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Isolation of the Polythene Degrading Bacteria from the Dumping Area of Jabalpur

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Abstract: ***Plastic pollution, particularly from polyethylene, poses a significant environmental challenge worldwide. In this study, bacterial isolates capable of degrading polyethylene were obtained from the dumping area of Jabalpur, India. The isolation process involved sampling soil and waste materials from the dumping site and culturing them on M9 media supplemented with Polyethylene glycol. Screening of bacterial isolates was performed using staining with Coomassie blue R - 250 to visualize zones of clearance around colonies, indicating potential polyethylene degradation. The selected bacterial isolates were further characterized using morphological, biochemical, and molecular methods to identify their species and assess their polyethylene - degrading capabilities. The findings of this study contribute to the understanding of microbial plastic degradation and provide insights into potential biotechnological applications for plastic waste management in Jabalpur and beyond.***

Keywords: polythene, Bacteria, Xenobiotic.

1. Introduction

Plastic pollution has emerged as one of the most pressing environmental challenges of our time, with polythene (polyethylene) being a major contributor to the global plastic waste crisis (Kibria et al., 2023). Despite its widespread use and convenience, polythene exhibits remarkable resistance to degradation, leading to its accumulation in landfills, water bodies, and ecosystems worldwide (Webb et al., 2013). The persistent presence of polythene poses significant threats to biodiversity, human health, and ecological balance, necessitating urgent and innovative strategies for its mitigation and management (Irouegbu et al., 2021). In recent years, the search for sustainable solutions to address plastic pollution has intensified, with a growing emphasis on harnessing the remarkable capabilities of microorganisms in biodegradation processes (Irouegbu et al., 2021). . Microbial degradation of polythene represents a promising avenue for mitigating plastic pollution, offering potential pathways for the eco - friendly disposal and recycling of plastic waste. Polythene - degrading microorganisms, including bacteria, fungi, and algae, possess unique enzymatic machinery capable of catalyzing the breakdown of polythene into environmentally benign byproducts (Olieveira et al., 2020).

Plastics, versatile polymers that can be molded into various shapes upon heating, are ubiquitous in modern society. These non - metallic compounds find extensive applications in industries, particularly in packaging sectors such as food, pharmaceuticals, and cosmetics (Narancic, et al., 2020). Among the plethora of plastics used, notable examples include polyethylene (LDPE, MDPE, HDPE, LLDPE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polybutyrene tetraphthalate (PBT), and nylon. Low - density polyethylene (LDPE) stands out as a significant contributor to environmental pollution (Olieveira et al., 2020). Composed of repeating ethylene monomer units, polyethylene is witnessing a staggering annual growth rate of 12%, with global production exceeding 140 million tons annually. However, its widespread use poses grave environmental threats both during manufacturing and post - consumer disposal. The accumulation of LDPE waste in landfills, water bodies, and ecosystems poses multifaceted challenges (Kumar et al., 2021). Plastic debris threatens marine life through ingestion and entanglement, disrupts terrestrial habitats, and leaches toxic chemicals into the environment, further compromising ecosystem health. The environmental impact of LDPE underscores the urgent need for sustainable solutions to mitigate plastic pollution. Initiatives aimed at reducing plastic consumption, promoting recycling and circular economy models, and developing alternative biodegradable materials are essential steps towards addressing this global challenge (Kibria et al., 2023, Kumar et al., 2021).

Microorganisms possess the remarkable ability to degrade plastics, with over 90 genera of bacteria and fungi identified as capable of this process. Among these, notable examples include *Pseudomonas* sp., *Bacillus megaterium*, *Azotobacter* sp., *Halomonas* sp., *Ralstonia eutropha*, and many others (Mohanan et al., 2020). These microbes initiate plastic degradation by cleaving the polymer chain using specific enzymes, resulting in the conversion of plastics into monomers and oligomers. Exploiting the diverse metabolic capabilities of microorganisms, bioremediation of plastic waste has emerged as a promising approach (Temporiti et al., 2020). This involves the development of microbial strains through processes such as selection, strain improvement, and genetic modifications. By harnessing the enzymatic activities of selected microbial strains, plastic waste can be efficiently degraded and remediated.

The primary objective of our study is to isolate bacteria from soil samples with the potential to degrade polyethylene plastics. Through systematic screening and characterization of microbial isolates, we aim to identify novel bacterial

strains capable of degrading polyethylene efficiently. This research endeavor contributes to the ongoing efforts towards developing sustainable solutions for plastic waste management and environmental remediation.

2. Material and Method

Sample collection: The sample was collected from soil dumping site of Jabalpur named kathoda land fill site. For microbial isolation the polythene which is degrading has benn taken and kept in sterile water.

Isolation of Microorganism: the polythene taken from the site was dissolved in sterile water and serially diluted. After serial dilution the dilution 10^{-5} and 10^{-6} were plated on to the Nutrient agar plate.

Biochemical identification of Microorganism: The isolates underwent identification based on their morphological, cultural, and biochemical characteristics following the protocols outlined in Bergey's Manual of Systematic Bacteriology. Gram staining and specific biochemical tests were conducted on all isolates (Jumaah, 2017).

Morphological Identification:

Gram Staining Method: A clean, grease - free slide was used to make a smear of bacterial culture, which was then air - dried and heat - fixed. The smear underwent staining with Crystal violet, Gram's Iodine, Gram's Decolorizer, and Safranin sequentially. The stained slide was examined under a microscope to observe cell morphology.

Colony Morphology: Selected strains were observed for their morphology based on shape, size, and color of colonies.

Biochemical Tests

Biochemical identification manual biochemical methods. The kit employs colorimetric identification through pH and substrate utilization changes.

Oxidase Test: To detect the presence of cytochrome 'c' oxidase, an oxidase test was conducted. A loopful of a 24 - hour - old bacterial culture was applied onto a filter paper treated with 1% Kovac's oxidase reagent. The paper was then observed for any color changes. A rapid change to dark purple within 5 to 10 seconds indicated a positive result, while no color change indicated a negative result.

Catalase Test: For the catalase test, trypticase soy agar medium was prepared, containing Trypticase, Phytone, Sodium chloride, Agar, and distilled water. Bacterial cultures were inoculated onto slants made with this medium, with one slant left uninoculated for comparison. After 24 hours of incubation at 37°C, 3 - 4 drops of hydrogen peroxide (H2O2) were added to each culture. The appearance of gas bubbles indicated a positive catalase test, as catalase enzymes present in some microorganisms break down hydrogen peroxide into water and oxygen.

Citrate Utilization Test: The citrate utilization test was conducted using Simmons citrate agar (SCA) as the medium. The SCA medium contained Ammonium dihydrogen phosphate, Dipotassium phosphate, Sodium citrate, Magnesium sulphate, Agar, Bromothymol blue, and distilled water. Test tubes were inoculated with isolated bacteria, with one tube left uninoculated as a control. After 48 hours of incubation at 37°C, the slant cultures were observed for growth and any color changes in the medium. A change in the medium's color to blue indicated a positive result, while no change indicated a negative result.

The Methyl Red and Voges Proskauer test (MRVP): both tests were conducted simultaneously using MR - VP broth. The composition of MRVP broth included 7.0gm/l Peptone, 5.0gm/l Dextrose/Glucose, 5.0gm/l Potassium phosphate, and 1000ml Distilled water.5ml of MRVP broth in each tube was inoculated with bacteria, and an uninoculated tube served as the control. All tubes were then incubated at 35˚C for 48 hours. For the MR test, 5 drops of Methyl Red indicator were added to each tube and observed for color changes. A positive test was indicated by the indicator remaining red, while a negative test was indicated by the indicator turning yellow. For the VP test, 12 drops of VP range reagent I and 2 - 3 drops of VP reagent II were added to each set of two tubes, including the control tube. The tubes were gently shaken for 30 seconds and left to complete the reaction for 15 - 30 minutes. A positive VP test was indicated by the development of a crimson to ruby pink (red) color, while no change in coloration indicated a negative test.

Indole production test: the was performed by inoculating bacteria into tryptone broth. The tryptone broth contained 10gm/l Tryptone, 10gm/l Peptone, and 1000ml distilled water.5ml of tryptone broth was poured into each tube, with one tube inoculated with bacteria and one serving as the control. All tubes were incubated for 48 hours at 35˚C. After incubation, 1 ml of Kovac's reagent was added to each tube, including the control, and gently shaken every 10 - 15 minutes. A positive indole production test was indicated by the development of a cherry red color in the top layer of the tube, while the absence of red coloration indicated a negative test.

Carbohydrate Fermentation Test: To investigate carbohydrate fermentation, Durham tubes were employed. The fermentation medium consisted of Trypticase/peptone (10.0g/l), distilled water (1000ml) adjusted to pH 7.3, and specific carbohydrates (2.5g/l) such as glucose, sucrose, and lactose. Phenol red served as the indicator, with the medium initially red at neutral pH and turning yellow at a pH below 7, indicative of acid production. Each fermentation tube was labeled with the respective carbohydrate. Bacteria were inoculated into the fermentation broths with different sugars, leaving one tube uninoculated as a control for each sugar type. Incubation was carried out at 35˚C for 24 - 48 hours, and color changes from red to yellow indicated carbohydrate fermentation.

Microbial Degradation of Plastics:
Weight loss of polythene discs in culture broth inoculated with different bacterial species was determined after one

month of incubation. Control discs in microbe - free medium were also weighed.

Zone of Clearance test

The obtained isolates underwent screening on M9 media supplemented with Polyethylene glycol (Botre *et al*., 2015). Following the designated incubation period, the plates were subjected to staining using 0.1% Coomassie blue R - 250 in a solution of 40% methanol and 10% acetic acid for 20 minutes. Subsequently, destaining was carried out using a mixture of 40% methanol and 10% acetic acid (v/v) for an additional 25 minutes. Bacterial isolates demonstrating zones of clearance were singled out for further investigation (Divyalakshmi and Subhashini, 2016)

3. Result

Identification of Microorganism

Five different samples were identified by micro and macroscopically and confirmed by various biochemical tests. Based on the colony morphology, gram staining, biochemical tests, and motility test (Table1) five different strains were identified. In Gram staining out of five sps three were grams negative and only two are grams positive. On the basis of grams staining and biochemical identification test the strain were probably identified as *Staphylococcus sps, Klebsiella sps Pseudomonas sps*, *E. coli sps,* and *Bacillus sps*. On the basis of Biochemical identification test s mentioned in table 1.

Table 1: Biochemical identification of the bacterial culture isolated from dumping site

Biochemical test	C1	C2	C3	C4	C5
Catalase test	+	+	+	+	+
Geltin Hydrolysis test	-	-	+	-	+
Indole test	-	-	+	-	
Methyl red test	-	-	+	-	
Voges - proskuer test	+	-	-	-	+
Starch hydrolysis test	-	+	-	-	+
Citrate Utilition test	+	+	-	-	
Motility test	-	-	+	+	+
Grams Staining	G+Ve	G - Ve	G - Ve	G - Ve	G+Ve
Probable sps.	*Staphylococcus sps,*	*Klebsiella sps*	**E. *coli Sps***	*Pseudomonas sps*	*Bacillus sps.*

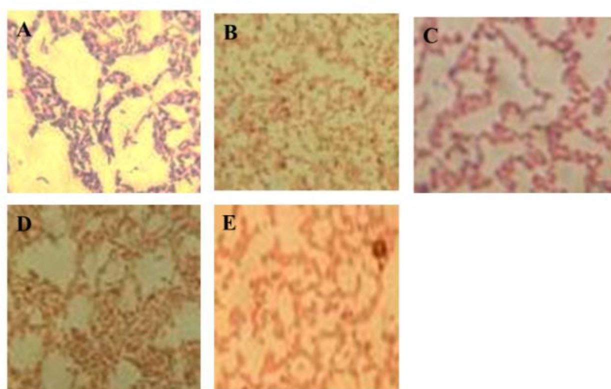

Figure 1: Gram Staining of Culture isolated A. & E is Gram Positive and other three are Gram Negative Bacteria.

Weight loss test of polythene

The polythene degradation ability of the polythene was estimated after 1 month of incubation and found that all five isolates (C1 to C5), are able to degrade the plastic as showed in table 2. The maximum degrading ability was shown by C2 followed by C1, C3, C4 and C5 respectively.

Table 2: Percentage change in weight of plastic after microbial degradation

Culture no.	Initial wt (mg)	Final wt (mg)	Difference (mg)	Weight loss/ Month (in %)
1	100	82	18	18%
2	100	71	29	29%
3	100	84	16	16%
4	100	86	14	14%
5	100	88	12	12%

Zone of Clearance test

In a zone of clearance test the C2 strain showed maximum zone of inhibition followed by C1 and C2 the other two strain (Figure 2) could not show any clearance.

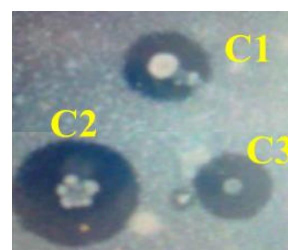

Figure 2: Zone of Clearance test of Bacterial strain isolated from the site

4. Discussion

The research focused on isolating bacteria capable of degrading polyethylene, a commonly used plastic with significant environmental implications due to its resistance to degradation. Through a series of methods outlined in the Materials and Methods section, 5 bacterial isolates were obtained and screened for their ability to degrade polyethylene.

The screening process involved the use of M9 media supplemented with Polyethylene glycol, providing a suitable environment for the growth of polyethylene - degrading bacteria. After the designated incubation period, staining with Coomassie blue R - 250 was employed to visualize zones of clearance around bacterial colonies on the plates. This method allowed for the selection of bacterial isolates demonstrating potential polyethylene degradation (Zhange et al., 2022).

The findings of this study indicate the presence of bacteria capable of degrading polyethylene, highlighting the potential for microbial biodegradation as a solution to plastic pollution. The ability of microorganisms to break down polyethylene into simpler compounds offers promising avenues for environmental remediation and waste management strategies.

Furthermore, the isolation of these polyethylene - degrading bacteria opens doors for further exploration into the mechanisms underlying plastic degradation. Future studies could focus on elucidating the metabolic pathways and enzymes involved in polyethylene degradation, ultimately leading to the development of biotechnological applications for plastic waste management. this research contributes to our understanding of microbial plastic degradation and underscores the importance of harnessing the capabilities of bacteria in addressing the global plastic pollution crisis. By identifying and characterizing polyethylene - degrading bacteria, this study lays the groundwork for the development of sustainable solutions to mitigate the environmental impact of plastic waste.

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