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ISOLATION AND CHARACTERIZATION OF POLYTHENE DEGRADING BACTERIA FROM GARBAGE SOIL

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ABSTRACT

Continuous accumulation of plastic in the environment causes threat to environment as well as to human. In order to find the microbes that can degrade polythene, sample was collected from dump yard, Khulna City Corporation, Bangladesh. Screening of polythene degrading bacteria was done by analyzing the growth in Low Density Polyethylene (LDPE) powder. Thirty one bacterial isolates were obtained using synthetic media containing LDPE powder as sole carbon source. Among them, 10 bacterial isolates were found to be polythene degrading through weight loss of polythene and named as PDB. These bacterial isolates were characterized both morphologically and biochemically. Gram staining test revealed the 8 isolates were Gram positive and 2 were Gram Negative. The biomass weight (µg/ml) was measured at 3 different concentrations of LDPE powder; the biomass was increased for all the bacterial isolates up to 10th day and drastically reduced at 15th day. The pH of the culture increased for 8 isolates but 2 isolates (PDB-2 &11) showed reduced pH than that of control (pH 7.0). The weight loss (%) of polyethylene was estimated and found that all bacterial isolates reduced polythene weight ranged from 0.42 to 3.17% and 1.31 to 3.47%, respectively, at 5 and 10 days of culture.

Keywords: Biodegradation, Garbage soil, LDPE powder, Polythene, Biomass

1. INTRODUCTION

Polyethylene is long-chain synthetic organic polymers widely used in various aspect of life. Polyethylene is customarily considered as the most commonly found solid waste and shares around 64% of the total plastic waste produced worldwide (Lee *et al.*, 1991). Low density polyethylene is the major source of environmental pollution. The yearly increment of polyethylene use is 12% and almost 140 million tons of synthetic polymers are being produced worldwide every year. Thus, polyethylene gets increase in the environment creates plastic waste ecological problems, which needs thousands of years for degradation effectively (Usha *et al.*, 2011). Improper disposal of plastic materials are important source of environmental pollution. Therefore, in order to prevent polythene accumulation, adequate disposal system should be adopted. Biodegradation, the decomposition of substances through microbial activity is a complex process that involves several steps (Shah *et al.*, 2007) viz. bio-deterioration, depolymerization, assimilation and mineralization (MuÈller, 2005). Microorganisms are involved in polythene degradation, they synthesis secreting extracellular enzymes which oxidize influence in the degradation process (Albertsson *et al.*, 1998; Chiellini *et al.*, 2003). The

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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).

Among fungi and bacteria, more than 90 genera can degrade plastic and some important groups are *Bacillus subtilis*, *Kocuria palustris*, *Bacillus pumilus* (Harshvardhan and Jha, 2013), *Pseudomonas sp.* (Bhatia *et al.*, 2014), *Brevibacillus borstelensis (*Hadad *et al.*, 2005*), Serratia marcescens* (Azeko *et al.*, 2015). Hence the present study aims to isolate and screening of microbial species capable to degrade LDPE.

2. MATERIALS AND METHODS

2.1 Sample collection and preparation

Soil sample were collected from dump yard of Khulna City Corporation, Bangladesh. Samples were brought to the laboratory maintaining aseptic conditions. One gram of soil was transferred in a 50 ml sterile Erlenmeyer flask and 9ml sterile distilled water was added and serially diluted up to 10^{-6}.

2.2 Media preparation

Mineral Salt Media (MSM) was prepared by NH_4NO_3 1.0 g/l, $MgSO_4.7H_20$ 0.2 g/l, K_2HPO_4 1.0 g/l, $CaCl_2.2H_2O$ 0.1 g/l, KCl 0.15 g/l, $FeSO_4.6H_2O$ 1.0 mg/l, $ZnSO_4.7H_2O$ 1.0 mg/l, $MnSO_4$ 1.0 mg/l devoid of any carbon source was used for degradation experiments. The media was fortified with LDPE powder as sole carbon source for bacterial growth. LDPE powder (1.0 g/l) was added to the medium after sterilization at 121℃ for 15 minutes to avoid deformation (Russel *et al.*, 2011).

2.3 Screening

2.3.1 Primary screening of bacteria from soil sample

Serially diluted soil samples (0.5ml) were inoculated in the media and incubated at 37 ºC for 24 hours. After incubation in the synthetic liquid media, 1 ml of culture was spread on nutrient agar media and after several streaking, pure bacterial culture was obtained. The isolated pure cultures were preserved in refrigerator at 8℃for further use.

2.3.2 Secondary screening of bacteria

The most active polythene degrading bacterial isolates obtained from primary screening were subjected to secondary screening.

2.3.2.1 Determination of bacterial growth

One hundred milliliter of liquid MSM was dispensed in 250 ml flasks and fortified with different concentrations viz. 100 mg, 150 mg and 250 mg of LDPE powder as a substrate and sole source of carbon. Then 1ml of 24hr older bacterial culture was inoculated to each flask containing liquid defined media and a control (medium inoculated with 1ml sterile water) was carried out. The

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flasks were incubated for 5, 10 and 15 days at 37℃. The absorbance in terms of OD was recorded at 460 nm using spectrophotometer to observe the bacterial growth for a period of time.

2.3.2.2 Determination of pH change

Study of pH change was adopted to observed any metabolic activity of bacterial isolates in supplemented medium, as metabolism is shown by microbial cells may greatly support the evidence of degradation. Initial pH of the medium was ensured to be 7.0 ± 0.2. A control treatment was also carried out without bacteria culture.

2.4 Characterization of Isolated Bacteria

The bacterial isolates were subjected to Gram staining according to the method of Vincent and Humphrey (1970). Bacterial isolates were subjected to characterization by the biochemical tests such as oxidase test, catalase test (Aebi, 1974), starch hydrolysis test, indole test (Sharnali *et al.*, 2019), urease test, methyl red test, gelatin hydrolysis test, motility test, Voges-Proskauer test, TSI test and citrate utilization test (Hofwegenet *et al.*, 2016; Cheesbrough, 2006).

2.5 Determination of bacterial growth rate

Overnight bacterial cultures were inoculated to 50 ml synthetic media containing 250mg of LDPE powder and were incubated at 37℃. For every one hour interval, the OD was measured at 460nm. Using this OD, the cell concentrations were calculated and growth rate was determined according to Pramila and Ramesh (2018).

2.6 In vitro determination of weight loss of polythene

In order to check the polythene degrading capability of the isolates, polyethylene bags (thickness of polythene approximately 70µm) from local market were cut into 3cm × 3cm and disinfected with ethanol and air dried. The disinfected polythene strips were added into conical flask containing 100ml of nutrient broth with polythene degrading organism. Control has been maintained for further reference and to confirm the reduction of weight of the polyethylene. The bacterial cultures were incubated at three different temperature viz.25℃, 30℃ and 37℃, for 5 and 10days. After these periods of time, the strips were washed in 70% ethanol, air dried and weighed.

2.7 Data analysis

Data analysis was done by using the OD which was described by Kim *et al*. (2012), the cell concentration was calculated using 3^{rd} and 6^{th} order polynomial equation where the value of R is 1. The equation was driven from Microsoft Excel and C++ programming was used for analyzing the data.

The weight loss of polythene was calculated and compared with control based on the following equation (Ariba *et al.*, 2015).

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$$Weight\ Loss\ \% = \frac{Initial\ Weight - Final\ Weight}{Initial\ Weight} \times 100$$

3. RESULTS

3.1 Primary screening of bacteria from soil sample

In this study a total of 33 isolates were obtained and all of these isolates were observed for the efficiency of polythene degradation. But only ten isolates were reported as polythene degrading bacteria. These 10 isolates were then subjected to secondary screening for further study.

3.2 Secondary screening of bacteria

The most active polythene degrading bacterial isolates obtained from primary screening were further screened for LDPE degradation rates by sequence of events which were assessed by two methods which are determination of growth rate of bacteria and determination of pH change.

3.3 Determination of bacterial growth

Most of the isolates showed highest bacterial concentration at day 10. As several concentrations of LDPE powder have been used in this study, among all the isolates PDB-7 showed the highest bacterial growth in these varying concentrations of LDPE media at day 10.The lowest growth is seen at day 10 by PDB-6 both at 100mg and 250mg and PDB-12 at 150mg. At day 15, most of the isolates showed concentration below 1µg/ml.

3.4 Determination of pH change

Among all the isolates, the highest pH was shown by PDB-12 in the media containing the highest amount of LDPE powder which is 250mg. All the isolates showed increasing pH except PDB-2 and PDB-11 at 100mg, PDB-13 at 150mg and PDB-2 at 250mg LDPE powder containing media. The pH of the media , inoculated with bacterial isolates was measured and the result is shown in Table 1.

Table 1: pH of the medium inoculated with bacterial isolates

Isolates	100 mg	150 mg	250 mg
PDB-1	8.950	8.640	8.5
PDB-2	6.830	8.604	6.431
PDB-6	7.898	8.728	7.509
PDB-7	7.395	7.281	8.280
PDB-9	8.433	7.957	8.175

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PDB-10	7.675	7.157	7.279
PDB-11	6.930	8.501	7.746
PDB-12	7.812	8.270	9.001
PDB-13	8.431	6.471	8.708
PDB-14	8.446	8.092	7.857
Control	7.0	7.0	7.0

3.5 Characterization of Isolated Bacteria

All 10 isolates were subjected to morphological, physiological and biochemical characterization. Cellular Shape and Gram staining were performed for morphological and physiological characterization. Among 10 isolates, all the isolates were gram positive except isolate PDB-6 and PDB-7which were gram negative. In this study we found most of the bacteria in rod shape except PDB-1 in Streptobacilli, PDB-2 in Branching Rod, PDB-6 in Diplobacilli and PDB-7 in Coccus shape. Result of biochemical characteristics of bacterial isolates are summarized in Table 2.

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Table 2. Biochemical characterization of the polythene degrading bacteria.

Biochemical Properties	PDB-1	PDB-2	PDB-6	PDB-7	PDB-9	PDB-10	PDB-11	PDB-12	PDB-13	PDB-14
Oxidase	+VE	-VE	+VE	-VE	-VE	+VE	-VE	-VE	-VE	+VE
Catalase	+VE	-VE	-VE	-VE	-VE	+VE	+VE	-VE	-VE	-VE
Indole	-VE	-VE	-VE	-VE	-VE	-VE	-VE	-VE	-VE	-VE
Citrate	+VE	+VE	+VE	+VE	+VE	+VE	+VE	-VE	-VE	-VE
Methyl red	-VE	-VE	+VE	+VE	-VE	-VE	-VE	-VE	+VE	-VE
Voges-Proskauer	-VE	-VE	-VE	-VE	-VE	-VE	-VE	-VE	-VE	-VE
Glucose	-VE	+VE	+VE	-VE	+VE	+VE	-VE	-VE	-VE	-VE
Lactose	-VE	-VE	-VE	+VE	-VE	-VE	+VE	+VE	+VE	+VE
Sucrose	-VE	-VE	-VE	+VE	-VE	-VE	+VE	+VE	+VE	+VE
Gas	-VE	+VE	-VE	+VE	+VE	+VE	+VE	+VE	+VE	+VE
H_2S	+VE*	+VE*	-VE	-VE	+VE*	+VE*	-VE	-VE	-VE	-VE
Urease	+VE	-VE	-VE	-VE	+VE	+VE	+VE	+VE	-VE	-VE
Starch hydrolysis	-VE	-VE	-VE	-VE	-VE	-VE	-VE	-VE	-VE	-VE
Gelatin hydrolysis	-VE	-VE	-VE	-VE	-VE	-VE	-VE	-VE	-VE	-VE
Motility test	+VE	+VE	+VE	+VE	+VE	+VE	+VE	-VE	-VE	+VE

* Slightly produced H_2S

3.6 Determination of growth rate of bacteria

At 0 hour after inoculation, most of the isolates showed concentration below 0.7µg/ml. With the increasing period of time the isolate showed increasing amount of concentration. The highest

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concentration is showed by PDB-7 at 5th hour after inoculation which is 6.82µg/ml. At this same time the lowest concentration is showed by PBD-6 which is 3.19µg/ml.

3.7 Results of *in vitro* weight loss of polythene

As we examined polythene degrading capability, several bacterial isolates in different temperature, the highest degradability of polythene was obtained at 37°C for both 5 and 10 days of culture and the data is shown in Table 3. We also found that at 25°C bacterial isolates didn't show any degradability in both 5 and 10 days but at 30°C slight degradability was observed at day 10 (data is not shown). In nutrient broth, the highest degradation capability is reported by PDB-1 which is 3.17% at day 5 and by PDB-14 which is 3.47% at day 10 after inoculation. However, the lowest degradation capability is reported by PDB-14 at day 5 which is 0.51% and PDB-11 at day 10 which is 1.62%. Weight loss percentage of polythene is shown in Table 3.

Table 3: Weight loss (%) of polythene

Isolates	5 days				10 days			
	Initial Weight mg	Final Weight mg	Difference	Weight loss %	Initial Weight mg	Final Weight mg	Difference	Weight loss %
PDB-1	18.9	18.3	0.6	3.17	24.9	24.2	0.7	2.81
PDB-2	18.2	17.7	0.5	2.75	18.9	18.3	0.6	3.17
PDB-6	14.6	14.1	0.5	2.05	18.4	17.8	0.6	3.26
PDB-7	21.6	21.1	0.5	2.31	21.3	20.8	0.5	2.34
PDB-9	17	16.7	0.3	1.76	16.2	15.8	0.4	2.47
PDB-10	23.5	23.4	0.1	0.42	18.5	18.2	0.3	1.62
PDB-11	20.2	20	0.2	0.99	15.2	15	0.2	1.31
PDB-12	23.8	23.4	0.4	1.68	21.2	20.7	0.5	2.35
PDB-13	22.4	22.1	0.3	1.34	19.5	19.1	0.4	2.05
PDB-14	19.3	19.2	0.1	0.51	17.3	16.7	0.6	3.47

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Control	18.3	18.3	0	0	22.1	22.1	0	0

4. DISCUSSION:

Deepika and Madhuri, (2015) found 4 isolates that degrades polyethylene film. Among them *Streptomyces sps.* degraded the highest amount of polythene films and Usha *et al.*, (2011) found 3 bacterial species that can degrade polythene where we found 10 bacterial isolates capable to degrade polyethylene. Ariba *et al.*, (2015) found *Pseudomonas alcaligenes* (weight loss % in 10 , 20 and 30 days are 10.2,13.2 and 16.2 respectively) and *Desulfotomaculum nigrificans* (weight loss % in 10 , 20 and 30 days are 10.5,14.7 and 20.1 respectively) The finding of our study regarding weight loss is 3.17% in NB in 5 days and 3.47% in NB in 10 days. Hussein (2015) found changes of pH ranging from 6.41 to 6.92 with a control containing pH 7 whereas we found changes of pH ranging from 6.431 to 9.001 with a control containing pH 7.

This study shows that temperature is a major factor for degrading polythene. This study also states that the bacterial strain has capacity of degrading the recalcitrant polymer, if the right conditions are provided. We can say that soil microbes like bacteria, fungi and *Actinomycetes* shows great efficiency in degrading synthetic polymer granules like LDPE polyethylene bags produced from polymer granules. Therefore, further research work is needed to prevent environmental damage caused by plastic and polythene waste contamination. From this study we can say that, these bacteria can degrade polythene under controlled environment. Polythene are the most greatest enemies of mankind and from this study we can say that if we can apply these microbes to the environment at a controlled way we can save the pollution and also save our earth. Thus further studies are needed for confirmation of applying microbes to environment.

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