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LIGNINOLYTIC ENZYMES PRODUCTION AND REMAZOL BRILLIANT BLUE R DECOLORIZATION BY TROPICAL BRAZILIAN BASIDIOMYCETES FUNGI

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ABSTRACT

Remazol Brilliant Blue R (RBBR) dye was used as substrate to evaluate ligninolytic activity in 125 basidiomycetous fungi isolated from tropical ecosystems. The extracellular RBBR decolorizing activity produced when selected fungi were grown in solid media and in soil contaminated with organochlorines was also evaluated. A total of 106 fungi decolorized the RBBR during the growth in malt extract agar (MEA, 2%); 96 fungi showed a mycelia growth and decolorization activity stronger than the *P. chrysosporium* used as reference. Extracellular extracts of 35 selected fungi grown on solid medium with sugar cane bagasse (BGS) were evaluated for RBBR decolorization and peroxidase activity. All fungi showed peroxidase activities, but 5 of those were unable to decolorize the RBBR. Different patterns of ligninolytic enzymes were detected in 12 fungi extracts. Mn-dependent peroxidase (MnP) was produced by *Peniophora cinerea, Psilocybe castanella,* three strains of *Trametes villosa, T. versicolor, Melanoporia nigra* and *Trichaptum byssogenum*. All 12 fungi had laccase activity. *Trogia buccinalis* showed the highest RBBR decolorization and did not produce MnP activity. RBBR decolorization without MnP production was also observed for three strains of *Lentinum* tested. Higher levels of peroxidase and laccase cannot be related to high RBBR decolorization. RBBR decolorization by extracellular extract was also detected during the growth of *P. castanella, L. crinitus, P. cinerea* and two strains of *T. Villosa* in pentachlorophenol- and hexachlorobenzene-contaminated soils. These fungi showed higher RBBR decolorization when grown in the presence of organochlorine compounds than when in non contaminated soil.

Key words: white rot fungi, peroxidases, laccase, MnP, pentachlorophenol, hexachlorobenzene

INTRODUCTION

The white rot fungi (WRF) seem to be the unique microorganisms which show capacities of degrading and mineralizing lignin and a series of organic pollutant compounds, highly toxic and recalcitrant. This capacity is, at least in some extent, caused by non-specific enzymatic system produced by these fungi during the lignin degradation, includes several isoenzymes of Lignin Peroxidase (LiP, EC 1.11.1.14), Manganese dependent Peroxidase (MnP, EC 1.11.1.13), laccases (EC 1.10.3.2) as well as H_2O_2-producing oxidases. The multienzymatic system involved in the lignin degradation and mineralization is constituted of different ligninolytic enzymes combinations, being the occurrence of MnP and Laccase higher than LiP (19).

Phanerochaete chrysosporium has been widely used as a model system to understand the process of lignin and some environmental pollutants biodegradation. However, there is a great diversity of basidiomycetes with different ligninolytic

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enzymes pattern, which also has great differences in their ability in xenobiotic degradation. The ligninolytic systems of other basidiomycetes and several methods involving dyes have been reported as screening methodologies for the selection of xenobiotic compounds degrading microorganisms (3,4,14). The use of dyes offers a series of advantages in relation to conventional substrate because they are stable, soluble and cheap substrates with high rates of molar extinction and low toxicity. These dyes can be applied in simple, quick and quantitative spectrophotometric assays. Remazol Brilliant Blue R dye (RBBR) is an antracene derivative and, thus, related to an important group of organopollutants.

There is evidence that the capacity of WRF in decolorizing dyes results in part from the activity of enzymes that take part in lignin depolymerization process. Low molar weight compounds, as metals and H_2O_2, can also be envolved in pesticides degradation (15). Peroxidative activity does not seem to be the only extracellular enzyme available to WRF for dyes degradation, and decolorization rate could not be related to one particular enzyme, but to the result of the ligninolytic mechanism action. The identity of enzymes envolved in the RBBR degradation is still incompletly known (2,10,14).

To understand the basidiomycetes complex enzymatic system and the use of the RBBR dye as indicative of the degradative potential fungi system, this paper studied the RBBR-decolorizing activity present in extracellular basidiomycetes tropical fluids in solid medium and in soil contaminated with organochlorines.

MATERIALS AND METHODS

Fungi

A total of 125 fungi were isolated from basidiomes collected in different ecosystems of tropical Atlantic rainforest, Brazil (12). They were deposited in the culture collection of Instituto de Botânica, São Paulo. *Phanerochaete chrysosporium* ATCC28326 was used as reference sample. All strains were preserved in malt extract agar medium (MEA) 2% (w/v) slants at 4ºC.

RBBR decolorization test

Agar plug (∅ 2mm) from 7 day-old mycelium of each test strain grown in MEA were inoculated in plates (∅ 90mm) containing 2% MEA with 0.05% RBBR (Sigma), in quadruplicate. Plates were incubated at 30ºC until they were totally colonized or for 14 days. The decolorization coefficient (A) was calculated by measuring the decolorization disc diameter and growth disc diameter (cm), of each species, according the formula (1):

$$A = \frac{\left(H_t / C_t - H_{ref} / C_{ref} \right)}{H_{ref} - C_{ref}}$$

where:
H - decolorization disc diameter
C - growth disc diameter
t - test lineage
ref - reference strain *Phanerochaete chrysosporium* ATCC 28326

Fungi growth velocity

The growth disk (∅ 2mm) of each fungus on MEA was placed in the center of a 90mm plate with MEA. Triplicate plates were kept at 30ºC in the dark. The growth velocity was determined by measuring the average increase of the colony diameter (cm), in two perpendicular directions.

Extracellular ligninolytic activity in solid medium BGS

The extracellular ligninolytic activity was determined for 35 native fungi. The growth disk (∅ 2mm) of each fungus on MEA was transferred to solid medium BGS containing $(NH_4)SO_4$ 2 g L^{-1}; $MnSO_4.4H_2O$ 0.004 g L^{-1}; NaCl 0.1 g L^{-1}; $CaCl_2.2H_2O$ 0.1 g L^{-1}; $KH_2PO_4.7H_2O$ 10 g L^{-1}; glucose 20 g L^{-1}; sugar cane bagasse power 10 g L^{-1}; agar 20 g L^{-1}. The fungi grew for 7 days at 30ºC, in triplicate. The solid agar was cut out, and the enzyme extraction was done with 30 mL of 50 mM sodium acetate buffer, pH 4.6, during 30 min. The mixture was filtered and assayed for extracellular ligninolytic activity.

Extracellular ligninolytic activity in organochlorine contaminated soils

The soils were collected in São Vicente, SP, in a region contaminated with organochlorine industrial residuals containing high concentrations of hexachlorobenzene (HCB) and pentachlorophenol (PCP). PCP-soil contains 6.50% of total organochlorines (6.04% of PCP, 0.15% of pentachlorobenzene, 0.04% of tetrachlorobenzene and 0.16% of hexachlorobutadiene). HCB-soil has 8.92% of total organochlorine (8.40% of HCB, 0.50% of pentachlorobenzene, and 0.02% of tetrachlorobenzene). The soils were diluted with non-contaminated soil (control-soil) to 0.18% and 1.2% of PCP and HCB, respectively. The concentrations of organochlorines were determined by high-resolution gas chromatography as described (9).

The soil was air dried and sieved through a mesh of 2 mm. A 100g (dry weight) mixture of soil-CaSO4-sugar cane bagasse (95:2.5:2.5 per dry weight) was put into an 800-mL bottle and sterilized for 1h at 100ºC for three consecutive days. The moisture content was adjusted at 75% and corrected weekly by gravimetry. The inoculum was prepared with wheat grain (8) and 10.0g were inoculated in the mixture of soil-gypsum-bagasse. As control, soils without fungus were used. All treatments were done in triplicate and incubated for 30 days at room temperature. For enzymes extraction, 50 mL sodium acetate buffer (50 mM, pH 4.6) were added to each flask, mixed thoroughly and shaken for 30 min (90 rpm). The mixture was filtered and assayed for extracellular ligninolytic activity.

Enzymatic assays

RBBR decolorization was determined with the reaction mixture of 5.0 mL of the enzymatic extract and 50 µL of RBBR 2% (18). After an hour, 100 µL of the mixture were diluted 1:10 and the absorbancy reading was done at 500 and 585 nm. The decolorization was determined by the difference between the absorbance rates of the test sample and of a control prepared with enzymatic extract inactivated by boiling.

Peroxidase and laccase activities were determined by the oxidation of 0-dianisidine ($\in_{460}$ = 29.400 M^{-1} cm^{-1}) with and without the adding of hydrogen peroxide (16). The Manganese Peroxidase activity was determined by the phenol red oxidation ($\in_{610}$ = 4.460 M^{-1} cm^{-1}) (5). All of the enzymatic units were defined as the quantity of enzyme which oxides 1 µMol of substrate per minute per liter.

RESULTS AND DISCUSSION

Out of the 125 fungi evaluated 106 were able to decolorize the RBBR in solid medium. *Ceriporiopsis aneirina* CCB261 and *Oligoporus* sp. CCB164 decolorized the dye without a clear decolorization halo. Fourty four fungi showed the decolorization halo bigger than the growth ring (Table 1). *Collybia dryophila* CCB308, *Climacodon pulcherrimus* CCB191, *Fomitela supina* CCB414, *Fomitopsis spraguei* CCB214, *Ganoderma lucidum* CCB168, *Gloeophyllum striatum* CCB188, *Gymnopilus earlei* CCB249, *Lentinus crinitus*, CCB212, *Lentinus* sp. CCB174, *Oudemansiella canarii* CCB241, *Phellinus gilvus* CCB485, *P. umbrinellus* CCB386, *Pleurotus flabelatus* CCB210 and CCB197, *Psilocybe silvatica* CCB443, *Schizophyllum commune* CCB473, *Trametes*

Table 1. Remazol Brilliant Blue R (RBBR) decolorization and mycelial extension rate of Basidiomycetes during growth on agar malt (2%).

Fungi	CBB	mycelial extension rate (cm.day-1)	RBBR decolorization A	RBBR decolorization halo D/ haloC	Fungi	CBB	mycelial extension rate (cm.day-1)	RBBR decolorization A	RBBR decolorization halo D/ haloC
Agrocybe perfecta	161	0.90±0.13	1.37	1.1	*Oudemansiella canari*	179	1.18±0.35	1.17	1.0
Agrocybe platensis	211	0.95±0.15	1.63	1.1	*Panaeolus papilionaceus*	187	0.84±0.12	0.28	0.6
Antrodiella americana	206	5.28±0.22	1.20	0.7	*Peniophora cremea*	286	1.07±0.14	0.43	0.7
Antrodiella semisupina	446	1.22±0.21	1.24	0.1	*Peniophora utriculosa*	282	1.95±0.32	0.30	0.8
Antrodiella sp.	426	1.76±0.21	1.28	1.1	*Peniophora utriculosa*	282	1.95±0.63	0.67	0.8
Auricularia fuscosuccinea	43	0.95±0.16	1.43	1.1	*Phellinus gilvus*	182	0.76±0.17	2.15	1.5
Auricularia fuscosuccinea	44	0.95±0.23	1.30	1.1	*Phellinus gilvus*	186	0.87±0.14	2.20	1.5
Auricularia fuscosuccinea	265	1.00±0.14	1.41	1.1	*Phellinus gilvus*	317	0.64±0.20	1.46	1.1
Calvatia cyathiformis	173	nd	2.20	1.5	*Phellinus gilvus*	254	0.99±0.32	1.22	1.1
Cladoderris dendritica	306	1.86±0.31	0.78	0.8	*Phellinus lividus*	305	1.39 ±0.31	2.24	1.5
Coprinus comatus	53	0.45±0.12	1.24	1.0	*Phellinus spiculosus*	335	0.90±0.20	0.57	0.7
Coprinus jamaicensis	318	1.14±0.33	3.89	2.3	*Pheniophora cenirea*	204	1.15±0.19	1.15	1.0
Dictyopanus pusillus	263	0.45± 0.11	1.28	1.1	*Pleurotus flabelatus*	394	0.42±0.09	1.20	1.0
Ganoderma annularis	477	0.49 0.16	1.39	1.1	*Pleurotus flabellatus*	396	nd	3.04	1.9
Ganoderma australe	169	0.76±0.17	2.59	1.7	*Pleurotus ostreatoroseus*	440	0.64±0.33	4.54	2.6
Ganoderma lipsiensis	352	0.43±0.17	1.24	1.0	*Pleurotus ostreatoroseus*	412	1.69±0.37	0.24	0.6
Ganoderma lipsiensis	364	0.70±0.20	1.26	1.0	*Pleurotus* sp	68	0.58±0.30	1.67	2.4
Ganoderma lobatum	209	1.41±0.25	0.74	1.2	*Polyporus arcularius*	266	0.34±0.27	1.24	0.9
Gloeoporus dichrous	400	0.18±0.08	1.13	1.0	*Polyporus tricholoma*	242	1.15±0.21	0.96	2.4
Gymnopilus aureobrunneus	373	0.62±0.14	0.50	0.7	*Psathyrella tenuis*	376	1.50±0.28	0.30	0.6
Gymnopilus chrysopellus	381	1.44±0.18	7.74	4.0	*Psilocybe castanella*	300	0.59±0.11	1.54	1.2
Gymnopilus earlei	199	nd	1.26	1.0	*Psilocybe castanella*	444	1.33±0.17	2.74	1.7
Gumnopilus earlei	375	1.12±0.15	1.33	1.1	*Psilocybe castanella*	259	0.20±0.05	0.52	0.6
Hydnopolyporus fimbriatus	289	1.25±0.17	0.59	0.7	*Psilocybe singeriana*	357	nd	1.15	1.0
Hygrocybe sp.	342	1.65±0.24	0.15	0.5	*Psilocybe* sp.	445	0.66±0.20	1.46	1.1
Hypholoma subviride	257	0.33±0.09	1.22	0.7	*Psilocybe subcubensis*	224	0.59±0.12	1.22	0.9
Inonotus ludovicianus	207	0.48±0.15	0.52	0.8	*Psilocybe subcubensis*	156	0.66±0.29	1.11	1.0
Irpex lacteus	196	1.89±1.67	0.70	0.8	*Psilocybe venezuelana*	367	0.42±0.14	1.11	1.0
Lentinus araucariae	377	0.92±0.20	1.26	1.0	*Pycnoporus sanguineus*	175	1.62±0.23	0.54	0.7

Lentinus bertieri	255	1.19±0.21	1.48	1.0	*Pycnoporus sanguineus*	273	1.73±0.23	1.54	0.5
Lentinus bertieri	260	1.42±0.27	0.28	1.1	*Pycnoporus sanguineus*	277	2.53±0.53	1.28	0.6
Lentinus crinitus	356	1.33±0.11	1.20	1.0	*Pycnoporus sanguineus*	294	1.58±0.30	0.50	0.7
Lentinus crinitus	217	1.47±0.26	1.54	1.0	*Pycnoporus sanguineus*	458	1.56±0.31	0.80	0.8
Lentinus striatulus	253	1.48±0.19	1.26	1.0	*Rigidoporus microporus*	334	0.51±0.15	2.22	1.5
Lentinus strigellus	380	1.23±0.21	1.02	0.9	*Ripartitella brasiliensis*	467	1.59±0.33	1.11	1.0
Lentinus strigosus	162	1.09±0.23	3.28	2.0	Russula hygrophytica	328	1.62±0.27	0.72	0.8
Lentinus strigosus	178	1.11±0.20	1.33	1.1	*Stereum ostrea*	267	1.28±0.11	0.91	0.6
Lentinus strigosus	250	0.22±0.08	4.22	1.0	*Trametes pubescens*	166	0.81±0.17	0.63	0.8
Lentinus velutinos	268	1.31±0.24	0.33	1.1	*Trametes versicolor*	202	1.42±0.21	2.22	1.5
Lentinus velutinos	288	nd	1.67	1.2	*Trametes versicolor*	372	1.10±0.12	1.33	1.1
Lentinus villosus	271	1.43±0.19	1.43	1.2	*Trametes villosa*	176	1.59±0.28	1.30	1.1
Lentinus zeyheri	247	1.44±0.19	0.11	1.1	*Trametes villosa*	213	1.43±0.28	1.39	1.2
Lepista sp.	110	0.84±0.16	1.30	1.1	*Trametes villosa*	291	1.53±0.20	1.43	1.1
Marasmius cladophyllus	361	1.69±0.21	1.30	1.1	*Trametes villosa*	295	1.57±0.33	1.43	1.1
Marasmius niveus	455	1.67±0.34	1.26	1.0	*Trametes villosa*	165	1.61±0.35	1.80	1.1
Marasmius smaragdinus	470	0.35±0.07	0.65	0.8	*Trichaptum bysogenum*	203	1.56±0.27	1.33	1.1
Marasmius sp.	378	1.26±0.15	1.15	1.0	*Trogia buccinalis*	390	1.39±0.24	1.20	1.0
Melanoporia nigra	184	1.31±0.35	1.15	1.0	*Tyromyces pseudolacteus*	193	1.40±0.31	1.33	1.1

CCB: culture collection number; A = coefficient of decolorization (1), using *Phanerochaete chrysosporium* ATCC2832 as reference strain; nd: not determined; halo D/halo C = ratio between the decolorization halo and growth halo; All values reported as means ± standard deviation for three replica cultures.

versicolor CCB158, *Trichaptum biforme* CCB297 and *Tubaria furfuracea* CCB374 were unable to decolorize the RBBR.

Ninety-six fungi showed more decolorization than *P. chrysosporium* (Table 1). *Agaricus porosporus* CCB299, *Ceriporiopsis aneirina* CCB261, *Collybia subpruinosa* CCB404, *Ganoderma Lipsiense* CCB321, *Merulius corium* CCB355, *Nothopanus hygrophanus* CCB216, *Oligoporus* sp. CCB164, *Pycnoporus sanguineus* CCB113, *Schizophyllum commune* CCB307 isolates showed decolorization activity equal or lower than *P. chrysosporium*.

A total of 55 fungi showed average growth velocity higher than or equal to 1cm day^{-1} (Table 1). The selection of fungi with high growth rates is one of the desirable characteristics in the application of bioremediation processes since this factor can make the strains more competitive with the native soil biota (6). Other facilities include the handling in laboratory and the production of spawn.

Thirty-four fungi grew in BGS solid medium and the enzymatic extracts of 30 showed RBBR decolorization and peroxidase activity (Table 2). *A. perfecta*, *Antrodiella* sp., *Hygrocybe* sp., *Psathyrella tenuis* and *Pycnoporus sanguineus* CCB175 extracts did not show RBBR decolorization, but showed extracellular peroxidase activity.

Twelve fungi with expressive RBBR decolorization capacity were tested for Mn-peroxidase and laccase activities (Table 3). Neither *Lentinus* species nor *Trogia buccinalis* showed MnP activity. Laccase activity was produced by all of the native fungi. Until this study, the production of laccase activity was not described in *Peniophora cinerea, Psilocybe castanella, Trichaptum byssogenum* and *Trogia buccinalis*.

Significant decolorization of RBBR by fungi which did not produce MnP activity evidences the involvement of other peroxidases, and even phenoloxidases as laccase in the degradation of dyes or the presence of low molar weight compounds with oxidative abilities. The decolorization of RBBR by *Pleurotus ostreatus* has been attributed to a new enzymatic peroxidase (14). Decolorization of RBBR by an enzyme-mediated process in which the main enzyme responsible is a recently described versatile peroxidase (VP) produced by *Bjerkandera sp.* was observed (10). Laccase was responsible for the RBBR decolorizing activity in the culture filtrate of *Funalia trogii* (2). Novotny *et al.* (11) observed RBBR, Drimaren Blue and Drimaren Red decolorization independent from the ligninolytic enzymes produced by *Irpex lacteus* (11).

The extracellular RBBR decolorization by 5 studied fungi able to degrade and mineralize PCP and HCB (8,9) changed along the time during the growth in soil (Figs. 1 and 2). *P. castanella* showed higher RBBR decolorization in HCB-soil than in control-soil ($P < 0.05$) which did not differ ($P < 0.05$) from the PCP-soil. *T. villosa* CCB176 showed higher RBBR decolorization ($P < 0.05$) in contaminated soils than control-soil (Fig. 1). *T. villosa* CCB213, *P. cinerea*, and *L. crinitus* showed the highest RBBR decolorization when grown in the presence of organochlorine compounds ($P < 0.05$) (Fig. 2). The extract of the soils without fungi failed to decolorize the RBBR.

Table 2. Extracellular Brilliant Remazol Blue R (RBBR) decolorization and peroxidose activity produced by Basidiomycetes during growth on BGS solid medium.

Fungi	RBB descolorization		Peroxidase activity[a]	
	5 days	7 days	5 days	7 days
Agrocybe perfecta CCB161	z	z	0.3±0.01	0.7±0.20
Antrodiella semisupina CCB446	0.18±0.04	0.63±0.10	1.2±0.30	2.7±0.61
Antrodiella sp. CCB426	z	z	0.9±0.00	1.0±0.00
Gymnopilus chrysopellus CBB381	0.40±0.10	1.25±0.09	1.6±0.30	2.4±0.00
Gymnopilus earlei CCB 375	0.09±0.03	0.12±0.01	0.1±0.00	0.6±0.12
Hidnopolyporus fimbriatus CCB289	-	0.10±0.06	z	2.4±0.00
Hygrocybe sp. CCB342	z	z	0.6±0.00	5.9±0.50
Irpex lacteus CCB196	0.24±0.09	0.11±0.04	z	0.5±0.00
Lentinus crinitus CCB217	0.23±0.08	0.69±0.08	0.3±0.30	2.3±1.2
Lentinus crinitus CCB356	0.98±0.39	1.70±0.14	2.0±0.35	4.8±0.67
Lentinus strigellus CCB380	z	0.60±0.05	0.6±0.00	0.9±0.86
Lentinus velutinus CCB268	-	0.86±0.13	-	21.9±1.33
Lentinus zeyheri CCB274	-	1.41±0.08	-	4.5±1.48
Marasmius sp. CCB378	-	0	-	0.6±0.12
Melanoporia nigra CCB184	-	0.91±0.06	-	16.9±0.50
Oudemansiella canarii CCB179	-	0	-	1.3±0.65
Panaeolus papilionaceus CCB187	0.10±0.06	0.08±0.08	0.1±0.05	0.2±0.00
Peniophora cinerea CCB204	2.10±0.17	2.01±0.18	-	58.9±11.32
Peniophora cremea CCB286	-	0.12±0.06	-	1.7±0.71
Phellinus lividus CCB305	0.27±0.04	0.65±0.05	2.9±0.70	4.3±0.60
Psathyrella tenuis CCB376	z	z	z	1.9±0.00
Psilocybe castanella CCB444	-	0.57±0.10	-	28.5±12.30
Pycnoporus sanguineus CCB175	z	z	0.3±0.00	2.7±0.45
Pycnoporus sanguineus CCB277	0.11±0.03	z	0.1±0.10	0.3±0.21
Pycnopous sanguineus CCB458	z	0.09±0.05	0	4.2±2.20
Ripartitella brasiliensis CCB467	0.09±0.08	0.09±0.05	0.5±0.30	0.6±0.44
Stereum ostrea CCB267	z	0.08±0.04	0.2±0.00	0.4±0.00
Trametes versicolor CCB202	0.31±0.07	0.88±0.08	2.1±1.10	12.3±2.05
Trametes villosa CCB165	0.61±0.14	0.94±0.12	1.3±0.49	14.0±4.92
Trametes villosa CCB176	0.98±0.18	1.95±0.06	3.3±1.40	31.0±2.25
Trametes villosa CCB291	0.69±0.09	1.11±0.09	58.9±11.32	90.2±15.35
Trametes villosa CCB213	0.68±0.12	1.50±0.27	3.3±0.70	20.6±7.73
Trichaptum byssogenum CCB203	0.58±0.07	2.13±0.14	3.2±1.05	28.4±4.12
Trogia buccinalis CCB390	0.71±0.07	6.39±0.30	3.1±0.70	7.6±1.36
P. chrysosporium ATCC28326	0.13±0.03	0.23±0.12	0.3±0.46	z

- = not determined; z = no activity; [a]: Enzymatic units = µmol of oxidate substrate L^{-1}, in the presence of H_2O_2; All values reported as means ± standard deviation for three replica cultures.

The enzymatic system involved in RBBR decolorization was produced by basidiomycete fungi in solid medium and in soils. This system was produced even in the absence of the RBBR and the decolorization was performed in the absence of added H_2O_2 showing that there are different combinations of extracellular ligninolytic enzymes produced in significant quantities under simple conditions of cultivation. Which enzymes are being stimulated or activated and their relation to organochlorine compounds degradation is not well known yet. RBBR-decolorizing activity is a simple indicative method for a multienzymatic system and can be a valuable approach to be used as a tool for xenobiotic biodegradation studies as well as an indication of the physiological conditions of basidiomycetes during bioremediation process.

Table 3. Manganese peroxidase and laccase activities during Brilliant Remazol Blue R (RBBR) decolorization by rot fungi.

Fungi	City of collect	RBBR decolorization	Enzymatic activity (U L^{-1})	
			MnP	Laccase
Lentinus crinitus CCB356	Ubatuba	++	0.00±0.00	5.8±0.87
L.velutinus CCB268[a]	São Vicente	+	0.00±0.00	23.8±0.30
L.crinitus CCB274[a]	São Vicente	+	0.00±0.00	4.3±1.58
Melanoporia nigra CCB184#	Maceió	+	3.77±2.39	1.6±0.10
Peniophora cinerea CCB204	São Vicente	++	17.57±9.51	6.5±0.80
Psilocybe castanella CCB444[a]	São Vicente	+	6.39±10.81	2.0±0.21
Trametes versicolor CCB202	São Paulo	+	25.15±12.66	10.7±0.10
T. villosa CCB165	Campinas	+	1.20±0.00	12.5±3.48
T. villosa CCB213	São Paulo	+	4.31±2.04	2.3±0.68
T. villosa CCB176	Assis	++	6.64±0.96	3.1±0.17
Trichaptum byssogenum CCB203[a]	São Vicente	++	10.13±7.45	4.4±1.28
Trogia buccinalis CCB203[a]	São Paulo	+++++	0.00±0.00	4.1±1.35

[a]: fungi collected in places where dumps of organochlorines residues ocorred; # brown rot fungi; Enzymatic units = µmol of oxidate substrate L^{-1}; +++++ = 100 % of decolorization (in relation to the highest decolorization = 6.39, *T. buccinales*); ++++ = 75 a 99% of decolorization; +++ = 50 a 74 % of decolorization; ++ = 25 a 49% of decolorization; + = até 24 % of decolorization; All values reported as means ± standard deviation for three replica cultures.

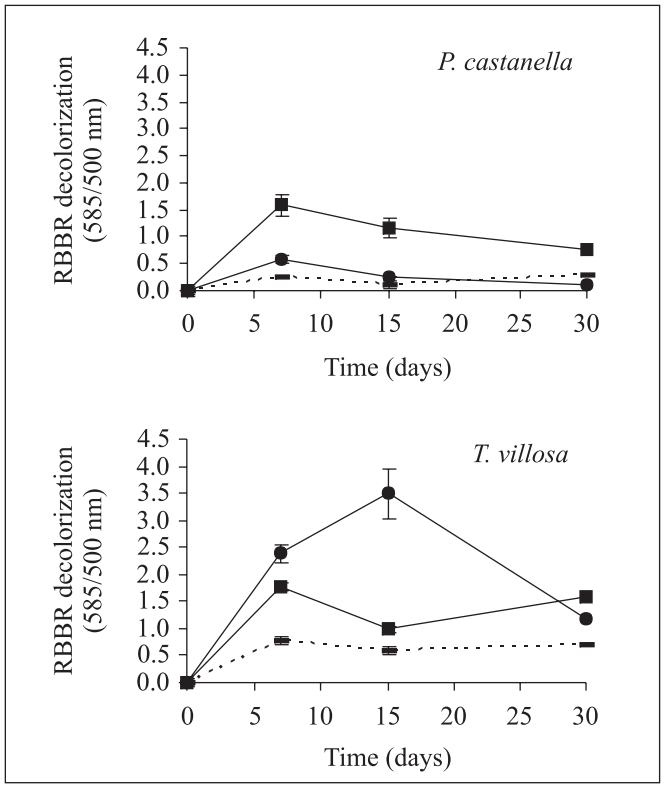

Figure 1. Brilliant Remazol Blue R (RBBR) decolorization by extracellular extracts of *Psilocybe castanella* CCB444 and *Trametes villosa* CCB176 during growth in organochlorine soils: (●) PCP-soil, (■) HCB-soil and (----) control-soil.

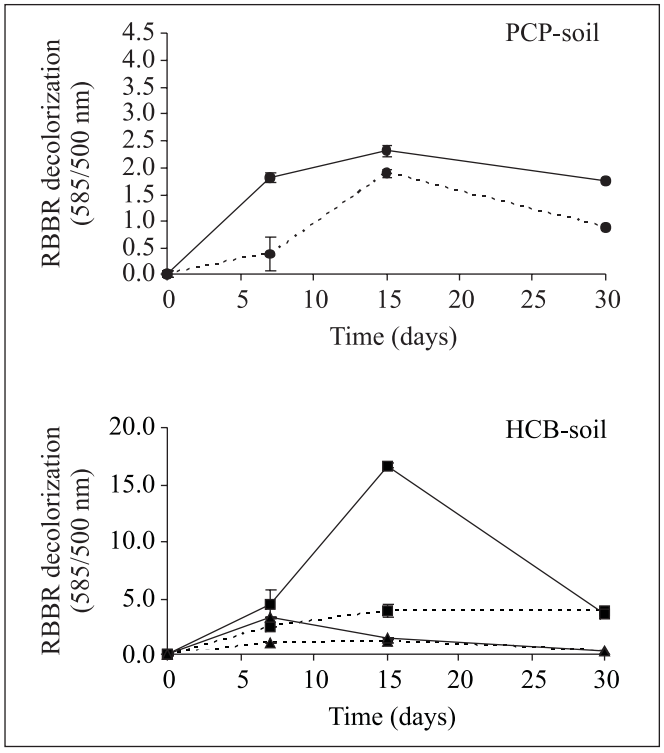

Figure 2. Brilliant Remazol Blue R (RBBR) decolorization by extracellular extracts of basidiomycetes during growth in organochlorine soils: (●) *Trametes villosa* CCB213, (■) *Peniophora cinerea* CCB204, (▲) *Lentinus crinitus* CCB274, and (----) control-soil (non-contaminated soil).

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RESUMO

Produção de enzimas ligninolíticas e descoloração do corante azul brilhante de Remazol R por fungos basidiomicetos tropicais brasileiros

O corante azul brilhante Remazol R (RBBR) foi usado como substrato para avaliar 125 fungos basidiomicetos isolados de ecossistemas tropicais brasileiros quanto a atividade ligninolítica. A descoloração do RBBR por extratos obtidos do crescimento de fungos em meio sólido e em solo contaminado com organoclorados foi também avaliada. Cento e seis fungos descoloriram o RBBR durante o crescimento em meio sólido (agar extrato malte); noventa e seis desses fungos mostraram crescimento micelial e descoloração superiores a uma cepa de *Phanerochaete chrysosporium* usada como referência. Trinta e cinco fungos foram crescidos em meio sólido contendo bagaço de cana-de-açúcar (BGS) e seus extratos foram avaliados quanto a descoloração do RBBR e a produção de atividade de peroxidases. Todos os fungos mostraram atividade de peroxidases, mas 5 foram incapazes de descolorir o RBBR. Diferentes padrões de enzimas lignolíticas foram detectados em 12 desses extratos fúngicos, porém todos mostraram atividade de laccase. Atividade de peroxidase dependente de manganês (MnP) foi produzida por *Melanoporia nigra*, *Peniophora cinerea*, *Psilocybe castanella*, três cepas de *Trametes villosa*, *T. versicolor* e *Trichaptum byssogenum*. *Trogia buccinalis* apresentou a maior descoloração do RBBR e não produziu MnP. Descoloração do RBBR sem produção de MnP foi também observada para as três cepas de *Lentinus* testadas. Altos níveis de atividades de peroxidases e laccases não mostraram relação com significativa descoloração do RBBR. Foi observada descoloração do RBBR por extratos obtidos de *P. castanella*, *L. crinitus*, *P. cinerea* e duas cepas de *T. Villosa* durante crescimento em solos contaminados com pentaclorofenol e hexaclorobenzeno. Estes fungos mostraram maior descoloração de RBBR na presença de compostos organoclorados do que em solos não contaminados.

Palavras-chave: fungos de podridão branca, peroxidases, lacase, pentaclorofenol, hexaclorobenzeno

REFERENCES

1. Antier, P.; Minjares, A.; Roussos, S.; Raimbault, M.; Viniegra-González, G. Pectinase-hyperproducing mutants of *Aspergillus niger* C28B25 for solid-state fermentation of coffee pulp. *Enzyme Microbiol. Technol.*, 15, 254-260, 1993.
2. Deveci, T.; Unyayara, A.; Mazmanci, M.A. Production of Remazol Brilliant Blue R decolourising oxygenase from the culture filtrate of *Funalia trogii* ATCC200800. *J. Mol. Catal. B: Enzymatic*, 30, 25-32, 2004.
3. Field, J.A.; Jong, E.; Feijoo-Costa, G.; De Bont, J.A.M. Screening for ligninolytic fungi applicable to the biodegradation of xenobiotics. *Trends Biotechnol*., 11, 44-49, 1993.
4. Glenn, J.K.; Gold, M.H. Decolorization of several polymeric dyes by the lignin-degradating basidiomycete *Phanerochaete chrysosporium*. *Appl. Environm. Microbiol.*, 45, 1741-1747, 1983.
5. Kuwahara, M.; Glenn, J.K.; Morgan, M.A.; Gold, M.H. Separation and characterization of two extracellular H_2O_2-dependent oxidases from ligninolytic cultures of *Phanerochaete chrysosporium*. *Febs Lett.*, 169, 247-250, 1984.
6. Lamar, R.T.; Larsen, M.J.; Kirk, T.K. Sensitivity to and degradation of pentachlorophenol by *Phanerochaete* spp. *Appl. Environm. Microbiol.*, 56, 3519-3526, 1990.
7. Machado, K.M.G.; Matheus, D.R.; Fabris, C.; Bononi, V.L.R. *Efeito da adição de substrato lignocelulósico no crescimento de fungos basidiomicetos em solo com baixo teor de matéria orgânica*. Workshop sobre Biodegradação, Campinas, 1996, p.243.
8. Machado, K.M.G.; Matheus, D.R.; Monteiro, R.T.R.; Bononi, V.L.R. Biodegradation of pentachlorophenol by tropical basidiomycetes in soils contaminated with industrial residues. *World J. Microbiol. Biotechnol.*, 21, 297-301, 2004.
9. Matheus, D.R.; Bononi, V.L.R.; Machado, K.M.G. Biodegradation of hexachlorobenzene by basidiomycetes in soil contaminated with industrial residues, *World J. Microbiol. Biotechnol.*, 16, 415-421, 2000.
10. Moreira, P.R.; Almeida-Vara, E.; Sena-Martins, G.; Polonia, I.; Malcata, F.X.; Duarte, J.C. Decolourisation of Remazol Brilliant Blue R via a novel Bjerkandera sp. Strain. *J. Biotechnol.*, 89, 107-111, 2001.
11. Novotyny, C.; Svobodova, K.; Erbanova, P.; Cajthaml, T.; Kasinath, A.; Lang, E.; Sasek, V. Ligninolytic fungi in bioremediation: extracellular enzyme production and degradation rate. *Soil Biol. Biochem.*, 36, 1545-1551, 2004.
12. Okino, L.K.; Machado, K.M.G.; Fabris, C.; Bononi, V.L.R. Ligninolytic activity of tropical rainforest basidiomycetes. *World J. Microbiol. Biotechnol.*, 16, 889-893, 2000.
13. Shah, V.; Nerud, F. Lignin degrading system of white-rot fungi and its exploitation for dye decolorization. Review. *Can. J. Microbiol.*, 48, 857-870, 2002.
14. Shin, K-S.; Oh, I-K.; Kim, C-J. Production and purification of remazol brilliant blue R decolorizing peroxidase from the culture filtrate of *Pleurotus ostreatus*. *Appl. Environm. Microbiol.*, 63, 1744-1748, 1996.
15. Sun, Y.; Pignatello, J.J. Activation of Hydrogen Peroxide by Iron (III) Chelates for Abiotic Degradation of Herbicides and Insecticides in Water. *J. Agric. Food Chem.*, 41, 308-312, 1993.
16. Szklarz, G.D.; Antibus, R.K.; Sinsabaugh, R.L.; Linkins, A. Production of phenol oxidases and peroxidases by wood-rotting fungi. *Mycologia*, 81, 234-240, 1989.
17. Thurston, C.F. The structure and function of fungal laccases. *Microbiology*, 140, 19-26, 1994.
18. Ulmer, D.C.; Leisola, M.S.A.; Fiechter, A. Possible induction of the ligninolytic system of *Phanerochaete chrysosporium*. *J. Biotechnol.*, 1, 13-24, 1984.
19. Wesenberg, D.; Kyriakides, I.; Agathos, S.N. White rot fungi and their enzymes for the treatment of industrial dye effluents. *Biotechnol. Adv*., 22, 161-187, 2003.