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RESEARCH ARTICLE

DETERMINATION OF POLYETHYLENE DEGRADABLE BACTERIAL STRAINS FROM PLASTIC WASTES DUMP YARD SITES

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ABSTRACT

Polythene wastes accumulating in the environment are posing an ever increasing ecological threat. Naturally colonized microorganisms play a vital role in biodegradation of polyethylene, depending on the environmental conditions. This study represents biodegradability of native bacterial strains in the municipal waste landfill sites. Samples from various landfill sites of polyethylene films were collected from Cuddalore district. The morphological characteristics of various colonies of bacteria were observed, the isolated were screened for the biodegradability and were identified using staining, biochemical characterization and 16SrRNA sequencing techniques. Twenty different bacterial strains were isolated among these, *Pseudomonas aeruginosa*, (37.56 %) recorded maximum weight loss were observed followed by *Bacillus* sp. (32.70 %), *Alcaligenes* sp. (31.33 %), *Bacillus anthracis* (26.83 %) and *Shigella sonnei* (25.30 %) were estimated. Two novel bacteria *Bacillus* sp and *Alcaligenes* sp. were ultimately degraded polyethylene films and posing important role in polymer degradation.

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INTRODUCTION

One of the wastes that cannot be easily destroyed is plastic waste, which is a type of polyethylene plastic. Plastics include polythene, propylene, polystyrene, polyurethane, nylon etc. polyethylene either LDPE (low density polyethylene) or HDPE (high density polyethylene) is a thermoplastic made by monomers of ethylene, used primarily as thin films and packaging sheets (Albertsson and Karlsson, 1990). Among these LDPE materials are strong, light-weight and durable thus are having wide uses. Polyethylene (PE) is the man made polymeric material using as the alternative source of other metals and glasses because of this flexibility and durability. Low density polyethylene is one of the major sources of environmental pollution. The use of polyethylene growing worldwide at a rate of 12% and about 140 million tons of synthetic polymers are produced worldwide each year. With such a large amount of polyethylene gets accumulated in the environment, generating ecological problems are needed thousands of years for efficiently degradation (Usha *et al.*, 2011). Microorganisms, such as bacteria, fungi and actinomycetes are having capable to degrade of both natural and synthetic plastics (Gu *et al.*, 2000) by the action of enzymes, chemical degradation with living organisms. Biodegradation resulting from the utilization of polyethylene as

nutrient may be more efficient if the degrading microorganism forms a biofilm on the polyethylene surface. The primary mechanism for the biodegradation of high molecular weight polymer is the oxidation or hydrolysis by enzyme to create functional groups that improves its hydrophilicity (Huang *et al.*, 1990) when a plastic degrades, these polymer bonds break. This research investigated the presence of bacteria in plastic wastes dump yard sites and to facilitate this degradation process by breaking up the polyethylene polymers.

MATERIALS AND METHODS

Isolation of bacterial strains from polyethylene wastes (Gupta *et al.*, 2010)

Polyethylene waste in the capture of various land fill polyethylene waste dump yard sites Cuddalore in Tamilnadu washed with sterile distilled water and then cut with sterile scissors. Samples were diluted up to 10^{-6} with normal saline (0.9 % NaCl) and from 10^{-5} to 10^{-6} sample of 0.1 ml dilution was inoculated into prepared media NA (Nutrient agar) for the isolation of bacteria. Plates were incubated at 37 °C for 24 to 48 hrs. After the incubation period colonies have been counted by CFU (colony forming unit) and colony morphology were observed.

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Screening the bacterial degradation(Kathiresan, 2003)

The initial weights of polyethylene films were noted. The minimal carbon free media were prepared the cultures were swabbed on plates about three replication per culture and placed the sterile polyethylene films

RESULTS AND DISCUSSION

More than 20 bacteria were isolated from various polyethylene waste dump yard sites of Cuddalore in NA media. All the isolates were purified and named as PBI, PBII, PBIII up to PBXX (PB-Polyethylene Bacteria) in these 20 bacteria, the five bacterial strains ultimately degraded the provided LDPE

Table 1. Screening of biodegradability of bacterial isolates

Bacteria	Initial weight (mg)	Weight of polyethylene			Average	Percentage (%)
		1 month	2 month	3 month		
PBIV	20.71	18.57	15.76	12.93	7.78	37.56
PBVI	19.98	18.19	16.60	13.72	6.26	31.33
PBIX	20.76	18.83	16.93	13.97	6.79	32.70
PBXV	20.53	18.32	17.87	15.02	5.51	26.83
PBXVI	20.27	18.03	17.27	15.14	5.13	25.30

Table 2. Physiological and biochemical characterizations of polyethylene degrading bacteria

S.No	Characters	PBIV	PBIX	PBVI	PBXV	PBXVI
1	Morphology	Rod	Rod	Short rod	Rod (large)	Rod
2	Arrangement	Single	Pairs, chain	single	Chain or filament	Single
3	Gram's reaction	-	+	-	+	-
4	Pigment	Blue/Green agar	no	No	no	No
5	Motility	motile	Motile	Motile	Non motile	Non motile
6	Indole	-	-	-	-	+
7	MR	-	-	-	+	+
8	VP	-	+	+	+	-
9	Citrate	+	+	+	+	-
10	Urease	+	-	-	-	-
11	Starch	-	+	+	+	-
12	Nitrate	-	+	+	+	+
13	Catalase	+	+	+	+	+
14	Oxidase	+	-	+	-	-
15	Glucose	-	+	-	+	+
16	H ₂ S	-	-	-	-	-

(2.5x2.5cm sq) and the minimal carbon free broth were prepared the LDPE (Low Density Polyethylene) films were mixed with these. Culture plates were maintained for incubation at 37°C for three months. The culture broth has been maintained on shaker for 130 rpm in same duration. After the incubation period, polyethylene films were recovered from the culture plates and broth rinsed by sterile distilled water and 77 % ethanol for the removal of microbes and media particles. Samples were air dried and maintained in hot air oven at 60°C for 10 minutes for dehydration. Recovered polyethylene films loss of weight was measured using weighing balance.

$$\text{Weight loss (\%)} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100$$

Selection and Identification of biodegradable bacterial strains

Dominantly degraded bacterial strains were performed to identify on the basis of macroscopic & microscopic examination and biochemical test according to Bergey's manual of determinative bacteriology (Holt *et al.*, 1994).

The genomic characterizations of biodegradable bacterial isolates have been identified by 16SrRNA sequence analysis.

(low density polyethylene) with carbon free minimal media. Among the five bacteria 37.56 % of weight loss were observed in PBIV which had utilized polyethylene as a sole source of carbon, low density polyethylene was used and the growth experiment were performed by inoculating the tested bacterial isolate in minimal carbon free minimal broth and incubated in shaker incubator (130 rpm) at 30°C for 3 months followed by 32.70 %, 31.33 %, 26.83 % and 25.30 % *viz.* PBIX, PBVI, PBXV and PBVI were observed in same period of incubation. This observation indicated that this bacterial isolate utilized polyethylene as a sole of carbon source resulting in partial degradation of plastics these details shown in Table-1. Dede Mahdiyah and Bayu Hari Mukti (2013) have screened and reported as the eight isolates had different percentages of degradation and the highest degradation obtained by isolate of 22 TSB (Tryptic Soy Broth) at 17 %, than the other isolates have the capacity degradation of plastics: isolate of 1 TSB (6 %), isolate of 6 TSB (12 %), isolate of 11 TSB (4 %), isolate of 17 NB (4 %), isolate of 15 NB (5 %), isolate of 30 NB (6 %), and isolate of 21 NB (5%). Identification of microorganisms: The identified bacterial species were *E.Coli*, *Staphylococcus*, *Pseudomonas*, *Klebsiella* and *Bacillus*. It is also based on the reports of (Kathiresan, 2003) that *Pseudomonas* sp degraded the plastic up to 8.16 % and 20.5 % of degradation was observed anaerobically.

Identification of the polyethylene degradable bacteria

Most of the bacterial strains of isolates have been rod shaped; formed pairs and chain, and other cultural physiological and biochemical characters were identified and shown in Table 2. The 16s rDNA sequences were matched for local alignment through NCBI-BLAST. Based on the scores and identity percentages, the isolates identified (Soni *et al.*, 2010). Similarly in the present study the isolates of bacterial strains were identified as PBIV-*Pseudomonas aeruginosa*, PBXV-*Bacillus anthracis*, PBXVI-*Shigella sonnei* & novel species of *Bacillus* sp., and *Alcaligenes* sp., were identified through 16SrRNA genomic sequence analysis and submitted in NCBI.

Conclusion

The isolated microbes were native to the site of polyethylene disposal and might show some degradability in natural conditions, they also exhibited biodegradation in laboratory conditions on carbon free minimal media. This study gives some suggestion that these microbes can be used in both natural and artificial conditions for the purpose of degradation of polymers. Our knowledge, microbes cause greatest degradation of polythene and plastics. Among the 20 isolated bacteria, these five *viz.* *Pseudomonas aeruginosa*, *Bacillus* sp., *Alcaligenes* sp., *Bacillus anthracis* and *Shigella sonnei* and among these *Pseudomonas aeruginosa*, having greater degradation ability when compared with other five bacteria. Hence, the further attention is required from microbiologists for commercial degradation and eco-friendly polyethylene with these five bacteria.

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