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Qualitative Screening of lignocellulolytic Enzymes in Wood Rotting Agaricomycetes from North Western Himalayas

Lalita* and I.B. Prasher
Mycology and Plant Pathology Lab, Department of Botany, Panjab University.

*Corresponding author: Lalita,
Mycology and Plant Pathology Lab, Department of Botany,
Panjab University, Chandigarh,
India- 160014.
E-mail: rinkubargujer22@gmail.com
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ABSTRACT
Six species of agaricomycetous fungi belonging to five genera of family Polyporaceae viz.: *Hexagonia tenuis*, *Trametes versicolor*, *Trametes* sp., *Pycnoporus coccineus* and *Pseudotrametes* sp. and to Meruliaceae viz.: *Flavodon flavus* which cause wood decay of timber trees of N.W. Himalayas were qualitatively screened for lignocellulolytic enzymes.. These were collected from different localities of North Western Himalayas. In the results, yellow opaque area around the mycelial growth shows Carboxymethyl cellulose (CMC) degradation whereas zone of discoloration of the medium show the ligninolytic enzymatic activity of the fungi.
Keywords: Agaricomycetes, cellulase, laccase

INTRODUCTION
Wood tissues are degraded by fungi and these wood- decay fungi are classified as White-rot fungi, soft-rot fungi and brown-rot fungi [1] according to their mode of attack on wood cell wall: White rot fungi are efficient degraders of lignocelluloses as they can degrade both cellulose and lignin, whereas, brown rot fungi decompose hemicelluloses, cellulose and modify or cleave lignin but do not metabolise it. The soft rot fungi degrade only cellulose and hemicelluloses.
Wood rotting fungi are capable of decomposing all the major components of wood due to production of hydrolytic and oxidative enzymes [3]. The major hydrolytic enzymes are endo 1, 4-β D glucanase, exo-1, 4- β-D-glucanase and xylanase [2]. The hydrolytic enzymes play a decisive role in the steady supply of nutrients to the growing fungi. These fungi play vital role in nature and particularly in the forests ecosystem, where they contribute significantly to carbon recycling, as the residues remaining from the harvest of trees and logs are attacked and degraded, particularly by white and brown rot fungi which are more aggressive colonizers and degraders of wood in such environment [4].
Qualitative estimation of lignocelluloses has been used for the study of systematic and biodiversity in fungi [5]. The assay for qualitative estimation are powerful tools used in screening of fungi for lignocelluloses degrading enzymes production [6, 7, 8].
Lignocellulose biodegradation is intiated by the multiple forms of lignin peroxidase, manganese peroxide and copper-containing phenoxidase, laccase, although the specific enzymes complements of different species vary considerably [9, 10]. During a survey of the different ecological zones of N.W. Himalayas in the states of Himachal Pradesh and Uttarakhand some of the poroid Agaricomycetes were collected which were found to be very common and causing wood decay of the important timber trees. In order to use these fungi for biotechnological application these were screened for production of enzymes related to wood degradation. The objective of the present work was to determine the ability of different wood rotting fungi to produce lignocellulolytic enzymes.

MATERIALS AND METHODS
Cellulolytic enzyme assay:
The cellulolytic activity of enzymes was detected by staining of Carboxymethyl cellulose .
Cellulolysis basal medium (CBM) consisting of $C_4H_{12}N_2O_6$ 5 g, yeast extract 0.1 g,KH_3PO_4 1 g, $MgSO_4$ ·$7H_2O$ 0.5 g, $CaCl_2$·$2H_2O$ 0.001 g, in 1 L distilled water with supplementations for screening for various enzymes was employed in screening of fungi.
Endoglucanase (CMC agar): 1.8% w/v agar was added to the CBM medium supplemented with 1% w/v low viscosity carboxymethylcellulose (CMC). The medium was autoclaved, dispensed into petri dishes, allowed to solidify and inoculated with discs of the test fungi and then incubated. After growth for 5–10 days, the plates were flooded with 2% aqueous Congo red (C.I.22120) and allowed to sit for 15 minutes. The stain then washed from the agar surface with distilled water and the plates

then flooded with 1 M NaCl to destain for 15 minutes. The NaCl solution then removed. CMC degradation around the colonies (as endoglucanase activity) was appeared as a yellow-opaque area against a red color for the un-degraded CMC.

Detection of Ligninolytic activity:

The ligninolytic activity of the enzymes was detected by using LME basal medium.

Lignin modifying enzyme assays (LME)

LME basal medium (LBM): This medium consists of KH_2PO_4 1 g, $C_4H_{12}N_2O_6$ 0.5 g, $MgSO_4.7H_2O$ 0.5 g, $CaCl_2.2H_2O$ 0.01 g, Yeast Extract 0.01 g, $CuSO_4.5H_2O$ 0.001 g, $Fe_2(SO_4)_3$ 0.001 g, $MnSO_4.H_2O$ 0.001 g in 1 litter distilled water. The basal medium may be conveniently stored as a 10 X sterilized stock. It is also possible to simplify the procedure by using less defined basal growth medium such as peptone plus yeast extract, or malt extract. Such basal media have been used in several studies in varying concentrations [11-16]. however, it should be noted that LME production in fungi is generally repressed under conditions of nutrient sufficiency [17]. Enzyme was detected through the use of the LBM medium with 0.01% w/v Azure B and 1.6% w/v agar and autoclaved. Aseptically added one ml of a separately sterilized 20 % w/v aqueous glucose solution to each 100 ml of growth medium prepared. The medium was aseptically transferred to petri plates. It was inoculated with the test fungus and incubated at 25°C in darkness. The plates were examined daily for 10 days. The production of lignin peroxidase and Mn dependent peroxidase as clearance of blue coloured medium has been recorded [8]

RESULTS AND DISCUSSION:

Six species(*Hexagonia tenuis*, *Trametes sp*, *Trametes versicolor*, *Flavodon flavus*, *Pycnoporus coccineus* and *Pseudotrametes sp* of wood rotting fungi were collected from different sites of North Western Himalayas (Kasauli-Himachal Pradesh, Dehara Dun, Mussoorie- Uttarakhand). These were identified morphologically with the help of standard literature (Figure-1) and isolated on Malt extract agar medium (MEA). The qualitative estimation of lignocellulolytic enzymes are depicted in table-1, Figure-2 and Figure -3.

Table 1 Qualitative estimation of lignocellulolytic enzymes by isolated wood rot fungi using CMC assay and LME assay.

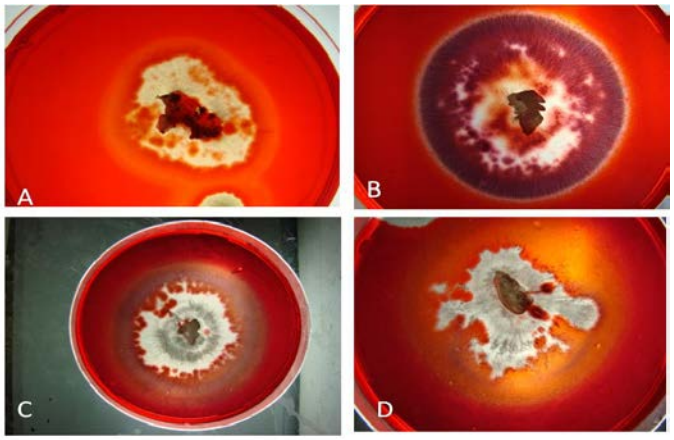
Sr. No.	Name of the Species	Cellulolytic activity	Ligninolytic activity
1	*Hexagonia tenuis*	++	++
2	*Trametes sp.*	+	++
3	*Trametes versicolor*	+++	+++
4	*Flavodon flavus*	+++	+++
5	*Pycnoporus coccineus*	+++	+++
6	*Pseudotrametes sp.*	++	++

(+= Minimum, ++= Medium, +++=Maximum activity of Cellulolytic and ligninolytic enzymes)

A study of table-1 exhibits the potential ability of lignin and cellulose degrading enzymes by these mentioned wood rotting fungi viz.: *Hexagonia tenuis*, *Trametes sp.*, *Trametes versicolor*, *Flavodon flavus*, *Pycnoporus cinnabarinus* and *Pseudotrametes sp*. It indicates that among the investigated species, there is variation in lignin and cellulose degrading ability. *Trametes versicolor*, *Flavodon flavus* and *Pycnoporus cinnabarinus* exhibited maximum cellulolytic activity whereas *Hexagonia tenuis*, *Pseudotrametes* sp. show medium and *Trametes* sp. exhibited minimum cellulolytic activity. Ligninolytic enzyme activity was detected in all the selected isolates. *Trametes versicolor*, *Flavodon flavus* and *Pycnoporus coccineus* produced maximium ligninolytic activity whereas *Hexagonia tenuis* and *Pseudotrametes* sp. and *Trametes sp*. show medium enzymatic activity.

Figure-1: Wood rotting agaricomycetous fungi A *Hexagonia tenuis*, B *Trametes sp*, C *Trametes versicolor*, D *Flavodon flavus*, E *Pycnoporus coccineus* and F *Pseudotrametes sp*

Figure-2: Qualitative estimation of cellulolytic enzymes in A *Hexagonia tenuis*, B *Trametes sp*, C *Trametes versicolor*, D *Flavodon flavus*, E *Pycnoporus coccineus* and F *Pseudotrametes sp*.



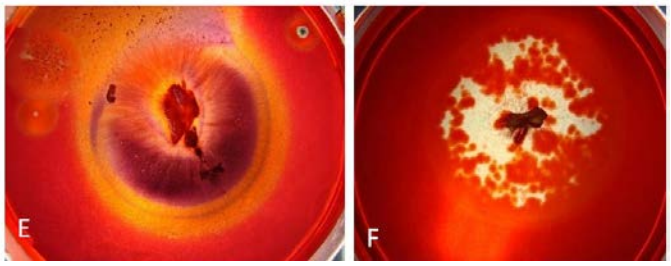
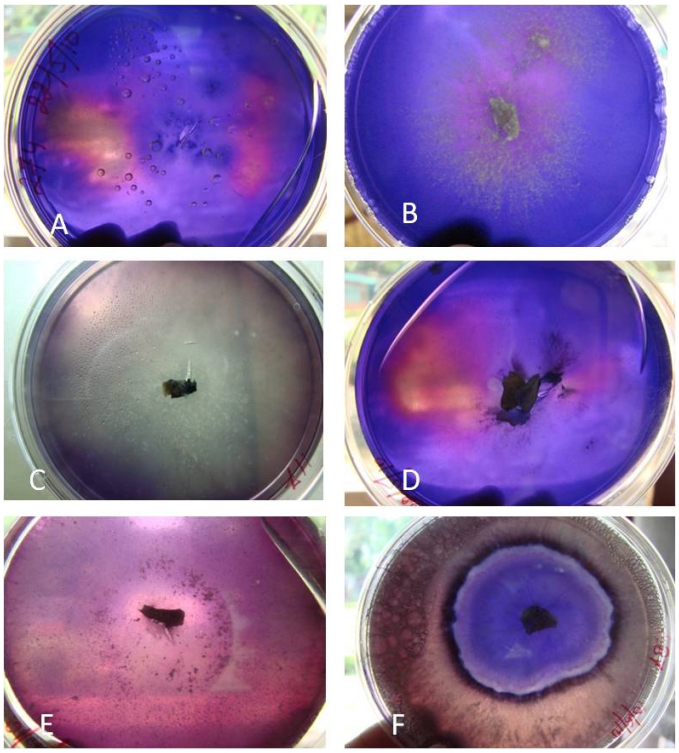


Figure-3: Qualitative estimation of cellulolytic enzymes in A *Hexagonia tenuis*, B *Trametes sp*, C *Trametes versicolor*, D *Flavodon flavus*, E *Pycnoporus coccineus* and F *Pseudotrametes sp*.



CONCLUSION

Six taxa of wood rotting fungi were collected and screened for lignocellulolytic enzymes using CMC and LME assay. This study clearly indicates the potential of production of lignocellulolytic enzymes by wood rotting agaricomycetous fungi which may promote further research for their biotechnological applications.

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REFERENCES

1. Deacon, Fungal Biology, Blackwell Publishers,2005.
2. Kobakhidze A., Elisashvili V., Irbe I., Tsiklauri N., Andersone, Andersons B. and Isikhuemhen O.S. Lignocellulolytic enzyme activity of new corticoid and Poroid basidiomycetes isolated from Latvian cultural monuments. Journal of Waste Conversion, Bioproducts and Biotechnology 1(1): (2012) 16-21.
3. Eriksson, K.E.L., Blanchette, R.A. and Ander P. Microbial and Enzymatic degradation of wood and wood components. Springer-Verlag, Berlin Heidelberg. 1990. pp 407.
4. Singh, A. P. and Singh, T.S. Biotechnological applications of wood-rotting fungi: A review. Science Direct. Elsevier, 2014.
5. Hyde, K.D.Biodiversity of tropical microfungi. Hong Kong University Press, Hong Kong, 1997.
6. Thurston, C. F. The structure and function of fungal laccase. Microbiol., 140: (1994)19-21.
7. Reddy, C. A. The potential for white-rot fungi in the treatment of pollutants. Current Opinion in Biotechnol., 6: (1995)320-328.
8. Pointing, S. B. Qualitative methods for the determination of lignocellulolytic enzymes production by tropical fungi. Fungal diversity 2: (1999) 17-33.
9. Hatakka, A. Lignin-modify ing enzymes from selected white-rot fungi: product on and role in lignin degradation. FEMS Microbiol. Rev., 13: (1994)125-135.
10. Maijala P. Heterobasidion annosum and wood decay: enzymology of cellulose, hemicelluloses and lignin degradation. Dissertation, university of Helsinki, pp 67, 2000.
11. Gessner, R. V. Degradative enzymes produced by salt marsh fungi. Botanica Marina, 23: (1980)133-139.
12. Egger, K.N. Substrate hydrolysis patterns of post-flre ascomycetes(Pezizales). Mycologia, 78: (1986) 771-780.
13. Niku- Paavola, M.L., Raaska and M. Itavaara, Detection of white-rot fungi by non-toxic stain. Mycol. Res., 94: (1990) 27-31.
14. Raghukumar, C., Raghukumar, S., Chinnaraj, A., Chandramohan, D., D'Souza, T.M. and Reddy, C.A. Laccase and other lignocellulose modifying enzymes of marine fungi isolated from the coast ofIndia. Botanica Marina 37: (1994) 515-523.
15. Rohrmann, S. and Molitoris, H.P. Screening for wood-degrading enzymes in marine fungi. Canadian Journal of Botany 70: (1992)2116-2123.
16. Pointing, S.B., Vrijmoed, L.L.P. and Jones, E.B.G. Laccase is produced as the sole lignin modifying enzyme in submerged liquid culture by the white-rot fungus Pycnoporus sanguineus L. Mycologia 91: 1999b (In press).
17. Reddy, C.A. and D'Souza, T.M. Physiology and molecular biology of the ligninperoxidases of Phanerochaete chrysosporium. FEMS Microbiology Reviews 13: ((1994)137-152.

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