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Short communication

Biodegradation of gamma irradiated low density polyethylene and polypropylene by endophytic fungi

Sana Sheik [a], K.R. Chandrashekar [a, b, *], K. Swaroop [b], H.M. Somashekarappa [b]

[a] Department of Applied Botany, Mangalore University, Mangalagangothri, 574199, Mangalore, Karnataka, India
[b] Centre for Application of Radioisotopes and RadiationTechnology (CARRT), Mangalore University, Mangalagangothri, 574199, Karnataka, India

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ABSTRACT

Biodegradation of plastic waste through fungal strains offer a solution to serious issues of pollution as fungi are known to release plastic degrading enzymes. In the present study, the isolated endophytic fungi from two endemic plants, *Psychotria flavida* and *Humboldtia brunonis* which produced laccase enzymes and grew profusely over hydrophobic surface of plastic films were tested for biodegrading ability. The fungi were inoculated on the polythene and polypropylene films irradiated with different doses of radiation, (0–1000 kGy for Low Density Polyethylene and 0–100 kGy for Polypropylene) and incubated for 90 days. The extent of biodegradation pattern of endophytic fungi was measured for the highest dose mainly by analyzing changes using FTIR spectroscopy, DSC, SEM, alteration in viscosity and thereby average molecular weight. The decrease in intrinsic viscosity and average molecular weight of gamma irradiated LDPE strips inoculated with *Aspergillus* sp., *Paecilomyces lilacinus* from *H. brunonis* and *Lasiodiplodia theobromae* from *Psychotria flavida* indicate fungal efficiency in plastic degradation. Only *L. theobromae* from *P. flavida* could degrade irradiated polypropylene film with 0.3 mg on actual weight loss basis. Further work on employing these endophytic fungi in biodegradation of plastics is warranted.

1. Introduction

Much of the nature remains to be explored, particularly microbial environments (Newman et al., 2003). One such unexplored and less studied microorganism is the endophytic fungi, those microbes that reside in internal tissues of living plants without causing any immediate overt negative effects or external symptoms (Aly et al., 2011). The enormous importance of studies on endophytic system is the ability to produce metabolites and innovation of microbial source which are valuable in bioremediation (Stępniewska and Kuzniar, 2013). The huge variety of the metabolic pathways employed by endophytes makes them valuable tools for bioremediation of pollutants and biotransformation of organic substances (Gai et al., 2009; Kim et al., 2012).

Semicrystalline low density polyethylene (LDPE) and polypropylene (PP) of extremely recalcitrant, high hydrophobic nature are major persistant plastics dumped in the environment. Polyethylene is a polymer consisting of long chains of the monomer ethylene (IUPAC name ethene). Polypropylene (IUPAC name polypropene), are synthesized propene monomers which are typically obtained from oil refinery as gaseous products. Nowadays, a wide variety of petroleum-based synthetic polymers are produced worldwide to the extent of approximately 140 million tons per year and remarkable amounts of these polymers are introduced in the ecosystem as industrial waste products (Shimao, 2001). Biodegradation is governed by different factors that include polymer characteristics, type of organism, and nature of pretreatment. The polymer characteristics such as its mobility, tacticity, crystallinity, molecular weight, the type of functional groups and substituents present in its structure, and plasticizers or additives added to the polymer all play an important role in its degradation (Artham and Doble, 2008; Gu et al., 1998; Gu et al. 2000b, 2011; Gu, 2003a,b).

The prerequisite condition for biodegradation is that the microorganism should be able to use the polymer as its sole source of carbon. LDPE, an extremely high molecular weight large sized polymer molecule is made up of methylene which microorganism is unable to transport directly into the cells. . In the current study, inorder to boost biodegradation of plastics, films were pretreated with gamma radiation to induce photo oxidation. Photo oxidation

* Corresponding author. Department of Applied Botany, Mangalore University, Mangalagangothri, 574199, Mangalore, Karnataka, India.
E-mail address: profkrchandrashekar@gmail.com (K.R. Chandrashekar).

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enhances the rate of biodegradation of polymer that results in the generation of large surface area due to its embrittlement and also produces a greater degree of hydrophilicity due to the introduction of carbonyl groups (Arkatkar et al., 2009). The biodegradation always follow photodegradation and chemical degradation (Shah et al., 2008). The initial breakdown of a polymer can result from a variety of physical and biological forces (Swift, 1997). Biodegradability of large molecules such as cellulose acetate and electronic insulation polyimides were studied previously by many workers (Gross et al., 1995; Gu et al., 1996a) A comprehensive study of polyolefin biodegradation has shown that some microorganisms could utilize polyolefins with low molecular weight (Yamada Onodera et al., 2001).

Realizing the capability of microorganisms to produce diverse bioactive molecules and the existence of unexplored microbial diversity, research is underway to isolate and screen microbes of diverse habitat and unique environment for discovery of novel metabolites (Mohanta et al., 2007). The unique biological niche of endophytes as endosymbionts of tissues rich in complex carbon polymers justifies the investigation of their wider metabolic capabilities (Russell et al., 2011). Plants that are endemic are also more likely to lodge endophytes with active natural products than other plants (Strobel and Daisy, 2003). The anticipated topic is to unlock the poorly investigated trapped microorganism of novel metabolic capabilities for polymer degradation inside two endemic plants viz. *Humboldtia brunonis* Wall. and *Psychotria flavida* Talbot. of Western Ghats, India.

2. Materials and methods

2.1. Isolation and selection of endophytic fungi for biodegradation

Healthy, disease free, mature leaf and stem samples of *P. flavida* Talbot and *H. brunonis* Wall collected from Western Ghats were transferred to sterile bags and processed within 24 h. Samples were surface sterilized, excised into small pieces (0.5 cm size), plated in potato dextrose agar medium for more than 10 days, and examined for endophytic fungal emergence. The emerging fungal mycelium was sub cultured in potato dextrose agar for 7 days or till sporulation. The spores along with the fruiting bodies were used for identification. Few of the fungi were deposited and morphologically identified by Agharkar Research Institute, Pune. Few of the representative morphotypes and non sporulating fungi of each leaf and stem samples were identified purely on molecular basis. DNA was extracted using CTAB method (HiPurA TMPlant DNA isolation kit-HIMEDIA). PCR was conducted to amplify the internal transcribed spacer (ITS) region of the extracted DNA, using the primers ITS 1 and ITS 4 (White et al., 1990) under the following conditions: 95 °C for 3 min followed by 35 cycles of 95 °C for 30 s, 50 °C for 45 s, 72 °C for 90 s, and final extension at 72 °C for 10 min. PCR amplicons were electrophorized in 1.2% agarose gel. The amplified PCR products were sequenced by BIOSERVE (Hyderabad) using ABI 3130 (48 capillary) or 3730Xl (96 capillary) electrophoresis instrument. A BLAST (Basic Local Alignment Search Tools) was used to search for closest match sequences in the GenBank database and the sequences were submitted to gene bank.

Among the consortia of endophytes isolated from two plant species, six fungi were selected on the basis of their ability: i) To produce laccase enzyme which was confirmed by the oxidation of colorless 1-naphthol amended medium to bluish violet color (Hankin and Anagnostakis, 1975) ii) To grow profusely forming biofilm over the control plastic films incubated for a month.

Estimation of Laccase: The Laccase activity was assayed at room temperature using 10 mM Guaiacol in 100 mM sodium acetate buffer (pH 5.0). For this, 1 ml of Guaiacol was mixed with 1 ml enzyme in the presence of 3 ml acetate buffer. The change in the absorbance of the reaction mixture containing guaiacol was monitored at 470 nm for 10 min of incubation using Spectrophotometer. A control test was conducted in parallel with absence of enzyme source. Enzyme activity is measured in U/ml which is defined as the amount of enzyme catalyzing the production of 1 μmol of colored product per min per ml.

2.2. Inoculation of endophytic fungi to gamma irradiated LDPE and PP films

LDPE (Thickness – 20 μm, density – 0.93 g/cm^2, melting point – 114 °C) and PP films (Thickness – 20 μm, density – 0.91 g/cm^2, melting point – 160 °C) were purchased from Janani Plastic industry, a commercial manufacturing unit, Baikampady, Mangalore, India. The films were cut into strips and irradiated at different doses of gamma radiation ranging from 0 to 1000 kGy for LDPE. LDPE films were not subjected to gamma radiation for more than 1000 kGy as films were slightly brittle and further inoculation with higher doses would cleave the films resulting in inaccurate weight loss measurements. In case of PP, strips were subjected to 0–100 kGy doses as the doses beyond 100 kGy lead to pyrolysis in these films. Individual pre weighed strips were surface sterilized using70% alcohol and aseptically transferred to the conical flasks containing 50 ml of Rose Bengal broth medium, to which fungal strains were inoculated separately. Unirradiated LDPE and PP inoculated with/without fungi served as control. The flasks were maintained for 90 days under aseptic conditions. Subsequently, strips were washed thoroughly in distilled water, shade dried and weighed for final weight. From the data collected, weight loss of the plastic film was calculated.

2.3. Characterization and analysis

Samples were characterized for changes in their viscosity, spectra, morphology and thermal behavior with reference to control films.

Average Molecular Weight Determination: Average molecular weight was determined by viscometric measurements using an Ostwald Viscometer (Gadag and Shetty, 2006). The intrinsic viscosity was determined by dissolving the samples in 20 ml of p-xylene for LDPE and 20 ml toluene for PP, followed by heating upto 80 °C. Inorder to find out viscosity, flow time measurements for various solvents (Xylene for LDPE, Toluene for PP) and polymer solvents system were measured using the following equation,

$$\eta_2 = \frac{\eta_1 \rho_2 t_2}{\rho_1 t_1}$$

where, t_1 and t_2 are the time of flow of the water and xylene/toluene and ρ_1 and ρ_2 are the respective densities and η_1 is the coefficient of viscosity of water. The average molecular weight (M) was determined following Mark–Houwink equation

$$\eta = KM^{\alpha}$$

where K and α are constants for a given polymer–solvent–temperature system (K = 0.000218, α = 0.725 for polypropylene – toluene and K = 0.000510, α = 0.725 for LDPE – xylene system).

Spectral analysis: The spectrum was taken from 400 to 4000 wavenumbers cm^{-1} using FTIR spectrophotometer (IR Prestige – 21 SHIMADZU).

Surface Morphology: Determinations of surfacial biofilm formation and degradation characteristics were observed using