Global J. Environ. Sci. Manage. 10(2): 805-820, Spring 2024, Serial #38

Global Journal of Environmental Science and Management
(GJESM)

Homepage: https://www.gjesm.net/

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ORIGINAL RESEARCH PAPER

Degradation of low-density polyethylene by a novel strain of bacteria isolated from the plastisphere of marine ecosystems

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ARTICLE INFO

Article History:
Received 20 August 2023
Revised 26 November 2023
Accepted 04 January 2024

Keywords:
Biodegradation
Fourier transform infrared
Low-density polyethylene
Marine bacteria
Plastic

ABSTRACT

BACKGROUND AND OBJECTIVES: Low-density polyethylene is one of the dominant recalcitrant plastic pollutants in the ocean, thus causing complicated problems. Biodegradation is an efficient, environmentally friendly, and sustainable option to overcome these problems. This study aims to quantitatively and qualitatively analyze the ability of marine bacterial isolates to degrade low-density polyethylene plastic.

METHODS: Bacteria were isolated from plastic samples using serial dilution technique and inoculated on media containing low-density polyethylene powder. Bacterial degradation ability was analyzed quantitatively based on weight loss percentage and energy-dispersive X-ray spectroscopy values, as well as qualitatively based on changes in physical and chemical structures using Scanning Electron Microscopy and Fourier transform infrared spectroscopy. Meanwhile, bacterial isolates were identified based on gene sequence and phylogenetic analyses.

FINDINGS: Four bacterial isolates were isolated from low-density polyethylene plastic samples. Quantitative analysis found that the low-density polyethylene film experienced weight loss up to 10-15 percent during 35 days of incubation, with a maximum daily weight loss rate of 0.004 milligrams per day, meaning that the four bacterial isolates have the potential to degrade plastic. Meanwhile, qualitative analysis based on Scanning Electron Microscope observations revealed changes in the physical structure of the film surface in the form of a rough surface, formation of holes, and breakdown into clumps across the film surface. Variations in these changes were tested. In the control, no changes occurred and the film surface remained flat and smooth. Conversely, the results of the energy dispersive X-ray spectroscopy spectrum analysis showed that the low-density polyethylene film broke down into smaller fragments, characterized by a decrease in mass from 98.51 percent to 98.23 percent. Fourier transform infrared observations showed variations in transmittance and wavenumbers, indicating changes in chemical bonds or functional groups in the low-density polyethylene film which caused it to become brittle and break down into smaller fragments with a lower molecular weight, making it easier for bacteria to digest. The results of the gene sequence analysis identified four bacterial isolates, namely *Lysinibacillus* sp. IBP-1, Bacillus sp. IBP-2, *Bacillus paramycoides* IBP-3, and Bacillus cereus IBP-4. Based on the quantitative and qualitative analyses, the ability of the bacterial isolates to degrade low-density polyethylene film was shown in the following order: *Bacillus paramycoides* IBP-3 > Bacillus cereus IBP-4 > *Lysinibacillus* sp. IBP-1 > *Bacillus sp*. IBP-2.

CONCLUSION: All four marine bacterial isolates can use low-density polyethylene as the sole carbon source. Based on quantitative and qualitative analyses, *Bacillus paramycoides* IBP-3 has the best potential for degrading low-density polyethylene film. This study provides information on potential bacterial isolates that can be developed to control low-density polyethylene plastic waste.

DOI: 10.22034/gjesm.2024.02.24

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
57	8	2

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Note: Discussion period for this manuscript open until July 1, 2024 on GJESM website at the "Show Article".

INTRODUCTION

Plastic is a synthetic polymer composed of long carbon chains with stable chemical bonds. It is lightweight, resistant to moisture and certain chemicals, flexible, malleable, and hydrophobic (Sekhar *et al.,* 2016). Due to its practical nature, plastic has become an important part of human life with its widespread use, automatically affecting plastic production (Danso *et al.,* 2019). World plastic production reaches 335 million tons per year (mt/y) (Gupta and Devi, 2019). According to data from the Director General of Chemical, Pharmaceutical, and Textile Industries of the Ministry of Industry, Indonesia's plastic production target in 2020-2024 is estimated to reach 6.85-10.03 million tons, with consumption of around 25-40 kilograms per capita per year (kg/capita/year) (IKFT-KP, 2019). However, the use of plastic without proper management accelerates the rate of plastic waste generation in the environment. Based on the National Waste Management Information System of the Ministry of Environment and Forestry of the Republic of Indonesia, Indonesia generates 12.83 million tons of waste per year, with a plastic waste composition of 19.11 percent (%) (SIPSN KLHK, 2023). Commonly used plastic waste management methods include incineration (12%) and recycling (9%), while the remaining 79% accumulates in the environment (Khandare *et al.,* 2022; Geyer *et al.,* 2017). Nonetheless, incineration results in air pollution as it releases harmful gases such as carbon monoxide, furans, dioxins, sulfur dioxide, nitrous oxide, and carbon dioxide in the air, thus causing respiratory and immune disorders (Gangwar *et al.,* 2019; Cheng *et al.,* 2020). Furthermore, around 32% of plastic waste accumulated in landfills ends up in the ocean (Delacuvellerie *et al.,* 2019). Low-density polyethylene (LDPE) is one of the most widely used types of plastic in the world and is most commonly found in the ocean (Pinto *et al.,* 2022). LDPE has a higher molecular weight, so it takes a long time to decompose in the environment (Gupta and Devi, 2020; Li *et al.,* 2020; Raddadi and Fava, 2019). The presence of LDPE in the marine environment can cause marine pollution, endanger marine animals, disrupt the ecological balance, and damage marine ecosystems (Varó *et al.,* 2021; Yang *et al.,* 2021). Several studies have reported solutions for degrading LDPE plastic waste using bacteria which are considered more effective and environmentally friendly (Taghavi *et al.,* 2021; Asiandu *et al.,* 2021). Among these methods is the potential use of *Bacillus cereus* NJD 1 (strain code) isolated from landfills to degrade LDPE with a weight loss percentage (W%) of 43 for 120 days (Jayan *et al.,* 2023). A study of *Marinobacter* sp H-244, *Marinobacter* sp H-246, and *Bacillus subtilis* H-248 found that these three identified marine bacterial isolates can degrade LDPE film, with a maximum W% of up to 1.68 within 90 days of degradation by *Marinobacter* sp H-246 (Khandare *et al.,* 2022). In addition, another previous study reported that three of the six tested marine bacterial isolates were able to degrade LDPE film after incubation for 30 days, namely *Kocuria palustris* M16, *Bacillus pumilus* M27, and *Bacillus subtilis* H1584, with W% of 1%, 1.5%, and 1.75%, respectively (Sangeetha Devi *et al.,* 2019).

Based on the above explanation, the development of a microbial-based plastic waste management method is a wise and environmentally friendly option. In recent decades, studies on the biodegradation of plastic waste have gained great popularity and have been widely conducted. Bacteria that have been proven to be able to degrade various types of plastics, such as PE, LDPE, and others, include *Bacillus, Rhodococcus, Chelatococcus, Comamonas, Pesudomonas, Paenibacillus,* and *Ideonella* isolated from various polluted locations. These bacteria can degrade plastics by producing diverse extracellular enzymes, such as esterase, protease, glycoside, and hydrolases (Yuan *et al.,* 2020). Enzymatic degradation does not harm the environment because it works on specific substrates, thus being considered better, more environmentally friendly, and safer (Roohi *et al.,* 2017). So far, information regarding the degradation of LDPE plastic, both physically and biologically, by marine microorganisms is still limited. Therefore, it is crucial to conduct a study on this topic to contribute to the existing literature. This study aims to discover new bacterial isolates from LDPE plastic waste floating in the ocean in Indonesia. Marine bacteria were chosen as they are more tolerant of various physical and chemical environmental conditions, with high variability. In addition, marine bacteria are more adaptive to exposure to plastic waste accumulating in the ocean. In this study, marine bacterial isolates that have the potential to act as biodegradation agents were tested quantitatively and qualitatively in the laboratory to be further developed and widely used for environmental bioremediation of LDPE plastic

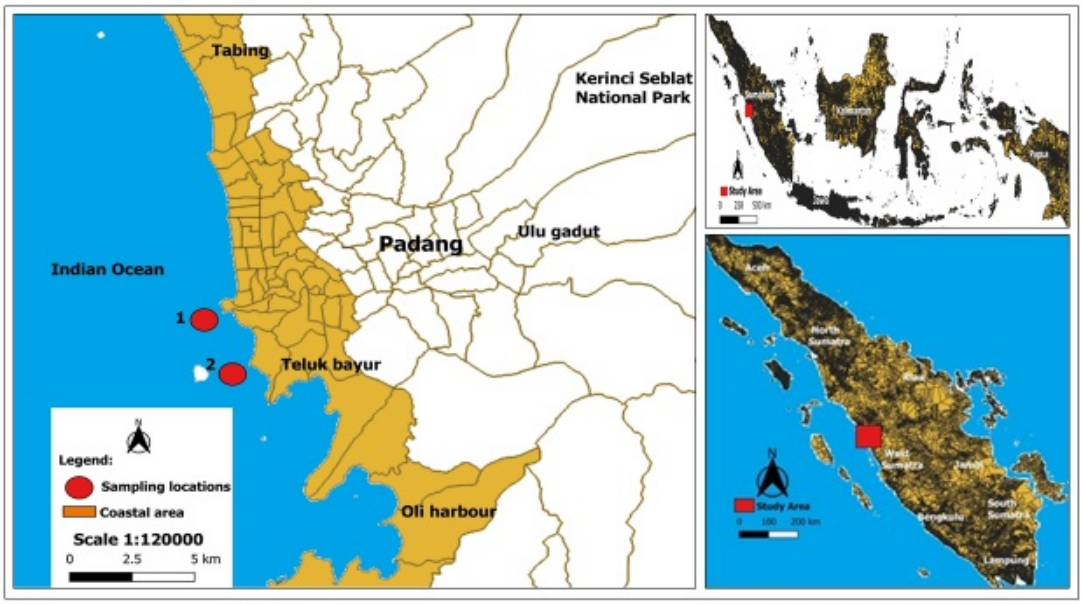


Fig. 1: Geographic location of the study area for marine plastic sampling at Padang Beach, Indonesia

contamination. This study was conducted in coastal Padang City, West Sumatra, Indonesia in 2023.

MATERIALS AND METHODS

Sampling and isolation of marine bacteria for LDPE degradation

In this study, seawater and LDPE plastic sediment samples were taken from two stations (S1 and S2), namely Purus Beach (0°55'57.8"S 100°35'00.1"E) and Padang Beach (0°56'01.3"S 100°21'00.9"E) located on the coast of Padang City, West Sumatra, Indonesia, as shown in Fig. 1. The description of sampling stations is as follows: Purus Beach (S1) is a tourist attraction which is the estuary where the Bandar Purus River meets the Padang Beach. Meanwhile, Padang Beach (S2) is the center of Padang City beach tourism activities which is visited by many domestic and foreign tourists. Samples were taken in April 2023, during the dry season in the region. Samples were taken from sea depths of 0-30 cm and stored in a cool box for further analysis.

Marine bacteria were isolated using media in grams per liter (g/L) consisting of 1 g/L LDPE powder, 0.05 g/L peptone, 15 g/L bacto agar, and 3.5% NaCl. Isolation was performed using a serial dilution technique and inoculation with a pour plate, and incubation was carried out at room temperature, approximately 25 degrees Celsius (25°C). Morphologically distinct colonies were purified on streak plates to obtain pure isolates (Khandare *et al*., 2022). The flowchart of the complete stages of this study procedure can be seen in Fig. 2.

Preparation of LDPE film biodegradation by marine bacterial isolates for quantitative and qualitative analyses

LDPE plastic was collected from the sea with a size of about 10 x 15 cm. Then, the LDPE plastic was prepared for testing by reducing its size to 1x1 square centimeter (cm^2) and sterilizing it using a washing solution of 7 milliliters (mL) tween 80, 10 mL bleach, and 983 mL distilled water for one hour. After that, the LDPE film was rinsed with sterile distilled water 2-3 times to remove any remaining washing solution. The LDPE film was surface sterilized with 70% isopropanol and aseptically transferred into a sterile petri dish to dry overnight (Khandare *et al*., 2022; Sudhakar *et al*., 2008).

Biodegradation of LDPE film by marine bacterial isolates

Sterile LDPE film that has been previously weighed as initial weight data was aseptically inserted into Bushnell Haas medium with the following

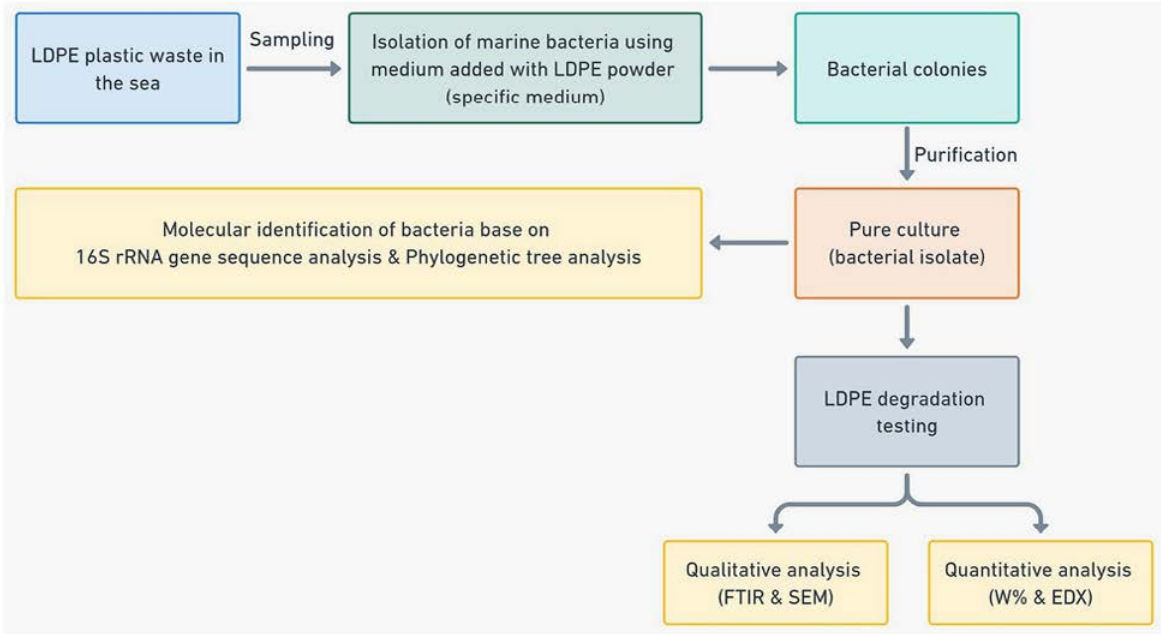


Fig. 2: Flowchart of the current study

composition (g/L): 1.0 ammonium nitrate (NH_4NO_3), 0.2 magnesium sulfate heptahydrate ($MgSO_4$ $7H_2O$), 1.0 dipotassium phosphate (K_2HPO_4), 0.1 calcium chloride dihydrate ($CaCl_2.2H_2O$), and 0.15 potassium chloride (KCl). Then, 3.5% NaCl was added to the medium. Each marine bacterial isolate was inoculated separately at 10% volume per volume (v/v). The inoculum density was adjusted to 1.5 x 10^6 colony-forming units per milliliter (cfu/mL), and a control study was carried out without adding inoculum. The study was conducted in 3 replicates, incubated for 35 days on a shaker with a rotation speed of 120 per minute (120 rpm) at room temperature (Khandare *et al*., 2022).

Harvesting of LDPE film after biodegradation

After the biodegradation process, the LDPE film was removed and rinsed with 2% sodium dodecyl sulfate (SDS) solution to remove the residual cells and medium. Then, the LDPE film was rinsed with sterile distilled water three times and dried overnight. The LDPE film was weighed to obtain its final weight after biodegradation for the quantitative and qualitative analyses (Harshvardhan and Jha, 2013; Sudhakar *et al*., 2008).

Quantitative analysis of the ability of marine bacterial isolates to degrade LDPE film

The ability of marine bacterial isolates to degrade LDPE film was analyzed quantitatively based on the weight loss (%W) of the LDPE film after the biodegradation process calculated using Eq. 1 (Khandare *et al*., 2022).

$$\text{Weight loss (\%)} = [(Iw - fw) \div Iw] \times 100 \quad (1)$$

where:

Iw = Initial weight of LDPE film before the degradation process

Fw = Final weight of LDPE film after degradation

Qualitative analysis of the ability of marine bacterial isolates to degrade LDPE film

During the degradation process, marine bacterial isolates form biofilms on the surface of the LDPE film, resulting in changes in its physical and chemical structures. In this study, the qualitative analysis of LDPE plastic degradation by marine bacterial isolates with the identification of LDPE plastic functional groups applied the FTIR method. The frequency range of the spectrum was observed at a wavelength

per centimeter of 4000/cm–500/cm (Deswati *et al*., 2023a; Deswati *et al*., 2023b; Khandare *et al*., 2022). After the biodegradation process, a qualitative analysis of the morphology of the LDPE plastic surface was carried out by coating the LDPE film with a thin layer of gold nanoparticles. SEM was employed to observe the physical structure of the samples in the form of holes or cracks due to bacterial activity on the surface of the LDPE film (Jayan *et al*., 2023).

Molecular identification of marine bacterial isolates by analyzing the 16S rRNA gene sequence

Isolation of genomic deoxyribonucleic acid (gDNA) utilized the GeneJET Genomic DNA Purification Kit (ThermoFisher Scientific, USA), while gene amplification used a Polymerase Chain Reaction (PCR) machine (Biometra, Germany), KOD One™ PCR Master Mix -Blue-, and a primer pair of 16S rRNA_27F (5'AGA GTTTGATCMTGGCTCAG3') and 16S rRNA_1525R (5'AAGGAGGTGWTC CARCC3') at 35 cycles of PCR (Samimi and Shahriari-Moghadam, 2021). The PCR products were analyzed by 1% agarose gel electrophoresis using GeneRuler 1 kilobase (kb) DNA Ladder (ThermoFisher Scientific, USA). After that, the PCR products were purified, and gene sequencing was performed by a sequencing service provider (1st Base, Singapore) using the Sanger method. Then, the sequencing result in the form of a chromatogram was edited and contigged using the SeqMan™ application. The 16S rRNA gene sequence of each bacterium was BLASTed at the NCBI website (Zhang *et al*., 2000). A total of 15 BLAST sequence data were taken for alignment using the Clustal W algorithm, phylogenetic tree construction using the Neighbor-joining method, determination of evolutionary distance analyzed using the Kimura 2-parameter method, and determination of genetic distance using the MEGA X program. Furthermore, genetic distances were analyzed using the Pairwise Distances method (Kimura, 1980; Kumar *et al*., 2018; Saitou and Nei, 1987) and the bootstrap value used was 1000 (Felsenstein, 1985).

RESULTS AND DISCUSSION

Isolation of plastic-degrading microorganisms

The results of isolation and purification found four marine bacterial isolates that grew in media containing LDPE powder as a selection factor (selective media). Only specific bacterial isolates can live, move, and adapt naturally in the selective medium by producing certain enzymes to use the selective medium as a source of energy (Febria *et al*., 2023; Qubra *et al*., 2023). Four bacterial isolates can produce various enzymes to break down the complex bonds of LDPE and use them as a single carbon source to support the life of microorganisms (Delacuvellerie *et al*., 2019; Jayan *et al*., 2023). Some isolates may include hydrolase, alkane monooxygenase, rubedoxin reductase, and other enzymes (Roager and Sonnenschein, 2019).

Biodegradation of LDPE film by marine bacterial isolates

The result of the LDPE biodegradation process using the four marine bacterial isolates showed that all bacterial isolates grew in Bushnell Haas media containing LDPE film, appearing rather cloudy. In contrast, the control (before the addition of bacterial inoculum) remained clear. This proves that the bacterial isolates can utilize LDPE as the only carbon source. The degradation ability of the four bacterial isolates was quantitatively analyzed based on the weight loss percentage of the LDPE film; the average weight loss of the LDPE film reached 3.4-3.6 milligrams (mg) or about 10-15% during 35 days of incubation, with a daily weight loss rate of 0.004 mg/day. Of the four bacterial isolates, IBP-3 and IBP-4 quantitatively have the best ability, with a maximum weight loss of 15% (Fig. 3). Conversely, the control showed no weight loss.

The weight loss is caused by a degradation process by bacterial isolates which enzymatically break the bonds of the LDPE film and use it as a sole carbon source. LDPE bond-breaking enzymes include laccase, lipase, and esterase (Jayan *et al*., 2023; Khandare *et al*., 2022). The best order of isolate ability in degrading LDPE film is IBP-3>IBP-4>IBP-1>IBP-2. Table 1 shows a comparison of the ability of bacterial isolates to degrade LDPE plastic based on the weight loss percentage of the LDPE film.

In this study, the four marine bacterial isolates showed good weight loss at 35 days (5 weeks) of incubation, compared to the results of several previous studies (Table 1). While this result is the best discovery of this study, further studies are needed to obtain the maximum W% and incubation time of the four bacterial isolates in the LDPE degradation process. The four bacterial isolates have great

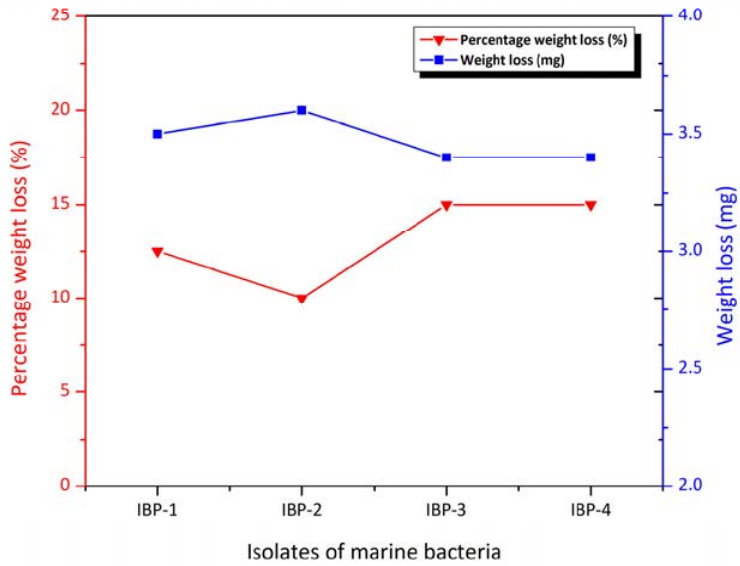


Fig. 3: Weight loss percentage of LDPE film degraded by marine bacterial isolates after biodegradation process for 35 days

Table 1: Comparison of the ability of bacterial isolates to degrade LDPE plastic based on weight loss percentage

Isolates	Sources	Plastic type and size	Weight loss (%)	Day	Sources
Kocuria palustris M16, *Bacillus pumilus* M27 *Bacillus subtilis* H1584	Sea water samples	LDPE	1 1.5 1.75	30	Harshvardhan and Jha, 2013
Bacillus amyloliquefaciens (BSM-1) *Bacillus amyloliquefaciens* (BSM-2)	Municipal solid soil	LDPE 1.5x1.5cm	11 16	60	Das and Kumar, 2015
Stenotrophomonas sp., *Serratia* sp., and *Pseudomonas* sp.	Solid waste-dumping sites	LDPE 10 mg	32 40 21	150	Nadeem *et al*., 2021
SARR1 bacteria	Soil	LDPE 3×3 cm	38.3	30	Rani *et al*., 2021
Bacillus cereus	Mangrove sediment	PE PET PS	1.6 6.6 7.4	40	Auta *et al*., 2017
Bacillus sp. strain 27 *Rhodococcus* sp. strain 36	Mangrove sediment Mangrove sediment	PP	4.0 6.4	40	Auta *et al*., 2018
Alcanivorax borkumensis	Marine	LDPE 1.5 x 1.2 cm	3.5	80	Delacuvellerie *et al*., 2019
Bacillus sp.	Marine	LDPE 1x1 cm	1.26	75	Kumari *et al*., 2019
Isolate IBP-1 Isolate IBP-2 Isolate IBP-3 Isolate IBP-4	Marine plastic waste	LDPE 1 x 1 cm	12.5 10 15 15	35	The current study

potential as a biodegradation agent in reducing LDPE plastic waste.

Qualitative analysis of the ability of marine bacterial isolates to degrade LDPE film

The ability of the isolates to degrade LDPE plastic was confirmed through qualitative analysis of the physical and morphological changes that occurred on the surface of LDPE plastic, which were visualized using high-resolution SEM (Fig. 4).

SEM analysis aims to determine the morphology of the sample's surface (Putra *et al*., 2022). As seen in Fig. 4, there are morphological changes on the surface of LDPE plastic before and after degradation

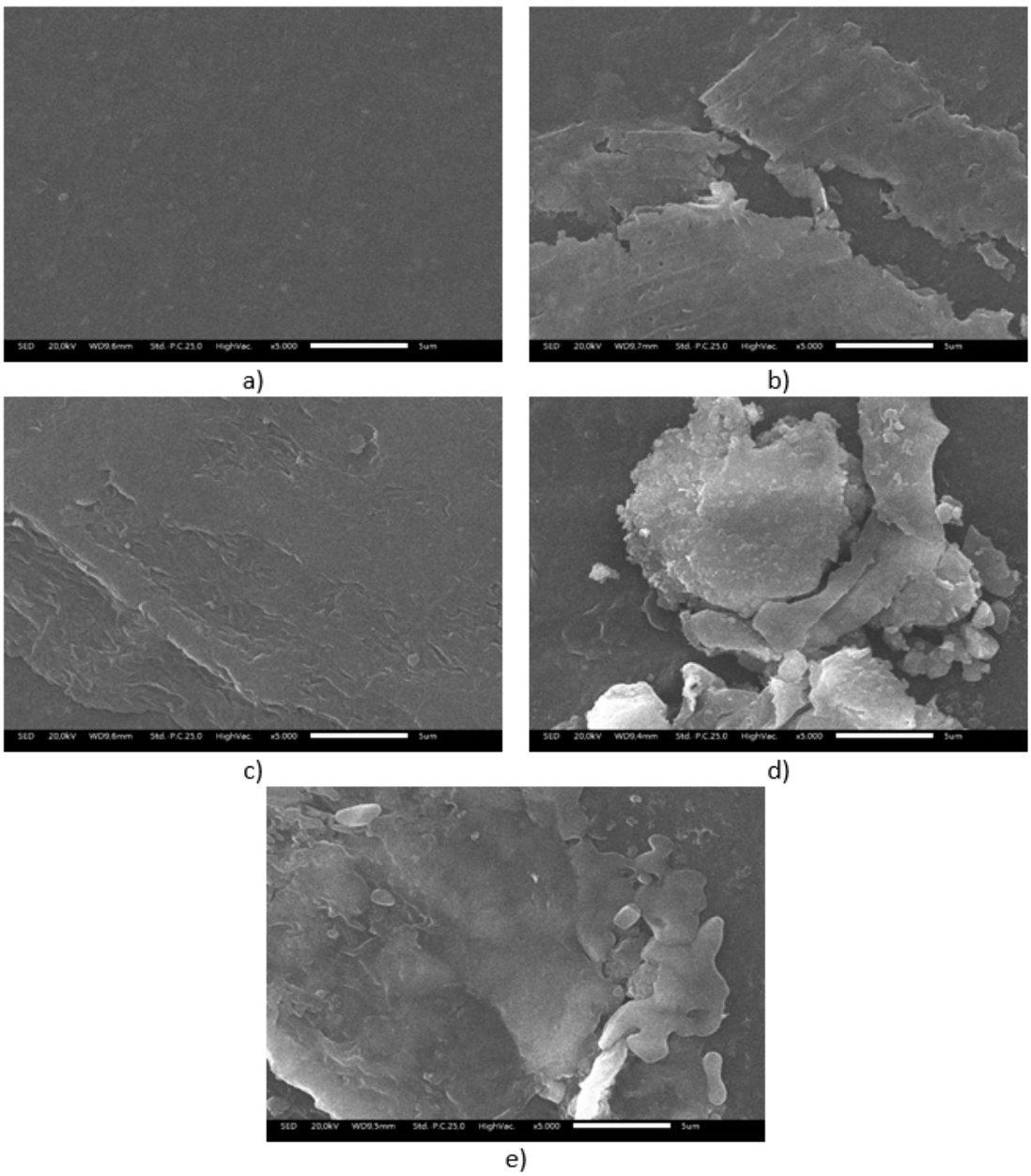


Fig. 4: SEM morphology image of LDPE plastic biodegradation: a) Control (before biodegradation), b) IBP-1, c) IBP-2, d) IBP-3, and e) IBP-4 (after biodegradation)

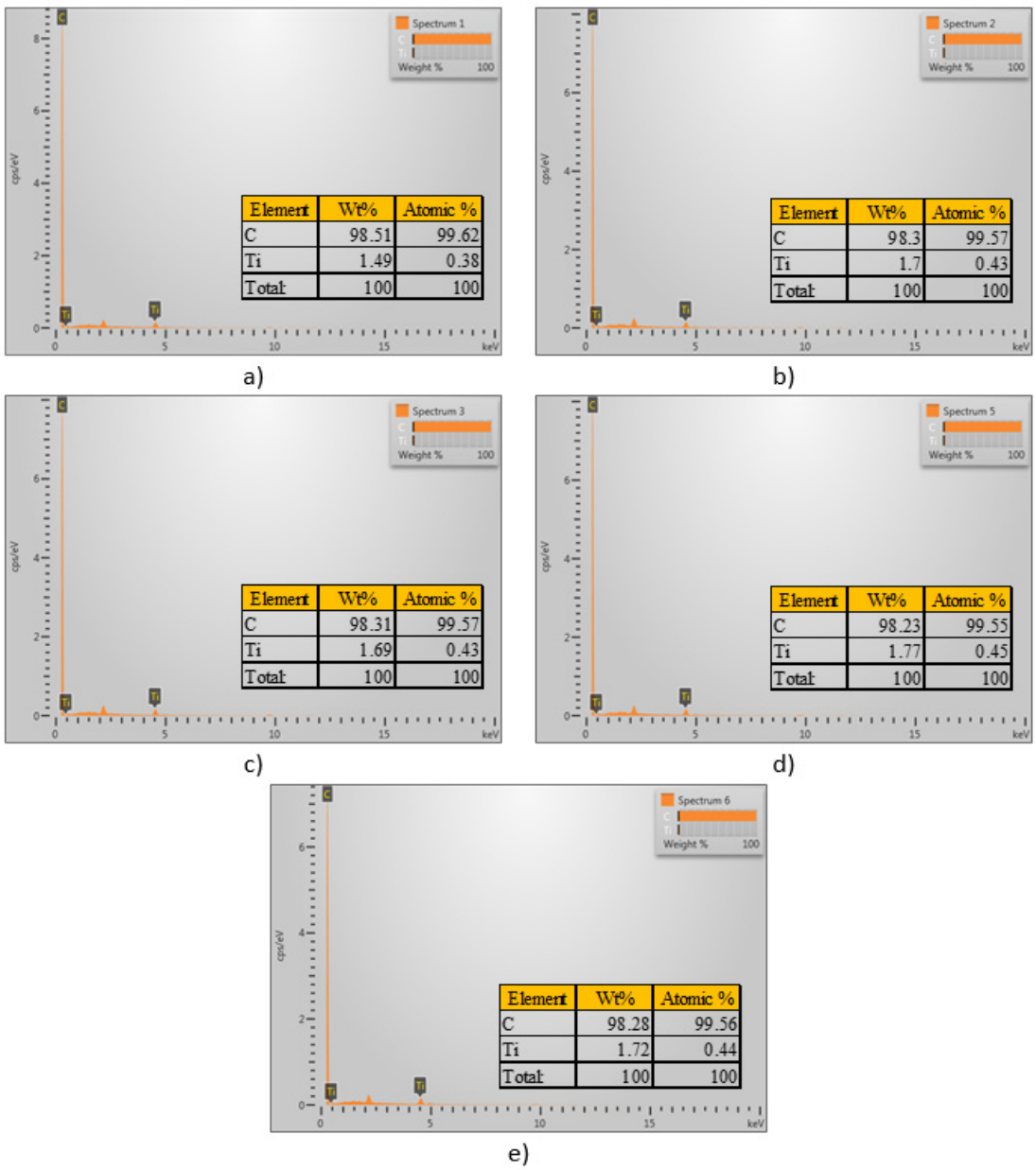


Fig. 5: EDX spectra of LDPE plastic biodegradation: a) Control (before biodegradation), b) IBP-1, c) IBP-2, d) IBP-3, and e) IBP-4 (after biodegradation)

by bacteria. Before degradation, the LDPE plastic has a smooth surface (Fig. 4a). However, after exposure to bacteria, damages and irregularities occur on the surface, indicating a biodegradation process by enzymes. Variations in the degradation results from different types of bacteria can be observed in the resulting images. As presented in Fig. 4b, the polymers that make up the LDPE plastic break into fragments. Fig. 4c displays the rough and pitted surface of LDPE plastic, while Fig. 4d shows the LDPE polymer breakdown into large clumps. Lastly, Fig. 4e shows the evenly distributed clumps on the surface

Table 2: Changes in the wavenumbers of LDPE plastic biodegradation

Wavenumber (/cm)					
Control	IBP-1	IBP-2	IBP-3	IBP-4	Bonding
3835.31	3836.35	3836.85	3836.31	3852.59	C-H
3740.41	3742.98	3742.07	3743.75	3743.52	C-H
3615.35	3616.54	3616.66	3617.2	3615.69	C-H
2914.7	-	2914.69	2914.58	2914.26	C-H
2849.03	2849.05	2849.13	2849	2848.8	C-H
2305.7	2308.9	2305.9	2306.68	2301.12	C=C
2032.01	-	2028.62	2038.98	2028.69	C=C
1972	1976.79	1976.37	1973.02	1972.46	C-H
1641.42	1700.07	1699.01	1660.49	1645.29	C=C
1463.89	1463.97	1463.96	1463.62	1463.41	C-H
1367.94	1369.04	1368.62	1369.68	1370.72	C-H
1049.38	1052.68	1052.44	1052.9	1048.75	C-O
717.62	717.04	717.41	717.6	717.05	C-H
645.28	642.78	645.19	-	-	C-H

of LDPE plastic. Similar findings have been reported in several prior studies (Asiandu *et al*., 2020; Gan and Zhang, 2019; Kim *et al*., 2021; Urbanek *et al*., 2018).

Energy Dispersive X-ray (EDX) spectroscopy is an analytical technique that uses SEM to analyze the elemental composition of observed samples. Based on study data and EDX spectra in Fig. 5, the final result of the LDPE plastic degradation process shows a decrease in %W from 98.51% (Control) to 98.23% (Fig. 5d). This proves that the biodegradation process converts the LDPE plastic polymer into smaller fragments which are eventually oxidized to CO_2 and H_2O. These smaller fragments have a lighter mass than the original plastic polymer, thus causing a decrease in plastic mass (Amobonye *et al*., 2021; Sarkhel *et al*., 2020). Based on existing data, the optimality of several types of bacteria used can be sorted as follows: IBP-3>IBP-4>IBP-1>IBP-2. Of the four bacterial isolates, IBP-4 can degrade LDPE plastic more optimally, as seen in Figs. 4 and 5.

FTIR is used to identify a compound based on the wavenumber of the pure compound from its functional groups (Deswati *et al*., 2023c; Syamsu *et al*., 2024; Samimi, 2024). Fig. 6 shows that the samples degraded by potential bacterial isolates for five weeks experience visible changes in the spectrum, wavenumbers, and transmittance compared to LDPE before degradation. This signifies changes in chemical bonds or functional groups in LDPE plastic due to interactions with bacteria or degradation products (Khandare *et al*., 2021; Abraham *et al*., 2016; Rajandas *et al*., 2012), among which are wavenumbers 835.31-3615.35/cm, 2849.03/cm, 1972/cm, 1463.89/cm, 1367.94/cm (C-H), 1049.38/cm (C-O), and 2032.01/cm, 1641.42/cm (C=C). The changes in the wavenumbers of LDPE plastic biodegradation are displayed in Table 2. According to Webb *et al*., (2013), microorganisms that degrade plastic waste convert carbon in polymer chains into carbon dioxide or incorporate it into biomolecules. The biodegradation process causes plastic waste to become brittle and break down into smaller fragments until the polymer chains in the plastic waste have a molecular weight low enough to be metabolized by microorganisms. This aligns with the EDX analysis data in Fig. 5, where the W% of the control (before degradation) decreases, compared to W% after degradation. In addition, the samples also experience deletion or loss of frequencies. For example, the missing frequency in LDPE plastic is the wavenumber 2032.01/cm, a type of C-H rock vibration from the C=C bond. All missing chemical bonds contain carbon, nitrogen, hydrogen, and oxygen compounds. This is in line with a statement by Yuan *et al*. (2020) that the reduction or addition of hydroxyl groups indicates that monooxygenase enzyme activity has occurred. Nevertheless, initiating polymer chain cleavage is the longest and most challenging step in the degradation process. Thus, a long incubation time is required to produce enough carbonyl groups (C=O) to proceed with the degradation process.

Mechanism of plastic biodegradation

Bacteria can degrade LDPE plastic through

biodegradation. In this regard, LDPE is utilized by bacteria as a source of carbon and energy. Based on study data and characterization, the main stages of the LDPE plastic biodegradation process by bacteria are as follows: 1) Bacterial attachment: bacterial isolates attach to the surface of LDPE plastic (Fig. 6); 2) Extracellular enzyme development: bacterial isolates produce extracellular enzymes that attach to the LDPE plastic polymer (Fig. 7); 3) LDPE polymer breakdown: the extracellular enzymes create smaller fragments of LDPE plastic (Fig 4); 4) Carbon oxidation: the smaller fragments are oxidized to carbon dioxide (CO_2) and water (H_2O); and 5) Plastic weight reduction: the biodegradation process of LDPE plastic leads to a reduction in plastic weight, which can be observed through a decrease in mass loss percentage (Fig. 3) (Ali *et al*., 2021; Amobonye *et al*., 2021; Asiandu *et al*., 2020).

Identification of marine bacterial isolates based on 16S rRNA gene sequence analysis

The identification results of the four marine bacterial isolates based on 16S rRNA gene sequence analysis (Samimi and Shahriari Moghadam, 2020) and phylogenetic tree analysis can be seen in Fig. 8.

The position of Isolate IBP-1 in cluster B of the phylogenetic tree is adjacent to *Lysinibacillus* sp. WTXJ1-4 (KP150574.1). Isolate IBP-2 is adjacent to *Bacillus* sp. VZ1M (JQ618102.1). Isolate IBP-4 is adjacent to *Bacillus paramycoides* strain Alaa5 (OM984660.1), while Isolate IBP5 is adjacent to *Bacillus cereus strain* fg33 (ON715736.1). Based

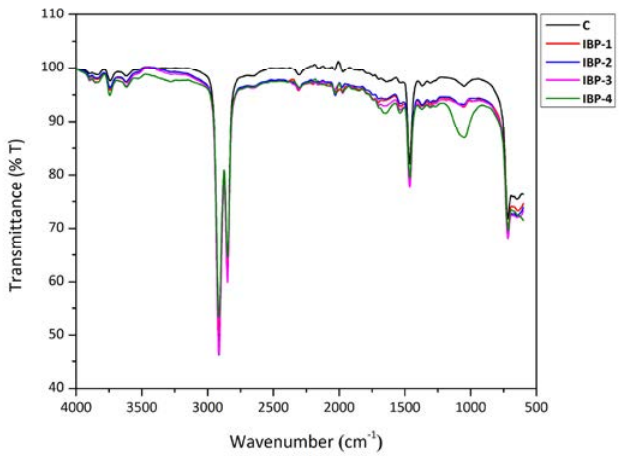


Fig. 6: FTIR spectra of LDPE plastic biodegradation: a) Control (before biodegradation), b) IBP-1, c) IBP-2, d) IBP-3, and e) IBP-4 (after biodegradation)

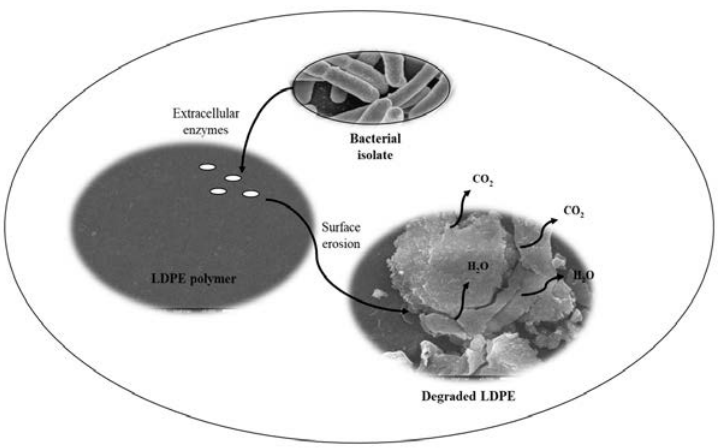


Fig. 7: Biodegradation mechanism of LDPE plastic by bacteria

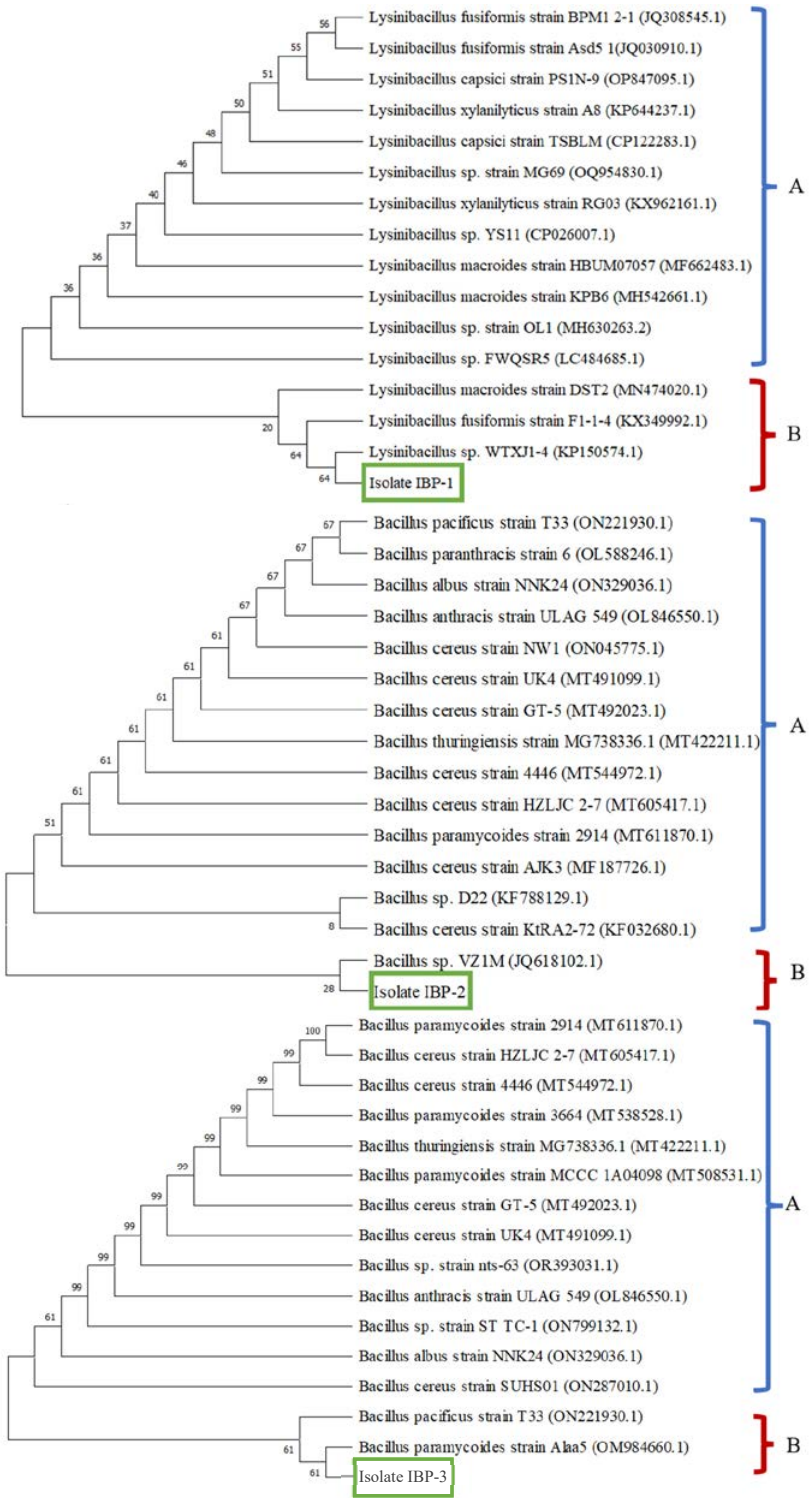
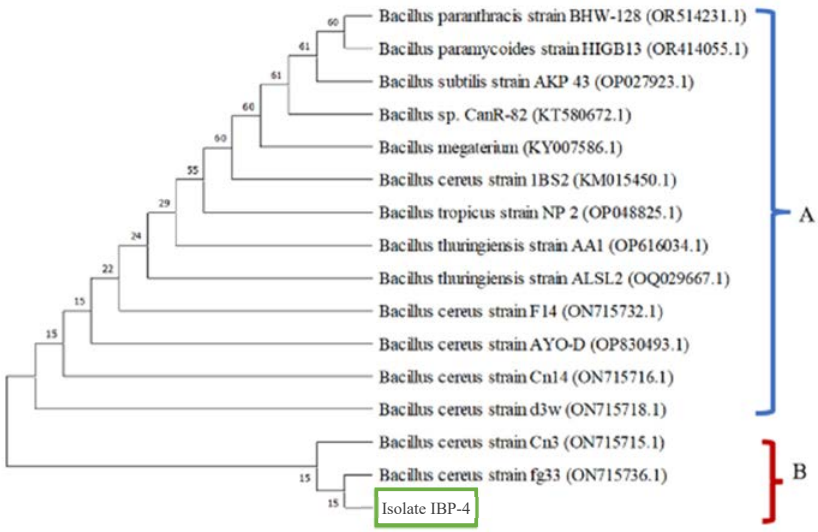


Fig. 8: Identification of LDPE-degrading marine bacteria based on 16S rRNA gene sequence analysis and phylogenetic tree analysis using the Neighbor-joining method with a bootstrap value of 1000 replicates



Continued Fig. 8: Identification of LDPE-degrading marine bacteria based on 16S rRNA gene sequence analysis and phylogenetic tree analysis using the Neighbor-joining method with a bootstrap value of 1000 replicates

on the results of BLAST analysis, genetic distance calculation, and phylogenetic tree construction, the four bacterial isolates are identified as *Lysinibacillus* sp. IBP-1, *Bacillus* sp. IBP-2, *Bacillus paramycoides* IBP-3, and *Bacillus cereus* IBP-4.

CONCLUSIONS

Four bacterial isolates are found from isolated marine plastic debris; they grow in media containing LDPE powder as the sole carbon source. These bacteria have the potential to degrade LDPE. Based on the quantitative study using the weighing process and EDX analysis, the four bacterial isolates showed the best ability to degrade LDPE plastic compared to the results of several previous studies. From the quantitative analysis of the biodegradation test during five weeks of incubation, the four isolates were found to experience a weight loss of 3.4-3.6 mg or about 10-15%, with a daily weight loss rate of 0.004 mg/day. EDX data also showed a decrease in LDPE mass from 98.51% (Control) to 98.23%. This proves that biodegradation has converted the LDPE plastic polymer into smaller fragments which are eventually oxidized to CO_2 and H_2O. Additionally, a qualitative analysis was conducted comprehensively using SEM and FTIR. Changes in the morphology and structure of the plastic surface after degradation were visualized using high-resolution SEM. The surface of the LDPE film was smooth and flat before the degradation process (control). After the biodegradation process by the four bacteria, damages occurred to the LDPE film as follows: the LDPE film broke into several parts (IBP-1), the surface of the LDPE film became rough and pitted (IBP-2), the LDPE film decomposed into large clumps (IBP-3), and evenly-distributed clumps formed on the surface of the LDPE film (IBP-4). Furthermore, the results of the FTIR analysis revealed a change in the wavenumber frequency. Changes in morphology, surface structure, and wavenumbers indicate the activity and performance of bacterial extracellular enzymes in degrading LDPE. Overall, the results of the quantitative and qualitative analyses are interrelated in explaining the biodegradation process of LDPE film by bacteria. Based on both analyses, the four bacterial isolates found in this study are found to be potential LDPE plastic degraders. From the identification, three of the four bacterial isolates were >90% identified as *Lysinibacillus* sp. IBP-1, *Bacillus paramycoides* IBP-3, and *Bacillus cereus* IBP-4, whereas IBP-2 showed a percent identity of only 78.56-83.85% (<90%). This also signifies that IBP-2 is a new strain and species in the genus *Bacillus*. In this study, the four isolates showed the discovery of new strains with the best order of ability to degrade LDPE film polymer (IBP-3>IBP-4>IBP-1>IBP-2). The results of this study can be further developed as an alternative method for LDPE plastic degradation to reduce plastic waste pollution in the future. Therefore, future studies

are highly recommended to involve single isolates and consortia, optimization of the number of bacterial inoculums and environmental factors (incubation time, salinity, and other factors), and examination of the degradation mechanism and enzymes involved in the LDPE degradation process.

AUTHOR CONTRIBUTIONS

F.A. Febria, the corresponding author, conducted literature review, designed and carried out experiments, analyzed and interpreted data, prepared the manuscript, and edited the manuscript. A. Syafrita was responsible for the sampling, experiments, and data collection. A. Putra conducted data analysis, interpreted the results, and prepared the discussion and conclusion sections of the manuscript. H. Hidayat performed secondary data collection, carried out supporting analysis, linked the findings with existing literature, interpreted data, and arranged the layout of the manuscript. C. Febrion assisted in drafting, reviewing, and revising the manuscript.

ACKNOWLEDGMENTS

The authors would like to thank the Chancellor and the Head of the Institute of Research and Community Service (*Lembaga Penelitian dan Pengabdian kepada Masyarakat*/LPPM) of Andalas University for the opportunity and approval to fund this study through the Andalas University Indexed Publication Research scheme grant for the 2023 fiscal year, with contract number: [T/26/UN.16.19/PT.01.03/Energi-RPT/2023].

CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the publication of this manuscript. Ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been completely observed by the authors.

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PUBLISHER'S NOTE

ABBREVIATIONS	DEFINITION
%	Percentage
°C	Degree Celsius
AFM	Atomic force microscopy
ATR-FTIR	Attenuated Total Reflectance-Fourier Transform Infra-Red Spectroscopy
Bacillus cereus NJD 1	NJD 1 strain code bacteria *Bacillus cereus*
Bacillus subtilis H-248	H-248 strain code bacteria *Marinobacter* sp
Bacillus subtilis H-248	H-248 strain code bacteria *Marinobacter* sp
BLAST	Basic local alignment search tool
$CaCl_2.2H_2O$	Calcium chloride dihydrate
cfu/mL	Colony form unit per milliliter
cm	Centimeter
CO_2	Carbon dioxide
DNA	Deoxyribonucleic Acid
EDX	Energy dispersive x-ray spectroscopy
FTIR	Fourier Transform Infra-Red
fw	Final weight
g/L	Gram per liter
GCMS	Gas chromatography-mass spectrometry
H_2O	Dihydrogen oxide

IBP-1	Plastic bacterial isolate code 1
IBP-2	Plastic bacterial isolate code 2
IBP-3	Plastic bacterial isolate code 3
IBP-4	Plastic bacterial isolate code 4
IKTF-KP	*Direktur Jenderal Industri Kimia, Farmasi, dan Tekstil (IKFT) Kementerian Perindustrian (IKFT-KP)* (Director General of Chemical, Pharmaceutical, and Textile Industries, Ministry of Industry of Indonesia)
Iw	Initial weight
K_2HPO_4	Dipotassium phosphate
Kb	Kilobase pair
KCl	Potassium chloride
kg/capita/y	Kilogram per capita per year
LDPE	Low-density polyethylene
Marinobacter sp H-244	H-244 strain code bacteria *Marinobacter* sp
mg/L	Milligram per liter
$MgSO_4.7H_2O$	Magnesium sulfate heptahydrate
mL	Milliliter
mt/y	Million tons per year
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NCBI	National center for biotechnology information
NH_4NO_3	Ammonium nitrate
PCR	Polymerase Chain Reaction
rpm	Rotations per minute
SDS	Sodium dodecyl sulfate
SEM	Scanning electron microscope
SIPSN KLHK	*Sistem Informasi Pengelolaan Sampah Nasional Kementerian Lingkungan Hidup dan Kehutanan* (National Waste Management Information System, Ministry of Environment and Forestry of Indonesia)
W%	Weight loss percentage

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HOW TO CITE THIS ARTICLE

Febria, F.A.; Syafrita, A.; Putra, A.; Hidayat, H.; Febrion. C., (2024). Degradation of low-density polyethylene by a novel strain of bacteria isolated from the plastisphere of marine ecosystems. Global J. Environ. Sci. Manage., 10(2): 805-820.

DOI: 10.22034/gjesm.2024.02.24
URL: https://www.gjesm.net/article_709798.html