

LIGNINASES PRODUCTION BY BASIDIOMYCETES STRAINS ON LIGNOCELLULOSIC AGRICULTURAL RESIDUES AND THEIR APPLICATION IN THE DECOLORIZATION OF SYNTHETIC DYES

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ABSTRACT

Wood rotting Basidiomycetes collected in the “Estação Ecológica do Noroeste Paulista”, São José do Rio Preto, São Paulo State, Brazil, concerning Aphyllophorales order and identified as *Coriolopsis byrsina* SXS16, *Lentinus strigellus* SXS355, *Lentinus* sp SXS48, *Picnoporus sanguineus* SXS 43 and *Phellinus rimosus* SXS47 were tested for ligninases production by solid state fermentation (SSF) using wheat bran or rice straw as culture media. *C. byrsina* produced the highest laccase (200 U mL⁻¹) and *Lentinus* sp produced the highest activities of manganese peroxidase (MnP) and lignin peroxidase (LiP) (7 and 8 U mL⁻¹, respectively), when cultivated on wheat bran. The effect of N addition on enzyme production was studied in medium containing rice straw and the data showed an increase of 3 up to 4-fold in the laccase production compared to that obtained in SSF on wheat bran. The laccases presented optimum pH at 3.0-3.5 and were stable at neutral pH values. Optimum pH for MnP and LiP activities was at 3.5 and between 4.5 and 6.0, respectively. All the strains produced laccase with optimum activities between 55-60°C while the peroxidases presented maximum activity at temperatures of 30 to 55°C. The crude enzymes promoted decolorization of chemically different dyes with around 70% of decolorization of RBBR and cybacron blue 3GA in 6h of treatment. The data indicated that enzymes from these basidiomycetes strains are able to decolorize synthetic dyes.

Keywords: laccase, manganese peroxidase, lignin peroxidase, Basidiomycetes, decolorization, synthetic dye

INTRODUCTION

Basidiomycetes fungi are the most efficient lignin-degrading organisms that produce mainly laccases (EC 1.10.3.2), lignin peroxidase (EC 1.11.10.14) and manganese peroxidase (EC 1.11.1.13). These enzymes present a non-specific biocatalyst mechanism and have been used for bioremediation process due to their ability to degrade azo, heterocyclic, reactive and polymeric dyes (2,12). Prospection for fungi able to secrete high levels of lignin-degrading enzymes and novel enzyme variants, with desirable properties for biotechnological applications, is

aimed. On the other hand, alternative low cost substrates like agricultural residues for enzyme production using solid state fermentation (SSF) offer economic and environmental advantages.

Around 10 thousand types of synthetic dyes are widely used in the textile, paper, printing and leather tanning industries, corresponding to 8 x 10⁵ tons per year (28). The chemical classes of synthetic dyes mostly used in industrial process are azo, anthraquinone, sulfur, indigoid, triphenylmethyl (trityl) and phthalocyanine derivatives, although other types have many applications such as triazine (12). Besides the color effect, the

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majority of these compounds are toxic, carcinogenic and highly persistent in the environment. Conventional biological treatment of wastewaters is not effective for dyes degradation, so that a number of chemical and physical techniques have been used to remove them, including adsorption to inorganic or organic matrices, decolorization by photocatalysis and oxidation process (3,4,16). However some ligninolytic microorganisms are capable of degrade a wide variety of pollutant substances resembling lignin or its derivative, being is an attractive method due to low cost, specificity and the possibility of a total mineralization of these compounds (32).

In this study, agricultural wastes and industrial by-products were used to evaluate ligninases production by different strains of Basidiomycetes isolated from decaying wood in forests in Brazil and their enzymes were evaluated by decolorizing different synthetic dyes.

MATERIAL AND METHODS

Isolation of microorganisms

Wood-rotting Basidiomycetes were collected at the “Estação Ecológica do Noroeste Paulista”, located between São José do Rio Preto and Mirassol, in the São Paulo State, Brazil. The strains cultures were isolated and purified as described previously (33).

Media, cultivation of fungi and enzyme production

Pure cultures were tested for production of laccase, manganese peroxidase and lignin peroxidase by solid state fermentation (SSF) in 250 mL Erlenmeyer flasks containing 5g of sterilized wheat bran or 3g of rice straw both with approximately 70% moisture content. Each flask was inoculated with three disks (1.5 cm diameter) of mycelium grown on PDA (potato dextrose agar) medium. The flasks were incubated at 27°C for five weeks. The solid fermented material from one Erlenmeyer flask was mixed with 40 mL distilled water, stirred for 40 min, filtered under vacuum and centrifuged (10 min, 20,000 x g). The supernatant was used as the crude enzyme solution.

The effect of nitrogen concentration of the culture media on ligninase production was evaluated using rice straw as media for SSF and by addition of 10 mL of (NH₄)₂SO₄ solution in concentration sufficient to obtain 0.3, 1.0, 3.0, 6.0 or 21 milligram of N per gram of solid substrate. The effect of different Nitrogen sources was evaluated by addition of 10 mL of (NH₄)₂SO₄, (NH₄)₂HPO₄ or a mixture of (NH₄)₂HPO₄ and (NH₄)₂SO₄ solutions in concentration necessary to obtain 3 mg of N per gram of solid substrate (rice straw). The control was carried out using distilled water instead of nutrient solution.

Enzyme assays

Laccase activity was determined *via* the oxidation of 2,2'-azino-bis(3-ethylbenzthiazoline)-6-sulfonate (ABTS). The reaction mixture, containing 0.1 mL of 0.3 mM ABTS in 100

mM of sodium acetate (pH 3.5) and 0.1 mL of crude enzyme solution, was incubated at 40°C for 1 min. The ABTS oxidation was monitored by the increase in absorbance at 420 nm ($\epsilon = 36,000 \text{ M}^{-1} \text{ cm}^{-1}$). One Unit was defined as 1 μmol of ABTS oxidized per minute and activity was expressed in U per mL per min (22).

Manganese peroxidase (MnP) was assayed in a mixture of 0.9 mL of 50 mM of sodium lactate buffer (pH 4.0) containing 0.3 mM of manganous ions (Mn²⁺) and 0.1 mL of crude enzyme solution, at 40°C. The reaction was started by addition of 40 μM H₂O₂ and absorbance at 270 nm ($\epsilon = 8100 \text{ M}^{-1} \text{ cm}^{-1}$) was monitored (13). One Unit was defined as 1 μmol complex Mn³⁺-lactate formed per mL per min.

Lignin peroxidase (LiP) activity was measured by monitoring the oxidation of veratryl alcohol at 310 nm (31) at 40°C. The reaction mixture contained 0.1 mL of enzyme solution, 0.8 mL of 2 mM veratryl alcohol in 10 mM sodium acetate pH 3.5. The reaction was started by adding 0.5 mL of 0.1 mM H₂O₂. One Unit was defined as 1 μmol of veratraldehyde released per minute using extinction coefficient of 9300 M⁻¹ cm⁻¹.

Enzyme characterization

The optimum pH was determined by measuring the activity at 40°C in various buffers: sodium-malonate (pH 3.0-5.0), citrate-phosphate (pH 5.0-7.0) and tris-HCl (pH 7.0-8.5). The optimum temperature was assayed by incubating each reaction mixture at 20-90°C in the optimum pH.

The enzymes thermostability was evaluated by incubating the enzymes solutions at various temperatures (10°C-90°C) for 1h at pH 5.0. An aliquot was withdrawn and placed on ice before assaying for remaining enzyme activity at the optimum pH and temperature.

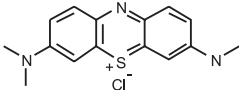
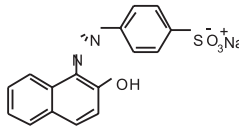
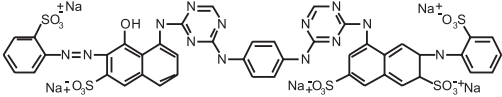
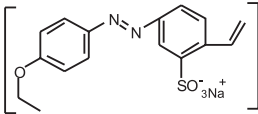
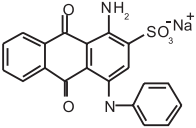
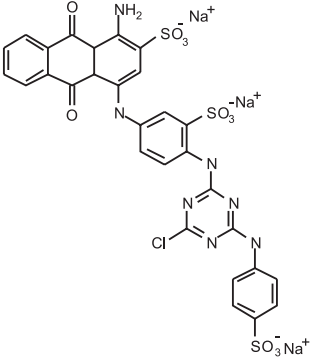
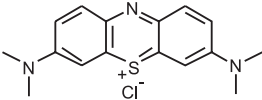
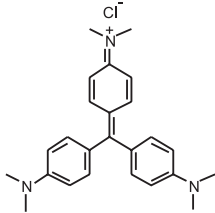
The enzyme stability under different pH values was determined dispersing the crude enzyme (1:1) in 0.1M buffer solutions pH 3.0-5.0 (sodium acetate), pH 5.0-7.0 (citrate-phosphate), pH 7.0-8.5 (tris-HCl) and pH 8.5-11.0 (glycine-NaOH) and maintaining it at 25°C for 24h. An aliquot was used to determine the remaining activity at the optimum pH and temperature.

Decolorization of synthetic dyes by crude enzyme solution

Decolorization of dyes was monitored in the mixture containing 0.8 mL of dye solution, 0.99 mL of 0.2 M acetate buffer pH 3.5, 0.1 mL of crude enzyme and 0.01 mL of deionized water or ABTS solution (concentration in reactive mixture 0.3 mM), at 40°C. The enzyme solution obtained from SSF on wheat bran for 3 weeks was diluted or concentrated so as to the enzyme activities were around 100U·mL⁻¹ of laccase and around 5 U·mL⁻¹ for LiP and MnP.

Visible spectra were recorded using a DU spectrophotometer (Beckman). The rate of decolorization was expressed as the percentage decrease of maximum visible wavelength of

Table 1. Chemical and structural properties of dyes.

Name	Chemical structure	Type	λ of max. Abs..
Azure B		Triazine	645
Orange II		Azo	485
Reactive Red 120		Azo	535
Chrysophenine		Azo	400
Remazol Brilliant Blue R		Antaquinona	590
Cybacron blue 3GA		Triazine	600
Methylene blue		Triazine	660
Crystal violet		Triphenylmethane	590

Concentration of dyes solutions (mg l^{-1}) Azure B=5/ orange II=10/ Reactive red= 25, chrysophenine =10, remazol brilliant blue R =60, cybacron blue 3GA=75 methylene blue=7, crystal violet =5.

absorbance (Table 1). Control tests were conducted with crude enzyme replaced by deionized water. Experiments were performed in triplicate and results were expressed as the mean values.

Chemicals

Veratryl alcohol and ABTS were purchased from Fluka (Buchs, Switzerland). Synthetic dyes (Table 1) were purchased from Sigma.

RESULTS AND DISCUSSION

Fungi and ligninases production in SSF

Seventy-five strains were isolated as pure cultures which were distributed in 7 orders, 17 families, and 42 species of basidiomycetes as presented previously (33). Five strains of white rot of the Aphyllophorales order, *Corioliopsis byrsina* SXS16 (Polyporaceae), *Lentinus strigellus* SXS355 and *Lentinus* sp SXS48 (Lentinaceae), *Picnoporus sanguineus* SXS 43 (Polyporaceae) and *Phellinus rimosus* SXS47 (Hymenochaetaceae) were selected for subsequent ligninase production assays.

The highest laccase activity ($200 \text{ U}\cdot\text{mL}^{-1}$) was obtained from *C. byrsina* in the 3th week of fermentation in medium containing wheat bran, although a good activity was detected in crude enzyme from *P. sanguineus* in the same medium ($93\cdot\text{U}\cdot\text{mL}^{-1}$ after 3th week) (Fig. 1a). When rice straw was used as substrate, laccase production was lower than in wheat bran for all strains (Fig. 2b). In terms of growth, there was no visible difference in the colonization of the substrates among the strains since all the media were completely colonized by mycelial biomass after two weeks. The laccase activity detected in the culture media was 5-fold higher than those obtained by Baldrian, Snajdr (2) using SSF of barley bran and wheat bran, respectively.

The *Lentinus* sp was a good producer of peroxidases (MnP and LiP) on both substrates (Fig. 1 c-f). The values of MnP and LiP obtained from culture media of *Ph. rimosus*, *P. sanguineus* and *C. byrsina* were very high compared to those obtained from others fungi such as *Pleurotus ostreatus* and *Trametes versicolor* (2,35), *T. modesta*, *T. hirsuta* and *T. versicolor* (24), *Phebia tremellosa* and *Coriolus versicolor* (29). In relation to substrate colonization stages, it was observed that the beginning of the fungal growth was associated with peroxidases production while higher laccase activities were detected after 3th week of cultivation. These results were opposite of those obtained by Lkounougou *et al.* (21) with *T. versicolor* which the laccase expression was observed during the first days of colonization of wood.

The production of the extracellular ligninolytic enzymes is strongly affected by the nature and amount of the nutrients in the substrate, specially nitrogen, and microelements (5,7,9). Agricultural wastes such as wheat bran and rice straw have

been used for enhanced ligninase production in submerged and solid state fermentation (1,25). However, data about strict effect of available N sources in the substrate on extracellular ligninolytic enzyme induction in wood-rotting basidiomycetes have been conflicting. For some species, the ligninolytic enzyme production is suppressed by high nitrogen concentration while for other, high concentration of this nutrient stimulate ligninase production (14,18- 20).

In order to valuate the influence of content of available nitrogen in the substrate on ligninase production by the fungi in SSF was carried out assay using the basidiomycetes strains that presented the most homogeneous and fast growth in medium composed by rice straw (*Ph. rimosus* and *C. byrsina*) and only data about laccase were showed. Different sources and concentrations of this nutrient was added to rice straw since this substrate contained a lower nitrogen concentration (1.1%) than wheat bran (2.4%) and afforded a rapid growth of the microorganisms but low ligninase activities.

The data in Fig. 2 demonstrate that the laccase production by *Ph. rimosus* and *C. byrsina* was stimulated in concentration until 6 and 3 $\text{mg}\cdot\text{g}^{-1}$ respectively, reaching around 4-fold the activity obtained in the control medium for *C. byrsina* and 2-fold for *Ph. rimosus*. For both species laccase production was decreased with 21 $\text{mg}\cdot\text{g}^{-1}$ of nitrogen. The laccase activity in medium of *C. byrsina* cultivation was higher than that *Ph. rimosus* for all N concentrations, except for 21 $\text{mg}\cdot\text{g}^{-1}$.

Laccase production by *C. byrsina*, *Ph. rimosus* was greatly influenced by type of N source ($3 \text{ mg}\cdot\text{g}^{-1}$) (Fig. 3). For the both fungi, the highest enzyme activity was observed in medium on which $(\text{NH}_4)_2\text{HPO}_4$ was added with a maximum activity of 3.8-fold of the control for *C. byrsina* and 4-fold for *Ph. rimosus*. The data indicated that *C. byrsina* and *Ph. rimosus* responded, in terms of laccase induction, mainly the P addition. Although, there was not apparent difference between grown of the fungi in the substrates, the biomass was not quantified and therefore, the stimulation of the grown by N and P with consequently higher enzyme production can not to be discarded.

Enzyme characterization

The laccase activities in the crude filtrates obtained from basidiomycetes cultures were characterized in terms of pH and temperature optima and stability (Table 2). The laccases produced by all the fungi exhibited maximum activity at pH 3.0-3.5 and stability in acidic to alkaline pH. It is common for basidiomycetes laccases pH optima in the acidic range, although they are more stable at neutral to alkaline pH values (23,27,34). pH optimum for MnP and LiP activities were variable in function of the strains producer with optimum values between 4.5 to 6.0 for enzymes from *C. byrsina* and *Ph. rimosus*.

The laccases showed thermophilic properties with optimum activity at 55-65°C, similar to thermostable laccases from basidiomycetes strains (POXA1) (17) and from *Peniophora* sp.

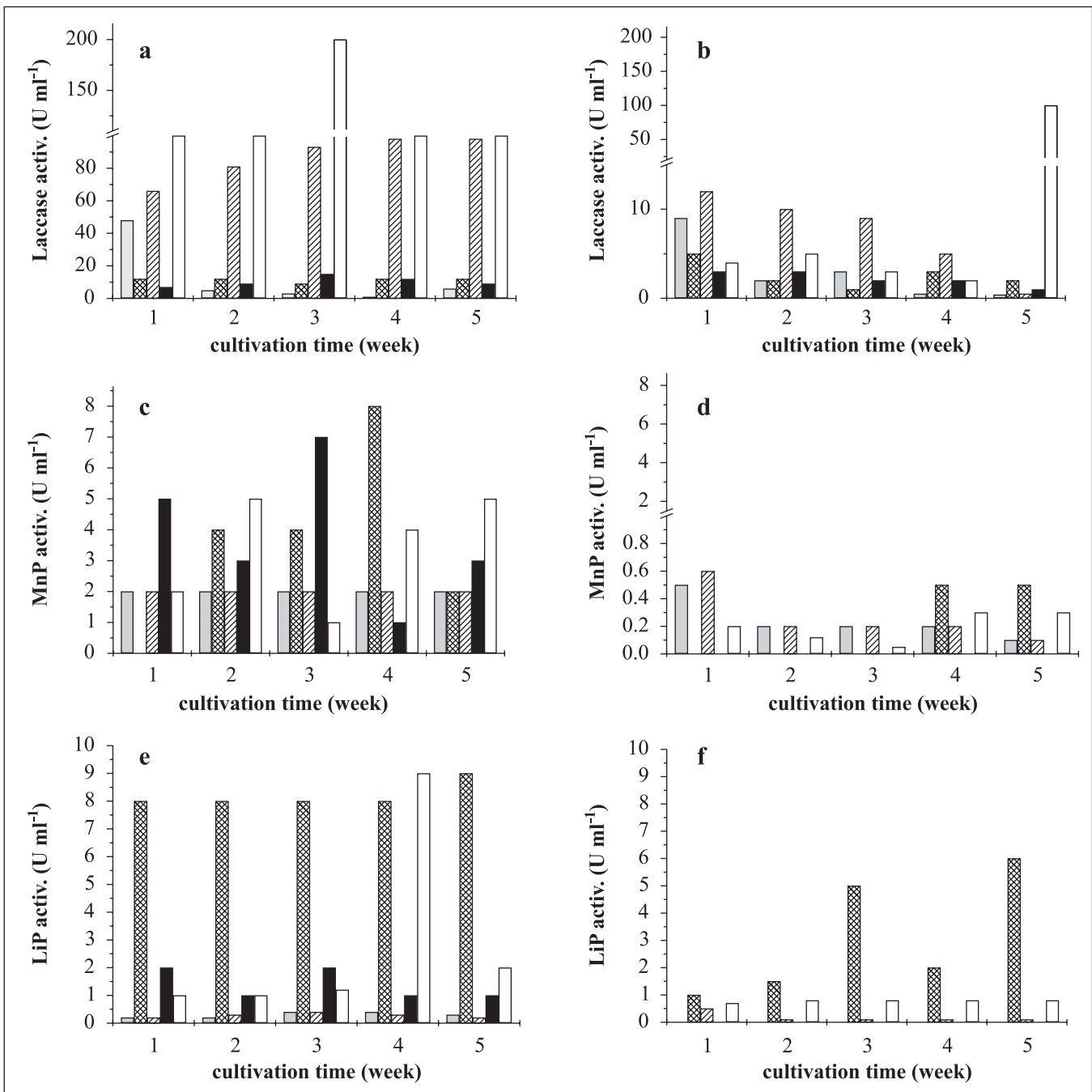


Figure 1. Production of laccase (a,b), MnP (c,d) and Lip (e,f) by solid state fermentation using wheat bran (a,c,e) and rice straw (b,d,f) as substrate by basidiomycetes strains. Light gray = *Lentinus strigellus*; = *Lentinus* sp.; = *Picnosporus sanguineus*; = *Phellinus rimosus*; = *Coriolopsis byrsina*.

(19) although they were more sensitive to temperatures above 40°C when in absence of substrate when compared to that mentioned laccases.

The maximum peroxidase activities did not exceed 40°C but MnP from majority of strains were stable up to 70°C when in absence of substrate.

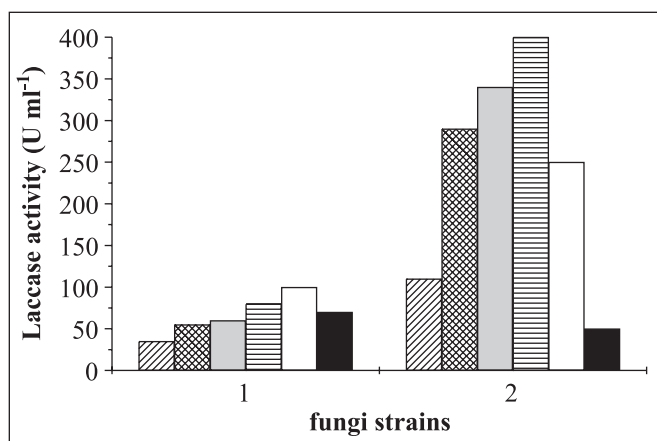


Figure 2. Effect of nitrogen addition on laccase production by *Phellinus rimosus* (1) and *Coriolopsis byrsina* (2) in SSF using rice straw as substrate, after 3 weeks of fermentation. Nitrogen addition: ▨ none (control); ▩ 0.3 mg.g⁻¹; □ 1.0 mg.g⁻¹; ▧ 3.0 mg.g⁻¹; ▦ 6.0 mg.g⁻¹; ■ 21.0 mg.g⁻¹.

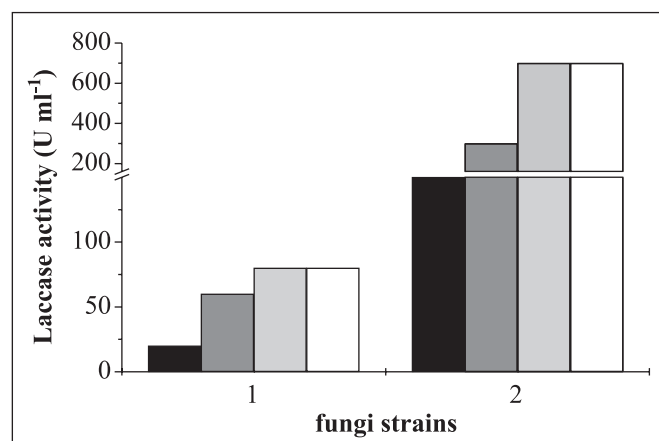


Figure 3. Effect of nitrogen source on laccase production by *Phellinus rimosus* (1) and *Coriolopsis byrsina* (2) in SSF using rice straw as substrate, after 3 weeks of fermentation. Nitrogen source (6 mg.g⁻¹): black= none (control); gray=(NH₄)₂SO₄; light gray=(NH₄)₂HPO₄; white=(NH₄)₂SO₄ + (NH₄)₂HPO₄.

Table 2. Biochemical properties of ligninases from basidiomycetes strains.

Enzyme/ Microorganism	Optimum pH	Optimum temperature (°C)	pH range preserving 70-100% of initial activity	Temperature (°C) range preserving 70-100% of initial activity
Laccase				
<i>Lentinus</i> sp.	3.0	65	4.0-6.0	10-55
<i>L. strigellus</i>	3.5	55-60	5.0-11	10-55
<i>P. sanguineus</i>	3.0	60-70	4.5-11	10-60
<i>C. byrsina</i>	3.5	60	7.5-9.0	10-45
<i>Ph. rimosus</i>	3.5	60	6.0-8.0	10-35
MnP				
<i>Lentinus</i> sp.	3.5-4.0	55	5.0-6.0	10-50
<i>L. strigellus</i>	3.0	40	3.0-10	10-75
<i>P. sanguineus</i>	3.5	40	3.0-11	10-75
<i>C. byrsina</i>	4.5-5.0	35	7.0-8.0	10-70
<i>Ph. rimosus</i>	5.0-6.0	30	5.0-7.0	10-45
LiP				
<i>Lentinus</i> sp.	3.5	40	5.0-6.0	10-40
<i>L. strigellus</i>	3.5	40	4.5-5.5	10-40
<i>P. sanguineus</i>	4.0-4.5	40	4.5-6.0	10-40
<i>C. byrsina</i>	4.5-5.0	40	5.5-7.0	10-70
<i>Ph. rimosus</i>	5.0-6.0	30	6.0-7.0	10-70

The important properties observed for crude ligninases were the stability in the large pH and temperatures range that permits the application of which in several environmental conditions.

Decolorization of synthetic dyes by crude enzymes

Crude enzymes obtained from cultivation of *Lentinus* sp., *L. strigellus*, *P. sanguineus* and *C. byrsina* on wheat bran for 3 weeks was tested for decolorization of structurally different

Table 3. Effect of crude ligninases from Basidiomycetes strains on discoloration of synthetic dyes.

Fungi producer of enzyme	Mediator	dyes															
		Orange II		Reactive Red 120		Chryso phenine		RBBR		Cybacron blue 3GA		Azure B		Methylene blue		Crystal violet	
		h	%	h	%	h	%	h	%	h	%	h	%	h	%	h	%
<i>Lentinus strigellus</i>	ABTS	48	74	48	27	48	0	48	66	48	66	48	22	48	32	24	65
	none	48	18	48	8	48	0	6	67	6	67	48	0	48	21	48	45
<i>Lentinus</i> sp.	ABTS	48	64	48	0	48	28	48	50	48	50	48	12	48	23	24	39
	none	48	15	48	0	48	27	6	58	6	58	48	24	48	13	24	51
<i>Picnoporus sanguineus</i>	ABTS	6	72	48	0	48	0	48	65	48	65	48	5	48	32	24	50
	none	48	40	48	30	48	0	24	75	24	75	48	0	48	0	48	45
<i>Coriolopsis byrsina</i>	ABTS	48	55	48	8	48	0	48	46	48	46	48	0	48	9	24	45
	none	48	17	48	0	48	0	24	50	24	50	48	19	48	19	24	33

RBBR= Remazol Brilliant Blue R; % value represents percentage of color reduction in relation to maximum absorbance of dye and the minimal time when this reduction was obtained.

synthetic dyes (Table 1). Table 3 shows the highest percentage of decolorization at the lowest treatment time necessary to obtain it. The data indicated that the enzyme were able to decolorization of different chemical classes of dyes, since efficiently decolorization was observed for orange II, RBBR, cybacron 3GA and crystal violet, which are azo, anthraquinone, triazine and tri methyl methane, respectively. The same results were obtained for enzyme from all the strains tested. Nyanhongo *et al.* (24) reported similar results in relation to dyes chemical type, such as anthraquinone, azo, indigo and triarymethane, however, these authors observed that crude laccase from different strains presented remarkably difference in their dyes decolorization. These results suggest that the structural chemical complexity of the dyes is more important than the chromophore group type for enzymes actions. High steric impediment of dyes could difficult the electron transference or enzyme approach to reduced substrate. According to Meyer (23), due to the structural variety of azo dyes they are not uniformly susceptible to biodegradation. The substituted groups such as nitro and sulpho are frequently recalcitrant to biodegradation, whereas aromatic substitution 2-methyl, 2-methoxy, 2,6-dimethyl and 2,6-dimethoxy-substituted 4-(4-sulfophenylazo)-phenol were preferred for azo dyes degradation by peroxidase from *Streptomyces* spp. and *Phanaerochaete chrysosporium* (30).

Maximum decolorization for orange II was 74% in 48 h using enzyme from *L. strigellus*, although enzyme from *P. sanguineus* decolorized 72% this dye in 6 h. RBBR and cybacron blue 3GA lost 75% of colorization in 48h of treatment with enzyme from *P. sanguineus* in absence of ABTS. The maximum decolorization of crystal violet was 65% in 48h of treatment with enzyme

from *L. strigellus* and ABTS. Reactive red 120, chrysophenine, Azure B and methylene blue were little susceptible to enzymes action.

The presence of ABTS in the reactive mixtures increased the orange II decolorization when enzymes from all fungi were used. RBBR and cybacron 3GA decolorization decreased or was not affected when ABTS was added to reaction mixture depending on the enzyme used. The decolorization of crystal violet increased in presence of ABTS when enzymes from *L. strigellus*, *P. sanguineus* and *C. byrsina* were used and was reduced with enzyme from *Lentinus* sp.

Since was not used H₂O₂, dyes decolorizing can be attributed to the laccase activity and this enzyme was able to oxidize RBBR, cybacron 3GA and crystal violet without mediator participation. Biodegradation of several persistent compounds such as dyes, pesticides and lignin derivatives has been attributed to the oxidative enzymes, mainly laccase (28,29).

The efficiency in terms of time and percentage of decolorization of the anthraquinone and azo-based dyes obtained in this work was higher than those described for enzymes from *P. chrysosporium* and *Pleorotus sajorcaju* (6), *Dichimitus squalens*, *Ischnoderma resinsum* and *P. calyptratus* (10), *Trametes trogii* (36), *Funalia trigii* (26), *Pleorotus* spp. (11) and similar to obtained by Chulhwan *et al.* (8). Nevertheless, attempts for better understanding of enzyme attack of these compounds and their derivative is necessary, since more toxic products might be released.

In conclusion, the white rot fungi grown in SSF using an inexpensive substrate to get high ligninase activity and the high level of synthetic dyes decolorization was achieved in vitro by these crude enzymes.

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RESUMO

Produção de ligninases por linhagens de fungos Basidiomicetos usando resíduos agrícolas lignocelulósicos e aplicação das enzimas na descoloração de corantes sintéticos

Fungos decompositores de madeira, do grupo Basidiomicetes, coletados na “Estação Ecológica do Noroeste Paulista”, São José do Rio Preto, São Paulo, Brasil, pertencentes a ordem Aphyllophorales e identificados como *Corioliopsis byrsina* SXS16, *Lentinus strigellus* SXS355, *Lentinus* sp. SXS48, *Picnoporus sanguineus* SXS 43 e *Phellinus rimosus* SXS47 foram estudados para a produção de ligninases por FES (fermentação em estado sólido) usando farelo de trigo ou palha de arroz como meio de cultura. A espécie *C. byrsina* produziu a maior quantidade de lacase (200 U mL^{-1}) enquanto que *Lentinus* sp. foi o melhor produtor de manganês peroxidase (MnP) e lignina peroxidase (LiP) (7 e 8 U mL^{-1} , respectivamente), quando cultivados em meio composto por farelo de trigo. A avaliação do efeito da suplementação de nitrogênio do substrato sólido lignocelulósico (palha de arroz) indicou um aumento de 3 a 4 vezes na produção de lacase. A caracterização das enzimas mostrou que as lacases apresentaram atividade ótima em pH 3,0-3,5 e foram estáveis em pH de neutro a alcalino. O pH ótimo para atividade de MnP e LiP foi de 3,5 e entre 4,5 e 6,0, respectivamente. Todas as linhagens produziram lacase com atividade ótima a 55-60°C, enquanto as peroxidases apresentaram atividades máximas entre temperaturas de 30 e 55°C. A aplicação das soluções enzimáticas brutas, obtidas pelo cultivo das linhagens em meio de farelo de trigo, em testes de descoloração de corantes sintéticos de diferentes grupos químicos levou a mais 70% de perda de cor dos corantes RBBR e de cybacron blue 3GA, em 6h de tratamento. Os dados obtidos indicaram que as soluções enzimáticas contendo ligninases produzidas pelas linhagens de basidiomicetos estudadas promoveram a descoloração de corantes sintéticos.

Palavras-chave: lacase, manganês peroxidase, lignina peroxidase, Basidiomicetes, descoloração, corantes sintéticos

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