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Potential Microplastic-Degrading Bacteria from Mangrove Sediment in The Paluh Getah Area, Percut Sei Tuan District

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Abstract: Microplastic pollution poses a severe threat to marine ecosystems due to its persistence and toxicity, necessitating innovative biodegradation strategies. This study explored the potential of bacteria isolated from mangrove sediments of the Paluh Getah mangrove forest, North Sumatra, to degrade common microplastics, including LDPE, HDPE, PET, PP, and PS. Ten bacterial isolates were obtained and screened for their ability to grow on microplastic-supplemented mineral salt medium over 20 days. Three isolates (BPM 5, BPM 9, and BPM 10) demonstrated robust growth, with biochemical tests confirming catalase activity critical for managing oxidative stress during plastic degradation. Molecular identification via 16S rRNA gene sequencing revealed the isolates as *Enterobacter* sp. and *Brevibacillus parabrevis*, both known for their biodegradation capacities. The findings underscore the diverse metabolic pathways these bacteria employ to utilize microplastics as carbon sources. This research highlights the promise of mangrove sediment bacteria as eco-friendly bioremediation agents to mitigate microplastic pollution in coastal environments. Further studies are required to optimize degradation conditions and elucidate enzymatic mechanisms to enhance practical applications.

Keywords: Biodegradation; *Brevibacillus parabrevis*; *Enterobacter* sp; Mangrove; Microplastic; Sediment Bacteria.

Introduction

Plastic is a popular material because it is versatile, durable, and inexpensive. Its continued use is inversely proportional to its difficulty in decomposing. Plastic has low degradability, so over time, it becomes a dangerous pollutant. Most microplastic pollution originates from land, where humans live. After arriving on land, approximately 4.8–1.27 tons of microplastics move to marine ecosystems annually (Dissanayake et al., 2022). Microplastic particles, measuring less than 5 mm, originate from various sources such as domestic waste, industry, and larger plastic degradation sites. The presence of microplastics in waters can affect marine organisms, from plankton to top predators, through ingestion and accumulation in body tissues. Microplastics can cause physiological disorders, tissue damage, and even death in marine biota.

Furthermore, microplastics also have the potential to act as vectors for dangerous chemical pollutants, such as heavy metals and persistent organic compounds, which can accumulate in the food chain and impact human health (Rochman et al., 2015). Therefore, research related to microplastic degradation needs to be continuously developed. One way is to utilize microorganisms. Mangrove sediments are a reservoir of unique microbial diversity. These sediments serve as an accumulation site for organic and inorganic materials

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derived from mangrove ecosystem activities, such as the decomposition of leaves, roots, and other organisms (Putri et al., 2021). These processes create a nutrient-rich but often extreme environment, enabling the formation of highly adaptive microbial communities. Studies of mangrove sediments can provide insights into the ecological relationships of microbes with their environment, including how they contribute to biogeochemical cycles such as carbon, nitrogen, and sulfur. Furthermore, microorganisms in mangrove sediments have the potential to produce unique bioactive compounds that can be used in medical, industrial, and environmental applications (Ni'am et al., 2024).

North Sumatra is a region with a long coastline. One of the ecosystems found in the coastal areas of North Sumatra is the mangrove ecosystem. Several previous studies have demonstrated the significant potential of microorganisms in mangrove sediments. This potential can be used as an antimicrobial (Newaz et al., 2023; Rajan et al., 2020). Another potential that is still being explored is as bioremediation agents (Ren & Ni, 2023; Tiralerdpanich et al., 2021; Zhang et al., 2023). However, much of this research still focuses on basic aspects, such as initial isolation or basic bioactivity testing, without involving in-depth molecular approaches. Based on this background information, research on mangrove sediment bacteria with the potential to degrade microplastics is warranted. Expected to contribute to the development of knowledge and information on microplastic management, particularly in mangrove areas.

Method

Time and Place of Research

This research was conducted from March 2025 to July 2025 at the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, State University of Medan. Mangrove sediment isolated from the Paluh Getah mangrove forest area, Percut Sei Tuan District.

Mangrove Sediment Sampling

Mangrove sediment sampling was conducted using a purposive random sampling method, where samples were taken based on specific objectives. Sampling points were determined by dividing the mangrove ecosystem into three zones: the outer zone, the middle zone, and the inner zone, with each zone divided into three sample points. Five hundred grams of samples were taken from a depth of 0-5 cm, placed in ziplock bags, and stored in a cooler box. The samples were then sent to the microbiology laboratory at Medan State University for observation (Febriyanti et al., 2024).

Microplastic Sample Preparation

Microplastic samples used for screening bacteria with the ability to degrade microplastics included LDPE, HDPE, PET, PP, and PS. Each sample was cut to 1 cm x 1 cm and cleaned using aquabidest. Each microplastic was soaked in a solution of 70% alcohol and 5% H_2O_2 for 4 hours. Next, the microplastic samples were air-dried and then sterilized using UV light for 45 minutes (Fang et al., 2024).

Isolation of Potential Microplastic-Degrading Bacteria

A total of 10 grams of sediment samples were placed in 200 mL of Mineral Salt Medium (MSM) (K_2HPO_4 1 g; KH_2PO_4 0.2 g; NaCl 1 g; $CaCl_2$ 0.002 g; $(NH)_42SO_4$ 1 g; $MgSO_4$ 0.5 g; $CuSO_4$ 0.001 g; $ZnSO_4$ 0.001 g; $MnSO_4$ 0.001 g; $FeSO_4$ 0.01 g). The medium was adjusted to pH 6-8, and the previously prepared microplastic samples were added and incubated for 20 days. After incubation, each culture was continued in a serial dilution. A total of 1 mL of each culture was placed in 9 mL of 0.9% NaCl to obtain a 10-1 dilution. Dilution was carried out up to 10-6. Each suspension was spread on MSM media in a volume of 20 µL using the spread plate method. Incubation was carried out at 28°C for 24 hours (Fang et al., 2024; Novitasari et al., 2023) Each growing colony was selected and purified using the same isolation media.

Macroscopic Identification of Bacteria

Macroscopic identification was performed on successfully purified bacteria. Identification included form, surface, texture, colour, and elevation. The characteristics of each colony were recorded and labelled accordingly (Novitasari et al., 2023).

Biochemical Assay of Mangrove Sediment Bacteria
Oxidase Test

The oxidase test is performed by placing 1–2 drops of oxidase solution on sterile filter paper, then adding one loop of bacteria to the filter paper and observing the color change. A positive result indicates a purple color change within 10-30 seconds, while a negative result shows no color change (Fauziyah et al., 2022).

Catalase Test

The catalase test is performed by smearing 1 loop of bacteria onto a glass slide and adding 1–2 drops of 3% H_2O_2. A positive result is indicated by the formation of gas in the bacterial smear (Fauziyah et al., 2022).

Indole Test

The indole test is performed using the method of Mardalisa et al. (2021), using a medium containing tryptophan. One loop of bacteria is added to the medium, followed by Kovacs' reagent, and the sample is

homogenized. After 1-2 minutes, the color change was observed. A positive result was indicated by the formation of a red to pink color on the sample, while no color change indicated a negative outcome.

Motility Test

The motility test was conducted by inoculating one loop of bacterial isolate into Sulfide Indole Motility medium. The bacteria were then incubated for 24 hours at 37°C (Apriliya et al., 2021). Indicators of motile and non-motile bacteria were observed by the growth and distribution of bacteria in the medium. Motile bacteria were indicated by bacterial growth spreading across the surface of the medium and a cloudy medium. In contrast, non-motile bacteria grew only in the inoculated area and did not spread to other areas of the medium.

Methyl Red–Voges Proskauer (MR-VP) Test

The MR-VP test was conducted by adding one loop of bacteria to the MR-VP medium and incubating it at 37°C for 24 hours. Then, 5 drops of methyl red are added to the medium, and the color change is observed. A red color indicates a positive bacterial result, while a yellow color indicates a negative result. The Voges-Proskauer test is performed by adding 3 drops of alpha-naphthol solution and 2 drops of 40% KOH and observing the color change from pink to dark red, indicating a positive result (Mayanti et al., 2023).

TSIA Test

The TSIA test is performed by pricking a loop of bacterial isolate and then streaking the isolate onto the TSIA medium. A positive indicator is a yellow color change, indicating acidic conditions, while a red color indicates alkaline conditions (Septiana et al., 2024).

H_2S Test

The H_2S test is performed by inoculating bacteria onto the TSIA medium by pricking the vertical portion (butt). Incubation is carried out at 35–37°C for 24–48 hours. The presence of a black precipitate on the medium indicates that the bacteria are producing H_2S gas (Septiana et al., 2024).

Simmons Citrate Test

The Simmons citrate test is performed by streaking bacteria onto Simmons citrate media, incubating them for 24 hours, and observing the color change. A positive result indicates a color change from green to blue, indicating the bacteria are utilizing citrate as a carbon source (Alam et al., 2019).

Molecular identification of bacteria isolates based on 16S rRNA gene

The genomic DNA of bacteria isolates was extracted using Quick-DNA™ Fungal/Bacterial Miniprep (Zymo Research, Irvine, USA), following the manufacturer's guidelines. 16S rRNA gene was amplified using the primer set designed by Marchesi et al. (1998) , 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACCTTGTTACGACTT-3'). Each PCR reaction was conducted in a total volume of 50 µl, containing 25 µl GoTaq Green® Master Mix 123 (Promega), 5 µl of forward primer (10 pmol), 5 µl of reverse primer (10 pmol), 4 µl DNA template (100 ng/ µl), and nuclease-free water. A negative PCR reaction was prepared as above, but without a DNA template. The PCR conditions used were predenaturation at 94°C for 5 minutes, denaturation at 94°C for 30 seconds, annealing at 55°C for 45 seconds, elongation at 72°C for 1 minute 45 seconds, and after elongation at 72°C for 10 minutes. PCR was performed in 35 cycles. The PCR products were visualized using 1% agarose gel at 75 V for 30 minutes and sequenced in First Base (Applied Biosystems 3730xl) (Selangor, Malaysia). The 16S rRNA gene sequences generated were compared to the sequences in the National Center for Biotechnology Information (NCBI GenBank) database through the BLAST program (http://www.ncbi.nlm.nih.gov). The parameter thresholds for the species identification included the identity value (≥99% identity), query cover (≥99%), and e-value of <10-5. The phylogenetic tree construction was carried out using MEGA 11 software, and bootstrap analysis of 1,000× repetitions was carried out using the neighbor-joining method.

Result and Discussion

Ten isolates were successfully isolated and purified from mangrove sediments. These isolates were then characterized morphologically (Table 1). The morphological characteristics showed quite diverse characteristics in terms of shape, edges, color, and elevation of each isolate. Several morphological characters observed previously were reported by Hening et al. (2025), who succeeded in isolating microplastic-degrading bacteria from a landfill with macroscopic characteristics of a circular shape, small size, creamy white, with smooth edges (entire), transparent, and convex elevation, which was later identified as *Bacillus circullans*. Wiratno et al. (2024), also reported microplastic-degrading bacteria from the Brantas River, Malang City, with several characteristics similar to this study, namely flat elevation and transparent color. This isolate was then reported as *Enterobacter cloacae*.

Table 1. Morphological characteristics of potential microplastic-degrading bacteria from Mangrove Sediment

Isolate Code	Morphological Characteristics			
	Form	Margin	Color	Elevation
BPM 1	Irregular	Undulate	Cream Yellow	Flat
BPM 2	Irregular	Undulate	White	Flat
BPM 3	Spindle	Undulate	White	Flat
BPM 4	Irregular	Entire	White	Flat
BPM 5	Irregular	Entire	Transparent	Flat
BPM 6	Irregular	Entire	White	Flat
BPM 7	Circular	Entire	Cream Yellow	Flat
BPM 8	Circular	Entire	White	Raise
BPM 9	Circular	Entire	White	Flat
BPM 10	Circular	Entire	White	Convex

The potential of the ten isolated bacteria to degrade microplastics was determined by observing the growth of bacterial colonies in media supplemented with microplastics. The types of microplastics used were *Low-Density Polyethylene (LDPE), High-Density Polyethylene (HDPE), Polypropylene (PP), polyethylene terephthalate* (PET), and *Polystyrene* (PS). Of the 10 isolates previously isolated, three grew well in all microplastic treatments. The three bacteria were identified as isolates with the codes BPM 5, BPM 9, and BPM 10 (Table 2). These three isolates were then subjected to biochemical and molecular identification.

Table 2. Initial screening of bacterial growth isolated from mangrove sediment using several types of microplastics

Isolate Code	Bacterial growth				
	LDPE plastic	HDPE plastic	PP plastic	PET plastic	PS plastic
BPM 1	-	-	-	-	-
BPM 2	-	+	-	-	-
BPM 3	-	-	+	+	-
BPM 4	-	-	+	-	+
BPM 5	+	+	+	+	+
BPM 6	-	-	-	-	-
BPM 7	+	+	-	-	-
BPM 8	-	-	-	+	-
BPM 9	+	+	+	+	+
BPM 10	+	+	+	+	+

Note: (+) indicates bacteria grow well and degrade microplastics; (-) indicates that bacteria do not grow and are unable to degrade microplastics

Through a series of biochemical tests, the three isolates with the potential to degrade microplastics from mangrove sediments showed positive results in the catalase test, indicated by the formation of gas bubbles (Table 3). The ability to produce catalase is essential for bacteria that degrade microplastics. Catalase is an enzyme that breaks down hydrogen peroxide (H_2O_2), a harmful byproduct of cellular metabolism, into water and oxygen. Bacteria often experience oxidative stress during their degradation process, resulting in the production of free radicals and other reactive oxygen species (ROS). Catalase helps the bacteria survive these conditions by eliminating H_2O_2 (Yuan et al., 2021). In addition, the three bacteria demonstrated the ability to utilize different types of sugars. These differences indicate different metabolic pathways for obtaining energy and carbon sources. These differences indicate different metabolic pathways for obtaining energy and carbon sources. Sugar substrates are utilized as the primary nutrient, which is in line with increased growth and degradation rates. Uthra et al. (2023), reported that glucose was the carbon source that had the most influence on the rate of microplastic degradation, followed by fructose, sucrose, maltose, lactose, citric acid, and starch.

Table 3. Biochemical characteristics of three selected bacteria

Biochemical Test	BPM 1	BPM 5	BPM 9
TSIA	K/A	K/K	A/A
Gas	+	+	+
H_2S	-	-	-
Motility	-	-	-
Indol	-	-	-
Simmon citrate	+	-	+
Methyl red	-	-	-
Voges Proskauer	-	-	-
Catalase	+	+	+
Oxidase	-	-	-

Note: (+) indicates a positive result; (-) indicates a negative result; K/A indicates that the bacteria only ferment glucose, but not lactose or sucrose; K/K indicates that the bacteria being tested cannot ferment glucose, lactose, or sucrose; A/A indicates that the bacteria being tested can ferment the three sugars contained in the media, namely glucose, lactose, and sucrose.

In this study, two potential microplastic-degrading bacteria from mangrove sediments were identified molecularly using the 16S rRNA gene. *Enterobacter* sp. BPM 5 isolate showed the closest homology to *Enterobacter* sp. MFM with a similarity level of 100%

(Figure 1). Phylogenetically, *Enterobacter* is a genus belonging to the phylum *Proteobacteria*. Previous research reported that *Enterobacteriaceae*, as the dominant bacteria, were isolated from mangrove plants (*Sonneratia caseolaris* and *Avicennia marina*) (Alifia et al., 2024). Bacteria are microorganisms that can migrate into plant tissue. Therefore, it is highly likely that the bacteria present in mangrove sediments are the same as those living in mangrove plants. The microplastic degradation ability of *Enterobacter* bacteria has been reported in *Enterobacter* sp. HY1 isolate, isolated from a plastic waste treatment station, capable of degrading BHET, through the conversion of BHET to MHET and then to landfill (Qiu et al., 2020). *EstB esterase* cloned from *Enterobacter* sp. HY1 specifically hydrolyzed BHET to MHET, demonstrating enzymatic specificity. This finding suggests that *Enterobacter* bacteria utilize their *EstB esterase* enzyme as a biocatalyst for the degradation of BHET and PET intermediates. Other studies have also reported a bacterial consortium of the *Enterobacter* genus as the best combination for significantly degrading LDPE and PP with biofilm formation (Skariyachan et al., 2021).

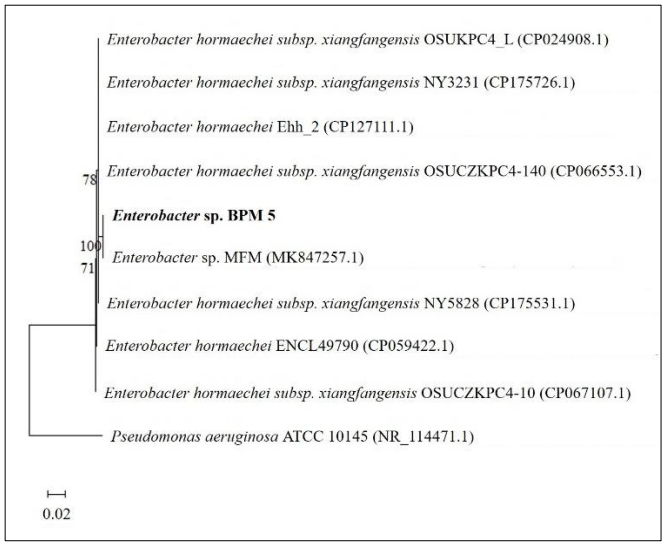

Figure 1. Phylogenetic tree showing the position of 16S rRNA gene sequences of potential microplastic-degrading bacteria from mangrove sediment (BPM 5) among reference species (reference species obtained from GenBank database) using the neighbor-joining method. The phylogenetic tree was constructed using MEGA 11 with 1000 bootstrap replications and a bar scale indicating a genetic distance of 0.02. *Pseudomonas aeruginosa* acts as the outgroup.

The isolate *Brevibacillus parabrevis* BPM 9 showed the closest homology to *Brevibacillus parabrevis* NBRC 12374 with a similarity level of 35% (Figure 2). Phylogenetically, *Brevibacillus* is a genus included in the phylum *Firmicutes*. A previous study reported that *B. parabrevis* was successfully isolated from mangrove sediments and possessed antimicrobial activity (Arumugam et al., 2018). *B. parabrevis* bacteria from this mangrove sediment were also reported as potential bacteria for the degradation of *polyethylene microplastics* (PEMPs) by reducing the dry weight of microplastics by 19.8% over 35 days of treatment. The byproducts of PEMP's degradation also produced small molecules such as *2-hexadecanone* and *decanone*. Interestingly, *B. brevis* can utilize these small molecules for its metabolic processes (Tiwari et al., 2023).

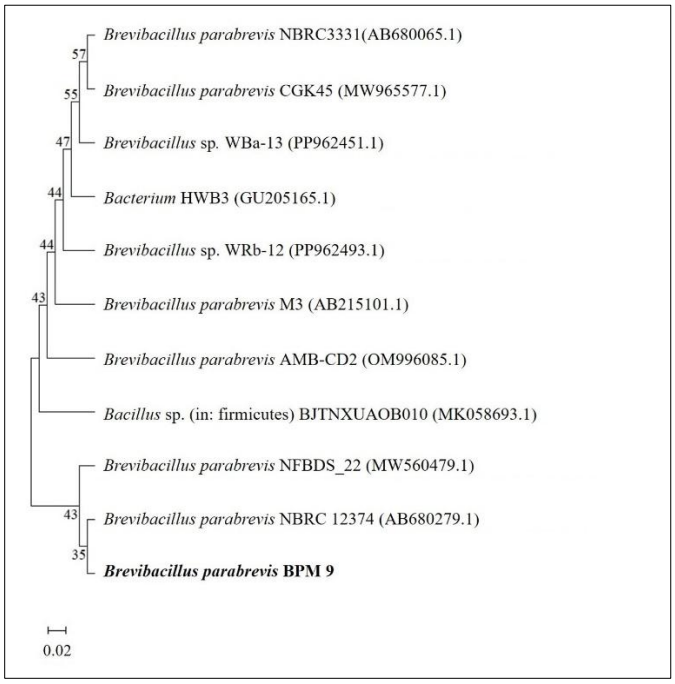

Figure 2. Phylogenetic tree showing the position of 16s rRNA gene sequences of potential microplastic-degrading bacteria from mangrove sediment (BPM 9) among reference species (reference species obtained from the GenBank database) using the neighbour-joining method. The phylogenetic tree was constructed using MEGA 11 with 1000 bootstrap replications and a bar scale indicating a genetic distance of 0.02.

Conclusion

This study reveals that mangrove sediment bacteria in North Sumatra possess significant potential to degrade prevalent microplastic polymers. The identification of *Enterobacter* sp. and *Brevibacillus parabrevis* as effective microplastic degraders highlights the ecological importance of mangrove microbial communities in mitigating plastic pollution. Their catalase activity suggests these bacteria can withstand oxidative stress associated with microplastic breakdown. Utilizing these bacteria for bioremediation could offer an eco-friendly solution to reducing microplastic accumulation in marine ecosystems, thereby protecting marine life and human health. Future research should focus on optimizing degradation

conditions and elucidating molecular mechanisms to harness these bacteria effectively for environmental management.

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Author Contributions

A.S.S.P: formulates research ideas, prepares designs, and revision of manuscript content. L.A.U: performs data analysis, interpretation of results, and writes the main manuscript. M.N.R: interpretation of results and revision of manuscript content. A.P.N: responsible for literature review, documentation, and reference preparation. C.S.M: collects data. All authors read and approve the final manuscript to be published.

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Conflicts of Interest

The authors declare no conflict of interest.

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