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An approach to Low density polyethylene (LDPE) biodegradation by *Xylaria* sp. from termite garden

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Abstract
Plastics are the most commonly used polymers for many applications. Plastic wastes accumulating the environment are causing ecological threat. Biodegradation of these plastic wastes using effective microbial strains could provide a solution to the problem. The plastic degrading fungi was isolated from fungal garden of termite ecosystem. The isolates were screened for effective plastic degradation by enrichment method and the best isolates were identified as (*Xylaria* sp.) ascomycetes and endosymbiotic fungi. The characterization based on the 18S rRNA gene sequencing revealed that the isolated culture belonged to *Xylaria* sp. their effectiveness in degrading low density polyethylene (LDPE) was studied over a period of six weeks.

Keywords: biodegradation- low density polyethylene – *Xylaria*- enzymes

Introduction
Plastic is one of the important and indispensable material in the present world because of their flexibility, toughness, strong excellent barrier, light weight and ease of fabrication. Now a days plastic uses increasing in all kind of business, it plays an important role in packaging of food materials, medicine and garbage bags and used as a wrapping materials, sheathing materials and stationery materials etc. But this plastic waste can't be degraded in soil is becoming a great threat and cause of environmental pollution [14]. Increased consumption of plastic can become wastes and can contaminate the soil environment because of its low degrading nature [6]. According to the municipal administrators carry bags are the main cause of blocked drains and thus municipal wastes cannot be incinerated leading to accumulated gargage, sludge, junk. They release toxic chemicals which contaminate food items. A very general estimate of worldwide plastic waste generation is annually about 57 million tons [4].
In which low density polyethylene (LDPE) is one of the major sources of environmental pollution. Because the LDPE is a polymer made up of repeating units of ethylene monomers. The worldwide utility of polyethylene was expanding at a rate of 12% per annum [17]. Microorganisms such as bacteria, fungi and actinomycetes are a widely distributed groups in ecosystem, play a significant role by degrading both natural and synthetic plastics [9]. Biodegradation is a process which includes microorganisms that can have ability to degrade the polythene. The microbial enzymes increase the rate of degradation of plastics without causing any harm to the environment [3].
The microbial degradation of plastic is carried out by enzymatic activities which lead to the breakdown of polymer into monomers and oligomers and metabolized by microbial cells. Aerobic metabolism leads to the production of carbon dioxide and water [19] and the contrary anaerobic metabolism production of carbon dioxide, water and methane as the end products [10]. So, here we have assessed the different environmental factors which were used for increasing the activity of plastic degrading fungi, *Xylaria* sp. Among the microorganisms, *Xylaria* sp. has been found to be the best in degrading the plastic sheets. Under optimum conditions the fungus isolate was able to grow in mineral salt medium with 0.5 per cent glucose and using plastic strips as co-carbon source. The plastic in the medium decreased in the weight, indicate that fungus was utilize the plastic as a carbon sources.

Materials and Methods
Collection of sample and isolation of plastic degrading microorganism
The collected termite comb was incubated at room temperature (25±2ºC). After four days of incubation, the termite comb develops numerous stromata spikes. The elongated stromata were carefully picked up and washed with sterile water and placed in paper bags after removal of piece of 2×2 mm of the fruiting body was aseptically transferred to the Malt

Extract Agar medium (MEA) aseptically and incubated for 10 days at room temperature.

Pre-treatment of LDPE plastic

A set of three different pre-treatment (heat, UV and chemical treatment) was given to LDPE pieces (2x2cm). The plastic are cut in to small pieces (2x2 cm) with the weight of 500mg they were put into the petridish were exposed to heat treatment at 70°C hot air oven, for a period of 20 days. The heat treated polyethylene were taken and kept under the UV light for 1 to 2hr [18] After the UV treatment, the plastic strips were suspended in 10 ml of concentrated nitric acid to enhance per cent elongation kept for 10 days. To enhance the biodegradation of polyethylene, pre-treatment strategies were carried out [11].

Screening of polythene and plastic degrading microorganisms by clear zone method

Polythene and plastic powder were added in mineral salt medium at a final concentration of 0.1% (w/v) respectively and the mixtures were sonicated for 1 hour at 120 rpm in shaker. After sonication, the medium were sterilized at 121°C and 15 lbs pressure for 20 minutes. About 15 ml of sterilized medium was poured before cooling in each plate. The isolated organisms were inoculated on LDPE polymer containing agar plates and then incubated at 22-30°C for 2-4 weeks. The organisms producing zone of clearance around the colonies were selected for further analysis [5].

Characterization and identification of *Xylaria* sp.

Each isolates is examined on the basis of the morphological characters with host specificity and microscopically characters of perithecia, asci and ascopores. The macro fungus was identified by micro and macroscopic observation. Molecular characterization was done by 18S rRNA gene sequence comparisons [1]. The *Xylaria* fungi were identified by infer the phylogeny of *Xylaria* and fungus garden of several fungus-growing termite species by using their ITS1-5.8S-ITS2 region sequences. The phylogenetic results were used as the basis for host specificity and distribution of termite-associated *Xylaria* species.

Results and Discussion

The disposal of plastic by land-filling, incineration and recycling is an inefficient method of plastic waste management and hence there is growing concern for the use of efficient microorganisms used in biodegradation of plastics. The present investigation was performed to provide the information deals with the isolation, identification and degradative ability of *Xylaria* fungi isolated from termite comb which degrade the LDPE plastic. About twenty isolates and their mycelium from each comb were individually collected from five different sampling sites of Tamilnadu (Aanaikatti, Pudhukottai, Maangarai, Thasanur and Thanjavur) having different morphological characters. After 4 days of incubation the vegetative growth was observed over the comb was excised and cultured in malt extract medium. Isolates were purified and designated as (Xtc_1-Xtc_{20}) and their respective isolate numbers as represented in Table 1.

The morphological characters of isolates were characterized based on perithecial, ascocarps and stromata. Among the twenty isolates, Xtc_1 is an effective plastic degrader. The most common type of fungi known to be associated with termites is the *Termitomyces* and *Xylaria* [15]. The genus *Xylaria* is a fungus with mostly upright clavet of strap like stromata collected from the termite comb of the fungal garden and used in this study. Stromata of the ascomycetous genus *Xylaria* sp was isolated from fungus combs [2].

Micro and Macro morphological characterization

Colony morphology of the genus *Xylaria* showed mostly upright, clavet or strap like stromata. The stromata was cylindrical, unbranched or infrequently branched up and 5 cm long by 1 mm dia at stipe. The mycelia on the culture were aggregated to elevated forms. Colonies were initially white in colour and later turned blackish at the tips due to production of conidia. Cylindrical stromata arising from the centre of colonies had tapering upward aggregation and their stromata are cylindrical, branched or unbranched, up to 5 cm long and 1 mm dim (Fig.1). The microscopic observation of the isolate *Xylaria* showed conidiospores and hypha [16]. The hyphae with many septa were multi-branched. Conidiophores were upright, dichotomously branched several times from base and smooth hyaline (Fig.2).

Pre-treatment of LDPE plastic

Physio-chemically treated polythene films were found to be effectively degraded by the fungal isolates than untreated films. Three different pre-treatment strategies were employed for the present study. Thermal treatment and UV treatment followed by chemical treatment were given to the plastic for 30 days of incubation. The pre-treated sample as the sole carbon source for isolation of LDPE degrading strain and proved that enhancement in the biodegradation process was due to pre-treatment [12]. The treated (heat, UV, chemical) LDPE plastic was found to be effectively degraded by the *Xylaria* isolate (Xtc_1) than the untreated LDPE films (20 micron). The physicochemical treatment of polythene leads to its oxidation and subsequent breakdown of the polymer chain and hence the easy assimilation by the *Xylaria* isolates.

Screening of *Xylaria* sp. isolated for biodegradation of LDPE

Among the twenty isolates five were selected for further study by using clearing zone method. *Xylaria* capable for degrading LDPE displayed a zone of clearance around the growing culture (Fig.3). The eight fungal strain (*A.alternata, A. flavus, A. Niger, C. globosum, F. moniliforme, F. solani, P. funiculosm* and *P. chrysosporium*) was identified and out of which only four fungal species showed clear zones [13]. The clear zones varied in clarity and initial appearance, where *A. Niger* showed maximum clearing zone having ability to degrading starch based plastic. The 144 isolates were selected for screening their growth ability in the mineral medium supplemented with 2% LDPE powder, out of which only 5 isolates had the ability to grow in the medium and produce the clearing zone [8]. The formation of a clear halo around the colony indicates the organisms were able to depolymerize the polymer, which is the first step of biodegradation.

Molecular characterization and identification of the *Xylaria* sp.

Xylariaceae is a large family of 40 genera and although it has representatives in most countries of the world the xylariaceae exhibits its greatest diversity in the tropics (Laessoe 1994, Ju and Rogers 1996). However based on the morphological and sequence analysis, the isolates are found to be different species of *Xylaria*, hence they named as *Xylaria* sp. Molecular characterization was performed for Xtc_1 isolates based on 18s rRNA gene sequencing. 18S rRNA gene

sequencing revealed that the Xtc_1 isolates belong to *Xylaria* sp. phylogenetic analysis revealed that Xtc_1 showed 97% similarity with species of *Xylaria* (Fig.4) *Xylaria furcata* has highly branched stromata and long tapering acute apices, as well as highly evident perithecial elevations in most collections [7].

Conclusion

Biodegradation is one of the best, low cost, efficient and eco-friendly. Degradation treatments capable of reducing and even eliminating plastics are of great environmental interest. Among biological agents, microbial enzymes are one of the most powerful tools for the biodegradation of plastics. There is a huge demand in exploring these microbes which can grow in different conditions and, under specific stress conditions, may be directed to grow and use the plastic carbon polymers as energy source. The current research indicates that *Xylaria* (fungi) is capable of degrading plastic. They are cable of producing different enzymes which is degrading the plastic structures.

Table 1: Isolates obtained from termite mound collected from different place of Tamil Nadu.

Place of collection	Isolates	Colony characters
Aanaikatti	Xtc_1	White stromata surface
	Xtc_2	Short cluster stromata
	Xtc_3	Partial branched stromata
	Xtc_4	Highly branched stromata
Pudukottai	Xtc_5	Sparingly branched
	Xtc_6	Cylindrical stromata
	Xtc_7	Unbranched stromata
	Xtc_8	Dusty white with cylindrical stromata
Maangarai	Xtc_9	Glossy stromata
	Xtc_{10}	Dark mycelia
	Xtc_{11}	cylindrical stromata
	Xtc_{12}	glossy stromata
	Xtc_{13}	cylindrical stromata
Thasanur	Xtc_{14}	cylindrical stromata
	Xtc_{15}	glossy stromata
	Xtc_{16}	Dark mycelia
Tanjore	Xtc_{17}	glossy stromata
	Xtc_{18}	cylindrical stromata
	Xtc_{19}	glossy stromata
	Xtc_{20}	Dark mycelia

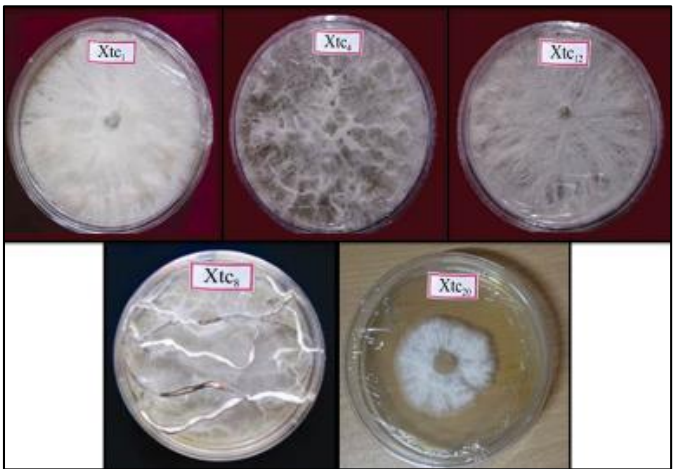

Fig 1: Isolation of plastic degrading fungi *Xylaria* sp. isolated from fungal garden

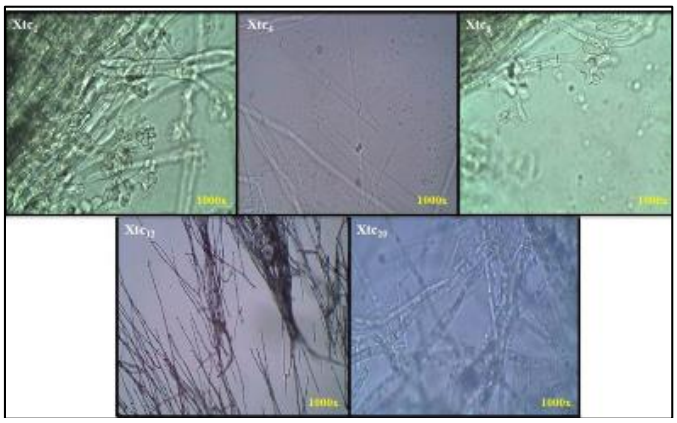

Fig 2: Microscopic view (1000x) of mounted mycelia of *Xylaria* sp

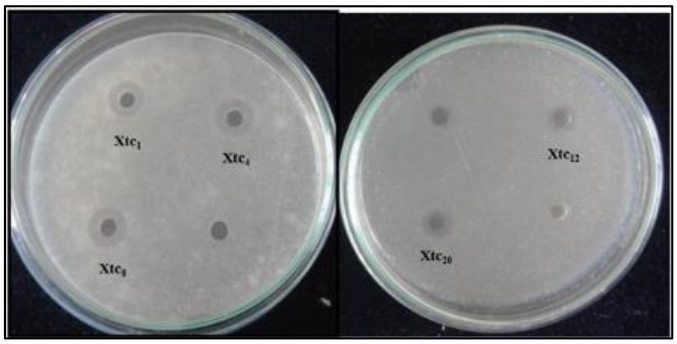

Fig 3: Screening of plastic degrading fungi *Xylaria* sp. isolated from fungal garden

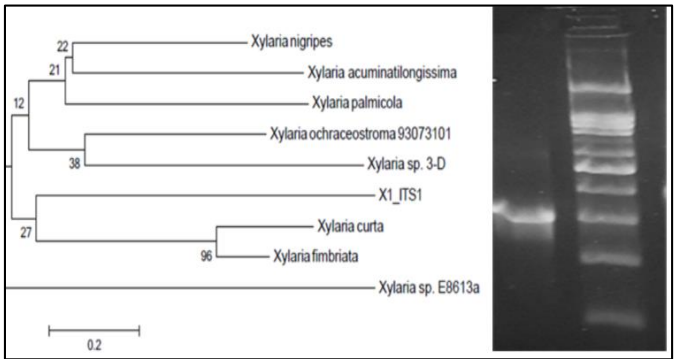

Fig 4: Neighbor joining tree was constructed based on 18S rRNA gene identification of isolated *Xylaria*

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