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Effect of *Pseudomonas fluorescens* and *Bacillus cereus* on the Biomass Production and Bioactivity of *Lentinus tigrinus* and *Lentinus strigosus* in Submerged Culture

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Abstract

Mushrooms have gained global recognition for their nutritional and therapeutic importance, as they are rich in bioactive metabolites that contribute to disease prevention and treatment. This study underlined the submerged cultivation utilizing *Pseudomonas fluorescens* BIOTECH 1123 and *Bacillus cereus* BIOTECH 1509 as biostimulants in the mycelial biomass production of *Lentinus tigrinus* and *Lentinus strigosus*. The antibacterial properties of the ethanolic extracts of *L. tigrinus* and *L. strigosus* were assessed using the disc diffusion technique, while their antioxidant potential was evaluated through the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Both *L. tigrinus* and *L. strigosus* exhibited higher mycelial dry weights when cultured with *P. fluorescens* and *B. cereus*, compared to the control setup. All ethanolic extracts obtained from *L. tigrinus* and *L. strigosus* demonstrated antioxidant activity, indicating the presence of compounds with potential health benefits. Notably, the ethanolic extracts of *L. tigrinus* cultures co-inoculated with B. cereus on the same day as the fungal mycelia exhibited antibacterial activity against *Staphylococcus aureus* BIOTECH 1582, producing an inhibition zone of 15.60 mm. Similarly, extracts from *L. strigosus* that were inoculated with sterile distilled water on day 0 also showed antibacterial activity, producing a 6.54 mm inhibition zone against the same bacterial strain. Moreover, the ethanolic extract of *L. strigosus* showed an 8.11 mm zone of inhibition after 5 days of mycelial incubation prior to *B. cereus* introduction. In conclusion, co-cultivating *L. tigrinus* and *L. strigosus* with beneficial bacteria enhanced mycelial yield and produced extracts with notable antioxidant and antibacterial activities, supporting their potential in sustainable mushroom biotechnology and functional product development.

Keywords: Antibacterial; Biostimulants; DPPH radical scavenging assay; Submerged fermentation

Introduction

Mushrooms have been utilized worldwide because of their nutraceutical and pharmaceutical significance. In addition to nutrient-rich foods, mushrooms also contain several bioactive chemicals that help to prevent and treat different diseases. Apart from being healthy diet sources, mushrooms are also known for their abundance of bioactive antioxidant compounds. Among these are ascorbic acid, tocopherols, carotenoids, and a variety of phenolic compounds including catechin, gallic acid, tannic acid, caffeic acid, resveratrol and benzoic acid (Kim *et al*. 2008a). Furthermore, mushrooms include bioactive compounds like polysaccharides, sesquiterpenes, and glycolipids that have been said to show antibacterial, antiviral and antifungal potentials, respectively (Dharmaraj *et al*. 2014).

Lentinus mushrooms are edible macrofungi that naturally grow in various terrestrial habitats. A wood-rotting edible basidiomycete with leathery flesh and a notably appealing aroma and flavor, *Lentinus tigrinus* is considered the local equivalent of shiitake (*Lentinula edodes*), which was brought to the Philippines because of its suitability and use for gourmet preparation (Dulay *et al*. 2012a). Conversely, *Lentinus strigosus* shows different

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morphological characteristics including circular caps (pilei) covered with light to brownish scales, sawtoothed margins, gills on the underside ranging from white to yellowish, and white stripes with a scaly texture (Dulay *et al*. 2017b). *L. strigosus* is also second to the recently domesticated *Lentinus* species (Dulay *et al*. 2020). The growing of these mushrooms indicated a promising future in the production technology of the mushroom industry. Recent studies have shown the successful and effective domestication of the mycelial cultures of these mushrooms. Moreover, the optimized and ideal growing conditions have been established (Dulay *et al*. 2012a; Dulay and Garcia 2017). Various commercial mycological culture media are widely employed in laboratories for mycelial growth, providing essential nutrients for mushrooms (Dulay and Garcia 2017). These commercially available media, however, are expensive. Alternative culture media derived from indigenous sources such as coconut water for submerged cultivation, rice bran, corn grit, sorghum seeds, and potato have been developed and introduced. These alternatives are cheaper and cost-efficient and advantageous for mushroom farmers as well as researchers (Kalaw *et al*. 2016). Highlighting the biological properties of these mushrooms, it has proteins, lipids, carbohydrates, minerals, dietary fibers, and reducing sugars; the lyophilized water extract of *L. tigrinus* has been shown to have hypoglycemic effects in alloxan-induced mouse models (Dulay *et al*. 2014). Moreover, several studies have underlined the antioxidant potential of extracts derived from *Lentinus* species (Dulay *et al.* 2015; Dulay *et al*. 2017a). These species have also shown antibacterial activity highlighting their importance in the development of pharmaceutical and nutraceutical products (Dulay *et al*. 2014, 2017a).

L. tigrinus and *L. strigosus* cultivation has been studied in relation to critical factors influencing mushroom growth including nutritional and physical factors (Dulay *et al*. 2012b; Leon *et al*. 2013). Beneficial bacteria such as *Pseudomonas fluorescens* and *Bacillus* spp. are known for their ability to promote plant growth and suppress diseases by producing a variety of bioactive compounds (Javed *et al.* 2021; Sharf *et al.* 2021; Zohaib *et al.* 2024). The utilization of biological elements specifically the introduction of bacteria in mushroom cultivation, particularly in submerged cultivation, was underexplored. Herein, the cultivation potential utilizing bacteria and the bioactivities of *L. tigrinus* and *L. strigosus* have been investigated. *Pseudomonas fluorescens* BIOTECH 1123 and *Bacillus cereus* BIOTECH 1509 was the bacteria used in this study. Studies have shown the use of beneficial microorganisms to increase edible mushroom yield (Kim *et al*. 2008a; Zarenejad *et al*. 2011), particularly product quality and consistency (Choudhary 2011). Several bacteria can potentially influence the proliferation of mycelia and edible mushroom production through the synthesis and release of biologically active compounds such as phytohormones (Kaneko 2009). For instance, Carrasco *et al*. (2018) reported that *Bacillus*, *Pseudomonas*, *Paenibacillus* and *Azotobacter* have been utilized as nutritional supplements or biofertilizers that have been used for the cultivation of mushrooms. In addition, beneficial interactions were seen during cocultivation of *Pleurotus florida* (a white-rot fungus) and a *P. fluorescens* bacterium (Cho *et al*. 2003). Further research by Kim *et al*. (2008b) demonstrated that *Pseudomonas* strain P7014 could enhance the production of fruiting bodies in *Pleurotus eryngii* grown in bottle cultures, primarily by stimulating mycelial development. This effect has been attributed to the production of indole-3-acetic acid by the bacterium, a growth-promoting compound essential to the fungus, as discussed by Kang and Cho (2014). Similarly, Chen *et al*. (2022) found that fermentation broth from *Bacillus cereus* Bac1 facilitated the growth of *P. eryngii* hyphae. In the present study, the influence of biostimulants on the submerged cultivation of *L. tigrinus* and *L. strigosus* was examined. Additionally, the antioxidant and antibacterial properties of ethanolic extracts derived from their mycelia were assessed, underscoring their potential for application across various industrial sectors.

Materials and Methods

Source of mushroom culture

The pure cultures of *L. tigrinus* and *L. strigosus* used in this study were obtained from the culture collection of the Center for Tropical Mushroom Research and Development (CTMRD) at Central Luzon State University, situated in the Science City of Muñoz, Nueva Ecija, Philippines. Each strain was inoculated under sterile conditions onto freshly prepared potato dextrose agar (PDA) plates. These were then incubated at a temperature of 30°C for a period of seven days. Following incubation, 10 mm diameter mycelial discs were excised using a flame-sterilized cork borer from the 7-day-old cultures of each mushroom to serve as inoculum for subsequent experimental procedures

Source of bacteria

Bacillus cereus BIOTECH 1509 and *Pseudomonas fluorescens* BIOTECH 1123 were obtained from the Philippine National Collection of Microorganisms (PNCM), located at the National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños (UPLB), Los Baños, Laguna, Philippines. With a minor modification, the methods described by Dulay *et al*. (2021) were used to prepare the bacterial suspensions. Separate cultures of each bacterial strain were grown in 9 mL of nutrient broth and incubated for 24 h. Each cultures cell density was adjusted after incubation to satisfy the 0.5 McFarland turbidity standard, or approximately 1.5×10^8 cells mL^{-1}. The mycelial biomass production of *L. tigrinus* and *L. strigosus* was then evaluated using these standardized bacterial suspensions as inoculants.

Submerged culture of *L. tigrinus* and *L. strigosus*

Coconut water obtained from mature coconuts (*Cocos nucifera)* was utilized as the basal medium for submerged cultivation. A volume of 50 mL was dispensed into sterile 250-mL ketchup bottles, which were sealed using polypropylene bags. The culture media were sterilized through autoclaving at 121°C and 103.4 kPa for 30 min. Following sterilization, each medium was inoculated with mycelial discs of *L. tigrinus* and *L. strigosus* and incubated at 30°C to promote mycelial development. A 0.5 mL aliquot of bacterial suspension was introduced into the respective cultures on days 0, 5 and 10 of incubation. In contrast, control cultures received an equal volume of sterile distilled water. All experimental and control cultures were incubated for 15 days following mycelial inoculation. After the incubation period, the mycelial was harvested, air-dried, and subsequently measured in milligrams. Moreover, the volume of the spent culture (mL) was also measured. The dry mycelial biomass was extracted for bioactivity evaluation.

Ethanol extraction

The functional component of *L. tigrinus* and *L. strigosus* mycelia were extracted using ethanol. *L. tigrinus* and *L. strigosus* 10 g powdered mycelia were dispensed separately in 300 mL of 95% ethanol and soaked for 72 h. Using Whatman No. 2 filter paper, extracts were filtered and concentrated to dryness using a rotary evaporator. The concentrated extracts were then subjected to bioactivity profiling.

DPPH radical scavenging assay

To assess the antioxidant activity of the sample, the DPPH free radical scavenging assay was employed, following the method described by Kolak *et al*. (2006). The procedure began with the preparation of a concentrated extract, from which a stock solution was obtained. This stock was subsequently diluted to achieve a final concentration of 1000 ppm. As a reference, Catechin was used, prepared at an identical concentration of 1 mg mL^{-1}.

For the assay, 1 mL of the sample solution was mixed with 4 mL of a 0.1 m*M* DPPH solution in separate plastic cuvettes. Each test was performed three times to ensure the reproducibility and accuracy of the results. The reaction mixtures were incubated in the dark at 37°C for 30 min, minimizing the influence of light on the reaction. After incubation, the absorbance of each mixture was read at 517 nm using a UV-Visible spectrophotometer (Spectrumlab 752S, Hinotek Instrument Co., Ltd., China). The radical scavenging activity was expressed as a percentage, calculated with the formula:

$$RSA\ (\%) = [(Ac - As) / Ac] \times 100$$

Where, Ac denotes the absorbance of the control and As represents that of the sample.

Antibacterial assay

L. tigrinus and *L. strigosus* antibacterial activities were investigated employing the disc diffusion method by Bauer *et al*. (1966). The test microorganisms namely: *Staphylococcus aureus* BIOTECH 1582 and *Escherichia coli* BIOTECH 1634 was obtained from the Philippine National Collection of Microorganisms, at the National Institute of Molecular Biology and Biotechnology, University of the Philippines Los Baños, Los Baños, Laguna, Philippines. Each bacterial strain was cultured in 9 mL of nutrient broth and incubated for 24 h at 37°C. Following incubation, the turbidity of the cultures was standardized to match the 0.5 McFarland turbidity standards which approximates 1.5×10^8 cells mL^{-1}. Sterile discs, 5 mm in diameter, were punched from Whatman No. 1 filter paper and sterilized at 121°C less than 103.4 kPa for 15 min. Subsequently, the standardized bacterial suspensions were inoculated onto Mueller-Hinton agar plates using sterile cotton swabs. The inoculated plates were then air-dried for about 5 min prior to the placement of test discs.

Following bacterial inoculation on the agar's surface, discs soaked in *L. tigrinus* and *L. strigosus* ethanolic extracts, with streptomycin sulfate as the positive control and 95% ethanol (the solvent used for the extraction) serving as the negative control, were positioned equidistantly on it. All tests were conducted in triplicate and incubated at 37°C for duration of 24 h. Following the incubation period, the diameter of the inhibition zone was determined using a Vernier caliper. The presence of an inhibition zone suggests that the extract is effective against the test culture, whereas the absence of one indicates that the microorganisms are resistant to the extracts and have no antibacterial properties.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) following a completely randomized design at a 5% level of significance using Minitab® 21 Statistical Software. To determine significant differences among treatment means, Tukey's Honest Significant Difference (HSD) test was employed.

Results

Mycelial biomass production

The effect of introduction of bacteria on the growth and mycelial biomass production of *L. tigrinus* and *L. strigosus* was investigated. Table 1 shows the mycelial biomass yield of *L. tigrinus* in submerged culture.

As per *L. tigrinus*, *P. fluorescens*-introduced cultures exhibited significantly higher mycelial dry weights (328, 415 and 370 mg) on days 0, 5, and 10 of mycelial incubation when compared to distilled water, indicating

growth stimulatory effect on mushroom. On the other hand, the introduction of *B. cereus* in submerged culture on days 0, 5, and 10 showed a significant difference compared to distilled water (300, 382 and 356 mg, respectively). The control recorded the lowest mycelial dry weight values (280, 365 and 306 mg). Likewise, *P. fluorescens*-introduced and *B. cereus*-introduced cultures of *L. strigosus* exhibited higher mycelial dry weights when compared to control, as shown in Table 2. Both biostimulants enhance growth performance of the mushrooms. Moreover, these results suggest distinct responses to treatments, highlighting the influence of specific conditions or components on mycelial growth and biomass production.

The study also evaluates the volume loss of the spent culture of *L. tigrinus* and *L. strigosus* submerged culture. In relation to mycelial biomass, it is evident that the two bacterial treatments with the highest volume loss generated the highest mycelial biomass for both of the mushroom, as shown in Table 3 and Table 4. Subsequently, the observed relationship between mycelial dry weight and volume loss of the spent culture is consistent with the results recorded. The biostimulants, particularly *P. fluorescens*, resulted in highest volume losses in 0 day, 5 days and 10 days of *L. tigrinus* mycelial incubation (8.80, 8.90 and 9.90 mL, respectively) than the control group. Furthermore, *B. cereus*-introduced cultures recorded the highest volume losses in 3 different incubation periods in *L. strigosus* (6.80, 8.80 and 13.60 mL). However, the two treatments are not significantly different from each other in both species. Moreover, these results demonstrate conclusively that *L. tigrinus* and *L. strigosus*, when inoculated with biostimulants, efficiently utilizes the liquid medium to produce mycelial biomass.

Table 1: Mycelial dry weight of *L. tigrinus* inoculated with bacteria at different incubation periods

Treatment	Mycelial biomass (mg/50 mL of the media)		
	0 day	5 days	10 days
P. fluorescens	328 ± 14.65*	415 ± 5.48*	370 ± 6.69*
B. cereus	300 ± 13.67*	382 ± 6.43*	356 ± 8.58*
Distilled water (control)	280 ± 15.85	365 ± 9.82	306 ± 13.00

Values are the mean ± standard deviation of ten replicates. Asterisk (*) indicate significant differences with the control at the $P < 0.05$ significance level

Table 2: Mycelial dry weight of *L. strigosus* inoculated with bacteria at different incubation periods

Treatment	Mycelial biomass (mg/50 mL of the media)		
	0 day	5 days	10 days
P. fluorescens	288 ± 64.50*	231 ± 30.80*	418 ± 46.40*
B. cereus	279 ± 61.60*	300 ± 77.70*	379 ± 73.40*
Distilled water (control)	252 ± 62.00	215 ± 44.90	330 ± 48.80

Values are the mean ± standard deviation of ten replicates. Asterisk (*) indicate significant differences with the control at the $P < 0.05$ significance level

Table 3: Volume loss of spent culture of *L. tigrinus* inoculated with bacteria at different incubation periods

Treatment	Mycelial biomass (mg/50 mL of the media)		
	0 day	5 days	10 days
P. fluorescens	8.8 ± 0.92*	8.90 ± 0.74*	9.50 ± 0.53*
B. cereus	8.3 ± 0.48*	8.70 ± 0.68*	8.80 ± 0.92*
Distilled water (control)	6.7 ± 0.95	7.70 ± 0.48	7.90 ± 0.88

Values are the mean ± standard deviation of ten replicates. Asterisk (*) indicate significant differences with the control at the $P < 0.05$ significance level

Table 4: Volume loss of spent culture of *L. strigosus* inoculated with bacteria at different incubation periods

Treatment	Mycelial biomass (mg/50 mL of the media)		
	0 day	5 days	10 days
P. fluorescens	6.60 ± 0.55*	8.80 ± 0.45*	12.00 ± 0.71*
B. cereus	6.80 ± 0.45*	8.80 ± 0.45*	13.60 ± 0.90*
Distilled water (control)	5.60 ± 0.55	7.80 ± 0.45	8.80 ± 1.30

Values are the mean ± standard deviation of ten replicates. Asterisk (*) indicate significant differences with the control at the $P < 0.05$ significance level

Table 5: Antioxidant activity of the ethanolic extracts of *Lentinus* species

Mushroom	Extract	Scavenging activity (%)		
		0 day	5 days	10 days
L. tigrinus	*P. fluorescens*	27.44[b]	22.03[b]	25.56[b]
	B. cereus	27.56[b]	20.38[c]	24.73[c]
	Distilled water	28.39[b]	24.03[d]	26.38[d]
	Catechin (control)	83.04[a]	83.04[a]	83.04[a]
L. strigosus	*P. fluorescens*	29.29[b]	30.19[b]	24.17[b]
	B. cereus	29.74[b]	29.65[c]	26.06[c]
	Distilled water	33.96[c]	28.30[d]	37.65[d]
	Catechin (control)	83.74[a]	83.74[a]	83.74[a]

Scavenging activities of ethanolic mycelial extracts of *L. tigrinus* and *L. strigosus* compared with catechin. Means with different superscript letters in the same column differ significantly at the 5% significance level

DPPH radical scavenging activity

Following the evaluation of mycelial biomass production, the harvested mycelia underwent extraction, after which the antioxidant activity was investigated through the DPPH radical scavenging assay. After the evaluation of the mycelial biomass production, the produced mycelia were subjected to extraction and the antioxidant activity using DPPH radical scavenging assay was investigated. In this work, mean absorbance values and corresponding scavenging activity percentages were provided for each extract. Compared to the control sample of catechin, the *L. tigrinus*and *L. strigosus* ethanolic extracts displayed varying percentages of scavenging activity (Table 5).

Among all of the extracts of *L. tigrinus*, 0 day of mycelial incubation before distilled water introduction showed the highest scavenging activity of 28.39%, suggesting that the immediate inoculation with distilled water provided favorable conditions for the growth and development of the mycelium, resulting in enhanced scavenging activity. On the other hand, with 5 days of mycelial incubation before *B. cereus* introduction recorded the lowest scavenging activity of 20.38%. Moreover, with 0 day of mycelial incubation before *P. fluorescens* and *B. cereus* introduction have reported higher scavenging activity (27.44% and 27.56%, respectively) compared to 5 days and 10 days of mycelial incubation before *P. fluorescens* and *B. cereus* introduction. Meanwhile, differences in scavenging

activity in different ages of mycelial culture are not statistically significant at the 5% level of significance within any treatment except for 5 days and 10 days of mycelial incubation.

On the other hand, findings at the early stage (0 day) of *L. strigosus* mycelial development inoculated with *P. fluorescens* and *B. cereus* shows lower radical scavenging activity (29.29% and 29.74%, respectively) than the distilled water (33.96%). The *L. strigosus* inoculated with distilled water showing much higher antioxidant activity compared to bacterial inoculants suggesting that *P. fluorescens* and *B. cereus* contributed to the decreased on the antioxidant activity.

A change in radical scavenging activity was observed on the extract with 10 days of mycelial incubation inoculated with distilled water demonstrating an increased in scavenging activity of 37.65% being the highest scavenging activity percentage. On contrary, the radical scavenging activity of *L. strigosus* with *P. fluorescens* and *B. cereus* as biostimulants was decreased, falling to 24.17% and 26.06%, respectively. The data suggest that the presence of bacteria in the submerged culture media has an effect on the radical scavenging activity of *L. strigosus*. In contrast, 5 days of mycelial incubation prior *P. fluorescens* and *B. cereus* introduction exhibited increased radical scavenging activity (30.19% and 29.65%, respectively). These results suggest that the timing of bacterial inoculations affects the scavenging ability of the *L. strigosus* mycelia.

Antibacterial activity of the ethanolic extracts

This study investigated the antibacterial properties of ethanolic extracts derived from *L. tigrinus* and *L. strigosus* mycelia against *Staphylococcus aureus* BIOTECH 1582 and *Escherichia coli* BIOTECH 1634 through the disc diffusion assay. The results, as presented in Table 6, illustrate the inhibition zone diameters, indicating the potential of the extracts in suppressing the growth of the Gram-positive bacterium *S. aureus* BIOTECH 1582. As per *L. tigrinus*, except with 0 day of mycelial incubation before *B. cereus* introduction (15.60 mm), the study reports that no zone of inhibition against *S. aureus* BIOTECH 1582. The presence of inhibition zones indicates that the extract with 0 day of mycelial incubation before *B. cereus* introduction has antibacterial activity and may suppress the proliferation of the tested microorganism (Fig. 1). On the other hand, the ethanolic extract of *L. strigosus* inoculated with distilled water on day 0 showed zone of inhibition of 6.54 mm against *S. aureus* BIOTECH 1582, indicating the presence of antibacterial activity (Fig. 2). Additionally, the antibacterial activity of *L. strigosus* ethanolic extract with 5 days of mycelial incubation before *B. cereus* introduction was observed against *S. aureus* BIOTECH 1582, having a inhibition zone of (8.11 mm).

In terms of the antibacterial activity of *L. tigrinus* and *L. strigosus* ethanolic extracts against the Gram-negative bacterium *E. coli* BIOTECH 1634, all ethanolic extracts consistently exhibited no zone of inhibition. This suggests that the ethanolic extracts of these mushrooms was ineffective in inhibiting the growth of this Gram-negative bacterium (Fig. 3).

Table 6: Antibacterial activity of the ethanolic extracts of *Lentinus* species against Gram-positive *Staphylococcus aureus* BIOTECH 1582

Mushroom	Treatment	Diameter zone of inhibition (mm)		
		0 day	5 days	10 days
L. tigrinus	*P. fluorescens*	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
	B. cereus	15.60 ± 0.14^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
	Distilled water	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
	Streptomycin (+)	29.47 ± 0.33^{a}	29.16 ± 0.10^{a}	28.41 ± 0.30^{a}
	Ethanol (-)	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
L. strigosus	*P. fluorescens*	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}
	B. cereus	0.00 ± 0.00^{c}	8.11 ± 1.60^{b}	0.00 ± 0.00^{b}
	Distilled water	6.54 ± 0.95^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}
	Streptomycin (+)	28.02 ± 0.75^{a}	27.75 ± 1.96^{a}	29.82 ± 1.12^{a}
	Ethanol (-)	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}

Each value represents the mean ± SD of tests conducted in triplicate (n = 3) at the 5% level of significance. Values within the same column that do not share the same superscript letter indicate statistically significant differences. Note: streptomycin (+ control), ethanol (- control)

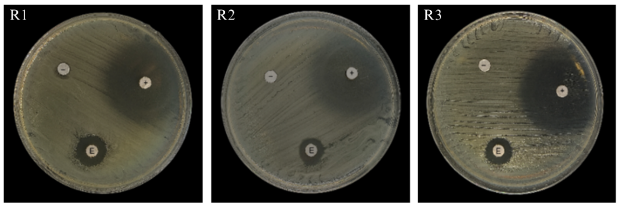

Fig. 1: Zones of inhibition of *L. tigrinus* ethanolic extracts with 0 day of mycelial incubation before *B. cereus* BIOTECH 1509 introduction against Gram-positive *S. aureus* BIOTECH 1582

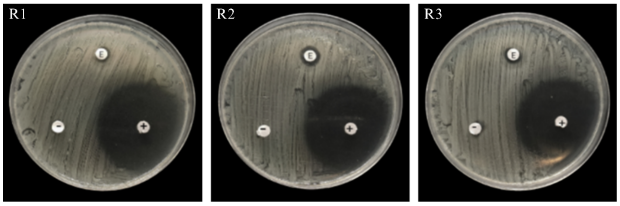

Fig. 2: Zones of inhibition of *L. strigosus* ethanolic extracts with 0 day of mycelial incubation before distilled water introduction against Gram-positive *S. aureus* BIOTECH 1582

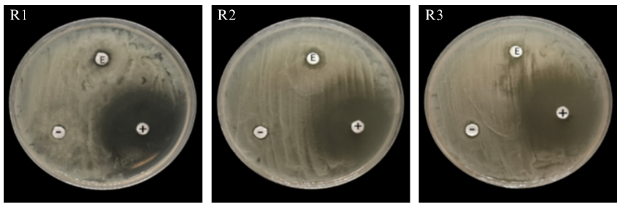

Fig. 3: Zones of inhibition of *L. strigosus* ethanolic extracts with 5 days of mycelial incubation before *B. cereus* BIOTECH 1509 introduction against Gram-positive *S. aureus* BIOTECH 1582

Discussion

Mushrooms have been valued for centuries not only as food but also for their medicinal properties. Known for their rich nutritional profile, they contain significant amounts of protein, fiber, carbohydrates, and essential vitamins and minerals. Because of these attributes, mushrooms are widely used in both pharmaceutical and nutraceutical fields. Their health-promoting potential is largely due to the diverse bioactive compounds they possess (Wang *et al*. 2014; Kozarski *et al*. 2015). In this study, submerged cultures of *Lentinus tigrinus* and *Lentinus strigosus* were explored to determine how biostimulants influence their growth. An evaluation of the mycelial biomass was measured through mycelial dry weight and observed volume loss of the spent culture, alongside conducting bioactivity assessments to better understand their therapeutic and nutraceutical potential.

To better understand how the timing of bacterial treatment influences the growth and development of *L. tigrinus* and *L. strigosus* mycelia, biostimulants were applied at different stages of mycelial incubation. By introducing these treatments on days 0, 5 and 10, the study aimed to determine whether the effectiveness of the bacterial application depends on the specific growth phase of the mycelia. This strategy allows for a clearer observation of how the mushrooms interact with the biostimulants at different developmental points (Zhang *et al.* 2018).

The findings revealed that *P. fluorescens* contributed to significantly higher mycelial dry weights in *L. tigrinus* across all time points—days 0, 5, and 10—compared to untreated controls, which consistently showed the lowest dry weights. For *L. strigosus*, cultures treated with *P. fluorescens* and *B. cereus* also resulted in greater mycelial biomass than the control group. These results align with those of Cho *et al*. (2003), who reported that fluorescent *Pseudomonas* species enhanced not only the mycelial growth of *Pleurotus ostreatus* but also stimulated primordia formation and basidiome development.

Furthermore, Kang and Cho (2014) emphasized the role of indole-3-acetic acid (IAA), produced by *Pseudomonas* sp. P7014, in promoting the development of *Pleurotus eryngii* mycelia. The addition of beneficial bacteria to mushroom-growing substrates appears to support both the growth and reproductive stages of fungal development. These microbial treatments likely exert their effects through the production and release of biologically active compounds such as phytohormones, which can influence various aspects of fungal physiology (Kaneko 2009). Zarenejad *et al*. (2011) suggested that growth-stimulating bacteria play a role in promoting mushroom growth and productivity through an array of mechanisms, including the breakdown of inhibitory volatiles produced by vegetative mycelia, growth hormone secretion, phosphate solubilization, 1-aminocyclopropane-1-carboxylate (ACC deaminase) and siderophore production.

Among the treatments applied, *P. fluorescens* caused the greatest volume loss of the spent culture during *L. tigrinus* mycelial incubation periods of 0, 5, and 10 days, when compared to *B. cereus* and the control group. Correspondingly, both bacterial treatments also led to a greater volume loss in *L. strigosus* than the control, although no statistically significant difference was observed between *P. fluorescens* and *B. cereus* treatments.

The findings of this study support existing knowledge indicating that *B. cereus* and *P. fluorescens* play active roles in breaking down organic materials through enzyme production. Their presence likely hastened the decomposition of the spent substrate, which could explain the significant volume loss observed. One possible factor contributing to this enhanced breakdown is the use of a liquid culture medium enriched with biostimulants. These may have encouraged stronger metabolic activity in the spent cultures, leading to increased mycelial growth and utilization of the substrate. This interpretation is consistent with the work of Dulay *et al*. (2015), who found that *L. tigrinus* and *Lentinus sajor-caju* produced considerable amounts of mycelial biomass and substantially reduced spent culture volume when grown in rice bran broth. Similar trends have been observed where the loss of weight in lignocellulosic materials following mushroom fruiting on artificial logs, highlighting the link between mycelial growth and substrate utilization. However, the particular biological mechanisms that drive these changes are still not fully understood. Future research is needed to clarify how bacterial inoculants interact with *Lentinus* species, particularly to identify the key processes that lead to variability in biomass production and substrate utilization.

The antioxidant activity was evaluated in addition to the growth performance. The ethanolic extracts of *L. tigrinus* and *L. strigosus* exhibited varying percentages of scavenging activity. When distilled water was added immediately on day 0 of *L. tigrinus* mycelial incubation, the highest scavenging activity (28.39%) was observed, suggesting that the conditions were favorable for mycelial growth and increased scavenging activity. Furthermore, the five days of *L. tigrinus* mycelial incubation prior to *B. cereus* introduction had the lowest scavenging activity (20.38%). Significantly, scavenging activity was higher in mycelial cultures incubated for 0 days prior to the introduction of *P. fluorescens* and *B. cereus* (27.44% and 27.56%, respectively) than in cultures incubated for 5 and 10 days prior to the introduction of these bacteria. However, the extract with 10 days of mycelial incubation before distilled water introduction had the highest scavenging activity in *L. strigosus* (37.65%), followed by 0 days of mycelial incubation before distilled water introduction (33.96%). Following bacterial treatment along with 0 and 10 days of mycelial incubation, *L. strigosus* extracts showed a decrease in scavenging activity. In contrast to distilled water, *L. strigosus* ethanolic extracts treated with bacteria and incubated for five days showed higher scavenging

activities. The extracts, the age of the mycelial culture and the particular treatment all considerably affected the scavenging abilities of these mushrooms. A related study explored the antioxidant potential of ethyl acetate extracts derived from the mycelia of submerged cultures of two *Lentinus* species, *L. tigrinus* and *L. sajor-caju*. It was found that the culture of *L. tigrinus* demonstrated a higher scavenging activity than that of *L. sajor-caju* (Dulay *et al*. 2015). Further investigation by Dulay *et al*. (2017a) showed that extracts from *L. tigrinus* obtained using hexane and acetonitrile exhibited free radical scavenging activities of 35.5% (with an EC_{50} of 710.23 mg L^{-1}) and 39.2% (with an EC_{50} of 637.75 mg L^{-1}), respectively.

These findings underline the importance of the extraction method in determining antioxidant potentials. Factors such as the timing of inoculation, the type of culture medium, and the presence of specific microorganisms during submerged fermentation can all affect the antioxidant properties of ethanolic extracts. Overall, the demonstrated ability of mycelial extracts from *L. tigrinus* and *L. strigosus* to neutralize free radicals suggests potential applications in therapeutic and pharmaceutical settings. Nonetheless, it is worth noting that all tested extracts exhibited lower scavenging activity compared to the control substance, catechin, which recorded a scavenging percentage of 83.04%.

Lastly, the antibacterial potential of ethanolic extracts derived from *L. tigrinus* and *L. strigosus* was evaluated against two bacterial strains: *Staphylococcus aureus* BIOTECH 1582 and *Escherichia coli* BIOTECH 1634. In this study, the extract from *L. tigrinus* exhibited a measurable inhibitory effect on *S. aureus* BIOTECH 1582, producing a zone of inhibition measuring 15.60 mm in diameter. In contrast, *L. strigosus* ethanolic extracts introduced with distilled water at day 0 produced a 6.54 mm inhibition zone, while those treated with *B. cereus* after 5 days of incubation recorded an 8.11 mm diameter zone.

These findings affirm that the ethanolic extracts possess antibacterial capabilities, particularly against *S. aureus*, as demonstrated by the formation of inhibition zones, which signal microbial growth suppression. Comparable outcomes were reported by Dulay *et al*. (2017a), where a 9.48 mm inhibition zone was observed against *S. aureus* using an acetonitrile-based extract of *L. tigrinus*. Similarly, Dulay *et al*. (2014) confirmed that fruiting body extracts of *L. tigrinus* in ethanol effectively inhibit the growth of *S. aureus*.

Conversely, neither *L. tigrinus* nor *L. strigosus* ethanolic extracts showed inhibitory activity against the Gram-negative *E. coli* BIOTECH 1634, suggesting limited efficacy of these extracts against this particular bacterial strain. These observations are in line with those reported by Dulay *et al*. (2017a), where acetonitrile and hexane extracts of *L. tigrinus* also showed no antibacterial action against *E. coli*. Dulay *et al*. (2014) further supported this with findings indicating the ineffectiveness of ethanol-based extracts from *L. tigrinus* fruiting bodies against *E. coli*.

The antimicrobial activity observed in some extracts may be attributed to the presence of bioactive compounds capable of penetrating bacterial cell walls and membranes, leading to cellular disruption, as noted by Fischer *et al*. (2012). However, the specific compounds responsible for these effects have not yet been identified and require further investigation. Overall, the findings highlight the need for additional research to optimize the extraction processes and enhance the antibacterial properties of these mushroom-derived extracts, especially in relation to their activity against Gram-negative bacteria.

Conclusion

The present study demonstrated a significant enhancement in the mycelial dry weight of *Lentinus tigrinus* and *Lentinus strigosus* caused by *P. fluorescens* BIOTECH 1123 and *B. cereus* BIOTECH 1509. Notably, the highest reduction in liquid culture volume was observed in the same treatments, which corresponded to the highest production of mycelial biomass in *L. tigrinus* and *L. strigosus*, respectively. Furthermore, ethanol extracts of mycelia from these mushrooms showed different DPPH radical scavenging activities. *L. tigrinus* antibacterial activities were observed against *Staphylococcus aureus* BIOTECH 1582 in the extract produced with 0 days of mycelial incubation prior to the introduction of *B. cereus* BIOTECH 1509. Furthermore, the antibacterial activity of *L. strigosus* ethanolic extracts with five days of mycelial incubation prior to the introduction of *B. cereus* BIOTECH 1509 was observed against *S. aureus* BIOTECH 1582. Meanwhile, ethanolic extracts for both mushrooms showed no significant antibacterial activity against *Escherichia coli* BIOTECH 1634. Overall, the positive influence of specific bacteria on *L. tigrinus* and *L. strigosus* mycelial growth suggests the need for further exploration. Future studies could focus on detailed compound analyses and comprehensive bioactivity assessments to unlock the full therapeutic potential of these mushrooms.

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Author Contributions

EMM, GCMM and RMRD conceptualized and designed the study. EMM and GCMM gathered the data. EMM, GCMM, RMRD and AMDL analyzed and interpreted the findings. EMM and GCMM prepared the initial draft of the

manuscript. All authors critically reviewed the findings and collectively approved the final version for submission.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Data Availability

The datasets produced and/or utilized in this study are not publicly accessible due to proprietary restrictions. However, they may be made available by the corresponding author upon reasonable request.

Ethics Approval

The Ethical Clearance of the study from the CLSU Ethics Review Committee was issued and received by the authors on January 23, 2023.

References

Bauer AW, WMM Kirby, JC Sherris, M Turck (1966). Antibiotic susceptibility testing by a standardized single disk method. *Amer J Clin Pathol* 45:493–496

Carrasco J, DC Zied, JE Pardo, GM Preston, A Pardo-Giménez (2018). Supplementation in mushroom crops and its impact on yield and quality. *Appl Microbiol Biotechnol* 8:146–157

Chen L, M Yan, X Qian, Z Yang, Y Xu, T Wang, J Cao, S Sun (2022). Bacterial community composition in the growth process of *Pleurotus eryngii* and growth-promoting abilities of isolated bacteria. *Front Microbiol* 13:1–11

Cho YS, JS Kim, DE Crowley, BG Cho (2003). Growth promotion of the edible fungus *Pleurotus ostreatus* by fluorescent pseudomonads. *FEMS Microbiol Lett* 218:271–276

Choudhary DK (2011). First preliminary report on isolation and characterization of novel *Acinetobacter* spp. in casing soil used for cultivation of button mushroom, *Agaricus bisporus* (Lange) Imbach. *Intl J Microbiol* 2011:1–6

Dharmaraj K, T Kuberan, R Mahalakshmi (2014). Comparison of nutrient contents and antimicrobial properties of *Pleurotus djamor*, *Agaricus bisporus*, and *Ganoderma tsugae*. *Intl J Curr Microbiol Appl Sci* 3:518–526

Dulay RMR, EJB Garcia (2017). Optimization and enriched cultivation of Philippine (CLSU) strain of *Lentinus strigosus* (BIL1324). *Biocatal Agric Biotechnol* 12:323–328

Dulay RMR, EC Cabrera, SP Kalaw, RG Reyes (2021). Optimization of submerged culture conditions for mycelial biomass production of fourteen *Lentinus* isolates from Luzon Island, Philippines. *Biocatal Agric Biotechnol* 38:102–226

Dulay RMR, EC Cabrera, SP Kalaw, RG Reyes (2020). Nucleotide sequencing and identification of wild mushrooms from the Philippines. *Biocatal Agric Biotechnol* 27:1–7

Dulay RMR, LA Miranda, JS Malasaga, SP Kalaw, RG Reyes, CT Hou (2017a). Antioxidant and antibacterial activities of acetonitrile and hexane extracts of *Lentinus tigrinus* and *Pleurotus djamour*. *Biocatal Agric Biotechnol* 9:141–144

Dulay RMR, AGC Rivera, EJB Garcia (2017b). Mycelial growth and basidiocarp production of wild hairy sawgill *Lentinus strigosus*, a new record of naturally occurring mushroom in the Philippines. *Biocatal Agric Biotechnol* 10:242–246

Dulay RMR, KS Flores, RC Tiniola, DHH Marquez, AGD Cruz, SP Kalaw, RG Reyes (2015). Mycelial biomass production and antioxidant activity of *Lentinus tigrinus* and *Lentinus sajor-caju* in indigenous liquid culture. *Mycosphere* 6:659–666

Dulay RMR, MC Arenas, SP Kalaw, RG Reyes, EC Cabrera (2014). Proximate composition and functionality of the culinary-medicinal tiger sawgill mushroom, *Lentinus tigrinus* (Higher Basidiomycetes), from the Philippines. *Intl J Med Mushr* 16:85–94

Dulay RMR, SP Kalaw, RG Reyes, EC Cabrera, NF Alfonso (2012a). Optimal growth conditions for basidiospore germination and morphogenesis of Philippine wild strain of *Lentinus tigrinus* (Bull.) Fr. *Mycosphere* 3:926–933

Dulay RMR, SP Kalaw, RG Reyes, EC Cabrera, NF Alfonso (2012b). Optimization of culture conditions for mycelial growth and basidiocarp production of *Lentinus tigrinus* (Bull.) Fr., a new record of domesticated wild edible mushroom in the Philippines. *Philipp Agric Sci* 95:278–285

Fischer CL, DR Drake, DV Dawson, DR Blanchette, KA Brogden, PW Wertz (2012). Antibacterial activity of sphingoid bases and fatty acids against Gram-positive and Gram-negative bacteria. *Antimicrob Agents Chemother* 56:1157–1161

Javed S, A Javaid, U Hanif, S Bahadur, S Sultana, M Shuaib, S Ali (2021). Effect of necrotrophic fungus and PGPR on the comparative histochemistry of *Vigna radiata* by using multiple microscopic techniques. *Microsc Res Tech* 84:2737–2748

Kalaw SP, DO Alfonso, RMR Dulay, AMD Leon, JQ Undan, JR Undan, RG Reyes (2016). Optimization of culture conditions for secondary mycelial growth of wild macrofungi from selected areas in Central Luzon, Philippines. *Curr Res Environ Appl Mycol* 6:277–287

Kaneko M (2009). Auxin-regulation of hyphal elongation and spore germination in arbuscular mycorrhizal fungus, *Gigaspora margarita*. *Asian J Agric Rur Dev* 3:199–204

Kang YM, KM Cho (2014). Identification of auxin from *Pseudomonas* sp. P7014 for the rapid growth of *Pleurotus eryngii* mycelium. *Kor J Microbiol* 50:15–21

Kim MK, R Math, KM Cho, KJ Shin, JO Kim, JS Ryu, YH Lee, HD Yun (2008a). Effect of *Pseudomonas* sp. P7014 on the growth of edible mushroom *Pleurotus eryngii* in bottle culture for commercial production. *Bioresour Technol* 99:3306–3308

Kim MY, P Seguin, JK Ahn, JJ Kim, SC Chun, EH Kim, SH Seo, EY Kang, SL Kim, YJ Park, HM Ro, IM Chung (2008b). Phenolic compound concentration and antioxidant activities of edible and medicinal mushrooms from Korea. *J Agric Food Chem* 56:7265–7270

Kolak U, M Öztürk, F Özgökçe, A Ulubelen (2006). Norditerpene alkaloids from *Delphinium linearilobum* and antioxidant activity. *Phytochemistry* 67:2170–2175

Kozarski M, A Klaus, D Jakovljevic, N Todorovic, J Vunduk, P Petrovic, M Niksic, MM Vrvic, LV Griensven (2015). Antioxidants of edible mushrooms. *Molecules* 20:19489–19525

Leon AMD, RG Reyes, TEED Cruz (2013). Enriched cultivation of three wild strains of *Lentinus tigrinus* (Bull.) Fr. using agricultural wastes. *Intl J Agric* 9:1199–1214

Sharf W, A Javaid, A Shoaib, IH Khan (2021). Induction of resistance in chili against *Sclerotium rolfsii* by plant growth promoting rhizobacteria and *Anagallis arvensis*. *Egypt J Biol Pest Contr* 31:1-11

Wang XM, J Zhang, LH Wu, YL Zhao, T Li, JQ Li, YZ Wang, HG Liu (2014). A mini-review of chemical composition and nutritional value of edible wild-grown mushrooms from China. *Food Chem* 151:279–285

Zarenejad F, B Yakhchali, I Rasooli (2011). Evaluation of indigenous potent mushroom growth-promoting bacteria (MGPB) on *Agaricus bisporus* production. *World J Microbiol Biotechnol* 28:99–104

Zhang B, L Yan, Q Li, J Zou, H Tan, W Tan, W Peng, X Li, X Zhang (2018). Dynamic succession of substrate-associated bacterial composition and function during *Ganoderma lucidum* growth. *Peer J* 6:1-23

Zohaib KA, U Bashir, IH Khan, A Javaid, W Anwar (2024). Synergistic effect of plant growth promoting rhizobacteria and *Cirsium arvense* against black scurf disease of potato. *Sains Malays* 53:3071–3083