LIGNINOLYTIC ENZYME PRODUCTION BY LENTZITES BETULINUS ON SELECTED PLANT RAW MATERIALS

ABSTRACT: To get a better insight into the ligninolytic system of Lenzites betulinus, the effect of wheat straw and oak sawdust, as carbon sources, on production of Mn-oxidizing peroxidases and laccase, under solid-state and submerged fermentation, was studied. Obtained results revealed considerable differences related to the both factors affecting enzyme activities. Wheat straw was more favorable carbon source for Mn-oxidizing peroxidases and oak sawdust for laccase activity. Solid-state fermentation of wheat straw was optimal for Mn-dependent peroxidase activity (72.1 U/l). In contrary to this, submerged fermentation of the same residue gave the highest level of versatile peroxidase activity (25.4 U/l). The peak of laccase activity was noted during solid-state fermentation of oak sawdust (32.3 U/l), while this enzyme was not detected under submerged fermentation of any plant residues.

KEY WORDS: laccase, Lenzites betulinus, oak sawdust, Mn-dependent peroxidase, versatile peroxidase, wheat straw

INTRODUCTION

Lenzites betulinus (L.) Fr. is a plant pathogen and common white-rot species grows scattered or clustered on deciduous wood such as birch, beech and oak, and less frequently on coniferous woods. This species has been used in traditional Chinese medicine for haunch and femoral pain, acropathy, apoplexy, and cold (Ren et al., 2006), and a wide range of medicinal effects were confirmed. A water extract of L. betulinus demonstrated mild antitumor activity against Sarcoma 180 (Ikekawa et al., 1968), and the methanol extract showed free-radical scavenging (Lee et al., 1996), immunomodulating (Fujimoto et al., 1994) and antimicrobial activity (Yamac and Bilgili, 2006).

Nowadays, the ligninolytic enzyme systems of different mushrooms present an important area of interest which aim is to introduce the types and characteristics of produced enzymes, as well as the possibilities of their application in various biotechnological processes. Considering the literature data where ligninolytic enzyme system of L. betulinus has been only rarely reported, the aim of this study was to discover whether cultivation conditions...
affect the production and activity of the selected enzymes during solid-state and submerged fermentation of different types of agricultural wastes by this species.

MATERIAL AND METHODS

Organism and growth conditions

*Lenzites betulinus* BEOBF 500 was collected from *Populus* sp. in Permksiy Kray, Russia, and maintained on malt agar medium (MA) in the culture collection of the Institute of Botany, Faculty of Biology, University of Belgrade.

The inoculum was prepared by inoculation of 100 ml of synthetic medium (glucose, 10.0 g l\(^{-1}\); NH\(_4\)NO\(_3\), 2.0 g l\(^{-1}\); K\(_2\)HPO\(_4\), 1.0 g l\(^{-1}\); NaH\(_2\)PO\(_4\) \(\times\) H\(_2\)O, 0.4 g l\(^{-1}\); MgSO\(_4\) \(\times\) 7 H\(_2\)O, 0.5 g l\(^{-1}\); yeast extract, 2.0 g l\(^{-1}\); pH 6.5) with 25 mycelial agar plugs (0.5 cm in diameter) of 7-day-old *L. betulinus* culture on MA. The Erlenmayer flasks (250 ml) were incubated at room temperature (22 ± 2°C) on a rotary shaker (100 rpm) for 7 days. The obtained biomass was washed (3 times) by sterile distilled water (dH\(_2\)O) and homogenized with 100 ml of dH\(_2\)O in laboratory blender.

The ligninolytic enzyme activity was determined after solid-state (SSF) and submerged (SF) fermentation of wheat straw and oak sawdust. SSF was carried out at 25°C in 100 ml flasks containing 2 g of wheat straw or 5 g of oak sawdust soaked with 10 ml of modified synthetic medium (without glucose, with nitrogen in a concentration of 25 mM, and pH 6.5). Homogenized suspensions (of 5 ml) were used for inoculation of one flask. On the other hand, SF was performed in 250 ml flasks containing 2 g of wheat straw or 5 g of oak sawdust, grounded to pass at least 40-mash screen, and 50 ml of modified synthetic medium, at room temperature (22 ± 2°C) on a rotary shaker (100 rpm). Prepared flasks were inoculated with 3 ml of homogenized inoculum. Samples from flasks were harvested successively after 7 and 10 days of cultivation. Extraction of ligninolytic enzymes obtained under SSF conditions was performed by stirring samples with 50 ml of dH\(_2\)O on magnetic stirrer for 10 minutes at the temperature of 4°C and centrifugation (4°C, 3000 rpm, 10 min). Obtained biomasses after SF fermentation were separated by centrifugation (4°C, 3000 rpm, 10 min) and supernatants were used to estimate the enzyme activity. Five replicates for each plant residue were analyzed.

Enzyme activity assays

Laccase (Lac) and Mn-oxidizing peroxidases [Mn-dependent peroxidase (MnP) and versatile peroxidase (VP)] were determined spectrophotometrically using CECIL CE 2501 spectrophotometer. Lac activity was estimated by monitoring the A\(_{436}\) change related to the rate of oxidation of 50 mM 2,2’-azino-bis-[3-ethylthiazoline-6-sulfonate] (ABTS) (ε\(_{436}\) = 29300 M\(^{-1}\) cm\(^{-1}\)) in 0.1 M
phosphate buffer (pH 6.0), at 35°C. The reaction mixture contained: buffer, ABTS, and sample ($V_{tot} = 1$ ml). Mn-oxidizing peroxidases activities were determined with 3 mM phenol red ($\varepsilon_{610} = 22000$ M$^{-1}$cm$^{-1}$) as a substrate in a buffer with the following content: succinic acid disodium salt, albumin from bovine serum, and DL-lactic acid sodium salt (pH 4.5). The reaction mixture ($V_{tot} = 1$ mL) contained: buffer, sample, 2 mM H$_2$O$_2$ and phenol red, with or without 2 mM MnSO$_4$ (for MnP and VP, respectively). Reaction was stopped by 2M NaOH.

Enzymatic activity of 1 U is defined as the amount of enzyme that transforms 1 $\mu$mol of substrate/min.

RESULTS AND DISCUSSION

Studied species was able to grow at both substrates and produce all three assayed enzymes. The calculated enzyme activity at different time points shows significant differences. Lower MnP activity was associated with initial phases of experiment while Lac showed the opposite effect. Production of ligninolytic enzymes was dependent on the carbon source and cultivation type. Wheat straw appeared to be a better carbon source for activity of Mn-oxidizing peroxidases, while oak sawdust was more favorable for Lac production. SSF of wheat straw was optimal for MnP activity (72.12 U$^{-1}$), while VP activity reached peak after 10 days of wheat straw SF (29.09 U$^{-1}$) (Figures 1, 2). *L. betulinus* was able to produce Lac by solid state cultivation on both wheat straw and oak sawdust substrates, but its activity was not noted in liquid medium (Figures 1,2). The obtained results demonstrated that initial stages of SSF are associated with considerable Lac production, and the highest level was noted after oak sawdust SSF (32.25 U$^{-1}$) (Fig. 1).

![Fig. 1 – Effect of carbon source and duration of cultivation on Mn-oxidizing peroxidases and laccase activity in solid state cultivation of *Lenzites betulinus*](image-url)
Lekoûnougou et al. (2008) came to the same conclusion research- ing *Trametes versicolor*. Namely, the authors suggested that under conditions of beech SSF Mn-oxidizing peroxidases are not involved in initial phases of wood material colonization. The lack of Lac production could be strongly related with aeration level, i.e. the oxygen deficit under SF conditions, as oxygen is required for the enzyme catalytical processes (Reinhammer, 1984; Thurston, 1994; Egger et al., 1996; Solomon et al., 1996). In contrary to this, solid-state cultivation has proved to be particularly suitable for the Lac production since it reproduces the natural living conditions of fungi (Pandey et al., 1999; Moo–Young et al., 1983). Based on this fact, higher enzyme activities during SSF in comparison to SF could also be explained.

Although numerous studies of different medicinal effects of *L. betulinus* were done, data on its ligninolytic enzyme system have not been reported until now. This type of study is significant because, recently, special attention in biotechnology has been given to obtaining large amounts of low-cost enzymes by usage of various agricultural and food industry residues, which can often be serious environmental pollutants. Residues could be mineralized to low-molecular weight compounds by various ligninolytic enzymes, which are better digested by animals and could be used in further processing, such as in producing feeds and basic commodities for different industrial purposes.

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ПРОДУКЦИЈА ЛИГНИНОЛИТИЧКИХ ЕНЗИМА LenzaTes BETULINUS НА ОДАБРАНОМ БИЉНОМ ОТПАДНОМ МАТЕРИЈАЛУ

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Резиме

Многобројна истраживања су показала различите медицинске ефекте екстраката Lenzytes betulinus. Међутим, проучавање лигнинолитичког ензимског система ове врсте до сада није рађено. Да ли услови култивације утичу на производњу и активност Mn-оксидујућих пероксидаза и лаказе у току чврсте и течне ферментације пшеничне сламе и пиљевине храста овом врстом било је питање на које смо желели да добијемо одговор. Добијени резултати су показали да и тип култивације и извор угљеника утичу на ензимску активност. Пшенична слама је била знатно бољи извор угљеника за активност Mn-оксидујућих пероксидаза, а пиљевина храста за активност лаказе. Чврста ферментација пшеничне сламе је била оптимална за активност Mn-зависне пероксидазе (72.1 Ul^-1), док је при течној ферментацији истог материјала добијен највиши ниво активности верзатил пероксидазе (25.4 Ul^-1). Пик лаказне активности је забележен 10. дана чврсте ферментације пиљевине храста (32.3 Ul^-1). Међутим лаказна активност није била детектована након течне ферментације тестираног биљног материјала.