ISSN: 0973-4929, Vol. 20, No. (**3**) 2025, Pg. 1330-1342

Current World Environment

www.cwejournal.org

Isolation, Screening and Characterization of Plastic-Degrading Bacteria from Soil for PWM

SUMIT KUMAR DUBEY, PUSHPLATA CHANDRA and NEHA BEHAR*

Department of Biotechnology, DLS Postgraduate College, Affiliated to Atal Bihari Vajpayee University, Bilaspur, Chhattisgarh, India.

Abstract

Plastic pollution causes a potential environmental challenge due to its perseverance and detrimental effects on ecological system. The problem statement addresses the urgent need for biological solutions to mitigate plastic waste degradation for effective PWM as traditional methods like recycling and incineration are insufficient. In this study, bacterial strains have been reported for their capability of degrading plastic collected from soil samples from garbage. An opaque method was used for screening plastic-degrading bacteria. The SEM analysis of the plastic surface was conducted to evaluate the penetration efficacy of bacterial isolates. The SEM results revealed significant damage (e.g., notable holes and cracks) caused by bacteria on the surface of the test plastic strip under experimental conditions. The results demonstrated that the Isolate B-8 (*Bacillus* sp.) exhibited notable plastic degradation capabilities, as evidenced by a 37.5% reduction of LPDE in weight (from an initial weight of 0.08 g to a post-degradation weight of 0.05 g). SEM provided critical qualitative evidence linking bacterial colonization to the biodegradation of the LDPE films. In contrast to the smooth, homogenous surface of the untreated control, the treated samples exhibited extensive morphological damage, including cracks, fissures, and surface erosion. This confirms that the physical breakdown of the plastic was directly driven by Isolate B-8 (*Bacillus* sp.) infiltrating material to metabolize it, rather than just surface-level abrasion. Further visual evidence of structural damage validates the gravimetric data where weight was reduced. This indicates that Isolate B-8 (*Bacillus* sp.) utilized the plastic as a metabolized carbon source. The future prospects involve exploring consortium to synergistically break down different types of plastics. This research underscores the potential of microbial solutions in addressing plastic pollution, paving the way for sustainable environmental management strategies.

Article History
Received: 17 November 2025
Accepted: 06 January 2026

Keywords
Plastic Pollution;
Plastic Waste Management;
Plastic-Degrading Bacteria
Sustainable Environmental.

CONTACT Neha Behar nehа1_biotech@yahoo.com Department of Biotechnology, DLS Postgraduate College, Bilaspur University, Bilaspur, Chhattisgarh, India.

Doi: http://dx.doi.org/10.12944/CWE.20.3.29

Abbreviation

PWM	Plastic Waste Management
SEM	Scanning Electron Microscope
PP	Polypropylene
PS	Polystyrene
PVC	Polyvinyl chloride
PBT	Polybutylene terephthalate
LDPE	low-density polyethene
FTIR	Fourier-transform infrared spectroscopy
XRD	X-ray Diffraction
RTO	Regional Transport Office
NP	Non-degradable plastics
DP	degradable plastics
VP	Voges–Proskauer
MR	Methyl Red
GC-MS	gas chromatography-mass spectrometry
XRD	X-ray Diffraction
EPS	extracellular polymeric substances
MSM	Minimal Salt Medium

Introduction

Plastics are non-metallic substances, derived from fossil fuels that can be moulded into any desired shape. They are durable, resilient, moisture-resistant, lightweight polymers composed of carbon combined with hydrogen, nitrogen, sulphur, and other organic and inorganic elements. Owing to their adaptability, robustness, and low weight, they are widely used in medical, agriculture, and packaging industries. PVC, PBT, PP, PS, Polyethene and nylon are the most widely used polymers in the industry.[1] One of the main contributors to pollution in the environment is low-density polyethene (LDPE). Approximately, 140 million tonnes of synthetic polymers are produced each year worldwide and the usage is expanding at a pace of 12% annually. The buildup of plastic bags is posing a serious threat to the environment due to its tough biodegradation. Since its initial introduction in the 1950s, plastic has grown to be indispensable part of daily life. According to the Central Pollution Control Board, India, around 26,000 tonnes of plastic waste are produced every day. Biological agents, such as bacteria, can break the polymer link in plastic, therefore, microbial degradation is the ecofriendly and cost-effective alternative technique to address the environmental problems caused by plastic waste.[2] The microbes break down polymers into smaller components like monomers, oligomers, and water-soluble intermediates, which is then integrated into the cells of the bacteria.[3] The mechanisms by which bacteria degrade plastics involve the secretion of extracellular enzymes such as hydrolases, oxidases, and depolymerases.[4] For instance, polyethylene-degrading bacteria produce enzymes like laccase and manganese peroxidase, which oxidize the polymer backbone, facilitating its fragmentation.[5] Microbes with varied metabolic capacities can be used to bioremediate plastic wastes by utilising microbial strains that have been improved and genetically modified.[6] Moreover, bacterial consortia, consisting of different bacterial species with complementary enzymatic activities, have been found to enhance plastic degradation. Synergistic interactions within these consortia can lead to more efficient breakdown of complex polymers compared to individual strains.[7] In a study, the bacteria (*Lysinibacillus fusiformis*) degraded polythene by 21.87% at pH 3.5, with SEM and FTIR analysis, confirming surface damage and a 4.14% carbonyl index reduction. Similarly, *Bacillus cereus* also reduced tensile strength significantly at pH 9.5.[8] In recent study, seven bacteria were isolated from waste disposal sites in which *Bacillus* spp. showed 47.46% degradation of black plastic.[9] Additionally, they found *Escherichia coli*, *Corynebacterium spp., Micrococcus spp., Azotobacter spp*., *Pseudomonas spp*., and *Staphylococcus spp*. as plastic degraders. The analysis of structural changes in plastic using high end instruments: FTIR peak shifts, SEM-visible biofilms, and XRD confirmed the biodegradation process.[9] These studies exhibit potential of environmental bacteria as eco-friendly agents for mitigating plastic pollution. Numerous previous studies have confirmed that biofilm formation also plays a critical role in bacterial plastic degradation. This was usually examined with methods like SEM, FTIR, and XRD. SEM is widely used for surface characterization, FTIR for molecular bonds and functional groups, and XRD for the crystal structure of the sample. This study pioneers a dual-validation framework that integrates rapid opacity screening with confirmatory SEM analysis to rigorously establish the biodegradation potential of bacterial isolates relative to gravimetric data. However, primary objective of present study was to isolate and screen bacterial strains from soil samples and to conduct a detailed surface characterization of plastic materials degraded in the presence of bacterial isolates.

Materials and Methods

Soil samples were collected aseptically from the RTO road dumping area in Telipara, Bilaspur, and transported to the laboratory for subsequent experiments in accordance with the research protocol. We have used Low-Density Polyethylene (LDPE) derived plastic carry bags for assessing plastic degradation efficacy of bacterial isolates. Thickness of test plastic material was 45 µ. We have collected it from the waste dumping site.

The study was carried out in Central Laboratory of D.L.S P.G. College, Bilaspur, Chhattisgarh. The present course of research involved sampling, isolation of bacteria, screening of bacteria for plastic-degrading efficacy, followed by surface characterization of plastic to evaluate biodegradation potential using SEM. The detailed methodology is described as follows.

Isolation of Bacteria

The bacteria were isolated from soil samples collected from areas where garbage was dumped.[10,11] A soil sample of 1.0 g was mixed with 50 mL distilled water. Then, 1.0 mL of this mixture was diluted into 10 mL of sterile distilled water. The solution was serially diluted aseptically to reach dilution of 10^{-9}. Nutrient agar was poured into sterilized petri dishes and allowed to solidify. After solidification, 0.1 mL of the 10^{-6} dilution was aseptically spread onto agar plates. These plates were then incubated for 24 hours at 37°C followed by observation for bacterial growth.

Characterization of Bacteria

The grown bacterial colonies were further processed for pure culture preparation, and the prepared pure cultures were subjected to colony, morphological, microscopical and biochemical characterization using the standard protocol mentioned in Bergey's Manual of Systematic Bacteriology[12-14] and relevant literature works.[15,9]

Screening of Plastic-Degrading Bacteria

Plastic-degrading bacteria were qualitatively screened using the opacity (clear zone) method.[16] Sterilized agar media were supplemented with 1% starch to examine the enzymatic activity present in culture extracts of bacteria. The solidified agar plates were allowed to surface-dry overnight, and wells were meticulously cut to accommodate the loading of culture filtrates from isolated bacterial strains in their pure form. The plates containing bacterial culture filtrates were subsequently incubated at temperature of 37°C for a duration of five hours. Following incubation, the opacity surrounding the well surfaces was carefully observed. The reduction of opacity around colonies defines the potency of the bacterial strain for positive results. The bacterial strains with positive results were further selected for quantitative assay.

Microbial Degradation of Plastic in Laboratory Conditions

The quantitative determination of the plastic-degrading efficacy of bacteria was estimated.[17] A pre-weighed, clean, and washed plastic disc (2×2 cm) was taken. It was again wiped by acetone. Then plastic strips were dried and their dry weight was measured. Sterilized Minimal Salt Medium (MSM) was prepared by dissolving KH_2PO_4 (2.27 g), Na_2HPO_4 (5.97 g), NH_4Cl (0.5 g), $MgSO_4$ (0.25 g), $CaCl_2$ (0.0025 g), $FeSO_4$ (0.001 g), $MnSO_4$ (0.0005 g), and $ZnSO_4$ (0.001 g) in 500 mL of distilled water. The plastic disc and pure culture of bacteria (6.25 ml) were added to 100 ml of MSM. Inoculated MSM was incubated in an incubator shaker at 30°C temperature, with agitation of 150 rpm for a month. Plastic degradation was carried out by fermentation using a shaking incubator. To estimate plastic degradation using the weight-loss method, plastic samples were first accurately weighed to obtain their initial weights. These pre-weighed plastic strips were then exposed to experimental conditions involving different bacterial isolates. After a defined incubation period, the plastic samples were carefully retrieved for final analysis. To ensure accuracy and prevent contamination, each plastic strip was washed thoroughly with sterile distilled water, then sprayed with alcohol, and finally air-dried before its final weight was recorded. The plastic degradation percentage was found by measuring the weight loss with this formula:

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

Determination of Plastic Degradation of Plastic Strips by SEM

The SEM analysis of the plastic strips was conducted to assess the change in the plastic ssurface in the presence of bacterial isolates. The SEM analysis of non-degradable plastics (NP) as control (fig. 1) and NP after bacterial degradation (fig. 2) were outsourced from Sophisticated Test and Instrumentation Centre under Cochin University of Science and Technology (Cochin-682 022, Kerala, India) with Ref. No SAIF/SM/26274/250428. The analysis was conducted by SEM (INSTRUMENT JSM-6390) in Vacuum, and the analysis conditions are mentioned in Table 1.

Table 1: SEM Analysis Condition

Sample	Accelerating voltage	Magnification	Micron Marker	Spot Size
NP-1	20	250	100 µm	50 µm
NP-2	20	500	50 µm	50 µm
NP-3	20	1000	10 µm	50 µm
NP-4	20	3000	5 µm	50 µm
DP-1	20	250	100 µm	50 µm
DP-2	20	500	50 µm	50 µm
DP-3	20	1000	10 µm	50 µm
DP-4	20	3000	5 µm	50 µm

All biodegradation experiments were performed in triplicate (n=3) and data are presented as the mean (±) standard deviation (SD). Statistical analyses were conducted using MS Office 2021. To compare the degradation efficacy across different bacterial isolates, one-way Analysis of Variance (ANOVA) was performed. Significant differences between specific group means were identified using Tukey's Honest Significant Difference (HSD) post-hoc test.

Results

Bacterial strains have been reported for their capability of degrading plastic collected from soil samples were collected from garbage. A total of twelve different bacterial colonies were isolated from a soil sample collected from the RTO road dump area in Telipara, Bilaspur (C.G.). Colony, microscopic, and biochemical characterization of bacterial isolates are shown in Table 1. Based on the phenotypic characterization, the four plastic-degrading bacterial isolates viz., B-3, B-4, B-8, and B-9, exhibited close resemblance with *Streptococcus* sp., *Staphylococcus* sp., *Pseudomonas* sp. and *Bacillus* sp. respectively (Table 2).

Table 2: Morphological, Colony, and Biochemical Characteristics of Plastic-Degrading Bacterial Isolates

S. No.	Characteristics	Isolate B-3 (*Staphylococcus* sp.)	Isolate B-4 (*Pseudomonas* sp.)	Isolate B-8 (*Bacillus* sp.)	Isolate B-9 (*Streptococcus* sp.)
1	Gram Staining	Gram-positive	Gram-negative	Gram-positive	Gram-positive
2	Cell Shape	Cocci	Rods (Bacilli)	Rods (Bacilli)	Cocci (Chains)
3	Colony Morphology	Creamy, opaque, convex, smooth	Greenish, flat, irregular margins	Dry, irregular, rough	Pinpoint, translucent, circular
4	Nitrate Reduction	+	+	+	+
5	Urease Activity	-	-	-	-
6	H_2S Production	-	-	-	-
7	Citrate Utilization	-	+	-	-
8	VP	+	-	+	-
9	MR	+	-	-	+
10	Oxidase	-	+	-	-
11	Catalase	+	+	+	-
12	Starch Hydrolysis	-	-	+	-
13	Motility	Non-motile	Motile	Motile	Non-motile
14	Hemolysis on Blood Agar	No result	γ hemolysis	β hemolysis	No result
15	Indole Production	-	-	-	-
16	Growth at NaCl (7.5%)	+	-	+	-

Positive (+); Negative (-)

The identification of plastic-degrading bacterial isolates B-3, B-4, B-8 and B-9 was performed through systematic evaluation of their morphological, colony and biochemical characteristics following taxonomic keys provided in Bergey's Manual of Systematic Bacteriology and corroborated by recent studies on plastic-degrading microbes. Isolate B-3, tentatively identified as *Staphylococcus* sp. It was Gram-positive and coccoid in shape, formed creamy, opaque and convex colonies. Biochemically, it tested positive for catalase and nitrate reduction, and showed positive results for MR and VP tests while being negative for urease, H_2S, citrate, oxidase and indole production. Importantly it exhibited growth in 7.5% NaCl define trait of *Staphylococcus* species such as S. aureus and S. epidermidis. The lack of motility and catalase activity further supports its classification as per Holt (1994) and distinguished it from morphologically similar to *Streptococcus*.

Isolate B-4 identified as *Pseudomonas* sp. It was exhibited Gram-negative rod morphology and produced flat, greenish colonies with irregular margins and characteristic feature of pigment-producing *Pseudomonas* aeruginosa. It was oxidase and catalase-positive, motile, and capable of citrate utilization and nitrate reduction, aligning well with the genus description in Vos *et al*. (2009). The isolate tested negative for MR, VP, indole, urease, H_2S and starch hydrolysis which is typical for *Pseudomonas* spp. The non-hemolytic (γ hemolysis) nature and robust motility further confirmed its identity. These features also align with previous literature[15] which was involved with plastic-degrading using *Pseudomonas* isolates from waste sites. Isolate B-8 was identified as *Bacillus* species based on its Gram-positive, rod-shaped morphology and formation of dry, irregular, and rough colonies. These traits are commonly associated with spore-forming *Bacillus* spp. It showed positive results for catalase, VP, and starch hydrolysis tests, and was motile and capable of growing in 7.5% NaCl. It was negative for urease, H_2S, indole, citrate and oxidase. The β-hemolytic activity on blood agar and starch hydrolysis are diagnostic markers showed in Bergey's Manual for differentiating *Bacillus* subtilis and related species. These findings are in line with earlier literature which describes similar biochemical traits in *Bacillus* strains capable of polyethene degradation.[9]

Isolate B-9 was attributed to genus *Streptococcus*. It was a Gram-positive coccus occurring in chains and formed pinpoint, translucent and circular colonies. It was catalase-negative, non-motile and exhibited positive reactions for the MR and nitrate reduction tests. All other tests including VP, citrate, H_2S, oxidase, urease and starch hydrolysis were shown negative results. The absence of growth in high salt concentration (7.5% NaCl) and catalase negativity differentiate it from *Staphylococcus*. This confirms it as *Streptococcus* genus as described by Holt (1994). The lack of hemolytic activity (no result recorded) limits precise species-level identification, though the MR-positive, catalase-negative profile supports the tentative classification. Similar traits have been documented in environmental *Streptococcus* spp. involved in biofilm formation and bioplastic interaction.[15] This results strongly support the tentative identification of the bacterial isolates using classical microbiological techniques in agreement with the systematic taxonomy approaches outlined in Bergey's Manual and peer-reviewed literature. These bacterial isolates viz., *Staphylococcus*, *Pseudomonas*, *Bacillus* and *Streptococcus* spp. are not only taxonomically distinct but also represent genera previously reported for their potential roles in the biodegradation of plastics and other recalcitrant polymers.

The results divulged that bacterial isolates exhibited notable plastic degradation capabilities. Four bacterial isolates were evaluated for 30-day. Among them, Isolate B-8 (*Bacillus* sp.) demonstrated the highest degradation efficacy, reducing the initial plastic weight (0.08 g) by 37.5% (Table 2). This was followed by Isolate B-3 (*Staphylococcus* sp.) and Isolate B-4 (*Pseudomonas* sp.), which exhibited 33.8% and 28.8% reduction, respectively. Isolate B-9 (*Streptococcus* sp.) showed the least activity with 16.3% reduction.

Table 3: Plastic Degradation Efficacy of Bacterial Isolates

Bacterial Isolates	Days	Initial Weight (g)	Final Weight (g)	Reduction (%)
Isolate B-3 (*Staphylococcus* sp.)	30	0.08 ±0.004	0.053 ±0.002	33.8
Isolate B-4 (*Pseudomonas* sp.)			0.057 ±0.002	28.8
Isolate B-8 (*Bacillus* sp.)			0.05 ±0.001	37.5
Isolate B-9 (*Streptococcus* sp.)			0.067 ±0.003	16.3

The study revealed that the bacterial isolates possessed notable plastic degradation capabilities. Preliminary screening utilized opacity zones as a qualitative proxy for extracellular enzymatic activity; the formation of clear halos around colonies confirmed the secretion of depolymerases capable of hydrolyzing the polymer matrix. Following this, a biodegradation assay was conducted in conical flasks containing nutrient broth inoculated with the respective test bacteria and plastic strips. Over a 30-day incubation period, four bacterial isolates were evaluated. Among them, Isolate B-8 (*Bacillus* sp.) demonstrated the highest degradation efficacy of 37.5% representing the most significant reduction in the initial weight of the plastic polymer.

Prior to statistical evaluation, data were screened to ensure assumptions of normality and homogeneity of variance were met. Subsequent statistical assessment confirmed significant variation in the biodegradation potential of the tested isolates [$F(3,8) = 21.68$, $p < 0.001$]. Since a lower residual weight corresponds to greater polymer consumption, Isolate B-8 (*Bacillus* sp.) demonstrated the highest degradation efficacy, achieving the lowest final weight (0.050 ±0.003g). Post-hoc analysis (Tukey's HSD) confirmed that this result was significantly better than that of the least efficient strain, Isolate B-9 (*Streptococcus* sp.), which retained the highest final weight (0.067 ±0.002 g, $p < 0.01$). However, differences in final weights among Isolates B-3, B-4, and B-8 were not statistically significant ($p > 0.05$). This indicated that these strains possess comparable degradation capabilities. This high performance across taxonomically diverse isolates presents a compelling opportunity to evaluate a microbial consortium. Such a consortium could be pivotal in targeting recalcitrant Non-degradable Plastics (NP), such as LDPE, potentially achieving degradation rates comparable to those typically seen with engineered Degradable Plastics (DP).

Similar work was reported where *B.brevis* degraded microplastic's by 19.8% in 35 days.[16] Polethylene and Plastic degrading microbes isolated from salt-tolerant mangrove soil and revealed that microbial counts in the degrading materials reached up to 79.67×104 per gram for total heterotrophic bacteria.[17]

Discussion

The isolation of plastic-degrading bacteria from soil involves collecting soil samples from environments exposed to plastic waste and then subjecting them to enrichment culture techniques to promote the growth of plastic-degrading bacteria by incubating the soil samples with plastic polymers.[18] After enrichment, bacterial colonies exhibit clear zones of polymer and degradation on solid media can be isolated and purified for further characterization.[19] Qualitative screening can be performed using the clear zone method, as strains that produce clear zones around their colonies indicate polymer degradation by secreting extracellular enzymes,[20] while quantitative screening involves measuring the weight loss of plastic films or particles incubated with bacterial cultures over time. This can be further detected using high-throughput analytical instruments viz., FTIR, SEM and GC-MS to monitor changes in the chemical structure and surface morphology of the plastics, as well as the identification of degradation by-products.[21] Soil samples were collected from various dumping sites in five districts (including Solan, Bilaspur, Hamirpur, Mandi, and Kangra) of Himachal Pradesh. The plastic-degrading microorganisms were isolated on M9 media enriched with polyethylene glycol (sole carbon source).[22] A similar study was conducted and they observed *S. aureus*, *Streptococcus* sp, *Bacillus* sp, and *E. coli* as plastic degraders.[23] The biodegradation of low-density polyethene by microorganisms from garbage soil was reported that *Pseudomonas* sp. showed 24.22% plastic degradation in six months.[24] Biodegradation potential of polythene-degrading *Avicennia marina* isolated from the rhizosphere soil has been evaluated. VASB14 (*Lysinibacillus fusiformis*, confirmed by 16S rRNA gene sequencing) showed highest weight loss in polythene with average of 21.87 ± 6.37% recorded at pH 3.5 after two months of shaking at room temperature.[8] They also reported reduction in tensile strength (87.50 ± 4.8%) with VASB1 (*Bacillus cereus*, confirmed by 16S rRNA gene sequencing) at pH 9.5 and further plastic degradation was confirmed by SEM and FTIR. SEM revealed visible scions and cracks on the surface of degraded polythene and FTIR analysis demonstrated a maximum reduction of 4.14% in carbonyl index in untreated polythene strips inoculated with L fusiformis strain VASB14/WL.[8]

Recently, researchers successfully isolated seven bacterial species namely *Azotobacter* spp., *E. coli*, *Bacillus* spp., *Corynebacterium* spp., *Micrococcus* spp., *Staphylococcus* spp. and *Pseudomonas* spp. from waste disposal site soils. Among them, *Bacillus* spp. showed highest degradation efficiency for black plastic (47.46% reduction in weight).[9] *E. coli* and *Corynebacterium* spp. effectively degraded pink plastic by achieving 46.42% and 45.76% reduction, respectively.[9] *Pseudomonas* spp. and *Micrococcus* spp. demonstrated significant degradation of white plastic (46.43%) and CSD biodegradable plastic (56.60%), highlighting their potential for plastic bioremediation. The significant optical density during the degradation experiment indicated robust bacterial growth in the presence of CSD plastic which showed its potential for plastic biodegradation. Further, the FTIR analysis confirmed plastic degradation with a notable shift in peak from 2916 cm^{-1} to 2914 cm^{-1}, which is an indication of alterations in alkane and alkene bonds. Moreover, SEM observation revealed the bacterial colonization, surface cracks, and spots on degraded plastics, while XRD analyses exhibited the significant structural changes with peak intensities at 4.083, 3.705, 3.020, and 1.909 Å. These findings collectively demonstrate that waste disposal sites harbor potent plastic-degrading bacteria, which could serve as effective biological agents for reducing environmental plastic pollution.

The current study aligns with prior findings that support the use of the zone of clearance (opacity) technique as an effective screening method for identifying plastic-degrading bacteria.[15,16] In the present investigation, phenotypic characterization of four isolates, designated B-3, B-4, B-8, and B-9, suggested their close resemblance to *Streptococcus* sp., *Staphylococcus* sp., *Pseudomonas* sp. and *Bacillus* sp. respectively. These genera have been widely reported in literature [9,15,25] for their capacity to degrade synthetic polymers due to their enzymatic versatility and adaptive metabolic pathways.

The ability of bacteria to form biofilms on plastic surfaces is critical in the degradation process, as biofilm formation facilitates stable attachment, nutrient acquisition, and the localized concentration of EPS which enhance the biodegradation efficiency.[26] In agreement with the present findings, bacterial strains *Pseudomonas* spp. have been previously been recognized for their roles in polymer breakdown and surface colonization.[27] The development of biofilms over the 28-day incubation period underscores the importance of extended observational durations in assessing microbial plastic degradation.[9,28] Furthermore, the morphological alterations observed on plastic surfaces post-incubation, including microbial colonization, surface pitting, and structural cracking, were consistent with earlier findings [4] where SEM revealed comparable degradation features following 30 days of bacterial exposure. These surface changes provide strong visual evidence of the biodegradation process and validate the effectiveness of the isolated strains in initiating physical and chemical breakdown of plastic polymers. Collectively, the integration of phenotypic identification, biofilm dynamics, and structural analyses through SEM contributes to a comprehensive understanding of bacterial-mediated plastic degradation and reinforces the ecological potential of these microorganisms in mitigating plastic pollution. Bacterial strains have been reported for their capability of degrading plastic that has been isolated from soil samples collected from garbage.[29,10,11] Plastic waste management is a critical environmental challenge due to the persistence of plastics in the environment and their adverse ecological impacts. Traditional methods of plastic waste disposal, such as landfilling and incineration, have limitations and contribute to secondary pollution problems. Therefore, bioremediation using plastic-degrading bacteria offers a promising and eco-friendly alternative for mitigating plastic pollution.

Besides notable results, the differences in final weights among Isolates B-3, B-4 and B-8 were not statistically significant ($p > 0.05$) which indicated that these strains possess comparable degradation capabilities. Henceforth this comparable high performance of these taxonomically diverse isolates presents a compelling opportunity to evaluate a microbial consortium towards further extension of present work. Since these bacteria belong to distinct genera, it might be possible that these bacterial isolates utilize different metabolic pathways and enzymatic systems to break down polyethylene. Therefore, co-culturing of these isolates could potentially induce a synergistic effect and lead to accelerated degradation rates and more complete mineralization of the plastic waste.

Additionally, genetic engineering techniques can be utilized to enhance the plastic-degrading capabilities of these bacteria, making them more efficient and useful.[30] Furthermore, understanding the natural occurrence and distribution of plastic-degrading bacteria in various ecosystems can build strategies for *in situ* bioremediation. This approach uses microbial communities present in polluted environments, reducing the need for introducing exogenous strains and minimizing ecological disruptions.[31] Despite the promising potential of plastic-degrading bacteria, several challenges need to be addressed. Additionally, the efficiency of plastic degradation by bacteria can be influenced by factors such as the type of plastic, environmental conditions, and the presence of inhibitory substances.[32-35] One of the main challenges is the relatively slow degradation rates of plastics compared to their production and accumulation in the environment. Future research should focus on optimising the conditions for bacterial degradation of plastics, exploring microbial consortia for synergistic effects, and identifying novel plastic-degrading enzymes through metagenomic approaches in relation to the present work. Furthermore, high-throughput transcriptome-based methods can facilitate the identification of differential gene expressions under various growth conditions during plastic biodegradation, thereby providing valuable insights.[36] Recent trends focused on understanding the interactions between genes and proteins, as well as elucidating the functions of the involved genes which can augment our comprehension of the plastic degradation process.[37,38]

Recently, G. *mellonella* has been reported for average 28.9% reduction of LDPE in 36 hours at 25°C.[39] Likewise, strain KKU-LDPE4 has been documented for highest biodegradation efficiency of 2.66% weight loss after 30 days of incubation with LDPE particles of plastic sheets.[40] During the present work, Isolate B-8 (*Bacillus* sp.) exhibited notable plastic degradation efficacy of 1.6-fold (37.5%) reduction of LDPE. The present SEM result provided critical qualitative evidence linking bacterial colonization to the biodegradation of LDPE films. In contrast to the smooth, homogenous surface of the untreated control, the treated samples exhibited extensive morphological damage, including cracks, fissures, and surface erosion. Most notably, the micrographs captured the active penetration of bacterial cells into the polymer matrix, creating deep pits and cavities; this confirms that the physical breakdown of the plastic was directly driven by the bacteria infiltrating the material to metabolize it, rather than just surface-level abrasion. The quantitative weight loss directly correlated with qualitative SEM observations. This establishes a clear correlation where significant mass reduction aligns with extensive surface erosion. Isolate B-8, which achieved the highest degradation efficacy, correspondingly exhibited the most severe pitting and deep bacterial penetration in micrographs.

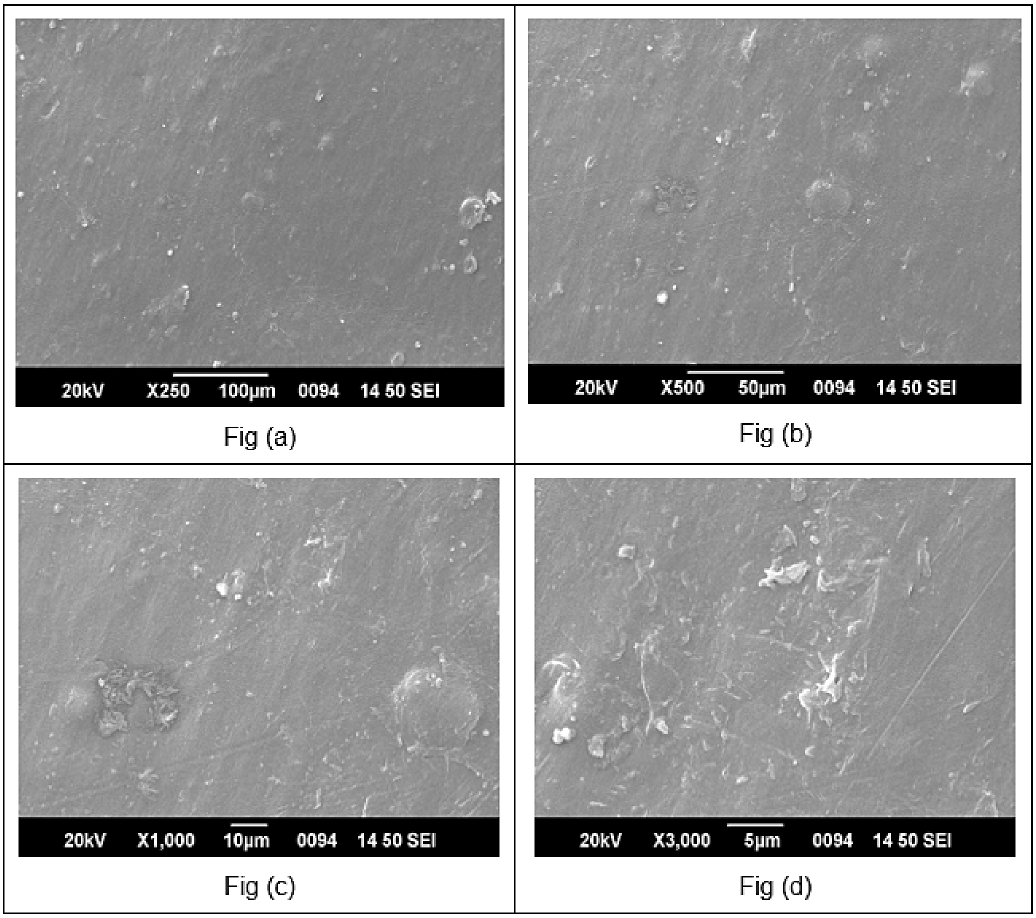

Fig. 1: (a-d) SEM image of non-biodegradable plastic as control (without bacterial degradation) (a) SEM 250×; (b) SEM 500×; (c) SEM 1000×; (d) SEM 3000×

Finally, plastic waste treatment technologies (e.g., sustainable valorization, biological degradation and enhanced sorting techniques) must be both robust and practical for large-scale deployment of microbial adaptability within the environment.[41-43] Thus, scaling up microbial-based plastic waste treatment technologies could serve as a highly effective solution to address the global issue of plastic waste.

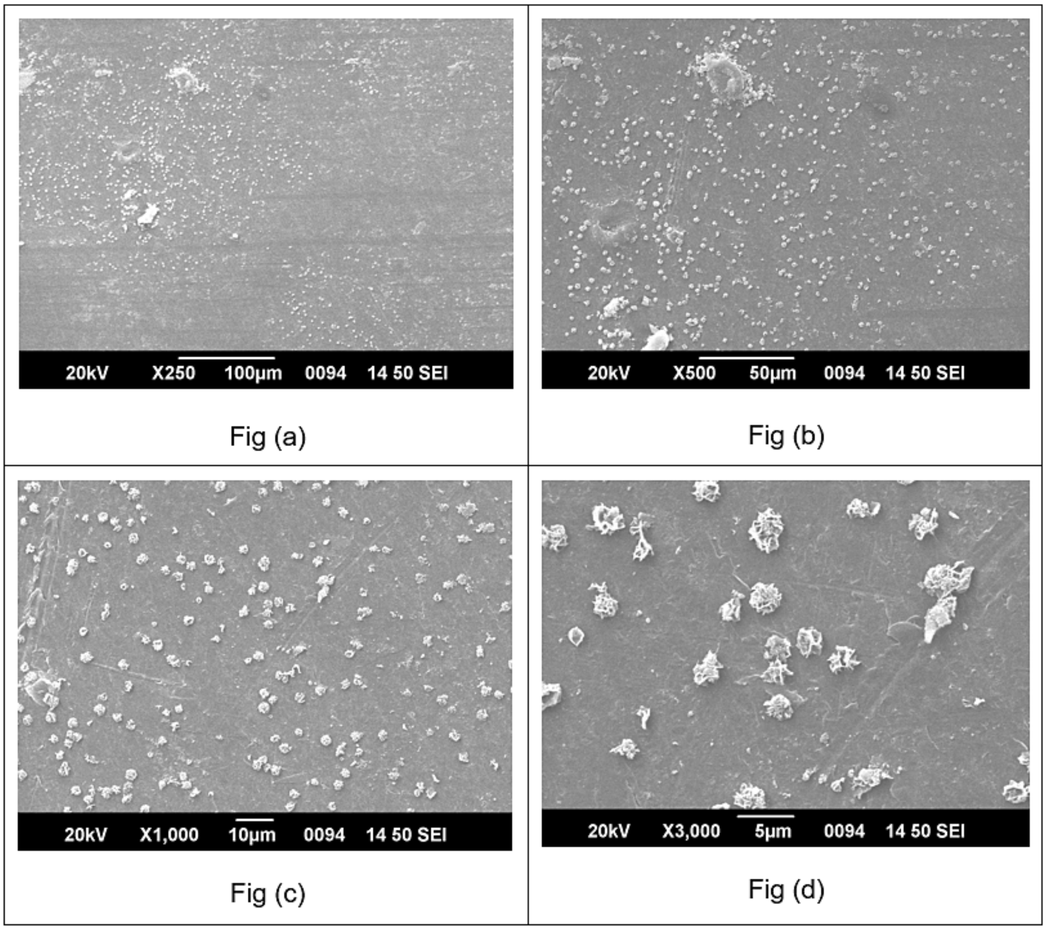

Fig. 2: (a-d) SEM image of non-biodegradable plastic after bacterial degradation (a) SEM 250× (b) SEM 500× (c) SEM 1000× (d) SEM 3000×

Conclusion

The isolation and screening of bacteria that degrade plastic from soil represent a promising approach in addressing the global plastic waste crisis. While significant progress has been made in understanding the mechanisms of bacterial plastic degradation, further research and technological innovations are needed to harness the full potential of these microorganisms for sustainable plastic waste management. Presently, there is lack of data on the capacity and potential of enzymes involved in the breakdown of plastics. Therefore, optimization studies should be carried away for enzymatic conditions, enzyme structure and the catalytic mechanism to get desired result. The pretreatment methods for microbiological breakdown of synthetic plastics may also play important role. Plastic biodegradation is an environment friendly and also economical approach for PWM. But no studies are carried away in the actual field. So, the Lab to Land approach is needed for actual evaluation. The future prospects involve exploring consortium approaches to synergistically break down different types of plastics. In the future, the focus should also be on genetically modifying bacteria to enhance their capacity to degrade plastic. However, limitations include the variable degradation rates among isolates and the incomplete breakdown of plastic polymers, highlighting the complexity of microbial plastic degradation. This research underlined the potential of microbial solutions in addressing plastic pollution and paving the way for sustainable environmental management strategies.

Acknowledgement
The authors are grateful to D.L.S. P.G. College, Bilaspur, for providing the necessary infrastructure and well-equipped laboratory facilities to carry out this research work. We also extend our sincere thanks to STIC India for providing the Scanning Electron Microscopy (SEM) analysis facilities.

Funding Sources
The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest
The authors do not have any conflict of interest.

Data Availability Statement
This statement does not apply to this article.

Ethics Statement
This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement
This study did not involve human participants, and therefore, informed consent was not required.

Permission to reproduce material from other sources
Not Applicable

Author Contributions
- **Sumit Kumar Dubey:** Methodology design, Supervision, data validation and manuscript editing.
- **Pusplata Chandra:** Experimental work and initial manuscript writing.
- **Neha Behar:** Conceptualization, compiling and final manuscript drafting.

References

1 Ali, A., Bahadar, A., Khan, A., & Sanaullah, K. Role of agricultural waste in recycled plastic biocomposites. *Recycled Plastic Biocomposites.* 2022: 165–94. https://doi.org/10.1016/B978-0-323-88653-6.00002-X

2 Cai, Z., Li, M., Zhu, Z., Wang, X., Huang, Y., Li, T., Gong, H., & Yan, M. Biological degradation of plastics and microplastics: A recent perspective on associated mechanisms and influencing factors. *Microorganisms.* 2023; 11(7): 1661. https://doi.org/10.3390/microorganisms11071661

3 Mohanan, N., Montazer, Z., Sharma, P. K., & Levin, D. B. Microbial and enzymatic degradation of synthetic plastics. *Front Microbiol.* 2020; 11: 580709. https://doi.org/10.3389/fmicb.2020.580709

4 Putcha, J. P., & Kitagawa, W. Polyethylene biodegradation by an artificial bacterial consortium: Rhodococcus as a competitive plastisphere species. *Microbes Environ.* 2024; 39(3): ME24031. https://doi.org/10.1264/jsme2.ME24031

5 Suresh, V., Shams, R., Dash, K. K., Shaikh, A. M., & Béla, K. Comprehensive review on enzymatic polymer degradation: A sustainable solution for plastics. *J Agric Food Res.* 2025; 20: 101788. https://doi.org/10.1016/j.jafr.2025.101788

6 Lokesh, P., Shobika, R., Omer, S., Reddy, M., Saravanan, P., Rajeshkannan, R., Saravanan, V., & Venkatkumar, S. Bioremediation of plastics by the help of microbial tool: A way for control of plastic pollution. *Sustainable Chemistry for the Environment.* 2023; 3: 100027. https://doi.org/10.1016/j.scenv.2023.100027

7 Al Balushi, A., Amara, Y., Al Balushi, K., Mannai, S. E., Ben Saber, R., Masmoudi, A. S., Cherif, A., & Souissi, Y. Harnessing the plastic-degrading potential of *Pseudomonas* species for environmental sustainability. IOP *Conf Ser Earth Environ Sci.* 2024; 1401: 012009. https://doi.org/10.1088/1755-1315/1401/1/012009

8 Shahnawaz, M., Sangale, M. K., & Ade, A. B. Rhizosphere of Avicennia marina (Forsk.) Vierh. as a landmark for polythene

degrading bacteria. *Environmental Science and Pollution Research International.* 2016; 23(14): 14621–14635.

9 Javid, F., Ali, G., Rehman, A., Naeem, R., Ali, I., & Naz, I. Assessment of plastic degradation by indigenous bacteria from waste disposal sites. *Emerging Contaminants.* 2024; 10(3): 100323.

10 Shruthi S.D., Ghosh, S., Chowdary, M. Isolation and molecular characterization of plastic Degrading bacteria from dumped garbage soil. *Innovare Journal of Life Sciences.* 2020; 8(5): 1-5.

11 Usha, R., Sangeetha, T., & Palaniswamy, M. Screening of Polyethylene Degrading Microorganisms from Garbage Soil. Libyan *Agric Res Center J Internati.* 2011; 2(4): 200-204.

12 Williams, S. T., Sharpe, M. E., & Holt, J. G. Bergey's *Manual of Systematic Bacteriology,* 1st ed., vol. 4. Williams and Wilkins, Baltimore. 1989.

13 Vos, P., Garrity, G. M., Jones, D., Krieg, N. R., Ludwig, W., Rainey, F. A., Schleifer, K.-H., & Whitman, W. B. (Eds.). *Bergey’s manual of systematic bacteriology:* Volume 3: The Firmicutes (2nd ed.). Springer. 2009.

14 Holt, J. G. Bergey's *Manual of Determinative Bacteriology,* 9th ed. Williams & Wilkins, Baltimore. 1994.

15 Jia, J., Liu, Q., Zhao, E., Li, X., Xiong, X., & Wu, C. Biofilm formation on microplastics and interactions with antibiotics, antibiotic resistance genes and pathogens in aquatic environment. *Eco-Environ Health.* 2024; 3(4): 516–28. https://doi.org/10.1016/j.eehl.2024.05.003

16 Tiwari N, Santhiya D, Sharma JG. Degradation of polyethylene microplastics through microbial action by a soil isolate of *Brevibacillus brevis. Polym Degrad Stab.* 2023;215:110436. doi:10.1016/j.polymdegradstab.2023.110436

17 Kathiresan, K. Polythene and plastic degrading microbes from mangrove soil. *International Journal of Tropical Biology and Conservation.* 2003; 51: 629-633.

18 Gupta, K. K., Yadav, S. S., & Devi, S. Biodegradation of plastics: A state of the art review. *Frontiers in Microbiology.* 2021; 12: 792340.

19 Yuan, J., Ma, J., Sun, Y., Zhou, T., Zhao, Y., Yu, F., & Dang, Z. Microbial degradation and other environmental aspects of microplastics/plastics. *Science of the Total Environment.* 2020; 715: 136968.

20 Ghatge, S., Yang, Y., Ahn, J.-H., & Hur, H.-G. Biodegradation of polyethylene: a brief review. *Applied Biological Chemistry.* 2020; 63(1). https://doi.org/10.1186/s13765-020-00511-3

21 Auta, H. S., Emenike, C. U., & Fauziah, S. H. Distribution and importance of microplastics in the marine environment: A review of the sources, fate, effects, and potential solutions. Environment International. 2017; 102: 165-176.

22 Rana, K., & Rana, N. Isolation and Screening of Plastic Degrading Bacteria From Dumping Sites of Solid Waste. *Int.J.Curr.Microbiol.App.* Sci. 2020; 9(07): 2611-26.

23 Hossain, K. S., Das, S., Kundu, S., Afrin, S., Nurunnabi, T. R., & Rahman, S. M. M. Isolation and characterization of polythene degradating bacteria from garbage soil. *International journal of agriculture Environment and bioresearch.* 2019; 4(5): 254-263.

24 Deepika, S., & Jaya Madhuri, R. Biodegradation of low density Polyethylene by micro-organisms From garbage soil. *Journal of Experimental Biology and Agricultural Sciences.* 2015; 3(1): 15-21.

25 Grigary, S., & Umesh, M. Floral waste as a potential feedstock for polyhydroxyalkanoate production using halotolerant *Bacillus cereus* TS1: optimization and characterization studies. *Biomass Conversion and Biorefinery.* 2023.

26 Flemming, H.-C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A., & Kjelleberg, S. Biofilms: an emergent form of bacterial life. *Nature Reviews. Microbiology.* 2016; 14(9): 563–575.

27 Yang, Y., Yang, J., Wu, W.-M., Zhao, J., Song, Y., Gao, L., Yang, R., & Jiang, L. Biodegradation and mineralization of polystyrene by plastic-eating mealworms: Part 1. Chemical and physical characterization and isotopic tests. *Environmental Science & Technology.* 2015; 49(20): 12080–12086.

28 Lebeaux, D., Ghigo, J.-M., & Beloin, C. Biofilm-related infections: bridging the

gap between clinical management and fundamental aspects of recalcitrance toward antibiotics. *Microbiology and Molecular Biology Reviews:* MMBR. 2014; 78(3): 510–543.

29 Chen M, Li J, Zhang L. A novel plastic-degrading bacteria isolated from farmland soil utilizes non-sole carbon sources to enhance the degradation of poly(butylene adipate-co-terephthalate). *J Environ Chem Eng.* 2025;13(2):115461. doi:10.1016/j.jece.2025.115461

30 Martín-González D, de la Fuente Tagarro C, De Lucas A, Bordel S, Santos-Beneit F. Genetic modifications in bacteria for the degradation of synthetic polymers: a review. *Int J Mol Sci.* 2024;25(10):5536. doi:10.3390/ijms25105536

31 Tiwari, A., Manickam, N., & Ramachandra, T. V. Synergistic microbial consortia for plastic biodegradation: A review of the current status and future prospects. *Environmental Technology & Innovation.* 2021; 23: 101565.

32 Montazer, Z., Habibi-Najafi, M. B., Mohebbi, M., & Oromiehie, A. Microbial degradation of UV-pretreated low-density polyethylene films by novel polyethylene-degrading bacteria isolated from plastic-dump soil. *Journal of Polymers and the Environment*. 2020; 28(3): 1100-1112.

33 Pilapitiya PGCNT, Ratnayake AS. The world of plastic waste: a review. *Clean Mater.* 2024;11:100220. doi:10.1016/j.clema.2024.100220

34 Ekanayaka AH, Tibpromma S, Dai D., Ruifang X., Suwannarach N., Stephenson S.L., Dao C., Karunarathna, S.C. A review of the fungi that degrade plastic. J Fungi (*Basel*). 2022;8(8):772. doi:10.3390/jof8080772

35 Dimassi SN, Hahladakis JN, Yahia MND, Ahmad MI, Sayadi S, Al-Ghouti MA. Degradation-fragmentation of marine plastic waste and their environmental implications: a critical review. *Arab J Chem.* 2022;15(11):104262. doi:10.1016/j.arabjc.2022.104262

36 Roy A, Chakraborty S. A comprehensive review on sustainable approach for microbial degradation of plastic and its challenges. *Sustain Chem Environ.* 2024;7:100153. doi:10.1016/j.scenv.2024.100153

37 Reddy CN, Kallem P, Mounika KVSSN, Muqeet A, Raj JCJ, Aishwarya CVS, Gupta RK, Polisetti V, Mishra B, Yadavalli R, Mandal SK, Hedenqvist MS, Banat F. Review of microplastic degradation: Understanding metagenomic approaches for microplastic degrading organisms. *Polym Test.* 2023; 128(108223): 108223. doi:10.1016/j.polymertesting.2023.108223

38 Qian K, Wang L, Teng J, Liu G. Strategies and technologies for sustainable plastic waste treatment and recycling. *Environ Funct Mater*. 2025. doi:10.1016/j.efmat.2025.01.004

39 Poma O, Ricce B, Beraún J, Perez Carpio JE, Fernandez H, Soria J. Biodegradation of Low-Density Polyethylene-LDPE by the Lepidopteran Galleria *Mellonella* Reusing Beekeeping Waste. *Front Bioeng Biotechnol.* 2022; 10: 915331. doi:10.3389/fbioe.2022.915331

40 Wanapat, M., Muslykhah, U., Matra, M., Dagaew, G., Suriyapha, C., Sommai, S., Pongsub, S., Phupaboon, S. Biodegradation of low-density polyethylene plastics by cellulolytic *Pseudomonas* aeruginosa isolated from the rumen of Swamp buffalo and the in vitro end-product characteristics. *Environmental Technology & Innovation.* 2025; 38(104175): 104175. https://doi.org/10.1016/j.eti.2025.104175

41 Newrick BA, Valdés D, Laca A, Laca A, Díaz M. Enhanced biodegradation of high-density polyethylene microplastics: study of bacterial efficiency and process parameters. *J Hazard Mater.* 2025;485:136822. doi:10.1016/j.jhazmat.2024.136822

42 Alabi OO, Akande TO, Joseph Gbadeyan O, Deenadayalu N. Advanced technologies for plastic waste recycling: examine recent developments in plastic waste recycling technologies. *RSC Adv.* 2025;15(48):40541-40557. doi:10.1039/d5ra06715d

43 Saxena S. Pyrolysis and beyond: sustainable valorization of plastic waste. *Appl Energy Combust Sci.* 2025;21:100311. doi:10.1016/j.jaecs.2024.100311