



Isolation and identification of LDPE degrading fungi from municipal solid waste

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ABSTRACT

Plastic causes serious pollution to the environment, both during its production and disposal. In-vitro biodegradation of plastic waste through microbial strains could offer a solution to this problem because will be compelled to take the polymer as carbon source and possibly because of their diverse metabolic capability, adaptability to different adverse environment it will be able to degrade it in due course of time. In the present investigation, potential fungal strains to degrade LDPE were isolated and assessed for its capability in-vitro. Eight fungal strains namely FSM-1, FSM-3, FSM-4, FSM-5, FSM-6, FSM-8, FSM-9, FSM-10 were isolated and identified. Among these six were *Aspergillus sp.* and two were *Fusarium sp.* This study was showed the microscopic analysis for its ability to adhere and grow on hydrophobic surface of LDPE film. The active enzymes produced by this fungal strain were responsible for the biodegradation.

Keywords: Solid waste; fungal strain; LDPE; biodegradation.

INTRODUCTION

Plastic materials are strong, light-weight and durable and thus are widely used in food, clothing, shelter, transportation, construction, medical and recreation industries [1]. A very general estimate of worldwide plastic generation is annually about 57 million tons [2]. Because of its xenobiotic origin and recalcitrant nature, its biodegradation is problematic and thus accumulates in the environment for a long time. Plastics include polythene, propylene, polystyrene, polyurethane, nylon etc. polyethylene either LDPE (low density polyethylene) or HDPE (high density polyethylene) is a thermoplastic made by monomers of ethylene, used primarily as thin films and packaging sheets [3]. Among these LDPE materials are strong, light-weight and durable thus are having wide uses.

The biodegradable polymers are designed to degrade it fast using microbes since microorganisms are capable degrading most of the organic and inorganic materials, including lignin, starch, cellulose and hemicelluloses [4], there is lot of interest in the microbial degradation of polyethylene waste material [5]. Biodegradation resulting from the utilization of polyethylene as nutrient may be more efficient if the degrading microorganism forms a biofilm on the polyethylene surface [6]. The microbial species are associated with the degrading materials were identified as bacteria (*Pseudomonas*, *Streptococcus*, *Staphylococcus*, *Micrococcus*, *Moraxella*), fungi (*Aspergillus niger*, *Aspergillus glaucus*), *Actinomycetes sp.* and *Saccharomonospora* genus [7]. Specifically some white rod fungi also can degrade toxic compounds by the secretion of extracellular enzymes [8]. Microbial degradation of plastic caused by oxidation or hydrolysis using microbial enzymes that lead to chain cleavage of the high molecular weight polymer into low molecular weight oligomer and monomer by aerobic or anaerobic metabolism.

The aim of this study was to isolate the efficient fungal strain from municipal solid waste, screening and identifying the high potential fungi that degrade the LDPE.

EXPERIMENTAL SECTION

Preparation of LDPE powder

Low density polyethylene glycol (LDPE) was obtained from B.N. Polymers, Bangalore, India. LDPE films were cut into small pieces, immersed into xylene and boiled for 15 min followed by crushing with blender at 3000rpm. The obtained powder of LDPE was later washed with ethanol and dried in hot air oven at 60⁰C for overnight to get dry powder, stored at room temperature for further use.

Collection of sample

Municipal solid waste is the rich source of plastic sample as it contains domestic waste, other waste. The soil samples were collected from the municipal solid waste landfill area, Pallikaranai, Chennai, India at a depth with of 2-3 cm in a sterile container and air dried at room temperature.

Isolation and screening of LDPE degrading fungi

One gm of soil sample was suspended in 99 ml of sterile distilled water. This suspension was incubated for 10 min and subjected for serial dilution. To isolate the LDPE degrading fungi, the plating was done through spread plate technique using 10⁻⁶ dilution on synthetic medium agar plates containing 0.1% LDPE powder. The composition of medium was as follows: (g/l: K₂HPO₄ 1, KH₂PO₄ 0.2, (NH₄)₂SO₄ 1, MgSO₄ 7H₂O 0.5, NaCl 1, FeSO₄.7H₂O 0.01, CaCl₂ 2H₂O 0.002, MnSO₄ H₂O 0.001, CuSO₄ 5H₂O 0.001, ZnSO₄ 7H₂O 0.001 Agar 15) with pH 7.0. The plates were incubated at 37⁰C for 7 d. The pure fungal mats were isolated and subcultured on Saboraud's dextrose agar and then preserved in slant at 4⁰C.

Identification of fungal strain

Fungal strains were identified by using standard identification techniques such as Lactophenol cotton blue (LPCB) staining, colony morphology and microscopic examination. Structure of hyphae and conidiophores were observed microscopically.

Measurement of biodegradation

The measurement of biodegradation of the fungi on LDPE film was studied by growing the fungi in petriplates. Synthetic medium was aseptically poured into petriplates. LDPE sheets were cut into small pieces 1cm× 1cm of similar weight, disinfected with 70% ethanol for 30 min and transferred to sterile distilled water for 20 min. Nine LDPE sheets were placed on those plates and they were inoculated with eight identified fungal mat, one plate was kept as control without fungal sample. The petriplates were incubated at 37⁰C and results were observed after 30 d [9].

RESULTS AND DISCUSSION

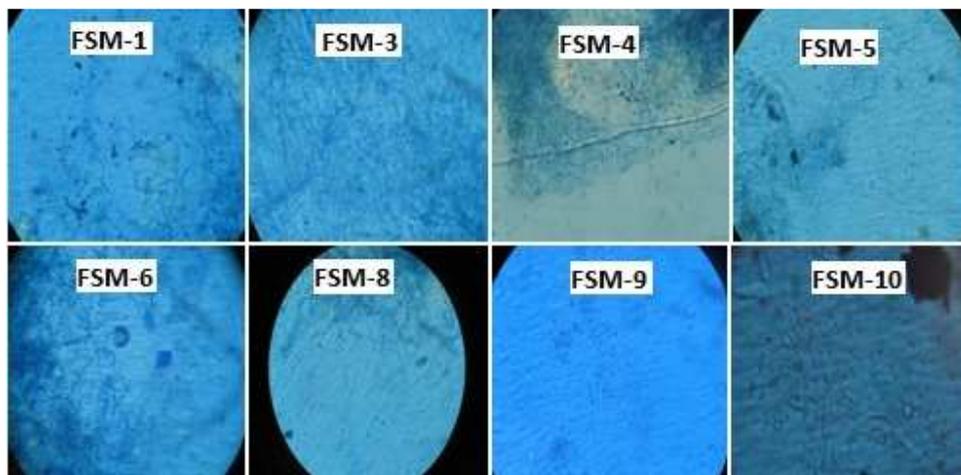
This research work deals with the isolation and identification of polythene degrading fungi from the municipal solid waste. For this study low density polyethylene was used as powder form and as film. The synthetic medium was prepared with 0.1% of LDPE and the eight fungal strains were grown on it. These observations indicate that these fungal samples utilized LDPE as an only carbon and energy source resulting partial degradation of plastics. In addition, the pure fungi were also able to attach with LDPE film forming a biofilm. Cell surface hydrophobicity of the fungi was found to be an important factor in the formation of biofilm on polyethylene surface, which consequently enhanced biodegradation of polymers [10].

On the basis of microscopic examination and morphological characteristics the fungal samples were identified (Table1). Out of eight fungal samples, six were *Aspergillus* sp. and two were *Fusarium* sp. The fungi FSM-1 and FSM-9 were having long, septate hyphae. Both FSM-3 and FSM-6 were found with septate, branched hyphae and but spores shapes were different. For FSM-5, the shape of hyphae was very thin, branched, fingers with spore and for FSM-8 was Long and short, irregular. In case of FSM4 was with septate unbranched hyphae whereas FSM-10 was containing very thin branched hyphae. Fungal isolate FSM1, FSM-4, FSM-5, FSM-6 and FSM-8 was having ascospores. In case of FSM-9 and FSM-10 were both containing chlamydospores.

Table 1. Identification of LDPE degrading fungal isolates

Fungal Isolate	Identified fungi
FSM-1	<i>Aspergillus niger</i>
FSM-3	<i>Aspergillus</i> sp.
FSM-4	<i>Aspergillus</i> sp.
FSM-5	<i>Aspergillus</i> sp.
FSM-6	<i>Aspergillus</i> sp.
FSM-8	<i>Aspergillus</i> sp.
FSM-9	<i>Fusarium</i> sp.
FSM-10	<i>Fusarium</i> sp.

The eight fungal samples and the measurement of degradation were identified under light microscope. The extensive network of hyphae was observed on the LDPE film surface after the incubation of 30 d (Figure 1).

**Figure 1. Microscopic view of the attachment of fungi on LDPE film**

The microscopic observation revealed that the fungal attachment on the surface of plastic, indicating the utilization of plastic as nutrient and energy source.

CONCLUSION

The present study gives the evidences for biodegradation of plastics. Here eight fungal strains were isolated from the solid waste and were identified. All these fungal strains were capable to adhere on the surface of LDPE film and to grow in the synthetic medium supplemented with 0.1% LDPE as they utilized it as a sole carbon and energy source. Further study is required to get more supportive data for this biodegradation.

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