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Original Research Article

Plastic Degrading ability of *Aspergillus oryzae* isolated from the garbage dumping sites of Thanjavur, India

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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. Plastic and polythene waste accumulating in the environment are posing an ever increasing ecological threat. Municipal solid waste contains high amounts of cellulose, which is an ideal organic waste for the growth of most of microorganism as well as composting by potential microbes.

Keywords

Biodegradation, Polythene, Microbes, Waste, Fungal Biodiversity.

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, polytetraflouroethylene, polyurethane and polyvinyl chloride (Usha *et al*., 2011) are being continuously used in our day-to-day life. Polythene finds a wide range of applications in human s daily use because of its easy processing for various products used for carrying food articles, for packaging textiles, for manufacturing laboratory instruments and automotive components (Arutchelvi *et al.*, 2008).

Polythene constitutes 64% of the total synthetic plastic as it is being used in huge quantity for the manufacture of bottles, carry bags, disposable articles, garbage containers, margarine tubs, milk jugs, and water pipes (Lee *et al.*, 1991). Polythene bags are made of polyethylene. The synthetic polymers are high hydrophobic level and high molecular weight. Annually 500 billion to 1 trillion polythene bags are being used routinely all over the world. The worldwide utility of polyethylene is expanding at a rate of 12%

annum and approximately 140 million tonnes of synthetic polymers are produced worldwide each year (Roy *et al.,* 2008; Vatseldutt and Anbuselvi, 2014). With such huge amount of polyethylene getting accumulated in the environment and their disposal evokes a big ecological issue. To the marine life polythene waste is recognized as a major threat. Sometimes, it could cause intestinal blockage in the fishes, birds and marine mammals (Spear *et al.,* 1995; Secchi and Zarzur, 1999; Denuncio *et al.,* 2011). Due to plastic pollution in the marine environment minimum 267 species are being affected which includes all mammals, sea turtles (86%) and seabirds (44%) (Coe and Rogers, 1997). The death of terrestrial animals such as cow was reported due to consumption of polythene carry bags (Singh, 2005). The widely used packaging plastic (mainly polythene) constitutes about 10% of the total municipal waste generated around the globe (Barnes *et al.,* 2009). Only a fraction of this polythene waste is recycled whereas most of the wastes enter into the landfills and taken hundreds of years to degrade (Lederberg, 2000; Moore, 2008). The use of polythene is increasing every day and its degradation is becoming a great challenge. 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 (Deepika *et al*., 2015). 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. 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 (Kawai, 1995). Consequently, in the present study was intended to evaluate the fungal biodiversity capable of plastic biodegradation from plastic contaminated soil.

Biodegradation of Synthetic Plastics

The degradation of most synthetic plastics in nature is a very slow process that involves environmental factors, which follows the action of wild microorganisms (Albertsson, 1980; Cruz-Pinto et al., 1994; Albertsson et al., 1994). The oxidation or hydrolysis by enzyme to create functional groups that improves the hydrophylicity of polymer is the the primary mechanism for the biodegradation of high molecular weight polymer. Consequently, the main chain of polymer is degraded resulting in polymer of low molecular weight and having feeble mechanical properties, which makes it more accessible for further microbial assimilation (Albertsson and Karlsson, 1990; Albertsson et al., 1987; Huang et al., 1990). Poly(vinyl alcohol), poly(lactic acid), polycaprolactone, and polyamides are some examples of synthetic polymers along with oligomeric structures that biodegrade. The rate of degradation is affected by several physical properties such as crystallinity and orientation and morphological properties such as surface area (Huang et al., 1992).

Sources of the Polythene Degrading Microbes

Following sites were reported to be rich source of polythene degrading microbes:

a. Rhizosphere soil of mangroves.
b. Polythene buried in the soil.

c. Plastic and soil at the dumping sites.

d. Marine water.

Materials and Methods

Estimation of Heterotrophic Fungi

The soil samples were collected in sterile zip lock bags from plastic contaminated places in Thanjavur, Tamil Nadu. The samples were serially diluted and pour plated in sterile Potato Dextrose Agar to estimate and isolate heterotrophic fungi respectively (Kathiresan 2003). 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 by Lacto phenol Cotton blue staining technique.

Among all the soil samples collected Aspergillus oryzae is found to be the dominant fungi and was further tested for their ability for degradation of plastics in laboratory conditions.

Degradation of Plastic Strips by *Aspergillus oryzae*

About 1gm weighed strips were used for the degradation process. The strips were tested for degradation by burying in soil pits. Sterile soil pits were prepared and the strips were placed in layers with soil alternatively. The pits were then enriched with the inocula of A. oryzae in intervals. The strips were allowed to degrade for three months. The degradation by the fungus was analysed by determining the dry weight at an regular interval of 30 days. The plastic strips were collected, washed thoroughly using distilled water, shade-dried and then weighed for final weight. From the data collected, weight loss of the plastics was calculated.

Results and Discussion

The present study deals with the isolation, identification and degradative ability of plastic degrading fungi from soil. Synthetic plastic and soil sample was collected from the garbage dumped soils at Thanjavur was used in this study. This plastic was used to study their biodegradation by fungi isolated from them.

Microbial degradation of a solid polymer like polyethylene requires the formation of a biofilm on the polymer surface to enable the microbes to efficiently utilize the non-soluble substrates by enzymatic degradation activities. Development of multicellular microbial communities known as biofilm, attached to the surface of synthetic wastes have been found to be powerful degrading agents in nature.

When the total biodegradation process of any organic substrate is considered the formation of microbial colony is critical to the initiation of biodegradation. Thus, the duration of the microbial colonization is an important factor that effects total degradation period.

The Fungal species found associated with the degrading materials were identified as *A. Niger, A. Oryzae, A. Japonicus, Penicillium sp.,etc.,* The dominant species foung in all the soil samples was found to be *A.Oryzae* (Fig. 2).

Table.1 Degradation of Plastic by *A. oryzae*

Fungal Strain	Incubation Days	Initial weight (mg)	Final weight (mg)	Difference (mg)	% of Weight Loss
Aspergillus Oryzae	15	50	40	10	20
	30	50	38	12	24
	45	50	37	13	26
	60	50	35	15	30

Fig.1 *Aspergillus oryzae*

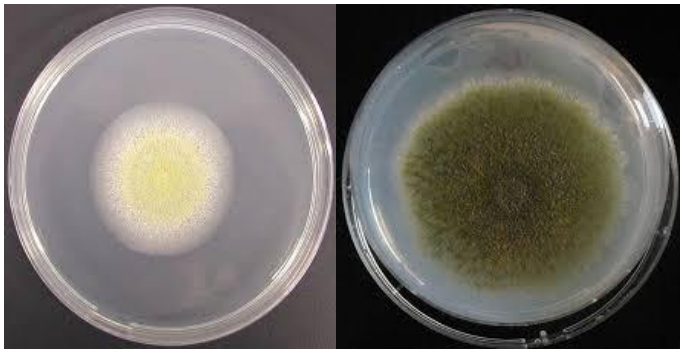
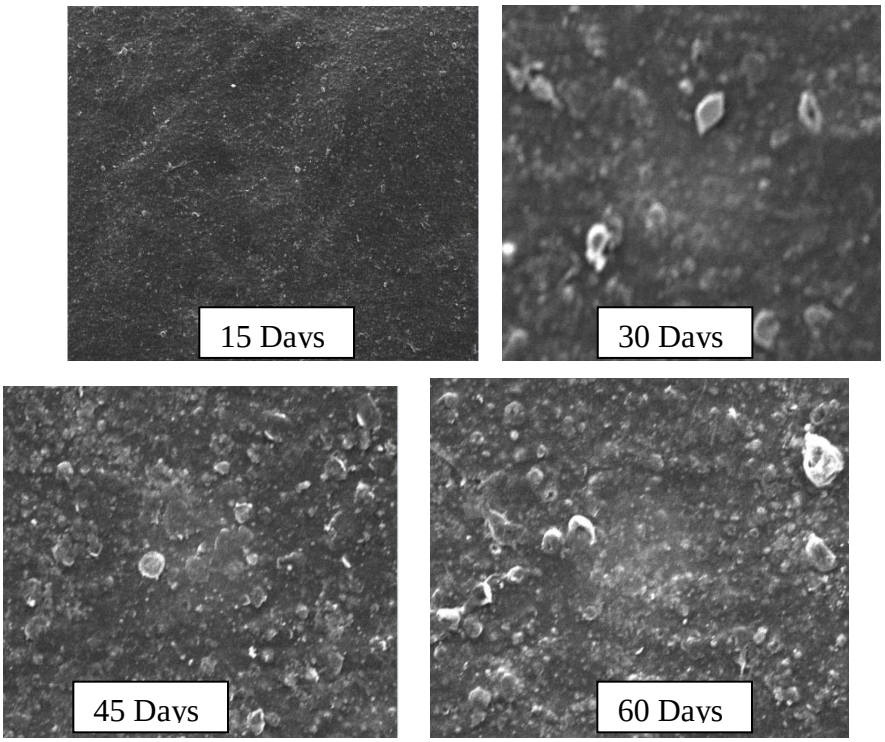


Fig .2 Degradation of Plastic by SEM Analysis



In the present study pieces of plastics were buried in the soil culture medium containing *A.oryzae* isolates and kept for 2 months to observe the percentage of weight loss. The result shows the degradative ability of the microorganisms after two months of incubation. *Aspergillus oryzae* shows considerable degradation of plastic as that of other species of *Aspergillus* (Table 1). This shows it also has the greater potential of degradation compared to other fungal species. The physical properties of the plastic strips were monitored for the morphological changes like microcracks, embrittlement by SEM analysis was given in Fig. 3. Therefore through these results among all the fungal species that are identified to degrade the plastics *A. Oryzae* also involves in effective in Exsitu degradation of plastics.

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