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ISOLATION AND SCREENING OF LOW DENSITY POLYETHYLENE (LDPE) DEGRADING BACTERIAL STRAINS FROM WASTE DISPOSAL SITES

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ABSTRACT

Synthetic polymers such as polyethylene are widely used in agriculture, construction, packaging, health care, and medicine. These are resistant to microbial attack, hence create serious threat to the environment. The aim of this research was to isolate and screen bacterial strains for the biodegradation of LDPE. For this purpose, 42 bacterial strains isolated from different waste disposal sites of Haridwar region were screened on minimal salt media plates. The biodegradation activity of the isolates was investigated with the help of clear zone method by using LDPE powder as the sole carbon source in minimal salt medium. Zone of clearance was detected by the use of coomassie blue solution. 7 isolates demonstrated positive results for clear zone around the bacterial colonies. On the basis of morphological and biochemical characterization the bacterial isolates were probably identified as *Bacillus* sp., *Pseudomonas* sp. and *Micrococcus* sp.

KEYWORD: Low density polyethylene, Plastic waste, Biodegradation, Clear zone.

INTRODUCTION

Approximately 311 million tonnes of synthetic polymers are being produced in the world each year.[1] Polyethylene, are the inert synthetic polymers composed of long chain monomers of ethylene, that accumulates in landfills and natural habitats in huge amount therefore contaminates the environment.[2,3,4] Waste management of low density polyethylene (LDPE) is a growing concern of present time because of its non-degradable nature due to the presence of hydrophobic backbone.[5] India produces approximately 12 million tonnes of

plastics every year with quantum of plastic waste generated and recycled per day is estimated to be 15342.6 and 9205 tonnes respectively. Out of the total plastic waste generated in India per day, 6137 tonnes remains uncollected and littered.[6,7] Because of the inadequate disposal of polyethylene, the pollution has risen to unprecedented level in the environment. From an ecological point of view, the plastic debris accumulates in the environment causing harmful effect on all major types of biomes. Total plastic waste polluting marine as well as terrestrial environment is around 25 million tonnes, out of which 64% are synthetic plastic.[8] Almost all types of terrestrial ecosystems are affected by polyethylene pollution like deserts,[9] forest, grassland[10] and Polar Regions.[11] Polyethylene and plastics have also been known for its deleterious effect on the aquatic environment.[12] A decrease in marine fauna population has been observed due to the plastic waste.[13,14] Owing the plastic waste pollution minimum 267 marine species is being suffered from impact of plastic waste. This includes 86% of all sea turtle species, 44% of all sea bird species, and 43% of all marine mammal species.[12] Currently four options are available i.e. incineration, landfilling, recycling and biodegradation for the disposal of plastics, however, all the methods have certain inherent limitations.[7] 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 done for the degradation 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. A major problem associated with land filling treatment is the release of pollutants in the form of toxic compounds, which may cause several diseases in humans such as various types of cancer.[8] Many plastics can be recycled, but this is not a conventional approach due to difficulties with the collection and storage of plastic waste.[15,16,17a] Therefore, biodegradation can be considered as the most suitable, eco-friendly, and inexpensive method for polyethylene waste treatment, involving biological activity leading to the degradation and assimilation of polymers by living organisms. It has been reported that over 90 genera of bacteria and fungi are able to degrade plastic waste.[18,19,20,21,22,23,24,25,26] Properties that make polyethylene resistant to microbial attack includes not only the hydrophobicity and high molecular weight of polyethylene but also the inability of microbial enzymatic system to recognize functional groups on LDPE.[27] However, microorganisms have been isolated from various sources showing the ability to degrade polyethylene waste.[28,29,30,31,32] Some of the important bacteria reported for the biodegradation of LDPE includes, *Bacillus* spp., *Pseudomonas* spp., *Streptomyces* spp., *Rhodococcus* sp., *Acinetobacter* sp., *Brevibacillus* sp., *Flavobacterium* spp., *Ralstonia* spp., *Staphylococcus* spp., *Stenotrophomonas* spp.,

Micrococus spp., *Microbacterium* sp. and *Nocardia* sp.[33] However, the success rate is not satisfactory for the biodegradation of polyethylene.[31] Hence exploration of microorganisms from different sources is needed to get compatible strains with high potential of polyethylene degradation. There are a number of habitats and reservoirs in which unusual microbial niches are present, for example, waste disposal sites. The diverse physico-chemical and biological nature of solid waste indicates that screening of microorganisms from these sites has a profound scope in finding more bacterial strains having potential of LDPE degradation.[34,35] Therefore, in the present study, an attempt has been made to isolate potential polyethylene degraders from waste disposal sites.

MATERIALS AND METHODS

Preparation of polyethylene powder

LDPE samples were collected from VSPN Packaging industries, Bhagwanpur, (Haridwar) India. The polyethylene sheets were cut into small pieces and immersed in xylene followed by boiling for 5- 15 minutes to dissolves completely. The resulting residue was crushed by hands wearing gloves.[36] The crushed residue was washed with ethanol 2-3 times to remove the residual xylene. The polyethylene powder thus obtained was kept for evaporation of ethanol and then dried overnight in hot air oven at 60^{o}C. Finally, the polyethylene powder was stored at room temperature.[37]

Sample collection

Five polyethylene contaminated soil samples were collected from waste disposal sites located in different regions of Haridwar, India (Table 1). Partially degrading polyethylene films with adhered soil samples were collected in a sterile container at a depth of 3-5 cm[38]. The samples were labelled, sealed properly and transported to the laboratory. All the samples were processed within 24 hours of collection.

Isolation and Screening of LDPE degrading bacteria

Initially bacteria from the soil samples were isolated on nutrient agar medium by standard serial dilution method.[39] The nutrient agar plates were incubated at 37 ^{o}C for 24 hours. All morphologically distinct colonies were selected and streaked on minimal salt medium containing LDPE powder as a sole carbon source. The growth medium was prepared by adding 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.[40] The bacteria were allowed to grow at 30-35^{o}C for 2-4 weeks.

Isolates were screened for LDPE degrading efficiency by zone of clearance method.[31] After the completion of incubation period, agar plates were flooded with 0.1% (w/v) Coomassie blue R-250 solution in 40% (v/v) methanol and 10% (v/v) acetic acid for 20 minutes. The solution of coomassie blue was then poured off, and the plates were flooded with 40% (v/v) methanol and 10% (v/v) acetic acid for 20 minutes. The organisms producing zone of clearance in a blue background were selected as the utilizer of polyethylene.[41]

Microbial characterization of isolated bacteria- The morphological characterization of the isolates was done by Gram's staining method.[42] Further the bacterial isolates were identified according to the criteria given in bergeys's manual of Determinative Bacteriology.[43] The various biochemical tests such as IMViC, carbohydrate fermentation, gelatin liquefaction, starch hydrolysis and catalase production were performed.

RESULTS

Isolation of bacteria- In this study 42 bacterial isolates were obtained from five polyethylene contaminated soil samples collected form waste disposal sites (Table 1).

Table 1: Screening of LDPE degrading bacteria

S. No.	Sample collection site	Type of area	Total isolates	Isolates +ve for zone of clearance
1	Solanibridge, Roorkee	Urban	ISJ1, ISJ2, ISJ3, ISJ4, ISJ5, ISJ6, ISJ7, ISJ8	ISJ3
2	Ganeshpur, Roorkee	Rural	ISJ9, ISJ10, ISJ11, ISJ12, ISJ13	NIL
3	Civil lines, Roorkee	Urban	ISJ14, ISJ15, ISJ16, ISJ17, ISJ18, ISJ19, ISJ20, ISJ21, ISJ22, ISJ23, ISJ24, ISJ25, ISJ26, ISJ27	ISJ14
4	Balmiki temple, Jhabrera	Rural	ISJ28, ISJ29, ISJ30, ISJ31, ISJ32, ISJ33, ISJ34	ISJ30, ISJ33
5	Jatol	Rural	ISJ35, ISJ36, ISJ37, ISJ38, ISJ39, ISJ40, ISJ41, ISJ42	ISJ36, ISJ38, ISJ40

Screening and visualization of LDPE degrading bacteria

Out of 42 bacterial strains, 7 isolates ISJ3, ISJ14, ISJ30, ISJ33, ISJ36, ISJ38 and ISJ40 showed zone of clearance around the colonies, indicating the ability to utilize polyethylene as the sole carbon source in the minimal salt medium.

Characterization of isolates

On the basis of morphological and biochemical characterization it was concluded that out of all, One isolate was identified as gram negative bacterial strain. ISJ14 was probably identified as *Pseudomonas* sp. Six bacterial strains were identified as gram positive. In which ISJ3, ISJ33, ISJ36, ISJ38, ISJ40 were probably identified as *Bacillus* sp., whereas strain ISJ30 was identified as *Micrococcus* sp. Results of morphological and biochemical tests have been summarized in table 2.

Table 2: Morphological and biochemical characterization of selected bacterial isolates

Isolates	Gram's reaction	Shape	Motility	G[a]	S[b]	M[c]	G[d]	S[e]	I[f]	MR[g]	VP[h]	C[i]	C[j]	Probable identify
ISJ3	+ve	Rod	Present	+	-	+	+	+	-	+	-	+	+	*Bacillus sp.*
ISJ14	-ve	Rod	Present	-	-	-	+	-	-	-	-	+	+	*Pseudomonas* sp
ISJ30	+ve	Cocci	Absent	+	-	-	-	-	-	+	-	-	+	*Micrococcus sp.*
ISJ33	+ve	Rod	Present	+	-	-	-	+	-	+	-	-	+	*Bacillus sp.*
ISJ36	+ve	Rod	Present	+	+	-	-	+	-	+	-	+	+	*Bacillus sp.*
ISJ38	+ve	Rod	Present	+	-	+	+	-	+	+	-	-	+	*Bacillus sp.*
ISJ40	+ve	Rod	Present	+	+	+	-	+	-	-	-	-	+	*Bacillus sp.*

Keys – -ve (Negative), +ve (positive)

[a] Glucose fermentation; [b] Sucrose fermentation; [c] Mannitol fermentation; [d] Gelatin liquefaction; [e] Starch hydrolysis; [f] Indole; [g] Methyl Red; [h] Vogeus-proskeur; [i] Citrate utilization; [j] Catalase production

DISCUSSION

Industrialization as well as social development is playing a significant role in the generation of solid waste. Soil found in waste disposal sites is rich in organic matter and contains a lot of microorganisms such as bacteria and fungi, which uses waste materials as the source of nutrients.[44,45,46] From the literature it can be concluded that there are a huge number of microorganisms isolated from contaminated soil have the ability to degrade synthetic plastic efficiently.[17,37,47,48] Current study focuses on the isolation and screening of bacterial strains capable of degrading LDPE from five waste disposal sites of Haridwar region. Screening of LDPE degrading bacteria was done by clear zone method using LDPE powder as the sole

carbon source in minimal salt medium. Nisida (1993) suggested that clear zone technique is a convenient method for the investigation of plastic degrading microorganisms.[49] Microorganisms secrete the extracellular enzymes which degrade the polymeric substances into water soluble materials, resulting into the formation of a clear zone around the microbial culture, indicating utilization of polyethylene powder as the sole carbon source.[19,50,51] In present study formation of clear zone around 7 isolates confirmed the polymer decomposing ability of the bacterial strains. Characterization of bacterial isolates was performed by standard morphological and biochemical techniques and the bacterial isolates were probably identified as *Bacillus* sp., *Pseudomonas* sp. and *Micrococcus* sp. The majority of isolates belonged to the genus *Bacillus*. In addition Gouri et al.(2015) isolated *Bacillus* sp. and *Pseudomonas* sp. from contaminated soil form halo zones around the bacterial colonies indicating the utilization of polyethylene.[52] Skariyachan et al. (2015) reported *Pseudomonas* sp. isolated from waste soil showed tiny zone of clearance around the colonies after the treatment with commasie blue.[31] A strain of *Bacillus amyloliquefaciens* having the ability to degrade low density polyethylene have been isolated from municipal solid soil.[37] Besides this, these bacterial sp. were also been reported to involve in the biodegradation of different types of plastics. Shah et al. (2008) reported biodegradation of polyurethane by *Bacillus* sp., *Pseudomonas* sp. and *Micrococcus* sp. isolated from soil.[17b] Nishida and Tokiwa (1993) reported degradation of polyurethane-diole by *Pseudomonas aeruginosa*.[49] Patil (2012) isolated *Micrococcus* sp. from municipal solid waste having the ability to degrade polyvinyl chloride.[53] 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. Further analysis concerning weight loss method, evaluation of cell surface hydrophobicity, assessment of bacterial biomass and viability and extraction of extracellular enzymes which are responsible for the biodegradation process.

CONCLUSION

The microbial diversity in the environment is far greater than is reflected in most strain collections due to the microbes which cannot be cultured by using standard techniques. Therefore, a huge number of microbes remain unexplored in the sample. The following work reveals that waste disposal sites may contains a vast majority of microbes which can degrade polyethylene sufficiently. The clear zone method seems to be a simple method for the screening of polyethylene degrading bacteria. The present study reveals that 7 isolates have the ability to use LDPE as the soul carbon source and probably identified by morphological

and biochemical characterization as *Pseudomonas* sp., *Bacillus* sp. and *Micrococcus* sp. Bacterial strains which showed zone of clearance around the colonies were selected and further study is required for the effective application of bacterial strains as a tool in biodegradation process. Additional evaluation using several environmental samples is needed to isolate microorganism with high polyethylene degrading activity.

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