ENZYME SCREENING FROM *Pleurotus* spp. STRAINS UNDER SUBMERGED CONDITIONS AS A BASIS FOR FUTURE AGRO-WASTE BIOCONVERSION STUDIES

Doan Chi Cuong[1,*], Munehiro Tanaka[2], Vo Chau Tuan[1], Bui Duc Thang[1], Nguyen Thi Bich Hang[1]

[1] *Faculty of Biology, Agriculture and Environmental Science, University of Science and Education, The University of Danang*

[2] *Faculty of Agriculture, Saga University, Japan*

[*] *Email: dccuong@ued.udn.vn*

ABSTRACT

Bioconversion efficiency of agricultural waste into value-added products relies on robust microbial strains capable of producing a broad of extracellular enzymes. Among edible mushrooms, *Pleurotus* species are recognized for their enzymatic diversity and potential in solid-state fermentation processes. This study aimed to evaluate and compare the extracellular enzymatic profiles of four *Pleurotus* species, including *P. ostreatus*, *P. pulmonarius*, *P. djamor*, and *P. citrinopileatus* to identify the most suitable strain for agro-waste bioconversion. Each species was cultivated under identical conditions and the activities of four key enzymes (laccase, cellulase, protease, and chitinase) were quantified using standardized colorimetric assays. Statistical analysis was performed using one-way ANOVA followed by Tukey's HSD test ($p<0.05$). The results showed that *P. pulmonarius* exhibited the most balanced enzymatic profile, with high laccase (59.9 ± 5.5 U/mL) and cellulase (5.39 ± 0.38 U/mL) activities, moderate protease activity (14.4 ± 3.4 U/mL) and strong chitinase activity (7.59 ± 0.85 U/mL). Unlike other species that showed specialization in one or two enzymes, *P. pulmonarius* performed consistently across all categories, enabling it to degrade lignin, cellulose, proteins and chitin. Hence, *P. pulmonarius* is identified as the optimal candidate for integrated agro-waste valorization via SSF, offering significant potential for applications in circular bioeconomy frameworks and sustainable bioprocess development.

Keywords: *Agro-waste, bioconversion, enzyme activity, enzyme screening, Pleurotus* spp.

Received: 10 September 2025; revised: 6 November 2025; accepted: 2 December 2025.

1. INTRODUCTION

The increasing global demand for sustainable agricultural practices has encouraged a transition toward circular bioeconomy models that emphasize waste minimization and biomass valorization. Agricultural residues, which are generated in vast quantities worldwide, represent an abundant resource of carbon-based polymers such as lignocellulose and chitin. Mismanagement of these residues contributes significantly to environmental degradation through greenhouse gas emissions, soil nutrient imbalances and water pollution. Therefore, developing eco-friendly and cost-effective bioconversion strategies to transform agro-waste into high-value products are important [1].

Solid-state fermentation (SSF) has been recognized as a viable biotechnological platform for the transformation of agricultural byproducts into valuable compounds due to its low moisture requirements, high productivity, and compatibility with filamentous fungi [2]. Among these fungi,

members of the *Pleurotus* genus (oyster mushrooms) are of interest due to their fast growth, adaptability to diverse lignocellulosic substrates, and high yield of extracellular enzymes [3]. These mushrooms produce an extensive range of hydrolytic and oxidative enzymes, including laccase, cellulase, protease and chitinase, which decompose the complex organic matter such as straw, husks, bran and seafood waste [4, 5].

Laccases are multi-copper oxidase that catalyzes the oxidation of phenolic and non-phenolic substrates, playing a central role in lignin depolymerization. Due to broad substrate specificity, laccases have applications in paper pulping, dye decolorization, wastewater treatment and biosensor development [6]. Cellulases are a group of synergistic enzymes that hydrolyze cellulose into glucose, making them essential for biomass saccharification, bioethanol production and animal feed processing [7]. Proteases break down proteinaceous materials into peptides and amino acids and have important roles in organic waste valorization, feed enhancement, and pharmaceuticals [8]. Chitinases degrade chitin, a major component of crustacean shells and fungal cell walls, contributing to waste management, antifungal biocontrol and biomedical applications [9].

While the enzyme-producing potential of *Pleurotus* spp. is well documented with numerous advantages regarding enzyme production levels, substrate preferences and environmental tolerance. Recent studies have screened wild and commercial *Pleurotus* strains to identify those with superior enzyme productivity. For example, Mikiashvili *et al.* (2006) demonstrated strain-dependent variability in cellulase and laccase production when *P. ostreatus* grew on different lignocellulosic substrates [10]. Kachlishvili *et al.* (2006) observed that *P. eryngii* and *P. pulmonarius* exhibited distinct enzymatic profiles under SSF, underscoring the need for targeted screening and selection [11]. However, studies on investigating multi-enzyme production, particularly for combinations of laccase, protease, cellulase and chitinase, which together enable the comprehensive degradation of mixed agro-waste streams are still limited. Moreover, the majority of enzyme-related studies focus on enzyme extraction for industrial use rather than in situ application of enzyme-producing fungi in SSF systems. Utilizing native *Pleurotus* strains that exhibit high enzymatic activity in SSF could enhance the efficiency of agro-waste bioconversion while reducing processing costs. Such approaches not only yield nutrient-enriched fermented products but also align with sustainability goals through circular material flows [12, 13].

Herein, the present study aims to isolate and characterize native *Pleurotus* strains capable of producing high levels of laccase, protease, cellulase and chitinase. The selected strains were also evaluated for their suitability in SSF of agricultural residues, which are rich in lignocellulose and protein/chitin components but remain largely underutilized in many developing countries. This research supports the principles of circular agriculture, which promotes the reuse and recycling of organic matter within agricultural systems. Incorporating enzyme-rich fungi into SSF offers a practical, low-cost and decentralized strategy for managing waste, improving soil health and producing feed additives or biofertilizers. It also contributes to local bioeconomy development, particularly in regions where agricultural residues and seafood byproducts are plentiful but poorly exploited [14].

2. MATERIALS AND METHODS

2.1. Materials

The strains *P. ostreatus*, *P. pulmonarius*, *P. djamor* and *P. citrinopileatus* were purchased from the Lam Dong Center for Science and Technology Application (Hamlet 5, Cam Ly Ward, Da Lat city, Lam Dong province). After acquisition, the strains were reactivated, cultivated to produce fruiting bodies and subsequently re-isolated to ensure purity. Strain identities were confirmed through ITS region sequencing using primers ITS1/ITS4. BLASTn comparison against GenBank revealed > 99% sequence similarity to the corresponding *Pleurotus* species, which was further supported by morphological observations.

Then *P. pulmonarius*, *P. citrinopileatus*, *P. ostreatus* and *P. djamor* were cultured and preserved at the Mushroom Technology Laboratory, Faculty of Biology. Agriculture and Environment Science, University of Science and Education, The University of Danang.

2.2. Propagation on agar and liquid media

After pouring the prepared agar medium into test tubes in appropriate volumes, sterilization is carried out at 121°C for 20 minutes. The tubes are then allowed to cool before inoculation. The fungal cultures are subsequently incubated at 25 - 28°C for 5 to 7 days. Primary spawn of *Pleurotus* was propagated in glass test tubes (F1, Ø8 cm, 180 mm) containing a medium composed of potato extract (200 g/L), glucose (20 g/L) and agar (15 g/L) and incubated at 27°C for 10 days. Subsequently, mycelial cultivation experiments were conducted by transferring 150 mL of PDB+ medium (composed of potato extract: 200 g; glucose: 20 g; yeast extract: 2 g; peptone: 2 g; distilled water: 1 L) into 250 mL Erlenmeyer flasks. The media were sterilized using autoclaving at 121°C for 30 minutes. After cooling, equal amounts of mycelial inoculum from the primary spawn tubes were transferred into each flask. The cultures were incubated on a rotary shaker at 150 rpm, at a temperature ranging from 25 - 27°C.

2.3. Determination of laccase activity

Laccase activity was determined spectrophotometrically by measuring the rate of oxidation of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), a commonly used chromogenic substrate. The assay was conducted according to the method described by Niku-Paavola *et al.* (1990) with slight modifications. The standard reaction mixture (total volume: 3.0 mL) consisted of: 2.7 mL of 100 mM sodium acetate buffer (pH 4.5), 0.2 mL of 1.0 mM ABTS solution, and 0.1 mL of appropriately diluted enzyme sample [15]. The reaction was initiated by the addition of the enzyme extract and monitored by the increase in absorbance at 420 nm using Shimadzu UV-1800UV-Vis spectrophotometer. The oxidation of ABTS by laccase results in the formation of a green-colored cation radical (ABTS$\bullet^+$), which exhibits strong absorbance at 420 nm (ε = 36,000 $M^{-1}cm^{-1}$). 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 equation (1):

$$\text{LacA}\left(\frac{\text{U}}{\text{mL}}\right) = \frac{\Delta A_{420} \times V_{total}}{\varepsilon \times d \times V_{enzyme} \times T} \quad (1)$$

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); T: incubation time (minutes).

2.4. Determination of protease activity

Protease activity was determined based on the method recommended by Sigma-Aldrich, which measures the hydrolysis of casein as a substrate under alkaline conditions. The assay quantifies the release of soluble peptides and amino acids, particularly tyrosine, which remain in solution after precipitation of undigested proteins with trichloroacetic acid (TCA). This method is widely used for estimating general proteolytic activity in crude enzyme extracts. The standard reaction mixture contained: 2.5 mL of 0.65% (w/v) casein solution prepared in 50 mM glycine-NaOH buffer (pH 9.0); 0.5 mL of appropriately diluted enzyme extract [16]. The reaction was initiated by adding the enzyme solution to the prewarmed substrate mixture and incubated at 37°C for 10 minutes. The enzymatic reaction was terminated by the addition of 2.5 mL of 110 mM trichloroacetic acid (TCA) to precipitate the undigested casein. The mixture was then allowed to stand for 30 minutes at room temperature to ensure complete precipitation, followed by centrifugation at 10,000 g for 10 minutes. The absorbance of the supernatant was measured at 280 nm against a reagent blank using Shimadzu UV-1800UV-Vis spectrophotometer. One unit (U) of protease activity was defined as the amount of enzyme that liberates 1 µmol of

tyrosine equivalent per minute under the assay conditions. Protease activity (U/mL) was calculated using the equation (2):

$$\text{ProA}\left(\frac{\text{U}}{\text{mL}}\right) = \frac{\Delta A_{280} \times V_{total}}{\varepsilon \times d \times V_{enzyme} \times T} \quad (2)$$

Where: ΔA_{280}: change in absorbance per minute at 280 nm; V_{total}: total reaction volume (mL); ε: extinction coefficient for tyrosine at 280 nm (~1.1 mLU'µmol^{-1}U'cm^{-1}); d: path length of the cuvette (1 cm); V_{enzyme}: volume of enzyme extract added (mL); T: incubation time (minutes).

2.5. Determination of cellulase activity

Cellulase activity was determined using the filter paper assay described by Ghose (1987), which is the internationally recognized standard protocol for measuring total cellulase activity. This method estimates the amount of reducing sugars released from a standardized strip of filter paper by the action of cellulase enzymes under defined conditions [17]. The assay mixture contained: 1.0 mL of appropriately diluted enzyme solution; 1.0 mL of 0.05 M citrate buffer (pH 4.8); 1 pre-weighed strip of Whatman No. 1 filter paper (50 mg). The reaction was initiated by immersing the filter paper into the reaction tube and incubating at 50°C for 60 minutes in a water bath. After incubation, the reaction was terminated by adding 3.0 mL of DNS reagent to the tube and placing it in a boiling water bath for 5 minutes to develop color. After cooling to room temperature, the absorbance of the reaction mixture was measured at 540 nm using Shimadzu UV-1800UV-Vis spectrophotometer. The amount of reducing sugar released was determined by comparing to a glucose standard curve. One unit (U) of cellulase activity is defined as the amount of enzyme required to release 2.0 mg of glucose equivalents from filter paper in 60 minutes under the specified assay conditions. If the released glucose is less than 2.0 mg, the enzyme is diluted further and the cellulase activity is calculated using the equation (3):

$$\text{CellA}\left(\frac{\text{U}}{\text{mL}}\right) = 0.37 \times \frac{reducing\ sugar\ (mg)}{\text{volume of enzyme used (mL)}} \quad (3)$$

Where: The factor (0.37) is a constant derived from the IUPAC standard that normalizes the unit to the amount of enzyme that releases exactly 2.0 mg of glucose in 60 minutes under assay conditions. The reducing sugar released (in mg) is calculated using a glucose standard curve, typically via DNS assay (absorbance at 540 nm). The volume of enzyme used is usually 0.5 or 1.0 mL.

2.6. Determination of chitinase activity

Chitinase activity was assayed by quantifying the amount of N-acetyl-β-D-glucosamine (GlcNAc) released from colloidal chitin as substrate during enzymatic hydrolysis. The method is based on colorimetric detection of reducing sugars using the 3,5-dinitrosalicylic acid (DNS) reagent and is adapted from established protocols with modifications to increase specificity for chitinolytic activity [18]. Colloidal chitin was prepared by slowly adding 10 g of purified chitin powder to 100 mL of cold concentrated HCl (12 N) under constant stirring at 4°C. After overnight incubation, the mixture was filtered and washed repeatedly with distilled water until the filtrate reached neutral pH. The final colloidal chitin suspension was stored at 4°C and adjusted to 1% (w/v) concentration before use. The standard reaction mixture (final volume: 1.0 mL) contained: 0.5 mL of 1% (w/v) colloidal chitin in 50 mM sodium acetate buffer (pH 5.0); 0.4 mL of 50 mM sodium acetate buffer (pH 5.0); and 0.1 mL of enzyme extract.

The reaction mixture was incubated at 37°C for 60 minutes. The enzymatic reaction was terminated by adding 1.0 mL of DNS reagent, followed by boiling the tubes in a water bath for 5 minutes to color development. After cooling to room temperature, the absorbance was measured at 540 nm using Shimadzu UV-1800UV-VIS spectrophotometer. The amount of reducing sugar released was determined using a GlcNAc standard curve (ranging from 0 to 500 µM). The GlcNAc standard curve regression equation: y = 0.0003x - 0.0317, with R^2 = 0.99. One unit (U) of chitinase activity is defined as the amount of enzyme required to release 1 µmol of GlcNAc per minute

under the assay conditions, the chitinase activity is calculated using the equation (4):

$$ChiA\left(\frac{U}{mL}\right) = \frac{C \times K \times V_{total}}{t \times V_{enzyme} \times 10^3} \quad (4)$$

Where: C: Concentration of GlcNAc released (µM), determined from the GlcNAc standard curve using the absorbance at 540 nm; K: dilution factor; 10^3: conversion factor from L to mL; V_{total}: total reaction volume (mL), typically 1.0 mL; t: incubation time (min), typically 60 min; V_{enzyme}: volume of enzyme extract used (mL), typically 0.1 mL.

2.7. Data analysis

All enzyme activity assays (laccase, protease, cellulase and chitinase) were conducted in triplicate and results are expressed as mean Ơ standard deviation. Enzyme activities were calculated using appropriate equations based on the amount of product formed, as determined from standard curves prepared for each assay. Statistical analyses were performed using IBM SPSS v20. Differences in enzyme activities among fungal isolates or treatment conditions were assessed using one-way ANOVA, followed by Tukey's HSD post hoc tests to identify statistically significant differences at $p < 0.05$. Graphs and data visualizations were generated using MS Excel 365, with error bars representing standard deviations.

3. RESULTS AND DISCUSSION

3.1. Laccase activity

Laccase activity among the *Pleurotus* species showed substantial interspecies variation (Figure 1). The highest activity was recorded in *P. ostreatus* and *P. pulmonarius*, both about 60 U/mL. One-way ANOVA revealed a statistically significant difference in laccase activity among the species ($F = 1146.38$, $p < 0.001$). Post hoc analysis using Tukey's HSD indicated that *P. ostreatus* and *P. pulmonarius* belonged to the same group "a", significantly different from *P. djamor* (group "b") and *P. citrinopileatus* (group "c"). This finding aligns with trends observed by Couto and Herrera, where basidiomycetes like *Pleurotus* typically exhibit robust laccase production [6]. *P. ostreatus* and *P. pulmonarius*'s high laccase level suggests strong ligninolytic potential, positioning it aptly for pretreatment of lignin-rich substrates such as straw, husks, or wood chips, consistent with the oxidative polymer breakdown reported by Baldrian [19].

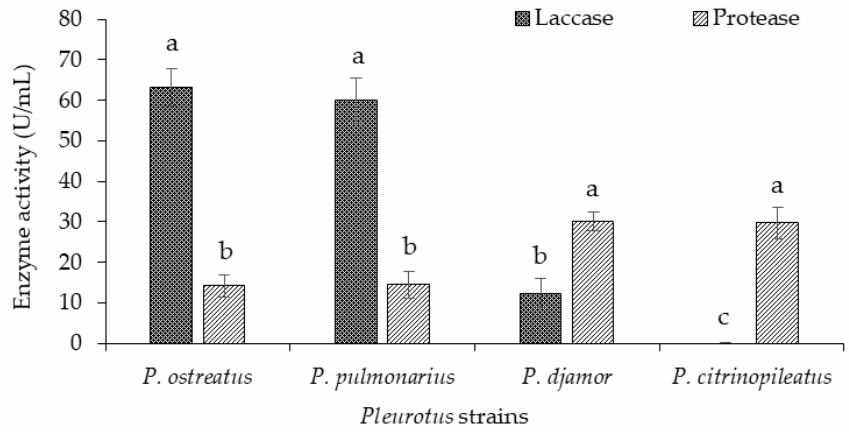

Figure 1. Comparative enzyme activities of laccase and protease among different *Pleurotus* strains (Different letters (a, b, c, d) in the same pattern chart indicate statistically significant differences at $p < 0.05$ by Tukey's HSD)

High laccase activity in *P. ostreatus* and *P. pulmonarius* holds significant promise for biomass pretreatment applications. Laccases are known to oxidize phenolic lignin substructures, reducing structural recalcitrance and enhancing enzymatic hydrolysis efficiency in downstream processes [20]. Crude laccases from both *P. ostreatus* and *P. pulmonarius* have been successfully applied to

degrade bisphenol A, demonstrating their robust oxidative capacity and potential for lignin modification [21]. Moreover, *P. ostreatus* exhibits superior laccase production on lignocellulosic substrates under solid-state fermentation, underlining its suitability for low-cost enzyme sourcing [22]. While direct comparisons of *P. pulmonarius* are limited, both species clearly function as efficient ligninolytic enzyme sources, with *P. pulmonarius* likely matching *P. ostreatus* in laccase-driven biomass deconstruction, particularly when considering its broader enzymatic capacities observed in preliminary studies [23].

3.2. Protease activity

The protease activity assay (Figure 1) demonstrated significant variation ($F = 42.62$, $p < 0.001$). *P. djamor* and *P. citrinopileatus* exhibited the highest protease activities (~30 U/mL), forming group "a" in the Tukey post hoc test. *P. pulmonarius* and *P. ostreatus* showed moderate protease activity (~14 U/mL), categorized as group "b".

In this study, general protease activities reached approximately 14 U/mL for *P. ostreatus* and *P. pulmonarius*, and around 30 U/mL for *P. djamor* and *P. citrinopileatus*. This is broadly consistent with previously reported values of Ravikumar *et al.* documented about 28 U/mL protease activity from *P. sajor-caju* fermentation on finger millet, whereas corn flour substrate produced about 45 U/mL [24]. By contrast, a study by Santana *et al.* (2024) measured a much higher fibrinolytic protease activity of 71.5 U/mL for *P. ostreatus*, though differences in assay specificity and enzyme type likely account for this discrepancy [25]. Meanwhile, an engineered *P. sajor-caju* strain (CTM10057) demonstrated an extraordinarily high output of 10,500 U/mL under optimized conditions, highlighting how strain selection and process optimization can drastically amplify enzyme yield [26]. Overall, our findings support moderate-level protease production in *Pleurotus* species under typical liquid fermentation, while also indicating the potential for markedly higher yields with targeted strain and condition enhancements.

Despite not being the highest protease producer, *P. pulmonarius* displayed consistent protease activity sufficient to hydrolyze proteinaceous biomass in mixed-substrate fermentation systems. The presence of protease within high ligninolytic and cellulolytic enzymes is beneficial for degrading composite agro-residues, where lignin and proteins often occur. This enables more holistic substrate valorization and protein enrichment of fermented materials. In addition, *P. pulmonarius* did not lead in protease production, its moderate activity is comparable to commercial strains used in fungal protein enrichment [24]. Furthermore, the presence of ligninolytic and cellulolytic enhances substrate breakdown efficiency in composite feedstock, pathogenically beneficial in strain selection for mixed agro-residue fermentation [27].

3.3. Chitinase activity

The chitinase activity of all four fungal species is presented in Figure 2. In contrast to the pronounced variability observed for the other enzymes, the chitinase activity among the strains remained relatively uniform, ranging from 7.0 to 8.0 U/mL. One-way ANOVA revealed no statistically significant differences among species ($F = 1.29$, $p > 0.05$), indicating that chitin hydrolysis capacity is a common and stable characteristic within the surveyed *Pleurotus* genus.

This quantitative assessment provides an important reference dataset and represents the first report of specific chitinase activity for *P. ostreatus*, *P. pulmonarius*, *P. djamor*, and *P. citrinopileatus* under liquid fermentation conditions. Previous literature on *Pleurotus* chitinase has been largely qualitative or focused on gene identification, with very few studies offering quantitative enzyme activity measurements. Compared with the limited earlier study on *P. eryngii* (approximately 3.57 U/mL), the values obtained here are more than twice as high, reinforcing the notion that Pleurotus species possess an inherent chitinase system with moderate basal activity. In another study involving a different fungal group, under optimized

environmental conditions, *Aspergillus sydowii* exhibited the highest reported chitinase activity, reaching 1,667 U/mL.

The consistent production of chitinase across all strains carries important practical implications. This trait enhances their potential applications, particularly for *P. pulmonarius*, the strain exhibiting a well-balanced overall enzymatic profile in the bioconversion of chitin-rich waste from the aquaculture industry (e.g., crustacean shells) or insect biomass. Ecologically, this capability enables *Pleurotus* species to participate actively in nutrient cycling and to compete effectively within natural ecological niches.

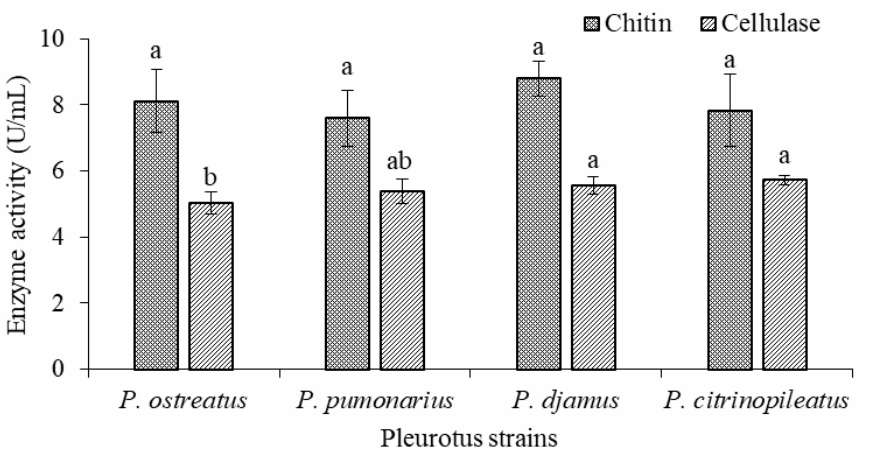

Figure 2. Comparative enzyme activities of chitinase and cellulase among different *Pleurotus* strains (Different letters (a, b, c, d) in the same pattern chart indicate statistically significant differences at p < 0.05 by Tukey's HSD)

3.4. Cellulase activity

The data presenting in figure 2 showed that, *P. djamor* and *P. citrinopileatus* exhibited the highest cellulase activity (about 5.03 - 5.73 U/mL), followed by *P. pulmonarius* (about 5.39 U/mL), and *P. ostreatus* (~5.03 U/mL). One-way ANOVA confirmed significant differences ($F = 7.022$, $p < 0.001$), with Tukey's HSD grouping *P. djamor*, *P. citrinopileatus* and *P. pulmonarius* together as group "a" and *P. ostreatus* as group "b". The cellulase performance of *P. pulmonarius* is notably within 3% of the highest producers, affirming its efficacy in saccharifying cellulosic feedstocks. Ghose's standardized method supports this quantitative analysis, ensuring reproducibility [17]. While not the top cellulase producer, *P. pulmonarius* exhibits high enough activity to be grouped statistically with the leading strains. This, in combination with its laccase output, enables efficient decomposition of both lignin and cellulose, a critical requirement for full exploitation of lignocellulosic residues.

Comparative enzymatic assessments consistently identify *P. pulmonarius* as the optimal strain for valorizing agro-industrial waste. Its balanced yet potent ligninolytic, chitinolytic, cellulolytic and proteolytic activities collectively establish its suitability for integration into circular agriculture and biorefinery systems.

3.5. Selection strain for agro-waste bioconversion

Degradation of complex agro-waste relies on enzyme synergy: Laccase initiates lignin removal; cellulase hydrolyzes cellulose; protease processes proteins; chitinase attacks chitinous content. *P. pulmonarius* presents a balanced multi-enzyme profile: top-tier laccase, robust cellulase, and sufficient protease and chitinase. This contrasts with the other species: *P. ostreatus*, which shows exceptionally high laccase but subpar protease and cellulase; *P. djamor*/*P. citrinopileatus*, which excel in protease and cellulase but have deficient ligninolytic ability. The integrated profile endorses *P. pulmonarius* for solid-state fermentation setups

aimed at valorizing heterogeneous biomasses. Previous studies underscore the importance of such balanced enzymatic systems in improving bioconversion yields in SSF [28, 29].

The comprehensive performance of *P. pulmonarius* across all four enzymatic activities distinguishes it from the other strains. *P. ostreatus*, despite high laccase activity, had the lowest cellulase output. *P. djamor* and *P. citrinopileatus*, while excelling in protease and cellulase, had significantly lower laccase activity, limiting their efficiency in delignification. In contrast, *P. pulmonarius* maintains high laccase and chitinase levels, along with moderately high cellulase and protease activities. Its enzyme profile is therefore balanced, ensuring synergistic degradation of structurally diverse agro-residues, including those rich in lignin, cellulose, proteins, and chitin. The enzyme synergy is critical in solid-state fermentation, where moisture content is low and nutrient availability is restricted. Under SSF conditions, strains with balanced extracellular enzyme systems are more likely to adapt and maintain high bioconversion efficiency. The multifunctionality of *P. pulmonarius* also reduces the need for strain consortia, simplifying process control and reducing production costs.

From a bioeconomic perspective, the adoption of *P. pulmonarius*-based bioprocesses aligns with circular bioeconomy principles by converting diverse agro-residues into valuable bioproducts such as biofertilizers, enzyme-rich feed and compost [30]. FAO emphasizes transforming conventional waste streams through local biotechnology to enhance rural livelihoods and promote environmental sustainability [14]. The multifunctional enzymatic capability may also generate value-added compounds like antioxidants or polysaccharides, boosting economic outcomes. Moreover, its generally recognized as safe status, rapid mycelial growth and adaptability to a variety of lignocellulosic substrates make it scalable and industrially viable [31]. Its ability to produce bioactive compounds during fermentation further enhances the functional value of the bioproducts.

Integrating comparative enzyme activity profiles with the corresponding statistical evaluations indicates that *P. pulmonarius* represents the most promising strain for broad-spectrum bioconversion of agro-industrial residues. Its diverse enzymatic repertoire that is characterized by robust ligninolytic and chitinolytic capacities alongside consistently high cellulase and protease activities confers a distinct functional advantage, underscoring its potential as a key biological agent in circular agricultural systems and biorefinery platforms.

4. CONCLUSION

This study evaluated four *Pleurotus* species for their extracellular enzyme activities to identify a suitable strain for agro-waste biodegradation. Among them, *P. pulmonarius* exhibited a well-balanced enzymatic profile, with high laccase and cellulase activities, moderate protease production, and strong chitinase activity. These findings indicate its superior capacity for degrading lignin, cellulose, proteins and chitin - major components of agro-industrial residues. Statistical analyses confirmed that *P. pulmonarius* performed comparably or significantly better than the other species in most enzyme categories. Unlike strains that excelled in only one or two enzyme types, *P. pulmonarius* demonstrated consistent activity across all four, highlighting its potential for integrated biomass conversion. Given its enzymatic versatility and known adaptability in cultivation, *P. pulmonarius* is a strong candidate for use in solid-state fermentation systems aimed at converting agricultural by-products into value-added materials. Its application supports circular bioeconomy goals by facilitating waste valorization and sustainable bioprocess development. Future research should focus on scaling up fermentation, optimizing process parameters and exploring downstream applications of the resulting products.

ACKNOWLEDGEMENT

This study was conducted with financial support from the Vietnam Ministry of Education and Training. Grant code: B2024.DNA.11.

REFERENCES

1. Liu, R., Yu, Z., Chen, C. (2021). Circular bioeconomy: Current status and future direction. *J Clean Prod*, 280, 124278. doi: 10.1016/j.jclepro.2020.124278.

2. Pandey, A., Soccol, C. R., Mitchell, D. (2000). New developments in solid state fermentation: I - bioprocesses and products. *Process Biochemistry*, 35, 1153 - 1169. doi: 10.1016/S0032-9592(00)00152-7.

3. Zervakis, G. I., Koutrotsios, G., Katsaris, P. (2017): Mushroom breeding and cultivation. In: Petre, M. (ed.) Advances in mushroom biotechnology, 1 - 20. IntechOpen.

4. Adebayo, E. A., Oloke, J. K., Ayandele, A. A. (2021). Screening of *Pleurotus* species for extracellular enzyme production. *J Environ Biol*. 42, 563 - 571. doi: 10.22438/jeb/42/3/MRN-1596.

5. Isikhuemhen, O. S., Mikiashvili, N. A. (2009). Lignocellulolytic enzymes activity during vegetative growth of *Pleurotus ostreatus* on different lignocellulosic materials. Mycoscience. 50, 439 - 445. doi: 10.1007/s10267-009-0015-5.

6. Couto, S. R., Herrera, J. L. T. (2006). Industrial and biotechnological applications of laccases: A review. Biotechnol Adv, 24, 500 - 513. doi: 10.1016/j.biotechadv.2006.04.002.

7. Singhania, R. R., Sukumaran, R. K., Patel, A. K., Larroche, C., Pandey, A. (2010): Advancement and comparative profiles in the production technologies using solid-state and submerged fermentation for microbial cellulases. Enzyme Microb Technol, 46, 541 - 549. doi: 10.1016/j.enzmictec.2010.02.002.

8. Rao, M. B., Tanksale, A. M., Ghatge, M. S., Deshpande, V. V. (1998). Molecular and biotechnological aspects of microbial proteases. Microbiology and Molecular Biology Reviews, 62, 597 - 635. doi: 10.1128/MMBR.62.3.597-635.1998.

9. Patil, R.S., Ghormade, V., Deshpande, M. V (2000). Chitinolytic enzymes: An exploration. Enzyme Microb Technol, 26, 473 - 483. doi: 10.1016/S0141-0229(00)00134-4.

10. Mikiashvili, N., Elisashvili, V., Wasser, S. P., Nevo, E. (2006). *Lentinus edodes* and *Pleurotus* species lignocellulolytic enzyme activity. Bioresour Technol. 97, 775 - 781. doi: 10.1016/j.biortech.2005.04.013.

11. Kachlishvili, E., Penninckx, M.J., Tsiklauri, N., Elisashvili, V. (2006). Effect of nitrogen source on enzyme production by white-rot fungi. *World J Microbiol Biotechnol*, 22, 391 - 397. Doi: 10.1007/s11274-005-9054-7.

12. Mata, G., Savoie, J. M., Royse, D. J. (2017). Recycling of spent mushroom substrate. In: Zied, D. C. and Pardo-Gim'enez, A. (eds.) Mushroom biotechnology: developments and applications, 313 - 332. John Wiley& Sons.

13. Shankar, T., Gopinath, R.M., Sridevi, M. (2021). Solid-state fermentation of agro-wastes by white-rot fungi: Enzyme production and biotransformation potential. *Bioresour Bioprocess*, 8, 27. doi: 10.1186/s40643-021-00367-3.

14. FAO (2021). The state of the world's land and water resources for food and agriculture - Systems at breaking point.

15. Niku-Paavola, M. L., Karhunen, E., Salola, P., Raunio, V. (1988). Ligninolytic enzymes of the white-rot fungus Phlebia radiata. *Biochem J*, 254, 877 - 883. doi: 10.1042/bj2540877.

16. Sigma-Aldrich (1999). Protease from *Aspergillus oryzae* - Product Information and Assay Procedure. Technical Bulletin.

17. Ghose, T. K. (1987). Measurement of cellulase activities. Pure & Appl. Chem, 59, 257 - 268. doi: 10.1351/pac198759020257.

18. Monreal, J., Reese, E. T. (1969). The chitinase of *Serratia marcescens*. *Can J Microbiol*, 15, 689 - 696. doi: 10.1139/m69-122.

19. Baldrian, P. (2006): Fungal laccases - occurrence and properties. FEMS Microbiol Rev. 30, 215 - 242. doi: 10.1111/j.1574-4976.2005.00010.x

20. Haq, I. ul, Saleem, A., Chaudhary, R., Alessa, A.H., Nawaz, A., Du, C. (2024). Role of

microbial laccases in valorization of lignocellulosic biomass to bioethanol. Front Bioeng Biotechnol. 12. doi: 10.3389/fbioe.2024.1441075.

21. De Freitas, E. N., Bubna, G. A., Brugnari, T., Kato, C. G., Nolli, M., Rauen, T. G., Peralta Muniz Moreira, R. de F., Peralta, R.A., Bracht, A., de Souza, C. G. M., Peralta, R. M. (2017): Removal of bisphenol A by laccases from *Pleurotus ostreatus* and *P. pulmonarius* and evaluation of ecotoxicity of degradation products. *Chemical Engineering Journal*. 330, 1361-1369. doi: 10.1016/ j.cej.2017.08.051.

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

23. Stajić, M., Persky, L., Friesem, D., Hadar, Y., Wasser, S. P., Nevo, E., Vukojević, J. (2006): Effect of different carbon and nitrogen sources on laccase and peroxidases production by selected *Pleurotus* species. Enzyme Microb Technol, 38, 65 - 73. doi: 10.1016/j.enzmictec.2005.03.026.

24. Ravikumar, G., Gomathi, D., Kalaiselvi, M., Uma, C. (2012). A protease from the medicinal mushroom *P. sajor-caju*, production, purification and partial characterization. *Asian Pac J Trop Biomed*, 2, S411 - S417. doi: 10.1016/S2221-1691(12) 60198-1.

25. Santana, R. da S., Mendes, F. de S., Paula da Silva, B. J., Lima, E. S., Nascimento, T. P., Carneiro da Cunha, M. N., Porto, A. L. F., Teixeira, M. F. S., Carvalho, R. P., Gomes, W. R. (2024). Recovery and partial purification of fibrinolytic protease from *P. ostreatus* and *P. eryngii* and cytotoxic and antioxidant activity of their extracts. Prep Biochem Biotechnol. 54, 545 - 552. doi: 10.1080/10826068.2023.2253466.

26. Omrane Benmrad, M., Mechri, S., Zaraî Jaouadi, N., Ben Elhoul, M., Rekik, H., Sayadi, S., Bejar, S., Kechaou, N., Jaouadi, B. (2019). Purification and biochemical characterization of a novel thermostable protease from the oyster mushroom *P. sajor-caju* strain CTM10057 with industrial interest. BMC Biotechnol, 19, 43. doi: 10.1186/s12896-019-0536-4.

27. Pandey, A. (2003). Solid-state fermentation. Biochem Eng J. 13, 81 - 84. doi: 10.1016/S1369-703X(02)00121-3.

28. Dao T. M. L., Nguyen, T. Q. M., Bui, T. K. T., Nguyen, D. T. V., Nguyen, D. K., Son, T. N., Kieu, Y. V., Tran, Q. H. (2019). Selecting and investigating the effects of environment on characteristics of chitinase from soil fungi. *Journal of Science, Technology and Food*, 19, 38 - 49.

29. Zhou, S., Raouche, S., Grisel, S., Navarro, D., Sigoillot, J. -C., Herpoël-Gimbert, I. (2015). Solid-state fermentation in multi-well plates to assess pretreatment efficiency of rot fungi on lignocellulose biomass. Microb Biotechnol. 8, 940 - 949. Doi: 10.1111/1751-7915.12307.

30. Gkountani, V. A., Tsoulfas, G. T. (2023): Circular Bioeconomy: A Review on the Current State and Future Opportunities BT - Digital Economy and the Green Revolution. Presented at the.

31. Wu, N., Tian, F., Moodley, O., Song, B., Jia, C., Ye, J., Lv, R., Qin, Z., Li, C. (2019). Optimization of agro-residues as substrates for *P. pulmonarius* production. AMB Express. 9, 184. doi: 10.1186/s13568-019-0907-1.