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Qualitative Estimation of Cellulases and Lignin Modifying Enzymes in Five Wild *Lentinus* Species Selected from North West India

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Abstract: Present investigations on five wild selected fungal species of Genus *Lentinus* {Fr. namely *L. sajor-caju* (Fr.), *L. connatus* Berk. *L. torulosus* (Pers. Fr.) Lloyd, *L. cladopus* Lèv, *L. squarrosulus* (Mont.) Sing.} collected from different localities of North West India have been studied for the qualitative estimation of cellulose and lignin degradation enzymes through colorimetric assays. The results obtained indicated that the production of these enzymes started from the fourth to sixth day of mycelial growth on petriplate. Whereas, the lignin modifying enzymes were detected on the third day of mycelial growth. The degradation of carboxymethyl cellulose (CMC) was observed with a yellow opaque layer formation around the colony in the concentric manner in case of cellulase enzymes detection. The enzymes responsible for the modification of lignin were observed with the formation of brown oxidation zones around the colonies. The findings clearly indicate that as like other wood degrading fungi, these wild fungal species of *Lentinus* despite of occurring on different hosts contain the enzymes responsible for cellulose and lignin degradation.

Key words: Lentinus species · Cellulase · Lignin modifying enzymes

INTRODUCTION

The genus *Lentinus* (Fr.) Elench. belongs to class Agaricomycetes, order Polyporale*s* and family Polyporaceae [1]. Species of *Lentinus* (Fr.) Elench. are wood-decaying basidiomycetes and causes white rot disease in plants. White rot basidiomycetous fungi produces three major classes of enzymes designated lignin peroxidases (LIPs), manganese dependent peroxidases (MNPs) and laccases, which play an important role in the fungal degradation of lignin [2-11]. Qualitative estimation of lignin and cellulose has been used for the study of systematics and biodiversity in fungi [12]. The study of the enzymes responsible for the degradation of cellulose and lignin is essential for understanding the ecological and also in the biotechnology potential of enzymes involved in this process [13]. Lignocellulose is a heteropolymer consisting mainly of three components, cellulose, hemicellulose and lignin [14-16]. Lignocellulose degrading enzymes plays a vital role in the basic research on classification of enzymes [17-18]. Qualitative assays are powerful tools used in screening fungi for lignocellulose degrading enzyme production [19-21]. The importance of the qualitative tests in the detection of these enzymes further provide the basis of other classification of enzyme occurring in fungi and they are also useful in screening large numbers of fungal isolates for several classes of enzyme, where definitive quantitative data are not required [15]. In the present investigation five wild species of Genus *Lentinus* collected from the different localities are studied for their capability to degrade lignin and cellulose which is quite useful as the basic need for enzyme profiling.

MATERIALS AND METHODS

Qualitative Detection of Cellulase Enzymes: Activity of cellulase enzymes was detected by dye staining of carboxymethyl cellulose (CMC). The composition of Cellulose Basic medium (CBM) (g L^{-1}) is given below:

$C_4H_{12}N_2O_6$	5
KH_2PO_4	1
$MgSO_4.7H_2O$	0.5
Yeast Extract	0.1
$CaCl_2.2H_2O$	0.001

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CBM medium was supplemented with 2% w/v low viscosity CMC and 1.6% w/v agar and then autoclaved. Subsequently it was inoculated with test fungus after pouring. The test fungus was incubated at 25°C in darkness. When the colony diameter reached 30 mm, the agar plates were stained firstly by flooding with 2% w/v aqueous congo red which were then left to stand undisturbed for fifteen minutes. The stain was poured off and then plates were flooded with 1M NaCl so as to destain for another fifteen minutes. The activity then was observed as yellow opaque area against a red color of undegraded CMC.

Qualitative Detection of Lignin Modifying Enzymes: Activity of lignin modifying enzymes was detected by dye staining of lignin modifying basal medium (LME). The composition of Lignin Basic Medium (LBM) (g L^{-1}) is given below:

KH_2P0_4	1
Yeast Extract	0.01
$C_4H_{12}N_20_6$	0.5
$CuS0_4.5H_20$	0.001
$MgS0_4•7H_2$	00.5
$Fe(S0_4)_3$	0.001
$CaCl_2•2H_20$	0.01
$MnS0_4.H_20$	0.001

LBM was supplemented with 1.6% w/v agar and autoclaved. To this added 1 ml of separately sterilized 20% aqueous glucose solution and 1 ml of 1% w/v aqueous tannic acid solution to each 100 ml growth medium prepared. Then this medium was transferred aseptically into the petriplates and inoculated with test fungus after solidification of medium. The petriplates were incubated at 25 °C in darkness and examined plates daily for ten days. LME production was recorded as the appearance of brown oxidation zones around the colony.

RESULTS AND DISCUSSION

Five wild species collected from different hosts and from different localities and altitude is presented in Table 1 and Figure 1. The species of genus *Lentinus* Fr. are all wood inhabitating as these contain cellulase as well as other wood rotting enzymes. The five wild *Lentinus* species were checked for cellulase activity qualitatively by dye diffusion method. In this carboxymethycellulose (CMC) is used as a substrate which is also a substrate for endoglucanase and so can be used as a test for endoglucanase and glucosidase activity. This assay is a good indicator of cellulolytic ability since endoglucanase is generally produced in larger amount by fungi than cellobiohydrolase [22-24]. In addition many fungi that successfully degrade cellulose on wood produce no detectable cellobiohydrolase [25]. In this assay after growth of the fungus on CMC, a dye is used to differentiate between intact CMC and degraded substrate. CMC degradation around the colonies appear as a yellow-opaque area against a red colour for undegraded CMC. All the five species of *Lentinus* exhibited good amount of cellulase activity. The activity is checked on the earlier stages of culture growth, when it was just five days old. All the species showed considerable activity against the lignocelluloses substrates. The prominent zone of yellow opaque area in the cellulase activity was observed on the fourth to sixth day of the mycelial growth on petriplate. The zone was in concentric manner around the mycelial colony. The formation of such zone was constant from sixth day till the maturation of mycelium. This assay is a well established procedure and has been used in many areas like systematics and biodiversity [26-31]. Lignin modifying enzyme assay can be useful in determining the ability of a fungus to utilize a lignin substrate. The method indicates degradation of phenolic components in lignin. Degradation of the more recalcitrant non-phenolic lignin components. Laccase production in *P. chrysosporium* appears to be relatively low (1.7 nkat/ml of concentrated extracellular fluid) even with ABTS, which is considered one of the more sensitive substrates for laccase assay[32,33]. These procedures offer a powerful research tool for obtaining data on possible methods of lignocellulose substrate utilization, or the production of commercially important enzymes (Figure 2 and Figure 3).

Table 1: Showing associated natural host and location with altitude and forest type

Species	Host	Location	Altitude (m)	Type of forest
Lentinus sajor-caju	*Bauhinia variegata*	Sirmour (H.P)	672	Mixed
Lentinus connatus	*Mangifera indica*	Chandigarh (Pb.)	200	Plains
Lentinus torulosus	*Pinus roxburghii*	Palampur (H.P)	850	Pine forest
Lentinus cladopus	*Albizzia chinensis*	Palampur (H.P)	1200	Mixed
Lentinus squarrosulus	*Albizzia chinensis*	Palampur (H.P)	1200	Mixed

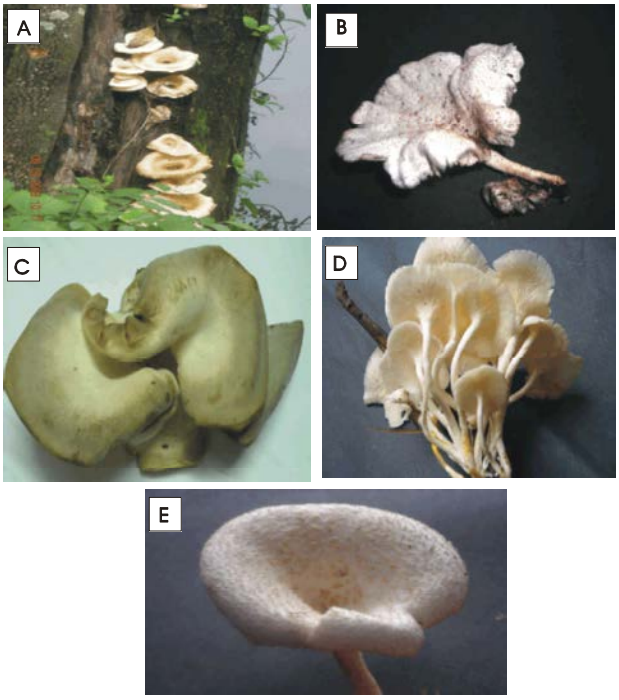

Fig. 1: A. Lentinus sajor - caju B. Lentinus connatus. C. Lentinus torulosus D. Lentinus cladopus and E. Lentinus squarrosulus.

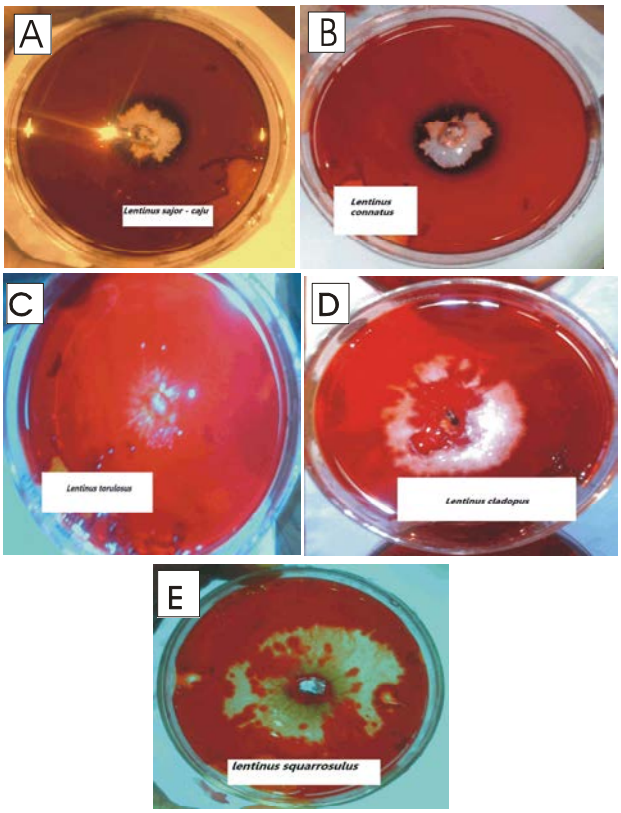

Fig. 2: A. Cellulase activity of A. L. sajor - caju B Cellulase activity of L. conatus. C. Cellulase activity of L. torulosus D. Cellulase activity of L. cladopus E. Cellulase activity of L. squarrosulus.

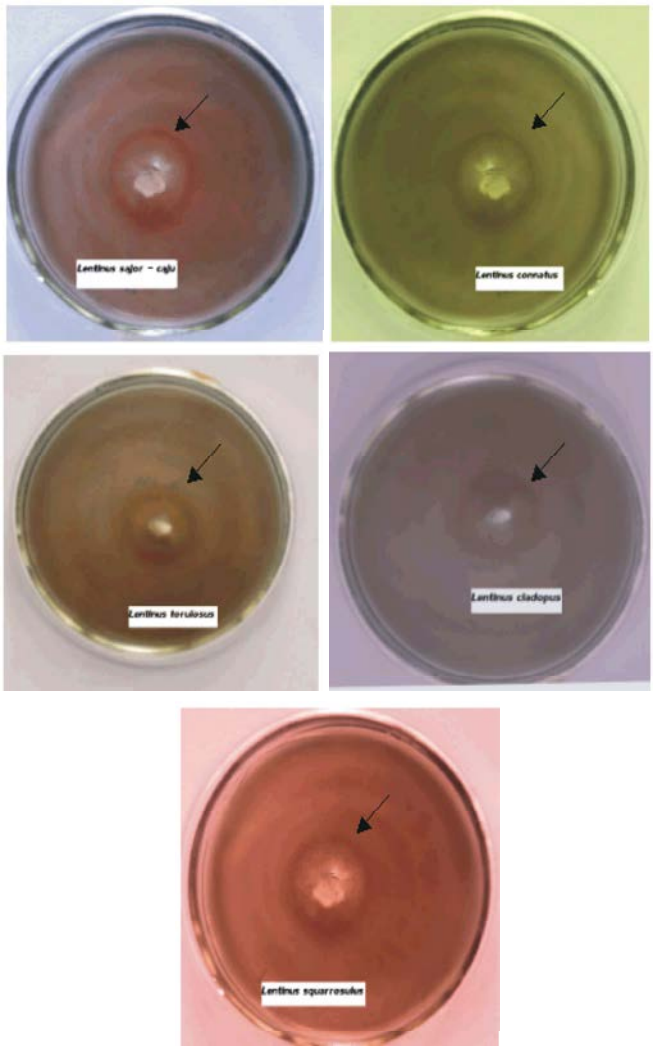

Fig. 3: A. Lignin modifying enzyme activity A. L. sajor - caju B L. connatus. C. L. torulosus D. L. cladopus E L. squarrosulus.

CONCLUSIONS

Qualitative estimation of the lignocellulytic enzymes provides the basic information for the presence of enzymes in the wood inhabitating mushrooms. This is quite useful in the fields of systematics and biodiversity. Further their role in the ecological aspects can be best understood with these basic tools.

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