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## Isolation and Evaluation of Plastic Degrading Bacterial Species from Municipal Waste Dumping Site

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### ABSTRACT

The process of biodegradation involves the breakdown of organic materials by microorganisms, primarily bacteria and fungi. Multiple experiments are conducted to determine optimal conditions for biodegradation in various environments. Proper degradation of biodegradable compounds significantly contributes to the reduction of environmental pollution. This study investigates the microbial degradation of plastic waste, employing soil samples collected from a municipal waste disposal site. Bacterial cultures were prepared in Muller Hinton (MH) medium following serial dilution of the collected samples. After incubation, pure bacterial cultures were isolated, and bacterial morphology was identified via Gram staining. The study identified Gram-positive *Bacillus spp.* and Gram-negative *Pseudomonas* and *Moraxella spp.* as effective plastic-degrading microorganisms. Pure strains of these bacteria were co-incubated with polyethylene discs in nutrient broth for at least one month. Following co-incubation, the polyethylene discs exhibited a 46% reduction in weight, compared to their initial weight, per month. The results indicate that *Bacillus spp.* exhibit the highest degradation potential among the tested microorganisms, achieving 46% and 44% weight reduction of polyethylene discs monthly, underscoring their effectiveness in plastic breakdown.

**KEYWORDS:** Biodegradation, Plastic waste, Environmental pollution, Polyethylene degradation, Gram staining

### INTRODUCTION

Plastic is a primary polymer utilized in everyday life and commonly discarded into sewage systems (Evode *et al.*, 2021). The extensive use of synthetic polymers, particularly plastics, has created persistent environmental issues due to their durable composition, which hinders biodegradation (Lim and Thian, 2022). Plastics accumulate in ecosystems, especially aquatic environments, where they fragment into microplastics that contaminate water sources, harm marine life, and infiltrate the food web, ultimately impacting human health (Thompson *et al.*, 2009; Swain *et al.*, 2025; Eze *et al.*, 2024). It causes aquatic species to become contaminated with microplastics, which enter the food chain and negatively affect human health (de Sousa, 2021). These resilient polymers are not readily degradable and take years to break down in the environment. This problem contributes to the accumulation of plastic waste, putting many species of life including people in peril. Only two of the many environmental problems brought on by decades of unrestrained plastic use are plastic waste management and pollution (Moshood *et al.*, 2022). The annual global

production of plastic exceeds 300 million tons, and plastic waste has become one of the leading pollutants worldwide, imposing challenges in waste management and environmental preservation (Geyer *et al.*, 2017). Pakistan wastes more than 3.3 million tons of plastic annually, according to the United Nations Development Programme (UNDP) (Ahmad *et al.*, 2025). Therefore, reducing its lifespan is a crucial action that must be taken in order to save the environment (Hashmi *et al.*, 2023). Traditional plastic disposal methods, such as landfilling and incineration, exacerbate pollution and release harmful chemicals into the soil and atmosphere (Thompson *et al.*, 2009). The process by which microorganisms (bacteria and fungi) break down organic substances is known as biodegradation (Choi *et al.*, 2023). Microbial biodegradation offers an environmentally sustainable solution. Microorganisms, including bacteria and fungi, facilitate biodegradation by breaking down complex polymers into simpler monomers through enzymatic action, a process occurring naturally in ecosystems (shah *et al.*, 2008). Biodegradation comprises three steps: bio deterioration, bio fragmentation, and assimilation.

Different factors affect the biodegradation process at open dumping sites, which occurs in all materials and chemical substances (Gorish *et al.*, 2025). These factors are light, temperature, oxygen, and water. Various tests were performed for confirmation of the ideal biodegradation process in a provided environment (Haider *et al.*, 2019). Biodegradable polymers are intended to decompose when discarded through the activity of living microorganisms. Biodegradability of plastics is caused by enzymatic activities that result in the polymer chain breaking into monomers (Tania and Anand, 2025). They colonize the surfaces of polyethylene films, generating a biofilm. The hydrophobicity of these organisms' cell surfaces was discovered to be a significant factor in the creation of biofilm on the polythene surface, which improves polymer biodegradation (Ghatge *et al.*, 2020). Once the organisms attach to the surface, they begin to proliferate by utilizing the polymer as a carbon source (Shen *et al.*, 2013). Microorganisms affect the degree of biodegradation in a natural ecosystem (Thakur, 2012). Microbial degradation of plastic has been reviewed by several studies, which demonstrated the abiotic and biotic degradation of synthetic plastic. The fungus species *Penicillium simplicissimum* was supposed to produce an extracellular enzyme capable of degrading polyethylene PE (Zhang *et al.*, 2022). Various classes of fungi degraded specific types of plastic (PHA and PHB) isolated from soil and marine environments (Kim and Rhee, 2003). The bacterial strains *Pseudomonas* and *Bacillus* degraded the synthetic plastic. *Bacillus amylolyticus* degraded plastic by up to 32% when isolated from garbage-contaminated soil. The *Pseudomonas fluorescens* degraded 7.8% and 7.9% of the polythene at 30°C and 37°C temperature during one month (Kotova *et al.*, 2021). These bacterial strains were isolated and identified microscopically by examining colony pigmentation, size, and shape. Dumping soil is rich in plastic degrading bacteria because there is a very suitable environment for the growth of these bacteria. *Bacillus subtilis* along with its biosurfactant (surfactin) was revealed to be the most productive in 30 days of polyethylene breakdown, with a weight loss percentage of 9.26 percent (Chauhan and Ambechada, 2025). The aim of this study was to isolate and evaluate plastic-degrading bacterial species capable of degrading plastic waste from soil collected at a municipal dumping site. The selected dumping site was 25–30 years old, providing a greater likelihood of the presence of plastic-degrading bacteria due to long-term accumulation of plastic waste. The objectives of the study included isolation of bacterial strains from soil obtained from the municipal waste dumping site, evaluation of the isolated bacterial strains, and investigation of their biodegradative ability against plastic waste.

## MATERIALS AND METHODS

**Sample collection and Isolation:** The soil sample was collected from the I-12 Islamabad dumping site, which was approximately a 15–20-year-old dumping site. The soil was taken from dumped plastic with the help of safety gloves. The soil samples were collected in a flask containing 100 mL of distilled water and five test tubes, each with 9 mL of sterile diluents, (Ben-David and Davidson, 2014) which consisted of distilled water. Sterile pipettes were used. A pipette was filled with 1 mL of adequately mixed material. After that, the sample was added to the first tube to make a total volume of 10 mL. This resulted in an initial  $10^{-1}$  dilution. The dilution was well homogenized by repeatedly emptying and refilling the pipette. The pipette tip was removed, and a new one was attached to the pipette. Then, from the  $10^{-1}$  dilution, 1 mL of the liquid was poured into the second tube. The total dilution factor in the second tube was  $10^{-2}$ . The same procedure was followed for the remaining tubes, with 1 mL from the preceding tube added to the following 9 mL of diluents. Because five tubes were utilized, the final bacterial dilution was  $10^{-5}$ . In scientific experiments, a negative control is used as a baseline reference to evaluate the validity of the results. For an experiment, a negative control is a sample that does not contain the substance being tested or the necessary experimental conditions necessary for the reaction to occur. For the negative control, one dilution was prepared. After serial dilution, bacterial cultures were grown in MH medium (Irina *et al.*, 2025) was used for bacterial culturing and it was left for incubation overnight (24-36 h). Bacterial pure cultures are frequently established by streaking bacteria (Sanders, 2012) across the surface of a solid substrate. After culturing the bacteria, colonies were selected based on their color and appearance, and eight different pure culture plates were prepared. This guaranteed that each plate had a single cell in an isolated zone that could grow into a visible colony of clones or identical cells. The streaking techniques were used to produce the pure culture.

**Identification by Gram Staining Method:** Identification was made through morphological and cultural characteristics. Gram staining of all the isolated strains was performed (Tripathi *et al.*, 2025). On a clean glass slide, a smear of bacterial culture was developed using a sterile loop with water droplets. The smear was air-dried before being heat fixed. It was then stained using the following reagents: One drop of crystal violet was applied for one minute. After that, washed with running distilled water. The smear was flooded with Gram's iodine for one minute, then washed with flowing distilled water. The slide was then treated with a Gram decolorizer for 30 seconds. The slides were counterstained with safranin for 30 seconds before being washed with flowing distilled water. The slide was air-dried before the cell morphology was observed under the

microscope. All captured images were included in the results section.

#### Biochemical Tests

**Catalase Test:** The catalase test is a biochemical test that is used to determine the presence of the enzyme catalase in bacterial cells (Hadwan *et al.*, 2024). Catalase is an enzyme that facilitates the breakdown of hydrogen peroxide into water and oxygen gas. This test is useful in identifying different bacterial species as catalase production varies between species. To perform the catalase test, a small amount of bacterial growth is added to a microscope slide or petri dish. A few drops of hydrogen peroxide are then added to the bacterial growth. If the bacteria produce catalase, oxygen gas bubbles are observed.

**Mannitol test:** Mannitol test is a laboratory procedure used to identify bacteria that can ferment mannitol sugar (Saleena *et al.*, 2023). To perform the Mannitol test, a small amount of mannitol salt agar is inoculated with the bacterial sample and incubated for 24-48 hours. If the bacteria can ferment mannitol, they produce acid, which lowers the pH of the agar and causes it to turn yellow. If the bacteria cannot ferment mannitol, the agar remains red. To ensure

accurate results, it is important to follow the proper technique when performing the Mannitol test. The agar should be properly sterilized, and the bacterial sample should be properly inoculated onto the agar. Additionally, the incubation conditions should be carefully controlled to ensure optimal growth conditions.

**Weight loss determination:** Pre-weighted polyethylene bag discs of varying thicknesses were prepared in aseptic conditions and placed in a cylindrical flask each carrying 50 mL of culture broth medium containing various bacterial strains. A bacteria-free medium served as the control for the study (Thakur, 2012). All flasks prepared for different bacterial strains were placed in a shaker for 20–30 days. The incubator shaker was maintained at 37°C temperature and 30 rpm speed. Plastic discs were collected and washed with flowing distilled water after one month of shaking. The final weight was recorded and compared with the original weight after it has been air-dried. Weight loss was determined using the collected data. However, to determine the degradation of plastic the percentage weight loss was calculated using the formula mentioned below.

$$\% \text{weight loss} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

## RESULTS and DISCUSSION

**Colony morpho-physiology:** Researchers employ colony morphology to characterize the features of individual bacterial colonies growing on agar in a Petri dish. It can be utilized to assist in bacterial identification. Colonies vary in shape, size, color, and texture. Each different colony represents a single bacterial cell or group that has repeatedly split. As a result of being held in one location, the resultant cells have gathered to create a visible patch. **Table 2** categorizes various bacterial strains based on their morphological characteristics, specifically focusing on shape and size. The bacterial strains are labelled with unique codes (W1, W2, X1, X2, Y1, Y2, Z1, and Z2) for identification purposes. The data indicates that the majority of the bacterial strains studied exhibit irregular shapes, with only a subset displaying the more defined rod morphology. The uniform small size across all strains suggests a degree of consistency in their physical scale, despite their differences in shape. These morphological characteristics can be critical in determining the bacteria's classification, environmental adaptability, and potential pathogenicity. Further analysis, including staining techniques, may provide additional insights into their taxonomic classification and functional roles.

**Gram-Positive Bacteria:** Thick cell walls are a characteristic of gram-positive bacteria. These organisms are identified as "Gram-positive" by the Gram stain test, which employs a chemical dye to make their cell walls purple. Four strains of gram-positive bacteria, W1, W2, Z1, and Z2, were used in this investigation.

**Gram-Negative Bacteria:** In this investigation, four gram-negative bacteria strains were used: X1, X2, Y1, and Y2. **Table 2** provided categorizes eight distinct bacterial strains based on their morphological and biochemical properties. Each strain is identified by a unique code (W1, W2, X1, X2, Y1, Y2, Z1, and Z2), and their characteristics are described in terms of shape, color, and Gram staining reaction. These features are critical for the preliminary identification and classification of bacterial species. The bacterial strains demonstrate two primary morphological forms: irregular and rod-shaped. Strains W1, W2, Y1, Y2, Z1, and Z2 exhibit an irregular shape, indicating a lack of uniform structure, which may indicate pleomorphic morphology. Strains X1 and X2 are rod-shaped, a typical morphology associated with many Gram-negative bacteria. The Gram staining color also correlates with the composition of the bacterial cell wall, strains W1, W2, Z1, and Z2 are Gram-positive in appearance and therefore have a thick layer of peptidoglycan in the cell wall. Strains X1, X2, Y1, and Y2 appeared pink in color that is associated with Gram-negative bacteria possessing a thinner peptidoglycan layer and an outer membrane composed of lipopolysaccharides. Shape, color, and Gram-staining properties of bacterial strains are important aspects to facilitate their categories in terms of structure and possible behavior in various places. The bacterial population exhibited multiple irregular morphologies (Gram-positive strains: W1, W2, Z1, Z2) and irregular shapes along with rod-shaped Gram-negative strains (X1, X2) Gram-positive and Gram-negative strains.

The microscopic images presented depict two bacterial strains, (**Figure 1**) labelled W1 and W2, as observed under high magnification. Both strains exhibit a densely packed arrangement of cells with an irregular shape, characteristic of certain Gram-positive *Bacillus* spp. The purple coloration suggests a positive reaction to the Gram staining procedure, indicative of a thick peptidoglycan layer in their cell

walls. Notably, W1 shows a more uniform distribution of cells, while W2 displays clusters of cells with areas of apparent aggregation. These morphological observations align with the previously noted characteristics of these strains, supporting their classification within the Gram-positive category and *Bacillus* spp.

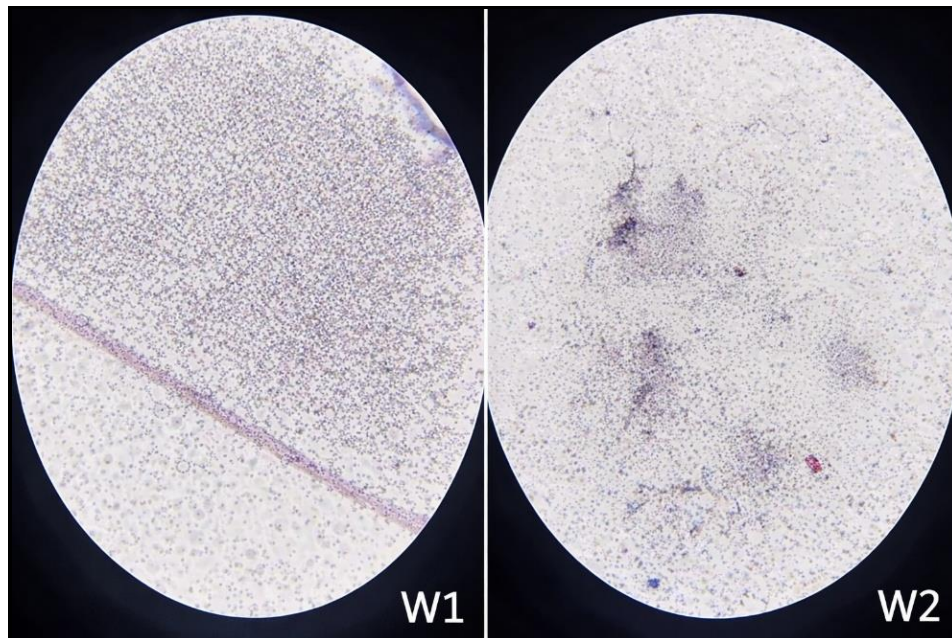


**Figure 1** Gram staining of bacterial strains W1 and W2 under 40X. Microscopic images (W1 and W2) display *Bacillus* spp. colonies at different stages of growth. In W1, a high density of *Bacillus* cells can be evenly distributed across the field. W2 shows bacterial clumping and darker areas, suggesting aggregation or increased bacterial biomass in certain regions

These two microscopic images, (**Figure 2**) identified as X1 and X2, show two different bacterial strains having their own morphological and staining properties. These two strains are rod-shaped resembling the morphology of *Moraxella* spp. The pink coloration of the two samples under the microscope indicates that they are Gram-negative as the reaction is to the staining due to a thinner peptidoglycan layer and the presence of an outer membrane. The X1 strain appears uniformly distributed, whereas the X2 strain exhibits some regions where it is oriented with a dense streak-like concentration, possibly due to unequal spreading on the shiny area or increased cellular aggregation. These findings agree with common characteristics of Gram-negative rods, that are typical of *Moraxella* spp

Morphological characteristics of the Gram-stained bacterial strains (**Figure 3**) Y1 and Y2 can be observed in the microscopic form. The two strains exhibited irregular morphology and pink staining, which is representative of Gram-negative and *Pseudomonas* spp. This implies that there is a thinner peptidoglycan layer in Gram-negative bacteria and an additional outer membrane. Uneven distribution of clusters of cells can be observed in the images especially in Y2 where the clusters of cells are more evident in their aggregates. The streak appearance can be due to the method of preparation so that differences in density of bacteria in different parts of the slide can be used in diagnosis. These features demonstrate the possible differences between the two strains regarding the properties and adhesion of their cells and cell surfaces

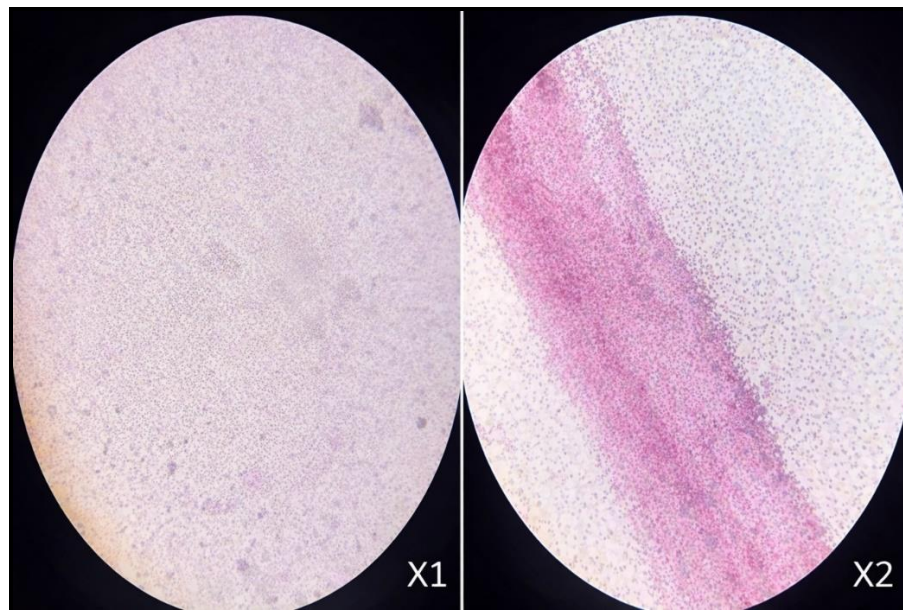


**Figure 2** Gram staining of bacterial strains X1 and X2, under 40X magnification. Microscopic images (X1 and X2) depict *Moraxella spp.* colonies under different staining conditions. X1 shows a uniform distribution of cells, indicating even growth across the field. In X2, a prominent, pink-stained streak is visible, with bacteria densely lining the edges, suggesting possible colony formation along a nutrient source or streaking pattern.

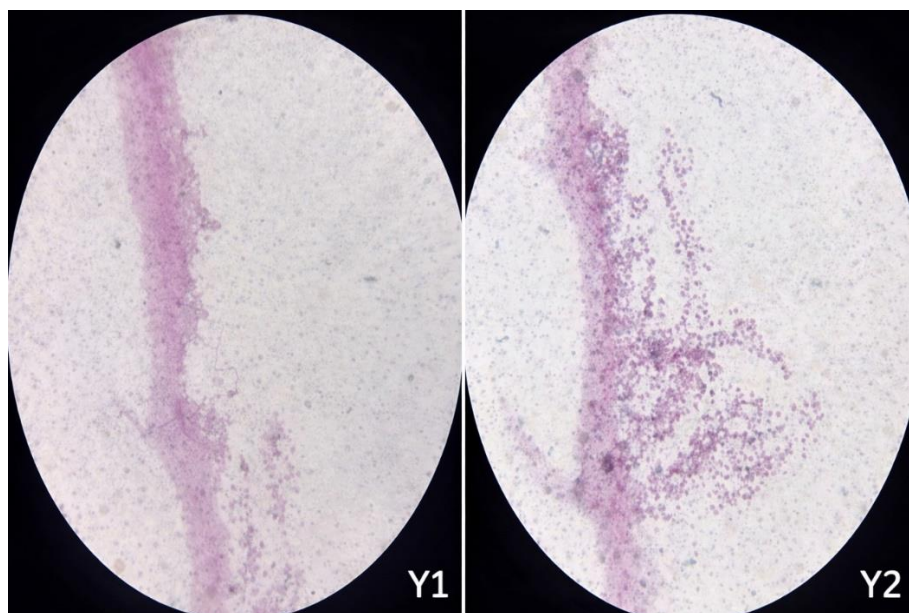


**Figure 3** Gram stains of *Pseudomonas spp.* bacterial strains (Y1) and (Y2) at 40 x magnification. In Image Y1, there is a common linear arrangement of colony growth whereas, in Image Y2, there is scattered branching bacterial growth. These disparities could be observed because of the dissimilarity in biofilm formation and adhesion capabilities of the bacteria strains.

Gram-stained images observed under 40× magnification, of *Bacillus spp.* bacteria strain (Z1) and (Z2) (**Figure 4**). The presence of a dense and evenly distributed pattern of bacterial cells is observed in Image Z1 whereas Image Z2 depicts a

more irregular level of those irregularly distributed clusters of different densities. Such structural variations could represent a different growth rate and shape of colonies of the *Bacillus spp.*

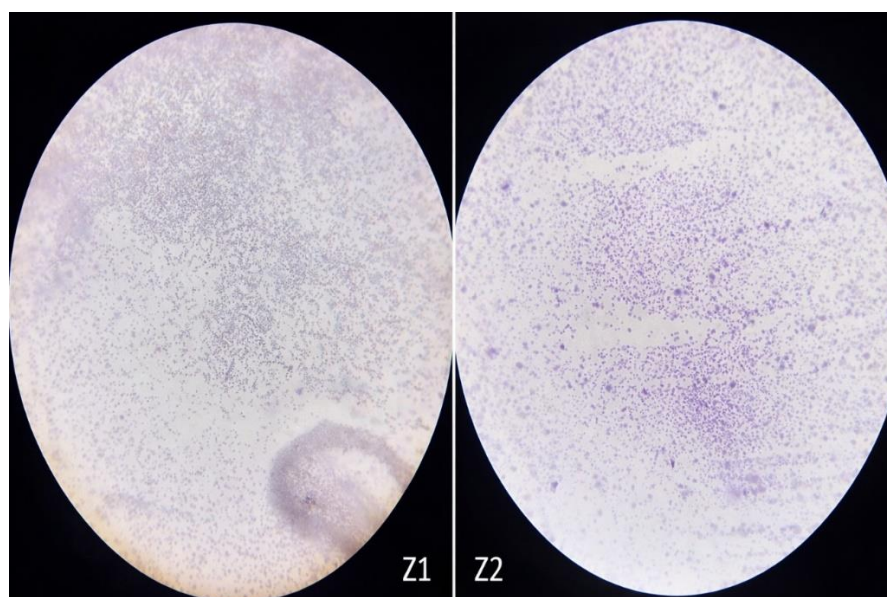


**Figure 4** *Bacillus spp.* bacterial strains (Z1) and (Z2) as seen under Gram stain magnification 40x. In Image Z1, a large number and even distribution of bacterial cells are observed whereas in Image Z2 the pattern is quite different, with some clumps of high and some of low density. Observed structural differences between them could reflect different growth profiles and colony morphologies among *Bacillus spp.*

**Biochemical tests:** The **table 1** presents the findings of the Catalase and Mannitol tests which were performed on eight different samples which were labelled as W1, W2, X1, X2, Y1, Y2, Z1, and Z2. It is depicted by the +VE (positive) and -VE (negative) symbols which mark the results of each of the tests. All samples included in W1, W2, X1, X2, Y1, Z1 and Z2 (**Table 1**) showed positive results on the presence of catalase, which means that the enzymes were active in these samples. Sample Y2 was the only sample that tested negative for catalase indicating that it did not exhibit catalase activity. All these samples

showed positive Mannitol test results W1, W2, X2, Y1, Y2, Z1, and Z2 indicating their ability to ferment mannitol. Sample X1 tested negative in the Mannitol test, and this means it could not ferment mannitol. The consistency of these results in the table confirms once again the consistency of the findings in the provided samples. The presence (+VE) or absence (-VE) of catalase and the ability (+VE) or inability (-VE) (**Table 1**) to ferment mannitol are indicative of the biochemical characteristics of the samples under examination

**Table 1** Catalase and Mannitol Test of all isolated strains. Overview of biochemical results with positive catalase and mannitol reactions in all strains of bacteria including W1, W2, X1, X2, Y1, Y2, Z1, Z2 as well as a negative catalase reaction in Y2.

| Tests         | W1  | W2  | X1  | X2  | Y1  | Y2  | Z1  | Z2  |
|---------------|-----|-----|-----|-----|-----|-----|-----|-----|
| Catalase Test | +VE | +VE | +VE | +VE | +VE | -VE | +VE | +VE |
| Mannitol Test | +VE | +VE | -VE | +VE | +VE | +VE | +VE | +VE |

**Weight Loss:** After one month of bacterial treatment in the culture, the broth media biodegradation rate was calculated, and the results are summarized in **Table 2** shows that bacterial strains W1, W2, and Y2 exhibited more biodegradation of plastic samples as compared to others. Their differences were 23 mg, 22 mg, and 22 mg respectively. According to **Table 2**

strains Z1 and Z2 showed moderate biodegradation because their weight differences were 20 mg and 15 mg. Bacterial strains X1, X2, and Y1 showed lower plastic degradation. In these strains, the weight differences were 4 mg, 10 mg and 10 mg respectively.

**Table. 2.** Morphological characteristics, Gram staining results, and plastic biodegradation potential of bacterial strains isolated from municipal waste soil collected from the municipal waste dumping site.

| Bacterial Strain Code | Shape     | Color  | Characteristic | Initial Weight (mg) | Final Weight (mg) | Weight Loss/Month (%) |
|-----------------------|-----------|--------|----------------|---------------------|-------------------|-----------------------|
| W1                    | Irregular | Purple | Gram +ve       | 50                  | 27                | 46                    |
| W2                    | Irregular | Purple | Gram +ve       | 50                  | 28                | 44                    |
| X1                    | Rod       | Pink   | Gram -ve       | 50                  | 46                | 8                     |
| X2                    | Rod       | Pink   | Gram -ve       | 50                  | 40                | 20                    |
| Y1                    | Irregular | Pink   | Gram -ve       | 50                  | 40                | 20                    |
| Y2                    | Irregular | Pink   | Gram -ve       | 50                  | 28                | 44                    |
| Z1                    | Irregular | Purple | Gram +ve       | 50                  | 30                | 40                    |
| Z2                    | Irregular | Purple | Gram +ve       | 50                  | 35                | 30                    |
| Mean $\pm$ SE         | —         | —      | —              | 50.0 $\pm$ 0.00     | 34.25 $\pm$ 2.51  | 31.5 $\pm$ 2.51       |

\*Values are presented as mean  $\pm$  standard error (SE).

The data presented in **table 2** summarizes the weight loss analysis of eight different strains, designated as W1, W2, X1, X2, Y1, Y2, Z1, and Z2. Each strain was initially weighed, and after a period of one month, the final weight was recorded to calculate the percentage weight loss after one month. Strain W1 showed a reduction in weight from 50 mg to 27 mg, resulting in 46% weight loss after one month (**Figure 5**). Similarly, strain W2 showed a reduction from an initial weight of 50 mg to a final weight of 28 mg, resulting in a weight loss of 44% per month. The initial weight of strain X1 decreased from 50 mg to 46 mg to final weight of 46 mg yielding a loss of 8 percent a month. The starting weight is 50 mg with an end weight of 40 mg, and the weight loss came out to be 20 percent per month. For strain Y1, the initial weight of 50 mg decreased to a final weight of 40 mg thus giving a weight loss of 20 percent monthly. For strain Y2, the original weight of 50 mg decreased to smallest weight of 28 mg leading to loss of 44 percent

of weight per month. For strain Z1, the original weight of 50 mg became a final weight of 30 mg translating to a weight loss of 40 percent per month. For strain Z2, the initial weight of 50 mg decreased to final weight of 35 mg thus a reduction of weight was measured by 30 percent per month. The weight loss analysis of these strains shows a lot of differences with the highest weight loss brought about by strains W1 and W2 at 46% and 44% respectively, and the least by strain X1 at 8 percent loss. These variations are noted as differences in differences in strain performance (metabolically/biochemically) under comparable conditions. Graphical interpretation of plastic degradation efficiency among the bacterial strains W1, W2, X1, X2, Y1, Y2, Z1, and Z2 which were expressed in terms of percentage weight loss (**Figure 5**) shows important results that may have important implications for the development of biotechnological approaches to plastic waste management.

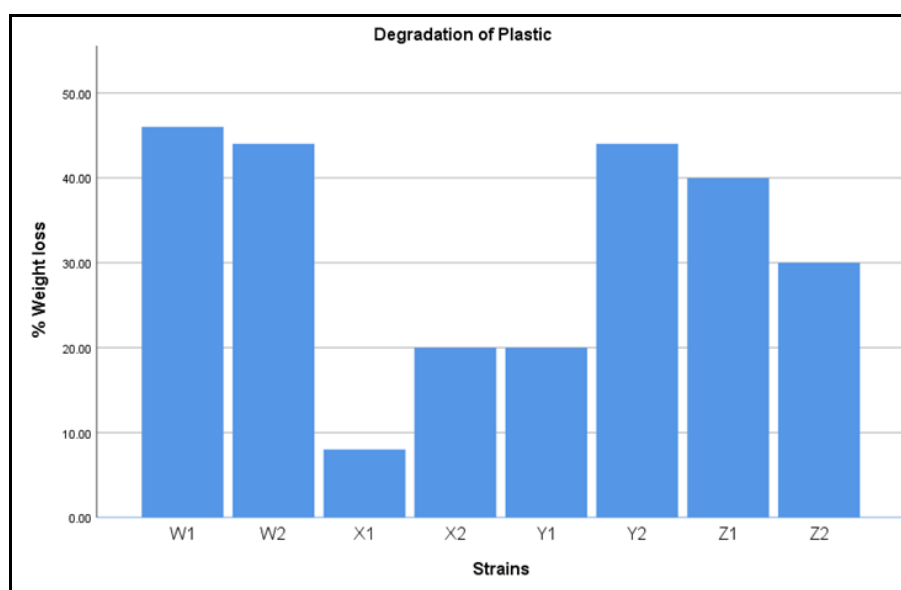


**Figure 5** Plastic degradation efficiency Graphical representation of the percentage weight loss of bacterial strain W1, W2, X1, X2, Y1, Y2, Z1, and Z2, the efficiency of these bacteria were evaluated for plastic degradation by measuring percentage weight loss. The strains W1, W2, and Y2 have the greatest degradation rates suggesting high potential applications of the strains should be screened on plastic as possible degrading agents, and X1 has very low activity. A control treatment was also included.

**Photographic evidence of degraded Plastics:** The strains exhibited rough and irregular pieces of plastic debris, indicating a substantial level of degradation and fragmentation of the plastic material (**Figure 6**). The presence of cracks, surface erosion, and fragmented particles suggests that the polymer

structure was significantly affected during the treatment process (**Figure 6**). These visible alterations are strong indicators of an advanced stage of plastic deterioration and may reflect a high potential of the strains for effective biodegradation of plastic materials.

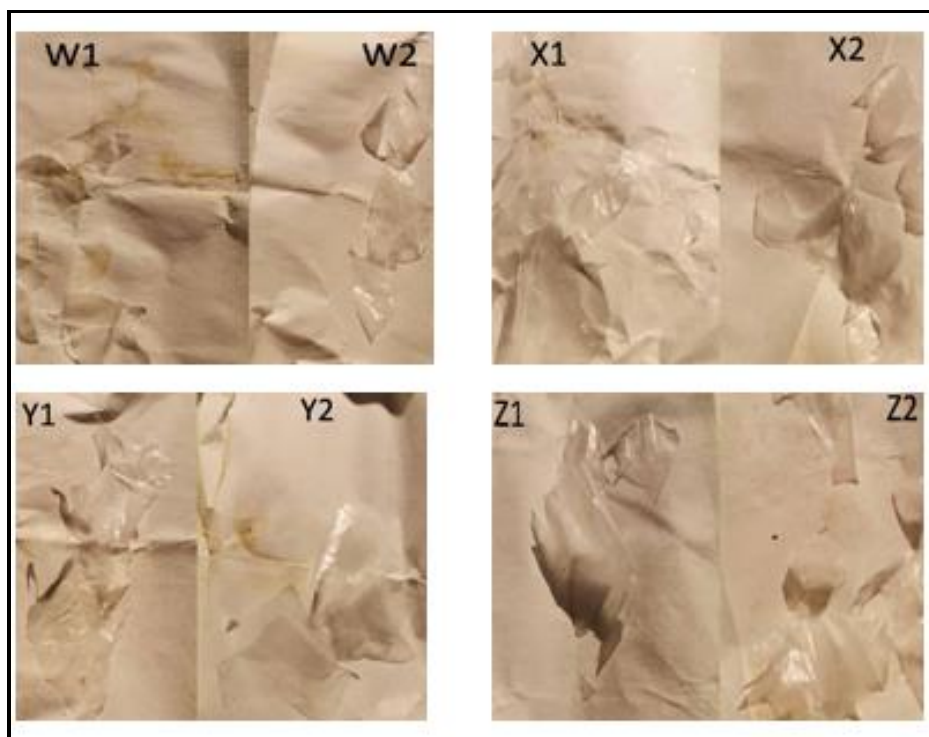


**Figure 6** Plastic degradation visual inspections with one-month exposure to bacterial strains W1, W2, X1, X2, Y1, Y2, Z1 and Z2. Degradation (within the samples) and fragmentation is recognizable towards W1, W2, and Y2, which provides evidence of high biodegradation capacity. In comparison, degradation is little on X1 and Z2.

Biofilm growth on a solid polymer, for example polyethylene, requires microbial colonization of polymer surfaces so that the bacteria can efficiently use non-soluble materials through biodegradation functions (Ali *et al.*, 2023). Biofilms, which are multidimensional microbiological colonies that attach to the surface of pollutants, have now been discovered in nature to be potent degrading agents (Thakur, 2012). This research addressed significant issues related to synthetic plastics, including their types, the isolation, and identification of degrading microorganisms. It also investigated disposal methods and the standards for assessing polymers breakdown. The study also investigated plastic biodegradation using the liquid culture technique (Saira *et al.*, 2022). Under suitable conditions and concentrations, most refractory polymers can be partially degraded. The present study is focused on isolating, characterizing, and assessing the plastic-degrading activity of bacteria isolated from soil. During morphological analysis, the microorganism exhibited various modifications. In this investigation, soil from the dumping site was collected and employed to investigate the biodegradation potential of the soil-isolated microorganisms. When assessing the

complete biodegradation process of any organic substrate, the formation of a microbial colony is critical for initiating bioremediation. Moreover, the longevity of microbial populations plays a significant role in total degradation time (Selvakumar *et al.*, 2021). Four Gram-positive and four Gram-negative bacterial strains were associated with the degrading materials. In the current study, plastic pieces were exposed to a broth medium to measure the extent of weight loss caused by bacterial action. A liquid culture medium containing bacterial isolates was held for one month. Biochemical characterization further supported the identification of the isolated bacterial strains. Positive catalase activity indicated the ability of the isolates to decompose hydrogen peroxide into water and oxygen, while the mannitol fermentation test differentiated bacterial strains based on their ability to utilize mannitol sugar through acid production (Arimurti *et al.*, 2023). The results demonstrated the microorganisms' capacity to degrade after one month of incubation. W1 and W2 species exhibited a higher proportion of weight loss due to degradation. The bacteria identified through microscopic morphology were *Bacillus spp.* (2 strains) based on microscopic morphology (Dörr *et*

al., 2019). This is due to the accumulation of microbial cells and the production of enzymes by microorganisms during the surface degradation process. In the case of polythene, enzymes utilize carbon, hydrogen, and oxygen during degradation by microorganisms to break it down. The polymer-monomer bond is easily cleaved by the microbiological cells (Hu *et al.*, 2023; El-Shafei *et al.*, 1998). An increase in the incubation period results in a significant weight loss of polythene bags demonstrating that it has a higher degrading capacity than other bacteria. Polyethylene was degraded at a moderate rate in our isolated bacterial strains. These strains Z1 and Z2 (*Bacillus spp.*, 2 strains), strains Y1 and Y2 (*Pseudomonas spp.*, 2 strains) also showed a moderate rate of degradation as compared to previous studies these bacterial strains showed a moderate rate of biodegradation (Kotova *et al.*, 2021), which observed only 12% and 15% biodegradation. Some other bacterial strains were able to degrade the plastic under laboratory conditions but in very low percentage. These strains were X1, X2 (*Moraxella spp.*) showed minimal polythene degradation. The study shows that in a shaker incubator for a month, bacteria from the soil of a waste dumping site were employed to degrade polyethylene plastic. The strains of *Bacillus spp.* W1 and W2 degraded plastic more effectively than *Moraxella spp.* (X1, X2), *Pseudomonas spp.* (Y1, Y2), and *Bacillus spp.* (Z1 and Z2). As daughter compounds were produced over time, the size and weight of the plastic decreased. This demonstrates significant biodegradation potential. These potential plastic degrading organisms can be further analyzed by genome sequencing studies.

## CONCLUSION

Microbial degradation of polyethylene relies on biofilm formation, enabling bacteria to utilize non-soluble substrates. This study isolated and characterized plastic-degrading bacteria from soil at the dumping site. Strains W1 and W2 (*Bacillus spp.*) showed the highest polyethylene weight loss after one month, attributed to enzyme production and polymer bond cleavage. Strains Z1, Z2 (*Bacillus spp.*), and Y1, Y2 (*Pseudomonas spp.*) exhibited moderate degradation, while X1, X2 (*Moraxella spp.*) showed minimal activity. The findings highlight the potential of *Bacillus spp.* for plastic degradation, supported by reduced plastic size and weight over time. Further analysis using Scanning Electron Microscope (SEM), Fourier transform infrared (FTIR) spectroscopy, and genome sequencing is recommended to optimize degradation mechanisms. This study underscores the promise of soil-isolated microorganisms in addressing plastic pollution, offering a sustainable approach for future bioremediation efforts.

## AUTHORS CONTRIBUTIONS

Conceived and designed the experiments:  
**M.Y.M., H.M.M., H.A.H.** Performed the

experiments: **M.Y.M., H.M.M., H.A.H.** Analyzed the data: **M.A., A.M., Q.A.Q., M.M., M.S.N.U.R.** Contributed reagents/materials/analysis tools: **Q.A.Q., M.Y.M.** Wrote the paper: **M.Y.M., A.M.**

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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