

OVERVIEW OF SUBMERGED CULTURE AND THE BIOLOGICAL ACTIVITIES OF MEDICINAL MUSHROOM SPECIES

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Abstract

Submerged cultivation of medicinal mushrooms is receiving increasing attention and is considered an effective alternative to traditional substrate cultivation methods for producing fungal mycelial biomass and bioactive metabolites with diverse applications. This method allows for the control of culture environment conditions, enabling more efficient synthesis of bioactive compounds such as polysaccharides, triterpenoids, cordycepin, polyphenols, etc. Furthermore, the bioactivity of these compounds, including antioxidant, anticancer, antibacterial, and immunomodulatory effects, further emphasizes the potential of producing medicinal mushroom biomass by submerged cultivation in the pharmaceutical and functional food industries. Submerged cultivation is considered a promising alternative to traditional mushroom fruiting body cultivation because it offers better control over culture conditions and product quality, as well as shorter cultivation times. Submerged fungal cultivation has significant industrial potential; however, there are still challenges in optimizing production yield and scaling up the process for industrial application. The successful application of this method on a commercial scale depends on increasing product yield and developing new production systems to address the issues related to submerged mushroom cultivation techniques. Although many researchers are making efforts to produce bioactive metabolites from fungi, the physiological and technical aspects of submerged cultivation still require extensive and long-term research.

Keywords: Bioactive compounds, medicinal mushrooms, mycelial biomass, submerged cultivation

1. Introduction

Medicinal mushrooms have long been known for their immense potential in medicine, with studies confirming the presence of highly bioactive compounds in medicinal mushroom species (Venturella et al., 2021). These fungi synthesize a variety of molecules, including polysaccharides, peptides, proteins and protein complexes, phenolic compounds, polyketides, triterpenoids, steroids, alkaloids, nucleotide terpenes, alkaloids (Zhao et al., 2020), and other secondary metabolites (Yadav & Negi, 2021) exhibiting

remarkable biological activities ranging from immune support and modulation to antioxidant (Khatua, Paul, & Acharya, 2013), antibacterial (Vallavan et al., 2020) and anticancer effects (Nandi, Sikder, & Acharya, 2019).

Currently, commercial products from medicinal mushrooms are mostly obtained through fruiting body cultivation. However, in many cases, this method makes it difficult to control the quality of the final product. In recent years, the submerged culture method for producing edible and medicinal mushroom mycelium has achieved success with various mushroom species. Laboratory experiments have shown that most mycelia grow well under suitable liquid culture conditions (Alarcon et al., 2003). Submerged cultivation is a biological method currently used to cultivate certain medicinal mushroom species in a liquid nutrient medium, where mycelia are cultured under controlled conditions such as temperature, pH, oxygen concentration, and agitation speed in a bioreactor or shaking speed in Erlenmeyer flasks (Tang et al., 2007). Agitation and oxygen supply must be carried out continuously throughout the cultivation process to ensure that biological cells and nutrients are evenly dispersed in the medium. During this process, nutrients are dissolved and evenly distributed in the liquid medium, and oxygen is supplied by agitation or aeration to prevent anaerobic conditions, which can inhibit growth or cause mycelial death, while simultaneously supporting mushroom growth and metabolite production. This method allows the mycelium to disperse evenly throughout the nutrient medium, so that the entire cell surface has direct contact with the nutrient solution, thereby optimizing nutrient utilization and biomass yield. This technique enables the efficient production of fungal biomass and bioactive compounds (Fazenda et al., 2008; Luan et al., 2022). The submerged cultivation method has the potential for industrial-scale application for mass production of biological preparations and helps to save time, labor, and reduce the steps of processing raw materials compared to traditional methods. Submerged cultivation demonstrates superiority over traditional cultivation in terms of yield and consistency (Berovic, Podgornik, & Gregori, 2022), especially for rare mushroom species (Elisashvili, 2012). Research on optimizing parameters such as nutrient composition, pH, and oxygen concentration has opened up significant advances in the production of medicinal mushroom biomass with high bioactivity.

2. Submerged culture in a bioreactor and optimization of nutrient environment

Submerged cultivation in bioreactors involves growing fungal mycelia in a liquid medium inside bioreactors, where parameters such as temperature, pH, agitation, and aeration are tightly controlled. Bioreactors are frequently used due to their effectiveness in homogenizing culture conditions and facilitating the dissolution process. For example, *Pleurotus ostreatus* cultivated in a bioreactor produced 19 bioactive metabolites, including alkaloids and phenolic compounds, under optimal agitation speed conditions (Papaspnyridi et al., 2012). The pH of the medium is one of the key factors determining the ability to synthesize bioactive compounds (Alarcon et al., 2003). pH affects cell membrane function, nutrient absorption, cell structure and shape, the solubility of salts, the ionic state of substrates, and the activity of enzymes that synthesize substances (Kane, 2016). The strain *Cordyceps militaris* produced 7.3g/L of exopolysaccharide (EPS) when cultured at pH 6.0, while deviations reduced both biomass and EPS yield. Temperature variations also impact the metabolite content in fungal biomass. *C. militaris* achieved maximum biomass at 20°C but required 25°C for EPS production, highlighting the need to adjust parameters according to specific stages (Kim et al., 2003). Carbon and nitrogen

sources play a crucial role in shaping metabolic output. Nitrogen is another essential nutrient source for mycelial growth and substance synthesis, being a vital factor for the synthesis of fungal enzymes, including enzymes involved in primary and secondary metabolism (Antia, Harrison & Oliveira, 1991). Yeast extract and peptone are two commonly used organic nitrogen sources for submerged cultivation of many medicinal mushroom species. Carbon is the primary nutrient component, constituting a large proportion of the elements necessary for fungal life and development. Most fungal species can utilize some simple carbon sources such as glucose and sucrose. The carbon-to-nitrogen ratio (C/N) further regulates biosynthesis; a C/N ratio of 18 increased the production of intracellular polysaccharide (IPS) in *Lentinula edodes* to 940mg/L, tripling the yield from the basal medium (Bisko et al., 2020). Nitrogen limitation often triggers the production of secondary metabolites, as observed in *C. militaris*, where the addition of peptone increased EPS synthesis (Kim et al., 2003).

3. Bioactive metabolites of medicinal mushrooms

3.1. Polysaccharides and β -Glucan

Polysaccharides from mushrooms, especially β -glucans, are known for their immunomodulatory and antitumor properties (Şengül & Ufuk, 2022). IPS with antioxidant activity has been demonstrated, showing resistance to degradation by gastric proteases, enhancing bioavailability in therapeutic applications (BARroS et al., 2016). Exo-polysaccharides (EPS) exhibit hypoglycemic and hypolipidemic effects due to their ability to regulate glucose metabolism and lipid peroxidation (Pyclik et al., 2020). The structural diversity in these polysaccharides, such as (1 $\rightarrow$ 3)- β -D-glucan with (1 $\rightarrow$ 6)- β -D-glucopyranosyl branches, is closely related to their ability to activate macrophages and dendritic cells (Edo et al., 2024). β -glucans, the most studied polysaccharides in medicinal mushrooms, are characterized by β -(1 $\rightarrow$ 3) and β -(1 $\rightarrow$ 6) glycosidic linkages forming helical or branched configurations. These structural features determine their solubility and biological activity (Barsanti et al., 2011). For example, lentinan from *Lentinula edodes* has a triple helix structure, allowing it to bind to macrophage receptors, enhance phagocytosis, and support immunity (Chakraborty et al., 2023). Similarly, schizophyllan from *Schizophyllum commune* activates dendritic cells, promoting antitumor responses in breast and lung cancer (Komatsu et al., 1969). The β -glucan content varies significantly between mushroom species. *Lentinula edodes* contains 25.3g of β -glucan /100g of dry weight (Finimundy et al., 2014), while mushroom caps have 8.6g/100g (Atila, Owaid, & Shariati, 2017). This variability influences their therapeutic efficacy; higher β -glucan concentrations correlate with stronger immune activation. Oral administration of β -glucan reduces upper respiratory tract infections in clinical trials, possibly due to their prebiotic effects on lymphoid tissue (Atila, Owaid, & Shariati, 2017).

3.2. Heteropolysaccharides and Glycoconjugates

Beyond β -glucans, medicinal mushrooms also produce heteropolysaccharides linked to proteins or peptides. *Ganoderma lucidum* (reishi) synthesizes ganoderans—proteoglycans with α -(1 $\rightarrow$ 4) glucan cores and mannose-rich side chains—that lower blood glucose in diabetic models by enhancing insulin sensitivity (Hikino et al., 1989). *Cordyceps militaris* glycoproteins, such as cordyglucans, exhibit antihypertensive effects by inhibiting angiotensin-converting enzyme (ACE), offering potential for cardiovascular disease management (Lee et al., 2015).

3.3. Triterpenoids and Ganoderic Acids

Triterpenoids are a major class of bioactive secondary metabolites found in *Ganoderma* (Reishi) species. Ganoderic acids are a subgroup of triterpenoids characterized by a tetracyclic lanostane skeleton with various oxidation patterns. *Ganoderma lucidum* synthesizes more than 140 ganoderic acids (Boh et al., 2007), which have cholesterol-lowering (Hajjaj et al., 2005), antihypertensive, and anticancer activities (Sliva, 2003). These compounds inhibit tumor cell proliferation by inducing apoptosis via mitochondrial pathways and suppressing NF- κ B signaling (Berovic, Podgornik & Gregori, 2022).

3.4. Cordycepin

Cordycepin (3'-deoxyadenosine), a characteristic metabolite of *Cordyceps militaris*. Structurally, it is an adenosine analog but lacks a hydroxyl group at the 3' position of the ribose moiety, which is crucial for biological activity. It exhibits antibacterial (Jiang et al., 2019), antiviral (Songprakhon et al., 2024), and immunomodulatory activity (Lin & Li, 2011). Cordycepin acts primarily by mimicking adenosine, interfering with nucleic acid synthesis, intracellular signaling pathways, and other pathways involved in cell proliferation, apoptosis and immunomodulation (Liu et al., 2015).

3.5. Lectins and Enzymatic Proteins

Lectins are a type of bioactive protein or glycoprotein abundant in medicinal and edible mushrooms. They have the unique ability to bind specifically to carbohydrate components on cell surfaces, leading to a series of biological effects. For example, lectins from *Pleurotus ostreatus* exhibit specificity toward N-acetylgalactosamine (GalNAc) residues on cancer cell surfaces (Wang, Gao & Ng, 2000). Lectin from *Agaricus bisporus* (ABL) induces programmed cell death in colorectal cancer cell lines (HT-29) by activating caspase-3 and suppressing Bcl-2 expression (Koyama et al., 2002).

3.6. Alkaloids and Phenolic Compounds

Alkaloids and Phenolic compounds are secondary organic metabolites found in many species of mushrooms and plants. *Hericium erinaceus* (lion's mane) synthesizes hericenones and erinacines that stimulate nerve growth factor (NGF) synthesis, showing potential in the treatment and prevention of neurodegenerative diseases such as Alzheimer's (Mori et al., 2008).

4. Submerged vs. Solid-State Fermentation

Submerged fermentation enables rapid mycelial growth (7-14 days) and high metabolite titers but requires stringent sterility and energy input for agitation (Elisashvili, 2012). Solid-state fermentation, using lignocellulosic substrates like sawdust, aligns closer to natural mushroom growth and yields enzymes like laccase and peroxidases, which are less abundant in submerged systems (BarroS et al., 2016). However, solid-state methods face scalability challenges, making submerged cultivation preferable for industrial bioreactor setups (Elisashvili, 2012). Traditional Farming with Biotechnological Approaches Fruiting body farming on wood logs remains cost-effective but is time-intensive (months) and season-dependent (Elisashvili, 2012). In contrast, submerged cultivation ensures year-round production of standardized mycelial biomass, though it lacks the complex secondary metabolites found in mature fruiting bodies. Hybrid approaches, such as combining submerged mycelial propagation with solid-state maturation, are being explored to balance efficiency and metabolite diversity (Elisashvili, 2012).

5. Conclusion

Submerged cultivation of medicinal mushrooms stands at the intersection of traditional mycology and modern biotechnology, providing a sustainable platform for bioactive metabolite production. Advances in bioreactor design, metabolic engineering, and clinical validation have positioned this method as a cornerstone of nutraceutical and pharmaceutical innovation. Future efforts must prioritize cost reduction, metabolite diversification, and translational research to fully harness the therapeutic potential of mushroom biomass.

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