HAYATI
Journal of Biosciences

Vol. 31 No. 5, September 2024 867-879
DOI:10.4308/hjb.31.5.867-879
ISSN: 1978-3019
EISSN: 2086-4094

Characterization of Microplastic Degrading Indigenous Bacteria from Ambon Bay Waters

Idham Halil Zakaria[1], Dian Siswanto[2], Irfan Mustafa[2*]

[1]*Master Program of Biology, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, Kota Malang 65145, Indonesia*
[2]*Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, Kota Malang 65145, Indonesia*

ARTICLE INFO

Article history:
Received January 15, 2024
Received in revised form March 22, 2024
Accepted May 2, 2024

KEYWORDS:
16S rDNA,
Ambon Bay,
bacteria,
degradation,
microplastics

ABSTRACT

Microplastic degradation by bacteria can degrade low-density polyethylene (LDPE). This study aimed to analyze the potential of Ambon Bay bacteria for microplastic degradation, the condition of microplastics after degradation, and identification of the potential for microplastic degradation. The results of isolation revealed as many as 20 bacterial isolates, which correlated with physicochemical conditions in the waters of Ambon Bay. Nine of them could degrade microplastics as indicated by the presence of a clear zone, namely KA1, KA2, KA3, KA4, KA5, KA9, KA10, KS6, and KS8. They were checked for biofilm formation, microplastic hydrophobicity, and percentage of microplastic weight reduction. Four isolates with the highest percentage of microplastic weight reduction on day 40 were KA1, KA2, KA3, and KA10 at 36.19%, 10.16%, 28.39%, and 17.07%, respectively. The results of LDPE microplastic degradation showed differences using field emission scanning electron microscopy-energy dispersive spectroscopy (FESEM/EDS), attenuated total reflection-fourier transform infrared (ATR-FTIR), and X-ray diffraction (XRD). The bacterial isolates identified were KA1 (*Bacillus cereus*), KA2 (*Bacillus toyonesis*), KA3 (*Bacillus paramycoides*), and KA10 (*Escherichia coli*). Indigenous bacteria from the waters of Ambon Bay have the potential to degrade LDPE microplastics, which causes structural changes, decreased crystallinity, weight, and C=C groups in microplastics after degradation, with bacterial isolate KA1 identified as *Bacillus cereus* showing the best potential with degradation of LDPE microplastics by 36.19%.

1. Introduction

Ambon Bay is located at 128°1'33,6''-128°18'7.20'' East Longitude and 3°34'4.80''-3°47'38.4'' South Latitude. These waters consist of two main parts: Outer Ambon Bay and Inner Ambon Bay (Gemilang *et al.* 2017). The Inner Ambon Bay has a serious problem due to the accumulation of plastic waste in its waters of Ambon Bay. These waters have serious problems due to the accumulation of plastic waste in the waters of Ambon Bay. The plastic waste accumulated in these waters originates from the activities of coastal communities that throw garbage in coastal waters. The circulation patterns influence the transportation pattern of marine debris in these waters. Morphologically, these waters are semi-enclosed bays; therefore, the water circulation pattern is strongly influenced by tidal parameters. This results in the accumulation of plastic waste (Noya and Tuahatu 2021).

Plastic pollution remains a problem because plastics can break down into smaller particles, known as microplastics (Manullang 2019). It can be caused by physical, chemical, or biological processes (Meng *et al.* 2023). Microplastics are hydrocarbon-based synthetic particles with sizes between 5 mm and 1 µm. Microplastic waste comes from cosmetics, synthetic textiles, packaging, larger fragments of plastic items (Liu *et al.* 2021), and wastewater (Habib *et al.* 2020).

Microplastics have a negative impact on the balance of marine ecosystems (Fachrul and Rinanti 2018). Microplastics have sizes and colors resembling those of food; therefore, many marine organisms consider microplastics as food (Gurjar *et*

* Corresponding Author
E-mail Address: irfan@ub.ac.id

al. 2023). According to Auta *et al.* (2017), microplastic degradation by bacteria has the potential to degrade LDPE-type microplastics to reduce the risk of negative impacts on marine biota, the environment, and humans. LDPE was chosen for this study because of its wide availability and resistance to environmental degradation.

Microplastic biodegradation consists of three steps: colonization of the polymer surface by microbes, biofilm formation, and depolymerization by extracellular enzymes (Meng *et al.* 2023). Several species of marine bacteria that can degrade microplastics are *Kocuria palustris* M16, *Bacillus pumilus* M27, *Bacillus subtilis* H1584, *Cucumis sativus*, and *Pseudomonas stutzeri*, which are capable of producing microbial flocculants and have cell biocatalysts for microplastic biodegradation (Gong *et al.* 2018). The microplastic content in the waters of Ambon Bay needs to be addressed immediately so that an effective solution can be found, which is to utilize bacteria as microplastic degraders.

This research was conducted as an initial step to overcome the microplastic pollution found in the waters of Ambon Bay. This study aimed to analyze the potential of indigenous bacteria for microplastic degradation, the condition of microplastics after degradation, and the bacteria with the best potential for microplastic degradation in the waters of Ambon Bay.

2. Materials and Methods

2.1. Sampling and Physicochemical Measurements of Sea Water and Sediments

This study was conducted from March 2023 to December 2023. Sampling was carried out in Inner Ambon Bay, Indonesia (Figure 1). This location was chosen based on the amount of plastic waste. Seawater and sediment samples were collected from four locations in Inner Ambon Bay waters using purposive sampling.

The samples were composited into seawater and sediment samples, and the bacteria were isolated and identified. Physicochemical measurements of the sampling locations were performed, including water temperature, water pH, light intensity, wind speed, water salinity, and water depth. Water samples were taken from surface seawater, and sediment samples were taken at a depth of 3-5 cm from the surface. The samples were stored in an ice box under cold conditions (4°C) prior to laboratory analysis.

2.2. Isolation and Identification of Bacteria

Bacterial isolation was performed on Nutrient Agar (NA) medium (Merck, Germany) using serial dilution and spread plates and incubated for 48 h to obtain morphologically distinct colonies. The colonies were purified using the streak plate method (quadrant)

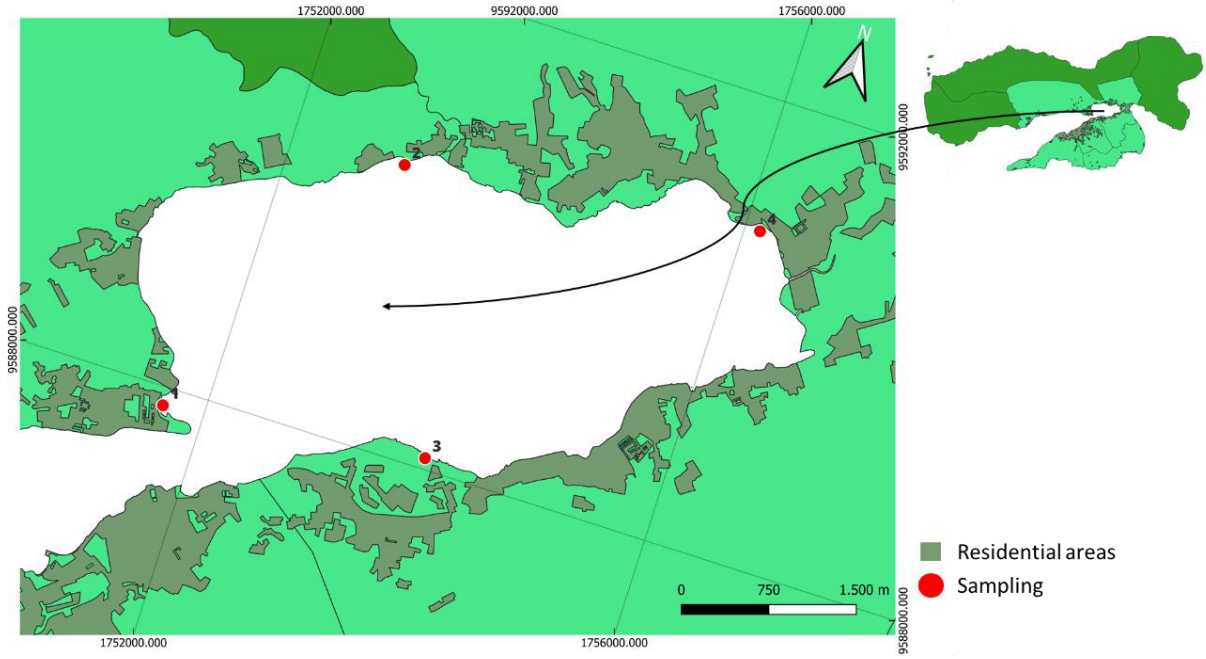

Figure 1. Sampling location

and confirmed by microscopic observation following Gram staining (Leboffe and Pierce 2012).

2.3. Measurement of Clear Zone of Microplastic Degrading Bacteria

Test the ability of bacteria to degrade microplastics using Mineral Salt Media (MSM) agar (yeast extract: 1 g/L, peptone: 2.5 g/L, $NaNO_3$:1 g/L, $CaCl_2$:0.02 g/L, K_2HPO_4:0.3 g/L, $MgSO_4$:0.05 g/L, $FeSO_4$:0.03 g/L, NaCl: 20 g/L, LDPE powder: 5 g/L, and agar 17 g/L) (Dey *et al.* 2020). The bacterial isolates were inoculated onto MSM and incubated for 3-5 days at room temperature using a paper disk. The clear zone in the qualitative test was calculated using the formula (Surjowardojo *et al.* 2016):

$$\text{Clear zone index} = \text{Average of clear zone diameter (mm)} - \text{disk paper} \quad (1)$$

2.4. Measurement of Biofilm Formation, Hydrophobicity, and OD Value of Bacterial Isolates

Bacterial isolates with clear zone values were further analyzed by measuring biofilm formation, hydrophobicity, and OD values simultaneously on days 0, 10, 20, 30, and 40. The microplastic weight percentage was determined on Day 40. These measurements were performed by growing the bacterial isolates on MSM (Dey *et al.* 2020).

2.4.1. Biofilm Formation of Bacterial Isolates

Biofilm formation on the LDPE surface in NA medium and LDPE (5 g/L) was assessed using the spread plate method of 0.1 ml. The number of colonies was counted after overnight incubation at room temperature, and the following formula was used to determine the biofilm cell growth. Biofilm growth was measured on days 0, 10, 20, 30, and 40 (Dey *et al.* 2020).

2.4.2. Measurement of Bacterial Hydrophobicity

The hydrophobicity of the bacterial cells was measured using the bacterial adhesion to hydrophobicity (BATH) method (Rosenberg *et al.* 1980). Bacteria were cultured with 1 ml in phosphate-buffered saline (PBS) (NaH_2PO_4:1.44 g/L, K_2HPO_4, 8 g/L; NaCl, 8 g/L) and washed twice. The washed cell pellet was resuspended in 4 ml PBS, and the initial optical density (OD) was plotted. The samples whose absorbance had been measured were supplemented with 0.2 ml of hexane (Samet and Icen 2022), and the organic phase was discarded. The liquid phase was used for the second hydrophobicity value (final OD) measurement. The absorbance was measured using a spectrophotometer at a wavelength of 400 nm. PBS without cells was used as blank. This formula was used to measure the percentage hydrophobicity of the bacteria (Adithama *et al.* 2023).

2.4.3. Measurement of OD Value of Bacterial Isolates

Measurement of OD values under treatment and control conditions using spectrophotometry at a wavelength of 600 nm (Auta *et al.* 2017).

2.5. Microplastic Degradation Ability

The degradation test was carried out by growing the bacterial isolates on MSM + LDPE powder (5 g/L) and incubating for 40 days at room temperature. In each experiment, the OD values were measured on days 0, 10, 20, 30, and 40. The microplastics were separated from the biofilm, washed using 70% alcohol and Tween 80 (1%), dried in an oven at 50°C for 24 h, and the final weight was taken. The final weight of the microplastics was determined by measuring their degree of degradation. Based on Habib *et al.* (2020), the percentage of microplastic weight loss was calculated using the following equation:

$$\text{Microplastic weight (\%)} = \left(\frac{\text{initial weight } (W_0) - \text{Final weight } (W)}{\text{initial weight } (W_0)}\right) \times 100 \quad (2)$$

The data were further analyzed to determine the rate constant of microplastic polymer reduction using a first-order kinetic model based on the equation of Auta *et al.* (2017). After the microplastic polymer removal rate constant was obtained, the half-life ($t_{1/2}$) with an ln value of 0.69 was calculated based on the equation from Auta *et al.* (2017).

2.6. Characterization of Microplastics After Degradation

The sample used to observe the changes in chemical groups was a 5% LDPE sample from MSM after 40 days. Microplastics with bacterial isolates that show potential degradation ability were analyzed using FESEM/EDS, ATR-FTIR, and XRD.

2.7. Identification of Bacteria Based on 16S rDNA Sequences

Isolates with the potential to degrade microplastics were identified by 16S rDNA sequencing. Genomic DNA was extracted from the isolates using a Quick-DNA Magbead Plus Kit (Zymo Research, D4082). The 16S rRNA gene was amplified by PCR using universal bacterial primers (27F: 5'–AGAGTTTGATCMTGGCTCAG–3' and 1492R: 5'–TACGGYTACCTTGTTACGACTT–3'). Phylogenetic tree analysis was performed using the bootstrap neighbor-joining (NJ) method with a sample repetition of 1.000 times on Molecular Evolutionary Genetics Analysis (MEGA) 11.0.

2.8. Data Analysis

Various tests were analyzed using ANOVA ($p<0.05$). Physicochemical analysis and importance value index were examined using CCA and Diversity Index in PAST 4.12 software.

3. Results

3.1. Physicochemical Conditions and Microplastic Waste in Ambon Bay Waters

Samples from the four sites were analyzed for temperature, pH, light intensity, wind speed, salinity, and depth using multiple ANOVA ($p>0.05$) (Table 1).

3.2. Importance Value Index (IVI)

Isolation of bacteria from the waters of Inner Ambon Bay amounted to twenty isolates. Ten bacterial isolates from seawater samples were KA1-KA10. Ten bacterial isolates from sediment samples were KS1-KS10. The importance value indices (IVI) in the two areas, seawater and sediment, were different. This index represents the relative proportion of each bacterial isolate in the sample. The difference in index values in the two areas showed variations in the composition and diversity of bacteria in the waters of Ambon Bay. Isolate KA3 showed the highest IVI (24.35%), and isolate KA1 showed the smallest IVI (3.47%) in the seawater samples. Isolates KS6 and KS10 had the highest and smallest IVI, which were 16.66% and 4.32%, respectively, in the sediment samples (Figure 2).

The diversity index of bacterial diversity was determined using the Simpson and Shannon indices (Table 2). The Simpson index values were 0.6963 (seawater) and 0.7374 (sediment). The Shannon index values were 1.564 (sediment) and 1.676 (seawater).

3.3. Correlation between Bacteria and the Aquatic Environment

Isolates KA5, KA7, KA10, and KS5 were negatively correlated with depth but not with pH, temperature, salinity, wind speed, and light. Isolates KA2, KA3, KA6, KS1, KS2, and KS10 were positively correlated with pH, temperature, salinity, and light and negatively correlated with wind speed, with no depth correlation. Isolates KA1, KA9, KS6, and KS9 have a positive correlation with wind speed and a negative correlation with pH, temperature, salinity, and light without light correlation. Isolates KA4, KA8, KS3, KS4, KS7, and KS8 have a positive correlation with depth without correlation with pH, temperature, salinity, wind speed, or light (Figure 3).

3.4. Ability of Microplastic Degradation Bacteria Based on Clear Zone

Bacteria that show clear zones have the potential as LDPE microplastic degraders. Nine out of the 20 bacterial isolates formed clear zones. The bacterial isolates are KA1, KA2, KA3, KA4, KA5, KA9, KA10, KS6, and KS8 (Table 3).

3.5. Biofilm Formation, Hydrophobicity, and Bacterial Growth

Nine bacterial isolates with clear zones showed potential for microplastic degradation. The

Table 2. Bacterial diversity index

	Simpson	*Shannon*
Seawater (KA)	0.6963	1.676
Sediment (KS)	0.7374	1.564

Table 1. Physicochemical conditions

Location	Temperature (°C)	pH	Light Intensity (lux)	Wind Speed (m/s)	Salinity (‰)	Depth (m)
1	29.9±1.46^{a}	8.5±0.37^{a}	6707±692.5^{a}	0.7±10.17^{a}	15±0.7^{a}	0.8±0.04^{a}
2	29±0.15^{a}	8.4±0.15^{a}	1731±361.73^{b}	2.9±1.62^{b}	14.8±0.46^{a}	0.8±0.3^{a}
3	29.3±0.12^{a}	8.4±0.04^{a}	1850±71.65^{b}	1.4±0.36^{ab}	14.7±0.47^{a}	1±0.05^{a}
4	29.6±0.08^{a}	8.4±0.04^{a}	1888±12.81^{b}	1.5±0.16^{ab}	16.7±0.47^{b}	1.1±0.45^{b}

Numbers followed by the same letter in the same parameter are not significantly different based on ANOVA ($p<0.05$)

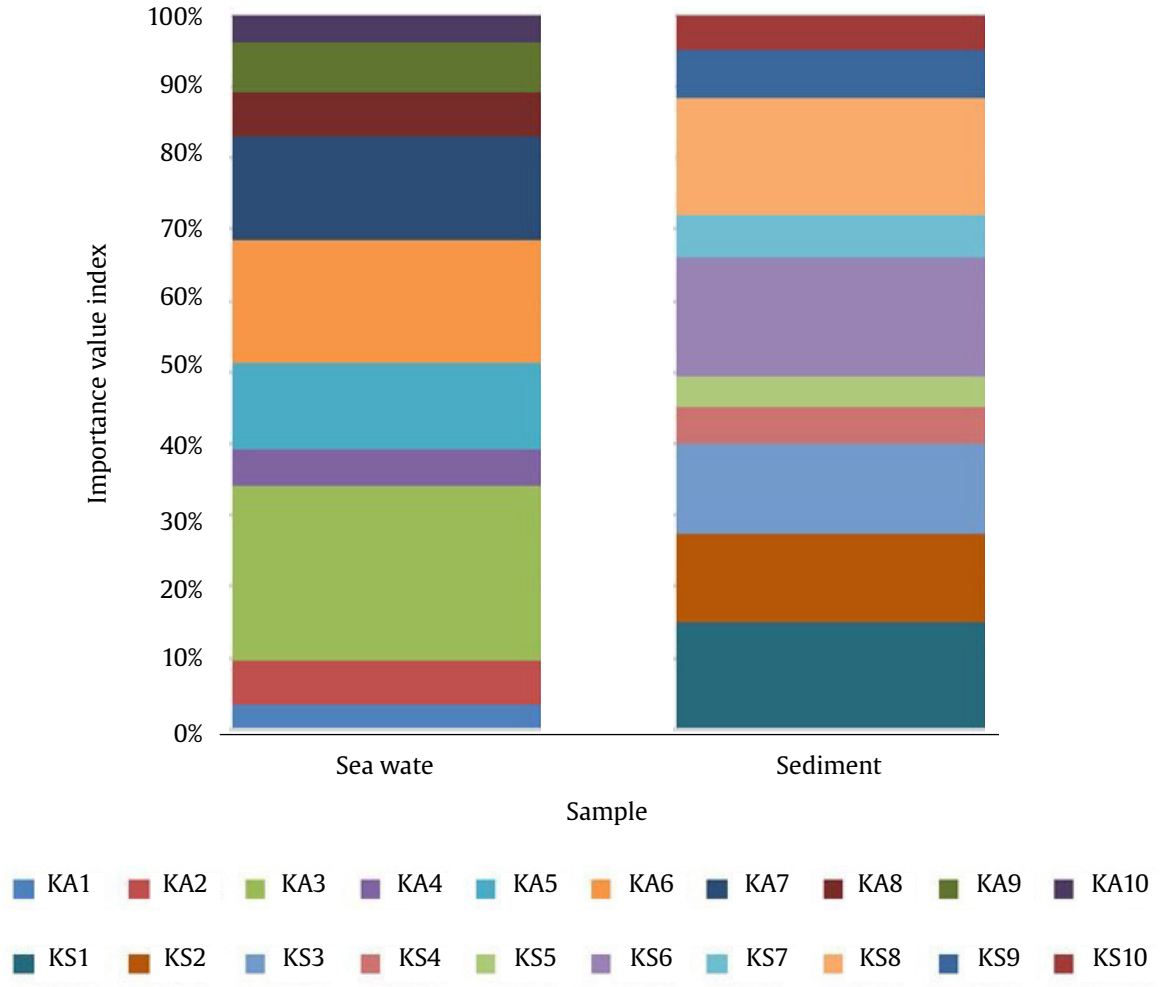

Figure 2. Importance value index of bacteria in seawater and sediment samples

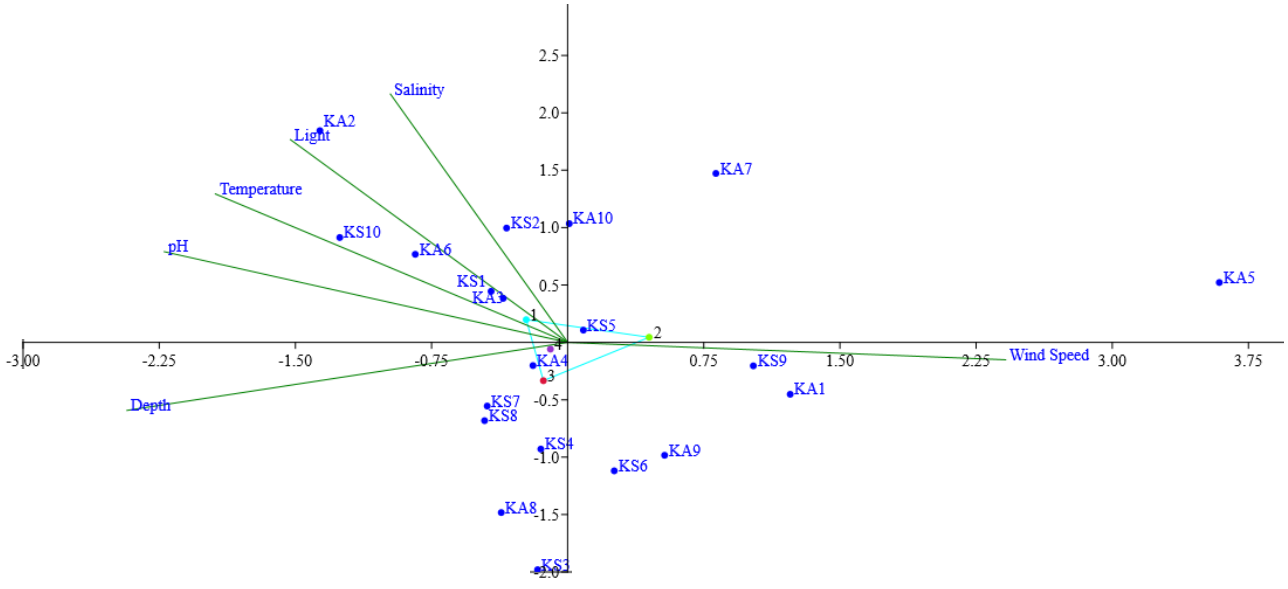

Figure 3. Correlation between bacterial isolates and physicochemical conditions

isolates were tested for biofilm formation ability, hydrophobicity, and bacterial growth on days 0, 10, 20, 30, and 40.

3.5.1. Biofilms Formation on Bacterial Isolates

Isolates KA1, KA2, KA3, KA4, KA5, KA10, KS6, and KS8 showed biofilm formation ability on days 0, 10, 20, 30, and 40. Isolate KA5 showed the highest biofilm value of 3.73, while the control (without bacterial inoculation) only reached a biofilm value of 0.84 on day 40 (Figure 4).

3.5.2. Hydrophobicity in Bacterial Isolates

Isolates KA1, KA2, KA3, KA4, KA5, KA10, KS6 and KS8 showed hydrophobicity. Isolate KA10 showed the highest hydrophobicity value of 11.22%, and the control showed the lowest hydrophobicity value of 0.17% on day 40 (Figure 5).

3.5.3. Growth of LDPE Microplastic Degradation Bacteria

The growth of nine bacterial isolates was increased from day 0 to day 20. Isolates KA4 and KA9 decreased from day 20 to 40. Isolates KA1, KA2, KA3, KA5, KA10, KS6, and KS8 increased on day 30. The growth of the nine isolates decreased by day 40. Isolate KA10 had the highest OD value on day 40 of 0.64. The control had the lowest OD value of 0.011 (Figure 6).

3.6. Microplastic Weight Percentage

Four of the nine bacterial isolates with the highest percentage of microplastic degradation were used for 16S rDNA identification. The isolates were KA1, KA2, KA3, and KA10. The KA1 bacteria reduced the plastic weight by 36.19%, KA2 by 10.16%, KA3 by 28.39%, and KA10 by 17.07% from the initial weight (Table 4).

Table 3. Clear zone index

Isolate	Clear zone (mm)	Isolate	Clear zone (mm)	Isolate	Clear zone (mm)
KA1	9.33±3.171^{b}	KA8	0^{a}	KS5	0^{a}
KA2	6.5±2.858^{ab}	KA9	2.17±2.4836^{ab}	KS6	2.67±3.171^{ab}
KA3	4.5±1.224^{ab}	KA10	2.67±1.886^{ab}	KS7	0^{a}
KA4	3.5±6.276^{ab}	KS1	0^{a}	KS8	3.33±2.867^{ab}
KA5	2±4.027^{ab}	KS2	0^{a}	KS9	0^{a}
KA6	0^{a}	KS3	0^{a}	KS10	0^{a}
KA7	0^{a}	KS4	0^{a}	K	0^{a}

Numbers followed by the same letter in the same parameter are not significantly different based on ANOVA ($p<0.05$)

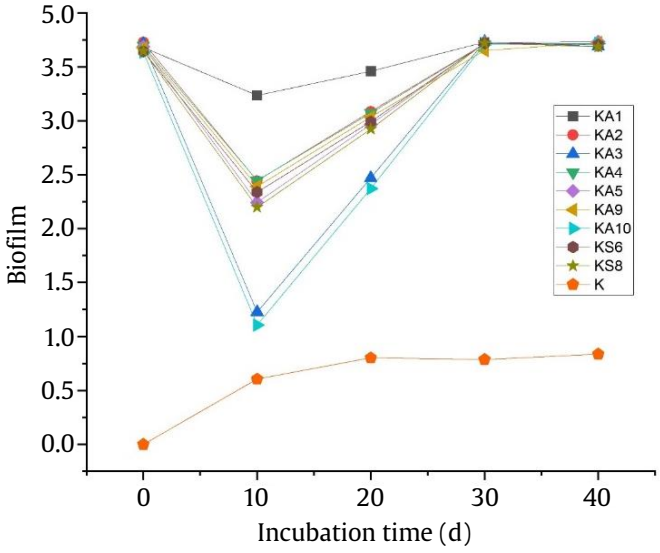

Figure 4. Biofilm formation rate

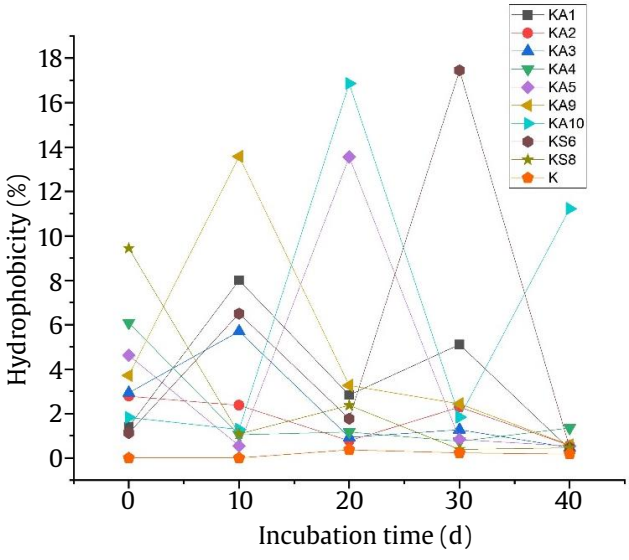

Figure 5. Hydrophobicity of bacterial isolates

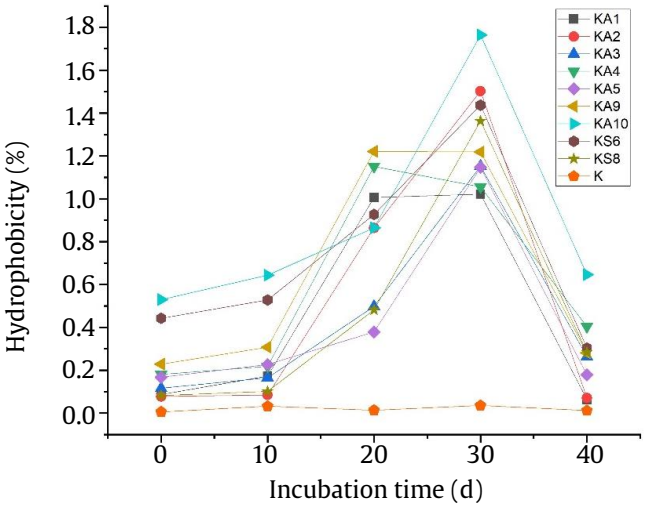

Figure 6. Bacterial growth rate

Table 4. Weight reduction efficiency, reduction rate, and half-life of bacterial isolates in MSM containing microplastics

Isolate	Percentage of weight loss (%)	Reduction rate constant (k) day^{-1}	Half-life (ln 2/k) (days)
KA1	36.19^{f}	0.0033	91.98
KA2	10.16^{c}	0.00087	11601.73
KA3	28.39^{e}	0.002	382.85
KA4	6.82d^{bc}	0.00045	22126.67
KA5	10.01^{c}	0.00099	12247.5
KA9	4.57^{dabc}	0.00037	187130.74
KA10	17.07^{d}	0.0012	2684.15
KS6	1.76^{ab}	0.00016	2261195.26
KS8	9.09^{c}	0.00067	23037.42
K	0.22^{a}	1.56E-05	10687395229

Numbers followed by the same letter in the same parameter are not significantly different based on ANOVA ($p<0.05$)

3.7. Characteristics of Post-Degradation Microplastics

The characteristics of the microplastics in the four potential isolates were analyzed using FESEM/EDS, FTIR-ATR, and XRD. The isolates were identified as KA1, KA2, KA3, and KA10.

3.7.1. Morphological analysis using FESEM and EDS

FESEM observations showed morphological changes on the surface of microplastics caused by KA1, KA2, KA3, and KA10 compared to the control (Figure 7). This was confirmed by the EDS results, which showed the elements in each treatment and

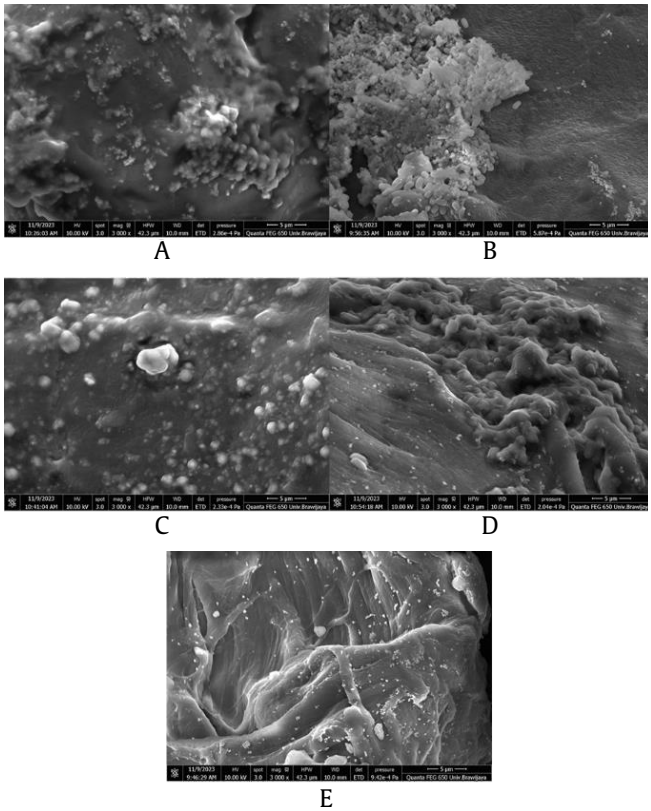

Figure 7. FESEM analysis of isolates KA1 (A), KA2 (B), KA3 (C), KA10 (D), and control (E) with 3,000 times magnification

the control (Figure 8). Observations were performed at a magnification of 3000 ×.

3.7.2. Changes in Microplastic Function Groups Using ATR-FTIR

KA1, KA2, KA3, and KA10 exhibited changes in transmittance values, including the addition and removal of several absorbances. New indentations were formed in the single-bond (2,500-4,000 cm^{-1}) and double-bond (1,500-2,000 cm^{-1}) regions, as well as in the fingerprint region (500-1,500 cm^{-1}). The treatment of KA1, KA2, KA3, and KA10 produced new peaks at 1465 cm^{-1}, 1636 cm^{-1}, 1646 cm^{-1}, and 1648 cm^{-1}, which are related to the C-O (carbonyl) functional group. KA2 had a higher percentage transmittance than KA1, KA3, KA10, and the control, indicating a weakening of the C-H bond. A new peak was detected at 1078 cm^{-1} for KA1, KA2, KA3, and KA10, whereas no peak was detected in the control. Loss of absorption at 853 cm^{-1} in the treatments.

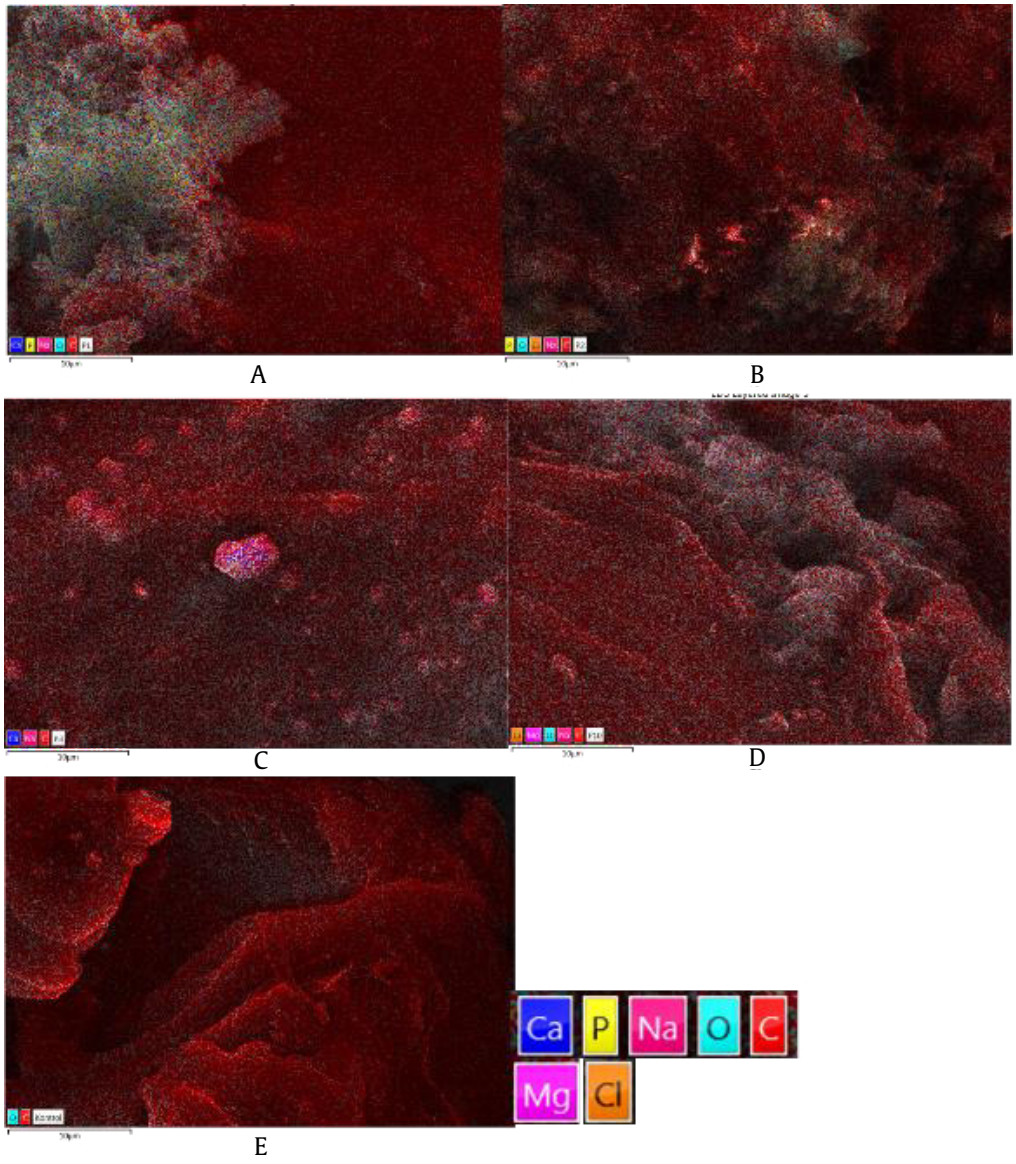

Figure 8. EDS analysis of isolates KA1 (A), KA2 (B), KA3 (C), KA10 (D), and control (E)

Waves 717 cm^{-1} (KA1), 717 cm^{-1} (KA2), 715 cm^{-1} (KA3), and 719 cm^{-1} (KA10), but not in the control (Figure 9).

3.7.3. Crystallinity Change Using XRD

The crystallinity index measured by X-ray diffraction (XRD) was 28.27% for the control and between 24.01% and 27.84% for treatments KA1, KA2, KA3, and KA10. The crystallinity index value decreased in treatments KA1, KA2, KA3, and KA10, with the largest decrease of 4.26% in KA1 and the smallest of 0.43% in KA10 (Figure 10). The strongest peak positions for the control and treatment groups were in the range of 21-36 θ.

4. Discussion

The temperature in the waters of Ambon Bay was in accordance with the optimum growth of bacteria. This is based on a study by Lu *et al.* (2020). The pH of the waters of Ambon Bay was not in accordance with the optimum growth of the bacteria. This was based on the study by Krause *et al.* (2012). High pH is caused by the presence of carbonate minerals, photosynthesis, and human activities such as waste disposal (Hu *et al.* 2021). The light intensity at location one shows the optimum bacterial growth. This was based on the study by Hotos and Avramidou

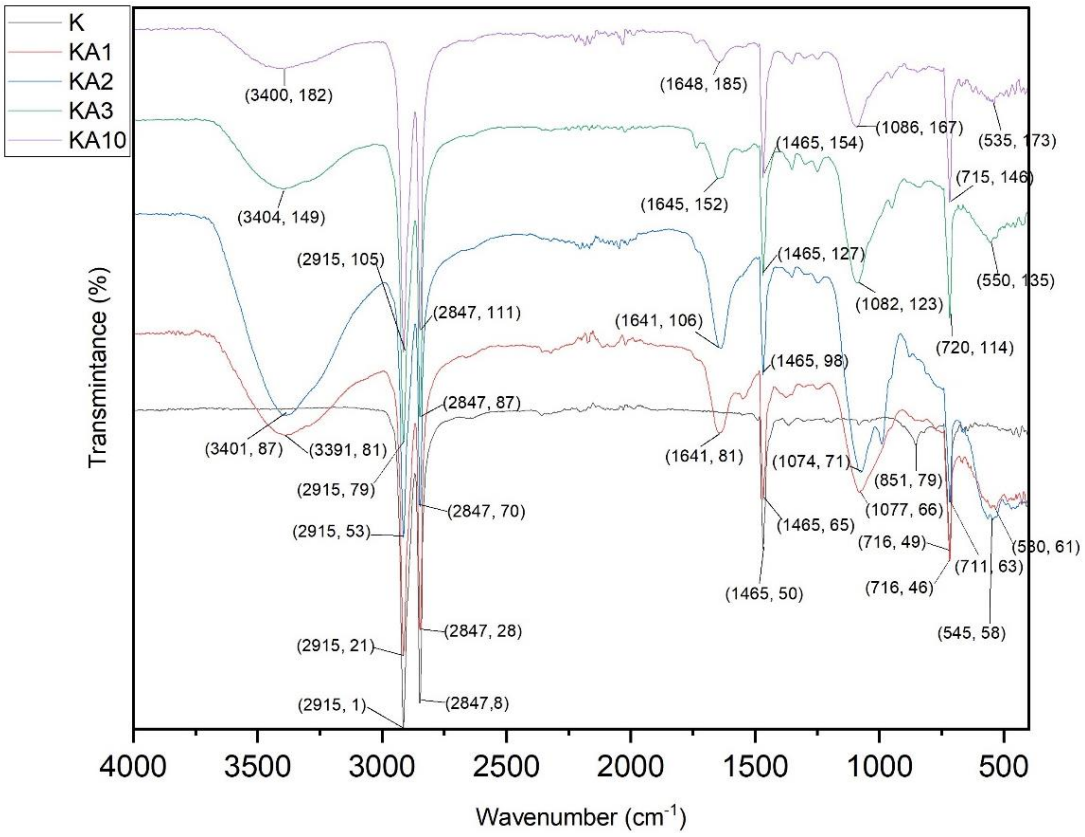

Figure 9. LDPE microplastic FTIR spectra after 40 days of incubation

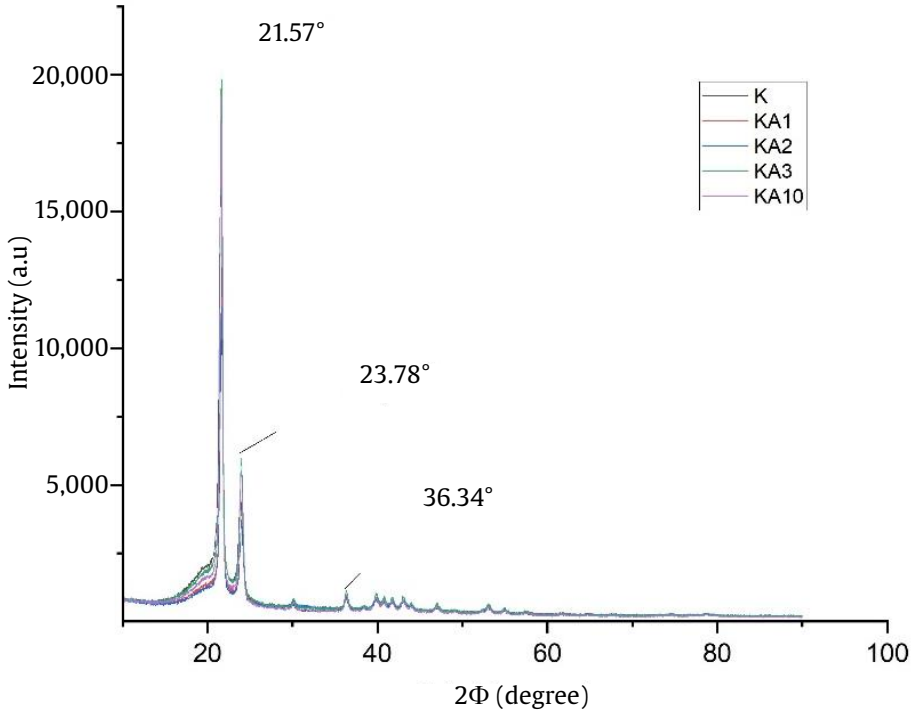

Figure 10. XRD spectra of LDPE fragments

(2021), which found that light intensity at locations two, three, and four tends to be low. This is likely due to the weather conditions during physicochemical measurements, which indirectly affect the light intensity received by the bacteria. The wind speed in the waters of Ambon Bay ranged from 0.7-2.9 m/s. This was due to the weather conditions during the physicochemical measurements. Salinity in these waters ranged from 14.7-16.7‰, and Ambon was not in accordance with the optimum growth of bacteria. Weather conditions caused this during physicochemical measurements, namely, high rainfall and water input from rivers (Li *et al.* 2021). The water depth in Ambon Bay ranges from 0.8 to 1.1m. This depth plays a role in the transport and accumulation of microplastics (Bertoldi *et al.* 2023; Fardami *et al.* 2023).

Bacterial isolates from seawater and sediment samples showed different Simpson and Shannon indices. The sediment samples had the highest Simpson's index value of 0.7374, indicating the dominance of certain species. The seawater samples had the smallest Simpson's index value of 0.6963, indicating that many species were evenly distributed without dominance. The seawater samples had the highest Shannon index value of 1.676, indicating greater species diversity with balanced proportions. Sediments had the smallest index value (1.564), indicating a lower species diversity. Bacterial isolates from seawater and sediment samples were correlated with physicochemical conditions. Positive, negative, and no correlations indicated a relationship between physicochemical conditions and bacterial isolates.

Nine of the 20 bacterial isolates formed clear zones. The bacterial isolates were KA1, KA2, KA3, KA4, KA5, KA9, KA10, KS6, and KS8. This indicates that these isolates have the potential for microplastic degradation. According to Gupta *et al.* (2012), the clear zone value of each bacterial isolate can be different because of the genetic makeup of the isolate, growth conditions, and specific substrates, causing variations in the size of the clear zone around the bacterial colony.

The isolates were tested for biofilm formation ability, hydrophobicity, and bacterial growth on days 0, 10, 20, 30, and 40. Isolates KA1, KA2, KA3, KA4, KA5, KA10, KS6, and KS8 on day 10 showed decreased biofilm formation ability. On days 20, 30, and 40, these isolates showed increased biofilm-forming ability. Isolate KA5 showed the highest biofilm value of 3.73, while the control (without bacterial inoculation) only reached a biofilm value of 0.84 on day 40. According to Wagner *et al.* (2021), variability in biofilm formation by bacterial isolates is caused by several factors, including genetic diversity, microcolony formation, and surface specificity.

Isolates KA2, KA4, KA5, KA10, and KS8 on day 10 showed decreased hydrophobicity. Isolates KA1, KA2, KA3, KA9, and KA6 on day 20 showed decreased hydrophobicity ability. Isolates KA5, KA9, KA10, and KS8 on day 30 showed decreased hydrophobicity. Isolate KA10 showed the highest hydrophobicity value of 11.22%, and the control showed the lowest hydrophobicity value of 0.17% on day 40. The percentage of hydrophobicity indicates the tendency of bacteria to attach to the surface of the LDPE microplastics due to the penetration of bacteria into the LDPE surface facilitated by enzymatic activities present in bacterial strains, including laccase, esterase, monooxygenase, and peroxidase (Ru *et al.* 2020). The greater the hydrophobicity of bacteria, the more bacterial cells adhere to the surface of hydrophobic objects (Krasowska and Silger 2014).

The growth of the nine bacterial isolates increased from day 0 to 20. Isolates KA4 and KA9 decreased from day 20 to 40. Isolates KA1, KA2, KA3, KA5, KA10, KS6, and KS8 increased on day 30. The growth of the nine isolates decreased by day 40. Isolate KA10 had the highest OD value on day 40 of 0.64. The control condition had the lowest OD value (0.011). This increase in bacterial growth is thought to be related to nutritional and other factors that support bacterial growth (Adithama *et al.* 2023).

Nine bacterial isolates showed the ability to degrade microplastics based on the percentage of microplastic weight. Four of the nine bacterial isolates with the highest percentage of microplastic degradation were used for 16S rDNA identification. The isolates were KA1, KA2, KA3, and KA10. Microplastics from these isolates were further analyzed using FESEM/EDS, ATR-FTIR, and EDS. The KA1 bacteria reduced the plastic weight by 36.19%, KA2 by 10.16%, KA3 by 28.39%, and KA10 by 17.07% from the initial weight. Based on Dey *et al.* (2020), *Stenotrophomas* sp. isolates were only able to reduce the weight of LDPE microplastics by 8% for 100 days. *Stenotrophomonas maltophilia* and *Enterococcus* sp. were able to reduce weight by

5% and 8%, respectively, for 90 days (Adithama *et al.* 2023). The 36.19% weight loss in this study was much greater than that reported in previous studies. This difference is thought to be caused by several factors, including enzymatic activity and surface colonization (Akarsu *et al.* 2023).

The bacterial isolates showed significant potential to affect the weight of microplastics on day 40, which can be seen from the percentage of weight lost, the constant rate of reduction (K), and the calculated half-life. Isolate KS6 showed the highest rate of reduction, at 0.00014 day^{-1}, and a half-life of approximately 2261195.262 days in the LDPE degradation process. The results showed that isolate KS6 could degrade 0.00014 g of LDPE microplastics daily. KS6 required approximately 2261195.262 days to degrade the LDPE microplastics fully. This removal rate may stem from the genetic traits of the isolate, which may have a significant capacity to degrade polymers (Auta *et al.* 2017).

The observation results show that morphological changes were observed on the surfaces of LDPE microplastics treated with KA1, KA2, KA3, and KA10 bacteria. These changes show the significant effect of bacterial treatment on the surface structure of microplastics. The EDS analysis results revealed the elemental composition of the LDPE microplastics. The control treatment only found the elements carbon (C) and oxygen (O), indicating that no bacteria were attached to the LDPE microplastics in the control treatment. Bacteria KA1, KA2, KA3, and KA10 were detected. This was indicated by the presence of other elements on the surface of the microplastics analyzed using EDS. Calcium (Ca), phosphorus (P), sodium (Na), oxygen (O), and carbon (C) elements were found in KA1. Phosphorus (P), oxygen (O), chlorine (Cl), sodium (Na), and carbon (C) were detected in KA2. Calcium (Ca), sodium (Na), and carbon (C) were detected in KA3. In comparison, Chlorine (Cl), magnesium (Mg), oxygen (O), sodium (Na), and carbon (C) were detected in KA10.

Treatment with bacteria KA1, KA2, KA3, and KA10 showed the addition of IR absorption peaks, indicating a change in chemical structure (Bhatia *et al.* 2014). Treatment of LDPE microplastic powders with KA1, KA2, KA3, and KA10 bacteria resulted in the loss of the absorption peak at 853 cm^{-1}, indicating the loss of the C=C group. The addition of absorption peaks in KA1, KA2, KA3, and KA10 at 1078 cm^{-1}, 1081 cm^{-1}, 1074 cm^{-1}, and 1071 cm^{-1}, respectively, indicate the stretching of the C-O group, which occurs due to the presence of alkyl groups in ester groups, carboxylates, and hydroxy alcohols. The addition of C-O (carbonyl) groups in KA1 (1646 cm^{-1}), KA2 (1641 cm^{-1}), KA3 (1645 cm^{-1}), and KA10 (1648 cm^{-1}) indicates the presence of ester groups (Adithama *et al.* 2023). The oxidation of -OH on the C-O group occurs as a result of microbial activity (Esmaeili *et al.* 2013). The surface deterioration of LDPE microplastics begins with bacterial adhesion, which leads to the formation of biofilms and new structures. These structures trigger hydrolysis to produce hydroxyl and ester intermediates. These intermediates undergo oxidase and dehydrogenase reactions, converting fatty acids, esters, ketones, hydroxyls, alkanes, and other functional groups into simpler molecules (Dey *et al.* 2020; Ru *et al.* 2020).

After degradation, the LDPE microplastics exhibited a decrease in the crystallinity index. According to Esmaeilli *et al.* (2013), this decrease signifies structural changes that affect the particle size of the LDPE. This degradation is related to the carbonyl index and double bonds in the LDPE powders. Changes in the carbonyl index indicate the presence of ketones and aldehydes in LDPE as well as the production of CO_2 and H_2O as products of the β-oxidation process (Adithama *et al.* 2023).

Based on the DNA BLAST results, the bacteria KA1, KA2, KA3, and KA10 were identified as *Bacillus cereus* strain DN-55, *Bacillus toyonesis* strain BCT7112, *Bacillus paramycoides* strain 3664, and *Escherichia coli* strain NCTC9085, respectively (Figure 11).

Bacteria KA1, KA2, KA3, and KA10 have an INP of 3.47%, 6.18%, 24.35%, and 3.79%, respectively, thus requiring biostimulation to enhance the growth of microplastic degrading bacteria. Indigenous bacteria from the waters of Ambon Bay have the potential to degrade LDPE microplastics. The characteristics of microplastics after degradation by bacteria included changes in the surface structure of the microplastics, decreased crystallinity, decreased weight of LDPE microplastics, and decreased C=C groups that made up LDPE monomers. The KA1 bacterial isolate had the best potential for LDPE microplastic degradation (36.19 %) and was identified as Bacillus cereus.

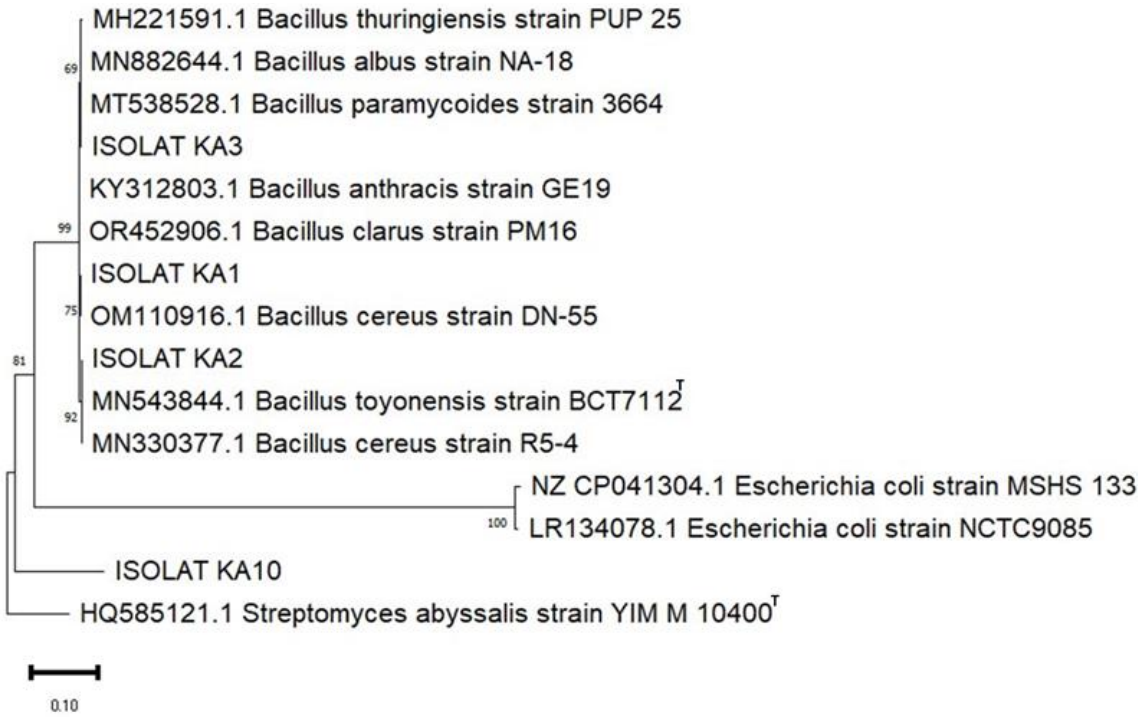

Figure 11. Phylogenetic tree of bacteria KA1, KA2, KA3, and K10, grouping based on Neighbor Joining DNA barcoding of 16S rRNA gene

Acknowledgements

The authors would like to thank the Biotechnology and Microbiology Laboratory of the Biology Department, Faculty of Mathematics and Natural Sciences at Universitas Pattimura, the Analysis and Measurement Services Laboratory of the Chemistry Department and Lembaga Sentral Ilmu Hayati (LSIH) at Universitas Brawijaya, and PT. Genetika Science Indonesia.

References

Adithama, R.M., Munifah, I., Yanto, D.H.Y., Meryandini, A., 2023. Biodegradation of low-density polyethylene microplastic by new halotolerant bacteria isolated from saline mud in Bledug Kuwu, Indonesia. *Bioresource Technology Reports*. 22, 101466. https://doi.org/10.1016/j.biteb.2023.101466

Akarsu, C., Özdemir, S., Ozay, Y., Acer, Ö., Dizge, N., 2023. Investigation of two different size microplastic degradation ability of thermophilic bacteria using polyethylene polymers. *Environmental Technology*. 44, 3710-3720. https://doi.org/10.1080/09593330.2022.2071638

Auta, H.S., Emenike, C.U., Fauziah, S.H., 2017. Screening of *Bacillus* strains isolated from mangrove ecosystems in Peninsular Malaysia for microplastic degradation. *Environmental Pollution*. 231, 1552-1559. https://doi.org/10.1016/j.envpol.2017.09.043

Bertoldi, C., Lara, L.Z., Fernandes, A.N., 2023. Revealing microplastic dynamics: the impact of precipitation and depth in urban river ecosystems. *Environmental Science and Pollution Research*. 30, 111231-111243. https://doi.org/10.1007/s11356-023-30241-0

Bhatia, M., Girdhar, A., Tiwari, A., and Nayarisseri, A., 2014. Implications of a novel *Pseudomonas* species on low density polyethylene biodegradation: an *in vitro* to *in silico* approach. *Springerplus*. 3, 497. https://doi.org/10.1186/2193-1801-3-497

Dey, A.S., Bose, H., Mohapatra, B., Sar, P., 2020. Biodegradation of unpretreated low-density polyethylene (LDPE) by *Stenotrophomonas* sp. and *Achromobacter* sp., isolated from waste dumpsite and drilling fluid. *Frontiers in Microbiology*. 11, 603210. https://doi.org/10.3389/fmicb.2020.603210

Esmaeili, A., Pourbabaee, A.A., Alikhani, H.A., Shabani, F., Esmaeili, E., 2013. Biodegradation of low-density polyethylene (LDPE) by mixed culture of *Lysinibacillus xylanilyticus* and *Aspergillus niger* in soil. *Plos one*. 8, e71720. https://doi.org/10.1371/journal.pone.0071720

Fachrul, M.F., Rinanti, A., 2018. Bioremediation of microplastic pollutant in aquatic ecosystem by indigenous bacteria. *Prosiding Seminar Nasional Kota Berkelanjutan.* 2018, 302-312. https://doi.org/10.25105/psnkb.v1i1.2910

Fardami, A.Y., Magami, I.M., Yarima, A.A., Sabitu, M., 2023. Microbes associated with bioremediation of microplastic waste in Nigerian freshwater bodies: a review. *UMYU Scientifica.* 2, 140-150. https://doi.org/10.56919/usci.2123.017

Gemilang, W.A., Rahmawan, G.A., Wisha, U.J., 2017. The quality of the waters of Inner Ambon Bay is based on physical and chemical parameters during Transition Season I. *EnviroScieteae.* 13, 79-90. https://doi.org/10.20527/es.v13i1.3518

Gong, J., Kong, T., Li, Y., Li, Q., Li, Z., Zhang, J., 2018. Biodegradation of microplastic derived from poly (ethylene terephthalate) with bacterial whole-cell biocatalysts. *Polymers.* 10, 1326. https://doi.org/10.3390/polym10121326

Gupta, P., Samant, K., Sahu, A., 2012. Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential. *International Journal of Microbiology*. 2012, 1-5. https://doi.org/10.1155/2012/578925

Gurjar, U.R., Xavier, K.M., Shukla, S.P., Takar, S., Jaiswar, A.K., Deshmukhe, G., Nayak, B.B., 2023. Seasonal distribution and abundance of microplastics in the coastal sediments of north eastern Arabian Sea. *Marine Pollution Bulletin.* 187, 114545. https://doi.org/10.1016/j.marpolbul.2022.114545

Habib, S., Iruthayam, A., Shukor, A.M.Y., Alias, S.A., Smykla, J., Yasid, N.A., 2020. Biodeterioration of untreated polypropylene microplastic particles by Antarctic bacteria. *Polymers.* 12, 2616. https://doi.org/10.3390/polym12112616

Hu, C., Li, X., He, M., Jiang, P., Xu, J., 2021. Effect of ocean acidification on bacterial metabolic activity and community composition in oligotrophic oceans, inferred from short-term bioassays. *Frontiers in Microbiology*. 12, 583982. https://doi.org/10.3389/fmicb.2021.583982

Hotos, G.N., Avramidou, D., 2021. The effect of various salinities and light intensities on the growth performance of five locally isolated microalgae [*Amphidinium carterae, Nephroselmis* sp., *Tetraselmis* sp. (var. red pappas), *Asteromonas gracilis* and *Dunaliella* sp.] in laboratory batch cultures. *Journal of Marine Science and Engineering.* 9, 1275. https://doi.org/10.3390/jmse9111275

Krasowska, A., Sigler, K., 2014. How microorganisms use hydrophobicity and what does this mean for human needs? *Front. Cell. Infect. Microbiol.* 4, 1–7. https://doi.org/ 10.3389/fcimb.2014.00112

Krause, E., Wichels, A., Giménez, L., Lunau, M., Schilhabel, M.B., Gerdts, G., 2012. Small changes in pH have direct effects on marine bacterial community composition: a microcosm approach. *PloS one.* 7, e47035. https://doi.org/10.1371/journal.pone.0047035

Leboffe, M.J., Pierce, B.E., 2012. *Brief Microbiology: Laboratory Theory and Application,* second ed. Morton Publishing, Colorado, Englewood.

Li, Z., Lian, T., Ying, J., Zhu, H.X., Papa, F., Xie, H., Long, Y., 2021. The cause of an extremely low salinity anomaly in the Bay of Bengal during 2012 spring. *Journal of Geophysical Research: Oceans*. 126, e2021JC017361. https://doi.org/10.1029/2021JC017361

Liu, S.Y., Leung, M.M, Fang, J.K., Chua, S.L., 2021. Engineering a microbial 'trap and release' mechanism for microplastics removal. *Chemical Engineering Journal*. 404, 127079. https://doi.org/10.1016/j.cej.2020.127079

Lu, P., Wang, W., Zhang, G., Li, W., Jiang, A., Cao, M., Zhang, X., Xing, K., Peng, X., Yuang, B., Feng, Z., 2020. Isolation and characterization marine bacteria capable of degrading lignin-derived compounds. *PLoS One*. 15, e0240187. https://doi.org/10.1371/journal.pone.0240187

Manullang, C.Y., 2019. The abundance of plastic marine debris on beaches in Ambon Bay. *In IOP Conference Series: Earth and Environmental Science.* 253, 012037. https://doi.org/10.1088/1755-1315/253/1/012037

Meng, F., Harkes, P., van Steenbrugge, J.J., Geissen, V., 2023. Effects of microplastics on common bean rhizosphere bacterial communities. *Applied Soil Ecology*. 181, 104649. https://doi.org/10.1016/j.apsoil.2022.104649

Noya, Y.A., Tuahatu, J.W., 2021. Density and transport patterns of floating marine waste on the west coast of Outer Ambon Bay. *Jurnal Penelitian Sains*. 23, 19-27. https://doi.org/10.56064/jps.v23i1.594

Rosenberg, M., Gutnick, D., Rosenberg, E., 1980. Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity. *FEMS Microbiol. Lett.* 9, 29-33. https://doi.org/10.1111/j.1574-6968.1980.tb05599.x

Ru, J., Huo, Y., Yang, Y., 2020. Microbial degradation and valorization of plastic wastes. *Front. Microbiol.* 11, 442. https://doi.org/10.3389/fmicb.2020.00442

Samet, K., Icen, T.I., 2022. Screening of potential probiotic properties of *Lactobacillus* and *Lactococcus* strains. *Research Journal of Biotechnology*. 17, 119-126.

Surjowardojo, P., Susilorini, T.E., Benarivo, V., 2016. The inhibitory power of Manalagi apple peel decoction (*Malus sylvestris* Mill) against the growth of *Escherichia coli* and *Streptococcus agalactiae*, the causes of mastitis in dairy cows. *Journal of Tropical Animal Production.* 17, 11-21. https://doi.org/10.21776/ub.jtapro.2016.017.01.2

Wagner, E.M., Fischel, K., Rammer, N., Beer, C., Palmetzhofer, A.L., Conrady, B., Roch, F.F., Hanson, B.T., Wagner, M., Rychli, K., 2021. Bacteria of eleven different species isolated from biofilms in a meat processing environment have diverse biofilm forming abilities. *International Journal of Food Microbiology*. 349, 109232. https://doi.org/10.1016/j.ijfoodmicro.2021.109232