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# IDENTIFICATION AND CHARACTERIZATION OF MICROPLASTIC DEGRADING BACTERIA IN THREE LANDFILLS OF LAMPUNG PROVINCE

Identifikasi dan Karakterisasi Bakteri Pendegradasi Mikroplastik di Tiga Tempat Pembuangan Akhir (TPA) Provinsi Lampung

Jeni Latri Hening\*, Marlina Kamelia, Andri Jaya Kesuma

Biologi Study Program, Faculty of Saince and Technology, University of Islam Negeri Raden Intan Lampung, Indonesia

\*Email: jenilatrihening12@gmail.com

#### **ABSTRACT**

Microplastics are durable and contain harmful compounds that can be absorbed into the soil and enter the food chain, posing a risk to human health and the environment. One of the efforts to reduce its impact is to utilize bacteria as biodegradation agents. The purpose of this study was to isolate and characterize microplastic degrading bacteria from three landfills in Lampung Province, and determine their degradation rate. The research method used a quantitative descriptive approach, including the isolation stage with the pouring technique, degradation tests using Polystyrene, Polyethylene Terepthalate, and Polyethylene measuring 1x1 cm and identification using Vitek-2. The results of the study from nine bacterial isolates that were successfully purified, three of them showed the highest ability to degrade. The weight reduction result on PS plastic sample was 22.2% by *Bacillus circulans*, PET sample was 15.9% by *Pandoraea* spp. and PE sample was 36.2% by *Pseudomonas aeruginosa* bacteria.

**Keywords**: Bacteria, Biodegradation, Characterization, Landfill, Microplastic

## **ABSTRAK**

Mikroplastik memiliki sifat tahan lama dan mengandung senyawa berbahaya yang dapat terserap ke dalam tanah, memungkinkan zat berbahaya tersebut masuk ke rantai makanan dan berpotensi memberikan dampak buruk terhadap kesehatan manusia serta lingkungan. Salah satu upaya untuk mengurangi dampak mikroplastik adalah dengan memanfaatkan bakteri sebagai agen biodegradasi. Tujuan dari penelitian ini adalah untuk mengisolasi dan mengkarakterisasi bakteri pendegradasi mikroplastik dari tiga Tempat Pembuangan Akhir (TPA) di Provinsi Lampung, serta mengetahui laju degradasinya. Metode yang digunakan dalam penelitian ini adalah deskriptif kuantitatif yang mencakup tahap isolasi dengan teknik tuang, uji degradasi menggunakan *Polystyrene*, *Polyethylene Terepthalate*, dan *Polyethylene* berukuran 1x1 cm dan identifikasi bakteri dilakukan melalui uji biokimia otomatis menggunakan alat Vitek-2. Hasil penelitian dari sembilan isolat bakteri yang berhasil dimurnikan, tiga diantaranya menunjukkan kemampuan paling tinggi dalam mendegradasi. Hasil pengurangan berat pada sampel plastik PS sebesar 22,2% oleh *Bacillus circulans*, sampel PET sebesar 15,9% oleh *Pandoraea* spp. dan sampel PE sebesar 36,2% oleh bakteri *Pseudomonas aeruginosa*.

Kata kunci: Bakteri, Biodegradasi, Karakterisasi, Mikroplastik, Tempat Pembuangan Akhir

### INTRODUCTION

Plastic waste is a problem that is very difficult to overcome. This is due to the high level of use, indiscriminate disposal, and lack of effective management to reduce its amount in the environment (Kapo et al. 2020). Various types and sizes of plastic can break down into microplastics, which are small fragments less than 5 mm in size (Azizah et al. 2020). Microplastics have long-lasting properties and harmful compounds that can seep into the soil, allowing them to enter the food chain and potentially cause negative impacts on human health and the environment (Fachrul and Rinanti, 2018). In terrestrial ecosystems, microplastics have been shown to reduce soil ecosystem viability as well as worm body weights. Furthermore, microplastics have been detected far from their source of origin, including in human blood and on the tops of high mountains (Dewi, 2022).

An alternative to overcome the problem of microplastics is through bioremediation methods that utilize microorganisms (Pikali et al. 2020). Microorganisms such as bacteria play a crucial role in the decomposition of organic matter (Jekti, 2018). Bacteria are single-celled prokaryotic microorganisms that reproduce asexually through cell division. These organisms can live freely, as parasites, saprophytes, or pathogens, and can be found in various habitats such as soil, atmosphere. and oceans (Survani and Taupiqurrahman, 2021). The use of bacteria as bioremediation is often chosen due to their diversity in morphology, physiology, and biological potential. One of the main advantages is the ability to utilize various materials in the environment, including pollutants as a source of nutrients (Jekti, 2018).

Some bacterial species are capable of decomposing natural polymers such as lignin and cellulose, as well as synthetic polymers such as *Polyethylene* and *Polyurethane* as carbon sources. A promising candidate for bioremediation is *Pseudomonas* sp., which is known as a gram-negative, rod-shaped and generally aerobic bacterium (Chofifawati et al. 2022).

Common microbial groups detected in landfills include cellulose degraders, acidogens, acetogens, and methanogens. Some of the bacteria that play a role include *Staphylococcus sciurii*, *Staphylococcus xylosus*, *Escherichia coli*, and *Proteus mirabilis* (Frączek et al. 2017; Nair 2021). Research at the Jabon Sidoarjo landfill identified five bacterial genera, namely *Alcaligenes*, *Actinobacillus*, *Pseudomonas*, *Acinobacter*, and *Neisseria*, which showed potential in decomposing plastic waste (Wati, 2020).

Landfills represent the final stage of waste management, from collection, transportation, processing, and finally disposal (Harjanti and Anggraini, 2020). In general, landfills are large-scale landscape elements, accommodating millions of tons of both organic and inorganic waste (Meyer-Dombard et al. 2020). The management system in landfills is still open, which only involves dumping waste on the ground, which has caused unrest in the surrounding community. This condition causes various problems, such as the accumulation of waste that is not handled optimally and the emergence of pungent odors that disturb the comfort of the environment (Tabrani et al. 2021).

This situation supports the growth of plastic degrading bacteria, so it is an interesting topic to study as a first step in the utilization of biodegradation agents. This study was conducted with the aim of isolating and characterizing microplastic degrading bacteria from three landfill sites, as well as determining their degradation rate.

### **MATERIALS AND METHODS**

### Place and time of research

This research was conducted in December 2024-April 2025, at the INALAB Laboratory, Gedong Meneng, Bandar Lampung City and identification of bacteria using the Vitek-2 compact tool was carried out at the Palembang Public Health Laboratory Center (BBLKM).

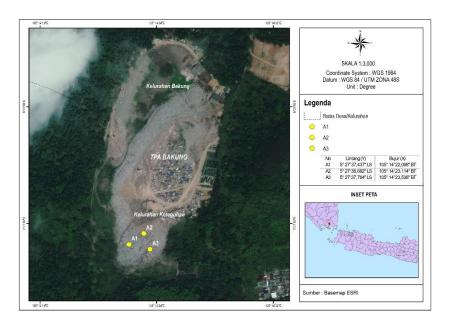
### **Materials**

The tools used were standard laboratory equipment, analytical balance,

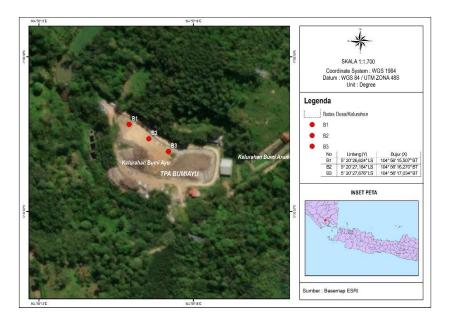
microscope, and Vitek-2 Compact Biomeriux. The materials used in this study include *Mineral Salt Medium* (MSM), *Nutrient Agar* (NA), *Nutrient Broth* (NB), and agar. The types of plastic used include *Polystyrene* (PS), *Polyethylene Terepthalate* (PET), and *Polyethylene* (PE). In addition, sterile distilled water, spritus, 70% alkohol, crystal violet, 96% alcohol, iodine, and safranin were also used as other supporting materials.

# Methods Sampling

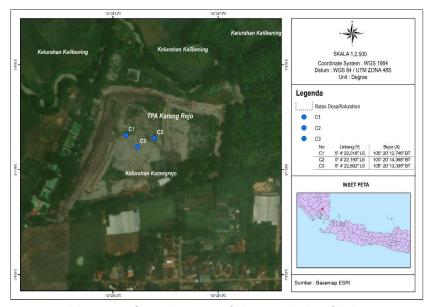
The sampling locations were divided into three areas, namely Bakung, Bumiayu and Karangrejo landfills. Samples were taken using *purposive random sampling* method (Pratiwi et al. 2019; Novitasari et al. 2023). Three sample points were taken at a soil depth of 10-15 cm, which were selected based on the presence of plastic waste piles that were buried and began to decompose.



Picture 1. Sampling map of Bakung landfill site



Picture 2. Sampling map of Bumiayu landfill site



Picture 3. Sampling map of Karangrejo landfill site

#### **Bacterial** isolation

The sample was weighed as much as 5 grams, then 1 mL was taken to be diluted until the concentration became 10<sup>-9</sup> (Rifa Nur Azizah, 2022), Next, the samples were inoculated into *Mineral Salt Medium* (MSM) agar media using the pour plate method, then PS, PET, and PE powders were added as much as 1%, then the mixture was poured into a Petridish and incubated at 37°C for approximately four weeks (Nursyahid et al. 2022).

# Plastic sample preparation

The plastics used are *Polystyrene*, *Polyethylene* Terepthalate and *Polyethylene*. Each plastic is cut into 1x1 cm² size (Sari et al. 2020) and then sterilized by immersing them in 70% alcohol for 30 minutes. After the sterilization process, the samples were washed using distilled water and UV irradiated under laminar airflow within 30 minutes. The initial dry weight was determined by drying the plastic pieces in an oven at 80°C for 12 hours to obtain a weight that did not contain water (Riandi et al. 2017). Next, the initial weight of the plastic was measured with an analytical balance (Octavianda et al. 2016).

# Microplastic degradation test with bacteria

Bacteria that have been isolated are transferred into 10 mL of Nutrient Broth media as much as 1 ose. The bacterial culture was then placed in a shaker at 150 rpm for

18 hours at room temperature. Furthermore, 0.5 mL of inoculum was taken and put into 10 mL of *liquid* MSM media (Sari et al. 2020). After that, the test plastic that has been prepared is placed in a culture medium (*triplo*) containing the bacteria. Then incubated at 37°C for 30 days (Dey et al. 2016; Istiqomah 2020; Okta Vianti et al. 2020).

After the incubation period, the tested plastics were taken out of the test tubes, washed and dried. After this process, the plastic was weighed and the percentage was calculated using the degradation formula.

# **Bacterial characterization**

a. Morphological identification

Morphological identification is carried out to determine the characteristics of colony growth on agar media in petri dishes. In the research conducted, the morphological characteristics considered include color, shape, edge and height of the colony (Rifa Nur Azizah, 2022).

Morphologic identification of bacterial cells

Bacteria isolated and proven capable of degrading microplastics were then examined microscopically. The examination process with gram staining uses four types of solutions, namely crystal violet, iodine, 96% alcohol, and safranin (Rifa Nur Azizah, 2022).

### c. Biochemical identification

Bacterial identification using the Vitek-2 Compact automated instrument is based on determining the minimum inhibitory concentration as a standard when species matching, through barcodes on identity cards to the most specific level. The identity card is placed in a tube containing NaCl solution, then incubated with the bacterial isolate for 24 hours. After incubation, the identification results are automatically printed and analyzed using the Advanced Expert System (AES) device, with data validation and result interpretation equivalent to conventional biochemical tests (Okta Vianti et al. 2020).

# **Data analysis**

Data were analyzed using descriptive analysis of the results of isolation and identification of bacteria that have the potential to degrade microplastics. Meanwhile, quantitative analysis by calculating the percentage of plastic weight loss before and after incuba-

tion. The following is the formula for calculating the percentage of plastic weight loss (Riandi et al. 2017):

% Degradation= 
$$\frac{wi-wf}{wi}$$
 x 100 %

Description:

Wi = initial dry weight (gram)

Wf = final dry weight (gram)

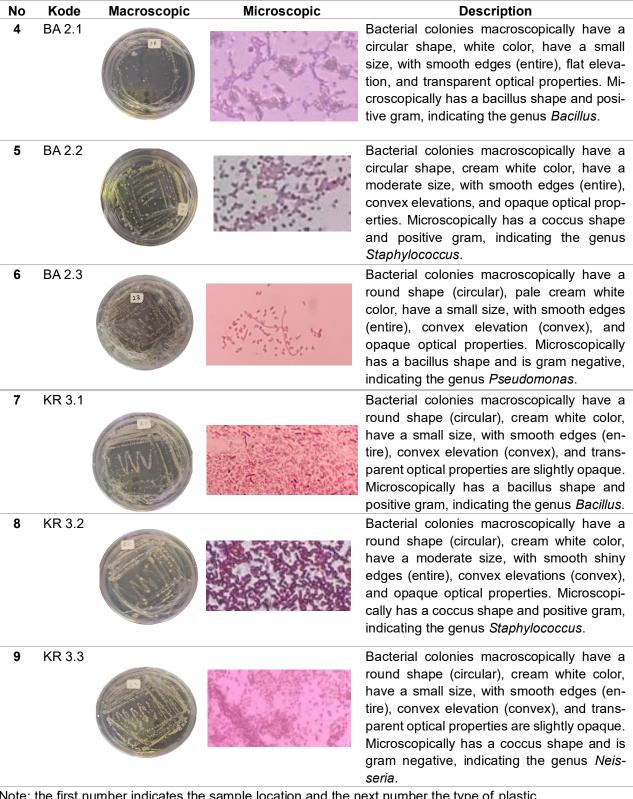
### **RESULTS AND DISCUSSION**

# Identification and characterization of bacteria

Nine bacterial isolates from three different locations were obtained through culture and purification. Each isolate showed differences in macroscopic characteristics such as colony shape, color, elevation and colony edges, and microscopically, such as cell shape and gram staining. Each isolate was coded according to the origin of the sample, namely BK (Bakung), BA (Bumiayu), and KR (Karangrejo). Detailed isolate characteristics are provided in Table 1.

Table 1. Macroscopic and Microscopic characterization of bacterial isolates

No	Kode	Macroscopic	Microscopic	Description
1	BK 1.1			Bacterial colonies macroscopically have a circular shape, cream white color, have a small size, with smooth edges (entire), convex elevation, and transparent optical properties. Microscopically has a rod shape and positive gram, indicating the genus <i>Bacillus</i> .
2	BK 1.2			Bacterial colonies macroscopically have a round shape (circular), cream white color, have a moderate size, with smooth edges (entire), flat elevation (flat), and transparent optical properties. Microscopically has a rod and negative gram, indicating the genus <i>Pandoraea</i> .
3	BK 1.3			Bacterial colonies macroscopically have a round shape (circular), cream white color, have a moderate size, with smooth edges (entire), flat elevation (flat), and opaque optical properties. Microscopically has a coccus shape and positive gram, indicating the genus <i>Staphylococcus</i> .



Note: the first number indicates the sample location and the next number the type of plastic.

Morphological observations showed that isolates BK 1.1, BA 2.1 and KR 3.1 showed characteristics similar to the genus Bacillus, with white or cream colored colonies, round shape, wrinkled edges, straight rod cells or short rods, small to

medium size, flat or convex elevation, aerobic, and able to form endospores. These bacteria are known to produce various degradation enzymes such as amylase, laccase, αglucanase, β-levansucrase, xylanase, chitinase, and protease (Diarti et al. 2017;

Napitupulu et al. 2019; Asmi et al. 2022; Handayani et al. 2023).

Isolate BK 1.2 shows colony morphology that resembles the *genus Pandoraea*, characterized by white color, smooth edges, flat surface, and gram-negative bacillus shape (Yosmaniar et al., 2017). This identification is further supported by previous research, which states that *Pandoraea* is aerobic, motile, does not form spores, and has a short stem shape (Wang et al. 2015).

Isolates BK 1.3, BA 2.2 and KR 3.2 display consistent characteristics with round colonies, flat to convex elevations, intact margins, white in color, with gram-positive coccus cells and grow in irregular groups resembling a bunch of grapes, in accordance with the characteristics of the *genus Staphylococcus* (Khairunnisa et al. 2018; Putri 2022).

Isolate BA 2.3 has similarities with the *genus Pseudomonas*, with the characteristics of circular colonies, convex elevation, yellow-greenish or cream color, and cells that are rod-shaped and gram-negative (Rahmadian et al. 2018; Sari et al. 2020). The *genus Pseudomonas* is an obligate aer-

obic bacterium capable of degrading proteins, carbohydrates, and other organic compounds through the production of enzymes such as protease, amylase, and lipase (Rahmadian et al. 2018). Meanwhile, isolate KR 3.3 shows characteristics consistent with the *genus Neisseria*, in the form of small round colonies, white in color, entire edges, convex elevations, with coccus-shaped cells and is gram-negative (Fauziah and Ibrahim, 2020; Wati, 2020).

# **Bacterial degradation ability**

The ability of bacteria to degrade microplastics in this study is supported by the fact that the isolates obtained come from an environment polluted with microplastics. This is further strengthened by the results of research (Meitri Widya Pangestika, 2024) that the types of microplastics found were fragment, fiber, film, foam, pellet from the Piyungan landfill site in Bantul.

Degradation testing was conducted by comparing the initial and final weight of plastic samples after bacterial inoculation for 30 days. The observation results show that all isolates have the ability to degrade, as shown in Table 2.

Table 2. Degradation ability test results of polystyrene, polyethylene terepthalate, and polyethylene

No	Isolate code	Re- play	Sam- ple	Initial Weight (gr)	Final Weight (gr)	Weight differ- ence (gr)	Degrada- tion results	Aver- age	To present degradation (%)
1.	BK 1.1	1	PS	0.015	0.013	0.002	0.133333	0.1362	13.6%
		2	PS	0.019	0.016	0.003	0.157894	91	
		3	PS	0.017	0.015	0.002	0.117647	-	
2.	BK 1.2	1	PET	0.032	0.028	0.004	0.125	0.1590	15.9%
		2	PET	0.028	0.022	0.006	0.214285	72	
		3	PET	0.029	0.025	0.004	0.137931	-	
3.	BK 1.3	1	PE	0.011	0.008	0.003	0.272727	0.3290	32.9 %
		2	PE	0.007	0.005	0.002	0.285714	04	
		3	PE	0.007	0.004	0.003	0.428571	=	
4.	BA 2.1	1	PS	0.014	0.012	0.002	0.142857	0.1190	11.9%
		2	PS	0.014	0.013	0.001	0.071428	47	
		3	PS	0.014	0.012	0.002	0.142857	-	
5.	BA 2.2	1	PET	0.031	0.029	0.002	0.064516	0.0815	8.15%
		2	PET	0.027	0.024	0.003	0.111111	30	
		3	PET	0.029	0.027	0.003	0.068965	-	
6.	BA 2.3	1	PE	0.006	0.003	0.003	0.5	0.3619	36.2%
		2	PE	0.007	0.005	0.002	0.285714	04	
		3	PE	0.01	0.007	0.003	0.3	-	

No	Isolate code	Re- play	Sam- ple	Initial Weight (gr)	Final Weight (gr)	Weight differ- ence (gr)	Degrada- tion results	Aver- age	To present degradation (%)
7.	KR 3.1	1	PS	0.015	0.009	0.006	0.4	0.2221	22.2%
		2	PS	0.019	0.018	0.001	0.052631	052	
		3	PS	0.019	0.015	0.004	0.210526		
8.	KR 3.2	1	PET	0.033	0.028	0.005	0.151515	0.1191	11.9%
		2	PET	0.034	0.032	0.002	0.058823	32	
		3	PET	0.034	0.029	0.005	0.147058		
9.	KR 3.3	1	PE	0.006	0.004	0.002	0.333333	0.2222	22.2%
		2	PE	0.006	0.004	0.002	0.166666	21	
		3	PE	0.006	0.005	0.001	0.166666		

Isolate KR 3.1 showed the highest degradation activity against *Polystyrene* plastic of 22.2%, higher than the degradation activity against *Polystyrene* plastic (Hidayat et al. 2020) by 18.23% by several *Bacillus species*. Identification with Vitek-2, confirmed this isolate as *Bacillus circulans*. The *genus Bacillus* is known to be commonly used in plastic degradation (Asmita et al. 2015; Miloloza et al. 2022; Mohan et al. 2016), and *Bacillus circulans* have specifically been reported to have the potential to degrade *Polyethylena* (PE) plastic (Chizike et al. 2022).

The isolate coded BK 1.2 showed the highest degradation activity towards *Polyethylene Terepthalate* (PET) plastic at 15.9%. Based on Vitek-2 identification, this isolate is *Pandoraea* spp., which is known to degrade complex aromatic compounds such as p-xylene (Wang et al. 2015) and microplastics including *Polyhydroxybutyrate* (PHB), *Polyethylena* (PE), *Polyhydroxy alkanoate* (PHA), and *Polystyrene* (PS) (Ren and Ni, 2023). Its efficiency is also noted to be high in breaking down harmful compounds such as *Dibutyl-phthalate* (DBP) (Yang et al. 2018) and 2,4 *Diterbutylphenol* (2,4-DTBP) showed significant ecological potential (Adolf et al. 2024).

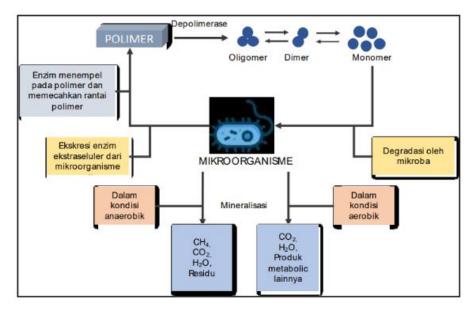
Then isolate BA 2.3 showed the highest activity against *Polyethylene* (PE) plastic at 36.2%. Based on Vitek-2 identification, this isolate is *Pseudomonas aeruginosa*. This ability surpassed previous reports, which recorded PE degradation ranging from 1.71% to 27.3% (Sari et al. 2020; Chizike et al. 2022) and 18.75% for LDPE in Karangasem-Bali landfill (Riandi et al. 2017), indicating a stronger biological potential in this isolate.

The loss of polymer weight in this study is most likely due to the enzymatic activity of the bacteria, which works through a hydrolysis mechanism on the polymer surface. This process is gradual and leads to a decrease in plastic mass with incubation time (Okta Vianti et al. 2020). During the 30-day incubation, the biodegradation process was carried out in MSM (Mineral Salt Medium), a low-carbon medium designed to stimulate the utilization of plastic as the sole carbon source. Under these conditions, bacteria form biofilms (a layer of microorganisms attached to the polymer surface) on the plastic surface to optimize energy efficiency (Shovitri and Marjayandari, 2015), which is also an indicator of the growth and activity of degrading bacteria (Sriningsih and Shovitri, 2015).

The process of plastic degradation by microorganisms takes place through complex mechanisms and is influenced by various factors. Microbial activity causes changes in the molecular integrity of the polvmer, with variations in the mechanism depending on the characteristics of each microorganism (Dwicania et al. 2014). The effectiveness of degradation is also highly dependent on environmental conditions, such as plastic type, pH, temperature, and humidity (Islami 2019; Fatwa and Yoswaty 2021). In addition, substrates that are small in size and have a simple structure will be easier to degrade (Fatwa and Yoswaty, 2021).

Intrinsic characteristics of plastics, such as crystallinity level, molecular weight, functional groups, and additive content also determine the effectiveness of the biodegradation process. These factors can inhibit the penetration of plastics into the bacterial cell membrane, requiring an initial stage of

depolymerization (the process of breaking down polymers into smaller fragments) by microbial enzymes before they can be absorbed (Sari et al. 2020).



Picture 4. Mechanism of microplastic degradation (Fachrul et al. 2021).

(Picture 4.) In general, the biodegradation process is initiated by abiotic mechanisms, such as photodegradation, which results in the formation of carbonyl groups (C=O) on the polymer chain. These groups then undergo oxidation and produce lowmass compounds such as ketones, carboxylic acids and hydrocarbons. This transformation changes the polymer properties from hydrophobic to hydrophilic, which facilitates water absorption and bacterial colonization (Arifina, 2019).

After colonization, the bacteria begin to adhere to the plastic surface and secrete extracellular enzymes that break down the polymer into oligomers, dimers, and monomers. These molecules are then absorbed into the cell and further degraded by intracellular enzymes, until they are finally mineralized into CO<sub>2</sub>, H<sub>2</sub>O, CH<sub>4</sub> and other compounds that can be utilized as carbon and energy sources (Sari et al. 2020).

Table 3. Degradation time comparison

Location	Type of plastic	% degradation in 30 days	Estimated exhaustion (year)	Natural degradation time (year)
Bakung	PS	13.6 %	5.9	500
	PET	15.9%	20.5	70-450
	PE	32.9%	1.5	10-20
Bumiayu	PS	11.9%	6.8	500
	PET	8.15%	32.9	70-450
	PE	36.2%	1.4	10-20
Karangrejo	PS	22.2%	3.74	500
	PET	11.9%	27.4	70-450
	PE	22.2%	2.24	10-20

Based on table 3. above, *Polyethylene* (PE) plastic shows the fastest degradation time with an estimated depletion in 1.4-2.24 years. *Polystyrene* (PS) plastic takes 3.74-5.9 years, while *Polyethylene Terepthalate* (PET) takes longer, 20.5-32.9 years. These estimates are much faster than the natural degradation time of plastics in the environment, which ranges from 10-500 years (Chamas et al. 2020; David et al. 2021; Wahyu Utomo and Arfiana 2023), This insdicates the potential of bacteria in accelerating the plastic biodegradation process.

### CONCLUSION

Plastic pollution is an ever-increasing environmental problem, mainly due to the difficult nature of plastic to biodegrade. Landfills are potential habitats for microorganisms that are able to adapt to plastic polluted conditions. This study successfully separated and tested nine bacterial isolates from three landfills in Lampung Province. Three isolates showed the highest microplastic degradation potential, namely BK 1.2 (Pandoraea spp.) which was able to degrade Polyethylene Terepthalate (PET) by 15.9%, BA 2.3 (Pseudomonas aeruginosa) against Polyethylene (PE) by 36.2% and KR 3.1 (Bacillus circulans) against Polystyrene (PS) by 22.2%. These findings show significant potential in the development of bacteria-based bioremediation strategies to reduce the impact of microplastic pollution in a more environmentally friendly manner.

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