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The succession of microfungi in the compost during the cultivation of *Agaricus bisporus* (Lange) Lnbach

I.Milenkovic
INIPKB Agroekonomik, Belgrade, Yugoslavia
M.Ljaljevic Grbic & J.Vukojevic
Institute of Botany and Botanical Garden, Faculty of Biology, University of Belgrade, Yugoslavia

ABSTRACT: More than 20 species of thermophilic fungi were found at the end of the composting process in the substrate. Growing of *A. bisporus* under control conditions in a growing room changed the quality and quantity of microfungi in the compost. In a study aimed to investigate those changes, compost samples were taken during the growing cycle of *A. bisporus* in a commercial mushroom farm. After 7 days incubation of the samples on sterile, wet filter paper and malt agar, isolation and identification of fungi was carried out. The following species were identified: *Acremonium* sp., *Chaetomium olivaceum, Trichoderma harzianum, Mucor mucedo, Rhizopus stolonifer, Epicoccum purpurascens, Trichotecium roseum, Chromelosporium* stage of *Peziza ostracoderma, Coprinus* sp. and *Doratomyces stemonitis.* All of the species identified, except for *Chromelosporium* sp., are very common and their isolation from soil, air, water, live and dead plant material etc., can be done routinely. However, *Chromelosporium* sp., according to the literature data, is specific for *A. bisporus* growing rooms.

1 INTRODUCTION

Cultivation of edible mushrooms is not a traditional branch of agriculture in the Balkan peninsula region. However, an increase in production in the recent period can be observed, especially of species *Agaricus bisporus.* The majority of Champignon growers purchase compost prepared in the standard way (Sinden & Hauser 1950, Lambert & Ayers 1951) based on wheat straw and chicken litter, and very seldomly on horse manure.

Mushroom growing rooms are frequently located in adapted spaces of quite various previous purpose. The most common growing method in the Balkan peninsula region is cultivating in bags (Stamets et al. 1983).

Despite the relatively badly equipped growing rooms, as well as the fact that the compost is transported under inappropriate conditions several hundreds of kilometers, growing houses manage to produce yields between 23% and 25%. In cases of correctly prepared composts, which ensure successful mycelium growth and start of fruiting in *A.bisporus,* a large yield reduction can occur due to frequent occurrence of diseases particularly by *Mycogone perniciosa.*

As emphasized in many papers, the purpose of composting is to prepare a substrate of such characteristics that the growth *of A. bisporus* mycelium is promoted to the exclusion of other microorganisms (Fermor et al. 1985).

The whole process of composting proceeds through the presence of microorganisms. In Phase I, the components are mixed and a temperature of 80° C is obtained inside the mass (Straatsma et al. 1995). Phase II proceeds in the tunnels in which after initial heating to 60°C a process of controlled aerobic composting at 45°C is carried out for 6 days (Gerrits 1988). In this phase ammonium is removed by airing and bioconversion, and compost becomes selective for the growth *ofA.bisporus* mycelium (Fermor 1985).

The thermophilic microorganisms that survive the process of composting have been studied extensively. Papers (Straatsma et al. 1994) describe in detail the correlation of *A. bisporus* my-

celium growth rate with the coexistent species *Scytalidium thermophilum.* Detailed research has also been done into the process of substitution of microorganisms in the substrate subjected to composting.

The literature, however, holds little information on the succession of the mycopopulation in the compost during the growth and development of *A. bisporus.* Morphogenesis of the fruit body, as well as the mechanism and stimulants of this process, are also little known (Griensven 1996).

There is no question about whether, and to what extent, the presence of some microorganisms affects the formation of the fruit bodies, since it has already been proven that the presence *of Scytalidium thermophilum* is positively correlated with the yield of *A. bisporus.*

The question is to what extent the number of the species that constitute the mycopopulation in the compost during the growth of *A.bisporus* changes. Namely, since the very development of *A. bisporus* mycelium, together with many technology factors, affects the quality, i.e. chemical composition, of compost (Gerrits 1994), it is expected that a succession of mycopopulation in the medium occurs too.

As emphasized, the occurrence of pathogenic fungi is more than evident in growing rooms. Our interest was focused on investigating the change of the mycopopulation in the compost during the growth of *A. bisporus* in a growing room in which no fungal disease was observed.

2 MATERIAL AND METHODS

The compost inoculated with *A. bisporus* mycelium was brought in bags into a 20 t growing house, and, after covering of the surface by a plastic foil, was left for mycelial growth. After 2 days the temperature reached 20°C. During the following 14 days the temperature ranged between 22°C and 30°C. After full colonization of the compost *by A.bisporus* mycelium, 16 days after the introduction into the growing room, casing was applied.

The usual casing soil was used, consisting of 80% peat, 20% lime, disinfected by a 2% formalin solution. Each bag was given 8 1 of the casing soil, which was treated with Sporgon fungicide (Prochloraz Manganese - 4 g/m^ of growing area) (Fletcher et al. 1989).

During the casing colonization the temperature in the compost ranged between 26°C and 22°C. Seven days after casing, the whole casing layer was ruffled.

Three days after ruffling, introduction of fresh air was commenced to decrease the temperature to 17°C in the compost. Fresh air constituted 1/3 of the total volume of air that circulated in the growing room. Such a regime was maintained throughout the whole period of fruiting.

Sampling was done on day 31 (SI) after filling at the beginning of the first flush, as well as on day 58 (S2), when the second flush was completed. The samples were taken from variously placed compost bags.

All compost samples were placed on Petri dishes under aseptic conditions, in triplicate, on: potato dextrose agar (PDA), malt agar (MA), and wet filter paper (Booth, 1971). The incubation was carried out at 22°C (± 2) under a day-night light regime. The first observation was performed after 7 days, and subsequent changes were observed until day 30. The identification of the isolated microfungi was done on the basis of macroscopic and microscopic characteristics. Microscopy slides were stained in lactophenol and fuchsin acid.

The following keys and publications were used for identification of the fungi: Ellis (1971, 1976), Rifai (1969), Morton & Smith (1963) and Gravesen et al. (1994).

3 RESULTS

From the compost sampled at the beginning of fruiting (SI), two thirds of the samples showed no presence of filamentous microfungi, regardless of the substrate type. *A. bisporus* mycelium normally grew through the substrate. The following fungi were isolated and identified from the other samples (S2): *Acremonium sp., Chaetomium olivaceum, Trichoderma harzianum, Epicoccum purpurascens, Trichotecium roseum* and *Chromelosporium* state of *Peziza ostracoderma. T. harzianum* was isolated from a large number of samples, while the other species were isolated only in individual cases.

4 DISCUSSION

Species isolated and identified from the compost did not originate from the growing room compost. Their growth in the laboratory was stimulated by the optimal substrate and growing conditions. The tests thus showed the presence of isolated fungi, which did not appear under regular growing room conditions at all. This supports the understanding that a well prepared compost is selective after the second phase of composting. Its selectivity is reflected in the stimulation of *A. bisporus* growth and in the suppression of the growth of competitors (Overstijns 1981).

One species in the compost, observed in the analysis of the mycopopulation, which could suggest inadequate composting is *Chaetomium olivaceum.* The fact that this fungus did not develop in the growing room implies that the control of the conditions in Phase II of the composting was adequate.

Trichoderma harzianum is the only species present in both samplings. This species has been described as a potential mycoparasite, possibly producing toxins and antibiotics (Fletcher et al. 1989). All species of this genus are known for a high production of spores, which can explain the highest number of isolates of this species in the samples tested. Since no visible *Trichoderma* disease symptoms were observed in this growing room, it is possible that spores of this fungus were not introduced via inoculation with *A. bisporus,* but in some other way - by air, during compost manipulation, etc.

All other microfungi appeared sporadically, and their role in the growing room is minor.

The only macromycete, *Coprinus.* sp., recorded only in one sample, does not seriously affect the development and fruiting of *A. bisporus,* even when it is present in the growing room, since it belongs to the category of non-antagonistic fungi, found on poor compost (Fletcher 1987).

The results shown clearly indicate a succession of compost mycopopulation during the fruiting of *A. bisporus.* Since no treatment was applied during the fruiting, either in the growing room or in the casing, the presence of various species in the mycopopulation, found by sampling in different fruiting phases, is a sole consequence of spontaneous changes. It is particularly interesting that the majority of the species developed in the phase (SI) when compost was at the beginning of the first fruiting flush, while completely different species, although fewer, were found in the samples taken after the second flush (S2).

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