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Enhancing Laccase and Manganese Peroxidase Activity in White-Rot Fungi: The Role of Copper, Manganese, and Lignocellulosic Substrates

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Abstract: White-rot fungi (WRF) are increasingly recognized for their biotechnological potential due to the wide range of applications of ligninolytic enzymes. The addition of different metals involved in the functioning of ligninolytic enzymes, mainly copper and manganese, has been widely studied to maximize the enzymatic activities of the WRF. This review aims to provide information on the effect of metal-fungi interaction mechanisms that justify the effects of enzymatic activity. The addition of copper is associated with increased laccase activity, with reported improvements in the laccase activity compared to controls without metal addition of up to 100% at doses between 0.5–1 mM. The addition of manganese resulted in an improvement in manganese peroxidase activity with respect to the control at the wide range of 1–18.2 mM. Furthermore, enzymatic activity was generally favored by using substrates with lignocellulosic fibers with respect to synthetic culture medium. Quantifying the concentration of metals in the substrate is required to monitor bioavailable metals for fungi in these assays accurately, making an external contribution less necessary.

Keywords: white-rot fungi; ligninolytic enzymes; laccase; manganese peroxidase; lignocellulosic substrates

1. Introduction

White-rot fungi (WRF) are microorganisms with a high biotechnology potential thanks to their capability of producing extracellular oxidative enzymes for the degradation of lignocellulosic fibers [1,2]. WRF are saprotrophic fungi well known for their remarkable lignocellulose-decaying capabilities and are the responsible to the bleached white aspect to the wood [3]. The ecological function of WRF consists in its decomposition of plant residue, wood and organic material. Lignocellulose represents the largest renewable

organic carbon pool in the terrestrial biosphere [4,5]. Hence, WRF play a key role in the recycling of carbon and provide insights into the global carbon cycle. At the same time, these enzymes constitute an important topic of research in the biotechnological field to enhance their extraction and exploitation. The potential degrading enzymatic machinery allows for the division of this group into two groups according to whether they selectively or simultaneously (non-selectively) degrade lignin. For instance, *Trametes versicolor* and *Fomes fomentarius* are species that are able to degrade all components [5]. There are about 10,000 species of WRF belonging to the *Polyporales*, *Agaricales*, *Auriculariales*, *Hymenochaetales*, and *Russulales* orders, the first being the order with the most members. WRF can produce a variety of extra-cellular ligninolytic enzymes that act as a catalyst for reactions including oxidation, reduction, hydrolysis, esterification, synthesis, and molecular inter-conversions [6]. The enzymes produced by these fungi are denominated as "auxiliary activities" and include four main enzymes: lignin peroxidases (LiP); manganese-dependent peroxidases (MnP); laccases (Lac); or versatile peroxidase (VP) [7]. These extracellular oxidatives secreted by WRF act by breaking down lignocellulosic materials into simpler molecules. Therefore, the result is the depolymerization of lignin and its metabolization into carbon dioxide and water [5].

The wide range of applications of WRFs has made them a broadly studied topic over the last 50 years (Figure 1). As can be seen, scientific interest in the field continues to grow, with the number of publications in the area increasing year after year. Most of the published studies in relation to WRFs focus on application processes, either in evaluating the degradation capacity of xenobiotics or [8–10] bioremediation processes [11–13], and to a lesser extent, on the integration of WRF with other technologies such as hydrogen production [14] or composting [15,16].

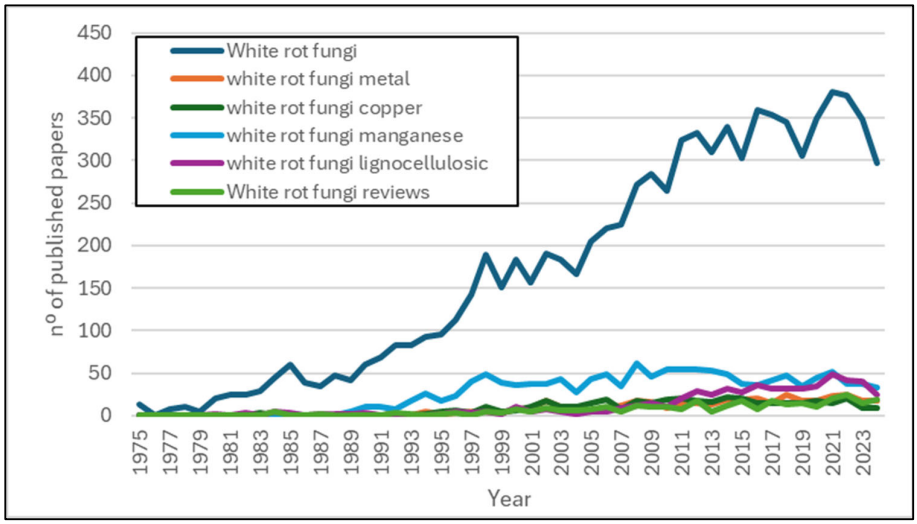

Figure 1. Number of publications per year for different search terms (Data source: Scopus, on 22 October 2024).

Regardless of the specific technological application of WRF, the use of these organisms is based on taking advantage of the characteristics of their enzymatic machinery. Therefore, it is desirable to optimize operational conditions with a view to maximizing fungal enzymatic activity and, therefore, to optimize the efficiency of the desired processes.

In practical terms, the use of lignocellulosic substrates and/or incorporating different inducers and cofactors has proven essential for boosting the activities of lignin-degrading enzymes in fungi [17]. Metals considered inducers act as mediators, expediting the conversion of numerous compounds and thereby enhancing reaction kinetics through increased enzymatic activity [18]. Cofactors, operating at the transcriptional level, constitute integral components of enzyme architecture, serving diverse functions including electron transfer, O_2 binding, activation and reduction, NO_2^- and N_2O reduction, and substrate

activation [19]. Although certain metals, like copper and manganese, are indispensable for the life of all organisms, their concentrations in aerobic and aqueous environments may not always be sufficient to ensure that they are bioavailable to fungi. Therefore, it is sometimes necessary to supplement these metals to meet the physiological requirements of the fungi [20].

The effectiveness of metal supplementation is typically evaluated based on the dosage employed. However, it is worth noting that bioavailable metal fractions may influence the microbial response more or, if substrates contain a significant metal concentration, by the total metal concentration in the medium [21,22]. Defining the mechanism of action of different metals in their interaction with fungal strains can help optimize the addition of metals as a strategy to improve fungal enzymatic activity, being able to explain the processes in a deeper way than a cause–effect relationship.

2. Scope of the Review

The review aims to provide information on the effect of metal–fungi interaction mechanisms that justify the effects of enzymatic activity. Given their major relevance in the literature, the metals reviewed have been copper and manganese. The review also includes a discussion of the effect of metal addition depending on the type of substrate used, differentiating between synthetic models and lignocellulosic substrates. This review also highlights the biotechnological potential of basidiomycete fungi in enzyme production under metal supplementation, which could significantly enhance biodegradation and the acquisition of biomass-derived products. Additionally, it provides a summary of the research conducted over the last two decades, underscoring basidiomycetes as promising candidates for biotechnological applications.

In contrast to the present work, previous reviews on WRF published in the last five years mainly focused on the biotechnological applications of WRF. In that sense, several highly cited reviews focused on lignin degradation processes [5,23], removal of specific xenobiotic and/or pollutants [24,25], or the coupling of WRF with valorization processes [26,27]. Thus, this review addresses a gap in the available information by analyzing the improvement of enzyme production, an aspect that is transversal to all WRF applications.

3. Copper

Copper is an essential micronutrient for fungal growth, acting as a metal activator of fungal enzymes like oxidases. Based on its generally accessible I/II redox couple and bioavailability, copper plays a wide variety of functions in nature that mostly involve electron transfer, O_2 binding, activation, and reduction, NO_2^- and N_2O reduction, and substrate activation [28]. In WRF, copper is crucial for lignin breakdown, acting as a cofactor in the laccase catalytic center [29]. Laccases (EC 1.10.3.2) catalyze the oxidation of a wide variety of organic and inorganic substrates, including phenols, ketones, phosphates, ascorbate, amines, and lignin [30]. In fungi, laccases are transcriptional regulators, and they carry out a variety of physiological roles, including morphogenesis, fungal plant–pathogen/host interaction, stress defense, and lignin degradation [31,32]. Laccase (Lac) is considered an ideal "green catalyst" because its oxidizing property is a vast variety of compounds using O_2 and releasing H_2O as the only by-product [33].

Fungal laccases are extracellular and monomeric glycoproteins with ~520–550 amino acids and a typical weight of ~60–70 kDa in their glycosylated form [33] and have accounted for the most important group of blue multicopper oxidases concerning the number and extent of characterization [34]. These enzymes are multi-copper oxidoreductases that catalyze the one-electron (e^-) oxidation of the substrates and sequentially transfer four electrons to the catalytic copper atoms, which are used to reduce O_2 to two water molecules [28,31]. The copper architecture has two separate copper sites: a mononuclear site (T1 copper ion) and a trinuclear site (a cluster of T2, T3, and T3′ copper ions). Reducing substrates are oxidized close to the mononuclear site, and then electrons are transferred through the Cys-His pathway to the trinuclear site, where dioxygen is reduced to water molecules [32]. These

oxidases are unique in that they effectively perform this reaction in one step, indicating that the free energy barrier for the second two-electron reduction of the peroxide product of the first two-electron step is very low [28].

According to the reviewed literature, the cooper addition in a range between 1.6×10^{-8} and 150 mM permitted the enhancement of ligninolytic enzyme activity, with Lac being the main enzyme reported in the literature, followed by manganese peroxidase (MnP) (Table 1). The supplementation of copper has yielded notable enhancements, ranging from 14.3% to 100% in Lac activity and 34.1% to 100% in MnP activity, as delineated in Table 1. However, the improvements are variable and depend on other parameters, such as the strains, the culture medium, and operational conditions (Table 1). Several studies have underscored the impact of different copper dosages added to culture media on the production of ligninolytic enzymes across a diverse array of fungi, particularly those within the basidiomycetes group. For instance, the addition of a minimal dose of 1.6 × 10−8 mM copper to glucose-peptone culture media elicited a 20% improvement in Lac activity from *Grammothele fuligo* [35], while supplementation of lignocellulosic culture media with 0.007 mM copper resulted in a 37.4% enhancement in MnP activity from *Pleurotus eryngii* [36]. Likewise, the addition of 0.19 mM copper to the culture medium comprising potato dextrose broth (PDB), wood chips, and rice bran led to a 61.3% increase in laccase activity by *Ganoderma lucidum* [37]. These authors also demonstrated varying sensitivities to copper supplementation, highlighting that excess copper does not significantly contribute to laccase production. Additionally, Jain et al. [38] showed that including 7 mM copper in a malt extract broth culture medium resulted in a remarkable increase of up to 87.2% in laccase activity from *G. lucidum* compared to cultures without this metal.

Among the extensive array of fungi documented in the literature, Basidiomycetes stand out for their exceptional ability to degrade highly recalcitrant wood constituents, including lignin. This group has also been extensively studied for enzyme production and agricultural waste treatment, with particular attention given to assessing the effects of copper, as outlined in Table 1. This review identified the following important genera: *Pleurotus*, *Grammothele*, *Pycnoporus*, *Dichomitus*, *Ceriporiopsis*, *Lentinus*, *Ganoderma*, *Trametes*, *Daedaleopsis*, *Phlebia*, and *Fomes*. Within this group, some fungi such as *Trametes versicolor* [39], *Tramates pubescens* [40], *Phlebia radiata* [41], *Fomes fomentarius* [20], *T. suaveolens* [20], *G. lucidum* [42], *T. versicolor* [20], *Daedaleopsis confragosa* [20], *Trametes gibbosa* [20], and *Trametes hirsuta* [43] have demonstrated the highest enhancements in Lac activity, with improvements of more than 90% compared to Lac activity without copper addition. Moreover, MnP activity has also been extensively enhanced, reaching improvements above 95% for different fungal strains such as *T. hirsuta* [43], *T. versicolor* [20], *D. confragosa* [20], *F. fomentarius* [20], and *T. gibbosa* [20].

Several authors have evaluated the supplementation of copper using complex lignocellulosic biomasses as substrates [43–48]. In these studies, the effect of adding copper would be more limited than using synthetic media because (i) enzymatic activity was already favored by the presence of the lignocellulosic fibers [39], and (ii) the substrate would already contain relevant concentrations of copper that makes less necessary an external metal contribution. However, there are examples where the combination of synthetic media such as malt extract agar (MEA), glucose-yeast extract-peptone (GYP), malt extract broth (MEB), or potato dextrose broth (PDB) with lignocellulosic biomasses has led to large improvements in enzyme activity.

Relevant improvements in the fungal enzymatic activities by copper addition using lignocellulosic substrates have been reported in the literature. For example, cassava waste supplemented with 0.5 mM of copper [49], corn silage supplemented with 100 mM of copper [44], and sorghum supplemented with 0.1 mM of copper [43] led to enhancements in Lac activity of more than 80% by *P. ostreatus*, *T versicolor*, and *T. hirsuta*, respectively.

Notwithstanding the above indicated benefits, it is important to acknowledge that high copper concentrations might substantially impede fungal growth, perhaps resulting in negative reactions during the bioprocesses being conducted. For example, stress conditions

due to copper toxicity can trigger a more prolonged adaptation phase in the microorganism, as demonstrated by studies on *T. pubescens* [40]. This can possibly be explained by the fact that once the microorganism has colonized most of the substrate or culture medium, it undergoes, to a lesser extent, a growth inhibition due to copper consumption and bioadsorption in the mycelium [40]. Moreover, the excess copper supplement may delay the enzyme kinetics due to a possible consequence of changes to the pH in the substrate or due to effects on microorganism growth on the substrate portion that is not colonized. Likewise, this addition can cause the production of other compounds, like organic acids, that may inhibit catalysis by ligninolytic enzymes [50]. In that sense, Mäkelä et al. [41] reported that 0.5 mM L^{-1} of Cu suppressed the production of MnP from *P. radiata* grown under semi-solid conditions. Likewise, Gassara et al. [51] associated *Phanerochaete chrysosporium* loss of viability with the inhibition of MnP as a consequence of the toxicity exerted by the addition of copper to a solid-state fermentation process.

These negative effects can be controlled in some cases thanks to microorganisms having developed different strategies to mitigate excess copper in the medium. For example, to avoid the long periods of adaptation that can occur, it has been shown that copper addition must appear at the beginning of the exponential phase to achieve satisfactory levels of Lac activity [29]. Thus, the enzyme activity can be restored in time, under conditions of solid-state fermentation, mainly given the ability of WRF to use organic compounds generated in various metabolic pathways, consequently preventing the accumulation of copper and new compounds [50]. According to Merino et al. [46], in solid-state fermentation using corn cob, the addition of copper to the fungus adaptation phase can reduce the toxicity due to the microorganism having grown enough. In this way, the deleterious effects of copper on microorganism growth are prevented, and hence, copper acts as an inducer in ligninolytic enzyme production during this time. Another reported natural strategy in fungi to mitigate the toxic effects of copper is the generation of natural pigments, such as melanin. This was demonstrated in *T. versicolor*, where the addition of copper progressively decreased its radial growth and biomass production over time, along with subsequent color changes in the fungus [52]. However, this strategy may have limited applicability in the biotechnology application of fungi.

Table 1. Enzyme activity in the presence of copper as an inducer.

Strains	Culture Medium/Substrate	Added Concentration (mM)	Doses Defined as Optimal (mM)	Lac (U/L) * ABTS ** DMP *** Other	Improvement Compared to Control (%)	MnP (U/L) * ABTS ** DMP *** Other	Improvement Compared to Control (%)	Reference
T. pubescens MB89	Glucose-based medium	1	1	47,500	95.8	NA	ND	[40]
P. radiata	Peptone-yeast extract-glucose-succinate (PYGS) medium+milled alder	0.025, 0.1, 0.5, 1, 1.5, 2, 3		25	98	6.5	0	[41]
P. eryngi F019	Agar nutrient	10	10	1500 *		250	ND	[53]
Lentinus polychrous	Glucose-yeast peptone (GYP) medium	0.1, 1.0, 2.0, 3.0	2	2000 *	85	NA	ND	[54]
P. ostreatus	Mineral salts	0, 5, 10, 15, 20, 25, 30 and 50	15	NA	ND	NA	ND	[55]
Dichomitus squalens	1.2% glucose, 0.1% arabinose, 20 mM sodium nitrate, 0.5% casein	0.1, 0.31, 0.25, 0.4, 0.5, 0.55	0.5	1815 ** U/g	65.1	NA	ND	[56]
Ceriporiopsis subvermispora	2.5% glucose, 0.05% arabinose, 30 mM sodium nitrate, 0.5% casein	0.1, 0.31, 0.25, 0.4, 0.5, 0.55	0.25	1242 ** U/g	56.5	NA	ND	[56]
G. lucidum	Glucose-yeast extract	0, 0.5, 1, 1.5, 2	0.5	1 ** U/g	100	NA	ND	[42]
T. versicolor	Malt extract liquid medium	0.1, 0.05	0.05	NA		NA	ND	[57]
T. versicolor	Potato dextrose broth (PDB)	0.1, 0.3, 0.5 and 1.0	1	1291.7	100	0.09	100	[20]
T. suaveolens	Potato dextrose broth (PDB)	0.1, 0.3, 0.5 and 1.0	1	2108.3	98.3	0.08	87.5	[20]
D. confragosa	Potato dextrose broth (PDB)	0.1, 0.3, 0.5 and 1.0	1	750	100	0.38	100	[20]
F. fomentarius	Potato dextrose broth (PDB)	0.1, 0.3, 0.5 and 1.0	1	1551.4	97.7	0.69	100	[20]
T. gibbosa	Potato dextrose broth (PDB)	0.1, 0.3, 0.5 and 1.0	1	156.9	100	0.25	100	[20]
Coriolus versicolor (current *T. versicolor*)	Sweet sorghum bagasse	0, 5.5×10^{-4}, 5.5×10^{-3}, 1.1×10^{-2}, 2.2×10^{-2}, 4.4×10^{-2}, 0.11	1.1×10^{-2}	NA	ND	NA	ND	[45]
T. versicolor	Corn silage	50, 100, 150	100	1539.4	91	231.5	61.1	[44]

Table 1. *Cont.*

Strains	Culture Medium/Substrate	Added Concentration (mM)	Doses Defined as Optimal (mM)	Lac (U/L) * ABTS ** DMP *** Other	Improvement Compared to Control (%)	MnP (U/L) * ABTS ** DMP *** Other	Improvement Compared to Control (%)	Reference
G. fuligo	Glucose-peptone medium	0, 1.6×10^{-8}, 1.6×10^{-7}, 1.6×10^{-6}, 1.6×10^{-5}, 1.6×10^{-4}, 1.6×10^{-3}, 1.6×10^{-2}, 0.16, 1.6, 6.3	1.6×10^{-8}	653.5 ***	20	1.65	−50	[35]
T. versicolor	Corn cob	0, 0.5, 1.0	0.5	18 U/g *	17	3.6 U/g	0	[46]
P. ostreatus	Switchgrass	1	1	0.21 ** U/g	14.3	0.7	−42	[47]
G. lucidum	Malt extract broth (MEB)	7		0.705	89.2	NA	ND	[38]
G. lucidum	Potato dextrose broth+ wood chip + rice bran	0.038; 0.076; 0.114; 0.152; 0.19; 0.228; 0.304	0.19	574.9	61.3	NA	ND	[37]
P. eryngii	Cherry waste	0, 0.035, 0.07, 0.1, 0.5, 1, 2	1	3403	79.4	821.36	87.8	[48]
T. hirsuta	Sorghum 2,5%	0.1	0.1	19,758.5	99.9	3315	97	[43]
P. ostreatus	10 g of cassava waste moistened with 20 mL of MYG (1% malt extract, 0.4% yeast extract, and 0.4% glucose) and 100 mM acetate buffer (pH 6)	0, 0.25, 0.5, 0.75, 1, 1.25	0.5	15,400	87.3	NA	ND	[49]
Pycnoporus sanguineus 2512.	Glucose-casein medium	0, 3.9, 7.8	3.9	64,580 *	79	80.72	34.1	[58]
P. eryngii	Peach waste+stajic basal medium without Tween 80	0, 0.035, 0.07, 0.1, 0.5, 1, 2, 3, 5	0.07	NA		732.23	37.4	[48]

Substrates used to measure laccase activity (U L^{-1}): * ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)); ** DPM(2,6-Dimetilfenol); *** other substrates. ND: not determined; NA: not available.

4. Manganese

Manganese has important roles in fungal biology, i.e., mycelial growth, fruiting body development, and fungal biomass production, as it is adsorbed and accumulated at different concentrations [59]. In WRF, manganese has been shown to play a key role in the transcriptional phase as well as in the activation of the peroxidase enzyme and directly participates in the process of lignin degradation through the reaction cycle of Mn-dependent peroxidase (MnP) [29]. Manganese peroxidase (E.C.1.11.1.13) is a heme-containing enzyme that consists of Mn-binding sites and catalyzes the oxidation of Mn^{2+} to Mn^{3+} in the presence of H_2O_2 [47]. Manganese binding sites are constituted by three acidic amino acid residues that coordinate Mn^{2+} (i.e., Glu-35, Glu-39, and Asp-179), as revealed in *P. chrysosporium* [60,61]. The highly reactive Mn^{3+} combines with organic chelating compounds such as oxalic acid and acts as a low-molecular-weight, diffusible redox mediator targeting the oxidation of the phenolic components of lignin [56]. In lignin degradation, chelated Mn^{3+} is diffused into cell wall lignin through the micropores, which are less accessible for larger enzymes [61,62]. MnP has lower redox potential, which was estimated at around 1.0–1.2 V [63]. However, its action on non-phenolic lignin is generally mediated by MnP-lipid systems of lipid peroxidation, which generates reactive intermediates of peroxyl radicals [60].

Manganese has an important role in regulating either laccase and/or peroxidase activity, causing either induction or suppression of the enzyme activity, depending on the concentration of nutrients [47]. Therefore, it is possible that, at higher Mn^{2+} concentrations, the production of MnP increases and Lac decreases [64] or vice versa [56]. In addition, manganese plays an important role in other physiological activities, such as its nutritional value during *G. lucidum* growth [65].

The manganese addition in a range between 0.1 and 27 mM permitted the enhancement of ligninolytic enzyme activity, with laccase and MnP being the main enzymes reported in the literature. However, most of the best results have been reported when doses lower than 4 mM were added (Table 2). However, similar to copper, improvements can be variable and depend on other parameters such as the strains, the culture medium, and/or operational conditions (Table 2). For example, according to Kannaiyan et al. [35], the effects were controlled by the type and relative ratios of carbon and nitrogen sources. The prevailing enzyme in a fungal strain and its nutritional regulation are strain-dependent; for instance, the highest peroxidase activity in *C. subvermispora* was enhanced to a greater extent in the presence of arabinose and sodium nitrate along with $MnSO_4$.

Some authors have reported that minimal doses of manganese influence ligninolytic enzyme production, i.e., when 0.1 mM of manganese was added to sorghum straw, the laccase activity by *T. hirsuta* achieved a maximum value of 10,215.6 U L^{-1}. In contrast, manganese peroxidase achieved 1280 U L^{-1} [43]. Likewise, when 0.12 mM of manganese was added to the basal medium with 1% glucose and 1.1 mM ammonium tartrate, the values of MnP activity by *P. chrysosporium* were around 176,000 U L^{-1} [66]. In addition, Scheel et al., [67] reported an improvement in both laccase activity (between 50 and 80%) and MnP activity (between 93.3 and 100%) when 0.13 mM of manganese was added to the Hofrichter medium, showing maximum enzyme activity values of 40,000 U L^{-1} of laccase by i63-2 (unidentified strain) and 300,000 U L^{-1} by *Clytocybula dusenii*. It is worth noting that there is no consistency in the literature regarding the relationship between manganese addition and enzymatic response. However, excess manganese addition usually does not significantly impact enzyme production. From this perspective, when manganese was added to Corncob and Kirk's medium at a dose of 18.2 mM, the Laccase and MnP activity by *P. chrysosporium* only achieved a value of 290 and 2400 U L^{-1}, respectively [68]. Adding a dose of 27 mM to a medium with glucose, peptone, and yeast extract resulted in the MnP activity by *P. ostreatus* (PC9) only achieving a value of 1300 U L^{-1} [69].

Table 2. Enzyme activity in the presence of manganese as an inducer.

Strains	Culture Medium/Substrate	Added Concentration (mM)	Doses Defined as Optimal (mM)	Lac (U/L) * ABTS ** DMP *** Other	Improvement Compared to Control (%)	MnP (U/L) * ABTS ** DMP *** Other	Improvement Compared to Control (%)	Reference
P. chrysosporium	Basal medium with 1% glucose and 1.1 mM ammonium tartrate	0, 0.06, 0.03, 0.119, 0.237	0.12	NA	NA	176,000 ***	ND	[66]
P. chrysosporium	Basal medium	0, 0.06, 0.03, 0.119, 0.237	0.24	NA	NA	90,000 ***	ND	[66]
P. chrysosporium	Corncob and Kirk's medium with modifications	18.2	18.20	290	100	2400	100	[68]
C. dusenii	Hofrichter medium	0.13	0.13	25,000	80	300,000	96.7	[67]
i63-2	Hofrichter medium	0.13	0.13	40,000	75	130,000	100	[67]
Nematoloma frowardi	Hofrichter medium	0,133	0.13	10,000	50	150,000	93.3	[67]
F. sclerodermeus	Glucose, tiamine, asparagine	0.4, 0.5, 0.6, 0.7, 08, 0.9, 1.0	0.70	2,508,000	ND	212,600	ND	[70]
P. ostreatus	Wheat straw	1	1.00	580,000	ND	200,000	ND	[71]
P. ostratus	Wheat straw Kirk's medium with modifications	1	1.00	455,110 *	ND	210,770 **	ND	[71]
P. ostreatus (PC9)	Glucose, peptone, yeast extract	27	27.00	NA	ND	1300 **	ND	[69]
P. ostreatus	GYP medium.	0.2, 0.5	0.20	159.1	ND	NA	ND	[72]
P. eryngii	Cherry waste + basal medium	0.18, 0.25, 0.5, 0.75, 1	0.75	43,761.33	45,87	NA	ND	[48]
P. ostreatus HAUCC162	GPY medium	0.2 0.5	0.20	160 *	93.75	NA	ND	[72]
G. lucidum	Cottonseed hull (90%), wheat bran (5%), cornflour (4%), and gypsum (1%)	0.295, 0.591, 0.887, 1.183, 1.479, 1.775, 2.071	1.18	NA	ND	82.4 ***	62.8	[65]
P. ostreatus	Switchgrass	1	1.00	6	100	26 **	100	[47]
P. eryngii	Cherry waste + basal medium	0.18, 0.25, 0.5, 0.75, 1	0.18	4677 *	ND	2064 **	ND	[48]
T. hirsuta	sorghum straw	0,1	0.10	10,215.6	ND	1280	ND	[43]
P. chrysosporium	Norfloxacin, glucose, ammonium tartrate, and mineral salts	0, 0.21, 0.66, 1.5, 4.0	4.00	1249.6	95.8	7398.2	89.6	[73]
Microbial comunities from soil	Wheat straw, urea, and soil suspension	2.2, 8.7, 17.5	2.20	7000 *	40	1900 **	36.8	[74]
P. eryngii	Peach waste + basal medium	0.18, 0.25, 0.5, 0.75, 1	0.75	NA	ND	5036.82	ND	[36]

Substrates used to measure laccase activity (U L−1): * ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)); ** DPM(2,6-Dimetilfenol); *** other substrates. ND: not determined; NA: not available.

Basidiomycota was the main phylum reported for enzyme production and agricultural waste treatment induced by manganese. The most representative genera in this field are *Phanerochaete*, *Pleurotus*, *Ganoderma*, *Trametes*, *Fomes*, *Nematoloma*, and *Clytocybula* (Table 2). However, the best results for improving laccase and MnP activity (100%) have been demonstrated using *P. ostreatus* [47] and *P. chrysosporium* [68]. Regarding culture medium, their composition is variable. It includes reagents that are very common in the preparation of culture media, ranging from glucose, peptone, yeast extract, mineral salts, and other compounds to the use of complex matrices such as agricultural waste including corncob, wheat straw, cherry waste, cottonseed hull, wheat bran, switchgrass, sorghum straw, and peach waste (Table 2). Peach waste has been used as a growth substrate and supplementary inducer, i.e., when corncob supplemented with 18.20 mM of Mn was used, the laccase and MnP activity by *P. chrysosporium* increased by 100% [68]. Likewise, with switchgrass and 1mM of Mn, the laccase and MnP activity from *P. ostreatus* increased by 100% [47].

5. Conclusions

The addition of metals is an effective strategy to improve laccase and manganese peroxidase enzyme activities in white-rot fungi. In particular, the addition of manganese at a wide range of 0.2–18.2 mM improved the activity of manganese peroxides by more than 90% compared to controls without metal addition. Similarly, copper doses of 0.1–100 mM increased the activity of laccase by more than 90% compared to controls. As can be seen, defining a specific optimal metal addition range is difficult as it depends on many factors, including the fungal strain or the type of substrate used. Furthermore, enzymatic activity was generally favored by using substrates with lignocellulosic fibers with respect to synthetic culture media. Quantifying the concentration of metals in the substrate is required to monitor bioavailable metals for fungi in these assays accurately, making an external contribution less necessary. Despite this, synergistic effects can be observed between adding metals and using lignocellulosic substrates to improve enzymatic activity.

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