ORIGINAL RESEARCH PAPER
Volume - 15 | Issue - 04 | April - 2026 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

LITTER AS A POTENTIAL SOURCE OF FUNGAL ENZYMES INVOLVED IN LIGNIN DEGRADATION

Microbiology

Dr. Geethanjali P.A*
Department of Microbiology, Field Marshal K M Cariappa College, Mangalore University, Madikeri, Kodagu (D), Karnataka, India*Corresponding Author

ABSTRACT

Lignin is the prominent woody tissues, the most abundant structural polymer of plant residue and is thus particularly important in forest ecosystem. Litter contains 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 *Artocarpus heterophyllus Lam*. About 55% of the total fungi isolated were found to be potential lignin degraders. *Phanerochaete sp, Chaetomium sp, Humicola sp, Pleurotus sp* showed maximum ligninase activity. The isolates were tested for their efficiency to degrade areca and coffee husk. The *Humicola sp* and *Phanerochaete* sp were found to degrade areca and coffee husk effectively.

KEYWORDS

Degradation, Screening, Lignin, Lignolytic activity and Litter.

INTRODUCTION

The annual input of litter from the forests is very high and contributes to the biomass of the ecosystem (Glazer and Nikaido, 1995). Litter forms the major portion of the net primary production in natural ecosystems and in most managed forests and grasslands. The litter contains plant residues having cellulose, hemicelluloses, lignin, proteins, sugars, amino acids and organic acids (Rao, 2008). The litter supports several 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).

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 CO2, by secreting extracellular lignin peroxidase and manganese –dependent peroxidase isozyme (Kumar and Gupta, 2006). The important lignin degrading fungi are *Clavaria, Clitocybecollybia, Flammula, Hypholoma, Lepiota, Mycena, Pleurotus, Agaricus, Polyporus, Fusarium, Arthrobotrys, Poria, Pholiota, Cephalosporium, Collybi, and Humicola* (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.

Agriculture produces significant number of wastes, which contain high quantity of organic matter. Coffee husk and areca husk, the two important agricultural wastes are highly resistant to degradation because of their complex structure. Areca husk contains 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 studying diversity of lignin degrading fungi in the litter of Artocarpus heterophyllus and their application in degrading coffee husk and areca husk respectively.

Methodology

Screening of Lignin Degrading Fungi

Artocarpus heterophyllus Lam. trees were selected randomly from the region of Madikeri, Kodagu (D).1g of litter sample was collected, serially diluted, and were inoculated on META (malt extract tannic acid) medium. Plates were incubated at 26° 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° C for 5-7 days. The colonies showing clear zone around them were considered as positive for lignin degradation (Wagianto, 2008).

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°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).

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°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 up to 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).

Results and discussion

About 15 different types of colonies were observed and isolated on META medium. Total 9 isolates showed very clear zones on Low Nitrogen Media. These isolates were used for studying Ligninase enzyme activity.

The enzyme activity estimated by tannic acid method showed the result as in Figure 1. Among the 9 isolates subjected for studying enzyme activity, AI5, AI2, AI6, AI4, AI3, AI7, AI1, AI9 and AI8 showed maximum activity. These isolates were identified as *Phaenerochaete sp, Chaetomium sp, Humicola sp, Penicillium sp, Pleurotus sp, Talaromyces sp, Trichoderma sp, Fusarium sp, Aspergillus sp*, respectively.

The isolates which showed maximum enzyme activity were selected to study their efficiency to degrade the husks using solid state fermentation technique. After incubation, the amount of phenol found in the substrate was measured using standard graph of phenol. Padmaja and Lavanya (2006) extracted phenol from the coir substrate inoculated with H. grisea fungi and estimated the quality of phenol using Folin- Ciocalteau reagent.

Coffee husk inoculated with *Humicola* sp showed maximum amount of phenol followed by *Phaenerochaete sp, Chaetomium sp, Pleurotus sp and Penicillium sp*, respectively as shown in

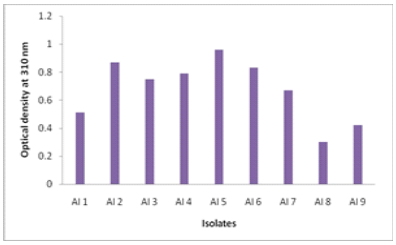

Fig 1: Enzyme activity of the isolates.

Figure 2.

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

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 Eraaen*. 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).

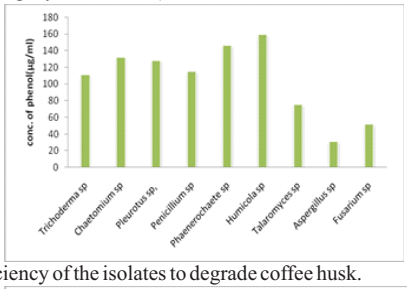

Fig 2: Efficiency of the isolates to degrade coffee husk.

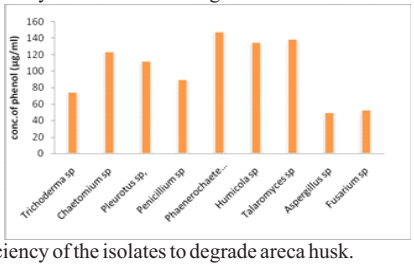

Fig 3: Efficiency of the isolates to degrade areca husk.

CONCLUSION

The litter of *Artocarpus heterophyllus* harbors a diverse group of lignin degrading fungi. The isolates showed high ligninolytic activity. Hence these isolates can be exploited to produce industrially important ligninase enzymes and for degradation or bio softening of complex agricultural wastes. Since the husk itself provides nutrition to fungi, they can also be used as cheap raw material to produce Lignolytic enzymes of industrial importance.

Acknowledgement

The author is very grateful to Mangalore and Kodagu University, for providing necessary facilities and to everyone who had given their support.

REFERENCES:

1. Atlas, R.M., and Bartha, R. 1998.Biogeochemical cycling. In: Microbial Ecology (4th Ed.). An imprint Addison Wesley Longman, Inc., Sydney. pp. 403-405.
2. Brady, N.C., and Well, R.R. 2005. Formation of soils from parent materials. In: The Nature and Properties of Soil (13th Ed.). Pearson Prentice Hall, New Delhi. pp.85.
3. Duran, N., and Esposito, E. 2000. Potential applications of oxidative enzymes and phenol oxidase- like compounds in waste water and soil treatment: a review. Appl. Catal. B. Environ., 28:83-99.
4. Kumar, D., and Guptha, R.K. 2006. Biocontrol of wood rotting fungi. Indian Journal of Biotechnology. 5: 20-25.
5. Mueller-Harvey, I., Reed, J.D., and Hartley, R.D. 1987. Characterization of phenolic compounds, including tannins of ten Ethiopianbrowse species by high performance liquid chromatography. J. Sci. Food Agric., 39: 1-14.
6. Padmaja, C.K., and Lavanya, D.L. 2006. Efficiency of Humicola grisea Eraan on the biodegradation of coir waste. Asian J. of Microbiol. Env. Sc., 8: 259-262.
7. Rao, N.S.S. 2008. Organic matter decomposition. In. Soil Microbiology (4th Ed.). Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi. pp. 252-258.
8. Reddy, R.M., Vijay, C.H., and Singaracharya, M.A. 2008. Acceptability of biologically treated paddy straw through solid state fermentation using soil fungi. Asian J. of Microbiol. Env. Sc., 10: 23-28.
9. Setti, L., Giuliani, S., Spinozzi, G., and Pifferi, P.G. 1999. Laccase catalyzed oxidative coupling of 3-methyl 2-benzothiozolinon hydrazone and methoxyphenols. Enzyme microb. Technol., 25: 285-289.
10. Shanmugam, S., Rajasekaran, P., and Kumar, T.S. 2007. Laccase the state of art of versatile catalyst a review. Advanced Biotech., 9-12.
11. Suganya, D.S., Pradeep, S., Jayapriya, J., and Subramanian, S. 2007. Bioleaching in coir for value addition. Asian J. of Microbiol. Biotech. Env. Sc., 9: 263-265.
12. Wagianto, S.P. 2008. Materials needed for extracting Lignin Degrading fungi. Mycology Research, Cambridge University Press. Oxford and IBH publishing. Co.Pvt. Ltd, New Delhi. pp.4-7.