

Isolation and Identification of Indigenous Polypropylene-Degrading Bacteria Isolated From Bestari Landfill of Probolinggo

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ABSTRACT

Polypropylene (PP) is a linear hydrocarbon polymer, which is the most widely used among other synthetic polymers, has high chemical resistance and is difficult to decompose naturally, making it a major challenge in handling plastic waste. This study aims to identify and evaluate the degradation ability of PP by indigenous bacteria from passive cell zone at Bestari Landfill, Probolinggo City. Isolation is carried out by the enrichment method on Mineral Salt Media (MSM) which contains 0.5% PP granules. The research stages included sampling, isolation, and screening of bacteria for the most effective degrading agents. Six isolates of PP-degrading bacteria were discovered at the Bestari Landfill of Probolinggo City, including B1UM1, B1UM2, B1UM3, B2UM1, B2UM2, and B2UM3. After 15 days of incubation, potential isolates B1UM1, B1UM2, and B2UM1 showed the highest polymer reduction of 8.25%, 7.15%, and 6.25%. Gram staining results showed that isolate B1UM1 was coccus and a Gram-positive bacterium, while isolates B1UM2 and B2UM1 were basil and Gram-positive. Genotypic identification through 16S rRNA sequence analysis showed that isolate B1UM1 had 100.0% similarity with *Staphylococcus haemolyticus*, B1UM2 had a 100% similarity to *Bacillus cereus*, and B2UM1 had a 99% had a similarity to *Bacillus* sp.

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Introduction

Plastics are an important group of organic, synthetic polymer materials, derived from petroleum, which are widely used in agriculture, commerce, and industry, because they are lightweight, and have stable chemical and physical properties [1]. Plastics have a long degradation time, because the polymer structure of plastic has a long repeating chain. Polypropylene (PP) is one of the second most widely produced types of thermoplastics in the world (after Polyethylene), often used in

packaging and labeling, especially in the packaging of food, textiles, laboratory equipment, and automotive [2]

PP expressed as C_nH_{2n} , a polymer with a C-C main chain, is difficult to degrade due to its high molecular weight, stable covalent bonds, and lack of reactive functional groups. Consequently, PP dominates the highest plastic waste in Indonesia [3]. As PP consumption increases, so does the amount of PP waste discharged into the environment.

Several methods have been applied to deal with microplastics in the environment, including incineration, disposal to landfill, and photodegradation, but they are not effective in reducing PP accumulated in the environment [4], [5]. Biodegradation of microplastics by soil microbes, especially indigenous bacteria, is an alternative to PP waste treatment, due to the potential of bacteria to convert microplastics into intermediate compounds such as short-chain fatty acids, some of which can be utilized as a carbon source for microbial metabolism [6]. From a chemical point of view, the main challenge in PP degradation lies in the non-polar nature of PP, which causes microbes to be unable to degrade. However, certain bacteria are known to produce oxidative enzymes that are able to catalyze oxidation reactions on the C-C chain, resulting in hydroxyl groups. This change increases the polarity and reactivity of the polymer [7].

The chemical principles underlying the degradation process of PP by indigenous bacteria are oxidative reactions and enzymatic processes. The degradation starts from the main chain C-C being broken down into aliphatic hydrocarbons with low molecular weight, then the main chain C-C is hydroxylated by hydroxylase enzymes to produce primary and secondary alcohols, which are then oxidized into carboxylic acids. Carboxylic acid will be further oxidized into fatty acids that will enter the bacterial metabolic system and the citric acid cycle (TCA) to be released into the environment as a final result [8]. Therefore, this process is one of the sustainable solutions to address PP waste in the environment. So, to support this treatment, the isolation of indigenous bacteria degrading PP plastic is carried out.

The Probolinggo City Bestari Landfill is a potential habitat to find indigenous bacteria. The passive cell zone at Bestari landfill shows the presence of brittle and cracked plastic, influenced by environmental conditions such as high temperature, pH, aeration and low humidity. In 2023, research on the exploration of soil bacterial diversity in the Bestari Probolinggo landfill was conducted using the Next Generation Sequencing (NGS) method. The result showed that soil samples from the Bestari Landfill in Probolinggo showed high

diversity of bacterial communities [9]. This high diversity indicates the potential of bacteria in degrading xenobiotic compounds, including microplastics. To confirm the potential of these bacteria in degrading PP, genotype analysis through 16S rRNA sequencing was conducted. Because, the NGS method has limitations, it can only detect microbial diversity without providing information on specific superior indigenous bacterial species and their physiological characteristics. Therefore, further approaches are needed through direct bacterial isolation and characterisation to determine the specific species capable of degrading microplastics, especially PP [10], so that the genotypic characteristics and chemical biodegradation potential can be understood more deeply [11].

Several previous studies have shown that indigenous bacteria can degrade plastics. *Bacillus sp* and *Pseudomonas sp* are some of the bacteria that can degrade High Density Polyethylene (HDPE) [12]. Helen et al. (2017) reported that indigenous bacteria isolated from mangrove sediments identified by genotypes *Bacillus cereus* and *Sporosarcina globispora* were able to degrade PP grains by 12% and 11%, respectively, in MSM media during 40 days of incubation [13]. The ability of indigenous bacteria was also proven in the study (Arimurti et al. 2023), that strain 17 (K2D3U1 MWJ), isolated from the Wonorejo Mangrove Forest, Surabaya which was identified as *Pseudomonas sp.* was able to degrade LDPE by 21.64% during 45 days of incubation [12]. Another study reported that *B. cereus* bacteria isolated from the Okhla landfill in New Delhi were able to degrade PP sheets by up to 12% over 40 days [14].

Research on the ability of indigenous bacteria to degrade PP is still limited, so this research is interested in isolating and identifying PP degrading bacteria from the passive cell zone of Bestari landfill in Probolinggo City, by conducting phenotyping and genotyping tests, this research is expected to identify bacterial species that have the potential to be in PP degradation. This identification will be an initial understanding for further research about the potential chemical interactions that occur between bacterial

metabolic activities and polymer structures. By directing this research to the microbial-based bioremediation pathway, the results are expected to be the basis for the development of plastic waste treatment strategies that are aligned with the principles of green chemistry, especially in the context of using biological agents as biological catalysts for the degradation process.

Material and Methods

Material

Polypropylene granules were obtained from Sigma Aldrich. The granules were round and white. The Mineral Salt Media (MSM) used for isolation and biodegradation studies of polypropylene-degrading bacteria had the following composition (per liter of distilled water): NH_4NO_3 1.0 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2 g; K_2HPO_4 1.0 g; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.1 g; KCl 0.15g; yeast extract 0.1 g; and 1.0 mg of each of the following microelements; $\text{FeSO}_4 \cdot 6\text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, and MnSO_4 [15].

Samples Collections

Soil sampling took place at 5 locations within the passive cell zone of the Bestari Landfill, located in Sukabumi Village, Mayangan Subdistrict, Probolinggo City. Samples were collected from a soil depth of 7 cm to 10 cm using a shovel. Each point was collected up to 250 grams, then the samples were mixed until homogeneous, so that a total of 1 kg of soil samples were obtained. The samples were then transferred to a dark bottle and stored in an ice box with ice gel. The purpose of the ice gel is to protect the sample from temperature changes.

Isolation and Identification of Bacteria by Enrichment Techniques

The isolation technique by (Auta et al. 2018) and (Jain et al. 2018) serves as the foundation for the isolation procedure. One gram of soil sample was combined with 10 mL of sterile physiological saline (0.9% NaCl). The mixture was vortexed and then allowed to settle for 15 minutes. Subsequently, 5 mL of the resulting suspension was added to a 250 mL Erlenmeyer flask containing 100 mL a minimal selective medium (Enrichment), followed by

incubation on a shaker at 37°C and 120 rpm for 15 days. Aseptically, 5 mL of culture was transferred to 100 mL of Enrichment media containing 0.5% PP and shaker for 15 days. The Enrichment procedure was repeated under the same conditions. 100 μL of culture was plated on 0.5% PP and incubated for 15 days at 37°C (observed daily for growth). Colonies were then plated in four quadrants on nutrient agar plates and incubated for 24 hours at 37°C [14], [16]

In vitro Biodegradation Studies

The study investigated the biodegradation of potential PP-degrading isolates. The experiment was carried out in six 250 mL Erlenmeyer flasks, each containing 100 mL of selective medium supplemented with 0.5% glucose. To each conical flask, 0.5% PP polymer granules and one ounce of bacterial isolate were added. The flasks were then incubated for 15 days. After 15 days of incubation, the PP granules were collected, and the bacterial layer attached to the granules was removed by washing them with distilled water and 70% ethanol solution. Subsequently, the residue was dried and weighed to determine the percentage of weight loss. The following formula is used to calculate the remaining film weight:

$$\text{Percentage weight loss} = \frac{\text{Initial granules weight} - \text{Final granules weight}}{\text{Initial granules weight}} \times 100$$

Phenotypically Identification of Potential Polypropylene Degrading Bacteria

Potential bacteria were identified phenotypically through the use of Gram staining. This process involves inoculating one loop of ose syringe of a single colony into 10 mL of nutrient broth media and incubating it on a shaker for 3 hours. After incubation, a glass slide was used to fix 1 ounce of the bacterial culture by heating it with a spirit lamp. The pure isolate cells on the slide were then stained by adding 1 drop of crystal violet and waiting for 1 minute before rinsing with sterile distilled water. The cells were stained with iodine, incubated for 2 minutes, and rinsed with sterile distilled water. They were then treated with 95% alcohol, left for 1 minute, and rinsed with sterile distilled water. Finally, the cells were stained with safranin solution, incubated for 30 seconds, and rinsed one last time with sterile

distilled water. Observation of the potential bacterial cell shape and color, resulting from Gram staining, was executed at 1000x magnification using microscope.

Identification of Potential Bacteria by 16S rRNA Sequence Analysis

The potential bacterial isolates that have been purified are genetically analyzed at PT Genetika Science. 16S rRNA analysis is performed to identify the isolated bacterial species and their relationship to other bacteria. The identification process is carried out by isolating bacterial DNA, DNA quantification using nanodrops, PCR using primers 27F 5'AGA GTT TGA TCM TGG CTC AG3', 1492R 5'TAC GGY TAC CTT GTT ACG ACT T3', sequencing, blasting and phylogenetic tree synthesis. Bacterial genomic DNA was extracted using Quick-DNA Bacterial Miniprep Kit (Zymo Research, D6006). Amplification was performed in two reactions using MyTaq Red Mix (Bioline, BIO-25048). The amplified DNA was checked for purity and quantity using a Nanodrop. Furthermore, the sequencing process was carried out. The sequencing results between the forward and reverse primers were carried out by Contig using Bioedit software to reconstruct a series of overlapping DNA segments, and then analyzed using the BLAST (Basic Local Alignment Search Tool) online program to compare the isolate sequences obtained with the comparison bacterial sequences obtained at NCBI GenBank. The selected sequences were then used to construct a phylogenetic tree to identify the closest relatives of the test isolates. Phylogenetic tree construction was performed using the software Molecular Evolutionary Genetics Analysis 11 (MEGA 11) to determine the closest kinship of the most promising indigenous bacteria. The phylogenetic tree was constructed using the Neighbor-Joining Tree and the Kimura-2 model parameters [17].

Result and Discussion

Isolation and Screening of Polypropylene Degrading Bacteria from Passive Cell Zone of Bestari Landfill, Probolinggo City by Enrichment Method

Landfill is a final waste disposal site with a

dynamic environment characterized by high nutrient levels. Due to the high nutrient content, the landfill has a diverse group of microbial communities, especially soil bacteria. In nature, bacteria function primarily as decomposers and are therefore abundant in such environments. Bacteria can serve as an effective agent for the degradation of plastics. *Brevibacillus sp.* and *Aneurinibacillus sp.* isolated from landfills and sewage treatment plants have been shown to degrade PP [16]. Another study also tested *Pseudomonas aeruginosa*, a bacterium isolated from landfills, which was also found to be capable of degrading PP [18]. In this research, indigenous PP-degrading bacteria have been isolated from the soil of the passive zone of the Bestari Landfill, Probolinggo City [9]. This is done with the understanding that the conditions in the soil are favorable for the growth and development of the bacteria. While certain bacteria are capable of using plastic as a carbon source, most bacteria prefer to use the simpler carbon sources found in soil. The site of the soil sample collecting point is surrounded by an old mound of plastic debris, some of which has begun to break down. The soil samples were subjected to physical and chemical characterization tests, which included measurements of pH, temperature, moisture, organic C content and N content. The results of the physicochemical characterization of soil samples from the passive zone of the Bestari landfill are presented in Table 1. These physicochemical results are important to understand the environmental conditions of the passive zone, to identify and isolate indigenous bacteria capable of degrading polypropylene, and to assess the potential for microbial activity under these specific environmental conditions.

Table 1. Chemical physics data of Bestari Landfill passive cell soil samples

Parameters	Bestari Landfill of Probolinggo City
Temperature	34°C
Soil moisture	50%
pH	6.9 – 7
C organic	2.85%
N total	0.15%

The temperature in the passive zone is 34°C and mesophilic bacteria are known to thrive in

these conditions. Mesophilic bacteria have an optimum growth temperature between 20-45°C, bacteria thrive in this environment as it allows them to carry out metabolic processes efficiently and replicate rapidly. The water content of the soil sample was 50%. Soil moisture is a key factor influencing microbial activity in the soil and serves as a critical medium for various biochemical reactions and the composition of soil communities. In general, soil bacteria require a relative humidity (RH) of 60% WHC [19]. Another environmental factor affecting bacterial survival is pH. The pH measurements of soil samples ranged from 6.9 to 7. Most bacteria growing at pH 6.9-7 are neutrophilic bacteria. In addition, analysis of organic C content showed a result of 2.85% and N content of 0.15%, giving a C:N ratio of 19%. Microbial growth requires 2 important elements, C as an energy source and N as a cellular component. The best C:N ratio for microbial growth is in the

range of 10-20%. The C:N ratio is a determinant of the stability and composition of the microbial community in a system, which has an impact on microbial growth [20].

Based on the analysis of the physicochemical characteristics of the Bestari Landfill soil, it appears that the Bestari Landfill in Probolinggo City is not conducive to the isolation of PP-degrading bacteria. Therefore, the Enrichment method will be used for bacterial culture. Bacterial colonies will be grown on solid minimal media containing PP granules. This is an initial screening to identify or select bacterial isolates for the degradation test. Colonies of PP degrading bacteria grown on Enrichment media can be seen in Figure 1. Bacteria can grow on media containing PP granules as a carbon source Figure 1. The bacteria began to grow around the PP granules within 15 days of incubating.

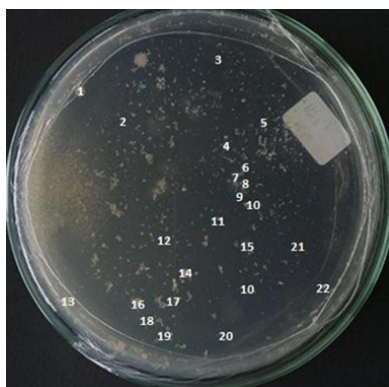


Figure 1. Colonies of polypropylene-degrading bacteria grown on selective medium

Isolates that grew on selective media were separated into two large groups labelled isolate B1UM and isolate B2UM which can be seen in Figure 2. This grouping was based on the morphological properties of the microbes which included colour, shape, edges, height, surface, and

size of the detailed colonies. Group B1UM has a yellowish white morphology, with a round shape, smooth edges, and flat elevation. While isolated, B2UM is white, round in shape, with smooth edges, and raised elevation.

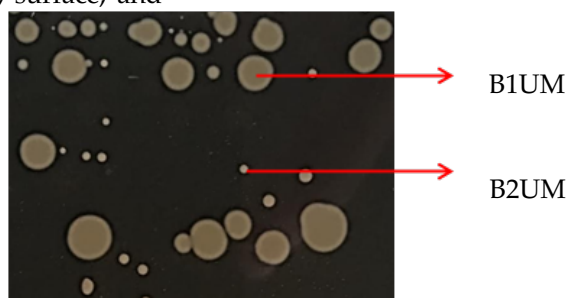


Figure 2. Polypropylene degrading bacteria B1UM and B2UM

A four-quadrant streak was performed on isolated strains B1UM and B2UM, which resulted in 6 bacteria colonies. The purpose was to obtain a pure single colony for further testing and identification. Based on the findings, it can be observed that the colonies separated to form a single colony, confirming the isolated purity. Thus, there were 6 bacterial strains successfully isolated and identified from the landfill passive zone soil samples. The isolates were designed as B1UM1, B1UM2, B1UM3, B2UM1, B2UM2, and B2UM3 (Figure 4) with their respective morphological characteristics shown Table 2. The bacterial isolates were subsequently tested for PP biodegradation.

Determination of Reduction Rate of Polypropylene by Bacterial Isolates

Biodegradation tests were conducted to assess the capability of indigenous bacterial isolates to break down PP polymer in a selective medium containing 0.5% polypropylene granules. The percentage weight reduction after 15 days of incubation determined the biodegradation level of PP. The weight loss of PP polymers observed during the incubation process with indigenous bacteria, indicates the presence of biodegradable activity. From a chemical perspective, this process can be explained through enzymatic interactions that affect the chemical structure of PP. PP is a saturated hydrocarbon with a chemical structure consisting of repeating propylene units $[-CH_2-CH(CH_3)-]_n$. This stability is supported by strong C-C covalent bonds and the absence of polar functional groups, making partial dissolution of PP surfaces by secondary metabolite compounds [21].

In this study, the characterization of compounds resulting from PP degradation has not been carried out, but chemically it can break down PP polymers through oxidation and enzymatic processes that have the potential to produce various low-weight monomer compounds. The initial process of PP degradation, is the breakdown of PP polymers into aliphatic hydrocarbons with a lower molecular weight first, so that it will reduce the size of the molecule into a range that can be further attacked by enzymes. Chemically, the degradation of PP begins with the oxidation of

PP hydrophobic and difficult to degrade. However, some microorganisms have the ability to initiate oxidative reactions against those PP polymer chains.

The result of percentage weight loss after 15 days and the degradation potential of isolates, as presented in Table 3. The PP degradation ability of the six bacterial isolates can be determined by performing gravimetric analysis on day 15, to calculate the dry weight of PP and the percentage of degradation. This analysis can identify bacterial isolates with the highest and lowest percentage of degradation. Based on the biodegradation test result, the percentage of PP degradation was obtained in six selected bacterial isolates. Bacterial isolates B2UM1, B1UM1, and B1UM2 showed high percentage degradation values of 8.25%, 7.15%, and 6.25%, respectively. In contrast, bacterial isolates B2UM2, B1UM3, and B2UM3 showed low percentage degradation values of 5.7%, 5.3%, and 4.35%, respectively. These results indicate that the weight loss of PP is caused by biological processes in the medium. The results of the biodegradation test showed that the treatment groups experienced a reduction in plastic weight, indicating that biodegradation of PP plastic granules had occurred. In addition, the six isolates showed the potential to use plastic as a carbon source for growth for 15 days. These results were supported by a control group in which there was no weight loss of PP plastics, which could confirm two main factors causing PP degradation. The first is the metabolic of microbes, through oxidation and hydrolysis, and the second is the potential for hydrocarbon chains by monooxygenase enzymes to form primary or secondary alcohols. Monooxygenase catalyzes oxidation at the terminal or subterminal of the hydrocarbon chain of the polymer. Terminal oxidation monooxygenase converts the aliphatic chain into a primary alcohol, while in subterminal oxidation it becomes a secondary alcohol. The alcohol that is formed is subsequently converted into aldehydes or ketones by alcohol dehydrogenase enzymes. The formation of these aldehydes or ketones introduces a more reactive carbonyl group thereby increasing the solubility and accessibility of the substrate for enzymes. Ketones are further converted into

esters and further broken down by esterases, keratinases and lipases into fatty acids and then enter the metabolic system (β -oxidation) and citric acid cycle (TCA) as a source of energy for

microorganisms. [8]. Chemical reactions gradually break down the carbon bonds in PP and converts them into simple, soluble compounds, seen below in Figure 3.

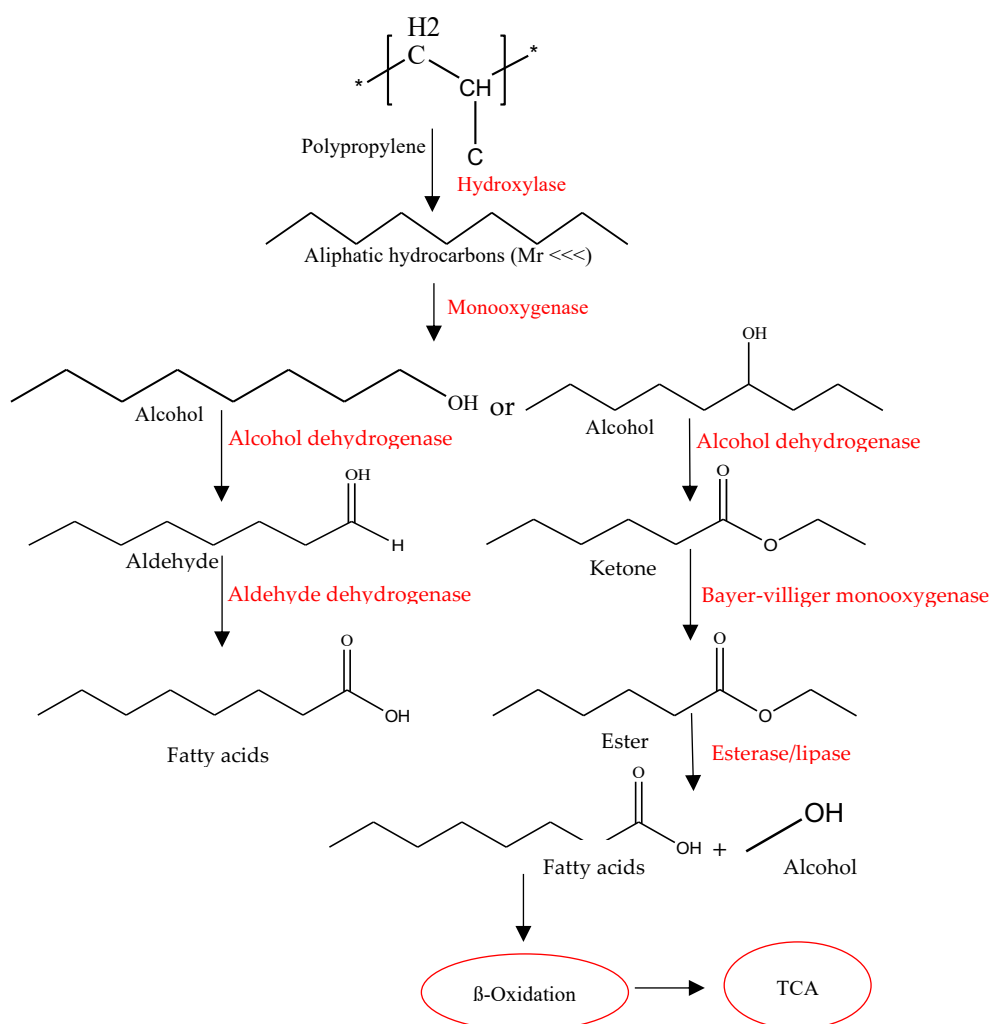


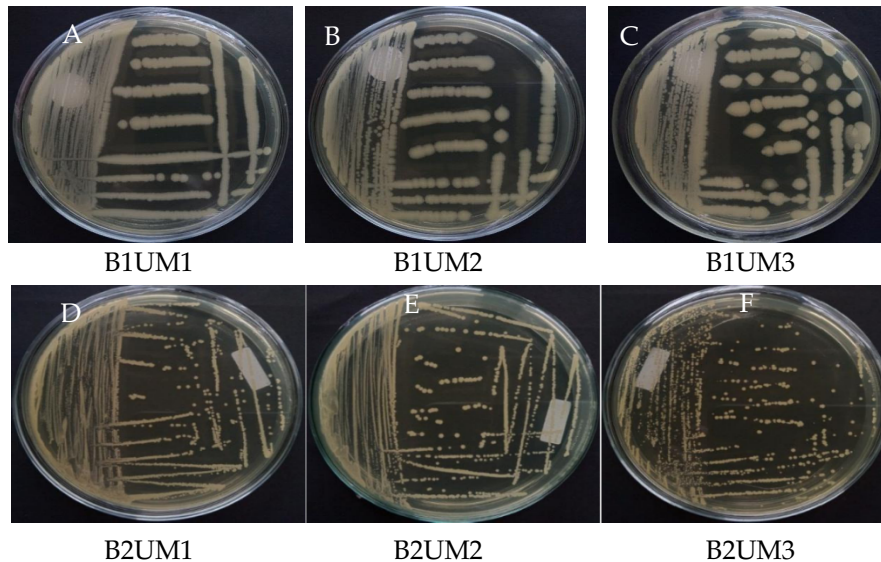
Figure 3. Chemical Reaction of Polypropylene (PP) Degradation by Bacteria

The reaction illustrates how the inert structure of PP can undergo conversion into smaller, easily metabolized chemical compounds. PP polymers are broken down into smaller fragments and partially converted into gases or water-soluble compounds, which are chemically excreted out of the system as a result of microbial metabolism. The weight loss also reflects the success rate of microbes in utilizing

plastic fragments as an alternative source of carbon. The percentage of weight loss also shows the enzymatic potential of this indigenous bacterium in starting the chemical degradation process of PP. Although characterization of degraded compounds has not been performed, significant weight loss may be an early indicator of chemical changes in the macromolecular structure of PP

Table 2. Morphological Characteristics of Polypropylene Degrading Bacterial Isolates

Character	B1UM1	B1UM2	B1UM3	B2UM1	B2UM2	B2UM3
Color	Yellow	White	White	Yellow	White	White
Size	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
Shape	Round	Round	Round	Round	Round	Concentric
Margin	Serrate	Serrate	Entire	Entire	Entire	Serrate
Elevation	Flat	Flat	Flat	Arise	Arise	Arise
Edge	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth

**Figure 4.** Streak results of polypropylene degrading bacterial isolates: A) isolate B1UM1, (B) isolate B1UM2, (C) isolate B1UM3, (D) isolate B2UM1, (E) isolate B2UM2, (F) isolate B2UM3**Table 3.** Percentase weight loss PP granules after 15 days of incubation with bacterial isolates

Bacterial isolates	% Reduction		$\bar{x} \pm SD$
	Repition 1	Repition 2	
B1UM1	7.44	7.42	7.15 $\pm$ 0.01
B1UM2	6.48	6.39	6.35 $\pm$ 0.04
B1UM3	5.45	5.34	5.39 $\pm$ 0.05
B2UM1	8.33	8.22	8.28 $\pm$ 0.05
B2UM2	5.99	5.51	5.75 $\pm$ 0.24
B2UM3	4.65	4.61	4.64 $\pm$ 0.01
Control	0	0	0

Analysis based on Table 3 data, showed that strains B1UM1, B1UM2, and B2UM1 showed higher potential compared to strains B1UM3, B2UM2, and B2UM3. The results of this study show a greater potential in comparison to the previous research by (Helen et al. 2017) on PP plastic degradation by indigenous bacteria in Mangrove Peninsular Malaysia. Two PP plastic degrading bacteria isolated from mangrove sediment were *B. cereus* and *S.*

globispora. *B. cereus* was able to degrade PP granules up to 12%, and *S. globispora* degraded 11% PP granules during 40 days of incubation [13]. Another study conducted by Trikurniadewi et al. (2022) successfully screened indigenous PP degrading bacteria isolated from Landfill, Ngipik Gresik with strain NG2 identified as *Bacillus* able to degrade PP by 0.47% for 28 days [22].

Another comparison, from the study of

Arkatkar et al. (2010) which evaluated the degradation of PP plastic in vitro using *Bacillus flexus* obtained from landfills, showed a weight reduction of 2.5% over 365 days. It can be concluded that strains B1UM1, B1UM2, and B2UM1 from this study have potential as biological agents for PP degradation. The bacteria exhibit a gradual activation process, which makes them increasingly adept at utilizing carbon sources. As a result of acclimatization, there was a substantial reduction in the weight of the plastic material during the experiment. This shows that, B1UM1, B1UM2, B2UM1 if given sufficient time and supportive conditions, have the potential to contribute effectively to the degradation of PP plastics. The superior B1UM1, B1UM2, B2UM1 bacteria were further selected for morphological identification and genotyping using Gram staining and 16S rRNA analysis, following an objective and precise method.

Phenotypically Identification Results of Potential Polypropylene Degrading Bacteria

Phenotypic identification of isolates is conducted through the Gram staining produced by potential bacteria. Observations were made using a light microscope with a 1000x magnification showing bacterial morphology. This method utilizes the ability of the bacterial cell wall to retain its crystalline purple color after being treated with solvent, Figure 5 presents the Gram staining results of the potential bacterial isolates, which show that strain B1UM1 has a coccus shape, while bacterial strains B1UM2 and B2UM1 have a basil shape, as seen in Figure 5. The three potential strains of bacteria identified are all Gram-positive. Gram-positive bacteria are bacteria that can retain the purple color of methyl purple (crystal violet) during the Gram staining process. Under a microscope, these bacteria appear purple due to their thick, peptidoglycan-rich cell walls. The purple colour results from strong binding to methyl purple, the main dye used in the Gram staining technique. This binding is a product of the compound's affinity for the cell wall and cytoplasm, allowing a strong interaction with the methyl purple-iodine complex that is resistant to colour fading.

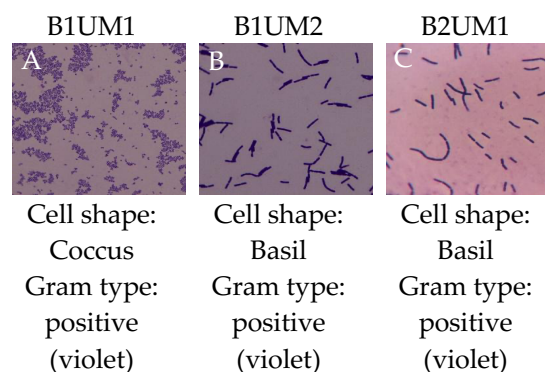


Figure 5. Gram Stain Test Results: A) isolate B1UM1, (B) isolate B1UM2, (C) isolate B2UM1

Results of identification of potential bacteria by genotyping with 16S rRNA sequence analysis

Bacteria capable of PP degradation, specifically strains B1UM1, B1UM2, and B2UM1, were identified through 16S rRNA sequence analysis. Genotyping analysis was conducted by PT Genetika Science. The sequencing results of each isolate in the form of forward primer and reverse primer sequences were obtained in FASTA format, which was then carried out Contig. Bioedit software was used to run the sequencing data sequentially using Contig to avoid DNA segment breaks and promote the development of unique genes. The final results of Contig were evaluated using BLAST software, which identifies and compares nucleotide patterns among species in the NCBI database that have relationships with B1UM1, B1UM2 and B2UM1. After identifying 10 consecutive nucleotide species, the maximum identity was approximately 99-100%. The bacterial species identified using the BLAST algorithm in the NCBI database are listed in Table 4. BLAST analysis compared the DNA sequences of all isolated bacteria with those in the NCBI database to determine their similarity. The result showed that isolate B1UM1 has 100% similarity with *Staphylococcus haemolyticus*, isolate B1UM2 has 100% similarity with *Bacillus cereus*, and isolate B2UM1 has 99 % similarity with *Bacillus sp.* According to Montazer et al. (2018), similarity of more than 97% in the base sequence of the 16S rRNA coding gene indicates that the organisms are not new species and belong to the same species [23].

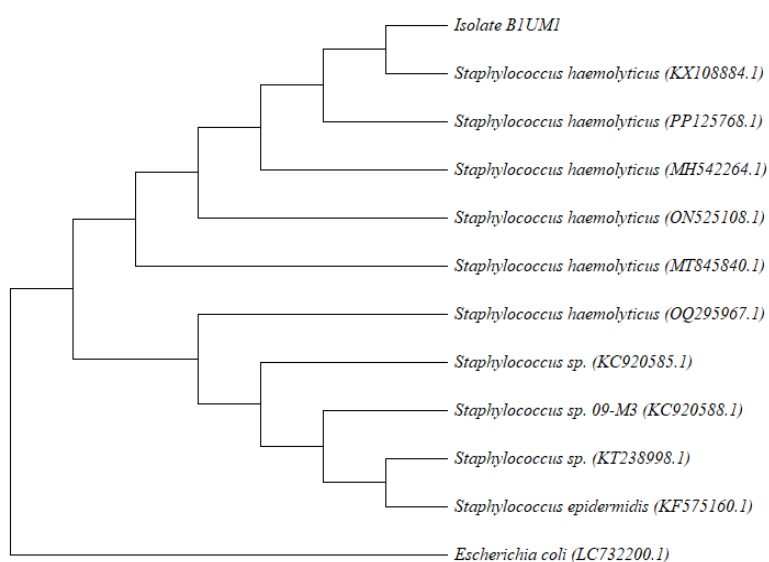
Table 4. Names of Bacteria and Percentage of Gene Similarity

Isolate	Name of Bacteria	%Similarity
B1UM1	<i>Staphylococcus haemolyticus</i>	100,0%
B1UM2	<i>Bacillus cereus</i>	100%
B2UM1	<i>Bacillus sp</i>	99%

MEGA 11 software was used for phylogenetic analysis, based on contig alignment and BLAST results. This aims to understand the relationships and evolutionary history of a group of organisms that require phylogenetic analysis. To serve as an outgroup, *E. coli* sequences were used. Outgroup species show close kinship to the species under study, but not as close as the target species to each other. Outgroup species need to be used to understand how features evolve along the lineage. The length of tree branches can be used to estimate the duration of evolution. Sequences occupying adjacent branches are assumed to be the most closely related due to their nucleotide sequence similarity and evolutionary distance. The 16S rRNA gene alignment analysis resulted in a phylogenetic profile that matched the percentage similarity found in BLAST Figure 6.

The phylogenetic tree for bacterial isolate

B1UM1 shows a close relationship with *Staphylococcus haemolyticus*, while indicating a more distant relationship with the outgroup species. Bacteria strain B1UM2 showed closeness to the species *Bacillus cereus*. Isolate strain B2UM1 was closely related to *Bacillus sp.* (in *Firmicutes*). Based on the results of the phylogenetic tree kinship analysis, it can be seen that the B2UM1 indigenous bacteria are closely related to *Bacillus sp* (which is included in the *Firmicutes* phylum). *Firmicutes* belong to the phylum of gram-positive bacteria. The results were reinforced by several previous studies that have also reported the ability of *Bacillus sp.* and *Staphylococcus sp.* bacteria isolated from soil, to degrade various types of plastics [24], [25]. *Bacillus sp.* bacteria are more commonly found to degrade plastics compared to *Staphylococcus sp.*, the superior ability of *Bacillus sp.* can be analyzed due to its enzymatic and spore-forming abilities. *B. cereus* bacteria were successfully isolated from Mangrove ecosystems in Peninsular Malaysia and has the ability to degrade PP reaching 12% after 40 days of incubation [13]. Thus, the results of this study reinforce that the microbial community in Bestari landfill does have the potential to be used in microbial-based plastic bioremediation strategies.



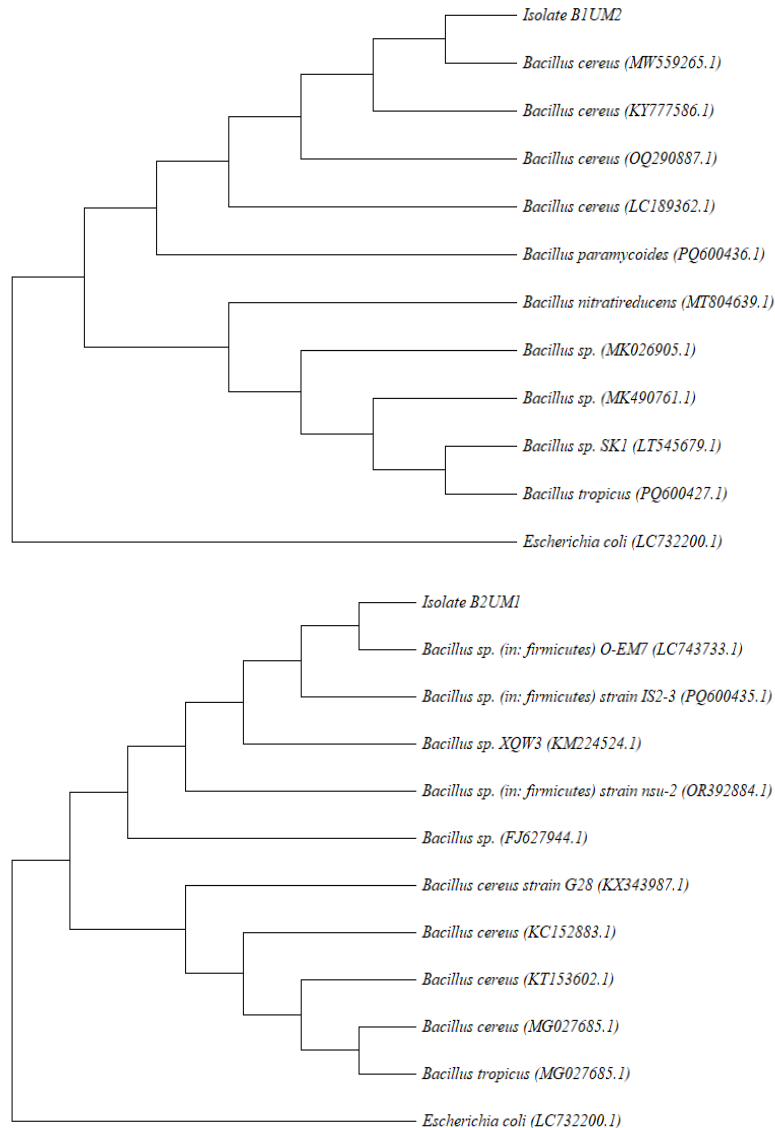


Figure 6. Phylogenetic tree of indigenous bacterial species: B1UM1 (top), B1UM2 (middle), B2UM1 (bottom)

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Conclusions

Six strains of soil bacteria B1UM1, B1UM2, B1UM3, B2UM1, B2UM2, and B2UM3 were successfully isolated and identified from passive cells of Bestari landfill, Probolinggo City for this study. Three of these isolates (B1UM1, B1UM2, and B2UM1) had the potential ability to degrade PP and could grow on media

containing PP granules as the sole carbon source. Through 16S rRNA sequence analysis, B1UM1 has 100% similarity percentage with *Staphylococcus haemolyticus*, B1UM2 has 100% similarity percentage with *Bacillus cereus*, while B2UM1 has 99% similarity with *Bacillus sp.* Future research is expected to optimize degradation conditions and explore genetic mechanisms to improve the efficiency and specificity of polypropylene degradation. These findings provide important opportunities for bioremediation applications in sustainably managing plastic waste.

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