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Exploration of Plastic Degrading Bacteria in the Musi River, Palembang City

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Abstract

Musi River is a waterway in South Sumatra that faces increasing plastic pollution, threatening aquatic ecosystems and human life. This study aims to identify and evaluate plastic-degrading bacteria from Musi River using phenotypic and molecular approaches. The methods of this study include bacterial isolation, plastic degradation test, and Scanning Electron Microscopy (SEM) analysis were carried out to assess degradation efficiency and changes in surface morphology, phenotypic and molecular identification. The results showed that isolates S1I3, S1I5, and S2I1 had high plastic degradation potential, with levels of 38.03%, 34.73%, and 30.46%, respectively. SEM observations showed changes in surface morphology, including pores and cracks. Molecular identification confirmed that S1I3 was Bacillus proteolyticus (99.87%), while S1I5 and S2I1 matched Bacillus cereus (100%).

Keywords*: Bacillus; Bacteria; Biodegradation; Musi River; Plastic Pollution.*

Introduction

Plastic is the most widely used material for food packaging, with polyethylene (PE) being one of the most common types. According to data from Plastic Europe, global plastic production reached more than 368 million tons in 2019, with PE being one of the dominant types used in various industries. The widespread use of PE contributes to the increasing accumulation of plastic waste in the environment, primarily due to its resistance to natural degradation. This results in plastic residues persisting in ecosystems and posing serious threats to aquatic life and overall environmental quality [1].

PE degradation occurs through two main mechanisms: abiotic and biotic degradation. Abiotic degradation is triggered by environmental factors such as temperature and ultraviolet radiation, while biotic degradation involves microbial activity that alters the properties of the plastic [2]. Based on their polymer composition, plastics are categorized into several types,

including PE (polyethylene), PP (polypropylene), PVC (polyvinyl chloride), PS (polystyrene), PET (polyethylene terephthalate), and PA (polyamide) [3].

Poorly managed plastics can pollute the environment and increase the number of microplastics entering the food chain of humans and animals, posing long-term health risks [4]. PE is widely used in plastic production due to its resistance to acids, water, alkalis, and most organic solvents. PE is produced at high temperatures and pressures, depending on the desired characteristics of the final product [5]. However, plastic pollution has severe impacts on aquatic ecosystems. At the population level, plastic presence can reduce species numbers and biomass, while at the individual level, it can affect survival, reproduction, growth, and overall organism health. Plastic exposure also contributes to physiological disturbances, such as increased oxygen consumption and oxidative damage to tissues [6]. The direct impact of plastic waste in waters can be seen from the many cases of death of marine ecosystems due to ingestion of plastic waste. Marine organisms that accidentally consume plastic face serious risks, such as blockage of the digestive tract, organ complications, and death. This condition not only threatens the sustainability of marine species populations, but also disrupts the balance of the aquatic ecosystem as a whole. In addition to having an impact on the ecosystem, the use of plastic that does not meet safety standards also risks causing health problems in humans. Plastic contains hazardous compounds that can be carcinogenic, potentially trigger cancer, and damage body tissue. Plastic is also difficult to degrade naturally by microorganisms, increasing the potential for accumulation of toxic substances in the environment. Several studies have linked low-dose exposure to Bisphenol-A (BPA) with a variety of health effects, such as increased prostate gland size, decreased testosterone levels, increased risk of breast and prostate cancer, and a tendency toward hyperactivity [7].

Degradation of polyethylene (PE) by bacteria occurs in four stages. Biodeterioration involves the formation of carbonyl groups due to oxidative enzymes or external factors such as sunlight. Biofragmentation breaks down polymer chains through hydrolysis or oxidation, producing monomers or oligomers. Bioassimilation allows microorganisms to absorb small fragments as a source of energy and carbon. Mineralization converts degradation products into microbial biomass and releases carbon dioxide and water into the environment [8].

Biodegradation is influenced by several factors including the chemical structure of the polymer, molecular weight, and solubility. In addition, the presence of hydrolyzed and oxidized compounds also has a significant effect. Other factors that also affect the biodegradation process are hydrophilicity between the microorganism and the surface of plastic film, polymer bonds, as well as the level of roughness of plastic surface [9].

One of the most effective methods for observing bacterial morphological changes in detail

is Scanning Electron Microscopy (SEM). The advantages of this visualization technique include its ability to display high resolution surface structures, provide a large depth of field, and generate three-dimensional images. This allows for the observation of changes such as the formation of holes, cracks, or surface degradation, which indicate bacterial degradation activity. According to research conducted in [10] observations using Scanning Electron Microscopy (SEM) revealed significant changes on the surface of low density polyethylene (LDPE) plastic after treatment with marine bacteria. SEM showed that the plastic surface became rough and perforated, indicating the occurrence of biodegradation due to bacterial enzymatic activity. Additionally, SEM demonstrated morphological changes in certain areas of the polyethylene plastic, resulting from interactions between the bacteria and the plastic.

To address the issue of plastic pollution, the isolation of plastic-degrading bacteria, supported by phenotypic characterization and molecular identification, is a crucial step. Phenotypic characterization provides an initial overview of the bacteria's physical and biochemical properties. However, for more accurate identification, especially in distinguishing morphologically similar species, molecular identification through genetic analysis, such as 16S rRNA gene sequencing, is necessary. According to research conducted in [11], isolating polyethylene plastic degrading bacteria from plastic contaminated soil was conducted. Subsequently, phenotypic characterization and molecular identification were performed using 16S rRNA gene sequencing. The results showed that the isolated bacteria belonged to the genus *Bacillus*, which is known for its ability to degrade plastic. This highlights the importance of phenotypic characterization and molecular identification in determining bacterial species with potential for plastic biodegradation.

Research on plastic degrading bacteria in the Musi River is currently still very limited. Bacteria found in the waters of the Musi River, Palembang City, are suspected of having the potential as plastic degrading agents, which can be characterized phenotypically and molecularly to obtain more accurate and in-depth identification.

Materials and Methods

1. Materials

The materials used in the study were cotton, brown paper, rubber bands, tissue, labels, aluminum foil, distilled water, 70% alcohol, spirits, Nutrient Agar, Tryptic Soy Broth, Mineral Salt Medium (MSM), NaCl, crystal violet, iodine solution, 96% alcohol, safranin, Congo red dye, 30% hydrogen peroxide (H_2O_2) solution, peptone, permanent marker, and polyethylene plastic. The quantities of materials were adjusted according to the needs during the study.

2. Sampling

The sampling locations were determined through a purposive sampling method, carefully considering the unique conditions of the research area in relation to specific objectives [12]. Samples were taken from three strategically chosen stations, selected for their high population activity and proximity to markets—factors that significantly contribute to the volume of plastic waste in the water. The selection criteria included the density of human activity, the amount of plastic waste produced, ecological considerations, and nearby sources of pollution. To enhance the representativeness of the data, samples were collected from five different points at each station, reflecting the overall research population, and then composited. The sampling was conducted at a depth of 0. 5 meters from the surface of the Musi River waters.

3. Isolation and Purification of Bacteria

The process of isolating and purifying degrading bacteria was conducted using Nutrient Agar (NA) media. Bacteria were isolated via the spread plate method, where 1 ml of river water sample was combined with 9 ml of 5% NaCl, achieving a dilution ranging from 10^{-1} to 10^{-6}. The petri dishes were then incubated at 37°C for 24 to 48 hours, allowing for the observation of bacterial growth [13].

Subsequent to this, various colonies were purified on the same media until a single, distinct colony was achieved. The purification process utilized the streak plate method, which involved inoculating the NA media with one loop of the bacterial isolate. This was followed by another incubation at 37°C for 24 hours. This procedure was repeated until a pure culture was attained, distinguished by variations in color and colony morphology [14].

4. Plastic Degradation Test

A plastic biodegradation test was carried out to evaluate the percentage reduction of polyethylene (PE) plastic by degrading bacteria. Square samples measuring 1 cm × 1 cm with an initial weight of 0.0068 g were rinsed using sterile distilled water followed by 70% ethanol, then aseptically transferred into glass bottles containing 50 ml of MSM (Mineral Salt Medium). The bacterial suspension was prepared according to McFarland standard 0.5 (1.5×10^{8} CFU/ml), using bacterial cultures grown on NA medium incubated for 24 hours. These bacteria were suspended in physiological saline (NaCl), and 5 ml of the suspension was added to each sample bottle. The samples were incubated at 30°C on a shaker set to 150 rpm for a period of 60 days [15]. A negative control was included by treating the plastic in MSM medium without adding any bacteria, to ensure that any observed degradation was not due to environmental factors alone. As a positive control, known plastic-degrading bacteria were

used to benchmark the effectiveness of the tested isolates.

After the incubation period, the plastic samples were filtered, washed, dried, and reweighed to obtain the final weight The degradation percentage was then determined using the following formula:

$$\% \text{ degradation} = [(Wi - Wf) / Wi] \times 100\% \quad (1)$$

Description:

Wi: Initial dry weight (grams)

Wf: Final dry weight (grams)

5. Characterization and Identification

The bacteria slated for characterization and identification are those that demonstrate a high rate of degradation. Initially, macroscopic characterization involves observing colony morphology on various forms of nutrient agar media—namely upright, slanted, and plate forms. Following this, microscopic characterization is conducted through gram staining and endospore staining techniques. To further refine the identification of the genus and species, a series of biochemical tests are performed. These include the catalase test, motility test, oxidative/fermentative test, indole test, methyl red test, Voges-Proskauer (VP) test, and the Triple Sugar Iron Agar (TSIA) test, which together assess the fermentation capabilities of glucose, lactose, and sucrose, as well as gas production and hydrogen sulfide (H_2S) formation [16].

The identification process for the genus and species of bacteria relies on a combination of macroscopic, microscopic, and biochemical test results, referencing established sources such as Bergey's Manual of Systematic Bacteriology or other relevant databases.

The molecular identification phase begins with the isolation of bacterial DNA, following the specific procedures dictated by the bacterial DNA extraction kit. Samples are taken from bacteria cultured in suitable growth media for DNA extraction. The bacterial cells are then lysed using either enzymatic methods (such as lysozyme or proteinase K) or mechanical methods (such as sonication or bead-beating). Once the cell walls are disrupted, the resulting solution undergoes purification, using either a phenol-chloroform mixture or a commercial DNA extraction kit that utilizes silica-based membranes. After purification, the quality and quantity of the extracted DNA are assessed using a spectrophotometer (like NanoDrop) or agarose gel electrophoresis to confirm its integrity and purity. With pure DNA ready, the next step is the amplification of target gene fragments using the Polymerase Chain Reaction (PCR) technique. This process employs specific primers, like the universal 16S rRNA primer, to

facilitate bacterial identification. The PCR process consists of DNA denaturation, primer binding, and DNA extension by the Taq polymerase enzyme, conducted through repeated thermal cycles to amplify the desired sequence. The resulting PCR products are subsequently purified before being forwarded for sequencing and bioinformatics analysis. This sequencing data is then compared to existing bacterial DNA sequences in GenBank via the Basic Local Alignment Search Tool (BLAST) accessible at http://www. ncbi. nlm. nih. gov. The relationships among the bacteria are visually represented through images generated using MEGA 7 software [17].

6. Visualization with SEM

The morphology of degraded plastic particles was examined using Scanning Electron Microscopy (SEM) after a 30-day incubation period with bacterial isolates. Following incubation, the samples were carefully removed from the culture media and washed with 70% ethanol and distilled water to eliminate adhering cells without compromising the integrity of the plastic surface. Subsequently, the samples were forwarded to the Central Laboratory of the Engineering Faculty at Sriwijaya University for SEM visualization [18],[19]. The SEM analysis aimed to investigate the morphology of the degraded plastics, with a particular focus on identifying holes and cracks. Prior to examination, the samples were dried and coated with a thin layer of conductive metal, either gold or platinum, through sputter-coating. Observations were conducted at acceleration voltages ranging from 5 to 15 kV. A lower voltage of 5 kV was employed to minimize excess electron penetration, while a higher voltage of 15 kV was used to enhance resolution. The magnifications selected for the study included 50x, 1000x, and 1500x. The 50x magnification offered a general overview of the degradation, while the 1000x magnification revealed more nuanced texture changes. The highest magnification of 1500x provided detailed visualization of small holes and cracks. To improve topographic contrast, secondary electron (SE) mode was utilized, and high vacuum conditions were maintained to prevent any distortion in the images [20].

Results and Discussions

According to the data presented in Table 1, the results of bacterial isolation from the waters of the Musi River in Palembang City reveal a noteworthy variation in the number of bacterial isolates across different sampling stations. Specifically, Station 1 generated 11 isolates, Station 3 yielded 10, while Station 2 had the highest count, totaling 12 isolates. This disparity in isolates across the stations highlights the diversity of bacteria present in the environment, suggesting that these microorganisms can adapt to the unique conditions found in each location.

Supporting this observation, research cited in[21], ndicates that environmental factors, including nutrient availability and physical conditions, significantly affect bacterial growth. Key nutrients such as carbon, hydrogen, oxygen, and nitrogen, coupled with physical factors like temperature, are crucial for bacterial proliferation and overall physiology. During the sampling period, the water temperature was approximately 30°C, a condition conducive to the survival of mesophilic bacteria, which thrive at moderate temperatures. This environmental context enhances the effectiveness of bacterial isolation methods tailored to the local conditions. As noted in the study referenced in [22], mesophilic bacteria are commonly found in various environments, including soil, water, and within vertebrates and humans. To facilitate their metabolic activities, a suitable growth medium is essential for the degradation processes carried out by these bacteria. Typically, mesophilic bacteria achieve optimal reproduction and activity within a temperature range of 30-37°C, further underscoring the importance of environmental factors in their life cycles.

Table 1. Results of bacterial isolation from the Musi River, Palembang City

Sampel Point	Isolate Code	Number of Isolates
Station 1	S1I1, S1I2, S1I3, S1I4, S1I5, S1I6, S1I7, S1I8, S1I9, S1I10, S1I11	11
Station 2	S2I1, S2I2, S2I3, S2I4, S2I5, S2I6, S2I7, S2I8, S2I9, S2I10, S2I11, S2I12	12
Station 3	S3I1, S3I2, S3I3, S3I4, S3I5, S3I6, S3I7, S3I8, S3I9. S3I10	10
Total		33

At the time of sampling, the water's pH level was recorded at 7, which indicates a neutral condition—an ideal environment for the growth of various bacteria. Research outlined in [23], indicates that residential activities, particularly the disposal of household waste, can significantly influence pH levels. Additionally, industrial wastewater discharge into the river further contributes to these pH fluctuations. Changes in pH and temperature not only affect bacterial growth but also influence the efficiency of degradation processes. Specifically, temperature plays a crucial role in the activity of enzymes responsible for biodegradation, as each enzyme operates optimally within a specific temperature range. Low temperatures can slow enzyme activity, hindering the degradation rate, while excessive heat may lead to enzyme denaturation, impairing the bacteria’s ability to break down compounds effectively.

Furthermore, pH is a critical factor in the degradation process because it influences enzyme stability and the permeability of bacterial cell membranes. An excessively acidic or basic pH

can hinder enzyme activity and disrupt the transport of essential nutrients required for effective degradation. Mesophilic bacteria tend to thrive optimally in a neutral to slightly alkaline pH (ranging from 6. 5 to 8). Therefore, pollution-induced pH changes can adversely affect degradation efficiency.

Examining the results of the plastic degradation tests presented in Table 2 over a 30-day period, the bacterial isolates demonstrated degradation capabilities that ranged from 19. 02% to 36. 19%. This indicates that, during the initial phase, the bacterial isolates began to exhibit plastic degradation activity. After 60 days, this percentage increased to between 20. 90% and 38. 03%. These findings suggest that the bacterial isolates require an extended duration to enhance the effectiveness of the enzymes involved in the plastic degradation process. Research cited in [24], highlights the variability in degradation percentages and incubation times for polyethylene plastic when exposed to different bacterial strains. For instance, one study revealed that a bacterial isolate from the genus Pseudomonas achieved a weight loss of 3. 87% after 40 days of incubation. Another investigation utilizing the Winogradsky column method, which included Lactobacillus bulgaricus and Streptococcus thermophilus, recorded a maximum degradation percentage of 19. 47% after just 20 days of incubation. The discrepancies in degradation rates are likely attributed to differences in bacterial species, incubation conditions, and the types of plastic being studied.

Table 2. Percentage Results of Degradation Test

Isolate Code	Initial Weight (Wi)	Final Weight (Wf) 30 Days	Final Weight (Wf) 60 Days	30 Day Degradation Percentage	60 Day Degradation Percentage
Control	0,0165	0,0165	0,0165	0,000	0,000
S1I2	0,0171	0,0132	0,0130	22,80%	23,97%
S1I3	0,0174	0,0125	0,0121	28,16%	30,46%
S1I5	0,0167	0,0112	0,0109	32,39%	34,73%
S2I1	0,0163	0,0104	0,0101	36,19%	38,03%
S3I1	0,0143	0,0111	0,0109	22,38%	23,80%
S3I5	0,0163	0,0132	0,0129	19,02%	20,90%

The S2I1 isolate exhibited the highest degradation capability, achieving a remarkable rate of 38. 03%. This suggests that the bacterial strain produces enzymes with enhanced activity, effectively breaking down the polymer bonds in plastics. As noted in [25], the process of plastic degradation by bacteria involves enzymatic activity that dismantles polymer chains into smaller

oligomers and monomers, which are then metabolized by the bacterial cells. Moreover, the research presented in [26], several enzymes involved in plastic degradation include extracellular enzymes such as hydrolases, oxidases, and dehydrogenases, which break the plastic polymer bonds into simpler monomers or oligomers. These monomers are then metabolized by the bacterial cells via catabolic pathways to produce energy, biomass, and final products such as CO_2 and H_2O, a process known as mineralization.

Figure 1 illustrates the morphological changes observed in plastic samples after 30 and 60 days of exposure to bacterial activity from the Musi River in Palembang City. This degradation is characterized by alterations in shape and a reduction in dimensions, including length, width, and weight. According to the findings in [27], biodegradation is the process through which complex compounds are broken down into simpler substances, such as water and carbon dioxide, through the action of bacteria. Each bacterial species possesses unique characteristics, leading to differing rates of degradation among them. Generally, degradation occurs when these compounds are utilized by bacteria as a nutrient source for growth.

Figure 1. Results of degraded plastic samples.

Figure 1 illustrates the percentage of plastic degradation achieved by six bacterial isolates over periods of 30 and 60 days, alongside a control group for comparison. The control group demonstrated minimal degradation, underscoring the pivotal role of bacteria in this process. Among the isolates, S15 and S21 exhibited the highest levels of degradation after 30 days, with

S21 surpassing 35% degradation by the 60-day mark. These findings suggest that extended incubation time enhances biodegradation efficacy, aligning with previous research [28], that revealed bacteria from specific environments are particularly effective at breaking down polyethylene.

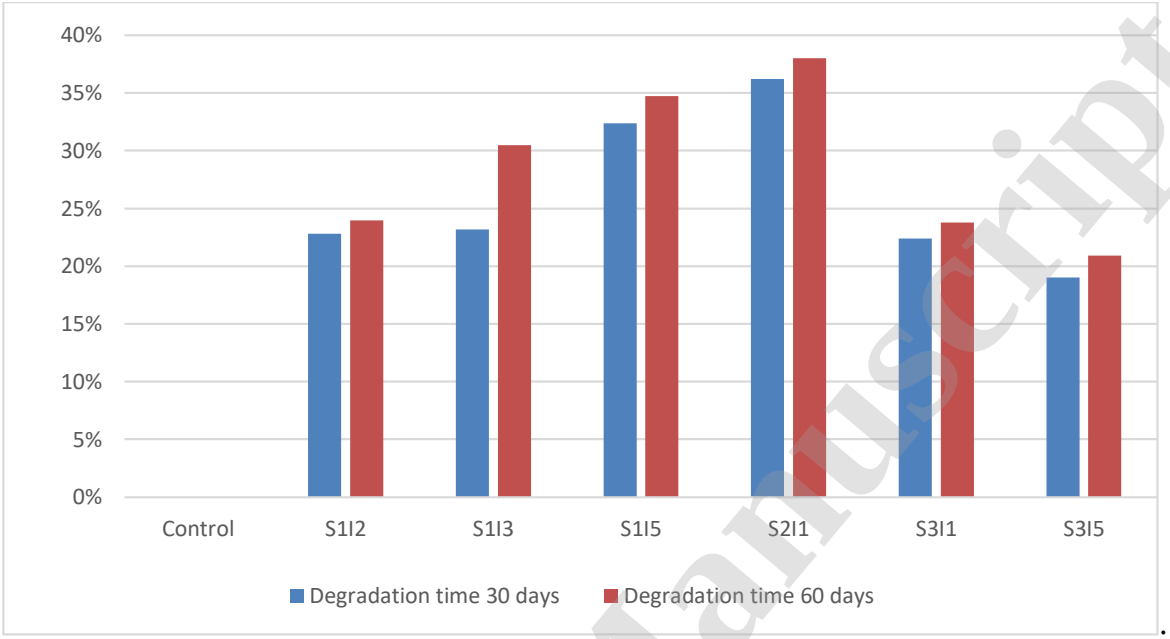

Figure 2. Plastic Degradation Percentage Chart.

Bacterial colonization on the surface of polyethylene (PE) plastic leads to significant alterations in its mechanical properties, including increased surface roughness, heightened brittleness, and a reduction in molecular weight. According to [29], the chemical and physical structure of plastics plays a crucial role in their biodegradation. From a chemical perspective, the elasticity and susceptibility of plastics to degradation are largely influenced by the type of monomer used in their composition. Polymers that contain ester or amide bonds, such as polyesters (like polycaprolactone and polylactic acid), tend to degrade more readily because these bonds are susceptible to hydrolysis. In contrast, polymers with strong covalent bonds, such as polyethylene (PE) and polypropylene (PP), require substantial energy to break these bonds. The presence of polar groups, such as carbonyl and hydroxyl, accelerates biodegradation by enhancing the affinity for water and enzymes. Conversely, the hydrophobic structures observed in polyolefins can impede this process. Physically, the degree of crystallinity is a vital factor in biodegradation; plastics with lower crystallinity (amorphous) are more readily degraded, as the molecules in these regions are more accessible to enzymes and microorganisms. Higher molecular weight contributes to a greater resistance to degradation, while plastics with high porosity enable improved penetration of water and enzymes, thereby

facilitating the biodegradation process.

The enzymatic biodegradation of plastics is contingent upon both the type of polymer and the specific enzymes employed by microorganisms. This process unfolds in three primary stages: (1) Oxidative Initiation – where the plastic is oxidized by enzymes such as laccase and peroxidase, forming carbonyl and hydroxyl groups that promote degradation, particularly in hydrophobic polymers like PE; (2) Depolymerization – during which the polymer is disassembled into oligomers and monomers by enzymes like esterases, cutinases, and proteases. While polyolefins (PE and PP) present greater challenges to degradation due to their robust C-C bonds, oxidative enzymes such as alkane monooxygenases facilitate the initial breakdown; (3) Mineralization – in which the monomers resulting from depolymerization are converted into CO_2, H_2O, and biomass by enzymes such as dehydrogenases and dioxygenases. This mineralization process varies depending on whether the environment is aerobic or anaerobic. The rate of degradation ultimately hinges on the compatibility of the enzymes with the chemical structure of the plastic; polymers with reactive groups like esters and amides degrade more easily than polyolefins, which require initial oxidation [30].

Figure 3 illustrates the morphology of plastic samples as observed through a scanning electron microscope (SEM). The findings reveal that the control plastic maintained a smooth surface, showing no signs of damage, while the plastic subjected to bacterial treatment exhibited varying degrees of degradation. Specifically, Image S1I3 displays small holes, S1I5 presents more numerous holes along with structural changes, and S2I1 features prominent cracks, indicating the most significant level of degradation. This disparity in effectiveness can be attributed to the type of bacteria involved and the specific degradation enzymes they produce [31]. The SEM analysis highlights notable degradation after a 60-day period, with a weight reduction of polyethylene by as much as 20%. This underscores the potential of Bacillus cereus in managing plastic waste. Research conducted in [32], emphasizes that SEM offers a detailed visualization of plastic degradation, showcasing a transition from a smooth to a rough surface due to microbial activity. Numerous studies have identified Bacillus species as promising agents for plastic biodegradation. Their effectiveness is linked to their unique physiological and metabolic traits, which facilitate the degradation process. As Gram-positive bacteria, Bacillus species can secrete extracellular enzymes such as laccase, cutinase, lipase, and protease, all of which play a crucial role in breaking down complex polymers through oxidation and hydrolysis.

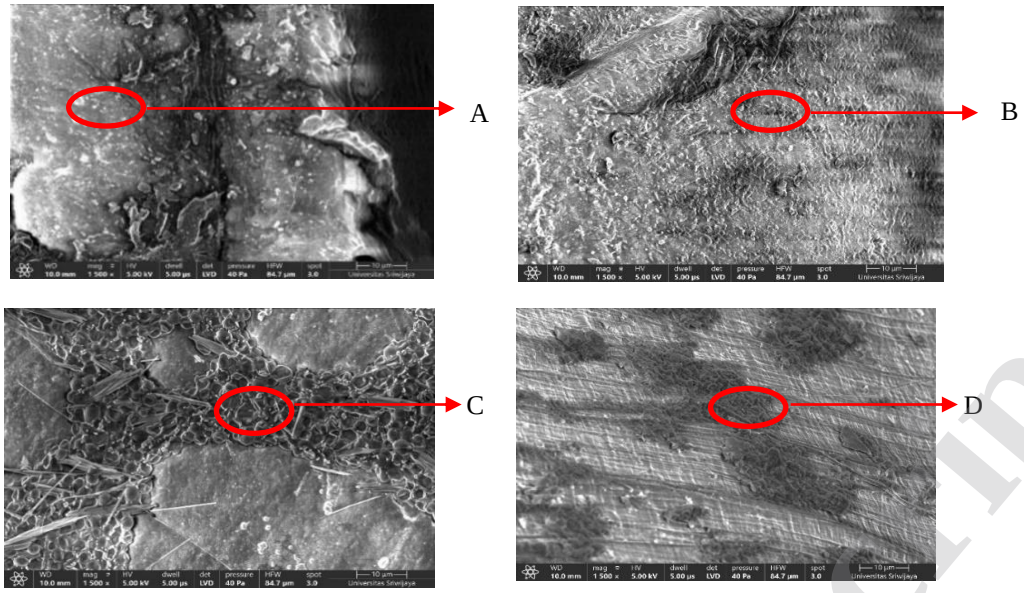

Figure 3. SEM visualization results. a) Control: smooth surface b) Isolate S1I3: presence of fine cracks c) Isolate S1I5: larger holes d) Isolate S2I1: more significant damage and uneven texture.

According to Table 3, the phenotypic identification of isolates demonstrating high degradation capabilities, as outlined in Bergey's Manual of Systematic Bacteriology, reveals that isolates S1I3, S1I5, and S2I1 are classified within the genus Bacillus. This genus comprises gram-positive, rod-shaped bacteria known for their ability to form endospores. The identified isolates were positive for catalase, motility, and Voges-Proskauer (VP) tests, and they exhibited the capacity to ferment both sucrose and glucose, aligning with the biochemical test results. As highlighted in the study referenced in [33], Bacillus species exhibit considerable potential attributed to their distinctive physiological characteristics, including resilience against various physical and chemical factors, the capability to produce antibiotics, and a broad spectrum of enzymatic activities. Notably, certain Bacillus species can degrade a variety of compounds, including complex polymers like plastics. The enzymatic mechanisms employed by Bacillus spp. in plastic degradation involve the production of specific enzymes that effectively dismantle polymer chains. Enzymes such as cutinase and PETase, classified as polyesterases, are instrumental in hydrolyzing synthetic polyesters like polyethylene terephthalate (PET) and polycaprolactone (PCL) by cleaving ester bonds to yield simpler monomers. Moreover, oxidative enzymes such as laccase and peroxidase aid in the degradation of polyolefins, like polyethylene (PE), through an oxidation mechanism that introduces carbonyl and hydroxyl groups into the polymer chain, thereby promoting further depolymerization.

Additionally, some species of Bacillus can produce lipases and proteases, which facilitate the breakdown of plastics containing lipid or protein components. The degradation

process initiates with the adsorption of these enzymes on the polymer surface, followed by the cleavage of chemical bonds, resulting in smaller oligomers and monomers that can subsequently be metabolized by other microorganisms.

Table 3. Phenotypic characteristics of plastic degrading bacteria

Isolate Character	S1I3	S1I5	S2I1
Macroscopic morphology of colonies	Cream colored colonies with round shape, flat margins	Yellowish white colonies with round shape, flat margins	Colonies are white and circular in shape, and the margins are entire
Microscopic morphology of cells	Rod shaped, gram positive cells produce endospores	Rod shaped, gram positive cells produce endospores	Rod shaped, gram positive cells produce endospores
Motility	-	-	-
Biochemical Test			
Glucose fermentation	+	+	+
Sucrose fermentation	+	+	+
Lactose fermentation	+	+	+
Indole production	-	-	-
Catalase production	+	+	+
Methyl red test	+	+	+
Voges proskauer test	-	-	-
TSIA Test	+	+	+
Conclusion	*Bacillus*	*Bacillus*	*Bacillus*

Bacillus exhibits significant promise for bioremediation in natural water systems, owing to its resilience to environmental fluctuations and impressive metabolic capabilities. Firstly, in the biodegradation of organic pollutants, Bacillus generates a variety of enzymes—including proteases, lipases, and amylases—that effectively break down harmful substances like petroleum hydrocarbons, detergents, and agricultural waste. Secondly, certain Bacillus species can tackle microplastic pollution by producing enzymes such as cutinase and laccase, which decompose plastics like polyethylene (PE) and polystyrene (PS).
In terms of heavy metal bioremediation, Bacillus has the ability to absorb and precipitate heavy metals, including cadmium (Cd), lead (Pb), and mercury (Hg), thereby mitigating their toxic effects in aquatic ecosystems. Additionally, Bacillus contributes to water quality enhancement and pathogen control through the production of biosurfactants that emulsify pollutants and

antimicrobial compounds that inhibit aquatic pathogens. For practical applications in fostering sustainable ecosystems, Bacillus can be utilized via bioaugmentation (the direct addition of microorganisms) or biostimulation (promoting in-situ microbial growth) to treat industrial waste and purify water before its discharge. Given these numerous advantages, Bacillus offers a sustainable strategy for addressing water pollution and preserving the balance of aquatic ecosystems [34].

Turning to the findings illustrated in Figure 3, which presents the electrophoregram results of PCR amplification, it is evident that the DNA bands from isolates S1I3, S1I5, and S2I1 fall within the size range of 1000–1500 base pairs. The agarose gel electrophoresis results depict single, clear, and intact DNA bands without any signs of smearing, indicating the successful and specific amplification of the 16S rRNA gene. The DNA bands align with the marker (M) at around 1000 bp, corroborating the expected target size. This aligns with the observations noted in reference [35], which states that prominent bands reflect high DNA purity, while smeared bands could indicate damage likely resulting from suboptimal extraction techniques.

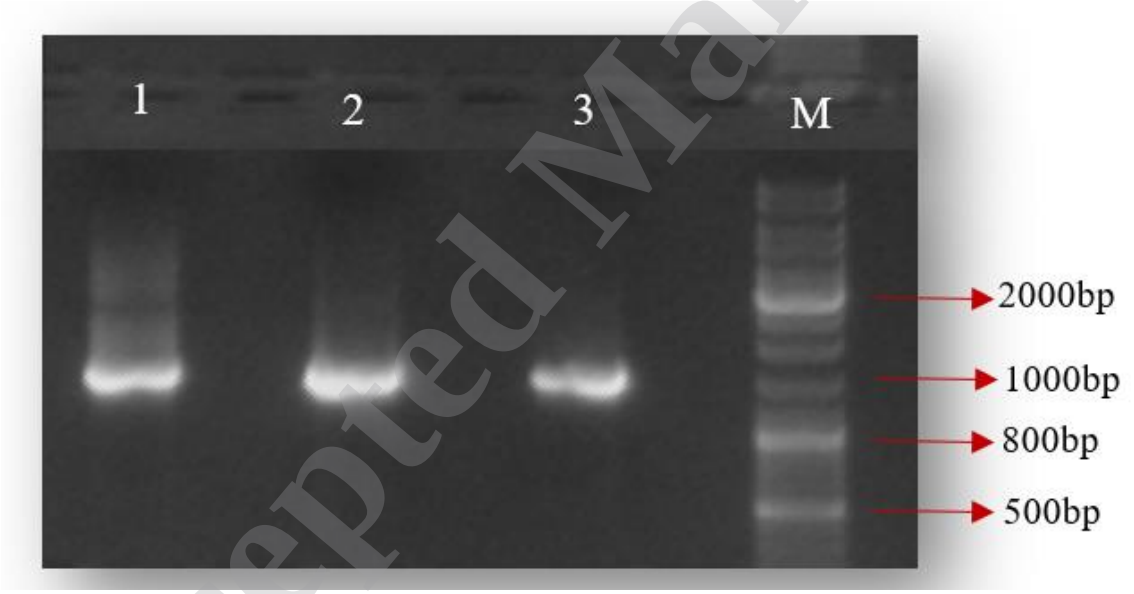

Figure 4. Electrophoregram of PCR results using the 16S rRNA gene. M = Ladder 100 bp; 1 = Isolate S1I3; 2 = Isolate S1I5; 3 = Isolate S2I1.

Based on the BLAST analysis results from NCBI, isolate S1I3 shows a 99.87% similarity to *Bacillus proteolyticus* strain MCCC 1A00365, with a query cover of 99% and a maximum score of 2741 (Table 4).

Table 4. BLAST results of plastic degrading bacteria isolate S1I3

Description	Max Score	Query Cover	E.value	Per.Ident
Bacillus proteolitycus strain MCCC 1A00365 16S ribosomal RNA	2741	99%	0.0	99.87%
Bacillus wiedmannii strain FSL W8-0169 16S ribosomal RNA	2736	99%	0.0	99.80%
Bacillus cereus ATCC 14579 16S ribosomal RNA	2734	99%	0.0	99.87%
Bacillus albus strain MCCC 1A02146 16S ribosomal RNA	2730	99%	0.0	99.73%
Bacillus paramycoides strain MCCC 1A04098 16S ribosomal RNA	2724	99%	0.0	99.66%

Meanwhile, the BLAST results indicate that isolates S1I5 and S2I1 exhibit a high degree of similarity with *Bacillus cereus*. Isolate S1I5 has a maximum score of 2748, an expectation value of 0.0, and both a query cover and percent identity of 100% (Table 5).

Table 5. BLAST results of plastic degrading bacteria isolate S1I5

Description	Max Score	Query Cover	E. Value	Per.Ident
Bacillus cereus ATCC 14579 16S ribosmal RNA	2748	100%	0.0	100%
Bacillus albus strain MCCC 1A02146 16S ribosmal RNA	2743	100%	0.0	99.93%
Bacillus paranthracis strain MCCC 1A00395 16S ribosomal RNA	2732	100%	0.0	99.80%
Bacillus pacifius strain MCCC 1A06182 16S ribosomal RNA	2726	100%	0.0	99.73%
Bacillus clarus strain ATCC 21929 16S ribosomal RNA	2721	100%	0.0	99.66%

Similar results were observed for isolate S2I1, which had a query coverage of 99% and a percentage identity of 100% with similarity to *Bacillus cereus* (Table 6).

Table 6. BLAST results of plastic degrading bacteria isolate S2I1

Description	Max Score	Query Cover	E. Value	Per.Ident
Bacillus cereus ATCC 14579 16S ribosomal RNA	2739	99%	0.0	100%
Bacillus toyonensis strain BCT-7112 14579 16S ribosomal RNA	2712	99%	0.0	99.66%
Bacillus mobilis strain MCCC 1A05942 16S ribosomal RNA	2706	99%	0.0	99.60%
Bacillus thuringiensis strain ATCC 10792 16S ribosomal RNA	2704	99%	0.0	99.73%
Bacillus pseudomycoides strain NBRC 101232 16S ribosomal RNA	2695	99%	0.0	99.59%

Bacillus proteolyticus is believed to possess the potential for degrading polyethylene plastic, although comprehensive studies on this capability are limited. Several Bacillus species are known to produce enzymes such as lipase and esterase, which contribute to the breakdown of polyester plastics, including polyethylene terephthalate (PET) [36]. Notably, Bacillus cereus has demonstrated the ability to produce enzymes like protease and lipase that facilitate plastic biodegradation. This species can effectively disrupt polyethylene chains, leading to morphological changes on the plastic surface, including the formation of small holes and cracks[37].

The analysis of the 16S rRNA gene sequences from isolates S1I3, S1I5, and S2I1 reveals nucleotide lengths that are consistent with their respective species identities. As shown in Table 7, the typical length of the 16S rRNA gene ranges from 1,400 to 1,550 bp, encompassing both conserved and variable regions. Specifically, isolate S1I3 features a 1,501 bp fragment, which aligns with a 1,491 bp sequence in GenBank, identifying it as Bacillus proteolyticus. In contrast, isolates S1I5 and S2I1 have fragments measuring 1,492 bp and 1,499 bp, respectively, both matching a 1,488 bp sequence in GenBank and classified as Bacillus cereus. The minor variations in fragment sizes may be due to differences in the hypervariable regions (V1-V9) of the 16S rRNA gene, which may exhibit species-specific insertions or deletions. According to research referenced in [38], the length of the 16S rRNA gene differs among bacterial species because of variations in nucleotide composition in these hypervariable regions. The interplay of highly conserved and variable regions within this gene is crucial for phylogenetic analysis and bacterial identification, as conserved sequences enable accurate alignment while variable

regions allow for differentiation at the species level.

Table 7. Size of DNA fragments from isolates of plastic-degrading bacteria used 16S rRNA gene

Sample	Isolate Fragment Size	Genbank	Identified Species
S1I3	1501	1491	*Bacillus proteolyticus*
S1I5	1492	1488	*Bacillus cereus*
S2I1	1499	1488	*Bacillus cereus*

According to Figure 4, the phylogenetic tree reveals two primary clusters at a genetic distance of 0. 150. Cluster I, identified as the ingroup, includes isolate S1I3 and several bacterial species: *Bacillus proteolyticus, Bacillus wiedmannii, Bacillus cereus, Bacillus albus*, and *Bacillus paramycoides*. In contrast, Cluster II acts as the outgroup, comprising *Lactobacillus laiwuensis*. Notably, isolate S1I3 is closely aligned with *Bacillus proteolyticus*, as indicated by a genetic distance of 0. 000 and a complete 100% DNA sequence similarity, highlighting their close genetic connection. Additional sequence homology data further reinforce this relationship, showcasing a 99% query cover and a 99. 87% identity (see Table 4), which suggests that isolate S1I3 is likely *Bacillus proteolyticus* or a closely related species.

The resemblance between isolate S1I3 and *Bacillus proteolyticus* is also evident in its phenotypic characteristics. The colony morphology, color, and fermentation abilities of isolate S1I3 correspond with those typical of the Bacillus genus. As noted in reference [39], *Bacillus proteolyticus* has small to medium-sized colonies (2–3 mm), which are white or pale pink in color, round in shape with smooth edges. Microscopically, this bacterium is gram-positive, non-motile, rod-shaped, and capable of forming endospores. Biochemical analysis shows that this isolate is catalase-positive, methyl red-positive, and grows optimally at 37°C with a neutral pH (pH 7). These phenotypic similarities further support the identification of isolate S1I3 as *Bacillus proteolyticus* or a species that is phylogenetically very closely related.

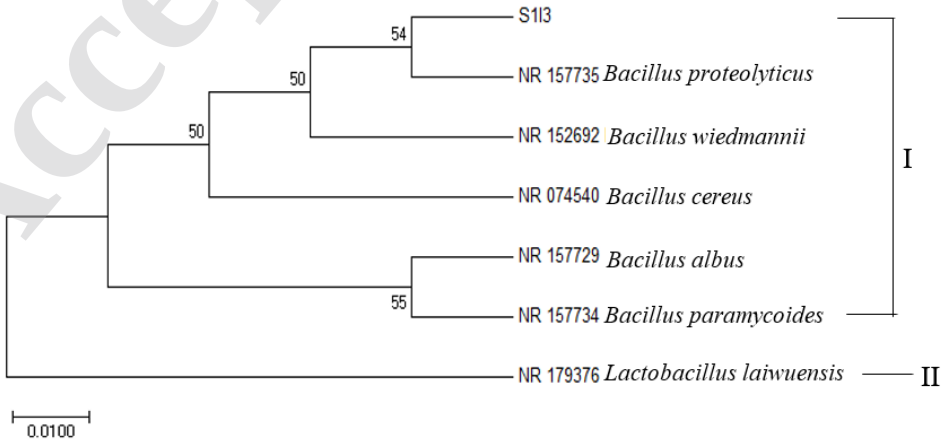

Figure 5. Phylogenetic tree of isolate S1I3.

Based on Figure 5, the phylogenetic tree forms two main clusters at a genetic distance of 0.150. Cluster I is the ingroup, which includes isolate S1I5 along with several bacterial species, including *Bacillus cereus*, *Bacillus paranthracis*, *Bacillus pacificus*, *Bacillus albus*, and *Bacillus clarus*. Meanwhile, Cluster II serves as the outgroup, consisting of *Lactobacillus laiwuensis*. Isolate S1I5 forms a sister taxon with *Bacillus cereus*, supported by a bootstrap value of 68, a genetic distance of 0.000, and a 100% DNA sequence similarity, indicating a very high genetic proximity. Sequence homology results also show a 100% query cover and percent identity (Table 5), suggesting that isolate S1I5 is most likely *Bacillus cereus* or a close relative. This close genetic relationship is further reinforced by the phenotypic characteristics of isolate S1I5, which align with those of the *Bacillus* genus, particularly *Bacillus cereus*. The study in [40], *Bacillus cereus* typically has round colonies with irregular edges, cream to white in color, dry and rough in texture, with a raised elevation. Microscopically, this bacterium is gram-positive, rod-shaped with varying sizes, and capable of forming endospores that play a role in its resistance to extreme conditions. Biochemical analysis shows that *Bacillus cereus* is catalase-positive and can ferment glucose and lactose. These phenotypic characteristics further support the identification of isolate S1I5 as *Bacillus cereus* or a closely related species, consistent with the phylogenetic analysis results.

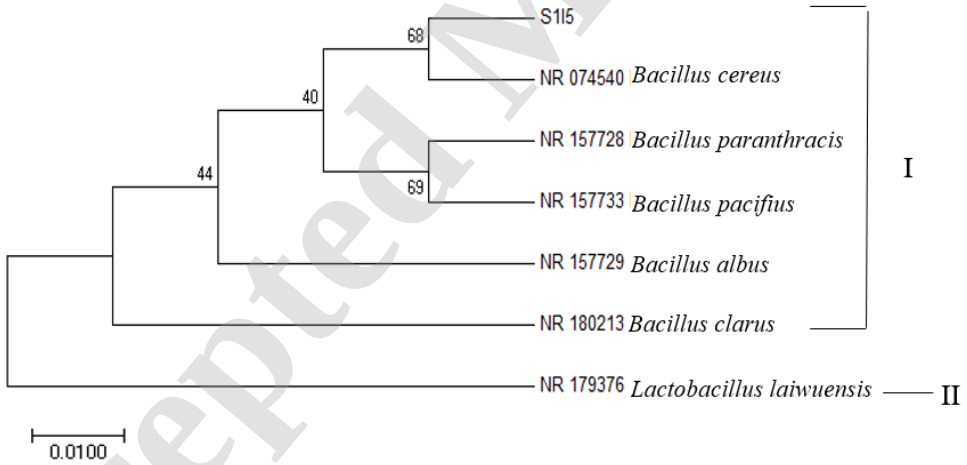

Figure 6. Phylogenetic tree of isolate S1I5

According to Figure 6, the phylogenetic tree illustrates two primary clusters, separated by a genetic distance of 0. 761. Cluster I represents the ingroup, which includes the isolate S2I1 along with various bacterial species such as *Bacillus cereus*, *Bacillus pseudomycoides*, *Bacillus mobilis*, *Bacillus toyonensis, and Bacillus thuringiensis*. *Lactobacillus laiwuensis* serves as the outgroup in this analysis. Notably, isolate S2I1 is closely related to *Bacillus cereus*, forming a sister taxon relationship. This connection is underscored by a bootstrap value of 95, a genetic distance of 0. 000—indicating no observed sequence divergence—and 100% DNA sequence similarity. Furthermore, sequence homology results indicate a 99% query cover and 100%

identity (refer to Table 6), strongly suggesting that isolate S2I1 is either *Bacillus cereus* or a closely related species. This high level of genetic similarity is further corroborated by the phenotypic characteristics of isolate S2I1, which are consistent with those of the *Bacillus* genus, particularly *Bacillus cereus*. As noted in reference [41], *Bacillus cereus* colonies typically appear medium to large in size, have a round shape with a rough surface, exhibit white to cream coloring, and possess irregular edges. Gram staining reveals that *Bacillus cereus* is a gram-positive, rod-shaped bacterium measuring 3–5 µm in length and 1–1. 5 µm in width, and it is capable of forming endospores. Biochemical tests demonstrate that this bacterium can ferment glucose and fructose and is catalase positive. These phenotypic traits align well with the molecular identification results, further reinforcing the classification of isolate S2I1 as *Bacillus cereus.*

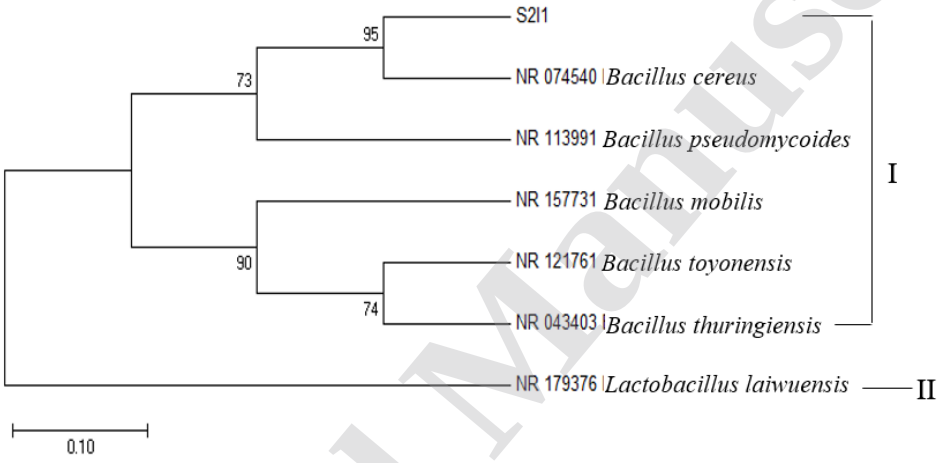

Figure 7. Phylogenetic tree of isolate S2I1.

All three phylogenetic trees above exhibit a monophyletic nature, indicating that members of Cluster I share a common ancestor. A stated in [42], genetic, morphological, and biochemical similarities within a monophyletic group suggest a close evolutionary relationship, as observed in these phylogenetic trees, where all members in the group belong to the *Bacillus* genus.

Conclusions

The results of this study show that bacterial isolation from the Musi River yielded 33 isolates with varying distribution across different stations. Some isolates exhibited high plastic degradation potential, with S2I1 reaching 38.03%, S1I5 at 34.73%, and S1I3 at 30.46%. Phenotypic and genotypic characterization identified isolate S1I3 as *Bacillus proteolyticus*, while isolates S1I5 and S2I1 were confirmed as *Bacillus cereus*.

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