The potential of bioinoculants in enhancing the mushroom productivity

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Abstract. Nowadays, environmentally friendly and sustainable food production is gaining importance day by day. The most important factor in achieving the goal of sustainable food production is limiting the use of chemicals that pose risks to human and environmental health, such as fertilizers and pesticides. One of the methods used to reduce the use of chemicals in agriculture is the use of bioinoculants. Bioinoculants are formulations comprised of microorganisms such as bacteri and fungi. They have potentials to increase the agricultural productivity and to control pests and diseases. Mushrooms are among the sustainable foods that stand out with their high nutritional values and medicinal properties. However, the excessive use of chemicals in the production of some edible mushroom species may make their consume risky, despite rich nutritional and medicinal values of the mushroom. The use of biological agents replacing chemicals can provided a great advantage to the mushroom industry in this regard. *Azotobacter, Bacillus, Paenibacillus* and *Pseudomonas* are most important bacteria genera used in mushroom cultivation. Generally, they increase the mycelial growth of mushroom species while exhibiting competition against harmful molds and stimüle the mushroom yield. On the other hand, use of microorganisms instead of additive materials in the mushroom growing media is highly new and interesting issue in the sector of mushroom cultivation. The aim of this study is to provide a view of the possibility of use of bioinoculants in enhancing the mushroom yield through the agency of the growth encouragement, and their potential as biocontrol agents to prevent various diseases in the mushroom cultivation.

1 Introduction

Nowadays, the use of sustainable techniques in agricultural production has started to gain value with the understanding of the negative effects of chemicals such as pesticides and fertilizers on environment and human health. In this process, biological approaches, in particular the use of growth-promoting bacteria and fungus to enhance plant growth and yield, are widely used worldwide as one of the most environmentally friendly and sustainable techniques.

Many of the bacteria inoculated into the plant rhizosphere are known to have a positive effect on plant growth [1]. Today, these bacterial species can be used in organic agriculture. Beneficial microorganisms not only support plant growth, but also contribute to the protection of crops from the effects of diseases and pests. Although biological agents to replace chemicals have long been used in various fields of agriculture, it is a newer topic for the mushroom cultivation sector.

All living organisms have close relationships with microorganisms throughout their life cycle [2]. The continuous interaction between human, plants, the environment and microorganisms ensures ecological balance and sustainability. Microorganisms, collectively referred to as microbiomes, can associate with hosts in a beneficial, detrimental, symbiotic or opportunistic manner and affect positively the growth and development of hosts [2, 3].

Mushrooms are valuable food sources due to their high nutritional value and medicinal properties and they are widely consumed worldwide. World mushroom production was 44.207.117 tons in 2021 [4]. The largest mushroom producer is China with 41 million tons, followed by Japan, Poland and the United States. Button mushroom (*Agaricus bisporus*), oyster mushrooms (*Pleurotus* spp.) and shiitake (*Lentinula edodes*) are the most commercially produced species [5]. Total mushroom production in the world is expected to continue to increase in the next years.

Mushrooms cannot photosynthesize and therefore are described as heterotrophic. They need external nutrients for mycelial development, pinhead formation and fruitbody development [6]. Therefore, most cultivated mushooms are saprophytic species. They degrade lignocellulosic contents by secreting various lignocellulosic enzymes to break down substrates and in this way they can grow on lignocellulosic substrates [7].

Mushrooms are grown on composts and substrates rich in microorganisms, where a wide range of mutually beneficial interactions as well as competition between bacteria and fungi are established [8].

In mushroom cultivation, the growing medium mainly consists of two different groups: basal substrate and

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additive materials. In addition to these, auxiliary materials such as $CaCO_3$ and $CaSO_4$ are added to regulate the texture and pH of the growing medium [6].

As a basal substrate, agricultural and forestry wastes that are the easiest and cheapest to find in the region where mushrooms will be grown are preferred. The most commonly used basal substrates are straw types, sawdust and cotton wastes. These materials are rich in lignocellulosic content such as cellulose, hemicellulose and lignin, but low in nitrogen. Therefore, in order to achieve the desired yield, additives with high nitrogen content such as cotton meal, rice bran, various meal such as corn meal, etc. should be added to the growing medium [9].

Cultivated mushroom species are basically grown on 2 different substrates prepared in different ways;

1. Fermented-pasteurized substrates (*Agaricus bisporus*)
2. Steam sterilized agricultural and forestry waste mixtures (*Lentinula edodes, Hericium erinaceus*, etc.) [10].

Different substrate materials used in the preparation of mushroom growing media affect mushroom yield [11-13]. Therefore, growing media formulas used in mushroom production sector should be improved depending on the mushroom species.

Identification of the microbial communities involved in the processes of the mushroom production process can help to entirely understand the functional structure of these microbial communities and their impact on mushroom growth and biological efficiency (BE%). Nowadays, many researchs have focused on the impact of microbial communities on mushroom yield and substrate quality [3, 14, 15].

The aim of this study was to review the possibility of using microorganisms in mushroom cultivation to increase mushroom growth and yield, and their potential for use as biocontrol agents to protect from pests and diseases in mushroom cultivation.

1.1 Microorganisms growing naturally in mushroom compost and growing media

Mushrooms are produced in microorganism-rich composts and growing media where a wide range of mutually beneficial as well as competitive interactions between bacteria and fungi are established [8]. Fermentable substrates are produced by composting various animal manures such as chicken and horse and plant material such as wheat straw [10].

There is a rich diversity of microorganisms in compost, which play important roles in digesting the lignocellulosic content of mushroom growing media, minimizing competitor organisms in the growing media and triggering fruitbody formation [16, 17]. *Agaricus bisporus* is the most important species grown on fermented substrates worldwide, where microorganisms play an important role in pinhead formation and fruitbody development [18]. In the pasteurization and maturation phase of *A. bisporus* compost preparation, the amount of bacteria belonging to the order *Bacillales* increases in connection with the high ammonia emission. The role of the natural microbiota during pasteurization and maturation is to provide to the elemination of ammonia in the compost [18]. In oyster mushroom growing media, the main part of bacterial species detected belonged to the genera *Paenibacillus* and *Bacillus*, while the majority of fungal species belonged to the genera *Wallemia* and *Verticillium* [19]. The interactions between bacteria and fungi in the natural structure of the growing media and cultivated mushrooms may not always be positive, and some bacterial and fungal species can cause various diseases and cause significant yield losses [20]. Expanding research on such microorganisms and identifying bacterial and fungal antagonists against various pests could be helpful in increasing mushroom yields.

Several factors such as temperature, oxygen, humidity, substrates used in growing medium preparation and C/N ratio are known to influence the type and amount of microbial communities present in growing media [21]. Analysis and search of microbial diversity in mushroom compost will supply important knowledge for the preparation of effective growing media and an important contribute to mushroom production sector.

1.2 Bioinoculants that can be used to increase mushroom productivity

The presence of beneficial microorganisms in substrates used in mushroom cultivation promotes mycelial growth and pinhead formation. Many researchs have suggested the use of beneficial microorganisms to improve the productivity of edible mushrooms, especially product quality and uniformity [22- 24].

The preparations used to promote yield in mushroom cultivation are usually nutritional supplements based on rich protein mixtures added to compost or cover soil [10]. There are also a variety of commercial preparations prepared from microorganisms that are used in mushroom cultivation to protect the crop against diseases and pests, as well as to provide the nutritional needs of the mushroom, stimulate host growth or increase disease resistance. For example, fungi are used as insecticides to control fungus flies or two strains identified as *Bacillus subtilis* QST 713 and *Bacillus amyloliquefaciens* MBI 600 to control green mold disease. There is no commercial formulation available on the market for mushroom growers based solely on microorganisms that promote mushroom growth. Therefore, the selection and identification of new bacterial isolates for increased mycelial development and mushroom yield in an environmentally friendly approach and their commercialization into commercial preparations could make significant contributions to the mushroom growing industry. In general, the association of mushroom mycelia with beneficial bacteria present in the mushroom compost results in an initial increase in the rate of hyphae elongation followed by stimulation and formation of primordia [25]. Some bacteria present in mushroom compost or soil cover secrete some biologically active compounds such as phytohormones. These compounds

secreted by the bacteria can promote mycelial growth and mushroom yield [26].

Azotobacter, Bacillus, Paenibacillus and *Pseudomonas* have been reported to be used as biosupplements in mushroom cultivation [10]. The addition of these bacteria to mushroom growing media can potentially promote the growth and fruiting of *Pleurotus* spp. and *Agaricus bisporus* mushrooms [27].

Microorganisms can be used to increase mushroom yields and shorten the production process in mushroom cultivation. The interactions identified between beneficial microorganisms and mushroom are as follows:

1. shortening the composting process to facilitate the degradation of lignocellulose while improving the quality of the substrate [18].
2. promoting mycelial growth in mushroom growing media/compost by releasing nutrients [28, 29]
3. Stimulation of fungal pinhead formation [30].
4. In truffle species and other mycorrhizal fungi, promoting the growth of mycorrhizal fungi by stimulating the establishment of symbiotic associations [31].

Many bacterial species provide fungal substrate benefits to fungal mycelia and affect fruiting and BE of fungi. Preparations from some microorganisms can also produce phytohormones such as auxin, cytokinin and ethylene that stimulate mycelial growth [32].

IAA secreted by *Pseudomonas* sp. P7014 plays an important role in promoting the growth of mycelial growth of king oyster mushroom (*Pleurotus eryngii*) [33,34]. Kim et al. [22] reported that *Pseudomonas* sp. P7014 promoted the growth of *P. eryngii* in bottle cultures. A positive interaction was reported to occur during co-cultivation of *Pleurotus florida* and fluorescent *Pseudomonas* bacteria [15].

Bacteria are also involved in the degredation of cellulose by secreting cellulase, providing nutrients to the mushroom mycelium [35]. Actinobacteria has an important role in the biodegradation of cellulose and lignin [36]. Kues and Liu [37] and Hultberg et al. [38] reported that laccases have important functions in stimulating mycelial growth and pinhead formation. Actinomycetals are abundant in the medium during the thermophilic stages of compost preparation and can degrade lignin and cellulose. *Bacillus* are the most abundant bacteria in composting process and can provided to the breakdown of lignocellulosic materials in the process due to their thermotolerance properties [39, 40].

Chen et al. [41] reported that *Bacillus cereus* isolated from *P. eryngii* promoted the mycelial growth rate by 1.15 times. Young et al. [42] reported that the application of the actinobacterium *Microbacterium hum*i in the covering soil during the cultivation of A. subrufescens contributed to shorten the time to harvest up to 26 days and increased the mushroom yield up to 215%. Ekinci and Dursun [43] reported that composts supplemented with bacteria and organic fertilizers increased mushroom yield and quality in *A. bisporus* cultivation.

Bacillus subtilis increased mushroom yield when applied at lower concentrations [44]. Addition of *Pseudomonas* sp. P7014 to cultures resulted in earlier initiation of primordia compared to the control of *P. eryngii*. Similarly, Young et al. [45] reported a significant increase of 64% in *Agaricus blazei* yield when two bacterial isolates were inoculated with *Exignobacterium* sp. Mohammad and Sabaa [46] reported an increase in mushroom yield (26.6%) when inoculated with *Pseudomanas putida* compared to uninoculated sets.

Cho et al. [15] determined that inoculation of isolated fluorescent *Pseudomonas* spp. from the mycelial surface of *P. ostreatus* exhibited positive effects on pinhead formation and fruitbody development. Similarly, inoculation of *Actinobacteria* and *Glutamicibacter arilaitensis* into the growing substrate of *Pleurotus florida* mushroom resulted in increased mushroom yield and BE. Kumar and Narian [23] reported that *Glutamicibacter arilaitensis* MRC119 bacterial isolate used in oyster mushroom production increased mushroom yield by 28% and BE by 58%. The study showed that the bacteria were strictly attached to the mycelial surface and as a consequence increased mycelium growth. This relation between mushroom mycelia and bacterial cells has an important role in stimulating mycelium growth through beneficial secretions from both sides [23].

Ascomycota, especially the thermophilic fungi *Chaetomium thermophilum, Malbranchea sulphrea, Thermomyces lanuginosus* and *Torula thermophila* (*Mycothermus thermophilus*) support the growth of *A. bisporus* [47].

It was noted that inoculation of covering soil with *Bacillus subtilis* and *Alcaligenes faecalis* exhibited significantly higher yield compared to non-inoculated [48]. Çetin et al. [49] also reported the productive effect of various bacteria on the yield of *A. bisporus* mushroom, which increased by 8-40%.

Complex carbohydrates in the structure of mushroom growing substrates are degraded by beneficial bacteria into simple sugars, which can easily taken up by oyster mushrooms. This effect of bacteria on the growing medium may leads to good mycelial development and increased mushroom yield [14].

1.3 Use of biocontrol agents for the control of fungal parasites

Although the microbiome naturally present in compost and covering soil, bacteria such as *Bacillus* and *Pseudomonas*, produce antifungal and insecticidal compounds, the natural microbiota is not generally effective in suppressing mycoparasites, especially at low temperatures and conditions of high disease pressure [3]. Therefore, the control of diseases and insects that occur during mushroom production relies on the application of chemical pesticides and insecticides as well as integrated pest management programs [50].

However, the reports on the effects of overuse of chemicals on human health and the emergence of resistant strains [51] support the thesis of developing new sustainable strategies for the conservation of mushroom crops. Several natural biomolecules such as aromatic plant extracts have been tested to reduce fungicide

dependence. But, studies have shown that these extracts have variable efficacy in inhibiting fungal diseases in agricultural crops and sometimes even have detrimental effects on the plants [52]. On the other hand, promising results have been obtained that the use of bacterial species as an alternative against fungal diseases in agricultural crops can be a potentially sustainable solution to these problems. Several species of bacteria belonging to the genus *Bacillus*, naturally present in covering soil, show a selective inhibitory effect on fungal diseases and their application to compost or cover soil can prevent green mold and losses due to this disease [53, 54].

Numerous species or strains of *Bacillus* have been developed as commercial products because they are not normally considered human pathogens. *Bacillus* species have a number of desirable properties, such as rapid growth, ability to thrive in inexpensive culture media, production of various antifungal substances and some plant nutrients. In addition, preparations prepared from *Bacillus* are resistant to unfavorable environments and can be stored for a long time [55]. The actinobacterium *Streptomyces flavovirens* A06, isolated from a Phase I substrate, was described to be effective in controlling green mold disease while minimizing production losses and showed no deleterious effects against the host mycelium of *A. bisporus* [56].

Chittihunsa et al. [55] reported that Bl 01-SU1, a *Bacillus* isolate, was very effective against *Trichoderma* sp. in *Lentinula edodes* and *Pleurotus sajor-caju*, reducing infection by 58.23% and 80%, respectively. Bacterial strains isolated from compost, covering soil, *A. bisporus* fruitbody and wild mushrooms were also effective against bacterial diseases such as brown spot (*Pseudomanas tolaasii*) and internal stripe necrosis (*Ewingella americana*) [57, 58]. Similarly, bacteria isolated from the fruitbodies of *A. bisporus* exhibited broad-spectrum antimicrobial activities [35].

1.4 Limiting factors related to the use of biocontrol agents

The mechanisms by which bacterial agents inhibit disease and pests in mushroom cultivation are still poorly characterized. Due to limited knowledge of the ecology of the mushroom crop and the difficulties of producing a reliable microcosm in the laboratory, there are also significant challenges in translating results from in vitro to in vivo. Often successful results obtained in vitro may not translate into crop productivity [54, 58].

Determining the timing and doses of bacterial applications for different mushroom species are important issues. For this purpose, the use of molecular markers and the application of functional imaging techniques to monitor the behavior of the biocontrol agent in a growing media will be useful.

Suitable formulations of bioactive compounds, the timing and the dose of application are important point to be determined for different mushroom species. For this purpose, it is necessary to apply tools to monitor the behavior of the biocontrol agent in a growing media, such as the use of molecular markers and the application of functional imaging techniques to monitor the distribution of bacteria.

2 Conclusion

Microorganisms can be used in mushroom cultivation as an environmentally friendly alternative to chemical preparations to increase mushroom yields. Further investigation of microbial communities that help or inhibit fruitbody formation may provide important insights into mushroom cultivation practices. Understanding the microorganisms that associated with mushrooms may contribute to the development of methods that will make possible to improve both yield and mushroom quality. A deeper understanding of bacteria-fungus interactions can support the development of strategies to increase both the profitability and sustainability of the industry in the medium and long term.

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