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RESEARCH ARTICLE

ISOLATION OF POLYTHENE DEGRADING BACTERIA FROM GARBAGE SOIL

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ABSTRACT

Polythene is a polymerized ethylene monomer which is resistant to degradation accumulates in an environment. Polythene used in everyday life as a packaging material. Release of toxic chemicals from polythene creates negative impacts on environment and life threatening problems to many aquatic and terrestrial organisms. The present study focused on biodegradation of polythene by soil microorganisms .Isolated microorganisms were identified based on biochemical characterization as *Staphylococcus aureus* and *Bacillus cereus*. Biodegradation of polythene by isolated microorganisms were tested *in vitro* in both nutrient and minimal medium. After 30 days incubation period, the biodegradation of polythene was measured in terms of weight. The percentage of weight loss by *staphylococcus aureus* and *Bacillus cereus* in nutrient medium was 32.2% and 9% respectively. The percentage of weight loss by *staphylococcus aureus* and *Bacillus cereus* in minimal medium was 57.3% and 26% respectively. The results depict that *Staphylococcus aureus* are found active in degrading polythene than *Bacillus cereus*.

INTRODUCTION

A plastic is the general term given to polymerized xenobiotic compounds that are resistant to degradation. They accumulate in an environment and creating many problems. Commercially produced plastic materials like Polyethylene, polypropylene, polystyrene, Polyvinyl chloride and polyethylene terephthalate have wide applications in industries and human life. Polyethylene is a synthetic long chain polymer of ethylene which contains carbon, hydrogen and oxygen derived from petrochemicals (Mukherjee.S and Chatterjee.S, 2014). This complex makes the polythene to be less degraded and accumulated in the environment causing great threat. It is estimated that 500 billion to 1 trillion tones of polythene bags are consumed worldwide. This increased commodity of polythene bags are attributed to their elasticity, strength, durability and cheapness (Chee *et al*., 2010)**.** Thus polythene is used in manufacture of bottles; carry bags, disposable articles, garbage containers, margarine tubs, milk jugs, and water pipes (Sangale *et al*., 2012). Polyethylene is a chemically inert and hydrophobic in nature its water resistant property is suitable for producing packaging materials. Approximately 30% of plastic materials were used for producing packaging materials. The usage of polythene bags were increased every day. Every year 25 million polythene bags were accumulated in an environment (Chee *et al*., 2010). Polyethylene cannot be broken down easily in an environment, their accumulation in a land affect the water percolation and fertility of the soil. In aquatic environment these polythene bags are ingested by many aquatic animals and thus causing threat to their life.

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These problems have thrown the light on the polythene a major solid waste that has to be managed (Dey *et al*., 2013). Biodegradation is an alternative approach for waste management. The attractive part of biodegradation is they do not produce secondary pollutants associated with incineration and landfills. Polythene is resistant to biodegradation due to its high molecular weight, three dimensional structures and its hydrophobicity that interferes with polythene availability to soil microorganisms. Physical pre-treatment such as weathering, UV irradiation and thermal treatment was employed to raise the hydrophilicity of polyethylene by introducing polar groups such as carbonyl groups to the polyethylene backbone chain and cause mechanical damage to the polymer thus facilitates the microbes to metabolize the plastics (Gu *et al*., 2000; Nanda *et al.,* 2010). In the present study two bacterial strains were isolated from polythene dumped garbage soil. In vitro biodegradation of LDPE was performed with the isolated bacterial strains by shake –flask incubation method. The percentage of biodegradation was evaluated by comparing the initial and final dry weights of polyethylene before and after incubation. The hypothesis of the study was microorganisms will utilize polyethylene as the sole source of carbon and energy when the rest of the nutrients in the broth were limited.

MATERIALS AND METHODS

Sample Collection

Garbage soil sample (waste disposable sites dumped with polythene bags and plastic cups) was collected from THE AMERICAN COLLEGE campus, Madurai, Tamil Nadu. The

sample was collected at the depth of 3-5cm, in a sterile container and then air dried at room temperature (Fig.1).

Fig. 1. Collection of garbage soil sample

Polythene film

The LDPE films used for this study were collected from local market where it is sold as 40 micron thick carry bags [Fig. 2].

Fig. 2. Polythene bag purchased from local supermarket

Isolation of microorganisms from garbage Soil

One gram of soil sample was transferred into a conical flask containing 99ml of sterile distilled water and serially diluted. To isolate the microorganisms, pour plate method was adopted by using nutrient agar medium. For each dilution three replicates were made. The plates were then incubated at 30◦C for 2-7 days. The developed colonies were isolated and sub cultured repeatedly to get pure colonies and then preserved in slant at 4◦C.

Identification of microorganisms

Isolated organisms were identified based on their morphological, cultural and biochemical characteristics. All the isolates were subjected to Gram staining, Mannitol fermentation test on Mannitol-Salt agar, hemolytic activity on blood agar and Catalase tests were performed. Species level of identification was done by biochemical analysis in Bose clinical laboratory in Simmakkal, Madurai, Tamil Nadu 625 001, India.

Microbial degradation of polythene in laboratory condition

Pretreatment of polyethylene

LDPE films were cut into (3X3 cm) strips and then washed with 70% ethanol for 30 min, washed with a fresh solution of universal disinfectant containing 7 ml of Tween 80, 10 ml of bleach, and 983 ml of sterile water, and stirring for 30 to 60 min. Each film was removed with sterile forceps and placed into a covered beaker of sterile water, where it was stirred for 60 min at room temperature. The films were then aseptically transferred into a standing 70% (vol/vol) ethanol solution and left for 30 min. Each film was then placed into a preweighed sterile petridish. The dishes with films were placed into an incubator at 45 to 50°C to dry overnight, allowed to equilibrate to room temperature, and weighed to +0.1-mg accuracy; the weight of the film was then determined [Fig. 3].

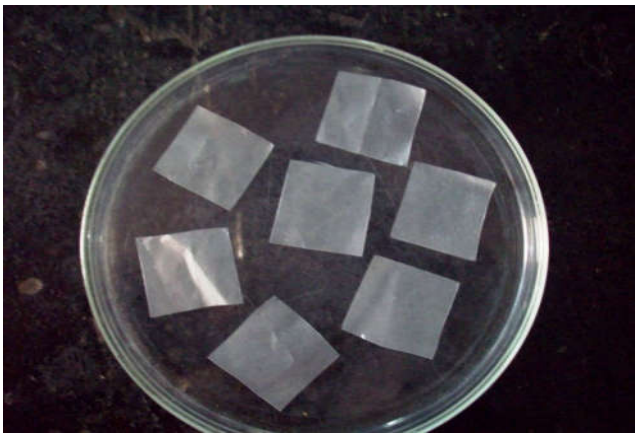

Fig. 3. Polythene strips (3×3cm) before degradation

Determination of Weight Loss

Pre-weighed strips of 2-cm length and breadth prepared from polythene bags were aseptically transferred to the conical flask containing 50 ml of culture broth medium, inoculated with isolated bacterial species. Control was maintained with plastic discs in the microbe-free medium. Different flasks were maintained for each treatment and left in a shaker. After one month of shaking, the plastic discs were collected, washed thoroughly using distilled water, shade-dried and then weighed for final weight. From the data collected, weight loss of the polyethylene was calculated (Thakur.P, 2005). The percentages of weight loss of each plastic material used in this study were determined by using the formula (Saminathan *et al*., 2014).

RESULTS

Isolation of microorganisms from garbage soil

Two different microorganisms were isolated from garbage soil and identified based on biochemical characterization.

Identification of microorganisms

Biochemical characterization such as Gram staining, Mannitol fermentation test on M.S agar, hemolytic activity on blood agar

and Catalase tests were performed. As per results, such as Gram staining [Fig.4 A], Hemolysis on blood agar [Fig.7 A], Mannitol fermentation on Mannitol salt agar medium [Fig.5] and positive Catalase reaction [Fig.6 A] indicates that the isolated organism was *Staphylococcus* sp [8]. Similarly second organism was identified as *Bacillus* sp based on Gram staining [Fig.4B], Creamy white colonies [Fig .8] and Partial hemolysis on blood agar [Fig.7B] and positive Catalase reaction [Fig.6 B] (Al-Bager.S.D, 2005). And species level identification was done by biochemical analysis in Bose clinical laboratory in Madurai, Tamil Nadu, India. Thus based on biochemical characterization the isolated organisms were identified as *Staphylococcus aureus* [Table 1] and *Bacillus cereus*. [Table 2].

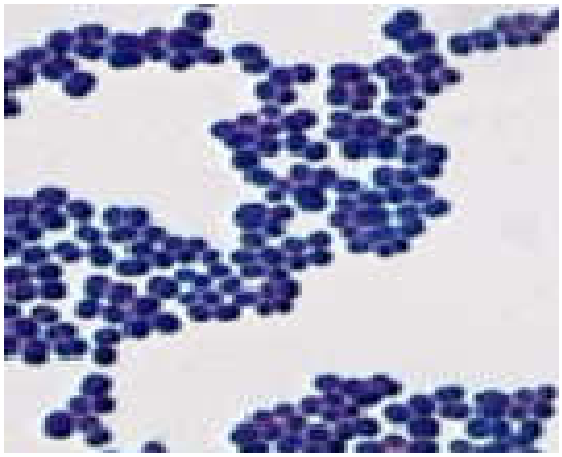

Fig. 4A. Gram staining of *Staphylococcus aureus*

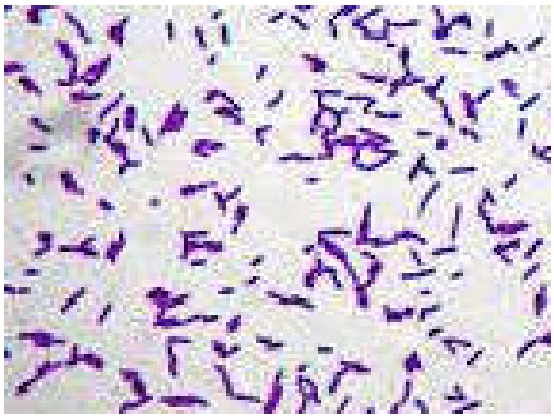

Fig. 4B. Grams staining of *Bacillus cereus*

Fig. 5. Mannitol fermentation test for *Staphylococcus aureus*

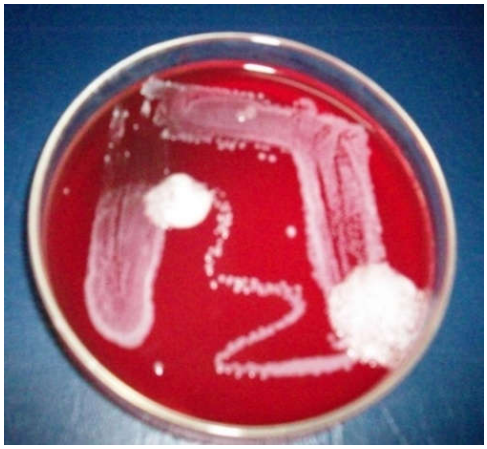

Fig. 6A. Catalase test for *Staphylococcus aureus*

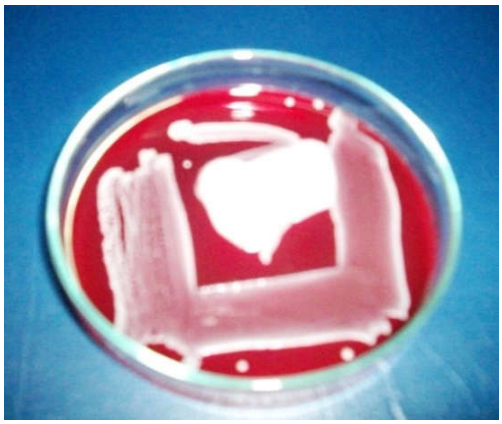

Fig 6B. Catalase for *Bacillus cereus*

Fig. 7A. Hemolysis test for *Staphylococcus aureus*

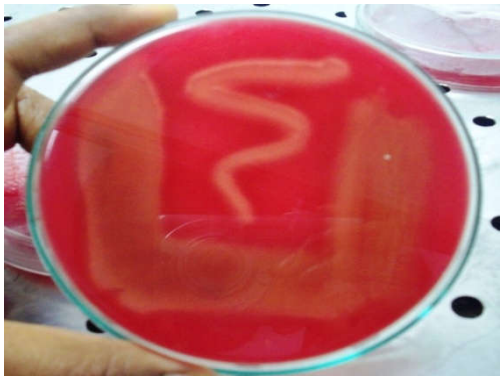

Fig. 7B. Hemolysis test for *Bacillus cereus*

Fig. 8. Creamy white colonies of *B.cereus* on blood agar

Table 1. Biochemical tests for *staphylococcus aureus*

Sl.no	Biochemical Tests	Results
1	Urea	Positive
2	Sorbitol	Negative
3	Aesculin	Negative
4	Rhamnose	Negative
5	Trehalose	Negative
6	Glucose	Positive
7	B-methyl glucoside	Positive
8	Arginine	Negative
9	Glycerol	Positive
10	Sucrose	Positive
11	Mannitol	Positive
12	Maltose	Positive

Table 2. Biochemical tests for *Bacillus cereus*

Sl.no	Biochemical tests	Results
1	Urea	Positive
2	Sorbitol	Negative
3	Aesculin	Negative
4	Rhamnose	Negative
5	Trehalose	Negative
6	Glucose	Positive
7	B-methyl glucoside	Positive
8	Arginine	Negative
9	Glycerol	Positive
10	Sucrose	Positive
11	Mannitol	Positive
12	Maltose	Positive

Morphological changes in polythene bag

In addition to decreased weight of polythene strip, surface deformations such as increased transparency and reduced thickness of polythene strips were observed. This indicates the partial biodegradation of polyethylene [Fig. 9, 10, 11 and 12].

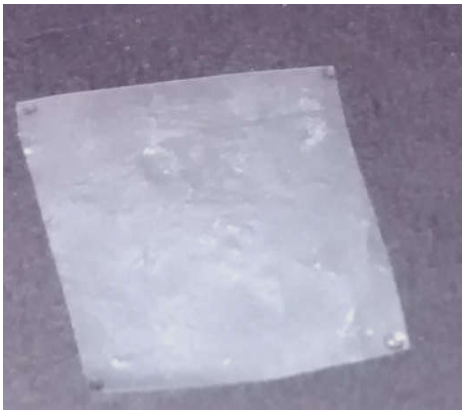

Fig 9. After degradation by *S. aureus* in Nutrient medium

Fig. 10. After degradation by *S. aureus* in minimal medium

Fig. 11. After degradation by *B. cereus* in Nutrient medium

Fig. 12. After degradation by *B.cereus* in minimal medium

Degradation of polythene by isolated microorganisms in nutrient medium

The weight loss of polythene by *Staphylococcus aureus* and *Bacillus cereus* in nutrient medium was 21.3±1.15 and 27.3±0.57 respectively. And the percentage of weight loss by *Staphylococcus aureus* and *Bacillus cereus* was 32.2% and 9% respectively [Table 3].

Table 3. Weight of a polythene strip (mean ±S.D) before and after biodegradation by isolated microorganisms in nutrient medium for 30 days

Microorganisms	Weight of a polythene strip (mg)		Percentage of Weight loss (%)
	Initial	Final	
Staphylococcus aureus	30	21.3±1.15	32.2
Bacillus cereus	30	27.3±0.57	9

Degradation of polythene by isolated microorganisms in minimal medium

The weight loss of polythene by *Staphylococcus aureus* and *Bacillus cereus* in minimal medium was 12.8±0.76 and 22±1 respectively. The percentage of weight loss by *Staphylococcus aureus* and *Bacillus cereus* in minimal medium was 57.3% and 26% respectively [Table 4].

Table 4. Weight of a polythene strip (mean±S.D) before and after biodegradation by isolated microorganisms in minimal broth for 30 days

Microorganisms	Weight of a Polythene strip (mg)		Percentage of Weight loss (%)
	Initial	Final	
Staphylococcus aureus	30	12.8±0.76	57.3
Bacillus cereus	30	22±1.0	26

Among these bacterial strains *Staphylococcus aureus* are found active in degrading polythene than *Bacillus cereus*.

Conclusion

Plastics are non metallic polymers which on heating become mobile and cast into moulds. Plastics are widely used to make items like automobile parts, furniture, packaging materials *etc*. About 30% of plastics were used for packaging worldwide. Plastics can be disposed through land filling, incineration and recycling. These plastic wastes remains in the environment for a long time and causes harmful effects on many living organisms (Thakur.P, 2005). Hydrocarbons in the polymers were used as a carbon source by microorganisms like bacteria and fungi .These microorganisms they degrade the polymer by producing enzymes like mono-oxygenase, di- oxygenase and dehydrogenase. In biodegradation, hydrocarbons are oxidized by these enzymes by taking oxygen from air; in addition photo catalytic oxidation by Ultraviolet radiation facilitates the process (Gu *et al*., 2000). Five different types of bacterial strains (i.e.) *E.coli, Staphylococcus* sp*, Pseudomonas* sp*, Klebsiella* sp and *Bacillus* sp will degraded Low Density Polythene was identified (Pandey and Anbuselvi.S , 2014).

In the present study two different bacterial species were isolated from polythene dumped garbage soil. The identification of the isolated strains was done by gram staining, Mannitol fermentation, Catalase test *etc*. The results indicated that the isolated organisms are *Staphylococcus aureus* and *Bacillus cereus* (Al-Bager.S.D, 2005; Li.Zhu.L *et al*., 2016). After one month of incubation period, a thin bio film was observed over the surface of polyethylene film. The transparency was increased more in a polythene strip degraded by *Staphylococcus aureus* than *Bacillus cereus.* The reduction in the thickness of the polythene strip was also more in the polythene strip treated with *S. aureus* than *B. cereus.* Thus, surface deformations such as increased transparency and reduced thickness of degraded polythene strips were observed indicates the biodegradation of polyethylene by the isolated microorganisms. Thus it confirms that microbes utilized polyethylene as sole source of carbon. These bacteria caused nearly 30 to 50% of biodegradation of polythene strips. In the study maximum amount of weight loss was observed in polythene strips degraded by *Staphylococcus aureus* in minimal medium than *Bacillus cereus*. In nutrient medium also the effect of degradation of polythene strips by *Bacillus cereus* was less compared to the *Staphylococcus aureus*. Thus *Staphylococcus aureus* was comparatively good in degrading polythene than *Bacillus cereus*. Therefore more reduction in the weight was seen in the *Staphylococcus aureus* treated polythene strips than the *Bacillus cereus*. Similarly maximum degradation of 40 micron size polythene strip by *Staphylococcus sp* up to 10% and *Bacillus sp* up to 5% was observed upon 30 days of incubation (Singh.M.J and Padmavathy Sedhuraman 2015). This observation is also in correlation with the work of Thakur.P (2005) who reported that *Bacillus* sp has less capacity to degrade polythene than other bacteria. Pandey.S and Anbuselvi.S, (2014) also observed that the maximum amount of polyethylene was degraded by *Staphylococcus* sp up to 52% and 11% of degradation was by *Pseudomonas* sp. Pandey.S and Anbuselvi.S (2014) was confirmed from their experiment that *Staphylococcus* sp was found to be very effective and less amount of biodegradation was observed in *Pseudomonas* sp. However, the loss in weight of polythene bags increases with increasing period of incubation. Polythene degradation by microbes is an eco-friendly and acceptable method. Microbes produce extracellular enzymes such as lignin peroxidase, manganese peroxidase to degrade the polythene. *Bacillus cereus* showed a positive result for the presence of laccase enzyme which is responsible for polythene degradation (Singh.M.J and Padmavathy Sedhuraman, 2015). Some microorganisms they produce an extracellular secretion called biosurfactant which dissolves a hydrophobic contaminant and makes the degradation process easy. These isolated microorganisms were also qualitatively analyzed for its biosurfactant production and it was confirmed by performing various assays like hemolysis on blood agar, C-TAB agar plate assay and penetration assay (Archana.B *et al*., 2017). Microbes from various sources can degrade the polythene, but efficient polythene degrading microbe and extracellular enzymes and its relation with degradation process are needed to be screened (Sangale *et al.,* 2012).

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