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Ligninase and Cellulase Activity of *Lentinula edodes* (Berk.) Pegler Strains in Different Culture Media

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This study was conducted with the objective of evaluating the growth of six strains of *Lentinula edodes* (LE1, LE2, LE3, LE4, LE5 and LE6) in five culture media and characterizes strains for enzyme production. The culture medium that provided the best mycelial growth of the strains was used for semi-quantitative tests of ligninase and cellulase activity. Samples for enzyme assays and determination of total proteins were collected at 7, 14, 21 and 28 days of incubation to building an enzymatic curve. The strain LE5 is the most aggressive, with higher mycelial growth rate and mycelial mass production in the basic medium added of 0.01% yeast extract. The addition of biotin and thiamine to the culture medium is not sufficient to substitute yeast extract. The performance of the strains with respect to the production of cellulase and ligninase was different. The LE6 strain excelled in the production of laccase, manganese peroxidase and β-glucosidase with 28 days of incubation and endo-β-1,4-glucanase with 14 days of incubation, while strain LE5 excelled in the production of exo-β-1,4-glucanase with 7 days of incubation.

Keywords: shiitake, biotin, thiamine, ligninase, cellulase.

Mushroom production has increased in recent decades and the shiitake (*Lentinula edodes*) occupies a prominent position currently being the second most cultivated edible mushroom, comprising about 25% of world production[1,2]. The production can be improved by the development of new cultivation technologies, but the selection of strains for commercial cultivation as well as for genetic breeding is an important step in this process.

The selection of strains is a factor of great importance, since they may differ to the mycelial growth rate, optimal spawning-run temperature, growing temperatures, nutritional needs and others[3]. The knowledge of the nutritional requirements of each strain is necessary for the definition of an ideal culture medium that enables a higher mycelial growth rate, which is important to avoid contamination and a faster step of spawn production.

The production of shiitake is conducted using substrates rich in lignocellulose so that the colonization of these substrates by *L. edodes* depends on the production of hydrolytic (i.e. cellulases and ligninases)[4,5]. Ligninase is a general term that refers to laccase, manganese peroxidase (MnP), and lignin peroxidase (LiP). White-rot fungi produce different combinations of enzymes; some species produce LiP and MnP, MnP and laccase, LiP and laccase while others species do not

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produce LiP or MnP[6]. The widely accepted mechanism for the enzymatic hydrolysis of cellulose involves the synergistic actions of endoglucanase (EC3.2.1.4), exoglucanase or cellobiohydrolase (EC 3.2.1.91) and β-glucosidase (EC 3.2.1.21)[7-10]. Since the secretion of these enzymes is essential for the substrate colonization, enzymatic characterization of *L. edodes* strains is an important step in selecting strains for mushroom cultivation.

The objective of the present work is (i) to evaluate the growth of *L. edodes* strains in different media, (ii) select those with the highest potential for producing cellulases and ligninases by semi-quantitative tests and (iii) quantitatively characterize these strains for the production of enzymes laccase, manganese peroxidase, lignin peroxidase, endo-1,4-β-glucanase, exo-1,4-β-glucanase and β-glucosidase in the growth medium selected as best for the strains.

MATERIALS AND METHODS

Microorganisms and cultivation media

Six strains of *L. edodes*: LE1, LE2, LE3, LE4, LE5 and LE6, belonging to the collection of microorganisms of the University of Lavras (UFLA) were evaluated. The stock cultures were maintained on malt agar medium (15 g L^{-1} of glucose; 15 g L^{-1} of malt extract; 15 g L^{-1} agar) and incubated at 25°C.

The strains were inoculated onto five different culture media: basic medium (10 g L^{-1} glucose; 1 g L^{-1} potassium phosphate monobasic; 0.5 g L^{-1} magnesium sulfate heptahydrate, 1 g L^{-1} ammonium sulfate, 0.5 g L^{-1} calcium chloride, 15 g L^{-1} agar); basic medium plus 0.01% yeast extract; basic medium and biotin at a concentration of 5 mg L^{-1}; basic medium and thiamine at a concentration of 100 mg L^{-1} and the basic medium with biotin and thiamine at concentrations of 5 mg L^{-1} and 100 mg L^{-1}, respectively.

Each culture medium was inoculated with a 10 mm diameter disc containing the mycelium in the center of the Petri dish. The plates were incubated at 25°C and the diameter of colonies was measured daily until the faster colony had grown throughout the entire plate. At the end, each culture were transferred to a flask containing 200 ml dH_2O and melted in micro-wave oven, washed with dH_2O using a 200 mesh sieve and the mycelial mass was dried at 65°C until constant weight. We did determine the dry weight of the mycelial mass to compare it to the growth rate (mg day^{-1} vs mm day^{-1}).

The experiment was carried out using three replicates and the means were compared by the Scott-Knott test using the SISVAR software for determining the culture medium that provided greater mycelial growth.

Semi-quantitative evaluation of cellulase and ligninase production

We performed a semi-quantitative evaluation of cellulase and ligninase production in order to select the strains for the following enzymes induction and production curves. The strains were selected according to the enzymatic index (EI)[11], defined by the following equation:

$$EI = \frac{\text{halo diameter}}{\text{colony diameter}}$$

A semi-quantitative evaluation of laccase was conducted with modifications of the Pointing method[12]. To the basic medium with 0.01% yeast extract, 0.1% ABTS, 2.2 azino-bis (3-ethylbenzothiazoline-6-sulfonic acid acid) was added and a 10 mm disc containing mycelia was inoculated in center of the plate. The incubation was conducted at 25°C for 5 days. Laccase production was observed by the formation of a green color in the culture medium. Diameters of the green halo and colony were measured daily to calculate the enzymatic index (EI).

Semi-quantitative evaluation of lignin peroxidase and manganese peroxidase was made according Pointing[12] with modifications. In the basic medium with yeast extract 0.01% Azure-B was added. A 10 mm disc containing mycelia was inoculated in the center of the plate. The incubation was conducted at 25°C for 14 days. The discoloration of the Azure B dye (Cl 52010) by fungi is positively correlated with the production of lignin peroxidase and manganese peroxidase[13].

The production of cellulase was made according Kasana *et al.*[14], with modifications. The basic medium with 0.01% yeast extract was prepared by replacing glucose by carboxymethylcellulose. A 10 mm disc containing mycelia was inoculated in the center of the plate. The incubation was carried out at 25°C for 3 days. After this incubation period, a solution of Lugol (6.67 g L^{-1} potassium iodide, 3.33 g iodine L^{-1}) was

added to cover the entire plate. The formation of an opaque yellow zone indicated the production of cellulase.

Cellulase induction curve

The basic medium plus 0.01% yeast extract was used, substituting glucose in the medium by carboxymethylcellulose, Avicel and cellobiose to induce endo-1,4-β-glucanase, exo-β-1,4 glucanase and β-glucosidase, respectively. For this 500 ml Erlenmeyer flask containing 250 ml of liquid medium was used.

The inoculation was made by adding 12-10 mm disks containing the mycelium of each strain selected from the semi-quantitative evaluation per vial. The incubation was performed at 25°C, at 110 rpm for 28 days. We conducted a completely randomized design (CRD) with a 3×4 factorial scheme, plots subdivided in time, withdrawing 8.3 ml from each sample at 7, 14, 21 and 28 days for enzyme assays and total protein determination.

Determination of the endo-1,4-β-glucanase activity (EC 3.2.1.4)

The endo-1,4-β-glucanase activity was determined in a reaction mixture containing 0.05 ml of the enzyme source, and 0.45 ml of 1% carboxymethylcellulose in a 0.05 mol L^{-1} (pH 5.0) sodium acetate buffer. The reaction mixture was incubated for 30 minutes at 50°C. The reaction was stopped by adding 1.5 ml of 1% p-hydroxybenzoic acid hydrazide. The mixture was kept at 100°C for 5 minutes and then chilled on ice. Readings were taken at 410 nm in a spectrophotometer by monitoring the liberation of glucose molecules[15]. The blank was made with all the components of the reaction mixture, replacing the enzyme source by the culture medium without inoculation.

The absorbance readings were plotted against a standard glucose curve and specific enzyme activity was expressed as m mol min^{-1} mg $protein^{-1}$ (U mg $protein^{-1}$).

Determination of the exo-1,4-β-glucanase activity (EC 3.2.1.91)

The exo-1,4-β-glucanase activity was determined in a reaction mixture containing 0.05 ml of the source enzyme and 0.45 ml of 1% microcrystalline cellulose in a 0.05 mol L^{-1} (pH 5.0) sodium acetate buffer. The reaction mixture was incubated for 30 minutes at 50°C. The reaction was stopped by adding 1.5 ml of 1% p-hydroxybenzoic acid hydrazide. The mixture was kept at 100°C for 5 minutes and then chilled on ice. Readings were taken at 410 nm in a spectrophotometer by monitoring the liberation of glucose molecules[15]. The blank was made with all the components of the reaction mixture, replacing the enzyme source by the culture medium without inoculation.

The absorbance readings were plotted against a standard glucose curve and specific enzyme activity was expressed as m mol min^{-1} mg $protein^{-1}$ (U mg $protein^{-1}$).

Determination of β-glucosidase activity (EC 3.2.1.21)

The β-glucosidase activity was determined in a reaction mixture containing 0.3 mL of p-nitrophenyl-β-D-glucopyranoside (0.02 mol L^{-1}) in a 0.05 mol L^{-1} sodium acetate buffer (pH 5.0) and 0.2 ml of the enzyme source. The reaction mixture was incubated for 30 minutes at 50°C. The reaction was stopped by adding 0.5 ml of sodium carbonate (1 mol L^{-1}). Readings were taken at 405 nm in a spectrophotometer by monitoring the liberation of p-nitrophenol molecules[16]. The blank was made with all the components of the reaction mixture, replacing the enzyme source by the culture medium without inoculation.

The absorbance readings were plotted against a standard p-nitrophenol curve and specific enzyme activity was expressed as m mol min^{-1} mg $protein^{-1}$ (U mg $protein^{-1}$).

Ligninase production curve

The basic medium was supplemented with 0.01% yeast extract for the production of ligninases (laccase, manganese peroxidase and lignin peroxidase). For this, we used 500 ml Erlenmeyer flasks containing 250 mL of liquid medium.

The inoculation was made by adding 12-10 mm disks containing the mycelium of each strain per vial. The incubation was performed at 25°C, at 110 rpm for 28 days. We conducted a completely randomized design (CRD) with a 3x4 factorial scheme, plots subdivided in time, withdrawing 8.3 ml from each sample at 7, 14, 21 and 28 days for enzyme assays and total protein determination.

Determination of laccase activity (EC 1.10.3.2)

The laccase activity was determined by indirect spectrophotometric method using 2.2 azino-bis ethylbenzothiazoline (ABTS) in a 1 mL reaction mixture containing 0.3 mL of 0.1 mol L^{-1} (pH 5.0) sodium acetate buffer, 0.1 ml of 1 mmol L^{-}

[1] ABTS (in water) and 0.6 ml of the enzyme source. The reaction mixture was incubated for 5 minutes at 37°C and the oxidation of ABTS was measured by the increase in absorbance at 420 nm[17]. The blank was made with all the components of the reaction mixture by replacing the enzyme source by the culture medium without inoculation.

One unit of enzyme specific activity was defined as the amount of enzyme capable of oxidizing 1 mmol of ABTS per minute (ε_{420} = 3.6 x 10^4 mol^{-1} L cm^{-1}) per mg of protein.

Determination of manganese peroxidase activity (EC 1.11.1.13)

The manganese peroxidase activity was measured using the phenol red (1.0 g L^{-1}) as the substrate (= 4,460 mol^{-1} L cm^{-1})[18]. The 1 ml reaction mixture containing 0.5 ml of enzyme extract, 0.1 ml of phenol red solution (1.0 g L^{-1}), 0.1 ml of pH 4.5 sodium lactate (250 mmol. L^{-1}), 0.2 ml bovine albumin (0.5%), 0.05 ml of manganese sulfate (2 mmol. L^{-1}) and 0.05 ml of H_2O_2 (2 mmol.L^{-1}) in succinate buffer (20 mmol.L^{-1}), pH 4.5 was incubated for 15 minutes at 37°C and the reaction was stopped by adding 0.04 ml of NaOH (2 mol L^{-1}). The absorbance was measured at 610 nm against a blank made with all the components of the reaction mixture, replacing the enzyme source by the culture medium without inoculation.

One unit of enzyme specific activity was defined as the amount of enzyme capable of oxidizing 1 mmol of phenol red per minute per mg protein.

Determination of lignin peroxidase activity (EC 1.11.1.14)

The lignin peroxidase activity was determined by monitoring the absorption at 310 nm of veratraldehyde formtion (ε = 9,300 mol^{-1} L cm^{-1}) through the oxidation of veratryl alcohol[19]. The 2 ml reaction mixture consisted of 0.5 ml of enzyme source, 0.5 ml of H_2O_2 (2 mmol L^{-1}), 0.5 mL of veratryl alcohol (10 mmol L^{-1}) and 1 ml of sodium tartararo (0.125 mol L^{-1}) pH 3.0, incubated for 5 minutes at 37°C. The blank was made with all the components of the reaction mixture, replacing the enzyme source by the culture medium without inoculation.

One unit of enzyme specific activity was defined as the amount of enzyme capable of oxidizing 1 mmol of the veratryl alcohol to veratraldehyde per minute per mg protein.

Determination of total protein

Protein content was determined by the Bradford method[19] with some modifications, which consists of a reaction with 0.2 ml sample and 0.8 ml of concentrated Bradford reagent plus stirring. After 5 minutes, spectrophotometer readings were taken at 595 nm. Protein concentration was obtained by plotting on standard bovine serum albumin curve (BSA).

RESULTS AND DISCUSSION

Mycelial growth and dry weight in different culture media

Significant differences were observed for mycelial growth in the five culture media tested for *L. edodes* strains (Tables 1 and 2). LE4 was the slowest growing strain in all tested media. Considering the mycelial mass production from the same media, similar results were obtained. LE5 was, generally, the fastest growing strain, with some exceptions, but this high growth rate did not result in a higher mycelial mass production, except for the basic medium added of 0.01% yeast extract. In

Table 1. Mycelial growth (mm day^{-1}) of *Lentinula edodes* strains in different culture media

Culture medium	Mycelial growth (mm dia^{-1})											
	LE		LE2		LE3		LE4		LE5		LE6	
Basic medium	6.1	A b	6.0	C b	6.0	D b	4.8	A c	6.7	D a	6.9	B a
Basic medium and 5 μgL^{-1}biotin	6.7	A c	6.4	B d	7.3	A a	4.8	A e	7.0	B b	6.9	B c
Basic medium and 100 μgL^{-1} thiamine	6.4	A c	5.9	C d	6.9	B a	4.4	B e	7.0	B a	6.6	C b
Basic Medium biotin and thiamine	6.7	A b	5.2	D d	7.0	B a	4.5	B e	6.9	C a	6.4	C c
Basic medium and 0.01% yeast extract	6.6	A c	7.4	A b	6.6	C c	4.4	B d	7.8	A a	7.5	A b

Means followed by the same uppercase letters in the columns and lowercase in the rows do not differ by Scott-Knott test at 5% probability

Table 2. Dry mass (mg day^{-1}) of *Lentinula edodes* strains in different culture media

Culture medium	Dry mass (mg dia^{-1})											
	LE		LE2		LE3		LE4		LE5		LE6	
Basic medium	1.07	A a	0.71	A b	1.14	A a	0.50	A b	1.14	B a	1.00	B a
Basic medium and 5 ìgL^{-1}biotin	0.71	B b	0.93	A a	0.57	C c	0.50	A c	0.57	B c	0.64	C b
Basic medium and 100 ìgL^{-1} thiamine	0.50	B d	0.93	A a	0.50	C d	0.36	A e	0.64	B c	0.79	C b
Basic Medium biotin and thiamine	1.29	A a	1.07	A b	0.79	B c	0.41	A d	0.93	B c	0.64	C c
Basic medium and 0.01% yeast extract	1.07	A c	1.36	A c	1.21	A c	0.86	A c	3.86	A a	2.43	A b

Means followed by the same uppercase letters in the columns and lowercase in the rows do not differ by Scott-Knott test at 5% probability

this culture medium, LE5 produced 3.86 mg day^{-1} of mycelial mass, which is very high, compared to the other strains, followed by LE6, with 2.43 mg day^{-1}. It is important to make a comparison between the mycelial growth rate (mm day $^{-1}$) and mycelial mass production (mg day^{-1}) since fast mycelial growing do not always correspond to a higher mycelial production.

Surprisingly, we did not observe a clear evidence for a positive effect from the addition of biotin, thiamin or biotin and thiamin to the culture media, compared to the basic medium. Only LE3 and LE5 showed a higher mycelial growth (mm day^{-1}) in media containing those vitamins, however, the same effect it was not observed for the mycelial mass production (mg day^{-1}). Considering that some basidiomycete fungi are deficient in thiamin and biotin[20], it was expected that the media supplemented with biotin and thiamine, separately or in combination, would provide a higher growth of *L. edodes* strains, compared to the basic medium. In opposite, we observed a clear evidence for a positive effect for the addition of yeast extract to the basic medium, for both, mycelial growth (mm day^{-1}) and mycelial mass production (mg day^{-1}). This result suggests that *L. edodes* needs more vitamins than biotin and thiamin, which are provided by yeast extract.

Considering the best results of mycelial growth rate (mm day^{-1}) and mycelial mass production (mg day^{-1}), the basic medium supplemented with yeast extract was selected for the semi-quantitative ligninase and cellulase production tests and the enzyme curve.

Semi-quantitative evaluation of the cellulase and ligninase production

According to Pointing[12], the color of the medium becomes green from oxidation of ABTS 2,2 ‘azino-bis (3-ethylbenzothiazoline-6-sulfonic acid acid) in the presence of laccase, indicating laccase activity. Strains LE4 and LE6 had a higher enzymatic index with five days of incubation, while the LE5 strain showed the lowest enzymatic index (Table 3). Strains LE1, LE2 and LE3 had intermediate values with no difference between them.

The strains LE1, LE3, LE4 and LE5 were able to decolorize the Azure B dye, indicating the production of lignin peroxidase and/or manganese peroxidase. The dye discoloration was not observed at the same incubation time for these strains, being observed after seven days for strains LE3 and LE5, eight days for LE1 and twelve days for strain LE4. The discoloration occurred only under the colonies, so that the enzymatic index value was <1.

In the semi-quantitative evaluation of cellulase for all strains tested, the opaque yellow color occurred only under the colonies, resulting in an enzymatic index <1. According Kasana *et al*.[14], the method using Lugol, besides allowing observation of rapid results when compared with

Table 3. Enzymatic index for laccase at five days of incubation

Strain	Enzymatic index
LE5	2.09 c
LE2	2.64 b
LE3	2.95 b
LE	3.25 b
LE6	5.00 a
LE4	5.33 a

Means followed by the same letter do not differ by Scott-Knott test at 5% probability

other methods, can be used for bacteria, actinomycetes and fungi. The authors just reported the observation of positive or negative results using this method, not mentioning any relationship about the quantity of cellulase production.

Hankin and Anagnostakis[21] reported a study about cellulase production in solid medium containing CMC by four species of fungi (*Trichoderma viride*, *Penicillium* sp., *Peziza ostracoderma* and *Fusarium* sp.) and only *Penicillium* showed zones of cellulolytic activity beyond the edge of the colony, in function of its slower growth. In our work, the clear areas did not exceed the edge of the colonies, possibly, due to the rapid growth of the *L. edodes* strains. Therefore, these results not necessarily indicate low cellulase production.

Lealem and Gashe[22] recommended an enzymatic index ≥ 2 to select potential enzyme producers, but the enzymatic index was not an

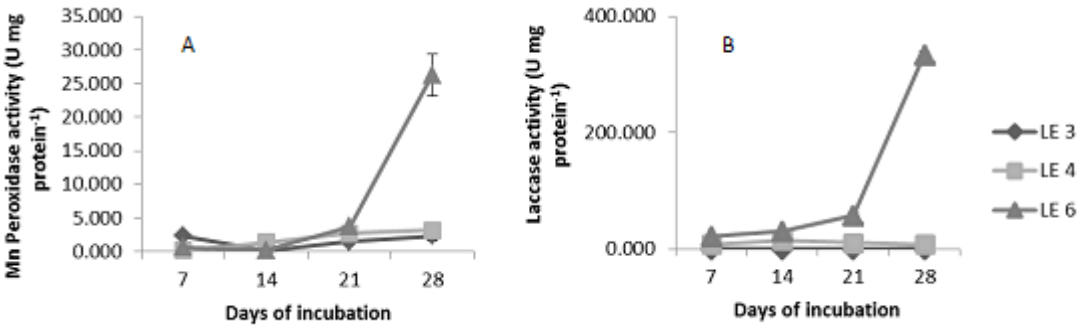

Fig. 1. Manganese peroxidase (A) and laccase (B) production curve for strains LE3, LE4 and LE6 of L. edodes

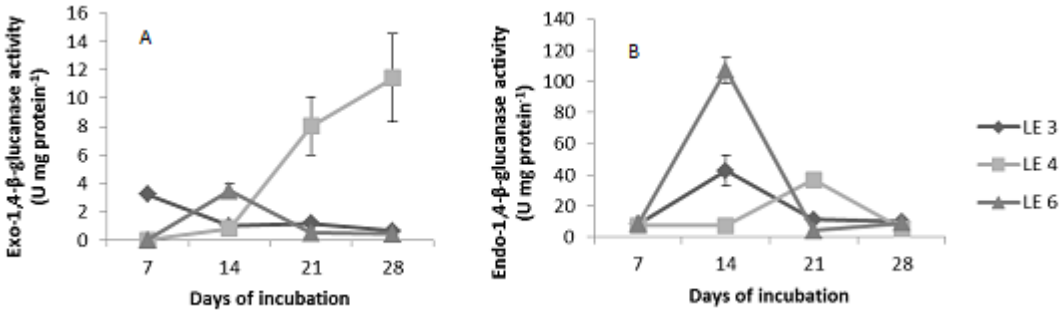

Fig. 2. Induction curve of exo-1,4-β-glucanase (A) and endo-1,4-β-glucanase (B) for strains LE3, LE4 and LE6 of *L. edodes*

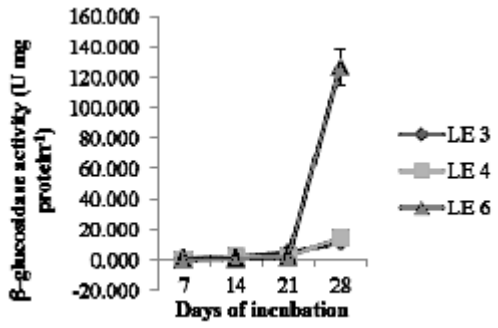

Fig. 3. Induction curve of β-glucosidase for strains LE3, LE4 and LE6 of *L. edodes*

appropriate parameter to compare the production of cellulase, lignin peroxidase and/or manganese peroxidase enzymes from *L. edodes* strains.

LE4 and LE6 strains were selected to develop the ligninase production curve because these strains have shown the highest enzymatic index for laccase. Furthermore, the LE4 strain showed ability to decolorize the Azure B dye, indicating production of lignin peroxidase and/or manganese peroxidase. The strain LE3 showed, statistically, the same behavior as LE1 and LE2 strains in the semi-quantitative evaluation of laccase. Among these strains, LE1 and LE3 decolorized the azure B dye, but the reaction with

the ABTS test for laccase was immediate for LE3, so this strain was also selected to perform the ligninase production curve.

Since it was not possible to select the strains for carrying out the induction curve of cellulases by semi-quantitative evaluation, the same strains used in the ligninase production curve were chosen for the cellulase induction curve too.

Enzymatic curve

Strains LE3 and LE4 had a low activity with a slight tendency to increase manganese peroxidase activity until 28 days of incubation, but for strain LE6, which show a similar activity until 21 days, it was observed a sudden increase at the end. The activity increased from 0.642 U mg protein^{-1} at 7 days to 26.2 U mg protein^{-1} at 28 days incubation (Fig. 1A). Similar results were observed for laccase activity, with a sudden increase of activity for LE6, reaching maximum activity of 333.7 U mg protein^{-1} at 28 days of incubation, while strains LE3 and LE4 showed low activity throughout the experiment (Fig. 1B). No activity was detected for lignin peroxidase from any of the strains evaluated.

L. edodes has been previously described as a good producer of laccase and manganese peroxidase, while lignin peroxidase apparently does not play a significant role in its enzymatic ligninolytic system[4,15,23-25]. Research results have shown that incubation of veratryl alcohol with *L. edodes* enzyme extract produces several products, very different from those identified from other *white-rot fungi*[26]. Thus, the absence of LiP in extracts of *L. edodes* raise the following question: does this fungus really not produce LiP enzyme or can it not be detected by conventional analytical methods?[27].

Makkar *et al*.[4] who evaluated the production of manganese peroxidase and laccase from *L. edodes* in medium containing sawdust and rice bran after forty days of incubation, observed activities of 11.3 U mg protein^{-1} for laccase and 10.2 U mg protein^{-1} for manganese peroxidase. In our work, the strain LE4, with 21 days of incubation, showed laccase activity of 11.7 U mg protein^{-1}, approximately equal to that found by Makkar *et al*.[4]. The strain LE6, however, showed higher laccase activity than that found by these authors in all stages of cultivation evaluated. Strains LE3 and LE4 presented manganese peroxidase activity below that reported in the literature, in all stages of cultivation evaluated, while strain LE6 showed higher activity of this enzyme than that reported by these authors after 28 days of incubation.

Orth, Royse and Tien[28] obtained an extract to evaluate the activity of manganese peroxidase, laccase and lignin peroxidase from 12 species cultivated in oak sawdust for 30 days at 23ºC. Some of the tested species, *L. edodes*, *Pleurotus eryngii* and *Pleurotus pulmonarius* showed manganese peroxidase activity of 11.8, 19.0 and 4.47 U mg protein^{-1}, respectively. These authors reported that lignin peroxidase activity was not observed and, for all fungi species tested, the laccase activity was much lower in relation to manganese peroxidase. The opposite was observed in our study for the strains LE4 and LE6, which showed laccase activity superior to manganese peroxidase activity.

Strains LE4 and LE6 showed no activity of exo-1,4-β-glucanase with seven days of incubation, while for the strain LE3, the peak activity of this enzyme (3.228 U mg protein^{-1}) was obtained at seven days of incubation. For LE6, the peak was obtained at 14 days, followed by a decrease until the end. The LE4 strain was the highest exo-1,4-β-glucanase producer, with increasing activity until reach 11.4 U mg protein^{-1} of activity at the end of incubation (Fig. 2A).

The activity of endo-1,4-β-glucanase for LE3 and LE6 strains was maximum at 14 days of incubation, with values of 42.8 U mg protein^{-1} and 107.2 U mg protein^{-1}, respectively. The LE4 strain showed peak activity of this enzyme at 21 days of incubation (Fig. 2B). Thus, for induction of endo-1,4-β-glucanase, incubation time could be reduced to 14 days for the strains LE3 and LE6, and to 21 days of incubation for LE4.

None of the strains started to produce β-glucosidase until 21 days, suggesting a need of a longer period of adaptation to the medium containing cellobiose. As observed for laccase and manganese peroxidase, LE6 showed a sudden increase of activity, standing out from the other strains with an activity of 126.7 U mg protein^{-1} (Fig. 3).

Pereira Júnior *et al*.[29] evaluated the production of endoglucanase, exoglucanase and β-glucosidase of *L. edodes* for ten days in three different culture media, SC medium containing Avicel (0.5%) or carboxymethylcellulose (0.5%),

peptone, calcium nitrate, monobasic potassium phosphate and magnesium sulfate heptahydrate and JP medium containing Avicel (1.7%) and peptone. It was observed that *L. edodes* showed endoglucanase activity in the supernatant of the three media tested, however, exoglucanase activity was detected only in the SC medium. The β-glucosidase enzyme was not detected in any of the media. The endoglucanase activity in the JP medium 820 U mg protein^{-1}. In SC medium containing Avicel, the peak production of endoglucanase was 620 U mg protein^{-1} with 48 h of incubation and the exoglucanase was 74 U mg protein^{-1}. In SC medium containing carboxymethylcellulose, there was a reduction in endoglucanase activity to 200 U mg protein^{-1} and the exoglucanase activity reduced to less than 50%.

Makkar *et al.*[4], evaluating the β-glucosidase production of *L. edodes* in medium containing rice bran and sawdust, after 40 days of incubation, obtained an extract with an enzyme activity of 240 U mg protein^{-1}. The values found by these authors are much higher than those found in the present work, which can be explained by the different composition of the medium, since it has been demonstrated that the activity of these enzymes varies accordingly.

Therefore we conclude that the strain LE5 of *L. edodes* is the most aggressive, with higher mycelial growth rate and mycelial mass production in the basic medium added of 0.01% yeast extract. The addition of biotin and thiamine to the culture medium is not sufficient to substitute yeast extract in the culture medium. The performance of the strains with respect to the production of cellulase and ligninase was different. The LE6 strain excelled in the production of laccase, manganese peroxidase and β-glucosidase with 28 days of incubation and endo-β-1,4-glucanase with 14 days of incubation, while strain LE5 excelled in the production of exo-β-1,4-glucanase with 7 days of incubation.

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