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ISOLATION, CHARACTERIZATION, AND IDENTIFICATION OF PLASTIC DEGRADING BACTERIA FROM LANDFILL SITES IN SURAT, GUJARAT

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ABSTRACT

Plastic pollution remains a critical environmental challenge due to the recalcitrant nature of synthetic polymers. This study aimed to isolate and identify indigenous bacterial strains capable of degrading plastics from three dumping sites in Surat, Gujarat: Khajod (Site-1 & 2), and Althan (Site-3). A total of 18 bacterial isolates were screened using Polyethylene Glycol (PEG-400 and PEG-1500) as a sole carbon source. Quantitative analysis via weight loss and spectrophotometric methods (OD 600nm) was conducted over a 37-day period using Low-Density Polyethylene (LDPE). Five potential isolates; PDBK-3, PDBK-5, PDBKC-14, PDBA-15, and PDBA-18 showed significant degradation capabilities. Isolate PDBA-15 (*Pseudomonas* sp.) emerged as the most potent isolate, representing a weight loss of 8.70%. Morphological and biochemical characterization identified these potential degraders as species of *Klebsiella, Bacillus, Pseudomonas,* and *Proteus*. These findings suggest that indigenous landfill bacteria possess significant potential for the bioremediation of plastic waste.

KEYWORDS: Plastic degradation, LDPE, PEG, Bioremediation, Landfill.

1. INTRODUCTION

Plastic is a versatile, durable, and cost-effective material, but its accumulation in the environment has led to a major ecological crisis. Low-density polyethylene (LDPE) is one of the most widely used synthetic polymers in packaging and single-use bags, yet it is highly resistant to natural degradation (Yao et al., 2022). Due to its high molecular weight and hydrophobic nature, LDPE remains in ecosystems for decades, causing physical harm to wildlife and leaching toxic chemicals into soil and water (Thushari & Senevirathna, 2020).

Traditional waste management strategies, such as mechanical recycling and incineration, often suffer from high energy costs or the release of harmful by-products (Damayanti et al., 2022). Consequently, biological degradation or bioremediation has emerged as a sustainable and eco-friendly alternative. This process involves the enzymatic breakdown of polymers by microorganisms like bacteria and fungi, which utilize the carbon backbone of plastics as an energy source (Lim & Thian, 2022).

Landfills and dumping sites are considered "hotspots" for plastic-degrading microbes. Over time, indigenous bacteria in these nutrient-deprived environments undergo selective pressure, evolving metabolic pathways to utilize synthetic polymers (Auta et al., 2017). Previous studies have identified genera such as *Bacillus, Pseudomonas,* and *Rhodococcus* as effective degraders of unpretreated LDPE (Dey et al., 2020). However, isolating localized strains remains crucial for developing site-specific remediation strategies. This study aims to isolate and characterize LDPE-degrading bacteria from Surat's landfill sites, evaluating their efficiency through weight loss and spectrophotometric analysis.

2. METHODOLOGY

2.1 Sample Collection and Screening

For the isolation of plastic degrading bacteria, the waste plastic samples were collected from three distinct locations; Sites 1 and 2 (21.10993° *N*, 72.804083° *E*), situated within the Khajod waste disposal facility, a primary municipal solid waste landfill and site 3 (21.158986° *N*, 72.819473° *E*) is located near the Uma Bhawan Society in the Althan area of Surat, Gujarat, India. Soil samples were scrapped off from the waste plastics and processed by standard microbiological method and aliquots were plated on Minimal Salt Media (MSM) plates containing PEG-400 and PEG-1500 as the sole carbon source. From the initial screening, 18 isolates were selected based on the formation of a clearance zone around the colony (Patinge & Zodpe, 2020).

2.2 Quantitative Plastic Degradation Analysis

2.2.1 Weight Loss Method: Biodegradation was assessed via the weight loss method, following the protocol described by Usha et al. (2011) with minor modifications. Pre-weighed strips of shredded low-density polyethylene (LDPE) were aseptically introduced into Mineral Salt Medium (MSM) broth and inoculated with the selected isolates. The cultures were maintained under shaking conditions for 37 days. Following incubation, the strips were recovered, washed, and dried to a constant weight. The percentage of weight loss was calculated using the following equation; Weight loss (%) = Initial Weight – Final Weight × 100/Initial Weight.

2.2.2 Spectrophotometric Method: The growth of isolates in MSM broth containing LDPE was monitored by measuring Optical Density (OD) at 600nm at intervals of 1, 7, and 37 days (Usha *et al.*,2011).

2.3 Identification of Isolates

Potential degraders were identified through Gram staining, colony morphology, and an array of biochemical tests including Citrate, Catalase, Oxidase, and sugar fermentation (Cappuccino, J. G., & Sherman, N., 2014).

3. RESULTS AND DISCUSSION

3.1 Initial Screening and Distribution of Isolates:

The investigation into landfill sites at Khajod & Althan, Surat and the screening process successfully yielded 18 bacterial isolates (PDBK-1 to PDBA-18). These isolates demonstrated a clear ability to utilize Polyethylene Glycol (PEG-400 and PEG-1500) as a sole carbon source, evidenced by visible growth and the formation of clearance zones around some of the colonies within 48 hours at 37°C. The population density of plastic degrading microorganisms is reported in table-1. The majority were sourced from the Khajod Site 1 (72%); thus, it was identified as a high-activity zone for polymer-degrading microbes.

Table 1: Distribution of Isolates across three sampling sites:

Name of Location	Number of Isolates	Isolate Codes
Khajod 1 Site	13	PDBK-1 to PDBK-13
Khajod 2 Site	1	PDBKC-14
Althan	4	PDBA-15 to PDBA-18

All 18 isolates showed positive growth on both PEG substrates within 48 hours of incubation at 37°C (Fig.-1 & Fig.-2) and demonstrated zone of clearance around the colony.

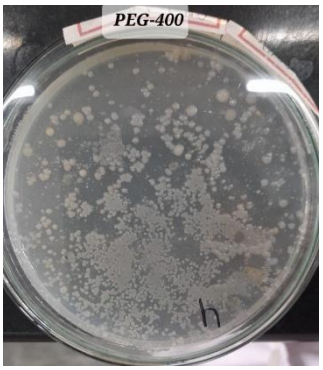

Fig.1: Microbial growth on PEG-400-MSM.

Fig. 2: Microbial growth on PEG-1500-MSM.

The results of this study highlights that indigenous bacteria from Surat's landfill sites possess a significant capacity to degrade LDPE and landfill environments are rich reservoirs for specialized plastic-degrading bacteria. The presence of plastic-degrading bacteria in landfill sites is a testament to microbial adaptability. Such harsh environments act as selective pressure chambers, favouring the survival of microbes capable of metabolizing complex polymers like LDPE and PEG. The ability of the 18 isolates to grow on PEG-MSM plates and form the clearance zones indicates the production of extracellular enzymes, likely esterases or oxidoreductases, which initiate polymer chain scission. strains around the colonies (Kavita & Neerja, 2020). This is consistent with findings by Pathak & Navneet (2017), who noted that while many bacteria can degrade low-molecular-weight PEG, the high molecular weight and hydrophobic nature of LDPE present a much greater barrier to microbial enzymatic attack.

3.2 Quantitative Plastic Degradation Analysis

3.2.1 Quantitative Biodegradation by Weight Loss method:

Weight loss analysis of LDPE strips over a 37-day period revealed varying levels of metabolic activity and degradation efficiency across the isolates. While many isolates showed baseline growth, only five demonstrated significant weight reduction in LDPE strips which are mentioned in figure-3 and figure-4. Isolate PDBA-15 demonstrated the most significant degradation (8.70% weight loss) followed by PDBK-3 (6.15%) and PDBKC-14 (3.28%) representing moderate degradation while the other isolates showed very low degradation. Approximately 50% of the isolates showed 0% LDPE weight loss, despite their initial growth on PEG during the screening phase.

Fig. 3: Comparative analysis of LDPE weight loss after 37 days.

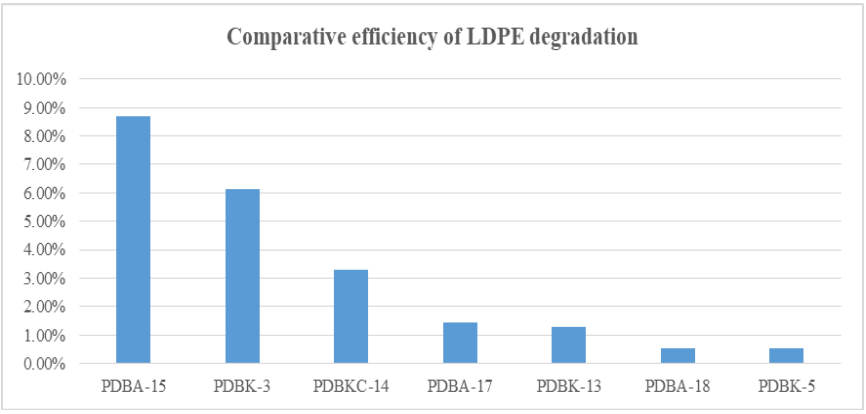

Fig. 4: Comparative efficiency of LDPE degradation.

3.2.2 Spectrophotometric Analysis (Growth Kinetics):

The optical density measurements at 600nm MSM broth inoculated with isolated strains confirmed a consistent increase in microbial biomass over the 37-day period. The result indicate that the bacteria were actively assimilating the plastic components of LDPE strips (Figure-5). The highest microbial density after 37 days was observed in PDBA-18 and PDBKC-14 (0.779 and 0.731 respectively. Most isolates showed a steady increase from Day 1 to Day 37, indicating that the LDPE was not toxic to the bacteria and served as a sustainable energy source over the long term.

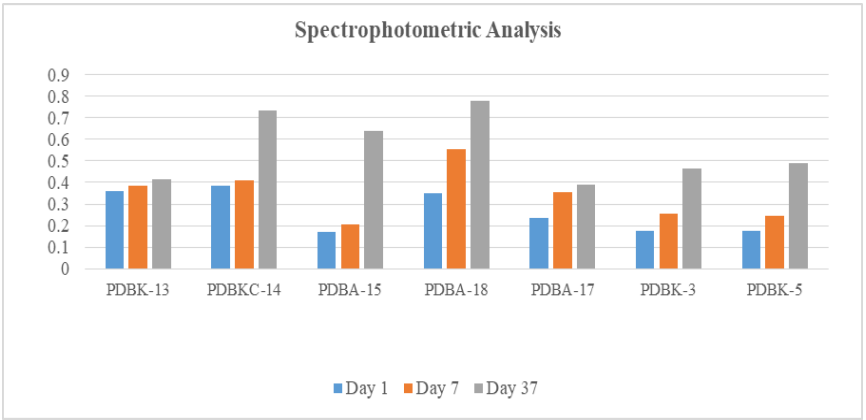

Fig. 5: Spectrophotometric Analysis of LDPE-MSM inoculated broth.

3.3 Identification of Potential Isolates

The potential polymer degraders were identified using Gram staining, growth on differential media and an array of biochemical profiling tests. Based on the results as presented in table-2, PDBK-3 is identified as *Klebsiella* sp., PDBK-5 as *Bacillus* sp., PDBKC-14 & PDBA-15 as *Pseudomonas* sp. and PDBA-18 as *Proteus* sp.

Table 2: Biochemical Characterization of Potential Plastic Degrading Isolates.

Isolate code	PDBK-3	PDBK-5	PDBKC-14	PDBA-15	PDBA-18
Gram stain	Neg., Rod	Pos., Rod	Neg., Rod	Neg., Rod	Neg., Rod
Mac Conky agar	Lactose fermenting	-	Lactose non-fermenting	Lactose non-fermenting	Lactose non-fermenting
King's agar	-	-	+	+	+
Indole test	-	-	-	-	+
MR test	-	-	-	-	+
VP test	+	-	-	-	-
Citrate	+	+	-	-	+
H_2S	-	-	-	-	+
Gelatinase	+	-	+	+	+
Catalase	+	+	+	+	+
Urease	+	-	-	-	+
TSI: Slant	A	AK	AK	AK	A
Butt	A	AK	AK	AK	A
H_2S	-	-	-	-	+
Gas	+	-	-	-	+
Oxidase test	-	-	+	+	+
Starch hydrolysis	-	+	-	-	-
Glucose	AG	AG	AG	AG	A
Lactose	AG	L	-	-	-
Xylose	AG	+	-	-	AG
Mannitol	AG	+	-	-	-
Maltose	AG	+	-	-	AG
Sucrose	AG	+	-	-	AG
Probable Organism	***Klebsiella sp.***	***Bacillus sp.***	***Pseudomonas sp.***	***Pseudomonas sp.***	***Proteus sp.***

Comparative LDPE Degradation Efficiency:

The most efficient isolate in this study, *Pseudomonas* sp. (PDBA-15), achieved an 8.70% weight loss in 37 days. This performance is comparable to, or exceeds, several studies published in the last decade. For instance, Rajandas et al. (2012) highlighted the efficiency of *Pseudomonas* species in breaking down the long-chain hydrocarbons of polyethylene. Skariyachan et al. (2018) demonstrated that bacterial consortia, often including *Pseudomonas* and *Bacillus*, are highly effective at LDPE degradation due to synergistic enzymatic activity.

The steady increase in optical density (OD) observed in PDBA-18 (*Proteus* sp.) and PDBKC-14 (*Pseudomonas* sp.) throughout the 37-day incubation period confirms that the plastic strips were not toxic to the bacteria. Instead, the isolates were actively assimilating the plastic components into their biomass. The identification of *Klebsiella* sp. (PDBK-3) and *Bacillus* sp. (PDBK-5) further supports the diversity of indigenous plastic-degraders available in landfill sites investigated in this study. *Bacillus* species are well-documented for their ability to form biofilms on plastic surfaces, a critical step that precedes enzymatic degradation (Ghosh et al., 2013).

CONCLUSION

This study successfully demonstrated that indigenous bacteria from Surat's landfill sites (Khajod and Althan) not merely comprises of passive residents but possess active bioagents that have a significant capacity to degrade LDPE. PDBA-15 (*Pseudomonas* sp.) emerged as the most potent isolate, representing a weight loss of 8.70%. These findings highlight the potential of using native microbial strains for the development of eco-friendly bioremediation strategies to manage the growing crisis of plastic waste in urban environments.

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