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Isolation and Molecular Characterization of Polythene-Degrading Bacteria from Dumping Sites in Bhopal District, Madhya Pradesh

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ABSTRACT: Low-density polyethylene (LDPE) is a plastic material that is widely used due to its resistance to natural degradation, leading to severe environmental pollution. The microbial biodegradation of LDPE presents a potential solution to this issue. However, the efficiency and mechanisms of this process are not yet fully understood. In this study, we isolated and identified several bacterial strains from a dumpsite in the Bhopal district of Madhya Pradesh, India. These strains demonstrated growth on LDPE as the sole carbon source, indicating their potential for degradation. We further evaluated their ability to degrade LDPE sheets under laboratory conditions by measuring the weight loss after 16 weeks of incubation at 37˚C and 28˚C. Our results revealed that the isolated strains belonged to four genera: Pseudomonas, Bacillus, Alcaligenes, and Priestia. These strains exhibited varying degrees of LDPE degradation. The most efficient degraders were *Bacillus cereus* and *Priestia megaterium*, which achieved weight losses of 29.31± 2.77 % and 22.54 ± 1.39 %, respectively, at 37˚C. Our findings suggest that certain bacterial strains could be effective in mitigating plastic pollution through biodegradation. Factors such as temperature, biofilm formation, and surface oxidation play significant roles in the biodegradation process. This study provides new insights into the microbial degradation of LDPE and its implications for environmental biotechnology.

Keywords: Polythene-Degrading Bacteria, low-density polyethylene, *Priestia megaterium, Bacillus cereus.*

INTRODUCTION

Polythene is a synthetic polymer that is widely used in various applications, such as packaging, agriculture, and construction (Rajendran *et al.,* 2016; Bhat *et al.,* 2021). However, polythene is also a major source of environmental pollution, as it is resistant to natural degradation and accumulates in landfills and water bodies (Sharma & Chatterji 1998). Polythene poses a threat to the ecosystem, biodiversity, and human health, as it can release toxic chemicals, entangle or suffocate wildlife, and disrupt the food chain (Shah *et al.,* 2008). Therefore, there is an urgent need to find effective and sustainable ways to dispose of polythene waste (Kumar *et al.,* 2020). One of the promising approaches to deal with polythene pollution is biodegradation, which is the breakdown of organic substances by microorganisms (Singh & Sharma 2008). Biodegradation of polythene involves the action of enzymes that can cleave the carbon-carbon bonds in the polymer chain and convert it into simpler compounds that can be assimilated or mineralized by the microorganisms (Sharma & Chatterji 1998). Several studies have reported the isolation and characterization of polythene-degrading bacteria from various sources, such as soil, compost, and marine environments (Shah *et al.,* 2008). However, the diversity and potential of polythene-degrading bacteria from dumping sites, where polythene is exposed to various environmental factors and mixed with other wastes, are still largely unknown (Sahu *et al.,* 2019). One of the possible solutions is to use microorganisms, such as bacteria and fungi, that can degrade polythene by producing enzymes that break down its molecular bonds. Several studies have reported the isolation and identification of polythene-degrading bacteria from different sources, such as soil, compost, landfill, marine water, etc. Some of the common genera of bacteria that can degrade polythene are Pseudomonas, Bacillus, Staphylococcus, Streptomyces, Rhodococcus, Sphingomonas etc.

The mechanism of polythene degradation by bacteria involves several steps, such as adhesion, colonization,

biofilm formation, oxidation, and mineralization. The bacteria adhere to the surface of polythene and form colonies or biofilms that secrete enzymes, such as oxygenases, lipases, esterases, etc., that initiate the oxidation of polythene. The oxidation results in the formation of hydroxyl, carbonyl, carboxyl, and other functional groups on the polythene surface, making it more hydrophilic and susceptible to further degradation. The oxidized polythene is then cleaved into smaller fragments, such as alkanes, alkenes, alcohols, acids, etc., that can be assimilated and metabolized by the bacteria into carbon dioxide and water (Ghatge *et al.,* 2020; Yang *et al.,* 2020). The rate and extent of polythene degradation by bacteria depend on various factors, such as the type and concentration of bacteria, the molecular weight and crystallinity of polythene, the presence of additives or fillers in polythene, the temperature and pH of the environment, the availability of oxygen and nutrients, etc. Some studies have reported that the degradation of polythene by bacteria can be enhanced by pretreating polythene with physical, chemical, or biological methods, such as UV irradiation, oxidation, blending with starch, etc., that increase the surface area and reduce the molecular weight of polythene (Ghatge *et al.,* 2020; Yang *et al.,* 2020).

In this study, we aimed to isolate and characterize polythene-degrading bacteria from dumping sites in Bhopal district, Madhya Pradesh, India. Bhopal is one of the largest and most populous cities in central India, and generates a huge amount of solid waste, including polythene, every day. The dumping sites in Bhopal are often open and unmanaged, and pose a serious environmental and health hazard to the surrounding areas. We hypothesized that these dumping sites harbor a rich and diverse community of polythene-degrading bacteria that can be exploited for bioremediation purposes. To test this hypothesis, we collected soil samples from different dumping sites in Bhopal, and screened them for polythene-degrading bacteria using low-density polyethylene (LDPE) as the sole carbon source. We then identified the polythene-degrading bacteria based on their morphological, biochemical, and molecular characteristics, and evaluated their polythene degradation efficiency under different conditions. The results of this study provide new insights into the diversity and functionality of polythene-degrading bacteria from dumping sites, and suggest their potential applications in biodegradation of polythene waste.

MATERIAL AND METHODS

Sample Collection: Samples were collected from various dumping sites in the Bhopal district of Madhya Pradesh, India. The samples consisted of soil and waste materials that were in contact with low-density polyethylene (LDPE) materials.

A. Isolation of Bacteria

The collected samples were enriched in a minimal salt medium (MSM) with LDPE as the sole carbon source. The cultures were incubated at 37˚C for a week. After incubation, the cultures were streaked on MSM agar plates and incubated at 37˚C for 24-48 hours. The distinct colonies were picked and purified by repeated streaking on MSM agar plates.

B. Identification of Bacteria

The isolated bacterial strains were identified using a combination of morphological and molecular techniques. The process involved the following steps:

16S rRNA Gene Amplification: The 16S rRNA gene was amplified using polymerase chain reaction (PCR).

(i) The universal primers used in the study are given in Table 1.

Table 1: Universal Primer sequence used in present study are given below.

Primer	Sequence	PCR product	Reference
16S rRNA 27F	AGAGTTTGATCMTG GCTCAG	≈ 1500 bp	Weisburg *et al.* (1991)
16S rRNA 1492R	GGTTACCTTGTTAC GACTT		

(ii) Purification and Sequencing: The PCR products were purified and sequenced using the Sanger sequencing method.

(iii) Sequence Analysis: The 16S rRNA gene sequences were further analyzed using the Basic Local Alignment Search Tool (BLAST) available at the National Center for Biotechnology Information (NCBI) for identification. The sequences were compared with the available sequences in the GenBank database using the BLAST algorithm (Altschul *et al.,* 1990).

This comprehensive approach allowed for the accurate identification of the isolated bacterial strains based on their genetic profiles. The use of 16S rRNA gene sequencing, in particular, provided a reliable method for bacterial identification due to its highly conserved nature across different bacterial species. This method can be used to identify bacteria at the genus and species level, providing valuable information for further studies on their potential applications in polythene degradation.

C. LDPE Degradation Assay

The ability of the isolated strains to degrade LDPE was evaluated under laboratory conditions. LDPE sheets were sterilized and weighed before being introduced to the bacterial cultures. The cultures were incubated at 37˚C and 28˚C for 16 weeks. After incubation, the LDPE sheets were washed, dried, and weighed. The weight loss was calculated to determine the degradation efficiency of the bacterial strains.

D. Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation.

The data were analyzed using one-way ANOVA, and $p<0.05$ was considered statistically significant.

This methodology provides a comprehensive approach to isolate and characterize polythene-degrading bacteria from dumping sites. It also allows for the evaluation of their degradation efficiency under controlled laboratory conditions. The findings from this study could contribute to the development of effective strategies for mitigating plastic pollution.

RESULTS AND DISCUSSION

The results of this study showed that some bacterial cultures isolated from plastic waste samples had the ability to degrade different types of plastics. The weight loss percentage of the plastic samples was used as an indicator of the biodegradation activity of the bacterial cultures. The bacterial cultures that showed the highest weight loss percentage were selected for further isolation and purification. The isolation was done by serial dilution and spread plate method on nutrient agar plates. The pure colonies were obtained by repeated streaking and subculturing.

The pure bacterial isolates were identified by morphological, biochemical, and molecular methods. The findings revealed that bacteria from four genera - Pseudomonas, Bacillus, Alcaligenes, and Priestia - exhibited plastic degradation abilities. These genera belong to the phylum Proteobacteria, which is known to contain many plastic-degrading bacteria (Ahmed *et al.,* 2018). The most common plastic-degrading enzymes produced by these bacteria are esterases, lipases, and cutinases, which hydrolyze the ester bonds in the polymer chains. The biodegradation activity of the pure bacterial isolates were quantified by measuring the weight loss percentage of the plastic samples after incubation with the bacterial cultures for 16 weeks, the results are shown in Table 2.

Table 2: Biodegradation activity of the pure bacterial isolates on different types of plastics.

Bacterial isolate	Plastic type	Weight loss percentage (%)
Bacillus cereus	LDPE	29.31 ± 2.77
Priestia megaterium	LDPE	22.54 ± 1.39
Pseudomonas aeruginosa	LDPE	18.62 ± 2.01
Alcaligenes faecalis	LDPE	16.45 ± 1.86

Notably, the *Bacillus cereus* strain showed the highest degradation activity at 29.31± 2.77%, followed by *Priestia megaterium* at 22.54 ± 1.39%. These results are comparable to those reported by other studies on plastic biodegradation by bacterial strains (Acero *et al*., 2011). These results suggest that certain bacterial strains could be effective in mitigating plastic pollution through biodegradation, thereby contributing to environmental conservation efforts. This study explored the biodegradation of different types of plastics by four bacterial genera isolated from various sources. The biodegradation activity was quantified by measuring the weight loss percentage of the plastic samples after incubation with the bacterial isolates. The results showed that *Bacillus cereus* and *Priestia megaterium* had the highest biodegradation activity on low-density polyethylene (LDPE) and polyethylene terephthalate (PET), respectively, and suggested that these bacterial strains could be applied for plastic waste management. This study on the isolation and molecular characterization of polythene-degrading bacteria from dumping sites in Bhopal, Madhya Pradesh, aligns with previous research on microbial degradation of plastics. A study by Devi *et al.* (2021) found that four bacterial isolates from the Vaigai River, Madurai, India, were effective in degrading UV-treated polyethylene (PE) and polypropylene (PP). The most efficient were Bacillus paramycoides (BP) and *Bacillus cereus* (BC), achieving degradation rates of 78.99±0.005% and 63.08±0.009% respectively. Similarly, Singh *et al.,* (2016) reported that three bacterial isolates (Staphylococcus sp. (P1A), Pseudomonas sp. (P1B), and Bacillus sp. (P1C)) were able to degrade 10 and 40 micron polythene. Bacillus sp. (PIC) showed the highest degradation at 42.5%. In a study on the biodegradation of polycaprolactone (PCL), Ariole and George-West (2020) found that three bacterial strains and one fungal strain were able to achieve degradation rates of 53-62% at 30°C. These findings, along with our results, suggest that certain bacterial strains could be effective in mitigating plastic pollution through biodegradation. Factors such as temperature, biofilm formation, and surface oxidation are important in the biodegradation process.

CONCLUSIONS

The study isolated bacterial cultures from plastic waste, capable of degrading various plastics. Weight loss in plastic samples indicated biodegradation activity. Bacteria from the genera Pseudomonas, Bacillus, Alcaligenes, and Priestia, all part of the Proteobacteria phylum, showed notable plastic degradation abilities. These findings contribute to potential solutions for plastic waste management.

FUTURE SCOPE

Further research is needed to optimize these factors and enhance the efficiency of plastic degradation. However, the study also acknowledged some limitations and future directions, such as the need to test more types of plastics, to measure the complete mineralization and biodegradation of the plastics, to investigate the molecular mechanisms and pathways of plastic degradation by the bacterial isolates, and to evaluate the environmental and ecological impacts of the plastic-degrading bacteria.

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Conflict of Interest. None.

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