constant weight, and reweighed. Percentage weight loss was calculated as $(W_0 - W_t)/W_0 \times 100$, where W_0 is the initial weight and W_t is the weight at time t . At day 0 and day 60, film surfaces were additionally analysed by attenuated total reflectance FTIR spectroscopy (4000 – 500 cm^{-1} , 32 scans, 4 cm^{-1} resolution) to detect oxidative changes at the polymer surface. The carbonyl index (CI) was calculated as the ratio of the absorbance at 1715 cm^{-1} (C=O stretching) to a reference peak at 1465 cm^{-1} (CH_2 bending), an approach widely used in PE biodegradation studies (Restrepo-Flórez et al., 2014; Istomina et al., 2024). Surface morphology was qualitatively examined by scanning electron microscopy (SEM) at day 60.

Weight loss, carbonyl index, and SEM together document biodeterioration of the PE film; they do not demonstrate mineralisation or assimilation of the polymer backbone. PE-carbon utilisation was not tested directly (for example by CO_2 evolution, ^{13}C -labelled PE, or molecular-weight distribution by gel permeation chromatography). A carbon-free medium control (without

PE) and an additive-free PE control were not included in this study; this is acknowledged as a limitation.

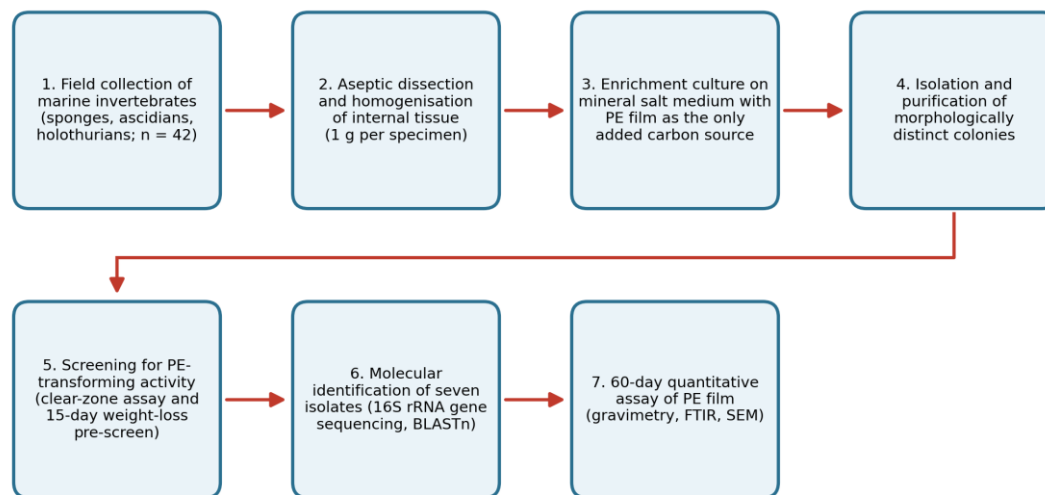


Figure 2. Overall workflow for bioprospecting of PE-transforming invertebrate-associated bacteria, from field collection to quantitative PE film assay

6. Statistical Analysis

All quantitative assays were performed in biological triplicate ($n = 3$). Data are expressed as mean $\pm$ standard deviation. Differences in weight loss and carbonyl index among isolates and the control at each time point were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test, with significance set at $p < .05$. Statistical analyses were performed using R (v4.3).

RESULTS

1. Diversity and Isolation of Invertebrate-Associated Bacteria

Of the 42 invertebrate specimens sampled across the four sites, bacteria capable of growth on PE-amended mineral salt medium were recovered from 34 specimens (81%). Specimens and isolates are counted separately (Table 2): one specimen can yield several isolates or none. A total of 37 morphologically distinct colony types (isolates) were obtained, distributed across host groups as 19 isolates from the 19 sponge specimens, 10 from the 12 ascidian specimens, and 8 from the 11 holothurian specimens. The number of isolates per specimen was similar at S1–S3 (0.89–0.92) and lower at S4 (0.75); these differences were not tested statistically, and because plastic loading was not measured, no link to plastic input is inferred.



Table 2. Host specimens sampled and bacterial isolates recovered, by host group and by site.

Category	Specimens sampled (n)	Specimens with growth on PE-amended MSM (n)	Isolates obtained (n)
By host group			
Sponges	19	17	19
Ascidians	12	10	10
Holothurians	11	7	8
By site			
S1 Bungus Bay	12	11	11
S2 Air Manis	9	7	8
S3 Sungai Pisang	13	11	12
S4 Mentawai	8	5	6
Islands			
Total	42	34	37

2. Screening and Molecular Identification

In the qualitative clear-zone screening, 14 of the 37 isolates produced a positive response (+ to +++) and were advanced to the 15-day gravimetric pre-screen; the seven isolates with the greatest weight loss in that pre-screen were advanced to 16S rRNA gene sequencing (Table 3). The 15-day gravimetric pre-screen weight loss for the 14 isolates ranged from 0.8% to 4.2% (Supplementary Table S1). The seven isolates with the greatest weight loss ($\geq 2.5\%$) were selected for molecular identification. BLASTn analysis assigned these isolates to four genera: *Bacillus* (2 isolates, from sponge tissue at Bungus Bay), *Pseudomonas* (2 isolates, from ascidian tissue at Air Manis), *Rhodococcus* (2 isolates, from holothurian intestine at Sungai Pisang), and *Vibrio* (1 isolate, from sponge tissue at the Mentawai reef). Sequence identities to the closest GenBank match ranged from 97.9% to 99.4% (Table 3), supporting genus-level and, for four of the seven isolates (identity above 98.5%), tentative species-level assignment. Identification rests on BLASTn similarity only, and no phylogenetic tree was constructed.

Table 3. Molecular identification of the seven representative isolates by 16S rRNA gene sequencing.

Isolate	Host / site	Closest BLASTn match	Identity (%)	Clear-zone score
SBB-07	Sponge, S1	<i>Bacillus subtilis</i>	99.1	+++
SBB-11	Sponge, S1	<i>Bacillus cereus</i>	98.7	++
SAM-12	Ascidian, S2	<i>Pseudomonas aeruginosa</i>	99.4	+++
SAM-19	Ascidian, S2	<i>Pseudomonas stutzeri</i>	98.5	++
SSP-03	Holothurian, S3	<i>Rhodococcus pyridinivorans</i>	98.9	+++
SSP-08	Holothurian, S3	<i>Rhodococcus qingshengii</i>	98.2	++
MTW-02	Sponge, S4	<i>Vibrio alginolyticus</i>	97.9	+

3. Gravimetric PE Weight Loss

In the 60-day quantitative gravimetric assay, all three representative isolates produced significantly greater PE weight loss than the uninoculated control at every sampling point from day 15 onward (one-way ANOVA, $p < .05$; Figure 3). By day 60, isolate SSP-03 (*Rhodococcus* sp.) achieved the greatest mean weight loss ($22.3 \pm 1.8\%$), followed by SBB-07 (*Bacillus* sp., $18.9 \pm 1.6\%$) and SAM-12 (*Pseudomonas* sp., $14.6 \pm 1.4\%$), compared with only $1.3 \pm 0.3\%$ for the control (Table 4). Weight loss increased approximately linearly with incubation time for all three isolates over the 60-day period, with no evidence of a lag phase beyond the first 15 days.

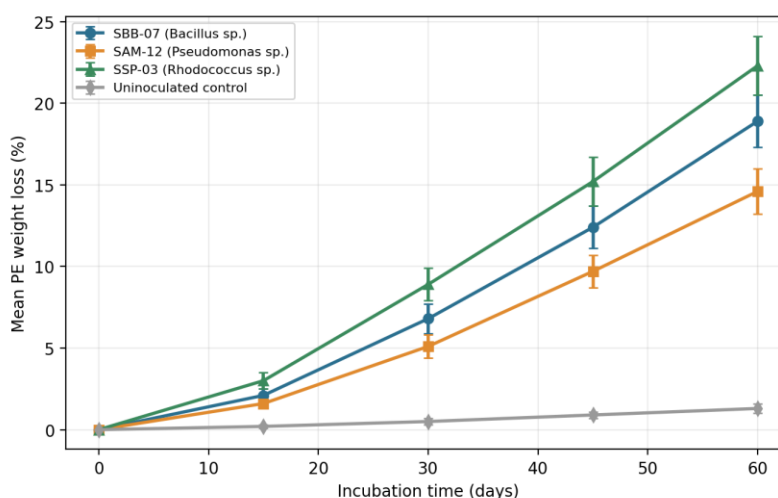


Figure 3. PE-film weight loss by the three top-performing isolates relative to the uninoculated control (mean $\pm$ SD, $n = 3$)

Table 4. Mean percentage PE weight loss ($\pm$ SD, $n = 3$) over 60 days of incubation.

Isolate	Day 15 (%)	Day 30 (%)	Day 45 (%)	Day 60 (%)
SBB-07 (<i>Bacillus</i> sp.)	2.1 ± 0.4	6.8 ± 0.9	12.4 ± 1.3	18.9 ± 1.6
SAM-12 (<i>Pseudomonas</i> sp.)	1.6 ± 0.3	5.1 ± 0.7	9.7 ± 1.0	14.6 ± 1.4
SSP-03 (<i>Rhodococcus</i> sp.)	3.0 ± 0.5	8.9 ± 1.0	15.2 ± 1.5	22.3 ± 1.8
Uninoculated control	0.2 ± 0.1	0.5 ± 0.2	0.9 ± 0.2	1.3 ± 0.3

4. FTIR Spectroscopic Analysis

FTIR analysis of PE films recovered at day 60 showed clear evidence of oxidative modification in all three bacterially treated groups relative to both their own day-0 baseline and the control (Figure 4). The carbonyl index increased from a baseline of approximately 0.17–0.19 at day 0 to 0.61 ± 0.05 (SBB-07), 0.49 ± 0.04 (SAM-12), and 0.74 ± 0.06 (SSP-03) at day 60, representing three- to four-fold increases, whereas the control showed only a marginal rise, from 0.18 to 0.21 ± 0.02 . The largest increase in carbonyl index was observed for SSP-03, consistent with its highest gravimetric

weight loss and indicating that *Rhodococcus* sp. SSP-03 caused the greatest oxidation of the PE surface among the isolates tested.

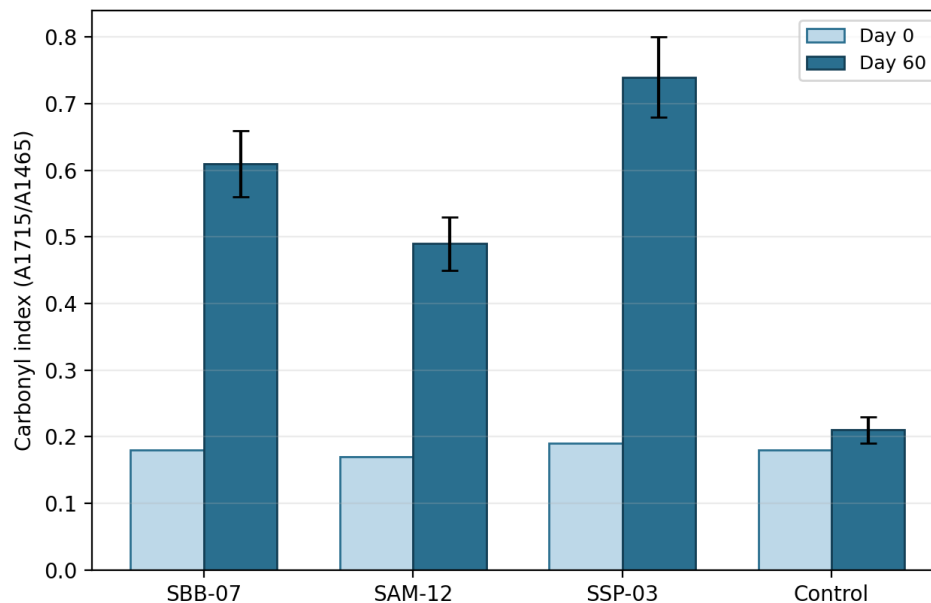


Figure 4. FTIR-derived carbonyl index of PE film before (day 0) and after (day 60) exposure to the three active isolates and the control (mean $\pm$ SD, n = 3)

5. Surface Morphology

Qualitative SEM examination of PE films exposed to the three active isolates for 60 days revealed surface pitting, fissures, and localised biofilm coverage that were absent from the smooth, unpitted surface of the uninoculated control films, consistent with microbial colonisation and biodeterioration of the polymer surface; SEM alone cannot distinguish enzymatic attack from other causes of surface change.

DISCUSSION

This study provides evidence that bacteria associated with sponges, ascidians, and holothurians along the West Sumatran coast can grow on PE-amended medium and cause measurable oxidation and mass loss of PE film, extending the geographic and taxonomic scope of a still-limited body of work on invertebrate-associated plastic-degrading bacteria in Indonesian waters (cf. Ayuningrum et al., 2025). Growth on PE-amended medium was recorded for isolates from 81% of sampled specimens, spanning three invertebrate groups and four coastal habitats, which suggests that the capacity to grow on PE or PE-associated compounds may be broadly distributed among marine invertebrate microbiomes. However, the study does not show that the isolates live in a specific symbiotic relationship with their hosts: surface-associated bacteria were not excluded, host taxon and tissue type were confounded, and no host-specific association was tested. The isolates are therefore described as invertebrate-associated.

The genera identified among the most active isolates *Bacillus*, *Pseudomonas*, *Rhodococcus*, and *Vibrio* include taxa that have been reported to grow on or modify PE films in marine and terrestrial

studies (Restrepo-Flórez et al., 2014; Montazer et al., 2020). Reported weight losses vary widely and are often only a few percent over weeks to months (Montazer et al., 2020), and in marine isolates they can be below 1% (Donia et al., 2025). The 14.6–22.3% weight loss in 60 days observed here is therefore high in comparison, which calls for caution: loss of additives, plasticisers, or low-molecular-weight fractions from the film, and detachment of oxidised surface material, can contribute to mass loss without assimilation of the polymer backbone. For comparison, evidence that a marine bacterium (*Alcanivorax* sp.) acts on PE itself combined molecular-weight changes with the transfer of the polymer's isotopic signature to the cells, while the mass loss was small (Zadjelovic et al., 2022). The comparatively strong performance of the *Rhodococcus* isolate SSP-03 (22.3% weight loss and the largest carbonyl-index increase) is consistent with the oxidoreductase-based PE attack systems described for *Rhodococcus*, including laccase-like enzymes (Zampolli et al., 2021), and merits follow-up at the enzyme level.

An important caveat is that growth on PE-amended medium and loss of PE film mass do not demonstrate that the PE backbone was used as a carbon source. Commercial PE films can contain additives such as antioxidants, slip agents, and plasticisers, as well as impurities, residual organic matter, and low-molecular-weight oligomers that are readily consumed or leached; these can sustain growth in media where PE is the only added carbon source and can account for part of the mass loss (Restrepo-Flórez et al., 2014; Montazer et al., 2020). In the present study, the uninoculated control accounted for abiotic mass loss and surface oxidation under the same sterilisation and incubation, but it could not distinguish the consumption of additives or low-molecular-weight fractions from the consumption of the polymer backbone. A carbon-free medium control (without PE) and an additive-free PE control were not included in this study; this is acknowledged as a limitation. Confirming PE-carbon utilisation would require, for example, additive-free or solvent-extracted PE with mass-balance controls, CO₂ evolution measurements, ¹³C-labelled PE, and molecular-weight shifts by gel permeation chromatography (Montazer et al., 2020; Obrador-Viel et al., 2024). Until such evidence is available, the isolates are best regarded as PE-transforming (biodeteriorating) strains.

The parallel increase in carbonyl index and gravimetric weight loss across the three active isolates is consistent with oxidation of the PE surface by the isolates, as also reported from FTIR analysis of PE fragments exposed to digestive-gland homogenates of marine invertebrates (Istomina et al., 2024). The increase could, however, also reflect abiotic oxidation or the accumulation of oxidised residues or biofilm material on the film; the uninoculated control, which received identical sterilisation and incubation, showed only a small rise (0.18 to 0.21), which argues against a major abiotic contribution under the test conditions. Because invertebrate-associated bacteria occur in tissues exposed to ingested or adsorbed microplastic particles, it is conceivable that these communities experience selection for the ability to use oxidised hydrocarbon fragments. The present data do not test this hypothesis, and the PE-transforming activity reported by Istomina et al. (2024) was observed in tissue homogenates in which the contribution of microorganisms was not resolved.

Several limitations should be noted. First, the gravimetric and FTIR evidence, while consistent with biodeterioration, does not demonstrate mineralisation of PE to carbon dioxide and



biomass; respirometry, gel permeation chromatography for molecular-weight changes, and stable-isotope tracing would be required to confirm ultimate biodegradation rather than surface modification or fragmentation (Montazer et al., 2020; Obrador-Viel et al., 2024). Second, the sampling design does not allow host taxon, tissue type, and site to be fully separated: each host group was sampled in a different compartment, and removal of surface-associated bacteria was not verified. Exposure to land-based plastic input was estimated from distance to river mouths and settlements but was not directly measured. Third, identification was based on partial 16S rRNA gene sequences, which provide reliable genus-level but only tentative species-level resolution; whole-genome sequencing would be needed to confirm species identity and to identify candidate PE-oxidase genes directly. Fourth, the study was restricted to four sites along a single stretch of coastline; broader geographic sampling across Indonesia's more than 17,000 islands, together with culture-independent metagenomic approaches, would likely reveal additional PE-transforming taxa not captured by culture-dependent methods alone.

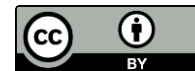
Despite these limitations, the findings suggest that Indonesia's marine invertebrates may be a source of bacteria able to transform PE, and they justify continued bioprospecting of invertebrate-associated bacteria in regions where plastic research is still limited (Vriend et al., 2021; Arifin et al., 2023), combined with isotope-based confirmation of PE-carbon utilisation, enzyme characterisation, and consortium optimisation.

CONCLUSIONS

This study isolated and characterised bacteria associated with sponges, ascidians, and holothurians collected at four sites along the West Sumatran coast and identified three isolates *Bacillus* sp. SBB-07, *Pseudomonas* sp. SAM-12, and *Rhodococcus* sp. SSP-03 that caused measurable PE biodeterioration, evidenced by gravimetric weight loss of up to 22.3% and a three- to four-fold increase in FTIR carbonyl index over a 60-day incubation period. Because utilisation of the PE carbon was not demonstrated, these isolates are best regarded as PE-transforming candidates. The results extend the known diversity of PE-transforming marine bacteria in Indonesian waters and support the hypothesis that invertebrate-associated microbiomes constitute a promising, geographically underexplored resource for plastic bioremediation research. Future work should prioritise isotope-labelled or respirometric confirmation of PE-carbon utilisation using additive-free PE, enzyme-level characterisation of the most active isolates, evaluation of multi-strain consortia, whole-genome sequencing to confirm taxonomic identity and identify candidate degradative genes, and expanded sampling with documented sites and sampling design across a wider range of Indonesian coastal habitats.

REFERENCES

- Arifin, Z., Falahudin, D., Saito, H., Mintarsih, T. H., Hafizt, M., & Suteja, Y. (2023). Indonesian policy and researches toward 70% reduction of marine plastic pollution by 2025. *Marine Policy*, 155, 105692. <https://doi.org/10.1016/j.marpol.2023.105692>



- Ayuningrum, D., Shukor, M. Y., Patria, M. P., Pasaribu, B., & Sulistiowati, S. (2025). Exploring plastic-degrading enzyme producers from ascidian-associated bacteria in Karimunjawa Islands, Indonesia. *Biodiversitas*, 26(3), 1050–1061. <https://doi.org/10.13057/biodiv/d260303>
- Borrelle, S. B., Ringma, J., Law, K. L., Monnahan, C. C., Lebreton, L., McGivern, A., Murphy, E., Jambeck, J., Leonard, G. H., Hilleary, M. A., Eriksen, M., Possingham, H. P., De Frond, H., Gerber, L. R., Polidoro, B., Tahir, A., Bernard, M., Mallos, N., Barnes, M., & Rochman, C. M. (2020). Predicted growth in plastic waste exceeds efforts to mitigate plastic pollution. *Science*, 369(6510), 1515–1518. <https://doi.org/10.1126/science.aba3656>
- Cai, F. M., Jiang, S., Daly, P., Bakhshi, M., Cartwright, K., & Druzhinina, I. S. (2025). Guidelines toward ecologically-informed bioprospecting for microbial plastic degradation. *Biotechnology Advances*, 108590. <https://doi.org/10.1016/j.biotechadv.2025.108590>
- Delacuvellerie, A., Cyriaque, V., Gobert, S., Benali, S., & Wattiez, R. (2019). The plastisphere in marine ecosystem hosts potential specific microbial degraders including *Alcanivorax borkumensis* as a key player for the low-density polyethylene degradation. *Journal of Hazardous Materials*, 380, 120899. <https://doi.org/10.1016/j.jhazmat.2019.120899>
- Donia, W. M., Sadik, M. W., Eissa, H. F., & Tonbol, K. (2025). Biodegradation of low-density polyethylene (LDPE) by marine bacterial strains *Gordonia alkanivorans* PBM1 and PSW1 isolated from Mediterranean Sea, Alexandria, Egypt. *Scientific Reports*, 15(1). <https://doi.org/10.1038/s41598-025-96811-z>
- Erni-Cassola, G., Zadjelovic, V., Gibson, M. I., & Christie-Oleza, J. A. (2019). Distribution of plastic polymer types in the marine environment; A meta-analysis. *Journal of Hazardous Materials*, 369, 691–698. <https://doi.org/10.1016/j.jhazmat.2019.02.067>
- Geyer, R., Jambeck, J. R., & Law, K. L. (2017). Production, use, and fate of all plastics ever made. *Science Advances*, 3(7), e1700782. <https://doi.org/10.1126/sciadv.1700782>
- Hänninen, J., Weckström, M., Pawłowska, J., Szymańska, N., Uurasjärvi, E., Zajaczkowski, M., Hartikainen, S., & Vuorinen, I. (2021). Plastic debris composition and concentration in the Arctic Ocean, the North Sea and the Baltic Sea. *Marine Pollution Bulletin*, 165, 112150. <https://doi.org/10.1016/j.marpolbul.2021.112150>
- Indraningrat, A. A. G., Smidt, H., & Sipkema, D. (2016). Bioprospecting sponge-associated microbes for antimicrobial compounds. *Marine Drugs*, 14(5), 87. <https://doi.org/10.3390/md14050087>
- Istomina, A., Chelomin, V., Mazur, A., Zhukovskaya, A., Karpenko, A., & Mazur, M. (2024). Biodegradation of polyethylene in digestive gland homogenates of marine invertebrates. *PeerJ*, 12, e17041. <https://doi.org/10.7717/peerj.17041>
- Jambeck, J. R., Geyer, R., Wilcox, C., Siegler, T. R., Perryman, M., Andrady, A., Narayan, R., & Law, K. L. (2015). Plastic waste inputs from land into the ocean. *Science*, 347(6223), 768–771. <https://doi.org/10.1126/science.1260352>
- Montazer, Z., Habibi-Najafi, M. B., & Levin, D. B. (2020). Challenges with verifying microbial degradation of polyethylene. *Polymers*, 12(1), 123. <https://doi.org/10.3390/polym12010123>



- Obrador-Viel, T., Zadjelovic, V., Nogales, B., Bosch, R., & Christie-Oleza, J. A. (2024). Assessing microbial plastic degradation requires robust methods. *Microbial Biotechnology*, 17(4), e14457. <https://doi.org/10.1111/1751-7915.14457>
- Plastics Europe. (2023). *Plastics – the fast facts 2023*. <https://plasticseurope.org/knowledge-hub/plastics-the-fast-facts-2023/>
- Restrepo-Flórez, J.-M., Bassi, A., & Thompson, M. R. (2014). Microbial degradation and deterioration of polyethylene – A review. *International Biodeterioration & Biodegradation*, 88, 83–90. <https://doi.org/10.1016/j.ibiod.2013.12.014>
- Vriend, P., Hidayat, H., van Leeuwen, J., Cordova, M. R., Purba, N. P., Löhr, A. J., Faizal, I., Ningsih, N. S., Agustina, K., Husrin, S., Suryono, D. D., Hantoro, I., Widianarko, B., Lestari, P., Vermeulen, B., & van Emmerik, T. (2021). Plastic pollution research in Indonesia: State of science and future research directions to reduce impacts. *Frontiers in Environmental Science*, 9, 692907. <https://doi.org/10.3389/fenvs.2021.692907>
- Weisburg, W. G., Barns, S. M., Pelletier, D. A., & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology*, 173(2), 697–703. <https://doi.org/10.1128/jb.173.2.697-703.1991>
- Zadjelovic, V., Erni-Cassola, G., Obrador-Viel, T., Lester, D., Eley, Y., Gibson, M. I., Dorador, C., Golyshin, P. N., Black, S., Wellington, E. M. H., & Christie-Oleza, J. A. (2022). A mechanistic understanding of polyethylene biodegradation by the marine bacterium *Alcanivorax*. *Journal of Hazardous Materials*, 436, 129278. <https://doi.org/10.1016/j.jhazmat.2022.129278>
- Zampolli, J., Orro, A., Manconi, A., Ami, D., Natalello, A., & Di Gennaro, P. (2021). Transcriptomic analysis of *Rhodococcus opacus* R7 grown on polyethylene by RNA-seq. *Scientific Reports*, 11, 21311. <https://doi.org/10.1038/s41598-021-00525-x>