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Decolorization and biodegradation of reactive blue 220 textile dye by *Lentinus crinitus* extracellular extract

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ABSTRACT

Studies were carried on the decolorization of the textile dye reactive blue 220 (RB220) by a novel isolate of *Lentinus crinitus* fungi. The optimal conditions for the production of destaining activity were obtained in media containing intermediate concentrations of ammonium oxalate and glucose (10 g L^{-1}) as nitrogen and carbon sources, respectively, at 28 °C and pH 5.5. Maximum decolorization efficiency against RB220 achieved in this study was around 95%. Ultra-violet and visible (UV–vis) spectrophotometric analyses, before and after decolorization, suggest that decolorization was due to biodegradation. This effect was associated with a putative low molecular weight laccase (41 kDa) displaying good tolerance to a wide range of pH values, salt concentrations and temperatures, suggesting a potential role for this organism in the remediation of real dye containing effluents.

1. Introduction

The textile dye industry is far from being ecologically correct and sustainable. The dyeing process consumes large volumes of water and the resulting effluent introduces high loads of dyes, a complex mixture of other carbon-based pollutants and salts into water reservoirs [1]. Textile effluents are usually treated by physical and chemical methods, such as coagulation, flocculation, adsorption, membrane filtration and irradiation [2,3]. Although these treatments achieve high levels of mineralization and decolorization, they have two main constraints: high cost (e.g. photocatalysis and advanced oxidation processes), and the production of significant amounts of sludge that requires a final destination, such as incineration or landfill disposal [2,4]. In this context, biological based approaches, such as enzyme-mediated decolorization, are a promising alternative. Biological catalysis detoxifies the original pollutant and also enables its re-introduction into the normal nutrient cycle in nature, reducing drastically the amount of sludge at the end of the process. A further advantage is that the organisms or enzymes utilized in these processes can be grown using cheap and abundant carbon and nitrogen wastes derived from agro-industry [5–7].

A wide range of microorganisms, including bacteria, fungi, yeast and algae, have the ability to destain and degrade several classes of dyes [8–10]. Among fungi, a particular group of species associated with the decay of wood, the so-called white rot fungi (WRF), has been used to biodegrade various pollutants, including textile dyes. White rot fungi possess a group of enzymes (phenol oxidases and peroxidases) that participate in lignin degradation and which allow them to access cellulose as a carbon source [7]. These enzymes have a relatively low specificity and are therefore able to oxidize man-made pollutants such as pesticides, drugs, polycyclic aromatic hydrocarbons (PAHs) and textile dyes, among many others [11–13]. Although there are already many reports of the use of WRF and their enzymes in the biodegradation of textile dyes, each enzyme or isolate usually displays a limited range of action as a result of the wide variety of chemical dyes (there are currently more than 10,000 different dyes and there is a constant introduction of new dyes with new chemical attributes) and because of the extremely variable dyeing conditions that result in effluents with quite different values of pH, temperature and salt concentration. Thus, it is necessary to search for novel enzymes to expand the range of applications of enzymatic treatment of textile effluents.

The aim of the present work was to characterize the biodegradation of the textile dye Remazol reactive blue 220, by the action

Abbreviations: PAGE, polyacrilamide gel electrophoresis; SDS, sodium dodecyl sulfate; ABTS, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); SMM, solid minimal medium; LMM, liquid minimal medium; RB220, reactive blue 220.

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of soluble extracts from the white rot fungus *Lentinus crinitus*. We describe an *in vitro* optimization process for the production of an extract with destaining activity and its behavior under different conditions of carbon and nitrogen sources, pH, temperature and substrate concentration.

2. Materials and methods

2.1. Dyes and chemicals

Textile Remazol dyes: reactive blue 220 (RB22), reactive red 198 (RR198) and reactive orange 107 (RO107) were supplied by Dystar (São Paulo, Brazil). 2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and guaiacol were obtained from Sigma Chemical Company (St. Louis, MO, USA). All chemicals used were of the highest purity available and of analytical grade.

2.2. Organisms and culture conditions

L. crinitus IOC 4579 was originally collected in Campina Grande do Sul (Paraná, Brazil) and maintained by serial cultivation on potato dextrose agar at 28 °C in the dark. It is actually deposited in the Fungal Collection of the Oswaldo Cruz Institute (Rio de Janeiro, Brazil). For each assay, it was grown on plates of solid minimal medium (SMM), containing (per liter): $NaNO_3$ 6.0 g; KH_2PO_4 1.5 g; KCl 0.5 g; $MgSO_4$ 0.5 g; $FeSO_4{\cdot}7H_2O$ 0.01 g; $ZnSO_4{\cdot}7H_2O$ 0.02 g; glucose 10.0 g; bacteriological agar 10.0 g; pH 6.8. Liquid minimal medium (LMM) had an identical composition with the exception that it did not contain agar. After 7–10 days of growth on SMM, a 4-mm diameter biopsy punch was used to remove four mycelial plugs, which were used to inoculate 5 mL replicates of different sterilized culture media. Cultures were incubated at 28 ± 0.2 °C for all the subsequent assays.

2.3. Preparation of soluble culture-derived extracts

For the analysis of liquid cultures, samples were centrifuged at 150 rpm for 5 min, the supernatant was collected for further analysis and the mycelium (pellet) was used for the determination of biomass. For cultures on solid media, two extractions with 2 mL of salt solution (LMM with neither glucose nor $NaNO_3$) for 30 min in an orbital shaker (150 rpm) were performed. The resulting volume was centrifuged and the supernatant used for direct analysis or stored at −10 °C. Protein content was determined using the Bradford assay [14]. For biomass calculation, mycelia were filtered through previously dried and tared Whatman No. 1 filter papers, washed with distilled water and dried at 50 °C to constant weight.

2.4. Decolorization assays

Two kinds of tests were performed: the *in culture* decolorization assay and the destaining activity assay. In the *in culture* decolorization assay, cultures containing RB220 were monitored by measuring the absorbance of the dye in supernatants (liquid cultures) and in extracts (from solid cultures) at 280 and 600 nm on a Shimadzu UV-160A UV–vis spectrophotometer, after 10–30 days of fungal growth. The percentage of *in culture* decolorization was calculated as

$$\text{in-culture decolorization } (\%) = \frac{A_{\text{CONTROL}} - A_{\text{CULTURE}}}{A_{\text{CONTROL}}} \times 100 \quad (1)$$

where A_{CULTURE} is the absorbance (at either 280 or 600 nm, as specified in the text) of the supernatant or extract and A_{CONTROL} is the absorbance, at the same wavelength, of a supernatant or extract from a non-inoculated culture containing the dye. Results are the mean of at least three replicates.

The destaining activity assay measured the consumption of the dye, over a 90-min period, when supernatants or soluble extracts (derived from liquid and solid cultures, respectively, grown for 15 days) were added to an assay solution containing RB220. The supernatant or extract samples (1 mL) were added to 0.1 mL of a salt medium containing RB220, giving a final concentration of RB220 of 0.1 g L^{-1}. The destaining activity was calculated as

$$\text{destaining activity } (\%) = \frac{A_0 - A_{90}}{A_0} \times 100 \quad (2)$$

where A_{90} is the absorbance (at 600 or at 280 nm, as specified in the text) after 90 min incubation at 28 °C and A_0 is the initial absorbance (at the same wavelength) of the assay mixture.

2.5. Optimization of carbon and nitrogen content for enzyme production

Six different carbon substrates (glucose, sucrose, starch, maltose, fructose and glycerol) and nitrogen sources (sodium nitrate, urea, ammonium tartrate, ammonium carbonate, ammonium oxalate and peptone) were used to substitute the original carbon and nitrogen sources in SMM and LMM. The assessed concentrations were 5, 10 and 15 g/L. All inoculated media were incubated for 15 days at 28 °C in the dark and the supernatant or eluted extracts collected for further analysis.

2.6. Effect of pH, temperature and copper supplementation on enzyme production

Cultures optimized for carbon and nitrogen content were supplemented with copper sulfate (0.1–5 mM) and incubated for 15 days at 28 °C. Supernatants or soluble extracts were then submitted to the destaining activity assay. Additionally, destaining activity was measured in samples derived from cultures grown at 28, 35 and 40 °C for 15 days and at pH values from 5.5 to 7.0 at 28 °C for 15 days.

2.7. Lignin modifying enzymes, LMEs

The activities of manganese peroxidase (MnP), manganese-independent peroxidase (MiP) and laccase (Lac) were determined by spectrophotometric assays performed on soluble extracts from cultures as previously described: Lac activity was determined by monitoring the oxidation of ABTS at 420 nm [15]; MiP activity was determined by assessing veratryl alcohol oxidation at 310 nm [16] and MnP activity was determined by following the oxidation of phenol red at 440 nm [17]. To correlate dye destaining with Lac, MnP or MiP activity, the substrate was substituted with RB220. All assays were carried out at 28 °C with a minimum of three replicates. Alternatively, experiments were performed in the presence of 20 mM sodium azide.

2.8. Effect of pH, temperature and salt concentration on destaining activity

Soluble extracts derived from 15-day-old solid or liquid cultures, carried out under optimized conditions, were tested for destaining activity (see Section 2.4). Destaining of RB220 (0.1 g L^{-1}) was carried out at pH values from 2 to 9 (intervals of 0.5 units), adjusted by using 50 mM citrate-phosphate buffer. The temperatures assessed were 20, 30, 40, 50, 60 and 70 °C. For testing susceptibility to salt, different NaCl concentrations (0.05, 0.1, 0.2, 0.4 and 0.6 M) were used in the assay. In all experiments UV–vis absorbance was monitored in each condition after 90 min at 28 °C.

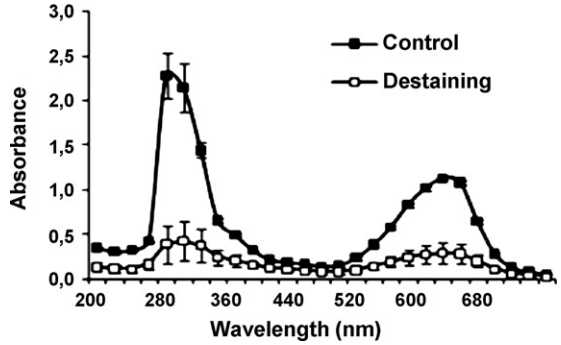

Fig. 1. UV–vis spectrum of a solution of RB220 before and after incubation with *L. crinitus* cultures.

2.9. Zymograms

Supernatants and soluble extracts from *L. crinitus* cultures were subjected to electrophoresis on 12% SDS-PAGE gels [18], without previous heating and without reducing agent. After electrophoresis, gels were washed in distilled water (2× 15 min) and then soaked in renaturation buffer (0.1 M sodium phosphate pH 6.0; 1% Triton X100) for three cycles of 1 h. Gels were then soaked in saline solution containing 5 g L^{-1} RB220. The excess of saline solution was removed and the gels were then incubated at 28 °C overnight. Identical gels were soaked in 50 mM acetate buffer pH 5.0 containing either 0.05 mM ABTS or 0.01% guaiacol and incubated under the same conditions. The appearance of destained bands on a blue background (for RB220) or colored bands on a white background (for ABTS and guaiacol) was verified. In parallel, a replicate gel was developed by silver staining to check the protein expression profile.

2.10. Statistical analysis

Data were evaluated by one-way analysis of variance (ANOVA) followed by the Tukey test, through the ASSISTAT 7.5 software (assistat.com). Readings were considered significant when p was <0.01.

3. Results

3.1. Screening using reactive dyes

L. crinitus IOC 4579 cultures were initially exposed to three textile reactive dyes: Remazol red (RR198), Remazol orange (RO107) and Remazol blue (RB220). *In culture* decolorization occurred only with RB220. The total flattening of the UV–vis spectra of the samples indicates that decolorization was accompanied by biodegradation since the smoothening of absorbance peaks at 600 and at 280 nm is consistent with the reduction of azo linkages and the loss of aromatic rings (Fig. 1).

3.2. Effect of dye concentration and water content on fungal growth, in culture decolorization and destaining activity

When *L. crinitus* was cultured in LMM containing 0.1 g L^{-1} RB220, the percentage decolorization increased over time, reaching a peak of 90% decolorization after 10 days of incubation (Fig. 2). The decolorization was not directly associated with fungal growth since over the period of 7–10 days the biomass level fell while the percentage decolorization was still increasing rapidly. *In culture* decolorization tests were then done with increasing concentrations of RB220. Although these concentrations delayed fungal growth slightly, *L. crinitus* was able to develop in RB220 concentrations as high as 2 or even 5 g L^{-1}, giving percentage *in culture* decolorizations of 100% and 70%, respectively, after 30 days (Fig. 3).

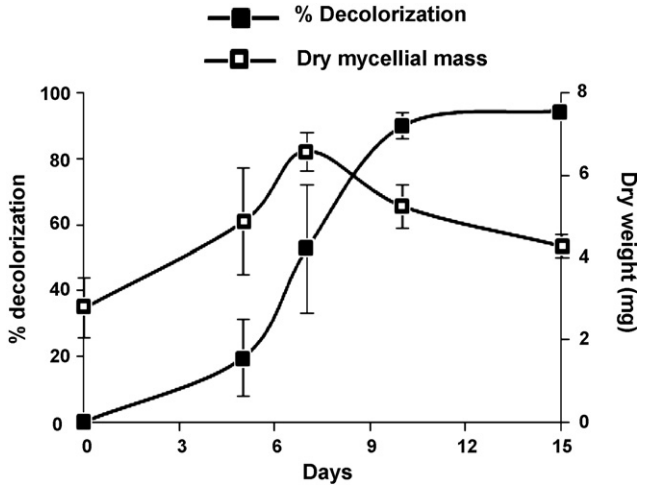

Fig. 2. *In culture* decolorization of RB220 and growth of *L. crinitus* over time in liquid minimal medium containing 0.1 g L^{-1} dye. The absorbance of the culture supernatant was determined at 600 nm.

In order to determine whether the fungal products responsible for dye decolorization were secreted or mycelium-associated, supernatants and mycelia from 30-day liquid cultures and eluted extracts from 30-day solid cultures were subjected to the UV–vis 90-min destaining activity assay. Activity was highest in supernatants and eluted extracts, with relatively low activity being detected in mycelia (results not shown). The destaining activity eluted from solid cultures was very similar to that obtained in the supernatants obtained from liquid cultures. Since our aim is to develop future applications based on solid-state fermentation, from this point on we worked with solid cultures. Also, an RB220 concentration of 0.1 g L^{-1} was selected. At this concentration the dye was totally consumed and slightly higher activities were obtained in the destaining activity assay performed on the culture extracts.

3.3. Effect of carbon and nitrogen content on the production of destaining activity

In the case of white rot fungi, it has been reported that the production of lignin modifying enzymes (LMEs) and biodegradation activity varies greatly, according to the type and concentration of carbon and nitrogen sources *in culture* media. These effects were therefore investigated.

L. crinitus was grown on solid minimal media that contained glucose, fructose, maltose, starch, sucrose or glycerol as the sole carbon source. For each substrate, three concentrations (5, 10 and 15 g L^{-1})

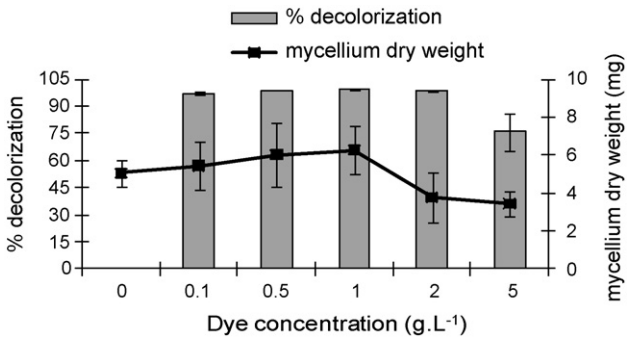

Fig. 3. Effect of dye concentration on fungal growth and *in culture* decolorization. Absorbance at 600 nm was assessed after 30 days of culture.

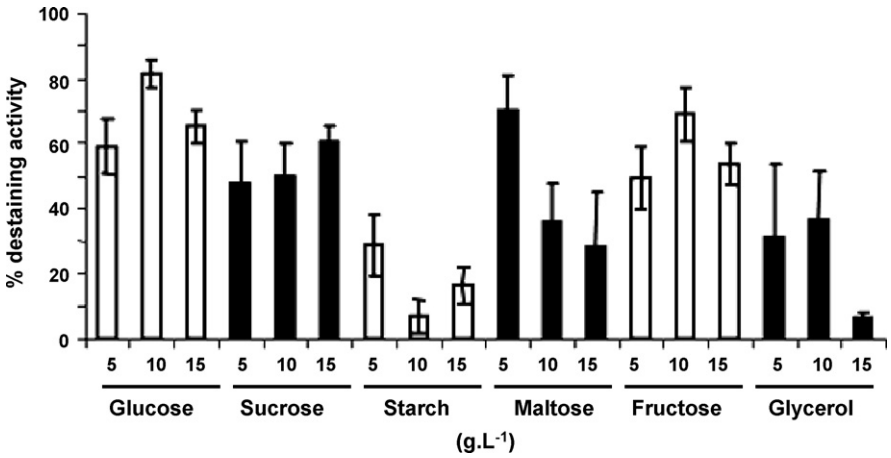

Fig. 4. Effect of carbon type and concentration on the production of destaining activity at 600 nm in solid medium.

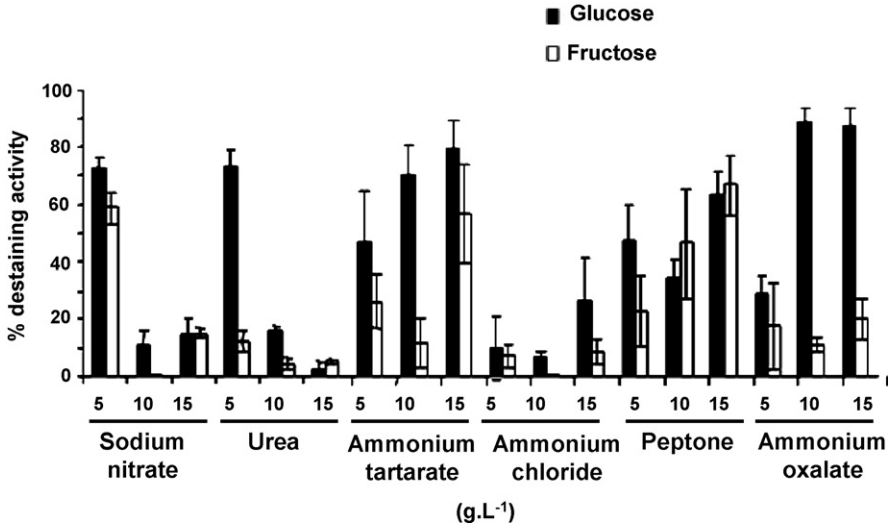

Fig. 5. Effect of nitrogen source and concentration on the production of destaining activity at 600 nm in solid media containing fructose (10 g L^{-1}) and glucose (10 g L^{-1}) as carbon source.

were tested. After 15 days of incubation, the destaining activity assay was performed using extracts from the solid medium. Drastic differences in the production of destaining activity were observed. *L. crinitus* produced the highest levels of destaining activity when grown in 10 g L^{-1} glucose; 5 g L^{-1} maltose or 10 g L^{-1} fructose, with the destaining activity ranging from 65% to 85%. Higher concentrations of these carbon sources did not lead to higher production of destaining activity. With some carbon sources the yield of destaining activity was very low. For example, with 15 g L^{-1} glycerol and 10 g L^{-1} starch destaining activities of only 5–10% were produced (Fig. 4).

When nitrogen sources were assessed, using fructose (10 g L^{-1}) and glucose (10 g L^{-1}) as carbon sources (maltose was omitted due to its high cost), soluble extracts derived from cultures containing 5 g L^{-1} sodium nitrate, 15 g L^{-1} ammonium tartrate and 10 g L^{-1} ammonium oxalate produced the highest destaining activities (Fig. 5). Again, some substrates promoted the production of destaining activity and some of them, such as urea and ammonium chloride, were inhibitory. A significant observation is that the effect of a nitrogen source can depend on the accompanying carbon substrate. Thus, soluble extracts derived from cultures containing 5 g L^{-1} urea led to a 10% dye-destaining activity when combined with 5 g L^{-1} fructose in the culture medium, but when combined with 5 g L^{-1} glucose, this same concentration of urea gave a 7-fold higher destaining activity. A similar effect was observed for 10 and 15 g L^{-1} ammonium oxalate with fructose and glucose.

3.4. Effect of pH, temperature and copper supplementation of cultures on the production of destaining activity

Cultures were done in solid medium with optimized carbon and nitrogen contents (10 g L^{-1} glucose and 10 g L^{-1} ammonium oxalate) in five different initial pH values (at 28 °C) and three

Table 1
Effect of pH, temperature and copper concentration on the production of destaining activity.

T (°C)	28	35	40
% d.a	91.0 ± 0.6	15.5 ± 4.5	0.03 ± 0.03

Cu^{2+} (mM)	0	0.1	1.0	5.0
% d.a	77.8 ± 4.7	80.7 ± 5.7	49.6 ± 13.3	63.7 ± 8.6

pH	5.5	6.0	6.5	7.0	7.5
% d.a	98.8 ± 3.2	84.06 ± 5.8	86.6 ± 5.6	6.0 ± 1.6	10.5 ± 1.6

All values correspond to % destaining activity (d.a). Values are mean of three experiments ± SEM.

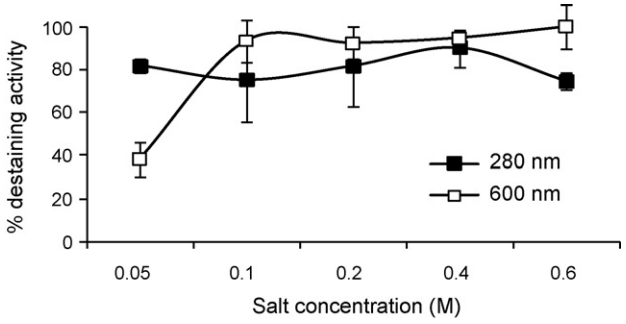

Fig. 6. Effect of salt concentration within the assay solution on destaining activity at 280 and 600 nm.

different temperatures (with initial pH 5.5). Other cultures were performed (at 28 °C and initial pH 5.5) in the presence of three different copper sulfate concentrations. Extracts from 15-day-old cultures were tested for destaining activity. Dye-destaining activity was better in extracts derived from cultures incubated at 28 °C and with initial pH values ranging from 5.5 to 6.5 (Table 1). Extracts derived from copper supplemented cultures did not display an improvement in destaining activity and at copper sulfate concentrations above 1 mM the destaining activity produced was relatively low (Table 1).

3.5. Effect of pH, temperature and salt concentration on destaining activity

The destaining activity assay was carried out at several pH values, salt concentrations and temperatures. Destaining activity was not sensitive to salt concentration since reduction of UV–vis absorbance, which indicates degradation of the dye, took place over the whole range of NaCl concentrations tested (0.05–0.6 M) (Fig. 6). When pH variation was assessed, soluble extracts retained more than 70% of the original activity in pH values ranging from 3.5 to 8.0 (Fig. 7). Destaining activity was relatively insensitive to temperature since it remained high from 20 to 70 °C, for a reaction time of 90 min (Fig. 8).

3.6. Activity of lignin modifying enzymes (LMEs)

Assays for lignin modifying enzymes in soluble extracts revealed the presence of a putative phenol oxidase (laccase) since neither Mn^{2+} nor H_2O_2 were necessary for ABTS oxidation or RB220 degradation. Further, both the destaining activity and ABTS oxidation were reduced (by 80% and 85%, respectively) by the addition of sodium azide, a well known inhibitor of laccases (data not shown). *In gel* activity assays and silver stained SDS-PAGE gels revealed a unique sharp band of 41 kDa displaying dye destaining and phenol oxidase activity, suggesting that only one enzyme is involved in the process (Fig. 9).

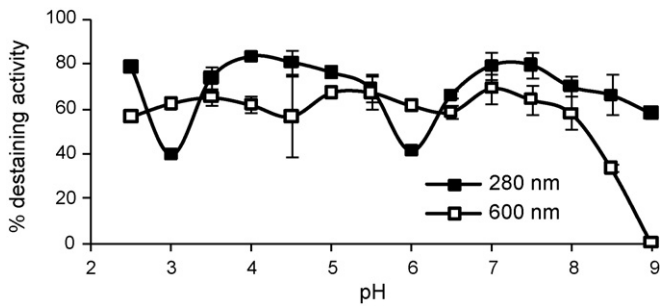

Fig. 7. Effect of pH on RB220 destaining activity, measured at 280 and 600 nm.

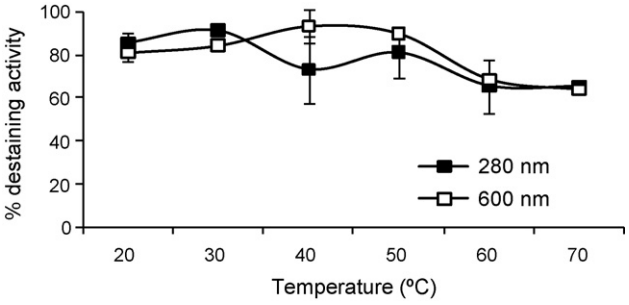

Fig. 8. Effect of temperature on RB220 destaining activity measured at 280 and 600 nm.

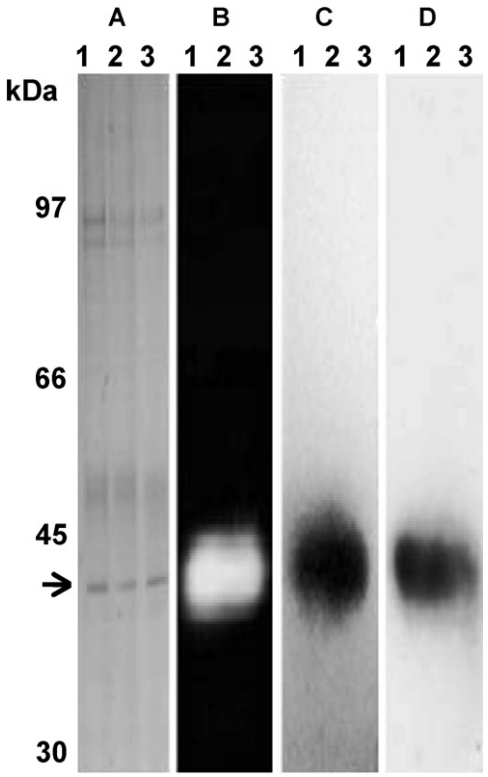

Fig. 9. Protein profile and *in gel* destaining activity of soluble extracts from liquid cultures of *L. crinitus*. Lanes: soluble extract replicates (1–3). Gels: (A) 12% SDS-PAGE after silver staining. (B and C and D) *In gel* destaining activity on renaturated 12% SDS-PAGE gels containing RB220, ABTS and guaiacol, respectively, as substrates. Mw markers are described on the left.

4. Discussion

The genus *Lentinus* comprises a group of edible fungi with important medicinal and biotechnological applications. Several species and strains have been assessed for biodegradation of different pollutants such as crude oil [19], pentachlorophenol [20], DDT [21], trinitrotoluene [22] and some textile dyes [23,24]. Here we report the expression of a putative laccase in *L. crinitus*, a relatively unexplored *Lentinus* species, and its participation in the destaining and biodegradation of the textile dye reactive blue 220.

Remediation of reactive dyes is an important issue since cotton occupies the first place among dyed textile fibers and more than 50% of it is dyed with reactive dyes. Use of reactive dyes is ecologically troublesome since it results in heavily colored effluents, containing high concentrations of salts and alkali and high BOD/COD values [25]. Reactive blue 220 (RB220) is a vinyl sulfone azo dye. Previous attempts to degrade it have focused on photo-

catalytic and chemo-oxidative processes [26–28]. Although these processes may degrade the dye partially or even totally, they have several drawbacks such as the generation of by-products, including chemical sludge, and high investment and operating costs [2]. Three reports of enzyme-mediated degradation of RB220 have been published: two using a laccase-containing crude extract from *Pleurotus sajor caju* [29,30] and the other using bilirubin oxidase [31]. Concentrated crude extracts derived from *P. sajor caju* achieved 90% decolorization of 0.05 and 0.1 g L^{-1} RB220 solutions, after 5 h and 30 min incubation periods, respectively, in two separate reports [29,30]. In our case, similar destaining values were obtained by *L. crinitus* soluble extracts without previous concentration, after 90 min of incubation. Moreover, these previous reports did not confirm the complete loss of dye chromophores, either because destaining was monitored only through changes in the vis-spectra [30], or because the decay in the 600 nm absorbance peak was accompanied by a shift to the 400 nm region [29], but no changes in the UV-spectrum. This suggests that decolorization in these previous studies was simply due to partial reduction of double bonds or changes in the substitutions around them [29,31].

A putative laccase activity in *L. crinitus* was firstly described by Ullah et al. [32] and more recently in the crude extract of a Colombian strain displaying destaining activity against the dyes azure B and Coomassie blue [33]. In the latter work, decolorization depended on the activity of laccase and manganese peroxidase, the production of which was enhanced by supplementing the culture medium with Mn^{2+}, ethanol or Cu^{2+}. We did not detect MnP activity in our Brazilian isolate, nor an increased production in destaining activity due to copper supplementation. Copper supplementation can stimulate laccase synthesis since the enzyme uses copper as cofactor, but the ion can also inhibit the growth of the organism [34].

It used to be generally accepted that carbon and nitrogen limitation favored the production of lignolytic enzymes in white rot fungi [35]. However, more recent results are somewhat contradictory. For example, in *L. edodes*, Buswell et al. [36] obtained 5-fold higher laccase levels under high nitrogen conditions than in low-nitrogen cultures, while Hatvani and Mécs [37], working on the biodegradation of dyes by *Lentinus* sp. grown in solid media, found that faster decolorization occurred at very low NH_4Cl, peptone and malt extract concentrations. On the other hand, the lignolytic activity of *L. edodes* grown in liquid culture was stimulated by high N concentrations [38]. In the present work, the production of destaining activity was affected by the type and concentration of the nitrogen source, but different trends occurred for different sources: higher concentrations of ammonium salts resulted in higher production of destaining activity, while for sodium nitrate and urea higher production levels were obtained at the lower concentrations. Moreover, the final result varied drastically with the type of carbohydrate present in the culture medium. For example, the high destaining activity obtained in cultures containing 5 g L^{-1} urea was almost lost when glucose was replaced with fructose as the main carbon source. A similar effect was observed for ammonium oxalate.

Some studies have been conducted on the effect of carbon sources on lignolytic enzyme production by white rot fungi, but to the best of our knowledge, not one using *Lentinus* species. In studies of other genera of white rot fungi, Mansur et al. [39] showed that changing the carbon source from glucose to fructose results in a 100-fold increase in the specific Lac activity of a *Trametes* sp. isolate. Mikiashvili et al. [40] tested seven different carbon sources with two *Trametes versicolor* isolates. Mannose and cellobiose were the best substrates for Lac production, with a five fold difference in yield between the most and least efficient carbon sources. Also, Galhaup et al. [41], in testing six carbon sources with a strain of *T. pubescens*, observed that the sources that were efficiently and rapidly utilized by the organism resulted in higher production of laccase activity. Our results are in agreement with these reports. It seems that complex interactions among signals regulating the expression of lignolytic enzymes take place, depending on the type and concentration of the carbon and nitrogen sources, as well as the lignocellulosic substrate *in culture* medium and the organism being utilized [42–45].

According to our results, the destaining of RB220 is mediated by a putative laccase with a molecular mass of 41 kDa. This activity displayed a good tolerance to temperature and pH, since more than 70% of it was retained in temperatures ranging from 20 to 70 °C and pH values from 3.5 to 8.0. Previously characterized laccases from *Lentinus* species displayed molecular masses from 65 to 74 kDa, good activity between 50 and 65 °C and optimal pH between 3.0 and 7.0 [46–48]. Cadimaliev et al. [47] reported a *L. tigrinus* laccase displaying around 70–100% of maximal activity at temperatures between 25 and 75 °C and over the pH range of 5.0–8.5, while Gomes at al. [24] related a similar pattern in two laccases from *Lentinus* sp. and *L. strigellus*. Our results are in accordance with these reports. Nevertheless, the molecular mass that we describe here is lower than those described in laccases from other *Lentinus* species. Laccases with low molecular mass are less common but have been described in other white rot fungi such as *G. lucidum* (43 and 40 kDa) [49,50].

5. Conclusions

Soluble extracts derived from *L. crinitus* IOC 4579 cultures were able to decolorize and biodegrade the textile dye reactive blue 220. The production of the destaining activity was highly influenced by medium composition and culture conditions, being higher in media containing intermediate concentrations of ammonium oxalate and glucose. Dye destaining was associated with a putative low molecular weight laccase displaying good tolerance to a wide range of pH values and temperatures, suggesting a potential role for this organism and enzyme in the remediation of real dye containing effluents.

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