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Effect of Culture Conditions on Production Laccase from *Trametes versicolor* and Partial Purification Extracellular Enzyme

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Abstract. Laccase is a multicopper oxidase enzyme which oxidizes a wide range of substrates, including phenols, ketones, phosphates, ascorbate, amines, and lignin. This study was conducted to evaluate the effects of medium composition, cultivation time, and culture method on laccase production by *Trametes versicolor*. The results showed that the optimal medium for laccase production consisted of potato extract, glucose, KH_2PO_4, $MgSO_4$, NH_4^+, Cu^{2+}, and ABTS. Under these conditions, laccase activity reached 2.858 U/mL after 8 days of cultivation. Additionally, the study investigated the effects of different precipitation agents on the efficiency of laccase purification from the culture broth. The ratios of enzyme solution to organic solvents (ethanol, acetone) varied from 1:1 to 1:5 (v/v), and the saturation level of ammonium sulfate ranged from 40% to 80%. The results indicated that ethanol was the most effective precipitating agent, with the highest laccase yield obtained at a ratio of enzyme to ethanol of 1:3 (v/v) and a treatment time of 45 minutes. The molecular weight of the purified laccase was approximately 100 kDa as determined by SDS-PAGE analysis. These findings highlight the potential of *T. versicolor* culture broth as a source for laccase enzyme production, supporting its application in industrial bioprocesses such as bioremediation, food processing, and the development of sustainable biotechnology solutions.

Keywords: Laccase; *Trametes versicolor*; purification; enzyme activity

1 Introduction

Trametes versicolor, commonly known as turkey tail mushroom, is a medicinal fungus highly valued for its therapeutic properties and widely consumed in countries such as China, Japan, and various European and American nations. This mushroom contains biologically active polysaccharide–protein complexes, primarily two types: PSP (polysaccharide peptide) and PSK (polysaccharide krestin). Both PSP and PSK have been reported to inhibit the proliferation of various cancer cells, including epithelial and hematologic malignancies. These compounds are recognized for their potential in cancer therapy, enhancement of immune responses, mitigation of the adverse effects of chemotherapy and radiotherapy, and suppression of HIV replication [1]. In Vietnam, the mushroom cultivation industry, particularly the production of *T. versicolor*, has recently received increasing attention, contributing to the annual increase in mushroom yield [2].

Mushrooms are traditionally cultivated on solid substrates. In order to reduce production time, space, and costs, submerged fermentation is increasingly adopted. Liquid-state cultivation allows for higher biomass yields in a shorter period, with lower contamination risk compared to conventional solid-state methods [3].

Consequently, this fermentation method is widely applied in the cultivation of *T. versicolor* [4]. The products obtained from fermentation include fungal mycelial biomass and culture filtrate. The biomass can be utilized for the extraction of bioactive compounds, preparation of first- and second-generation fungal inocula, or even directly used as third-generation inoculum. In contrast, the post-fermentation culture medium, after biomass removal, is often underutilized economically, despite its potential to contain valuable enzymes such as laccase [5].

Laccase is an enzyme belonging to the oxidoreductase group and catalyzes the one-electron oxidation of a broad range of organic and inorganic substrates. These include aromatic compounds bearing hydroxyl groups such as mono-, di-, poly-, and methoxy-phenols, as well as aliphatic and aromatic amines, hydroxyindoles, benzenethiols, carbohydrates, and inorganic metals [6,7]. A notable feature of laccase is its ability to oxidize both toxic and non-toxic compounds. In the paper industry, laccase is employed for lignin removal during pulp bleaching and for treating chlorine-based bleaching effluents. In the food processing industry, it is used to eliminate undesirable phenolic compounds during cooking, juice clarification, and for stabilizing wine and beverages. In textiles, laccase contributes to enhancing cotton whiteness while reducing chemical, energy, and water consumption. Furthermore, laccase has applications in the bioremediation of industrial wastewater, where it degrades phenolic compounds generated from processes such as petrochemical manufacturing and soil remediation. It is also used in the biosynthesis of complex pharmaceuticals, including anesthetics, anti-inflammatory agents, antibiotics, sedatives, and more recently, in cosmetics [8].

In the present study, the effects of medium composition, culture duration, and cultivation method were investigated to evaluate the laccase production potential of *T. versicolor*. Additionally, the study assessed the efficiency of enzyme precipitation and purification using ammonium sulfate ($(NH_4)_2SO_4$) and organic solvents such as ethanol and acetone.

2 Materials and methods

2.1 Materials

The *T. versicolor* strain used in this study was provided by the Mushroom Biotechnology Laboratory, Faculty of Biology, Agriculture and Environmental Science, University of Science and Education – The University of Danang.

2.2 Effect of culture medium composition

Compositions of culture media under various treatments for laccase production in *T. versicolor* presented at Table 1. A volume of 1000 mL of culture medium was dispensed into each of 250 mL Erlenmeyer flasks for each tested condition. The media were sterilized by autoclaving at 121°C for 30 minutes. After cooling to room temperature, each flask was inoculated with a 0.5 cm^2 mycelial agar plug taken from a first-generation seed culture tube. The cultures were incubated at room temperature (26°C) and the shaking speed was 150 rpm for 7 days. Following incubation, the fungal biomass was harvested and weighed. The culture broth was filtered and centrifuged at 9000 rpm for 10 minutes at 4°C to obtain a clear supernatant for enzyme analysis.

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Table 1. Composition of culture media used in different treatments for laccase production by *Trametes versicolor*.

Treat-ments	Medium compositions
CT1	200g potato powder + 20g glucose + 1g $MgSO_4.7H_2O$ + 1g KH_2PO_4 + 0,02g $CuSO_4$
CT2	200g potato powder + 20g glucose + 1g $MgSO_4.7H_2O$ + 1g KH_2PO_4 + 0,27g NH_4Cl + 0,5 mL ABTS 0,01%
CT3	200g potato powder + 20g glucose + 1g $MgSO_4.7H_2O$ + 1g KH_2PO_4 + 0,02g $CuSO_4$ + 0,27g NH_4Cl
CT4	200g potato powder + 20g glucose + 1g $MgSO_4.7H_2O$ + 1g KH_2PO_4
CT5	200g potato powder + 20g glucose + 1g $MgSO_4.7H_2O$ + 1g KH_2PO_4 + 0,02g $CuSO_4$ + 0,27g NH_4Cl + 0,5 mL ABTS 0,01%

2.3 Effect of cultivation time

T. versicolor was cultured in the optimized medium identified from the previous experiment. The culture broth was collected at various time points: 7, 8, 9, 10, and 11 days of incubation at room temperature (26°C) [9]. At each time point, the broth was filtered and centrifuged at 9000 rpm for 10 minutes at 4°C to obtain the supernatant for enzyme activity analysis.

2.4 Evaluation of cultivation methods

Two cultivation methods were applied: Aerated culture in which oxygen was continuously supplied using an air pump at a flow rate of 0.6 L/min per liter of medium at 26°C; and shaking culture, where flasks were incubated on a shaker at 150 rpm and 26°C.

After cultivation, the broth was filtered and centrifuged at 9000 rpm for 10 minutes at 4°C. The collected supernatant was used to evaluate laccase production.

2.5 Determination of laccase activity

Laccase activity was determined based on the enzyme-mediated oxidation of ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) into a green-colored radical cation, which exhibits a strong absorbance at 420 nm. The reaction mixture consisted of 800 µL of phosphate buffer (pH 5.0), 100 µL of 1 mM ABTS, and 100 µL of enzyme solution. The mixture was incubated at 27°C for 10 minutes. The reaction was then terminated by the addition of 50% (v/v) trichloroacetic acid (TCA). After thorough mixing and filtering, the optical density (OD) of the reaction mixture was measured and compared with a control sample in which distilled water was used instead of the enzyme solution [10].

The assay was performed at 27°C, and the change in absorbance was recorded for 3 minutes. One unit (U) of laccase activity was defined as the amount of enzyme required to oxidize 1 µmol of ABTS per minute under the assay conditions. Laccase activity (U/mL) was calculated using the following equation:

$$Laccase\ activity\ \left(\frac{\mathrm{U}}{\mathrm{mL}}\right) = \frac{\Delta \mathrm{A}_{420} \times \mathrm{V}_{\mathrm{total}} \times F \times 10^3}{\varepsilon \times \mathrm{d} \times \mathrm{V}_{\mathrm{enzyme}} \times T}$$

Where: ΔA_{420}: change in absorbance per minute at 420 nm; V_{total}: total reaction volume (mL); ε: molar extinction coefficient of ABTS at 420 nm (36,000 $M^{-1}cm^{-1}$); d: path length of the cuvette (1 cm); V_{enzyme}: volume of enzyme extract used (mL); 10^3 : conversion factor to convert from mol/L to µmol/mL; F is the initial dilution. T is the reaction time.

2.6 Effect of ammonium sulfate on enzyme precipitation

The efficiency of ammonium sulfate ($(NH_4)_2SO_4$) precipitation was assessed to evaluate its effectiveness in concentrating and partially purifying the laccase enzyme from the culture filtrate. The enzyme solution was precipitated using the inorganic salt ammonium sulfate

($(NH_4)_2SO_4$) at concentrations of 40%, 50%, 60%, 70%, 80%, 85%, 90%, and 100% [9]. The appropriate amount of solid $(NH_4)_2SO_4$ was added to the supernatant obtained after initial centrifugation, corresponding to the desired saturation levels. A magnetic stirrer was used to ensure complete dissolution of the salt. The precipitation process was carried out over a period of 2 hours. Following precipitation, the mixture was centrifuged at 6000 rpm for 10 minutes at 4°C to collect the pellet. The collected pellet was then re-dissolved in phosphate buffer (pH 5.0), and laccase activity was subsequently determined.

2.7 Effect of organic solvents on enzyme precipitation

The experiment was conducted using two types of organic solvents—ethanol and acetone—at various crude enzyme solution to solvent ratios: 1:1, 1:2, 1:3, 1:4, and 1:5 (v/v) [11]. Both the enzyme solution and the solvents were pre-chilled prior to use. The organic solvent was slowly added to the enzyme solution, and the precipitation process was carried out at 4°C for 40 minutes. After precipitation, the mixture was centrifuged at 6000 rpm for 10 minutes at 4°C to collect the precipitate. The pellet was then re-dissolved in phosphate buffer (pH 5.0), and laccase activity was measured to determine the optimal solvent ratio for maximum precipitation efficiency.

2.8 Effect of precipitation time

The experiment was conducted at five different time intervals: 15, 30, 45, 60, and 75 minutes. Ethanol was used as the precipitating solvent. After each precipitation period, the mixture was centrifuged to collect the precipitate, which was then re-dissolved in phosphate buffer (pH 5.0). Laccase activity was subsequently measured to assess the effect of precipitation time on enzyme recovery efficiency.

2.9 Zymogram electrophoresis

Native polyacrylamide gel electrophoresis (PAGE) was performed to analyze laccase activity. A volume of 20 µL of the enzyme sample was mixed with 5 µL of 4X loading dye containing 0.05 M Tris-HCl (pH 6.8), 10% glycerol, and 0.1% bromophenol blue, and incubated at 27°C for 10 minutes. Subsequently, 25 µL of the prepared sample was loaded into each well alongside a protein molecular weight marker (Fermentas). Electrophoresis was conducted at a constant current of 14 mA for 2 hours at 4°C.

Following electrophoresis, the gel was used to detect laccase activity bands through the following steps: the gel was first rinsed with phosphate buffer (pH 5.0), then stained with 0.01% ABTS solution for 10 minutes. The reaction was terminated upon the appearance of distinct green bands, which corresponded to laccase activity zones. These bands were used to determine the active form and approximate molecular weight of the laccase enzyme [9].

2.10 Data analysis

All experiments were arranged in a completely randomized design with three replicates. Data were collected and analyzed using Microsoft Excel and IBM SPSS Statistics software. Analysis of variance (ANOVA) was performed, and the Least Significant Difference (LSD) test was applied to determine statistically significant differences among treatment means.

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3 Results and discussion

3.1 Effect of cultivation conditions on enzyme production

Nutrient composition greatly affects *T. versicolor* growth and laccase production, though growth does not always reflect enzyme yield. Five media formulations were tested to identify the most effective one for laccase production. The best-performing medium was then used to evaluate the effect of cultivation time over 7, 8, 9, 10, and 11 days.

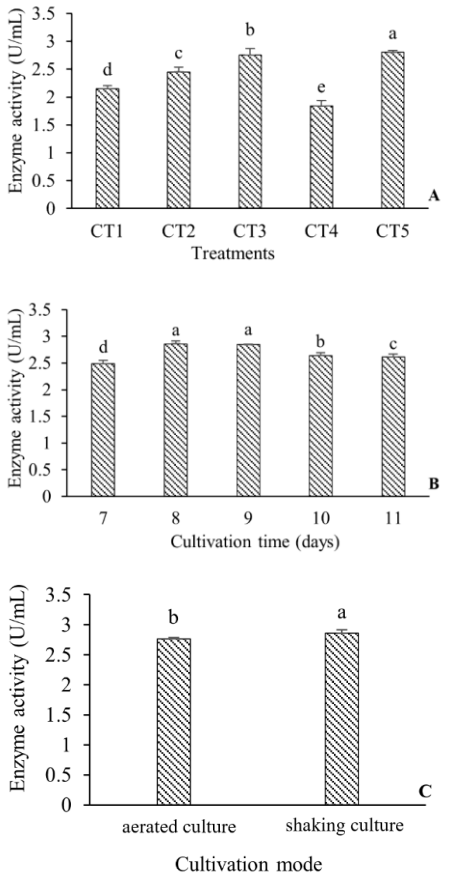

Fig. 1. Effect of cultivation conditions on enzyme production. A: Effect of medium composition; B: Effect of cultivation time; C: Effect of cultivation mode. Different letters (a, b, c, d) indicate statistically significant differences at the 5% level based on the LSD test

The results demonstrated that cultivation conditions significantly influenced laccase production by *T. versicolor*. Variations in culture media led to different levels of enzyme synthesis. Among the tested treatments, CT5 yielded the highest enzyme activity at 2.80 U/mL, followed by CT3 at 2.76 U/mL, whereas the lowest activity was recorded in CT4 at only 1.84 U/mL (Fig.1A). The low laccase activity in the minimal medium containing only potato extract, glucose, and mineral salts was likely due to insufficient nutrients for fungal growth and enzyme biosynthesis. In contrast, the other media formulations, which included additional components such as inorganic nitrogen (NH_4^+), copper ions (Cu^{2+}), and ABTS, enhanced laccase production. These findings suggest that these factors play a stimulatory role in laccase biosynthesis in various fungal strains, including *T. versicolor*.

Fig. 2. Growth of *T. versicolor* in liquid culture at different cultivation time points. The images show temporal changes in biomass and culture appearance

These results are consistent with those reported by Duonng Minh Lam, who investigated the effects of carbon sources on laccase production by *T. maxima* CPB30 and identified glucose as the most suitable sugar source [10]. Similarly, a study by Nguy Thi Mai Thao demonstrated the positive influence of metal ions on laccase biosynthesis in *Pleurotus* sp. (oyster mushroom), showing that the addition of Cu^{2+} to the medium increased laccase activity by 105% compared to the control without Cu^{2+} [12].

Cultivation duration also had a significant effect on laccase activity. The maximum enzyme activity was observed on day 8 at 2.86 U/mL. However, prolonged cultivation beyond this point led to a gradual decline in enzyme activity, with activity decreasing to 2.61 U/mL by day 11

(Fig.1B). This decline may be attributed to nutrient depletion and the transition of the fungus into the stationary or senescent phase after 8–9 days, during which biomass peaked and metabolic activity stabilized. Laccase production tends to peak during this equilibrium phase. Beyond day 10, hyphal structures began to show signs of disintegration and morphological deformation, contributing to the reduction in enzyme activity. These findings indicate that laccase production does not necessarily correlate with the growth rate of *T. versicolor*. Therefore, the optimal cultivation period for obtaining high laccase activity under shaking conditions is between 8 and 9 days, which is consistent with the findings of Katarzyna Litwińska et al. (2019), who reported maximum enzyme recovery on day 9 [13].

6 days 7 days 8 days

Fig. 3. Mycelial growth of *T. versicolor* in aerated liquid culture at different cultivation time points. The images illustrate changes in mycelial density and morphology over time.

Oxygen supply is another critical factor in submerged fungal cultivation. During fermentation, microorganisms rapidly consume oxygen, causing a decline in dissolved oxygen levels. To ensure sufficient oxygen availability and enhance fungal contact with oxygen, both aerated and shaking culture methods were evaluated. As shown in Fig.1C, there was no substantial difference in laccase activity between the two methods. However, this difference was statistically significant ($p<0.05$). Shaking cultivation resulted in slightly higher activity (2.86 U/mL) compared to aerated cultivation (2.77 U/mL). This may be attributed to more efficient oxygen transfer under shaking conditions, which facilitated better fungal growth and enzyme synthesis.

3.2 Crude laccase purification efficiency by ammonium sulfate

The degree of ionization is a critical factor for each enzyme, as it affects hydration capacity and water affinity, thereby altering the enzyme's physical state. Among the influencing factors, solvents play an important role in modifying the ionic strength of enzymes. Based on this principle, we investigated the effects of ammonium sulfate ($(NH_4)_2SO_4$) and organic solvents on the precipitation and recovery of laccase for purification purposes.

Table 2. Crude laccase purification efficiency using ammonium sulfate.

No.	$(NH_4)_2SO_4$ concentration	Total laccase activity (U/mL)	Efficiency (%)
1	40%	0.000^{a}	0.000^{a}
2	50%	14.58 ± 0.06^{b}	17.01 ± 0.45^{b}
3	60%	25.27 ± 0.10^{d}	29.48 ± 0.16^{d}
4	70%	31.20 ± 0.08^{g}	36.39 ± 0.6^{g}
5	80%	31.29 ± 0.07^{g}	36.50 ± 0.54^{g}
6	85%	27.89 ± 0.08^{f}	32.53 ± 0.65^{f}
7	90%	26.73 ± 0.11^{e}	31.18 ± 1.17^{e}
8	100%	21.83 ± 0.06^{c}	25.45 ± 0.42^{c}
9	control	87.74 ± 0.05^{h}	100^{h}

The results at Table 2 indicated that no visible precipitate formed at ammonium sulfate ($(NH_4)_2SO_4$) saturation levels below 40%. Precipitation was observed at saturation levels ranging from 50% to 100%; however, at 50% saturation, laccase activity was relatively low,

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reaching only 14.58 U/mL. The highest precipitation efficiency was obtained at saturation levels between 70% and 80%, with the maximum laccase activity recorded at 80% saturation (31.29 U/mL). When the ammonium sulfate concentration exceeded this level, precipitation efficiency gradually declined.

This observation may be explained by the salting-out effect: when salt is added to the crude laccase solution, the salt dissociates into ions, which compete for water molecules, thereby disrupting the hydration shell surrounding the enzyme. As a result, enzyme molecules aggregate and precipitate out of solution. However, at very high salt concentrations (> 80% saturation), the excess ionic strength may cause irreversible denaturation of the enzyme, thereby reducing precipitation efficiency. These results are consistent with those reported by Qin et al., who achieved a laccase precipitation yield of 38% from *T. versicolor* using a 40–80% saturated ammonium sulfate fraction (w/v) after overnight incubation [14]. Similarly, Nguyen Thi Phuong Mai et al. reported a 39% precipitation efficiency of laccase from *Aspergillus niger* D15#26 lcc1 1.8B using the same 40–80% $(NH_4)_2SO_4$ saturation range [9].

Overall, the results demonstrate that both specific activity and recovery yield are not linearly correlated with the ammonium sulfate concentration. The optimal salt saturation range for laccase precipitation from *T. versicolor* was found to be between 70% and 80% ammonium sulfate.

3.3 Effect of organic solvents on crude enzyme purification efficiency

Figures 4A and 4B present a comparative analysis of the effects of ethanol and acetone as precipitation solvents on the activity and purification efficiency of crude laccase extracted from *T. versicolor*. Both solvents significantly influenced the recovery of enzymatically active protein, but distinct trends were observed in their efficiency profiles and optimal precipitation conditions.

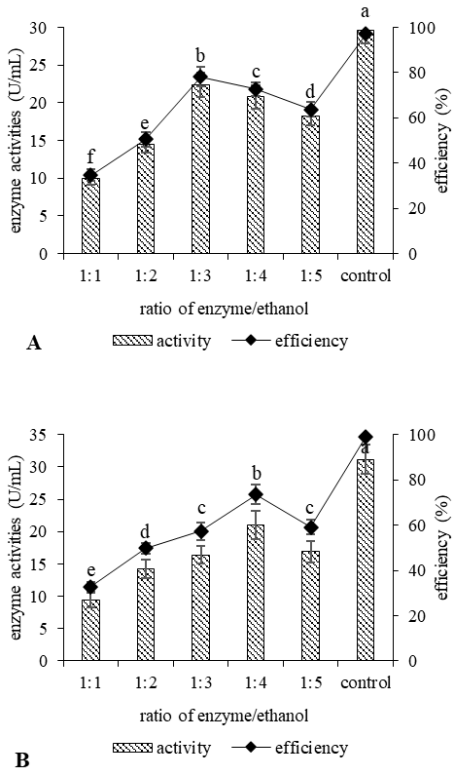

Fig. 4. Purification efficiency of the enzyme using different organic solvents. A: Ethanol; B: Acetone. Different letters (a, b, c, d, e, f) indicate statistically significant differences at the 5% level based on LSD test

In both cases, the untreated control group consistently exhibited the highest enzymatic activity (29.58–31.17 U/mL) and purification efficiency (~97–99%), serving as a baseline for evaluating solvent-induced losses. Among the tested solvent ratios, ethanol precipitation at a 1:3 enzyme-to-solvent ratio yielded the highest laccase activity (22.33 U/mL) and efficiency (78.12%), suggesting that this condition offers the most favorable balance between solubility suppression and enzymatic stability. In contrast, acetone precipitation achieved its peak activity and efficiency at a 1:4 ratio, reaching slightly higher enzymatic activity (21.01 U/mL) and

comparable efficiency (73.58%). This indicates that acetone may require a higher solvent concentration to achieve optimal protein aggregation, potentially due to its higher polarity and lower dielectric constant compared to ethanol.

At lower solvent ratios (1:1 and 1:2), both ethanol and acetone showed reduced precipitation efficiency, likely due to insufficient solvent content to disrupt protein solvation and promote aggregation. However, the degree of reduction in activity was more pronounced with acetone at the 1:1 ratio (9.38 U/mL) compared to ethanol (9.92 U/mL), suggesting that acetone may induce less protein denaturation at low concentrations. Conversely, at higher solvent ratios (1:5), both solvents showed a decline in activity, indicating potential over-precipitation or irreversible protein aggregation. The enzyme/ethanol showed a slightly higher activity (18.23 U/mL) than enzyme/acetone (16.88 U/mL) at this ratio, though both were significantly lower than their respective optima.

Overall, while both ethanol and acetone effectively precipitate laccase with moderate retention of activity, ethanol appears to achieve the highest efficiency at a lower volume ratio, making it potentially more economical and less denaturing under controlled conditions. However, acetone precipitation may preserve higher activity at slightly more diluted ratios, indicating greater compatibility with some enzyme isoforms or co-solutes. These findings demonstrate the importance of optimizing solvent type and ratio in crude enzyme purification, as the physicochemical interactions between protein structure and solvent environment directly influence yield and functional recovery.

3.4 Effect of precipitation time on crude enzyme purification efficiency

In addition to the choice of precipitating agents, precipitation time also plays a crucial role in determining enzyme recovery efficiency. In this experiment, the effect of precipitation duration on laccase recovery was evaluated at time intervals of 15, 30, 45, 60, and 75 minutes. Ethanol (96°) was used as the precipitating solvent at a crude enzyme solution to ethanol ratio of 1:3 (v/v).

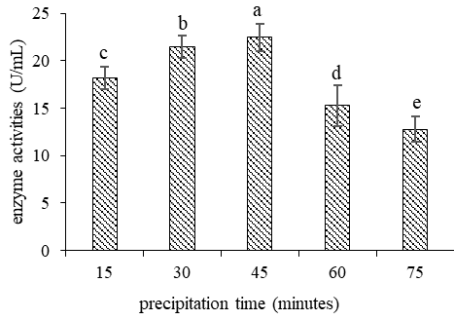

Fig. 5. Effect of precipitation time on the activity of laccase from *T. versicolor*. Different letters (a, b, …) indicate statistically significant differences at the 5% level based on the LSD test

Figure 5 illustrates the effect of different ethanol precipitation durations (15–75 minutes) on the enzymatic activity of laccase extracted from *T. versicolor*. The data show a significant increase in laccase activity with increasing precipitation time up to 45 minutes, where maximal activity (~22.5 U/mL) was recorded. This suggests that a 45-minute ethanol precipitation period provides optimal conditions for efficient aggregation and recovery of the active laccase enzyme. Beyond this point, a significant decline in enzymatic activity was observed at 60 and 75 minutes (~16 and ~13 U/mL, respectively; $p < 0.05$). This reduction may be attributed to ethanol-induced denaturation, excessive protein aggregation, or loss of soluble active enzyme. Statistical differences among time points are denoted by distinct superscript letters (a–e), indicating that each treatment group differs significantly ($p < 0.05$). These findings highlight

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the critical role of precipitation time in ethanol-based protein recovery protocols, and confirm that a 45-minute duration is optimal for maintaining laccase activity during downstream processing of *T. versicolor* fermentation products.

3.5 Molecular weight of laccase

The structural and functional integrity of purified laccase from *T. versicolor* was evaluated using native Tris–Glycine PAGE, which preserves the enzyme's quaternary and functional structures. Zymogram analysis (Fig. 6) revealed a distinct green band at approximately 100 kDa, confirming that enzymatic activity was maintained after purification. This finding indicates that solvent-based methods (ethanol/acetone) offer a rapid and effective approach for partial purification without significant structural disruption. The estimated molecular mass (~100 kDa) further suggests that the enzyme predominantly exists as a dimer under native conditions, which is consistent with the characteristic molecular weights of intact fungal laccases reported previously (100–130 kDa).

Comparable results were reported by Mun-Jung Han (2005), who identified a molecular weight of ~97 kDa for *T. versicolor* laccase using SDS-PAGE [15]. In contrast, Ramayanam BM (2025) reported a molecular weight of 66 kDa for purified laccase using native PAGE, likely reflecting a smaller subunit and a predominance of the monomeric form under their experimental conditions [16]. Such differences may arise from variations in cultivation conditions, inducers, or purification strategies, which can favor the expression of distinct isoforms or alter the enzyme's quaternary state. Collectively, these findings underscore the structural diversity of laccase isoforms produced by *T. versicolor*.

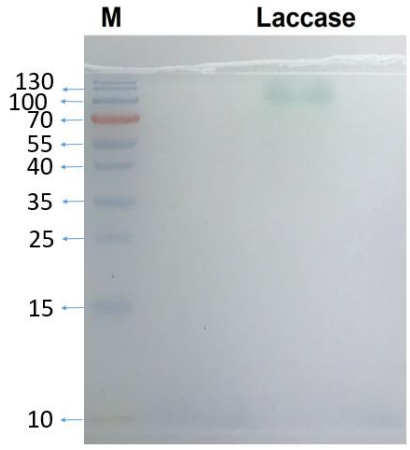

Fig. 6. Zymogram analysis of partial purified extracellular laccase from *T. versicolor*

4 Conclusion

Nutritional conditions, cultivation duration, and culture methods significantly influenced the enzyme production capacity of *T. versicolor*. The most effective culture medium (CT5 treatment) consisted of 200 g potato extract, 20 g glucose, 1 g $MgSO_4 \cdot 7H_2O$, 1 g KH_2PO_4, 0.02 g $CuSO_4$, 0.27 g NH_4Cl, and 0.5 mL of 0.01% ABTS. This medium supported the highest laccase production. The optimal shaking cultivation time for maximum laccase activity was 8 days, with an enzyme activity of 2.86 U/mL. Shaking cultivation yielded higher laccase activity than aerated cultivation. Ethanol precipitation resulted in the highest laccase recovery efficiency at a crude enzyme to ethanol ratio of 1:3 (v/v), with the optimal precipitation time ranging from 30 to 45 minutes. The molecular weight of laccase from *T. versicolor* was approximately 100 kDa. These findings highlight the potential of *T. versicolor* culture broth as a valuable source of laccase, in addition to fungal biomass, for the extraction of bioactive compounds to serve various human needs.

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