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RESEARCH ARTICLE

Biodegradation of plastic by *Aspergillus* spp. isolated from polythene polluted sites around Chennai

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Abstract

The diversity and load of heterotrophic fungi associated with the polythene degradation in polythene polluted sites around Chennai, Tamil Nadu was isolated and identified by plating and staining technique. Isolated fungal strains were identified as *Aspergillus niger*, *A. japonicus*, *A. terreus A. flavus* and *Mucor* sp. Predominant fungal strains *Aspergillus niger* and *A. japonicus* were selected for polythene degradation under laboratory conditions. Their effectiveness on the degradation of commercial polythene carry bags of low density polyethylene (LDPE) was studied over a period of 4 weeks in shaker culture under laboratory conditions. Biodegradation was measured in terms of mean weight loss, which was nearly 8 to 12% after a period of 4 weeks. Further SEM (Scanning electron microscopy) analysis confirmed the degradation by revealing the presence of porosity and fragility of the fungal degraded polythene surface. *Aspergillus japonicus* showed 12% degradation potential when compared to *A. niger* of 8% degradation in one month period.

Keywords: Polythene, *Aspergillus niger,* low density polyethylene, scanning electron microscopy.

Introduction

Plastic is the most versatile synthetic 'manmade' substance created out of the fossil fuel resources that enable most of the industrial and technological revolutions of the 19th and 20th centuries. During the past 25 years, plastic materials have gained widespread use as they have been increasingly used in food, clothing, shelter, transportation, construction, medical and leisure industries Plastics are composed of petroleum based materials called resins (e.g., polythene and polypropylene) materials that are resistant to biodegradation. Due to this resistance, plastics that are disposed in landfills remain in their original form in perpetuity. Plastics offer a number of advantages over alternative materials– they are lightweight, low cost, extremely durable and relatively unbreakable. Production of plastics has grown significantly in the last 30 years averaging an annual growth rate of 10%. A general estimate of worldwide plastic waste generation is annually about 57 million tons (Shristi Kumar *et al*., 2007). However, plastic materials have several disadvantages, the most important being that they do not break down in the environment. Due to their buoyancy, long term persistence and ubiquity in the marine environment, plastic waste poses a variety of hazards to marine life (Spear *et al*., 1995). In the recent years there has been growing public concern over environmental deterioration associated with the disposal of conventional plastics. Discarded plastics, besides being highly visible are a rapidly increasing percentage of solid waste in landfills, resistant to biodegradation leading to pollution, harmful to the natural environment.

Most of the biodegradation studies on plastics are being carried out using microorganisms. Most of the organic wastes undergo microbial degradation and contribute to the biological productivity either directly or indirectly. Since microorganisms are capable of degrading most of the organic and inorganic materials, there is a lot of interest in the microbial degradation of plastic and polythene waste material. Kambe *et al*. (1995) isolated and characterized a bacterium from soil which utilizes polyester polyurethane as a sole carbon and nitrogen source. Two strains with good polyurethane degrading activity were isolated and identified as *Comamonas acidovorans*. Oda *et al*. (1998) studied polycaprolactone depolymerase produced by the bacterium *Alcaligenes faecalis*and isolated several bacteria capable of degrading polycaprolactone (PCL) from soil and activated sludge. Webb *et al*. (2000) studied the fungal colonization and biodeterioration of plasticized polyvinyl chloride in *in situ* and *ex situ* conditions and suggested that microbial succession may occur during the long periods of exposure in *in situ* conditions. They also identified *Aureobasidium pullulans* which was the principle colonizing fungus and a group of yeast and yeast like fungi, including *Rhodotorula aurantica* and *Kluyveromyces* spp. Incidence of marine and mangrove bacteria accumulating polyhydroxy-alkanoates on the mid west coast of India has been reported by Rawte *et al.* (2002). Microbial degradation of poly (caprolactone) (PCL)-poly vinyl butyral (PUB) blends has been studied by Rohindra *et al.* (2003).

Kathiresan (2003) has revealed that the high diversity of microorganisms in mangrove soil is capable of degrading plastics, although at a slower rate. Low–density polyethylene (LDPE) accounts for 60% of the total plastic production and the most commonly found solid waste is the non-degradable polythene carry bags. The indiscriminate use of polythene shopping bags by the public is increasingly becoming an environmental problem in India. Most of the municipal and garbage sites are littered with large quantity of this highly recalcitrant waste material. Against these backdrops, this study was aimed to estimate the diversity of heterotrophic fungi with special emphasis on determining their ability to degrade the polythene waste.

Materials and methods

Estimation of heterotrophic fungi in the polythene samples: The polythene bags were collected in a sterile plastic box from the polluted areas of Chennai, Tamil Nadu. The samples were serially diluted and pour plated in sterile Potato Dextrose Agar to estimate and isolate heterotrophic fungi respectively. The plates were incubated at 37°C for 48 h. After incubation, plates with 30-300 colonies were chosen for counting and the total plate count for fungi was expressed as number of colony forming units per gram of soil.

Characterization of the heterotrophic fungi

After counting and estimation of total, morphologically different colonies were picked up using sterile needle and forceps and aseptically transferred to sterile PDA agar slants for further characterization. Fungi were chosen for characterization and identified by macroscopic and microscopic observation (staining technique).

Screening and identification of polyethylene degrading fungi: Agar amended with substrates like 1% starch, 1% gelatin, 1% tween-80 served as the suitable medium for the action of enzymes present in culture extracts of fungi. Agar amended with each substrates were separately sterilized at 15 lbs for 15 min. About 5-20 mL of sterilized substrate was poured into sterile Petri-dish. The plates were surface dried over night and the wells were cut aseptically to load the culture filtrates of isolated fungi. The plates loaded with culture filtrates were incubated at 37°C for 3-4 h. After incubation, opacity was observed around the well surface which indicated the positive result for the respective substrates. Further quantitative assay was performed.

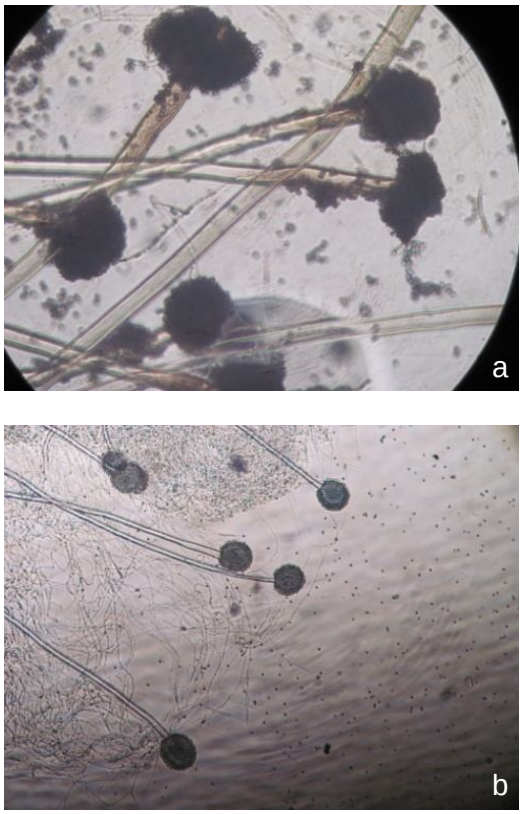
Microbial degradation of plastics: The pre-weighed low density polyethylene (LDPE) strips of 1 cm dia were aseptically transferred to the conical flask containing 50 mL of Rose Bengal broth medium, and separately inoculated with the selected fungal strains. Control was maintained with low density polyethylene (LDPE) strip in the microbe free medium. Four flasks were maintained for each treatment and left in a shaker.

After one month of shaking, the polythene strips were collected, washed thoroughly using distilled water, shade-dried and weighed for final weight. From the data collected, weight loss of the polythene strip was calculated. Further the surface of degraded low density polyethylene (LDPE) was analyzed SEM.

Results

Fungi identified from polluted site were identified as *Aspergillus niger*, *A. japonicus*, *A. terreus, A. flavus* and *Mucor* sp. Two predominant fungi *Aspergillus niger* and *A. japonicus* were selected for further studies (Fig.1). *Aspergillus niger* showed degradation of low density polyethylene up to 5.8% in one month while *A. japonicus* showed more capability to degrade low density polyethylene up to 11.11% in one month under laboratory conditions (Fig. 2). For further confirmation, SEM analysis was done at different magnification. The control polythene strips displayed a normal surface view but the polythene strips treated with *A. niger* and *A. japonicus* showed appreciable surface corrosion, folding and cracks. This may be due to the fungal extracellular metabolites and fungal enzymes (Fig. 3 and 4). Both control and test fungi treated with polythene strips were heated to 0-200°C at the heating rate of 10°C/min. The melting point was reduced in the fungal treated polythene strips (161ºC) when compared to control (162.2ºC) polythene strips (Fig. 5).

Fig. 1. Isolated fungal cultures under microscopy view.



a. *Aspergillus niger*
b. *Aspergillus japonicus*

Fig. 2. Percentage of plastic degradation by the fungal strains.

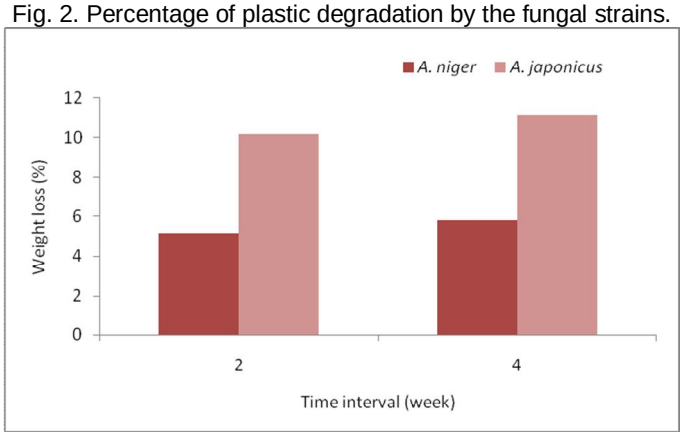
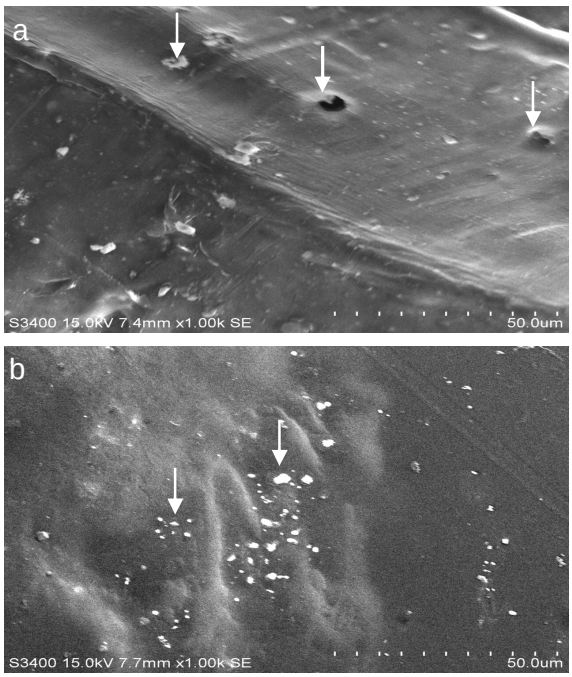


Fig. 3. SEM of polythene film treated with *A. japonicus.*

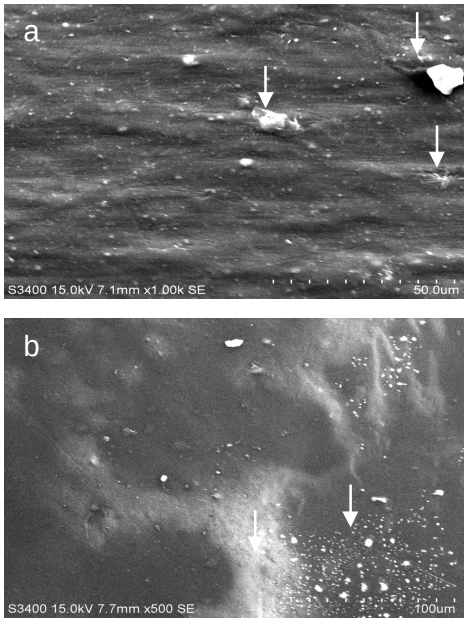


a. Two weeks; b. Four weeks.

Discussion

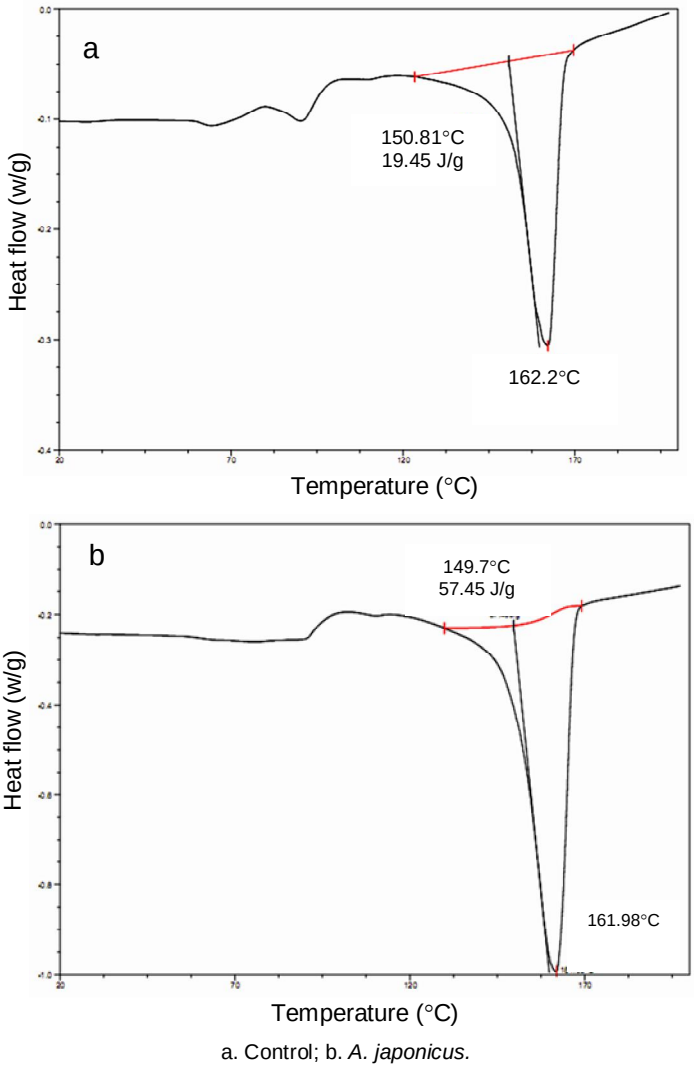
Microorganisms play a significant role in biological decomposition of materials, including synthetic polymers in natural environments. High-density and low-density polyethylenes are the most commonly used synthetic plastics and they are slow in degradability in natural environments, causing serious environmental problems. In this regard, there is a growing interest in non-degradable synthetic polymer biodegradation using effective microorganisms (Lee *et al.,* 1991; Gu, 2003). There is no report on polythene degradation by *Aspergillus japonicus* so far. This is the first experimental report of low density polythene (LDPE) degradation under laboratory conditions by showing effective ability of *A. japonicus.* The potency of degradation by *A. japonicus* is twice than that of *A. niger* i.e., *A. japonicus* degraded 11.11% per month while *A. niger* degraded 5.8% per month.

Fig. 4. SEM of polythene film treated with *A. niger.*



a. Two weeks; b. Four weeks.

Fig. 5. Melting point of *A. japonicus* treated polythene strips.



a. Control; b. *A. japonicus.*

The polythene bags in the soil of polluted sites have been degraded by the presence of fungi besides abiotic factors such as moisture, heat, temperature, etc of the soil (Anonymous, 1999). The mechanism of degradation is not known exactly and the surface of plastic material has turned from smooth to rough with cracking and the molecular weight reduction, increase in carbonyl double bond groups, erosion on the surface of polyethylene is due to the microorganisms (Weiland *et al*., 1995). In the process of depolymerization at least two categories of enzymes are actively involved in biological degradation of polymers: extracellular and intracellular depolymerases (Gu *et al*., 2000). During degradation, exo-enzymes from microorganisms break down complex polymers yielding smaller molecules of short chains, e.g., oligomers, dimers, and monomers, that are smaller enough to pass the semi-permeable outer membranes of the microbes, and then to be utilized as carbon and energy sources (Frazer, 1994; Hamilton *et al.,* 1995). Hence, further study on microbial enzymes or organic acids in degradation of the polythene and plastic will pave way for finding technology for degrading these environmentally hazardous plastic materials.

Conclusion

Fungal strains *Aspergillus niger* and *A. japonicus* were selected for polythene degradation under laboratory conditions. Their effectiveness on the degradation of commercial polythene carry bags of low density polyethylene was studied over a period of 2 and 4 weeks. Biodegradation was measured in terms of mean weight loss, which was nearly 8 to 12% after a period of 4 weeks. Further, SEM analysis confirmed the degradation revealing the presence of porosity and fragility of the fungal degraded polythene surface. *Aspergillus japonicus* showed 12% degradation potential when compared to *A. niger* of 8% degradation in one month period.

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