Isolation of High Density Polyethylene Degradating Bacteria from Soil

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ABSTRACT

Plastics are the most commonly used polymers for routine applications. These plastics are less biodegradable in the environment. So the plastics accumulated drastically in the environment and had bad effects on it. High-density polyethylene (HDPE) is a major cause of persistent and long-term environmental pollution. The main objective of the present study is the isolation and screening of bacterial species with the capability to degrade high-density polyethylene (HDPE). The two bacterial isolates were identified from soil collected from the dumping yard of village Atake (Tal-Karad, Dist-Satara). These two bacterial isolates were obtained on minimal agar medium incorporated with HDPE. These isolates are further tested for degradation of the plastic. The isolate 1 (Bacillus cereus) could degrade the plastic to the extent of 16% in terms of % weight loss, while the isolate 2 (Pseudomonas halobacterium) could degrade the plastic to the extent of 10% in terms of % weight loss, thus the isolate 1, which was identified as Bacillus cereus was found to be the best among the two isolates.

Key words: Polyethylene, HDPE, Soil.

Introduction

Plastics are organic polymers of high molecular mass. The name plastic is derived from its property of plasticity. Plasticity is the property of any material by which the material is able to irreversibly deform without breaking. The term plastic is derived from the Greek word “plastikos which means “capable of being shaped or molded (Divyalakshmi et al., 2016). Plastic products have brought tremendous convenience to our lives. Polyethylene (PE) has been utilized in making grocery bags, food packaging films, and toys; polypropylene (PP) for the creation of straws, car seats and container caps; and lastly, polystyrene (PS) for making disposable food trays, and laboratory wares. The exceptional mechanical properties of PE and its relatively low cost led to the abundance and popularity of the mentioned polymer to the general public. It is also known that plastics, specifically PE, are inert materials (Bolo et al., 2015)

The most common types of polyethylene are HDPE (High-density polyethylene), LLDPE (Linear low-density polyethylene) and LDPE (Low-density polyethylene). Polyethylene waste accumulation in the environment causes blockages in fishes, mammals, and birds, endangering a large number of species (Alamer et al., 2023). Polyethylene (PE) is man-made polymers used in many aspects of human life. It is widely used due to its light weight, inexpensive, strong and durable nature. The worldwide production rate of synthetic polymers is about 140 million
tons and its utility is increasing at the rate of 12% per annum (Maroof et al., 2021).

The by products of plastic degradation by physical and chemical methods are toxic irritants and harmful gaseous foams that not only damage the ozone layer in the upper atmosphere but also destroy the underground water resources, cause infertility of soil, prevent degradation of normal substances and are proved dangerous to human, animals and the ecosystem (Nadeem et al., 2021). Microbes are naturally performing degradation on complex organic matters, converting them into simpler organic substances and gaining nutrients during this process. Plastic waste has energy and carbon sources that help microbes in their survival. Plastics can degrade in aerobic or anaerobic conditions, and they can be used as a major natural clean up at locations where toxic waste is disposed. Additionally, biodegradation of polymers is defined as any change in polymer characteristics, such as molecular weight, mechanical strength, and surface features, or, in other words, the breakdown of material into pieces through microbial digestion into a simpler form via biochemical transformation (Alamer et al., 2023).

Research on microbial degradation of plastic wastes, especially polyethylene which is highly resistant to biodegradation has been steadily growing. Some microorganisms have been shown to produce enzymes capable of polyethylene plastic degradation, including moulds such as Aspergillus and Fusarium, yeast such as Cryptococcus and bacteria in the genera Pseudomonas and Bacillus (Montazer et al., 2018).

Plastic wastes accumulating in the environment pose an ever-increasing ecological threat. Plastics that are biodegradable can be considered environmentally friendly. So the present project work was undertaken with the following principal objectives as Isolation of bacteria from plastics dumped in soiltheir plastic biodegradative ability.

**Material and Methods**

**Collection of soil sample from dumping yard**

The Soil samples were collected from the dumping yard of village Atake (Tal-karad, Dist-Satara) in a clean polythene bag and brought to the laboratory, where they were kept at room temperature for further processing.

**Enrichment and isolation of plastic degrading bacteria**

The plastic bottles were cut into pieces using scissors. The pieces and soil samples were added to minimal broth for enrichment at room temperature. After the 10 days of incubation, the 0.1 mL enrichment culture was spread and inoculated on the minimal agar plate and incubated at room temperature for 6-7 days for the isolation of plastic-degrading bacteria. Well-isolated representative colonies from minimal agar were picked up, studied for morphological and cultural characteristics, and aseptically transferred to sterile minimal agar slants.

**Characterization and identification of plastic degrading bacteria**

Bacteria were chosen for characterization and identification by microscopic and morphological observation and biochemical tests with respect to Bargey’s Manual of determination bacteriology.

**Studies on plastic degradation capacity of bacterial isolates**

The pre-weighted high density polythene strips of 1.00 g were aseptically transferred to the conical flask containing 100 ml of minimal broth medium and separately inoculated with the selected bacterial strains. Control was maintained with high density polythene strips in the microbe free medium. Four different flasks were maintained for each treatment.

**Dry weight determination of plastic (Nadeem et al., 2021)**

After 3 weeks of incubation, the polythene strips were collected, washed thoroughly using distilled water, and shade dried till a constant weight was obtained. From the collected data, the weight loss of the polythene strip was calculated as follows:

\[
\frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100
\]

**Determination of percentage weight of degraded (HDPE)**

From the calculated value of polyethylene degradation the percentage of degradation was determined.

**Results and Discussion**

As per the cultural, morphological, and biochemical characteristics, (Table 1) the bacterial isolates were
designated as isolate 1, isolate 2, and tentatively identified as shown in Table 1. The selected isolate was characterised according to Bergey’s Manual of Systematic Bacteriology, Volume II, Section 12.

Bacterial isolate 1 was found to be *Bacillus cereus*. Bacterial isolate 2 was found to be *Pseudomonas halobacterium*.

It can be seen from Table 1 that all two bacterial isolates associated with plastic that was dumped in the dumping yard were isolated and were designated as Isolate 1 and Isolate 2. It is further seen from Table 1 that the isolates were identified as *Bacillus cereus* (isolate 1) and *Pseudomonas halobacterium* (isolate 2). It becomes clear from Figures 1 and 2 that all two isolates were capable of degrading the plastic (HDPE). It can be also observed from Fig. 1 and 2 that the isolate 1 (*Bacillus cereus*) could degrade the plastic to the extent of 16% in terms of % weight loss, while the isolate 2 (*Pseudomonas halobacterium*) could degrade the plastic to the extent of 10% in terms of % weight loss, thus the isolate 1, which was identified as *Bacillus cereus*. *Bacillus cereus* was found to be the best among the two isolates.

**Conclusion**

The present study deals with the isolation, identification, and degradative ability of plastic-degrading bacteria from plastic dumped in soil. The organisms identified were further inoculated into different culture media, and their biodegradative ability was determined by weight loss after a period of 21 days. The plastic strip was degraded by using isolate 1 (*Bacillus cereus*) at approximately 16% and isolate 2

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**Table 1. Identification of isolates**

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Isolate</th>
<th>Tentatively identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Isolate 1</td>
<td>Bacillus cereus</td>
</tr>
<tr>
<td>2</td>
<td>Isolate 2</td>
<td>Pseudomonas halobacterium</td>
</tr>
</tbody>
</table>

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**Fig. 1. Weight of HDPE before and after degradation**

**Fig. 2. Percentage of weight loss HDPE strips after treatment**
(Pseudomonas halobacterium) at 10%. From this project, we conclude that the bacterial strains isolated from different soil samples can have the ability to degrade the plastic.

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Conflicts of interest

Authors hereby declare that there is no any conflict of interest among authors.

References


