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Isolation, Identification and Analysis of Plastic Degrading Bacteria from Dumped Soil Area

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Abstract

Environment plays a crucial role in all survival of all living organisms that they live in. The need to utilize and protect it simultaneously is a degree that has to be achieved. One of the major threats that are causing an imbalance in the nature is the disposal of plastics. This has been one of the biggest challenges faced by the environmentalists from past two decades after which the usage of the plastics became a need. Plastics are long chain hydrocarbon polymers with higher molecular mass. The plastic accumulates in environment and threatens the terrestrial and marine ecosystem. In this study, the plastic degrading bacteria was screened from the soil in the dump yards and the biodegradation of plastics type HDP (high density polymer), LDP (low density polymer) was analysed up to 2 months of incubation in liquid culture (Bushnell Haas Broth) medium with a periodic analysis of every 15 days. The percentage reduction of plastic dry weight was found to be 12.121, 15.151, 19.819 and 23.076 in LDP- 10µ, LDP-40µ, HDP-10µ and HDP-40µ respectively. The microbial species associated with the degrading plastics was identified as Gram-positive bacteria. The quantitative analysis of carbon content in culture medium showed periodic increased from 0.196 to 3.039, 0288 to 5.689, 0.039 to 1.875, and 0.089 to 5.731 in LDP 10µ, LDP40µ, HDP10µ and HDP40µ respectively.

Keywords

Biodegradation, Environment, HDP, LDP

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INTRODUCTION

Plastics are defined as the polymers (solid materials) and non-metallic mouldable compounds which can be given any desired shape and sizes [1]. Plastics are use all over the world extensively. Various sectors like agriculture, telecommunication, building and construction, consumer goods, packaging, health and medical are all high growth areas that ensure present demand for plastics. Plastic is the mother industry to hundreds of components and used in our daily life like

automobiles parts, electrical goods, plastic furniture, defence materials, agriculture pipes, packages and sanitary wares, pipes and fittings, tiles and flooring, artificial leathers, bottles and jars, PVC shoes and sleepers hundreds of household items. Plastics are used in packaging of products such as food, pharmaceuticals, cosmetics, detergents and chemicals. Approximately 30% of plastics are used worldwide for packaging applications and the most widely used plastics used for packaging are polyethylene (LDPE, MDPE, HDPE, LLDPE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyurethane (PUR), polybutylene terephthalate (PBT), nylons. Plastics consists of organic polymers made up of chains of carbon atoms alone or with sulfur, oxygen or nitrogen [2,3]. The backbone is the part of the chain contains large number of repeat units together. In order to customize the properties of a plastic, different molecular groups "hang" from the backbone (usually they are "hung" as part of the monomers before linking monomers together to form the polymer chain). This property of the polymer by repeating unit's molecular structure has allowed plastics to become an indispensable part of the twenty-first century world. Important groups include acrylics, silicones, polyesters, polyurethanes, halogenated plastics. Plastics can be classified by the chemical process that is used in their synthesis. Plastics can also be classified depending on various physical properties, such as density, high tensile strength, and resistance to various chemical products. The compounds leaching from polystyrene food containers have been proposed to interfere with hormone functions and are suspected human carcinogens [4,5]. The polythene is the most typically found non-degradable solid waste that has been recently recognized as a major threat to marine life. The polythene might cause blockage in intestine of fish, birds and marine mammals [6,7]. Degradation of polythene is a great challenge as the materials are increasingly used by humans. A very general estimate of worldwide plastic waste generation is annually about 57 million tons [8]. These solid waste related problems pose threat to megacities especially the coastal ones. 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 tons of synthetic polymers are produced worldwide each year [9]. 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. The microbial biodegradation is widely accepted and is still underway for its enhanced efficiency. 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 for metabolism. Aerobic metabolism results in carbon dioxide and water [10], and anaerobic metabolisms results in the production of carbon dioxide, water and methane and are called end products, respectively [11]. The degradation leads to breaking down of polymers to monomers creating an ease of accumulation for further degradation by the microbial cells.

Microorganisms utilize polythene film as a sole source of carbon resulting in partial degradation of plastics. They colonize on the surface of the polyethylene films forming a biofilm. Cell surface hydrophobicity of these organisms was found to be an important factor in the formation of biofilm on the polythene surface, which consequently enhances biodegradation of the polymers. Once the organisms get attached to the surface, starts growing by using the polymer as the carbon source. In the primary degradation, the main chain cleaves leading to the formation of low-molecular weight fragments (oligomers), dimers or monomers. The degradation is due to the extra cellular enzyme secreted by the organism [12]. These low molecular weight compounds are further utilized by the microbes as carbon and energy sources. The resultant breakdown fragments must be completely used by the microorganisms, otherwise there is the potential for environmental and health consequences. The purpose of this study was to isolate microorganism from dumped soil area and screening of the potential polyethylene degrading microorganisms and identifying the high potential microorganism that degrade the plastics.

MATERIALS AND METHODS

Sample collection: Dumped soil was collected from South part of city dump yard. **Microbial Degradation of Plastics in Laboratory Condition:** The type of plastics used for the experiments were LDP-10µ, LDP-40µ, HDP-10µ and HDP-40µ. The plastics were cut into 3x3cm sizes. Bushnell Haas Broth medium was prepared and sterilized. 10gms of collected soil was weighed and suspended into 90ml of sterile saline.5ml of soil suspension was inoculated as source of plastic degrading microorganisms along with 3strips of 3x3cm size of each plastic into 50ml of sterile medium. 4sets of medium was prepared for each type of plastics. The flasks were incubated for 37^{0}C for up to 2months with a periodic observation for every 15 days. Control was maintained with plastic strips in the microbe-free medium. After specific incubation period the plastic strips 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 [13].

$$\text{Weight Loss \%} = \left(\frac{\text{Initial Weight-Reduction in Weight}}{\text{Initial Weight}}\right) \times 100$$

Identification of Bacteria

Identification of the isolates was performed according to their morphological, cultural characteristics and biochemical tests by following Bergey's Mannual of Systematic Bacteriology [14]. All the isolates were subjected to Gram staining and specific biochemical tests.

Determination of available Organic Carbon [15] in the medium

1ml of incubated sample was taken. 50ml of 1N $K_2Cr_2O_7$ solution was added and shake to mix it. Then 10 ml Conc. H_2SO_4 was added and the flask was swirl 2 or 3 times. 10ml of orthophosphoric acid was added and 1ml of Diphenylamine indicator. The control flask contained only plastics without soil suspension. The solution was titrated with 1N Ferrous Ammonium Sulphate till the colour changes from violet through blue to bright green. The volume of Ferrous Ammonium Sulphate utilized was noted.The blank titration (without soil) was carried out in a similar manner.

$$\text{Organic Carbon \%} = \left(\frac{0.003\times10\times\text{Blank-Sample reading}}{\text{Blank}\times\text{Volume of the sample}}\right)\times100$$

RESULTS AND DISCUSSIONS

Identification of bacteria

The present study deals with the isolation, identification and degradative ability of plastic degrading microorganisms from soil collected from dumping area. This study has covered the major concerns about synthetic polymers, their types and degradability by assessing polymer degradation in terms of percentage reduction of plastics. Another area examined was the biodegradation of plastics in the liquid culture method.The microbial species found associated with the degrading plastics were identified as Gram positive *Bacillus subtilis* bacteria (Table1).

Table1: The Morphological and Biochemical Identification of Bacteria

Sl.No	Test	Result
01	Gram's Reaction	Gram Positive bacilli with spore former
02	Colony Morphology	Large Round and White
03	Oxidase Test	Positive
04	Catalase Test	Positive`
05	Fermentation of Carbohydrates	Glucose Positive with gas production

In the present study plastics were inoculated in the liquid culture medium containing bacterial isolates and kept for 2 months to observe the percentage of weight loss by bacteria (Fig1). The result showed the degradative ability of the microorganisms. Microbial degradation of a solid polymer like polyethylene requires the formation of a biofilm on the polymer surface to enable the microbes to efficiently utilize the non-soluble substrates by enzymatic degradation activities and this is critical to the initiation of biodegradation. Thus, the duration of the microbial colonization is an important factor that effects total degradation period [17] (Fig 2).

Microbial Degradation of Plastics in Laboratory Condition (Fig 3)

The percentage degradation of plastic increased with respect to time. The degradation of HDP-40 plastic was highest by *Bacillus subtilis* followed by HDP-10µ, LDP-40µ and LDP-10µ.The Synthetic plastics of various measurements were used to study their

biodegradation by microorganisms isolated from soil. The bacteria degraded 12.121% of LDP-10µ, 15.151% of LDP-40µ, 19.819% of HDP-10µ and 23.076% of HDP-40µ plastic types. This shows it has the greater potential of degradation of higher plastic compared to other types [18,19].

Determination of Available Organic Carbon (Fig4)

The organic carbon content decreased maximally at 60th day and availability of organic carbon was negligible of all the types of plastics at 15th day. HDP-40 and HDP-10 showed more organic carbon during 30th day followed by sharp decrease on 45th and 60th day.LDP-10 and LDP-40 showed more organic carbon on 45th day and less on 30th day and 60th day. Probably the decrease in organic carbon content in the medium was due to utilization of bacteria *Bacillus subtilis* for their own growth and multiplications [20].

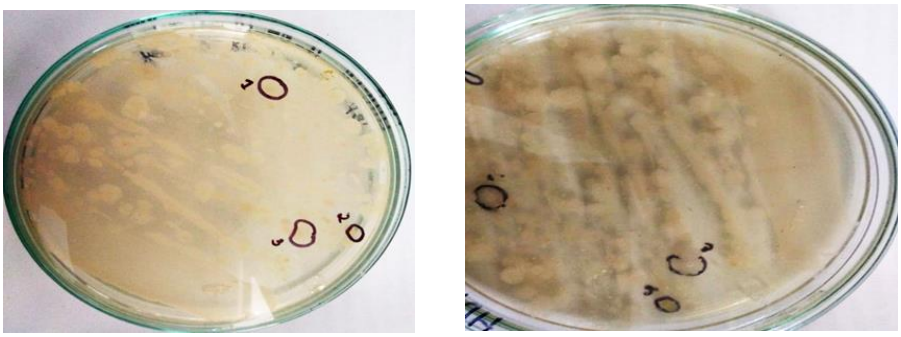

Fig1: Plastic Degrading Bacteria in Nutrient Agar Medium

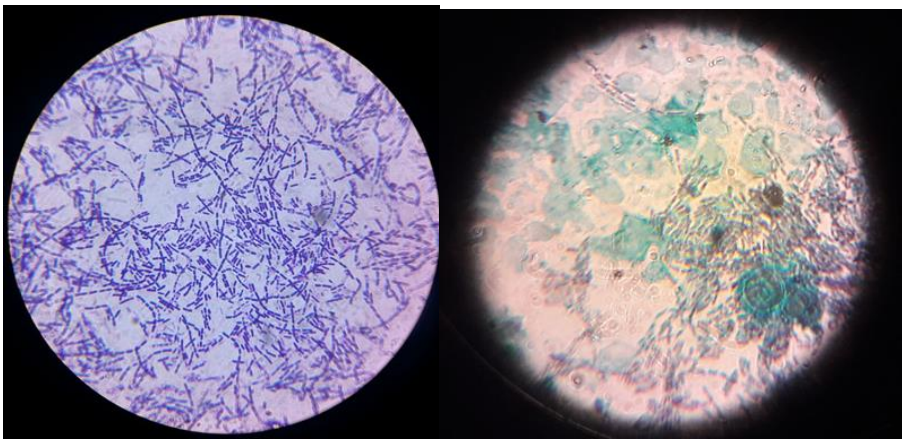

Fig2: Plastic Degrading Bacteria under 100x Biofilm of Bacteria attached to plastic under100x

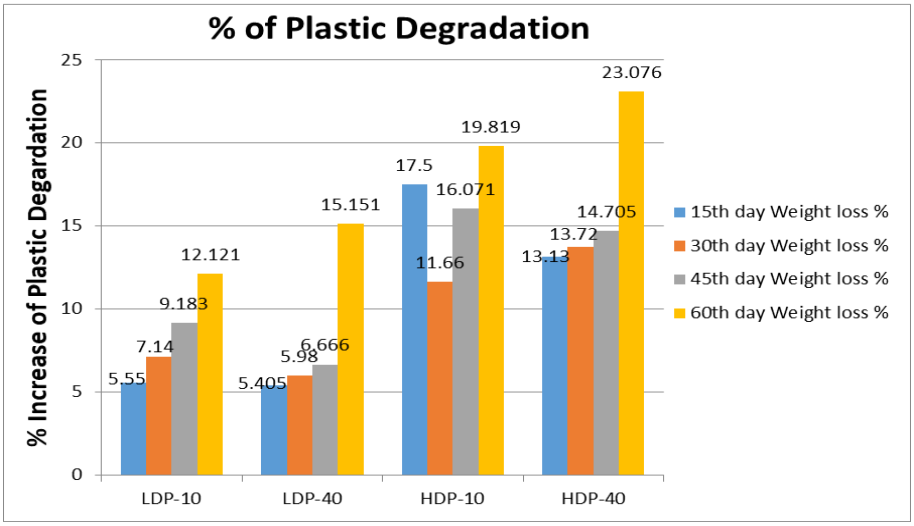

Fig3: Percentage Reduction of Plastics by Bacteria

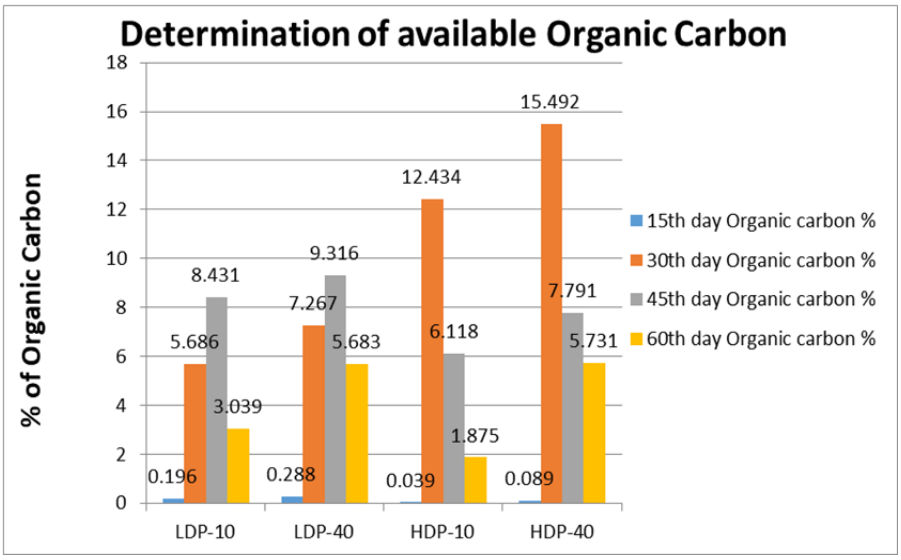

Fig4: Quantification of Organic Carbon in Growth Medium

CONCLUSION

Current investigation reveals that *Bacillus subtilis* which was isolated has the potential to degrade synthetic plastics especially HDP-40 compared to other grades. The isolated microbes were native to the site of plastics disposal and shown some degradability in natural conditions, yet they also exhibited biodegradation in laboratory conditions on synthetic media.

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Conflict of interest

The authors declare that there is no conflict of interest in this study.

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