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Overproduction of Laccase by *Trametes versicolor* and *Pycnoporus sanguineus* in Farnesol-Pineapple Waste Solid Fermentation

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Abstract: The effect of farnesol, a sesquiterpene alcohol, on the production of laccases by *Trametes versicolor* and *Pycnoporus sanguineus* in pineapple waste solid-state fermentation was evaluated. Extracellular laccase production reached a maximum of 77.88 ± 5.62 U/g (236% above control) in farnesol-induced cultures of *T. versicolor* on the 17th day, whereas in a similar *P. sanguineus* culture, a maximal laccase activity of 130.95 ± 2.20 U/g (159% increase) was obtained on the 17th day. A single 45 KDa laccase was produced by both fungi under the influence of farnesol. These and other data allow us to conclude that farnesol acted as an inducer of the same form of laccase in both fungi. Farnesol disfavored fungal growth by increasing the lag phase, but it also clearly improved the oxidative state of the cultures. Contrary to the results obtained previously in submerged cultures, farnesol did not promote hyperbranching in the fungal mycelia. This is the first demonstration that farnesol is an excellent inducer of laccases in *T. versicolor* and *P. sanguineus* in solid-state cultivation. In quantitative terms, the results can be regarded as an excellent starting point for developing industrial or at least pre-industrial procedures to produce laccases using *T. versicolor* and *P sanguineus* under the stimulus of farnesol.

Keywords: lag phase; fungal biomass; white-rot fungi; growth inhibition

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1. Introduction

Laccases (EC 1.10.3.2, benzenediol:oxygen oxidoreductase) possess the capacity of catalyzing the oxidation of a wide variety of organic and inorganic substrates, including mono-, di-, and polyphenols, methoxyphenols, aromatic amines, and ascorbate via reduction of oxygen to water [1,2]. In consequence, the enzyme has great biotechnological importance, plays a significant role in a wide range of eco-friendly processes, and is in high demand in several areas. Laccases are useful for application in different biotechnological processes, thanks to their characteristics of low substrate specificity, use of oxygen as an electron acceptor, and the fact that they do not require cofactors or hydrogen peroxide for their catalytic activity. These characteristics make them suitable for transforming and degrading toxic compounds found in polluted soils and wastewater (environmental area). Additionally, laccases have been used as a tool in building advanced lignin-based materials as well as in the pre-treatment of lignocellulosic biomass (bioenergy area) [3–6]. The growing demand for this enzyme has generated the need for developing new strategies to obtain higher amounts, including new cultivation methods for microorganisms, the

use of new growth substrates, and the discovery of new laccase inducers. Laccases can be produced in both submerged and solid-state fermentation (SSF) using lignocellulosic materials as substrates, sometimes supplemented with laccase inducers, such as phenolic compounds and aromatic amines structurally related to lignin and lignin derivatives [7–10]. Although submerged fermentation is traditionally the preferred technique for enzyme production, the use of SSF has increased in recent decades, especially to produce fungal enzymes, including laccases [7]. The interest in SSF arises from its closeness to the natural living conditions of most fungi and the possibility of using agricultural and forestry industrial wastes as support substrates for fungal growth, thus giving an added value to these otherwise underutilized or nonutilized wastes [7,11]. To enhance laccase productivity, optimization strategies, based on the one-factor-at-a-time method or based on statistical approaches, can be used. The most frequent factors studied are (1) the growth substrate, (2) the cultivation time, (3) the initial moisture, and (4) laccase inducers. One such inducer is farnesol ($C_{15}H_{26}O$), a sesquiterpenoid alcohol synthesized by various bacteria, fungi, plants, and animals which has a wide variety of biological activities [12]. In addition to its various activities, the compound also exerts a remarkable inductive effect on laccase production in submerged cultures. Increases of 1.92- [13] and 6.8-fold [14] in laccase yield have been observed, for example, in farnesol-induced submerged cultures of *Trametes versicolor*, both related to the hyper-ramification of hyphae. To date, however, no study has been carried out to evaluate the inductive effect of farnesol in solid-state fermentation (SSF). For this reason, the objective of the present study was to evaluate a possible inductive effect of farnesol on the production of laccases by *T. versicolor* and *Pycnoporus sanguineus* in solid-state fermentation using pineapple crown leaves as the main growth substrate. Attempts were also made to evaluate the alterations in the mycelial morphology and in the levels of oxidative stress caused by farnesol.

2. Materials and Methods

2.1. Chemicals

ABTS (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) and farnesol were purchased from Sigma Chemical Co. (Jurubatuba, SP, Brazil). All other chemicals were of the highest purity and analytical grade.

2.2. Microorganisms

Trametes versicolor and *Pycnoporus sanguineus* were obtained from the Basidiomycete Collection of the Laboratory of Biochemistry of Microorganisms and Food Science, Department of Biochemistry, State University of Maringá. Strains were maintained in the laboratory on Potato Dextrose Agar (PDA) Petri dishes. The microorganisms are deposited in the Basidiomycete Collection of the Laboratory of Biochemistry of Microorganisms and Food Science (Department of Biochemistry, State University of Maringá UEM) and are available to the scientific community.

2.3. Evaluation of Mycelium Growth and Laccase Activity on PDA Cultures

To evaluate the influence of farnesol on the growth rate and production of laccase in PDA cultures, the average diameter of the colonies of *T. versicolor* and *P. sanguineus* grown in Petri dishes was monitored daily. PDA media prepared with the desired farnesol concentration were used for the experiments and inoculated with one agar plug (17 mm of diameter) taken from an actively growing part of a colony from another Petri dish. All measurements were done in triplicates. Laccase was extracted from the cultures with 0.1 M citrate buffer, pH 3.0.

2.4. Production of Laccase in Solid-State Fermentation (SSF) in the Presence and Absence of Farnesol

The production of laccase in SSF was performed in 0.25 L Erlenmeyer flasks containing dry pineapple crown (5.0 g) and Vogel salt solution [15] supplemented with 1.0% glucose and 0.1% yeast extract to obtain an initial moisture of 90%. This mixture was sterilized by autoclaving for 15 min at 121 °C. Three agar plugs (17 mm diameter) of each fungus were used as inoculum. The cultures were incubated for 7 days under air at 28 °C and in the absence of light. To evaluate the effects of farnesol, the latter was directly added to the culture's medium before fungi inoculation to reach the desired concentration. For enzyme extraction, 30 mL of distilled water at 10 °C was added to each flask, which was kept under agitation for 30 min at 10 °C. The samples were centrifuged (6000 rpm for 5 min) and the supernatant was considered as crude enzyme extract.

2.5. Laccase Enzyme Assay

The laccase activity was measured as described previously [4]. Briefly, the substrate used for the determination of laccase was 2,2′-azino bis (3-ethylbenzthiazoline-6-sulphonic acid; ABTS) in 0.1 M citrate buffer (pH 3.0). The rates of ABTS oxidation were determined as the increase in A_{420} nm (ε = 36 mM^{-1} cm^{-1}). The enzyme activity was determined at 40 °C and expressed in (U) international enzyme units (mol × 10^{-6} min^{-1}). The results were expressed in international units (U) per gram of substrate on a dry basis, where one unit of enzyme activity is the amount of enzyme that catalyzes the transformation of 1 µmol of substrate per minute.

2.6. Scanning Electron Microscopy

The morphological characterization of microorganisms was performed using the Scanning Electronic Microscope (SEM) FEI Quanta 250 from the Microscopy Center of the User Support Center Complex at the State University of Maringá. *T. versicolor* and *P. sanguineus* were grown in Petri dishes with PDA media prepared with or without 5 mM farnesol. Cuts of both agar materials covered with the fungi colony were used as samples. Samples were fixed on coverslips in 2.5% glutaraldehyde with 0.1 M sodium cacodylate buffer, pH 6.8, for 12 h in a refrigerator. The samples were dehydrated by increasing series of ethanol proportions (30, 50, 70, 80, 90, 95, and 3 times 99%). The fragments were dried in a Critical Point Dryer BAL-TEC: CPD 030 drying equipment, passing through 12 cycles. After drying, the material was adhered to sample holders (stubs) using a conductive carbon adhesive tape suitable for electron microscopy. The fragments were covered with a thin layer of gold in a Sputter Coater BAL-TEC: SCD 050 metallizer and later observed in a scanning electron microscope to study morphology.

2.7. Determination of Catalase, Superoxide Dismutase, and Reactive Oxygen Species (ROS)

The activities of catalase (CAT), superoxide dismutase (SOD), and total ROS contents (quantified via the 2′–7′-dichlorofluorescein-diacetate, DCFH-DA assay) were quantified as previously described [16].

2.8. Physico-Chemical Properties of Laccases Produced in the Absence and Presence of Farnesol

Non-denaturing electrophoretic characterization of the laccases was carried out as previously described [17]. The apparent molecular mass of laccase was computed by comparing its electrophoretic mobility with those of appropriate standards (Bio Rad proteins ranging from 10 to 250 kDa). The kinetic parameters V_{max} and K_M were computed from the initial reaction rate measurements using ABTS (0.01–1.00 mmol/L) as substrate. The pH of the reaction medium was 3.0 (0.1 M citrate buffer) and the temperature 40 °C. The GraphPad Prism version 8.0 (GraphPad Software, Inc., San Diego, CA, USA) was used to estimate

the kinetic parameters by fitting the Michaelis–Menten equation to the experimental data using a nonlinear least-squares procedure:

$$v_o = \frac{V_{max}[S]}{K_M + [S]} \quad (1)$$

In Equation (1), v_o is the initial rate and [S] the substrate concentration.

2.9. Statistical Analysis

The GraphPad Prism software (version 8.0) was used. The results were expressed as the mean ± standard errors and were submitted to one-way variance analysis, followed by post hoc Student–Newman–Keuls testing. Differences between two means were assessed by Student's *t* test. The 5% level was adopted as a criterion of statistical significance.

3. Results and Discussion

3.1. Inhibitory Effect of Farnesol on Mycelial Growth

The effect of farnesol on the growth of *T. versicolor* and *P. sanguineus* was evaluated firstly in PDA medium (Figure 1). No inhibition of growth or visual modification of the mycelium was caused by the addition of farnesol up to 1.0 mM. For both fungi, however, farnesol at concentrations above 1 mM clearly created unfavorable conditions for growth as evidenced by the increased lag phase. In this respect, *T. versicolor* appears to be more resistant than *P. sanguineus*. Both fungi, however, were able to completely colonize the medium even in the presence of 50 mM farnesol. Despite the evident inhibition of biomass production, the inductive effect of farnesol on laccase production was evident in PDA cultures (Table 1).

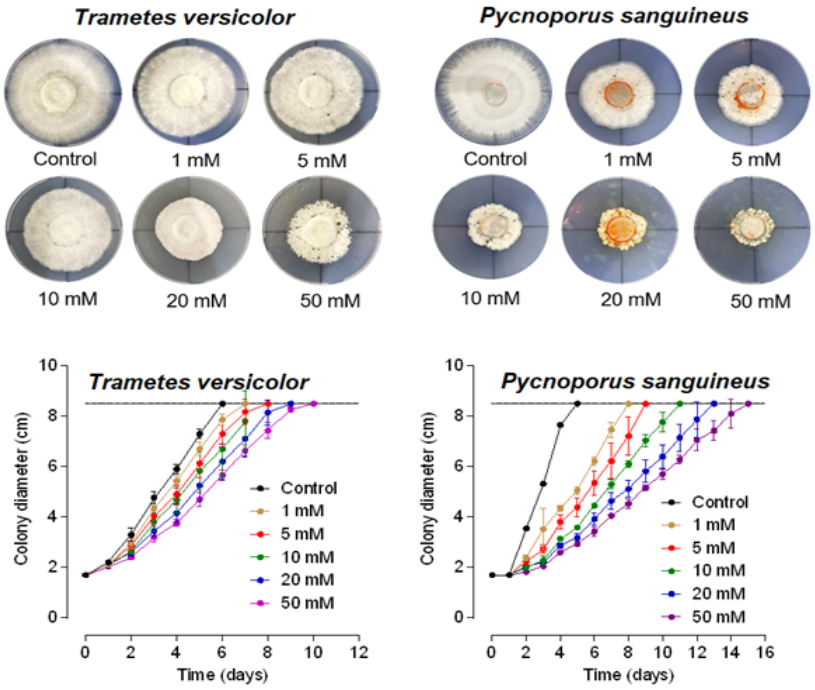


Figure 1. Effect of the farnesol concentration on *T. versicolor* and *P. sanguineus* grown in PDA media. Photos were taken on the fifth day of incubation at 28 °C. Control: without farnesol. Effect of farnesol concentration on the average diameter of the colony of *T. versicolor* and *P. sanguineus* grown in PDA medium. Petri dish diameter: 8.5 cm (dotted line). Inoculum diameter: 1.7 cm.

Table 1. Effects of farnesol concentration on the production of laccase by *T. versicolor* and *P. sanguineus* in PDA cultures. The cultures were developed with two different amounts of farnesol for 7 days at 28 °C.

	Laccase Activity U/mL		
	Control	**1 mM Farnesol**	**5 mM Farnesol**
T. versicolor	0.61 ± 0.09 [a]	1.10 ± 0.08 [b]	2.07 ± 0.12 [c]
P. sanguineus	0.71 ± 0.06 [a]	1.56 ± 0.12 [b]	0.88 ± 0.07 [c]

Values labeled with the same letter in each line are not statistically different ($p > 0.05$).

According to previous reports, farnesol can cause a delay in the growth of diverse microorganisms [18,19]. The growth of *Grifola frondosa*, for example, was inhibited by farnesol at levels from 0.2 to 1.0 mM in liquid fermentation [19]. On the other hand, both the growth extent and growth rate of *Candida dubliniensis* were unaffected by 0.15 mM farnesol, although the hyphae formation was inhibited at the same level [20]. In the same way, *Candida albicans* growth was not inhibited in the presence of 0.05 mM farnesol, even though its morphological transition to hyphae was greatly reduced [18]. In liquid cultures of *T. versicolor*, the addition of farnesol at low concentrations (up 80 µM) did not cause any inhibition of growth or growth rate [13]. In this work, the inhibition of fungal growth by farnesol in the solid-state condition varied with the species and appears to be dose-dependent, but it only occurred at high concentrations (up to 1 mM).

3.2. Effect of Farnesol on Laccase Production by T. versicolor and P. sanguineus in Solid-State Conditions

Both fungi, *T. versicolor* and *P. sanguineus*, produced laccase in the pineapple waste solid-state cultures, reaching 31.46 ± 2.45 U/g and 37.35 U/g, respectively (Figure 2). Pineapple waste has already been successfully used in solid-state cultures of *T. versicolor* with high laccase production [4]. This substrate is rich in fibers (79–83% cellulose, 19% hemicelluloses, and 5–15% lignin) [21].

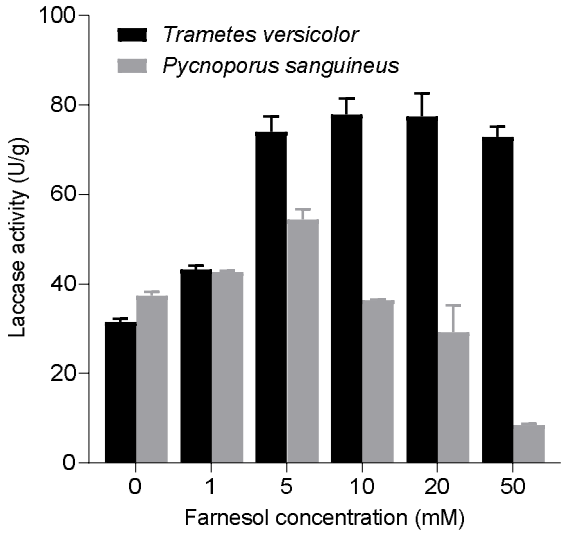
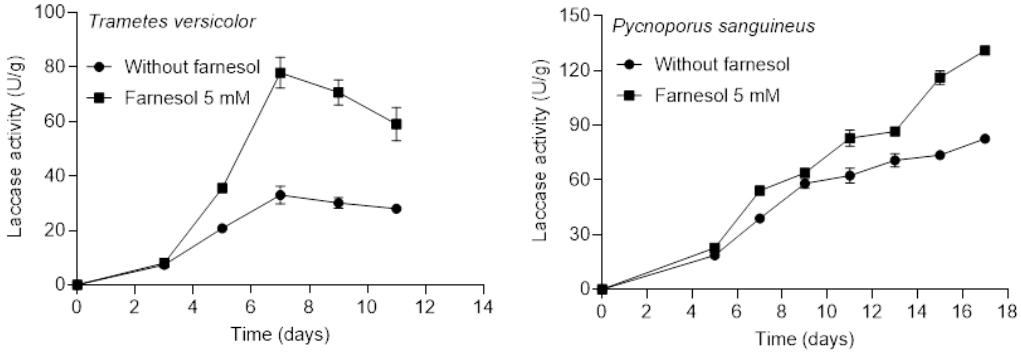


Figure 2. Effect of farnesol concentration on laccase production by *T. versicolor* and *P. sanguineus.* The cultures were developed for 7 days at 28 °C.

The production of laccase by the two fungi was differently affected by the addition of increasing amounts of farnesol (Figure 2). For *T. versicolor*, the best results in 7-day cultures were obtained using 5 mM farnesol (77.88 ± 3.6 U/g) and the laccase production was not significantly altered ($p > 0.05$) when the farnesol concentration was further increased. For *P. sanguineus*, the highest production of laccase was also obtained with 5 mM farnesol (54.42 ± 2.20 U/g). However, contrary to *T. versicolor*, the production of laccase by *P. sanguineus* was negatively affected when the farnesol concentration was raised above 5 mM.

The influence of farnesol addition on the time course of extracellular laccase production by *T. versicolor* and *P. sanguineus* is shown in Figure 3. In the presence of farnesol, the *T. versicolor* cultures reached maximal enzyme production at the same time as the control cultures, but at a considerably higher level. Laccase production reached the highest value of 77.88 ± 5.62 U/g on the seventh day, a 236% improvement compared to the control without farnesol (32.97 ± 1.99 U/g), with a subsequent reduction in activity. On the contrary, both control and farnesol-induced cultures of *P. sanguineus* showed improvements in laccase production until the last day of cultivation. On the 17th day, a laccase production of 130.95 ± 2.20 U/g was observed in the farnesol cultures, against 82.64 ± 1.99 U/g in the control cultures, which represents an improvement of 159%.



(**a**) (**b**)

Figure 3. Time course of laccase production by *T. versicolor* (**a**) and *P. sanguineus* (**b**) grown on pineapple crown SSF supplemented with farnesol or not.

It should be remarked that the farnesol concentrations mentioned in Figures 2 and 3 actually correspond to the concentration in the solution that was added to the culture medium. For a solid medium it may be useful to know the mass ratio of farnesol to substrate. Each culture medium contained 5 g pineapple waste and 0.05 g (50 mg) farnesol when the latter was added as a 5 mM solution. This means a farnesol/substrate ratio of 0.01. Such a ratio characterizes farnesol in the present context much more as an inducer rather than a quorum-sensing molecule [19,22].

3.3. Effect of Farnesol on Hyphae Morphology and on Oxidative Stress

In submerged cultures, some studies have suggested that farnesol acts as an inducer of complex cellular events by two distinct mechanisms: mycelial morphology alterations (hyper-branched) in filamentous fungi [13,14] and improvement in oxidative stress [19,22]. Both factors can be considered responsible for the increase in the production and biological activities of extracellular polysaccharides and several secretory proteins, including laccase [13,14,19,22–24]. In submerged cultures of *Coriolus versicolor* (synonymy of *T. versicolor*), Wang et al. [14] suggest that farnesol influenced both the expression of several morphogenesis-related genes, as well as significantly stimulated the expression of laccase genes.

Scanning electron microscopy was used to evaluate the alterations caused by farnesol in the fungal morphology (Figure 4). The hyphal morphologies of both fungi are clearly different. *P. sanguineus* has segmented and short hyphae when compared to *T. versicolor.* For both fungi, the presence of farnesol did not cause well-defined changes in their hyphae. Efforts were made to evaluate especially the hyphal ramifications, but only small differences were observed when comparing the mycelial masses of cultures grown in the presence or absence of 5 mM farnesol.

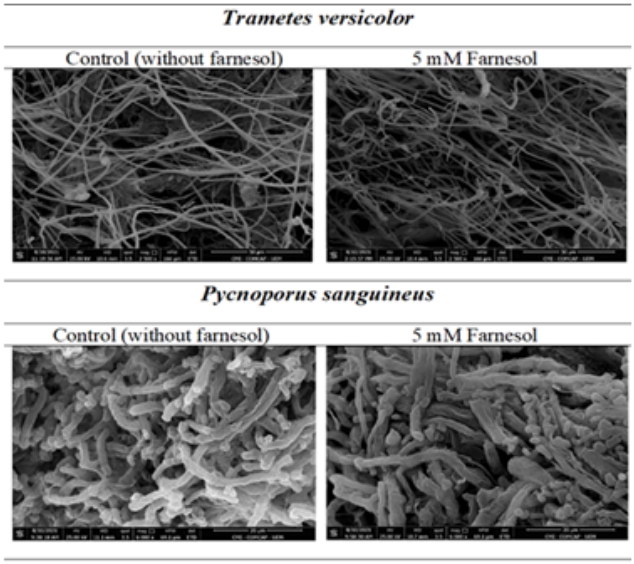


Figure 4. SEM analysis of *T. versicolor* and *P. sanguineus* hyphae. Fungi were grown in the absence (control) and presence of 5 mM of farnesol.

The branching of hyphae is critical for filamentous fungi, as it is necessary for mycelium spreading and substrate colonization [25]. Furthermore, the growth sites of the hyphae are located at their tips and the secretion of proteins occurs in specialized tips traditionally known as Spitzenkörper [14]. It is worth emphasizing that enzyme secretion undergoes simultaneous space- and time-dependent control. Although enzyme secretion may occur preferentially at the hyphae apex [26], the whole mycelium may eventually be involved in this activity [13]. The hyper-branching of several *Trichoderma reesei* strains increased cellulase secretion by 66% [27] and 22% [26] compared with the parental strain—revealing a direct relationship between fungal morphology and protein biosynthesis. Thus, the more branched the hyphae, the greater the surface area for protein secretion. Additionally, disruption of the *Trichoderma reesei* gul1 gene stimulates hyphal branching and reduces broth viscosity in cellulase production [26]. Similar effects were observed in the production of exo-polysaccharides and laccases by other white-rot fungi in submerged cultures. Examples are the production of exopolysaccharides by *Grifola frondosa* and *T. versicolor,* which was improved by farnesol at concentrations around 1.0 mM [19,28], and the production of laccase by *Coriolus versicolor*, which was increased by 4 mM farnesol [14]. Much lower concentrations, on the other hand, namely 60 µM, were described as the most suitable ones for improving laccase production by *T. versicolor* [13,21]. These data, however, were all obtained with submerged cultures, which emphasizes the distinct fungal behavior according to the cultivation conditions.

In solid-state conditions, the growth mode of filamentous fungi is a combination of the apical extension of hyphal tips through branching, the hyphal uptake of materials from the substrate, and the uptake of oxygen from the surrounding atmosphere to support the growth of the organism. Fungal hyphae extend branches to cover the surface area of the solid substrate or extend between substrate particles in response to substrate concentration and other conditions, such as nutrients, aeration, pH, and available surface area. The pictures obtained by scanning electron microscopy clearly show that the *P. sanguineus* mycelium is much more branched than the *T. versicolor* mycelium. However, the presence of farnesol did not cause any hyper-branching in the fungi. It is clear, therefore, that the higher levels of laccase produced in cultures with farnesol cannot be explained by a hyper-branching of the hyphae.

Reactive oxygen species (ROS) contents in the fungal extracts as well the activities of catalase (CAT) and superoxide dismutase (SOD) were determined in order to obtain information about the oxidative status of the cultures in the absence and presence of farnesol

(Table 2). Farnesol caused significant increases in the ROS contents of the cultures with both fungi, especially on the 7th and 10th days. The activities of catalase and superoxide dismutase were also increased by farnesol. For both fungi and under control conditions, the peak of the superoxide dismutase activity preceded the peak of the catalase activity. Farnesol modified the time course of the superoxide dismutase activities of both fungi. The most notable modification was the appearance of oscillations with maxima and minima at various times. These observations and the increased lag phase of the growth curve caused by farnesol are evidently indicative of toxicity. However, it should be stressed that it might be exactly this phenomenon that considerably enhances the laccase production, as previous investigations have suggested that fungal laccase may be involved in the defense against oxidative stress [16,29,30]. The heterologous expression of the laccase gene in the yeast *Pichia pastori* significantly enhances the resistance of the yeast to H_2O_2-mediated oxidative stress by stimulating the glutathione-dependent antioxidative system [31]. Other works have also reported the increase in the antioxidant defense system during the production of laccases by different filamentous fungi [32] and yeast [31]. In the latter, the expression of laccase confers a strong ability to scavenge intracellular H_2O_2 and to protect cells from lipid oxidative damage. More recently, it was suggested that *Coprinopsis cinerea* uses laccase Lcc9 as a defense strategy to eliminate oxidative stress during fungal–fungal interactions [33]. Additionally, some research has also suggested that other stress factors, including herbicides and pesticides, increase the laccase activity in association with improved activities of antioxidant enzymes [16,34].

Table 2. Effect of farnesol concentration on the production of ROS, catalase, and superoxide dismutase by *T. versicolor* and *P. sanguineus* in SSF.

Fungus/Condition	**Time Course**		
	4 Days	**7 Days**	**10 Days**
	ROS (nmols/mg Protein)		
T. versicolor			
Control	20.00 ± 3.00 ^{a,*}	34.00 ± 6.00 ^{a,*}	28.00 ± 5.00 ^{a,*}
5 mM farnesol	42.00 ± 7.00 ^{a,*}	68.00 ± 8.00 ^{a,*}	93.00 ± 10.00 ^{b,*}
P. sanguineus			
Control	38.00 ± 4.00 ^{a}	32.00 ± 5.00 ^{a,*}	32.00 ± 5.00 ^{a,*}
5 mM farnesol	45.00 ± 6.00 ^{a}	75.00 ± 5.00 ^{b,*}	103.00 ± 8.00 ^{c,*}
	Catalase (U per mg of protein)		
T. versicolor			
Control	180 ± 24 ^{a}	390 ± 45 ^{b,*}	330 ± 40 ^{b,*}
5 mM farnesol	255 ± 32 ^{a}	880 ± 100 ^{b,*}	560 ± 50 ^{c,*}
P. sanguineus			
Control	140 ± 18 ^{a,*}	270 ± 32 ^{b,*}	510 ± 22 ^{c,*}
5 mM farnesol	315 ± 45 ^{a,*}	460 ± 35 ^{a,*}	730 ± 58 ^{b,*}
	Superoxide dismutase (U per mg protein)		
T. versicolor			
Control	9.00 ± 0.50 ^{a,*}	7.10 ± 0.90 ^{a}	5.15 ± 0.80 ^{b,*}
5 mM farnesol	19.63 ± 0.72 ^{a,*}	12.42 ± 2.50 ^{a}	12.64 ± 1.41 ^{a,*}
P. sanguineus			
Control	13.00 ± 0.83 ^{a,*}	8.52 ± 0.60 ^{b,*}	7.00 ± 0.50 ^{b,*}
5 mM farnesol	27.24 ± 1.00 ^{a,*}	22.30 ± 1.40 ^{a,*}	14.80 ± 2.10 ^{b,*}

Values labeled with the same letter in each line are not statistically different ($p > 0.05$). Asterisks (*) were used to identify statistical difference between pairs of values (control versus 5 mM farnesol) in each column and for each parameter.

Our results have shown clearly that farnesol increased the levels of catalase and SOD in association with the increased laccase production. This fact suggests that laccase, as well as catalase and SOD, may play an important role in fungal defense against oxidative stress, which acts as an element of the stress response. In line with this reasoning, it was found that heterologous expression of the laccase gene in *Pichia pastoris* can enhance the resistance of this yeast to H_2O_2-mediated oxidative stress by stimulating the glutathione-dependent antioxidative system [31]. These observations strengthen the suggestion of an important role for laccase in the adaptive response to oxidative stress in white-rot fungi.

3.4. Physico-Chemical Properties of Laccases Produced in the Absence and Presence of Farnesol

Non-denaturing SDS-PAGE followed by incubation of the gel with ABTS showed only one band, indicating that a single 45 KDa laccase was produced by both *T. versicolor* and *P. sanguineus* in the presence and absence of farnesol (Figure 5A). These results suggest that farnesol addition increased the laccase biosynthesis in SSF of *T. versicolor* and *P. sanguineus* by promoting the secretion of a higher amount of the same laccase, rather than other isoenzymes. Additionally, the affinities (in terms of K_M values) of the laccases produced in the presence or absence of farnesol for the substrate ABTS did not present any statistically significant alteration (Figure 5B). The K_M values for *T. versicolor* laccase produced in the absence and presence of farnesol were 24.87 ± 5.47 and 30.99 ± 6.13 μM, respectively ($p > 0.05$). The K_M values for the *P. sanguineus* laccase produced in the absence and presence of farnesol were 13.06 ± 4.32 and 14.03 ± 1.77 μM, respectively ($p > 0.05$). These data reinforce the idea that farnesol acted as an inducer of the same form of laccase.

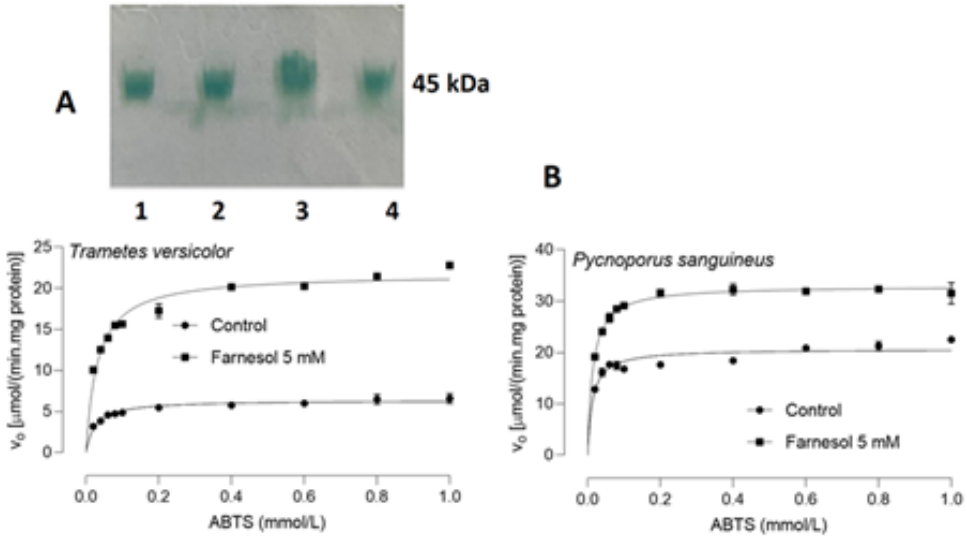


Figure 5. In (**A**): Laccase zymogram after non-denaturing SDS-PAGE of the crude enzymatic extracts. Line 1: *T. versicolor* laccase obtained without farnesol; Line 2: *T. versicolor* laccase obtained with 5 mM of farnesol; Line 3: *P. sanguineus* laccase obtained without farnesol; Line 4: *P. sanguineus* laccase obtained with 5 mM of farnesol. In (**B**): Effect of ABTS concentration on the initial velocity of the laccase obtained from *T. versicolor* and *P. sanguineus* in the presence or absence (control) of farnesol.

Supplementation of solid-state cultures with laccase inducers has been used as a frequent strategy for improving the laccase production of white-rot fungi. In this respect, Table 3 allows us to compare the results obtained in the present study with those reported previously with *T. versicolor* and *P. sanguineus* cultures in which solid substrates were enriched with well-known laccase inducers such as copper, phenolic acids, and ethanol. Examination of the results clearly reveals that farnesol can be considered an efficient inducer of laccase in both *T. versicolor* and *P. sanguineus* cultures.

Table 3. Laccase production (U/g) by *T. versicolor* and *P. sanguineus* in SSF with or without supplements.

White-Rot Fungi	Lignocellulosic Material (Substrate)	Inducer	Laccase Activity (U/g Substrate)	Reference
T. versicolor ATCC 20869	Horticultural waste	Veratryl alcohol	8.60	[35]
T. versicolor CICC 14001	Tea residues	Copper sulfate	25.70	[36]
T. versicolor ATCC 20869	Apple pomace	Copper sulfate	49.16 ± 4.50	[37]
	Pulp and paper solid waste		52.40 ± 2.20	
T. versicolor PSUWC 430	Oak sawdust, coffee husk, and corn bran	Copper and manganese sulfate	6.37	[38]
Coriolus versicolor MTCC 138	Sweet sorghum bagasse	Copper sulfate	58.20 ± 4.30	[39]
		Gallic acid	42.10 ± 3.60	
		Syringic acid	67.40 ± 7.70	
T. versicolor	Pineapple crown leaves	Control	32.97 ± 1.99	This study
		Farnesol	77.88 ± 5.62	
P. sanguineus SYBC-L1	Flower stems	Copper sulfate and Gallic acid	32.02	[24]
P. sanguineus	Sago 'hampas'	Urea	46.50	[40]
P. sanguineus	Wheat bran and corncob	NH_4Cl and $CuSO_4$	138.60	[41]
P. sanguineus	Pineapple crown leaves	Control	82.64 ± 1.99	This study
		Farnesol	130.95 ± 2.19	

All activities were evaluated using ABTS as substrate.

4. Conclusions

In conclusion, the results obtained in this work establish that farnesol is an excellent inducer of laccases from *T. versicolor* and *P. sanguineus* in SSF. This type of cultivation is frequently praised for its advantages over conventional submerged fermentation: the substrates are simple and inexpensive, there is no need for solubilization of nutrients from within the solid substrates, and a rigorous control of several variables during fermentation is dispensable. For all these reasons, the results of this work can be regarded as an excellent starting point for attempts at developing industrial or at least pre-industrial procedures to produce laccases using *T. versicolor* and *P sanguineus* under the stimulus of farnesol. Once further characterized in terms of activity, thermal stability, and pH dependence, their use in the degradation of xenobiotics such as bisphenol A or industrial dyes will be tested.

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