INFLUENCE OF THERMOPHILIC FUNGI *HUMICOLA INSOLENS* ON THE GROWTH OF *AGARICUS BRASILIENSIS (A. BLAZEI)*

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ABSTRACT

Cultivated medicinal mushrooms are a good source of bioactive substances that may be useful for human medicine. Some of these mushrooms are edible and contain valuable nutrients that are good for human health.

During our previous investigation of influence of thermophilic fungi *Humicola insolens* on the growth of *Agaricus bisporus*, *Agaricus bitorquis* and some other edible mushrooms we have found out that it stimulated the mycelium growth of some these tasted mushrooms. Our experiments on the interaction of *H. insolens*on with *Agaricus brasiliensis* showed that investigated thermophilic fungi stimulated the growth of this medicinal mushroom on straw agar media (SA). On this media the growth of *A. brasiliensis* on the surface of the *H. insolens* colony was twice or more faster than on the media without thermophilic fungi. Preliminary experiments on straw which was previously inoculated by *H. insolens* (grew during 3-5 days, temperature 42ºC), showed that fruit bodies of *A. brasiliensis* were produced the on obtained substrate.

Keywords: *Humicola insolens, Agaricus brasiliensis (A.blazei),* nutrition media, interaction, substrate, cultivation.

INTRODUCTION

During preparation (fermentation) of mushroom compost more than 20 species of thermophilic fungi were isolated from it [1-5]. Some of them play the main role in bioconversion of initial ingredients to selective substrate for development of *Agaricus bisporus* and some other mushrooms. Thermophilic fungi at optimum for them conditions (temperature, pH, humidity etc.) grow on different cellulose-lignin substrates [6-7].

During Phase I and II of compost fermentation and beginning of spawn run some thermophilic and mesophilic fungi are able to produce antibiotics which can protect mushrooms against negative effects of some fungi and bacteria [8-10]. They also produce some important substances for mushrooms development, like growth regulators - auxins, cytokines, gibberellins etc.; vitamins, amino acids; volatile compounds, such as carbon dioxide and are efficient in humus formation [11-13].

Our experiments showed different interactions on agar nutrition media of thermophilic fungi from mushroom compost [14] and it was found out that among them only *Humicola grisea var. thermoidea*, *H. insolens* and *Scytalidium thermophilium* (syn. *Torula thermophila),* so-called *Scytalidium* (*Torula) Humicola* complex, have been mentioned as dominant species at the end of

fermentation and beginning of *A. bisporus* spawn run [1, 4, 9]. The taxonomy and nomenclature of this thermophilic species are specified [15, 16]. The role of thermophilic fungi in mushroom compost preparation and formation of selective substrate for *A. bisporus* mycelium growth have been studied extensively [17-22]. It was found that in the nutrition media, sterilized mushroom compost and grain spawn, pre-incubated by *S. thermophilium*, *H.insolens* and some other thermophilic fungi *(Chaetomium* sp., *Myriococcum thermophilium)* promoted mycelium growth of *A. bisporus* but also of such mushrooms as *A. bitorquis, Coprinus cinereus, C. comatus, Lentinus edodes, Pleurotus ostreatus* [5, 9, 21-23].

Mushrooms are widely recognized as good sources of bioactive substances that may be useful for human medicine. Some of these mushrooms are edible and contain valuable nutrients that are good for human health. *Agaricus brasiliensis* is known by his medicinal properties (anticarcinogenic, antimutagenic, antitumour effects etc.) [24-28].

The cultivation of *A. brasiliensis* was started first in Brazil in the field (outdoors), but this type of production of this mushroom was risky because of uncontrolled factors in the environment. On the other hand, fruit bodies of *A. brasiliensis*, which where obtained in this way, now are in demand of Japanese importers, who use this mushroom for production of different medical additives etc. At present most of Brazilian growers of *A. brasiliensis* use the technology similar to growing of *A. bisporus* (the substrate formulation, its composting, pasteurization, and conditioning, inoculation, and incubation at controlled conditions [25, 29-30].

The aim of the present work was to study the interaction of thermophilic fungi *H. insolens* with medicinal mushroom *A. brasiliensis* on SA medium and growth of its mycelium and fruit body formation on straw pre-inoculated by this thermophilic fungi.

MATERIALS AND METHODS

Strain. *Humicola insolens* Cooney et Emerson, IBKF-519, (IMI-354859, ATTC-201434), *Agaricus brasiliensis* S. Wasser et al. (*A. blazei* Murrill) commercial strain.

Media. Two solid agar media were used. Yeast glucose agar (YGA) comprising yeast extract («Serva»), 4.0 g; peptone («Sigma»), 2.0 g; glucose monohydrate («Merck»), 10.0 g; agar, 15.0 g; 1000 ml distilled water. Autoclaved for 1h at the temperature of 120°C. Straw agar (SA) comprising milled air-dried wheat straw (mesh size 0.3-0.5 cm), 200.0 g; agar, 20.0 g; 1,000 ml distilled water. Autoclaved for 1h at the temperature of 120°C.

The pure culture of thermophilic fungi *H. insolens* and cultivated mushrooms with medicinal properties *A. brasiliensis* were grown on YGA medium in tubes (for subculture and storage) and YGA, SA (on which *H. insolens* formed more conidia) media in Petri dishes (for inoculation) in the dark at 42±1°C and 28±1°C, accordingly.

For the observation in the scanning electron microscope to study the growth and interaction of *H. insolens* and *A. brasiliensis* the sterile pieces (0.9 x 0.9cm) of the cover glass or straw were put on SA medium on different distance from the inoculum of thermophilic fungi and mushroom. After overgrowth and interaction of their colonies on glass or straw they were collected at different times (days) and fixed [32, 33]. For the research we use scanning electron microscope (SEM) Jeol JSM-35 and Jeol JSM-6060.

Grain for obtaining grain spawn in the flasks was prepared by standard technology [29]. After sterilization and cooling grain in flask was inoculated by pure culture of *A. brasiliensis* from Petri dishes with YGA medium and grown in the dark at 28±1°C.

For studying the interaction of *H. insolens* with *A. brasiliensis* in agar nutrition medium one part of Petri dishes with SA medium was inoculated by thermophilic fungi (diameter of inoculum 1.0 cm) from the YGA. The Petri dishes with *H. insolens* were cultivated for 3-5 days

at 42±1°C °C in the dark. After that another part of Petri dishes (opposite of *H. insolens* colony) was inoculated by pure culture of *A. brasiliensis*. These Petri dishes with thermophilic fungi and mushrooms were incubated in the dark at temperature 28±1°C. As control we used Petri dishes with pure culture of *A. brasiliensis* or *H. insolens* on SA medium that also were kept at 28±1°C.

For the study of the growth of mycelium and fruit body formation of *A. brasiliensis* on the experimental substrate (straw pre-inoculated by *H. insolens*) straw was meshed to the size 1.0-3.0 cm, and watered up to the 60-75%, and then put into polypropylene boxes (approx.1.0-1.2 kg of watered straw per box), covered by folia and autoclaved for 1h at 120°C. Boxes with sterile straw were inoculated by conidia of *H. insolens* (water suspension from the surface of SA medium from one Petri dishes per one box). After that inoculated boxes again covered by folia and shook for the better mixture of thermophilic fungi conidia in the straw. The boxes with straw which was inoculated by conidia of *H. insolens* (treatment) and control substrate - without inoculation with them (not treatment-sterile straw) were put into the thermostat cabinet at temperature 42±1° C for 3-5 days in the dark. There were six replications of the each experiment with Petri dishes and boxes. At the end of the growth of *H. insolens* on the straw (treatment substrate) and straw without treatment by thermophilic fungi (control) were inoculated with grain spawn of *A. brasiliensis* (10-12 g/kg of substrate), covered and arranged in growing room. Depending on the used substrate spawning run lasted 10-15 days, without light, at temperature of 28±1°C and relative humidity between 85 and 90%.

The cover paper was removed and the two types of substrates were covered by 4-5 cm of casing soil (mixture of 80% black and 20% brown peat) which was used in mushroom complex "Valentina Ltd.". Then these boxes were re-covered and placed on the shelves in growing room. The incubation lasted 10 days at 28±1°C, in the dark and a relative humidity of 90%.

In our experiments any type of supplements, like soybean meal, ChampFood etc., were not used and a traditional mushroom compost was used as control.

Under environmental control in growing room (which uses in "Valentina" mushroom complex), the first fruit body of *A. brasiliensis* started to appear at 18-25 days after casing.

RESULTS AND DISCUSSIONS

After the partially growth (aprox. 3.0 cm from the edge of inoculum) of colony of *H. isolens* on SA medium at temperature 42±1° C these Petri dishes were inoculated by pure culture of *A. braziliensis* and incubated at temperature 28±1° C (Fig. 1, 2). At this temperature, growth of the colony of *H. isolens* decelerated and continued to grow, but much more slowly. During this period, on the surface of the tested colony of thermophilic fungi a great number of conidia were formed. It is a very important moment, because our previous examinations have shown that mycelium of some species of genus *Agaricus* grow faster on the colony of *H. isolens* which formed a lot of conidia [21, 22]. During the contact of the colony of *H. isolens* with *A. braziliensis* the latter inhibited the growth of thermophilic fungi (Fig. 1). After said contact the colony of tested mushrooms started to grow on the surface of the colony of *H. isolens*. During first days of growth of the colony of *H. isolens*, *A. brasiliensis* grew rapidly, but it formed rare, thin mycelium (Fig. 1). On the twelfth – fifteenth day of the interaction of tested thermophilic fungi and mushrooms, the colony of *A. braziliensis* almost completely overgrew on the colony of thermophilic fungi with formation of a typical colony for these mushrooms with mycelium which formed the strands (Fig. 2). At the same time, mycelium of *H. isolens* continued to grow on those areas of the medium which had no contact with *A. brasiliensis* (Fig. 1 a-black line with bulbs). In the sequel, after the contact of thermophilic fungi with mushrooms, the latter overgrew on *H. isolens* (Fig. 2b).

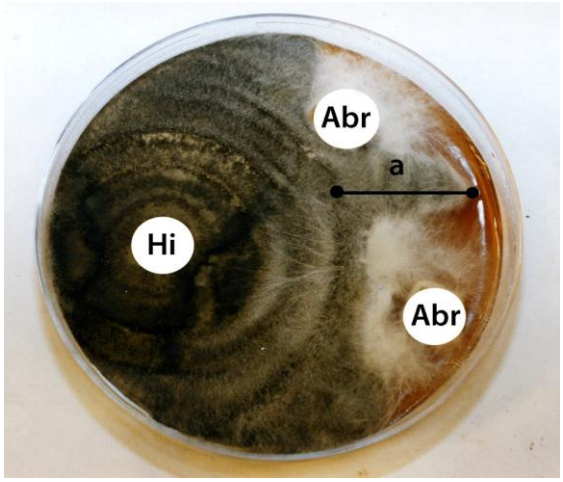

Figure 1: 5 days growth of *A.brasiliensis* (Abr) Colony on the surface of colony of *H. insolens* (Hi)

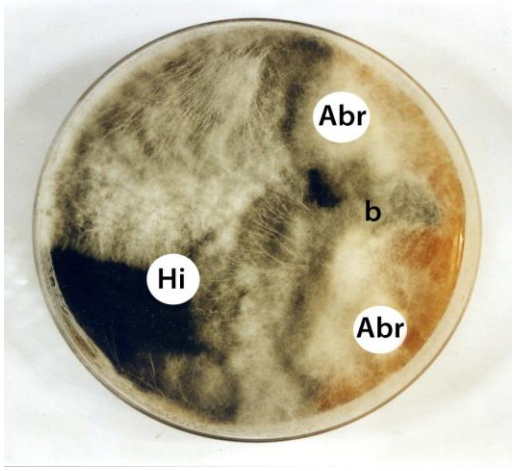

Figure 2: 14 days growth of *A.brasiliensis* (Abr) colony on the surface of colony of *H. insolens* (Hi)

Examination of interaction of *H. isolens* with *A. braziliensis* using the SEM is shown on Fig. 3, 4. During first days after the contact of the colony of thermophilic fungi with mushrooms, mycelium of *A. brasiliensis* started to grow on the surface of conidia *H. isolens* (Fig. 3). During subsequent interaction (12-15 days) of the thermophilic fungi with the tested mushroom, on the surface of the colony of *H. isolens* growth of mycelium and strands formation is observed (Fig. 4).

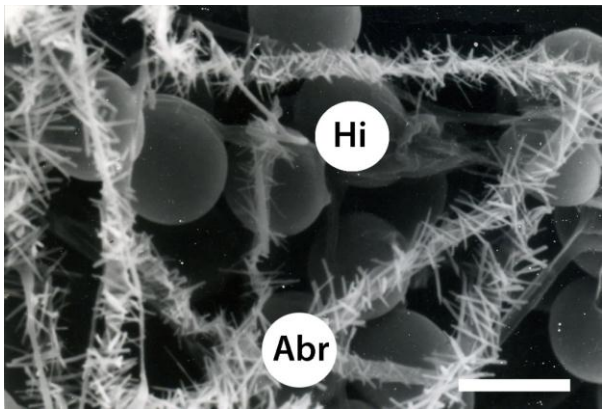

Figure 3: Growth of mycelium of *A.brasiliensis* (Abr.) on the conidia of *H. insolens* (Hi). SEM. Bar = 10 µm

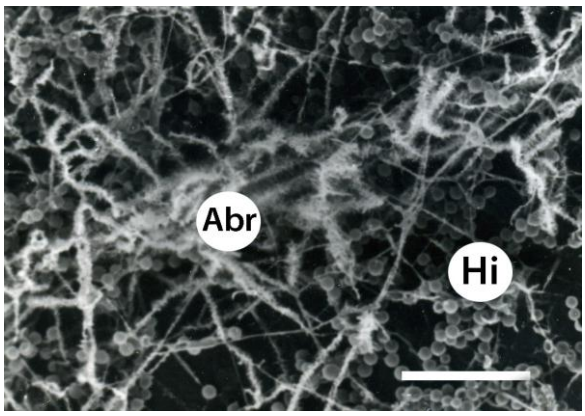

Figure. 4: Growth of mycelium and strands formation of *A.brasiliensis* (Abr.) on the conidia of *H. insolens*(Hi). SEM. Bar = 100 µm

Our preliminary experiments on the influence of *H. insolens* on the growth of *A.brasiliensis* showed that these thermophilic fungi stimulated the growth of mycelium of this mushroom on SA medium.

Next stage of our work was to study the possibility of mycelium growth and fruit body formation of *A. brasiliensis* on the straw pre-inoculated by *H. insolens* (experimental substrate) and development of this mushroom without pre-inoculated straw (control). Our study has shown that the mycelium growth of *A .brasiliensis* on the experimental substrate was 1.5-2 times more rapid (Fig. 5) than on the control one. After covering of the experimental substrate with casing

soil, the mycelium growth of *A. brasiliensis* was typical for this mushroom (Fig. 6). On the control substrate, the growth of mycelium of tested mushrooms was very slow or there was no growth at all through the casing soil. And only on the experimental substrate, fruit bodies of *A. brasiliensis* were formed (Fig. 7 a,b).

The task of our preliminary experiments was to study the influence of the thermophilic fungi *H. insolens*, strain IBKF-519, on the growth of mycelium and fruit body formation of the medicinal mushrooms *A. brasiliensis* on cellulose-lignine containing substrates, in our case it was the straw, and we obtained a positive result. On the other hand, we did not have a task to examine the impact of these thermophilic fungi on productivity of *A. brasiliensis.* We understand that it is necessary to conduct additional studies using different mixtures for the selective substrate preparation, various supplements (soybean meal, ChampFood, etc.), increasing density of the experimental substrate and its C/N relation, etc.

Figure 5: Spawn run of *A.brasiliensis* on the straw pre-inoculated with *H. insolens*

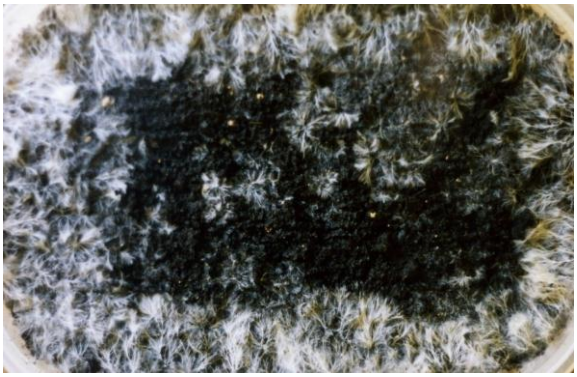

Figure 6: Growh of *A.brasiliensis* on the casing the substrate pre-inoculated by *H. insolens*

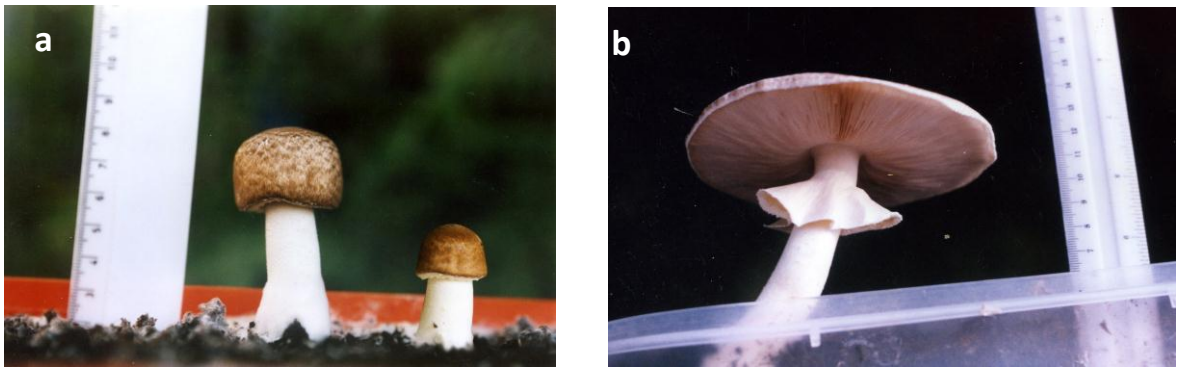

Figure 7: Different stages of fruit body formation of f *A.brasiliensis* on the substrate with *H. insolens*

CONCLUSIONS

In our previous investigation of influence of strain *H. insolens* IBKF-519, selected by us, on the growth of *A. bisporus* and *A. bitorquis* we have found out that this thermophilic fungi stimulated the growth of these two mushrooms and some others [21, 22]. We obtained the same results during the present studies. They showed that on SA medium *A. brasiliensis* inhibited the growth of *H. insolens* colony. After that these thermophilic fungi stimulated the growth of mycelium of tested mushrooms on its sporulated colony. The growth of *A. brasiliensis* on surface of the colony of *H. insolens* on SA medium was twice faster than on this media without thermophilic fungi. On the straw pre-inoculated with *H. insolens* (experimental substrate) this thermophilic fungi positive influence on the growth of mycelium and fruit body of *A. brasiliensis* formed on

it. That impact was not observed on straw without pre-inoculation of thermophilic fungi (control).

Further studies are needed to look for new initial components, waste of plants, food, etc., as well as supplements and formulation of different substrate (compost) for development of *A. brasiliensis*, including the use of thermophilic fungi *H. insolens*, strain IBKF-519 or another ones.

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