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LACCASE PRODUCTION BY *LEPISTA SORDIDA*

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SHORT COMMUNICATION

ABSTRACT

A *Lepista sordida* laccase has been characterized. Laccase and manganese peroxidase were detected in liquid medium with ammonium phosphate, yeast extract and ammonium molybdidate as nitrogen sources after 3 days of cultivation. Laccase optimal temperature and pH were 45ºC and 3.5, respectively.

Key words: *Lepista sordida,* laccase, enzyme.

Laccases are oxidases that transfer electrons to dioxygen, yielding water with the concomitant oxidation of a wide range of reducing substrates, including phenolic compounds and aromatic amines. Laccases have been found in many fungi, higher plants and insects (9,29) and are believed to be involved in several microbial and cellular events, such as fungal virulence (22), conidial pigmentation (31), lignification (6) and plant defense (18). However, their most important role is in lignin degradation and humification processes (19). Laccases have been the subject of much research for a broad variety of practical applications because of their low specificity. Some practical applications include: removal of xenobiotics from aqueous streams (4), removal of phenolic compounds from wine (26), biosensors (34), decolorisation of dyes (27) and effluents contaminated with industrial wastes (23), drug analysis (1), ethanol production (14) and removal of lignin from woody tissues (2). The wide range of substrates that can be attacked by laccases has led to a search for new sources of the enzyme (11,20,21). *Lepista sordida* (Schum.: Fr.) Singer is a basidiomycete fungus that produces an excellent tasting light purple mushroom. It grows in bare locations such as waysides and parks. No information on its laccase activity has yet been reported in the literature, as far as we know. In this study, the production and characterization of a laccase produced by *L. sordida* are presented.

Lepista sordida was isolated from a mushroom found at the campus of Federal University of Viçosa, Minas Gerais State, Brazil. The fungal culture was maintained through periodic transfer onto potato dextrose agar (PDA) plates at 25ºC and pH 5.5. Agar disks taken from the active borders of PDA cultures were transferred into 60 mL of sterile liquid medium (pH 5.5) containing $CaCl_2$ (0.5 g/L; MERCK, Darmstadt, Germany), $FeCl_3$ (0.0012 g/L; MERCK, Darmstadt, Germany), $(NH_4)_2HPO_4$ (0.15 g/L; MERCK, Darmstadt, Germany), NaCl (0.025 g/L; MERCK, Darmstadt, Germany), KH_2PO_4 (0.5 g/L; SIGMA, MO, USA), $MgSO_4.7H_2O$ (0.15 g/L; SIGMA, MO, USA), glucose (2.5 g/L; SIGMA, MO, USA), yeast extract (0.1 g/L; SIGMA, MO, USA), $MnCl_2.4H_2O$ (0.14 g/L; MERCK, Darmstadt, Germany), H_3BO_3 (0.118 g/L; SIGMA, MO, USA), $ZnSO_4.7H_2O$ (0.57 g/L; MERCK, Darmstadt, Germany) and $(NH_4)_6Mo_7O_{24}.4H_2O$ (0.011 g/L; SIGMA, MO, USA). Erlenmeyers flasks (125 mL) were incubated at 25ºC in the dark without shaking. Periodically, samples were collected for analysis and the volume of liquid medium withdrawn was replenished from a sterile stock. The liquid cultures were filtered using Millipore membranes (0.45 mm) and the filtrates were used for enzyme assays. Laccase (EC 1.10.3.2) activity

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was determined by the oxidation of 2,2'-azino-*bis* (3-ethylthiazoline-6-sulfonate) (ABTS; SIGMA, MO, USA) at 37ºC according to Buswell *et al.* (3). The reaction mixture (1 mL) contained 600 µL enzyme extract, 300 µL sodium acetate buffer pH 5.0 (0.1 M) and 100 µL ABTS solution (1 mM). Oxidation was followed via the increase in absorbance at 420 nm (ε_{420} = 36,000 $M^{-1} . cm^{-1}$). One unit of enzyme activity is defined as the amount of enzyme oxidizing 1 mmol of ABTS per minute. Manganese-dependent peroxidase (EC 1.11.1.13) activity was assayed with phenol red as the substrate (ε_{610} = 4460 M^{-1} cm^{-1}) according to Kuwahara *et al.* (13). The reaction mixture (1 mL) contained 500 µL enzyme extract, 100 µL phenol red solution (1.0 g/L), 100 µL sodium lactate pH 4.5 (250 mmol/L), 200 µL bovine serum albumin solution (0.5%), 50 µL manganese sulfate (2 mmol/L) and 50 µl H_2O_2 (2 mmol/L) in sodium succinate buffer pH 4.5 (20 mmol/L). One unit of enzyme activity is defined as the amount of enzyme oxidizing 1 µmol of substrate per minute. A control sample was tested using all the method conditions without manganese in order to demonstrate the manganese-dependency of the peroxidase. Lignina peroxidase (EC 1.11.1.14) activity was determined by monitoring the oxidation of veratryl alcohol to veratraldehyde at 37ºC as indicated by an increase in A_{310} (ε = 9300 M^{-1} cm^{-1}) following the methodology by Tien and Kirk (30). The reaction mixture (2.5 mL) contained 500 µL enzyme extract, 500 µL H_2O_2 (2 mmol/L), 500 µL veratryl alcohol solution (10 mmol/L) and 1.0 mL sodium tartarate buffer pH 3.0 (10 mmol/L). One unit of enzyme activity is defined as the amount of enzyme oxidizing 1 µmol of substrate per minute. Temperature was held constant at 37ºC to investigate optimal pH, while the pH was held at 5.0 to determine the optimal temperature. Kinetic tests were carried out at 37ºC and pH 5.0, and were calculated based on a Lineweaver-Burk plot (17). The kinetic properties were obtained in the stationary state by non-linear regression using Enzifitter (16) software.

L. sordida was cultivated for 57 days without shaking in the dark. In the early stages of growth, mycelia were white, but turned purple with the time. Laccase and manganese peroxidase activities were detected (Fig. 1), but no lignin peroxidase activity could be detected. The optimal temperature for laccase activity was 45ºC. This is in agreement with other research findings. Laccase from *Phellinus ribis*, for instance, reached highest activity at 50ºC (20), and laccase from *Pycnoporus cinnabarinus* presented maximum stability below 50ºC (7). *L. sordida* laccase presented highest activity at pH 3.5, with a second activity peak at pH 6.0. These data are consistent with other studies showing optima pH values for other fungal species, like *Pycnoporus cinnabarinus* (pH 4.0) (7), *Lentinula edodes* (pH 4.0) (5) and *Coprinus cinereus* (4.0) (25). The two activity peaks detected suggest the presence of an isozyme or indicate that this laccase presents two distinct pH optima, like observed in *Agaricus bisporus* (32) and *Monocillium saxena* (28). No laccase activity was detected at pH values higher than 6.5, which is in

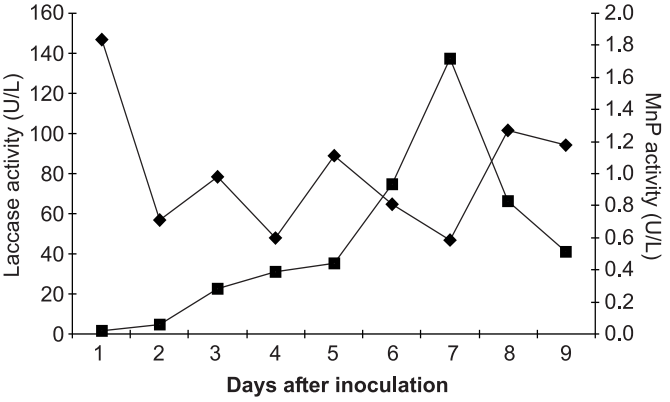

Figure 1. Time course of laccase (■) and manganese peroxidase (◆) production by *L. sordida* at 25ºC and pH 5.5 under static cultivation.

agreement with findings for other fungal species, such as *L. edodes* (5,33). The Km_{app} was 101.6 µM and $Vmax_{app}$ was 0.84 $\mu M.s^{-1}$. Similar values were found in other fungal laccases (11,20,24,25). It can be observed in Fig. 1 that laccase activity peaked at day 42 (137 U/L), and then started to decrease, indicating that the culture medium used was efficient in inducing laccase activity. It is known, however, that the activity profile can be altered by varying carbon and nitrogen sources. Galhaup *et al.* (8) investigated the effect of carbon sources on laccase production by *Trametes pubescens* and observed peaks of 5 and 10 U/mL when cultivated with α-cellulose and lactose, respectively. Addition of glucose and frutose stimulated laccase activity, generating values as high as 60 U/mL. Some species, like the well-studied *Phanerochaete chrysosporium,* present laccase activity only when nitrogen concentration falls to limiting levels (10,12,15). On the other hand, *L. edodes* exhibits decreasing laccase activity following the depletion of nitrogen (3,15). The culture media used in the present study was efficient to stimulate laccase activity in *L. sordida* with the nitrogen sources used ammonium phosphate (0.15 g/L), yeast extract (0.1 g/L) and ammonium molibdate (0.011 g/L). We have reported for the first time laccase activity of *L. sordida*, a fungal species about which little information is available. Laccase is a very important enzyme, with many applications, and the search for new laccase sources is important given the large quantities necessary for all its potential uses.

RESUMO

Produção de lacase por *Lepista sordida*

Uma lacase de *Lepista sordida* foi caracterizada. O fungo produziu lacase e manganês peroxidase em meio líquido com fosfato de amônio, extrato de levedura e molibdato de amônio

como fontes de nitrogênio 3 dias após a inoculação. Temperatura e pH ótimos para lacase foram 45ºC e 3,5, respectivamente.

Palavras-chave: *Lepista sordida,* lacase, enzima

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