EFFECT OF ANTIBIOTICS ON LIGNINOLYTIC ENZYMES PRODUCTION FROM STEREUM OSTREA AND PHANEROCHAETE CHRYSOSPORIUM UNDER SUBMERGED FERMENTATION

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ABSTRACT

The effect of different antibiotics (chloramphenicol, ampicillin, neomycin, erythromycin and tetracycline) on production of ligninolytic enzymes-laccase (Lac), lignin peroxidase (LiP) and manganese peroxidase (MnP) from Stereum ostrea (S. ostrea) and Phanerochaete chrysosporium (P. chrysosporium) have been studied. All the antibiotics tested at a concentration of 200 ppm affected the fungal growth, release of extracellular protein content and ligninolytic enzymes production to different extent. Though growth and secretion of extracellular protein by S. ostrea were comparable to those of P. chrysosporium in presence of tetracycline, but yields of laccase enzyme by S. ostrea were higher than laccase titres of P. chrysosporium by more than 18 folds on the peak production. S. ostrea yielded titres of 55 units of laccase/ml as against 3.0 units of laccase/ml on the 6th day of incubation. S. ostrea also exhibited activities of other ligninolytic enzymes – LiP and MnP higher than the reference culture. Increasing the concentrations of tetracycline enhanced ligninolytic enzymes production from S. ostrea and P. chrysosporium.

Keywords: Ligninolytic enzymes, S. Ostrea, P. Chrysosporium

INTRODUCTION

Lignin is an abundant natural aromatic polymer occurring in the woody tissue of higher plants. Due to its hydrophobicity and complex random structure lacking regular hydrolysable bonds, lignin is poorly biodegraded by microorganisms. The best degraders of lignin are white-rot fungi that produce laccase (Lac), lignin peroxidase (LiP) and manganese peroxidase (MnP) (EC. 1.11.1.13) [1]. Lignin degrading white-rot fungi and their ligninolytic enzymes find potential applications in the pulp and paper industry, detoxification of environmental pollutants, decolorization of dyes, cosmetics (including hair dyes), food and beverages, clarification/stabilization of fruit juices, clinical diagnostics, enzymatic conversion of chemical intermediates and upgrading of animal feeds (Mnussi et al., 2002). Some wood-degrading fungi contain all three major ligninolytic enzymes such as Lac, LiP and MnP, while others may contain only one or two of these enzymes (Hatakka, 1994; Pelaez et al., 1995). Certain organisms of white-rot fungi – Phanerochaete chrysosporium, Trametes versicolor, Pleurotus ostreatus, Bjerkandera adusta, Irpex lacteus, Lentinus tigrinus, Phanerochaete sordida, Phlebia radiata, Pleurotus eryngii, and Polyporus ciliatus have drawn more attention for lignolytic enzymes [3]. S. ostrea known as false turkey-tail fungus is a white-rot fungus belonging to family Stereaceae and most common in hard wood forests and is relatively characterized for lignolytic enzymes. S. ostrea has been recently shown to secrete laccase into extracellular medium [4]. There was confusion prevailing about ability of S. ostrea to secrete MnP in extracellular medium in view of contradictory results of two studies [5, 6]. According to our recent study [3], S. ostrea has been recognized to produce all three major ligninolytic enzymes – Lac, LiP and MnP. Low levels of extracellular ligninolytic enzymes production by white-rot fungi are not sufficient for their commercial applications. Therefore, in order to enhance production of ligninolytic enzymes various conventional and modern experimental approaches like mutagenesis and recombinant DNA technology are being targeted. Many workers have reported the use of different antibiotics such as ampicillin, tetracycline (Garber and Yoder, 1983), streptomycin sulfate and penicillin G (Rogers et al., 1989) for successful prevention of bacterial proliferation to get luxuriant fungal growth. In the present study, attempts have been made to understand the growth, secretion of extracellular protein content and production of Lac, LiP and MnP from S. ostrea and P. chrysosporium in the liquid medium in the presence of different antibiotics.

MATERIALS AND METHODS

S. ostrea was kindly supplied by Prof. M.A. Singaracharya, Department of Microbiology, Kakatiya University, Andhra Pradesh, India and was isolated from wood logs. The reference culture, Phanerochaete chrysosporium was obtained from IMTECH, India. The fungal cultures were maintained at 300 C on 2% Koroljova-Skorobogat'ko medium [8] because of good growth [3]. The maintenance medium was prepared according to Koroljova et al., [8] containing the following composition (g/l): 3.0 peptone, 10.0 glucose, 0.6 KH2PO4, 0.001 ZnSO4, 0.4 K2HPO4, 0.0005 FeSO4, 0.05 MnSO4, 0.5 MgSO4, 2.0 agar (pH 6.0). The stock solutions of antibiotics (chloramphenicol, ampicillin, neomycin, erythromycin and tetracycline) were filter sterilized and added to the sterilized medium at a final concentration of 200 ppm Sterile Koroljova-Skorobogat'ko medium was dispersed into sterile 250 ml Erlenmeyer flasks at a rate of 50 ml of broth supplemented with different antibiotics per flask. The flasks were inoculated with homogenized mycelial suspension and incubated in an orbital shaker (Orbitek, Chennai, India) at 30°C and speed of 150 rpm. All the flasks with growing cultures of S. ostrea and P. chrysosporium were withdrawn for processing with analytical methods.

Analytical methods

Fungal biomass estimation

The cultures - S. ostrea and P. chrysosporium in the flasks at regular intervals were aseptically filtered through pre-weighed Whatman No-1 filter paper to separate mycelial mat and culture filtrate. The filter paper along with mycelial mat was dried at 70°C in an oven until constant weight and this weight was recorded. Difference between the weight of the filter paper bearing mycelial mat and weight of only filter paper represented biomass of fungal mat. Fungal growth was expressed in terms of g/flask. Filtrate collected was used as a source of MnP.

Protein estimation

An aliquot of culture filtrates of S. ostrea and P. chrysosporium with appropriate dilution was used for estimation of soluble protein content according to Lowry et al., [9]. Bovine serum albumin was used as protein standard.

Enzyme Assay

Activities of ligninolytic enzymes in the cultural filtrate of both fungal cultures were estimated following the standard protocols. Laccase activity was assayed using 10mM guaiacol in 100mM acetate buffer...
(pH 5.0) containing 10% (V/V) acetone. The change in absorbance of the reaction mixture containing guaiacol was monitored at 470 nm ($\varepsilon = 6740 \text{ M}^{-1} \text{ cm}^{-1}$) for five minutes of incubation (12). Laccase activity was expressed in International Units (IU) where one unit corresponded to the amount of enzyme that oxidized one micromole of guaiacol per minute. Lignin peroxidase activity was determined by oxidation of veratryl alcohol at 310 nm ($\varepsilon = 9,300 \text{ M}^{-1} \text{ cm}^{-1}$) (39). The reaction mixture was composed of 0.5 ml culture filtrate, 0.4 mM H$_2$O$_2$ and 50 mM tartaric acid (pH 2.5) and 2 mM veratryl alcohol. The enzyme activity was expressed in IU where one unit of LiP corresponded to the amount of enzyme that oxidized one micromole of veratryl alcohol per min. MnP activity was determined by oxidation of phenol red at 610 nm (28). The assay mixture includes 0.5 ml culture filtrate, 0.25 M sodium lactate (pH 4.5), 0.5% bovine albumin, 200 mM MnSO$_4$, 2.0 mM H$_2$O$_2$ (prepared in 0.2 mM sodium succinate buffer pH 4.5) and 0.1% phenol red. The changes in absorbance of reaction mixture was monitored at 610 nm ($\varepsilon = 22,000 \text{ M}^{-1} \text{ cm}^{-1}$) for 5 min. MnP activity was expressed in IU where one unit of MnP was defined as the amount of enzyme that oxidized one micromole of phenol red per min.

RESULTS AND DISCUSSIONS

Since media composition and growth conditions are important factors affecting the production of extracellular ligninolytic enzymes and consequently lignin degradation [11], the influence of antibiotics on Lac, LiP and MnP production by fungal cultures in the culture medium was examined in the present study. Though cultures were monitored for growth, secretion of extracellular protein and MnP at 2-day interval for period of 10 days in all experiments, only results of 6th day incubation are presented in the present study because of peak production at this sampling time.

Effect of antibiotics on biomass, extracellular protein content and ligninolytic enzyme production

Cultivation of $S$. ostrea and $P$. chrysosporium on Koroljova medium supplemented with different antibiotics did not improve the growth of both cultures as reflected by biomass in comparison to biomass of the same cultures on unsupplemented medium (control) (Fig. 1). $S$. ostrea produced maximum biomass of 1.114 g/flask on the 10th day of incubation as against 1.000 g/flask in respect of $P$. chrysosporium.

Growth of both organisms on the medium with supplementation of antibiotics resulted in secretion of protein into the medium in considerable proportions (Fig. 2). Among antibiotics supplemented in this study, only tetracycline caused more release of extracellular protein of 800 and 710μg/ml into the medium by $S$. ostrea and $P$. chrysosporium on 8th day of incubation (Fig. 2).

Both cultures exhibited Lac, LiP and MnP activity when grown on medium in the presence of antibiotics (Table 1). Production of enzymes by both cultures touched peak on 6th day of incubation and there afterwards declined (data not shown). Maximum yields of Lac, LiP and MnP were 55, 0.897, 5.45 U/ml was recorded as against only 3.0, 0.864, 22.51 U/ml in respect of $S$. ostrea and $P$. chrysosporium on 6th day of incubation in presence of tetracycline (Table 1). $S$. ostrea displayed activity of even LiP and MnP on higher side than $P$. chrysosporium. Thus results clearly shows that $S$. ostrea was better than the reference culture on the score of Lac, LiP and MnP production.
The following conclusions can be drawn from the results of the present study. The white-rot fungus *S. ostrea* produces a complete ligninolytic system Lac, LiP, and MnP in the presence of antibiotics. Lac appears to be a dominant component in the ligninolytic system of *S. ostrea* and *P. chrysosporium*. For production of ligninolytic enzymes, *S. ostrea* culture is more promising and potential culture than the reference culture *P. chrysosporium*.

**REFERENCES**


