International Journal of Microbiological Research 11 (1): 24-29, 2020
ISSN 2079-2093

DOI: 10.5829/idosi.ijmr.2020.24.29

Isolation of Polyethylene Degrading Bacteria from Plastic Landfill Site and its Role in Crop Development

Suraj Katole and G. Sibi

Department of Biotechnology, Indian Academy Degree College-Autonomous, Bengaluru, India

Abstract: This study focused on isolation and identification of potential polyethylene degrading bacteria from plastic dumping region. In preliminary screening, the bacterial isolates had produced clear zones in the polymer emulsion containing mineral medium indicating their polymer degrading ability. Inoculation of the isolates into liquid mineral media containing polyethylene as the only carbon source resulted in weight loss of polyethylene film resulted by the biological activity of bacteria. The percentage weight loss of polyethylene sheets by the isolates were in the range of 12.7-58.5%. The potential polyethylene degrading isolate was identified as *Zymomonas mobilis* through morphological and biochemical characterization with optimum growth pH and temperature of 7 and 40°C respectively. Effect of isolated bacteria on wheat growth indicated that there was no adverse effect on crop development. The results suggest that isolated microorganism can be used in laboratory conditions as well as in situ for degradation of polyethylene.

Key words: Polyethylene · Plastic · Biodegradation · Mineral Media · *Zymomonas*

INTRODUCTION

Synthetic polymers are commonly used because of their low cost, reproducibility and resistance to physical aging and biological attack. However, extensive use of polymeric materials has made plastic pollution as significant environmental issue [1] and represents a major threat to ecological systems [2]. The accumulation of the plastic is responsible for the most unique and long-lasting changes to the environment [3]. Mostly used plastics are polyethylene (36.3%), polyethylene terephthalate, polybutylene terephthalate, nylons, poly-propylene (21%), polystyrene (7.6%), polyvinyl chloride (11.8%) and polyurethane [4-6]. Polythene comprises of 64% of total plastic, which is a linear hydrocarbon polymers consisting of long chains of the ethylene monomers [7]. Polyethylene either low density or high density is a thermoplastic polymer made by monomers of ethylene, used mostly as thin films and packaging sheets [8]. Environmental factors such as light, heat, moisture, chemical conditions and biological activity are involved in plastic degradation. The burning of waste plastic material produces toxic gases [9]. Plastic degraded by sunlight into smaller toxic parts contaminating soil and water [10].

Biodegradation of plastics involves microorganisms like fungi and bacteria by using plastic as a substrate for their growth [11]. Various factors which are responsible for biodegradation are type of polymer, organism characteristics and the type of treatment required [12, 13]. Biodegradation of plastic waste and the use of microorganisms to degrade the polymers have gained notable importance because of the inefficiency of the chemical and physical disposal methods used for these pollutants and the environmental problems they cause. The objective of this study was to isolate potential polyethylene degrading bacteria from plastic dumping region. Plastic degradation by the isolate was determined by calculating percentage loss of polyethylene film by the isolates.

MATERIALS AND METHODS

Sample Collection: Soil sample were collected from different plastic dumping regions in and around Jalgaon, Maharashtra (21°0’18.5905” N, 75°33’50.1912”E).

Corresponding Author: G. Sibi, Head, Department of Biotechnology, Indian Academy Degree College-Autonomous, Bengaluru, India. Tel: +91 99864 52875.

Fig. 1: Sample collection site from Jalgaon, Maharashtra

Isolation of Microorganisms: One g of soil sample was transferred to a flask containing 50 ml of sterile 0.85% physiological saline. The soil solution was shaken for 1 h on a rotary shaker at 37°C. The culture (100 µl) was then spread on Luria Bertani agar plates to isolate microorganisms. The plates were incubated at 28°C for 5-7 days in a bacteriological incubator. The developed colonies were isolated and sub-cultured repeatedly to obtain the pure cultures and then preserved in agar plates at 4°C.

Mineral Medium: A mineral medium (0.2g NaH_2PO_4, 0.05g $MgSO_4 \cdot 7H_2O$, 0.02g KH_2PO_4, 0.1g yeast extract) was prepared (100 ml) for isolation and examination of plastic degrading microorganisms. PBSA emulsion (0.1%) was added as the only source of carbon for the growth of bacteria in the mineral medium [14].

Preparation of Polymer Emulsion: Polymer emulsions were prepared by following the procedure described by Uchida *et al*. [15]. 0.5% PBSA [poly (butylene succinate-co-butylene adipate)] emulsion was prepared by dissolving about 2 g PBSA pellet in 40-60 ml of dichloromethane. This was followed by addition of 100 ml distilled water and 2 ml of 2% sodium lauroyl sarcosinate. The mixture was sonicated for 10 min and the dichloromethane was evaporated by stirring at 80°C for 2 h in a draft chamber. The emulsion was filled up to 400 ml with distilled water and the pH was adjusted to 7 with KOH.

Bacterial Degradation of Polyethylene: The microbial colonies cultivated on LB agar plates were tested on mineral agar plates containing 0.1% emulsified PBSA. The mineral agar plates were incubated at 37°C for 2-3 days and the polyethylene-degrading microorganisms were identified by zone of clearance method.

Colonies forming clear zones were selected and further analyzed for polyethylene degradation. Polyethylene films were cut into small squares (1 cm × 1 cm) and were sterilized using 70% ethanol and UV radiation (5 min). Approximately 0.1 g of polyethylene films was aseptically transferred into the conical flask containing 100 ml of sterile mineral medium. Then medium was inoculated with the selected strain incubated in rotary shaker at 37°C, 220 rpm for a 1-month period. The control was maintained with films in a bacteria-free medium.

At the end of the process, polyethylene films were collected, washed thoroughly using distilled water and shade dried. Degradation was determined by measuring the residual weight of the polymers. The percentage weight change was calculated by comparing the dry weight of residual films with the original weight of the films [16] using the formula [17].

$$Percentage\ of\ weight\ loss = \frac{Initial\ weight - final\ weight}{initial\ weight} X\ 100$$

Identification of Polyethylene Degrading Isolate: Bacterial growth resulted in highest weight loss of polyethylene sheet was further identified by morphological and biochemical tests.

Growth Optimization of Polyethylene Degrading Isolate: In order to determine the optimal growth conditions of the polyethylene degrading isolate, varying pH and temperature were tested. Minimal media with pH 3, 4, 5, 6, 7 and 9 were prepared and inoculated with the isolate. To determine the optimal growth temperature, the

inoculated flasks were incubated at 25°C, 30°C, 35°C, 40°C and 45°C. Growth of the isolate was determined by calculating the OD values at the end of log phase.

Bacterial Activities on Soil and Crops: After the degradation experiments, bacterial suspension (10^6 cells / ml) was added into soil and sowed with wheat grains to determine the effect of isolated bacteria on germination and crop development. Wheat seedlings were observed after 10 days for their growth characteristics and compared with control plants.

RESULTS AND DISCUSSION

Bacterial colonies grown in LB agar plates were inoculated into mineral agar medium containing polymer emulsion. A total of 8 colonies were observed with clear zone around their growth indicating their polymer degradation ability. The colonies were purified and the isolates (S1, S2, S3, S4, S5, S6, S7 and S8) were further characterized through morphological and biochemical tests (Table-1, 2 and 3).

The isolates were grown in liquid mineral media inoculated with polyethylene sheets for the loss of residual weight at the end of 1 month incubation period (Fig-2). The percentage weight loss of polyethylene sheets by the isolates were in the range of 12.7 -58.5%. Loss of weight in the control flask was tested and weight loss was higher in all the experimental flasks indicating that percent weight loss was not due to chemicals in the medium but because of biological process by the isolates. Among the isolates, S1 had produced greater weight loss of 58.5% followed by S4 (49.2%). The isolate was further identified as *Zymomonas mobilis* based on Bergey's manual (Fig-3).

The isolate, *Z. mobilis* was further tested to optimize the growth conditions under varying pH and temperature. Highest optical density of the isolate was recorded at pH 6 and 7 with the readings 0.014 and 0.029 respectively. Both 35°C and 40°C resulted in highest OD value of 0.041 and 0.048 of the isolate. Germination experiment involving the effect of polyethylene degrading isolate on wheat indicated there was no negative effect on the crop (Fig-4).

Polythene is the most commonly found non-degradable solid waste that has been recognized as a major threat to terrestrial and marine life. Several studies conducted on landfills and other terrestrial sites focused on the isolation and characterization of polyethylene degrading strains [18-20]. Polythene degradation by *Pseduomonas* and *Moraxella* sp were reported by Kathiresan [21]. Bacteria belong to the genera *Pseudomonas, Comamonas* and *Bacillus* have been

Table 1: Colony characteristics of plastic degrading isolates

Isolates	Shape	Configuration	Margin	Elevation	Surface	Pigment	Opacity
S1	Round	Circular	Filamentous	Convex	Smooth	White	Shiny
S2	Round	Circular	Punciform	Convex	Smooth	Buttery	Translucent
S3	Round	Circular	Punciform	Convex	Smooth	Pink	Opaque
S4	Round	Circular	Filamentous	Convex	Smooth	Buttery	Opaque
S5	Round	Circular	Undulate	Flat	Smooth	Buttery	Opaque
S6	Round	Circular	Entire	Convex	Smooth	Buttery	Opaque
S7	Round	Circular	Punciform	Convex	Smooth	White	Opaque
S8	Round	Circular	Entire	Convex	Smooth	Blackish	Opaque

Table 2: Biochemical characteristics of plastic degrading isolates

Test	S1	S2	S3	S4	S5	S6	S7	S8
Amylase	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+
Citrate	+	+	+	+	+	+	+	+
Endospore	+	-	-	-	-	-	-	-
Gram's reaction	-	+	-	-	-	-	-	+
H_2S production	-	-	-	-	-	-	-	-
Indole	-	-	-	-	-	-	-	-
Methyl red	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+	+
Urease	+	+	+	+	+	+	+	+
Voges-Proskauer	+	+	+	+	+	+	+	+

(+) positive; (-) negative

Table 3: Carbohydrate fermentation by the plastic degrading isolates

Carbohydrate fermentation		S1	S2	S3	S4	S5	S6	S7	S8
Glucose	Acid	-	+	+	-	-	-	-	+
	Gas	-	-	-	-	-	-	-	-
Maltose	Acid	-	-	+	-	-	-	-	-
	Gas	-	-	-	-	-	-	-	-
Sucrose	Acid	-	-	-	-	-	-	-	+
	Gas	-	-	-	-	-	-	-	-
Cellulose	Acid	-	-	-	-	-	-	-	-
	Gas	-	-	-	-	-	-	-	-
Starch	Acid	+	+	+	+	+	+	+	+
	Gas	-	-	-	-	-	-	-	-

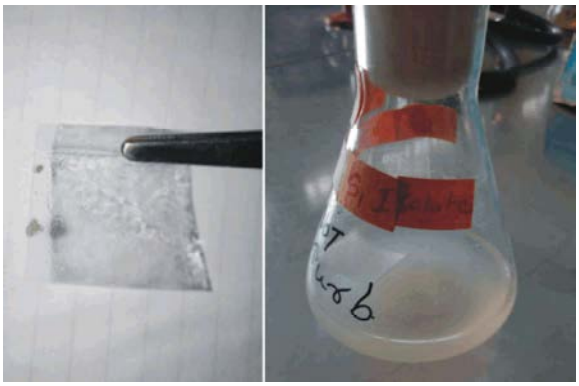

Fig. 2: Biodegradation experiments of polyethylene film

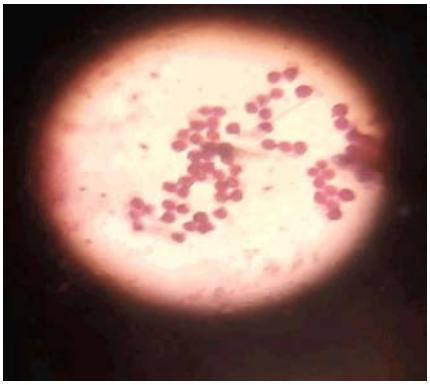

Fig. 3: Microscopic appearance of S1 isolate (*Zymomonas mobilis*)

Fig. 4: Effect of *Z. mobilis* on wheat plant growth

found to utilize polyester and polyurethane as sole carbon source [22, 23]. Incubation of *Brevibacillus borstelensis* with polyethylene revealed the reduction in molecular weight of polyethylene by 30% [24]. The ability of *Bacillus* species and *Lysinibacillus xylanilyticus* to utilize polyethylene was evaluated earlier [25, 26].

Microbial degradation of plastics is caused by enzymatic activities which lead to a chain cleavage of the polymer into oligomers and monomers. These water soluble enzymatically cleaved products are further absorbed by the microbial cells where they are metabolized. In this study, the degradation of polyethylene sheets by the isolates was confirmed by the weight loss of polyethylene after one month incubation period. The results were in accordance with the preliminary screening findings of this study in which clear zones were produced by the isolates in the polymer emulsion containing mineral medium. Treatment of soil with the isolate to cultivate wheat plants indicated the isolate has no negative effect on crop development.

CONCLUSION

Microorganisms isolated from specific environments have developed many unique features which help them to survive under unfavorable conditions. For this reason, we focused on the plastic waste dumping sites to isolate microorganisms which are able to rapidly decompose polyethylene materials. In the present study, we sought to isolate, test and identify polyethylene degrading microorganisms which possess high ability for biodegradability of plastics. A total of 8 isolates were isolated and the bacteria with highest degradation ability were identified as *Zymomonas mirabilis*. The results suggest that isolated microorganism can be used in laboratory conditions as well as in situ for degradation of polyethylene. Further optimization of growth conditions might significantly accelerate the process of decomposition of plastics. To clarify the degradation mechanism in more detail, research on the produced enzymes should be conducted.

REFERENCES

1. Guo, W., J. Tao, C. Yang, C. Song, W. Geng, Q. Li, Y. Wang, M. Kong and S. Wang, 2012. Introduction of environmentally degradable parameters to evaluate the Biodegradability of Biodegradable Polymers. PLoS ONE, 7(5): e38341.
2. Shah, A.A., S. Kato, N. Shintani, N.R. Kamini and T. Nakajima-Kambe, 2014. Microbial degradation of aliphatic and aliphatic-aromatic co-polyesters. Appl. Microbiol. Biotechnol., 98(8): 3437-3447.
3. Sivan, A., 2011. New perspectives in plastic biodegradation. Curr. Opin Biotechnol., 22: 422-426.
4. Rivard, C., L. Moens, K. Roberts, J. Brigham and S. Kelley, 1995. Starch esters as biodegradable plastics: effects of ester group chain length and degree of substitution on anaerobic biodegradation. Enzym Microb Technol., 17: 848-852.
5. Geyer, R., J.R. Jambeck and K.L. Law, 2017. Production, use and Fate of All Plastics Ever Made. Sci. Adv., 3: e1700782.
6. Puglisi, E., F. Romaniello, S. Galletti, E. Boccaleri, A. Frache and P.S. Cocconcelli, 2019. Selective bacterial colonization processes on polyethylene waste samples in an abandoned landfill site. Scientific Reports, 9: 14138.
7. Sangale, M.K., M. Shahnawaz and A.B. Ade, 2012. A review on Biodegradation of Polythene: The microbial approach. J. Bioremed. Biodeg., 3: 164.
8. Kumar, S., M.L. Das, J. Rebecca and S. Sharmila, 2013. Isolation and identification of LDPE degrading fungi from municipal solid waste. J. Chem. Pharm. Res., 5(3): 78-81.
9. Ali, M.I., Q. Perveen, B. Ahmad, I. Javed, R. Razi-Ul-Hussnain, S. Andleeb, N. Atique, P.B. Ghumro, S. Ahmed and A. Hameed, 2009. Biodegradation studies of cellulose Blended Polyvinyl Chloride Films. Int. J. Agric. Biol., 11: 577-580.
10. Denuncio, P., R. Bastida, M. Dassis, G. Giardino and M. Gerpe, 2011. Plastic ingestion in Franciscana dolphins, Pontoporia blainvillei (Gervais and d'Orbigny, 1844), from Argentina. Mar. Pollut. Bull, 62: 1836-1841.
11. Glass, J.E. and G. Swift, 1989. Agricultural and synthetic polymers, biodegradation and utilization, ACS symposium series, 433. American Chemical Society, Washington, pp: 9-64.
12. Artham, T. and M. Doble, 2008. Biodegradation of aliphatic and aromatic polycarbonates. Macromol. Biosci., 8(1): 14-24.
13. Gu, J.D., T.E. Ford, D.B. Mitton and R. Mitchell, 2000. Microbial degradation and deterioration of polymeric materials. In: Review (Ed) The Uhlig Corrosion Handbook, 2nd edn. Wiley, New York, pp: 439-460.
14. Kitamoto, H.K., Y. Shinozaki, X.H. Cao, T. Morita, M. Konishi and K. Tago, 2011. Phyllosphere yeasts rapidly break down biodegradable plastics. AMB Express 1:44.

15. Uchida, H., T. Nakajima-Kambe, Y. Shigeno-Akutsu, N. Nomura, Y. Tokiwa and T. Nakahara, 2000. Properties of a bacterium which degrades solid poly(tetramethylene succinate)-co-adipate, a biodegradable plastic. FEMS Microbiol. Lett., 189(1): 25-29.
16. Prema, S. and M.D. Uma, 2013. Degradation of poly lactide plastic by mesophilic bacteria isolated from compost. Int. J. Res. Pure. Appl. Microbiol., 3(4): 121-126.
17. Kyaw, B.M., R. Champakalakshmi, M.K. Sakharkar, C.S. Lim and K.R. Sakharkar, 2012. Biodegradation of low-density polythene (LDPE) by Pseudomonas species. Indian J. Microbiol., 52(3): 411-419.
18. Kowalczyk, A., M. Chyc, P. Ryszka and D. Latowski, 2016. *Achromobacter xylosoxidans* as a new microorganism strain colonizing high density polyethylene as a key step to its biodegradation. Environ. Sci. Pollut. Res. Int., 23: 11349-11356.
19. Skariyachan, S., M. Megha, M.N. Kini, K.M. Mukund, A. Rizvi and K. Vasist, 2015. Selection and screening of microbial consortia for efficient and ecofriendly degradation of plastic garbage collected from urban and rural areas of Bangalore, India. Environ. Monit. Assess., 187: 4174.
20. Jeon, H.J. and M.N. Kim, 2013. Isolation of a thermophilic bacterium capable of low-molecular-weight polyethylene degradation. Biodegradation, 24: 89-98.
21. Kathiresan, K., 2003. Polythene and Plastics-degrading microbes from the mangrove soil, Rev. Biol. Trop., 51: 629-634.
22. Howard, G.T. and R.C. Blake, 1998. Growth of *Pseudomonas fluorescens* on a polyester-based polyurethane and the purification and characterization of a polyurethanase-protease enzyme. Int. Biodeterior. Biodegradation, 42: 7-12.
23. Rowe, L. and G.T. Howard, 2002. Growth of *Bacillus subtilis* on polyurethane and the purification and characterization of a polyurethanase-lipase enzyme. Int. Biodeterior. Biodegradation, 50: 33-40.
24. Hadad, D., S. Geresh and A. Sivan, 2005. Biodegradation of polyethylene by the thermophilic bacterium *Brevibacillus borstelensis*. J. Appl. Microbiol., 98: 1093-1100.
25. Abrusci, C., J.L. Pablos, T. Corrales, J. Lopez-Marin and L. Marin, 2011. Biodegradation of photo-degraded mulching films based on polyethylene and stearates of calcium and iron as pro-oxidant additives. Int. Biodeterior. Biodegradation, 65: 451-459.
26. Esmaeili, A., A.A. Pourbabaee, H.A. Alikhani, F. Shabani and E. Esmaeili, 2013. Biodegradation of low-density polyethylene (LDPE) by mixed culture of *Lysinibacillus xylanilyticus* and *Aspergillus niger* in soil. PLoS ONE, 8(9): e71720.