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# Phytohormone Induced Submerged Fermentation for Mycelia Biomass and Exopolysaccharide Production of *Agrocybe cylindracea* (DC.) Maire and *Pleurotus djamor* (Rumph. Ex Fr.) Boedijn

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**Abstract**

Mushrooms are an excellent source of promising nutritional and pharmaceutical attributes. Several mushrooms from the wild are being investigated for their promising effect on health risk reductions. However, some mushrooms remain to be underutilized due to improper cultivation techniques, ineffective nutrient sources and extrinsic factors. Thus, the mycelial biomass and exopolysaccharide (EPS) production of *Agrocybe cylindracea* and *Pleurotus djamor* in submerged fermentation conditions was determined using selected commonly discarded fruits at varying concentrations. Saba banana puree (30%) at pH 7 yielded the highest mycelial biomass of both mushrooms. Supplementation of 5 ppm 6-BAP on saba banana puree (30%) at pH 7 for both mushrooms increased the mycelial biomass yield at 58% for *A. cylindracea* and 61% for *P. djamor*. In varying agitation conditions, *A. cylindracea* greatly increased its mycelial biomass yield and EPS production at 100 rpm and *P. djamor* at 150 rpm. The fermentation period highly influenced the mycelial biomass and EPS production of both mushrooms. The highest mycelial biomass yield was obtained by a fermentation period of 20 days for *A. cylindracea* and 15 days for *P. djamor*, but the highest EPS yield can be precipitated at a fermentation period of 10 days for *A. cylindracea* and 15 days for *P. djamor*. Overall, maximum mycelial biomass and EPS yield were achieved on optimum submerged fermentation conditions, which can be further used for mass production and bioactivity profiling, which can be utilized in the nutraceutical, cosmeceutical and pharmaceutical industries.

**Keywords** – exopolysaccharides – mushrooms – mycelia biomass – phytohormones – submerged fermentation

## Introduction

Mushrooms are spore-bearing fruiting bodies of fungi that typically grow on soil or any food surfaces. They are either edible, poisonous or indigestible (Surekha et al. 2011). Aside from its culinary value due to its nutritional and unique umami taste, it is also loaded with carbohydrates, proteins, fibers, vitamins, and minerals and has low-fat content (Martinezmedina et al. 2021,

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Beelman et al. 2019). Moreover, due to its bioactive compounds of nutritive and medicinal value, many rescued wild macrofungi were cultivated and utilized. Recently, Despe et al. (2023) reported wild mushrooms that were utilized by the Subanen Tribes in Misamis Occidental. Furthermore, these wild mushrooms were harvested and grown, supplying wholesome food for human consumption, guaranteeing food security, creating jobs, and advancing environmental preservation (Dulay et al. 2023).

Moreover, as mushrooms are loaded with bioactive metabolites with medicinal and therapeutic values, it is being consumed as hot water extracts, powders, concentrates, health tonics, tea, soup, and herbal formulations (Chang & Zhao, 2002). However, mass production of fruiting bodies takes months prior to consumption to maximize its potentialities. Recent studies reported optimized production of mycelial biomass using submerged fermentations (Garcia et al. 2020, Lopez et al. 2022). A variety of variables, such as temperature, light intensity, aeration, and agitation, in addition to the source of nutrients, have a significant impact on the biomass production of mushroom mycelia in submerged cultures (Dulay et al. 2015). Recently, Aguilar et al. (2023a, b) and Mendoza et al. (2020) successfully optimized mycelial biomass production of different wild mushroom species using indigenous media and physical factors along with their biological activities.

Phytohormones, commonly referred to as plant growth regulators, are organic compounds that, when applied in small amounts, regulate plant growth in addition to several nutrients and vitamins supplemented (Prajapati et al. 2015). However, only several studies have reported the effect of phytohormones on the increase of the growth response of different basidiomycetes (Jonathan & Fasidi 2001, Dey et al. 2007, Guo et al. 2009, Wu et al. 2012) and recently, Momi & Sodhi et al. (2020) reported that the maximum colony diameter of *Calocybe indica* was observed on a media supplemented with Indole-3-acetic acid, Indole-3-butyric acid and Gibberellic acid. For the liquid media, biomass growth was maximum in media supplemented with GA. Additionally, mycelial biomass and exopolysaccharides from *Pellinus linteus* with the addition of 1-Naphthaleneacetic acid, a synthetic auxin, enhanced by 15.98 and 56.36% compared to the control, respectively (Guo et al. 2009).

Furthermore, the Philippines, being known as a mega-biodiverse hotspot and a tropical country (Posa et al. 2008), common diets of Filipinos are plant-based foods like fruits and vegetables, which are essential in the human diet due to its role in sustaining life and health (Del Rio et al. 2013). Nonetheless, several fruits are discarded when over-ripe due to their unpleasing taste and texture, like the Indian mango *(Mangifera indica* L.*)*, saba banana (*Musa acuminata var. balbisiana)* and mature coconut water *(Cocos nucifera* L.*)*, yet they are reported to have many functional activities like antioxidant and antidiabetic (Shah et al. 2025), anti-ulcer and antimicrobial (Ahmad et al. 2019) and sugar lowering, antibacterial and anticancer properties (Tuyekar et al. 2021), respectively.

One of the simplest mango varieties to grow in the Philippines, particularly in rural areas, is the Indian mango. Months of November through February is when it flowers, and March through June is when it is harvested. This mango variety, which is typically eaten as a green mango, is not particularly appetizing when ripe. Since there are currently no consumer goods made from Indian mangoes on the market, they are commonly thrown out when they are ripe (Bronce & Ona 2015). Conversely, saba bananas can be planted at any time of year and are grown all over the nation. The saba banana is starchy and is typically consumed cooked and as a vegetable, but any part of the banana can be used. Since saba bananas are consumed as vegetables rather than desserts, many vendors tend to discard overripe saba bananas from the market due to their high production (Department of Agriculture 2013). In the meantime, the Philippines is among the world's top producers of coconuts, and each part of the fruit has significant economic value (Gürbüz & Manaros 2019). Aside from the flesh, which is frequently used for cooking and desserts, the endosperm of mature coconuts is frequently discarded in the Philippine public markets, which is why many studies have documented their use as indigenous culture media and supplements for the growth of various fungi and plants during micropropagation (Dulay et al. 2021, Bautista &

Valentino 2023). Valorization of these commonly discarded fruits creates a great impact on sustainable food production, food security, and the circular economy, to lessen agricultural waste and to generate innovative solutions to its greater use (Nirmal et al. 2023, Leong & Chang 2022).

Nevertheless, as mushroom mycelia are being exploited and several studies reported different optimized submerged liquid conditions to maximize mycelial biomass production along with its bioactivities, the liquid spent culture is commonly discarded. However, different studies reported optimized conditions in submerged fermentation for exopolysaccharides production (Guo et al. 2023, Supramani et al. 2023), along with its potential bioactivities like hypoglycemic activity (Xia et al. 2023), anticancer (Atlagić et al. 2023) and antioxidant activity (Chaudhary & Rahi 2023). A range of polysaccharides derived from various natural sources are becoming more and more popular as supplements to improve health outcomes. Molecular biology, immunology, biotechnology, and pharmaceutical chemistry are among the fields in which mushroom polysaccharides have been found to have enormous potential (Wang et al. 2015).

Thus, this study was done to evaluate the use of selected commonly discarded fruits as a medium for the optimal submerged fermentation conditions of *Agrocybe cylindracea* and *Pleurotus djamor* with reference to different intrinsic and extrinsic factors. Establishing an effective cultivation strategy for these mushrooms allows effective utilization of their mycelia and exopolysaccharides. This is the first report in the country to use discarded fruits as liquid fermentation substrate for mycelia biomass and exopolysaccharide production, which can be used in the food, nutraceutical, and pharmaceutical industries.

## Materials & Methods

### *Mushroom Source*

Mushrooms *A. cylindracea* and *P. djamor* were used in this study. Pure cultures were acquired from the culture collections of the Center for Tropical Mushroom Research and Development, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines.

### *Culture Inoculant*

An agar block from the pure culture of each mushroom was aseptically transferred into a previously prepared sterilized potato dextrose agar (PDA) plate. Revived plates were incubated at 28–30 $^{o}$C to allow the growth of mycelia. After seven days of incubation, mycelial discs were prepared using a flame-sterilized 10-mm diameter cork borer. Mycelial discs served as culture inoculants in the evaluation of the optimum submerged growth conditions.

### *Evaluation of Culture Media and pH*

The effect of liquid culture media from Indian mango puree, saba banana puree and mature coconut water on the mycelia production of the mushrooms in submerged fermentation was evaluated. Ripe Indian mangoes and saba banana were prepared by making a puree, and mature coconut water was filtered to remove dirt particles prior to the preparation of different concentrations. The liquid culture media was prepared in varying concentrations (10%, 20%, 30%, 40% and 50%) and was adjusted to pH 6. A 100 ml of each liquid culture medium was transferred into a 250 ml Erlenmeyer flask, sterilized in an autoclave at 121 $^{o}$C, 15 psi for 30 minutes, cooled down, inoculated with mycelia discs aseptically, and incubated at room temperature to allow mycelia growth. After 10 days of incubation, mycelia were harvested, rinsed with distilled water, and oven-dried for 24 hours at 35 °C to 40 °C. The mycelial dry weight was presented as milligrams dry weight (mg DW). The optimum liquid media was determined in terms of mycelial dry weight after 10 days of incubation.

For the pH of the optimum liquid culture medium, the best liquid culture medium was adjusted to pH 5.0 to 9.0 with 1.0 intervals using 1 M NaOH and 1 M HCl. The most suitable liquid medium with the best pH level was used for the evaluation of phytohormone induction, agitation conditions and fermentation periods.

***Evaluation of Induced Phytohormone, Agitation and Fermentation Period for Mycelia and Exopolysaccharide Production***

The influence of phytohormones, agitation and fermentation period on biomass production were evaluated using the method of Dulay et al. (2015) with modifications. To evaluate the influence of different phytohormones (indole-3-butyric acid, commercial gibberellic acid and 6-benzylaminopurine) on the mycelial growth, different concentrations (1, 5, 10, 15 and 20 ppm) of phytohormones and control were used. Prepared liquid culture media in best pH in culture bottles were sterilized, cooled down and supplemented with phytohormone via sterile filter filtration, swirled, and inoculated with 7-day-old mushroom mycelial discs. Incubation was done at room temperature (28–30 $^{o}$C) for 10 days and mycelial dry weight was recorded.

For the determination of the optimum agitation, the liquid culture medium with the best culture media, pH and phytohormone concentration were exposed to different agitation conditions which are static, 100 rpm, 150 rpm and 200 rpm. After 10 days, the mycelial biomass was harvested, and the supernatant was filtered and used for exopolysaccharide (EPS) precipitation. Mycelia and EPS dry weight were obtained and recorded.

The effect of the fermentation period (10, 15, 20 and 25 days) on the mycelia and exopolysaccharide production was also evaluated using the optimal liquid culture conditions.

***Isolation of Exopolysaccharides***

The protocol of exopolysaccharide (EPS) isolation was followed after El-Mahdy et al. (2023) with minor modifications. Exopolysaccharides (EPS) were isolated from the spent liquid culture. The spent liquid culture was centrifuged at 5000 rpm for 10 minutes at room temperature. After centrifugation, the supernatant was collected and cold 95% ethanol (1:2) was added and stored at 4 °C for 12 hours. After 12 hours, precipitated exopolysaccharides were harvested using a siever, washed gently with distilled water and oven-dried for 12 hours. The collected EPS was weighed (mg DW) and stored in a refrigerator prior to use.

***Statistical Analysis***

Each experiment was conducted with three replications. Analysis of variance (ANOVA) was used to analyze the data in a one-way classification analysis, and Tukey's HSD was used to compare the treatment means at the 5% significance level.

**Results**

***Evaluation of Culture Media and pH***

The effect of different fruit purees at varying concentrations is shown in Table 1. The highest mycelial biomass yield for the growth of A. *cylindracea* and *P. djamor* were recorded in banana puree at 30% concentration with 217.67 mg DW and 212.78 mg DW, respectively. Aside from 30% banana puree, varying concentrations of banana puree, mango puree and mature coconut water were also observed to be favorable for the mycelial growth of *A. cylindracea* and *P. djamor*. In contrast, several concentrations of mature coconut water were registered to have lower mycelial biomass yield in both mushrooms.

The effect of different pH levels in 30% banana puree is shown in Table 1. Culture media at pH 7 registered having the highest yield of mycelial biomass for *A. cylindracea* and *P. djamor* and were also found statistically comparable to other biomass yields of other pH values in both mushrooms, except for pH 5 in *A. cylindracea*, which is comparable to pH 7. The biomass yield of *A. cylindracea* and *P. djamor* at pH 7 is observed to be at 260.67 mg DW and 247.33 mg DW, respectively.

***Evaluation of Induced Phytohormone, Agitation and Fermentation Period for Mycelial and Exopolysaccharide Production***

The effect of induced phytohormones at several concentrations and percentage increases on

the mycelial biomass yield of *A. cylindracea* and *P. djamor* is shown in Table 2. The 6-benzylaminopurine (6-BAP) at 5 ppm reveals the highest mycelial biomass yield for *A. cylindracea* and *P. djamor* at 413.33 mg DW and 398.33 mg DW, respectively, compared to other concentrations of 6-BAP, as well as on the other phytohormones induced with varying concentrations. In addition, the mycelial growth percentage increase of *A. cylindracea* and *P. djamor* at 5ppm of 6-BAP shows the highest at 58.43% and 61.05%, respectively, compared to other phytohormone concentrations. It is also observed that higher phytohormone concentrations result in a decrease in mycelial biomass yield and negative mycelial biomass yield percentage change, as observed in indole-3-butyric acid (IBA) and commercial gibberellic acid.

The liquid culture flasks of *A. cylindracea* and *P. djamor* with the best culture media, pH and phytohormone concentration were subjected to different agitating conditions including static, 100 rpm, 150 rpm and 200 rpm as shown in Table 3. The highest mycelial biomass yield was observed at 100 rpm for *A. cylindracea* at 947 mg DW, and 150 rpm for *P. djamor* at 2582.33 mg DW. Furthermore, the exopolysaccharides of the supernatants of each mushroom liquid culture medium were also precipitated using 95% cold ethanol. The EPS at 100 rpm and 150 rpm of *A. cylindracea* and *P. djamor* were observed to have the highest yield at 416.67 mg DW and 105.67 mg DW, respectively. The amount of EPS precipitated is highly correlated to the amount of mycelial biomass yield of the mushrooms. The agitating conditions that yielded the highest mycelial biomass also have the highest amount of precipitated EPS. It is also noted that the EPS at the static condition significantly has lower EPS yields.

**Table 1.** Mycelial biomass yield of mushrooms at different submerged fermentation media and pH.

| **Factors** | | **Mycelial Dry Weight (mg DW)** | |
|---|---|---|---|
| **Culture Media** | | ***A. cylindracea*** | ***P. djamor*** |
| **Mango Puree** | 10% | $83.20\pm 2.21^{abc}$ | $55.70\pm7.50^{a}$ |
| | 20% | $117.00\pm3.00^{abc}$ | $96.00\pm9.16^{bc}$ |
| | 30% | $122.50\pm8.94^{abcd}$ | $175.30\pm4.51^{d}$ |
| | 40% | $128.50\pm4.04^{abcd}$ | $181.60\pm7.28^{d}$ |
| | 50% | $159.00\pm5.57^{cd}$ | $196.00\pm6.19^{d}$ |
| **Banana Puree** | 10% | $141.16\pm9.11^{bcd}$ | $26.90\pm8.92^{a}$ |
| | 20% | $154.00\pm7.21^{bcd}$ | $105.67\pm6.50^{c}$ |
| | 30% | $217.67\pm3.21^{d}$ | $212.78\pm6.00^{d}$ |
| | 40% | $178.67\pm2.51^{cd}$ | $111.08\pm3.73^{c}$ |
| | 50% | $173.33\pm3.79^{cd}$ | $106.70\pm9.97^{c}$ |
| **Mature Coconut Water** | 10% | $30.67\pm4.61^{a}$ | $21.60\pm1.08^{a}$ |
| | 20% | $50.67\pm4.62^{ab}$ | $33.43\pm2.34^{a}$ |
| | 30% | $107.67\pm4.61^{abc}$ | $59.60\pm8.45^{ab}$ |
| | 40% | $108.67\pm4.13^{abc}$ | $115.63\pm6.67^{c}$ |
| | 50% | $50.63\pm2.96^{ab}$ | $93.33\pm7.50^{bc}$ |
| **pH** | | | |
| | 5 | $94.00\pm4.58^{a}$ | $143.67\pm7.51^{a}$ |
| | 6 | $227.33\pm7.63^{ab}$ | $198.67\pm9.61^{a}$ |
| | 7 | $260.67\pm5.67^{b}$ | $247.33\pm8.19^{a}$ |
| | 8 | $225.33\pm8.72^{ab}$ | $212.22\pm7.77^{a}$ |
| | 9 | $216.33\pm6.43^{ab}$ | $197.67\pm9.07^{a}$ |

Values are expressed as mean ± SD. Means with the same letter superscripts are not significantly different at 5% level of significance.

After determining the optimum conditions for the mycelial biomass and production of *A. cylindracea* and *P. djamor*, the optimum fermentation period for mycelia and EPS production is observed as shown in Table 3. As noted, the mycelial biomass yield was found highest at 20 days

for *A. cylindracea* and 15 days for *P. djamor* at 3435 mg DW and 4576 mg DW, respectively. In addition, the mycelial biomass yields at each fermentation period show significant differences from each other. On the other hand, the total EPS produced was found highest at 10 days for *A. cylindracea* and 15 days for *P. djamor* at 416.67 mg DW and 177.33 mg DW respectively. The result of the study suggests that the mycelial biomass yield increases as the fermentation period increases, and decreases after reaching beyond the optimum fermentation days, in contrast to the EPS produced as it decreases as the fermentation period increases.

**Table 2.** Mycelial biomass yield at varying phytohormone concentrations and percentage change using 30% banana puree at pH 7.

| Phytohormone (ppm) | | Mycelial Dry Weight (mg DW) | | | |
|---|---|---|---|---|---|
| | | ***A. cylindracea*** | **% Increase** | ***P. djamor*** | **% Increase** |
| **IBA** | **1** | $289.33 \pm 10.50^{a}$ | 10.68 | $248.67 \pm 1.15^{ab}$ | 0.54 |
| | **5** | $343.00 \pm 10.00^{ab}$ | 31.58 | $251.67 \pm 2.51^{ab}$ | 1.75 |
| | **10** | $319.67 \pm 8.02^{ab}$ | 22.37 | $265.00 \pm 6.00^{ab}$ | 7.42 |
| | **15** | $316.33 \pm 3.21^{ab}$ | 21.26 | $312.67 \pm 3.21^{ab}$ | 26.42 |
| | **20** | $189.00 \pm 4.35^{a}$ | -27.49 | $106.67 \pm 8.14^{a}$ | -56.87 |
| **GA** | **1** | $300.67 \pm 4.04^{a}$ | 15.34 | $329.67 \pm 5.03^{ab}$ | 33.29 |
| | **5** | $326.67 \pm 10.21^{ab}$ | 29.15 | $297.33 \pm 10.50^{ab}$ | 20.22 |
| | **10** | $345.67 \pm 2.08^{ab}$ | 32.60 | $287.33 \pm 6.80^{ab}$ | 16.17 |
| | **15** | $337.67 \pm 7.02^{a}$ | 29.53 | $223.67 \pm 6.65^{ab}$ | -9.57 |
| | **20** | $280.33 \pm 9.71^{a}$ | 7.54 | $187.33 \pm 3.05^{ab}$ | -24.12 |
| **6-BAP** | **1** | $326.67 \pm 10.41^{ab}$ | 28.89 | $278.67 \pm 4.04^{ab}$ | 12.53 |
| | **5** | $413.33 \pm 2.51^{b}$ | 58.43 | $398.33 \pm 3.51^{b}$ | 61.05 |
| | **10** | $323.67 \pm 10.58^{ab}$ | 23.91 | $310.67 \pm 8.50^{ab}$ | 25.61 |
| | **15** | $310.67 \pm 2.08^{ab}$ | 18.92 | $304.67 \pm 7.09^{ab}$ | 23.05 |
| | **20** | $290.67 \pm 1.52^{ab}$ | 11.25 | $290.33 \pm 4.93^{ab}$ | 17.52 |
| **Control** | | $260.67 \pm 7.23^{a}$ | - | $247.33.33 \pm 5.85^{ab}$ | - |

Values are expressed as mean ± SD. Means with the same letter superscripts are not significantly different at a 5% level of significance. *Control- with no induced phytohormones, % Increase compared using the control.

**Table 3**. Mycelia biomass yield and EPS produced at varying agitating conditions using 30% banana puree at pH 7 induced with 6-bap at 5 ppm and fermentation period.

| **Factors** | ***A. cylindracea*** | | ***P. djamor*** | |
|---|---|---|---|---|
| **Agitation (rpm)** | **Mycelia (mg DW)** | **EPS (mg DW)** | **Mycelia (mg DW)** | **EPS (mg DW)** |
| 100 | $947.00 \pm 8.54^{a}$ | $416.67 \pm 6.67^{a}$ | $2011.67 \pm 6.62^{a}$ | $97.67 \pm 2.51^{a}$ |
| 150 | $457.67 \pm 2.08^{b}$ | $376.67 \pm 7.23^{a}$ | $2582.33 \pm 5.68^{b}$ | $105.67 \pm 9.07^{a}$ |
| 200 | $289.00 \pm 6.55^{b}$ | $337.00 \pm 9.64^{a}$ | $2308.00 \pm 6.24^{b}$ | $100.00 \pm 1.73^{a}$ |
| Static | $310.33 \pm 10.55^{b}$ | $4.00 \pm 1.00^{b}$ | $297.33 \pm 7.02^{c}$ | $2.66 \pm 1.53^{b}$ |
| **Fermentation Period (Days)** | | | | |
| 10 | $947.00 \pm 8.54^{a}$ | $416.67 \pm 6.66^{a}$ | $2582.33 \pm 5.68^{a}$ | $105.67 \pm 9.07^{a}$ |
| 15 | $1525.67 \pm 4.51^{b}$ | $325.00 \pm 6.55^{b}$ | $4576.00 \pm 7.55^{b}$ | $177.33 \pm 9.50^{b}$ |
| 20 | $3435.00 \pm 6.24^{c}$ | $73.00 \pm 4.58^{c}$ | $3925.33 \pm 6.66^{c}$ | $90.67 \pm 6.35^{a}$ |
| 25 | $2505.00 \pm 6.00^{d}$ | $17.67 \pm 7.37^{d}$ | $3323.00 \pm 5.29^{d}$ | $52.67 \pm 5.03^{c}$ |

Values are expressed as mean ± SD. Means with the same letter superscripts are not significantly different at a 5% level of significance.

**Discussion**

The growth of mushrooms varies among species due to various internal and external factors (Bellettini et al. 2019). These elements are crucial in identifying the ideal growth conditions that promote the productive growth of mushrooms. The formation of mycelia and bioactive compounds in a variety of mushrooms has been extensively facilitated by submerged fermentation. The method has several beneficial effects, including increased productivity, a small, controlled environment for consistency and quality, and a shorter production time (Chen et al. 2011, Tang et al. 2007, Rangel-Castro et al. 2002). In addition, the growth of a mushroom depends on the nutritional content of the culture media, as it affects its luxurious mycelial growth (Aguilar et al. 2023a).

The suitability of 30% banana puree for the mycelial growth of *A. cylindracea* and *P. djamor* could be attributed to the nutritional components of bananas that help the efficient growth of the mushrooms. Carbon and nitrogen are the primary nutrient requirements of the mushrooms. The presence of different nutrients in bananas, such as proteins, potassium, sodium and calcium (Robles et al. 2019), as well as vitamins (riboflavin, folate, Vitamin C, carotenoids), and minerals like magnesium, iron, manganese, zinc, copper and boron (Ashokkumar et al. 2018), provides essential carbon, sugar and other nutrients that support the luxuriant growth of mushrooms.

In addition, bananas also contain bioactive compounds like flavonoids, tannic acid, various amino acids and phytosteroids, in addition to antioxidant compounds like carotenoids and polyphenols (Sidhu & Zafar 2018). Meanwhile, a study reported by Silva et al. (2018) revealed that the growth of *P. djamor* on solid media using different banana varieties, such as Latundan, Lakatan and Saba, as a nutrient source was comparable to the positive control, potato dextrose agar. There are no more other reports that used banana fruit as a nutrient source for the mycelial growth of mushrooms, however, studies that reported the presence of different nutrients, vitamins and sugar suggest that it is a preferable choice for the mycelial growth of the mushrooms (Kumari et al. 2023).

The mycelial biomass of *A. cylindracea* and *P. djamor* is influenced by pH, which is an important intrinsic factor. Mushrooms have their optimal pH range for growth and development, and it also varies depending on the species. The direct impact of the hydrogen ions in the media can explain the biomass yield that was obtained at different pH levels. The pH has a significant impact on the entry of sodium ions and other essential molecules from the media into the individual cells (Elisashvili 2012). The capability of the mushrooms to grow on different pH levels is due to the activity of enzymes which exhibit strong activity and stability in pH 2–8 (Quiroz-Casteñada et al. 2009). The result of the study is aligned with the result of Aguilar et al. (2023b), wherein the mycelial biomass yield of *Pycnoporus sanguineus* at varying pH levels is not significantly different to each other. Also, the result of the study by Fabros et al. (2023a) reported that the growth of mycelia in indigenous solid media of *Coprinopsis cinerea, Coprinopsis vercillata* and *Leucoprinospsis cretaceous* reveals that mycelial growth is not affected by varying pH levels. In addition, *Cyclocybe cylindracea* mycelial growth at different pH levels shows faster mycelial growth in pH 6 compared to higher pH levels (Landingin et al. 2020) but some mushrooms like *Trametes versicolor* favorably grow faster in pH levels 7 to 8 as reported by Fabros et al. (2023b).

Phytohormones or plant growth regulators are organic compounds, that when applied in small amounts, regulate plant growth in addition to several nutrients and vitamins supplemented (Prajapati et al. 2015). It is also believed to be an intrinsic signal molecule that the plant produces in very small amounts. They optimize metabolic activity in particular cells both locally and during plant transportation, and they regulate a variety of physiological functions in cells (Upreti et al. 2014). The decrease in mycelial biomass yield in higher concentrations of phytohormones is due to its inhibiting effect in higher amounts, as they perform best when applied in smaller amounts. Similar results indicate a decrease in the amount of mycelial growth with increasing phytohormone concentrations (Wu et al. 2012, Islam et al. 2009, Mukhopadhyay et al. 2005). Moreover, similar studies have shown the efficacy of different induced phytohormones at lower concentrations to increase the mycelial growth of mushrooms (Jonathan & Fasidi 2001, Dey et al. 2007, Guo et al. 2009). Additionally, Wu et al. (2012) reported that IBA, GA3 and NAA at 2.0 to 10 mg/L promote

mycelial growth of *H. marmoreus*. Momi & Sodhi et al. (2020) revealed that the maximum colony diameter and growth rate of *Calocybe indica* were observed in a medium supplemented with IBA, IAA and GA from 10 to 25 mg/L. Furthermore, Dulay et al. (2020) reported that gibberellic acid stimulated the mycelial growth of *L. tigrinus* and *L. strigosus*, whereas other four evaluated phytohormones such as alpha-naphthaleneacetic acid, indole-3-acetic acid, gibberellic acid and furfurylaminopurine supported the rapid mycelial growth of *L. swartzii.*

Agitation generates a shear stress, which results in morphological changes and is a crucial parameter for the transfer of heat, dissolved oxygen, and substrate. Oxygen dissolved and shear stress are significant determinants of polysaccharide synthesis (Herbst et al. 1992, Dreventon et al. 1994, Lee et al. 2008). Also, agitation improves the mycelial mass, gas supply, and heat transfer (Cui et al. 1997). Agitation conditions had a significant impact on the mushrooms' biomass yield; however, once the ideal agitation condition was exceeded, further increasing the agitation (rpm) reduced both the mycelial biomass yield and EPS, consistent with the findings of Nguyen et al. (2012).

Moreover, fungal exopolysaccharides (EPSs) are naturally occurring biopolymers that have a wide range of potential uses in the food, cosmetic, packaging, and biomedical industries. According to Mahapatra & Banerjee (2013), fungal extracellular polysaccharides (EPSs) are easily extracted and purified polysaccharides possess various reactive groups, including hydroxyl, carboxyl, and amine, for chemical modification. They are also biodegradable, biocompatible, immunogenicity-free, bio-adhesion-capable, and have antibacterial activity. Fungal EPSs are secreted into the extracellular medium in the form of biofilm or capsules (Barcelos et al. 2020, Kagimura et al. 2015). The low EPS yield under static conditions, as shown in Table 4, is due to the reason that EPS secretion is highly influenced by the supply of oxygen and nutrients, which the agitation conditions can supply (Lee et al. 2004). The significant increase in mycelial biomass and EPS yield in mushrooms is due to agitation conditions, which enhance mixing in the culture broth, which is necessary to provide the nutrient and oxygen supply required for fungal growth (Cho et al. 2006), thus, increasing the production of biomass and EPS yield (Xu et al. 2006). Because there is an adequate supply of oxygen, the mycelia can effectively use the nutrients in the media (Peng et al. 2000). The significant increase in mycelia biomass of the mushrooms is due to the agitating conditions of the medium similar to the results obtained of Aguilar et al. (2023b). Moreover, Kachrimanidou et al. (2023) revealed that agitation conditions in *T. versicolor* proved beneficial for biomass and productivity and substrate consumption rates of the mushroom. Also, increased agitations reveal maximum polysaccharide and mycelial biomass in *Inonotus obliquus* (Xu et al. 2015).

The obtained results for the optimal fermentation period are similar to the result of Wu & Zhang (2012), as an increasing incubation period results in decreasing EPS and mycelial biomass yield, once reached beyond the optimum fermentation period. In addition, the decreasing yield of the EPS produced as the fermentation period increases is due to the reduction in culture filtrate, where EPS is suspended and will be precipitated. Nevertheless, as agitation conditions increase the oxygen supply and nutrient uptake of mushrooms (Cho et al. 2006), the amount of liquid media decreases, leading to a reduction in suspended EPS available for precipitation. The obtained results are consistent with the result of Wu & Zhang (2012), who reported that mycelial biomass and EPS production of *Grifola frondosa* decrease once the optimum incubation period is exceeded. Similarly, mycelia and EPS production of *Antrodia cinnamomea* showed a decreasing pattern beyond 20 days of incubation (Lin & Sung 2006). Furthermore, the mycelial biomass and EPS production of *G. lucidum* increased upon the addition of Tween 80 to the optimized culture media (Yang et al. 2021).

## Conclusion

The high mycelial biomass yield of *A. cylindracea* and *P. djamor* was observed in 30% Banana Puree at pH 7 at room temperature. Induction of phytohormone 6-BAP at 5 ppm increased the mycelia biomass yield of both mushrooms. The high mycelial biomass yield and EPS

production of the mushrooms were greatly influenced by different agitation conditions. *Agrocybe cylindracea* preferred agitation conditions at 100 rpm while *P. djamor* at 150 rpm. The fermentation period for high mycelial biomass and EPS production of each mushroom varies from each other, but choices could be made on which fermentation period should be followed, depending on whether mycelial or EPS is needed in future studies. A longer fermentation period could be used if mycelial biomass is needed, but a higher EPS yield can be precipitated in a shorter fermentation period.

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