DOI: https://doi.org/10.24297/jab.v12i0.8141

The microbial consortia directed evolution towards plastic degradation – the key to waste management?

Pooja Chandel[1], Gurpreet Kaur Sidhu[1*]
[1] Department of Microbiology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara
[*]gurpreet_sidhu28@yahoo.co.in

Abstract
The rampant use of plastics and their disposal into waste are adding to the problems of pollution. The resistance of plastics to bio-degradation is an added advantage for its significant use but the same property creates havoc when the plastic products are disposed off as waste in massive amounts. The property of micro-organisms to evolve quickly brings answers to even the most impossible situations. The current and several other reports show that the plastic is bio-degradable. The current report shows the action of consortia of microbes isolated from a plastic dumping site can lead to degradation of the polymer. The microbial consortia isolated from plastic dumping site when made to grow in controlled conditions in presence of basal media with plastic as sole source of carbon for an extended period of time, aberrations were observed on surface of the plastic. The proteins reported till date in plastic degradation when analysed *in-silico* for their homologs in all domains of life, they were found to be significantly similar to proteins of cutinase, hydrolase, lipase and some hypothetical proteins. This shows that the plastic degrading proteins have possibly evolved from these protein families.
Keyword: Plastic, Degradation, Biofilm, Bacteria, protein

Introduction

The rate of population growth is increasing which is directly leading to immense increase in the consumption rate of plastic. Plastic pollution is causing deterioration of environment leading to water and land pollution and also air pollution from combustion of plastics. Several reports show possibility of plastic degradation by physical, chemical and biological means. Biodegradation i.e. degradation of plastic by using microorganism like bacteria, fungi etc. is the most energy efficient method caused by degradation of plastics by microbial enzymatic activities that cleaves the polymer chain to monomers. Microbes form biofilm on the plastic surface and use it as carbon source hence causing the degradation of the plastic. The present study is an attempt to isolate such plastic degrading microbial consortia from the environment and evaluate their potential to cause plastic degradation.
Plastics resist deformation in natural conditions due to their inert nature and resistance against microbial attack (Yuksel orhan et al., 2004). Song et al (1998) have reported a low rate of recycling in plastic with higher amount of additives, hence further adding to the in-biodegradable pollution. Recent developments show that plastic materials can be degraded by using microbes (Chandra and Rustgi., 1998; Kumar et al., 2011). *Actinobacter* spp. has been reported to moderately degrade the LDPE. Further Polyester and polyurethane have also been reported to be used by several microbial communities, although at slow rate (Dey et al,. 2012; Schink et al,. 1992). Moreover, the exposure to U.V radiation, photo-oxidation and thermo-oxidation further facilitates synthetic polymer degradation (Singh and Sharma., 2008).
The biodegradation caused by microbes when analysed at molecular level was found to be caused by extracellular depolymerase and intracellular depolymerase (Gu., 2003). Biofilm is another term for microbial community in which the excretion of extracellular polymeric substances happens due to the contribution of each cell. The formation of EPS creates a complex matrix wherein the cell lives, develop and ultimately forms a biofilm that adheres to a surface. The nutrition requirement of biofilm is maintained by the heterogeneity of biofilm matrix. Biodegradation is enhanced due to biofilm formation in materials like synthetic polymers (Prakash et al,. 2003). Although extensive research is underway to identify plastic degrading bacteria, the marvel microbe is still a dream. Several reports have been published regarding identification of microbes involved in varying degree of plastic degradation (mentioned in table 1). The present study is a preliminary step in an attempt to identify such microbes in environment by directed evolution.

Material and Methods

Plastic and/or soil was collected from the sediment of lake or pond contaminated with plastic. For the isolation of prospective plastic degrading microbes, two approaches were followed, viz. isolation of microbes after serial dilution and directed evolution of microbial consortia in presence of plastic. For directed evolution, the microbial consortia present in collected sample were allowed to grow in presence of pre-weighed plastic bits. The plastic was cut in uniform sizes, sterilized by UV treatment and added into sterilized basal medium without sugar. The sample collected was added into plastic containing media at rate of 1% and incubated for 30 days at 30-37°C, 150rpm. Every week spent media was replaced by fresh media. After around 75 days the plastic bits added to the medium were analysed for any change in morphology. Protein BLAST of the sequences of reported plastic degrading proteins was done in order to identify diversity of potential homologs across all domains of life.

Results

For Directed evolution, fresh plastic bits (UV sterilized) were cut in uniforms sizes and were added in sterilized basal medium without sugar inoculated with the soil from sample collected and were kept for incubation. After 5 weeks of incubation the consortium was inoculated into basal media containing plastic but devoid of peptone/yeast extract or both. After 12 weeks of incubation, the bacterial consortia was analysed for ability to form biofilm formation (growth on Congo red agar) as well as plastic degradation (Physical examination against light). When the plastic was removed from the flasks the biofilm was visible by naked eyes. The plastic when observed against light revealed localized aberrations on the plastic surface showing signs of degradation.
The literature survey brought forth, three proteins (cutinase from *Paraphoma*, ethylene glycol terephthalate hydrolase from *Ideonella sakaiensis* 201-F6 and PeE from *Pseudozyma antatartica*) to show proven plastic degrading property as per the studies conducted by various research groups. Protein BLAST of the sequences was done in order to identify diversity of potential homologs across all domains of life. The analysis showed these proteins to have appreciable homology to cutinases (54-73%), hydrolases (53-99%), lipases (53-55%) and several hypothetical proteins (62-74%). The homologs were found in several bacteria and fungi, signifying the fact that there are several organisms in the environment having the ability to degrade the plastic polymer.

Conclusions

The current work is a stepping stone towards the hypothesis that the consortium of microbes directed for digestion of plastic waste can lead to providing a feasible solution to ever increasing plastic waste. The proteins identified till date have been found to close homology to proteins which have functions apart from digestion of plastics and hence are possible ancestors for these enzymes. Such a relationship is a yet another proof of the fact that microbes with their ability to evolve quickly are capable of developing a drastic diversity in their mode of nutrition. This ability of microbes is a blessing in disguise as identification and directed evolution of such microbes can be a boon to our society on several fronts, plastic degradation being one of them. The consortium isolated from plastic dumping site and used in current study, upon identification and further augmentation of their activity can be an important stepping stone in this direction.

Conflicts of Interest

Authors hereby declare no conflicts exist to interests of any individual.

Funding Statement

The study was carried out by funding obtained from Department of Microbiology of Lovely Professional University.

Acknowledgments

The authors are thankful to the Lovely Professional University for providing the finds and the infrastructure for conducting the study.

References

Ali Shah A, Hasan F, Hameed A and Akhter JI (2009) Isolation of Fusarium sp. AF4 from sewage sludge, with the ability to adhere the surface of polyethylene. African Journal of Microbiology Research 3(10):658-663.

Alshehrei F (2017) Biodegradation of Low Density Polyethylene by Fungi Isolated from Red Sea Water. Int. J. Curr. Microbiol. App. Sci 6(8): 1703-1709.

Chandra R, Rustgi R (1998) Biodegradable polymers. Perg 23:1273–1335.

Dey U, Mondal NK, Das K, Dutta S (2012) An approach to polymer degradation through microbes. J Pharm 2(3):385–388.

Gajendiran A, Krishnamoorthy S, and Abraham J (2016) Microbial degradation of low-density polyethylene (LDPE) by Aspergillus clavatus strain JASK1 isolated from landfill soil. 3 Biotech. 6(1): 52.

Gu JD (2003) Microbiological deterioration and degradation of synthetic polymeric materials: recent research advances. Int Biodeterior Biodegrad 52(2):69–91.

Kumar AA, Karthick K, Arumugam KP (2011) Biodegradable polymers and its applications. Int J Biosci Biochem Bioinform 1(3):173–176.

Kyaw BM, Champakalakshmi R, Sakharkar MK, Lim CS and Sakharkar KR (2012) Biodegradation of Low Density Polythene (LDPE) by Pseudomonas Species. Indian J Microbiol. 52(3): 411–419.

Prakash B, Veeregowda BM, Krishnappa G (2003) Biofilms: a survival strategy of bacteria. Curr Sci 85(9):1299–1307.

Schink B, Janssen PH, Frings J (1992) Microbial degradation of natural and of new synthetic polymers. FEMS Microbiol Rev 103(2/4):311–316.

Singh B, Sharma N (2008) Mechanistic implications of plastic degradation. Polym Degrad Stab 93:561–584.

Song JJ, Yoon SC, Yu SM, Lenz RW (1998) Differential scanning calorimetric study of poly (3-hydroxyoctanoate) inclusions in bacterial cells. Int J Biol Macromol 23:165–173

Suzuki K, Noguchi MT, Shinozaki Y, Koitabashi M, Sameshima-Yamashita Y, Yoshida S, Fujii T, Kitamoto HK. (2014) Purification, characterization, and cloning of the gene for a biodegradable plastic-degrading enzyme from Paraphoma-related fungal strain B47-9. Appl Microbiol Biotechnol. 98(10):4457-65.

Suzuki K, Sakamoto H, Shinozaki Y, Tabata J, Watanabe T, Mochizuki A, Koitabashi M, Fujii T, Tsushima S, Kitamoto HK (2013) Affinity purification and characterization of a biodegradable plastic-degrading enzyme from a yeast isolated from the larval midgut of a stag beetle, *Aegus laevicollis*. Appl Microbiol Biotechnol. 97(17):7679-88.

Yoshida S, Hiraga K, Takehana T, Taniguchi I, Yamaji H, Maeda Y, Toyohara K, Miyamoto K, Kimura Y, Oda K (2016) A bacterium that degrades and assimilates poly(ethylene terephthalate). Science. 351(6278):1196-1199.

Yüksel Orhan, Jasna Hrenović and Hanife Büyükgüngöra. (2004) Biodegradation of Plastic Compost Bags under Controlled Soil Conditions, Acta ChimSlov. 51:579−588.

Table 1: List of microbes identified by various research groups for plastic degradation

S. No	Microbe	Site of isolation	Polymer degraded	Reference
1	*Cryptococcus magnus*	Larval midgut of stag beetle (*Aeges laevicollis*)	Biodegradable plastic films	Suzuki et al 2013
2	*Paraphoma* related fungal strain B47-9	Barley	Biodegradable plastic	Suzuki et al 2014
3	*Aspergillus* and *Penicillium spp*	Red Sea water, Jeddah, Saudi Arabia	Polyethylene	Alshehrei F (2017)
4	*Fusarium* sp AF4	Sewage sludge	Polyethylene	Ali Shah et al 2009
5	*Ideonella sakaiensis* 201-F6	PET bottle recycling site	PET	Yoshida et al 2016
6	*Aspergillus*	Marine water	LDPE	Gajendiran et al 2016
7	*Pseudomonas*	Waste disposal site	Polyethylene	Kyaw et al 2012

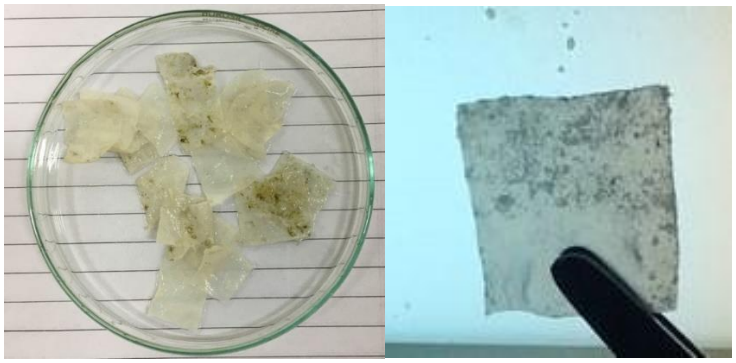

Figure 1. Formation of biofilm after 3 months on the plastic sample and aberration sites created on the surface when viewed against light