Research, Society and Development, v. 9, n. 11, e88191110643, 2020
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i11.10643

Coculture of white rot fungi enhance laccase activity and its dye decolorization capacity

O cocultivo de fungos da podridão branca aumenta a atividade da lacase e sua capacidade de descoloração de corantes

El cocultivo de hongos de pudrición blanca mejora la actividad de las lacasas y su capacidad de decoloración de colorantes

Received: 11/27/2020 | Reviewed: 12/02/2020 | Accept: 12/02/2020 | Published: 12/06/2020

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Abstract

Fungal cocultures can promote complex interactions that result in physiological and biochemical alterations that favor the synergic and more efficient action of extracellular enzymes such as laccase. Thus, coculture can be used as a strategy to increase enzymatic activity, dye degradation, and bioremediation of textile effluents. This study aimed to evaluate the coculture effect of *Lentinus crinitus, Pleurotus ostreatus, Pycnoporus sanguineus*, and *Trametes polyzona* on laccase activity, mycelial biomass production, and *in vitro* decolorization of azo, anthraquinone, and triphenylmethane dyes. The species were cultivated in liquid medium in monoculture and coculture in paired combinations for 15 days to determine the laccase activity and produced mycelial biomass. The enzymatic extracts of fungal cultivations were used in decolorization tests of reactive blue 220 (RB220), malachite green (MG), and remazol brilliant blue R (RBBR). *Pleurotus-Trametes*, *Lentinus-Pleurotus*, and *Lentinus-Trametes* cocultures increase laccase activity compared to respective monocultures. *Lentinus-Pycnoporus*, *Lentinus-Trametes*, *Lentinus-Pleurotus*, and *Pleurotus-Trametes* cocultures stimulate mycelial biomass production in relation to their respective monocultures. The enzymatic extracts of monocultures and cocultures promoted the decolorization of all dyes. RB220 dye presented fast decolorization. In 24 h, all extracts reached maximum decolorization and the greatest color reduction percentage was 90% for *Pleurotus-Trametes* coculture extract. *Pleurotus-Trametes* extract also increased the decolorization of MG and RBBR dyes when compared to their respective monocultures in 48

h and 72 h, respectively. However, RBBR dye presented the greatest resistance to decolorization.

Keywords: Basidiomycota; Cocultivation; Decolorization; Laccase; Textile dyes.

Resumo

Coculturas fúngicas podem promover interações complexas que resultam em alterações fisiológicas e bioquímicas que favorecem a ação sinérgica e mais eficiente de enzimas extracelulares, como a lacase. A cocultura pode ser uma estratégia para aumentar a atividade enzimática, degradação de corantes e biorremediação de efluentes têxteis. Este estudo teve como objetivo avaliar o efeito do cocultivo de *Lentinus crinitus*, *Pleurotus ostreatus*, *Pycnoporus sanguineus* e *Trametes polyzona* sobre a atividade da lacase, produção de biomassa micelial e descoloração in vitro de corantes azo, antraquinona e trifenilmetano. As espécies foram cultivadas em meio líquido em monocultivo e cocultivo em combinações pareadas por 15 dias para determinar a atividade da lacase e produzir biomassa micelial. Os extratos enzimáticos foram utilizados em testes de descoloração do azul reativo 220 (RB220), do verde malaquita (MG) e do remazol azul brilhante R (RBBR). Os cocultivos *Pleurotus-Trametes*, *Lentinus-Pleurotus* e *Lentinus-Trametes* aumentaram a atividade da lacase em comparação com as respectivas monoculturas. *Lentinus-Pycnoporus*, *Lentinus-Trametes*, *Lentinus-Pleurotus* e *Pleurotus-Trametes* estimulam a produção de micélio. Os extratos enzimáticos promoveram a descoloração de todos os corantes. O corante RB220, em 24 h, foi descolorido por todos os extratos e o maior percentual de redução da cor foi de 90% para *Pleurotus-Trametes*. *Pleurotus-Trametes* aumentou a descoloração do MG e RBBR em 48 he 72 h. Entretanto, o RBBR apresentou a maior resistência à descoloração.

Palavras-chave: Basidiomycota; Cocultivo; Corantes têxteis; Descoloração; Lacase.

Resumen

Los cocultivos de hongos pueden promover interacciones complejas que resultan en alteraciones fisiológicas y bioquímicas que favorecen la acción sinérgica y más eficiente de enzimas extracelulares como la lacasa. Por lo tanto, el cocultivo se puede utilizar como una estrategia para aumentar la actividad enzimática, la degradación de colorantes y la biorremediación de los efluentes textiles. Este estudio tuvo como objetivo evaluar el efecto del cocultivo de *Lentinus crinitus*, *Pleurotus ostreatus*, *Pycnoporus sanguineus* y *Trametes polyzona* sobre la actividad lacasa, la producción de biomasa micelial y la decoloración de colorantes azo, antraquinona y trifenilmetano. Las especies se cultivaron en monocultivo y

cocultivo en combinaciones pareadas durante 15 días para determinar la actividad lacasa y biomasa micelial producida. Los extractos enzimáticos se utilizaron en pruebas de decoloración del azul reactivo 220 (RB220), verde malaquita (MG) y remazol azul brillante R (RBBR). Los cocultivos de *Pleurotus-Trametes*, *Lentinus-Pleurotus* y *Lentinus-Trametes* aumentan la actividad de lacasa en comparación con los respectivos monocultivos. Los cocultivos *Lentinus-Pycnoporus*, *Lentinus-Trametes*, *Lentinus-Pleurotus* y *Pleurotus-Trametes* estimulan la producción de biomasa micelial en relación con sus respectivos monocultivos. Los extractos enzimáticos de monocultivos y cocultivos promovieron la decoloración de todos los colorantes. El colorante RB220, en 24 h, fue descolorido por todos los extractos y el porcentaje más alto de reducción de color fue del 90% de *Pleurotus-Trametes*. *Pleurotus-Trametes* aumentó la decoloración de los colorantes MG y RBBR en 48 hy 72 h. Sin embargo, el colorante RBBR presentó la mayor resistencia a la decoloración.
Palabras clave: Basidiomycota; Cocultivo; Colorantes textiles; Descolorización; Lacasa.

1. Introduction

In nature, fungi grow with other microorganisms and can establish symbiotic or competitive interactions. Concurrent interactions among fungi, mainly basidiomycetes, are common, competing for space/territory and nutrients. This interaction can be divided into two types: the interference competition and exploratory competition (Boddy, 2000). In interference competition, one species totally inhibits the other's growth while in exploratory competition there is partial inhibition by reduction of available resources. During exploratory competition, fungi can present morphological alterations in the mycelial branching with the formation of barriers that act as a defensive response to the invasion of other mycelia (Boddy, 2000). These morphological alterations of the mycelium can induce metabolic changes with the production of specific secondary metabolites (Hiscox et al., 2010).

Laccases (benzenediol: oxygen oxidoreductases, EC 1.10.3.2) catalyze the oxidation of a wide range of phenolic compounds to the corresponding free radicals using molecular oxygen as electron acceptor (Martínková et al., 2016). Usually, the use of redox mediators can extend the substrate range of laccases to non-phenolic compounds, polycyclic aromatic hydrocarbons, and dyes (Rivera-Hoyos et al., 2013). Redox mediators are expensive and toxic compounds and laccases that can cause substrate oxidation without the presence of mediators are of special interest to biotechnological applications (Husain & Husain 2008). Laccases are produced by basidiomycetes during the secondary metabolism, frequently induced by stresses

and limited levels of nutrients (Wesenberg et al., 2003). Laccase production during interactions among basidiomycetes has been believed to be a response to stress, representing a defensive action against the mycelial invasion and/or competition for nutrients (Score et al., 1997; Zhang et al., 2006). Cocultures may induce the enzymatic production or cause high levels of stress, which impair production (Chi et al., 2007). Therefore, combination of species and strains in cultivations are decisive to induce a positive effect on enzyme activity such as laccase.

Laccases have potential for industrial applications as in food, paper, cellulose, and textile industries, and in bioremediation (Sharma et al., 2018). The growth estimate for the world enzyme market is 6.7% a year, and 11 billion dollars in 2020 (Kumar et al., 2014). Thus, alternatives that aim to increase enzyme production for biotechnological applications to meet the growing demand in industrial and biotechnological processes are needed (Chander et al., 2004).

A lot of studies on fungal interactions are carried out to understand the general ecological aspects; however, it is important to understand these interactions in order to produce enzymes, mainly the ones that degrade dyes (Chi et al., 2007). The increasing utilization of dyes by several industrial sectors provided an increment in the production of colorful effluents, mostly due to dyes lost during the productive process (Vikrant et al., 2018). The utilization of conventional chemical and physical methods to decolorize effluents is not always efficient and may generate additional residues that also demand treatment (Sen et al., 2016). Fungal laccases have shown their capacity to degrade dyes from several chemical classes (Cardoso et al., 2018) and could represent an eco-friendly alternative to conventional treatments. Therefore, the axenic fungal coculture can be a viable method to increase enzymatic activity compared to the ones obtained by axenic monoculture (Bader et al., 2010; Chan-Cupul et al., 2016). Thus, this study aimed to evaluate the laccase activity, mycelial biomass production, and the *in vitro* decolorization of synthetic dyes by laccase obtained by mono and coculture of basidiomycetes.

2. Materials and Methods

2.1 Microorganisms and inoculum production

The species utilized in this study and their respective identifiers' GenBank accession numbers are *Lentinus crinitus* U9-1 (MG211674), *Pleurotus ostreatus* U2-11 (KJ010860),

Pycnoporus sanguineus U13-4 (MG211680), and *Trametes polyzona* U16-5 (MG211678). All strains belong to the culture collection of the Graduate Program of Biotechnology Applied to Agriculture of Paranaense University. The inoculum was produced by mycelial biomass growth in malt-extract-agar medium (MEA, 20 g L^{-1}) at 28 ± 1 °C for seven days in the dark. MEA disks (6 mm diameter) with mycelium without sectioning were utilized as inoculum for the coculture.

2.2 Coculture in liquid medium

The species were cultivated in conical flasks (250 mL) containing 100 mL of malt extract liquid 20 g L^{-1} previously autoclaved at 121 °C for 20 min (Valle et al., 2014). Paired combinations of the species were inoculated using three MEA disks containing mycelium of each species. The material was kept static at 28 ± 1 °C in the dark for 15 days (Marim et al., 2018). A monoculture of each fungus was used as control. At the end of the cultivation, the mycelial biomass was separated from the cultivation medium by filtration, and the filtrated was utilized to determine laccase activity. The produced mycelial biomass was kept in a stove with air circulation at 60 °C until constant mass to determine mycelial biomass.

The experimental design was completely randomized and all assays were carried out with three replicates. The results were evaluated by analysis of variance (ANOVA) and the significant differences among the arithmetical averages ($p \leq 0.05$) were determined by Scott-Knott test.

2.3 Laccase assay

The oxidation of 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS; Sigma; St. Louis, MO, USA) was used to determine laccase activity (Santana et al., 2018). ABTS (1 mM) in sodium acetate buffer (0.1 M, pH 5.0) was mixed with diluted liquid cultivation and ABTS oxidation rate was measured at 420 nm (ε = 36,000 M^{-1} cm^{-1}) at 30 °C. Two reaction mixtures were used as analytical control, one without the enzymatic extract and another without ABTS. Laccase activity was expressed in international units (U) defined as the amount of enzyme required to oxidize 1 μmol ABTS per minute.

2.4 Manganese peroxidase assay

The oxidation of $MnSO_4$ at room temperature was used to determinate manganese peroxidase (MnP) activity (Wariishi et al., 1992). $MnSO_4$ (10 mM) in sodium malonate buffer (50 mM, pH 4.5) was mixed with liquid cultivation and 0.5 mM hydrogen peroxide. The oxidation was monitored by absorbance increase at 270 nm (ε = 11,590 M^{-1} cm^{-1}) caused by the complex formed by Mn^{3+} ion with malonate. MnP activity was expressed in international units (U) defined as the amount of enzyme that oxidizes 1 μmol $MnSO_4$ per minute.

2.5 Lignin peroxidase assay

Lignin peroxidase (LiP) activity was determined by the oxidation of methylene blue at room temperature (Magalhães et al., 1996). Methylene blue (1.2 mM) in sodium tartrate buffer (0.5 M; pH 4) was mixed with liquid cultivation, and 2.7 mM hydrogen peroxide. The oxidation was monitored by absorbance increase at 664 nm (ε = 52.400 M^{-1} cm^{-1}) caused by methylene blue conversion to azure-C. LiP activity was expressed in international units (U) defined as the amount of enzyme that oxidizes 1 μmol methylene blue per minute.

2.6 *In vitro* decolorization of synthetic dyes by enzymatic extract

The color reduction of different classes of dyes was determined according to Cardoso et al. (2018). Azo dye reactive blue 220 (RB220), anthraquinone remazol brilliant blue R (RBBR), and triphenylmethane malachite green (MG) dyes were diluted in sodium acetate buffer (100 mM, pH 5), filtered (0.22-μm porous filter), and used in the assays. Aliquots of cultivation medium obtained on the 15th cultivation day were mixed with dye solutions in sufficient volume to obtain dye concentration of 0.1 mg mL^{-1} (mass/volume) in all assays. The decolorization reactions occurred in the absence of hydrogen peroxide and were kept at 28 ± 1 °C, in the dark, for 72 h. Aliquots of 300 μL were taken in aseptic conditions, and decolorization was followed in the maximum absorbance of RB220 (600 nm), RBBR (592 nm), and MG (620 nm), every 24 h in a spectrophotometer (SpectraMax Plus 384 Molecular Devices, San Jose, United States). The decolorization equal to or greater than 80% was considered efficient.

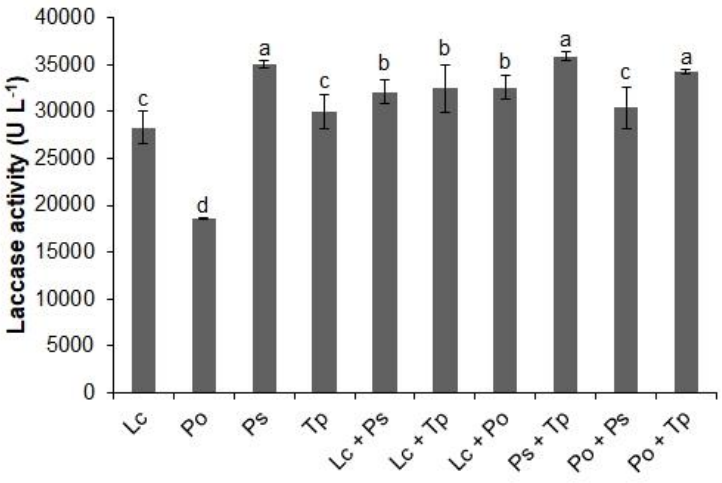
2.7 Statistical analysis

The research was based on quantitative methods developed with an experimental approach (Pereira et al., 2018). The assays had a completely randomized design (CRD) with three replicates. The results were evaluated using ANOVA, and significant differences among arithmetic means were determined by the Scott-Knott test at 5% of probability.

3. Results

Laccase activity was detected in all cultivations but there was no activity of MnP or LiP, except for *Pleurotus-Trametes* and *Pycnoporus-Trametes* cocultures (data not shown) in which traces of LiP (< 10 U L^{-1}) were detected. The greatest laccase activity ($p \leq 0.05$) in monoculture was for *P. sanguineus* (35050 ± 427 U L^{-1}) and in coculture ($p \leq 0.05$) it was for *Pycnoporus-Trametes* (35915 ± 437 U L^{-1}) and *Pleurotus-Trametes* (34279 ± 189 U L^{-1}) (Figure 1). In *Lentinus-Trametes* (32466 ± 2596 U L^{-1}), *Lentinus-Pleurotus* (32597 ± 1300 U L^{-1}) and *Pleurotus-Trametes* (34279 ± 189 U L^{-1}) cocultures, laccase activity was greater ($p \leq 0.05$) than their respective monocultures, whereas in *Lentinus-Pycnoporus* (32130 ± 1255 U L^{-1}) and *Pleurotus-Pycnoporus* (30441 ± 2190 U L^{-1}) cocultures, laccase activity had intermediate value compared to their respective monocultures. *Pycnoporus-Trametes* (35914 ± 437 U L^{-1}) coculture had laccase activity equal to one of monocultures (Figure 1). The greatest increase of laccase activity was observed for *Pleurotus-Trametes* coculture with an increase of 84% compared to *P. ostreatus* monoculture, and 14% in relation to *T. polyzona* monoculture.

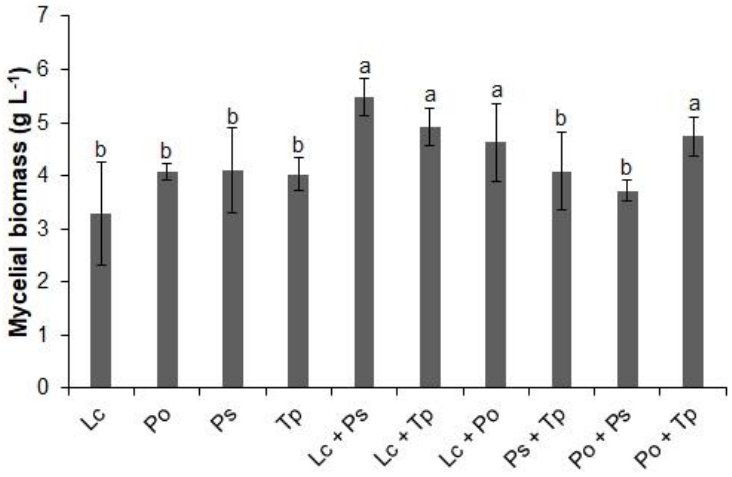
Figure 1. Laccase activity (average ± standard deviation) of basidiomycetes in mono or cocultures grown in liquid malt extract agar (20 g L^{-1}) for 15 days.



Different letters indicate differences among laccase activity according to Scott-Knott test ($p \leq 0.05$). Lc – *Lentinus crinitus*, Po – *Pleurotus ostreatus*, Ps – *Pycnoporus sanguineus* and Tp – *Trametes polyzona*. Source: Authors.

There was no significant difference in the mycelial biomass production between monocultures. Among cocultures the greatest mycelial biomass production occurred with *Lentinus-Pycnoporus* (5.5 ± 0.3 g L^{-1}), *Lentinus-Trametes* (4.9 ± 0.3 g L^{-1}), *Lentinus-Pleurotus* (4.6 ± 0.7 g L^{-1}), and *Pleurotus-Trametes* (4.1 ± 0.7 g L^{-1}) cocultures (Figure 2). In cocultures with *Lentinus-Pycnoporus*, *Lentinus-Trametes*, *Lentinus-Pleurotus*, and *Pleurotus-Trametes*, the mycelial biomass production was greater ($p \leq 0.05$) than their respective monocultures (Figure 2). *Pycnoporus-Trametes* and *Pleurotus-Pycnoporus* cocultures had mycelial biomass production equal to their respective monocultures (Figure 2). *Lentinus-Pycnoporus* coculture (5.5 ± 0.3 g L^{-1}) had the greatest increase in the mycelial biomass production of 67% compared to *L. crinitus* monoculture (3.3 ± 0.8 g L^{-1}) and 34% compared to *P. sanguineus* monoculture (4.1 ± 0.7 g L^{-1}).

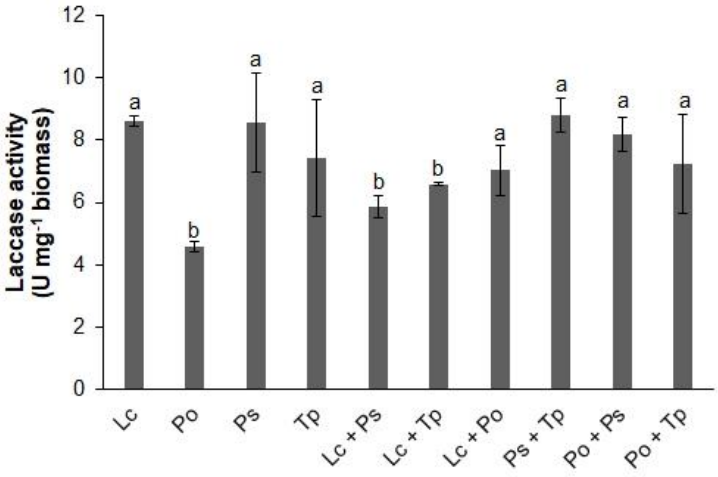
Figure 2. Mycelial biomass production (average ± standard deviation) of basidiomycetes in mono or cocultures grown in liquid malt extract agar (20 g L^{-1}) for 15 days.



Different letters indicate differences among laccase activity according to Scott-Knott test ($p \leq 0.05$). Lc – *Lentinus crinitus*, Po – *Pleurotus ostreatus*, Ps – *Pycnoporus sanguineus* and Tp – *Trametes polyzona*. Source: Authors.

In general, monocultures presented a better relationship of laccase activity divided by mycelial biomass than cocultures (Figure 3). Cocultures with *Lentinus-Pycnoporus* and *Lentinus-Trametes* were less effective than their respective monocultures for this relationship (Figure 3). Cocultures with *P. ostreatus* were better than *P. ostreatus* monoculture and the same when compared to the other respective monocultures (Figure 3). *Pycnoporus-Trametes* coculture had equal value to the respective monocultures and greater nominal values for the relationship of laccase with mycelial biomass (Figure 3). This suggests that the greatest laccase production can be reached by several combinations, mainly in *P. sanguineus* monoculture and *Pycnoporus-Trametes* coculture, without a significant advantage of the coculture utilization and with the interaction without losses in the laccase activity as in the case of *Pleurotus-Trametes* coculture compared to *T. polyzona* monoculture. This indicates that coculture can be directed to a greater or smaller mycelial biomass production with the same laccase activity, and with the possibility of choosing which product or process is of greater interest.

Figure 3. Laccase activity mycelial biomass production ratio (average ± standard deviation) of basidiomycetes in mono or cocultures grown in liquid malt extract agar (20 g L^{-1}) for 15 days.



Different letters indicate differences among laccase activity according to Scott-Knott test ($p \leq 0.05$). Lc – *Lentinus crinitus*, Po – *Pleurotus ostreatus*, Ps – *Pycnoporus sanguineus* and Tp – *Trametes polyzona*. Source: Authors.

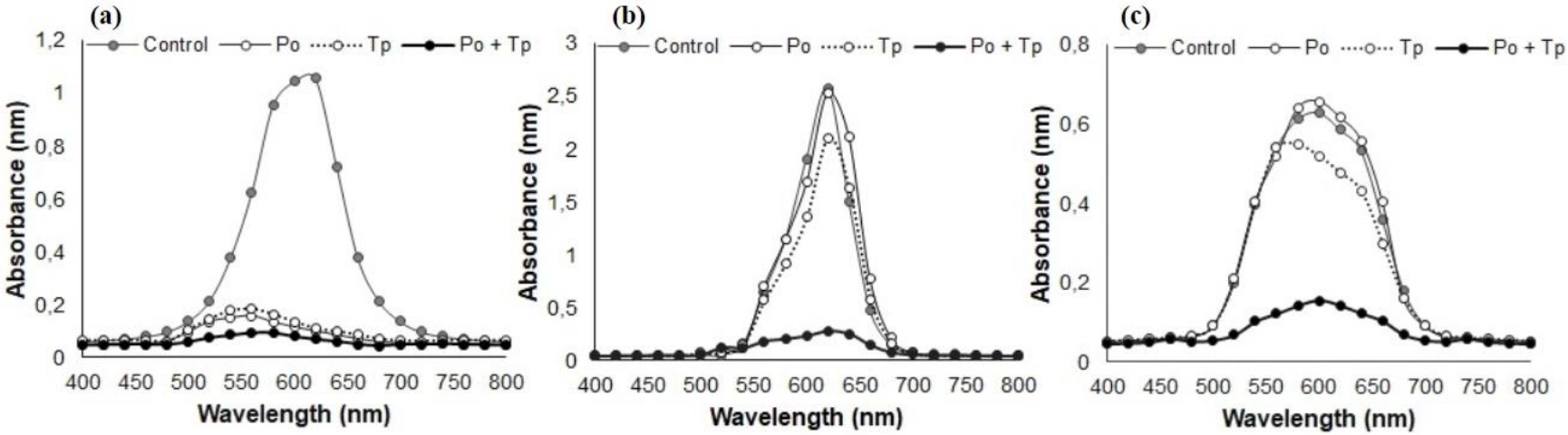
The enzymatic extracts obtained from all cultivations promoted efficient decolorization from 72 to 90% of RB220 in 24 h, without decolorization increase after 48 h or 72 h, (Table 1). Only the extract of *Pleurotus-Trametes* coculture had greater ($p \leq 0.05$) decolorization (90%) of RB220 in 24 h, while the monocultures with greater decolorization (87%) was *L. crinitus* (Table 1 and Figure 4). In 72 h of decolorization, the enzymatic extract from all cultivations had the same decolorization capacity ($p \leq 0.05$) ranging from 72 to 90% indicating the efficiency of the process and that coculture did not affect the decolorization capacity of RB220 (Table 1).

Table 1. Dye decolorization (0.1 mg mL^{-1}) for 24, 48 and 72 h with enzyme extract of basidiomycetes in mono or coculture grown in liquid malt extract medium (20 g L^{-1}) for 15 days.

	Decolorization (%)								
	Reactive Blue 220 (RB220)			Malachite Green (MG)			Remazol Brilliant Blue R (RBBR)		
Fungal mono and coculture	**24 h**	**48 h**	**72 h**	**24 h**	**48 h**	**72 h**	**24 h**	**48 h**	**72 h**
Lentinus crinitus	87 ± 0.91^{bA}	88 ± 0.69^{aA}	86 ± 5.38^{aA}	78 ± 2.26^{bC}	89 ± 0.68^{bB}	92 ± 0.53^{aA}	36 ± 9.80^{cB}	52 ± 4.54^{bA}	45 ± 10.1^{bA}
Pleurotus ostreatus	87 ± 0.77^{bA}	86 ± 2.24^{aA}	88 ± 0.79^{aA}	7 ± 2.56^{eB}	12 ± 2.68^{eA}	12 ± 3.90^{dA}	10 ± 2.55^{dB}	17 ± 2.89^{dA}	0^{dC}
Pycnoporus sanguineus	86 ± 0.74^{cA}	87 ± 0.56^{aA}	84 ± 6.19^{aA}	89 ± 0.96^{aB}	93 ± 0.73^{aA}	94 ± 0.56^{aA}	30 ± 8.65^{cA}	42 ± 5.18^{cA}	31 ± 12.8^{cA}
Trametes polyzona	85 ± 2.88^{cA}	85 ± 5.01^{bA}	72 ± 17.5^{aB}	14 ± 1.57^{dC}	34 ± 7.01^{dB}	46 ± 8.66^{cA}	13 ± 4.39^{dA}	8 ± 2.11^{eA}	11 ± 7.85^{dA}
Lentinus-Pycnoporus	86 ± 1.04^{cA}	86 ± 2.24^{bA}	77 ± 0.79^{aA}	67 ± 15.3^{cB}	82 ± 7.17^{cA}	86 ± 4.86^{bA}	13 ± 5.13^{dA}	20 ± 6.63^{dA}	22 ± 8.35^{cA}
Lentinus-Trametes	88 ± 0.50^{bA}	88 ± 1.53^{aA}	82 ± 11.9^{aA}	75 ± 5.01^{bB}	87 ± 2.32^{bA}	91 ± 1.44^{aA}	29 ± 5.50^{cA}	39 ± 6.32^{cA}	32 ± 12.8^{cA}
Lentinus-Pleurotus	87 ± 1.09^{bA}	87 ± 0.88^{aA}	88 ± 0.76^{aA}	78 ± 7.40^{bB}	89 ± 2.66^{bA}	$92 \pm 1,74^{aA}$	43 ± 10.5^{bA}	56 ± 6.83^{bA}	52 ± 14.9^{bA}
Pycnoporus-Trametes	87 ± 0.55^{bA}	88 ± 0.59^{aA}	78 ± 15.4^{aA}	88 ± 2.97^{aB}	92 ± 1.70^{aA}	93 ± 1.34^{aA}	34 ± 13.0^{cA}	41 ± 10.1^{cA}	36 ± 12.9^{cA}
Pleurotus-Pycnoporus	87 ± 0.60^{bA}	88 ± 1.40^{aA}	82 ± 9.42^{aA}	90 ± 1.80^{aB}	94 ± 1.25^{aA}	94 ± 0.93^{aA}	41 ± 8.61^{bB}	55 ± 6.73^{bA}	51 ± 6.00^{bA}
Pleurotus-Trametes	90 ± 0.69^{aA}	90 ± 0.91^{aA}	90 ± 1.18^{aA}	86 ± 2.79^{aB}	91 ± 1.41^{bA}	92 ± 0.99^{aA}	65 ± 10.7^{aA}	72 ± 9.51^{aA}	74 ± 8.54^{aA}

Different lowercase letters in the same column indicate statistical differences between mono and coculture, and different uppercase letters in the same line indicate statistical differences in decolorization time of each dye by Scott-Knott test ($p \leq 0.05$). Source: Authors.

Figure 4. Visible absorbance spectra of (a) azo dye reactive blue 220 (RB220), (b) triphenylmethane dye malachite green (MG), and (c) anthraquinone dye remazol brilliant blue R (RBBR), before (controls) and after 48 h decolorization with the enzymatic extract of *Pleurotus ostreatus* monoculture (Po), *Trametes polyzona* monoculture (Tp), and *Pleurotus*-*Trametes* coculture (Po+Tp).



Source: Authors.

The cultivation enzymatic extracts decolorized from 67 to 90% of MG in 24 h, except for *T. polyzona* (14%) and *P. ostreatus* (7%) monocultures (Table 1). The coculture extracts with *Pleurotus-Pycnoporus*, *Trametes-Pycnoporus*, and *Pleurotus-Trametes* presented the greatest ($p \leq 0.05$) decolorization varying from 86 to 90% (Table 1). Except for *Lentinus-Pycnoporus*, in 72 h of decolorization, all cocultures and *P. sanguineus* and *L. crinitus* monocultures ranged from 91 to 94%. The coculture extract with *Pleurotus-Trametes* increased ($p \leq 0.05$) MG decolorization in 48 h from 2.8 to 7.6 times compared to the respective monocultures. However, *Lentinus-Pycnoporus* coculture extract had lower decolorization (82%) in 48 h than the respective monocultures that ranged from 89 to 93% (Table 1 and Figure 4). Overall, the coculture extract was better than the respective monocultures, mainly for *Pleurotus-Trametes* and except for *Lentinus-Pycnoporus* (Table 1).

RBBR dye was the most resistant to decolorization. For enzymatic extract of *Lentinus-Trametes* coculture the decolorization was 30% for RBBR in 24 h, whereas for RB220 it was 88% and MG 75% (Table 1 and Figure 4). *Pleurotus-Trametes* coculture presented the greatest ($p \leq 0.05$) RBBR decolorization in 72 h (Table 1) compared to the respective monocultures and also had the greatest laccase activity (Figure 1). However, *P. sanguineus* monoculture, which had the greatest ($p \leq 0.05$) laccase activity (Figure 1) presented decolorization of 31% of RBBR in72 h. In general, the enzymatic extracts presented maximum ($p \leq 0.05$) decolorization in 48 h, varying from 17% for *P. ostreatus* to 72% for *Pleurotus-Trametes* monoculture (Table 1 and Figure 4). In general, the coculture extract was better than the respective monocultures for RBBR, except for *Lentinus-Pycnoporus* and *Lentinus-Trametes* (Table 1). *Pleurotus-Pycnoporus* coculture extract with RBBR decolorization in 48 h (55%) was greater ($p \leq 0.05$) than the respective monocultures (17 to 42%) (Table 1). On the other hand, *Lentinus-Pycnoporus* coculture extract had smaller ($p \leq 0.05$) RBBR decolorization in 48 h (20%) than the respective monocultures (42 to 52%) (Table 1). After 48 h there was an increase in absorbance, suggesting that a molecular rearrangement may have occurred for the enzyme to act on the dye.

4. Discussion

Laccase was the predominant enzyme in all cultivations, always with laccase activity above 18000 U L^{-1}. This is not an unusual situation as the predominance of laccase was reported (Moreira-Neto et al., 2013). The genetic expression and enzymatic activity of peroxidases are strongly affected by cultivation conditions as pH, temperature, and

carbon/nitrogen source (Fernández-Fueyo et al., 2014). Lignocellulose carbon sources can favor peroxidases activity and the malt extract medium used in our study (~60% reducing sugars) may have been a hindrance to ligninolytic peroxidase activity (Mali et al., 2017). However, laccases are associated with a defense response to competition and oxidative stress of cocultures, which can explain their greater activity in the cultivation conditions evaluated in our study (Giardina et al., 2010).

Two of three cocultures containing *P. ostreatus* promoted an expressive increase in laccase activity but *P. ostreatus* monoculture showed the smallest laccase activity among monocultures. Its presence in coculture with *L. crinitus* or *T. polyzona* seems to favor laccase activity. Our results are in accordance with Verma & Madamwar (2002) who demonstrated that *P. ostreatus* coculture with *Phanerochaete chrysosporium* resulted in higher ligninolytic activity than the respective monocultures. Chi et al. (2007) also reported that *P. ostreatus* cultivated with *Ceriporiopsis subvermispora* stimulated laccase activity in liquid cultivations, more than any other coculture between *C. subvermispora* with other fungus. Kumari |& Naraian (2016) verified greater laccase activity of *Pleurotus florida* (current name *Pleurotus ostreatus*) and *Rhizoctonia solani* (current name *Thanatephorus cucumeris*) cocultures.

During fungal coculture, fungi can compete for space and nutrients which can provoke oxidative stress, resulting in physiological and biochemical adaptations that can favor the synergic action of extracellular enzymes (Luo et al., 2017). The greater laccase activity during competitive interactions has been believed to be a response to stress, representing a defensive action against the mycelial invasion and the competition by nutrients (Zhang et al., 2006). On the other hand, cocultures can cause high levels of stress that negatively affect enzyme expression (Wesenberg et al., 2003). However, positive effects on the enzymatic activity depend on the species kept in coculture and the cultivation conditions (Chi et al., 2007). In our study, although most interactions did not increase laccase activity, there was still enzyme production and it suggests that interactions among the evaluated species could increase laccase activity if other cultivation conditions were optimized such as the presence of lignocellulosic substrate or laccase inducers (Mali et al., 2017).

Mycelial biomass production can be a parameter in the evaluation of growth and fungus biological efficiency as an indication of its capacity to convert nutrients in cells (Yang et al., 2013). However, the effects of basidiomycete cocultures have been little explored. *Lentinus-Pycnoporus*, *Lentinus-Trametes*, and *Pleurotus-Trametes* cocultures presented the greatest increases in mycelial biomass. Our results are in accordance with the ones by Kumari & Naraian (2016) that reported an increase in mycelial biomass during *P. florida* and *R. solani*

cocultures. The increase in mycelial biomass production in cocultures can be attributed to competitive interaction among fungi that promotes the increase of several enzymes. It is believed that a greater expression of amounts and variety of enzymes may favor the most efficient utilization of the substrate by the combined and/or synergic action of produced enzymes stimulating the mycelial biomass growth (Dwivedi et al., 2011).

Enzymatic extracts of all evaluated species, whether in monoculture or coculture, were able to decolorize the dyes, and decolorization efficiency varied according to the dye class (MG > RB220 > RBBR) and according to the species combination. It is important to note that the enzymatic extracts obtained in our study had laccase as the predominant enzyme and the decolorization reactions were maintained without hydrogen peroxide addition which excludes any other peroxidase activity. It implies that the decolorization occurred mainly, if not only, due to laccase activity.

In our study the level of laccase activity in the enzymatic extract apparently did not affect decolorization since *P. sanguineus*, the greatest producer of laccase, was cultivated alone and produced the greatest decolorization of MG in 72 h (94%) but produced one of the lowest decolorization of RBBR in 24 h (30%) and reasonable decolorization of RB220 in 24 h (86%). The same was observed with the coculture *Trametes-Pycnoporus* whose laccase activity was the greatest one, but the decolorization of RB220 and MG in 24 h was 87% and 88%, respectively. Our results are in accordance with Moreira-Neto et al. (2013) that reported similar decolorization rate with basidiomycetes with very different laccase activity.

In our study the combination of *Pleurotus-Pycnoporus* in coculture was more efficient in decolorizing the triphenylmethane dye MG achieving 94% of color reduction in 48 h. Our results are in accordance to the ones found by Kumari & Naraian (2016) who also reported that the enzymatic extract of *P. florida* and *R. solani* cocultivation was more efficient to decolorize triphenylmethane brilliant green dye than the extract of monocultures, reaching up 98% of decolorization when the dye was used at lower concentration.

The coculture of *Pleurotus-Trametes* promoted the greatest decolorization of azo dye RB220 and the anthraquinone dye RBBR among all cultivations. Krishnamoorthy et al. (2018) isolated fungi and bacteria from soil contaminated with azo dyes and reported that the coculture of two ascomycetes, *Dichotomomyces cejpii* (current name *Aspergillus cejpii*) and *Phoma tropica* (current name *Allophoma tropica*), produced the greatest decolorization rates of azo dyes assessed during four days (12 to 73%). On the other hand, Przystaś et al. (2013) reported the low efficiency of *P. ostreatus* and *Gloeophyllum odoratum* cocultures to decolorize the azo dye Evans blue and the triphenylmethane dye brilliant green. Instead, the

authors reported the color change and increase in the absorbance of decolorization reactions that were attributed to the interaction among strains metabolites and dyes and/or stress connected with presence of another strain in cultivation. In our study we also observed an increase in absorbance during the RBBR decolorization reactions of all the cultivations, particularly after 48 h. It suggests that the increase in absorbance could be due to the generation of intermediary metabolites or incomplete degradation of dye because after 72 h absorbance decreases (Eichlerová et al., 2007).

5. Conclusions

Pleurotus-Trametes, *Lentinus-Pleurotus*, and *Lentinus-Trametes* cocultures increase the laccase activity compared to monocultures. *Lentinus-Pycnoporus*, *Lentinus-Trametes*, *Lentinus-Pleurotus*, and *Pleurotus-Trametes* cocultures stimulate mycelial biomass production in relation to the respective monocultures while *Pycnoporus-Trametes* and *Pleurotus-Pycnoporus* produce the same mycelial biomass than the respective monocultures. The enzymatic extracts of monoculture and cocultures promote the decolorization of all dyes. The enzymatic extract of *Pleurotus-Trametes* coculture is the most efficient to decolorize RBBR dye. All cocultures are efficient to decolorize MG and RB220. The enzymatic extracts of *P. ostreatus* and *T. polyzona* axenic monocultures are not effective to decolorize MG and RBBR, but they are effective to decolorize RB220. Enzymatic extracts of *L. crinitus* and *P. sanguineus* monocultures are effective to decolorize RB220 and MG, but not to decolorize RBBR. RBBR is decolorized efficiently by enzymes only produced by *Pleurotus-Trametes* coculture and it is not effective for the other mono or cocultures. Laccase from different basidiomycetes produced during coculture in liquid cultivation medium is an option to decolorize dyes with different chemical structures and has potential for biotechnological applications in bioremediation. Further studies are needed to evaluate differences in the inoculation of cocultivations as well as to evaluate the decolorization of other textile dyes.

Acknowledgments

The authors thank Universidade Paranaense, Graduate Program in Biotechnology Applied to Agriculture, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) finance code 001, Fundação Araucária, and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, 307953/2017-3) for financial support and

fellowship.

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