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Screening and Isolation of Polyethylene degrading Bacteria from various sources

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Abstract

Plastics are the most commonly used polymers for routine applications. The accumulation of plastics is a threat to environment as it causes pollution, creating an imbalance in the ecosystem, thus proving to be hazardous. The ways to degrade plastic have not been successful. At the same time natural degradation of plastics is too time consuming. The most eco-friendly approach to resolve this ever growing and persistent issue is the microbial degradation route. The main objective of the present study is to isolate and screen for bacteria having the capability to degrade low density polyethylene (LDPE) which is a major cause of environmental pollution. The bacteria were isolated from various sources after serial dilution on M9 medium incorporated with LDPE. The selected isolates were comparatively screened by an Agar cup method to find strong LDPE degraders. These isolates were further tested for the extent of degradation using a film degradation assay. Total 20 cultures were obtained during the primary screening as probable LDPE degraders. In the agar cup method, 7 high LDPE degraders were selected. The percentage degradation was found out using the film degradation assay and the two most promising isolates were obtained from sewage and marine sources. These isolates were identified using biochemical methods as belonging to the Genus Staphylococcus.

Keywords: Plastics, degradation, eco-friendly, ldpe, agar cup etc.

Introduction

Plastic is the most useful synthetic 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. LDPE is the most commonly occurring non-biodegradable waste material which constitutes approximately 60% of the total plastic production[1]. The global usage of polyethylene is increasing at a rate of 12% yearly and around 140 million tones of synthetic polymers are produced worldwide each year[2]. Plastic wastes are one of the factors in causing environment pollution, because of their semi-permanent stability in the environment. There are many disadvantages of plastic like combustibility, deformation under load, embrittlement at low temperature etc and the major one is that they are not biodegradable and because of their high molecular weight, cross linkages, high number of aromatic rings and halogen substitution they clog in the environment[3]. The percentage of plastic waste has been increased in the landfills leading to pollution of the environment. A biological process, bioremediation breaks down various pollutants and reduces solid waste. Microbial degradation of plastic and polythene is a major interest in recent years. Biodegradable polymers are polymers which can be degraded naturally by micro-organisms such as fungi, bacteria, algae etc present in the environment[4]. There are various physical, chemical and biological forces which causes initial breakdown of polymer[5]. Microbial degradation of plastics converts polymer into oligomers and monomers. This microbial degradation may be based on aerobic and anaerobic metabolisms. The aim of the present study was to isolate microorganisms from varied natural sources and screen them for potential polyethylene degrading capability. The objective of the work carried out was to identify and characterize the high potential LDPE degraders. The isolation was carried out through serial dilution method of samples from sewage, oil sludge, marine, compost, tar sample and oil contaminated soil. The screening of potential LDPE degraders was carried out using a sequential screening procedure using an agar cup method followed by a film degradation assay. On identification the two selected bacteria were found to be belonging to genus *Staphylococcus*.

Material and Methods

Materials: Samples were collected in sterile test tubes, from varied sources like Compost (CC), Marine (M), Oil Sludge (OS), Sewage (S), Tar Sample (TS) and Oil Contaminated Soil (OCS). LDPE (Low Density Poly Ethylene) powder and LDPE films (Provided by Aegis Polymers). M9 agar and M9 broth (Prepared as per Maniatis, Manual for Molecular Biology)[6]. LB agar (Obtained from HI-Media, prepared as per manufacturer's recommendation)

Methodology: Primary screening and isolation of polymer degrading microorganisms: In primary screening the samples collected from the above mentioned sources were serially

diluted up to the range of 10^{-6} and alternate dilutions including the undiluted sample were spread plated on modified M9 agar containing 0.1% LDPE powder as the only source of carbon. The plates were kept for incubation at 37°C up to 72 hours. After incubation, growth was observed and the distinct colonies were chosen for each of the sources. These isolates were preserved on LB slants till further study.

Secondary screening for high capacity LDPE degraders: Next these probable plastic degrading isolates were screened further to select the ones which could withstand high LDPE concentration and thereby eliminate the weak LDPE degraders. Hence the LDPE concentration was increased to 1% as the only available source of Carbon. The method adopted for secondary screening was Agar Cup Assay. Wells were bored in the M9 agar and culture suspension with adjusted OD of 0.3(620nm) was added in the respective wells of the selected isolates. Plates were incubated up to 72 hours at 37°C and growth was observed on the plates. The colonies which could utilize the high concentration of LDPE grew around the periphery of the well and were shortlisted for the next study.

Film degradation assay: Pre-weighed alcohol sterilized 3*3 cm LDPE films were transferred to 50ml of respective M9 broth inoculated with isolates from secondary screening. A control flask was maintained with the film without any culture suspension. The flasks were put on shaker conditions at 37°C/150rpm for 3 weeks and the OD was checked at 620nm at regular intervals. After 3weeks, the LDPE films were weighed again and the percent plastic degradation by the microbes was determined and compared.

Results and Discussion

The current study deals with screening, isolation and extent of plastic degradation by the isolates from various sources. Twenty bacterial colonies were isolated from primary screening using 0.1%LDPE as the sole source of carbon in M9 Agar medium. In primary screening four bacteria were isolated from compost soil, three from marine, four from oil sludge, four from sewage and five from oil contaminated soil. The tar sample did not show any significant bacterial growth (figure-1).

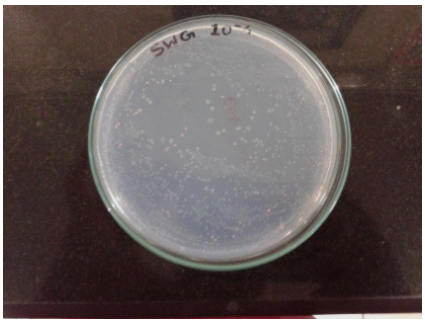

Figure-1
Microbial growth of sewage sample during Primary screening

The agar cup secondary screening method, which had 1% LDPE in M9 Agar, showed prominent growth of seven cultures around the periphery of the respective wells. These seven isolates were found to have a greater ability to withstand such high LDPE concentration as compared to the other thirteen isolates from primary screening. Thus agar cup secondary screening provided an efficient and quick method for comparative screening of LDPE degraders. The seven cultures named as CC1, OCS3, OCS5, OCS1, OCS4, M1 and SWG were selected for further film degradation assay (figure-2).

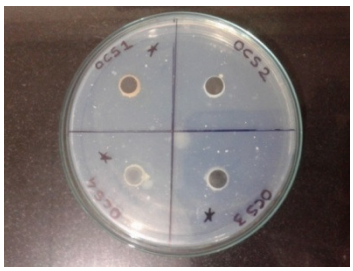

Figure-2
Microbial growth during secondary screening

Pre-weighed LDPE films were subjected to degradation with the above mentioned seven isolates. Out of the seven isolates M1 and S4 showed maximum degradation in liquid M9 medium, which confirmed higher plastic degrading capacity (table-1).

These two isolates were further characterized using Gram's staining and biochemical tests[7] .Gram staining of the two isolates showed Gram positive cocci in clusters (figure-3 and 4).

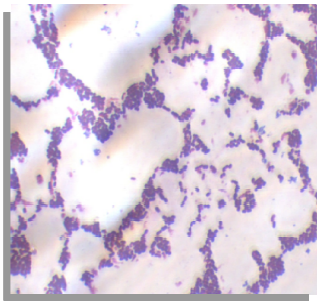

Figure-3
Gram positive cocci in Clusters for marine isolate

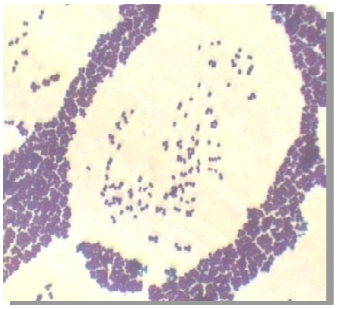

Figure-4
Gram positive cocci in Clusters for sewage isolate

Table-1
Result for extent of degradation of LDPE films by the isolates

Source	O.D (620nm) after 72 hours	Initial weight (g)	Final weight (g)	% degradation
Control	0	0.0225	0.0225	-
CC1	0.22	0.0207	0.0195	1.2
OCS3	0.12	0.0214	0.0210	0.4
OCS5	0.12	0.0220	0.0213	0.7
OCS1	0.11	0.0211	0.0207	0.4
OCS4	0.13	0.0222	0.0216	0.8
M1	0.25	0.0209	0.0184	2.5
S4	0.28	0.0237	0.0209	2.8

Table-2
Consolidated result for microscopy and biochemical tests for marine and sewage isolates

Test	M1 (Marine)	S4 (Sewage)
Gram nature	Gram positive cocci in clusters	Gram positive cocci in clusters
Catalase	Positive	Positive
Hemolysis	β	β
Carbohydrate Utilization	Positive	Positive
Vogues Proskeur's	Negative	Negative
Citrate	Positive	Positive
Urease	Negative	Negative
Nitrate reduction	Positive	Positive

The cultures were found to be catalase positive and β-hemolytic in nature (figure-5, 6 and 7).

Thus, both the bacteria were identified belonging to the genus *Staphylococcus*. On biochemical classification as per Bergey's Manual (table-2).

Figure-5
Catalse test for sewage isolate

Figure-6
Catalase test for marine isolate

Figure-7
Hemolysis test for sewage and marine isolates

Conclusion

Some degradability is present in natural conditions, but microbes also exhibit degradation in laboratory condition on culture media. The two strains of bacteria M1 and SWG isolated from marine and sewage sources respectively, were found to have higher ability to degrade LDPE and belonging to *Staphylococcus* genus.

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