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Optimization of Culture Condition for the Mycelial Growth of *Pleurotus ostreatus* (Sporeless Strain)

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Abstract. The cultivation of *Pleurotus ostreatus* mushrooms has gained popularity in the Philippines because of its ability to grow across a wide range of temperatures and to utilize diverse lignocellulosic substrates. However, optimizing culture conditions is essential for improving the growth efficiency of sporeless strains, which are preferred due to reduced health risks and better quality of mushroom products. This study aimed to determine the optimum cultural conditions for the mycelial growth of *P. ostreatus* (sporeless strain) by evaluating the effects of media type, pH level, aeration (sealed vs. unsealed plates), illumination (dark, light, or alternating dark/light), and incubation temperature. Mycelial growth was assessed across five different culture media and varying environmental conditions. Results revealed that rice bran decoction *gulaman* supported the most favorable growth, with near-neutral to slightly basic pH, under sealed or non-aerated conditions. Growth was consistent across dark, light, or alternating illumination, and was optimal at both room temperature and air-conditioned settings. These findings demonstrate that the sporeless strain exhibits strong adaptability to diverse conditions, reducing cultivation constraints compared with spore-forming counterparts. This research establishes the specific cultural requirements for the sporeless strain, which has been less studied despite its advantages. Optimizing its growth conditions can enhance mushroom production efficiency, minimize spore-related health risks for growers, and promote sustainable utilization of local agro-industrial byproducts, contributing to food security and rural livelihood development. This research supports SDGs by contributing to SDGs 2 (Zero Hunger) through improved mushroom production efficiency and food security; SDGs 3 (Good Health and Well-being) by reducing spore-related occupational health risks; SDGs 8 (Decent Work and Economic Growth) by strengthening livelihood opportunities in small-scale enterprises; SDGs 12 (Responsible Consumption and Production) through the sustainable use of agro-industrial byproducts; and SDGs 13 (Climate Action) by promoting resource-efficient cultivation practices aligned with the sustainability framework of the United Nations Sustainable Development Goals.

Keywords: Aeration; Illumination; Mycelial growth; optimization; *Pleurotus ostreatus* (sporeless strain)

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INTRODUCTION

Mushrooms are widely recognized as both nutraceutical and functional foods, offering not only nutritional benefits but also bioactive compounds with therapeutic properties. They are rich sources of carbohydrates, proteins, vitamins, minerals, and antioxidants, which make them an important component of balanced human diets (Jayachandran et al., 2017; Pandey et al.,2022; Rathore et al. 2017). Beyond their nutritional profile, mushrooms contain biologically active compounds such as polysaccharides, β-glucans, and phenolics that contribute to immune enhancement, cholesterol reduction, and anticancer activities (Ishimoto et al., 2017; Kumar et al., 2021). Given their ability to grow on decayed organic matter rich in lignin, cellulose, and hemicellulose (Bustillos et al., 2025; Dou et al., 2025), mushrooms also play an important role in natural ecosystems as decomposers and have been harnessed for sustainable agricultural production.

The growing global demand for affordable protein sources highlights the urgent need to identify alternative, sustainable, and accessible foods. Protein deficiency remains a persistent issue in developing countries where many populations face limited access to animal-based proteins (Agriculture Organization of the United States, 2013; Moini & Ferdowsi, 2024; Schönfeldt & Hall, 2012). In this context, mushroom

cultivation has emerged as a sustainable solution. The oyster mushroom (*Pleurotus ostreatus*), in particular, is one of the most extensively cultivated species worldwide due to its fast growth, high adaptability, and ability to utilize a wide variety of agro-industrial wastes (Desisa et al., 2024; Fufa et al., 2021). This feature allows mushroom production to not only contribute to food security but also to reduce environmental burdens by recycling agricultural residues that would otherwise contribute to waste accumulation.

Previous studies have demonstrated the versatility of *P. ostreatus* in growing on diverse substrates such as wheat straw, rice straw, maize stover, cotton waste, banana leaves, and sugarcane bagasse (Fanadzo et al., 2010; Girmay, 2016; Gebru et al., 2024). More recently, Nascimento Deschamps et al. (2024) demonstrated the successful cultivation of oyster mushrooms using brewer's spent grains and other agro-industrial residues, further proving its value as a low-cost and eco-friendly crop. The use of such substrates not only lowers production costs but also provides farmers with an additional source of income while supporting environmental sustainability. Moreover, the ability of oyster mushrooms to produce fruiting bodies in stacked clusters enhances their yield potential, which is particularly relevant for small-scale mushroom farmers seeking efficient use of limited space (Maseko et al., 2024).

Despite these benefits, as with other mushrooms, the mycelial growth and yield of oyster mushrooms are highly influenced by culture conditions. Mycelium development is dependent on several factors, including medium composition, pH, temperature, aeration, and illumination (Endes, 2021; Hoa & Wang, 2015; Kim et al., 2015; Zawadzka et al., 2022). The growth medium is especially critical as it provides the nutrients necessary for initiating and sustaining vigorous mycelial growth. Mycelial growth rate and density, in turn, directly affect the quality, quantity, and timing of fruiting body formation. For instance, studies have shown that near-neutral to slightly acidic pH and moderate temperatures are generally favorable for most *Pleurotus* species (Hoa & Wang, 2015; Sultana et al., 2018). However, variations exist across strains, and the lack of optimized parameters often results in delayed colonization, contamination problems, and inconsistent yields.

A key challenge in oyster mushroom cultivation lies in the limited understanding of sporeless strains, which are increasingly valued due to reduced spore-related health risks to workers and consumers. Airborne spores from mushroom farms have been associated with respiratory problems, allergies, and occupational hazards (Ficociello et al., 2019; Li et al., 2016). Sporeless strains thus provide a safer alternative, particularly for commercial-scale production, but have been less studied compared with their spore-producing counterparts (Krupodorova et al., 2024). This knowledge gap creates uncertainty in identifying the most suitable culture conditions to maximize their growth and productivity.

Addressing this gap, the present study was conducted to determine the optimum culture conditions for the mycelial growth of *P. ostreatus* (sporeless strain). Specifically, it aimed to evaluate the effects of media type, pH, aeration (sealed vs. unsealed plates), illumination (dark, light, or alternating), and incubation temperature. Establishing these parameters provides an evidence-based guide for maximizing growth efficiency under controlled conditions.

The outcomes of this study are expected to offer practical benefits to mushroom growers, researchers, and small-scale farmers. By identifying culture conditions that minimize production time, reduce contamination, and enhance yield, this research can improve the economic viability of mushroom cultivation at both household and commercial scales. On a broader level, promoting sporeless oyster mushroom production contributes to food security, sustainable waste management, and environmental protection by converting lignocellulosic agro-wastes into high-value protein-rich food products. Such innovations align with global efforts to develop low-cost, sustainable food systems that simultaneously address nutritional deficiencies and environmental challenges.

METHODS

To find the best conditions for mycelial growth, a pure culture of *P. ostreatus* (sporeless strain) was revived aseptically and was grown on five different media, namely Rice Bran Decoction Gelatin (RBDG), Corn Grit Decoction Gelatin (CGDG), Soybean Decoction Gelatin (SBDG), and Coconut Water Gelatin, while Potato Sucrose Gelatin was used as a control. Physical conditions were evaluated, laid out in a Completely Randomized Design, and analyzed using ANOVA.

Source of Pure Culture

Pure culture of *P. ostreatus (*sporeless strain*)* was obtained from the germplasm collection of the Center for Tropical Mushroom Research and Development (CTMRD) of the Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines. The culture was revived by aseptically transferring approximately 10 mm mycelial disc into a sterile potato sucrose gelatin plate. (provide references, please)

Preparation of Media

Five different culture media were used for growing mycelia and as a source of nutrients. These were the Rice Bran Decoction Gelatin (RBDG), Corn Grit Decoction Gelatin (CGDG), Soybean Decoction Gelatin (SBDG), Coconut Water Gelatin, and Potato Sucrose Gelatin was used as controls. Twenty-five grams of each source were brought to a boil with 500 ml of distilled water, to be strained and reconstituted with distilled water. Then it was added with 10 grams of gulaman bar (500 mL coconut water mixed with 10 grams of gulaman), dissolved in a low flame until the mixture was homogeneous. The prepared media in ketchup bottles were sterilized in an autoclave at 121ºC, at a pressure of 15 psi for 30 minutes (DeSantis, 2021; Krupodorova et al., 2024; Mihai et al., 2022; Sookwongse, 2014).

Influence of Five Different Media on Mycelial Growth

To determine the influence of these five different media on the mycelial growth of *P. ostreatus* (sporeless strain), an approximately 10 mm mycelial disc of a seven-day-old pure culture of *P. ostreatus* (sporeless strain) was aseptically inoculated into the center of sterile plates. The inoculated plates were sealed with parafilm and incubated at room temperature. The mycelial growth was measured using a vernier caliper after the seven-day incubation period. The mycelial growth rate (mm/day) and the average growth was note (Krupodorova et al., 2024). The mycelial density was determined qualitatively using the following scale: + (very thin (++) thin, (+++) thick, and (++++) very thick. The average growth rate values of the three replicates were reported for each treatment.

Evaluation of Physical Conditions

The influence of physical conditions such as pH, aeration, illumination, and temperature on the mycelial growth was evaluated following the protocol described by Lopez et al. (2022) with minor modifications. The best culture medium, among the five different media, was used as the assay medium. The initial pH of the medium was adjusted to varying levels, namely: 5, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0 using 0.1 M HCl or 0.1 M NaOH. Each plate with varying pH levels was inoculated with a 10 mm mycelial disc of the one-week-old culture of *P. ostreatus* (sporeless strain). The best medium and pH were used in the determination of the influence of aeration on mycelial growth. In this experiment, two aeration conditions were evaluated: sealed and unsealed conditions. In the sealed condition, the plates were wrapped with parafilm to prevent the entrance of air, while in the unsealed condition, the plates were not wrapped with parafilm. The petri plates were incubated at room temperature. The best medium, pH, and aeration were used in the evaluation of the effect of light on mycelial growth. The plates were incubated in alternating dark and light, dark and lighted conditions, at room temperature. Finally, the optimum medium, pH, aeration, and light conditions were used in determining the influence of temperature on the mycelial growth of *P. ostreatus* (sporeless strain). The culture plates were separately incubated at room temperature, air conditioning temperature, and refrigeration temperature. The mycelial diameter, incubation growth rate, and mycelial density were determined.

Statistical Analysis

The data collected were analyzed using the procedure used by Almoguera et al. (2024). The assumptions for Analysis of Variance (ANOVA) under a single-factor Completely Randomized Design (CRD) were tested prior to data analysis. Mean comparisons were performed using the least significant difference (LSD) test at a 5% significance level. The Statistical Tool for Agricultural Research (STAR) software was used for the statistical analysis.

RESULTS AND DISCUSSION

Influence of Five Different Media on Mycelial Growth

Every living organism requires food for its growth and reproduction, and *P. ostreatus*is no exception to this. The white thread-like structure in the substrate that provides nutrition to the mushroom is known as mycelium. To culture this sporeless strain of *P. ostreatus* in the laboratory, it

is necessary to furnish these compounds in the media that are required for its growth and other life processes. In the present study, *P. ostreatus* (sporeless strain) was cultured successfully in the laboratory environment. Five different media, i.e., PSG (Potato Sucrose Gulaman), Coco Water Gulaman, Rice Bran Decoction Gulaman, Corn Grit Decoction Gulaman, and SoyBean decoction Gulaman were used to identify their effect on mycelial growth.

Data (Table 1 and Figure 1) shows that the observed colony diameter of mushrooms on Rice Bran Decoction Gulaman is significantly different from four other media at ($p < 0.05$). Potato Sucrose Gelatin was not significantly different from corn grit decoction gelatin and significantly different from Coconut Water gelatin and Soybean Decoction gelatin. Results showed that the mycelial growth rate per day of *P.ostreatus* (sporeless strain) in Rice bran decoction gelatin is significantly higher than the control, which is Potato sucrose gelatin, and to the other four media used, the CWG, CGDG, and SBDG, respectively. The result indicated that Rice Bran decoction gulaman is media suitable for culturing of *P. ostreatus*(sporeless strain).

Table 1. Mycelial growth rate, Mycelial diameter (7-day incubation period), and Mycelial density of *P. ostreatus*(sporeless strain) on different culture media

Media	Mycelial growth rate (mm/day)	Mycelial diameter (mm)	Mycelial Density
Potato Sucrose Gelatin	10.48[b]	73.34[b]	+++
Coconut Water Gelatin	8.40[c]	58.86[c]	+++
Rice Bran Decoction Gelatin	12.27[a]	85.86[a]	++++
Corn Grit Decoction Gelatin	10.07[b]	70.50[b]	++
Soybean Decoction Gelatin	8.18[c]	57.30[c]	++

Means with the same superscript are not significantly different from each other at a 5% level of significance. The mycelial density was evaluated based on: very thin (+), thin (++), thick (+++), and very thick (++++).

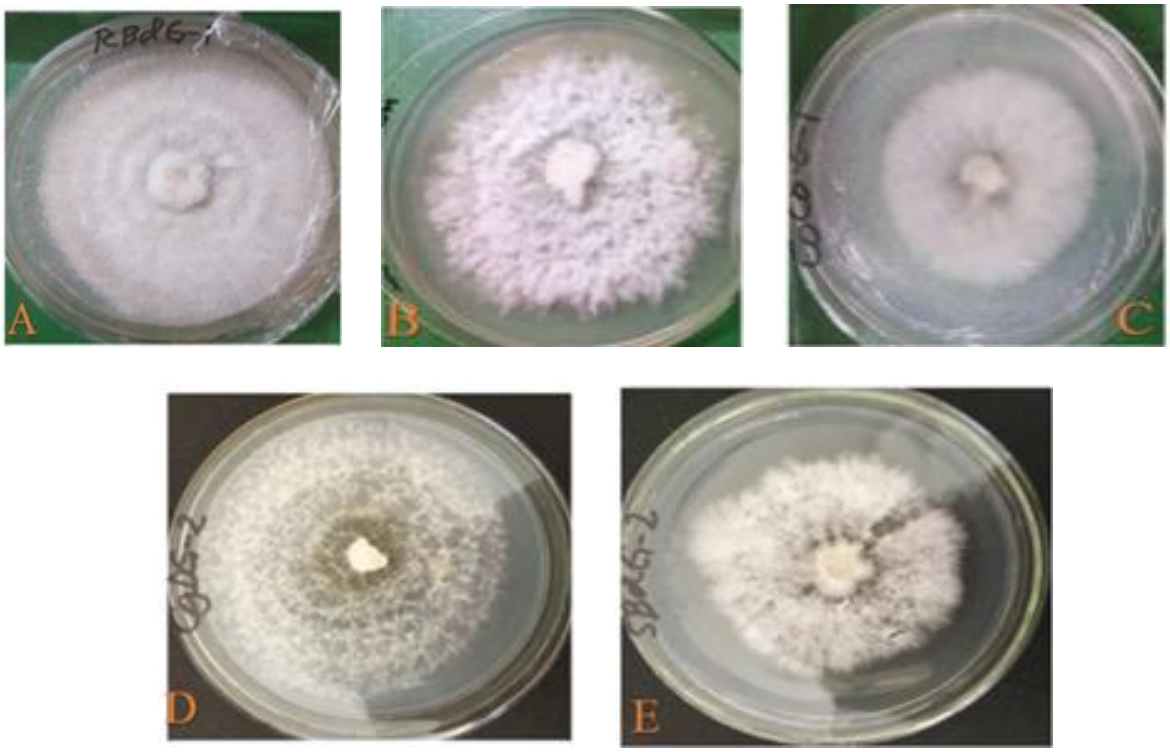

Figure 1. Mycelial growth and density of *P. ostreatus* (sporeless strain) in different media (A)Rice Bran decoction gulaman (B)Potato sucrose gulaman (C) Coconut water gulaman (D) Corngrit decoction gulaman (E) Soybean decoction gulaman.

The superiority of rice bran could be attributed to the variety of chemical components of rice bran that support efficient mycelial growth. Rice bran is a valuable source of carbohydrates, dietary fiber, sugar, sucrose, glucose, fructose, protein, and fat (Al-Doury et al., 2019; Sapwarobol et al., 2021; Sharif et al., 2014). In addition, the maximum mycelial growth of *P. ostreatus*was achieved in glucose as a source of carbon and peptone as a source of nitrogen (Nwokoye et al., 2010). Aside from the important nutritional components, minerals (calcium, iron, magnesium, phosphorous, potassium, sodium, zinc, copper, manganese, and selenium), and vitamins (riboflavin, thiamin, niacin, pantothenic acid, folate, choline, alpha-tocopherol, vitamin B6 and vitamin K) are also found in rice bran (Ryan, 2011; Zarei et al., 2017).

Minerals at certain concentrations stimulate the mycelial growth of mushrooms. Iron is essential for the vegetative growth of the mycelia, whereas calcium performs an important physiological role in the medium. Calcium supports the maximum growth of *Pleurotus,* which may be attributed to its role in the metabolic process, such as glycolysis and respiration (Yan et al., 2020; Zhu et al., 2019). Moreover, a good yield of *P. florida* can be obtained from a medium with a 5:1 carbon/nitrogen ratio, thiamine, gibberellin, calcium, and zinc. This supports the study of Chae and Ahn (2013) about the optimization of rice bran and food waste compost contents in a mushroom culture medium to maximize mycelial growth rate and fruit body yield of *Pleurotus ostreatus,* wherein the optimum conditions for maximum mycelial growth rate (14.8 cm 20 d^{-1}) were 9.3% RB and 24% FWC. According to them, the contents of rice bran (RB) and food waste components (FWC) significantly affect the mycelial growth of *Pleurotus ostreatus*. Dulay et al., (2015) reported that *L.tigrinus and L.sajor-caju,* edible wood-rotting basidiomycetes, have efficiently grown on rice bran (RB) decoction with the highest yield of mycelia, 11.53g and 9.75g, respectively.

pH of the Medium

Mycelium of fungi (mushrooms) obtains nutrients from substrate at a specific level of pH (Mardiana et al., 2021). To determine the optimum pH requirement of *P. ostreatus*(sporeless strain), the initial pH of the medium was adjusted to different pH ranges from 5.0 to 8.0 with an interval of 0.5. The mycelial growth response of PO (sporeless strain) is presented in Table 2 and Figure 2. Among the different pH levels evaluated, pH 8 recorded significantly faster mycelial growth with a mean of 88.78 mm in diameter and an incubation rate of 12.68 mm/day. As shown in the table, all pH ranges are not significantly different at 5% levels of significance, hence they are comparable to each other. On the other hand, pH 5.5 exhibited the lowest mycelial growth. This only shows that PO (sporeless strain) prefers slightly acidic and slightly alkaline medium. The present result is in agreement with the result of Nwokoye et al. (2010), who observed that the mycelium of *P. ostreatus* grew optimally at a temperature of 28 °C and pH of 9. Thus, the ability of the mycelia to tolerate this temperature and pH range of 3- 10 enabled them to flourish in agro wastes in the tropics. Bumanlag et al. (2018) recently reported that the mycelial growth of *Pleurotus djamor* is significantly higher at pH 8 and a shorter incubation period, which is comparable to pH 7.5, 6.5, and 6.0. In addition, three species of *Pleurotus,* namely *Pleurotus citrinopileatus, Pleurotus djamor, and Pleurotus salmoneostramineus*, have significantly higher mycelial growth at pH level 8 using mature coconut water gulaman media (Jacob et al., 2015). This result suggests that *Pleurotus* prefers a slightly alkaline medium.

Table 2. Mycelial growth rate, Mycelial diameter (7-day incubation period), and Mycelial density of *P. ostreatus*(sporeless strain) at different pH levels.

pH of the Medium	Mycelial growth rate (mm/day)	Mycelial diameter (mm)	Mycelial Density
5.0	11.46	80.21	+++
5.5	10.38	72.68	++
6.0	11.83	82.81	+++
6.5	12.26	85.85	+++
7.0	12.05	84.40	+++
7.5	12.55	87.84	+++
8.0	12.68	88.78	+++

The mycelial density was evaluated based on: very thin (+), thin (++), thick (+++), and very thick (++++).

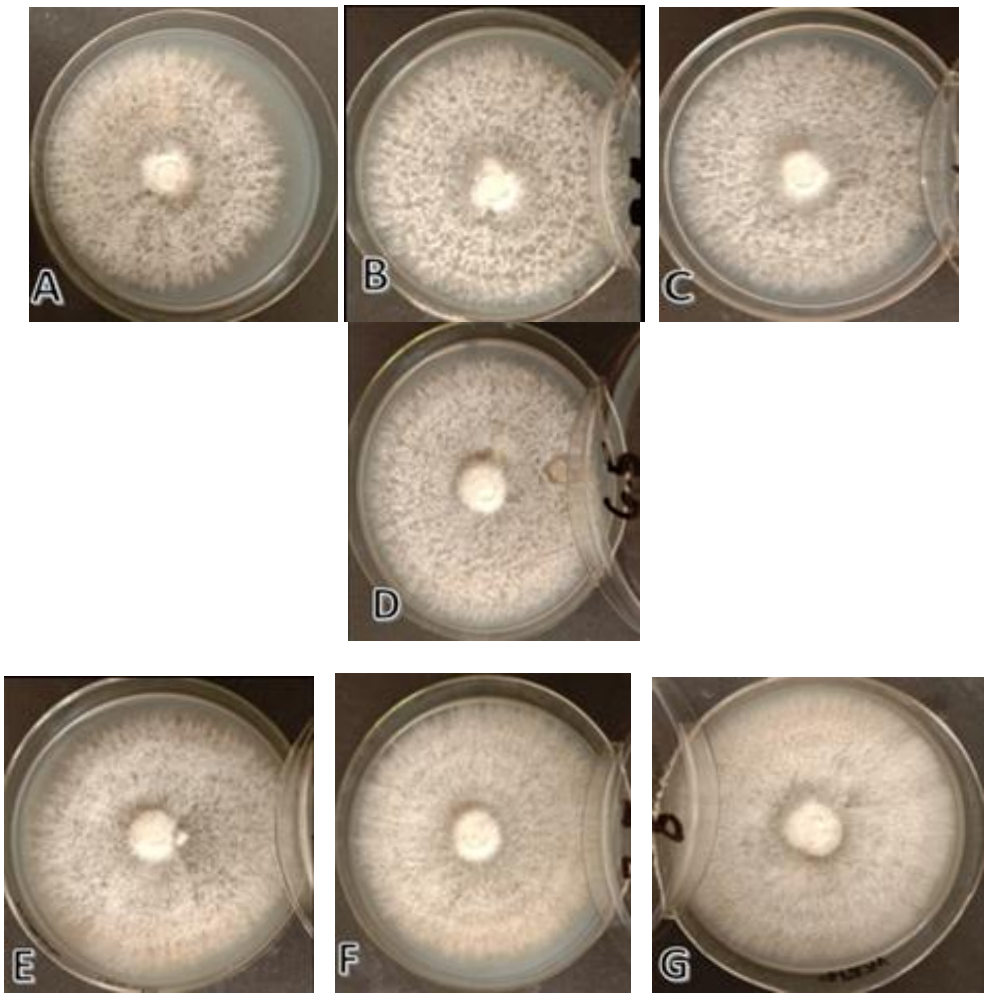

Figure 2. Mycelial growth and density of P. ostreatus (sporeless strain) at different pH levels: (A) 5.0 (B) 5.5 (C) 6.0 (D) 6.5 (E) 7.0 (F) 7.5 (G) 8.0.

Aeration

Oxygen and carbon dioxide are important gases for the growth of fungi. Most fungi are obligate aerobes, but many will also grow in reduced levels of oxygen (Bumanlag et al., 2018). The influence of aeration on the mycelial growth of P. *ostreatus* (sporeless strain) was evaluated by growing the mycelia in sealed and unsealed petri plates. Table 3 presents the mycelial growth rate, incubation period, and mycelial density in aeration conditions.

Table 3. Mycelial growth rate, Mycelial diameter (7-day incubation period), and Mycelial density of *P. ostreatus*(sporeless strain) by aeration (sealed and unsealed), illumination (lighted, dark, and alternating light and dark) and temperature (29 °C, 22 °C, and 10 °C) conditions**.**

Aeration Condition	Mycelial growth rate (mm/day)	Mycelial diameter (mm)	Mycelial Density
Sealed	13.54[a]	94.88[a]	++++
Unsealed	9.90[b]	69.32[b]	++
Lighted Condition	11.43	80.04	+++
Dark Condition	12.12	84.82	++++
Alternating Dark and Light	12.11	84.74	++++
Room temperature (29 °C)	10.90[a]	76.29[a]	+++
Air Condition (22 °C)	9.55[a]	66.83[a]	+++
Refrigerator (10 °C)	3.07[b]	21.52[b]	+

In each physical factor, means with the same superscript are not significantly different from each other at a 5% level of significance. The mycelial density was evaluated based on: very thin (+), thin (++), thick(+++), and very thick (++++).

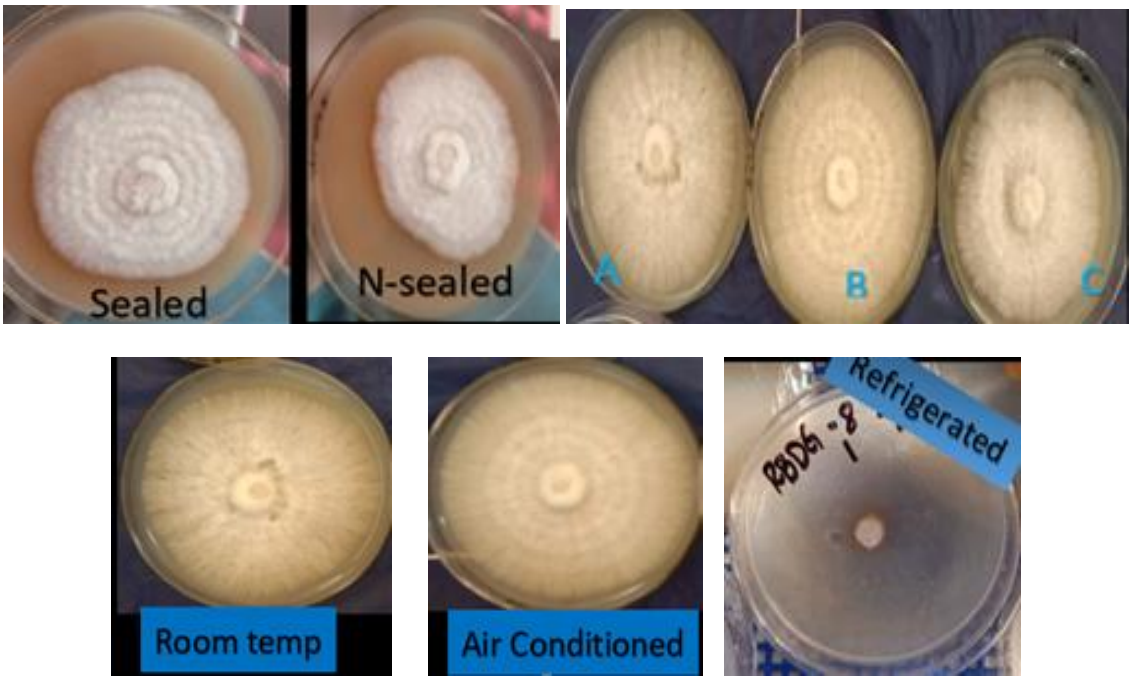

Figure 3. Culture plates of P. ostreatus(sporeless strain) as affected by Aeration (A) Sealed and (B) Non-sealed; Illumination (A) Dark (B) Alternating dark/light (C) Light Conditions; Temperature: Room Temperature, Air conditioned and Refrigerated.

Results showed significant differences in the growth of mycelia of the species and it favors sealed petri plates or non-aerated conditions. After the seven-day incubation period, the mean growth in diameter of the mushroom in sealed petri plates is 94.88^{+-}mm while the mycelial mean growth in unsealed conditions is 69.32 mm. This only shows that *P. ostreatus*(sporeless strain) has favorable mycelium growth in sealed petri plates condition. In the study of Jacob et al., 2015), *P. djamor and P. citrinopileatus* incubated in a non-aerated or oxygen-deprived plate produced larger mycelial diameter while *P. salmoneostramineus* exhibited a wider mycelial diameter in the aerated plate. According to them, the mycelia of these mushroom species can grow luxuriantly in both conditions. A similar result was obtained by Dulay et al (2012) who reported that *Lentinus tigrinus* can be incubated in either non-aerated or aerated conditions. However, Gu et al. (2024) explain that low rotational speed slows down growth due to insufficient oxygen content in the fermentation liquid, implying that aeration is beneficial.

Illumination

The mycelium of mushrooms from the *Pleurotus* genus does not require light for its growth (Kibar & Peksen, 2008), nevertheless, light is necessary for the proper development of carpophores (Ibrahim et al., 2024; Xie et al., 2018). In this study, the influence of light on mycelial growth was evaluated by separately incubating the culture plates in lighted and dark conditions. Table 3 also shows the mycelial growth, incubation period, and mycelial density of *P. ostreatus*(sporeless strain) in the three illumination conditions. Data shows no difference in the mycelial growth response, which indicates that the mycelial growth is not affected by light. This conforms to the observation of Bumanlag et al. (2018), and also with the study of Jacob et al. (2015) in their research about the Mycelial Growth Performance of Three Species of *Pleurotus* on Coconut Water Gelatin. Akkan et al. (2024) indicated that the mycelium of mushrooms from the *Pleurotus* genus does not require light for growth, but light is necessary for the proper development of carpophores, which are the fruiting bodies. De Leon et al. (2017) also reported that illumination conditions do not affect the mycelial growth of *Lentinus sajor caju*. On the other hand, Zhu et al. (2024) confirmed that light impacts the development of *P. ostreatus* fruiting bodies by identifying differentially expressed proteins and related pathways, such as oxidative phosphorylation, when comparing light treatments to darkness.

Temperature

Temperature is one of the most important and

critical factors affecting mycelial growth in mushroom cultivation (Hu et al., 2023). To determine the optimal temperature for mycelium growth, species of *P. ostreatus*(sporeless strain) were cultivated in three different temperature conditions, such as room temperature (29 °C), air-conditioned temperature (22 °C), and refrigerated conditions (10 °C). Data in Table 3 and Figure 3 show the mycelial growth rate, mycelial diameter of growth after a seven-day incubation period, and mycelial density as affected by three temperature conditions.

The result revealed that mycelial growth at room temperature and air condition have no significant differences from each other, but shows a highly significant difference from mycelial growth at refrigerated conditions. This only entails that the mycelium of PO (sporeless strain) can grow best both at room temp (29 °C) and air conditioning (22 °C), but low temperature (10 °C) can retard/inhibit the growth. This finding conforms with the study of Bumanlag (2018), who had a significant result that mycelial growth of *Pleurotus djamor* is best at 29 °C room temperature. Rout et al.(2015) reported that the mycelial growth of ten species of *Pleurotus* was reduced drastically beyond 30 °C and completely inhibited at a temperature of 40ºC while at 10 °C the growth declined drastically. Fletcher (2019) reported that the optimal temperature for mycelium growth of two Oyster mushrooms (*P. ostreatus*) was obtained at 22 °C, while Hu et al. (2023) noted that a previous study reported a maximum mycelial growth at 25°C for most *Pleurotus* strains, with maximum growth for many strains also reported at 30°C.

This optimum temperature result indicated that these *Pleurotus* species can grow better in tropical regions. These findings were also proven by Jacob, et al (2015) when they were able to grow *Pleurotus citrinopileatus, Pleurotus djamor*, and *Pleurotus salmoneostramineus* on coconut water gelatin at room temperature. Kong (2004) from his subsequent studies on the temperature requirement of *Pleurotus* mushrooms stated that the optimum temperature range to grow this kind of mushrooms is between 25-30°C, while the season to best grow these species is during summer and fall. The result of the present study shows that *P. ostreatus(*sporeless strain) has optimal nutrition and physical factors for efficient mycelial growth and has an opportunity to develop oyster mushroom production here in the Philippines as one of the developing countries in Asia.

This research supports Sustainable Development Goals (SDGs) by advancing SDGs 2 (Zero Hunger) through the optimization of cultural parameters (media composition, pH, aeration, illumination, and temperature) that enhance mycelial growth kinetics, production efficiency, and yield stability of *P. ostreatus*, thereby strengthening food system productivity and availability of nutrient-dense protein sources. It contributes to SDGs 3 (Good Health and Well-being) by promoting the adoption of sporeless strains, which significantly reduce bioaerosol exposure and associated respiratory health risks in occupational cultivation environments. The study supports SDGs 8 (Decent Work and Economic Growth) by improving process efficiency and reducing production variability, thereby increasing economic viability, value addition, and enterprise resilience among smallholder and micro-scale producers. It advances SDGs 12 (Responsible Consumption and Production) through the valorization of lignocellulosic agro-industrial residues (e.g., rice bran) as low-cost substrates, promoting circular bioeconomy principles, waste minimization, and resource recovery. Furthermore, it contributes to SDGs 13 (Climate Action) by enabling low-input, resource-efficient cultivation systems with reduced material intensity and environmental footprint, consistent with the sustainability framework of the United Nations.

CONCLUSION

This study determined the optimum culture conditions for the mycelial growth of *Pleurotus ostreatus* (sporeless strain) by evaluating the effects of media type, pH, aeration, illumination, and incubation temperature. The results showed that rice bran decoction gulaman was the most suitable medium, with optimal mycelial growth occurring at near-neutral to slightly basic pH levels. Growth was enhanced under sealed (non-aerated) conditions and was not markedly influenced by illumination, as comparable growth was observed under dark, light, and alternating light–dark regimes. In addition, satisfactory mycelial development was recorded at both room temperature and air-conditioned incubation. Overall, the findings confirm that media type, pH, aeration, illumination, and temperature significantly affect the mycelial growth of *P. ostreatus* (sporeless strain), and that the identified conditions provide an optimum environment for its in vitro cultivation. Future research should

evaluate fruiting body development and yield under these optimized conditions and examine the effects of nutrient formulation, particularly specific carbon and nitrogen sources.

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AUTHOR CONTRIBUTION STATEMENT

All authors made substantial contributions to the work reported in this manuscript. ZMCRD conceptualized the study, conducted the literature review, designed the methodology, led the data collection, and performed the data analysis. ALG contributed to data collection, assisted in data visualization and interpretation, and provided critical revisions and substantive feedback during the manuscript drafting and revision process. All authors reviewed and approved the final manuscript and agree to be accountable for all aspects of the work, ensuring its accuracy and integrity.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this paper.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI) tools were used in the generation, analysis, or writing of this manuscript. All aspects of the research, including data collection, interpretation, and manuscript preparation, were carried out entirely by the authors without the assistance of AI-based technologies.

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