Study on antimicrobial activities of *Agaricus bisporusa* with solid spawn vs liquid spawn

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Abstract

Mushrooms are one of the most important edible species grown in different parts of the world. It plays an important role in pharmaceutical industries due to the creation of intriguing compounds with bioactive qualities. Agaricus is the major significant mushrooms and used as a therapeutic drug. Due to its external elements, and low land requirement it grown in high number. The main diseases cured by the fungus are cardiovascular disease, atherosclerosis, cataracts, Alzheimer's and Parkinson's disease. The bacterial emergence is today's world is resisted by many antibiotics, which serves as a catalyst to find novel antimicrobial medicines to treat the infections. In present world, the medicines still face many problems, as modern study says that, prevalence of bacterial resistance to various antibiotics has significantly increased. The mushroom plays a vital role in ecology, due to its biodegradable activity over plant and agricultural waste. In this study, the mycelial liquid cultures of mushrooms is investigated in search of potent antibacterial substance, assuming that the cultures would generate the same substances as the fruiting bodies. The ability of the liquid culture to quickly and effectively inoculate the supplied substrate is one requirement for its employment in the spawn production process. It must have a high density of viable inoculum particles to accomplish the same.

Key word: Agaricus, disease, antibiotics, antimicrobial

1. Introduction

Numerous locations throughout the world have produced about 200 different edible mushroom species. Due to the creation of intriguing compounds with bioactive qualities, cultivated mushrooms are not only of significant interest to the food business but can also be viewed as being of considerable significance for pharmaceutical firms. The *Agaricus* genus is home to some of the most significant edible mushrooms that are grown today. *Agaricus bisporus*, the well-known "common mushroom," and *Agaricus brasiliensis*, which is grown all over the world for its therapeutic benefits, are two of them (Stojkovic et al., 2014),(Lin $ Sun,2019). Due to external elements that are common: short harvest intervals, extremely low input needs, limited land requirements, and an abundance of discarded agricultural biomass, the cultivation of edible mushrooms is becoming increasingly effective in many places (Higgins et al., 2017). The scientific community researched several mushrooms in an effort to find new medicinal options, and the findings demonstrated their bioactive characteristics (Barros et al., 2008) Since Ancient times, people have enjoyed eating mushrooms broadly because of its flavors, texture, and range of therapeutic and restorative capabilities. However, it has only lately become known that mushrooms are a significant source of biologically active compounds with therapeutic benefits. Over the past ten years, several mushroom species have been found to have high antioxidant potential la (Smolskaite et al., 2015). With a high concentration of dietary fibre, antioxidants, and vitamins C, B12, and D as well as folate, ergothioneine, and polyphenol, white button mushrooms may have potential anti-inflammatory, hypoglycemic, and hypocholesterolemic benefits. (Jeong et al., 2009),(Rashid HM et al.,2018)

Inflammation, cataracts, cardiovascular disease, atherosclerosis, and neurological illnesses like Alzheimer's and Parkinson's are all brought on by oxidative stress, which phytochemicals help to avoid. Antioxidant and other therapeutic chemicals are abundant in fungi (Ghahremani-Majd &

Farshad.,2015; Panigrahy et al. 2017). Mushroom natural chemicals have been the primary source of physiologically active substances. Many of these chemicals, which are employed in various ways, are still unidentified. Bacterial invasions in agriculture result in significant decreases in crop quality and output as well as significant financial losses (Waqas et al., 2019). Today, the emergence of a bacterium that is resistant to several of the widely used antibiotics serves as a catalyst for continued efforts to find novel antimicrobial medicines to treat infections. Modern medicine still faces challenges when treating infectious diseases with antimicrobial medicines, since numerous studies have found that the prevalence of bacterial resistance to various antibiotics has significantly increased (Ozturk et al.,2011). The continuous need for innovative antimicrobial drugs is prompted by the threat posed by antibiotic-resistant pathogens. Finding antimicrobial compounds from various natural sources, such as marine life, plants, prokaryotes, fungus, and mammals, is possible. (Rezaeian&Pourianfar, 2015). Moreover, mushrooms play a vital function in the ecology because mushroom myceliums may biodegrade agricultural and plant waste (Nadir et al., 2011). Due to the indiscriminate use of commercial antimicrobial medications frequently prescribed for the treatment of infectious diseases, multiple drug resistance in human pathogenic bacteria has emerged. This circumstance forced scientists to look for new antimicrobial compounds from a variety of sources that may serve as sources of novel antimicrobial chemotherapeutic drugs.The antibacterial activity of *Agaricus* species has not received significant research to yet. Therefore, assessing the antibacterial ability of *Agaricus* species against various Gram-positive and Gram-negative bacteria as well as against two yeast-like fungi, Candida albicans and *C. tropicalis*, is one of the study's objectives (Ozturk et al.,2011).

We investigated the mycelial liquid cultures of mushrooms in search of potent antibacterial substances, assuming that the cultures would generate the same substances as the fruiting bodies

(Imtiaj& Lee2007). The synthesis of higher fungi's mycelia using liquid culture technologies opens up the possibility of using this class of organisms on an industrial scale. These applications also apply to the creation of mushroom spawn, which is currently done through the solid state fermentation of cereal grains. On a substrate made of synthetic sawdust, Lentinula edodes have been used to study this kind of technology.Increased process control (growth rates and nutritional content), shorter production cycle times, increased automation in the spawn plant, inoculation of the substrate under stricter aseptic conditions, and more uniform distribution of the inoculum in the substrate are benefits of incorporating liquid culture technology into the spawn production process.The ability of the liquid culture to quickly and effectively inoculate the supplied substrate is one requirement for its employment in the spawn production process. It must have a high density of viable inoculum particles to accomplish this. To put it another way, the culture should be uniform and made up of unpelleted mycelia (Janpooret al., 2017).

2. Morphology

An edible species of *Basidiomycota* known as A.bisporus is widely farmed throughout Europe and North America, accounting for 35–45% of the world's total production of edible mushrooms(Ramos et al., 2019). *A. bisporus* is also known as the "button mushroom," and it is a good source of food and numerous significant bioactive chemicals. (Usman et al., 2021). Mushrooms are frequently used to describe the species variety since they have a shared innate anatomical structure but yet have diverse evolutionary histories. When mushrooms are fully developed, they have an outer cap or pileus structure that is frequently covered in scales to protect the internal gills or lamellae, which are responsible for spreading spores. For the purpose of transferring nutrients, the mushroom's stape/stem connects the cap to the mycelial threads and is made up of two additional structures: the ring/annulus and cup/colva. The transfer of absorbed nutrition, feedback of chemical signals, and the survival of the reproductive lamellae all depend on the inferior region of the mushroom, which refers to the structures below the ring or annulus. The characteristics of pileus of A. Bisporus species is its pale greyish brown color, broad flat scales which are hemispherical in shape and fade towards the borders with maturity, and its diameter ranges from 5 to 10 cm. Despite having the same inherent mushroom structure. The initially pink, tightly packed lamellae eventually turn brown with a whitish border as the mushroom ages. The spores are roughly 4.5-5.5 x 5-7.5 m2 in size and can be oval or spherical. This species can be identified by its unusual basidia, which bear just two spores instead of the normal four found in other Agaricus species. The cylindrical stipe has a narrow annulus that is frequently striped on the upper side and is up to 8 cm long and 3 cm wide. (Ramos et al., 2019).

3. Nutritional importance

Protein

Mushrooms are practically a complete food for humans, providing more good than milk but less protein than meat. They are a strong source of protein. The mushroom culture procedure determines the mushrooms' symbolic worth. Depending on the growth substrate, A. bisporus' protein concentration ranged from 11.01% to 25%, 17.7% to 24.7%, and 29.14%. Given the significant intake of animal food sources for protein, especially in industrialised nations, the amino acid composition of mushroom proteins is comparable to that of animal protein (Atila et al., 2021). The therapy provided the largest proportion of crud protein (43.693%) and the lowest percentage of protein (26.07%) (Nadir et al., 2020). The amino acids continually combine to form urea, which adds to the overall nitrogen content of the mushroom. Mushrooms that had been collected afterward reportedly displayed protease activity. Aspartic acid, serine, glycine, threonine, glutamine, valine, cysteine, alanine, leucine, isoleucine, lysine, histidine, proline, arginine, tyrosine, and norleucine are among the primary amino acids found in A. bisporus, according to Braaksma and Schaap. . (Atila et al., 2021)

Carbohydrate

The two most crucial digestible carbohydrates are glucose and mannitol, although they are only found in little amounts in *A. bisporus* (less than 1% of the dry weight). In addition, glycogen, which makes up 5 to 10% of A. bisporus's dry weight, is another digestible carbohydrate. Non-digestible carbohydrates like trehalose, mannans, and -glucan make up the majority of the carbohydrates in A. bisporus. When the carbohydrate content of A. bisporus was examined, the two sugars that were most prevalent were trehalose and mannitol. The sugar that is most prevalent in A. bisporus is mannitol. In the fresh fruiting bodies of this species, glucose, which ranged in concentration from 17.6-28.1 mg per g, was the primary soluble sugar type. (Usman et al.,2021)

Mineral content

It is well known that mushrooms are great mineral accumulators from their growing environment. According to Owaid (2015), A. bisporus is an excellent source of K, Fe, Zn, Cu, Na, Se,nM dCa oC. According to Guillamon et al. (2010) and Falandysz and Borovika (2013), the major components of mushroom fruiting bodies are potassium and phosphorus, which are typically followed by Ca, Mg, Na, and Fe, Zn. Mohiuddin and others (2015). The minaral content profile of Agaricus bisporus fruitbodies from various regions in Bangladesh was investigated. The total carbohydrate content of Pleurotus sajor caju and Agaricus bisporus mushrooms was 52.46 and 53.10 percent, respectively (Goyal et al., 2006). The samples' mineral contents ranged from 0.54 to 1.58% for potassium, 37.2 to 61.9 µg/g for sodium, 143.6-396 µg/g for iron, 54.6 to 163.4 µg/g for copper, 36.6 to 58.0 μg/g for zinc, and 56.2 to 91.1 μg/g for manganese. Caglarirmak (2009) determined that zinc (8.1-7.0 mg per kg), ferrum (7.4-7.9 mg per kg), phosphore (7.4-7.9 mg per kg), magnesium (88.0-76.3 mg per kg), potassium (213.3-238.8 mg per kg), sodium (2652- 2500 mg per kg), and calcium (534.2-554.8 mg per kg) contents of A. bisporus fruitbodies, while Ahlavat et al. (Atila et al., 2021).

Generally rich in copper (Cu), cobalt (Co), iron (Fe), selenium (Se), potassium (K), and manganese (Mn), A. bisporus is regarded as a significant source of minerals. Phosphorus (P) and potassium (K) are the two main minerals found in mushroom fruiting bodies, followed by calcium (Ca), zinc (Zn), iron (Fe), magnesium (Mg), and sodium (Na) (Na). Several minerals are present in A. bisporus and contribute to the health advantages of the mushroom. (Usman et al.,2021).

Vitamins

According to certain authors, mushrooms are a good source of vitamins. Vitamins A, B1 (thiamine), B2 (riboflavin), B3 (niacin acid), B5 (pantothenic acid), C (ascorbic acid), D, and folic acid are all abundant in A. bisporus. (Owaid et al., 2017).

Niacin and riboflavin are said to be the two vitamins present in Agaricus in the highest concentrations. Vitamin B1, B3, L-ascorbic acid, and -tocopherol are additional vitamins (Bernas & Jaworska, 2016). A. bisporus (brown portobello mushroom) is a good source of folic acid (0.09-0.08 mg/kg), riboflavin (0.27-0.29 mg/kg), niacin (3.6-2.9 mg/kg), and thiamin (0.085-0.09 mg/kg), although it is not high in vitamin C content, according to alarmak (2009). (Atila et al., 2021) Mohiuddin et al. studied the mineral content of A. bisporus growing in various environments. The samples' mineral contents (mg kg1) ranged from 37.2 to 61.9 mg kg1 for sodium (Na), 54.6 to 163.4 mg kg1 for copper, 56.2 to 91.1 mg kg1 for magnesium (Mg), 143.6-

396 mg kg1 for iron (Fe), and 36.6 to 58.0 mg kg1 for zinc (Zn). The mineral concentrations of A. bisporus were also measured by Caglarmak to be calcium (534.2-554.8 mg per kg), potassium (213.3-238.8 mg per kg), iron (7.4-7.9 mg per kg), zinc (8.1-8.7 mg per kg), sodium (2652-2500 mg per kg), magnesium (88.0-76.3 mg per kg), and phosphorus (7.4-7.9 mg per kg). Similar to this, Ahlavat et al. determined the themineral content to be Na (500.8 mg per kg) and Se (1.34 mg per kg) in the fruiting bodies of A. bisporus. Se in A. bisporus, according to Lu and Holmgren, is an essential micronutrient for both animals and people. (Usman et al.,2021)

Fat & Fatty acid

A mushroom sample weighing about 10 g was extracted with petroleum ether in an extraction device for 16 hours. The extract was weighed, its mass recorded, and it was then dried and chilled in desiccators. The mushroom was discovered to contain far less fat and more protein than carbohydrates. *Agaricus bisporous* has a total fat content of 2.1 2 g.(GA Teklit,2015)

Although having a low-fat content, Agaricus bisporus contains certain necessary fatty acids like linoleic acid revealed that due to the increased contribution of linoleic acid, wild Agaricus spp. had a lower value of monounsaturated fatty acids but a larger content of polyunsaturated fatty acids than the commercial species. The studied A. bisporus strains had total fatty acid contents ranging from 180 to 5818 mg/kg dry matter, with an average of roughly 90% linoleic acid stated that linoleic acid was the major fatty acid in *A. bisporus*, accounting for 44.19% of the total fatty acids identified. Other fatty acids found in A. bisporus were caprylic, palmitic, stearic, oleic, and eicosanoic acids. (Atila et al., 2021)

4. Health benefits of mushrooms

Antioxidant and immunomodulating activity

Antioxidant substances aid in protecting cells and tissues from oxidative damage. Another significant health problem is stress on the body brought on by ageing, obesity, and unfavorable lifestyle choices. This stress frequently manifests as oxidative damage to tissues (Panigrahy et al. 2017). Eating edible mushrooms meets the needs for natural sources of antioxidant foods (Rathod et al.,2021) Fourier-transform infrared spectroscopy (FTIR) indicated that the polysaccharides identified in A. bisporus contained a significant quantity of "a" and "b" glucans and had an immune-stimulating effect (Bhushan & Kulshreshtha ,2018). Concentration-dependent effects (i.e., an increase in the CuNPs' concentration causes an increase in anti-oxidant activities) were seen in the anti-oxidant effects of the CuNPs produced using *Agaricus bisporus* as determined by DPPH, ABTS, and Nitric oxide assays.

The exertion of radical scavenging effects varied significantly between the anti-oxidant tests, according to comparative study of the separate assays. The following list of tests might be used to rank the antioxidant activity using CuNPs: ABTS radical (82%), NO radical (76%) and DPPH radical (72%). Comparatively speaking to the CuNPs, standard (Ascorbic acid) showed less anti-oxidant properties.(Sriramulu et al.,2020)

Antimicrobial activity

Due to the growing trend of microbial resistance to the majority of presently used antimicrobial medications, the scientific interest in these metabolites has grown recently with the hunt for novel therapeutic compounds derived from mushrooms (Sharma et al.,2014) Two species of Gram-positive bacteria, three species of Gram-negative bacteria, and one species of yeast were used to assess the antibacterial properties of ethanol extracts of *A. bisporus*. The examined Gram-positive bacteria, including B. subtilis, were severely inhibited in their development by A. bisporus extract, which exhibited a limited antibacterial range against Gram-negative bacteria. The maximum zones of inhibition were in the 12–22 mm range. S. aureus, which had a diameter of 18 1 and 22 4 mm for the boiling and raw extracts, respectively, was the most vulnerable bacteria. Nevertheless, in the raw extract, a distinct zone measuring 15 1 mm in diameter was seen. The ethanol extract of A. bisporus did not exhibit any antibacterial action against K. pneumoniae. Both A. bisporus extracts had antibacterial activity against P. aeruginosa, however the range of activity was less in the cooked extract (12 0.3) than in the raw mushroom extract (16 0.2). This might be as a result of the molecule that causes the action being affected by temperature. (Jagadish et al.,2009), (Narasimha et al.,2011) ZnO NPs' ability to suppress some bacterial isolates, including E. faecalis, S. aureus, and B. subtilis as well as E. coli, *K. pneumoniae*, and P. vulgaris. Maximum inhibitory effect was discovered to be in the following order among the gram-positive isolates tested: E. faecalis (21 mm 1.05), S. aureus (28 mm 1.1), and *B. subtilis* (32 mm 1.05), respectively. The gram-negative organisms that were most affected by the ZnO NPs' inhibitory effect were E. coli (16 mm 0.8), K. pneumonia (18 mm 0.85), and P. vulgaris (22 mm 0.9). It was shown that ZnO NPs had a stronger inhibitory impact on gram-positive strains of pathogens than they did on gram-negative bacteria. Gram-positive and gram-negative bacteria that are affected by the conventional antibiotic streptomycin include B. subtilis (25 mm 1.25) S. aureus (26 mm 1.1) E. faecalis (36 mm 1.8); *K. pneumoniae* (28 mm 1.8) P. vulgaris (30 mm 1.5) and *E. coli* (32 mm 1.4), respectively. The morphological characteristics of the ZnO NPs were linked to their bactericidal capability. (t Mohana & Sumathi.,2020)

5. Conclusion

The high nutritional content of *A bisporus*, particularly in emerging and underdeveloped nations, may offer substantial protection against malnutrition. Consuming A. bisporus is not only beneficial for nutrition, but it also has medical benefits, particularly in regard to cancer, cardiovascular disease, diabetes, antioxidants, and antimicrobials. Edible mushrooms have gained popularity over the past few decades as a source of therapy or nutritional supplements. The majority of studies have demonstrated that nutraceutical therapy is a promising source of novel treatments for a variety of fatal illness. Being a practically complete food for humans, mushrooms, here *A. biosporus* has all the essential nutrients including protein, carbohydrates, several minerals, riboflavin, niacin etc. All these nutritional content makes *A. biosporus* a great and complete food for human consumption all over the globe. Extract of *A. biosporus* showed major antimicrobial property against the gram-positive bacteria like B. subtilis, P. aeruginosa, showing limited antibacterial range against Gram-negative bacteria. Although the ZnO NPs suppressed some bacterial isolates, including E. faecalis, S. aureus, and B. subtilis as well as *E. coli, K. pneumoniae, and P. vulgaris*.

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