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STUDIES ON THE LIGNOCELLULOSIC ENZYMES OF *PANUS TIGRINUS*

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Panus tigrinus, an edible mushroom, collected from decaying wood of *Magnifera indica* was studied for its capacity to degrade the lignocellulose complex. Various parameters influencing growth and production of lignicellulolytic enzymes in synthetic liquid medium were studied. The enzymes laccase and xylanase were isolated and purified by gel exclusion chromatography, and kinetics of enzyme activity determined.

The white rot basidiomycetes degrade lignin more rapidly and extensively than other microbial groups studied (1,2). Studies made on some of the white rot fungi indicate that different organisms depict varied enzymic activity on the lignocellulosic substrate. For instance, the much studied white rot *Phanerochaete chrysosporium* has a multi-enzyme ligninolytic system including lignin peroxidase, (LiP) (3), manganese peroxidase (MnP) (4) and xylanase (5), *T. versicolor* possesses both LiP and laccase activities in addition to MnP and xylanase (6-8) whereas *Lentinus edodes* shows MnP and laccase but not LiP activity (9).

Recent work on *Panus tigrinus* shows that the lignolytic system is composed of MnP and laccase, with the absence of Lip (10) in contrast to an earlier report indicting the presence of Lip (2). In the present study, a white rot fungus *Panus tigrinus* isolated from decaying mango wood was studied for the production of the various lignocellulolytic enzymes.

EXPERIMENTAL RESULTS AND DISCUSSION

The mycelial culture of the mushroom, *Panus tigrinus* was maintained on potato dextrose agar medium (PDA). Mineral salts medium pH 5.6 was used as culture medium and contained (gl^{-1}) ferrous sulphate 0.01, magnesium sulphate 0.2, sodium chloride 0.05, disodium hydrogen phosphate 0.1, potassium dihydrogen phosphate 0.7, ammonium sulphate 0.5g and 1ml of trace element solution consisting of (mgl^{-1}) copper sulphate 10.0, boric acid 50.0 cobalt sulphate 50.0, sodium molybdate 2.0 manganese sulphate 5.0 and zinc sulphate 11.0. The medium was supplemented with 0.2% malt extract and 1% glucose, and designated MMG.

An 18 mm diameter plug from mycelial mat grown at 28°C for 7-10 days was used as inoculum for 150 ml of MMG medium in a 500ml conical flasks in duplicate and incubated as per the experiment.

Enzyme assays: Laccase was assayed by a modified Hiroi-Eriksson method (11) and one unit of laccase activity was defined as the amount of enzyme that increased the absorbance by 0.1 at 420nm h^{-1}. Phenol oxidase was qualitatively detected by the Bevendamm test (12). The decolourization of the polymeric dye, Poly R-478 was used to detect MnP/Lip activity (13). Xylanase was assayed using oats spelt xylan (Sigma) as substrate (5).

Process parameters such as temperature, agitation and pH were optimised for maximum production.

Determination of the cellulolytic capacity of the fungal culture: A 12 mm diameter fungal mat grown on MMG agar was inoculated into 100 ml modified *Trichoderma viride* (MTV) liquid medium consisting of gl^{-1} of $NaNO_3$ 2.2, ;KH_2PO_4, 2.0; $MgSO_4.7H_2O$, 0.03; $CaCl_2$, 0.3; urea, 0.33; cellulose powder, 10.0 traces of peptone; 1 ml micronutrient solution, and a pH of 5.6. The flasks were incubated at 28°C under stationary conditions upto 12 days. Cellulase activity was determined by incubating 0.1 ml culture filtrate in 1.4 ml of 0.05 M acetate buffer, pH 5.6 and 0.5 ml of 1% carboxymethylcellulose as substrate. The reaction mixture was incubated at 50°C for 1 h; glucose liberated was estimated colorimetrically (14).

Laccase and xylanase were purified by ammonium salt precipitation. The pallets (7000 g, 4°C, 20 min) were redissolved in 0.02M Acetate buffer pH 4.2 and dialysed against 0.025 M buffer at pH 4.2 and 10°C. The dialysates were purified by gel filtration on a Biogel P200 column (9) and eluted with 0.02M acetate buffer, pH 4.2, in 1 ml fractions. The fractions were assayed qualitatively for enzyme activities. Temperature and pH conditions for laccase and xylanase activities were standardized in guaiacol in 0.02 M acetate buffer and 0.05M citrate Phosphate buffer respectively.

Laccase and xylanase enzymes showed a steady increase during 5-10 days of growth at 28°C under stationary conditions.

Factors affecting growth and enzyme production: In absence of malt extract,, growth was poor, and no enzyme activity was obtained. Addition of malt extract (HiMedia) supported growth only whereas both laccase and xylanase were synthesized when malt extract (CRL) was added to the medium. A temperature of 37°C and stationary culture conditions resulted in a maximum growth (Table 1) at pH 5.6. In earlier studies (1,2) a pH of 4-4.5 was reported optimum for lignin decomposers. Laccase synthesis increased appreciably when growth temperature was increased from 28°C to 37°C and 42°C but was adversely effected by increase in pH from 5.6 to neutral and by shake conditions.

However, change in pH and temperatures did not affect xylanase production which was adversely affected by agitation. Both, laccase and xylanase enzyme activities were maximum after 9-10 days of growth.

For phenol oxidase, the culture spot inoculated onto agar, gradually produced a brown zone around itself (Barendamm test) which increased in diameter indicating extracellular nature of phenol oxidase. Burges (16) attributed this brown zone due to melanin production.

Decolourization of Poly R-478: An agar extract in water showed a considerable decrease in A°max of the dye at 515 nm and 350 nm of decolourization by the fungus, indicating the presence of either LiP or MnP.

Enzyme kinetics: Xylanase was precipitated by addition of ammonium sulphate to 40%

Table 1 Optimisation of cultural conditions for growth of *P. tigrinus*

Cultural conditions	pH	Growth (g/flask)		
		28°C	37°C	42°C
Stationary	5.6	0.25	0.5	0.02
	7.0	0.10	-	-
Shaking	5.6	0.02	-	-

concentration, while laccase was obtained in both the 60% and 80% salt precipitated fractions.

A pH of 4.2, temperature of 42°C, and incubation period of about 1-2 were found optimum for maximum laccase activity while xylanase activity was maximum at pH 5.0, 60°C and 30-60 mins incubation.

The Km for laccase is 2.5×10^{-1} moles L^{-1}, and for xylanase is 1.26 gm L^{-1}.

Cellulase activity: Cellulase activity was obtained when the culture was grown in MTV medium however no activity was detectable when grown in MMG medium.

The data indicated that biomass production and enzyme activity are not correlated. A similar observation in *Phanerochate chysosporium* was also reported earlier (15).

The optimum pH of 4.2 for laccase activity in the present studies compares with a pH 4.0 in *Lentinus edodes* (9) and 3.5 in and *A. mellea*, pH for 4.5 for ligninolytic enzymes, viz. LiP of *P. chrysosporium* (6).

The lignocellulolytic capacity of the fungus *Panus tigrinus* indicates its potential use as a biodegrader besides nutritive supplement for foods.

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