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OPTIMIZATION OF MYCELIA GROWTH AND IMMOBILIZATION OF PLEUROTUS FLORIDA CELLS FOR ENHANCED BIOTECHNOLOGICAL APPLICATIONS

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ABSTRACT

Pleurotus species are commonly grown mushrooms that are crucial to the global economy. About 25 percent of all grown mushrooms worldwide are produced only from Pleurotus mushrooms. For the mushroom industry, producing high-quality spawn with a good capacity to colonize fruiting substrates with a low risk of contamination is essential. Pleurotus species are easily cultivated because of their 100% biological efficiency and ability to colonize a variety of agro-substrates rapidly. This study aims to optimize mycelium growth and find an approach to immobilizing it. The substrate jars were inoculated with both solid and liquid inoculum and incubated for 10 days in a dark condition at 21 °C. The coverage of mycelium in the jars was compared on the 10th day. It was demonstrated that the jars inoculated with liquid broth have covered 95% of the mycelium, while the jars of solid inoculum have only covered 70% of the mycelium. After the result was interpreted, the cells of the liquid broth of P. florida were immobilized using calcium chloride and sodium alginate. Once sodium alginate and fungal broth were added to a calcium chloride solution, spherical, glossy beads with an off-white color were produced. Research on bioremediation, pharmaceuticals, food technology, and environmental sustainability may find uses in these fields. This initiative seeks to leverage the biotechnological potential of Pleurotus florida to provide exciting and sustainable responses to current problems.

Keywords: P. florida, mycelium, cell immobilizing substrate, calcium chloride, sodium alginate

1. INTRODUCTION

For many years, people have utilized mushrooms as food, medicine, and nutraceutical products all throughout the world. Truffles belong to the phylogenetic sister group of Ascomycota, a phylogenetic phylum of fungi called Basidiomycota. These two phyla belong to the broad class known as "higher fungi." (Sandargo et al., 2019). Another name for Pleurotus is oyster mushroom. This macro fungus is distinguished by its unusual fruiting body and biota, which assembles its meal by the secretion of degrading enzymes. Pleurotus sp. is a member of the Agaricomycetes class, Phylum Basidiomycotina, and Family Pleurotaceae. High-grade mushrooms have a nutritious content that is nearly identical to that of milk, which accounts for their increased nutritional significance. (Chang et al., 1991).

In 1917, oyster mushroom cultivation was started in Germany on wood logs and tree stumps. Pleurotus species are extensively cultivated worldwide, mostly in Asia and Europe, due to their increased biological efficiency and simple, inexpensive production method. (Mane et al., 2007). It allows us to recycle waste, protect the environment, and get substrate material for little or no cost. Pleurotus species have many different therapeutic qualities and are useful in treating a number of serious illnesses since they are rich in medicinal values. The main medical benefits of oyster mushrooms are their anti-inflammatory, antiviral, anti-cancer, immune-modulating, and blood-lipid-lowering qualities. (Lavi et al., 2010). The substrate used in the cultivation of oyster mushrooms is made from crop residues, including wheat, soy, rice, beans, peas, cotton stems, and various industrial wastes like sugar cane bagasse, sunflower husks and stems, among others. These lignocellulosic wastes provide an inexpensive means of using waste materials; they also aid in the safe disposal of waste and the protection of the ecosystem (Jain, 2005).

The mushroom spawn industry, where the existing production process relies on the solid-state fermentation of cereal grain, can benefit from the application of liquid culture technology for the synthesis of mushroom mycelia. In the edible mushroom cultivation business, grain spawn is typically utilized. But compared to liquid spawn, it has been noted that the manufacture of grain spawn has a longer growing period and a greater risk of contamination. (Confortin et al., 2008). An alternate technique of producing spawn is through submerged fermentation, which results in mycelia, or liquid spawn. This process provides a larger yield and more homogenous mycelial biomass in a shorter amount of time. (Stamets, 2000), promotes mycelium dispersion, adaptability, and the ability to do inoculation under aseptic conditions that are comparatively more rigid. (Bettin et al., 2009). Primordial initiation and the spawn run phase were typically

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seen between 24 and 30 days. (Hoa HT et al.,2015, Girmay Z et al.,2016). For various Pleurotus species, spawn preparation needs to be standardized. Majorly cultivated species of Pleurotus are P. ostreatus (PO), P. sajor-caju (PSC), P. florida (PF) and P. ostretous (PEO), and, particularly, P. florida (PF) and P. sajor-caju (PSC) are the most popular (Kong WS.,2004, Directorate of Mushroom Research (ICAR) ,2011, Zmitrovich IV et al.,2016).

Many edible mushroom species have been cultured in liquid media in flasks or bioreactors over the past few decades. Several culinary mushroom genera, including Pleurotus, Agaricus, Lentinus, Cordyceps, Morchella, and Tuber, are well-known and can be grown underwater. Pleurotus spp., the genus of mushrooms sometimes referred to as "white rot fungi," is the second most popular genus in the mushroom market since it requires less time to cultivate than other genera. (Bellettini et al., 2019).

It has been cultivated submerged on a small or big scale with various carbon sources to study the process by which glucose is transformed into mycelial biomass. (Diamantopoulou et al., 2014; Smiderle et al., 2012). Due to a shortage of local mushrooms, there is a growing demand for them. (Mohd Zaffrie et al. 2104), It is much desired that the local mushroom business will adopt liquid seed technology in order increase productivity. Alternative spawn technology is required for local mushroom growing due to the numerous issues that mushroom producers face when using solid inoculum, particularly when produced eliminates get infected with contaminating agents and yield is unpredictable. When mycelium grows in liquid media, contaminants in liquid seed can be easily observed with the unaided eye, unlike with typical solid seed. Additionally, liquid seed has an easier time dispensing during the inoculation procedure than solid seed, and its maturity period for inoculation is shorter than that of standard solid medium seed. The mycelium of the mushroom, which consists of interwoven thread-like filaments, is propagated on a substrate of steam-sterilized cereal grain, often sorghum and wheat. We call this mixture of wheat grains and mycelium "spawn." Instead of using mycelium that was formerly a spore, most spawn is created from mycelium that has been preserved in culture. The reason for this is that every spore has the potential to produce a novel strain, whose characteristics would be uncertain. There are many benefits to using the liquid culture technique instead of solid spawns, including more automation in the spawn plant and more even inoculum distribution in the substrate. (Eyal, 1991), generating a lot of mycelia fast, year-round, a more uniform fungal growth, and less expensive (Rosado et al., 2002), early fruiting (Kawai et al., 1995), and high fruit body yield (Kirchhoff and Lelley, 1991).

This study examined an appropriate method for immobilizing oyster mushroom (Pleurotus florida) liquid spawn in order to increase its shelf life and make it easier to transport.

The immobilization approach has been extensively employed in agriculture to enhance the ecological competency of inoculum. (McLoughlin, 1994); A few investigations on edible mushroom spawn that has been immobilized have also been published. Compared to traditional grain spawn, coentrapped mycelial beads grew more quickly in pasteurized compost, had more inoculum sites, and had a shorter adaptation (lag) period—all while maintaining the same biomass levels. After being cultivated in malt extract broth for four days, the biomass levels increased threefold due to the entrapment of both the mycelium and a nutrient source in the beads. In this study, the fungal cell was immobilized using the entrapment approach. Entrapment is a commonly employed technique. This method has made use of calcium alginate and polyacrylamide gels.

In the first procedure, fungal cells are suspended in an aqueous solution of acrylamide monomers, which is then polymerized and divided into pellets to entrap the cells. Cell activity decrease can result from the process. Research has demonstrated that, in comparison to polyacrylamide, calcium alginate significantly increases the retention of cell activity. (Anderson JG.,1983). A solution of cold calcium chloride is agitated and a suspension of fungal spores in aqueous sodium alginate is poured into it to trap the fungi.

2. MATERIAL AND METHOD

2.1. Solid Culture preparation of P. Florida

Fresh culture was prepared by making slants of potato dextrose agar (PDA) to get multiple cultures for preservation as well as for further research purposes. The slants were inoculated with a strain of P. florida and incubated at 21 °C for 10 days in dark conditions. After the mycelium has covered the full medium, the slants are kept at 4 °C in the refrigerator for preservation.

2.2. Liquid Culture preparation in potato dextrose broth/Nutrient broth (PDB)

A fresh liquid culture was prepared from the fresh solid culture in potato dextrose broth. For preparing PDB, 0.97 gram of nutrient broth was weighed and 75 ml of distil water measured and mixed with the broth and distil water in a conical flask. The prepared media was autoclaved, and after proper cooling, the nutrient broth was inoculated with the desired culture and incubated for 10 days at 21 °C in a shaking incubator (120 rpm).

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2.3 preparation of substrate for quantitative analysis

2.3.1. Substrate preparation

The substrate used for the analysis was wheat grains. The grains were washed properly and boiled at 150 °C for 30 minutes until they cooked to 80%. Grains were removed from water and dried till 60% moisture was left, and $CaCO_3$ and $MgSO_4$ were mixed and autoclaved after filling jars.

2.3.2. Substrate inoculation with solid culture (PDA) and liquid culture (PDB)

After the substrate is sterilized properly and cooled to room temperature, it is inoculated with solid culture as well as liquid culture. Substrate was inoculated with a 2 cm-size cube of culture for inoculation of solid inoculum, and substrate was inoculated with 5 ml of liquid culture for inoculation with liquid inoculum by micropipette and incubated for 10 days in a dark condition with a RH of 75% and a temperature of 21 °C.

2.4. Immobilization of P. Florida cells

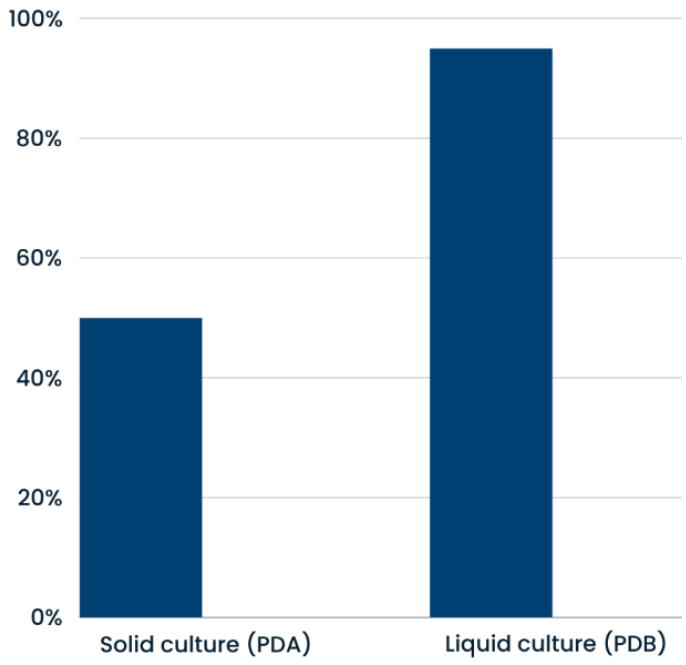
By using the entrapment approach, the liquid culture cells became immobile. In order to do it, 0.8 grams of sodium alginate and 1.8 grams of calcium chloride were combined with 10 and 20 milliliters, respectively, of distilled water. After combining 20 milliliters of liquid culture with a prepared sodium alginate solution, the cacl3 solution was added to autoclaved petri dishes. The sodium alginate solution and pre-mixed liquid culture were added drop-wise to cacl3 using a sterile syringe, resulting in the formation of entrapped beads.

3. RESULT

In this study, we have concluded that the substrate jars were inoculated with solid and liquid cultures and incubated at 21 °C for 10 days in a dark condition. The time needed for each form of inoculum to cover the entire substrate was compared after seven days of dark incubation at 21°C. The jar that was inoculated with liquid inoculum had 95% mycelial coverage after 7 days of incubation, but the jar that was inoculated with solid inoculum only had 70% mycelial coverage. Using a solution of calcium chloride and sodium alginate, P. florida cells were successfully immobilized. After the sodium alginate and fungal broth were dropped into the calcium chloride solution, the resultant beads were round, glossy, and off-white in color.

Table 1: Percentage of mycelial growth covered in 10 days by solid and liquid culture

S.no.	Type of culture	Days	Percentage
1.	Solid culture (PDA)	10 days	70%
2.	Liquid culture (PDB)	10 days	95%



Graph 1: Graph showing percentage of mycelial growth when inoculated with solid and liquid culture

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Figure 1: Jar 1 showing mycelial coverage inoculated with liquid inoculum and jar 2 showing mycelial coverage inoculated with solid inoculum

4. DISCUSSION

In this study, the investigation revealed that the substrate inoculated with the liquid inoculum shows a much faster rate of mycelium coverage as compared to the solid inoculum, as substrate jars inoculated with the liquid inoculum have shown 95% of mycelium coverage while the jars inoculated with solid inoculum have shown only 70% of the jars covering mycelium. Therefore, this revealed that liquid inoculum showcases the increased run time due to the extended reach of the culture to the grains.

An increased spawning area or volume can quicken the rate at which mycelium covers the substrate, decreasing the possibility of mold or other disease contamination. Because of this, it is commonly believed that during mushroom cultivation, inoculum that is easily and equally spread into substrates is desirable. The fungal cells can be immobilized due to their vast application. Cell immobilization is a useful and commercially significant technology that can be used on plant, algal, and microbial cells to produce beneficial metabolites or for other objectives (Liu YK et al., 1988; Iqbal M et al., 1993; Iqbal M et al., 1994; Santos DT et al., 2008; et al., 2015; Hu HJ, 2015). There are now four main ways to immobilize a substance: covalent binding, trapping, adsorption, and cross-linking (Wan-Mohtar WAAQI et al., 2016). In this study, entrapment techniques were used, and the cells of the liquid inoculum were trapped for their long-term usage.

5. CONCLUSION

Growing Pleurotus mushrooms is most profitable and suitable in tropical, subtropical, and temperate climates. Depending on what is easily available in various parts of the world, they can be cultivated on a variety of agricultural substrates. It promotes the recycling of agricultural wastes and their transformation into foods high in protein. They are an integral part of the forest ecology, which has the potential to stabilize and rebuild the forest communities. Particularly in underdeveloped nations, mushroom growing is a labor-intensive enterprise that can enhance income creation and sustain livelihoods. In this research, we have concluded that the suitable inoculum for the inoculation is the liquid culture, which showed 95% mycelium coverage in 10 days due to its uniform distribution in the substrate, faster mycelium coverage, and enhanced growing time.

This result will improve mushroom cultivation technology and will help the grower achieve faster and better-quality mushroom cultivation. Immobilization of the cells of P. florida by the entrapment method was done in this research. Immobilized fungal enzymes have enormous potential as biocatalysts for the removal of contaminants, the production of useful products, and the promotion of green industrial development. The process of immobilization offers a range of support materials and methods to improve the benefits of fungal enzymes, such as their catalytic capability, stability, and reusability. Enzymes from immobilized fungi can degrade a variety of substances for use in industry and the environment.

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