Autochthonous white rot fungi from the tropical forest of Colombia for dye decolourisation and ligninolytic enzymes production

Carolina Arboleda[1], Amanda I. Mejía[1], Ana E. Franco-Molano[2], Gloria A. Jiménez[3], Michel J. Penninckx[3] *

[1] Grupo Biopolimer, Facultad de Química Farmacéutica, Universidad de Antioquia, A.A. 1226, Medellín, Colombia
[2] Grupo de Taxonomía y Ecología de Hongos, Instituto de Biología, Universidad de Antioquia A.A. 1226, Medellín, Colombia
[3] Laboratoire de Physiologie et Ecologie Microbienne, Université Libre de Bruxelles, Belgium

Arboleda C., Mejía A. I., Franco-Molano A. E., Jiménez G. A., Penninckx M. J. (2008) Autochthonous white rot fungi from the tropical forest of Colombia for dye decolourisation and ligninolytic enzymes production. – Sydowia 60 (2): 165–180.

Nineteen different strains of white rot fungi, originating from the tropical forest in Colombia were screened for their ability to decolourise Azure B and Coomassie Blue included in solid media. *Collybia plectophyla, Pleurous djamor, Lentinus swartzii, Lentinus crinitus, Pycnoporus sanguineus, Auricularia auricula, Auricularia fuscosuccinea, Oudemansiella canarii, Ganoderma stipitatum and Collybia omphalodes* were selected on the basis of this screening. These ten strains were further characterized in liquid medium for decolourisation and production of Laccase, Manganese and Lignin peroxidases. The strains producing best decolourisation were *L. swartzii, L. crinitus, G. stipitatum*, and *O. canarii*. A correlation between dyes decolourisation, laccase and manganese peroxidase production, was shown. Lignin peroxidase was never detected in the cultivation conditions used. Enzyme induction by Mn^{2+}, Ethanol and Cu^{2+} was studied in more detail for *Ganoderma stipitatum, Lentinus crinitus* and *Lentinus swartzi*. The best increase of enzyme production after three weeks of cultivation was generally observed in the presence of Cu^{2+}, followed by ethanol and Mn^{2+} These three strains were apparently not previously characterized for production of specific ligninolytic activity, and *in fine* could find application in decolourisation technology.

Keywords: Laccase, Manganese Peroxidase, Dyes Decolourisation, Inducers, Fungi, Tropical Forest

Abbreviations: WRF: White rot fungi; PAHs: Polycyclic aromatic hydrocarbons; Lac: Laccase; LiP: Lignin Peroxidase; MnP: Manganese Peroxidase; VP: Versatile Peroxidase; HUA: Herbario Universidad de Antioquia; VA: Veratrylic alcohol; HNLM: High nitrogen level medium; LNLM: Low nitrogen level medium; ABTS: 2,2_-azino-bis-(3-ethylthiazoline-6-sulfonate)

* Laboratoire de Physiologie et Ecologie Microbienne, Université Libre de Bruxelles, 642 Rue Engeland, B-1180, Brussels, Belgium. Tel: 32 2 3733303; Fax: 32 2 3733309; Email: upemulb@resulb.ulb.ac.be

Introduction

As a consequence of varied climates, forests, deserts and savannas constitute important reservoirs of microbial diversity. Saprotrophic, in particular aphyllophoral fungi, contribute significantly to the maintenance of structure and function of the forest ecosystems. A great diversity of wood rotting – including white rotting – fungi has been described (Mueller & Schmit 2007 Lonsdale *et al.* 2008). However, detailed studies of these organisms have only sporadically been conducted in Latin America countries, in particular Colombia. Around 260 species have been inventoried in this country, making our knowledge very scanty on their presence, distribution and function (Ruiz & Varela 2006). In view of possible applications of fungal technology in wastewaters treatment (Wesenberg *et al.,* 2003) we decided to undertake more detailed studies on the potential of Colombian white rot fungal strains.

Wastewaters from dye and textile industries contain a variety of pollutants, including dyes. A great proportion of these dyes are not directly toxic for living organisms. Nevertheless, strong coloration can hamper photosynthetic processes in water, a reason why their presence must be controlled (O'Neill *et al.* 1999).

Dyes are difficult to remove by conventional treatment. A successful management of textile and dye factories effluents often necessitate expensive physico-chemical treatments (Moreira *et al.* 2000). Microbial decolourisation has been proposed as a less expensive and less environmentally intrusive alternative (Boer *et al.* 2004, Kariminiaae-Hamedaani *et al.* 2007). Wood rotting fungi, in particular white rot fungi (WRF) have been identified at several occasions as particularly suitable for this type of approach;

In order to depolymerise and mineralize lignin, WRF have developed oxidative and unspecific systems including extracellular enzymes, low molecular weight metabolites and reactive oxygen species. Ligninolytic enzymes of WRF can also be used for the degradation of a wide variety of organic pollutants, including polycyclic aromatic hydrocarbons (PAHs) (Clemente *et al.* 2001, Tekere *et al.* 2005), synthetic polymers or synthetic dyes (Novotný *et al.* 2001, Levin *et al.* 2003, Ramsay *et al.* 2005, Eichlerova *et al.* 2005), polyphenols in olive oil mill wastewater (Jaouani *et al.* 2003, 2005), chlorinated phenols, polychlorinated biphenyls (Sato *et al.* 2002, Young & Qing. 2004, Moeder *et al.* 2005) dioxins, pesticides, explosives (Wesenberg *et al.* 2003, Levin *et al.* 2004), etc. Several fungal species, e.g. *Phanerochaete chrysosporium* Burds, *Trametes versicolor* Lloyd, *Pleurotus ostreatus* P. Kumm and some others, have been proposed for this purpose. However, recently the interest in studying the lignin-modifying enzymes of a wider array of WRF is increasing,

not only from the point of view of comparative biology but also with the expectation of finding more effective lignin degrading systems adapted to the various biotechnological applications (Dhouib *et al.* 2005).

WRF produce various isoforms of extracellular ligninolytic enzymes: laccases (Lac), lignin peroxidase (LiP), manganese peroxidase (MnP) and versatile peroxidase (VP) (Martínez 2002), exhibiting differential characteristics depending upon species, strains and culture conditions (Heinzkill *et al.* 1996).

Many studies dedicated to bioremediation competence of WRF assess the properties of strains deposited in public culture collections (Jaouani *et al.* 2003) only. On the contrary, there have been fewer contributions attempting to exploit directly local biodiversity, most of them in tropical area (Pointing & Vrijmoed 2000, Tekere *et al.* 2001, Levin *et al.* 2004). However, this approach appears potentially promising for identifying new strains for biotechnological applications (Pointing *et al.* 2003).

Nineteen WRF autochthonous fungal strains, isolated from Colombian tropical forest, were tested in different physiological conditions with the purpose of determining the growth parameters favouring highest decolourisation activity and enzyme production. In order to evaluate decolourisation capacities and ligninolytic activities of these strains we used Azure B and Coomassie Brillant Blue. Azure B an azo dye, is a recalcitrant compound which is neither readily oxidized by MnP nor laccase, and has been used in a selective assay for detecting LiP (Archibald, 1992). Coomassie Brilliant Blue is also an heterocyclic recalcitrant dye used to evaluate the activities of lignin peroxidase,manganese peroxidase and laccase produced by the white-rot basidiomicetes (De Souza *et al.* 2005).

Material and methods

Strains

The fungal strains were isolated from fruiting bodies collected in the tropical forests of Colombia, in the departments of Antioquia and Caldas, and determined by the Laboratory of Taxonomy and Ecology of Fungi of the University of Antioquia, Colombia. The vouchers were deposited at the "Herbario Universidad de Antioquia" (HUA). Tissue cultures were made on Malt Agar and incubated at 20–22 °C. Once mycelia were obtained, the cultures were maintained at 4 °C (Table 1).

Dyes and chemicals

Azure B and Coomassie blue were purchased from Sigma. All other chemicals were of analytical grade.

Table. 1. – Decolourisation capacity of autochthonous WRF strains isolated from the Colombian tropical forest.

M 1: Malt Extract Agar added with 200 mg L^{-1} dye; M 2: with 400 mg L^{-1} dye. 0: no decolourisation, +: around 50% decolourisation, ++: total decolourisation after 15 days of growth. The strains were identified by a code number originating respectively from, THC (Laboratory of Taxonomy and Ecology of Fungi) and, HMC (Group Biodegradation and Bioconversion of polymers BIOPOLIMER).

Strains	**Decolourisation scale**				**Origin of the strain**
	Azure B		**Blue C**		
	M1	**M2**	**M1**	**M2**	
Pycnoporus sanguineus HCM3	+	0	++	+	Decaying wood
Pleurotus sp HCM4	+	0	++	+	Decaying wood
Lentinus crinitus HCM5	++	+	++	++	Decaying wood
Oudemansiella canarii HCM8	++	+	++	+	Decaying wood
Lepista subisabellina HCM9	0	0	0	0	Litter
Psathyrella sp HCM11	0	0	0	0	Trunk of Living Tree
Lentinus crinitus HCM14	++	+	++	+	Decaying wood
Collybia plectophyla HCM16	0	0	0	0	Trunk of Living tree
Lentinus swartzii HCM19	++	+	++	+	Decaying wood
Gyrodon exiguus HCM23	0	0	0	0	Trunk of Living Tree
Mycobonia flava HCM30	0	0	0	0	Decaying wood
Collybia ompholodes HCM32	0	0	0	0	Trunk of Living tree
Dictyopanus pusillus THC18	0	0	+	+	Wood
Psilocybe sp THC19	0	0	0	0	Dung
Auricularia auricula THC4	0	0	++	+	Decaying wood
Auricularia fuscosuccinea THC3	0	0	++	+	Decaying wood
Ganoderma stipitatum THC16	++	++	++	++	Wood
Pleurotus djamor HCM33	+	0	++	+	Wood
Trametes sp THC17	0	0	++	+	Wood

Dyes decolourisation in microplates

Tests were performed in CELLSTAR®); microplates with 24 wells of 2 mL, per fungus. Twelve wells for Azure B and the other twelve wells for Coomassie Blue were used at concentrations of 200 mg L^{-1} and 400 mg L^{-1} (six wells per concentration) included in malt extract agar media. Only four of each six wells, containing the solidified medium, were inoculated with one plug (2 mm diameter) of actively growing mycelium, the other two wells served as blank. The experience was repeated with addition of $MnSO_4$ 100 μM. Decolourisation activity on solid media was estimated by measuring the radial growth of the mycelium and the colour change of the agar. The wells were maintained at 26 °C.

Submerged cultivation

The basal medium of cultivation buffered at pH 5 contained, glucose (10 g L^{-1}), yeast extract (5 mg L^{-1}), potassium dihydrogenophosphate (1 g L^{-1}) and 0.2 mM veratrylic alcohol (VA). This medium was respectively added with 0.2 g L^{-1} of ammonium tartrate for preparation of the low nitrogen level medium (LNLM), and 2 g L^{-1} of ammonium tartrate for the high nitrogen level medium (HNLM; Jaouani *et al.* 2003).

The cultures were performed in 125-mL-Erlenmeyer-flasks with 50 mL of medium containing Azure B or Coomassie Blue at final concentration of 200 mg L^{-1}. The flasks were inoculated with 4 plugs (5 mm diameter) of active mycelium recently replicated on malt extract agar. The experiments were carried out at 30 °C in an orbital shaker operated at 150 rpm. Decolourisation of the liquid medium was measured in the filtrate after removing the mycelium on 0.22 μm glass fibres filter, and monitored by a Lambda 25 Perkin Elmer spectrophotometer at the maximum visible wavelength of absorbance of 595 nm for Coomassie blue and 648 nm for Azure B. Non-inoculated flasks served as control.

Enzyme assays

Lignin peroxidase (LiP): For measuring LiP activity, 200 μL to 400 μL of extracellular fluid were poured in a 1 mL quartz cell and mixed with 0.15 M sodium tartrate buffer (pH = 3) to obtain a final volume of 800 μL. 50 μL of 10 mM veratrylic alcohol (VA) was added just before the addition of 50 μl of freshly diluted H_2O_2 (1/1000 (v/v) to start the reaction. Absorbance vs. time was measured at 30 °C at λ = 310 nm. One unit of enzyme activity was defined as the amount of enzyme oxidising 1 μmol of VA min^{-1} (Paszczynski *et al.* 1986).

Manganese peroxidase (MnP): MnP was measured at 30 °C by monitoring oxidation of 0.1 mM vanillylacetone, in 100 mM sodium tartrate buffer (pH 5.0) added with 0.1 mM $MnSO_4$ and 50 mM H_2O_2 with a final volume of 1 mL. Decrease of absorbance was monitored at 336 nm in the presence of the enzyme (Paszczynski *et al.* 1986). One unit of enzymatic activity was defined as the amount of enzyme catalysing oxidation of 1 μmol of substrate per minute. Activities in the absence of H_2O_2 were subtracted from the values obtained in the presence of hydrogen peroxide to establish the true peroxidase activity.

Laccase: The enzyme activity was determined by monitoring the $A_{420\ nm}$ change related to the rate of oxidation of 1mM 2,2_-azino-bis-(3-ethylthiazoline-6-sulfonate) (ABTS) in 50mM Na-acetate buffer (pH 5) in the presence of 1,000 units of catalase (Boehringer) to

destroy any trace of endogenous H_2O_2. Assays were performed in 1-mL quartz cell at 20 ± 1 °C with 50 µL of culture supernatant. One unit activity was defined as the amount of enzyme, catalysing the oxidation of 1 µmol of ABTS per minute.

The experiments were performed at least twice using three replicates. The data presented in the tables corresponded to mean values with a standard error less than 7 %.

Enzyme induction

Enzyme induction was studied using the LNLM medium supplemented with 150 µM $CuSO_4$, 100 µM $MnSO_4$ or 500 mM ethanol as previously described (Jaouani *et al.* 2005). The inducers were added at day four of cultivation. The enzymatic activities of MnP and laccase were monitored every day for 20 days.

Results and Discussion

Sample screening

A collection of nineteen WRF strains was screened for growth and decolourisation on agar microplates containing 200 and 400 mg L^{-1} Coomassie Blue or Azure B, added to malt extract agar (Table 1). Among the fungi tested, *Ganoderma stipitatum* (Murrill) Murrill, two strains of *Lentinus crinitus* (Fr.) Fr, *Lentinus swartzii* Berk and *Oudemansiella canarii* (Jungh.) Höhn, were able to decolourise the dyes at both concentrations within 15 days. Three other strains, *Pycnoporus sanguineus, Pleurotus sp.,* and *Pleurotus djamor*, decolourised Coomassie Blue at both concentrations but only the lowest concentration of Azure B. *Trametes sp., Dictyopanus pusillus, Auricularia auricularia* and *Auricularia fuscosuccinea* decolourised Coomassie Blue only.

Dye structure has an important effect on the decolourisation ability of fungi; relatively low chemical differences lead to significant variations on the extent or rate of decolourisation (Vanhulle *et al.* 2007, D'Souza *et al.* 2006). In general, Azure B exhibited decolourisation resistance, even for that *Ganoderma sp.* that could decolourise the dyes in less time. This dye is mainly degraded by LiP, and more slowly by MnP and Laccase (Archibald 1992). Submerged culture experiments (reported below) verified that strains selected from agar plate test for their ligninases activities exhibited MnP and laccase activities, but no LiP.

The seven other strains, *Lepista subisabellina, Psathyrella sp., Collybia plectophyla, Gyrodon exiguous, Mycobonia flava, Collybia ompholodes* and *Psilocybe sp,* did not decolourise Coomassie Blue or

Azure B, in spite of abundant mycelial colonization of the media. The addition of $MnSO_4$ had apparently no significant effect on decolourisation, except for inhibition of Coomassie Blue decolourisation in the case of *Dictyopanus pusillus* (data not shown). Non-inoculated controls did not display any decolourisation. In general, it appeared that the most active strains were isolated from wood and decaying wood. With the exception of *Mycobonia flava*, all non-decolourising strains originated from living tree trunks, litter or dung. From tests on microplates it can be concluded that fungi growing in decomposed wood, may be potential producers of ligninolytic enzymes.

Decolourisation and enzyme production in submerged cultivation.

Ten strains, representing the four tendencies on solid media as described above, were selected for a study of decolourisation studies in liquid culture containing 200 mg L^{-1} of Azure B or Coomassie Blue. A comparison of decolourisation performances was made between the strains cultivated in the LNLM and HNLM media in the presence of dye (not illustrated). Only notable decolourisation was observed in the LNLM which corroborates previous observations of Jaouani *et al.* (2003) with strains degrading polyphenols The WRF strains producing higher decolourisation were *Lentinus swartzii, Lentinus crinitus, Ganoderma stipitatum,* and *Oudemansiella canarii* as illustrated for Coomassie blue. In general, WRF predominantly colonizing dead or living wood, are organisms reputed to degrade lignin and producing efficiently lignolytic enzymes under low nitrogen natural conditions (Kirk *et al.* 1978: Eriksson & Kirk 1985, Wong & Yu 1999). For the present study the selected fungi were collected from decaying wood.

In submerse cultures we observed that 5 days after addition of dye, *Ganoderma stipitatum* produced the highest enzymatic activities (Laccase and MnP – Fig. 1). A good correlation was also observed between dye elimination and enzymatic activities (Table 2). This result indicated that Laccase and MnP could be actively involved in dye decolourisation as shown for other strains (Wesenberg *et al.* 2003). Lignin peroxidase was never detected in the experienced situations. However, this does not preclude the intervention in decolourisation of other type of enzymes produced by WRF, as for example cellobiose quinone oxidoreductase (Van Hulle *et al.* 2007).

Enzyme Induction

Ganoderma stipitatum, Lentinus crinitus and *Lentinus swartzii*, were selected for their high production of MnP and laccase, and were

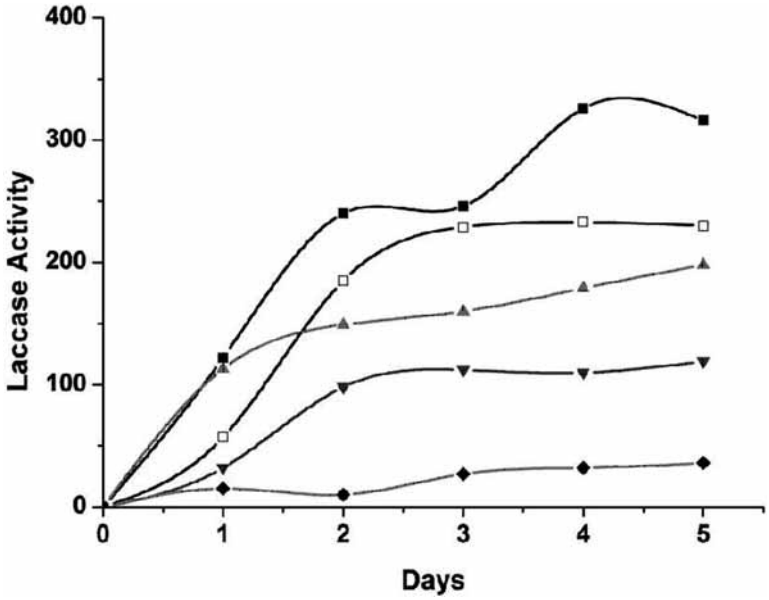

Fig. 1. Laccase activity (U L^{-1}) of strains in presence of 200 mg L-1 Coomassie blue under submerged cultivation on LNLM. (–■–) *Ganoderma stipitatum*, (–□–) *Lentinus crinitus,* (–▲–) *Lentinus swartsii,* (–▼–) *Oudiemansiella canarii,* (–◆–) *Pleurotus djamor.*

used for further induction experiments. Highest increase of enzyme production after three weeks of cultivation was generally observed in the presence of Cu^{2+}, followed by ethanol and Mn^{2+} (Figs. 2 a–c). In the case of *Lentinus swartzii*, laccase reached a level of around 3100 U L^{-1} after 20 days of cultivation in the presence of 150 μM Cu^{2+}; this is compared to a basal production level of 170 U L^{-1} (Fig. 2a). As a general rule, five to ten fold induction values were observed for both laccase and MnP. Copper showed a better inducing effect for laccase and MnP production in the three strains. Mn^{2+} was not apparently a good inducer for MnP or LiP. Ürek & Pazarlioğlu (2005) and Pappinuti *et al.* (2006) reported a stimulating effect for the production of MnP by *Phanerochaete chrysosporium* and *Fomes sclerodermeus,* with relatively low Mn^{2+} concentrations (between 10 to 174 μM). In our study the concentration of Mn^{2+} was 100 μM. However, no inductive effect on MnP production could be observed. Collins & Dobson (1996) reported high MnP activities when liquid cultures of the white-rot fungus *versicolor Trametes* were supplemented with 600 μM Mn^{2+}. Also Steffen *et al.* (2002) showed the stimulating effect of 200 μM Mn^{2+} on the removal of anthracene and pyrene by litter-decomposing fungi. This highlight the necessity to study more in detail the effect of manganese on MnP in a wider range of con-

centrations, in order to observe the real effect of Mn^{2+} on production of MnP by our fungi strains. As a general rule, requirement for manganese in the culture medium to increase MnP activity is a common characteristic among most of the white-rot fungi (Bonnarme & Jeffries 1990).It has been demonstrated that Mn^{2+} regulates the expression of the *mnp* gene in *Phanerochaete chrysosporium* (Brown *et al.* 1990). Yet, the level of manganese may be specific for each species.

Some dyes were found to induce laccase to different extent in fungi (D'Souza *et al.* 2006, Vanhulle *et al.* 2007; Sanchez-Lopez *et al.* 2008). This was also observed here, but studied only for Coomassie blue (Fig. 1). In the register of potential organic inducers we focused on ethanol. In fact, ethanol was identified as having positive inducing effects on laccase production in different fungal strains, for example by *Trametes versicolor* and *P. cinnabarinus* growing in submerged cultures (Lee *et al.* 1999, Lomascolo *et al.* 2003, Meza *et al.* 2005, Jaouani *et al.* 2005). Ethanol may favour protein excretion, by increasing membrane permeability and can also serve as carbon source for fungi (Lomascolo *et al.* 2003). On the other hand the ethanol addition may induce enzyme production indirectly, by causing oxidative stress (Meza *et al.* 2005). It was also advocated as a useful inducer for industrial enzyme production because of its abundance, cheapness and low toxicity.

Ethanol was also found to induce significantly laccase in *Lentinus swartzii, Lentinus crinitus* and *Ganoderma stipitatum* (Fig. 2). Very similar MnP activity was reached for *Lentinus swartzii* with ethanol or Cu^{2+} (Fig. 2a). Nevertheless, an inhibitory effect of the ethanol could be observed at high concentration. Beyond a threshold, the growth of the biomass become undetectable and no enzyme were produced.

In more detailed experiments, four concentrations of $CuSO_4$, ranging between 150–750 μM were tested for laccase and MnP induction in submerged cultures. The experiments (Figs. 3 and 4) showed that *Lentinus swartsi* was the most effective producer, attaining 11,650 U L^{-1} of laccase and 2,639 U L^{-1} of MnP. in the presence of 550 μM Cu^{2+}. Concentrations of 750 μM Cu^{2+} and higher altered enzyme production. A significant reduction of cell growth with a substantial decrease in laccase and MnP activity were also observed at these concentrations.

Copper as a micronutrient has a key role as metal activator for several fungal enzymes including laccases. It has been reported by several authors that copper activate laccase transcription but also increase catalytic activity and stability of the enzyme (Palmieri *et al.* 2000, Baldrian 2003.). Indeed, although the presence of copper in the catalytic center of the enzyme has been known for a very long time,

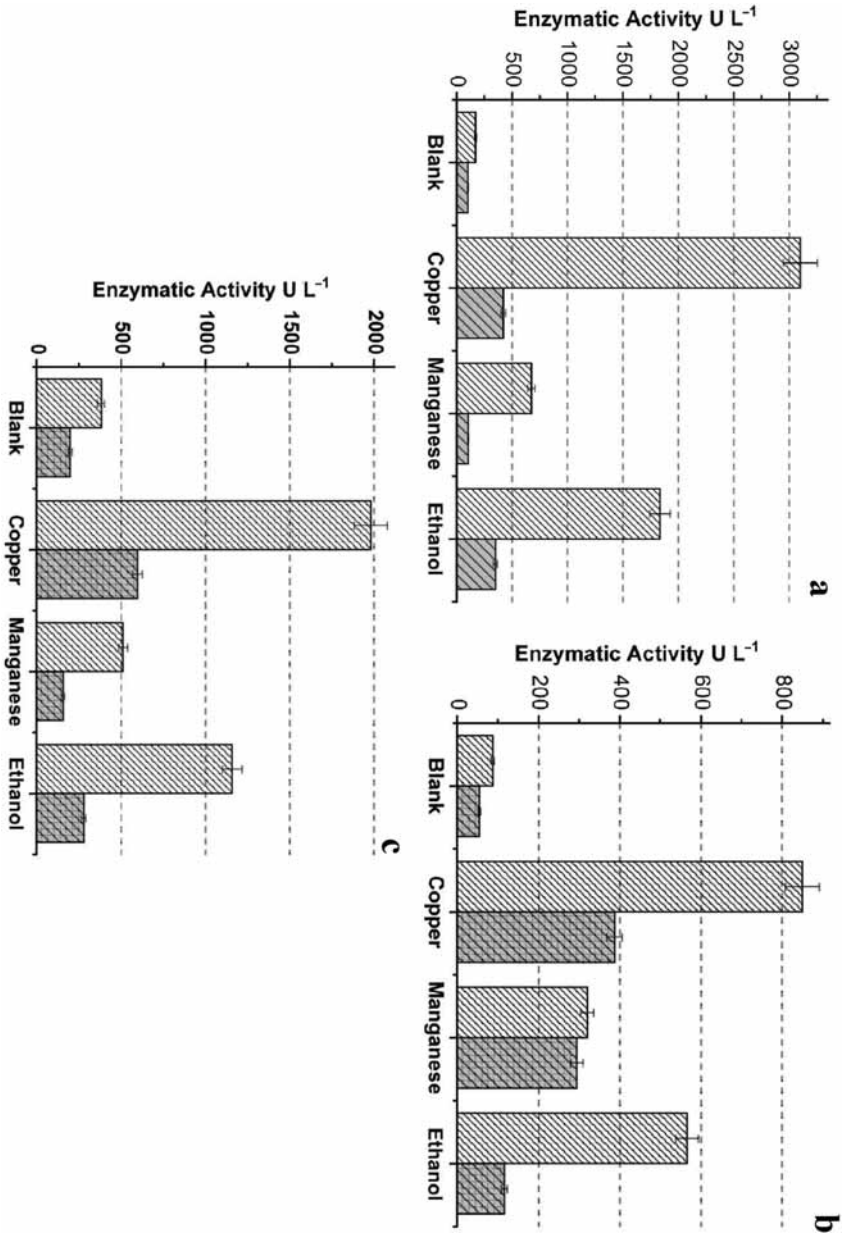

Fig. 2. Laccase (white lined columns) and MnP (gray lined columns) activities at 20th day of cultivation for (a). *Lentinus swartzii,* (b). *Lentinus crinitus* (c) *Ganoderma stipitatum.* Respective concentrations of 150 μM $CuSO_4$, 100 μM $MnSO_4$ or 500 mM Ethanol were present as inducers. Blank cultivation was without inducers.

the important regulatory role of copper in laccase production has been only addressed in the last ten years (Collins & Dobson 1997, Chen *et al.* 2003).

Maximal laccase production obtained for the strains investigated without inducers was around 100–200 U L^{-1} and remained stable for at least 20 days (Table 2). Higher peak levels were obtained in the presence of the different potential inducers, not only with copper. This could indicate that copper and others inducers used here may have a true inducing effect and are not only related to catalytic activation and stabilization of the enzyme.

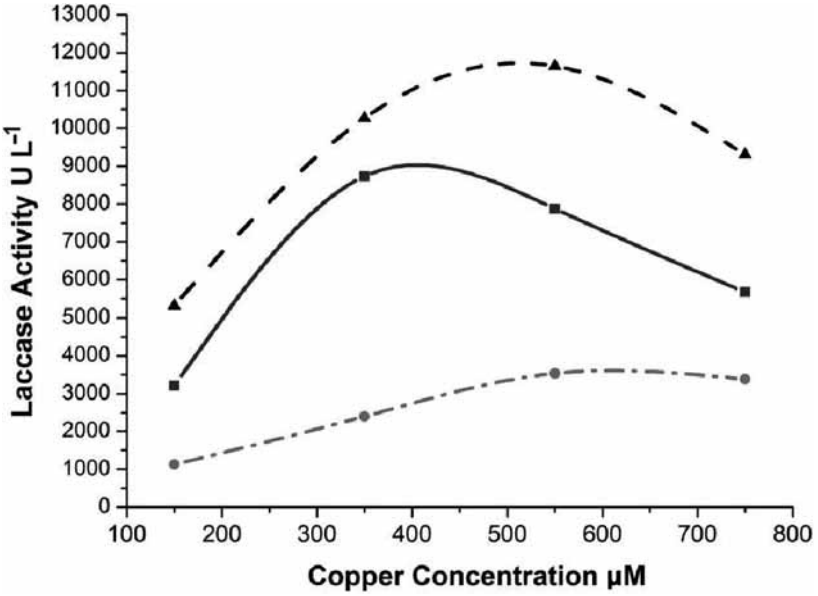

Fig. 3. Laccase production by (–■–) *Ganoderma stipitatum*, (–●–) *Lentinus crinitus*, (–▲–) *Lentinus swartsii*, at different Cu^{2+} concentrations under submerged cultivation at 30 °C and 20th day of cultivation.

Although it is not a common fact that MnP is induced by a metal different from Mn^{2+}, it has already been shown that in *Stereum hirsutum*, Cd^{2+} induces the synthesis of this enzyme (Baldrian *et al.* 1996). As a mater of fact, records about heavy metals effects on other ligninolytic enzymes are scarce. In *T. trogii*, addition of copper increased the activities of Mn-peroxidase and glyoxal oxidase, as well as the decolourisation of the polymeric dye Poly R-478. Highest enzyme activities and decolourisation rate was obtained with 1.6 mM Cu^{2+}, the highest concentration tested (Levin *et al.* 2002).

As a conclusion, the use of dyes has proven to be a practical method for selection of microorganisms potentially producing lig-

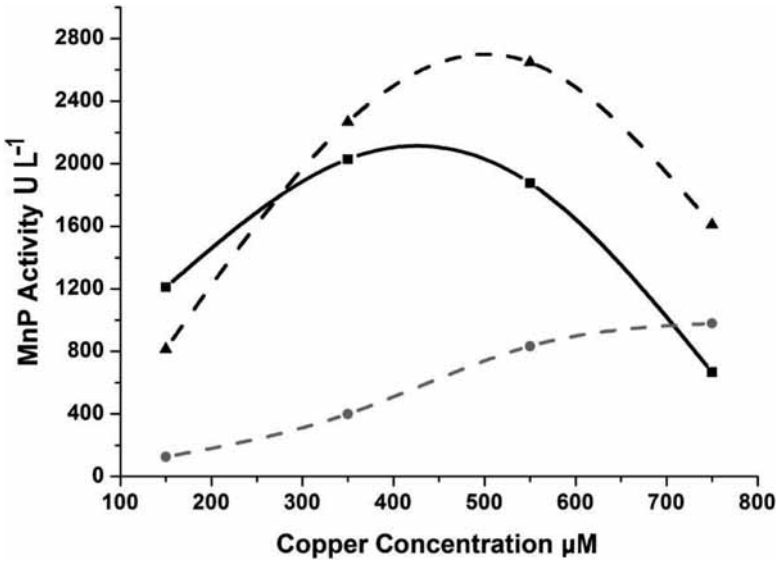

Fig. 4. Manganese peroxidase production by (–■–) *Ganoderma stipitatum*, (–●–) *Lentinus crinitus*, (–▲–) *Lentinus swartsii*, at different Cu^{2+} concentrations under submerged cultivation at 30 °C and 20th day of cultivation.

ninolytic enzymes. Following this approach, we focused our effort on three autochthonous strains, *Ganoderma stipitatum, Lentinus crinitus* and *Lentinus swartzii* that demonstrated important activities. These fungi were also the most efficient in the microplates tests for decolourisation of both. Coomasie Blue and Azure B. Moreover, Laccase production correlated with dye decolourisation. In similar way, in the test made by Pointing and Vrijmoed (2000) laccase showed to be efficient in the decolourisation of several dyes; partial decolourisation of two azo dyes (Orange G and Amaranth) and complete decolourisation of two triphenylmethane dyes (Bromophe-

Table 2. – Correlation between Laccase and MnP activities produced by different strains and the residual colour five days after dye addition. Initial concentration of Coomassie Blue was 200 mg L^{-1}. No decolourisation was observed in blank experiment without strain.

Strain	**Enzyme activity** [U L^{-1}]		**Residual colour [%]**
	Laccase	**Mn peroxidase**	
Pleurotus djamor	2	38	52
Oudiemansiella canarii	13	115	30
Lentinus swartzi	60	200	27
Lentinus crinitus	112	208	22
Ganoderma stipitatum	110	312	18

nol blue and Malachite green) was achieved by cultures in submerged liquid culture producing laccase as the sole phenoloxidase.

Little research attention has been paid until now for ligninolytic enzymes produced by *Ganoderma spp*. and *Lentinus spp*. (Hatvani & Mecs 2001; Songulashvili *et al*. 2007).

Several papers reported production of high laccase activity with other strains, however often using elaborate growth media, comprising quite expensive additives including vitamins, mix of several inducers, etc. (Chen *et al*. 2003, Baldrian 2004; Madhavi & Lele 2006; Lorenzo *et al*. 2006, Minussi *et al*. 2007). Here we have obtained comparably high laccase activity with the selected strains, while growing on a basal media with only one inducer. This could constitute a serious advantage in terms of competition for industrial applications. To our knowledge, this is also the first report of decolourisation and laccase and MnP production, specifically by *Ganoderma stipitatum, Lentinus swartzii and Lentinus crinitus*. In conclusion, these strains represent promising axes for biotechnological applications. Their description also highlights the advantage of the screening previously unexploited environments and could constitute a good incentive for better protection of fungal biodiversity.

Acknowledgements

This work was supported by the Red Alfa Caribiotec programme and the Universidad de Antioquia and Colciencias.

References

Archibald F. S. (1992) A new assay for lignin-type peroxidases employing the dye Azure B. *Applied and Environmental Microbiology* **58**: 3110–3116.

Baldrian P., Gabriel J., Nereud F. (1996) Effect of cadmium on the ligninolytic activity of *Stereum hirsutum* and *Phanerochaete chrysosporium*. *Folia Microbiologica* **41**: 363–367.

Baldrian P. (2003) Interactions of heavy metals with white-rot fungi. *Enzyme and Microbial Technology* **32**: 78–91.

Baldrian P. (2004) Increase of laccase activity during interspecific interactions of white-rot fungi. *FEMS Microbial Ecology* **50**: 245–253.

Boer C. G., Obici L., Giatti Marques de Souza C., Peralta R. M. (2004) Decolourisation of synthetic dyes by solid state cultures of *Lentinula (Lentinus) edodes* producing manganese peroxidase as the main ligninolytic enzyme. *Bioresource Technology,* **94**: 107–112.

Bonnarme P., Jeffries T. W. (1990) Mn(II) regulation of lignin peroxidases and manganese-dependent peroxidases from lignin-degrading white rot fungi. *Applied and Environmental Microbiology* **56**: 210–217

Brown J. A., Glenn J. K., Gold M. H. (1990) Manganese regulates expression of manganese peroxidase by *Phanerochaete chrysosporium*. *Journal of Bacteriology* **172**: 3125–3130.

Chen S., Ma D., Ge W., Buswell J. A. (2003) Induction of laccase activity in the edible straw mushroom, *Volvariella volvacea*. *FEMS Microbiology Letters* **218**: 143–148.

Clemente A. R., Anazawa T. A., Durrant L. R. (2001) Biodegradation of polycyclic aromatic hydrocarbons by soil fungi. *Brazilian Journal of Microbiology* **32**: 255–261.

Collins P. J., Dobson A. D. W. (1996) Oxidation of fluorene and phenanthrene by Mn(II) dependent peroxidase activity in whole cultures of *Trametes (Coriolus) versicolor*. *Biotechnology Letters* **18**: 801–804

Collins P. J., Dobson A. D. W. (1997) Regulation of Laccase Gene Transcription in *Trametes versicolor*. *Applied and Environmental Microbiology* **63**: 3444–3450.

De Souza C., Soares I., De Oliveira P. R. (2005) Ligninolytic enzyme production by *Ganoderma spp*. *Enzyme and Microbial Technology* **37**: 324–32.

Dhouib A., Hamza M., Zouari H., Mechichi T., H'midi R., Labat M., Martínez M. J. Sayadi S. (2005) Autochthonous fungal strains with high ligninolytic activities from Tunisian biotopes. *African Journal of Biotechnology* **4**: 431–436.

D'Souza D. T., Tiwari R., Kumar Sah A., Raghukumar C. (2006) Enhanced production of laccase by a marine fungus during treatment of colored effluents and synthetic dyes. *Enzyme and microbial Technology* **38**: 504–511.

Eichlerova I′., Homolka L., Frantisek, Nerud L. (2005) Orange G and Remazol Brilliant Blue R decolourisation by white rot fungi *Dichomitus squalens, Ischnoderma resinosum* and *Pleurotus calyptratus*. *Chemosphere* **60**: 398–404.

Eriksson K.-E., Kirk T. K. (1985) Biopulping, biobleaching and treatment of kraft bleaching effluents with white-rot fungi. In W. R. Campbell and J. A. Howell (eds.), *Comprehensive Biotechnology,* vol. IV. Pergamon Press, Toronto. p. 271–294.

Hatvani N., Mecs I. (2001) Production of laccase and manganese pezroxidase by *Lentinus edodes* on malt containing by-product of the brewing process. *Process Biochemistry* **37**: 491–496.

Heinzkill M., Bech L., Halkier T., Schneider P., Anke T. (1996) Characterization of laccases and peroxidases from wood-rotting fungi (Family *Coprinaceae*). *Applied and Environmental Microbiology* **64**: 1601–1606.

Jaouani A., Sayadi S., Vanthournhout M., Penninckx M. J. (2003) Potent fungi for decolourisation of olive oil mill wastewaters. *Enzyme and Microbial Technology*, **33**: 802–809.

Jaouani A., Guillén F., Penninckx M., Martínez A., Martínez M. (2005) Role of *Pycnoporus coccineus* laccase in the degradation of aromatic compounds in olive oil mill wastewater. *Enzyme and Microbial Technology* **36**: 478–486.

Kariminiaae-Hamedaani H.-R., Sakurai A., Sakakibara M. (2007). Decolourisation of synthetic dyes by a new manganese peroxidase-producing white rot fungus. *Dyes and Pigments* **72**: 157–162.

Kirk T. K., Schultz E., Connors W. J., Lorenz, L. F., Zeikus J. G. (1978) Influence of culture parameters on lignin metabolism by *Phanerochaete chrysosporium*. *Archives of Microbiology* **117**: 277–285.

Lee, I-Y., Jung, K-H., Lee, C-H., Park, Y-H. (1999) Enhanced production of laccase in *Trametes versicolor* by the addition of ethanol. *Biotechnology Letters* **21**: 965–968.

Levin L., Forchiassin F., Ramos A. M. (2002) Copper induction of lignin-modifying enzymes in the white-rot fungus *Trametes trogii*. *Mycologia* **94**: 377–383.

Levin L., Viale A., Forchiassin A. (2003) Degradation of organic pollutants by the white rot basidiomycete *Trametes trogii*. *International Biodeterioration and Biodegradation* **52**: 1–5.

Levin L., Papinutti L., Forchiassin F. (2004) Evaluation of Argentinean white rot fungi for their ability to produce lignin-modifying enzymes and decolorize industrial dyes. *Bioresource Technology* **94**: 169–176.

Lomascolo A., Record E., Herpoel-Gimbert I., Delattre M., Robert J. L., Georis J., Dauvrin T., Sigoillot J.-C., Asther M. (2003) Overproduction of laccase by a monokaryotic strain of *Pycnoporus cinnabarinus* using ethanol as inducer. *Journal of Applied Microbiology* **94**: 618–624.

Lonsdale D., Pautasso M., Holdenrieder O. (2008) Wood-decaying fungi in the forest;conservation needs and management options. *European Journal of Forest Research* **127**: 1–22.

Lorenzo M., Moldes D., Sanroman M. A. (2006) Effect of heavy metals on the production of several laccase isoenzymes by *Trametes versicolor* and on their ability to decolourise dyes. *Chemosphere* **63**: 912–917.

Madhavi S. R., Lele S. S. (2006) Enhanced production of laccase using a new isolate of white rot fungus WR-1. *Process Biochemistry* **41**: 581–588.

Martínez A. T. (2002) Molecular biology and structure-function of lignin degrading heme peroxidases. *Enzyme and Microbial Technology* **30**: 425–444.

Meza J. C., Lamascolo A., Casalot L., Sigoillot J. C., Auria R., (2005) Laccase Production By *Pycnoporus cinnabarinus* Grown On Suga-Cane Bagasse: Influence Of Ethanol Vapours As Inducer, *Process Biochemistry* **40**: 3365–3371

Minussi R. C., Pastore G. M., Durán N. (2007) Laccase induction in fungi and laccase/N-OH mediator systems applied in paper mill effluent. *Bioresource Technology* **98**: 158–164.

Moeder M., Cajthaml T., Koeller G., Erbanová P., and Šašek V. (2005) Structure selectivity in degradation and translocation of polychlorinated biphenyls (Delor 103) with a *Pleurotus ostreatus* (oyster mushroom) culture. *Chemosphere* **61**: 1370–1378.

Moreira M. T., Feijoo G., Lema J. M. (2000) Evaluation of different fungal strains in the decolorisation of synthetic dyes. *Biotechnology Letters* **22**: 1499–1503.

Mueller G. M., Schmit J. P. (2007) Fungal biodiversity: What do we know? What can we predict? *Biodiversity and Conservation* **16**: 1–5.

Novotný C., Rawal B., Bhatt M., Patel M.,Sasek V., Molitoris H. P. (2001) Capacity of *Irpex lacteus* and *Pleurotus ostreatus* for decolourisation of chemically different dyes. *Journal of Biotechnology* **89**: 113–122.

O'Neill C., Hawkes F. R., Lourenco N. D., Pinheiro H. M., Delee W. (1999) Colour in textile effluents-sources. Measurement, discharge consents and simulation: a review. *Journal of Chemical Technology and Biotechnology* **74**: 1009–1018.

Palmieri G., Giardiana P., Bianco C., Fontanella B., Sannia G. (2000) Copper induction of laccase isoenzymes in the ligninolytic fungus *Pleurotus ostreatus*. *Applied and Environmental Microbiology* **66**: 920–924.

Papinutti L., Martinez M. (2006) Production and characterization of laccase and manganese peroxidase from the ligninolytic fungus *Fomes sclerodermeus*. *Journal of Chemical Technology and Biotechnology* **81**: 1064–1070.

Paszczynski A., Huynh V. B., Crawford R. (1986) Comparison of ligninase-1 and peroxidase M-2 from the white-rot fungus *Phanerochaete chrysosporium*. *Archives of Biochemistry and Biophysics* **224**: 750–765.

Pointing S. B., Vrijmoed L. L. P. (2000) Decolourisation of azo and triphenylmethane dyes by *Pycnoporus sanguineus* producing laccase as the sole phenoloxidase. *World Journal of Microbiology and Biotechnology* **16**: 317–318.

Pointing S. B., Parungao M. M., Hyde K. (2003) Production of wood-decay enzymes, mass loss and lignin solubilization in wood by tropical Xylariaceae. *Mycological Research* **107**: 231–235.

Ramsay J. A., Mok W. H. W., Luu Y.-S., Savage M. (2005) Decoloration of textile dyes by alginate-immobilized *Trametes versicolor*. *Chemosphere* **61**: 956–964.

Ruiz A., Varela A. (2006) New reports of Aphyllophorales (Basidiomicota) in humid and cloudy mountain forests from Colombia. *Caldasia* **28**: 259–266.

Sanchez-Lopez M. I., Vanhulle S., Mertens V., Guerra G. *et al*. (2008) Autochtonous white rot fungi from the tropical forest : potential of cuban strains for dyes and textile industrial effluents decolourisation. *African Journal of Biotechnology* **7**: 1983–1900.

Sato A., Watanabe T., Watanabe Y., Harazono K., Fukatsu T. (2002) Screening for basidiomycetous fungi capable of degrading 2,7-dichlorodibenzo-p-dioxin. *FEMS Microbiology Letters* **213**: 213–217.

Songulashvili G., Elisashvili V., Wasser C. P., Nevo E., Hadar Y (2007) Basidiomycetes laccase and manganese peroxidase activity in submerged fermentation of industry wastes. *Enzyme and Microbial Technology* **41**: 57–61.

Steffen K. T., Hatakka A., Hofrichter M. (2002) Removal and mineralization of polycyclic aromatic hydrocarbons by litter-decomposing basidiomycetous fungi. *Applied Microbiology and Biotechnology* **60**: 212–217.

Tekere M., Zvauya R., Read J. S. (2001) Ligninolytic enzyme production in selected sub-tropical white rot fungi under different culture conditions. *Journal of Basic Microbiology* **41**: 115–129.

Tekere M., Read J. S., Mattiasson B. (2005) Polycyclic aromatic hydrocarbon biodegradation in extracellular fluids and static batch cultures of selected subtropical white rot fungi. *Journal of Biotechnology* **115**: 367–377.

Ürek R. Ö., Pazarlioğlu N. K. (2005) Production and stimulation of manganese peroxidase by immobilized *Phanerochaete chrysosporium*. *Process Biochemistry* **40**: 83–87.

Vanhulle S., Enaud E., Trosvalet M., Nouaimeh N. C., Bols C. M., Keshavarz T., Tron T., Sannia G., Corbisier A.-M. (2007) Overlap of laccase/cellobiose dehydrogenase activities during the decolourisation of anthraquinonic dyes with close chemical structure by *Pycnoporus* strains, *Enzyme and Microbial Technology* **40**: 1723–1731.

Wesenberg D., Kyriakides I., Aghatos S. N. (2003) White-rot fungi and their enzymes for the treatment of industrial dye effluents. *Biotechnology Advances* **22**: 151–187.

Wong Y., Yu J.(1999) Laccase-catalyzed decolourisation of synthetic dyes. *Water Research* **33**: 3512–3520

Young S. K., Qing X. L. (2004) Fungal laccase-catalyzed degradation of hydroxy polychlorinated biphenyls. *Chemosphere* **56**: 23–30.

(Manuscript accepted 3 Oct 2008; Corresponding Editor: M. Kirchmair).