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Review

Physiological regulation of laccase and manganese peroxidase production by white-rot *Basidiomycetes*

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ABSTRACT

This review integrates recent literature and our own data on the physiology of laccase and manganese peroxidase synthesis, focusing on the common characteristics and unique properties of individual fungi as well as on several approaches providing enhanced enzyme secretion. Firstly, the enzyme yield is species-dependent and strain-dependent and selection of new organisms with tremendous synthesis of these enzymes is possible. For example, in screening program the laccase activity of tested basidiomycetes varied from 0.5 U ml^{-1} to 75 U ml^{-1}. Secondly, the carbon source and lignocellulosic substrate play a crucial role in enzyme production. Thus, laccase activity of *Pseudotrametes gibbosa* varied from 0.3 U ml^{-1} (Avicel) to 13.7 U ml^{-1} (lactose), while the substitution of wheat bran with walnut pericarp increased *Cerrena unicolor* manganese peroxidase yield from 0.7 U ml^{-1} to 8.3 U ml^{-1}. Thirdly, aromatic compounds regulate the ligninolytic enzyme synthesis although their effect is very specific depending on fungi physiological peculiarities. 2,4,6-trinitrotoluene (TNT) supplemented to the medium at appropriate concentration significantly accelerated *C. unicolor* laccase production and 4-fold increased laccase specific activity. Fourthly, co-cultivation of appropriate fungi shows considerable promise as a strategy to highly enhance the enzyme production. For example, pairing of *C. unicolor* and *Phellinus robustus* 2-fold increased the total laccase yield.

Contents

1. Introduction

White-rot *Basidiomycetes* are unique in their ability to degrade all components of lignocellulose due to their capability to synthesize the relevant hydrolytic and oxidative extracellular enzymes (Eriksson et al., 1990; Aro et al., 2005). These fungi secrete one or more of three extracellular enzymes that are essential for lignin degradation: lignin peroxidase (EC 1.11.1.14), manganese-dependent peroxidase (MnP) (EC 1.11.1.13), and laccase (EC 1.10.3.2). The ligninolytic enzymes of Basidiomycetes are of fundamental importance for the efficient bioconversion of plant residues and they are prospective for the various biotechnological applications in pulp and paper, food, textile and dye industries, bioremediation, cosmetics, analytic biochemistry, and many others. It is evident that the potential applications of these enzymes in industrial and environmental technologies require huge amounts of these enzymes at low cost. The main issue delaying their implementation at industrial scale is the low yield of ligninolytic enzymes in most white-rot fungi. Although many recombinant organisms efficiently overproduce various industrial enzymes, high expression of laccase and peroxidases in heterologous systems has not been achieved yet and they still have to be obtained from natural sources (Weenink et al., 2006; Bailey et al., 2007). Moreover, ligninolytic enzymes of some basidiomycetes are formed in secondary metabolism; therefore, it

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is important to discover microorganisms effectively secreting these enzymes during primary metabolism and to develop strategies for their overproduction.

The structural and catalytic properties, molecular genetics, and biotechnological applications of lignin-degrading oxidases have recently been comprehensively reviewed (Martinez, 2002; Baldrian, 2006; Kersten and Cullen, 2007; Morozova et al., 2007), whereas an exhaustive overview of the basic aspects of the microbiology of ligninolytic enzymes production is still lacking in the literature. Recently, the cultural conditions for the production of lignin-degrading enzymes by *Phanerochaete chrysosporium* have been overviewed (Singh and Chen, 2008). In this review we summarize and integrate mainly our own data and recent literature reports on the physiology of laccase and manganese peroxidase synthesis, focusing on the common characteristics and unique properties of individual fungi as well as on several approaches providing enhanced secretion of laccase and manganese peroxidase by white-rot *basidiomycetes* (WRB).

2. Screening of fungi

A number of screening studies of WRB and other classes of fungi were conducted to discover promising producers of ligninolytic enzymes (Elisashvili et al., 2001, 2009; Tekere et al., 2001; Lomascolo et al., 2002; Kiiskinen et al., 2004; Songulashvili et al., 2007; Myasoedova et al., 2008). However, various nutrient media compositions and cultivation conditions have been used by different groups of investigators in addition to different activity assay procedures. Moreover, one of disadvantages of many studies was an evaluation of fungi enzyme activity without establishment of profile of their accumulation. Therefore, the direct comparison of data on WRB biosynthetic potential given in literature is difficult.

Recently, we have shown that several food industry wastes are appropriate growth substrates ensuring efficient production of ligninolytic enzymes (Elisashvili et al., 2006, 2008a, 2008b; Songulashvili et al., 2006). Moreover, the presence of lignocellulose was a prerequisite for the enzyme production by *Ganoderma lucidum* and *Pleurotus dryinus*. Therefore, in contrast to many other studies, we evaluated the ligninolytic enzyme activity of WRB under identical culture conditions in both glycerol and mandarin peelings containing media. The data submitted in Table 1 show that the levels of laccase and MnP activities significantly varied among the fungi tested, but several general features may be noted. Firstly, the enzyme yield is species-dependent and strain-dependent and selection of new organisms with significant laccase or MnP activity is possible. Thus, in fermentation of mandarin peelings the laccase activity of species belonging to genus *Ganoderma* ranged from 2.0 U ml^{-1} to 75.4 U ml^{-1} while the MnP activity of *Trametes versicolor* strains varied from 0 U ml^{-1} to 0.9 U ml^{-1}. In this respect, our results are in agreement with data of other authors reporting on quantitative variations of enzyme activity during cultivation of *Pycnoporus cinnabarinus* (Heproël et al., 2000), *Fomes fomentarius* and *T. versicolor* (Songulashvili et al., 2007). It is interesting that in the study made by Silva et al. (2005), four *Ganoderma* spp. strains possessed highly distinguished ligninolytic systems, accumulating 0.6–49.5 U l^{-1} of laccase activity; among them two strains exhibited MnP and lignin peroxidase activities.

In this study, *G. lucidum* 246 followed by *Cerrena unicolor* 300 produced extremely high laccase activity after 10 days of submerged fermentation of mandarin peelings. Several other fungi of genera *Cerrena*, *Ganoderma*, and *Trametes* also accumulated high levels of laccase and may be an appropriate source of this enzyme. Moreover, *C. unicolor* 300 appeared to be very promising producer of MnP in both synthetic and complex media. It is worth noting that these appreciable laccase and MnP activities were obtained in the absence of specific exogenous inductor. Secondly, all WRB secreted laccase activity independently on the nutrient medium, whereas the production of MnP was detected only in *Bjerkandera adusta* 47, *F. fomentarius* 38, *Cerrena* and *Trametes* genera. Thirdly, the tested WRB exhibited quite different responses to growth substrates used. While *G. lucidum* 246 and other strains of genus *Ganoderma* secreted only negligible laccase activity in submerged cultivation in glycerol-containing medium, strains of genus *Cerrena* and *Coriolopsis gallica* 1184 were capable to produce significant laccase activity in defined medium. Moreover, defined medium was preferable for the laccase production by *Pseudotrametes gibbosa* 17 and *T. versicolor* 775 and MnP secretion by *C. unicolor* 300. Fourthly, the tested fungi distinguished by the kinetics of enzyme accumulation. Depending on strain and medium used the peaks of laccase and MnP activities were observed after 5–14 days of fungi cultivation (Table 1).

3. Carbon source

After successful screening program, optimization of the ligninolytic enzyme production is extensively explored with the selected organisms. A large majority of the studies on the production of ligninolytic enzymes have been carried out with defined media. They clearly indicate that basidiomycetes display a wide diversity in their response to carbon sources and their concentration in nutrient medium (Elisashvili et al., 2002, 2006; Galhaup et al., 2002; Mikiashvili et al., 2005; Wang et al., 2008). In *P. chrysosporium*, the ligninolytic gene expression is triggered only by the depletion of nutrient carbon (Wang et al., 2008). Significant laccase secretion by *Trametes pubescens* started when the glucose concentration in the growth medium reached a certain low, critical concentration (Galhaup et al., 2002). Glucose and cellobiose that were efficiently and rapidly utilized by this fungus resulted in high levels of laccase activity. Both lactose and cellulose, which were only poorly utilized for growth, resulted in low laccase levels. The laccase activity obtained in cultivation of *Pleurotus sajor-caju* in media containing 0.5 g l^{-1}l fructose or glucose (37 U ml^{-1} and 36 U ml^{-1}, respectively) were significantly higher than those obtained with lactose (3 U ml^{-1}) (Bettin et al., 2008). Quite the contrary, lactose appeared to be the best carbon source for the laccase secretion by *P. gibbosa* and quite appropriate for the enzyme production by *C. unicolor* and *F. fomentarius* (Table 2). In addition, glycerol also provided significant accumulation of laccase by these three fungi. Unlike *C. unicolor* and *P. gibbosa* cultures, the easily metabolizable sugars, cellobiose and glucose, ensured the highest yield of laccase in submerged cultivation of *F. fomentarius*. All these compounds rather stimulated laccase production by fungi studied increasing the laccase specific activity from 363 U g^{-1} to 571 U g^{-1} biomass in control medium to 614–2454 U g^{-1} biomass. The second distinction of *F. fomentarius* concerns the capability of the fungus to secrete comparatively high levels of laccase in presence of polysaccharides with especially high specific laccase activity (2481 U g^{-1} biomass) in medium containing carboxymethyl cellulose. In another study, laccase activity of white-rot fungus *WR-1* varied from 44 U ml^{-1} with fructose as carbon source to 170 U ml^{-1} with starch as sole carbon source (Revankar and Lele, 2006). These observations indicate that some carbohydrates appear to regulate the laccase expression in white-rot *basidiomycetes* and fungus-specific carbon source should be elucidated to maximally enhance the enzyme synthesis.

4. Effect of lignocellulosic substrate

One of the appropriate approaches in fermentation technology development is to utilize the potential of lignocellulosic wastes,

Table 1
Basidiomycetes laccase and MnP activity in submerged cultivation in glycerol and mandarin peelings containing media.

Fungus and strain[a]	Glycerol based medium		Mandarin peels based medium	
	Laccase[b] (U ml^{-1})	MnP[b] (U ml^{-1})	Laccase (U ml^{-1})	MnP (U ml^{-1})
Bjerkandera adusta 47	0.4$^{(7)c}$	0.1$^{(7)}$	3.5$^{(10)}$	0.1$^{(7)}$
Cerrena maxima 401	8.6$^{(5)}$	0.4$^{(7)}$	25.2$^{(5)}$	0.6$^{(10)}$
Cerrena maxima 403	4.2$^{(5)}$	0.1$^{(5)}$	19.4$^{(5)}$	0.7$^{(10)}$
Cerrena unicolor 300	18.5$^{(10)}$	3.3$^{(10)}$	70.4$^{(10)}$	2.6$^{(7)}$
Coriolopsis gallica 1184	9.3$^{(10)}$	0	19.6$^{(10)}$	0
Fomes fomentarius 38	6.1$^{(14)}$	0.1$^{(10)}$	17.5$^{(7)}$	0.4$^{(10)}$
Ganoderma adspersum 845	0.3$^{(14)}$	0	22.5$^{(14)}$	0
Ganoderma applanatum 107	0.2$^{(10)}$	0	2.0$^{(10)}$	0
Ganoderma lucidum 158	0.3$^{(10)}$	0	20.8$^{(7)}$	0
Ganoderma lucidum 246	0.1$^{(7)}$	0	75.4$^{(10)}$	0
Ganoderma tsugae 1032	0.3$^{(14)}$	0	21.3$^{(7)}$	0
Pleurotus nebrodensis 1019	0.7$^{(7)}$	0	2.8$^{(10)}$	0
Pleurotus tuberregium 737	0.3$^{(7)}$	0	0.5$^{(10)}$	0
Pseudotrametes gibbosa 17	3.5$^{(7)}$	0	2.3$^{(7)}$	0
Trametes ochracea 7	1.8$^{(10)}$	0.1$^{(7)}$	3.9$^{(7)}$	0
Trametes pubescens 11	1.4$^{(7)}$	0.1$^{(10)}$	5.3$^{(7)}$	0
Trametes versicolor 235	7.8$^{(7)}$	0.1$^{(10)}$	16.3$^{(10)}$	0.9$^{10)}$
Trametes versicolor 775	14.8$^{(10)}$	0.2$^{(10)}$	7.3$^{(5)}$	0.6$^{(10)}$
Trichaptum biforme 117	1.9$^{(10)}$	0.1$^{(7)}$	5.0$^{(10)}$	0.6$^{(10)}$

[a] *C. gallica* 1184, *G. adspersum* 845, *G. lucidum* 158 and 246, *G. tsugae* 1032, *P. nebrodensis* 1019, *P. tuberregium* 737 have been received from Prof. Solomon P. Wasser (Haifa University, Israel), other strains were isolated from the forests of Georgia.

[b] Laccase activity was determined by monitoring the absorbance change at 420 nm related to the rate of oxidation of 1 mM ABTS in 25 mM Na-acetate buffer (pH 3.8) at room temperature (Bourbonnais and Paice, 1990). MnP activity was measured at 270 nm by following the formation of Mn^{3+}-malonate-complexes (Wariishi et al., 1992). One unit of laccase or MnP activity was defined as the amount of enzyme which leads to the oxidation of 1 μmol of substrate per minute.

[c] The numbers in parentheses indicate the day of peak activity.

some of which may contain significant concentrations of soluble carbohydrates and inducers ensuring an abundant growth of fungi and efficient production of ligninolytic enzymes (Sun et al., 2004; Rosales et al., 2005; Kachlishvili et al., 2006; Elisashvili et al., 2008a,b,2009; Levin et al., 2008; Winquist et al., 2008). This approach is attractive because of anticipated effects on production cost reduction and agro-industrial wastes reuse, besides enhanced enzyme production since many white-rot fungi produce very low enzyme activity when growing in defined medium (Bollag and Leonowicz, 1984; Elisashvili et al., 2002, 2006; Kapich et al., 2004; Mikiashvili et al., 2005; Songulashvili et al., 2007). Previously it was demonstrated that the water-soluble aromatic/phenolic compounds derived from different lignocellulosic substrates substantially increase the production of ligninolytic enzymes in white-rot fungi (Crestini et al., 1996; Kapich et al., 2004). Therefore, the selection of appropriate plant residue adequate for fungus growth and target enzymes synthesis plays an important role in the development of an efficient technology of enzyme production.

The regulatory role of lignocellulosic substrates in laccase and MnP production by *C. unicolor* and *Pellinus robustus* is shown in Table 3. Although all tested residues provided in submerged fermentation good growth of both fungi in form of pellets laccase activity of *C. unicolor* and *P. robustus* varied from 15.7 U ml^{-1} to 151.6 U ml^{-1} and from 0.9 U ml^{-1} to 8.4 U ml^{-1}, respectively. Wheat bran followed by ethanol production residue appeared to be excellent growth substrates for this enzyme production by *C. unicolor* whereas mandarin peels followed by kiwi and walnut pericarp favored to laccase accumulation by *P. robustus*. Analogically, walnut pericarp, used for the first time in this study, 12-fold augmented the yield of *C. unicolor* MnP as compared with wheat bran, while the fermentation of kiwi residue stimulated this enzyme secretion by *P. robustus*. Moreover, the ratio of individual enzymes in final preparations significantly depended on growth substrate. Thus, laccase/MnP ratio changed from 226 to 2 with substitution of wheat bran by walnut pericarp. Furthermore, the data received clearly show that the profiles of both enzyme accumulation significantly depended on growth substrate used. These results suggest that the type of lignocellulosic substrate appears to determine the types and amounts of ligninolytic enzymes produced by the WRB. However, it is not inconceivable that part of the secreted enzyme proteins (isoenzymes) may be differently immobilized on different types of lignocellulosic substrates and therefore it is impossible to reliably assess these substrates effects on the enzyme production.

Table 2
Effect of carbon source on fungi growth and laccase activity in submerged cultivation.

Carbon source	*Cerrena maxima*		*Fomes fomentarius*		*Pseudotrametes gibbosa*	
	Biomass (g l^{-1})	Laccase (U ml^{-1})	Biomass (g l^{-1})	Laccase (U ml^{-1})	Biomass (g l^{-1})	Laccase (U ml^{-1})
Control[a]	0.8	0.3	0.8	0.4	0.7	0.4
Avicel	3.5[b]	0.1	3.9[b]	1.9	3.4[b]	0.3
CMC[c]	1.2	0.2	1.6	7.0	1.3	0.7
Xylan	3.0	0.3	3.4	1.3	4.6	0.5
Glucose	4.9	0.5	6.4	9.0	7.3	1.0
Cellobiose	5.6	1.6	5.2	10.9	7.7	1.1
Lactose	6.8	4.4	4.0	7.6	5.6	13.7
Glycerol	9.5	5.2	4.6	5.8	6.5	6.2

This and other experiments were performed twice using three to five replicates. The data presented correspond to mean values, the standard deviation being lower than 15%.

[a] Without carbon source.

[b] Calculated from protein content.

[c] Carboxymethyl cellulose.

Table 3
Effect of lignocellulosic growth substrate on the laccase and MnP yield in submerged fermentation.

Growth substrate	*Cerrena unicolor*		*Phellinus robustus*	
	Laccase (U ml^{-1})	MnP (U ml^{-1})	Laccase (U ml^{-1})	MnP (U ml^{-1})
Ethanol production residue	108.1$^{(10)a}$	3.7$^{(7)}$	1.4$^{(7)}$	3.2$^{(10)}$
Wheat bran	151.6$^{(14)}$	0.7$^{(4)}$	2.3$^{(14)}$	1.4$^{(10)}$
Mandarin peels	78.5$^{(14)}$	2.2$^{(7)}$	8.4$^{(14)}$	2.3$^{(10)}$
Banana peels	74.3$^{(7)}$	3.1$^{(7)}$	2.3$^{(14)}$	1.1$^{(14)}$
Kiwi	57.0$^{(7)}$	4.0$^{(10}$	4.2$^{(14)}$	6.7$^{(10)}$
Walnut leaves	25.5$^{(7)}$	0.8$^{(7)}$	0.9$^{(10)}$	1.3$^{(14)}$
Walnut pericarp	15.7$^{(4)}$	8.3$^{(10)}$	3.8$^{(14)}$	1.9$^{(14)}$

[a] The numbers in parentheses indicate the day of peak activity.

Table 4
Effect of aromatic compounds on the *C. unicolor* laccase and MnP production.

Compounds	EPR[a] containing medium		Mannitol-containing medium	
	Laccase (U ml^{-1})	MnP (U ml^{-1})	Laccase (U ml^{-1})	MnP (U ml^{-1})
Control	117	4.1	15.0	2.0
Catechol	114	4.8	28.6	2.4
2,6-DMP	137	8.5	29.7	2.3
Ferulic acid	118	6.6	15.1	2.6
Pyrogallol	153	6.5	37.2	3.9
TNT	165	4.1	1.7	0
Vanillic acid	154	7.4	3.1	1.4
Veratric acid	83	6.4	24.1	3.2
Xylidine	103	4.2	14.2	0.9

[a] EPR: ethanol production residue; 2,6-DMP: dimethoxyphenol; TNT: 2,4,6-trinitrotoluene.

5. Aromatic compounds

One of the most effective approaches to increase the yield of ligninolytic enzymes is the supplementation of the nutrient medium with an appropriate inducer. The most widely reported potent inducer of laccase synthesis is 2,5-xylidine (Bollag and Leonowicz, 1984; Elisashvili et al., 2002; Revankar and Lele, 2006). However, the laccase production by *T. versicolor* doubled when veratryl alcohol or guaiacol was used instead of 2,5-xylidine (Lee et al., 1999). The best inducer for the *Lentinus strigosus* laccase synthesis is reported to be 2,6-dimethylphenol elevating the enzyme yield 8-fold compared to the control, whereas veratryl alcohol was inefficient or induced the enzyme production insignificantly (Myasoedova et al., 2008). Moreover, in *Trametes* sp. AH28-2 o-toluidine selectively induced the expression of laccase A while 3,5-dihydroxytoluene mainly stimulated the production of laccase B (Xiao et al., 2006). This means that fungus-specific inducer should be found in order to maximally express the target enzyme activity.

In our study, 2,4,6-trinitrotoluene (TNT) followed by vanillic acid, and pyrogallol at a concentration of 0.5 mM increased the laccase yield from 117 U ml^{-1} to (153–165) U ml^{-1} when *C. unicolor* was cultivated in medium containing ethanol production residue (Table 4). Under the same conditions, laccase activity in xylidine or veratric acid supplemented cultures was rather lower than that recorded in control medium. The supplementation of dimethoxyphenol (2,6-DMP) to the control medium 2-fold increased the MnP yield. Vanillic acid followed by ferulic acid, pyrogallol, and veratric acid increased *C. unicolor* MnP activity by more than 50%.

When *C. unicolor* was cultivated in the mannitol-containing medium the fungus grew well in the presence of all phenolic compounds with the exclusion of TNT. Pyrogallol followed by 2,6-DMP, catechol, and veratric acid caused between 1.6-fold and 2.5-fold increase of laccase activity as compared with the control culture. The aromatic compounds accelerated an enzyme production and shortened the time of peak activity appearance. Besides laccase, pyrogallol and veratric acid stimulated the MnP production by *C. unicolor*, respectively, 2-fold and 1.6-fold. Vanillic acid significantly repressed laccase synthesis, whereas xylidine and vanillic acid diminished MnP accumulation. It is interesting that significant laccase activity was observed (1.7 U ml^{-1} on day 11) in presence of 0.5 mM TNT in spite of complete inhibition of *C. unicolor* growth proving that an inoculated mycelium remained metabolically active.

Therefore, the effect of TNT concentration on the *C. unicolor* growth and laccase secretion was studied in short-term experiments. The concentrations between 0.03 mM and 0.1 mM TNT did not affect fungal growth. However, 0.3 mM TNT concentration delayed the biomass accumulation during the first 24–48 h of submerged cultivation. Afterwards, the fungal growth accelerated and by 96 h the biomass yield was comparable with that in control medium. TNT highly accelerated the laccase production increasing already after 8 h the enzyme activity 3–5-fold as compared with control medium (Fig. 1). Moreover, during the second day of fungus growth the differential rate of laccase synthesis in presence TNT reached 3125–8630 U mg^{-1} biomass significantly exceeding that in control medium (1267 U mg^{-1} biomass).

6. Co-cultivation

Several attempts have been made to enhance laccase production by the fungi co-cultivation strategy. Laccase activity in *T. versicolor* was increased several times when this fungus was co-cultivated with *Trichoderma harzianum* and other soil fungi or bacteria (Baldrian, 2004). Moreover, the induction of new isozymes was observed in dual cultures of *Pleurotus ostreatus* with *Trichoderma longibrachiatum* (Velazquez-Cedeño et al., 2004) and *Trametes* sp. AH28-2 with a *Trichoderma* strain (Zhang et al., 2006). Laccase was significantly stimulated in the co-culture of *P. ostreatus* with *Ceriporiopsis subvermispora* while manganese peroxidase activity was stimulated in co-cultures of *P. ostreatus* with *C. subvermispora* or with *Physisporinus rivulosus* (Chi et al., 2007). The data represented in Fig. 2 indicate that in submerged fermentation of wheat bran by monocultures *C. unicolor* produced as high as 98 U ml^{-1} laccase whereas *P. robustus* accumulated only 5 U ml^{-1} enzyme activity. Pairing of these two fungi in the same culture con-

Table 5
White-rot *basidiomycetes* laccase activity obtained in shake-flasks experiments.

Fungus	Major media components	Enzyme activity (U ml^{-1})	References
Cerrena unicolor	Tomato juice medium	19 (ABTS)*	Michniewicz et al., 2006
C. unicolor	Ethanol production residue (40 g l^{-1}), 0.5 mM TNT	165 (ABTS)	This study
Coriolopsis rigida	Barley bran (50 g l^{-1}), 2 mM $CuSO_4$, 10 mM xylidine.	40 (ABTS)	Alcántara et al., 2007
Ganoderma sp.	Glycerol (40 g l^{-1}), veratryl alcohol (0.85 mM)	240 (ABTS)	Teerapatsakul et al., 2007
Lentinus strigosus	Glucose (20 l^{-1}), 2 mM $CuSO_4$, 1 mM 2,6-dimethylphenol	67 (ABTS)	Myasoedova et al., 2008
Lentinus tigrinus	Birch sawdust (20 g l^{-1}), 1% butanol	24 (pyrocatechin)	Kadimaliev et al., 2008
Pycnoporus cinnabarinus	Maltose (20 g l^{-1}), ethanol (35 g l^{-1})	266 (ABTS)	Lomascolo et al., 2003
Pycnoporus coccineus	Glucose (10 g l^{-1}), 0.15 mM $CuSO_4$, 500 mM ethanol	100 (DMP)	Jaouani et al., 2005
WR-1	Starch (20 g l^{-1}), 1 mM $CuSO_4$, 0.8 mM xylidine	692 (ABTS)	Revankar, Lele, 2006
Trametes sp. +*Trichoderma* sp.	Xylose (15 g l^{-1}), 0.15% tryptone 2 mg l^{-1} $CuSO_4$	6.2 (guaiacol)	Zhang et al., 2006
C. unicolor +*P. robustus*	Wheat bran (40 g l^{-1}), 1 mM $CuSO_4$	184 (ABTS)	This study

* In parenthesis is the substrate in laccase assay; ABTS = 2,2-azino-bis-(3-ethyltiazoline-6-sulfonate); DMP = 2,6-dimetoxiphenol.

ditions caused almost 2-fold increase of total laccase activity yield. On the contrary, co-cultivation of *C. unicolor* with another efficient laccase producer, *G. lucidum*, did not stimulate enzyme production. Hence, the stimulating effect of fungal interaction on enzyme production is species-specific. Although the exact mechanism of this stimulation is not understood the interspecific interaction shows considerable promise as a strategy to highly enhance the ligninolytic enzyme production.

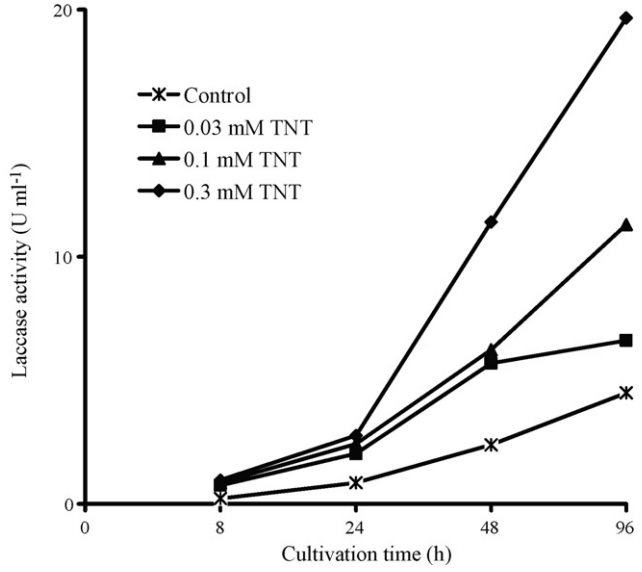

Fig. 1. Effect of 2,4,6-trinitrotoluene (TNT) concentration on *Cerrena unicolor* laccase production in mannitol-containing medium.

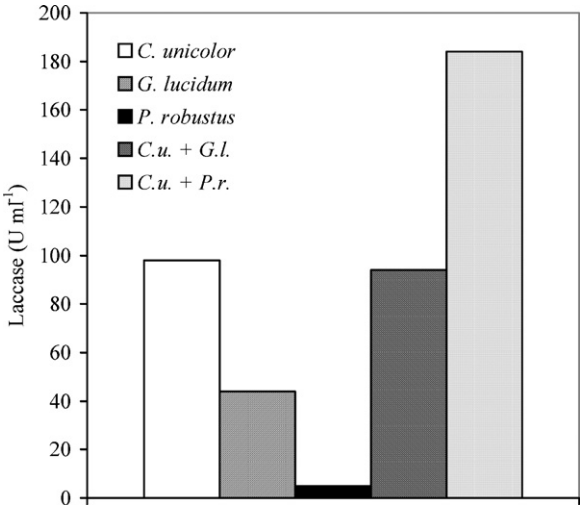

Fig. 2. Effect of submerged co-cultivation of *Cerrena unicolor* with *Ganoderma lucidum* or *Phellinus robustus* on the laccase yield.

Many fungal species were evaluated recently for their laccase activity. However, majority of them appeared to be the poor producers of enzyme accumulating less than 1 U ml^{-1} laccase activity. Nevertheless, in the appropriated culture conditions several basidiomycetes secreted exceptionally high levels of laccase. Data represented in Table 5 show that the researchers used various compounds as growth substrates and elicitors of laccase synthesis to achieve a high yield of enzyme in submerged cultivation of fungi. Our approaches permitted to select a promising laccase producer and significantly enhance the enzyme synthesis;

In conclusion, from the information reviewed here, it is evident that a specific feature of WRB is their extremely wide biodiversity and lack of universal conditions equally good for different fungi. It is not surprising taking into account that basidiomycetes studied so far were isolated from very different ecological niches and various lignocellulosic substrates. Moreover, WRB evolved to produce their particular set of enzymes that suit their natural substrate distinguishing with the large variety of the polymer structures. Lignocellulose degradation results to the formation of various sugars, acids and phenolic compounds. Undoubtedly therefore, there is significant and coordinated interrelationships and overlapping between ligninolytic and hydrolytic systems regulatory mechanisms. The understanding of these highly complex enzymatic systems and key regulatory factors is mandatory to realize the biosynthetic and degradation potential of WRB.

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