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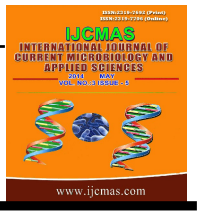

Original Research Article

A comparative study of commercially available plastic carry bag biodegradation by microorganisms isolated from hydrocarbon effluent enriched soil

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A B S T R A C T

Plastic is a broad name given to different polymers having high molecular weight and that can be degraded by various processes. However, degradation by physical and chemical means leads to innumerable environmental hazards. On the other hand, degradation of plastics by microorganisms seems to be more effective, considering their abundance in the environment, their specificity in attacking plastics and has very less environmental hazards. This study introspected the comparative extent of plastic biodegradation by employing one fungal species (*Aspergillus niger*) and three bacterial species (*Bacillus weihenstephanensis, Burkholderia cepacia* & *Escherichia coli*) which were isolated from hydrocarbon enriched soil from Purba Medinipur, West Bengal. Amongst the isolates *Bacillus weihenstephanensis* has been found to be a novel candidate having sound biodegradation potential.

Keywords

Aspergillus niger, *Burkholderia cepacia*, *Bacillus weihenstephanensis*, *Escherichia coli*, biodegradation, plastic.

Introduction

The term "plastic" is a term derived from the Greek word "plastikos" which means "able to be molded into different shape" (Joel. 1995) and is given to any synthetic or semi-synthetic organic polymers with high molecular mass and that are moldable. Plastics are man-made long chain polymeric molecules (Scott. 1999) mainly synthetically derived from petrochemicals. Plastic material such as polyethylene, polypropylene, polystyrene, polyvinyl chloride and polyethylene terephthalate have wide applications in industries and human life. However, most of these conventional plastics are non-biodegradable or biodegradable at a very slow rate (Sharon et al. 2012). Hence, the accumulation of these materials has been accountable for innumerable environmental hazards like air water and soil pollution and has been a threat to the planet (Sharon et al. 2012, Tokiwa et al. 2009). Nevertheless, plastics have multi-purpose use in human society and few have been summarized in Table 1 (Vona et al. 1965). Plastics are traditionally composed of petroleum based resins like polythene and polypropylene which are

very stable and do not get degraded in the ambient environment (Raaman et al. 2012), leading to its accumulation and as a result pollution. Though plastics made of natural polymers are inherently biodegradable, the synthetic ones are the real threat to the environment. Over the last two decades research has been concentrated on microbial degradation of natural and synthetic polymers. At present, there are several general guidelines concerning the structure and biodegradability of polymers (Kawai. 1995). Biodegradable and natural plastics are polyesters which are produced by a range of microbes, cultured under different nutritional and environmental conditions (Madison et al. 1999). Biodegradation of natural and synthetic plastics are carried out by microbes like bacteria, fungi and actinomycetes (Gu et al. 2000) under optimal growth conditions of the respective microbes in soil (Glass et al. 1989). Biodegradation depends directly upon the molecular weight of the polymers and the rate of biodegradation declines with an increase in the molecular weight of polymers (Gu et al. 2000).

In this context, *Aspergillus niger*, *Bacillus weihenstephanensis*, *Burkholderia cepacia* and *Escherichia coli* were isolated from hydrocarbon effluent enriched soil and pursued further for a comparative study on their efficiency of plastic biodegradation. *Aspergillus niger* has been reported to show biodegradation in plastics and polythene (Nayak et al. 2011). The ability to degrade the low density polyethylene (LDPE) of the commercially available plastic carry bags was almost up to 8% (Raaman et al. 2012) and almost 17% polythene (Kathiresan et al. 2003) in one month. Studies with *Pseudomonas* species like *Pseudomonas aeruginosa (PAO1), Pseudomonas putida* have revealed that they were able to reduce the weight of LDPE by 20% and 9% respectively after 120 days incubation. It has also shown a decline of - 0.00078 ± 0.00011 MPa and 79 mm ± 3% for *Pseudomonas aeruginosa (PAO1)* and 0.00057 ± 0.0002 MPa and 112 mm ± 2% for *Pseudomonas putida* in tensile strength (TS) and extention at break (EAB) respectively in comparison to 0.0032 ± 0.0003 MPa and 134 mm ± 3% before incubation (Kyaw et al. 2012). Moreover, it has also been found that *Pseudomonas putida* also plays a significant role in biodegradation of high density polyethylene (HDPE) plastic in combination with other microorganisms (Broshkevitch et al. in school article). Though there are earlier reports of *Burkholderia cepacia* using phthalate as their soul carbon source (Chang et al. 1998), there is no such report of it having a role in biodegradation of plastic carry bags as a whole when incubated. Bioengineered or recombinant *Escherichia coli* have been reported to secrete polyhydroxybutyrate which is a short-chain polyhydroxyalkanoate, a group of biodegradable plastics (Rahman et al. 2013). Recombinant *Escherichia coli BL21* have been used to produce alkB gene which expresses alkane degrading enzymes (Yoon et al. 2012). *Bacillus weihenstephanensis* has only been reported earlier showing degradation property of polycyclic aromatic hydrocarbons (PAH's).

Materials and Methods

Materials

All the reagents required to prepare different culture media were obtained from Sisco Research Laboratories (SRL). LDPE powder was purchased from Sigma Aldrich.

Sample Collection

Hydrocarbon effluent enriched soil samples were collected from petroleum refinery area of Purba Mednipur, West Bengal. The soil samples were collected from a depth of almost 5 cms in sterile containers and air dried in the laboratory at room temperature. The thick (thickness above 40 micron) and thin (thickness less than 40 micron) plastics were collected from the local market shops.

Isolation of microorganisms

A 1% (w/v) soil sample solution was prepared by adding 1gm of soil sample in 99 ml of sterile double distilled water. The soil solution was shaken properly and serially diluted. For each dilution triplicate luria bertani (LB) agar plates were made to isolate bacteria and potato dextrose agar (PDA) plates to isolate the fungi. The LB plates were incubated at 37ºC for 2-3 days and the PDA plates were incubated at 25ºC for 4-7 days. The developed colonies were isolated and sub-cultured repeatedly to get the pure culture and preserved as slants at 4ºC.

Screening for plastic degrading microorganisms

To select the biodegrading microorganism from the isolated ones, they were cultured in mineral salt agar plates containing LDPE powder at a concentration of 0.1% (w/v). The microorganisms that showed zone of clearance around their colonies were selected for further studies regarding their potency on biodegradation of plastic carry bags (Augusta et al. 1993).

Identification of plastic degrading microorganisms

From the isolated microorganisms, two bacteria and one fungus were successfully screened for plastic biodegradation property. The bacteria were further identified on the basis of macroscopic & microscopic examinations and biochemical analysis according to Bergey's manual (Holt et al. 1994). Fungi identification was done by lacto phenol cotton blue staining test following the keys Raper and Fennell (Raper et al. 1987).

Biodegradation of thick and thin plastic carry bags in liquid culture method

1.5gms of thick and 2gms of thin plastics carry bag strips were aseptically transferred to two conical flasks, each containing 50 ml of C-zopek-Dox broth respectively. The broth was then inoculated with identified polythene degrading fungi. A control was maintained without the fungi inoculum and left in shaker at 30ºC at 150 rpm for six months. The plastic strips were taken out from culture aseptically after 2, 4 and 6 months respectively, washed properly with double distilled water followed by 70% ethanol. The strips were then dried and weighed. The final weight loss were calculated and compared to the control. The same method was followed for the three identified polythene degrading bacteria in LB broth (Orhan et al. 2004).

Results and Discussion

Screening and Identification of plastic degrading microorganisms

LDPE containing mineral salt agar plates were inoculated by isolated bacteria and fungi. All the microbial isolates were screened for their potency to degrade polymer after an incubation period of 8-10 days at 25-30ºC. The efficiency of screening was based on the area of clear zone created by each colony around itself. Based on this screening, one fungus and two bacterial species with maximum degradation activity were chosen for

Table.1 Uses of Synthetic Plastics

Plastic	Use
Polyethylene	Plastic bags, milk and water bottles, food packaging film, toys, motor oil bottles.
Polystyrene	Disposable cups, packaging materials, laboratory wares and certain electronic uses.
Polypropylene	Bottle caps, drinking straws, medicine bottles, car seats and batteries, disposable syringes, carpet backings.
Polyvinyl chloride	Automobile seat covers, shower curtains, raincoats, bottles, visors, shoe soles, garden hoses and electricity, pipes.
Nylon	Small bearings, speedometer gears, windshield wipers, water hose nozzles, football helmets, race horse shoes, cell phone.
Polyurethane	Tires, refrigerator insulation, furniture cushions, sponges, life jackets.
Polycarbonate	Nozzles, street light, roofs of greenhouse, baby bottles, lens in glasses.
Polytetraflouro-ethylene (PTFE)	Electronics bearing, non-stick kitchen utensils.
Polyethylene terephthalate (PET)	Soft drink bottles, textile fibers, sleeping bag and pillow filling.

Table.2 Identification of LDPE degrading microorganisms

Microorganism - Bacteria	***Bacillus weihenstephanensis***	***Burkholderia cepacia***	***Escherichia coli***
Differential Media	Mannitol-egg yolk-polymyxin agar (MYP) (Pink colony and lecithinase positive)	PC Agar (Pink colonies)	EMB Agar (Green Metallic Sheen)
Gram Stain	+ve	-ve	-ve
Morphology	Rod	Rod	Rod
Indole Test	-ve	-ve	+ve
Methyl Red Test	+ve	+ve	+ve
Voges-Proskaur Test	-ve	-ve	-ve
Citrate Test	-ve	+ve	-ve
Urease	+ve	-	-ve
Gelatin Hydrolysis	+ve	-ve	-ve

Microoganism - Fungi	***Aspergillus niger***
Differential Media	Potato Dextrose Agar (PDA)
Morphology	Dark Brown to Black spores on PDA
Lacto Phenol Cotton Blue Test	+ve

Table.3 Comparative study of Loss of weight (%) of Thick and Thin plastic carry bags due to microbial biodegradation

Microorganism	2nd Month		4th Month		6th Month	
	Thick Plastic	Thin Plastic	Thick Plastic	Thin Plastic	Thick Plastic	Thin Plastic
Bacillus weihenstephanensis	5.95 ± 0.10	7.72 ± 0.31	23.05 ± 0.05	22.29 ± 0.10	32.61 ± 0.02	35.64 ± 0.08
Burkholderia cepacia	5.75 ± 0.16	8.67 ± 0.14	22.31 ± 0.18	23.80 ± 0.06	31.43 ± 0.09	36.34 ± 0.11
Escherichia coli	2.65 ± 0.05	4.51 ± 0.10	11.34 ± 0.07	15.76 ± 0.09	23.72 ± 0.03	23.57 ± 0.09
Aspergillus niger	4.46 ± 0.02	12.21 ± 0.14	14.57 ± 0.06	26.57 ± 0.32	30.82 ± 0.17	33.63 ± 0.20

Figure.1 Gram staining (A-C) and Lacto Phenol Cotton Blue Test (D) results of LDPE degrading microorganisms. **(A)** *Bacillus weihenstephanensis*. **(B)** *Burkholderia cepacia*. **(C)** *Escherichia coli*. **(D)** *Aspergillus niger*.

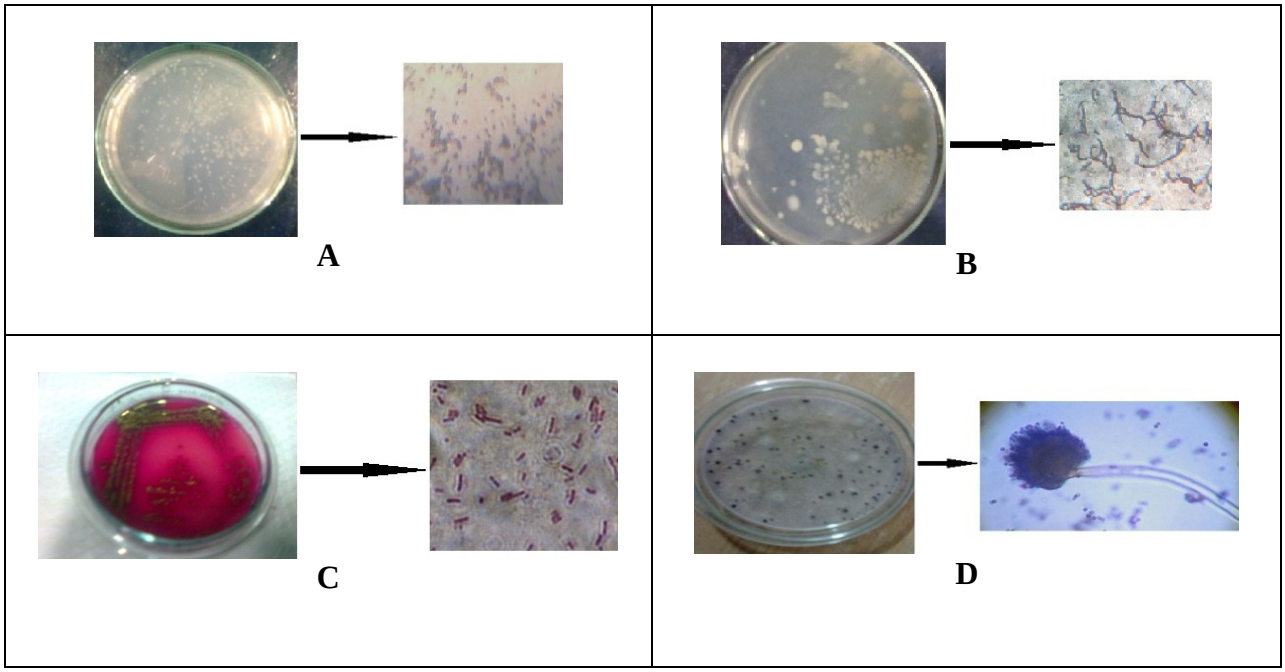

Figure.2 Comparative study of weight loss (%) of **(A)** Thick and **(B)** Thin plastic carry bags due to biodegradation by *Bacillus weihenstephanensis* (■) *Burkholderia cepacia* (■) *Escherichia coli* (■) *Aspergillus niger* (■).

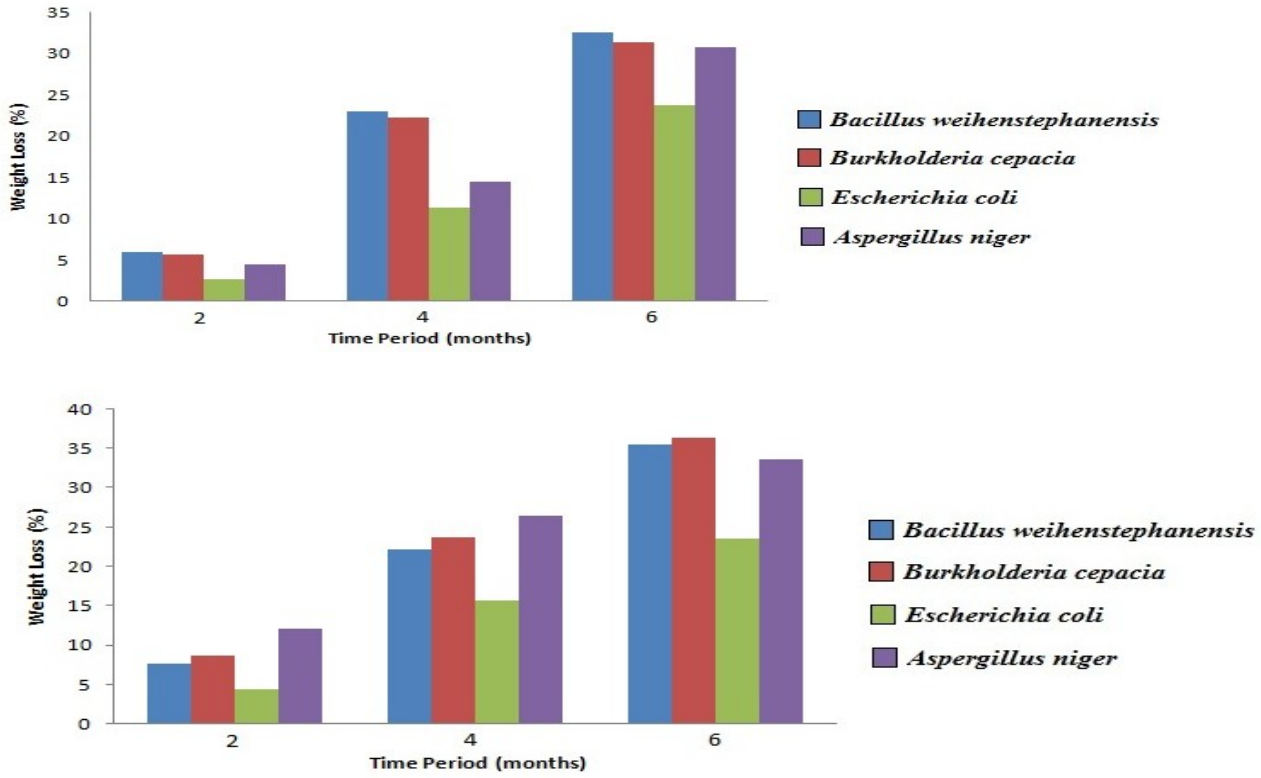

Figure.3 (A) Microbial culture with thick and thin plastic strips kept on shaker. **(B)** Thick plastic strips after 6 months of incubation with *Aspergillus niger*. **(C)** Thin plastic strips after 6 months of incubation with *Aspergillus niger*.

fermentation. Earlier studies on soil microorganisms reveal their active association in biodegradation of plastic films and polyethylene bags (Kathiresan. 2003). The zone of clearance around the bacterial colonies unveils hydrolysis of suspended polyester in turbid agar medium by the secreted hydrolyzing enzymes of the bacteria (Augusta et al. 1993). Gram staining and other biochemical assay according to Bergey's manual was used in identifying the three bacterial species, namely *Bacillus weihenstephanensis, Burkholderia cepacia* and *Escherichia coli* (Table 2) (Holt et al. 1994). Lacto-phenol cotton blue staining and following the keys of Raper and Fennell identified the screened fungus as *Aspergillus niger* (Raper et al. 1987).

Degradation of thin and thick plastic by the screened microorganisms

The screened microorganisms were further tested for their ability for degradation of plastics in laboratory conditions. The microorganisms were incubated in suitable broth culture under shaking condition for time period of 6 months having 1.5 gms and 2 gms of thick and thin plastic strips respectively. After a time interval of 2, 4 and 6 months, the plastic strips were collected from the culture, washed thoroughly with double distilled water and air dried. The strips were then weighed to study their final weight (Table 3). The three bacterial species revealed partial degradation of thick and thin plastic strips utilizing them as sole carbon source. On the other hand, *Aspergillus niger* exhibited the formation of a fungal mat and plastic lumps with time, which resulted due to its degradation. Cell surface hydrophobicity of these microorganisms was the leading factor for the formation of biofilm, consequently enhancing the degradation of plastics. The observations manifests that the thin plastics are more biodegradable than the thick plastics. This attributes to the fact that thin plastics are mainly composed of polyethylene as because they are 5-times thinner than the plastics. The organisms attach to the surface of the plastic strips and starts growing using the polymer as their only carbon source, degrading them into low molecular weight monomers and dimers (Vasile. 1993).

The microorganisms isolated were native to the hydrocarbon enriched petroleum refinery area of Purba Mednipur. Amongst the isolated microbes, *Bacillus weihenstephanensis, Burkholderia cepacia*, *Escherichia coli* and *Aspergillus niger* were identified and they exhibited plastic biodegradation in laboratory conditions on synthetic media. After six months of incubation with strips of plastic carry bags, *Bacillus weihenstephanensis* manifested greater potency towards degradation of thick plastics. On the other hand *Burkholderia cepacia* showed better activity against thin plastics. *Aspergillus niger* formed a fungal mat over the plastic strips and revealed a property to engulf them. These properties of microbes can help the present and future microbiologists to have a better insight into production of commercial biodegradable and eco-friendly plastic carry bags.

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