

Screening and Characterization of Isolated Fungi from Plastic Waste Dump Yard Sites



Microbiology

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ABSTRACT

*Disposal of polyethylene wastes leads to the crucial problems in our environment, also it is the major responsibility for global warming. The aim of this study was to investigate the biodegradability of polyethylene wastes inhabiting fungi its characterization identification. The fungal strains were isolated, screened using 2.5X2.5 cm. sq sterile low density polyethylene and fungal individuals were treated in carbon free synthetic media and broth has been carried out. From the isolates the maximum loss of weight in three months exposure were observed in *Aspergillus niger* (34.18 %) was utilized the polyethylene as the sole carbon source and the least value was observed in *Mucor* (6.08 %) considered as soil dwelling fungi.*

Introduction

The accumulation of polyethylene wastes contaminating the soil and water is very common now a days (Eubeler *et al.*, 2010). Low density polyethylene is one of the major sources of environmental pollution. Polyethylene is a polymer made up of long chain monomers of ethylene. The worldwide utility of polyethylene is expanding at a rate of 12 % per annum and approximately 140 million tonnes of synthetic polymers are produced worldwide each year (Shimao, 2001). One of the main problems of polyethylene is that not readily biodegradable, and thus accumulates in environment and being hazardous. Most of the commercial disposable polyethylene films and bags were made by LDPE (Low Density Polyethylene) the main reason is their durability. The denaturation of polyethylene as naturally it takes long times, but biodegradation is the only way to depolymerise the polyethylene and to reduce the accumulations. Microorganisms are involved in the biodegradation of polyethylene, the richness of microorganisms and their catalytic enzymes have able to degrade LDPE. The aim of this study is to ascertain the ability of isolated fungi from municipal landfill polyethylene wastes dump yard sites of various areas and identifications of the characterization. There are nine fungal spp. that were isolated and their potential involvements in the degradation of polyethylene films were revealed. Through this study it is known that the fungi can survive in environments of with low nutrient availability, low or high pH and low moisture as well.

Materials and Methods

Isolation of fungal strain: Samples were collected from the municipal plastic waste landfill sites of various areas in Cuddalore. 5gm of sample of each area was taken, samples were serially diluted and 10^{-4} and 10^{-5} dilution were taken for fungal isolation and inoculated in sterilized RBA medium. The plates were incubated at 27- 30°C for 3 – 5 days, after completion of incubation the colonies were counted and calculated based on CFU/g. Depending on the colony morphology the cultures were isolated and the sub-cultures were maintained for further investigations.

Screening the fungal isolates degradability:

The initial weights of polyethylene films were noted using laboratory digital weighing balance and the films were sterilized. The minimal carbon free media and broth (0.7g-KH₂PO₄, 0.7g-K₂HPO₄, 0.7g-MgSO₄, 1g-NH₄ NO₃, 0.0005g-NaCl, 0.002-FeSO₄, 0.0002g-ZnSO₄ and 0.001g-MnSO₄ for 1000 DW) were prepared, the isolated cultures were swabbed on media plates and the sterile polyethylene films (2.5x2.5cm) were placed over media and also the polyethylene films were mixed with the sterile minimal carbon free broth culture of individual. Culture plates have maintained for incubation at 37°C for three months. The culture

broth has been maintained on shaker for 130 rpm in same duration. After the incubation period, polyethylene films were recovered from the culture plates and broth rinsed by sterile distilled water and 77 % ethanol for removal of mycelia, spores and media particles and air dried then samples were maintained in hot air oven at 60°C for 10 minutes for removal of moisture. Polyethylene films were weighed and measured the loss of weight from initial weight.

Identification of polyethylene degrading fungi

The isolated fungi were identified according to the keys of taxonomy used for fungi (Raper and Fennell 1987). The observed grown mould were sub-cultured onto a fresh RBA plates and incubated at 28±2°C for 4-5 days and an accurate descriptions of the fungal colonial morphology was ascertained. In addition to the macroscopic colony morphology, the rate of growth, the texture, colour and colour changes both surface and reverse sides of colonies and details of structure of spores and conidial heads were observed fungi by using LPCB (lacto phenol cotton blue) slide culture methods (Buchanan and Gibbons, 1986; Barnett and Hunter, 1998; Barnett, 1960; Thom & Raper, 1945).

Results and Discussion

Colony morphology and identification of the fungal isolates

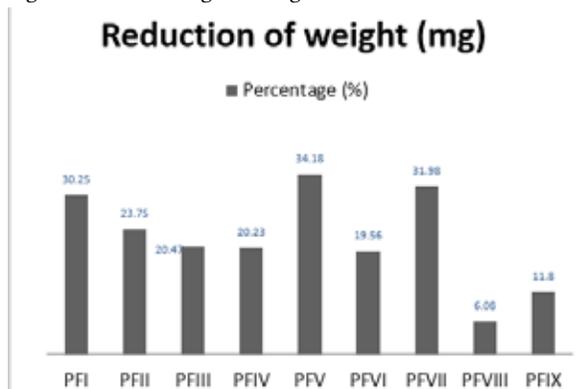
The maximum colonies have recorded in the sample of Cuddalore old town was recorded in 10^5 (1.5). Depending on the colony morphology nine fungal molds were isolated and named PFI, PFII up to PFIIX (PF-polyethylene fungi) and the colony morphology details were shown in table-1.

Table 1: Macroscopic identification of fungi

S. No	Fungal isolates	Colony morphology
1	PFI	When immature they are covered white fluffy aerial mycelia, mature colonies show salt and pepper effect, they are covered with black spores and hyphae are produced.
2	PFII	Rapidly growing colonies, Velvety in texture and yellowish green in color.
3	PFIII	The colonies of strains that produce predominantly conidia are green in color. Grow well at 37°C.
4	PFIV	Mature colonies have distinct margins they show blue – green, surface is granular, hyphae were produced.
5	PFV	Growth is slow to moderate, maturing in about 7 to 21 days. Colony size expands rather slowly. Colony colouration is media dependent but is described as a dull to deep green to a greyish turquoise, with yellow produced. They grew well at ambient room temperature (20°C).

6	PFVI	Initially white and fluffy, latter produce pigmented spores and turn into shades of green.
7	PFVII	Colonies are fast growing (reach 7cm in 10 days) as the observed and reverse color of the colonies has been white and cream to orange
8	PFVIII	Colonies grow rapidly in 1-2 days with high mycelium, do not produced rhizoides, colour may vary from brown to grey.
9	PFIX	Appear glassy with minute speck of black conidia, produced wheat straw, dark gray colonies with gray aerial mycelium.

Figure-1: Loss of weight on degraded LDPE films



Screening of isolates:

Among the nine fungal strains, maximum weight loss observed in PFV 34.18 % of the loss of weight was recorded, followed by PFVII – 31.98 % and PFI- 30.25 % showed maximum weight reduction, whereas the minimum degradation in the present study was recorded by PFVIII (6.08 %) were recorded within 3 months, each experimental results has been shown in Figure-1

Table-2: Microscopic identifications polyethylene degrading fungi

S.No	Isolates	Microscopic examination (LPCB)	Identified as
1	PFV	Conidiophore strips smooth walled, hyaline are pigmented. Vesicles sub-spherical, conidial heads radiate. Conidiogenous cells biseriolate. Medulla twice as long as the phialides. Conidia brown, ornamented with warts and ridges. Hyphae were septate.	<i>Aspergillus niger</i>
2	PFI	Conidiophore strips rough walled, hyaline vesicles spherical, conidial heads radiated unit and biseriolate. spherical or sub spherical, sclerotic may be present. Hyphae were septate.	<i>Aspergillus flavus</i>
3	PFVII	conidiophore are short, single, lateral monophialides in the aerial mycelium, later arranged in densely branched clustur, microconodia, septa fusiform, and pointed at the tip.	<i>Fusarium sp</i>

Out of nine fungal samples, five were identified as *Aspergillus* spp. (PFI-*Aspergillus niger*, PFII-*Aspergillus flavus*, PFIII-*Aspergillus nidulans*, PFIV-*Aspergillus fumigatus* PFV-*Aspergillus glaucus*), other PFVI- *Penicillium* sp., PFVII- *Fusarium* sp., PFVIII-*Mucor* sp., and PFIIX-*Alternaria* sp., were identified by having the microscopic examination under lacto phenol cotton blue, the degradability of polyethylene fungi results were mentioned in table-2.

Jyoti Singh and Gupta (2014) conducted the isolated fungi from polyethylene polluted site and screened for LDPE degradation under laboratory conditions. Fungal strain *Aspergillus japonicus* (36%), *Fusarium* sp (32 %), *Aspergillus flavus* (30 %) showed effective degradation results in 4 weeks as compared to *Penicillium* sp (24 %), *Aspergillus niger* (20 %), *Mucor* sp (16 %). Raaman *et al* (2012) focused the degradation potential of *Aspergillus japonicus* and noticed the degradation rate as 12 % but this rate that only 8 % by *A. niger* in one month. Kathiresan (2003) reported the *Aspergillus glaucus* and *A. niger*, as potent polythene degrading fungal sps. The *Aspergillus glaucus* was more efficient biodegrading agent in comparison to the *A. niger*. The rates were 28.8 % and 7.26 % respectively after one month of exposure.

Conclusion

The present study gives the evidences for biodegradation of low density polyethylene by fungi. Here nine fungal strains were isolated from the municipal polyethylene wastes dump yard sites were screened by their biodegradability and identified the strains. The three fungal strains (*Aspergillus niger*, *Fusarium* sp and *Aspergillus flavus* were capable to adhere on the surface of LDPE film and to grow in the minimal carbon free broth and medium supplemented with LDPE as they utilized it as a sole carbon and energy source. Among the three isolates *Aspergillus niger* have great ability to degrade LDPE and other polyethylene films followed by two used for the further biodegradation analytical studies.

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