Enhanced Growth and Production of Laccase and Peroxidases by *Pleurotus ostreatus* in an Enriched Natural Medium in Submerged Fermentation

Ariadna Denisse Andrade-Alvarado [a], Angel González-Márquez [b], Rosario González-Mota [c], and Carmen Sánchez [d,*]

Pleurotus ostreatus is a wood-decaying fungus capable of producing key enzymes for lignin degradation. Laccase (Lcc), manganese peroxidase (MnP), lignin peroxidase (LiP), and unspecific peroxygenase (UnP) activities of *P. ostreatus* grown on a wheat straw infusion medium enriched with malt extract and yeast extract (SMY) were studied. Growth kinetics and enzymatic parameters were also determined. Glucose medium was used as a control. The specific growth rate of *P. ostreatus* in SMY (0.62 h^{-1}) was more than twice as high as that shown in the control (0.24 h^{-1}). This fungus produced 5-fold higher biomass (15.7 g/L) in SMY medium than in the control (3 g/L). Lcc, MnP, LiP and UnP activities were approximately 67-,19-,11-, and 17-fold greater in SMY (3106, 4082, 1564, and 1883 U/L, respectively) than in the control (46, 213, 143, and 112 U/L respectively). *P. ostreatus* constitutively produced all the studied enzymes; however, the presence of wheat straw infusion components (*i.e.* phenolic compounds) in SMY enhanced the enzymes production. This is believed to be the first study to examine UnP production by *P. ostreatus* in an enriched natural medium. UnPs have been reported as promising enzymes for degradation of hazardous pollutants; however, there is still little information about their production.

DOI: 10.15376/biores.21.1.839-850

Keywords: Laccase; Lignin peroxidase; Manganese peroxidase; Pleurotus ostreatus; Submerged fermentation; Unspecific peroxygenase; Wheat straw medium

*Contact information: a: PhD Program in Engineering Sciences, Technological Institute of Aguascalientes, Aguascalientes, 20256, Mexico; b: Postdoctoral fellow, Laboratory of Biotechnology, Research Centre for Biological Sciences, Autonomous University of Tlaxcala, Ixtacuixtla, Tlaxcala, 90120, Mexico; c: Optoelectronics Laboratory, Technological Institute of Aguascalientes, Aguascalientes, 20256, Mexico; d: Laboratory of Biotechnology, Research Centre for Biological Sciences, Autonomous University of Tlaxcala, Ixtacuixtla, Tlaxcala, 90120, Mexico; *Corresponding author: carmen.sanchezh@uatx.mx*

INTRODUCTION

The filamentous wood-decaying fungi (*i.e.* basidiomycetes and ascomycetes) are unique organisms that break down lignocellulosic materials, which makes them essential for the carbon cycle in the earth (Sánchez *et al.* 2020; Corbu *et al.* 2023). In particular, the white rot fungus *Pleurotus ostreatus* is an edible mushroom belonging to the basidiomycete class. It can produce enzymes such as laccase (Lcc), manganese peroxidase (MnP), and lignin peroxidase (LiP) that are the key enzymes for lignin biodegradation (Sánchez 2009; Hernández-Domínguez *et al.* 2017; Martínez-Berra *et al.* 2018). *P. ostreatus* can be cultivated on several agricultural wastes (*e.g.* wheat straw, barley straw, rice waste, etc.) (Huang *et al.* 2024), since it is capable of converting complex lignin materials into

utilizable carbohydrates for its growth (Sánchez 2010; Zárate-Salazar *et al.* 2020). Lcc is a copper-containing oxidase that catalyzes the oxidation of various compounds using molecular oxygen, generating water as a byproduct. Laccases oxidize non-phenolic, and phenolic groups such as hydroxyindoles, benzenothiols and aromatic amines, which are commonly present in lignin (Basera *et al.* 2024). Oxidation of phenolic acids by laccases could produce various kinds of reactive oxygen species, among them hydrogen peroxide (H_2O_2) (Huang and Yang 2022). H_2O_2 production is a crucial role for Lcc, since it is necessary to activate other enzymes (*i.e.*, peroxidases) during lignin biodegradation. LiP (or ligninase) utilizes H_2O_2 as a cosubstrate to catalyze the oxidative cleavage of the phenolic and non-phenolic structures present in lignin (Singh *et al.* 2021). MnP or Mn-dependent peroxidase oxidizes Mn^{2+} to Mn^{3+} using H_2O_2 and the Mn^{3+} oxidizes a range of phenolic and non-phenolic aromatic moieties of lignin (Kumar and Arora 2022). In addition, unspecific peroxygenases (UnPs) are secreted fungal enzymes able to hydroxylate epoxidize C═C bonds, inactive C–H bonds, and sulfonate thioethers, which exhibit hybrid characteristics of P450 monooxygenases and heme peroxidases, showing a catalytic versatility (Karich *et al.* 2017). It has been suggested that a high nitrogen content, which can be supplied by plant-derived compounds (*e.g.* soybean meal or soybean peptone), may induce UnPs expression (González-Rodríguez *et al.* 2023). UnPs have been reported as promising enzymes for degradation of hazardous pollutants; however, there is still little information about their production. UnPs from the fungi *Agrocybe aegerita* and *Marasmius rotula* achieved conversion for 35 out of 40 U.S. EPA priority pollutants, such as nitroaromatic compounds, halogenated biphenyl ethers, polycyclic aromatic hydrocarbons and derivatives of phthalic acid and chlorinated benzenes (Karich *et al.* 2017). Fungi such as *Chaetomium globosum*, some species of *Corpinopsis* and *Marasmius*, *Psathyrella aberdarensis*, and *Coprinellus radians* have been reported as UnPs producers (Huang *et al.* 2024). *P. ostreatus* was able to secrete unspecific peroxidase (UnP) in a natural medium supplemented with polyethylene (Andrade-Alvarado *et al.* 2024; González-Márquez *et al.* 2024).

Ligninolytic enzymes have a wide range of industrial applications, such as bioremediation of pollutants, biofuel production, textile and paper pulp processing; clarification of musts and wines among others (Othman *et al.* 2023; Huang *et al.* 2024). Therefore, Lcc, MnP, LiP and UnP offer eco-friendly and sustainable alternatives for converting residues or waste lignocellulosic substrates into valuable products and can also be used as biocatalytic for detoxification of contaminated places.

In the present work, biomass production, Lcc, MnP, LiP, and UnP activities of *P. ostreatus* grown on a wheat straw infusion medium enriched with malt extract and yeast extract and on glucose medium (as control) were evaluated. Calculation of growth kinetics and enzymatic parameters were also carried out.

EXPERIMENTAL

Organism, Culture Media, and Fermentation Conditions

Pleurotus ostreatus was obtained from the culture collection at the Research Centre for Biological Sciences (CICB) at the Autonomous University of Tlaxcala (UATx, Mexico). Two different liquid culture media were prepared containing the following carbon sources: 1) wheat straw infusion enriched with 25 g/L of malt extract and 5 g/L of yeast extract (SMY medium), and 2) 10 g/L of glucose (control medium). Both culture

media also had a mineral salts medium, which consisted of (in g/L): K_2HPO_4, 0.19; $Mg(NO_3)_2$, 0.2; $Ca(H_2PO_4)_2$, 0.5; $MgSO_4$, 1; $FeSO_4$, 0.005; $ZnSO_4$, 0.007; $MnSO_4$, 0.009; and $CuSO_4$, 0.011. Wheat straw infusion was prepared by boiling 100 g of chopped wheat straw in 1 L of distilled water for 1 h, then filtered through cheesecloth, and completed with additional distilled water (100 mL approx.) to 1 L (González-Márquez *et al*. 2024). Erlenmeyer flasks of 125 mL capacity containing 50 mL of culture medium were used. Ten mycelial fragments (8 mm in diameter) of *P. ostreatus* were inoculated into each flask. The flasks were incubated in an orbital shaker (Prendo, Mexico) at 27 °C and 120 rpm for 26 d. Samples were taken in triplicate every 24 h.

A wheat straw infusion was prepared by boiling 100 g of chopped wheat straw in 1 L of distilled water for 1 h, filtering through cheesecloth, and bringing the final volume to 1 L with distilled water

Biomass Production and Supernatant Collection

The mycelial biomass from the liquid cultures was separated by vacuum filtration using pre-dried filter paper (Whatman No. 4) at 60 °C for 48 h with a vacuum pump (Millipore, Merck, Germany) (Ríos-González *et al.* 2019). Biomass production (X) was quantified by subtracting the original weight of the filter paper from the filter paper with the dried biomass. The supernatant was placed into Eppendorf tubes and stored in a freezer for later use.

Growth Kinetics of *P. ostreatus*

The specific growth rate (μ) was determined by fitting the logistic equation into the experimental data of biomass production over time (Ahuactzin-Pérez *et al.* 2016). X_{max} corresponds to the maximum biomass produced during the fermentation expressed in g/L.

Enzyme Activity of Laccases and Peroxidases, and Enzymatic Parameters

Lcc, MnP, LiP and UnP activities were evaluated as previously reported (Ocaña-Romo *et al.* 2024). Briefly, Lcc activity was measured using 2,6-dimethoxyphenol (DMP) as the substrate at 468 nm. The reaction mixture contained DMP and 100 µL of supernatant. MnP activity was determined using guaiacol as the substrate at 334 nm. The reaction mixture had guaiacol, tartrate buffer, $MnSO_4$, hydrogen peroxide, and 10 µL of supernatant. LiP activity was determined using veratryl alcohol as the substrate at 310 nm in a reaction mixture composed of veratryl alcohol, hydrogen peroxide, tartrate buffer, and 20 µL of supernatant. UnP activity was measured using veratryl alcohol as the substrate at 310 nm. The reaction mixture contained veratryl alcohol, hydrogen peroxide, citrate buffer, and 10 µL of supernatant. Enzymatic activity measurements were carried out using a UV-Vis spectrophotometer Jenway 7305 (Stone, Staffs, UK). In all cases, one enzyme unit (U) was defined as the amount of enzyme required to catalyze the conversion of 1 µmol of substrate per minute.

The enzymatic parameters, namely, the yield of enzyme per unit of biomass ($Y_{E/X}$) was determined by dividing the maximum enzyme activity (E_{max}) by the maximum biomass (X_{max}) produced by the fungus during the fermentation. Enzyme productivity (P_{RO}) was calculated by dividing E_{max} by the time to which it was achieved. Specific rate of enzyme production (q_p) was obtained by multiplying the μ value by the $Y_{E/X}$ value (Ahuactzin-Pérez *et al*. 2016).

Statistical Analysis

All data were analyzed to determine normality and homogeneity of variances using the Shapiro-Wilk and Levene tests, respectively. Statistical differences between groups were determined using one-way ANOVA, followed by Tukey's post-hoc test for multiple comparisons. The analyses were performed using the Statistical Analysis System (SAS® Free software Trials) program.

RESULTS AND DISCUSSION

Biomass Production and Kinetics of Growth

Biomass production of *P. ostreatus* was evaluated in glucose medium (control) and in SMY medium (Fig. 1). *P. ostreatus* showed the maximum biomass production in glucose medium (3 g/L) (Fig. 1a) and in SMY medium (15.7 g/L) (Fig. 1b) at 14 d and 10 d of growth, respectively. In both media, the fungus entered the exponential phase after 2 d and reached the stationary phase in glucose medium and in SMY medium at 15 d and 11 d, respectively. The biomass production of *P. ostreatus* was 5-fold higher in SMY medium than in the control medium. Studies on the composition of wheat straw infusion after soaking it in hot water showed that it contained glucose, xylose, lignin, protein, and essential minerals such as N, P, K and Ca (Mejía and Albert 2012; Tozluoğlu *et al.* 2015). The lignin detected in these studies might be traces of low molecular weight lignin. Therefore, the wheat straw infusion provided the fungus with a nutrient-rich natural medium that favored its growth. Similarly, Mejía and Albertó (2012) reported that the use in a culture medium of the residual water derived from wheat straw significantly enhanced mycelial growth in *P. ostreatus*.

P. ostreatus grown in a medium containing glucose, peptone, yeast extract, and mineral salts had a biomass production of 16.7 g/L, after 26 d in submerged fermentation (Argyropoulos *et al.* 2022). In the present research, *P. ostreatus* had a similar biomass production in a shorter period (10 d). In addition, *P. ostreatus* showed a biomass yield of 39.2 g/L in a medium containing xylose and corn steep liquor (Papaspyridi *et al.* 2010). Bakratsas *et al.* (2024) reported that *P. ostreatus* had a biomass production of 21 g/L when grown in a medium supplemented with wine lees and glucose. These findings show that agro-industrial by-products are rich in nutrients that can enhance the growth of *P. ostreatus*.

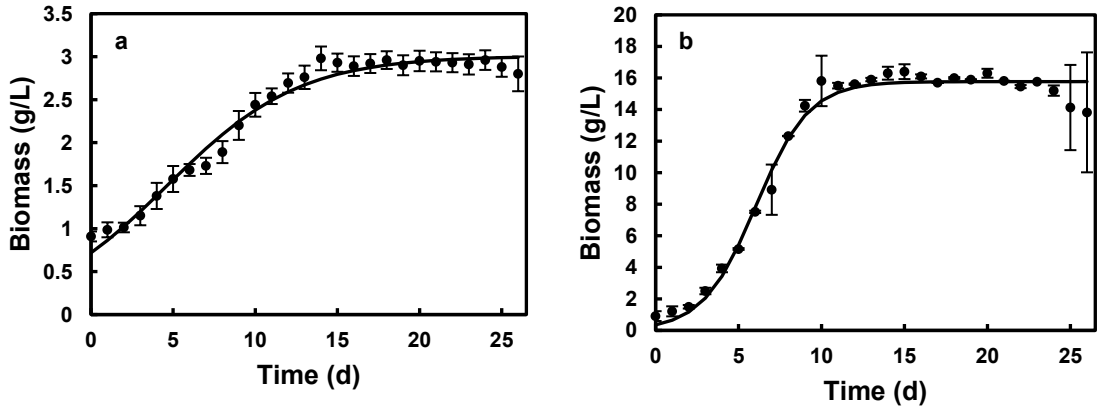

Fig. 1. Biomass production of *P. ostreatus* grown in glucose medium (a) and SMY medium (b) in submerged fermentation

Table 1 shows that the μ value for SMY medium (0.62 h^{-1}) was more than twice as high as that showed in the control (0.24 h^{-1}), indicating that the nutrient composition of SMY favored the growth of *P. ostreatus*. Bakratsas *et al.* (2023) reported μ values ranging from 0.8 to 1.8 d^{-1} (0.033-0.075 h^{-1} approximately) for *P. ostreatus* grown in media enriched with glucose and xylose. Furthermore, Bettin *et al.* (2010) observed a μ of 0.038 h^{-1} for *Pleurotus sajor-caju* cultivated in a stirred-tank bioreactor using sucrose and casein. The present results show that SMY represents a rich nutritional medium that enhanced mycelial growth, due to the presence of wheat straw hydrolysates in the wheat straw infusion.

Table 1. Growth Parameters of *P. ostreatus* Grown in Glucose Medium and SMY Medium Under Submerged Fermentation Conditions

Growth Parameters	Culture Media	
	Glucose	SMY
μ (h^{-1})	0.24^{b} (0.002)	0.62^{a} (0.005)
X_{max} (g/L)	3.00^{b} (0.012)	15.77^{a} (0.054)

Data are shown as mean of three independent experiments. The results presented in parentheses represent the standard deviation. Different superscript letters within the same row indicate significant differences (ANOVA, Tukey's test, $p < 0.05$). μ, specific growth; X_{max} maximum biomass.

Enzyme Production of *P. ostreatus*

The Lcc production by *P. ostreatus* grown in SMY medium and in control medium is shown in Fig. 2. In control cultures, Lcc was not produced during the first 5 d of growth, while a very low production level was observed from 6 to 12 d. Lcc production showed an increase after 13 d, reaching the highest level at 14 d (46.4 U/L) of fermentation (Fig. 2a). In SMY cultures, Lcc was produced after 3 d, showing an increase after 5 d, having the highest activity at 10 d (3110 U/L) (Fig. 2b).

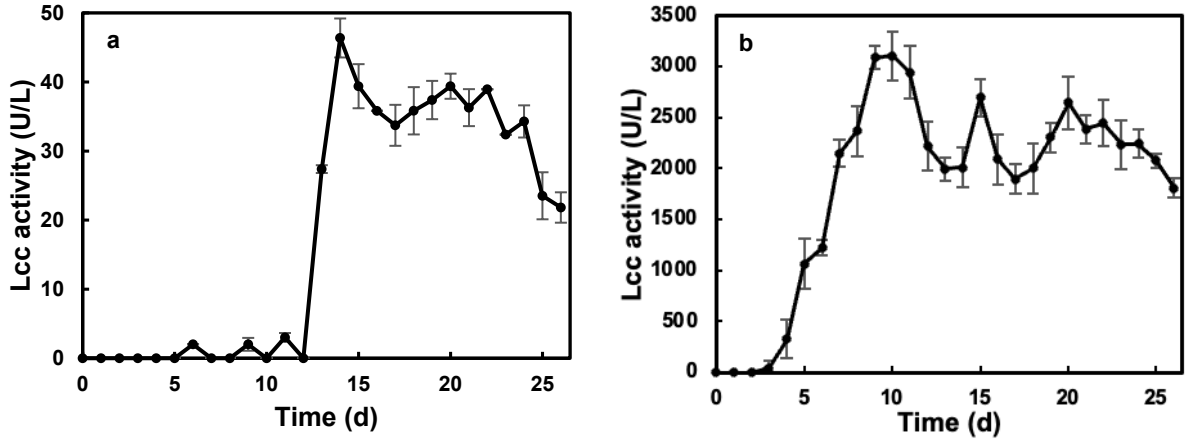

Fig. 2. Laccases activity of *P. ostreatus* grown in glucose medium (a) and in SMY medium (b) in submerged fermentation

An *et al.* (2021) reported that *P. ostreatus* grown on *Populus beijingensis* residues showed a Lcc activity of 304 U/L in solid-state fermentation, which is a lower activity than the reported in the present research in SMY. In addition, Ergun and Urek (2017) observed that *P. ostreatus* had a Lcc production of 6710 U/L using potato residues in solid-state fermentation. Furthermore, Bakratsas *et al.* (2024) found that *P. ostreatus* had a Lcc

activity of 74000 U/L in a medium containing 20% wine lees, glucose, and yeast extract in liquid fermentation. In addition, Deshmukh *et al.* (2025) reported that *P. ostreatus* had a Lcc activity of 2040 U/L in a medium added with sucrose, yeast extract, and malt extract, and it also showed a Lcc activity of 23900 U/L in a medium supplemented with cane molasses, corn steep liquor, yeast extract, and inducers. It is shown that agro-industrial residues and lignocellulosic materials induce laccase production, which is attributed to the components present in these materials (Mejía and Albertó 2012; Tozluoğlu *et al.* 2015).

MnP production by *P. ostreatus* in glucose medium and in SMY medium is shown in Fig. 3. In glucose medium, *P. ostreatus* showed a low MnP activity, which varied during the fermentation, having the highest MnP production (213 U/L) at 8 d (Fig. 3a). In SMY cultures, MnP activity increased continuously, showing the highest activity (4080 U/L) at 10 d, and then decreased at the end of the fermentation (Fig. 3b). Ergun and Urek (2017) reported that *P. ostreatus* had a MnP activity of 2050 U/L in a medium containing fresh potato peel in solid-state fermentation. Whereas *Pleurotus eryngii* showed a MnP activity of 4770 U/L in a medium added with peach peel residues (Akpinar and Urek 2022). It is suggested that lignin-based materials, which had phenolic compounds are crucial for the induction of MnP in this fungus.

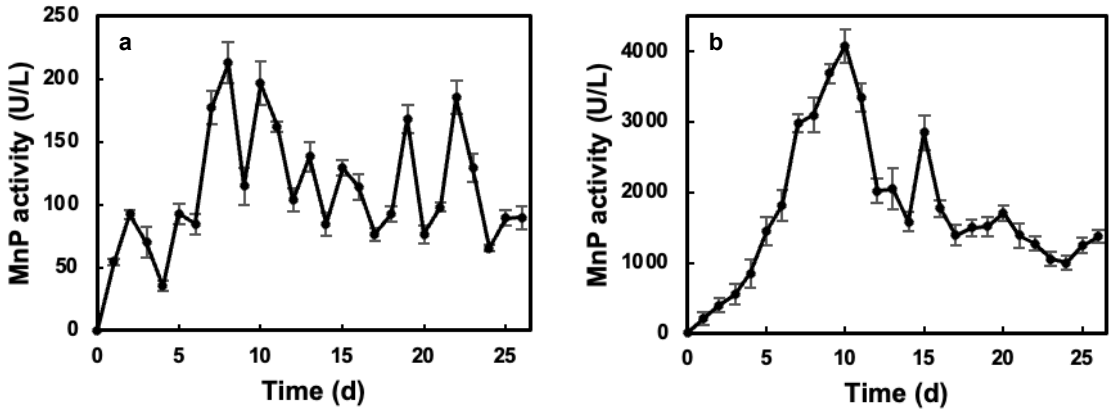

Fig. 3. Manganese peroxidase activity of *P. ostreatus* grown in glucose medium (a) and in SMY medium (b) in submerged fermentation

Figure 4 shows the LiP production by *P. ostreatus* grown in glucose medium and in SMY medium. In glucose medium, *P. ostreatus* produced a low activity of LiP, exhibiting its maximum activity (143.4 U/L) at 12 d, which then decreased at the end of the fermentation (Fig. 4a). In SMY cultures, this fungus showed low LiP activity during the first 9 d of growth, reaching the highest activity (1560 U/L) at 10 d of growth, which decreased at the end of the fermentation (Fig. 4b). The present results showed a better performance of LiP production by *P. ostreatus* in SMY medium as compared to other studies. For example, Ergun and Urek (2017) reported that *P. ostreatus* had a LiP activity of 231 U/L when grown in a medium added with potato peel residues in solid-state fermentation. Whereas *Inonotus obliquus* showed a maximum LiP activity of 123 U/mL in a medium containing wheat straw under submerged fermentation conditions (Xu *et al.* 2017).

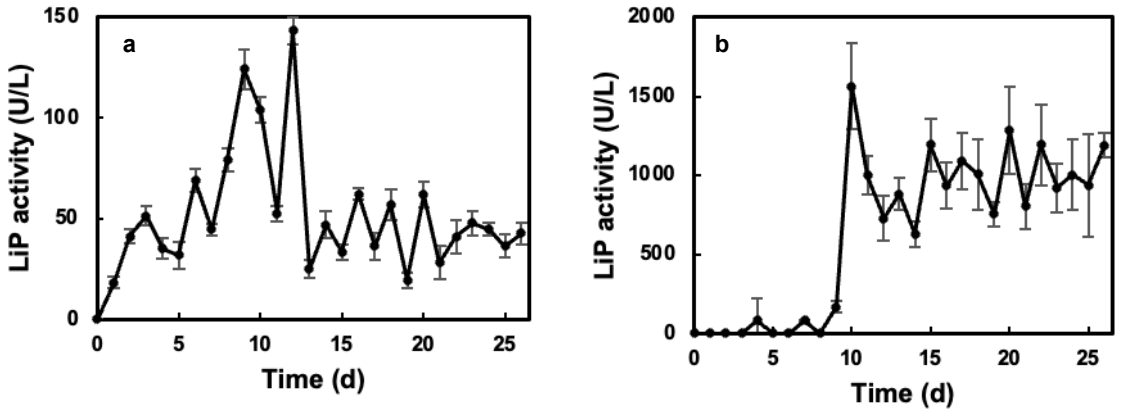

Fig. 4. Lignin peroxidase activity of *P. ostreatus* grown in glucose medium (a) and SMY medium (b) in submerged fermentation

Figure 5 shows the UnP production by *P. ostreatus* grown in glucose medium and in SMY medium. This fungus showed low and changing UnP activity during the fermentation, reaching the highest activity (112 U/L) at 15 d, which then decreased to 87 U/L at the end of the fermentation in medium added with glucose. In SMY medium, *P. ostreatus* produced low UnP activity during the first 7 d of growth, which increased to 1880 U/L at 10 d and then decreased to 500 U/L approximately at 16 d, reaching 1800 U/L at the end of the fermentation. The present results indicated that the composition of SMY medium significantly enhanced the UnP activity in *P. ostreatus*, which was higher than that activity (331 U/L) reported for *A. aegerita* grown in vinasse-supplemented medium after 23 d (González-Rodríguez *et al.* 2023), and that activity (530 U/L) showed by *Chaetomium globosum* in a rich glucose-based medium after 21 d (Kiebist *et al.* 2017).

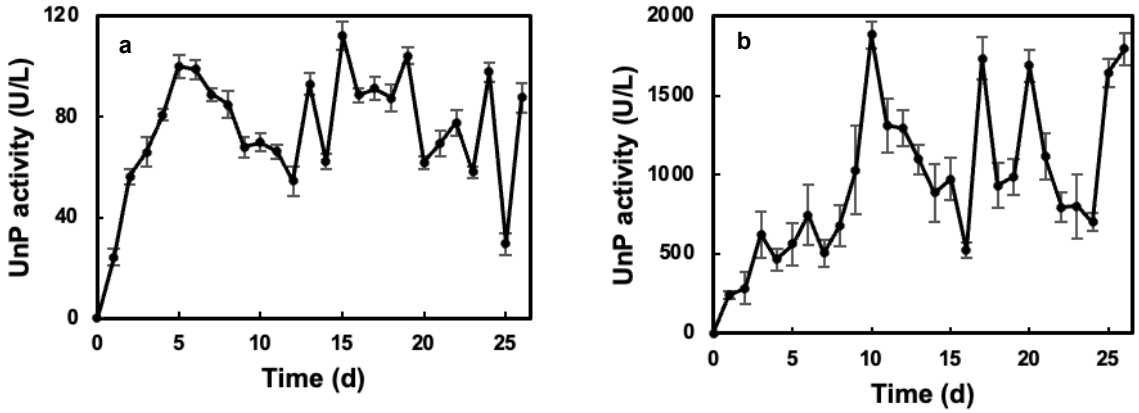

Fig. 5. Unspecific peroxygenase activity of *P. ostreatus* grown in glucose medium (a) and in SMY medium (b) in submerged fermentation

Enzymatic Parameters of Lcc, MnP, LiP and UnP

The enzymatic parameters E_{max}, $Y_{E/X}$, P_{RO}, and q_p were evaluated for Lcc, MnP, LiP and UnP (Table 1). *P. ostreatus* showed the greatest enzymatic parameters in the SMY medium for all enzymes. In SMY medium, this fungus presented the highest E_{max}, $Y_{E/X}$, P_{RO}, and q_p values for MnP (4082 U/L, 260 U/gX, 17 U/L/h, and 162.5 (U/gX/h), respectively), followed by Lcc, UnP, and LiP. In glucose medium, *P. ostreatus* also exhibited the highest E_{max}, $Y_{E/X}$, P_{RO}, and q_p values for MnP (213.1 U/L, 71 U/gX, 1.1

U/L/h, and 17.7 U/gX/h, respectively), followed by UnP, LiP, and Lcc. In fact, E_{max} for Lcc, MnP, LiP and UnP were 67-,19-,11-, and 17-fold greater in SMY than in the control. These results show that *P. ostreatus* constitutively produced all the studied enzymes, and the presence of wheat straw infusion components such as lignin (probably traces of low molecular weight lignin) and phenolic compounds (Mejía and Albertó 2012; Tozluoğlu *et al.* 2015) in SMY induced enzyme production. González-Márquez and Sánchez (2024) evaluated Lcc enzymatic parameters for both *P. ostreatus* and *A. aegerita* grown in a malt extract medium and found higher E_{max} (106.5 U/L), $Y_{E/X}$ (11.6 U/gX), P_{RO} (0.34 U/L/h) and q_p (0.25 U/gX/h) for *P. ostreatus* than for *A. aegerita.* However, *P. ostreatus* and *A. aegerita* did not show MnP, LiP, and UnP activities in malt extract medium (González-Márquez and Sánchez, 2024). It is shown that SMY is a more favorable medium for producing Lcc, MnP, LiP and UnP than malt extract medium.

Table 2. Enzymatic Yield Parameters of *P. ostreatus* Grown in Glucose Medium and in SMY Medium in Submerged Fermentation Conditions

Enzymatic Parameters	Culture Media	
	Glucose	SMY
Laccases		
E_{max} (U/L)	46.37^{b} (2.82)	3106.18^{a} (238.27)
$Y_{E/X}$ (U/gX)	15.45^{b} (1.82)	197.84^{a} (12.27)
P_{RO} (U/L/h)	0.13^{b} (0.001)	12.94^{a} (3.84)
q_p (U/gX/h)	3.84^{b} (0.68)	123.65^{a} (11.28)
Manganese peroxidases		
E_{max} (U/L)	213.11^{b} (16.49)	4081.96^{a} (236.92)
$Y_{E/X}$ (U/gX)	71.03^{b} (5.24)	259.99^{a} (30.52)
P_{RO} (U/L/h)	1.11^{b} (0.15)	17.01^{a} (2.94)
q_p (U/gX/h)	17.69^{b} (1.84)	162.50^{a} (21.27)
Lignin peroxidases		
E_{max} (U/L)	143.37^{b} (6.59)	1563.92^{a} (273.37)
$Y_{E/X}$ (U/gX)	47.79^{b} (4.92)	99.61^{a} (7.95)
P_{RO} (U/L/h)	0.50^{b} (0.008)	6.52^{a} (0.99)
q_p (U/gX/h)	11.90^{b} (2.85)	62.26^{a} (12.21)
Unspecific peroxigenases		
E_{max} (U/L)	112.18^{b} (5.56)	1882.82^{a} (84.48)
$Y_{E/X}$ (U/gX)	37.39^{b} (2.48)	119.92^{a} (15.93)
P_{RO} (U/L/h)	0.31^{b} (0.002)	7.84^{a} (1.05)
q_p (U/gX/h)	9.31^{b} (1.83)	74.95^{a} (14.96)

Data are shown as mean of three independent experiments. The results presented in parentheses represent the standard deviation. Different superscript letters within the same row indicate significant differences (ANOVA, Tukey's test, $p < 0.05$). E_{max}, maximum enzyme activity; $Y_{E/X}$, enzyme yield; P_{RO}, enzyme productivity; q_p, specific rate of enzyme production.

CONCLUSIONS

1. Agro-industrial by-products are rich in nutrients that can enhance the growth of *P. ostreatus*.
2. *P. ostreatus* constitutively produced Lcc, MnP, LiP and UnP; however, the presence of wheat straw infusion components in SMY enhanced enzyme production.

3. The highest Lcc, MnP, LiP and UnP activities were observed at that time to which the maximum biomass was achieved (10 d) in SMY medium.
4. SMY is a more favorable medium for producing Lcc, MnP, LiP, and UnP than glucose medium.

ACKNOWLEDGMENTS

The authors express their sincere gratitude to the Secretariat of Science, Humanities, Technology and Innovation (SECIHTI) of Mexico for granting a postdoctoral fellowship to AGM (No. 555469) and a PhD scholarship to ADAA (No. 1170027).

Conflict of Interest

The authors declare that they have no conflicts of interest.

REFERENCES CITED

Ahuactzin-Pérez, M., Tlecuitl-Beristain, S., García-Dávila, J., González-Pérez, M., Gutiérrez-Ruíz, M. C., and Sánchez, C. (2016). "Degradation of di(2-ethyl hexyl) phthalate by *Fusarium culmorum*: Kinetics, enzymatic activities and biodegradation pathway based on quantum chemical modeling," *Sci. Total Environ.* 566-567, 1186-1193. https://doi.org/10.1016/j.scitotenv.2016.05.169

Akpinar, M., and Urek, R. O. (2022). "Direct utilization of peach wastes for enhancements of lignocellulolytic enzyme productions by *Pleurotus eryngii* under solid-state fermentation conditions," *Chem. Pap.* 76(11), 6699-6712. https://doi.org/10.1007/s11696-022-02356-0

An, Q., Liu, Z. Y., Wang, C. R., Yang, J., Chen, S. Y., Chen, X., Zhang, Y. J., Bian, L., and Han, M. L. (2021). "Laccase activity from *Pleurotus ostreatus* and *Flammulina velutipes* strains grown on agro- and forestry residues by solid-state fermentation," *BioResources* 16(4), 7337-7354. https://doi.org/10.15376/biores.16.4.7337-7354

Andrade-Alvarado, A. D., González-Mota, R., González-Márquez, A., and Sánchez, C. (2024). "Biodegradation of low-density polyethylene films commercially labeled as biodegradable pretreated with UV-B radiation by *Pleurotus ostreatus* grown in liquid fermentation," *Mex. J. Biotechnol.* 9(4), 52-71. https://doi.org/10.29267/mxjb.2024.9.4.52

Argyropoulos, D., Psallida, C., Sitareniou, P., Flemetakis, E., and Diamantopoulou, P. (2022). "Biochemical evaluation of *Agaricus* and *Pleurotus* strains in batch cultures for production optimization of valuable metabolites," *Microorganisms* 10(5), article 964. https://doi.org/10.3390/microorganisms10050964

Bakratsas, G., Antoniadis, K., Athanasiou, P. E., Katapodis, P., and Stamatis, H. (2024). "Laccase and biomass production via submerged cultivation of *Pleurotus ostreatus* using wine lees," *Biomass* 4(1), 1-22. https://doi.org/10.3390/biomass4010001

Bakratsas, G., Polydera, A., Nilson, O., Chatzikonstantinou, A. V., Xiros, C., Katapodis, P., and Stamatis, H. (2023). "Mycoprotein production by submerged fermentation of the edible mushroom *Pleurotus ostreatus* in a batch stirred tank bioreactor using agro-

industrial hydrolysate," *Foods* 12(12), article 2295. https://doi.org/10.3390/foods12122295

Basera, P., Chakraborty, S., and Sharma, N. (2024). "Lignocellulosic biomass: Insights into enzymatic hydrolysis, influential factors, and economic viability," *Discov. Sustain.* 5, article 311. https://doi.org/10.1007/s43621-024-00543-5

Bettin, F., Da Rosa, L. O., Montanari, Q., Calloni, R., Gaio, T. A., Malvessi, E., Da Silveira, M. M., and Dillon, A. J. P. (2010). "Growth kinetics, production, and characterization of extracellular laccases from *Pleurotus sajor-caju* PS-2001," *Process Biochem.* 46(3), 758-764. https://doi.org/10.1016/j.procbio.2010.12.002

Corbu, V. M., Gheorghe-Barbu, I., Dumbravă, A. Ș., Vrâncianu, C. O., and Șesan, T. E. (2023). "Current insights in fungal importance – A comprehensive review," *Microorganisms* 11(6), article 1384. https://doi.org/10.3390/microorganisms11061384

Deshmukh, A., Sattikar, P., Sukhatankar, A., Wakade, G., Kumbhar, P., and Kommoju, P. (2025). "Optimized laccase production from the white rot fungi *Pleurotus ostreatus* and *Trametes versicolor*," *Protein Expr. Purif.* 237, article 106813. https://doi.org/10.1016/j.pep.2025.106813

Ergun, S. O., and Urek, R. O. (2017). "Production of ligninolytic enzymes by solid-state fermentation using *Pleurotus ostreatus*," *Ann. Agrar. Sci.* 15(2), 273-277. https://doi.org/10.1016/j.aasci.2017.04.003

González-Márquez, A., and Sánchez, C. (2024). "Mycelial growth and production of laccase and peroxidases by *Pleurotus ostreatus* and *Agrocybe aegerita* in liquid fermentation," *Mex. J. Biotechnol.* 9(3), 36-49. https://doi.org/10.29267/mxjb.2024.9.3.36

González-Márquez, A., Andrade-Alvarado, A. D., González-Mota, R., and Sánchez, C. (2024). "Enhanced degradation of phototreated recycled and unused low-density polyethylene films by *Pleurotus ostreatus*," *World J. Microbiol. Biotechnol.* 40(10), article 309. https://doi.org/10.1007/s11274-024-04116-6

González-Rodríguez, S., Trueba-Santiso, A., Lu-Chau, T. A., Moreira, M. T., and Eibes, G. (2023). "Valorization of bioethanol by-products to produce unspecific peroxygenase with *Agrocybe aegerita*: Technological and proteomic perspectives," *New Biotechnol.* 76, 63-71. https://doi.org/10.1016/j.nbt.2023.05.001

Hernández-Domínguez, E. M., Sánchez, C., and Díaz-Godínez, G. (2017). "Production of laccases, cellulases and xylanases of *Pleurotus ostreatus* grown in liquid-state fermentation," *Mex. J. Biotechnol.* 2(2), 169-176. https://doi.org/10.29267/mxjb.2017.2.2.169

Huang, Y., Sha, J., Zhang, J., and Zhang, W. (2024). "Challenges and perspectives in using unspecific peroxygenases for organic synthesis," *Front. Catal.* 4, article 1470616. https://doi.org/10.3389/fctls.2024.1470616

Huang, Y., and Yang, J. (2022). "Kinetics and mechanisms for sulfamethoxazole transformation in the phenolic acid-laccase (*Trametes versicolor*) system," *Environ. Sci. Pollut. Res.* 29, 62941-62951. https://doi.org/10.1007/s11356-022-20281-3

Karich, A., Ullrich, R., Scheibner, K., and Hofrichter, M. (2017). "Fungal unspecific peroxygenases oxidize the majority of organic EPA priority pollutants," *Front. Microbiol.* 8, article 1463. https://doi.org/10.3389/fmicb.2017.01463

Kiebist, J., Schmidtke, K., Zimmermann, J., Kellner, H., Jehmlich, N., Ullrich, R., Zänder, D., Hofrichter, M., and Scheibner, K. (2017). "A peroxygenase from *Chaetomium globosum* catalyzes the selective oxygenation of testosterone,"

ChemBioChem 18(6), 563-569. https://doi.org/10.1002/cbic.201600677

Kumar, A., and Arora, P. K. (2022). "Biotechnological applications of manganese peroxidases for sustainable management," *Front. Environ. Sci.* 10, article 875157. https://doi.org/10.3389/fenvs.2022.875157

Martínez-Berra, C., Díaz, R., Sánchez-Minutti, L., and Díaz-Godínez, G. (2018). "Biodegradation of azo dyes by *Pleurotus ostreatus*," *Mex. J. Biotechnol.* 3(1), 43-59. https://doi.org/10.29267/mxjb.2018.3.1.43

Mejía, S. J., and Albertó, E. (2012). "Heat treatment of wheat straw by immersion in hot water decreases mushroom yield in *Pleurotus ostreatus*," *Rev. Iberoam. Micol.* 30(2), 125-129. https://doi.org/10.1016/j.riam.2012.11.004

Ocaña-Romo, E., Rodríguez-Nava, C. O., and Sánchez, C. (2024). "Oxidases production by *Trametes versicolor* grown on green waste and on polyurethane foam in solid-state fermentation: A comparative study," *Mex. J. Biotechnol.* 9(2), 51-64. https://doi.org/10.29267/mxjb.2024.9.2.51

Othman, A. M., Mechichi, T., Chowdhary, P., and Suleiman, W. B. (2023). "Editorial: Ligninolytic enzymes and their potential applications," *Front. Microbiol.* 14, article 1235206. https://doi.org/10.3389/fmicb.2023.1235206

Papaspyridi, L., Katapodis, P., Gonou-Zagou, Z., Kapsanaki-Gotsi, E., and Christakopoulos, P. (2010). "Optimization of biomass production with enhanced glucan and dietary fibres content by *Pleurotus ostreatus* ATHUM 4438 under submerged culture," *Biochem. Eng. J.* 50(3), 131-138. https://doi.org/10.1016/j.bej.2010.04.008

Ríos-González, N. S., González-Márquez, Á., and Sánchez, C. (2019). "Growth and esterase activity of *Fusarium culmorum* grown in di(2-ethyl hexyl) phthalate in liquid fermentation," *Mex. J. Biotechnol.* 4(1), 51-60. https://doi.org/10.29267/mxjb.2019.4.1.51

Sánchez, C. (2009). "Lignocellulosic residues: Biodegradation and bioconversion by fungi," *Biotechnol. Adv.* 27(2), 185-194. https://doi.org/10.1016/j.biotechadv.2008.11.001

Sánchez, C. (2010). "Cultivation of *Pleurotus ostreatus* and other edible mushrooms," *Appl. Microbiol. Biotechnol.* 85(5), 1321-1337. https://doi.org/10.1007/s00253-009-2343-7

Sánchez, C., Moore, D., Robson, G., and Trinci, T. (2020). "21st century miniguide to fungal biotechnology," *Mex. J. Biotechnol.* 5(1), 11-42. https://doi.org/10.29267/mxjb.2020.5.1.11

Singh, A. K., Bilal, M., Iqbal, H. M. N., and Raj, A. (2021). "Lignin peroxidase in focus for catalytic elimination of contaminants – A critical review on recent progress and perspectives," *Int. J. Biol. Macromol.* 177, 58-82. https://doi.org/10.1016/j.ijbiomac.2021.02.032

Tozluoğlu, A., Özyurek, Ö., Çöpür, Y., and Özdemir, H. (2015). "Integrated production of biofilm, bioethanol, and papermaking pulp from wheat straw," *BioResources* 10(4), 7834-7853. https://doi.org/10.15376/biores.10.4.7834-7853

Xu, X., Xu, Z., Shi, S., and Lin, M. (2017). "Lignocellulose degradation patterns, structural changes, and enzyme secretion by *Inonotus obliquus* on straw biomass under submerged fermentation," *Bioresour. Technol.* 241, 415-423. https://doi.org/10.1016/j.biortech.2017.05.087

Zárate-Salazar, J. R., Santos, M. N., Caballero, E. N. M., Martins, O. G., and Herrera, A. A. P. (2020). "Use of lignocellulosic corn and rice wastes as substrates for oyster mushroom (*Pleurotus ostreatus* Jacq.) cultivation," *SN Appl. Sci.* 2, article 1904. https://doi.org/10.1007/s42452-020-03720-z

Article submitted: October 6, 2025; Peer review completed: November 22, 2025; Revised version received: November 28, 2025; Accepted: November 30, 2025; Published: December 10, 2025.
DOI: 10.15376/biores.21.1.839-850