Research Article

Basidiome Initiation in Medicinal Mushroom *Hypsizygus ulmarius* by Free Living Nitrogen Fixing *Azotobacter sp*

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ABSTRACT

Yield of fruit bodies in medicinal mushroom is considered as important criteria in the cultivation. Fruit body initiation in mushroom was controlled by several physical and metabolic parameters. Hence the current research was carried out to investigate the fungal bacterial interaction explored for the initiation of fruit body in medicinal mushroom. The antagonistic activity between the fungi and bacteria were studied on dual plate method and found to be compatible. We found that free living nitrogen fixing *Azotobacter* initiate the basidiome in *Hypsizygus ulmarius* and not in *Pleurotus aureovillosus, and Volvariella volvaceae.* The current research suggests that, in cultivation of medicinal mushroom *Hypsizygus ulmarius, Azotobacter* can be co-inoculated in the substrate as basidiome inducer. Further studies on molecular signaling between the fungi and bacteria have to be explored using recent molecular tools like transcriptome.

Keywords: *Azotobacter*, basidiome initiation, medicinal mushroom, *Hypsizygus ulmarius,* fungal-bacterial

INTRODUCTION

The investigation of fungal-bacterial interactions is an interesting field to explore modern microbial ecology. The function and interaction within the microbial consortia is not fully understood up to date. Associations between bacteria and fungi exist in many different contexts and can be considered from different perspectives. Both bacteria and fungi not only coexist on complex substrate but also on low molecular organic root exudates that would fetch both bacteria and fungi in the rhizosphere[1]. Not only the substrate plays a major role for co-existence, but the exudate from the partners also plays a major role in fungal-bacterial interaction. The bacterial-fungal communication is mediated by a specific molecule and communicates through physiochemical properties of their environment.

The researches have proved that, bacterial metabolites stimulate hyphal growth. Frey-Klett[2] reported the bacterial interaction with *Amanita muscaria* and *Streptomyces* sp unregulated the production of the secondary metabolite auxofuran, which promotes the extension of the fungal mycelium. In recent decades, several external inducers have been tested for their ability to stimulate the fruiting of edible mushrooms. In turn several reports on the fungal exudates serve major source of nutrients for bacteria[3]. Jensen[4] demonstrated the nitrogen fixing ability of *Azotobacter* sp. depends on organic matter as carbon source. In symbiotic, nitrogen fixing Rhizobium bacteria get the carbon source directly from the roots of the host plants. But in case of free-living diazotrophes it may get it from fungal exudates and from the soil in any form of organic matter. These fungal carbon exudates will enhance the growth of bacteria[5]. In turn the bacterial exudates such as biotin, thiamine, IAA, amino acids, phenylalanine methionine and proline are required for mycelial growth, which are growth promoters,[6,7]. Such growth promoters are produced by rhizospheric microorganism *Azotobacter chroococcum*[8]. Hence such growth promoters will enhance the mycelial growth that leads to basidia initiation. Rainey[9] examined the involvement of bacteria in basidiome initiation of *Agaricus bisporus.* For this reason, the current study was designed to find the influence of free living *Azotobacter* initiation of basidiome in commercially important mushrooms.

MATERIALS AND METHODS

Pleurotus aureovillosus was collected from Thirumal hills. *Hypsizygus ulmarius* was received from, TNAU and *Volvariella volvaceae* was collected from Keeriparai area of Western Ghats. All these belong to the fungi Basidiomycota. It was maintained on PDA slants (Potato-200gms, Dextrose 20gms, Agar 20gms dissolved in 1000mL and the pH was adjusted to 6.5). *Azotobacter sp* was isolated from the paddy straw which has been used for the cultivation of mushroom. The paddy straw was cut into small pieces and placed on nitrogen free Jensen's Agar Medium (Azotobacter Selective Medium) K_2HPO_4-1.0g, $MgSO_4.7H_2O$- 0.2g, Sucrose-20.0g, NaCl- 0.5g, FeSO4.4H2O-0.1g, $NaMoO_4$- 0.005g, $CaCO_3$- 0.1g, Agar-20g dissolved in 1000mL of distilled water and the pH was adjusted to 7.5[4]. The bacterial colonies formed were purified and identified using Bergy's manual of microbiology. The culture was maintained on Yeast Mannitol Agar medium Mannitol- 10g, Yeast extract-0.3g, K_2HPO_4 - 0.2g, $MgSo_4$- 0.2g, NaCl- 0.05g, Agar 20g dissolved in 1000ml of distilled water and the pH was adjusted to 7.0[10]. The following biochemical tests were performed for the initial characterization of bacteria such

as oxidase test, catalase test, indole test, methyl red test and voges-prausker test. Dual plate method was done to observe the compatibility between the bacteria and fungi. One half portion of the YMA medium plate the mycelium was grown. After 5 days of mycelium growth the *Azotobacter* culture was streaked on the other half of the Petri plate and incubated for the antagonistic activity.

RESULTS AND DISCUSSION

Azotobacter is an important free living nitrogen fixer in the rhizosphere of paddy field in India[11], so they may be present in the paddy straw which has been used for the cultivation of mushroom. Presence of nitrogen fixing bacteria in the fruit body of *Agaricus bisporus* was reported[12]. Hence this above information reveals that both fungi and bacteria can co-exist in these conditions. In view of this reason we have isolated the *Azotobacter* from the paddy straw used for the mushroom cultivation. Hence the interaction between the bacteria and fungi would be compatible.

The plate morphology and biochemical test confirm the genus as *Azotobacter* sp of the isolate (Table 1). In order to test the fungistatic effect, dual plate technique was performed. Mycelial growth of *Pleurotus aureovillosus, Hypsizygus ulmarius and Volvariella volvaceae* were not affected by the growth of *Azotobacter* sp when the organisms were cultured together in Petri plates. The fungal mycelium spread on the top of the bacterial streak without any antagonistic activity (Figure 1).

But *Pleurotus aureovillosus* well known edible mushroom suppressed the pathogenic bacteria like *Staphylococcus aureus* and *Bacillus sp*[13]. The interesting observation on dual plate was that *Hypsizygus ulmarius* over grew the *Azotobacter,* which showed the beneficial interaction by initiation of basidia on sides of the Petri plate and developed into fruit body. The mycelia that cross over the bacterial were started to initiate basidia whereas other side only mycelia mat were observed (Figure 2). This indicates that the association has exerted some signal or stress on nutrient front. Similar finding was also observed with *Pleurotus ostreatus* where fruit body was induced by *Pseudomonas putida*[14]. Further this free living Azotobacter is known to produce growth promoting substance like auxin which might also helpful in aggregation and the elongation of the mycelium which results in the formation of fruit bodies. This association may be exploited in the enhanced fruiting of edible mushroom. However, no induction of fruit body was observed with *Volvariella volvaceae* and *Pleurotus aureovillosus.* Similar induction of fruit body was also observed in *Agaricus bisporus* in the presence of *Pseudomonas putida*[9]. However the induction of fruit body by bacteria was found to be genus specific. Ahlawat[15] found that the mixing of 'Azotobacter' at spawning did not show any significant effect on the yield of *Agaricus bitorquis*. But, the broth culture of *Alcaligenes faecalis, Bacillus circulans-II* and *Bacillus thuringiensis* resulted in significantly higher yield than the uninoculated control. In our funding with *Azotobacter,* no induction of fruit body was observed with *Volvariella volvaceae* and *Pleurotus aureovillosus.* Hence this reveals that specific molecular conversation between the fungi and bacteria in the initiating of fruit body was confirmed. Ostreolysin, Veratryl alcohol, b-adenosine, phenol and saponin were found to initiate the growth and yield of *Pleurotus ostreatus* on nutrient medium plates[16-20]. According to Eger[21], the fruiting in mushrooms can be enhanced by the addition of a nitrogen source; this highly supports our finding, because the free living nitrogen fixing *Azotobacter* fixes atmospheric nitrogen available for the initiation of fruiting body. Sugar moieties as surface-activators also trigger the fruiting process[22,23]. 3-O-octyl- and 3-O-decyl-D-glucose have proved to be effective in enhancing the hyphal aggregation, primordial initiation and subsequent fruiting of the mushroom[22]. This finding strongly supports our report on *Azotobacter* induction of fruit body, because the extra cellular polysaccharide was estimated as 30µg /mL of the culture filtrate. Reports say that, *Azotobacter vinelandii* produces two polymers: the extra cellular polysaccharide alginate and the intracellular polyester poly-ß-hydro butyrate (PHB)[24]. Recently Sabina[25] found that Polymeric 3-alkylpyridinium salts (poly-APS), marine sponge, have been shown to stimulate the fruit body formation from *Pleurotus ostreatus* mycelium.

The inoculation of the mycelium with *Pseudomonas* strains promoted the formation of primordia and enhanced the development of the mushroom fruit bodies, suggesting that the compounds inducing the fruiting signals might be produced by the bacteria. The previous research finding states that, haemolytically active pseudomonads are often associated with cultivable mushrooms, including *P. ostreatus*, and are responsible for brown blotch disease of *Agaricus bisporus* or the yellowing of *Pleurotus eryngii*. In our case the non pathogenic *Azotobacter* was found to initiate the fruit body. Presence of nitrogen fixing bacteria in the fruit body of *Agaricus bisporus* was reported[12]. Hence this above information reveals that both fungi and bacteria can co-exist under natural conditions that mediate the basidia initiation. Hence free living nitrogen fixing *Azotobacter* would be promising bacteria in the enhancement of the fruit bodies in mushroom cultivation. In addition the antagonist activity of Azotobacter on the molds, which are competitive to the mushroom in the seed spawn substrate, was reported[26]. Fructification is a very important step in the cultivation of mushrooms and all the factors that influence this process are considered as potential biotechnological and commercial applications. *Hypsizygus ulmarius* is a promising mushroom with antidiabetic effect, excellent antioxidant potential and antitumor polysaccharides[27-29]. Hence the novel finding on *Azotobacter* the basidiome initiator in medicinal mushroom *Hypsizygus ulmarius*, has great impact and can extended in other medicinal mushrooms will be a great sight.

Table 1: Shows Biochemical Analysis of *Azotobacter* sp

Gram staining	Motility	Oxidase test	Catalase test	Indole test	Methyl red test	Voges-prausker
Gram negative	Motile	Negative	positive	Negative	Negative	Negative

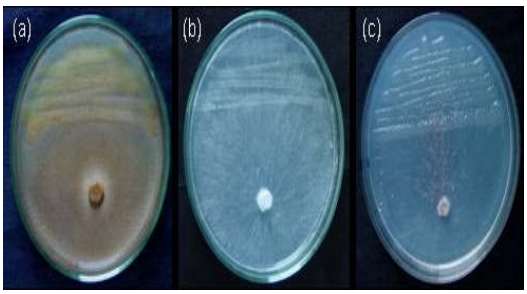

Figure 1: Dual culture of (a) *Pleurotus aureovillosus,* (b) *Hypsizygus ulmarius and* (c) *Volvariella volvaceae with Azotobacter* sp

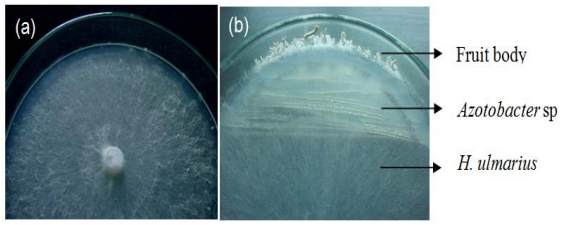

Figure 2: Induction of fruit bodies of *Hypsizygus ulmarius* by the *Azotobacter* sp in dual culture. (a) Control plate showing *H. ulmarius* and (b) dual plate showing basidia of *H. ulmarius* at the sides of the plate

CONCLUSION

The current finding suggest that non pathogenic free living nitrogen fixing *Azotobacter* can be mixed with substrate for the development of fruit body of *Hypsizygus ulmarius*.

Further standardization on culture inoculum and physical parameters has to be optimized. The molecular signaling between the fungi and bacteria has to be studied using recent molecular tools like transcriptome.

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