"Liquid Mycelium of Pleurotus Ostreatus, Ready For Cultivation and Optimized for Rural Use in Madagascar"

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ABSTRACT

This research focuses on enhancing the liquid mycelium of Pleurotus ostreatus for mushroom farming in rural areas of Madagascar. Four different liquid substrate mixtures were evaluated at three varying concentrations: honey, honey combined with potato, sucrose, and sucrose mixed with potato. The mycelium was cultivated at temperatures between 25-28°C with stirring for a period of 5-7 days, after which it was transferred to two types of fruiting substrates: coffee-rice straw and cotton-rice straw. The findings indicate that the potato and sucrose combination yielded the most robust growth (DO 0.901), whereas honey alone hindered development. Primordium formation occurred more rapidly on coffee-rice straw substrates (13-40 days) compared to cotton-rice straw (25-45 days). The highest biological efficiency was 19% with the 10% sucrose and potato mixture on coffee straw. These findings suggest that liquid mycelium offers a practical and accessible option for mushroom cultivation in rural settings, enhancing the transport and storage process while adding value to local agricultural by-products.

***Keywords*:** liquid mycelium, Pleurotus ostreatus, mushroom cultivation, lignocellulosic substrates, submerged fermentation, agricultural waste recovery

I. Introduction

In Madagascar, where malnutrition and limited access to animal protein are significant issues, *P. ostreatus* serves as a valuable sustainable resource. Its cultivation utilizes agricultural by-products like sugarcane bagasse, rice stalks, and corn cobs, while offering a food source with high nutritional value and natural medicinal benefits (Rambeloson, A. R. M., et al., 2024). (Chang, S. T., & Wasser, S. P,2017) Bellemain et al, 2019). Achieving a successful yield in oyster mushroom farming requires the production of high-quality mycelium to ensure quick and even substrate colonization, minimize contamination risks, and enhance mushroom production (Atagouddouanla, 2021). In this regard, producing liquid *P. ostreatus* mycelium emerges as an innovative and promising approach to address the limitations of traditional grain-based mycelium. This technique provides several benefits, such as significantly reducing contamination risks, simplifying substrate inoculation, and improving the biological quality management of the mycelium. It is a viable technical alternative in settings where laboratory facilities and sterile grain are unavailable, which is often the case in Madagascar (Kummer, 1871) (Rambeloson,

A. R. M. , 2024).. Therefore, the aim of this research is to tailor the formulation and production of liquid P. ostreatus mycelium to the local conditions in Madagascar to facilitate its production as a source of income.

II. Matériels

2.1 Fungal material

In Madagascar, the most extensively grown species is Pleurotus ostreatus, particularly in the central highlands of the Analamanga region Maevazafy, (E. G., Raharisoa, R. L., Rambeloson, A. R. M et al., 2023). This mushroom is primarily cultivated on rice straw, as this substrate is readily available in rural areas. Known as the oyster mushroom, Pleurotus ostreatus (Jacq.) P. Kumm. is part of the Basidiomycota division and is classified within the following taxonomic hierarchy:

Kingdom: Fungi
Division: Basidiomycota
Class: Agaricomycetes
Subclass: Agaricomycetidae
Order: Agaricales
Family: Pleurotaceae
Genus: Pleurotus
Species: *Pleurotus ostreatus*
Common name: Oyster mushroom

Photo 1 : *Pleurotus ostreatus*

2.2. Plant material for the formulation of liquid mycelium

The choice of liquid substrate directly affects productivity, biomass quality, and the economic viability of the process. Among the substrates commonly used, potatoes, sucrose, and honey are three distinct options, each with specific advantages and limitations. These will serve as the basis for determining the formulation best suited to local conditions. Although the dextrose-potato-broth (DPB) solution is the international reference medium for mycelial culture, producing exceptional mycelial biomass yields (average 2.1 ± 0.8 $g \cdot L^{-1}$ for Pleurotus spp.), its often prohibitive cost and low accessibility in developing regions such as Madagascar are major obstacles to semi-industrial applications, hence the need to use cane sugar or honey as alternative carbon sources Roy et al, 2014)

2.3 Plant material used for fruiting tests

The fruiting substrate is both the base and the medium on which the fungus will produce its fruit (carpophores). The substrate is chosen based on the agricultural by-products available according to the season, cost, and carbon/nitrogen ratio. Cotton hulls have a high crystalline cellulose content and a moderate C:N ratio, making them a structured and balanced substrate for mushroom cultivation. Coffee grounds are rich in extractable proteins and carbohydrates, and have an intermediate C:N ratio that is favorable for fungal growth. Finally, rice straw is characterized by a high cellulose content, a high C:N ratio, and a lignocellulosic composition suitable for fungi that require nitrogen-poor substrates (Bakratsas et al., 2023)

III. Methods

3.1. Preparation of liquid inoculum

The preparation of liquid inoculum follows a standardized two-step protocol. The P. ostreatus strain is first maintained on PDA (Potato Dextrose Agar) at 25°C for 7 days, then stored at 4°C. For liquid preculture, agar plugs containing active mycelium (5 mm in diameter or 1 cm²) are transferred to Erlenmeyer flasks containing 80-150 mL of liquid medium. The culture is then homogenized using a sterile grinder (10,000-11,000 rpm for 5-20 seconds) and incubated at 25-28°C under orbital shaking (150 rpm) for 5-7 days. This mycelial suspension, containing 6.3 g to 7 g of dry biomass per liter, is used as inoculum for the main culture at a seeding rate of 1.25-5% (v/v).[10] Tinoco-Valencia et al., 2014) (Antontceva et al., 2020)

3.2 Preparation of the liquid medium and sterilization

For our research, two types of carbon sources were used to formulate the base substrate: honey (H) and sugar (S), which were then mixed with distilled water to form a liquid solution with three different concentration ranges (3%, 5%, and 10%). To these formulations, 20 g of potato (P) per 100 ml was then added, also in the three previous concentration ranges. The potato and sugar-based substrate coded PS was used as a control substrate. (Rambeloson, A. R. M., 2024, mois)

3.3. Colonization rate of the different liquid media studied

Submerged fermentation conditions were standardized with an optimal temperature between 24 and 28°C and the initial pH of the medium adjusted between 5.0 and 7.0 [11,12,13]. The colonization rate of the different liquid media was observed by measuring the change in optical density of the liquid mycelium over time. (Oei, P, 2005) (Eurosubstrat,2025)

3.4. Preparation and inoculation of fruiting substrates

The dry substrates are chopped into small pieces in the case of rice straw, then soaked separately in water for 2 hours in a large tank so that the substrate is saturated with water in the case of rice straw and cotton seed hulls. The substrates are then drained on a sieve to obtain a relative humidity of between 50% and 60%. The addition of CaCO3 at 1% of the substrate weight increases the pH of the substrate to between 9 and 10. Culture bags are then filled to ⅔ with substrate and sealed tightly for sterilization in a tank at 90°C for 11 hours (Grenon, V, 2023)

.Once sterilized, the fruiting substrate is seeded under aseptic conditions at a rate of 10 ml per substrate bag, then covered with a filter to allow air exchange. The whole batch is then sent to an incubation chamber until the substrate is completely colonized. The colonized substrate is then sent to a fruiting greenhouse to stimulate the appearance of primordia and the growth of sporophores. The temperature (18 and 21°C) and humidity (80% and 90%) are adjusted to ensure rapid growth of the carpophores. (Lamycosphere, 2025) (ANDREAS JANOTTO, 2024) (Morel, 2022)

3.5. Colonization time of liquid mycelium inoculated on CAP and CP

In our research, we compared the colonization speed of liquid mycelium obtained from six different seed batches under the same conditions on different substrates, such as a mixture of rice straw and coffee grounds (CAP) and rice straw and cotton (CP).

3.6. Time to appearance of primordia in CP and CAP substrates

The time to appearance of the first primordium is the interval between the day the substrate is transferred to the fruiting chamber and the appearance of the first mushroom bud, and is counted in number of days.

3.7. Growth kinetics on the different selected substrates

Biological efficiency (BE) measures the fungus's ability to convert a given substrate (on a dry basis) into fresh fungal biomass (Janotto, A.,2021).

IV. Results and discussions

4.1. Colonization time of the different liquid media studied

After 21 days of incubation, the PD control medium reached maximum optical density (OD) (0.901), indicating optimal hyphal growth of P. ostreatus. The LPM3 medium achieved an OD of 0.657, showing significant growth but less than PD. The LS10 medium had an intermediate OD (0.522), indicating adequate but lower performance. PD remains the medium of choice due to its varied nutrients, providing a C/N ratio and compounds favorable to fungal growth (Morel et al., 2022). The LPM3 formulation offers an interesting alternative, with honey providing complex sugars and potatoes enhancing the nutritional effect. Media rich only in sucrose (LS10) are less effective, probably due to the absence of a complementary substrate. To maximize the production of liquid mycelium of P. ostreatus, it is therefore advisable to use PD or possibly LPM3. Substituting sucrose with honey remains possible but leads to slightly reduced efficiency compared to the PD control (Zervakis, 2013)

4.1 Colonization speed of liquid mycelium

In this research, the colonization time of liquid mycelium from six different seed batches was tested under the same conditions on different substrates, such as a mixture of rice straw and coffee grounds (CAP) and rice straw and cotton (CP).

Figure 1 shows the colonization time of the substrate for two treatments, CP (blue) and CAP (orange), according to different liquid mycelium (LM) formulations: LS10, LM3, LPS5, LPM5, LPS10, and LPM3. According to the data, CAP treatments generally show higher colonization times than CP for most formulations, except for LPS10, where CP exceeds CAP. The LPS5 and LPS10 formulations show lower values, indicating faster colonization of the substrate. The standard deviation, visible in the error bars, shows good reproducibility, although some variations exist.

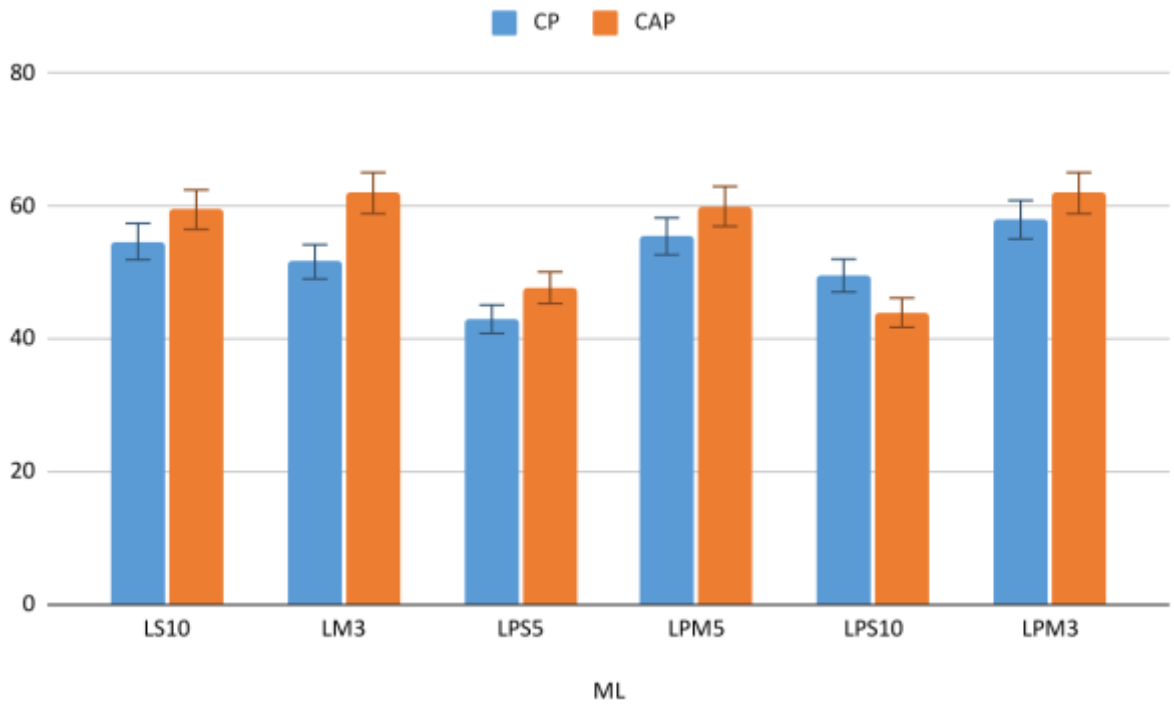

Figure 1 : Duration of mycelium colonization on CAP and CP

These results suggest that the type of liquid mycelium formulation significantly influences the speed of substrate colonization and that CAP treatment appears to optimize this process for certain formulations. This could guide the choice of the most effective formulations to improve the efficiency of myciculture, favoring LM3, LPM5, and LPM3 for maximum results, in line with observations from previous work (Pham, N. Q., 2018)

4.2 Time of appearance of primordia in CP and CAP substrates

The time of appearance of the first primordia is the interval between the day the substrate is transferred to the fruiting chamber and the appearance of the first mushroom bud.

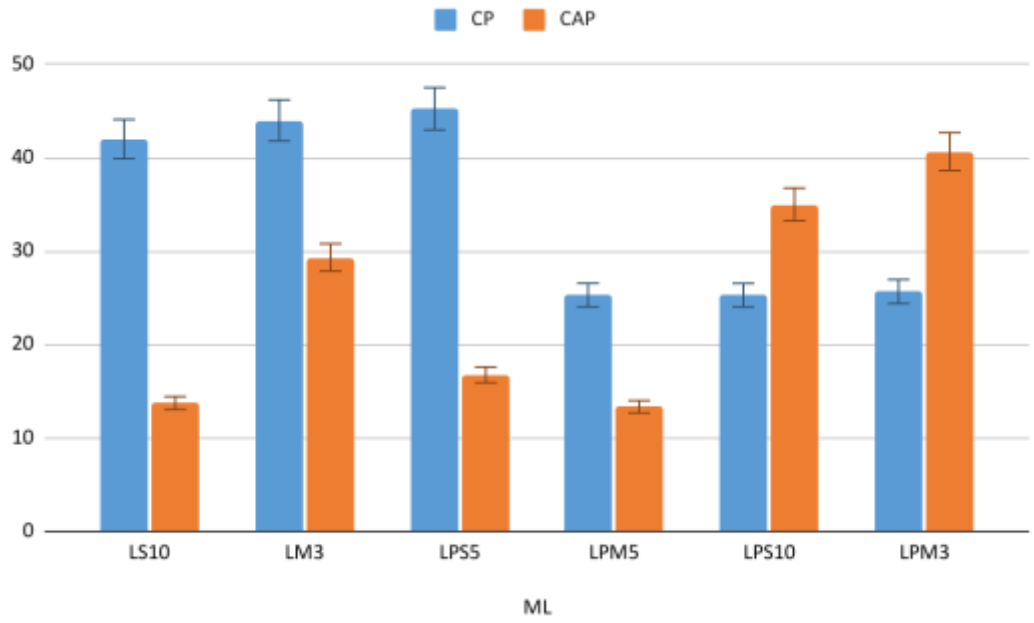

Figure 2: Average time to appearance of primordia on CP and CAP

Figure 2 shows a contrast between two groups of media: on the one hand, LS10, LM3, and LPS5 favor CP (40–45 days) over CAP (12–28 days), while on the other hand, LPS10 and LPM3 reverse this trend with a higher CAP. The LPM5 medium remains underperforming for both parameters. This dichotomy suggests that certain formulations accelerate the initiation of primordia, while others delay this phase while prolonging a period of mycelial growth. The differences observed probably reflect differences in nutritional balance (nitrogen/carbon, minerals) and physicochemical conditions. Here, optimization involves selecting the LS10, LM3, or LPS5 medium for rapid primordium induction, adapted to production objectives.

4.3 Growth kinetics on the different selected substrates

On CAP substrate (Figure 7), the biological efficiency of the fruit varies significantly depending on the type of liquid mycelium. LPS10 achieves the highest value, approaching 19%, indicating excellent conversion of substrate into fungal biomass. LPSS also performed well, reaching nearly 13%. LS10 and LPM5 achieved intermediate values (around 10–11%), while LM3 and LPM3 showed the lowest efficiencies, between 4 and 6%. This distribution highlights the importance of choosing the right liquid mycelium for the substrate in order to maximize the biological yield of oyster mushroom cultivation.

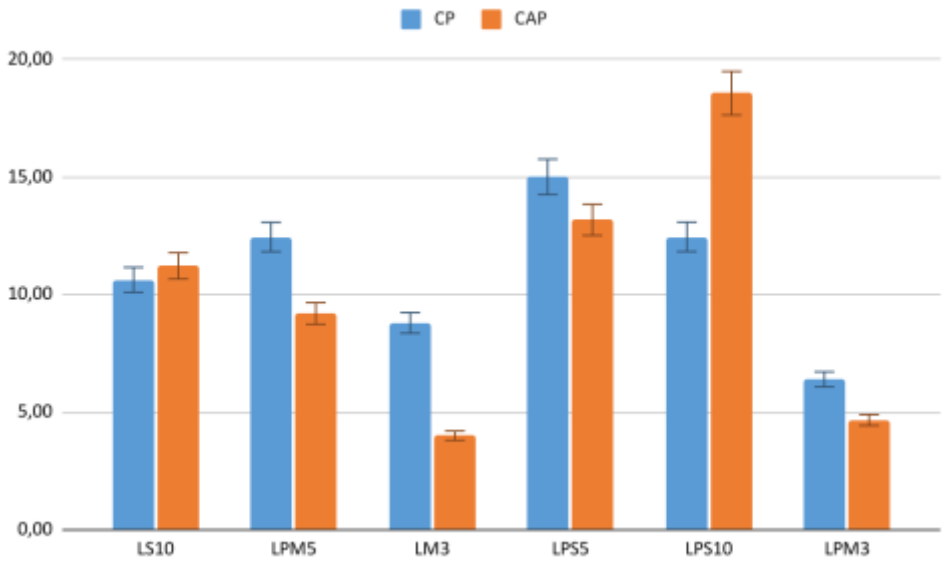

Figure 3: Biological efficacy of fruit on CP and CAP

The graph 3 illustrates the biological efficacy for CP and CAP treatments according to six liquid mycelium formulations (LS10, LPM5, LM3, LPS5, LPS10, LPM3). The results show significant variability in biological efficacy depending on the formulation used. The CAP treatment generally shows higher biological efficacy for the LPS5 and LPS10 formulations, reaching 13.02% and 18.38% respectively, while CP shows higher values for LM3 and LPM3. The LM3 formulation is notable for its low biological efficacy for both treatments, suggesting suboptimal performance. Conversely, the LPS5 and LPS10 formulations show greater potential, particularly with CAP treatment. These variations reflect the significant influence of liquid mycelium composition on substrate productivity. The standard deviation observed indicates good experimental repeatability. In conclusion, CAP treatment appears to be more effective in maximizing biological efficiency when combined with the LPS5 and LPS10 formulations, which are strategic options for optimizing mushroom cultivation yields (A A Shnyreva, A V Shnyreva, 2015).

V. Conclusion

This study demonstrated that optimizing Pleurotus ostreatus liquid mycelium is an effective and viable strategy for rural mushroom production in Madagascar. The results show that the potato + sucrose (PD) medium promotes optimal hyphal growth (DO 0.901), while the honey + potato (LPM3) and 10% sucrose (LS10) formulations offer interesting alternatives, although less effective. Primordia induction is faster on the coffee-rice straw substrate (13-40 days) compared to the cotton-rice straw substrate (25-45 days), demonstrating the importance of substrate choice for fruiting. Maximum biological efficiency reached 19% with the 10% sucrose + potato formulation inoculated on coffee-rice straw, validating its productive potential. These results highlight that liquid mycelium represents a technically viable and economically accessible alternative for rural mushroom growers, facilitating transport and storage and adding value to local agricultural residues. This innovative approach could contribute significantly to improving food security and income in rural areas, while promoting the sustainable use of local agricultural by-products.

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