STM JOURNALS

Research & Reviews: A Journal of
Microbiology & Virology

https://journals.stmjournals.com/rrjomv

ISSN: 2230-9853 (Online)
ISSN: 2349-4360 (Print)
Volume 14, Issue 2, 2024
May–August
DOI (Journal): 10.37591/RRJoMV

Research RRJoMV

Recovery of Mycelial Biomass of *Agaricus bisporus*, *Pleurotus ostreatus*, *Ganoderma lucidum*, and *Hericium erinaceus*: A Comparative Study

Apeksha Rathi[1], Neeraj Wadhwa[2,*]

Abstract

Mushrooms are the spore-bearing fruiting body of fungi with significant potential for numerous applications like pharmaceuticals, the textile industry, bioremediation, soil management, alternatives to toxic packaging materials, and environmental sustainability. Mushrooms generally belong to the class Basidiomycota or Ascomycota. They are typically made of hyphae, the tiniest threads which comprise most fungi. Mycelia are tiny thread-like structures that help in making the vegetative part of the fungus. In this study, the growth of mycelia from four edible mushroom species Agaricus bisporus, Pleurotus ostreatus, Ganoderma lucidum, Hericium erinaceus in Potato Dextrose Agar (PDA), and Potato Dextrose Broth (PDB) was compared. For identification of mycelia lactophenol cotton blue staining was performed. Interestingly, there was a noticeable difference in the mycelial biomass yield between (PDA) and (PDB). We report morphological characteristics of the mycelia based on color, texture, and growth time requirement. There was a significant difference in yield of mycelial biomass in the (PDA) and (PDB). We could recover 1.760 gm of Pleurotus ostreatus mycelia, of 0.260 gm Agaricus bisporus mycelia, 0.038gm of Ganoderma lucidum, and 0.427 g of Hericium erinaceus mycelial biomass after 15 days of culturing on (PDA) agar plates. Recovery of mycelial biomass on (PDA) was better than (PDB). One of the effective methods for producing fungal biomass is to cultivate it on PDA. Our findings showed that the two media differed significantly in the mycelial biomass output, with (PDA) generally outperforming (PDB). These findings underscore the importance of cultivation medium selection in maximizing mycelial biomass production. The utilization of fungal biomass can prove to be helpful in different areas such as medicine, nutrition, agriculture, and the sustainability of the environment.

Keywords: Mycelial growth, PDA, PDB, *Agaricus bisporus*, *Pleurotus ostreatus*, *Ganoderma lucidum*, *Hericium erinaceus*

***Author for Correspondence**
Neeraj Wadhwa
E-mail: neeraj.wadhwa@jiit.ac.in

[1]Research Scholar, Department of Biotechnology, Jaypee Institute of Information Technology, A-10, Sector-62, Noida, Uttar Pradesh, India
[2]Professor, Department of Biotechnology, Jaypee Institute of Information Technology, A-10, Sector-62, Noida, Uttar Pradesh, India

Received Date: April 19, 2024
Accepted Date: May 28, 2024
Published Date: July 03, 2024

Citation: Apeksha Rathi, Neeraj Wadhwa. Recovery of Mycelial Biomass of *Agaricus bisporus*, *Pleurotus ostreatus*, *Ganoderma lucidum*, and *Hericium erinaceus*: A Comparative Study. Research & Reviews: A Journal of Microbiology & Virology. 2024; 14(2): 12–18p.

INTRODUCTION

Mushrooms, which focus on providing many health benefits to humans, form a very long period. People eat mushrooms as they are very low in energy and fat, but simultaneously have high carbohydrate and dietary fiber contents [1]. Mushrooms have an umami flavor swell [2]. Various minerals and vitamins are also present in mushrooms, which helps to build good health [3]. *Agaricus bisporus*, *Pleurotus ostreatus*, *Ganoderma lucidum*, and *Hericium erinaceus* are traditionally used for their medicinal benefits, and pharmaceutical industries are also using them for different purposes [4]. Because of the presence of

bioactive constituents, various pharmacological effects can be observed, such as antiviral, antioxidant, hypocholesterolemic, hypoglycemic, and antitumor effects [5].

The fungal mycelium is a thread-like structure that is scattered throughout the soil and acts as a soil holder [6]. For culturing mycelia, it is important to consider various factors, such as the need for a particular growth environment along with specific nutritional requirements. The mycelia of different mushrooms grow differently with respect to color, texture, and other characteristics [7]. Mycelium ingests carbon and nitrogen for their growth [8]. The mycelial network connects and spreads all over by taking plant-based waste such as sawdust [9]. Hyphae were observed in the fungal cell walls. These hyphae were helpful in mycelia formation. The hyphae are thready-thin but comprise connections of chitin, which makes it quite sturdy [10]. Other polysaccharides, such as glucan cellulose and a small quantity of glycoproteins, are also present [11].

Agaricus bisporus and *Pleurotus ostreatus* mushrooms are known for their consumption, and mycelial growth is rapid. Although the growth of *Ganoderma lucidum* and *Hericium erinaceus* is quite slow, they are highly preferred for their medicinal uses [12]. Mushrooms are popular owing to their anti-inflammatory, anti-allergic, and antioxidant properties. Mycelia of mushrooms contain numerous bioactive compounds. These bioactive compounds are also anti-inflammatory, contain antioxidants, and can be utilized for consumption [13]. In addition, the anti-inflammatory, anti-oxidative, and immunostimulatory properties of mycelia have various promising health characteristics [14]. Mycelia can be grown in culture media. These culture media are gaining popularity for the easy incubation of growth environments [15]. Substrate availability is necessary, along with moisture, as mycelial growth requires a humid environment for growth, and suitable temperature, pH, and nutrient availability are required for mycelial growth [16]. The time required for mycelial growth varied according to the type of mushroom. Each type of mushroom has a different mycelial growth time. Usually, favorable temperature requirements are 25–27°C [17].

To obtain fungal biomass, growing mycelia over synthetic media is a suitable approach, as it is not very complicated. Mycelia contains many bioactive compounds. Fungal biomass has numerous applications in various sectors. First, it is an effective way for bioremediate mycelia to digest pollutants and is a good source of toxins from the soil. Mycelia destroy the pollutants caused by pesticides, heavy metals, etc., and the use of mycelia is an organic way to reduce soil pollution [18]. Second, it can be used as a packaging material. Plastic packaging is harmful to the environment because it does not easily decompose [19]. Mycelium-based packaging is a good alternative to reduce environmental pollution. Mycelia can also be used as a sustainable and non-toxic construction material [20]. In the textile industry, mycelia can be used as an alternative to leather [21]. Mycelia can be utilized by the food industry to produce sustainable consumable protein products for humans [22]. In pharmaceuticals, mycelia can be used to extract bioactive compounds, as these bioactive compounds have anti-cancer, anti-inflammatory, anti-allergic, and antioxidant properties [23]. Furthermore, mycelia can facilitate the production of biofuels, which can help reduce air pollution compared to fossil fuels [22, 23]. They also play a crucial role in maintaining soil health, effective waste management, decomposing organic waste, and mitigating landfill-related pollution [25]. This study compares the growth, physical characteristics, and morphological characteristics of four different mycelia (*Agaricus bisporus, Pleurotus ostreatus, Ganoderma lucidum,* and *Hericium erinaceus*) in two different media, PDA and PDB.

MATERIAL AND METHODS

Materials

Potato Dextrose Agar (PDA), Potato Dextrose Broth media, *Agaricus bisporus*, *Pleurotus ostreatus*, *Ganoderma lucidum*, *Hericium erinaceus* spawn Petri plates, flasks, cotton plugs (Potato Dextrose Agar), and Potato Dextrose Broth (PDB) are required for mycelium culture.

Culturing of Mycelia

To obtain the growth of mycelia along with morphological and physical characteristics in two different media, the mycelia were grown in two different media:

1. *Culturing of mycelia in PDA (Potato Dextrose Agar)* solidifies, unlike culturing in broth. PDA was used as the substrate for mycelial growth [26]. Media (200 ml) were autoclaved along with the Petri dishes. Four autoclaved Petri dishes were inoculated with four different spawns: *Agaricus bisporus*, *Pleurotus ostreatus*, *Ganoderma lucidum*, and *Hericium erinaceus*. Inside the laminar airflow under sterile conditions, the PDA medium was poured into four Petri plates, and the medium was allowed to harden. The individual plates were inoculated with individual spawns. The plates were then closed and maintained at 27°C for 15 d in an incubator. Table 1 shows the mycelia of *Agaricus bisporus*, *Pleurotus ostreatus*, *Ganoderma lucidum*, and *Hericium erinaceus* grown on PDA.
2. *Culturing of mycelia in PDB (Potato Dextrose Broth)*—four different mushroom spawns were prepared: *Agaricus bisporus*, *Pleurotus ostreatus*, *Ganoderma lucidum*, and *Hericium erinaceus*. Next, 200 ml of PDB was prepared. The right mushroom spawn was added to each of the four PDB flasks. To ensure sterility, the PDB medium was autoclaved before inoculation. To avoid contamination, inoculation was carried out under laminar airflow. After inserting cotton plugs into the flasks, the incubator was turned on at 27°C. After 15–20 days, the surface of the media inside each flask showed a white, thread-like structure that was indicative of mycelial growth. Table 2 shows the mycelia of *Agaricus bisporus*, *Pleurotus ostreatus*, *Ganoderma lucidum*, and *Hericium erinaceus* grown on PDB.

Lactophenol Cotton Blue Test

The lactophenol cotton blue test is highly suitable for mycology sample testing to identify and characterize a particular fungus. Mycelia can be easily characterized using (LCB) test. Figure 1 illustrates the filamentous structure of mycelia.

The requirement for making lactophenol cotton blue stain is 0.05 g; cotton blue, 20 g; phenol crystals 40 ml; glycerol 20 ml; lactic acid, 20 ml;- distilled water [27].

First, cotton blue was added to distilled water until it dissolved and kept overnight. Second, the phenol crystals were placed in a glass beaker along with lactic acid and then magnetically stirred until the phenol dissolved completely. When mixed with glycerol, the combination was filtered with cotton blue solution inside the phenol, glycerol solution, and lactic acid, and left to sit at room temperature [27].

On a clean glass slide. A drop of 95% alcohol was placed on a glass slide. With the help of an inoculating loop tease the mycelia growth, spread it, and wait for the alcohol to evaporate, a drop of lactophenol cotton blue stain was added. Wait for 3–4 minutes. It was then carefully rinsed with 95% ethanol and allowed to dry. A clean coverslip was placed on the slide. Slides were maintained for microscopic studies [27].

RESULTS AND DISCUSSION

Growth in Potato Dextrose Agar (PDA)

Potato Dextrose Agar—The growth of all four mycelia is shown in Table 1. The mycelia grew best at 25–27°C and pH 6, and minimum growth of mycelia was observed at pH 4.

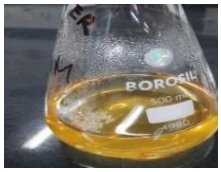
Growth in Potato Dextrose Broth (PDB)

The mycelia are grown on plates, by utilizing the substrate (PDA potato dextrose agar). The growth of all four mycelia is shown in Table 2. Mycelia grow best at 25–27°C with pH 6, and minimum growth of mycelia can be seen at pH -4 [28].

Table 1. Growth of mycelia in PDA (potato dextrose agar) after 15 days.

Mycelia growth in PDA	Texture	Color	Density	Growth time	Biomass recovered
Agaricus bisporus 	Fluffy-like in texture	White and a bit yellow in the center	Not very scarce density mycelia	15 days	0.260 gm
Ganoderma lucidum 	Fine cotton-like texture	white	The density of mycelia is very light and not scarce	15 days	0.038gm
Pleurotus ostreatus 	Flat cotton-like texture	Pure white	The density is scarce	15 days	1.760 gm
Hericium erinaceus 	Pure white fur-like texture	Milky white	The density is abundant and scarce	15 days	0.427 gm

Table 2. Growth of mycelia in PDB (potato dextrose broth) after 15 days.

Mycelia growth in PDB	Texture	Color	Density	Growth time
Agaricus bisporus 	Plain white cotton-like structure	White	Not very scarce density of mycelia	15 days
Ganoderma lucidum 	The sponge-like texture of mycelia	White	The density is scarce	15 days
Pleurotus ostreatus 	Less growth was visible	Foamy white	Very transparent	15 days
Hericium erinaceus 	White cloudy-like foam texture of mycelia	Foamy white	The density is abundant and scarce	15 days

Lactophenol Cotton Blue Test

The morphological characteristics of the mycelia were highlighted using lactophenol cotton blue staining. By using the lactophenol cotton blue test, the visibility of all the hyphae under a microscope

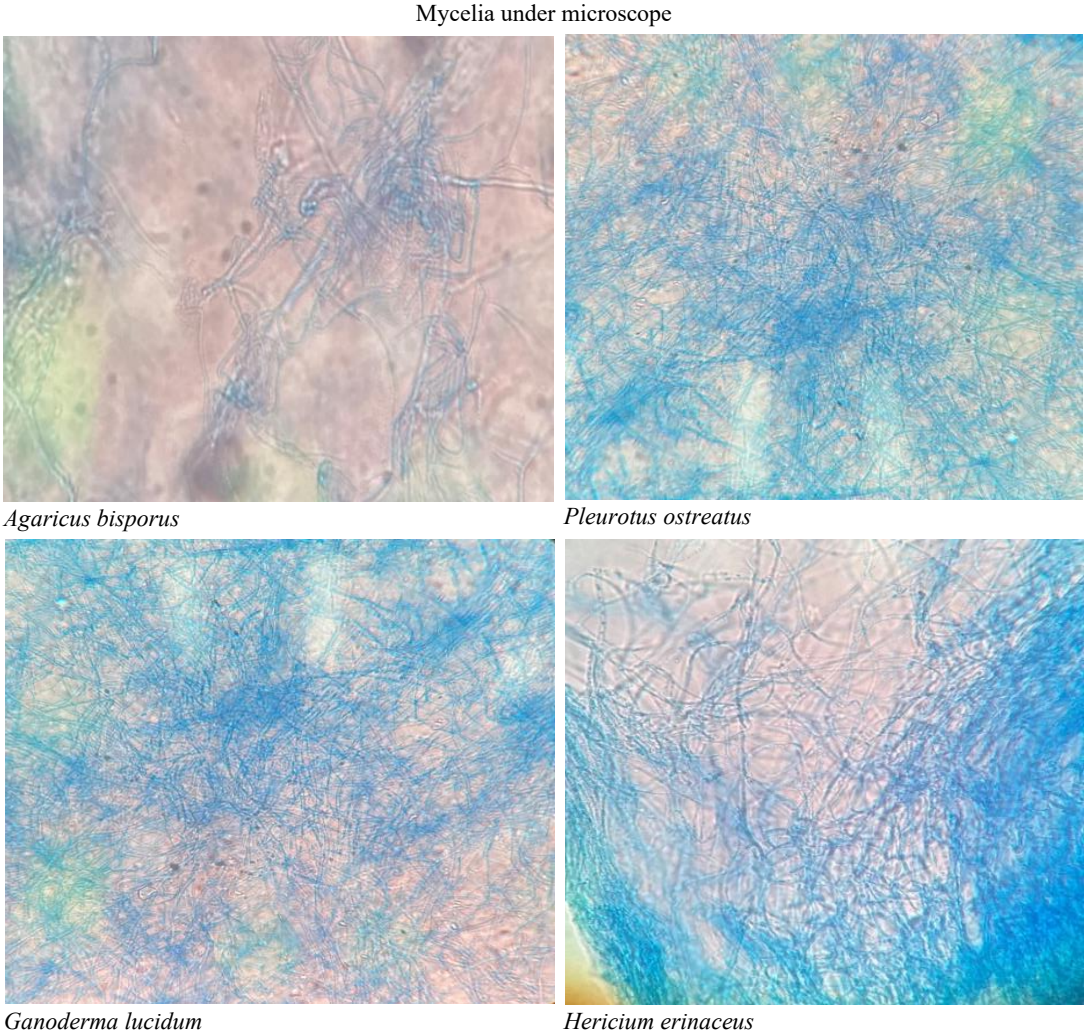


Figure 1. The figure represents the filamentous structure of mycelia stained with lactophenol cotton blue stain.

was better. The filamentous structure of the mycelia consisted of hyphae. Hyphae have a capability that is very important for nutrient absorption. The hyphae of all four mycelia, *Agaricus bisporus*, *Pleurotus ostreatus*, *Ganoderma lucidum*, and *Hericium erinaceus*, and their branching are shown in Figure 1. The mycelia of *Agaricus bisporus* showed less filamentous hyphae structure than those of *Pleurotus ostreatus*, *Ganoderma lucidum*, and *Hericium erinaceus* (Figure 1).

CONCLUSION

The growth of mycelia from four mushroom species, *Agaricus bisporus*, *Pleurotus ostreatus*, *Ganoderma lucidum*, and *Hericium erinaceus* in PDA and PDB were compared. PDA is a good medium for culturing fungal mycelia. The growth of mycelia on PDA was better than that on PDB. The physical characteristics of the mycelia, such as color, texture, and morphological characteristics, were optimized in the agar media. We report the recovery of 1.760 g of *Pleurotus ostreatus* mycelia, 0.260 g of *Agaricus bisporus* mycelia, 0.038 g of *Ganoderma lucidum*, and 0.427 g of *Hericium erinaceus* mycelial biomass in PDA within 15 days of culturing. Biomass recovery in PDA can be further optimized for suitable industrial applications. In the future, mycelia should be extracted to prepare appropriate products according to the needs of various industries. Extraction of bioactive compounds from mycelia can be performed and utilized by the pharmaceutical industry, as these bioactive compounds have anti-inflammatory, anti-allergic, and antioxidant properties and can be utilized for consumption as well.

REFERENCES

1. Nagulwar M, More D, Mandhare L. Nutritional properties and value addition of mushroom: A review. Pharma Innovation. 2020;9:395–8. DOI: 10.22271/tpi.2020.v9.i10f.5266.
2. Dermiki M, Phanphensophon N, Mottram DS, Methven L. Contributions of non-volatile and volatile compounds to the umami taste and overall flavour of shiitake mushroom extracts and their application as flavour enhancers in cooked minced meat. Food Chem. 2013;141:77–83. DOI: 10.1016/j.foodchem.2013.03.018, PubMed: 23768330.
3. Cheung PCK. The nutritional and health benefits of mushrooms. Nutr Bull. 2010;35:292–9. DOI: 10.1111/j.1467-3010.2010.01859.x.
4. Li H, Tian Y, Menolli Jr N, et al. Reviewing the world's edible mushroom species: A new evidence-based classification system. Compr Rev Food Sci Food Saf. 2021;20:1982–2014. DOI: 10.1111/1541-4337.12708, PubMed: 33599116.
5. Kumar K, Mehra R, Guiné RPF, et al. Edible Mushrooms: A comprehensive review on bioactive compounds with health benefits and processing aspects. Foods. 2021;10:2996. DOI: 10.3390/foods10122996, PubMed: 34945547.
6. Haneef M, Ceseracciu L, Canale C, et al. Advanced materials from fungal mycelium: Fabrication and tuning of physical properties. Sci Rep. 2017;7:41292. DOI: 10.1038/srep41292, PubMed: 28117421.
7. Jones M, Huynh T, Dekiwadia C, et al. Mycelium composites: A review of engineering characteristics and growth kinetics. J Bionanoscience. 2017;11:241–57. DOI: 10.1166/jbns.2017.1440.
8. Argyropoulos D, Psallida C, Sitareniou P, et al. Biochemical evaluation of Agaricus and Pleurotus strains in batch cultures for production optimization of valuable metabolites. Microorganisms. 2022;10:964. DOI: 10.3390/microorganisms10050964, PubMed: 35630408.
9. Harley JL, Waid JS. A method of studying active mycelia on living roots and other surfaces in the soil. Trans Br Mycol Soc. 1955;38:104–18. DOI: 10.1016/S0007-1536(55)80022-8.
10. Fricker MD, Heaton LLM, Jones NS, Boddy L. The mycelium as a network. Microbiol Spectrum. 2017;5:335–67. DOI: 10.1128/microbiolspec.FUNK-0033-2017, PubMed: 28524023.
11. Bartnicki-Garcia S. Cell wall chemistry, morphogenesis, and taxonomy of fungi. Annu Rev Microbiol. 1968;22:87–108. DOI: 10.1146/annurev.mi.22.100168.000511, PubMed: 4879523.
12. Kim SW, Hwang HJ, Park JP, et al. Mycelial growth and exo-biopolymer production by submerged culture of various edible mushrooms under different media. Lett Appl Microbiol. 2002;34:56–61. DOI: 10.1046/j.1472-765x.2002.01041.x, PubMed: 11849494.
13. Kandasamy S, Chinnappan S, Thangaswamy S, et al. Assessment of antioxidant, antibacterial activities and bioactive compounds of the wild edible mushroom Pleurotus sajor-caju. Int J Pept Res Ther. 2020;26:1575–81. DOI: 10.1007/s10989-019-09969-2.
14. Friedman M. Chemistry, nutrition, and health-promoting properties of Hericium erinaceus (Lion's Mane) mushroom fruiting bodies and mycelia and their bioactive compounds. J Agric Food Chem. 2015;63:7108–23. DOI: 10.1021/acs.jafc.5b02914, PubMed: 26244378.
15. Souilem F, Fernandes Â, Calhelha RC, et al. Wild mushrooms and their mycelia as sources of bioactive compounds: Antioxidant, anti-inflammatory and cytotoxic properties. Food Chem. 2017;230:40–8. DOI: 10.1016/j.foodchem.2017.03.026, PubMed: 28407928.
16. Dong CH, Yao YJ. Nutritional requirements of mycelial growth of Cordyceps sinensis in submerged culture. J Appl Microbiol. 2005;99:483–92. DOI: 10.1111/j.1365-2672.2005.02640.x, PubMed: 16108789.
17. Jones M, Mautner A, Luenco S, et al. Engineered mycelium composite construction materials from fungal biorefineries: A critical review. Mater Des. 2020;187:108397. DOI: 10.1016/j.matdes.2019.108397.
18. Houette T, Maurer C, Niewiarowski R, Gruber P. Growth and mechanical characterization of mycelium-based composites towards future bioremediation and food production in the material manufacturing cycle. Biomimetics. 2022;7:103. DOI: 10.3390/biomimetics7030103, PubMed: 35997423.

19. Lange J, Wyser Y. Recent innovations in barrier technologies for plastic packaging—A review [International journal]. Packag Technol Sci. 2003;16:149–58. DOI: 10.1002/pts.621.
20. Ly L, Jitjak W, editors. Formation of 3-dimensional bio-composite from local mushroom mycelia. In: The 5th TICC International Conference; 2020.
21. Bustillos J, Loganathan A, Agrawal R, et al. Uncovering the mechanical, thermal, and chemical characteristics of biodegradable mushroom leather with intrinsic antifungal and antibacterial properties. ACS Appl Bio Mater. 2020;3:3145–56. DOI: 10.1021/acsabm.0c00164, PubMed: 35025358.
22. Krupodorova TA, Barshteyn VY. Alternative substrates for higher mushrooms mycelia cultivation. J BioSci Biotechnol. 2015;4.
23. Badalyan SM, Zambonelli A. Biotechnological exploitation of macrofungi for the production of food, pharmaceuticals and cosmeceuticals. In: Advances in Macrofungi: Diversity, Ecology and Biotechnology. 2019. p. 199–230.
24. Karatay SE, Dönmez G. Evaluation of biotechnological potentials of some industrial fungi in economical lipid accumulation and biofuel production as a field of use. Prep Biochem Biotechnol. 2014;44:332–41. DOI: 10.1080/10826068.2013.829495, PubMed: 24320234.
25. Gujre N, Soni A, Rangan L, et al. Sustainable improvement of soil health utilizing biochar and arbuscular mycorrhizal fungi: A review. Environ Pollut. 2021;268:115549. DOI: 10.1016/j.envpol.2020.115549, PubMed: 33246313.
26. Tudses N. Isolation and mycelial growth of mushrooms on different yam-based culture media. J Appl Biol Biotechnol. 2016;4:33–6.
27. Dar B, Kyu MT, Nyunt KS, et al. Preparation of Fungal and Bacterial Spores' Formation Slides: MERAL Portal. 2015.
28. Sardar H, Ali MA, Ayyub CM, Ahmed R. Effects of different culture media, temperature and pH levels on the growth of wild and exotic Pleurotus species. Pak J Phytopathol. 2015;27:139–45.

