Microb Ecol (1990) 19:303–309

MICROBIAL ECOLOGY

Hyphal and Mycelial Interactions Between *Agaricus bisporus* and *Scytalidium thermophilum* on Agar Media

Huub J. M. Op den Camp,[1]* Claudius K. Stumm,[1] Gerben Straatsma,[2] Piet J. L. Derikx,[1] and Leo J. L. D. van Griensven[2]

[1]Department of Microbiology, Faculty of Science, University of Nijmegen, Toernooiveld, NL-6525 ED Nijmegen, The Netherlands; and [2]Mushroom Experimental Station, Postbus 6042, NL-5960 AA Horst, The Netherlands

Abstract. The interaction between *Agaricus bisporus* and *Scytalidium thermophilum* on agar media was studied by differential interference contrast and phase contrast microscopy. *A. bisporus* combatively replaces *S. thermophilum* in culture on agar media. The antagonistic effect of *A. bisporus* is transmissible through a cellophane membrane and causes irreversible disintegration of *S. thermophilum* protoplasm, resulting in a total loss of viability after prolonged interaction between the two fungi. On compost extract agar, but not on other media, the growth rate of *A. bisporus* increased from 2.7 to 5.3 $mm \cdot d^{-1}$ following contact with *S. thermophilum* mycelium.

Introduction

The edible white button mushroom, *Agaricus bisporus* (Lange) Imbach, is cultivated on a composted mixture of horse manure, wheat straw, chicken manure, and gypsum [22]. The composting is necessary to prepare a selective substrate on which the growth of *A. bisporus* is promoted to the practical exclusion of other microorganisms [9, 19]. During composting a succession of microorganisms takes place. Towards the end of the (composting) process thermophilic fungi become abundant, notably *Scytalidium thermophilum* (Cooney & Emerson) Austwick, *Humicola grisea* Traanen var. *thermoidea* Cooney & Emerson, and *Humicola insolens* Traanen [2, 6, 9, 16, 20], which were considered to be synonymous [20]. From a recent study on the population dynamics of *S. thermophilum* [20] the following could be concluded: (1) *S. thermophilum* promotes growth of *A. bisporus*; (2) the presence of *S. thermophilum* is important for successful colonization of the substrate by *A. bisporus* mycelium; and (3) precolonization of the compost by *S. thermophilum* results in a selective medium.

When the compost is fully colonized by *A. bisporus, S. thermophilum* can no longer be recovered [16, 20], even though *S. thermophilum* survives and

* Corresponding author.

grows at the optimum growth temperature of *A. bisporus* (24°C). It is generally accepted that the microbial biomass formed during composting has a role in the nutrition of *A. bisporus* [8, 24]. Growth of *A. bisporus* on heat-killed mycelium from *S. thermophilum* (synonymous to *Humicola grisea* var. *thermoidea*) and other fungi as sole C, N, and P source [5, 7] and the production of the appropriate extracellular depolymerizing enzymes have been reported [7, 14].

Since until now only dead biomass has been tested, the aim of the present study was to examine the interaction between living *A. bisporus* and *S. thermophilum* on agar where the hyphae could readily be examined microscopically.

Methods

Organisms and Media

Agaricus bisporus strain Horst® U1 and *Scytalidium thermophilum* CBS 671.88, isolated from compost [20], were used throughout. The fungi were cultivated on yeast/glucose agar [20], malt/peptone agar [30 g malt extract, 5 g mycological peptone, and 12 g agar per liter demineralized water], or on a compost extract/glucose agar (CEA) [21], at 24°C in the dark. When necessary, *S. thermophilum* was pregrown at 45°C.

Interactions between S. thermophilum *and* A. bisporus

The fungi were inoculated opposite to each other, 5 or 10 cm apart, on solid media in Petri dishes with diameters of 8.5 and 14 cm, respectively. Agar blocks (3 × 3 × 3 mm) taken from the mycelial growth front of the fungi pregrown on the same solid media were used as inocula. The bigger dishes were used to follow growth during a longer period of time. Growth was measured as mm radial extension. For microscopical examination, flat blocks of agar were cut from the region of the mycelial growth front and examined by means of Leitz differential interference contrast (DIC) and phase contrast. In order to preserve both the original position of the hyphae and the hyphal content, care was taken to prevent squashing of the agar. Experiments on all media were performed at least in triplicate and repeated three times.

In another experiment, *S. thermophilum* was pregrown at 45°C on yeast/glucose agar, while *A. bisporus* was cultivated at 24°C on a cellophane sheet lying on malt/peptone agar. *A. bisporus* was not able to penetrate the cellophane. When the mycelia had reached a diameter of about 5 cm, the cellophane with *A. bisporus* was peeled off the agar and layered onto the *S. thermophilum* mycelium. Incubation was continued at 24°C. In a control experiment the mycelium of *S. thermophilum* was covered with an autoclaved cellophane sheet without *A. bisporus*. After one day the *S. thermophilum* hyphae were checked microscopically and viability was tested by transferring agar blocks from the growth front into liquid agar medium at 45°C which was then allowed to solidify. The latter procedure allowed outgrowth of hyphae after incubation to be detected easily, since light refraction at the edges of the blocks was annihilated. The cellophane experiments were repeated in triplicate.

Results

Growth of *A. bisporus* and *S. thermophilum* opposed on compost/glucose agar is shown in Fig. 1. Prior to contact, the linear growth rate of *A. bisporus* and *S. thermophilum* was 2.7 ± 0.4 and 3.2 ± 0.1 $mm \cdot d^{-1}$, respectively (n = 5).

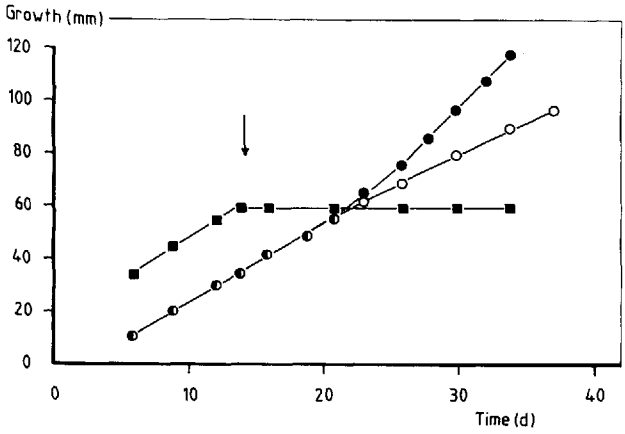


Fig. 1. Growth of *A. bisporus* (●) and *S. thermophilum* (■) during an interaction experiment in a large Petri dish with compost/glucose agar. The arrow indicates the point where mycelia of both fungi make contact. Growth of *A. bisporus* in the absence of *S. thermophilum* is plotted as a control (○).

When the growth fronts reached each other, extension of *S. thermophilum* stopped, while that of *A. bisporus* continued with the same rate, resulting in overgrowth of the *S. thermophilum* mycelium (Fig. 2). About 5 days after contact an acceleration of the growth rate of *A. bisporus* along the radius line between the two inocula points was observed (Fig. 2), resulting in a linear growth rate (after 25 d) of 5.3 ± 0.1 $mm \cdot d^{-1}$. In a control experiment *A. bisporus* grew at a rate of 2.7 ± 0.5 $mm \cdot d^{-1}$ for up to 30 d and thereafter decreased slightly. On the other agar media tested no growth stimulation was observed, although *S. thermophilum* was inhibited and covered by the *A. bisporus* mycelium.

At their optimal temperatures (24°C and 45°C for *A. bisporus* and *S. thermophilum*, respectively), the hyphal morphology of both fungi grown singly was similar (Figs. 3a and 3b). When *S. thermophilum* was grown at 24°C septation frequently increased and intercalary spore formation of the distal regions of the hyphae was observed (Fig. 3c). The growth rate was lower than at 45°C but remained constant (Fig. 1).

When the fungi were opposed to each other, growth of *S. thermophilum* stopped when the distance between the growth fronts had decreased to about 1 mm. Microscopical examination showed that the content of the *S. thermophilum* hyphae was coagulated and shrunk (Fig. 3d). After prolonged incubation (about 4 days) the *A. bisporus* hyphae penetrated into the disintegrated *S. thermophilum* mycelium (Figs. 3e and 3f).

In the cellophane overlay experiment, after one day of incubation, hyphae from control mycelium contained dense and rather homogeneous cytoplasm (Fig. 3h), while those covered with the *A. bisporus* overlay had a highly vacuolated, foam-like content (Fig. 3g). In phase contrast the cytoplasm appeared to be brighter compared to the control hyphae, probably due to loss of solutes and/or uptake of water, also indicated by the slight swelling of the affected hyphae.

In viability tests of agar blocks from the growth front of *S. thermophilum* mycelium covered by cellophane sheet for one day, the blocks from the control had a fur-like covering of growing hyphae after 2 days, whereas only single tiny mycelia developed from blocks cut from under *A. bisporus*. This indicated that

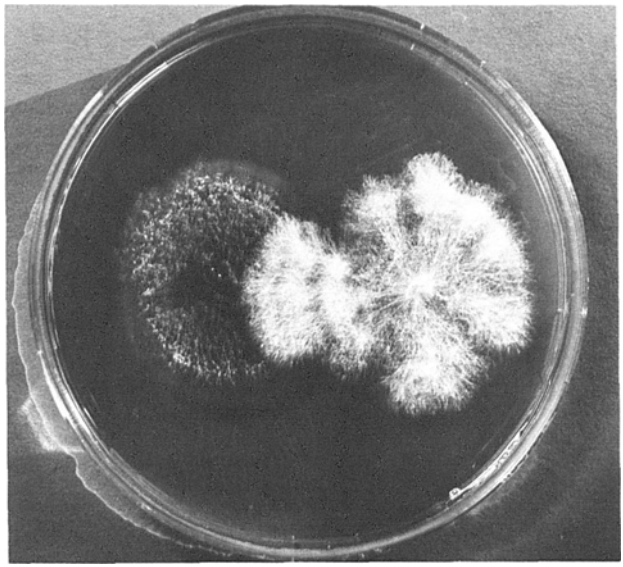


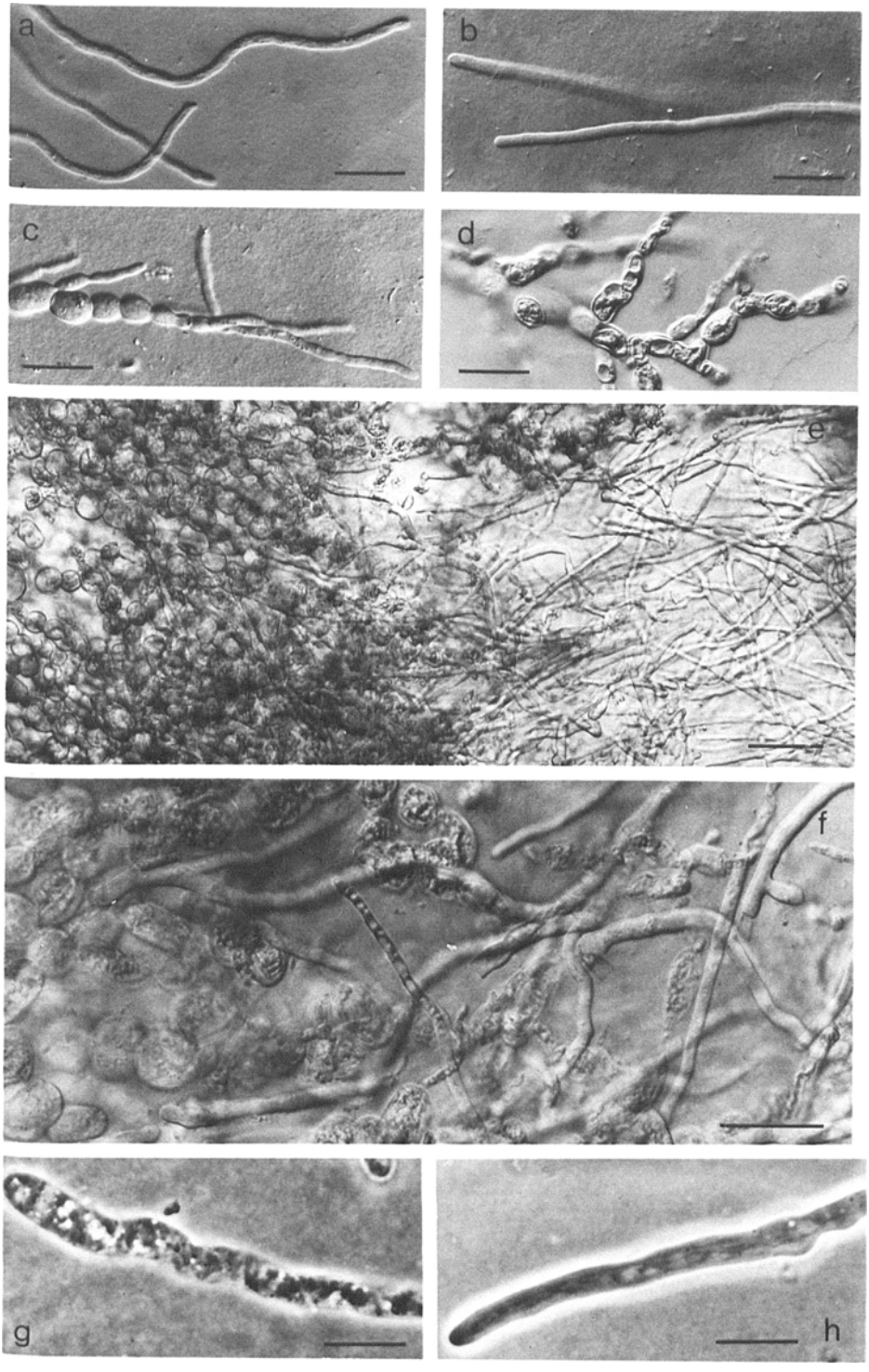
Fig. 2. *A. bisporus* (white mycelium) and *S. thermophilum* growing together on compost/glucose agar. The photograph was taken after 15 d of growth. A mycelial sector of *A. bisporus* penetrates and overlayers the *S. thermophilum* mycelium.

the majority of the *S. thermophilum* hyphae had been killed. After 7 days no viable *S. thermophilum* could be subcultured indicating complete killing of *S. thermophilum*. Similar antagonistic effects of *A. bisporus* were observed against some other thermophilic fungi isolated from compost, e.g., *Thermomyces lanuginosus* Tsiklinsky, *Rhizomucor miehei* (Cooney & Emerson) Schipper, and *Talaromyces thermophilus* Stolk (data not shown).

Discussion

The results presented in this report correspond well with those obtained studying the interaction of *A. bisporus* and *S. thermophilum* on compost substrate [20]. On agar medium *A. bisporus* mycelium encroached into the *S. thermophilum* mycelium, totally taking over its domain. After this "replacement", viability of *S. thermophilum* was strongly reduced.

Fig. 3. Microscopical observations on the interaction of *S. thermophilum* and *A. bisporus*. Hyphae from the growth front of *S. thermophilum* (**a**) and *A. bisporus* (**b**), singly grown at 45°C and 24°C, respectively. Differential interference contrast (DIC), bar = 25 μm. (**c**) Hyphae from the growth front of *S. thermophilum*, singly grown at 24°C. Note septation and intercalary spore formation. DIC, bar = 25 μm. (**d**) *S. thermophilum* hyphae from a mycelium about 1 mm distant from an *A. bisporus* growth front. The cytoplasm is shrunk and coagulated. DIC, bar = 25 μm. (**e**) *A. bisporus* hyphae (growing from the right to the left) penetrating *S. thermophilum* mycelium; not only the hyphal tips but also distal regions of the latter fungus are altered; (**f**) stronger magnification showing the difference between the light refractive, living hyphae of *A. bisporus* and the coagulated cell content of *S. thermophilum*. DIC, bar = 50 and 25 μm, respectively. (**g, h**) *S. thermophilum* hyphae from an overlay experiment. Whereas in the control hyphae (**h**) the thread-like mitochondria are visible, in the affected hyphae the cytoplasm is highly disintegrated (**g**). Phase contrast, bar = 10 μm.


a
b
c
d
e
f
g
h

Some classical literature [10, 25] already describes the behavior of ascomycete and basidiomycete fungi in mixed cultures. More recent studies have focused on interactions during successions of fungi involved in degradation of dung [13, 23] and wood [4, 11]. In an overview, Rayner and Webber [17] proposed a scheme to describe fungal interactions. Competitive, neutralistic and mutualistic interactions were distinguished. On basis of the definitions of these authors *A. bisporus* should be classified as a combative competitor of *S. thermophilum* leading to replacement of the latter (secondary resource capture). The antagonistic effect can be mediated at a distance. Hyphal interference [11, 12] and mycoparasitism [1] were not observed.

Although some preliminary results exist on the nature (diffusible and/or volatile) of the compounds involved in the antagonistic effects of *A. bisporus* [15], detailed studies were not performed. Previously [3] we reported on the presence of an alternative respiratory pathway in *A. bisporus*, while *S. thermophilum* lacked this pathway. The production of CO, an inhibitor of the normal respiratory pathway, by *A. bisporus*, however, cannot account for the killing phenomenon observed in this study since it is a reversible inhibition.

The growth promoting effect of *S. thermophilum* on *A. bisporus* was demonstrated only on compost/glucose agar. On other agar media *S. thermophilum* was inhibited by *A. bisporus* but no growth stimulation of the latter was found. Renard and Cailleux [18] also did not find growth stimulation on malt agar. Use of compost/glucose agar could be helpful in elucidating the mechanism of growth promotion.

Other thermophilic fungi were also reported to cause enhanced growth of *A. bisporus*, and *S. thermophilum* enhanced growth of different species of basidiomycete mushrooms [20] in compost. However, interactions were not studied in detail. On a potato agar plate, growth of *Pleurotus sapidus* was accelerated as soon as *Aspergillus glaucus* or *Aspergillus sydowi* colonies were reached [25]. There might be a correlation between medium composition and product formation by, or composition of, growth promoting fungi.

In the final stage of composting, thermophilic fungi colonize the substrate to a high density resulting in a selective substrate. *A. bisporus*, the next organism in the succession, has an ecological advantage in being competitive/combative, and furthermore this fungus is able to use the dead biomass as nutrient source [5, 7, 24].

References

1. Barnett HL (1963) THe nature of mycoparasitism by fungi. Ann Rev Microbiol 17:1–14
2. Bels-Koning HC, Gerrits JPG, Vaandrager MH (1962) Some fungi appearing towards the end of composting. Mushr Sci 5:165–169
3. Derikx PJL, Op den Camp HJM, Wagner A, Straatsma G, van Griensven LJLD, Vogels GD (1990) Respiratory pathways of *Agaricus bisporus* and *Scytalidium thermophilum*. FEMS Microbiol Lett 66:307–312
4. Dowson CG, Rayner ADM, Boddy L (1988) The form and outcome of mycelial interactions involving cord-forming decomposer basidiomycetes in homogeneous and heterogeneous environments. New Phytol 109:423–432
5. Eddy BP, Jacobs L (1976) Mushroom compost as a source of food for *Agaricus bisporus*. Mushr J 38:56–67

6. Fergus CL (1964) Thermophilic and thermotolerant molds and actinomycetes of mushroom compost during peak heating. Mycologia 56:267–284.
7. Fermor TR, Grant WD (1985) Degradation of fungal and actinomycete mycelia by *Agaricus bisporus*. J Gen Microbiol 131:1729–1734
8. Fermor TR, Wood DA (1982) Microbial biomass in compost: a mushroom nutrient? Mushr J 110:388–391
9. Fermor TR, Randle PE, Smith JF (1985) Compost as s substrate and its preparation. In: Flegg PB, Spencer DM, Wood DA (eds) The biology and technology of the cultivated mushroom. Wiley, Chichester, pp 81–109
10. Harder R (1911) Über das Verhalten von Basidiomyceten und Ascomyceten in Mischkulturen. Naturw Z Forst Landws 9:129–160
11. Ikediugwu FEO, Dennis C, Webster J (1970) Hyphal interference by *Peniophora gigantea* against *Heterobasidium annosum*. Trans Brit Mycol Soc 54:307–309
12. Ikediugwu FEO, Webster J (1970a) Anatagonism between *Coprinus heptemerus* and other coprophilous fungi. Trans Brit Mycol Soc 54:181–204
13. Ikediugwu FEO, Webster J (1970b) Hyphal interference in a range of coprophilous fungi. Trans Brit Mycol Soc 54:205–210
14. Kalisz HM, Moore D, Wood DA (1986) Protein utilization by basidiomycete fungi. Trans Br Mycol Soc 86:519–525
15. Olivier JM, Guillaumes J (1975) Effet antagoniste exercé in vitro par le mycélium de *Psaliota bispora* Lange. vis-à-vis de différentes espèces fongiques et bactériennes. Ann Phytopathol 8: 213–231
16. Olivier JM, Guillaumes J (1979) Evolution microbiologique des composts pendant la croissance mycélienne du champignon de couche. Mush Sci 10:311–334
17. Rayner ADM, Webber JF (1984) Interspecific mycelial interactions—an overview. In: Jennings DH, Rayner ADM (eds) The ecology and physiology of the fungal mycelium. Cambridge University Press, Cambridge, pp 383–417
18. Renard Y, Cailleux R (1973) Contribution à l'étude des microorganismes du compost destiné à la culture du champignon de couche. Revue Mycol 37:35–47
19. Stanek M (1972) Microorganisms inhabiting mushroom compost during fermentation. Mush Sci 8:797–811
20. Straatsma G, Gerrits JPG, Augustijn MPAM, Op den Camp HJM, Vogels GD, van Griensven LJLD (1989) Population dynamics of *Scytalidium thermophilum* in mushroom compost and stimulatory effects on growth rate and yield of *Agaricus bisporus*. J Gen Microbiol 135:751–759
21. Straastma G, Op den Camp HJM, van Griensven LJLD (1990) Synergistic interaction between *Scytalidium thermophilum* and *Agaricus bisporus* on an agar medium. Gen Microbiol (Submitted for publication)
22. Van Griensven LJLD (1987) The cultivation of mushrooms: its present status and future developments. Outlook Agric 16:131–135
23. Webster J (1970) Coprophilous fungi. Trans Brit Mycol Soc 54:161–180
24. Wood DA, Fermor TR (1985) Nutrition of *Agaricus bisporus*. In: Flegg PB, Spencer DM, Wood DA (eds) The biology and technology of the cultivated mushroom. Wiley, Chichester, pp 43–61
25. Zeller SM, Schmitz H (1919) Studies in the physiology of the fungi VIII. Mixed cultures. Ann Mo Bot Gard 6:183–191