7 Revealing the Features of the Oxidative Enzyme Production by White-Rot Basidiomycetes During Fermentation of Plant Raw Materials

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Abstract

White rot basidiomycetes (WRB), belonging to one of the most diverse and important groups of living organisms, have the unique ability to completely degrade lignin through an oxidative process catalyzed by extracellular and non-specific lignin-modifying enzymes (LME) consisting of laccase, manganese peroxidase, lignin peroxidase, and versatile peroxidase with the assistance of several auxiliary enzymes. Because LME are capable of oxidizing a wide variety of natural and synthetic compounds, these enzymes have received tremendous attention for a variety of industrial and biotechnological applications. Consequently, the demand for these enzymes has increased in recent years, leading to the search for cost-effective production systems. To increase LME yields, various approaches and strategies, such as exploitation of cheap plant raw materials as growth substrates, optimization of fermentation media and cultivation conditions, and development of better bioprocess technologies, have been widely exploited. Literature date evidence that many factors influence the synthesis and secretion of LME and their isoenzymes, but the effects of these factors differ among the fungal species and we still have to understand the whole spectrum of mechanisms that modulate LME production. In this chapter, we summarize recent literature reports and our data on the physiological features of LME production by WRB, focusing on the diversity, common characteristics, and unique properties of individual fungi as well as on several approaches and strategies that provide enhanced (or reduced) secretion of laccases and peroxidases.

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7.1 Introduction

White-rot basidiomycetes (WRB) belong to one of the most diverse and important groups of living organisms because of their role in ecosystem function and unique ability to degrade and completely metabolize all wood polymers due to their capability to synthesize required hydrolytic and oxidative enzymes. Lignin degradation is an oxidative process catalyzed by extracellular and non-specific lignin-modifying enzymes (LME) composed of laccase (EC 1.10.3.2), manganese peroxidase (MnP, EC 1.11.1.13), lignin peroxidase (LiP, EC1.11.1.14), and versatile peroxidase (VP, EC1.11.1.16) with the assistance of several auxiliary enzymes. Some WRB secrete several different LME, while others produce only one or two of them, usually as a set of isoenzymes encoded by multiple genes within one fungal species (Lundell et al. 2010; Piscitelli et al. 2011; Janusz et al. 2013; Peng et al. 2018).

Among various industrial enzymes, LME have attracted tremendous attention due to the wide range of industrial and biotechnological applications (Yadav and Yadav 2015; Mäkelä et al. 2016; Chowdhary et al. 2019). However, the wide implementation of LME at the industrial scale is delayed because of too high enzyme cost due to the relatively low yield in the hundreds of explored strains belonging to various taxonomic groups. To solve this problem, the exploitation of molecular biology approaches was widely considered. Various techniques have been utilized to modify enzymes for industrial purposes. Artificial intelligence and computational tools have provided better results for modification and utilization of enzymes (Kumar et al. 2019; Dixit et al. 2019). Nevertheless, although many industrial enzymes (amylases, cellulases, etc.) are efficiently overproduced in heterologous systems, high expression of LME by recombinant organisms has not been yet obtained (Mekmouche et al. 2014). The highest yields of laccase were obtained when the enzyme from *Moniliophthora roreri* and *Pycnoporus cinnabarinus* was expressed in *Pichia pastoris* (281 U/mL) (Bronikowski et al. 2017). At the same time, several wild strains of WRB overproducing individual LME have been discovered (Galhaup et al. 2002; Revankar and Lele 2006; Elisashvili et al. 2014; Coconi-Linares et al. 2014; Kachlishvili et al. 2014; Schneider et al. 2019).

To increase LME yields, various approaches and strategies such as exploitation of cheap plant raw materials as growth substrates, optimization of fermentation media and cultivation conditions, development of better bioprocess technologies have been widely exploited (Elisashvili and Kachlishvili 2009; Piscitelli et al. 2011; Li et al. 2016; Park et al. 2015; Martani et al. 2017). Literature date evidence that the synthesis and secretion of LME and their isoenzymes are affected by many factors, but the effects of these factors vary among the fungal species (Terrón et al. 2004;

Xiao et al. 2006; Piscitelli et al. 2011; Yang et al. 2013; Bertrand et al. 2013, 2014). The results and advances in strain engineering, transcriptional regulation in response to environmental conditions, and biotechnological applications of LME have been comprehensively and excellently reviewed (Yadav and Yadav 2015; Mäkelä et al. 2016; Martinez et al. 2017; Chowdhary et al. 2019), but an exhaustive overview of the basic aspects of modulating enzyme activity through alterations of the cultivation conditions is still lacking in the literature. In this chapter, we summarize recent literature reports and our data on the physiological features of LME production by WRB, focusing on the diversity, common characteristics, and unique properties of individual fungi as well as on several approaches and strategies that provide enhanced (or reduced) secretion of laccases and peroxidases.

7.1.1 Screening of LME Producers

Screening of WRB species is an important initial stage for selecting promising LME producers. It aims to discover novel fungal strains (1) producing high yield and (2) the right set of LME, (3) exhibiting enhanced physical, chemical, and catalytic properties, and (4) suitable for specific industrial applications. Moreover, LME of some basidiomycetes, such as *Phanerochaete chrysosporium* (Kirk and Farrell 1987), are formed during secondary metabolism; therefore, it is important to detect fungi that efficiently produce LME during primary metabolism.

A countless WRB belonging to different taxonomic groups have been screened to identify industrially significant producers of LME, but only a few were able to produce encouraging amounts of target enzymes (Galhaup et al. 2002; Revankar and Lele 2006; Songulashvili et al. 2012; Kachlishvili et al. 2014), MnP and LiP (Kapich et al. 2004; Singh and Chen 2008; Elisashvili and Kachlishvili 2009; Coconi-Linares et al. 2014). It is worth noting that in some cases the direct comparison and correct assessment of WRB biosynthetic potential and enzyme yields are difficult due to the differences in media composition and fermentation conditions as well as the use of various methods and analyses to evaluate the LME activity.

Sergentani et al. (2016) evaluated the enzyme activity of twenty-eight strains in liquid cultures with wheat bran as a suitable growth substrate. Laccase activity (0.12–30.14 U/mL) was detected in the culture supernatants of all strains except *Hydnum repandum*, *Pholiota adiposa*, and *Pycnoporus cinnabarinus*. Neither LiP nor MnP was detected even in *Trametes* spp., although these fungi are known producers of MnP and LiP (Levin et al. 2010; Kachlishvili et al. 2018). Recently, Kinnunen et al. (2017) performed an automated and miniaturized screening and comparison of 53 species of basidiomycetes for LME production in their cultivation in liquid mineral, soy, peptone, and solid-state oat husk media. Among the tested fungi, MnP (96%) and laccase (92%) producers were the most widespread. Strains of *Phlebia radiate* and *Trametes ochracea* showed the highest LiP activity; *P. radiate* appeared to be the best candidate for VP production in soy liquid medium. In this study, the highest enzyme activities were produced on lignocellulose-containing media. By contrast, low laccase activity (0.01–1.97 U/mL) was revealed in

34 basidiomycetes screened in the stationary cultivation using malt extract broth without lignocellulose (Bodke et al. 2012), while only 5 of 21 endophyte fungi were able to produce low laccase activity (0.01–0.04 U/mL) but not peroxidase activity in modified Kirk's liquid medium (Fillat et al. 2016). There is other evidence that not all WRB species readily express LME activity in a defined medium (Schlosser et al. 1997; Kapich et al. 2004; Elisashvili and Kachlishvili 2009).

Therefore, in our screening studies, we simultaneously evaluated the LME activity of fungi during their cultivation in media containing mandarin peels and glycerin, which provide abundant fungal growth and significant enzyme production (Elisashvili and Kachlishvili 2009; Kachlishvili et al. 2014, 2018; Elisashvili et al. 2017). These studies revealed several features that should be considered during screening experiments. Firstly, in concordance with available literature data, our results clearly showed a wide intra- and interspecies diversity in the ability of WRB to produce LME. Thus, in the submerged fermentation of mandarin peelings, the laccase activity of *Ganoderma* spp. varied from 2.0 U/m/L to 75.4 U/mL, while the MnP activity of *Trametes* spp. strains ranged from 0 to 0.9 U/mL. Secondly, the WRB manifested different responses to the used carbon sources. Among them, *Cerrena* spp., *Coriolopsis gallica*, *Pseudotrametes gibbosa*, and *T. versicolor* produced significant laccase activity in synthetic medium, whereas the presence of lignocellulosic material was a prerequisite for the enzyme production by *Ganoderma* spp., *Phlebia radiata*, *Pycnoporus coccineus*, and *Trametes ochracea*. Likewise, 13 fungal strains secreted laccase (0.2–9.4 U/mL) in the 1% glycerol-containing medium, but supplementation of this medium with 20 g/L milled mandarin peels 2 to 22-fold enhanced their laccase activity (to 1.2–38.3 U/mL) (Elisashvili et al. 2017). Moreover, only *Coriolopsis gallica* was capable to express LiP activity in the synthetic medium, whereas in the lignocellulose-based medium all fungi, with the exclusion of *Merulius tremellosus* produced appreciable levels of this enzyme. In another work (Kachlishvili et al. 2018), the substitution of glycerol with mandarin peels two- to tenfold increased the laccase activity of *Trametes* spp., although *T. versicolor* 775 better produced this enzyme in the synthetic medium. Besides, the use of mandarin peels as the fungal growth substrate stimulated or even induced MnP and LiP production. On the whole, an analysis of literature data shows that for the greatest production of individual LME by specific WRB, a certain composition of the nutrient medium is required, and the use of an inappropriate medium may lead to non-detection of promising enzyme producer.

7.1.2 Effect of Lignocellulosic Substrates

Understanding the mechanisms of regulation of the individual LMEs synthesis under specific growth conditions and elucidating the cultivation conditions ensuring their predominant production is a critical necessity. In particular, to ensure abundant fungal growth and efficient production of LME, it is important to select a suitable lignocellulosic substrate rich in readily available carbohydrates, nitrogen, trace elements, and inducers for the synthesis of target enzymes (Winquist et al. 2008;

Elisashvili et al. 2009; Kachlishvili et al. 2018). Thus, a study conducted by Mikiashvili et al. (2006) with *Pleurotus ostreatus* 98 showed that substitution of glucose with mandarin peels 40-fold and 18-fold increased the fungus laccase and MnP activities, respectively. Likewise, the cultivation of *P. radiata* 79 in low-nitrogen defined medium with milled alder as sole carbon source induced tenfold production of LiP and increased MnP activity, in comparison to the glucose-supplemented cultures (Mäkelä et al. 2013). Laccase and MnP activities were the highest in cultures of the *P. radiata* grown on birch wood (Villavicencio et al. 2020). On liquid ME medium, however, the production of laccase by *P. radiata* during 4 weeks of cultivation was very low, although distinct MnP activities were produced by the fungus. In another study, when the basal medium including 20 g/L glucose as a carbon source was supplemented by 10 g/L of orange peel, tea, bagasse, and corn cobs, the laccase production by *P. ostreatus* was improved 9-, 5-, 2-, and 1.1-fold, respectively (Zhao et al. 2017). It is worth noting that the proliferation capacity of the culture with orange peel extract was 0.5-fold higher than that of control. The more orange peel (ranged from 0 to 10 g/L) was added to the culture with glucose, the better was the fungus growth and enzyme production.

Undoubtedly, lignocellulosic biomasses differ greatly in their chemical composition, physical, mechanical, and other properties affecting fungal metabolism and productivity. For example, the tested residues provided equally good growth of *C. unicolor* and *Phellinus robustus* in their submerged fermentation, but the fungal laccase activity varied from 15.7 U/mL to 151.6 U/mL and from 0.9 U/mL to 8.4 U/mL, respectively (Elisashvili and Kachlishvili 2009). Wheat bran and residue after ethanol production appeared to be the best growth substrates for laccase production by *C. unicolor*, whereas mandarin peels and kiwi and walnut pericarp favored enzyme secretion by *P. robustus*. Simultaneously, as compared with wheat bran, the fermentation of kiwi residue stimulated MnP secretion by *P. robustus*, whereas walnut pericarp 12-fold augmented activity of this enzyme in *C. unicolor*.

Coffee husk (CH) and citric pulp pellet (CP) from an orange juice industry used as a carbon source for the submerged cultivation of *Lentinus crinitus* without an additional nitrogen source provided the secretion of 33.4 and 29.1 U/mL laccase activity, respectively(Almeida et al. 2018). Since CH contains caffeine, polyphenols, and tannins, while CP contains polyphenols (mainly flavonoids) soluble in water, the authors assumed that these compounds induced the laccase production by *L. crinitus*. Nevertheless, no MnP or LiP was detected in the fungus cultivation. On the contrary, Conceição et al. (2017) reported the production of MnP but not laccase in the cultivation of *L. crinitus* on a solid medium with barley and cassava residues (1:1). The stimulating effect of water-soluble aromatic compounds obtained from lignocellulosic substrates on the synthesis of LME has been shown by many authors (Crestini et al. 1996; Kapich et al. 2004; Adekunle et al. 2017). The induction of laccase isoforms by aqueous extracts from softwood and hardwood was shown in *T. versicolor* HEMIM-9; specifically, the pine, cedar, and oak extracts addition to the fungal cultures increased 4.6-, 4.5-, and 3.7-fold, respectively, the fungus laccase activity (Bertrand et al. 2014). The authors suggested that not only the concentration of phenolic compounds in aqueous extracts but also the origin or composition of the

extract may influence the induction of laccase. Moreover, native isoelectric focusing of laccases that were isolated from the control and the induced cultures revealed differences in isoform number (from 3 to 6) and pI values. According to the researchers, some isoforms of *T. versicolor* HEMIM-9 are downregulated, while others are upregulated in the presence of the tested inducers.

The available results suggest that kind and composition of lignocellulosic materials determine the set and yield of LME produced by the WRB. Thus, Janusz et al. (2018) compared the transcriptomes of *C. unicolor* FCL139 grown in the solid-state growth conditions on birch, ash, maple sawdust with those of fungus grown on mineral medium. It was found that the expression of MnP XLOC_004360 was upregulated during the fungus growth on maple sawdust, the expression of the MnP XLOC_004631 was specifically induced in the fungus cultivation on ash and maple but it was downregulated during *C. unicolor* growth on the birch medium. Analyses of laccase transcripts (XLOC_008955) revealed their reduced amounts in the fungus cultivation on the birch and ash sawdust-containing media.

It should be noted that the composition of some lignocellulosic substrates may not correspond to the nutritive demands of the fungus; then nutrient medium requires supplementation with an additional carbon source. Thus, the laccase activity of *P. ostreatus* in the fermentation of rice bran and other plant raw materials appeared to be lower as compared with that in the control culture with glucose (2.2 U/mL) (Selvaraj et al. 2014). However, the combinations of rice bran with sugarcane bagasse, corn stalks, or orange peelings showed laccase activity of 3.24 U/mL, 2.96 U/mL, and 2.6 U/mL, respectively, whereas supplementation of these media with 0.2 g glucose further increased laccase activity to 6.47 U/mL, 4.41 U/mL, and 5.71 U/mL, respectively.

Overall, the literature data demonstrate a clear regulatory role of individual lignocellulosic materials in LME activity expression. Undoubtedly, the chemical composition and other characteristics of lignocellulosic biomass play a decisive role in the production of LME; in particular, phenol-containing plant materials, such as coffee husks and by-products of citrus processing, provide the highest production of enzymes. However, the deconstruction of complex lignocellulosic substrates requires the participation of a consortium of various enzymes acting in synergy to provide the microorganism with the necessary nutrients. Therefore, LME producers simultaneously expressing high hydrolytic activity may be more attractive for the successful production of target enzymes during lignocellulose fermentation.

7.1.3 Effect of Carbon Source

Among the medium components, carbon sources and concentrations are the most important factors determining the rate and degree of fungal biomass accumulation, displaying diverse effects on the LME production, depending on the fungal strain (Galhaup et al. 2002; Elisashvili et al. 2002; Stajić et al. 2006; Elisashvili and Kachlishvili 2009; Piscitelli et al. 2011). For example, in the submerged cultivation of *Trametes pubescens* in the presence of glucose, the sugar was rapidly metabolized

and resulted in high laccase activity, but active enzyme secretion by the fungus was observed when the glucose concentration in the growth medium decreased to a certain low concentration (Galhaup et al. 2002). Slowly utilized lactose and cellulose resulted in poor laccase production. Likewise, laccase activity obtained in the cultivation of *Pleurotus sajor-caju* in media containing 0.5 g/L fructose or glucose was 12-fold higher than that obtained with lactose (Bettin et al. 2008). On the contrary, exactly lactose ensured the maximum laccase secretion by *P. gibbosa* (Elisashvili and Kachlishvili 2009). Interestingly, starch appeared to be the best carbon source for the laccase production by WRB*WR-1* fourfold increasing enzyme activity compared with fructose-containing medium (Revankar and Lele 2006). Myasoedova et al. (2015) observed the highest laccase activity of *Lentinus strigosus* 1566 when peptone-yeast extract medium was supplemented with galactose, arabinose, and xylose at a final concentration of 20 g/L. The presence of glucose, sucrose, or maltose in the medium led to a decreased laccase activity. The researchers demonstrated the selectivity of the *L. strigosus* 1566 towards mono- and disaccharides synthesizing different sets of laccase isozymes.

Early observations established that the production of LME in some fungi occurs in response to carbon depletion (Kirk and Farrell 1987). Then it was shown that individual laccase isozymes of basidiomycete I-62 (Mansur et al. 1998), *T. pubescens* (Galhaup et al. 2002), and *Trametes* sp. AH28-2 (Xiao et al. 2006) are differentially regulated by carbon and synthesis of some of them is subjected to catabolite repression by glucose or other easily metabolizable carbon sources. Moreover, many fungi such as *P. coccineus* (Kachlishvili et al. 2016), species of genus *Ganoderma* (Songulashvili et al. 2012; Elisashvili and Kachlishvili 2009) produce very low laccase activity in defined media containing glucose or glycerol. Therefore, in our study, to elucidate if there is a mechanism of catabolite repression of LME synthesis the mandarin squeeze-based medium (control) was supplemented with glucose before inoculation and after 4 days of the submerged fermentation (middle of logarithmic phase of growth). In addition to *P. coccineus* and *Ganoderma lucidum*, *Trametes trogii* and *T. versicolor*, which produce marked laccase activity in the presence of glucose, were studied for comparison. Laccase activity of *P. coccineus* in the control medium gradually increased to 4.2 U/mL peaking on day 5 (Fig. 7.1a). Supplementation of the control medium with 0.5% and 1% glucose delayed laccase secretion and gave lower enzyme activities after 3 days of the fermentation (2.1 and 1.0 U/mL vs. 2.6 U/mL in the control). However, subsequently, laccase activity rapidly increased to 5.6 and 7.5 U/mL, respectively, on day 8 significantly exceeding that in the control medium owing to higher biomass accumulation. When 0.5% glucose was added to the growing fungal culture no increase in enzyme activity was observed during 4 days of cultivation, then the enzyme secretion resumed. In the cultivation of *G. lucidum*, almost no delay in the laccase production was observed due to the supplementation of glucose in the initial medium, but enzyme activity was completely suppressed during 1 day when 0.5% glucose was added to the growing culture (Fig. 7.1b). However, without a transcriptomic analysis, the data obtained are insufficient to confirm the presence of catabolite repression of laccase synthesis in *P. coccineus* and *G. lucidum*, since

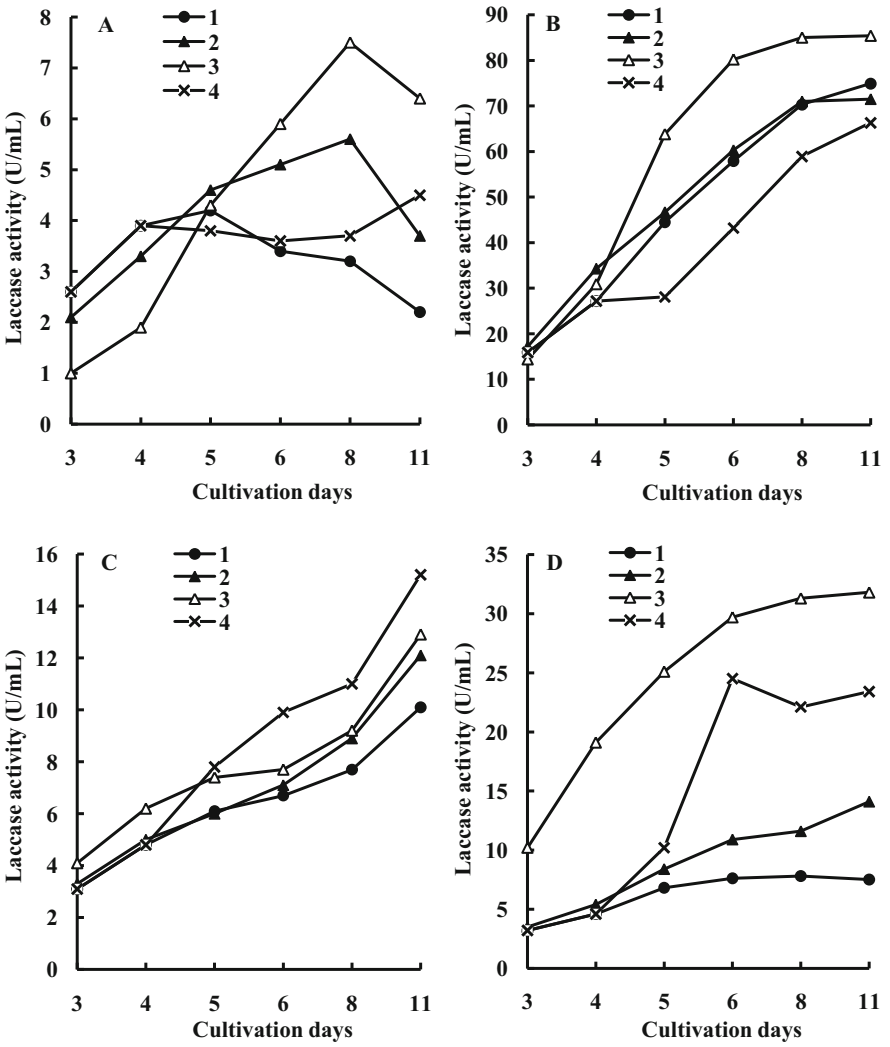

Fig. 7.1 The effect of glucose addition in the production of laccase during the submerged fermentation of mandarin squeeze by four basidiomycetes species. (**a**) *Pycnoporus cinnabarinus* 811; (**b**) *Ganoderma lucidum* 447; (**c**) *Trametes trogii* 146; (**d**) *Trametes versicolor* 159. 1 medium containing 40 g/L mandarin squeeze (control); 2 control + 0.5% glucose; 3 control + 1% glucose; 4 control + 0.5% glucose after 4 days of cultivation

the growth of exactly these fungi in the presence of glucose was accompanied by particularly strong acidification of the medium, which of course negatively affected the synthesis and activity of laccase. Finally, unlike these fungi, *T. trogii* (Fig. 7.1c) and *T. versicolor* (Fig. 7.1d) responded to the addition of glucose to the control medium by an accelerated secretion of laccase activity. This means the absence of

catabolite repression in the tested *Trametes* species and using an additional carbon source is the right strategy to increase the yield of target enzyme.

It is interesting that *Cerrena* sp. HYB07 showed especially distinctive features since very high carbon concentration (malt dextrin, 60 g/L) in the fermentation medium with a high concentration of nitrogen was beneficial for laccase production (280 U/mL) (Yang et al. 2016). Substitution of malt dextrin by glucose slightly increased laccase yield, whereas fructose decreased laccase production by 60%. Moreover, disaccharide maltose did not affect laccase yields, sucrose lowered laccase yield by 15.5%, whereas glycerol threefold decreased the fungus enzyme activity. At the same time, *Coriolopsis gallica* T906 expressed the highest laccase activity in medium with glycerol and ammonium dihydrogen phosphate as carbon and nitrogen sources, respectively (Xu et al. 2016).

Recently, Schneider et al. (2018) investigated LME activity of *Marasmiellus palmivorus* VE111 in media containing glucose, sucrose, or glycerol and showed that the medium prepared with glucose ensured the highest laccase and MnP activities. It is interesting that the effect of the carbon source on laccase secretion significantly depended on nitrogen source (casein was better than peptone). Usually, media that provided higher fungal biomass resulted in higher enzymatic activity, medium prepared with sucrose and casein ensured the best growth of the mushroom, but not the highest laccase activity. In our study with *Trametes multicolor*, the lowest laccase activity was detected in the fungus cultivation in the sodium gluconate-based medium (Kachlishvili et al. 2018). It turned out that mannitol, as well as cellobiose and xylose, were the best carbon sources for laccase production by *T. multicolor* but increased enzyme activities were not due to higher biomasses accumulated in the presence of these carbohydrates. In particular, calculation of the specific laccase activity showed that mannitol, cellobiose, and xylose favored enzyme production by the fungus, increasing more than two- to threefold the specific laccase activity compared with the control medium. Likewise, mannitol more than fourfold increased the *T. multicolor* MnP activity compared to the control medium. However, unlike laccase, it did not promote the secretion of MnP, since the specific activity of MnP in the medium with mannitol decreased 1.5-fold compared with the control medium.

These and many other observations indicate that some carbohydrates can modulate LME expression in WRB; therefore, to maximize the synthesis of LME, it is necessary to find out the carbon source optimal for the fungal growth and the target enzyme production. Yet, to correctly assess fungal enzymatic potential, it is necessary to take into account the amount of biomass accumulated in the presence of a specific carbon source. Moreover, in fungal cultures, the metabolism of various carbohydrates is accompanied by varying degrees of acidification of the nutrient medium. In turn, media with different pH differently affect both the synthesis of a target enzyme and the stability of the already synthesized enzyme.

It is clear that a carbon source, especially, in the form of lignocellulosic biomass, plays a principal role in modulation of basidiomycetes LME activity, but for each enzyme producer, fungus-specific carbohydrate ensuring the highest enzyme activity must be established. Some published data on the laccase activity of WRB, given in

Table 7.1 WRB laccase activity (ABTS) under submerged cultivation conditions

Enzyme producers	Media main components	Laccase (U/mL)	References
Agaricus blazei	10 g/L glucose, 2.8 g/L urea, 0.15 mM $CuSO_4$	45.7	Valle et al. (2015)
Arthrospira maxima	10 mM sucrose, 1 mM guaiacol	56.9	Afreen et al. (2018)
Cerrena unicolor	50 g/L ethanol production residue, 1 mM xylidine, 1 mM $CuSO_4$	507.0	Kachlishvili et al. (2014)
Cerrena sp. HYB07	60 g/L maltodextrin, 10 g/L peptone, 0.25 mM $CuSO_4$	280.0	Yang et al. (2016)
Ganoderma lucidum	50 g/L wheat bran	110.8	Songulashvili et al. (2012)
Ganoderma sp.	40 g/L glycerol, 0.85 mM veratryl alcohol	240.0	Teerapatsakul et al. (2007)
Lentinus crinitus	50 g/L coffee husk, 0.7 g/L urea, microelements	41.2	Almeida et al. (2018)
Lentinus strigosus	20 g/L glucose, 2 mM $CuSO_4$	186.0	Myasoedova et al. (2008)
Pleurotus ostreatus	Rice bran, sugarcane bagasse, 1 mM xylidine, 1 mM $CuSO_4$	37.5	Selvaraj et al. (2014)
Pleurotus pulmonarius	MEB, 0.1% xylidine	349.5	Lallawmsanga et al. (2019)
Pycnoporus cinnabarinus	20 g/L maltose, 35 g/L ethanol	266.0	Lomascolo et al. (2003)
Trametes pubescens	40 g/L glucose, 10 g/L peptone, 2 mM $CuSO_4$	743.0	Galhaup et al. (2002)
Trametes versicolor	2 g/L glucose, 1 mM 2,5-xylidine, 0.5 mM $CuSO_4$	33.6	Birhanlı and Yeşilada (2017)
WR-1	20 g/L starch, 1 mM $CuSO_4$, 0.8 mM xylidine	692.0	Revankar and Lele (2006)

Table 7.1, show their diversity in their requirements for a carbon source to achieve maximum laccase activity.

7.1.4 Effect of Nitrogen Source

Many studies verified the effect of organic and inorganic nitrogen sources in the fermentation medium on the WRB enzyme activity to create the best nutritional conditions for the maximum production of LME. Thus, the assessment of the effect of different nitrogen sources (20 mM as nitrogen) on the LME production by *T. multicolor* was assessed in the submerged fermentation of mandarin peels (Kachlishvili et al. 2018). The lowest activities of laccase, MnP, and LiP were revealed in the medium with potassium nitrate. This nitrogen source and yeast extract provided even lower specific laccase activity (2.4 and 2.9 U/mg, respectively) compared with that in the control medium. On the contrary, casein hydrolysate

favored the laccase production with the highest productivity (12.3 U/mg), obviously, due to available aromatic amino acids. Likewise, the casein hydrolysate ensured the highest specific activity of MnP activity increasing almost twofold specific activity of this enzyme compared with that in the control medium.

It is considered that compared with organic nitrogen sources inorganic ones provide lower enzyme yields despite sufficient biomass accumulation (Piscitelli et al. 2011). Indeed, laccase activity was not observed with any inorganic nitrogen source in the cultivation of *Grammothele fuligo* in glucose-based medium although it was detected in the control medium containing ammonium chloride as a nitrogen source in experiments with various trace elements (Chauhan 2019). It is useful to note that in this study, the enzyme activity was measured only after 12 days of fungus cultivation. At the same time, the fungus showed maximum LiP activity with ammonium chloride and MnP activity with ammonium acetate. In experiments with *T. multicolor*, ammonium sulfate and ammonium nitrate were suitable sources of nitrogen for the production of laccase and MnP, respectively (Kachlishvili et al. 2018). Besides, ammonium sulfate provided two times higher LiP activity compared with that in the control medium, although the calculations showed it was achieved due to the higher fungal biomass. Moreover, the highest values of laccase activity were observed for *P. eryngii* 616 and *P. ostreatus* 493 when $(NH_4)_2SO_4$ and NH_4Cl, respectively, were supplemented to the medium (Stajić et al. 2006). Likewise, inorganic nitrogen supported high laccase activity in the cultures of *Pleurotus dryinus*, *Pleurotus tuberregium*, and *Pleurotus pulmonarius* (Kachlishvili et al. 2006; Stajić et al. 2006), but organic nitrogen was favorable for high production of MnP in the culture of *P. ostreatus* 108 (Mikiashvili et al. 2006). These and many other data indicate the diversity of the nutritional needs of fungi for nitrogen sources necessary to express their enzymatic potential and also evidence that the nitrogen source optimal for one type of enzyme does not necessarily contribute to the production of another.

The highest value of *Coriolopsis gallica* T906 laccase activity was obtained with ammonium dihydrogen phosphate as nitrogen sources (Xu et al. 2016). Ammonium sulfate and casein were also good sources of nitrogen. Under the solid-state fermentation (SSF) of peach waste by *Pleurotus eryngii* supplementation of medium with 4.0 g/L ammonium nitrate resulted in 12.8 times higher laccase activity than in control culture (Akpinar and Urek 2017). Among nitrogen sources tested in the submerged fermentation of coffee husk (CH) and citric pulp pellet (CP) by *Lentinus crinitus*, only urea favored laccase production in both media, while the addition of yeast extract in CH medium and sodium nitrate in CP medium decreased enzyme activity as compared with control medium (Almeida et al. 2018). Interestingly, the urea concentration of 0.7 g/L was sufficient for maximum laccase production in CH medium and the enzyme production was completely inhibited at the urea concentration of 11.2 g/L whereas exactly this concentration of urea provided the highest laccase activity in the CP medium. The authors suggested that the effect of nitrogen on the laccase production by *L. crinitus* depends on the carbon substrate in the culture medium.

The production of fungal LME depends not only on the chemical nature but also on the concentration of nitrogen. In *P. chrysosporium* synthesis of MnP and LiP is suppressed by high-nitrogen concentrations in the medium and it is induced only during secondary metabolism in response to nitrogen limitation (Kirk and Farrell 1987). However, in the fermentation of lignocellulosic substrates, even a high concentration of organic nitrogen did not inhibit enzyme production in *P. chrysosporium* ME-446, but instead stimulated it (Kapich et al. 2004). Laccase, MnP, and LiP activities of *Coriolopsis polyzona* reached significantly higher values under low-nitrogen (2.2 mM) compared with high-nitrogen (20 mM) conditions (Jaouani et al. 2006). On the contrary, a tenfold increase in laccase production of *Ganoderma lucidum* was observed when nitrogen was increased from 2.4 to 24 mM (D'Souza et al. 1999). Interestingly, in the cultivation of *P. dryinus* only laccase activity increased with nitrogen concentration, whereas the MnP production was not affected (Kachlishvili et al. 2006). Especially distinctive features showed *Cerrena* sp. HYB07 since high carbon (malt dextrin, 60 g/L) and nitrogen (peptone, 10 g/L and ammonium tartrate, 1.6 g/L) concentrations in the fermentation medium provided the highest laccase production (280 U/mL), whereas low-nitrogen concentrations reduced laccase yields to 5.3 U/mL (Yang et al. 2016).

Finally, some above-mentioned and other studies indicate that the ratio of carbon to nitrogen (C/N) is important for the maximum production of the LME. Thus, a high carbon–nitrogen ratio favored laccase productions by *Trametes gallica* (Dong et al. 2005); in contrast, high laccase activity of *P. ostreatus* was observed with low carbon–nitrogen ratio (Hou et al. 2004). These data show a wide variety in the nitrogen requirements of WRB for the production of LME as well as some uncertainty regarding the selection of the optimal nitrogen concentration for enzyme production.

7.2 Aromatic Compounds

The effect of a wide variety of aromatic or phenolic compounds, especially of structurally related to lignin compounds, in the production of LME by WRB has been extensively studied (Eggert et al. 1996; Elisashvili and Kachlishvili 2009; Cambria et al. 2011; Bertrand et al. 2017; Daly et al. 2020). 2,5-Xylidine was the most widely used as a potent inducer of laccase synthesis (Elisashvili et al. 2002; Revankar and Lele 2006; Birhanlı and Yeşilada 2017; Lallawmsanga et al. 2019). However, the laccase production by *T. versicolor* increased twofold when veratryl alcohol or guaiacol was used instead of 2,5-xylidine (Lee et al. 1999). At the same time, in *Lentinus strigosus* veratryl alcohol insignificantly increased the laccase synthesis, whereas 2,6-dimethylphenol promoted eightfold compared to the control (Myasoedova et al. 2008). In the submerged fermentation of mandarin squeeze by *T. multicolor* 511, veratryl alcohol and guaiacol twofold increased the specific laccase activity compared with the control medium, guaiacol fourfold increased the fungus MnP activity, while veratryl alcohol favored LiP secretion (Kachlishvili et al. 2018). Kumari et al. (2019) observed a differential effect of inducers on the

secretion of LME by *Stereum ostrea* in a defined medium containing chlorpyrifos. Gallic acid induced maximum secretion of laccase and MnP; however, the highest production of LiP was recorded in the presence of veratryl alcohol. Interestingly, induction with 0.5 mM resveratrol showed the best laccase activity of *C. gallica*, followed by tannic acid and guaiacol, while ABTS and gallic acid did not affect laccase production (Xu et al. 2016). On the contrary, ABTS and guaiacol increased the *Cerrena* sp. HYB07 laccase production by 40.1 and 26.7%, respectively, whereas other aromatic compounds did not significantly affect enzyme production (Yang et al. 2016). It is interesting that in another strain of *C. unicolor* VKMF-3196 laccase production needed Cu^{2+} ions, but not aromatic compounds (Lisova et al. 2010). These results suggest that the effect of inducer for laccase production depends on the species and/or strains of the tested fungus.

In the early work, Soden and Dobson (2001) showed that 300 μM xylidine, 100 μM 1-hydroxybenzotriazole, 100 μM ferulic acid, and 500 μM veratric acid resulted in 21-, 16-, 10-, and 5-fold increases of laccase activity of *P. sajor-caju,* respectively, compared to the basal culture with no additions. Since one of the functions of fungal laccases is the polymerization of aromatic compounds formed during the degradation of lignin these authors, similarly to Eggert et al. (1996), proposed that laccase induction is a protective reaction of fungal culture to avoid the toxic effects of these compounds. Moreover, it turned out that in some WRB different laccase isoenzyme genes responded differently to the same aromatic inducer. Thus, in *Trametes* sp. AH28-2 o-toluidine induced expression of the laccase A gene, while 3,5-dihydroxytoluene favored laccase B production (Xiao et al. 2006). Guaiacol and p-coumaric acid selectively induced lcc1 and lcc2 expression in *Trametes* sp. I-62 but ferulic acid induced lcc3 expression (Terrón et al. 2004). In the cultivation of *P. ostreatus* HAUCC 162 ferulic, vanillic, coumaric, and cinnamic acids increased the expression of lacc8 and lacc11 genes, but not lacc1 and lacc2 (Zhuo et al. 2017).

Besides fungal species peculiarities, the effect of aromatic compounds on LME production depends on inducer concentration and adjusting the concentration of phenolic/aromatic compounds necessary for individual fungi is essential. In particular, a gradual increase in the concentration of ABTS from 0 to 0.05 mM and 2,5-xylidine from 0 to 1 mM in the glucose-yeast extract basal medium (SBM) increased *T. versicolor* ATCC 200801 laccase activity from 0.6 U/mL to 4.76 U/mL and 2.87 U/mL, respectively (Birhanlı and Yeşilada 2017). Higher concentrations of both compounds significantly decreased the enzyme yields. It is worth noting that under the same cultivation conditions, no induction of laccase production was observed in SBM with 0.025–0.5 mM syringaldazine.

Finally, Lallawmsanga et al. (2019) showed that the effect of aromatic compounds may depend on nutrient medium composition. Thus, the addition of xylidine in MEB medium (malt extract broth) resulted in 2.8-fold induction of laccase activity, while a 1.9-fold increase was observed when xylidine was added to potato dextrose broth medium (PDB). In contrast, the use of $CuSO_4$ as an inducer resulted in higher laccase activity in PDB than in MEB.

7.3 Effect of Microelements

The regulating effect of metal ions on LME activity and gene expression in WRB is well established (Piscitelli et al. 2011; Janusz et al. 2013). Copper is the most important and commonly used metal ion for laccase production. However, the effect of metal ions may vary depending on their concentration and fungal species/strain physiological peculiarities. Thus, in the cultivation of *P. coccineus*, the addition of 0.2 mM $CuSO_4$ to potato dextrose broth significantly upregulated the extracellular laccase activity in five strains of *P. coccineus*, whereas two strains did not respond to Cu^{2+} (Park et al. 2015). Supplementation of glucose-yeast extract medium with 0.5 mM Cu^{2+} increased laccase activity of *T. versicolor* ATCC 200801 from 0.60 to 10.25 U/mL (Birhanlı and Yeşilada 2017). Higher concentrations of copper decreased the enzyme yield with complete inhibition of laccase secretion at 5 mM Cu^{2+}. Supplementation of glucose-peptone-yeast extract medium with as high as 0.5–4 mM $CuSO_4$ led to a 13- to 22-fold increase in laccase activity of *L. strigosus* 1566 when compared with the control (Myasoedova et al. 2015). At the same time, low concentrations of copper ions (10–50 μM) were sufficient to 1.1 to 2.6-fold increase in the laccase activity of *G. lucidum* in comparison with control medium (Liu et al. 2020). When the concentration of added copper ions was increased to 60–80 μM, the laccase activity decreased. The optimal copper dose for the maximum laccase activity for *C. unicolor* C-139 in the glucose and L-asparagine containing culture was found to be only 10 μM (Janusz et al. 2007). Copper concentrations increase from 25 μM to 300 μM Cu^{2+} repressed laccase production (up to 90%) with no effect on fungal growth. The authors showed that the copper supplementation mode was also important. Adding 10 μM Cu^{2+} to the culture medium after 3 days and repeating on the 6th and 9th day of the fungus cultivation led to the greatest activity of laccase. Likewise, the time-dependent effect of copper was observed in *P. ostreatus* ACCC 52857 (Zhu et al. 2016). The laccase activity dramatically increased (more than 80-fold) upon the addition of copper to the medium from the 6th to the 9th day of cultivation. It is interesting that unlike *C. unicolor* C-139, the stimulating effect of even 100 times higher concentration of copper on laccase secretion was revealed in the cultivation of *C. unicolor* CBS 117347 (Kachlishvili et al. 2014).

Many studies proved that metal ions may control the expression of individual isozymes through activation of metal responsive elements (Janusz et al. 2013). Thus, the addition of 0.15 mM copper sulfate to the potato dextrose medium increased production (up to 500-fold) of *P. ostreatus* POXA1b laccase isoform, while POXA1w isoform was not affected (Palmieri et al. 2000). Klonowska et al. (2001) reported that *Marasmius quercophilus* produced only one laccase (LacI) in liquid medium with malt extract. Supplementation of the same medium with $CuSO_4$ induced three other isoforms, increasing the total activity 10 times. Concentrations of 0.05–1 mM Cu^{2+} induced Lac1 and Lac2 of *Coprinus comatus*, while Lac3 was strongly induced by the addition of 3 mM Cu^{2+} (Lu and Ding 2010).

The effect of metal ions other than Cu^{2+} on the LME activity was also widely studied. The addition of Mn^{2+} to the cultures of *C. subvermispora* (Manubens et al.

2007), *P. chrysosporium* (Brown et al. 1991), *T. versicolor* (Johansson et al. 2002), and several other WRB is required for the stimulation of MnP production. According to Brown et al. (1991), manganese is directly involved in the regulation of the mnp gene transcription through a mechanism specific for the growth stage and depending on the concentration. Manganese was a highly potent laccase inducer in *C. comatus* (Lu and Ding 2010). Supplemented to the cultures in the range of 0.05–0.8 mM, Mn^{2+} markedly increased the overall laccase activity and caused the synthesis of additional Lac2 and Lac3 isoforms compared with un-supplemented cultures. Increase of $CuSO_4$ and $MnSO_4$ concentrations from 1 to 3 mM as inducers for LME production by *Marasmiellus palmivorus* VE111 in the medium composed of 0.5% glucose, 0.18% casein, and potato broth led to increased production of the enzymes relative to the control (Schneider et al. 2019). Moreover, the presence of a third isoform in the zymogram of laccase was revealed after the fungus cultivation with inducers. For MnP, only 3 mM $MnSO_4$ significantly induced enzyme production over the control, 2 mM $CuSO_4$ also provided greater enzymatic activity as compared to the control medium, while veratryl alcohol rather decreased the fungus MnP activity.

Testing of the effects of eight microelements on *Grammothele fuligo* growth and enzyme production revealed that the fungus did not show any laccase activity with Ca^{2+}, Mn^{2+}, and Zn^{2+} and no MnP activity with Co^{2+} and Fe^{2+}, but it showed three LME activity at different concentration of B^{3+}, Cu^{2+}, and Mo^{6+} (Chauhan 2019). No effect on laccase production by *P. ostreatus* ACCC 52857was observed after the addition to the medium of K^+, Na^+, Mn^{2+}, Mg^{2+}, Ca^{2+}, Zn^{2+}, Fe^{2+}, and Fe^{3+}, whereas Cd^{2+}, Pb^{2+}, and Cu^{2+} caused a three- to sixfold increase in laccase activity (Zhu et al. 2016). Finally, it should be noted that the same metal ion may have the opposite effect depending on species or enzyme type. For example, Mn^{2+} induced laccase gene transcription in *P. sajor-caju* (Soden and Dobson 2001), but inhibited gene transcription in *C. subvermispora* (Manubens et al. 2007). Fe^{2+} significantly increased laccase activity in *P. eryngii* (Stajić and Vukojević 2011), while it inhibited laccase production in *G. lucidum* (Murugesan et al. 2009). Moreover, Mn^{2+} stimulated lacc3 and lacc12 genes transcription in *P. ostreatus* HAUCC, but decreased transcription of lacc2 and lacc4 (Zhuo et al. 2017). In the submerged fermentation of mandarin squeezes, an augmentation in copper concentration from 0 to 0.5 mM increased 18-fold the activity of *T. multicolor* laccase but did not significantly affect the activity of MnP and LiP, which indicated that the inductive effect of copper was specific for laccase (Kachlishvili et al. 2018). It is interesting that iron ions at the concentration of 0.1 mM increased almost eightfold both volumetric and specific activity of laccase compared with the control medium. In the same culture, increase in the concentration of manganese from 0 to 0.5 mM only slightly increased the activity of *T. multicolor* laccase, decreased the activity of the fungus LiP, but this metal 13–14 times specifically increased the volume and specific activity of MnP when it was added to the control medium at a concentration of 0.5 mm.

Several studies revealed the synergistic effect of different inducing agents on LME production. Manubens et al. (2007) found a synergistic effect between Mn^{2+} and syringic acid on laccase production in *C. subvermispora*. Laccase activity of

P. ostreatus HAUCC 162 increased from 205.5 U/L in the presence of Fe^{2+} alone to 250 and 363 U/L due to the addition of 0.5 mM vanillic or ferulic acid, respectively (Zhuo et al. 2017). In our study with *C. unicolor* CBS 117347, copper and xylidine added separately accelerated laccase secretion and 1.8-fold improved laccase yield. An additive effect and fourfold increase of laccase activity were observed upon supplementation of copper and xylidine to the medium simultaneously before inoculation. Likewise, supplementation of glucose-yeast extract basal medium (SBM) with 0.5 mM Cu^{2+} increased laccase activity of *T. versicolor* ATCC 200801 from 0.60 to 10.25 U/mL (Birhanlı and Yeşilada 2017). Enzyme production was enhanced considerably (to 33.61 U/mL) due to the synergistic effect of 1 mM 2,5-xylidine and 0.5 mM Cu. Interestingly, no induction of laccase production was observed in SBM with 0.025–0.5 mM syringaldazine alone, but laccase activity reached 22.23 U/mL in medium containing simultaneously 0.5 mM Cu and 0.1 mM syringaldazine. However, conflicting results were obtained when optimal concentrations of caffeic acid and Mn^{2+} were added to *C. comatus* culture, resulting in a decrease in total extracellular laccase activity compared to a culture supplemented with caffeic acid only (Lu and Ding 2010). Nevertheless, the supplementation of nutrient medium with different microelements is a suitable and effective tool to regulate the production of individual LME.

7.4 Effect of Co-cultivation

Co-cultivation of WRB may be a promising strategy to significantly improve LME production. Previous studies have shown that laccase activity of *L. edodes* (Savoie et al. 1998), *C. unicolor* (Elisashvili et al. 2014), *P. ostreatus*, *T. versicolor*, and several other basidiomycete species (Baldrian 2004) significantly increased in their co-cultivation with *Trichoderma* sp. The mixed cultivation of *Gongronella* sp. W5 with *Panus rudis* (Wei et al. 2010) and *Shiraia bambusicola* and *Phoma* sp. BZJ6 (Du et al. 2017) caused a 25-fold and 9.2-fold increase, respectively, of dual cultures laccase activity as compared with those in their monocultures.

Few studies were performed in the co-cultivation of basidiomycetes. Among four fungi tested, high stimulation of laccase production was observed only in the co-cultivation of *P. ostreatus* with *C. subvermispora*, while MnP activity was stimulated in the co-culture with both *C. subvermispora* and *Physisporinus rivulosus* and Western blotting revealed proteins from both fungi (Chi et al. 2007). However, in the co-cultivation of *C. subvermispora* with *P. rivulosus* low MnP activity was detected and the protein pattern resembled that of *P. rivulosus*, indicating that this fungus suppressed *C. subvermispora* growth. Similarly, among six WRB cultured in pairs for LME production, *Hypoxylon fragiforme* inhibited the expression of LME during co-cultivation with *P. ostreatus*, *Dichomitus squalens* inhibited MnP expression by *P. ostreatus* or *Phlebia radiata* (Qi-he et al. 2011). At the same time, the co-culture of *P. radiata* with *D. squalens* showed the maximum specific laccase activity but the co-culture of *P. radiata* with *P. ostreatus* was beneficial for MnP and LiP expression.

Table 7.2 Enzymatic activity of mono- and dual cultures of WRB in fermentation of mandarin squeeze on agar plates and in submerged cultures

	Laccase (U/mL)			MnP (U/mL)
	Solid medium		Liquid medium	Liquid medium
	Distance between colonies			
Fungi	1 cm	4 cm		
C. unicolor 305	4.9 ± 0.3	4.5 ± 0.2	140.3 ± 19.3	1.28 ± 0.20
L. betulina 141	4.3 ± 0.2	3.9 ± 0.2	16.1 ± 2.0	0.05 ± 0.01
P. lecometei 903	2.2 ± 0.1	2.6 ± 0.2	6.4 ± 1.0	0
P. coccineus 310	1.8 ± 0.1	1.5 ± 0.1	4.3 ± 0.6	0
T. hirsuta 82	2.2 ± 0.1	2.3 ± 0.2	27.2 ± 3.0	0.21 ± 0.02
T. versicolor 159	5.2 ± 0.2	5.3 ± 0.2	65.8 ± 10.4	0.58 ± 0.09
C. unicolor 305 + *L. betulina* 141	7.3 ± 0.5	6.0 ± 0.3	254.8 ± 32.9	0.35 ± 0.05
C. unicolor 305 + *P. lecometei* 903	2.3 ± 0.2	2.5 ± 0.3	224.4 ± 37.0	0.25 ± 0.05
C. unicolor 305 + *P. coccineus* 310	5.7 ± 0.4	5.1 ± 0.5	21.3 ± 3.8	0.44 ± 0.05
C. unicolor 305 + *T. hirsuta* 82	4.8 ± 0.2	5.6 ± 0.7	16.6 ± 2.1	0.10 ± 0.01
C. unicolor 305 + *T. versicolor* 159	6.5 ± 0.5	9.2 ± 0.5	318.0 ± 43.5	0.81 ± 0.15
P. coccineus 310 + *T. hirsuta* 82	1.5 ± 0.1	2.6 ± 0.2	51.8 ± 5.5	0.03 ± 0.01
P. coccineus 310 + *T. versicolor* 159	3.8 ± 0.2	7.7 ± 0.6	5.4 ± 0.6	0

In our work, we attempted to improve LME production through interspecific interaction of the best enzyme producer *C. unicolor* 305 with 5 different WRB species in the submerged fermentation of mandarin peels. Moreover, their growth and interactions were simultaneously verified on agar plates using the same medium composition. On the solid medium, a distance between inoculated fungal colonies was 1 and 4 cm. None of the dual cultures demonstrated invasion or replacement over 10 days after the creation of confrontation zones. All pairs inoculated at a distance of 4 cm formatted delineated barrages in mycelial contact zones although without brown pigmentation. Among closely inoculated fungi, only *C. unicolor* 305 and *L. betulina* 141 formed a faintly visible barrage.

The results presented in Table 7.2 show several distinctive features of the tested cultures. Firstly, like the above-mentioned data, the enzymatic activity of the dual cultures depended on the fungal species combination. Co-cultivation of *C. unicolor* 305 with *L. betulina* 141 and *T. versicolor* 159 on both solid and liquid media was the most beneficial for the laccase production and their synergistic interaction led to a much higher enzyme activity as compared with those produced by either monoculture. Secondly, it turned out that the cultivation method strongly affected the laccase activity of individual fungal pairs. Thus, pairs of *C. unicolor* 305 + *P. lecometei* 903 and *P. coccineus* 310 + *T. hirsuta* 82 demonstrated an upregulation of laccase activity when replacing SSF with submerged liquid fermentation. On the contrary,

interspecies interaction of *C. unicolor* 305 + *P. coccineus* 310 and *P. coccineus* 310 + *T. versicolor* 159 on the solid medium promoted laccase production, whereas in the submerged fermentation their co-cultivation inhibited laccase activity. Also, it should be noted that in monocultures grown on a solid medium, the laccase activity of the tested fungi differed less than three times, while in submerged cultures the enzyme activity varied from 4.3 to 140.3 U/mL. Thirdly, although no evident regularities were found between a distance of inoculation and enzyme activity values our findings indicate that in some pairs (for example, *P. coccineus*310 + *T. versicolor* 159) the distance between inoculated colonies can determine the outcomes of the interaction. Fourthly, no stimulation of MnP activity was observed in this study; on the contrary, a combination of *C. unicolor* 305 with other fungi 3 to 12-fold downregulated MnP activity of this fungus. Finally, the interaction of only live fungi is required to observe an increased outcome since the cultivation of *C. unicolor* 305 with a thermally inactivated mycelium of *T. versicolor* 159 did not increase laccase activity. Overall, it is clear that the process of interspecies interaction is complex and its result may depend not only on the particular microbe's combination, but also on physiological peculiarities and enzymatic systems of individual competitors, nutritional conditions in dual culture, and space they occupy. Compared with the SSF, in the submerged fermentation, mycelium of the dual culture has permanent access to the available nutrients, but the degree of fungal competition is closely related to the initial concentration of the nutrients. Undoubtedly, individual competitors' inoculum size, ratio, time, and sequence of their inoculation should be considered in the co-cultivation study since these parameters can significantly affect enzyme activities. An understanding of the peculiarities and mechanisms of interspecies interaction of WRB is essential for the development of improved technologies of LME production.

7.5 Conclusion

LME constitute one of the most important groups of versatile enzymes for industrial applications. Owing to the progress in recent research, screening of potent enzyme producers, genetic engineering, omics-based data, understanding of physiological and transcriptional regulation of individual LME synthesis, the production of these enzymes was maximized. Nevertheless, there are still many limitations that need to be overcome, and further research is needed, in particular, using various genetic methods and the recombinant technologies to improve the production of LME and to obtain these biocatalysts in quantity and quality, satisfying various fields of application. The use of cheap lignocellulosic materials, metals, and aromatic inducers, clarification of the molecular mechanisms triggering and regulating LME expression in a response to different stimuli may boost additional production of LME at low cost. Besides, elucidation of the factors that impede the production of enzymes is also one of the most challenging tasks. Mixed cultivation, as well as the development of process design in the lignocellulose SSF, should be considered as encouraging and cost-efficient strategies.

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References

Adekunle AE, Zhang C, Guo C, Liu CZ (2017) Laccase production from *Trametes versicolor* in solid-state fermentation of steam-exploded pretreated cornstalk. Waste Biomass Valoriz 8:153–159

Afreen S, Anwer R, Singh RK, Fatma T (2018) Extracellular laccase production and its optimization from *Arthrospira maxima* catalyzed decolorization of synthetic dyes. Saudi J Biol Sci 25:1446–1453

Akpinar M, Urek RO (2017) Induction of fungal laccase production under solid state bioprocessing of new agroindustrial waste and its application on dye decolorization. Biotech 7:98

Almeida PH, Oliviera ACC, De Sousa GPN, Friedrich JC, Linde GA et al (2018) Decolorization of remazol brilliant blue R with laccase from *Lentinus crinitus* grown in agro-industrial by-products. An Acad Bras Cienc 90:3463–3473

Baldrian P (2004) Increase of laccase activities during interspecific interactions of white-rot fungi. FEMS Microbiol Ecol 50:245–253

Bertrand B, Martínez-Morales F, Trejo-Hernández MR (2013) Fungal laccases: induction and production. Rev Mex Ing Quim 12:473–488

Bertrand B, Martínez-Morales F, Tinoco R, Rojas-Trejo S, Serrano-Carreón L, Trejo-Hernández MR (2014) Induction of laccases in *Trametes versicolor* by aqueous wood extracts. World J Microbiol Biotechnol 30:135–142

Bertrand B, Martínez-Morales F, Trejo-Hernández MR (2017) Upgrading laccase production and biochemical properties: strategies and challenges. Biotechnol Prog 33:1015–1034

Bettin F, Montanari Q, Calloni R, Gaio TA, Silveira MM, Dillon AJP (2008) Production of laccases in submerged process by *Pleurotus sajor-caju* PS-2001 in relation to carbon and organic nitrogen sources, antifoams and Tween 80. J Ind Microbiol Biotechnol 36:1–9

Birhanlı E, Yeşilada O (2017) The effect of various inducers and their combinations with copper on laccase production of *Trametes versicolor* pellets in a repeated-batch process. Turk J Biol 41:587–599

Bodke PM, Senthilarasu G, Raghukumar S (2012) Screening diverse fungi for laccases of varying properties. Indian J Microbiol 52:247–250

Bronikowski A, Hagedoorn PL, Koschorreck K, Urlacher VB (2017) Expression of a new laccase from *Moniliophthora roreri* at high levels in *Pichia pastoris* and its potential application in micropollutant degradation. AMB Express 7:73

Brown JA, Alic M, Gold MH (1991) Manganese peroxidase gene-transcription in *Phanerochaete chrysosporium*– activation by manganese. J Bacteriol 173:4101–4106

Cambria MT, Ragusa S, Calabrese V, Cambria A (2011) Enhanced laccase production in white-rot fungus *Rigidoporus lignosus* by the addition of selected phenolic and aromatic compounds. Appl Biochem Biotechnol 163:415–422

Chauhan R (2019) Nitrogen sources and trace elements influence laccase and peroxidase enzymes activity of *Grammothele fuligo*. Vegetos 32:316–323

Chi Y, Hatakka A, Maijala P (2007) Can co-culturing of two white-rot fungi increase lignin degradation and the production of lignin-degrading enzymes? Int Biodeterior Biodegrad 59:32–39

Chowdhary P, More N, Yadav A, Bharagava RN (2019) Ligninolytic enzymes: an introduction and applications in the food industry. In: Kuddus M (ed) Enzymes in food biotechnology, 1st edn. Academic, Cambridge, pp 181–195

Coconi-Linares N, Magaña-Ortíz D, Guzmán-Ortiz DA, Fernández F, Loske AM, Gómez-Lim MA (2014) High-yield production of manganese peroxidase, lignin peroxidase, and versatile peroxidase in *Phanerochaete chrysosporium*. Appl Microbiol Biotechnol 98:9283–9294

Conceição TA, Koblitz MGB, Kamida HM, Góes-Neto A (2017) Study of the production of *Lentinus crinitus* (L.) Fr. lignolytic enzymes grown on agro-industrial waste. Adv Biosci Biotechnol 8:259–272

Crestini C, D'Annibale A, Giovannozzi-Sermani G (1996) Aqueous plant extracts as stimulators of laccase production in liquid cultures of *Lentinus edodes*. Biotechnol Tech 10:243–248

D'Souza TM, Merritt CS, Reddy CA (1999) Lignin-modifying enzymes of the white-rot basidiomycete *Ganoderma lucidum*. Appl Environ Microbiol 65:5307–5313

Daly P, Peng M, López SC, Lipzen A, Ng V, Singan VR et al (2020) Mixtures of aromatic compounds induce ligninolytic gene expression in the wood-rotting fungus *Dichomitus squalens*. J Biotechnol 308:35–39

Dixit M, Gupta GK, Shukla P (2019) Insights into the resources generation from pulp and paper industry wastes: challenges, perspectives and innovations. Bioresour Technol 297:122496

Dong JL, Zhang YW, Zhang RH, Huang WZ, Zhang YZ (2005) Influence of culture conditions on laccase production and isozyme patterns in the white-rot fungus *Trametes gallica*. J Basic Microbiol 45:190–198

Du W, Sun C, Wang J, Xie W, Wang B, Liu X, Zhang Y, Fan Y (2017) Conditions and regulation of mixed culture to promote *Shiraia bambusicola* and *Phoma* sp. BZJ6 for laccase production. Sci Rep 7:17801

Eggert C, Temp U, Eriksson KEL (1996) The ligninolytic system of the white rot fungus *Pycnoporus cinnabarinus*: purification and characterization of the laccase. Appl Environ Microbiol 62:1151–1158

Elisashvili V, Kachlishvili E (2009) Physiological regulation of laccase and manganese peroxidase production by white-rot *Basidiomycetes*. Review. J Biotechnol 144:37–42

Elisashvili V, Kachlishvili E, Tsiklauri N, Khardziani T, Bakradze M (2002) Physiological regulation of edible and medicinal higher basidiomycetes lignocellulolytic enzymes activity. Int J Med Mushr 4:159–166

Elisashvili V, Kachlishvili E, Tsiklauri N, Metreveli E, Khardziani T, Agathos SN (2009) Lignocellulose-degrading enzyme production by white-rot *Basidiomycetes* isolated from the forests of Georgia. World J Microbiol Biotechnol 25:331–339

Elisashvili V, Kachlishvili E, Torok T (2014) Overproduction of ligninolytic enzymes. Patent No.: US 8,753,844 B2

Elisashvili V, Kachlishvili E, Asatiani MD, Darlington R, Kucharzyk KH (2017) Physiological peculiarities of lignin-modifying enzyme production by the white-rot basidiomycete *Coriolopsis gallica* strain BCC 142. Microorganisms 5:73

Fillat Ú, Martín-Sampedro R, Macaya-Sanz D, Martín JA, Ibarra D, Martínez MJ, Eugenio ME (2016) Screening of eucalyptus wood endophytes for laccase activity. Process Biochem 51:589–598

Galhaup C, Wagner H, Hinterstoisser B, Haltrich D (2002) Increased production of laccase by the wood degrading basidiomycete *Trametes pubescens*. Enzym Microb Technol 30:529–536

Hou H, Zhou J, Wang J, Du C, Yan B (2004) Enhancement of laccase production by *Pleurotus ostreatus* and its us. Process Biochem 39:1415–1419

Janusz G, Rogalski G, Szczodrak J (2007) Increased production of laccase by *Cerrena unicolor* in submerged liquid cultures. World J Microbiol Biotechnol 23:1459–1464

Janusz G, Kucharzyk KH, Pawlika A, Staszczak M, Paszczynski AJ (2013) Fungal laccase, manganese peroxidase and lignin peroxidase: gene expression and regulation. Enzym Microb Technol 52:1–12

Janusz G, Mazur A, Wielbo J, Koper P, Żebracki K et al (2018) Comparative transcriptomic analysis of *Cerrena unicolor* revealed differential expression of genes engaged in degradation of various kinds of wood. Microbiol Res 207:256–268

Jaouani A, Tabka MG, Penninckx MJ (2006) Lignin modifying enzymes of *Coriolopsis polyzona* and their role in olive oil mill wastewaters decolourisation. Chemosphere 62:1421–1430

Johansson T, Nyman PO, Cullen D (2002) Differential regulation of mnp2, a new manganese peroxidase-encoding gene from the ligninolytic fungus *Trametes versicolor* PRL 572. Appl Environ Microbiol 68:2077–2080

Kachlishvili E, Penninckx MJ, Tsiklauri N, Elisashvili V (2006) Effect of nitrogen source on lignocellulolytic enzyme production by white-rot basidiomycetes under solid-state cultivation. World J Microbiol Biotechnol 22:391–397

Kachlishvili E, Metreveli E, Elisashvili V (2014) Modulation of *Cerrena unicolor* laccase and manganese peroxidase production. Springerplus 3:463

Kachlishvili E, Asatiani M, Kobakhidze A, Elisashvili V (2016) Trinitrotoluene and mandarin peels selectively affect lignin-modifying enzyme production in white-rot basidiomycetes. Springerplus 5:252

Kachlishvili E, Asatiani MD, Kobakhidze A, Elisashvili V (2018) Evaluation of lignin-modifying enzyme activity of *Trametes* spp. (Agaricomycetes) isolated from Georgian forests with an emphasis on *T. multicolor* biosynthetic potential. Int J Med Mushr 20:971–987

Kapich AN, Prior BA, Botha A, Galkin S, Lundell T, Hatakka A (2004) Effect of lignocellulose-containing substrates on production of ligninolytic peroxidases in submerged cultures of *Phanerochaete chrysosporium* ME-446. Enzym Microb Technol 34:187–195

Kinnunen A, Maijala P, Järvinen P, Hatakka A (2017) Improved efficiency in screening for lignin-modifying peroxidases and laccases of basidiomycetes. Curr Biotechnol 6:105–115

Kirk TK, Farrell RL (1987) Enzymatic "combustion" the microbial degradation of lignin. Annu Rev Microbiol 41:465–505

Klonowska A, Le Petit J, Tron T (2001) Enhancement of minor laccases production in the basidiomycete *Marasmius quercophilus* C30. FEMS Microbiol Lett 200:25–30

Kumar S, Dangi AK, Shukla P, Baishya D, Khare SK (2019) Thermozymes: adaptive strategies and tools for their biotechnological applications. Bioresour Technol 278:372–382

Kumari BSS, Praveen K, Usha KY, Kumar KDG, Reddy GPK, Reddy BR (2019) Ligninolytic behavior of the white-rot fungus *Stereum ostrea* under influence of culture conditions, inducers and chlorpyrifos. Biotech 9:424

Lallawmsanga VV, Passari AK, Muniraj IK, Uthandi S, Hashem A et al (2019) Elevated levels of laccase synthesis by *Pleurotus pulmonarius*BPSM10 and its potential as a dye decolorizing agent. Saudi J Biol Sci 26:464–468

Lee IY, Jung KH, Lee CH, Park YH (1999) Enhanced production of laccase in *Trametes versicolor* by the addition of ethanol. Biotechnol Lett 21:965–968

Levin L, Melignani E, Ramos AM (2010) Effect of nitrogen sources and vitamins on ligninolytic enzyme production by some white-rot fungi. Dye decolorization by selected culture filtrates. Bioresour Technol 101:4554–4563

Li X, Qiu-Man X, Jing-Sheng C, Ying-Jin Y (2016) Improving the bioremoval of sulfamethoxazole and alleviating cytotoxicity of its biotransformation by laccase producing system under coculture of *Pycnoporus sanguineus* and *Alcaligenes faecalis*. Bioresour Technol 220:333–340

Lisova ZA, Lisov AV, Leontievsky AA (2010) Two laccase isoforms of the basidiomycete *Cerrena unicolor* VKMF-3196. Induction, isolation and properties. J Basic Microbiol 50:72–82

Liu SH, Tsai SL, Guo PY, Lin CW (2020) Inducing laccase activity in white rot fungi using copper ions and improving the efficiency of azo dye treatment with electricity generation using microbial fuel cells. Chemosphere 243:125304

Lomascolo A, Record E, Herpoel-Gimbert I, Delattre M, Robert J et al (2003) Overproduction of laccase by a monokaryotic strain of *Pycnoporus cinnabarinus* using ethanol as inducer. J Appl Microbiol 94:618–624

Lu X, Ding S (2010) Effect of Cu^{2+}, Mn^{2+} and aromatic compounds on the production of laccase isoforms by *Coprinus comatus*. Mycoscience 51:68–74

Lundell TK, Mäkelä MR, Hilden K (2010) Lignin-modifying enzymes in filamentous basidiomycetes-ecological, functional and phylogenetic review. J Basic Microbiol 50:5–20

Mäkelä MR, Lundell T, Hatakka A, Hildén K (2013) Effect of copper, nutrient nitrogen, and wood-supplement on the production of lignin-modifying enzymes by the white-rot fungus *Phlebia radiata*. Fungal Biol 117:62–70

Mäkelä MR, Bredeweg EL, Magnuson JK, Baker SE, de Vries RP, Hildén K (2016) Fungal ligninolytic enzymes and their applications. Microbiol Spectr 4:FUNK-0017-2016

Mansur M, Suárez T, González AE (1998) Differential gene expression in the laccase gene family from basidiomycete I-62 (CECT 20197). Appl Environ Microbiol 64:771–774

Martani F, Beltrametti F, Porro D, Branduardi P, Lotti M (2017) The importance of fermentative conditions for the biotechnological production of lignin modifying enzymes from white-rot fungi. FEMS Microbiol Lett 364:fnx134

Manubens A, Canessa P, Folch C, Avila M, Salas L, Vicuna R (2007) Manganese affects the production of laccase in the basidiomycete *Ceriporiopsis subvermispora*. FEMS Microbiol Lett 275:139–145

Martinez AT, Ruiz-Duenas FJ, Camarero S, Serrano A, Linde D, Lund H et al (2017) Oxidoreductases on their way to industrial biotransformations. Biotechnol Adv 35:815–831

Mekmouche Y, Zhou S, Cusano AM, Record E, Lomascolo A et al (2014) Gram-scale production of a basidiomycetous laccase in *Aspergillus niger*. J Biosci Bioeng 117:25–27

Mikiashvili N, Wasser S, Nevo E, Elisashvili V (2006) Effects of carbon and nitrogen sources on *Pleurotus ostreatus* ligninolytic enzyme activity. World J Microbiol Biotechnol 22:999–1002

Murugesan K, Kim YM, Jeon JR (2009) Effect of metal ions on reactive dye decolorization by laccase from *Ganoderma lucidum*. J Hazard Mater 168:523–529

Myasoedova NM, Chernykh AM, Psurtseva NV, Belova NV, Golovleva LA (2008) New efficient producers of fungal laccases. Appl Biochem Microbiol 44:73–77

Myasoedova NM, Gasanov NB, Chernykh AM, Kolomytseva MP, Golovleva LA (2015) Selective regulation of laccase isoform production by the *Lentinus strigosus* 1566 fungus. Appl Biochem Microbiol 51:222–229

Palmieri G, Giardina P, Bianco C, Fontanella B, Sannia G (2000) Copper induction of laccase isoenzymes in the ligninolytic fungus *Pleurotus ostreatus*. Appl Environ Microbiol 66:920–924

Park JW, Kang HW, Ha BS, Kim SI, Kim S, Ro HS (2015) Strain-dependent response to Cu^{2+} in the expression of laccase in *Pycnoporus coccineus*. Arch Microbiol 197:589–596

Peng M, Aguilar-Pontes MV, Hainaut M, Henrissat B, Hilden K et al (2018) Comparative analysis of basidiomycete transcriptomes reveals a core set of expressed genes encoding plant biomass degrading enzymes. Fungal Genet Biol 112:40–46

Piscitelli A, Giardina P, Lettera V, Pezzella C, Sannia G, Faraco V (2011) Induction and transcriptional regulation of laccases in fungi. Curr Genomics 12:104–112

Qi-he C, Krügener S, Hirth T, Rupp S, Zibek S (2011) Co-cultured production of lignin-modifying enzymes with white-rot fungi. Appl Biochem Biotechnol 165:700–718

Revankar MS, Lele SS (2006) Enhanced production of laccase using a new isolate of white rot fungus WR-1. Process Biochem 41:581–588

Savoie JM, Mata G, Billete C (1998) Extracellular laccase production during hyphal interactions between *Trichoderma* sp. and Shiitake, *Lentinula edodes*. Appl Microbiol Biotechnol 49:589–593

Schlosser D, Grey R, Fritsche W (1997) Patterns of ligninolytic enzymes in *Trametes versicolor*. Distribution of extra- and intracellular enzyme activities during cultivation on glucose, wheat straw and beech wood. Appl Microbiol Biotechnol 47:412–418

Schneider WDH, Fontana RC, Mendonça S, Siqueira FG, Dillon AJP, Camassola M (2018) High level production of laccases and peroxidases from the newly isolated white-rot basidiomycete *Marasmiellus palmivorus* VE111 in a stirred-tank bioreactor in response to different carbon and nitrogen sources. Process Biochem 69:1–11

Schneider WDH, Costa PC, Fontana RC, de Siqueira FG, Dillon AJP, Camassola M (2019) Upscale and characterization of lignin-modifying enzymes from *Marasmiellus palmivorus* VE111 in a bioreactor under parameter optimization and the effect of inducers. J Biotechnol 295:1–8

Selvaraj B, Sanjeevirayar A, Rajendran A (2014) Laccase production using mixed substrates containing lignocellulosic materials by *Pleurotus ostreatus* in submerged liquid culture. Int J Chem Tech Res 15:355–368

Sergentani AG, Gonou-Zagou Z, Kapsanaki-Gotsi E, Hatzinikolaou DG (2016) Lignocellulose degradation potential of Basidiomycota from Thrace NE Greece. Int Biodeterior Biodegradation 114:268–277

Singh D, Chen S (2008) The white-rot fungus *Phanerochaete chrysosporium*: Conditions for the production of lignin-degrading enzymes. Appl Microbiol Biotechnol 81:399–417

Soden DM, Dobson AD (2001) Differential regulation of laccase gene expression in *Pleurotus sajor-caju*. Microbiology 147:1755–1763

Songulashvili G, Jimenéz-Tobón GA, Jaspers C, Penninckx MJ (2012) Immobilized laccase of *Cerrena unicolor* for elimination of endocrine disruptor micropollutants. Fungal Biol 116:883–889

Stajić M, Vukojević J (2011) Interaction of trace elements and ligninolytic enzymes in *Pleurotus eryngii*. Biol Trace Elem Res 143:1202–1208

Stajić M, Persky L, Friesem D, Hadar Y, Wasser SP, Nevo E (2006) Effect of different carbon and nitrogen sources on laccase and peroxidases production by selected *Pleurotus* species. Enzym Microb Technol 38:65–73

Teerapatsakul C, Parra R, Bucke C, Chitradon L (2007) Improvement of laccase production from *Ganoderma* sp. KU-Alk4 by medium engineering. World J Microbiol Biotechnol 23:1519–1527

Terrón MC, González T, Carbajo JM, Yagüe S, Arana-Cuenca A et al (2004) Structural close-related aromatic compounds have different effects on laccase activity and on lcc gene expression in the ligninolytic fungus *Trametes* sp. I-62. Fungal Genet Biol 41:954–962

Valle JS, Vandenberghe LPS, Oliveira ACC, Tavares MF, Linde GA et al (2015) Effect of different compounds on the induction of laccase production by *Agaricus blazei*. Genet Mol Res 14:15882–15891

Villavicencio EV, Mali T, Mattila HK, Lundell T (2020) Enzyme activity profiles produced on wood and straw by four fungi of different decay strategies. Microorganisms 8:73

Wei F, Hong Y, Liu J, Yuan J, Fang W, Peng H, Xiao Y (2010) *Gongronella* sp. induces overproduction of laccase in *Panus rudis*. J Basic Microbiol 150:98–103

Winquist E, Moilanen U, Mettälä A, Leisola M, Hatakka A (2008) Production of lignin modifying enzymes on industrial waste material by solid-state cultivation of fungi. Biochem Eng J 42:128–132

Xiao YZ, Hong YZ, Li JF, Hang J, Tong PG, Fang W, Zhou CZ (2006) Cloning of novel laccase isozyme genes from *Trametes* sp. AH28-2 and analyses of their differential expression. Appl Microbiol Biotechnol 71:493–501

Xu X, Feng L, Han Z, Luo S, Wu A, Xie J (2016) Selection of high laccase-producing *Coriolopsis gallica* strain T906: mutation breeding, strain characterization, and features of the extracellular laccases. J Microbiol Biotechnol 26:1570–1578

Yadav M, Yadav HS (2015) Applications of ligninolytic enzymes to pollutants, wastewater, dyes, soil, coal, paper and polymers. Environ Chem Lett 13:309–318

Yang Y, Wei F, Zhuo R, Fan F, Liu H et al (2013) Enhancing the laccase production and laccase gene expression in the white-rot fungus *Trametes velutina* 5930 with great potential for biotechnological applications by different metal ions and aromatic compounds. PLoS One 8:79307

Yang J, Wang G, Ng TB, Lin J, Ye X (2016) Laccase production and differential transcription of laccase genes in *Cerrena* sp. in response to metal ions, aromatic compounds, and nutrients. Front Microbiol 6:1558

Zhao LH, Chen W, Wang LL, Sun HJ, Zhu Z (2017) Improvement of laccase production by *Pleurotus ostreatus* by means of agroindustrial waste and fermentation kinetics. Mycosphere 8:147–161

Zhu C, Bao G, Huang S (2016) Optimization of laccase production in the white-rot fungus *Pleurotus ostreatus* (ACCC 52857) induced through yeast extract and copper. Biotechnol Biotechnol Equip 30:270–276

Zhuo R, Yuan P, Yang Y, Zhang S, Ma F, Zhang X (2017) Induction of laccase by metal ions and aromatic compounds in *Pleurotus ostreatus* HAUCC 162 and decolorization of different synthetic dyes by the extracellular laccase. Biochem Eng J 117:62–72

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