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The Potential of Indigenous Bacteria from Various Soil Samples in Degrading Polymer Plastic Waste

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Abstract

The accumulation of plastic waste is a significant global issue encountered by many countries. Due to its resistance to natural degradation, plastic waste eventually finds its way into the soil, leading to alterations in soil composition. Plastic, a complex polymer with diverse applications, has become integral to various aspects of life. Correspondingly, the extensive use of High-Density Polyethylene (HDPE) and Low-Density Polyethylene (LDPE) plastics has escalated waste production. Hence, this research aimed to isolate indigenous bacteria from diverse soil samples obtained from Surabaya's landfill area and mangrove forests, specifically focusing on their capability to degrade HDPE and LDPE plastic polymers. Sampling was conducted at the Keputih bamboo forest in Surabaya and the Final Disposal Site (*TPA*). Bacteria were subsequently separated from the plastic waste and soil samples. Afterward, the isolated bacteria were purified and screened for their ability to degrade LDPE and HDPE polymers. The isolation process yielded 19 isolates, predominantly *bacilli*, Gram-negative, and catalase-positive bacteria capable of fermenting lactose and glucose, with cell sizes ranging from 1 to 5 µm. Screening tests revealed that Isolate 17 (K2D3U1 MWJ), originating from the Wonorejo mangrove forest, was identified as *Pseudomonas sp.* with an LDPE degradation ability of 21.64%. On the other hand, Isolate 2 (K1D2U2 KPH), obtained from the Keputih Final Disposal Site (*TPA*), was identified as *Bacillus sp.* with an HDPE degradation ability of 27.13%.

Keywords: Plastics, HDPE, LDPE, *Pseudomonas sp.*, *Bacillus sp.*

Introduction

Plastic is widely used in various industrial fields, including food, clothing, transportation, construction, medical, and recreation (Fadlilah & Shovitri, 2014). This material is formed through organic condensation and polymerization processes and is favored for its advantageous properties. Moreover, plastic is lightweight, transparent, water-resistant, and affordable, making it highly desirable for many applications. Its flexibility, durability, and aerodynamic properties have further contributed to its widespread use in various

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industries. However, from an environmental perspective, the increasing plastic usage poses problems for the environment and humans. Its waste is not easily decomposed, and the process involves toxicity and carcinogenicity, causing certain types of plastic to take hundreds of years to decompose naturally (Julina et al., 2022).

According to the World Economic Forum in 2020 (Widyanti, 2022), Indonesia's limited ability to manage and process domestic waste is the primary cause of plastic pollution. In this regard, a significant portion of domestic waste is not sorted before disposal, resulting in a mixture of plastics and other waste. Approximately 78% of uncollected plastic waste is burned, 12% is released into water bodies, and the remaining 10% is disposed of improperly, posing potential environmental pollution risks. Asmita et al. (2015) reported that the neighboring nations produced over 260 million tonnes of plastic, accounting for 8–12.7% of the world's total solid waste. Additionally, river currents carried eight million tonnes of plastic waste into the open sea, making it the largest source of solid waste in terrestrial environments (Eriksen et al., 2014).

Plastic waste has an unsightly appearance and risks releasing various toxic chemical compounds when interacting with biotic and abiotic elements in the environment. These compounds include styrene, vinyl chloride, acrylonitrile, methacrylonitrile, vinylidene chloride, and bisphenol A, all of which are known to be carcinogenic (Campanale et al., 2020). Permatasari and Radityaningrum (2020) argue that plastic exposure to organisms' bodies can harm the digestive system, hinder growth, impede enzyme formation, reduce steroid hormone levels, and affect reproduction. Moreover, plastic absorbs pollutants generated by chemicals in the environment and saltwater, indirectly entering harmful substances into organisms through the food chain. Consequently, contamination to humans can occur through consuming contaminated food (Rochman et al., 2016). The extensive use of plastic in food processing and packaging results in a significant volume of plastic waste. However, limited government regulations are currently in place to address this issue adequately.

Polyethylene, with an annual production of 140 million tonnes, is the most commonly used synthetic polymer in plastic manufacturing. According to data from the Ministry of Industry cited in the Daily Investor (2016), Indonesia consumed approximately 955,000 tonnes of polyethylene annually in 2012. It increased to nearly 1.03 million tonnes in 2013 and was projected to reach 1.11 million in 2014. Other data further reinforced the demand for plastic products. As the global industry expands, Low-Density Polyethylene (LDPE) is expected to rise to 9%, while High-Density Polyethylene (HDPE) is anticipated to reach 17%. Although these two types of plastic are still considered safe, their frequent and excessive use can adversely affect the environment and human health (Law & Thompson, 2014).

To minimize the amount of plastic waste, it is crucial to implement a low-risk control strategy. One viable approach entails harnessing indigenous bacteria, which can degrade plastic polymers. These bacteria are naturally found in habitats such as soil or landfills. By utilizing polyethylene as a carbon source for growth, they facilitate the mineralization process, breaking it down into small monomers (Sriningsih & Shovitri, 2015). Accordingly, recent studies conducted by Riandi et al. (2017) and Yao et

al. (2022) have demonstrated the plastic-degrading capabilities of various bacteria, including those belonging to the genera *Pseudomonas* and *Bacillus*.

Research Method

This study is classified as exploratory and experimental as it utilized two distinct sites for the sampling process: the Keputih bamboo forest in Surabaya and the area surrounding the Final Disposal Site (*TPA*). Correspondingly, the sampling location for the study was the Final Disposal Site (*TPA*). The investigation was carried out at the TLM Microbiology Laboratory of Muhammadiyah University in Surabaya. The activities were implemented from January to February 2023.

Soil sampling technique

Purposive sampling was employed to collect soil samples from two distinct areas: the mangrove forest and the Final Disposal Site. In order to obtain a total of 15 soil samples from each area, sampling was conducted three times at five different locations within the Final Disposal Site and mangrove forest. The collected samples contained naturally decomposed plastic present in the soil. Using a shovel, soils were extracted from the top layer of the excavation, reaching a depth of 10 cm and weighing up to 100 grams. Subsequently, those samples were placed into sterile ziplock bags labelled "Dn" and "Un," in which “Dn” represented the collection point, and “Un” indicated the number of replicates.

Preparation of plastic bags

The materials used in this study were a specific type of plastic bag, namely High-Density Polyethylene (HDPE) and Low-Density Polyethylene (LDPE). Three repetitions of each material were prepared, measuring 5 × 1 cm. The plastic bags were first sterilized using 70% alcohol for approximately 30 minutes. Subsequently, the materials were cleaned with sterile water and dried under a UV lamp in a Laminar Air Flow cabinet for 30 minutes. To determine the pure weight of the plastic, devoid of any water content, the pieces were dried in an oven at 80°C for 24 hours. This step allowed researchers to calculate the initial dry weight of the plastic, which was measured in a sterile environment using an analytical balance. Additionally, each plastic piece was labeled for easy identification. Subsequently, plastic powder and small-cut plastic were prepared using the respective types of plastic, HDPE and LDPE, which were cut into small pieces and boiled for 15 minutes in xylene solution to obtain a plastic powder. Once the plastic was transformed into powder form, it was further ground using a sterile mortar and pestle and thoroughly mixed. Afterward, it was immersed in 70% alcohol for approximately 30 minutes and dried under UV light in a laminar airflow cabinet for another 30 minutes. Finally, the plastic powder was incubated overnight at 60ºC (Hussein et al., 2015).

Tools and materials

The following equipment and materials were utilized in the research: Petri dishes, autoclaves, shaking

incubators, blenders, test tubes, test tube racks, microscope slides, plastic jars, microscopes, *Aqua* bottles, *YOU•C1000* glass bottles, Durham tubes, vortex mixer, oven, inoculation loop, micropipettes, tips, Erlenmeyer flasks, measuring cups, glass beakers, test tube clamps, 0.2 µL filter paper, Bunsen burner, cotton, filter paper, cooling box, ice cubes, spreader, hot plate, Eppendorf tubes, glass funnels, laminar airflow hood, analytical balance, rectangular wire, desiccator, anaerobic tubes, spectrophotometer, and black polybags. Additionally, high- and low-density polyethylene plastics, sterile distilled water, distilled water, sodium chloride (NaCl), nutrient broth media, xylene, glycerol, crystal violet solution, iodine salt solution, 70% and 95% alcohol, safranin, immersion oil, hydrogen peroxide solution (H_2O_2), mineral salt agar medium, mineral salt liquid medium, glucose powder, king's B agar medium, saline solution, and soil were used as materials.

Bacterial isolation

The first step in bacterial isolation involved plating using soil and plastic waste samples collected from the landfill and mangrove forests. The samples, weighing up to 10 grams, were suspended in 90 milliliters of 0.85% saline solution in a bottle, which was then placed in a shaking incubator at 37°C for 30 minutes. After the incubation period, the suspension was diluted to a dilution level of 10-6 using the serial dilution method. 2% Polyethylene Glycol (PEG) was added to each King's B agar medium to assess the isolates' ability to grow in a variable environment with a plastic substrate. Subsequently, 0.1 mL of the diluted suspension was pipetted onto each agar plate and spread using a sterile spreader.

Plastic-degrading bacteria purification stage

Following a 48-hour of incubation, the bacteria were purified by isolating each colony formed on King's B agar medium in a Petri dish. The morphological characteristics of the growing colonies, such as size, colour, and shift in media colour, shape, margins, surface, and elevation, were examined. A single colony was marked and isolated onto King's B agar medium using the streak method. The media was then incubated for 24 to 72 hours at 37°C and 40°C until a single, pure colony was visible on the Petri dish. The incubation occurred in an aerobic environment with the Petri dish upside down. Subsequently, the single colonies were transferred and placed in Nutrient Broth (NB) media to facilitate multiplication. The isolates underwent various identification tests, including Gram staining, lactose fermentation, gas production (oxidase) test, motility test, indole and hydrogen sulphide (H_2S) production test, and catalase. A pure isolate was also streaked on a King's B agar slant supplemented with glycerol to serve as a stock culture.

Preliminary identification of plastic-degrading bacteria

The obtained isolates were examined both macroscopically (visually) and microscopically. Several physiological traits were investigated or observed, including lactose fermentation, indole and hydrogen sulfide (H_2S) production,

motility, catalase, gas production (oxidase) test, Gram staining, and others. The identification procedure was based on the Bergey's Manual of Bacteriology.

Macroscopic observation

The present study involved macroscopic observations of the bacterial isolates, which included noting the shapes of the bacterial colonies on the surface of the media when viewed from above (dotted, threaded, irregular, root-like, or coil-shaped), observing the edges of the colonies when viewed from above (smooth, wavy, split, jagged, threaded, or curly), examining the surface textures of the colonies (smooth or rough), recording colony elevation, size, and colour, and documenting any changes in the media's colour.

Gram staining

A pure culture of bacteria was cultivated on an agar medium using a loop to perform Gram staining. The bacteria were then evenly spread on a glass slide moistened with sterile distilled water positioned 15-30 cm away from a Bunsen flame to allow the bacterial smear to air dry. Subsequently, the smear was stained with a crystal violet solution for one to three minutes, constituting the initial stage of Gram staining. Following this step, the slide was rinsed under running water while inverted. The bacterial smear was then immersed in an iodine solution (Lugol) for two minutes, followed by a 30-second wash in 95% alcohol to remove any residual colour. A final rinse under running water was performed. The bacterial smear was then counterstained with safranin for five to fifteen minutes. After cleaning the slide under running water, it was dried using a Bunsen burner. Finally, the bacterial smear was observed under a microscope, utilizing oil immersion and 1000-time magnification to visualize the bacteria's morphology.

Catalase test

After smearing the isolated material on a glass surface, two drops of 3% hydrogen peroxide (H_2O_2) were added. Ultimately, the appearance of oxygen gas bubbles resulting from the catalase enzyme breaking down H_2O_2 indicated a positive result.

Glucose fermentation test

Following the addition of glucose to the Nutrient Broth media with a Durham tube containing the isolate from King's B agar medium, a heated loop needle was inserted into the liquid culture. As a result, the presence of carbon dioxide gas bubbles, a byproduct of glucose metabolism, indicated a positive outcome.

Motility test

After transferring the isolates with an inoculation loop and inserting them approximately a quarter of the way into a test tube filled with SIM (Sulfur-Indole-Motility) media, the tube was incubated at 37ºC for a full day. Bacterial colonies extending beyond the original puncture path indicated a positive result.

Indole and Hydrogen Sulfide (H_2S) production test

The isolates were extracted using the prepared needle and placed approximately halfway up the media in a

test tube filled with SIM (Sulfur-Indole-Motility) media. The test tube was subsequently incubated at 37°C for a full day. A red or yellow colour on the surface of the media indicated a positive indole result, while a change in the media's colour to black indicated a positive H_2S result.

Lactose fermentation test

To suspend the isolate from King's B agar medium, a Durham tube-containing Lactose Broth medium was used, and a hot loop needle was then inserted into the liquid culture. The presence of carbon dioxide gas bubbles, a byproduct of lactose metabolism, suggested positive outcomes.

Screening of HDPE and LDPE polymer-degrading bacteria

The qualitative analyses of different types of bacteria capable of producing enzymes that break down plastic polymers is known as bacterial screening or a screening test. In this regard, the researchers compared the extent of plastic degradation by multiple bacterial isolates using a screening method derived from soil specimens. The intended screening procedure consisted of two testing phases: the first involved testing isolates on a liquid medium, and the second incorporated growth and clear zone formation tests on media containing polymers made of both high-density polyethylene (HDPE) and low-density polyethylene (LDPE) (Hussein et al., 2015).

Screening on liquid media (initial screening)

The liquid media screening method aims to determine the capability of bacterial isolates to degrade HDPE and LDPE polymers by measuring the optical density. One sample of each pure culture was obtained and inoculated into a Mineral Salt medium supplemented with 0.1% glucose as a carbon source for this procedure. The samples were then incubated at 30°C in a 150 rpm shaking incubator.

A 100 mL Erlenmeyer flask was filled with the previously prepared liquid Mineral Salt medium to conduct the screening. In addition, 0.1% HDPE and LDPE powder were added as the carbon source and substrate. The Erlenmeyer flask was sterilized through autoclaving. Subsequently, up to 1 mL inoculum was added to each liquid Mineral Salt medium containing HDPE and LDPE powders, along with glucose and the previous bacterial suspension. Negative controls consisted of Erlenmeyer flasks filled with sterile water and media controls, which included HDPE and LDPE powders without any inoculums. All Erlenmeyer flasks were incubated at 30°C in a shaking incubator at 150 rpm for four days. The degree of turbidity was used to determine the rate of bacterial growth.

Clear zone formation test (second screening)

The clear zone formation test method was employed to evaluate the ability of bacterial isolates to degrade HDPE and LDPE polymers by monitoring and quantifying the discoloration zones produced by bacterial colonies in media containing *Tween 80*. In this test, the researchers utilized agar media

containing HDPE and LDPE treated with *Tween 80*. Pure culture was added to the media and spread 1 cm in the centre—media without any inoculum served as the negative control in the clear zone test.

Bacterial growth test on media containing HDPE and LDPE (second screening)

To determine the growth rate of bacteria in media containing fragments of HDPE and LDPE polymers, the growth test method verified bacterial isolates capable of degrading these polymers. The procedure began with preparing a liquid Mineral Salt medium in advance. Approximately 25 mL of this medium was poured into a 100 mL Erlenmeyer flask, along with a small piece of plastic measuring 5 × 1 cm. After autoclave sterilization, 1 mL of bacterial suspension was added to each Erlenmeyer flask for inoculation. Following a 45-day incubation period at 30°C on a 150 rpm shaking incubator, the turbidity level of the media was assessed, and the final dry weight was recorded.

Dry weight loss percentage test

To evaluate the extent to which the bacterial isolates could break down HDPE and LDPE polymers, the dry weight percentage test method measured the difference between the initial and final weight of the plastic after degradation. The weight difference between the plastic pieces before and after degradation determined the weight lost. After extraction from the biofilm, the plastic fragments were dried and sterilized with 70% alcohol. Subsequently, the plastic pieces were dried and baked at 80°C for twenty-four hours. Weighing the baked plastic pieces provided their dry weight. The formula for calculating the percentage of weight lost from the plastic is as follows (Yao et al., 2022):

$$weight\ loss = \frac{Wi - Wf}{Wi}\ x\ 100\%$$

Notes:
Wi = Initial dry weight before degradation (grams)
Wf = Final dry weight after degradation (grams)

Identification of plastic-degrading bacterial species

To identify the bacterial isolates with the highest degradation ability at the genus level, the bacterial isolates' macroscopic and microscopic characteristics were compared and adjusted using Bergey's Manual of Determinative Bacteriology, Ninth Edition.

Research Results and Discussion

Isolation and Identification of Indigenous Bacterial Isolates from the Final Disposal Site and the Mangrove Forest

Nineteen bacterial isolates were obtained from soil samples collected from five distinct locations: Gunung Anyar mangrove forest, Benowo Final Disposal Site, Keputih Final Disposal Site, and Wonorejo mangrove forest. Table 1 presents the characteristics of the bacterial isolates collected during sampling after the completion of the biochemical test.

Table 1. Biochemical Test Results of Indigenous Bacterial Isolates

Isolate name	Catalase Test	Glucose Fermentation Test	Lactose Fermentation Test	Motility Test	Indole Production Test	Hydrogen Sulfide Production Test	Gram staining
Isolate 1 (K1D4U1 KPH)	+	-	-	motile	-	-	- (bacilli)
Isolate 2 (K1D2U2 KPH)	+	+	-	non-motile	-	-	- (bacilli)
Isolate 3 (K2D2U1 KPH)	+	-	-	motile	-	-	- (bacilli)
Isolate 4 (K1D3U3 KPH)	+	-	-	motile	-	-	- (bacilli)
Isolate 5 (K2D2U3 KPH)	+	-	-	motile	-	-	- (bacilli)
Isolate 6 (K3D1U3 KPH)	+	-	-	motile	-	+	- (bacilli)
Isolate 7 (K4D3U3 KPH)	+	-	-	motile	-	-	- (bacilli)
Isolate 8 (K1D2U3 FMUs)	+	-	-	motile	-	-	- (bacilli)
Isolate 9 (K2D4U1 KPH)	+	-	-	motile	-	-	- (bacilli)
Isolate 10 (K1D2U3 BNW)	+	+	+	motile	-	+	- (cocci)
Isolate 11 (K2D2U1 BNW)	+	-	-	motile	-	-	- (bacilli)
Isolate 12 (K1D4U2 BNW)	+	-	-	motile	-	-	- (bacilli)
Isolate 13 (K1D3U1 BNW)	+	-	+	motile	-	-	- (bacilli)
Isolate 14 (K3D3U2 MGA)	+	-	+	motile	-	-	- (bacilli)
Isolate 15 (K1D5U2 MGA)	+	-	-	motile	-	-	- (bacilli)
Isolate 16 (K1D2U2 MGA)	+	+	+	motile	-	-	- (bacilli)
Isolate 17 (K2D3U1 MWJ)	+	-	-	motile	-	-	- (bacilli)
Isolate 18 (K1D3U1 MWJ)	+	-	-	motile	-	-	- (bacilli)
Isolate 19 (K1D4U2 MWJ)	+	-	-	motile	-	-	- (bacilli)

Notes:
+ = Positive
- = Negative

Table 1 illustrates the distinct characteristics of the 19 different types of isolated bacteria. Most of them were categorized as Gram-negative bacilli, yielding positive results in the catalase test, exhibiting motility, and demonstrating an inability to ferment lactose or glucose and a lack of indole or hydrogen sulfide production. Conversely, one isolate exhibited a cocci form, produced hydrogen sulfide, displayed motility, and possessed the ability to ferment lactose and glucose. This isolate also yielded positive results in the catalase test while unable to produce indole compounds. Furthermore, certain isolates exhibited positive catalase activity and the capability to ferment glucose despite lacking motility.

Bacterial Liquid Media Screening Test Results: *Pseudomonas* and *Bacillus*

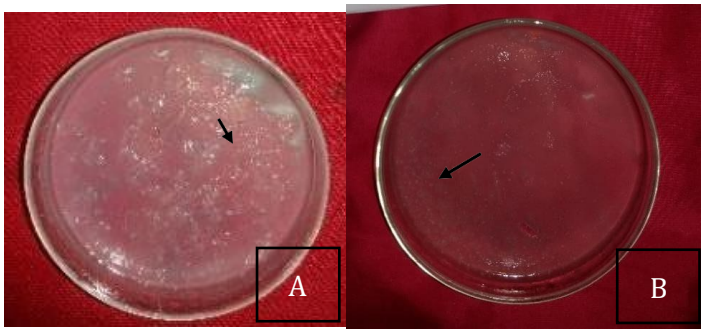
The screening test conducted on liquid media generated positive results for all 19 bacterial isolates. It indicates that the media, which contained High-Density Polyethylene (HDPE) and Low-Density Polyethylene (LDPE) plastics, underwent noticeable color changes and became hazy. The clear medium transformed into dark green, brown, and yellow, as evidenced by the observed color change. Figure 1 visually presents the alterations in the media's color.

Figure 1. *Color changes in the media exhibited by the bacterial isolates at 28°C after 14 days of incubation (A: High-Density Polyethylene Plastic; B: Low-Density Polyethylene Plastic)*

Bacterial Solid Media Screening Test Results: *Pseudomonas* and *Bacillus*

According to the test results, only four bacterial isolates could grow and form both opaque and clear zones in a medium containing HDPE plastic. Among the isolates, Isolate 2 (K1D2U2 KPH) exhibited the largest clear zone diameter, measuring 0.37 cm. Isolate 6 (K3D1U3 KPH) and Isolate 7 (K4D3U3 KPH) followed with clear zone diameters of 0.30 cm, while Isolate 15 (K1D5U2 MGA) measured 0.32 cm. On the other hand, five bacterial isolates demonstrated the capacity to proliferate and create opaque and clear zones in a medium containing LDPE plastic. Isolate 17 (K2D3U1 MWJ) displayed the largest clear zone diameter, measuring 0.38 cm. Figure 2 illustrates the resulting clear zone formation.

Figure 2. Bacterial isolates were cultured at 28°C for 14 days, forming a clear zone (A: High-Density Polyethylene Plastic in Media; B: Low-Density Polyethylene Plastic in Media).



Isolation and testing of plastic polymer-degrading microorganisms

In order to assess the capability of the isolates to degrade plastic polymers, supplementary screening procedures were conducted on the obtained isolates. Four bacterial isolates exhibiting the largest clear zone diameter were selected from the HDPE media. In comparison, three isolates were chosen from the LDPE media based on the preliminary test results. Notably, Isolate 2 (K1D2U2 KPH) demonstrated the most significant percentage of High-Density Polyethylene (HDPE) degradation, with a value of 27.13%. Likewise, Isolate 17 (K2D3U1 MWJ) exhibited the greatest percentage of Low-Density Polyethylene (LDPE) degradation, measuring 21.64%. These significant findings were derived from the plastic polymer degradation test described earlier. Detailed results of the degradation test can be found in Tables 2 and 3.

Table 2. HDPE plastic polymer degradation test results

Isolate name	Dry weight before treatment (grams)	Dry weight after treatment (grams)	Weight difference (grams)	Average (grams)	Percentage of degradation ability (grams)
Isolate 2 (K1D2U2 KPH)	Deuteronomy 1 = 0.0121 Deuteronomy 2 = 0.0102	Deuteronomy 1 = 0.0085 Deuteronomy 2 = 0.0077	Deuteronomy 1 = 0.0036 Deuteronomy 2 = 0.0025	0.0022	27.13%
Isolate 7 (K4D3U3 KPH)	Deuteronomy 1 = 0.0211 Deuteronomy 2 = 0.0197	Deuteronomy 1 = 0.0181 Deuteronomy 2 = 0.0159	Deuteronomy 1 = 0.0030 Deuteronomy 2 = 0.0038	0.00340	16.76%

Isolate 6 (K3D1U3 KPH)	Deuteronomy 1 = 0.0215 Deuteronomy 2 = 0.0201	Deuteronomy 1 = 0.0188 Deuteronomy 2 = 0.0165	Deuteronomy 1 = 0.0027 Deuteronomy 2 = 0.0036	0.00315	15.23%
Isolate 15 (K1D5U2 MGA)	Deuteronomy 1 = 0.0192 Deuteronomy 2 = 0.0208	Deuteronomy 1 = 0.0177 Deuteronomy 2 = 0.0189	Deuteronomy 1 = 0.0015 Deuteronomy 2 = 0.0019	0.00385	8.47%

Table 3. LDPE plastic polymer degradation test results

Isolate name	Dry weight before treatment (grams)	Dry weight after treatment (grams)	Weight difference (grams)	Average (grams)	Percentage of degradation ability (grams)
Isolate 6 (K3D1U3 KPH)	Deuteronomy 1 = 0.0226 Deuteronomy 2 = 0.0144	Deuteronomy 1 = 0.0209 Deuteronomy 2 = 0.0132	Deuteronomy 1 = 0.0017 Deuteronomy 2 = 0.0012	0.00145	7.92%
Isolate 17 (K2D3U1 MWJ)	Deuteronomy 1 = 0.0235 Deuteronomy 2 = 0.0133	Deuteronomy 1 = 0.0158 Deuteronomy 2 = 0.0119	Deuteronomy 1 = 0.0077 Deuteronomy 2 = 0.0014	0.00455	21.64%
Isolate 10 (K1D2U3 BNW)	Deuteronomy 1 = 0.0217 Deuteronomy 2 = 0.0131	Deuteronomy 1 = 0.0199 Deuteronomy 2 = 0.0112	Deuteronomy 1 = 0.0018 Deuteronomy 2 = 0.0019	0.00185	11.39%

Results of bacterial species identification

The findings of the manual identification of bacterial isolates capable of degrading HDPE and LDPE plastic polymers were obtained using Bergey's Manual of Determinative Bacteriology, Ninth Edition. In this context, Isolate 2 (K1D2U2 KPH) was identified as a member of the *Bacillus sp.* genus, whereas Isolate 17 (K2D3U1 MWJ) was recognized as a member of the *Pseudomonas sp.* genus.

Screening tests were conducted on 19 bacterial isolates of the genera *Pseudomonas* and *Bacillus*. These tests revealed that all 19 isolates could grow effectively on media containing HDPE and LDPE plastic polymers. The presence of bacteria caused the media to appear cloudy as their population multiplied and spread throughout the medium (Arimurti et al., 2022). In addition to the cloudiness, certain bacterial isolates also caused a change in the color of the media, resulting in shades of green, red, brown, or yellow. These color alterations are attributed to the release of pigments by bacteria, which react with the media's substrate to form chromophores, thereby altering the color of the medium (Bawazir et al., 2018).

The results of the microscopic

identification of the 19 isolated bacteria varied. Most were identified due to their motility, positive catalase test results, inability to ferment lactose or glucose, and inability to produce indole or hydrogen sulfide compounds. These bacteria were classified as *bacilli* with no Gram staining status. On the other hand, one isolate had a *cocci* form, produced hydrogen sulfide, was motile, could ferment lactose and glucose, and showed positive catalase test results. However, it was unable to produce indole compounds. Furthermore, certain isolates exhibited positive catalase activity and could ferment glucose despite being non-motile. Gram-negative bacteria are characterized by a thin cell wall structure with a thickness of 8–12 nm, and they are classified as multilayered or triple-layered. They possess a cytoplasmic membrane, are not resistant to physical disturbance, have a high-fat content in the cell wall (about 11–12 nm), exhibit reduced susceptibility to penicillin compounds, and have a high lipoprotein content (Aryal, 2022). Meanwhile, the positive catalase result of the isolates demonstrated their ability to produce the catalase enzyme, which converts hydrogen peroxide (H_2O_2)—a toxic byproduct of aerobic metabolism—into water and oxygen molecules. The catalase enzyme is believed necessary for aerobic growth, as it protects microbial cells from the harmful effects of H_2O_2 (Riandi et al., 2017).

Out of the 19 isolates, 18 were found to be motile, while 1 was non-motile. In this regard, motile bacteria are characterized by flagella, which enables them to move (Panjaitan et al., 2020). On the other hand, non-motile bacteria lack gliding motility or flagella and rely on other bacteria, animals, or water currents for movement. Furthermore, it was observed that 17 bacterial isolates could not produce both hydrogen sulfide (H_2S) and indole compounds, while 2 isolates could produce H_2S but not indole compounds. Arora et al. (2015) state that the breakdown products of L-tryptophan compounds, indole, and hydrogen sulfide, are highly toxic and can be fatal to humans and other microorganisms.

The development of a clear zone in the media indicates that bacteria can grow normally and utilize media substitutes, which include nitrogen elements and plastic polymers, as their primary source of nutrients for metabolic processes. Bacteria, aided by hydrolytic enzymes, can break the chemical bonds in plastic polymers. Moreover, they can degrade carbon elements into substrates to build internal cell structures and generate energy through internal metabolism. Additionally, they can use the added nitrogen in the media as their primary energy source for degrading plastic polymers (Agustien et al., 2016).

Isolate 2 (K1D2U2 KPH), obtained from the Keputih Final Disposal Site (*TPA*), was classified as a species of *Bacillus* based on the identification results. Isolate 17 (K2D3U1 MWJ), collected from the Wonorejo mangrove forest, was identified as a species of *Pseudomonas*. These findings are consistent with previous research conducted by Ainiyah and Shovitri (2014) and Sriningsih and Shovitri (2015). They demonstrated that the genera *Pseudomonas* and *Bacillus* could degrade

plastic polymers, primarily by reducing the dry weight of plastics and forming a transparent zone on media containing plastic polymers. According to Puiggené et al. (2022), *Pseudomonas* produces extracellular esterase, lipase, and cutinase enzymes, which enable the decomposition of plastic polymers. These enzymes can be secreted externally or bound to the cell membrane, participating in a catalytic process called hydrolysis that breaks down the polymer's ester bonds and forms short-chain compounds. Moreover, these substances are further mineralized by intracellular enzymes and metabolic pathways, which utilize carbon, nitrogen, and energy for growth. Meanwhile, Yao et al. (2022) found that bacteria in the *Bacillus* group could degrade plastic polymers due to the presence of enzymes such as laccase and alkane oxidase. Monooxygenases oxidise polyethylene molecules into alcohols, which are further oxidized to aldehydes by alcohol dehydrogenases.

Conclusion

This investigation revealed that Isolate 2 (K1D2U2 KPH), derived from the Keputih Final Disposal Site (*TPA*), was identified as a *Bacillus* species exhibiting a significant HDPE degradation ability of 27.13%. Additionally, Isolate 17 (K2D3U1 MWJ), obtained from the Wonorejo mangrove forest, was identified as a *Pseudomonas* species with a notable LDPE degradation ability of 21.64%.

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