International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS™ | Volume 8 | Issue 4 | OCT-DEC | 2018 | 53-55
Research Article | Biological Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

SCREENING AND IDENTIFICATION OF LOW-DENSITY POLYETHYLENE (LDPE) DEGRADING MICROORGANISM FROM MUNICIPAL LANDFILL SOIL

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ABSTRACT

Low density polyethylene (LDPE) is a recalcitrant thermoplastic used in manufacturing of plastic bags, disposable cups and so on. It is non-degradable substance and it is the environmental pollution causing agent. The current research focused on evaluation of degradation of Low-density polyethylene (LDPE) by bacteria isolated from municipal dump soil by performing baiting technique clear zone plate assay and BATH test. We identified the bacterial strain as Bacillus species by gram staining and endospore staining method.

KEY WORDS

LDPE, Clear Zone assay, BATH test, Bacillus species

INTRODUCTION

Plastics, such as polyethylene are used extensively in packaging and other industrial purposes. LDPE plays an important role where the materials are strong, light-weight and durable, they having wide uses. The polyethylene wastes are inert, recalcitrant and they accumulate in the environment and it takes several million years to degrade. This leads to global problem posing an ecological threat. Techniques were introduced to degrade polyethylene such as physical, chemical and biological degradation. Among these techniques, bio-degradation is one of the emerging techniques to degrade polyethylene. Microorganisms such as bacteria for example *Brevibacillus borstelensis, Pseudomonas fluorescens, Pseudomonas aeruginosa, Bacillus cereus, Bacillus pumilus* and fungi *Aspergillus* sps *Penicillium* species [(1-8)] are reported to be used in the biodegradation of LDPE with and without pretreatment of the substrate. Hence the present study concerned with isolation of potent microorganisms (bacteria and fungi) from solid waste.

MATERIALS AND METHODS:

Authenticated LDPE Sheets with 100 microns were purchased from Nature Pack Pvt. Ltd. Chennai.

Isolation of microorganism:

Soil sample was collected from the municipal solid waste landfill area, Pallikaranai, Chennai, at depth of 10cm. Samples were stored in sterile air tight container. 5 g of soil sample and 500mg LDPE powder was added in 100ml sterilized SM Broth (Synthetic medium). Incubated at 37°C for 24 hrs. After 24hrs, bacterial cultures were isolated in SM by streak plate method.

Determination of growth rate of bacteria colonized on LDPE:

Overnight culture was inoculated in 50ml SM with 250mg LDPE powder. The conical flask was incubated at 37˚C in rotary shaker. For every 30minutes OD was taken at 460nm. From the OD values generation time was calculated. SM without culture served as a control.

Determination of Bacterial hydrophobicity: (2)

Bacterial cell surface hydrophobicity was determined by the BATH test (Bacterial adhesion to hydrocarbon).

24hrs culture in 5ml nutrient broth was centrifuged; the pellet was washed twice with Phosphate-Urea-Magnesium (PUM) buffer and re-suspended in PUM buffer. The suspension was taken OD at 400nm. Added 0.2ml of hexadecane to the suspension and shaken for 20min. Test tubes were kept undisturbed for 5min. The separation of two phases into organic and aqueous was observed. Absorbance of aqueous layer was measured at 400nm. Cell free buffer served as the blank.

Identification of bacterial strain:

1. ***Gram staining:***

A smear was placed on clean slide; air dry and heat fix it. Crystal violet solution was flooded and kept for 1 minute. Washed off and flooded with iodine solution leave it for 30 seconds and washed off and destain with 80% alcohol. Again, flooded with safranin solution and allow remaining for 1 minute and then rinsing off the solution with distilled water. The slide was air dried and examined under 100x objective oil immersion microscope.

2. ***Endospore staining:***

Smears of bacterial species for prepared on clean slide, air dried and heat fix it. A small piece of blotting paper was placed over the smear. The slide was kept over the boiling water bath for steaming. As the paper begins to dry a drop of malachite green was added and kept for 5 minutes. After 5 minutes the slide was taken and the blotting paper was removed and cools it at room temperature. A drop of safranin was added and remains for 2 minute and rinse off the solution with distilled water. The slide was air dried and examined under 100x objective oil immersion microscope.

RESULTS AND DISCUSSION:

The present study deals with the degradation of Low-density polyethylene by microorganisms. We isolated microbes by performing baiting technique using LDPE as sole carbon source for growth. The strains which show clear around the colonies (Fig: 1) were selected and calculated generation time for biodegradation capability, the bacterial isolates PB1 revealed the doubling time of 17.91 (Table:1) by using LDPE as carbon source. BATH test revealed the percentage of 25.02% (Table: 2) were as in previous reports showed only 20 and 10% reduction in turbidity (1) (2).

Isolation of bacterial strain clear zone formation:

Fig: (1) Plate assay showing clear Zone

Table 1: Generation time

Culture	Generation time in minutes
PB1	17.91

Table 2: BATH test

Culture	Percentage of hydrophobicity
PB1	25.02%

Identification of bacterial strain:

1. ***Gram staining:***

Fig (2)

2. ***Endospore staining:***

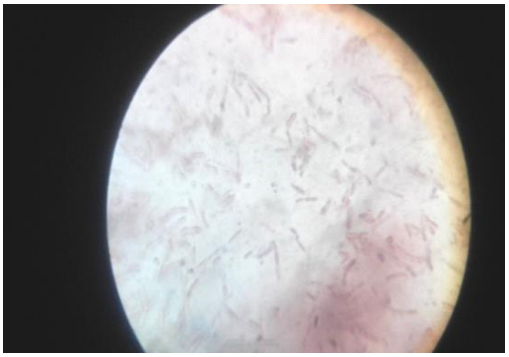

Fig (3)

From the above picture we identified that the bacterial species was gram positive, endospore forming *Bacillus* species.

CONCLUSION:

In order to facilitate the degradation of LDPE we isolated bacterial species from municipal landfill soil by performing clear zone plate assay. The present study proves that the bacterial strain has capacity to adhere on the hydrophobic surface of the polyethylene film and it can degrade the recalcitrant polymer, if the right conditions are provided.

ACKNOWLEDGEMENT:

The authors are grateful to the University grants Commission (F.no. 42-480/2013 SR dated march 2013) for providing financial assistance for the completion of this work.

REFERENCES:

1. Gilan, I., Y. Hadar., & A. Sivan. Colonization, biofilm formation and biodegradation of polyethylene by a strain of *Rhodococcus ruber*. *Appl. Micro. Biotech*. 65, 97-104 (2004).
2. Hadad, D., S. Geresh. & A. Sivan. Biodegradation of polyethylene by the thermophilic bacterium *Brevibacillus borstelensis*. *J. Appl. Microbiol*. 98, 1093-1100. (2005).
3. Hussein, A. A., Al-mayaly, I. K. & Khudeir, S. H. Isolation, Screening and Identification of Low-Density Polyethylene (LDPE) degrading bacteria from contaminated soil with plastic wastes. *Mesop. environ. j.* 1, 1–14 (2015).
4. Usha, R., Sangeetha, T. & Palaniswamy, M. Screening of Polyethylene Degrading Microorganisms from Garbage Soil. *Lib. Agr. Res. Cen. J. Int.* 2(4), 200-204 (2011).
5. Victorathisayam, T. & Sujatha, M. Biodegradation of High Density, Low Density and Linear Low-Density Polyethylene By Using Pseudomonas Aeruginosa And Staphylococcus Aureus. *Glob. J. Mod. Biol. Technol.* 2, 91–94 (2012).
6. Das, M.P.& Kumar, S. Microbial Deterioration of Low-Density Polyethylene by *Aspergillus* and *Fusarium*sp. *Int J. Chem. Tech Res*. 6(1), 299-305 (2014).
7. Harshvardhan, K. & Jha, B. Biodegradation of low-density polyethylene by marine bacteria from pelagic waters, Arabian Sea, India. *Mar. Pollut. Bull.*77(1), 100-106 (2013)
8. Singh, V., Dubey, M. & Bhadauria, S. Microbial Degradation of Polyethylene (Low Density) By *Aspergillius Fumigatus* and *Penicillium* sp. *Asian. J.Exp. Biol. Sci.* 3(3), 498-50 (2012).
9. Sowmya, H. V., Ramalingappa, B., Nayanashree, G., Thippeswamy, B. & Krishnappa, M. Polyethylene degradation by fungal consortium. *Int. J. Environ. Res.* 9, 823–830 (2015).
10. Latha, G., Ambikapathy, V. & Panneerselvam, A. Optimization of Culture Conditions to Enhance Polyethylene Enzyme Production by using Pseudomonas. *Int. J. Sci. Research*. 1–2 (2014).
11. Vatseldutt, S. & Anbuselvi. Isolation and Characterization of Polythene Degrading Bacteria from Polythene Dumped Garbage. *Int. J. of Pharm.Sci.* 25(2), 205-206 (2014).
12. Al-Jailawi, M. H., Ameen, R. S. & Al-Saraf, A. A. Polyethylene degradation by *Pseudomonas putida* S3A. *Int. J. Adv. Res. Biol.Sci.*. 2(1), 90–97 (2015).

Received:06.08.18, Accepted: 08.09.18, Published:01.10.2018

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