LIGNOCELLULOSE DEGRADATION USING LIMNOFUNGI

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ABSTRACT *Only a few organisms in nature are able to attack and destroy the lignin molecule. Both cellulose and lignin are rigid organic polymers for constructive and long-term preservation purposes and needs harsh conditions to attack or modify these compounds.There is little information available with regard to production of extracellular oxidoreductases by autochthonous fungal strains belonging to different ecophysiological and taxonomic groups and hence the study.All the fungi analysed showed varying levels of activity. Among the fungi that were analysed, the maximum activity was recorded by Penicillium commune followed by Penicillium olivicolorand Aspergillus oryzae.*

Keywords: *Lignin, Degradation, Limnofungi, enzymes*

1. INTRODUCTION

Lignocellulose is the major structural component of woody and non-woody plants and represents a major source and renewable organic matter (Banakar and Thippeswamy, 2014). One would therefore expect that the wood inhabiting organisms would produce exoenzymes that solubilize these complex organic substrates to form breakdown products that can be taken up and used for nutrition (Howard *et al*., 2003; Abdel-Raheem and Ali, 2004).

Only a few organisms in nature are able to attack and destroy the lignin molecule. Both cellulose and lignin are rigid organic polymers for constructive and long-term preservation purposes and needs harsh conditions to attack or modify three compounds (Banakar and Thippeswamy, 2014). Fungi being ubiquitous in distribution are highly successful in survival because of their great plasticity and physiological versatility to secrete a wide array of enzymes involved in the breakdown of complex polymers (Carlsen and Nielsen, 2001).

A large number of microorganisms have been reported to produce ligninases among which white rot fungi are the most efficient and extensive lignin degraders (Akin *et al*., 1995; Wu *et al*., 2005) as they have extracellular enzymes which has the ability to depolymerise lignin into lower molecule weight compounds (Bajpai,2004).

Decay fungi produce three major types of enzymes like ligninolytic, xylanolytic and pectinoloytic. Ligninolytic enzymes are involved in the degradation of lignin and the major enzymes include lignin peroxidases, manganese peroxidase versatile peroxidases an laccases (Mewada*et al*., 2017). Recently there has been a growing interest in studying the lignin modifying enzymes with the expectation of finding better lignin degrading systems. However, there is little information available with regard to production of extracellular oxidoreductases by autochthonous fungal strains belonging to different ecophysiological and taxonomic groups (Dhouib*et al*., 2005) and hence the study.

2. MATERIALS AND METHODS

Screening of Lignin Degrading Fungi: The litter samples were collected from the Kovilacheri Pond, Kumbakonam District, Tamil Nadu. One gram of sediment sample was 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 and 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 10000 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 310 nm using colorimeter and the amount of enzyme activity was determined (Padmaja and Lavanya, 2006).

Efficiency of the Isolate to degrade coir pith husk using SSF technique: Ten grams of coir pith husk was dried, powdered and sterilized. The moisture content was adjusted to 40%. 0.1 ml of spore suspension was added and incubated at 27°C for 10 days. After incubation, 1 g of husk was ground with 10 times the volume of 80% ethanol and centrifuged at 10000 rpm for 20 minutes. 0.2 ml of the supernatant volume was made upto 3 ml using distilled water, 0.5 ml of Folic-Ciocalteau reagent was added and incubated at room temperature for 30 minutes. To this 1.5 ml of 20% sodium carbonate was added, mixed well and incubated in boiling water bath for 1 minute. Optical density was read at 650 nm (Padmaja and Lavanya, 2006). The amount of phenol was calculated using the standard graph prepared from phenol (Mueller-Harvey *et al.*, 1987).

3. RESULTS AND DISCUSSION

Results of the enzymatic activity of the various fungi analysed for ligninolytic activity are presented in Table-1. As evident from the Table all the fungi analysed showed varying levels of activity. Among the fungi that were analysed, the maximum activity was recorded by *Penicillium commune* followed by *Penicilliumolivicolor*and *Aspergillusoryzae.*

Literature reveals that El-Fallal and El-Diasty (2006) suggested that all white rot fungi do not produce all the three extracellular enzymes involved in ligninolysis (lacase, lignin peroxidase and manganese peroxidase). They also suggested that laccase occupies an important place in ligninolysis. Arora *et al*. (2012) reported that either LiP or MnP production in association with laccase results in higher lignin losses which was proved earlier by Datta *et al*. (1991) while working on *Phelebia* sp. and *P. chrysogenium*. El-Fallal and El-Diasty (2006) also suggested that no single enzyme could be held responsible for ligninolysis as the distribution of the three ligninolytic enzymes varies a lot from fungus to fungus, while in one fungus LiP-laccase combination can cause ligninolysis, in others either MuP-laccase or LiP, MnP-laccase can do the same job. Thus is probably the reason why all the fungi showed differential breakdown of lignin. Vares (1996) also suggested that ligninolytic activity of other microbes indicted that the physiological conditions for lignin degradation and enzyme systems are fungal specific and can differ from other fungi. Thus, differences in enzyme system could be some extent reflect their phylogeny. Thus, evolution of fungal ligninolytic peroxidases requires further study. Nevertheless, from the present study, it is clear that all the fungi have the ability to degrade lignin.

Table-1
Degradation of Lignin using Limnofungi

	Enzymatic Activity		
Species	OD (5 days)	Con. Phenol (µg/ml) (30 days)	Con. Phenol (µg/ml) (60 days)
Aspergillus oryzae	1.24	252.2	308.0
Aspergillus wentii	1.26	238.6	278.0
Aspergillus corymbifera	1.07	96.5	142.0
Penicillium olivicolor	1.28	210.7	320.0
Penicillium commune	0.68	248.6	340.0
Fusarium culmorum	1.73	84.8	110.6
Fusarium oxysporum	1.62	158.6	198.6
Mucor luteus	1.44	92.3	160.4
Trichoderma harzianum	1.22	178.6	232.0
Trichoderma viride	0.98	198.7	241.6
Rhizopus stolonifer	0.90	172.4	228.6
Aspergillus oryzae + *Penicillium commune* + *Fusarium oxysporum* + *Trichoderma viride*	1.80	268.8	372.4

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