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Effects of Media Formulation, Temperature, and Preservation Period on the Viability of White Oyster Mushroom (*Pleurotus ostreatus*) Spores

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Abstract: This study aimed to evaluate the effects of growth medium formulation, storage temperature, and preservation period on the spore viability of the white oyster mushroom (*Pleurotus ostreatus*). The study employed an experimental method using a split-plot design involving three types of preservation media: Potato Dextrose Broth (PDB), PDB supplemented with 15% paraffin, and corn decoction medium. The treatments were evaluated under two storage temperature conditions, namely 4°C and room temperature at 26°C, across four preservation periods: 2, 4, 6, and 8 weeks. The observed parameters included the time of colony emergence, colony number, and microscopic hyphal morphology. The results showed that all media stored at 4°C were able to maintain spore viability for up to 8 weeks. PDB produced the highest colony numbers, ranging from 224 to 593 colonies, and showed the fastest colony emergence, occurring on days 1 to 2. PDB supplemented with 15% paraffin produced 128 to 248 colonies, with colony emergence on day 2, whereas the corn decoction medium produced 89 to 142 colonies, also with colony emergence on day 2. All treatments stored at room temperature at 26°C became contaminated and therefore failed to maintain spore viability. Microscopic observations indicated that hyphal structures remained normal in all treatments stored at 4°C. Based on colony number and growth rate, PDB stored at 4°C demonstrated better preservation performance than the other media in maintaining the spore viability of *Pleurotus ostreatus* for up to eight weeks of storage.
Keywords: *Pleurotus ostreatus*; preservation; growth medium; storage temperature; viability

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INTRODUCTION

White oyster mushroom (*Pleurotus ostreatus*) is an economically important edible mushroom belonging to the phylum Basidiomycota. This species is widely cultivated because of its high market value, broad adaptability to various lignocellulosic substrates, and relatively rapid mycelial growth (Rosmiah et al., 2020). Successful cultivation of *P. ostreatus* depends not only on substrate quality and environmental conditions but also on the biological stability of the starter culture, particularly mycelial viability, hyphal morphological integrity, and the ability of the culture to recover after storage (Krupodorova et al., 2024). Therefore, reliable preservation methods are essential for maintaining high-quality mushroom spawn and reducing losses of valuable fungal genetic resources. The development of practical culture preservation techniques also contributes to SDG 2 (Zero Hunger), SDG 9 (Industry, Innovation, and Infrastructure), and SDG 12 (Responsible Consumption and Production) by supporting the availability of high-quality spawn, promoting biotechnology-based innovation, and minimizing biological resource losses in mushroom cultivation systems (Lozada-Martinez et al., 2026).

Despite its importance, the production and distribution of mushroom spawn at the household level and in small-scale laboratories still face several constraints, particularly the decline in genetic stability and culture viability during storage. Conventional preservation through repeated subculturing on fresh solid media may increase the risk of somatic mutation, physiological degeneration, and reduced extracellular enzyme activity (Patricia et al., 2020a). Repeated transfer also increases handling frequency, which may elevate the risk of contamination and phenotypic variation in fungal cultures (Nakasone et al., 2004; Wiest et al., 2012). In addition, storage without adequate protective conditions can reduce culture viability and mycelial growth capacity by up to 40–50% within several months (Eichlerová et al., 2015), while contamination by wild microorganisms has been reported to reach 25–30% in laboratories with limited sterility facilities (Suryani & Carolina, 2017).

Several preservation strategies have been developed to overcome these limitations. Sterile mineral oil or paraffin overlay has been reported to slow metabolic activity and maintain culture purity by restricting oxygen diffusion (Surja et al., 2020). Meanwhile, nutrient-rich liquid media such as Potato Dextrose Broth (PDB) can support cellular recovery by providing readily available carbon sources for fungal metabolism. In the context of short-term preservation, liquid media may facilitate the recovery of viable mycelial fragments after storage, particularly when combined with low-temperature conditions that reduce metabolic activity (Rohadi et al., 2020). Corn boiling water has also been explored as a low-cost alternative medium because it contains soluble nutrients derived from maize, making it potentially suitable for simple laboratory or small-scale cultivation systems (Suryani & Carolina, 2017). Furthermore, storage at low temperature, particularly 3–4°C, is generally more effective in maintaining fungal culture viability than storage at room temperature, approximately 26°C, because reduced temperature suppresses respiration, enzymatic activity, and microbial contaminant growth (Fariani et al., 2021; Liu et al., 2024).

Previous studies have evaluated several preservation approaches for edible and filamentous fungi, but each approach has specific limitations. Eichlerová et al. (2015) applied cryopreservation and sterile-water protocols to *P. ostreatus* using Malt Extract Agar (MEA) at −80°C and 4°C for 12 months, with laccase activity as the main parameter. However, cryopreservation requires relatively high operational costs and may cause cellular injury due to ice crystallization when not properly controlled. Adhikari (2022) used periodic subculturing of *Pleurotus sajor-caju* on Potato Dextrose Agar (PDA) at 4°C for six months and evaluated mycelial growth rate, but this method remains vulnerable to cross-contamination and somatic mutation. Surja et al. (2020) evaluated mineral oil immersion for fungal isolates grown on PDA at 25–28°C for three months using culture recovery rate as the main parameter; however, prolonged oxygen restriction may increase the risk of anoxia and delay post-storage recovery. These findings indicate that preservation success is influenced by both the nutritional environment and storage conditions, but an integrated evaluation of medium composition, oxygen limitation, temperature, and storage duration remains limited.

Most previous studies have therefore addressed fungal preservation only partially. Studies on mineral oil or paraffin have not sufficiently optimized storage duration, studies using PDB have not fully considered the role of storage temperature, and the use of corn boiling water has mainly been discussed as a growth medium rather than as a liquid preservation medium. Moreover, few studies have compared nutrient-rich media, oxygen-limiting overlays, and low-cost local media within the same experimental framework for maintaining *P. ostreatus* culture viability. Consequently, the combined effects of preservation medium, oxygen limitation through paraffin,

storage temperature, and preservation duration on the viability and morphological stability of *P. ostreatus* cultures remain insufficiently understood.

Based on these research gaps, this study aimed to evaluate the effects of preservation medium, storage temperature, and preservation duration on the post-storage viability, growth initiation time, colony number, and hyphal morphological stability of *Pleurotus ostreatus*. It was hypothesized that low-temperature storage combined with a nutrient-rich medium would better maintain culture viability and hyphal integrity than room-temperature storage or a low-cost alternative medium. The findings are expected to clarify the potential of corn boiling water as an economical local preservation medium and assess the effectiveness of the PDB–paraffin combination in maintaining culture quality. Thus, this study may provide a practical basis for developing simple, applicable, and sustainable short-term preservation technology for mushroom spawn in small-scale and academic laboratories.

METHOD

Research Location and Period

This study was conducted at the Plant Protection Laboratory, Faculty of Agriculture and Business, Satya Wacana Christian University, and at the Mycology Laboratory, Jl. Sunanampel No. 3, RT01/RW04, Sidorejo District, Salatiga City, Central Java, Indonesia. The research was carried out from October 2025 to February 2026.

Materials and Preparation of Culture Isolates

The white oyster mushroom (*Pleurotus ostreatus*) isolate used in this study was obtained from the pure culture collection of the Mycology Laboratory of PT Mikoo Sijamur Cantik, Salatiga. The initial culture was grown on pure Potato Dextrose Agar (PDA) medium and maintained at 25°C under dark conditions to ensure that the mycelium remained in an active vegetative phase.

Inoculum preparation was performed aseptically in a Laminar Air Flow (LAF) cabinet. Pure cultures of white oyster mushroom grown on PDA medium were cut using a sterile cork borer with a standard diameter. Four mycelial plugs were carefully collected using a sterile scalpel and transferred into glass jars containing liquid preservation media according to each treatment.

After inoculation, the glass jars containing the mixture of preservation medium and fungal isolate plugs were immediately sealed tightly and covered with aluminum foil to minimize light exposure inside the jars. The media and fungal isolates were then homogenized using a magnetic stirrer at a constant speed for 3 days (72 hours). This stirring process was intended to break the mycelial plugs into smaller hyphal fragments and distribute them evenly throughout the preservation medium before the main storage or preservation stage.

Experimental Design, Media Formulation, and Aseptic Procedures

This study employed an experimental approach using a split-plot design. The main plot consisted of storage temperature with two levels: cold storage at 4°C (S1) and room temperature at 26°C (S2). The subplot consisted of preservation duration with four levels, namely 2, 4, 6, and 8 weeks (W1, W2, W3, and W4), combined with three liquid preservation media formulations, as follows:

M1: PDB medium, prepared by weighing 7.2 g of granulated PDB, adding 300 mL of distilled water, and stirring until homogeneous.

M2: PDB medium supplemented with 15% sterile liquid paraffin, which was poured to form a protective layer over the surface of the liquid PDB medium after homogenization of the mycelial inoculum.

M3: Corn boiling water medium, prepared by washing shelled corn, boiling it for 3 minutes, and collecting the resulting boiled water.

M1S1W1	M1S2W3	M2S2W1	M3S1W3
M1S1W2	M1S2W4	M2S2W2	M3S1W4
M1S1W3	M2S1W1	M2S2W3	M3S2W1
M1S1W4	M2S1W2	M2S2W4	M3S2W2
M1S2W1	M2S1W3	M3S1W1	M3S2W3
M1S2W2	M2S1W4	M3S1W2	M3S2W4

Figure 1. Treatment combinations of media formulation, storage temperature, and preservation duration for white oyster mushroom (*Pleurotus ostreatus*)

The treatment arrangement produced 24 treatment combinations with three replications (Figure 1), resulting in a total of 72 experimental units. The inoculum consisted of homogeneous mycelial fragments produced through magnetic stirring, with 10 mL of inoculum added to each treatment tube.

To ensure aseptic conditions, all glassware, centrifuge tubes, and growth media (M1, M2, M3, and PDA) were sterilized using an autoclave at 121°C for 20 minutes. Liquid paraffin was sterilized separately using an autoclave at 121°C for 30 minutes. All stages of media inoculation and inoculum transfer using a micropipette were carried out aseptically in a Laminar Air Flow (LAF) cabinet to minimize the risk of external contamination.

Post-Preservation Viability Testing

After the designated preservation periods according to each treatment had elapsed (2, 4, 6, and 8 weeks), sample tubes were removed from the storage conditions, either from a refrigerator at 4°C or from room temperature at 26°C. A 1 mL aliquot of homogeneous mycelial inoculum from each preservation medium was aseptically collected using a micropipette, inoculated onto PDA medium in a Petri dish, and evenly spread using an L-shaped glass spreader. The Petri dishes were then incubated in an incubator under dark conditions to promote renewed growth.

The observed parameters included the time of initial colony emergence, recorded as the day on which mycelial growth first appeared on the medium surface. Observations were conducted from day 1 to day 7. Colony density, expressed as the number of fungal colonies, was counted from day 2 to day 7 after inoculation using a digital colony counter based on the standard plate count method. The validity of plate counts was ensured by counting only plates with clearly separated colony growth. Microscopic hyphal morphology was examined on day 7 by collecting hyphae using an inoculating loop, placing them on a microscope slide, staining them with lactophenol cotton blue, and observing them under an Olympus microscope at a maximum magnification of 56× to determine the presence or absence of structural abnormalities in the hyphae.

Data Analysis

Although the initial experimental design adopted a quantitative split-plot approach, data analysis in this study was shifted to a descriptive qualitative method. This adjustment was made because all treatment units stored at room temperature

(26°C) experienced complete contamination (100%) beginning from the second week of observation. This condition resulted in the loss of data variance and an incomplete orthogonal matrix required for analysis of variance (ANOVA). Therefore, the evaluation of viability, mean colony number, and microscopic visualization of hyphae was systematically focused only on samples that survived under low-temperature storage at 4°C. The possible causes of complete contamination under room-temperature storage are discussed comprehensively in the Results and Discussion section as a basis for improving preservation protocols in future studies.

RESULTS AND DISCUSSION

Fastest Colony Emergence and Colony Density in Different Growth Media Formulations

Observation of the initial period of colony emergence and post-preservation colony density provided an initial indication of the effectiveness of each preservation medium formulation. Based on the observational data, the type of medium used was closely associated with the rate of cellular activity recovery in Pleurotus ostreatus spores after storage (Table 1).

Table 1. Fastest colony emergence and colony density in different growth media formulations

Treatment	Time of colony emergence (day)	Colony density (mean ± SD)
M1S1W1	1	517.00 ± 22.63 (n = 2)
M1S1W2	1	264.67 ± 12.58 (n = 3)
M1S1W3	2	224.00 ± 72.92 (n = 3)
M1S1W4	2	593.33 ± 17.62 (n = 3)
M1S2W1	Contaminated	Contaminated
M1S2W2	1	Contaminated
M1S2W3	Contaminated	Contaminated
M1S2W4	2	Contaminated
M2S1W1	2	165.67 ± 135.36 (n = 3)
M2S1W2	2	150.00 ± 70.71 (n = 2)
M2S1W3	2	247.50 ± 60.10 (n = 2)
M2S1W4	2	128.33 ± 30.14 (n = 3)
M2S2W1	Contaminated	Contaminated
M2S2W2	Contaminated	Contaminated
M2S2W3	Contaminated	Contaminated
M2S2W4	Contaminated	Contaminated
M3S1W1	2	116.67 ± 40.50 (n = 3)
M3S1W2	2	103.67 ± 24.44 (n = 3)
M3S1W3	2	142.00 (n = 1; SD not calculated)
M3S1W4	2	88.33 ± 11.50 (n = 3)
M3S2W1	2	Contaminated
M3S2W2	3	Contaminated
M3S2W3	Contaminated	Contaminated
M3S2W4	Contaminated	Contaminated

At 4°C (S1), all media were still able to maintain the viability of white oyster mushroom (*Pleurotus ostreatus*) spores for up to eight weeks, although colony numbers varied among treatments (Table 1). In contrast, all treatments stored at room temperature, 26°C (S2), were contaminated; therefore, further viability observations could not be conducted. Potato Dextrose Broth (PDB; M1) produced the highest number of *P. ostreatus* colonies, particularly in treatments S1W4 (593 colonies) and S1W1 (517 colonies). PDB supplemented with 15% paraffin (M2) produced lower colony numbers, ranging from 128 to 248 colonies, whereas the corn-boiling water medium (M3) produced the lowest number of colonies, ranging from 89 to 142 colonies.

Colony emergence in M1 occurred relatively earlier, on days 1–2, whereas colonies in M2 and M3 generally began to emerge on day 2. These findings indicate that the nutrient composition of the medium influenced the ability of *P. ostreatus* spores to maintain viability and form colonies after the preservation period (Singh et al., 2025). The dextrose content in PDB provides a simple carbon source that is readily metabolized, thereby supporting faster recovery of cellular metabolism compared with the other media (Singh et al., 2025).

These results are consistent with Abou Fayssal et al. (2023), who reported that media rich in simple carbohydrates improved the post-preservation viability of *P. ostreatus* cultures. Veerana et al. (2019) also reported that PDB effectively maintained spore germination capacity compared with alternative media derived from agricultural waste. Meanwhile, Patricia et al. (2020b) noted that the addition of paraffin oil can reduce metabolic activity by limiting oxygen availability, although it may also slow early recovery. The difference in colony number between M2 and M1 in the present study suggests that the paraffin layer had a protective function but delayed cellular reactivation (Surja et al., 2020). In contrast, the corn-boiling water medium contains more complex carbohydrates that require further enzymatic processing before utilization, resulting in fewer colonies (Silva et al., 2024).

Storage Temperature and Preservation Period

Storage temperature and duration showed contrasting effects on spore culture viability. Changes in viable colony numbers across treatments during the eight-week observation period are shown in Figure 2.

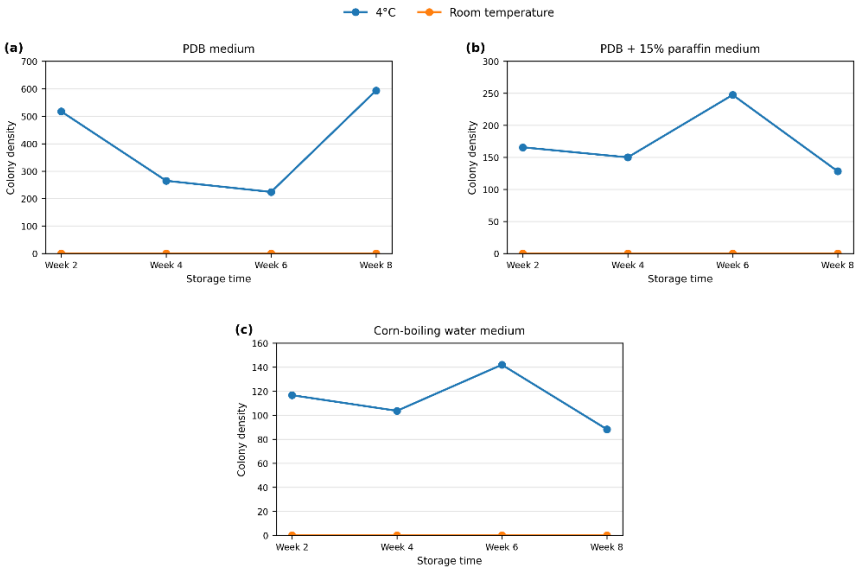

Figure 2. Number of white oyster mushroom colonies on day 7 at 4°C and room temperature: (a) PDB medium, (b) PDB+15% paraffin medium, (c) corn-boiling water medium

Storage period and temperature showed a tendency to be associated with changes in viability, as reflected by the number of colonies formed on day 7 (Figure 2). Across all media, storage at 4°C maintained colony growth, whereas no viable growth was observed at room temperature because all treatments were contaminated at all storage periods. This pattern was consistently observed in PDB (Figure 2a), PDB + 15% paraffin (Figure 2b), and corn-boiling water medium (Figure 2c), where the room-temperature line showed a value of zero. Under 4°C storage, colony numbers fluctuated with increasing preservation time, with the highest values tending to occur at week 6, followed by a decline at week 8 in several media.

In the PDB medium (Figure 2a), colony number was relatively high at week 2, decreased at weeks 4 and 6, and then increased again at week 8. Notably, PDB at week 8 produced the highest colony number, with 593.33 ± 17.62 colonies, although culture viability generally tends to decline as storage duration increases. This pattern indicates that the relationship between storage duration and colony number was not entirely linear. Because this study used a descriptive approach without inferential statistical testing, the increase in colony number at week 8 cannot yet be confirmed as a biologically meaningful difference. This variation may be related to differences in the density of spores successfully reactivated, variation among replicates, or technical factors during inoculation and colony counting.

In the PDB + 15% paraffin medium (Figure 2b), colony number peaked at week 6 but declined at week 8, indicating a possible upper limit for effective storage. Similarly, in the corn-boiling water medium (Figure 2c), colony numbers were generally lower than those in the other two media, with an increasing pattern up to week 6 followed by a decline at week 8. These data indicate that longer storage periods may increase the likelihood of viability loss, although low temperature can slow this process (Aftukha & Purbasari, 2021).

Physiologically, storage at 4°C may induce a partial dormancy phase in cells, characterized by reduced respiration rates and enzymatic activity (Aftukha & Purbasari, 2021). This condition helps maintain cell membrane integrity and protein stability. However, long-term storage may still induce the accumulation of reactive oxygen species (ROS), causing sublethal damage to membranes, mitochondria, and enzymatic systems (Marroha Doli Siregar et al., 2023). This possibility requires confirmation through physiological analyses, such as assessments of membrane integrity, enzymatic activity, ATP content, or oxidative stress. Media containing simple carbon sources, such as PDB, provide energy that can be used more rapidly during reactivation, resulting in higher colony numbers after dormancy (Singh et al., 2025). In media containing paraffin, restricted oxygen diffusion slows metabolism during storage but may also delay early recovery (Surja et al., 2020). Meanwhile, the corn-boiling water medium, which is based on more complex carbohydrates, requires additional hydrolysis before its nutrients can be utilized, resulting in a relatively slower growth response (Silva et al., 2024).

The findings of this study are consistent with Yan et al. (2020), who reported that *P. ostreatus* viability during cold storage is influenced by the balance between metabolic suppression and post-dormancy reactivation capacity. Abou Fayssal et al. (2023) reported that dextrose-based media showed higher germination success than alternative media based on plant extracts. Patricia et al. (2020a) explained that mineral oil or paraffin overlay techniques are effective in maintaining culture purity but often result in slower initial growth due to oxygen limitation. In addition, Liu et al. (2024) emphasized that long-term storage can reduce ATP production due to impaired mitochondrial function, even when hyphal morphology remains visually normal. The

findings of the present study are consistent with these reports, as PDB stored at 4°C showed the best viability stability up to week 8, whereas the other media appeared to reach their optimal preservation limit at week 6.

Macroscopic and Microscopic Morphology

In addition to colony number, macroscopic and microscopic morphological characteristics were observed to determine whether the preservation process caused changes in hyphal structure. The quality of white oyster mushroom cultures was evaluated through visual confirmation of colony morphology on Petri dishes and microscopic examination of the hyphal network structure (Figure 3).

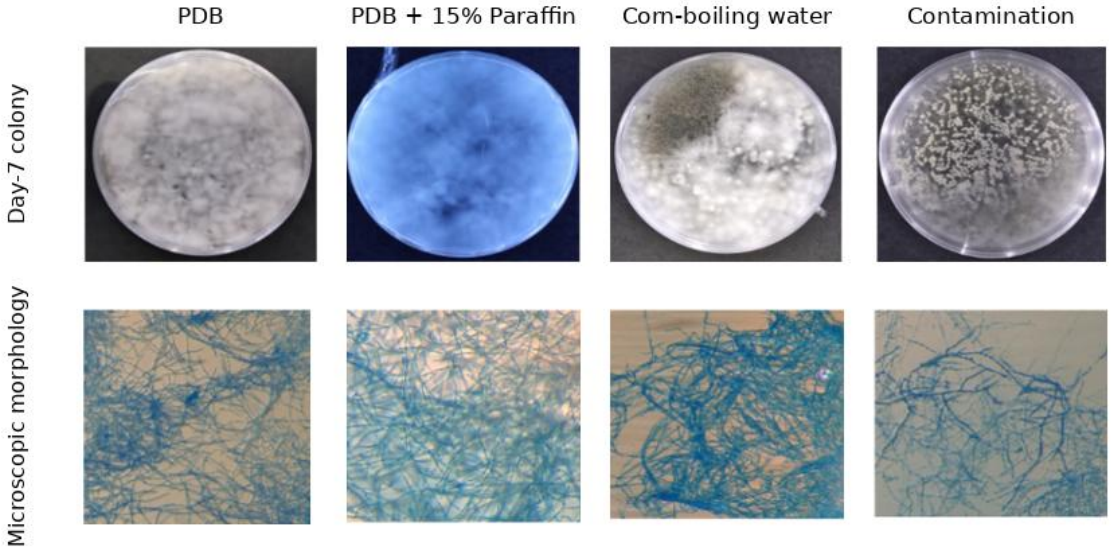

Figure 3. Macroscopic and microscopic morphology of Pleurotus ostreatus colonies in PDB, PDB + 15% paraffin, and corn-boiling water media on day 7

Macroscopic and microscopic observations of *P. ostreatus* colonies showed that all treatments stored at 4°C in all media had uniform hyphal structures, characterized by cylindrical, septate, and normally branched hyphae (Figure 3). No significant morphological differences were observed between treatments with high and low colony numbers. The reduction in viability was likely associated with submicroscopic damage to cell membranes or impaired mitochondrial function affecting ATP production (Zhang et al., 2025). However, this possibility requires confirmation through physiological analyses, such as assessments of membrane integrity, enzymatic activity, ATP content, or oxidative stress. Thus, morphological stability does not necessarily indicate physiological stability.

According to Krupodorova et al. (2024), morphological stability is often maintained even when physiological changes occur at the cellular level. Therefore, the possibility that viability loss was associated with metabolic impairment, membrane integrity disruption, or other physiological processes should be confirmed using more specific parameters, such as membrane integrity tests, enzyme activity assays, ATP quantification, and oxidative stress indicators. These parameters were not analyzed in the present study; consequently, the mechanism underlying viability reduction cannot be directly determined.

Patricia et al. (2020a) stated that low-temperature preservation can maintain morphological integrity but does not completely prevent a reduction in proliferative capacity. Liu et al. (2024) also reported that disruptions in energy metabolism during storage may reduce viability without altering hyphal structure. The results of the

present study are consistent with these findings, indicating that quantitative viability evaluation remains necessary even when morphology appears normal. The maintenance of stable hyphal structure up to week 8 suggests that preservation at 4°C was effective in maintaining the anatomical integrity of *P. ostreatus* cultures.

Effect of Room Temperature at 26°C on Culture Susceptibility to Contamination

Based on observations from week 2 to week 8, all experimental units of white oyster mushroom (Pleurotus ostreatus) preserved at room temperature, 26°C, experienced complete contamination (100%). Preservation failure under this condition indicates a critical limitation in environmental and procedural control. According to Gong et al. (2024), from a microbiological perspective, 26°C falls within a moderate thermophilic to mesophilic temperature range that is favorable for spore germination and rapid mycelial growth of various airborne microbial contaminants, particularly opportunistic molds such as Aspergillus spp., Penicillium spp., Trichoderma spp., and spore-forming bacteria.

Complete contamination at room temperature was most likely triggered by the high relative humidity of the uncontrolled tropical storage environment, which accelerated water vapor absorption through gaps in the container closures (Agustina et al., 2023). Microaerobic conditions and high moisture content in the liquid preservation tubes (M1, M2, and M3) at warm temperature may have stimulated contaminant spores that escaped during container sealing or subculturing to grow exponentially (Agustina et al., 2023). These contaminant spores have much shorter generation times than *P. ostreatus* mycelial fragments, resulting in competition for space and nutrients and ultimately causing complete suppression of the target fungus (Veerana et al., 2019).

In contrast, the treatment groups stored at low temperature, 4°C, showed substantially greater resistance, with minimal contamination. Cold storage acts as a static agent that inhibits metabolic activity, suppresses the germination of airborne contaminant spores, and slows the activity of hydrolytic enzymes produced by competing microorganisms (Rohadi et al., 2020). Therefore, low storage temperature serves as a crucial mechanical limiting factor for maintaining culture purity during the preservation period.

CONCLUSION

Based on colony number, time to initial colony emergence, and hyphal morphology, Potato Dextrose Broth (PDB) stored at 4°C showed the best preservation performance under the conditions of this study. However, this optimization claim should be interpreted cautiously because the data were analyzed using a descriptive qualitative approach without statistical testing. In addition, all treatments stored at room temperature experienced complete contamination and therefore failed to maintain spore viability. Scientifically, this study contributes to a better understanding of how dextrose-rich media combined with low-temperature storage can help maintain hyphal morphological stability and support post-dormancy cellular recovery. From a practical perspective, the findings indicate that PDB formulation under cold storage conditions may serve as a simple and efficient approach for the short-term preservation, up to eight weeks, of *Pleurotus ostreatus* spores in small-scale mushroom cultivation laboratories and academic settings.

RECOMMENDATION

Future studies should implement stricter laboratory control systems by including negative controls to validate aseptic conditions, pre-storage media sterility controls to

detect latent contaminants, and initial spore viability controls as a valid comparative baseline. In addition, observational criteria should be expanded to include more objective quantitative parameters, such as hyphal diameter measurement, hyphal branching counts, specific identification of colony color changes, and assessment of mycelial texture.

Each structural observation should be supported by standardized microscopic documentation that clearly reports lens magnification and includes an appropriate scale bar. Finally, further studies are recommended to apply inferential statistical analysis, particularly analysis of variance (ANOVA), to validate the significance of interactions among variables. Preservation periods should also be extended beyond eight weeks to determine the critical threshold at which spore viability is lost.

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