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ISOLATION AND CHARACTERIZATION OF LOW DENSITY POLYETHYLENE DEGRADING *BACILLUS* SPP. FROM GARBAGE DUMP SITES

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ABSTRACT

Present study investigates an ecofriendly approach for the degradation of Low Density Polyethylene that may persist in the environment for a long period of time due to its recalcitrant nature and creates major threat for the environment. Plastic contaminated soil samples were collected from three sampling sites and sixteen bacterial isolates were selected on the basis of cultural characteristics. All the isolates were studied for their biodegradability by clear zone method on minimal salt medium. Three isolates namely ISJ51, ISJ55and ISJ57 were considered positive for polyethylene degradation on the basis of halo zone produced around the bacterial colony after treatment with coomassie blue solution. By standard morphological and biochemical characterization, the isolates were probably identified as *Bacillus cereus, Bacillus subtilis* and *Bacillus megaterium.*

KEYWORDS: Plastics, Biodegradation, Screening, clear zone, *Bacillus* sp., LDPE.

1. INTRODUCTION

Polyethylene are synthetic organic polymers and used in a wide variety of applications in every aspect of life. Polyethylene is generally considered as the most commonly found solid waste, shares about 64% of total plastic waste produced (Lee et al. 1991). Due to their chemical structure containing strong single C−C and C−H bonds, waste management of Low density polyethylene (LDPE) is a growing concern today (Jia et al. 2016). Degradation of LDPE in the environment is difficult because in natural conditions, degradation of plastics is a slow and time consuming process, influenced by a number of environmental factor

including temperature, humidity of air and moisture in the polymer, pH and solar energy, polymer properties and biochemical factors followed by wild microorganisms (Albertsson et al. 1998; Arkatkar et al. 2009). Various chemical and physical methods used for the disposal of plastic waste are not feasible economically and environmentlly. For instance, during the thermal decomposition of plastic waste, toxic gases are evolved which may adversely affect the environment and resulting into breathing problem. Land filling is a conventional approach for the disposal of plastic waste but this method has its own hindrance such as land filling takes longer time for the degradation of polyethylene due to anaerobic environment and produces persistent organic pollutants (POP's) known as furans and dioxins which find their way into the human body, plant tissue and animals through absorption (Yang et al. 2011; Adams et al. 2014; Gupta et al. 2016). Many plastics can be recycled, but this is not a proficient method to manage the existing bulk of plastic waste generated (Shah et al. 2008; Hopewell et al. 2009; North and Halden 2013; Gupta et al. 2016). Hence there is an alarming importance to find out a suitable method for the ecofriendly disposal of plastic. Therefore, biodegradation is the eco-friendly and cost effective process which can solve the problem of plastic waste management easily. Biodegradation is the ability of micro-organism to influence the degradation of synthetic polymers through enzymatic action (Lee et al. 1991; Erlandsson et al. 1997; Albertsson et al. 1998; Chiellini et al. 2003). In this process, plastic reacts with oxygen from the air and then the microorganisms, facilitate the degradation by secreting extracellular enzymes to oxidize or break down the products for its energy into smaller byproducts such as carbon dioxide and water (Albertson, 1998). Although several microorganisms have been reported to degrade polyethylene such as *Rhodococcus ruber* (Orr. et al. 2004), *Brevibacillus borstelensis (*Hadad et al. 2005*), Bacillus subtilis, Kocuria palustris, Bacillus pumilus* (Harshvardhan and Jha 2013), *Pseudomonas sp.* (Bhatia et al. 2014), *Bacillus amyloliquefaciencs* (Das and Kumar 2015), *Serratia marcescens* (Azeko et al. 2015). The biodegradation of plastic materials is mainly depends upon its hydrophobicity and the size of the polymer molecules (Orr et al. 2004; Sivan et al. 2006). The hydrophobicity of polyethylene prevents the formation of biofilm by the microorganism which in return prevents the adhesion and colonization on the polyethylene (Ribitsch et al. 2012; Yoshida et al. 2016). Biodegradation of polyethylene attention to increase the hydrophobicity for the efficient microbial adherence and obtained low molecular weight compounds with modified mechanical properties (Palmisano and Pettigrew, 1992).

There is a growing interest in synthetic polymer biodegradation by using effective microorganisms. Developments of microbial communities attached to the synthetic wastes have been found to be powerful degrading agents in nature. Garbage soil was considered as a great reservoir for the isolation of plastic degrading microorganism because of the waste dumped on it. This study aims to isolate and identify the polyethylene degrading efficient bacterial strains from plastic waste dumped sites for the ecofriendly disposal of synthetic polymers and also attempts to understand the degradation ability of bacterial isolates by clear zone method.

2. MATERIAL AND METHODS

2.1 Collection of soil samples

Soil samples were collected from plastic dumping ground that has been used to dump plastic waste for many years. The samples were sealed properly, labelled and transported to the laboratory. All the samples were processed within 24 hours of collection.

2.2 Isolation of bacteria

Isolation of bacterial isolates was done by serial dilution method and spread plate technique on nutrient agar. The plates were incubated for 24-48 hr at 37^{o}C. Colonies having different morphology characteristics were selected and subculture for future characterization on nutrient agar (Pepper et al. 2004).

2.3 LDPE powder preparation

Low density polyethylene sheets were obtained from VSPN packaging industries, Bhagwanpur, Haridwar (Uttrakhand). The LDPE sheets were cut into small pieces and sterilized with 70% ethanol. Test samples were heated with xylene for 5- 15 minutes to dissolve completely. The resulting residue was washed with ethanol for 2-3 times to remove the residual xylene. The polyethylene powder thus obtained was sieved and kept for evaporation of ethanol and then dried overnight in hot air oven at 60^{0}C. Finally, the polyethylene powder was stored at room temperature (Rani and Singh 2015; Bhatia et al. 2014).

2.4 Screening of bacteria for biodegradability

Polyethylene degrading bacteria were screened on minimal salt medium (MSM) containing K_2HPO_4 (0.1g/L), KH_2PO_4 (3.0 g/L),) NaCl (5.0 g/L), NH_4Cl (2.0 g/L), $MgSO_4$ (0.2 (g/L), $CaCl_2.2H_2O$ (0.1 g/L), KCl (0.15 g/L) and agar powder (15 g/L) in distilled water. LDPE

powder (1.0 g/L) was added to the medium after sterilization to avoid deformation (Russel et al. 2011). All morphologically distinct colonies were selected and streaked on minimal salt medium containing LDPE powder as a sole carbon source. The bacteria were allowed to grow at 30-35^{0}C for 2-4 weeks (Skariyachan et al. 2015).

2.4 Visualization of clear zone

MSM agar plates were flooded with 0.1% solution of Coomassie blue R-250 for 20 minutes. The solution was prepared by dissolving 0.1% (w/v) of coomassie blue in 40% (v/v) methanol and 10% (v/v) acetic acid. After that the solution of coomassie blue was then poured off and the plates were flooded with destaining solution for 20 minutes, prepared by 40% (v/v) methanol and 10% (v/v) acetic acid. The organisms producing zone of clearance in a blue background were selected as the consumer of polyethylene. (Howard and Hilliard, 1999).

2.5 Identification of the bacterial isolates

The bacterial isolates were differentiated through colony morphology, microscopic examination and biochemical test (Shah et al. 2013). Morphological characterization of the isolates was done by Gram's staining method (Beveridge, 2001). Further the bacterial isolates were identified according to the criteria given in Bergeys's manual of Determinative Bacteriology (Holt et al. 1994).

3. RESULTS AND DISCUSSION

The present study deals with the isolation of LDPE degrading bacteria from the polyethylene waste disposal sites. The sampling site has been chosen to increase the probability of finding bacterial isolates capable of degrading polyethylene effectively because due to the absence of other carbon sources in these sites, microbes modify their metabolic pathways and the enzyme system to utilize LDPE as a nutrient. Sixteen bacterial colonies were selected on the basis of cultural characteristics and streaked on LDPE emulsified MSM agar plates followed by incubation at 35^{o}C for 28 days. After treatment with coomassie blue solution, clear halos were observed around three bacterial isolates designated as ISJ51, ISJ55 and ISJ57. The use of coomassie blue as an indicator for LDPE degradation in an agar medium provides the basis for the rapid and sensitive screening assay for polyethenolytic bacteria (Howard and Hilliard, 1998). Clear zone assay is a widely accepted technique for the screening of microorganisms for biodegradation of polyethylene. This assay is based on the secretion of microbial extracellular enzymes that convert polymer to a water soluble material in the agar medium

producing a clear zone around the bacterial colony (Fields et al. 1974; Pometto et al. 1992; Nishida and Tokiwa 1993; Tokiwa et al. 2009).

Table 1 - Isolation and screening of bacterial isolates from various waste disposal site.

Sampling site	Bacterial isolates	Positive for clear zone assay
Belda (Roorkee)	ISJ43, ISJ44, ISJ45, ISJ46	-
B.T ganj (Roorkee)	ISJ47, ISJ48, ISJ49 ISJ50, ISJ51, ISJ52	ISJ51
Nandvihar colony (Haridwar)	ISJ53, ISJ54, ISJ55 ISJ56, ISJ57	ISJ55, ISJ57

Identification of bacterial isolates showing positive results for LDPE utilization as a nutrient indicated the presence of Gram positive bacilli after Gram's staining (Beveridge, 2001). Biochemical characterization of bacterial isolates was done by standard microbial techniques. The microbiological feature of the isolates are shown in Table 2. From these studies it was concluded that the bacterial strains belong to the genus *Bacillus***.** Isolate ISJ51 was probably identified as *Bacillus* cereus while ISJ55 and ISJ57 as *Bacillus subtilis* and *Bacillus megaterium* respectively. Many previous studies reported that *Bacillus* is a major genus that can degrade polyethylene (Das and Kumar 2015; Singh et al. 2015; Gupta et al. 2016). Besides this a number of *Bacillus* sp. have also been reported for the degradation of other synthetic polymers e,g. polyurethane (Rowe and Howard 2002; Shah et al. 2008), polyester polyurethane (Shah et al. 2013). From this study, it was clear that the bacteria isolated from plastic waste processing areas are capable of utilizing polyethylene as nutritional sources. Our results recommend that each bacterial colony which is capable to form zone of clearance on minimal salt media plates should be considered for higher studies regarding to biodegradation activity.

Table 2. Morphological and biochemical characteristics of microorganisms.

Isolates	Morphology	I	Mr	V	C	Ct	Sh	Gl	G	M	F	S	Probably identified
ISJ51	Gram-positive bacilli	-	-	+	+	+	+	-	+	-	+	-	***Bacillus cereus***
ISJ55	Gram-positive bacilli	-	-	+	+	+	+	+	+	+	+	+	***Bacillus subtilis***
ISJ57	Gram-positive bacilli	-	-	-	+	+	+	+	+	+	+	+	***Bacillus megaterium***

I indole, Mr methyl red, V Voges-Proskauer, C citrate utilization, Ct catalase, Sh starch hydrolysis, Gl Gelatin liquefaction, G Glucose S sucrose, M mannitol, F fructose.

4. CONCLUSION

Present study conclude that three bacterial isolates consumes the LDPE powder as a carbon source. The isolates were identified as *B. cereus*, *B. subtilis* and *B. megaterium* by standard characterization method. Further analysis regarding mechanism of LDPE degradation by *Bacillus* sp. is necessary. Our next aim is to assess the hydrophobicity and biofilm formation ability of these bacterial strains.

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