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Growth of *Agaricus bisporus* on grain pre-colonized by *Humicola insolens* and growth of mushroom mycelium from this spawn on compost

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ABSTRACT: Good growth and conidium formation of *Humicola insolens* Cooney & Emerson on grain was obtained in flasks inoculated with a liquid culture of the thermophilic fungus. Mycelial growth of *Agaricus bisporus* (Lange) Imbach on grains pre-colonized by *H. insolens* was better than on sterile grains. Grain pre-colonized by *H. insolens* and subsequently colonized by *A. bisporus* (experimental spawn, EGS), provided for better growth of mushroom mycelium on compost than commercial grain spawn (GS). On the 40-th day of mushroom growth on compost, the grains of experimental spawn were reduced in width by 25.2% and had lost in weight by 68.2%, whereas these figures for commercial spawn were 4,4% and 41.2%, respectively. A considerable reduction of the nutrients in grains of experimental spawn could prevent the development of weed moulds on grain.

1 INTRODUCTION

The role of thermophilic fungi in mushroom compost preparation, the formation of a selective substrate and the stimulation of growth of *A. bisporus* mycelium on compost, straw and several nutrient agar media have been studied extensively (Olivier & Guillaumes 1979; Sparling et al. 1982; Ross & Harris 1983; Fermor & Grant 1985; Wood & Fermor 1985; Bilay 1986, 1994; Straatsma et al. 1989, 1991, 1994). Experiments on the interaction of *A. bisporus* with *H. insolens* have shown that the stimulating effect on the growth rate of mushroom mycelium occurred on different substrates, on nutrient agar media and on the biomass of the thermophilic fungus. The stimulating effect was observed if the surface of the substrates, the agar media or the fungus biomass, contained a high density of conidia *ofH. insolens* (Bilay & Lelley 1996).

For the inoculation of compost grain spawn of *A. bisporus* is used. If grain, that contains much carbohydrate, is not fully colonized by mushroom mycelium or if fully colonized spawn falls into non selective mushroom compost, grains can become the main nutrient source for weed moulds, especially species of the genus *Trichoderma* (Fletcher et al. 1986; Fletcher 1999). We studied i) the effect of inoculation of wheat grains by cultures of *H. insolens* in liquid medium or on nutrient agar on the growth rate of the thermophilic fungus and on the formation of its conidia, ii) the growth of *A. bisporus* mycelium on grain colonized by *H. insolens* and of mushroom mycelium on compost inoculated with the experimental spawn, iii) the changes of grain weight and size.

2 MATERIALS AND METHODS

2.1 *Strain*

We used *Humicola insolens* Cooney & Emerson strain IBKF 519 (=IMI 354859= ATCC 201434). This strain was isolated by us from mushroom compost at the end of Phase II and it

stimulated mycelium growth of *A. bisporus* (Bilay & Kholodov 1983; Bilay 1986). Further, we used commercial grain spawn of the cultivated mushroom *A. bisporus,* strain 130 ("Sylvan" spawn company).

2.2 *Preparation ofH. insolens inoculum*

The inoculum of *H. insolens* was grown on yeast glucose agar (YGA) in the dark at 45° C. For liquid culture *of H. insolens,* we placed 15.0 g of milled (size 3.0-7.0 cm) air-dried straw in wide-necked Erlenmeyer flasks (500 ml) filled with 300 ml of water. The flasks were autoclaved twice for Ih at 120° C. Flasks were inoculated with a 1 cm diam. disk of an YGA agar culture of *H. insolens* on the liquid surface. Flasks were incubated in the dark at 45° C and shaking for 1 minute, two times per day.

2.3 *Preparation of grain spawn and study of A. bisporus mycelial growth*

Mushroom spawn (based on wheat grain) was prepared in wide-necked Erlenmeyer flasks (500 ml) according to Elliott (1985). Five of these flasks were inoculated by 5 ml of a liquid culture *of H. insolens* with conidia, another five by 5 YGA agar disks (diameter 0.5 cm). Flasks were incubated in the dark at 45° C and part of them was shaken each day. Growth and conidia formation of *H. insolens* were recorded from the second day. To obtain experimental grain spawn (EGS) pre-colonized by *H. insolens* and traditional grain spawn (GS), flasks were inoculated by commercial mushroom spawn (10-15 grains per flask). Flasks were incubated in the dark at 25° C with shaking and used in further experiment as GS and EGS.

For studying the growth rate of *A. bisporus* on sterile grain and grain pre-colonized by *H. insolens,* 5-7 commercial spawn grains were carefully placed on the bottom and close to the wall of flasks. These flasks were also incubated in the dark at 25° C but without shaking and growth of *A. bisporus* mycelium was measured up to the 15-th day.

2.4 *Study of A. bisporus growth on mushroom compost inoculated by EGS and GS*

Fresh mushroom compost (after phase II) was dispensed in 14 cm Petri dishes (60-65 g per dish) and inoculated by EGS and GS (five Petri dishes for each type of the spawn). 10 grains were inoculated per Petri dish. One part of the grains was placed on the bottom of the dish, another on the surface of the compost. In order to prevent desiccation, Petri dishes were placed in plastic bags and they were incubated in the dark at 25° C. Growth of *A. bisporus* mycelium on compost was measured up to the 15-th day. Then Petri dishes were incubated in the dark at 18° C during 25 days.

2.5 *Size and weight changes ofEGS and GS grains*

On the 40-th day, the grains of EGS and GS were collected from compost and their length and width were measured. Grains were dried at 105 C for 24 h and their weights measured.

2.6 *Scanning electron microscopy ofH. insolens and of A. bisporus growth on grain*

Samples of the surface of grains and of cross sections of grains (with *H. insolens, A. bisporus* or after mutual growth) were fixed in vapor of 2% O_SO_4 for 48 h. Specimens were sputter coated with gold and examined in a JEOL JSM 35° C Scanning Electron Microscope (SEM).

3 RESULTS

Flasks with sterile grains inoculated with a conidiating liquid culture of *H. insolens* gave better results than flasks inoculated by agar disks. On the second day of incubation at 45° C, grains in flasks inoculated by liquid spawn were colonized by *H. insolens.* On the third day *H. insolens* started to sporulate on the surface of the grains and the flasks were inoculated by commercial mushroom grain spawn. On the grains inoculated with agar disks, *H. insolens* started to

Table 1. Changes of grain sizes and weights of GS and EGS after 40 days growth on compost (% to initial)

	size and weight of spawn grains		
	length	width	weight
initial value	100	100	100
after 40 days of growth			
GS	96.4	95.9	58.8
EGS	95.9	74.5	32.8

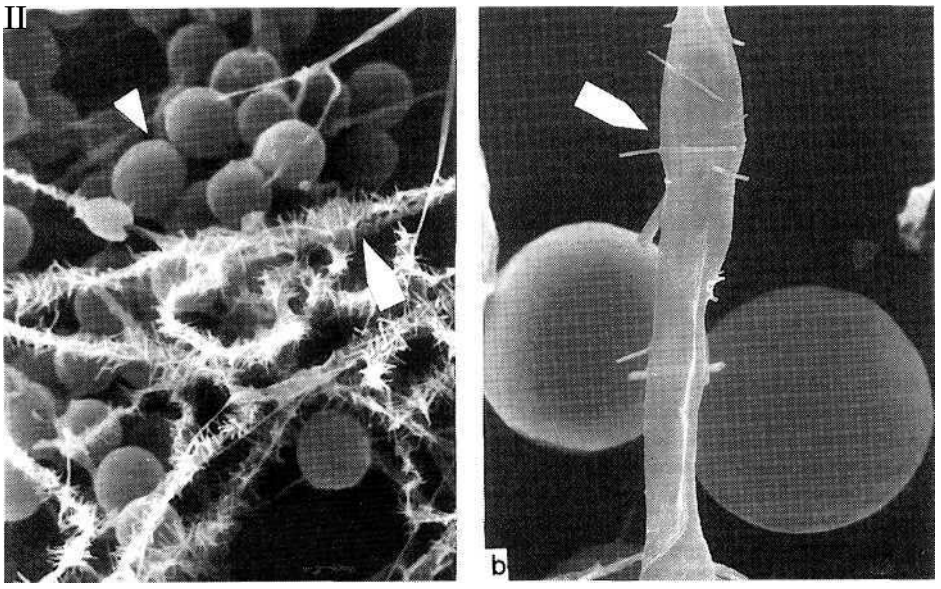

Figure la. Experimental Grain Spawn, growth of *A. bisporus* mycelium (arrow) on grain pre-colonized by *H. insolens* (arrowhead) -bar mark = 10 µm; b, part of Figure la - bar mark = 1 µm.

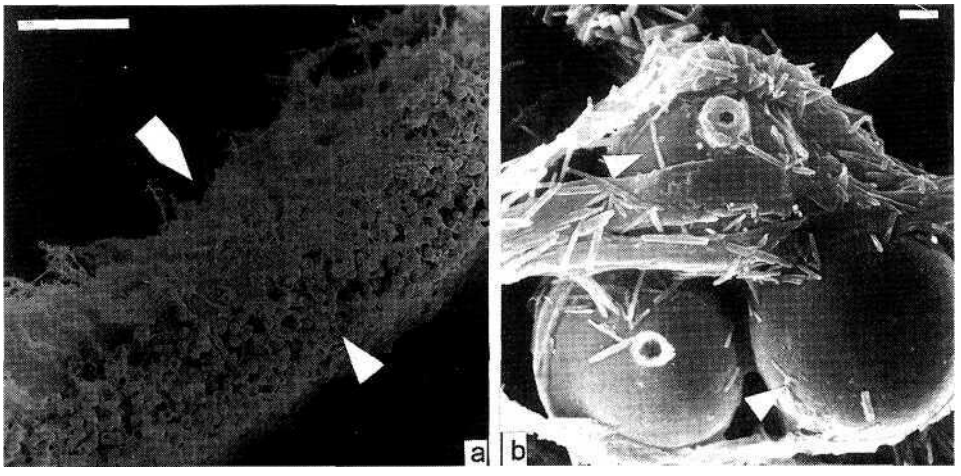

Figure 2a, EGS, growth of *A. bisporus* mycelium (arrow) on the layer of*//, insolens* conidia (arrowhead) - bar mark = 100 µm; b, part of Figure 2a - bar = 1 µm.

sporulate only after 5-7 days and growth of *A. bisporus* mycelium on it was unsatisfactory. Growth of *A. bisporus* mycelium averaged 2.0 mm per day and was faster on grains pre-colonized by *H. insolens* from a liquid culture than on sterile grains. After 12-15 days all grains were covered by mushroom mycelium.

Studies of *A. bisporus* growth on grains pre-colonized by *H. insolens* by using SEM showed that from the first day onwards mushroom mycelium preferred to grow on the surface of the conidia of the thermophilic fungus (Fig. la, b).

At 25 C, the growth rate of *A. bisporus* mycelium on compost from EGS grains (pre-colonized by *H. insolens)* was higher than from the GS and equaled 6.0 and 4.5 mm per day, respectively. Between the 15-th up to the 40-th day, when Petri dishes were incubated at temperature 18° C, mycelium of *A. bisporus* lay appressed on the compost and mycelial strands were formed. The same type of growth occurred on compost inoculated by GS.

Important changes in size and weight of GS and EGS grains after 40 days of *A. bisporus* growth were observed (table 1). On the 40-th day GS and EGS grains were identical in length, but the width of EGS grains had decreased by 25.5% whereas GS grains had decreased only by 4.4%. The dry weight of GS and EGS grains were reduced by 41.2% and 68.2%, respectively.

Cross sections and surfaces of the EGS grains examined by SEM (on the 40-th day) showed that *A. bisporus* grew on a layer of the grains formed by *H. insolens* (Fig. 2a) and mushroom mycelium was tightly attached to the conidia of *H. insolens* (Fig. 2b). Conidia of *H. insolens* and mycelium of *A. bisporus* were also detected inside the EGS grains which did not occur in GS grains.

4 CONCLUSION

The results show that it is possible to prepare mushroom spawn on grain pre-colonized by the thermophilic fungus *H. insolens.* The best results was obtained when sterile grains were inoculated with a liquid culture of *H. insolens* and an incubating period 2-3 days at 45° C. Mycelium of *A. bisporus* grew faster on grain fully colonized by *H. insolens* than on sterile grain. Inoculated into the mushroom compost, mycelium of *A. bisporus* from EGS grains started to grow faster than from GS. The thermophilic fungus grew slowly on and inside the grains during spawn run (25° C) and promoted *A. bisporus* mycelium to penetrate into the grain. These circumstances lead to the loss of nutrients from the grains. The nutrient source *for A. bisporus* in this period is the compost and possibly the thermophilic fungus on the grains (Sparling et al. 1982; Fermor & Grant 1985; Wood & Fermor 1985). The content of carbohydrates in the grains of EGS decreases and perhaps the nutrition source for the development of weed moulds on grain spawn is lacking.

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