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PLASTIC DEGRADING ABILITY OF LACCASE ENZYME ISOLATED FROM GARBAGE DUMPING SITES OF CHENNAI

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ABSTRACT

Plastics are used exhaustively worldwide, and its accumulation in the environment has become global concern because of ground water scarcity. Plastics cannot be degraded in land or water because of its features. The ability to degrade plastic enzymatically has been thought to be finite to a few bacterial and fungal species. On the other hand degradation of plastics by microorganisms seems to be more effective. Considering that we aimed to isolate bacteria from the waste dumping yard of Chennai, Tamilnadu, India. Thirteen bacteria were isolated using minimal salt agar medium amended with 1% of plastic powder. The microbiological and biochemical characteristics were studied for the 13 plastic degrading bacteria. The 13 Bacteria were screened for laccase enzyme assay in which PB1, PB-4, PB-7, PB-12, PB-13 showed brown color zone around the colonies resulting in production of the enzyme. 1g plastic were tested for degradation by burying in soil pits and enriched with the bacterial culture. Weight loss of the plastic is calculated after three months.

KEY WORDS : Plastic, Polymer, Laccase, Guaiacol, Biodegradardation, Waste dumping yard.

INTRODUCTION

Plastics are considered to be a major Global Pollution. Not only terrestrial it also affects the Marine environment in world wide. The plastics which on heating become mobile and can be cast into various forms such as nylon, polycarbonate, polyethylene –terephthalate and polyvinyl chloride (Usha *et al*., 2011) are being continuously used in our day-to-day. These polymers are based on the chains of carbon atom alone or oxygen or nitrogen as well. In order to customize the plastics different molecular groups of "hung: from the backbone. Usually they are "hung "as part of the monomer before linking monomers together to form polymer by repeating units molecular structure has allowed plastic to become an indispensable part of the twenty-first century world.

Plastics are made from inorganic and organic raw materials (Francis *et al*., 2010). The durability of plastics and their potential for diverse applications, including widespread use as disposable items, were predictable, but the problems associated with waste management and plastic debris was not solved. (Yuksel *et al*., 2004; Thompson *et al*., 2009). Biodegradation of natural and synthetic plastics are carried out by microbes like bacteria, fungi and actinomycetes (Gu *et al*., 2000). They produce extracellular enzymes which oxidize and influence the degradation process (Chiellini *et al*., 2003). Polymers degrading activity depends on the conversion of the polymers to oligomers and then to monomers by enzymes produced by the microbes. These enzymatically digested materials are utilized by the microbes as carbon source (Vasile, 1993).

Laccases have been studied since 1883 when extracted first time from the *Japanese lacquer* tree. (Giardina *et al*., 1995). Laccase catalyzes the oxidation of a broad number of phenolic compounds and aromatic amines by using molecular oxygen as the electron acceptor, which is reduced to water. The use of oxygen by laccases

makes these enzymes more adequate for industrial and environmental applications. (Poonam *et al*., 2000 and Tirupati *et al*., 2016). Therefore, the ability of laccases to oxidize a broad range of phenolic compounds employed in numerous industrial sectors has amplified their biotechnological potential. The aim of this study was to develop an eco-friendly method by exploring the potential of bacteria isolated from dumping yard of Chennai their laccase enzymes for plastic degradation.

MATERIALS AND METHODS

Sample Collection

The soil samples were collected from the waste dumping yard of perungudi and chrompet, Chennai, Tamilnadu, India. The soil was collected aseptically in clean sterilized containers without any contamination in it. The collected samples were straight away transferred to the laboratory in an ice box for bacteriological examination Fig. 1.

Fig. 1. Sample Collection Site

Isolation of Plastic Degrading Bacteria

One gram of ten soil samples was suspended separately in 99 mL of sterile water and these suspensions were serially diluted. Minimal salt agar medium were prepared. As it does not contains any carbon or nitrogen source of energy it is suspended with 1g of plastic powder was prepared and pour plate method was done for the isolation of plastic degrading bacteria. All the plates were incubated at 32 °C for 5 days.

Identification of Plastic Degrading Bacteria

The bacterial isolates were identified by morphological and biochemical characterization. In morphological characterization macroscopic characteristic were studied. Phenotypic characterization such as microscopic characterization of gram reaction, motility and biochemical test including catalase, oxidase, were performed the standard protocols (Bergey and Holt *et al*., 1994)

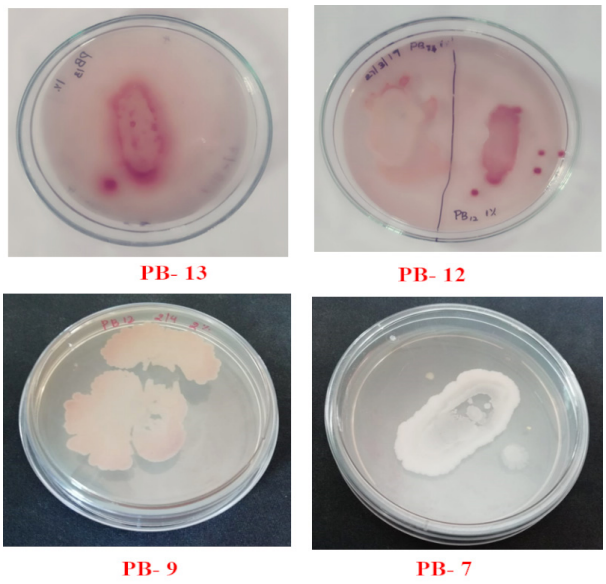

Fig. 2. Screening of Plastic Degrading Bacteria by Laccase Plate Assay

Screening of Plastic Degrading Bacteria by Laccase Plate Assay

The 13 bacterial isolates were screened for the production of Laccase enzyme, 0.01% of Guaiacol amended in nutrient agar medium before autoclaving. The bacterial cultures were streaked on the guaiacol nutrient agar medium and incubated at 32 °C for 3 days. Development of brown color zone, surrounding the bacterial growth, was indicating the production of laccase enzyme (Tirupati *et al*., 2016).

Investigation of Polythene Degradation by Culture Media

About 1 g weighed strips were tested for degradation process. By burying in soil pits and enriched with the culture. Sterile soil pits were made and the strips were placed in layers with soil alternatively. The pits were then nourished with the cultures isolated. The strips were allowed to degrade for three months. The degradation by the isolated cultures was analyzed by determining the dry weight at regular interval of 30 days. The plastic strips were collected, washed thoroughly using distilled water, shade-dried and then weighed for final weight. The weight loss of the plastic is calculated (Indhumathi and Gayathri, 2016).

RESULTS AND DISCUSSION

The present study deals with the isolation,

identification and degradative ability of plastic with the help of bacterial laccase enzyme. The soil samples are collected from the garbage dumped soils of perungudi and chrompet, Chennai, Tamilnadu, India. Minimal salt agar medium suspended with 1 gm of plastic powder used for isolation of bacteria. So the bacteria will utilizing the plastic power without any carbon and nitrogen source. Divyalakshmi and Subhashini (2016) used 1% concentrations of PEG in minimal salt agar media for the isolation of plastic degrading bacteria.

A total of 10 soil samples were collected 21 plastic degrading bacteria was isolated from the soil samples on further continuous sub culturing only 13 bacterial cultures grown well on nutrient agar plate (PB-1 – PB-13). Similarly 10 samples were collected from waste dump sites of Dhapa and Barrackpore Municipality and 9 isolates of bacteria were isolated using Nutrient Agar medium. Plastic strips are used to study their biodegradation by laccase enzyme isolated from the soil samples. Amrita and Chandra (2014) also used plastic strips for their study. Biochemical test for Grams staining 7 bacterial cultures has shown positive results. All 13 bacterial isolates were motile and showed positive result in oxidase. Eleven cultures were catalase positive Table 1.

In Laccase plate assay development of brown color zone, surrounding the bacterial growth was indicating the production of laccase enzyme. A clear brown color zone was found on PB-13 and PB-12, and light brown zone formed in PB-9 and PB-7 by which the production of laccase enzyme was confirmed (Fig. 2). Similar work has been carried out by Desai, (2017) used (0.01%) Guaiacol for the detection of production of Laccase. In plastic degradation test, by burying in soil pits and enriched with the culture. The weight loss of the plastic is calculated and tabulated as reported by Indhumathi and Gayathri, 2016 (Table 2).

CONCLUSION

One of the most ever-present and long-lasting recent changes to the surface of the planet is the accumulation and fragmentation of plastics, "starting from health problems from BPA and phthalates leaching in to water and drinks, to the great plastic patch in the oceans, the impact on the

Table 1. Biochemical Charaterization

S.No.	Gram Staining	Motility	Oxidase	Catalase
Pb-1	Positive	Motile	Positive	Positive
Pb-2	Positive	Motile	Positive	Positive
Pb-3	Positive	Motile	Positive	Positive
Pb-4	Negative	Motile	Positive	Positive
Pb-5	Positive	Motile	Positive	Positive
Pb-6	Negative	Motile	Positive	Negative
Pb-7	Positive	Motile	Positive	Positive
Pb-8	Positive	Motile	Positive	Positive
Pb-9	Negative	Motile	Positive	Positive
Pb-10	Negative	Motile	Positive	Positive
Pb-11	Positive	Motile	Positive	Positive
Pb-12	Negative	Motile	Positive	Positive
Pb-13	Negative	Motile	Positive	Negative

Table 2. Investigation of Polythene Degradation by Culture Media

S. No.	Bacterial Isolates	Intital Weight (Mg)	Incubation Days	Final Weight (Mg)	Weight Loss (%)
1.	PB-13	1000	15	997	3%
			30	990	10%
			45	986	14%
			60	981	19%
2.	PB-12	1000	15	998	2%
			30	994	6%
			45	990	10%
			60	987	13%

environment is profound. Toxic chemical release during manufacture is another significant source of negative environmental impact of plastics. As a consequence, the production of plastics has increased substantially over the last 60 years from around 0.5 million tonnes in 1950 to over 260 million tonnes till 2019. Even though Tamil Nadu Government has concern and reduced the usage of Non Biodegradable plastic, still small shops were using the carry bags and so on. So the overriding of plastic usage and to degrade the available plastic in landfill microbial degradation will be a best choice.

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