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Plastic (LDPE) Degradation by Induced Mutations In Pseudomonas Putida

Anantharam. H[1*], Muralidhar. S.Talkad[2]
*1*Research Scholar, Department of Biotechnology, Kalinga University, Campus: Village –Kotni, Near Mantralay, Naya Raipur, India*
2Professor and Research guide, Department of Biotechnology, Kalinga University, Campus: Village –Kotni, Near Mantralay, Naya Raipur, India
Corresponding Author; Anantharam. H

Abstract: *Polythene is considered as one of the important object used in daily life. Being versatile in nature and resistant to microbial attack, they effectively cause environmental pollution. The present investigation has focused the aspect through microbial assisted biodegradation of low-density polyethylene (LDPE) have been performed using Pseudomonas putida. The global utility of polyethylene is escalating at a velocity of 12% per annum. In recent times, the biodegradation of plastic waste and the use of microorganisms to degrade the polymers have gained remarkable magnitude because of the inefficiency of the chemical and physical disposal methods, and the ecological harms they cause. Environmental degradation of PE (polyethylene) proceeds by synergistic action of photo- and thermo-oxidative degradation and biological activity (i.e., microorganisms); in natural form it is not biodegradable. The making of the genetically engineered microbes for bioremediation, the latter being a strategy to develop an accelerated evolution of pathways by DNA restructuring the treatment of LDPE film with mutated Pseudomonas putida (MTCC NO: 2467) showed reduced tensile strength in 15 days were 0.22 when compared to 60 days 0.15. Media pH and viability and growth, concentration matters for ideal polymer degradation, in positive control it is 6.8, when compared to induced mutation strain is 8.5. When LDPE films were exposed to mutated Pseudomonas putida (60min), degradation rate based on weight loss, were noticed 34% against positive control 11% for 60 days, followed by DNA isolation for Gel electrophoresis, and Mutated DNA Stability analysis by Capillary Gel electrophoresis were carried out.*

Index Terms: *Polymer degradation, mutation induced (UV), Pseudomonas putida, LDPE, tensile Strength, weight loss and Capillary Gel electrophoresis*

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I. Introduction

One of the major environmental threats is the least rate of degradation or non-biodegradability of the organic materials under natural condition, e.g. plastics. The plastics of various forms such as nylon, polycarbonate, polyethylene-terephthalate, polyethylene, polypropylene, polystyrene, polytetraflouro ethylene, polyurethane and polyvinyl chloride[1] are being continuously used in our day-to-day life

The worldwide utility of polyethylene is expanding at a rate of 12% annum and approximately 140 million tons of synthetic polymers are produced worldwide each year[2, 3]. With such huge amount of polyethylene getting accumulated in the environment and their disposal evokes a big ecological issue.

The polythene is the most commonly found non-degradable solid waste that has been recently recognized as a major threat to life. The polythene could sometimes cause blockage in intestine of fish, birds and mammals. Degradation of polythene is a great challenge as the materials are increasingly used. An estimated one million birds and ten thousand marine animals die each year as a result of ingestion of or trapping by plastics in the oceans.

Microorganism – mediated biodegradation of synthetic plastics has been reported to have structural changes particularly with bacteria[4, 5]. The most involved bacterial species include Pseudomonas and fungal strains are Aspergillus and Penicillium[5]. The biodegradation is characterized by weight loss[5], change in mechanical and chemical properties[2].

Recently, the 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. Consequently, in the present investigation were designed to evaluate the biodegradation efficacy of mutation induced Pseudomonas putida, the bacteria in different duration on LDPE

The Microbial Degradation of Plastic (LDPE) polyethylene & domestic waste mixture with plastic when were induced with UV & EMS in Pseudomanas putida successfully revealed the beneficial response in Biomass reduction for better yield against growth, sugar conversion along with proteins utilization consistently proven in both normal and mutated organism as the days succeeded may be by more than a month, soil mixture and domestic waste with plastic : polyethylene bags dumping can be eco-friendly manageable to degrade and utilize the biomass for agricultural cultivation of crops[6]

II. Material And Methods

Materials: Low density polyethylene (LDPE) which is the major cause of environmental pollution was used for the study.

Microorganism collection

The bacteria Pseudomonas putida (MTCC NO: 2467) used in this study were procured from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh. Cultures were maintained on LB agar plate

Raw materials

Plastics is polyethylene (PE) as commercial plastic carry bags of LDPE were collected and cut into small strips and subjected for Chemical - alkali treatment.

Chemical - alkali treated polyethylene: polyethylene bags were cut into small strips & transferred to fresh solution containing 18ml tween, 10ml bleach, and 225ml of distilled water & stir it to 30-60mins. Bleach consists of 5gms of sodium chloride, 5gms of sodium hydroxide & 10 ml of glacial acetic acid. Strips were transferred to beaker with distilled water & stir it 2 one hour.

They were aseptically relocated to ethanol solution 70%v/v for 30min. Finally, the polyethylene strips were transferred to petridish and inoculated at 45°-50°c overnight. Ethanol was used as disinfectant to polyethylene & removes any organic matter adhering to its surface

Induction of mutation by UV

Materials required:-

- UV germicidal light bulb (Sylvania G15T8; 254 nm wavelength) or Stratagene UV Cross linker,
- Induction with ideal 60 min exposure (for mutation) and the viable growth culture were selected
- 37^0c incubator
- Pseudomonas putida (MTCC NO: 2467)
- LB agar plate

Grow and mutagenize cells

- Grow an overnight culture of the desired bacteria strain in 5 ml LB agar plate at 30^0c.
- Determine the density of cell in the culture and record this number Adjust concentration to ~2×10^8 cells/ml if necessary. Transfer 1 ml of the culture to a sterile micro centrifuge tube.
- Pellet cells in a micro centrifuge for 5 to 10 sec at maximum speed, room temperature. Discard supernatant and resuspend in 1 ml sterile water. Repeat wash. After the second wash, resuspend cells in 1ml of sterile water.

Plating:-

- Make serial dilutions of the culture in sterile water so that each plate has 200 to300 viable cells.
- Plate 0.1 and 0.2 ml of the diluted cells on separate sets of LB agar plate, using ten plates in each set. Incubate all plates for 3 to 4 days at room temperature.
- Irradiate all but two plates from each set with UV light using a dosage of 300 ergs/mm2 (there should be 40% to 70% survival), the non-irradiated plates will serve as controls to determine the degree of killing by the UV light.

Biodegradability Tests

Weight Loss Measurements

The plastic films after exposure to all four bacterial suspensions were taken and washed thoroughly with 2% SDS for 4 h. The strips were then dried at 60LC overnight and the percentage weight loss was determined using the following formula:

$$\text{Weight loss \%} = \frac{\text{initial weight - final weight}}{\text{Initial weight}} \text{ X } 100$$

Tensile Strength Testing

The films (5 cm 9 1 cm) were tested in materials testing machine (Model INSTRON 5566) with a crosshead speed of 10 mm/min. The testing conditions were maintained at a room temperature of 35–37LC with a relative humidity of 65%. The negative control maintained at similar incubation conditions was also tested and compared with the positive control. Three LDPE strips were tested for each group and the average was reported as the final result.

Gel electrophoresis:

Extraction and estimation of Genomic-DNA by gel electrophoresis- Amnion Bioscience KIT

Capillary Gel electrophoresis analysis:

Polyacrylamide gel-filled capillaries are usually employed, although new polymer formulations with greater stability to the applied electric field are likely to be introduced shortly. Agarose gels are unable to withstand the heating produced by the high voltages used in capillary gel electrophoresis (CGE). The instrument CGE Pro 9600 – CGE Lauf-Nr 15315(Machine 3)

Capillary Gel electrophoresis was used: to analyse DNA fingerprinting is a useful tool for identifying the genotype of living organisms by determining their DNA sequence. For this technique, genomic DNA must be amplified by PCR. Capillary electrophoresis separates this amplified DNA with a one base pair resolution and creates specific peaks for each nucleotide to map the DNA sequence.

III. Result

Table 1: weight loss of low density polyethylene film

Sample	Initial weight of the film	Mass of film after 60 days incubation (mg)	Percentage reduction in mass
Control(P.putida)	1.000	1.000	**0.00**
Positive Control (P.putida + LDPE)	1.000	0.890	**11%**
Test -01: P. putida (UV mutated :30 days) + LDPE	1.000	0.780	**22%**
Test -02: P. putida (UV mutated :60 days) + LDPE	1.000	0.660	**34%**

The dry weight of LDPE films were measured at different time points during the course of Incubation, the result represents the average of triplicate experimental data's

Table 2: change in pH of the media

Days	**Groups**	**pH**
0	Normal Control	6.5± 0.22
15	Positive Control	6.8 ± 0.24
30	P. putida (UV mutated :30 min) + LDPE	7.8 ± 0.31
60	P. putida (UV mutated :60 min) + LDPE	8.5 ± 0.34

The result represents the average of triplicate experimental data's

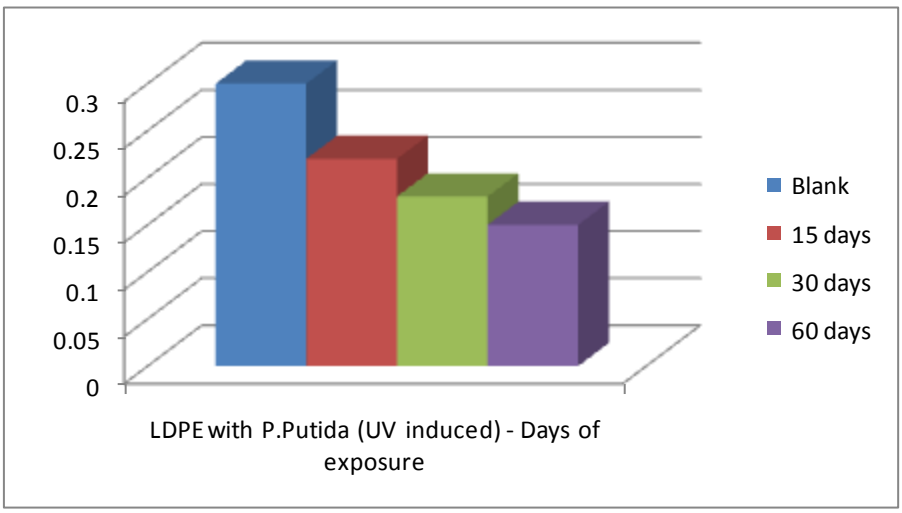
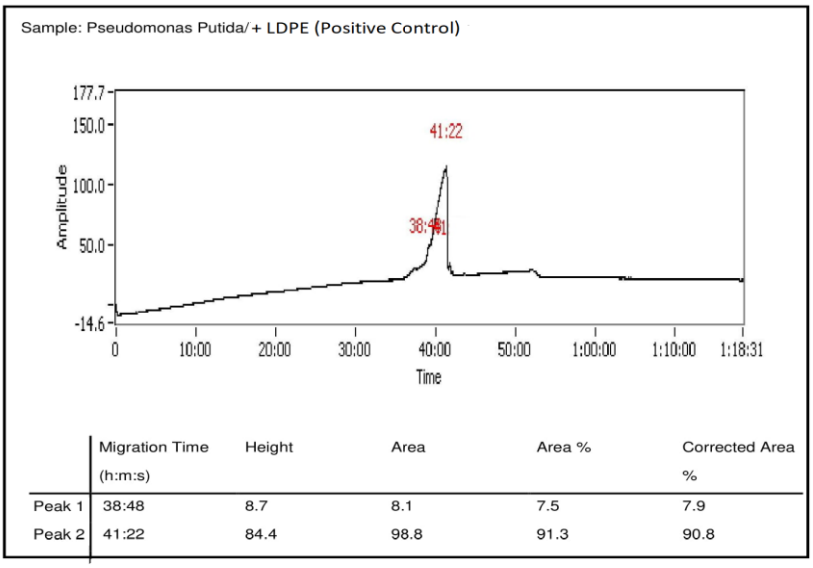


Fig. 1: Bar graph showing change in tensile strength of polyethylene film.

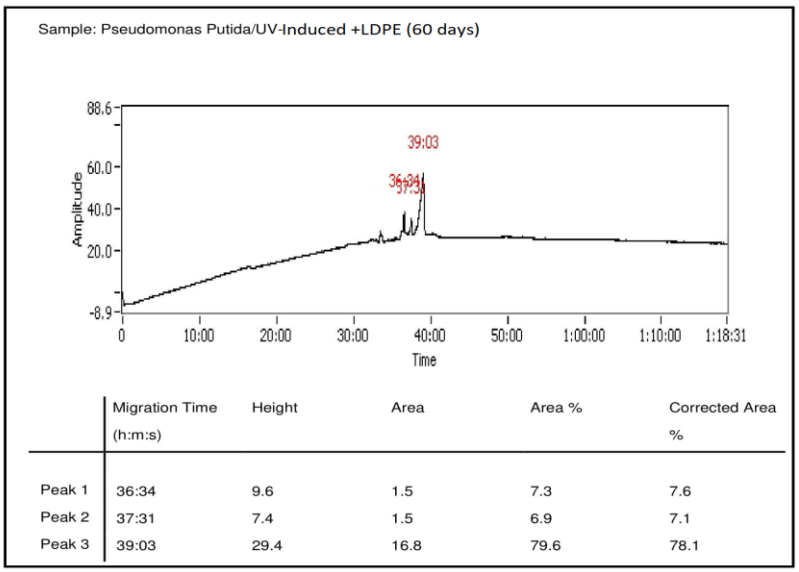
Treatment of the LDPE film with mutated Pseudomonas putida, Showing reduced tensile strength

Capillary Gel electrophoresis analysis:



	Migration Time (h:m:s)	Height	Area	Area %	Corrected Area %
Peak 1	38:48	8.7	8.1	7.5	7.9
Peak 2	41:22	84.4	98.8	91.3	90.8

Figure-2: Capillary Gel Electrophoresis -Genomic /DNA: Pseudomonas Putida + LDPE (Plastic)-60 days



	Migration Time (h:m:s)	Height	Area	Area %	Corrected Area %
Peak 1	36:34	9.6	1.5	7.3	7.6
Peak 2	37:31	7.4	1.5	6.9	7.1
Peak 3	39:03	29.4	16.8	79.6	78.1

Figure-3: Capillary Gel Electrophoresis - Genomic /DNA: Pseudomonas Putida (UV: induced + LDPE (Plastic) - 60 days)

Inferences: DNA of PP was found to be stable with a degradation capacity of 90.08 % With 2 STR repeats (Fig: 2).

Inferences: DNA of PP was found to be unstable with a degradation capacity of 78.01 % With 3 STR repeats (Fig: 3).

Moreover, biodegradation of polyethylene was studied and explored the propensity of fungi and Streptomyces strains to rush the degradable polyethylene consisting of disposed polyethylene bags[7]. Further, Aspergillus flavus, isolated from sanitary landfills was also found to be able to degrade polyethylene were identified a fungus, named Penicillium simplicissimum YK, which could degrade the untreated high-density polyethylene[8, 9]

This decrease in weight is in association to the others' findings carried out degradation of LDPE using Aspergillus fumigatus and Penicillium sp. According to their work, A. fumigatus was able to degrade 4.65% of polyethylene and Penicillium sp. degraded 6.58%. After bacterial treatment of thermally oxidised polyethylene, maximum weight loss of 7.006 ± 0.05% is achieved after 1 month for polyethylene oxidised in the presence of SDS.[10, 11, 12, 13]

In the present study weight loss will be Pseudomonas putida (UV mutated: 60 days) + LDPE were 34% against Positive Control (P.putida + LDPE) is 11% (Table 1).

The bacteria that degrade PE have been reported to Pseudomonas sp. (Balasubramanian et al. 2010), Bacillus sp. (Sudhakar et al. 2008), Mycobacterium sp, [14] and Nocardia sp.[15]

Change in the Tensile strength

Tensile strength was measured at 10 days interval. There is a clear pattern of reduction in tensile strength in comparison to blank polyethylene films in 60 days (Figure 1). Approximately 65% reduction in tensile strength was noted. This finding is in agreement with earlier studies showed tensile strength reduction of polyethylene film after incubation with microorganisms.[7,16] The percentage elongation of the LDPE film was reduced after thermal oxidation.[17] In addition, reported that biodegradability of disposable polyethylene was enhanced in controlled biological soil[18] and a reduction in the percentage elongation of poly-ethylene films after the biodegradation process[19] (Fig.1).

Change in the pH of biodegradation

The reduction in pH validates that the culture was still metabolically active and LDPE is utilized for its growth (Table 2). The reduction in pH not only affirms the consumption of the polyethylene film as their sole carbon source. [20, 21, 22]

Microorganisms secrete variety of intra and extracellular enzymes into the media which might be responsible for the degradation of polymer. During the polymer degradation process, complex polymers are first broken down into short chains or monomers by exoenzymes that are small enough to permeate through the cell walls to be utilized as carbon and energy sources by a process of depolymerisation.[23] Initial pH was 6.5± 0.22 while pH after 60 days incubation was measured as 8.5 ± 0.34 (Table 2).

IV. Discussion

Among the two isolates tested, Pseudomonas alcaligenes was found to be more effective in degradation of polythene films (LDPE) at 60 days. Previously, one more work reported on the biodegradability potential of Pseudomonas fluorescens and P. aeruginosa on synthetic plastics.[24, 25]

A thermophilic bacterial strain also isolated, identified as Brevibacillus borstelensis, which utilized standard and photo-oxidised polyethylene.[26]

Evidently, a biofilm isolated from strain of Rhodococcusruber (C208) that degraded polyethylene at a rate of 0.86% per week.[27] the ability of Bacillus species to utilize polyethylene, with and without pro-oxidant additives, was also evaluated.[28]

Recently, in another research work on Pseudomonas sp, has showed a significant plastic degradation capacity and it degrades up to 24.22% for the period of 6 months.[29] Similarly, that biodegradation of low density polythene (LDPE) were studied by Pseudomonas species.[5] They reported that after 120 days of incubation period, the percentage of weigh reduction was 20% in Pseudomonas aeruginosa (PAO1), 11% in Pseudomonas aeruginosa (ATCC) strain, 9% in Pseudomonas putida and 11.3% in Pseudomonas syringae strain.

The overall investigation of their work can be concluded that Pseudomonas alcaligenes exhibited significant polythene degradation ability and in the near future, Pseudomonas alcaligenes can be used to reduce the quantity of plastic waste, which is rapidly accumulating in the natural environment.[30]

These results are consistent with earlier observations[31] who reported that Pseudomonas spp. have slow rate of micro-colony formation when compared to other microorganisms. The biofilm formation presumably induced partial bio-degradation.

Spectrophotometric variations of LDPE films and the value of CI which determines the extent of degradation decreases with increase in incubation time and is maximum for Pseudomonas aeruginosa PAO1, The decrease in CI was complemented by decrease in weight and TS. These results suggest on the polythene degradation potential of Pseudomonas species.

They also provide novel ways of implementing plant pathogen for degradation purpose over a long run. Thus the information procured acts as an evidence for degradation capability of human and plant pathogens on LDPE which can be further enhanced with induced mutation to enhance their yield and performance in an industrial scale for degrading the recalcitrant materials.

May be Physico-chemically treated polyethylene films were found to be effectively degraded by the fungal isolates than untreated films. The hypothesis is that a physicochemical treatment of the polymer leads to its oxidation and subsequent breakdown assisting in the easy assimilation by the fungus and, hence, can be effectively used as a pre-treatment strategy before subjecting the polymer to biodegradation.[32] The oxidized polymer helps in adhesion of microorganisms (due to probable changes in the hydrophobicity of the polymer surface), which is a prerequisite for biodegradation.[33]

Similarly in the present study, a higher biomass was observed on the pre-treated samples. Because carbohydrates in the medium constitute the main energy source for their growth and metabolism during the non-availability of readily assimilating carbon source, microorganisms adhere to the polymeric surface during the formation of the biofilm, which is essential for bringing about degradation.[34]

It is also based on research[35] these bacteria Pseudomonas sp. able to degrade the plastic by 8.16% and was able to degrade the polythene by 20.54% within one month incubation anaerobically. While this type of fungi Aspergillus Glaucus able to degrade the plastic by 7.26% and was able to degrade the polythene by 28.80% within one month incubation anaerobically, from the results of the degradation of polythene faster and easier than plastic degradation. Earlier publications interpreted the growth of microorganisms on polyolefins, e.g. polyethylene as being limited to the microbial action on the surface of an inert support without impact on the polymers.[36]

However, it was found that polyethylene is not only colonized but also biodegraded by various fungi mostly belonging to the genera Aspergillus, Fusarium or Penicillium.[37]

Polythene and plastics are two polymers with wide application, both are recalcitrant and thus remain inert to degradation and damage that leads to accumulation in the environment, and create serious environmental problems. Therefore, further research is needed to prevent environmental damage caused by plastic and polythene waste contamination.[38]

Due to high durability, cheap cost, and ease of manufacture, 311 million tons of plastic-based products are manufactured around the globe per annum. The slow/least rate of plastic degradation leads to generation of million tons plastic waste per annum, which is of great environmental concern. Of the total plastic waste generated, polythene shared about 64 %.

Polythene degrading bacteria by using rhizosphere soil of Avicennia marina as a landmark. From 12 localities along the west coast of India, a total of 123 bacterial isolates were recorded. Maximum percent weight loss (%WL; 21.87 ± 6.37 %) was recorded with VASB14 at pH 3.5 after 2 months of shaking at room temperature.

Maximum percentage weight gain (13.87± 3.6 %) was reported with MANGB5 at pH 7. Maximum percentage loss in tensile strength (% loss in TS; 87.50 ± 4.8 %) was documented with VASB1 at pH 9.5. The results based on the % loss in TS were only reproducible. Further, the level of degradation was confirmed by scanning electron microscopic (SEM) and Fourier transform infrared spectroscopy (FTIR) analysis.

Their research work established similar biodegradation results, In SEM analysis, scions/crakes were found on the surface of the degraded polythene, and mass of bacterial cell was also recorded on the weight gained polythene strips. Maximum reduction in carbonyl index (4.14 %) was recorded in untreated polythene strip with Lysinibacillus fusiformis strain VASB14/WL. Based on 16S ribosomal RNA (rRNA) gene sequence homology, the most efficient polythene degrading bacteria were identified as L. fusiformis strainVASB14/WL and Bacillus cereus strain VASB1/TS.[39]

V. Conclusion

The overall investigation can be concluded that *Pseudomonas putida* exhibited significant polythene degradation ability and in the near future, *Pseudomonas putida* with induced mutation and establishing and enrichment of its activity can be used to reduce the quantity of plastic waste, which is rapidly accumulating in the natural environment.

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