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Isolation of Plastic Degrading Bacteria from Dump Yard Soil

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ABSTRACT

The soil samples were collected from Gadhinglaj, Maharashtra, and were subjected to isolate plastic degrading bacteria by the enrichment culture technique. The isolates were subjected to cultural and morphological characterization. The three isolates were designated PDB1, PDB2, & PDB3 and tentatively identified as Pseudomonas aeruginosa, Bacillus subtilis, & Bacillus cereus, respectively. These isolates were subjected to testing of the plastic biodegradation capacity for 21 days. The isolates were spread inoculated aseptically on plates and incorporated with a plastic sheet with a size of 0.01 mm. The plastic degradation by bacterial isolates was found to be 20 % by isolate PDB1, 40 % by isolate PDB2, and 50 % by isolate PDB3. The isolate PDB3 i.e.,Bacillus cereus was found to show maximum plastic degradation within 21 days and is a promising one and needs to be studied further.
***Key words**: Plastic Degrading Bacteria, Plastic, Bacillus cereus, Isolates*

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INTRODUCTION

Plastic is the most useful synthetic 'man made' substance, made up of elements extracted from the fossil fuel resources. It has made possible most of the industrial and technological revolutions of the 19th and 20th centuries. During the past 30 years plastic materials such as polyethylene (LDPE, MDPE, HDPE, LLDPE),polypropylene (PP),polystyrene (PS),polyvinyl chloride (PVC),polyurethane (PUR),polybutylene terephthalate (PBT),nylons have been used widely in food, clothing, shelter, transportation, construction, medical and leisure industries because they are lightweight, low cost, extremely durable and relatively unbreakable [4,5]. A very general estimate of worldwide plastic waste generation is annually about 57 million tons. They do not break down in the environment easily because they are resistant to microbial attack, due to their excessive molecular mass, high number of aromatic rings, unusual bonds, or halogen substitutions. As a result they remain in the environment for a very long time without any deterioration and the large-scale accumulation of waste plastics in the biosphere has given rise to the problem of severe environmental pollution. These problems have made plastic waste a major focus in the management of solid waste. Due to plastic's resilience against degradation and its proliferation in industry, the issue of plastic pollution has evolved to become a threat to global ecology [10]. Low density polyethylene is one of the major sources of environmental pollution. Polyethylene is a polymer made of long chain monomers of ethylene. The worldwide utility of polyethylene is expanding at a rate of 12% annum and approximately 140 million tonnes of synthetic polymers are produced worldwide each year. With such huge amount of polyethylene getting accumulated in the environment, their disposal evokes a big ecological issue. It takes thousand years for their efficient degradation. Since polymers are extremely stable, their degradation cycles in the biosphere are limited. In Western Europe alone it is estimated that 7.4% of municipal solid waste are plastic, which are classified as 65% polyethylene/polypropylene, 15% polystyrene, 10% PVC, 5% polyethylene terephthalate and remaining others. Environmental pollution by synthetic polymers, such as waste plastics and water-soluble synthetic polymers in waste- water has been recognized as a major problem. In view of this, energetic, chemical and biological polymer-degrading techniques have been studied extensively during the last three decades [11]. The energetic agencies can be either thermal or radiant. The radiant energy may be high energy radiation like gamma rays, ion beams, and electrons or even low energy radiation like ultraviolet (UV) rays. Chemical degradation is caused using certain chemicals like acids and alkalis, etc. Usage of certain microorganisms and enzymes to degrade polymers are classified as the biodegradation method of polymers. The microbial species are associated with the degrading materials. Microbial degradation of plastics is caused by certain enzymatic activities that lead to a chain cleavage of the polymer into oligomers and monomers. These water soluble enzymatically cleaved products are further absorbed by the microbial

cells where they are metabolized. Aerobic metabolism results in carbon dioxide and water, and anaerobic metabolism results in the production of carbon dioxide, water and methane and are called end products, respectively. The degradation leads to breaking down of polymers to monomers creating an ease of accumulation by the microbial cells for further degradation. Microorganisms can degrade plastic over 90 genera, from bacteria and fungi, among them; *Bacillus megaterium, Pseudomonas sp., Azotobacter, Ralstoniaeutropha, Halomonas sp.*, etc. Plastic degradation by microbes due to the activity of certain enzymes that cause cleavage of the polymer chains into monomers and oligomers. Plastic that has been enzymatically broken down further absorbed by the microbial cells to be metabolized. Aerobic metabolism produces carbon dioxide and water. Instead of anaerobic metabolism produces carbon dioxide, water, and methane as end products. The purpose of this study was to isolate microorganism from dumped soil area and screening of the potential plastic degrading microorganisms and identifying the high potential microorganism that degrade the plastics. [1]

MATERIALS AND METHODS

Sample Collection

Soil sample were collected from plastic dumping site at Gadhinglaj, Maharashtra, India. In clean polyethylene bag and brought to the laboratory where it was kept at room temperature for further processing.

Isolation of Bacteria from Soil Sample

1g of soil sample was inoculated in 100 mL of Minimal Salt Synthetic Agar Medium containing polyethylene granules as sole carbon source. Flask was incubated for 15 days at room temperature on the rotary shaker. After incubation 0.1mL of broth was spread inoculated on Minimal Salt Synthetic Agar Medium. The Minimal Salt Synthetic Agar plate was incorporated with 2cm X 2cm polyethylene sheet (plastic sheet 0.01mm). The Minimal Salt Synthetic Agar Medium plates were incubated for 21days at room temperature (28^0 C). After incubation the plates were observed for the colonies showing different morphology were subjected to Cultural, Morphological and Biochemical characterization. The isolates were preserved at 4^0 C on medium slants.[3, 6, 7]

Study of Cultural, Morphological and Biochemical Characteristics of the Isolates

a) Cultural Characteristics:

Cultural characteristics were determined by observing their colony size, shape, margin, consistency, opacity, elevation and results were noted.

b) Morphological Characteristics

Morphological identification was done by gram staining method and motility observation and result were noted.[8, 9]

C) Biochemical Characterization

The biochemical tests performed included fermentation of various sugars like glucose, lactose, maltose, sucrose, mannitol, hydrolysis of starch, urea, case in production of oxidase, catalase and IMViC tests. The identification of isolates was done on the basis of these above characteristics with standard references [16, 17].

Study of Evaluation of Plastic Degrading Ability of the Isolate

Plastic degrading ability of the isolates was studied by determination of weight loss method [12, 13, 14, 15].

Pre-weighed discs of 2 cm X 2cm diameter prepared from polythene bags were aseptically transferred to the conical flask containing 50 mL of Minimal Salt Synthetic Agar Medium, inoculated with 1mL (10^8CFU/mL) each of different bacterial isolates. Incubated the plates at 28^0 C for 21 days. Observed the bacterial growth on surrounding the 2cm X 2cm diameter plastic. Then proceeded to motility, gram staining, and biochemical characters of isolates. The plastic discs were collected, washed thoroughly using distilled water, shade-dried and then weighed for final weight. From the data collected, weight loss of the plastics was calculated by using following formula:

Weight loss = Initial weight -Final weight X100 / Initial weight

From these values of polyethylene degradation, the percentage of degradation was determined.

RESULTS AND DISCUSSION

Isolation of Plastic Degrading Bacteria

Three bacterial isolates were obtained from the plastic dumped soil on minimal salt synthetic agar medium after enrichment and they were purified and preserved for further studies. In all three isolates were obtained and they were designated as PDB_1, PDB_2 and PDB_3.

Identification of Isolates

According to cultural, morphological and biochemical characteristics of isolates, PDB1 was tentatively identified as stain of *Pseudomonas aeruginosa*, PDB2 was tentatively identified as strain of *Bacillus subtilis,* PDB3 was tentatively identified as stain of *Bacillus cereus.*

It can be also seen from above table that all the three isolates have plastic degrading ability. The isolate PDB1 found to be degrading plastic within 21 days, the percentage of plastic reduction was found to be 20%. The isolate PDB2 found to be degrading plastic within 21 days, the percentage of plastic reduction was found to be 40% while that ofPDB3 was found to be 50%and this capacity of degradation is fairly high.

Table 1: Results of Cultural and Morphological Characterization of The Isolates

Isolate	Size	Shape	Colour	Margin	Elevation	opacity	consistency
PDB1	2mm	Circular	Greenish	Irregular	Convex	Opaque	Moist
PDB2	1mm	Circular	White	Irregular	Flat	Opaque	Moist
PDB3	1mm	Circular	White	Irregular	Flat	Opaque	Moist

Isolate	Gram nature	Motility
PDB1	Gram negative rod	Motile
PDB2	Gram positive rod	Motile
PDB3	Gram positive rod	Motile

Table 2: Results of Biochemical Characterization of Isolates

Isolates	Sucrose test	Glucose test	Mannitol test	Lactose test	Maltose test	Nitrate reduction test	Methyl red
PDB_1	-	+	+	-	+	+	-
PDB_2	+	+	+	-	+	-	+
PDB_3	+	+	+	-	-	+	+

Isolates	Citrate dehydrogenase test	Oxidase test	Catalase test	Indole test	Casein hydrolysis	Starch hydrolysis	Urea hydrolysis
PDB_1	-	-	+	+	+	+	-
PDB_2	+	+	+	+	+	-	+
PDB_3	+	+	+	+	+	-	+

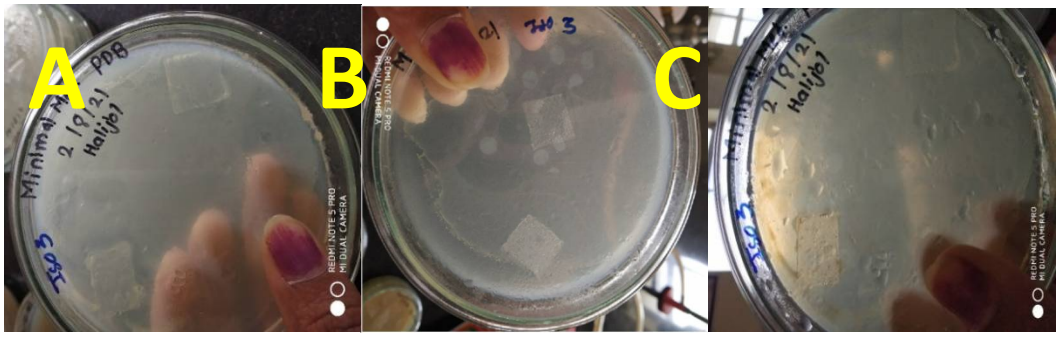
+ : Indicates positive test**; - :** Indicates negative test

Table 3: Results of Evaluation of Plastic Degrading Ability of the Isolate by Weight Loss Method

ISOLATES	Initial weight of plastic	Final weight of plastic	Difference in plastic weight	Weight loss of plastic in %
PDB_1	0.01	0.08	0.02	2%
PDB_2	0.01	0.06	0.04	4%
PDB_3	0.01	0.05	0.05	5%

Photograph 1: Sample of Polythene Pieces

Photograph 2: Photographs of various bacteria isolates obtained After 21 Days of incubation on Minimal Salt Synthetic Agar Medium



Growth of Isolates – (A) PDB1, (B) PDB2 and (C) PDB3on Minimal Salt Synthetic Agar Medium Supplemented With Polyethylene

CONCLUSION

The isolate PDB1, PDB2 and PD3 isolates found to be degrading plastic within 21 days at rate of 20%, 40% and50%. And this capacity of degradation is fairly high which further hints at their use in large scale degradation studies on the plastics.

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