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Biodegradation of lignin by fungal cultures

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Abstract
The lignocellulosic material of plants consists of three main components, namely cellulose, hemicellulose and lignin. After cellulose, lignin is the second most abundant renewable biopolymer in nature. It is the most abundant aromatic polymer in the biosphere. It is an essential part of the plant cell wall, imparting rigidity and protecting the easily degradable cellulose from attack by pathogens. Lignolytic fungi were cultivated on potato dextrose agar (PDA) plate with indicator compound 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) to screen for the laccase production ability. All the cultures produced dark brown coloured zone of lignin degradation, SI for fungal cultures ranged from 1.84 to 2.30.

Keywords: Lignin, fungi, laccase, ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid))

Introduction
Lignin is a very complex molecule constructed of phenyl propane units linked in a large three-dimensional structure. Three phenyl propionic alcohols exist as monomers of lignin *viz. p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. Lignin is closely bound to cellulose and hemicellulose and its function is to provide rigidity and cohesion to the material cell wall, to confer water permeability to xylem vessels and to form a physicochemical barrier against microbial attack. Due to its molecular configuration, lignin is extremely resistant to chemical and enzymatic degradation. Laccases (E. C. 1.10.3.2, p-diphenol:dioxygen oxidoreductase) are a group of multi-copper containing enzymes that catalyse one-electron oxidation of phenolic compounds with concomitant reduction of oxygen to water. Laccases find wide commercial applications within food industry, pulp and paper industries, textile industry, synthetic chemistry, cosmetics, soil bioremediation and biodegradation of environmental phenolic pollutants and removal of endocrine disruptors (Cuoto and Herrera, 2006) [3]. Laccases are widely distributed in fungi, higher plants, bacteria and insects. More than 60 fungal strains, belonging to various classes such as *Ascomycetes*, *Basidiomycetes* and *Deuteromycetes*, have been demonstrated to produce laccase (Gianfreda *et al*., 1999) [5]. The majority of laccases characterized so far have been derived from efficient lignin degraders such as white-rot fungi (Eggert *et al.*, 1996 and Niku-Paavola *et al*., 1990) [4, 9]. *Trichoderma spp*. also active participate in delignification and biodegradation of cellulose in nature and many strains have been studied extensively as sources of cellulase enzymes for potential commercially hydrolysis of cellulosic materials.

Material and Method
Fungal cultures used in the study
The fungal cultures *viz. Chaetomium globosum*, *Pleurotus ostreatus*, *Coriolus versicolor*, *Trichoderma viride*, *Trichoderma harzianum*, *Emericella nidulans*, *Aspergillus niger*, *Aspergillus wentii* and *Aspergillus terreus* were made available from Department of Microbiology, A.A.U., Anand for the study.

Maintenance and revival of microbial cultures
All the fungal cultures were maintained Potato dextrose agar medium for routine use. The fungi were sub cultured regularly. For long term storage, the fungal cultures were maintained on PDA slants and covered with glycerol after proper growth respectively and stored at 0 - 4˚ C. All the test fungi were revived on respective media before studies.

In vitro assessment of lignolytic activity of individual fungal cultures by plate and flask bioassay
In the first stage of screening, all the fungal cultures were tested for their potential for degrading lignin using tannic acid media in the laboratory.

Enzyme mediated microbial degradation of lignin

Lignin degradation by test bacteria and fungi was assessed by oxidative process and considering phenol oxidases as key enzyme (Kuhad *et al*., 1997; Leonowicz *et al*., 2001) [7, 8]. Of the enzymes, lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase have been studied.

The polyphenol oxidase (EC 1.10.3.1) activity of test microbial cultures was checked using tannic acid (0.01% w/v) in basal medium, according to modified procedure of Bavendamm (1928) [1] (Thormann *et al*., 2002) [11]. For determination of laccase activity, the agar medium supplemented with 2 mM 0.1% w/v ABTS (2, 2'azino- bis (3-ethylbenz-thiazoline-6-sulfonic acid) was used. Oxidation of ABTS to ABTS-azine in the presence of laccase gave green colour to colourless agar medium (Niku-Paavola *et al.*, 1990; Thurston, 1994) [10, 12] and considered as positive test.

Both the media were autoclaved at 121˚C for 20 minutes and poured approximately 20 ml into respective petri dishes. 5 mm mycelial discs of 5-day-old test fungal culture were transferred in the centre of the respective plates and incubated at in the dark room at temperature (28 ± 2 ˚C) for 3 to 5 days. The ligninolytic activity was recorded by observing color formation around the fungal growth. For polyphenol peroxidase (PPO) activity, formation of a dark brown colour and for laccase, green color was observed in respective plates (Thormann *et al.*, 2002; Kausar *et al*., 2010) [13, 6].

Result and Discussion

Lignin is a large stereo irregular polymers comprised of inter unit carbon-carbon and ether bonds, the lignolytic enzyme acting on are oxidative and extracellular. The major enzymes associated with lignin degradation are laccase, lignin peroxidase and manganese peroxidase. Some bacteria and fungi produce all three enzymes whereas majority produces only one or two enzymes.

All the fungal cultures exhibited ability to degrade lignin when tested on tannic acid (Bavedamm test) and ABTS-amended media.

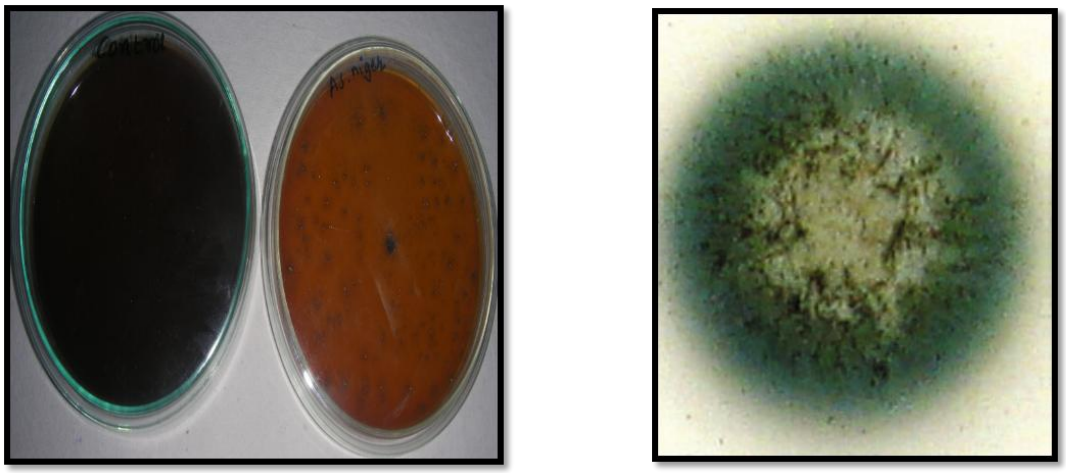
Table 1: Degradation of lignin by fungal cultures after 7 days

Sr. No.	Fungus cultures	Tannic acid agar plate				Laccase activity on ABTS agar
		Total zone diameter (mm)	Colony diameter (mm)	Zone diameter (mm)	Solubilisation index (SI)	
1	*P. ostreatus*	15.3	8.3	7.0	1.84	+
2	*C. versicolor*	20.0	12	8.0	1.67	+
3	*E. nidulans*	18.0	12.7	5.3	1.41	+
4	*A. wentii*	16.0	11.7	4.3	1.36	-
5	*A. terrus*	16.7	10.3	6.4	1.62	+
6	*A. niger*	23.3	11.3	12.0	2.06	+
7	*C. globosum*	13.7	8.7	5.0	1.57	-
8	*T. viride*	26.0	11.3	14.7	2.30	+
9	*T. harzianum*	24.3	12.3	12.0	1.97	+

Development of a dark brown zone on tannic acid medium confirmed the polyphenol oxidase (PPO) activity of fungal cultures. Polyphenol oxidase – a mixture of mono phenol oxidase and catechol oxidase catalysed the reaction between polyphenol and molecular oxygen to form dark brown complexes, which play a vital role in the degradation of the phenolic compound of lignin. All the fungi except *A. wentii*, do not produce a green color in ABTS agar plate for laccase activity. Fungi are known to produce lignolytic enzymes *viz.* lignin peroxidise and laccase during the biodegradation of lignocellulosic materials (Figure 1).

Christie and Shanmugam (2012) [2] isolated four ascomycetes for the laccase enzyme production. Initial screening of four fungi showed positive reaction when grown in the presence of Guaiacol and ABTS after 7 days of incubation. A colour zone produced by *A. niger* (20 mm), *F. oxysporum* (21.5 mm), *A. arborescens* (18 mm) and *P. marneffei* (22 mm).

Singh and Abraham (2013) [14] isolated a laccase producing fungi from compost soil and isolated 11 cultures which were cultivated on potato dextrose agar with indicator compounds ABTS to screen laccase activity and out of 11 isolates five fungal strains showed laccase, *Fusarium* sp. was potent laccase producer followed by F_3, F_4, F_5 and F_{11} (*Aspergillus* sp.).



Lignin degradation on tannic acid agar plate and laccase activity by *A. niger* and *T. viride*

Conclusion

Lignin degradation capacities of fungal cultures were tested on tannic acid agar plate and ABTS - amended media. All the cultures produced dark brown colored zone of lignin degradation, SI for fungal cultures ranged from 1.84 to 2.30. Whereas all the fungi except *A. wentii* and *C. globosum* does not showed laccase activity.

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