A study on lignin degrading fungi isolated from the litter of evergreen forests of Kodagu (D), Karnataka
Geethanjali. P.A
PG Department of Microbiology, Cauvery Campus, Madikeri, Pin-571201, Karnataka, India
geethanjalipa07@gmail.com
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ABSTRACT

Litter forms the major portion of the net primary production in natural ecosystems and in most managed forests and grasslands. Litter is the surface layer of the forest floor consisting of freshly fallen leaves, needles, twigs, stems, bark and fruits. Litter biota varies with depth and with stage of decay. Litter contain about 20-50% lignin. Because of the size and complexity of lignin, its decomposition rate is slow. Fungi especially Basidiomycetes are generally recognized as the major group responsible for lignin degradation. The lignolytic enzymes are being used in food, textile, paper industries and in the degradation of lignin rich agro-waste. In the present work lignin degrading fungi were isolated from the litter of evergreen forest of Kodagu D. About 58% of the total fungi isolated were found to be potential lignin degraders. The isolates were tested for enzyme activity and those showing maximum activity were tested for their efficiency to degrade areca and coffee husk. The *Chaetomium* sp, *Penicillium* sp, *Aspergillus* sp and *Trichoderma* sp were found to degrade areca and coffee husk effectively.

Keywords: Degradation, Screening, Lignin, Lignolytic activity, Evergreen forest and Litter.

1. Introduction

Kodagu district occupies a prominent position in the humid tropical belt of Western Ghats and is situated to the South West in Karnataka state. The district has a mountainous configuration with varied physical features. The Western Ghat ranges in this district in more or less crescent shaped and inculcate some of the loftiest peaks between the Himalayas and Nilgiris. It stretches to about 97 km from Pushpagiri in the North West to the Brahmagiri ranges in the South. Pushpagiri has dense Evergreen and semi evergreen vegetation with Shola forest and Grasslands in the higher elevation. The evergreen forest that covers almost 70% of the sanctuary has thick and dense vegetation (Murthy and Yoganarasimhan,1990).

The annual input of litter from the forests is very high and contributes to the biomass of the ecosystem (Glazer and Nikaido, 1995). The litter contains plant residues having cellulose, hemicelluloses, lignin, proteins, sugars, amino acids and organic acids (Rao, 2008). The litter supports a number of bacteria and fungi which enzymatically breakdown organic compounds and return them to the soil (Atlas and Bartha, 1998). The plant litter layer has 20-50% lignin which is the third most important component of plant residue. The basic building block of lignin is the phenyl propane unit that consists of a hydroxylated 6-C aromatic benzene ring (phenol) and a 3-C linear side chain (Brady and Well, 2005). Lignin occurs in infinite association with cellulose and hemicelluloses adding structural strength and protecting the polysaccharides by its biodegradation- resistant barrier (Atlas and Bartha, 1998).
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The degradation of lignin is brought about by fungi mainly belonging to Basidiomycetes (Rao, 2008). Over 600 species of Basidiomycetes have been found to be lignolytic converting lignin to CO$_2$, by secreting extracellular lignin peroxidase and manganese –dependent peroxidase isozyme (Kumar and Gupta, 2006). The important lignin degrading fungi are *Clavaria, Clitocybe, Flammula, Hypholoma, Lepiota, Mycena, Pleurotus, Agaricus, Polyporus, Fusarium, Arthrobotrys, Poria, Pholiota, Cephalosporium, Collybita*, and *Humiclola* (Atlas and Bartha, 1998). Lignin degrading enzymes have also been extensively used in textile industry, to decolourize textile effluents, to bleach and to synthesize dyes (Setti et al.1999). Also used in food industry to modify the colour and eliminate undesirable phenolics (Shanmugam et al. 2007). They can also be used in soil bioremediation (Duran and Esposito, 2000). The solid state fermentation treatment using lignin degrading soil fungi improves the nutritive value of paddy straw (Reddy et al. 2008). The fungi can be used for the bioleaching of coir, a lignin rich agro-waste.

Coffee husk and areca husk, the two important agricultural wastes are highly resistant to degradation because of their complex structure. Areca husk contain 13-24% of lignin. Coffee husk is rich in tannins and caffeine which makes it toxic in nature and also resistant to degradation. The present study was focused on isolation of lignin degrading fungi from litter of evergreen forests and their application in degrading coffee husk and areca husk respectively.

2. Materials and method

2.1 Screening of lignin degrading fungi

The litter samples were collected from the evergreen forests of Pushpagiri range, Kodagu (D). 1g of sample was serially diluted and were inoculated on META (malt extract tannic acid) medium. Plates were incubated at 26$^\circ$C for 5-7 days. The colony showing clear zone around them were selected. Confirmatory test for lignin degradation was done by streaking the isolates on Low Nitrogen Medium, incubated at 26$^\circ$C for 5-7 days. The colonies showing clear zone around them were considered as positive for lignin degradation (Wagianto, 2008).

2.2 Estimation of Enzyme activity

The enzyme activity was estimated by tannic acid method. The isolates were inoculated into META broth and incubated on rotary shaker, centrifuged at 10,000 rpm for 10 minutes and supernatant was collected and used as enzyme extract. The enzyme extract was mixed with 1% tannic acid solution. A blank was maintained. After 10 minutes of incubation at 30$^\circ$C, 90% ethanol was added to terminate the reaction. The optical density was read at 310nm using colorimeter. The amount of enzyme activity was determined (Padmaja and Lavanya, 2006).

2.3 Efficiency of the isolates to degrade coffee and areca husk using SSF technique.

10g of husk was dried, powdered and sterilized. The moisture content was adjusted to 40% 0.1ml of spore suspension was added and incubated at 27$^\circ$C for 10 days. After incubation 1g of husk was ground with 10 times the volume of 80% ethanol, centrifuged at 10,000 rpm for 20 minutes. To 0.2ml of the supernatant volume made upto 3ml using distilled water, 0.5ml of Folic-Ciocalteau reagent was added and incubated at room temperature for 30 min. To this 1.5ml of 20% sodium carbonate was added, mixed well and incubated in boiling water bath for 1min. Optical density was read at 650nm (Padmaja and Lavanya, 2006). The amount of
phenol was calculated using the standard graph prepared from phenol (Mueller-Harvey and Hartly, 1987).

3. Result and discussion

About 38 different types of colonies were observed and isolated from META medium. Total 18 isolates showed very clear zones on Low Nitrogen Media. These isolates were used for studying enzyme activity.

The enzyme activity estimated by tannic acid method showed the result as in Figure 1. Among the 18 isolates subjected for studying enzyme activity, EL1, EL2, EL4, EL9, EL8, EL6, EL5, EL11, EL10, EL7, EL3 showed maximum activity. These isolates were identified as Chaetomium sp, Humicola sp, Phaenerochaete sp, Talaromyces sp, Rhizopus sp, Pleurotus sp, Penicillium sp, Fusarium sp, Cephalosporium sp, Aspergillus sp, and Trichoderma sp respectively.

![Figure 1: Enzyme activity of the isolates](image)

Coffee husk inoculated with Humicola sp showed maximum amount of phenol followed by Penicillium sp, Chaetomium sp, Talaromyces sp and Trichoderma sp respectively as shown in figure 2.
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Figure 2: Efficiency of the isolates to degrade areca husk.

The phenol concentration of areca husk samples inoculated with *Chaetomium* sp was high, followed by *Humicola* sp, *Phanerochaete* sp and *Penicillium* sp, respectively as shown in Figure 3.

Figure 3: Efficiency of the isolates to degrade coffee husk.

According to Padmaja and Lavanya (2006) Lignin rich coir pith, inoculated with *Chaetomium globosum* showed reduced lignin content greatly. The lignin content of coir pith was found to be 31.14% and after composting it was found to be 20.47%, after 90 days of inoculation of *Humicola grisea Eraen*. The coir inoculated with *Phanerochate* sp, *Coriporiopsis* sp and *Pleurotus* sp showed drastic reduction in the lignin, because of the action of lignolytic enzymes of the fungi (Suganya et al. 2007).
4. Conclusion

Thus it can be concluded that, the litter harbours a diverse group of lignin degrading fungi. The isolates showed high ligninolytic activity. Hence these isolates can be exploited for the production of industrially important enzymes and for the degradation or biosoftening of complex agricultural wastes. Since the husk itself provides nutrition to fungi, they can also be used as cheap raw material for the production of enzymes.

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5. References


