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Degradation and detoxification of synthetic dyes and textile industry effluents by newly isolated *Leptosphaerulina* sp. from Colombia

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Abstract

Background: Wastewaters from the textile industry are an environmental problem for the well-known Colombian textile industry. Ligninolytic fungi and their enzymes are an option for the treatment of these wastewaters; however, the Colombian biodiversity has not been deeply evaluated for fungal strains with ligninolytic activities. In this research, 92 Colombian fungal isolates were collected from four locations around the Aburrá valley, Antioquia, Colombia. Their decolorizing activities were evaluated using Novacron Red, Remazol Black and Turquoise Blue in solid and liquid media at different culture conditions. The best fungal isolate was evaluated in the bioremediation of two real effluents and its enzymatic extracts were used in the decolorization of the three dyes.

Results: From 92 Colombian fungal isolates, *Leptosphaerulina* sp. exhibited the best decolorization percentage (>90 %) in solid and liquid cultures, and in agitated and un-agitated conditions. *Leptosphaerulina* sp. effectively decolorized the three dyes and two real effluents from textile industries. This decolorization was catalyzed by the production of significant quantities of laccase (650 U/L) and manganese peroxidase (100 U/L). *Leptosphaerulina* sp. enzymatic extracts exhibited decolorizing activity when ABTS as mediator was added. *Leptosphaerulina* sp. decolorized two real effluents from textile industries (>90 %) under conditions of low pH and glucose supplementation. Enzymatic degradation and decolorization products' innocuity was demonstrated by cytotoxic and chromatographic analyses.

Conclusion: *Leptosphaerulina* sp. was the best Colombian isolate. This fungal strain achieved a decolorization above 90 % for the three dyes and two real effluents from a textile industry. This decolorization was performed by producing significant amounts of laccase and manganese peroxidase. *Leptosphaerulina* sp. is an interesting prospect to treat waters polluted with dyes without the production of compounds dangerous for the environment.

Keywords: *Leptosphaerulina* sp., Ligninolytic enzymes, Dyes, Decolorization, Textile effluents, Colombia

Background

The Colombian textile industry has national and international acknowledgements by its quality and tradition. However, this industry faces different environmental problems, one of which occurs during the fabrics dyeing. Dyeing environmental problems are related to the large demand of water and the presence of remaining dyes in their effluents. In fact, 1 kg of dyed textile requires 100 L of water generating an effluent with a dye content between 5 and 50 % (Abadulla et al. 2000). These wastewaters are disposed in rivers and creeks all around the country, generating significant pollution. Chemical dyes pollute waters by changing its color and generating harmful effects to the aquatic ecosystems. Besides the anti-aesthetic effect produced in the effluents, the coloration reduces the solar light transmittance, affecting the aquatic organisms' photosynthetic activity (Michniewicz et al. 2008). Additionally, some dyes have exhibited

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mutagenic and carcinogenic effects (Weisburger 2002). The traditional technologies to degrade synthetic dyes (physical, physicochemical and biological) are not efficient enough. In these technologies, the dyes are transferred to other matrices (i.e., microbial cells) or the dyes degradation products become more toxic than the original dyes (Zouari-Mechichi et al. 2006).

An environmentally friendly strategy to degrade textile dyes is the application of ligninolytic fungi and their enzymes. Ligninolytic fungi developed an enzymatic system composed by three principal enzymes: laccase (E.C 1.10.3.2), lignin peroxidase (LiP) (E.C 1.11.1.14), and manganese peroxidase (MnP) (E.C. 1.11.1.13). Laccase or benzenediol: oxygen oxidoreductase belongs to the oxidoreductases class (Dias et al. 2007). The most referenced and well-known laccases belong to the wood-decay fungi or white-rot fungi. Wood decay fungi utilize these enzymes to degrade the recalcitrant structure that constitutes lignin and facilitate the access to hemicellulose and cellulose. Laccases have the advantages of being extracellular, inducible, low substrate specificity and an inexpensive cofactor (oxygen) (Zouari-Mechichi et al. 2006; Sergio 2006; Couto and Toca-Herrera 2006). On the other hand, LiP and MnP belong to the peroxidases subclass and can catalyze the degradation of a wide number of aromatic structures, they are produced by the white rot fungi to participate in the initial states of the lignin degradation (MnP) (Angel 2002; Hammel and Cullen 2008) or the degradation of aromatic rings moderately activated (LiP) (Khindaria et al. 1996). Therefore, ligninolytic fungi and their enzymes have been used in the degradation of different types of compounds as lignin, aromatic compounds, textile dyes, etc. (dos Santos et al. 2007).

Several fungi with ligninolytic activities (i.e., *Bjerkandera adusta, Trametes versicolor, Phanerochaete chrysosporium*) have proved their efficiency for treating textile dyes (dos Santos et al. 2007). However, each isolate generates a different enzymatic profile, a different response to environmental variables and different levels of dyes decolorization (Nyanhongo et al. 2002). Agitation, carbon and nitrogen source, and inducers (aromatic compounds, copper) are environmental factors that have been associated with the modification of the enzymatic production and/or the decolorization activity. However, the positive or negative effect of these factors is greatly related with the type of fungal strain evaluated. An example of this differences is the nitrogen concentration, low-nitrogen concentration has been found as favorable for *Cyathus bulleri* and *Phlebia tremellosa* (Salony et al. 2006; Robinson et al. 2001) whereas, *Pestalotiopsis* sp. and *Bjekandera adusta* showed greater enzymatic and decolorizing activity when the culture medium had nitrogen availability (Robinson et al. 2001; Hao et al. 2007).

In addition to fungal decolorization, synthetic dyes can be decolorized by using enzymatic extracts, purified or semi-purified enzymes, and commercial or native enzymes (Mattinen et al. 2011). Enzymatic decolorization have been performed by using individual or a mixture of ligninolytic enzymes, generally laccase is the main enzyme followed by MnP and LiP (Costa et al. 2005; Archibald et al. 1997). The principal advantage of enzymatic processes is the ability to obtain the same removal than microbial pretreatment in less time (between 2 and 48 h). However, some enzymes are not able to produce the same results when in vivo than in vitro. Therefore, some approaches have been utilized to increase the range of dyes degraded, the decolorization percentage, and the removal time (Zouari-Mechichi et al. 2006; Kokol et al. 2007). The principal strategy is the use of mediators. Mediators are chemical compounds, which act as electron carriers between the enzyme and the final substrate (Babot et al. 2011). The use of fungal laccases in presence of redox mediators is known as laccase-mediator system (LMS). The two most common mediators are 1-hydroxybenzotriazole (HBT) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). A greater degree of degradation using LMS is based in a high level of interaction between the enzyme and its mediator; in fact, not all the mediators produce the same effect with enzymes from different origins (Nyanhongo et al. 2002).

Enzymatic or fungal processes using ligninolytic fungi and their enzymes are a promising technology to solve the dyes pollution generated by the Colombian textile industry. However, the fungal strains evaluated in the treatment of textiles dyes have been isolated from environmental and geographical conditions different than Colombia. Therefore, to apply this type of technology in Colombia, it is necessary to evaluate the Colombian microflora and their ligninolytic and decolorizing activities. Therefore, this research aims were the isolation of native fungi from four locations around the Aburrá valley, in Antioquia, Colombia, the evaluation of fungal strains effectiveness over synthetic dyes and actual effluents from a Colombian textile industry, and the evaluation of enzymatic extracts in the removal of synthetic dyes.

Methods

Fungal strains

Ninety two fungi were isolated from four locations (cloud forest (CF), Montane rainforest (MRF), Medellin's Botanic Garden (BG) and an industrial and residential zone (IRZ)) around the Aburrá valley, Antioquia, Colombia. The strains were conserved in potato dextrose agar (PDA) at 4 °C until use.

Textile dyes

The dyes evaluated in this research were Novacron Red (azoic dye, $\lambda_{max} = 514$ nm), Remazol Black (azoic dye, $\lambda_{max} = 598$ nm), and Turquoise Blue (phthalocyanine dye, $\lambda_{max} = 622$ nm and 669 nm). Additionally, two types of real textile wastewaters were used. First, a dyeing bath effluent and second, the final effluent released into Medellin river. All dyes and effluents were kindly donated by Fabricato-Tejicondor S.A.

Evaluation of ligninolytic and decolorizing activity in solid media

The ligninolytic activity was measured in petri dishes with malt extract medium supplemented with 2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) also known as ABTS. The petri dish was inoculated with the isolated fungus and was incubated 2 weeks at 30 °C. The enzymatic activity was evaluated by the formation of a green or violet halo. The fungal strains that exhibited ligninolytic activity were selected for the decolorizing activity experiment. The decolorizing trials used malt extract agar supplemented with each dye. The fungi were cultivated during 20 days at 28 °C. The decolorizing activity was described as positive when the solid media exhibited partial or total loss of color. The fungal strains with decolorizing activity were selected for the liquid media evaluation.

Selection of decolorizing fungal strains

The fungal strains selection was performed in two phases. The first phase employed a completely randomized block design with three replicates. All the strains selected in the solid media experiment were evaluated in each dye. The culture was carried out in Erlenmeyer flasks with 50 mL of malt extract liquid media, 200 ppm of dye and an inoculum of four cylinders from the fungus. The fungal strains were cultured during 15 days at room temperature (20–25 °C) without agitation. At the end of the 15 days, the fungi with the largest decolorization percentage in each dye were selected for the next phase. The second phase of the selection process utilized the fungal strains selected in the unagitated culture, Kirk's media and agitation. The experiment employed a factorial design with three replicates. The experiment's factors were the selected fungi and ammonium tartrate concentration (0.22, 5, and 10 g/L). The fungal strains were cultivated during 15 days at room temperature and 150 rpm. The experiment's statistical analysis employed SAS 9.3 software. After that, a decolorization kinetic was performed for the treatments that exhibited the largest decolorization in each dye. The kinetic described the decolorization percentage, enzymatic activity, biomass, pH, protein concentration, glucose consumption, and adsorption. In order to determine the decolorization kinetic, Kirk's media was employed, with 200 ppm of dye, during 21 days at 25 °C and 150 rpm.

Selected fungus identification

The fungal isolate that presented the largest decolorization was molecular identified by analyzing the ITS and DNAr 28S sequences. The PCR used the following primers: ITS1 (5′ TCC GTA GGT GAA CCT GCG G 3′) and ITS4 (5′ TCC TCC GCT TAT TGA TAT GC 3′) and DNAr 28S LROR (5′ ACC CGC TGA ACT TAA GC 3′) and LR6 (5′ CGC CAG TTC TGC TTA CC 3′) (Chanagá Vera et al. 2012). The sequences analysis was performed in the BLAST software.

Enzymatic extracts experiment

The enzymatic extracts from the fungi with the largest enzymatic activity were evaluated in order to know their decolorizing activity. The cultures using the best fungus were stopped on the day with the largest enzymatic production. On this day, the media was centrifuged 10 min at 5000 rpm, after which, the supernatants were micro-filtrated and lyophilized. The enzymatic extracts experiment was performed for each dye, using a mixture of lyophilized extract (50 ppm), ABTS (2 mM), dye (50 ppm) and buffer pH 3. The enzymatic reaction was kept for 24 h at 25 °C and 150 rpm. The experiment had three replicates and the decolorization percentage as response variable. The statistical analysis was performed using the SAS 9.3 software.

Fungal decolorization of real textile wastewaters

The fungus with the greatest decolorization ability was evaluated with two types of textile wastewaters (the final effluent disposed into Medellin river and a fabrics dye bath). The final effluent was evaluated under a completely randomized factorial design $2 \times 2 \times 2$ with three replicates and using the decolorization percentage as response variable. The experiment's factors were medium supplementation (10 g/L of glucose and 5 g/L of ammonium tartrate), pH (as received and pH 5) and inoculum (4 and 8 cylinders). The fabrics dyeing bath was evaluated under a completely randomized factorial design $2 \times 2 \times 2$, using as factors medium supplementation (10 g/L of glucose and 5 g/L of ammonium tartrate), inoculum (4 and 8 cylinders) and effluent dilution [as received (6.8 g/L) and 200 ppm]. The decolorization was carried out in 250 mL Erlenmeyer flasks with 50 mL of medium, during 21 days at 25 °C and 150 rpm.

Enzymatic activities

The laccase activity was calculated using the enzymatic oxidation of ABTS. The reaction was followed

spectrophotometrically by employing the change in the absorbance at 420 nm and a molar extinction coefficient of ε_{420}, 36,000/M/cm. The LiP activity was obtained by following the formation of veratryl aldehyde from veratryl alcohol at 310 nm (ε_{310}, 9300/M/cm). The MnP activity was calculated by measuring the absorbance change at 469 nm produced by the oxidation of 2,6-dimethoxypenol (DMF) (ε_{469}, 27,500/M/cm). All the enzymatic activities were reported in units (U), which were defined as the quantity of enzyme that catalyze 1 µmol of substrate per minute.

Analytical methods

The decolorizing activity was calculated using the decolorization percentage $decolorization\,\% = \frac{A_0 - A_f}{A_0} \times 100$, where A_0 is the initial dye absorbance at the dye's λ_{max} and A_f is the final absorbance at the dye's λ_{max} (Mohorčič et al. 2006). The decolorizing activity was considered high when the decolorization percentage was above 80 %, medium when it was between 50 and 80 % and low when it was below 50 %. The error bars utilized in the decolorization percentage measurements corresponds to the standard error. To evaluate biomass adsorption, the biomass was re-suspended in methanol for 30 min, and the methanol solution was used to calculate the absorbance at the dye λ_{max}. In order to obtain the biomass production, after the methanol extraction, the biomass was dried at 105 °C for 24 h until obtain constant weight. The glucose determination was performed using the 3,5-dinitrosalicylic acid (DNS) methodology. The protein concentration was obtained by the Bradford's method. Dyes' degradation products were analyzed by thin layer chromatography (TLC) and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cytotoxicity test. Lyophilized extracts from fungal cultures were employed for both analyses. In the TLC, the lyophilized extracts were solubilized in different organic solvents (methanol, acetonitrile, ethyl acetate, and hexane). Each organic extract was spotted in the solid phase (silica gel U60 plates) and its respective extraction solvent was used as mobile phase. The degradation-products toxicity was evaluated using the MTT test. This test evaluated the effect of the lyophilized extracts in the cell-viability percentage of human cell line U937 (Gallego et al. 2006). The cytotoxicity was measured by comparing the absorbance (595 nm) produced by the control cells and the absorbance produced by the cells exposed to different concentration of the lyophilized extracts (500, 250, 125, 62.5, 31.2, 15.6, and 7.81 ppm). A compound was considered toxic, when the absorbance of the cells exposed to the compound exhibited an absorbance lower than the control's absorbance (Gallego et al. 2006).

Results

Evaluation of ligninolytic and decolorizing activity in solid media

In total, 92 fungi were collected from the four isolation locations. The strains were obtained from different types of lignocellulosic material (composted biomass, trees, discomposing trees etc.). The isolates distribution by site was IRZ (15), BG (32), CF (25), and MRF (20). After isolation and strains purification, the fungi were inoculated in solid malt extract media supplemented with ABTS. In solid media was possible to observe green and purple halos and a combination of both halos. The purple halos were observed after the production of small green halos. The halo formation fluctuated from hours (12–24 h) to days (2–12 days). In total, 30 of 92 isolates exhibited ligninolytic activity. The isolates ID and isolation location are described in Table 1. The total percentage of fungi with enzymatic activity was 32 % and the percentages of positive fungi by sites were IRZ (60 %), BG (15.6 %), CF (40 %), and MRF (32 %).

The 30 selected isolates were evaluated in malt extract agar with each of the three dyes (Novacron red, Remazol black, and Turquoise blue). In this case, an isolate was classified as positive for decolorizing activity when the media exhibited partial or total decolorization. In total, 15 fungi exhibited decolorizing activity which corresponds to 50 % of the preselected fungi and 16.3 % of the original isolated fungi. The percentage of positive isolates was relatively high. In fact, other reports with more than 50 isolates obtained 5.7 % (Hernández-Luna et al. 2008) and 10 % (Risna and Suhirman 2002) of fungi with decolorizing activity. The fungal isolates with decolorizing activity and their level of decolorization are reported in Table 2. Remazol black was degraded by 12 strains, two of which (LVN6 and LVN63) showed the greatest decolorization. Additionally, LVN6 and LVN63 obtained the fastest decolorization with 5 and 3 days, respectively. Similar decolorization velocity have been reported in *P. chrysosporium* (Máximo et al. 2003), *T. versicolor* (Mohorčič et al. 2006), *Bjerkandera adusta* and *T. versicolor* (Novotný et al. 2004). In contrast, the other fungal strains took from 8 to 15 days in decolorize Remazol Black. On the other hand, Turquoise blue obtained the largest number of fungal strains with decolorization (13 strains); however, the decolorization realized by the fungal strains had medium or low level (Table 2). In Turquoise Blue, the greatest decolorization was obtained after day 15. Opposite to Turquoise Blue, Novacron Red obtained the lowest number of fungal isolates (4) with decolorizing activity. This low quantity of positive strains was related to the dye's monoazoic structure. Monoazoic dyes have been reported in different studies to exhibit a high difficulty to be degraded by ligninolytic fungi. This difficulty is

Table 1 Fungal isolate and isolation location of the fungal strains with ligninolytic activity

Isolate	Isolation location	Isolate ID	Isolation location
LVN 4	Botanic garden	LVN 63	Cloud forest
LVN 5	Botanic garden	LVN 65	Cloud forest
LVN 6	Botanic garden	LVN 71	Cloud forest
LVN 31	Botanic garden	LVN 72	Cloud forest
LVN 32	Botanic garden	LVN 73	Cloud forest
LVN 35	Montane rainforest	LVN 74	Cloud forest
LVN 42	Montane rainforest	LVN 79	Industrial and residential zone
LVN 44	Montane rainforest	LVN 80	Industrial and residential zone
LVN 45	Montane rainforest	LVN 82	Industrial and residential zone
LVN 48	Montane rainforest	LVN 83	Industrial and residential zone
LVN 49	Montane rainforest	LVN 84	Industrial and residential zone
LVN 51	Montane rainforest	LVN 85	Industrial and residential zone
LVN 52	Montane rainforest	LVN 88	Industrial and residential zone
LVN 54	Cloud forest	LVN 91	Industrial and residential zone
LVN 61	Cloud forest	LVN 92	Industrial and residential zone

Table 2 Qualitative evaluation of the decolorizing ability in the selected fungal strains

Isolate	Dye		
	Turquoise Blue	Remazol Black	Novacron Red
LVN 6	Medium	High	Medium
LVN 35	Low	Low	Absence
LVN 44	Low	Low	Absence
LVN 45	Low	Low	Absence
LVN 48	Low	Absence	Absence
LVN 49	Medium	Absence	Absence
LVN 52	Absence	Medium	Absence
LVN 54	Medium	Low	Absence
LVN 61	Medium	Absence	Absence
LVN 63	Low	High	Medium
LVN 71	Low	Low	Absence
LVN 72	Low	Absence	Absence
LVN 74	Low	Medium	Absence
LVN 80	Low	Medium	Medium
LVN 88	Absence	Medium	Low

associated with the enzyme access to the dye's azo group (–N = N–) (Máximo et al. 2003; Park et al. 2007).

Selection of decolorizing fungal strains

The fungal strains selection was performed in two phases. The first phase was a preselection study employing malt extract medium without agitation. Whereas, the second phase was an experiment using agitation, Kirk's medium and variations in the nitrogen concentration.

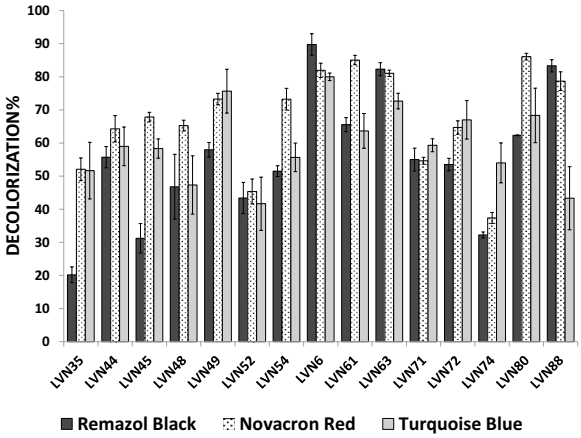

Fig. 1 *Barplot* of the decolorization percentage obtained by the fungal strains in the preselection experiment without agitation and malt extract media

Fungal strains preselection

The preselection experiment was carried out with the 15 isolates that exhibited decolorization activity in solid media (Fig. 1). This experiment evidenced that all the strains generated decolorization; however, the removal level varied among strains and dyes. The variation between solid and liquid media is explained by the changes in environmental conditions, it has been found that fungi behaves different between solid and submerged cultures, in fact, enzymes production is favored by growing in solid surfaces improving the enzymatic activity and the enzymatic profile (Novotný et al. 2004). In Remazol Black, the isolates LVN6, LVN88, and LVN63 exhibited the highest removal (>80 %), whereas the other fungi removal percentages varied between 20 and 80 %. The results between solid and liquid experiments were similar in LVN6 and LVN63; in both, they achieved the largest decolorization. In Novacron Red, the fungal isolates LVN80, LVN61, LVN6, LVN63 and LVN88 exhibited the most significant decolorization. From these fungi, LVN61 was the only strain with high decolorization in the liquid experiment and low decolorization in the solid experiment. The high removal observed in Novacron Red, under non-agitated conditions, is not common. In fact, monoazoic dyes had low or none decolorization under non-agitated conditions (Jarosz-Wilkołazka et al. 2002; Swamy and Ramsay 1999). Turning now to turquoise blue, five strains (LVN6, LVN49, LVN63, LVN80, and LVN72) obtained decolorization around 70 % or more. This result is promissory because under non-agitation conditions other phthalocyanines dyes have not been decolorized by other fungal strains (Swamy and Ramsay 1999).

The experiment's statistical analysis (see Additional file 1: Annex S1) was based in the decolorization

Table 3 Preselected fungal isolates

Turquoise Blue	Remazol Black	Novacron Red
LVN 6	LVN 6	LVN 6
LVN 63	LVN 63	LVN 63
LVN 80	LVN 88	LVN 80
LVN 72		LVN 88
LVN 49		LVN 61

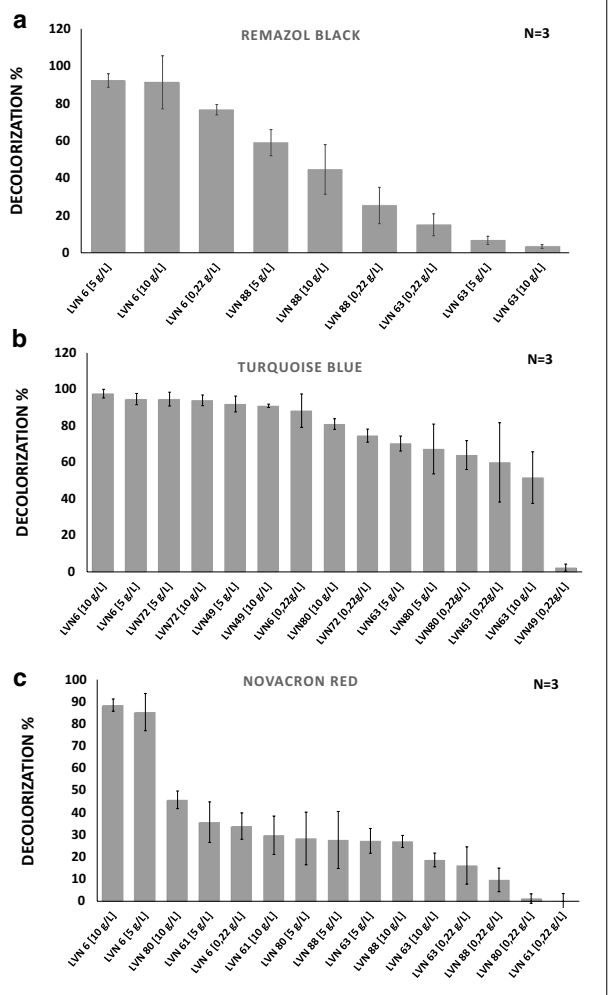

Fig. 2 *Barplot* of the decolorization percentage obtained in the selection experiment with Kirk's media and agitation; **a** Remazol Black, **b** Turquoise Blue, **c** Novacron Red

percentage, using the Duncan's test to select the best fungal isolates for each dye (Table 3). The isolates selected were LVN6, LVN63, LVN80, LVN88, LVN61, LVN49, and LVN72. The decolorization percentage of the selected fungal strains were around 85 % (see Fig. 1). These values were similar to the values achieved by cultures without agitation of *Phlebia tremellosa, Irpex lacteus,* and basidiomycete strain PV 002 (Kirby et al. 2000; Svobodová et al. 2008). The isolates LVN6 and LVN63 were the only strains with high removal in all the dyes evaluated. The ability of decolorize different types of dyes, under non-agitation conditions, was reported in a small number of fungal strains (Kirby et al. 2000; Erkurt et al. 2007).

Fungal strains selection

The fungal strains selection was performed in Kirk's medium with agitation and three different concentrations of ammonium tartrate. The fungal strains used in each dye are shown in Table 3. The experiment employed a factorial design, using as factors the selected microorganism for each dye and the three concentrations of ammonium tartrate. The factorial design produced nine treatments for Remazol Black and 15 treatments for Novacron Red and Turquoise Blue. The decolorization percentages obtained by each treatment are shown in Fig. 2.

Remazol Black experiments showed LVN6 as the best fungal strain (>90 %). In this dye, LVN6 at the three concentrations of ammonium tartrate realized decolorization greater than the other fungal strains (Fig. 2a). The statistical analysis showed interaction between the factors; therefore, the treatments selection was performed using the interaction plots (see Additional file 1: Annex S1). The interaction plots evidenced the greatest decolorization in the treatments with LVN6 and 5 and 10 g/L of ammonium tartrate. In contrast, LVN63 obtained the highest removal at 0.22 g/L of ammonium tartrate; this behavior was also detected in Novacron Red. The decolorizing activity observed in LVN6 [5 g/L] and LVN6 [10 g/L] was similar to *Bjerkandera adusta, Trametes versicolor, Funalia trogii* and *Irpex Lacteus,* strains recognized by their high decolorizing activity over Remazol Black (Mohorčič et al. 2006; Park et al. 2007; Máximo and Costa-Ferreira 2004). In Remazol Black, the selected treatment was LVN6 [5 g/L] because it produced the highest decolorization with the lowest consumption of ammonium tartrate.

The highest removals were achieved in Turquoise Blue. In this dye, almost all the treatments presented removals above 50 %. In this dye, three strains (LVN6, LVN72 and LVN49) obtained decolorizations above 90 % (Fig. 2b). The decolorization obtained by LVN6, LVN72 and LVN49 is comparable with the removal obtained by *P. simplicissimum* INCQS40211, *P. chrysosporium,* and *Ph. Tremellosa* over Turquoise Blue. In these strains, the removal was above 94 % (Kirby et al. 2000; Bergsten-Torralba et al. 2009). Similar to Remazol black, the statistical

analysis evidenced the interaction between isolates and ammonium tartrate concentration. The interaction plots indicated LVN49, LVN72 and LVN6 as the best fungal strains to decolorize Turquoise Blue and the concentrations of 5 and 10 g/L of ammonium tartrate as the best concentrations to obtain high decolorization. In this dye, the best treatments were LVN49 [5 g/L], LVN72 [5 g/L], and LVN6 [5 g/L]. However, the treatments LVN49 [5 g/L] and LVN72 [5 g/L] were not employed in further experiments because its lack of efficiency over the other two dyes.

Novacron Red experiment exhibited LVN6 [5 g/L] and LVN6 [10 g/L] as the treatments with the greatest decolorization percentages (>85 %) (Fig. 2c). On contrary, the other fungal strains had a reduction in the decolorizing ability, in comparison with the unagitated experiment. This reduction can be explained by the changes in the growth media and the inclusion of agitation. The statistical analysis (see Additional file 1: Annex S1) exhibited significant differences among the treatments and an interaction between the factors. The interaction plots revealed 10 and 5 g/L of ammonium tartrate and LVN6 as the best treatments for Novacron Red decolorization. The removal obtained by LVN6 [5 g/L] (85.3 %) and LVN6 [10 g/L] (88.5 %) was larger than the reported by other fungal strains at similar or greater concentrations (≥200 ppm) of monoazoic dyes (Jarosz-Wilkołazka et al. 2002). The treatment LVN6 [5 g/L] was selected as the best option for the decolorization of Novacron Red, because it reached a similar decolorization than LVN6 [10 g/L], using half of the concentration of ammonium tartrate.

Molecular identification of the isolate LVN6

The ITS region (DNAr) and DNAr 28S from LVN6 were amplified to identify the molecular identity of this strain. The amplification of these DNA sequences generated bands of 500–600 pair of bases (pb) for ITS, and 1100 pb for DNAr 28S. The BLAST analysis and sequence identity of ITS and DNAr 28S identified the fungi LVN6 as a member of the genre *Leptosphaerulina*. A specie determination using the blast analysis was not possible. In this strain, the sequences of the ITS region and DNAr 28S were homologous to two different species of *Leptosphaerulina*. The ITS region's sequence was homologue to *Leptosphaerulina Americana*, whereas DNAr 28S' sequence was homologous to *L. australis.* The phylogenetic and morphological analysis of these sequences did not allow the complete identification of this strain (Chanagá Vera et al. 2012). At this moment, this is the first report demonstrating the decolorizing activity in the genre *Leptosphaerulina.* The ability of *Leptosphaerulina* sp. to decolorize solutions with dyes can be related to the presence of ligninolytic enzymes. Likewise, *L. chartarum* and *L. briosiana* have been described as ligninolytic enzymes producers (Simon et al. 1979; Sajben-Nagy et al. 2014).

Decolorization kinetics

Figure 3a, b shown the Remazol Black decolorization kinetic. In the first 5 days, Remazol Black decolorization was approximately 30 %; however, after seven days, it increased above 90 %. The decolorization was correlated with the increment in the enzymatic production (Fig. 3a). *Leptosphaerulina* sp. produced laccase and MnP during all the kinetic. In Remazol Black, the greatest production of enzymes started at day 7 and had its maximum production around day 15. The highest production of laccase and MnP was 650 U/L and 100 U/L, respectively. Turquoise Blue decolorization kinetic exhibited a removal above 90 % in 3 days (Fig. 3c, d). The initial removal was performed by an adsorption process. The Turquoise Blue high adsorption can be related to the copper molecule in the core of the dye structure. After the day 5, the adsorption process was replaced by the enzymatic process. The enzymatic catalysis removed the dye particles attached to the fungal biomass. This removal was catalyzed by laccase and MnP production. The largest enzymatic production was observed at day 9 with a laccase activity of 512 U/L and a MnP activity of 100 U/L. The Novacron Red decolorization kinetic by *Leptosphaerulina* sp. [5 g/L] (Fig. 3e, f) showed a removal greater than 90 % and the production of ligninolytic enzymes. The highest decolorization achieved by *Leptosphaerulina* sp. was obtained in 9 days, the enzymatic production was observed during all the kinetic; nevertheless, the highest enzymatic activities of laccase and MnP were observed on the 30th day with 647 U/L and 90 U/L, respectively.

Enzymatic decolorization using *Leptosphaerulina* sp. extracts

The enzymatic degradation of the three dyes was evaluated by employing a commercial laccase and ABTS as mediator (see Additional file 1: Annex S1). The in vitro analysis showed that all the dyes were decolorized by the commercial laccase; however, the level of decolorization varied depending on the dye and ABTS concentrations. The highest decolorization was obtained in Turquoise Blue (83 %), followed by Remazol Black (80 %), and Novacron Red (56 %). The greatest decolorizations were achieved by the treatments with ABTS as mediator (2 mM) and the highest commercial enzyme concentration (2 mg/ml). Turquoise Blue was the only dye that exhibited any level of decolorization without using ABTS. The *Leptosphaerulina* sp. lyophilized-extracts (Fig. 4) decolorized enzymatically all the dyes. In Remazol Black,

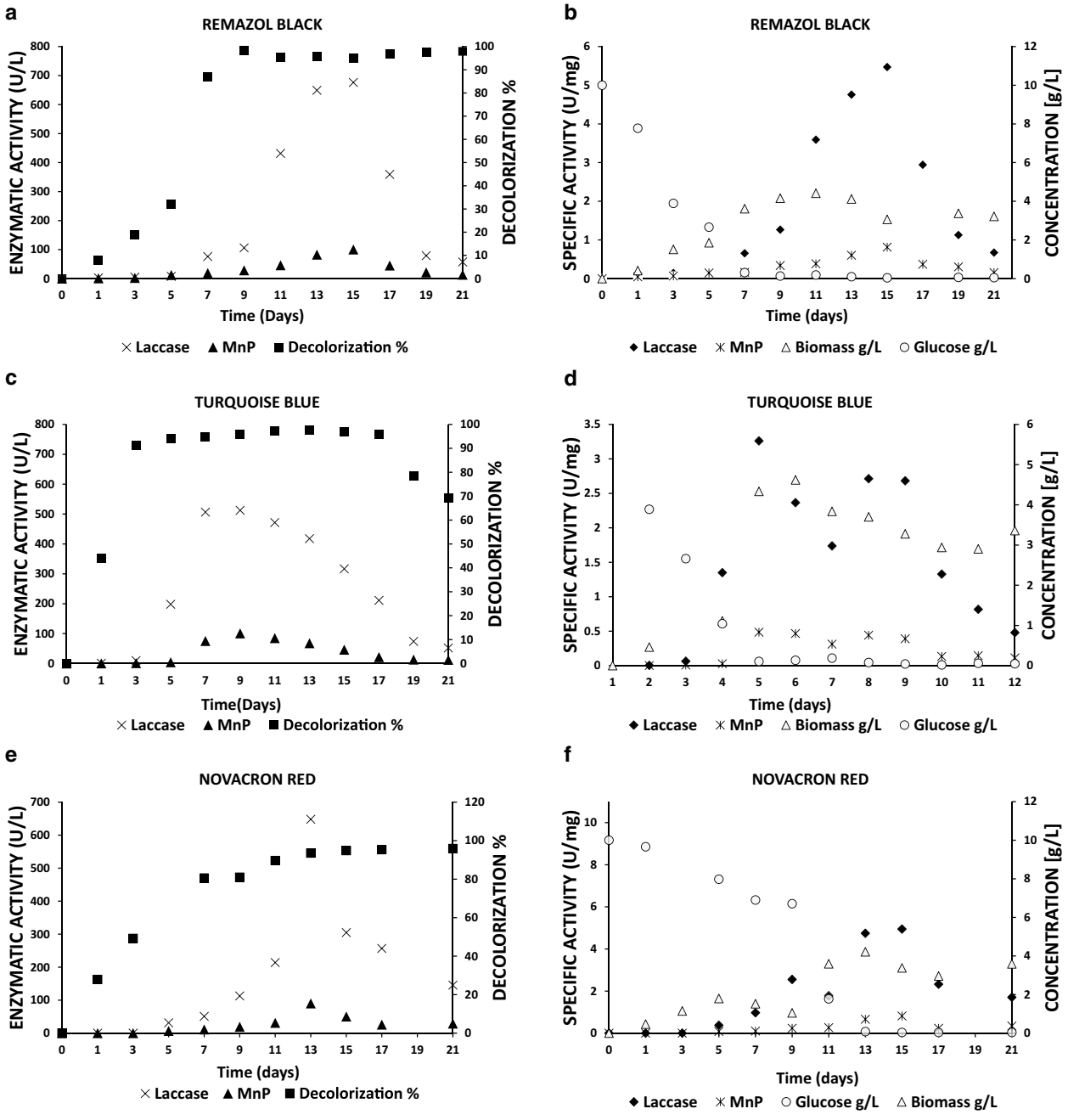

Fig. 3 *Leptosphaerulina* sp. decolorization kinetic. **a** Remazol Black kinetic showing decolorization % and enzymatic activities, **b** Remazol Black kinetic showing glucose concentration, biomass concentration, and specific enzymatic activities **c**Turquoise Blue kinetic showing decolorization percentage and enzymatic activities, **d** Turquoise Blue kinetic showing kinetic showing glucose concentration, biomass concentration, and specific enzymatic activities **e** Novacron Red showing decolorization percentage and enzymatic activities, **f** Novacron Red showing showing kinetic showing glucose concentration, biomass concentration, and specific enzymatic activities

the enzymatic decolorization was approximately 70 %. *Leptosphaerulina* sp. enzymatic extract transformed the reaction media color, from a deep black into a reddish color. *Leptosphaerulina* sp. enzymatic extracts achieved similar levels of decolorization than the commercial enzyme (80 %). In Turquoise Blue, the *Leptosphaerulina* sp. enzymatic extracts produced a decolorization of 56 % (Fig. 4). In Turquoise Blue, the decolorization difference between the commercial laccase (83 %) and *Leptosphaerulina* sp. enzymatic extracts (56 %) evidenced a necessity

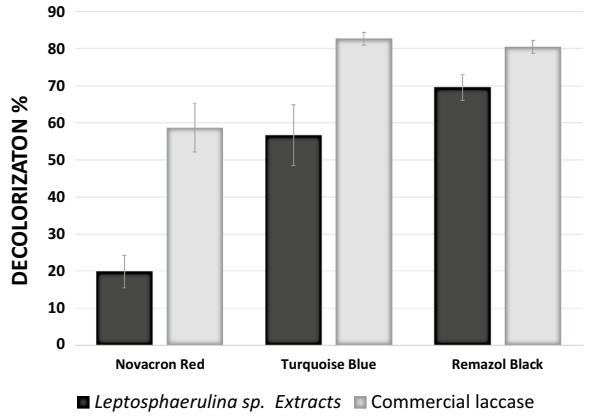

Fig. 4 *Barplot* of the decolorization percentage obtained by the in vitro study of *Leptosphaerulina* sp. enzymatic extracts

to improve the enzyme purification process. In Novacron Red, the enzymatic extracts achieved low removal (20 %); nevertheless, the commercial enzyme also got low removal (56 %).

Real textile effluents treatment using *Leptosphaerulina* sp.

The final discharge effluent had the following characteristics: alkaline pH (12), greyish color, low dyes concentration, and the presence of suspended particles. The effluent spectrophotometric analysis showed two strong signals at 520 and 680 nm. These signals were employed to calculate the effluent decolorization percentage. The decolorizing effect of *Leptosphaerulina* sp. was evaluated using a factorial design 2 × 2 × 2. The experiment's factors were: medium supplementation (10 g/L of glucose and 5 g/L of ammonium tartrate), pH (as received and pH 5) and inoculum (4 and 8 cylinders). After 21 days, the treatments with low pH achieved a decolorization above 70 %, whereas, the treatments with the original pH produced removals below 30 % (Fig. 5a). The experiment's statistical analysis evidenced a lack of interaction between the three factors (p value >0.05). Thus, the best treatment selection was carried out by doing the Duncan's test (see Additional file 1: Annex S1). The treatment pHm-Sup-8 achieved the best decolorization (95.5 %), followed by pHm-Sup-4 (71 %), pHm-woSup-4 (70 %), and pHm-woSup-8 (69 %). The treatments that produced the largest decolorization also showed laccase production. pHm-Sup-8 treatment achieved the greatest laccase production (24.86 U/L), followed by pHm-Sup-4 (5.27 U/L). The first and final days effluent's spectrum evidenced the complete reduction of the signals observed at the beginning of the experiment (Fig. 5c).

The dyeing effluent was evaluated using a factorial design 2 × 2 × 2. In this effluent, the experiment's factors were: medium supplementation (10 g/L of glucose and 5 g/L of ammonium tartrate), inoculum (4 or 8 cylinders), and dilution (as received and 200 ppm of dyes, the dye concentration was calculated based in Novacron Red's original concentration). The effluent had three dyes: Novacron Red (6.8 g/L), Novacron Blue (6.75 g/L), and Novacron Yellow (5.94 g/L). The effluent had a basic pH (12), suspended particles and deep military green coloration. The spectrophotometric analysis revealed two strong signals at 415 and 608 nm (Fig. 5d); these signals were used to calculate the decolorization percentage. *Leptosphaerulina* sp. only decolorized the effluent when it was diluted to 200 ppm (Fig. 5b), the undiluted samples did not show decolorization. The largest removal was obtained by Dil-Sup-8 (90 %), the other diluted treatments decolorized the effluent below 50 %. Dil-Sup-8 exhibited a considerable reduction in the signal at 415 nm (Fig. 5d). The absorbance reduction in this peak can be related with the production of laccases. In fact, Dil-Sup-8 presented the most significant laccase production (40 U/L). The absorbance reduction at 415 nm was observed in the treatment Dil-Sup-4, which also produced laccase activity (10 U/L). The statistical analysis evidenced an interaction between the inoculum quantity and the medium supplementation (see Additional file 1: Annex S1). The interactions plot described an inverse interaction between the factors. Additionally, the interaction plot confirmed the treatment Dil-Sup-8 as the best conditions to decolorize the dyeing effluent by *Leptosphaerulina* sp.

Decolorization products evaluation

The decolorized media (dyes and effluents) were lyophilized; then, the compounds in the lyophilized were extracted using four solvents (methanol, acetonitrile, ethyl acetate, and hexane). After that, the thin layer chromatography (TLC) was performed. The extracts were spotted and run it using the same solvent as mobile phase. The hexane extracts did not produce any type of signals under UV light (near and far) and visible light. By contrast, the methanol, acetonitrile and ethyl acetate extracts revealed signals under UV and visible lights (Table 4). The chromatograms of the three dyes and the effluents exhibited the disappearance of the signals corresponding to the dyes were present in dissolution and effluents (Rf 1). Additionally, the methanol, acetonitrile and ethyl acetate extracts showed a large number of signals. These signals were not observed in the controls (Kirk's media with and without dye). Turquoise Blue extracts generated a number of signals greater than Remazol Black and Novacron Red extracts. The use of TLC to evaluate the fungal or enzymatic degradation of dye have been reported in the enzymatic decolorization

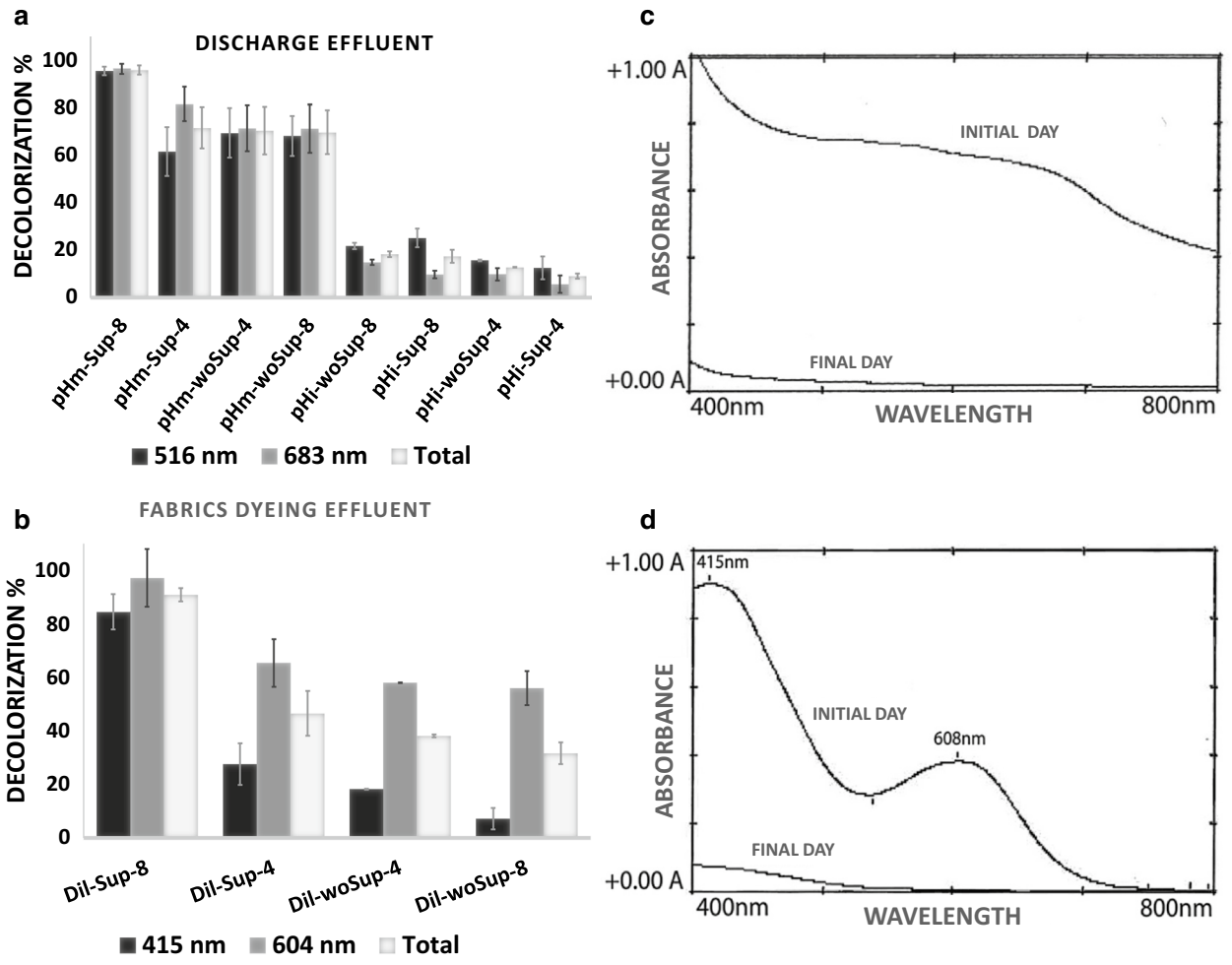

Fig. 5 Real textile industry effluents decolorization by *Leptosphaerulina* sp. **a** *Barplot* of the discharge effluent experiment. **b** *Barplot* of the dyeing effluent experiment. **c** Discharge effluent spectra from pHm-Sup-8 treatment at the initial and final day. **d** Dyeing effluent spectra Dil-Sup-8 treatment at the initial and final day

of Reactive Orange 16, Reactive Red 2 and Reactive Blue 4 (Axelsson et al. 2006; Telke et al. 2009). In these publications, the disappearance of the original dye's signal and the appearance of one or more signals with different Rf were correlated with fungal or enzymatic dye degradation. Using the same proposition, *Leptosphaerulina* sp. degraded the dyes in Kirk's media and the real effluents. However, it was not possible to identify how and which of the signals were related to the dye degradation.

The MTT test used lyophilized extracts from decolorized cultures of *Leptosphaerulina* sp. The MTT analyzed the cytotoxic effect of different concentrations of the lyophilized extracts over the human cell line U937. At the end of the test, the concentrations evaluated did not evidence cytotoxicity to the human cell line U937, because none of the concentrations generated a significant reduction in the absorbance at 595 nm (Table 5). These results evidenced that *Leptosphaerulina* sp. decolorized Novacron Red, Remazol Black and Turquoise Blue, without the production of toxic compounds.

Discussion

From 92 fungi isolated from different ecosystems, the selection process performed in this paper indicated that *Leptosphaerulina* sp. is the fungal strain with the best decolorizing activity among the fungal strains isolated. At this moment, this is the first report demonstrating the decolorizing activity in the genre *Leptosphaerulina.* The ability of *Leptosphaerulina* sp. to decolorize solutions with dyes can be related to the presence of ligninolytic

Table 4 Thin layer chromatography results of the lyophilized extracts obtained after the decolorization process

Dye/effluent	Solvent	Number of new bands			
		Visible	Near UV	Distant UV	Total
Remazol Black	Methanol	3 (Rf: 0.72, 0.78, 0.86)	3 (Rf: 0.72, 0.78, 0.86)	3 (Rf: 0.52, 0.72, 0.8)	9
	Acetonitrile	0	2 (Rf: band and 0.84)	2 (Rf: 1, 0.72)	4
	Ethyl acetate	0	1 (Rf: band between 0.70 and 0.79)	4 (Rf: 0.6,0.71, 0.79, 0.83)	5
Novacron red	Methanol	1 (Rf: 0.69)	3 (Rf: 0.67, band between 0.7 and 0.74, 0.79)	4 (Rf: 0.55, 0.75, 0.78, 0.87)	8
	Acetonitrile	0	3 (Rf: band, 0.52, 0.86)	3 (Rf: 0.68, 0.91, 1)	6
	Ethyl acetate	0	3 (Rf: 0.25, 0.31, 0.51, 0.75)	4 (Rf: 0.62, 0.79, 0.81, 0.85)	7
Turquoise blue	Methanol	1 (Rf: 0.69)	3 (Rf: 0.69, band between 0.71 and 0.77, 0.82)	4 (Rf: 0.55, 0.67, 0.7, 0.8)	8
	Acetonitrile	0	2 (Rf: barrido y 0.88)	5 (Rf: 0.76, 0.79, 0.83, 0.93, 1)	7
	Ethyl acetate	0	5 (Rf: 0.13, 0.19, 0.25, and 0.33 and a band between 0.66 and 0.8)	5 (Rf: 0.74, 0.78 0.81, 0.86, 0.9)	10
Discharge effluent	Methanol	0	2 (Rf: 0.65, 0.76)	1 (Rf: 0.65)	2
	Acetonitrile	0	1 (Rf: 1)	0	1
	Ethyl acetate	0	2 (band and Rf: 0.7)	6 (Rfs: 0.09, 0.16, 0.22, 0.5, 0.72, 0.84)	8
Dyeing effluent	Methanol	1 (Rf: 0.65)	1 (Rf: 0.65)	1 (Rf: 0.65)	1
	Acetonitrile	0	1 (Rf: 0.78)	0	1
	Ethyl acetate	0	3 (Rf: 0.65, 0.1, 0.2)	2 (Rf: 0.71, 0.76)	5

Table 5 Cytotoxicity analysis of the lyophilized extracts obtained after the decolorization process using the MTT test

Lyophilized extracts concentration (ppm)	Remazol Black Absorbance	Novacron Red Absorbance	Turquoise Blue Absorbance
500	1.09 ± 0.07	1.03 ± 0.04	1.02 ± 0.09
250	1.11 ± 0.10	0.68 ± 0.06	0.68 ± 0.10
125	0.99 ± 0.25	0.79 ± 0.05	0.68 ± 0.05
62.5	1.01 ± 0.05	0.81 ± 0.01	0.73 ± 0.07
31.2	1.04 ± 0.10	0.77 ± 0.02	0.68 ± 0.04
15.6	1.03 ± 0.05	0.64 ± 0.05	0.74 ± 0.08
7.8	1.15 ± 0.15	0.73 ± 0.09	0.63 ± 0.11
Control	0.91 ± 0.06	0.72 ± 0.06	0.72 ± 0.06

enzymes. Likewise, *L. chartarum* and *L. briosiana* have been described as ligninolytic enzymes producers (Simon et al. 1979; Sajben-Nagy et al. 2014).

The decolorizing activity observed by *Leptosphaerulina* sp. in remazol black was similar to *Bjerkandera adusta, Trametes versicolor, Funalia trogii* and *Irpex Lacteus,* strains recognized by their high decolorizing activity over Remazol Black (Mohorčič et al. 2006; Park et al. 2007; Máximo and Costa-Ferreira 2004). Similarly, *Leptosphaerulina* sp. decolorization percentage (>90 %) can be compared with the decolorization obtained by *P. simplicissimum* INCQS40211, *P. chrysosporium,* and *Ph. Tremellosa* over Turquoise Blue. In these fungal strains, the removal was above 94 % (Kirby et al. 2000; Bergsten-Torralba et al. 2009). Similarly, novacron red was decolorized by *Leptosphaerulina* sp. in a greater level than the reported by other fungal strains at similar or greater concentrations (≥200 ppm) of monoazoic dyes (Jarosz-Wilkołazka et al. 2002).

In terms of decolorization time, *Leptosphaerulina* sp. decolorized remazol black in times comparable with *Funalia trogii* (3 days); however, *F. Trogii* had a dye concentration (100 ppm) lower than *Leptosphaerulina* sp. (200 ppm) (Park et al. 2007). In other fungal strains, Remazol black decolorization was realized between 10 and 15 days and with a dye concentration lower than the used in this investigation (Mohorčič et al. 2006; Park et al. 2007). Turquoise Blue decolorization exhibited a high decolorization time (3 days) (Fig. 3b). However, in this dye, the initial removal was performed by an adsorption process. The Turquoise Blue high adsorption can be related to the copper molecule in the core of the dye

structure. The adsorption of copper-related dyes has been observed in other fungal strains, as *P. simplicissimum* and *Penicillium oxalicum* (Bergsten-Torralba et al. 2009; Xin et al. 2010). In contrast, novacron red was decolorized in less days (9 days, 90 %); however, this period is considered as a fast decolorization for monoazoic dyes. Other fungal strains decolorized monoazoic dyes at the same concentration, during a time between 12–20 days (Swamy and Ramsay 1999; Toh et al. 2003). Faster decolorizations have been reported; nevertheless, these decolorizations used concentrations (80–100 ppm) less than this study (200 ppm) (Swamy and Ramsay 1999). Similar to turquoise blue, Novacron Red decolorization by *Leptosphaerulina* sp. exhibited two decolorization mechanisms, adsorption and enzymatic catalysis. Adsorption was the predominant mechanism during the 1st days; however, after the 3rd day, the principal mechanism was the enzymatic catalysis. The combination of these two removal mechanisms has also been described in *I. lacteus* and *Penicillium simplicissimum* in the decolorization of reactive red 198 and methyl red (Novotný et al. 2004; Bergsten-Torralba et al. 2009).

Leptosphaerulina sp. produced laccase and MnP to decolorize the three dyes, LiP was not observed in the cultures. It is important to remark that these enzymes were produced during all the culture; however, they reached their maximum production at different times. In remazol black, the largest enzyme production of laccase (650 U/L) and MnP(100 U/L) by *Leptosphaerulina* sp. was observed at day 15. Ligninolytic microorganisms that decolorize solutions with Remazol Black can be grouped in two sets, high laccase producers and high MnP producers. *Leptosphaerulina* sp. belongs to high laccase producers. The largest laccase activity exhibited by *Leptosphaerulina* sp. (650 U/L) is comparable with *T. versicolor* (577 U/L) and *C. dryhophyla* (335 U/L) (Baldrian and Šnajdr 2006). The MnP activity is also comparable with those species; however, the MnP activity in *Leptosphaerulina* sp. was small compared with other microorganisms, such as *F. trogii* (559 U/L) or *Geotrichum* sp. (300 U/L) (Máximo et al. 2003; Kariminiaae-Hamedaani et al. 2007). In turquoise blue experiment, the largest enzymatic production was observed at day 9 with a laccase activity of 512 U/L and a MnP activity of 100 U/L. The laccase activity dominance, in the treatment of phthalocyanine dyes, has been described in other fungal strains, such as *Gloeophyllum odoratum* (104 U/L), *Trametes pubescens* (391 U/L) and *F. trogii* (400 U/L) (Anastasi et al. 2010; Yesilada et al. 2010). The enzymatic production was observed during all the kinetic; nevertheless, the highest enzymatic activities of laccase and MnP were observed on day 13 with 647 U/L and 90 U/L, respectively. In the degradation of monoazoic dyes is not common to see a laccase production greater than MnP. Monoazoic dyes have been decolorized by fungal strains (*T. versicolor ATCC 20869*, *T. versicolor CNPR 8107*, and *I. lacteus*) with greater MnP activities than laccase (Novotný et al. 2004; Toh et al. 2003).

The positive results obtained by *Leptosphaerulina* sp. and the differences obtained between the dyes are explained by the differences in the biochemistry of the decolorization process. The azoic dyes, Remazol black and Novacron red, are degraded principally by an enzymatic process (Michniewicz et al. 2008); whereas, phthalocyanine dyes (Turquoise Blue) are degraded by a mixture of biophysical and biochemical process. In this case, Turquoise Blue is initially absorbed by the cell wall and after that degraded by the ligninolytic enzymes (Swamy and Ramsay 1999; Kirby et al. 2000). Both types of degradation have a different physiological explanation. In the azoic dyes case, the chemical structure of azoic dyes is related with the presence of phenol groups. Phenol groups are the principal substrate for the ligninolytic enzymes; therefore, the enzymatic enzymes degrade the dye by using the structural similarity between azoic dyes and lignin (Kokol et al. 2007). On the other hand, phthalocyanine dyes are adsorbed in the membrane because they have a copper atom in their structure, copper atoms tend to be absorbed by ligninolytic fungus to further include them in the structure of their enzymes, in this case the dye molecule is absorbed and further degraded by the enzymatic enzymes (Jarosz-Wilkołazka et al. 2002).

The *Leptosphaerulina* sp. lyophilized-extracts (Fig. 4) transformed enzymatically all the dyes; however, this decolorization was dependent of the dye. *Leptosphaerulina* sp. enzymatic extracts achieved similar levels of remazol black decolorization than the commercial enzyme (80 %); however, this decolorization was less than the obtained by enzymatic extracts from *Pleurotus sajor-caju* and *Ganoderma lucidum*, using HBT as mediator (Murugesan et al. 2007). The enzymatic extracts transformed the remazol red into a reddish colour, this reddish coloration was described in the decolorization of Remazol Black by enzymatic extracts from *Pleurotus sajor-caju* and *Ganoderma lucidum* using N,N'-Bis-(1H-tetrazol-5-yl)-hydrazine (HBT) as mediator (Murugesan et al. 2007). In Turquoise Blue, the *Leptosphaerulina* sp. enzymatic extracts produced a decolorization of 56 % (Fig. 4). This enzymatic decolorization is similar to the obtained by extracts of *Dichomitus squalens* (60 %) over the copper (II) phthalocyanine dye (Svobodová et al. 2008). The low decolorization of monoazoic dyes by enzymatic extracts has been reported in acid yellow 49 and acid yellow 129 (Ciullini et al. 2008). In the future, the decolorization using *Leptosphaerulina* sp. enzymatic

extracts can be improved by incrementing the MnP activity in the extracts and employing other types of chemical mediators, as HBT or vanillin.

Leptosphaerulina sp. displayed high decolorizing activity in the discharge and dyeing bath effluent (>90 %). This level of decolorization was achieved by the optimization of different factors. The principal ones were pH and effluent concentration. The importance of neutral or acid pH to realize the decolorization has been observed in *P. sajor-caju*, *B. adusta*, and *I. lacteus* (Mohorčič et al. 2006; Novotný et al. 2011). The dye concentration in the effluent was 50 times larger than the dye concentration tested in the previous experiments. *Leptosphaerulina* sp. was inhibited by the large dye concentration; however, at lower concentrations, *Leptosphaerulina* sp. decolorized efficiently the dyeing effluent. Effluent dilution reduces the growth and enzymatic production inhibitions (Mohorčič et al. 2006). Other factors such as nitrogen and glucose supplementation and initial inoculum also affected the decolorization process; however, they were less relevant than pH and effluent concentration. Glucose supplementation has been proved as an important factor in order to bio-remediate textile effluents with ligninolytic fungi (Novotný et al. 2011; Pakshirajan et al. 2011). In contrast, nitrogen supplementation had contradictory effects. *Aspergillus fumigatus* XC6 (Jin et al. 2007) and *Phanerochaete chrysosporium* (Pakshirajan et al. 2011) exhibited large decolorization, when nitrogen was supplemented; whereas, *C. versicolor* showed less decolorization after nitrogen supplementation (Asgher et al. 2009).

Conclusions

From 92 Colombian fungal isolates, *Leptosphaerulina* sp. exhibited the best decolorization percentage (>90 %) in solid and liquid cultures, and in agitated and un-agitated conditions. Additionally, *Leptosphaerulina* sp. decolorized two real effluents from textile industries (>90 %) under conditions of low pH and glucose/nitrogen supplementation. These decolorizations were associated with the production of significant quantities of laccase (650 U/L) and MnP (100 U/L). *Leptosphaerulina* sp. enzymatic extracts decolorized (20–80 %) the three dyes; however, they needed the presence of ABTS as mediator. The cytotoxicity test and the chromatographic techniques demonstrated the dyes' biochemical transformation and the absence of negative effects in the compounds produced by this biochemical process. *Leptosphaerulina* sp. is an interesting microorganism for future biotechnological applications, specially to treat waters from the textile industry.

Additional file

Additional file 1. Statistical analysis.

Authors' contributions

JP was responsible of experiment's conception and design, acquisition of data, analysis and interpretation of data, and manuscript drafting. XC was responsible of microbiological and molecular experimentation. SO was responsible of microbiological and enzymatic experiments. MY was responsible of analysis and interpretation of data and manuscript drafting and approval. AM was responsible of final manuscript approval. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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