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Inoculation of *Scytalidium thermophilum* in Button Mushroom Compost and Its Effect on Yield

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***Scytalidium thermophilum* isolates in culture, as well as the endogenous strain(s) in mushroom compost, were inactivated at 70°C. This temperature was used to pasteurize composts for experiments. Of nine thermophilic fungal species, only *S. thermophilum* and *Myriococcum thermophilum* grew well on pasteurized compost in test tubes. The effect of both species on the crop yield of *Agaricus bisporus* mushrooms was studied. In solid-state fermentation rooms called tunnels, compost was pasteurized and inoculated. After incubation, the inoculated organisms were reisolated and counted, showing their successful colonization. The yield of mushrooms on inoculated composts was almost twice that on the pasteurized control. This result demonstrates the effectiveness of *S. thermophilum* in compost preparation. Inoculation is not necessary for traditional compost preparation. Naturally occurring strains of *S. thermophilum*, present in ingredients, readily colonize compost during preparation. Inoculation may be vital if compost is pretreated at a high temperature in tunnels. This finding is of relevance for the environmentally controlled production of high-yielding compost.**

Compost for the cultivation of the white button mushroom, *Agaricus bisporus*, is produced from wheat straw, straw-bedded horse manure, chicken manure, and gypsum. After mixing and moistening, these ingredients are subjected to a phase I composting process. Mixed ingredients are stacked in windrows in the open air for uncontrolled self-heating for 1 to 2 weeks. Temperatures in the windrows range from ambient to 80°C. During phase I, ammonia and foul-smelling compounds are emitted, causing environmental problems. Phase II is an aerobic process carried out by maintaining the compost at 45°C for 6 days in shallow layers in mushroom houses. After compost preparation, *A. bisporus* is seeded into the compost as spawn. The compost is colonized in 2 weeks at 24°C, and then the compost is covered with a casing material, based on peat, where the mycelium produces a crop of fruit bodies. The compost is partially converted into fruit body biomass for an additional 40 days (33). Compost can be treated in bulk, including the stage of colonization by *A. bisporus* mycelium, in solid-state fermentation rooms called tunnels (11, 33). Typically, the process area of a tunnel is 10^2 m^2 for the processing of 10^2 tonnes of phase II compost. Waste air from tunnels can be treated before discharge, preventing environmental problems with volatiles. For economic and environmental reasons, the use of tunnels in the mushroom industry is expected to increase.

Phase I is important to soften the straw, which allows for an optimal amount of compost being filled into the cropping rooms (13) and for an optimal conversion of substrate into mushroom fruit body biomass. Yield is 20% higher when phase I is applied than when it is not (13, 17, 18, 20), being about 400 kg of mushrooms per tonne of compost (fully colonized with *A. bisporus* mycelium).

During phase II, volatile ammonia disappears by emission and bioconversion and the compost becomes selective for the growth of *A. bisporus* mycelium (10). Ammonia disappearance and selectivity are linked with the presence of the thermophilic fungus *Scytalidium thermophilum* (≡*Torula thermophila*, =*Humicola grisea* var. *thermoidea*, =*Humicola insolens* [29]) (23, 24). A positive correlation was found between the density of *S. thermophilum* in compost, determined as the recovery efficiency from plated compost particles, and mushroom yield (26). Also, *S. thermophilum* strongly stimulates the extension rate of mushroom mycelium in vitro (27, 28). The fungus is present in straw and in horse droppings and is common in self-heating substrates. During phase II, it becomes the dominant, almost exclusive, thermophilic fungus (30). The optimum temperature for growth of *S. thermophilum* and for the disappearance of ammonia is exactly the same as the optimum for phase II processing, 45°C. The maximum temperature for growth of *S. thermophilum* is about 55°C (5, 6, 8, 22), hyphae are killed at 58°C (34), and conidiating colonies are killed at 68°C (9, 25). Inoculation of pasteurized compost has been done with activators (16) and with finished phase II compost (15, 18). Houdeau et al. (15) also inoculated with *S. thermophilum* cultures. However, their isolates did not grow well in the compost, and the subsequent yield of the mushroom crop was low.

Previous evidence for the significance of *S. thermophilum* in phase II compost for a good crop yield of mushrooms was a descriptive correlation only (26). The present study focuses on further experimental evidence obtained by cropping trials. Testing for selectivity and growth of mushroom mycelium only is insufficient because the rate of colonization of compost by *A. bisporus* is not necessarily the only determinant of mushroom yield. The partial conversion of the compost into fruit bodies takes about 40 days. Moreover, there is a physiological switch from laccase and manganese peroxidase to cellulase-mediated growth after colonization, at the onset of fruiting (2, 36). The second aim of this study is to explore application in commercial practice.

Isolates of nine species promoting *A. bisporus* mycelial growth (30) were screened for the ability to grow on pasteurized compost in test tubes. Selected isolates were inoculated

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TABLE 1. Analytical data of composts used for the 13 inoculation trials in the pilot plant of tunnels before phase II composting

Trials	Water (%, wt/fresh wt)	Nitrogen (%, wt/dry wt)	NH_4-nitrogen (%, wt/dry wt)	Ash (%, wt/dry wt)
1–4	76.5	1.45	0.47	19.9
5–7	78.7	1.28	0.41	12.0
8–10	80.0	1.48	0.57	13.4
11–13	77.4	1.40	0.39	22.9

into compost in tunnels, and these composts were used for cropping trials of *A. bisporus*.

MATERIALS AND METHODS

Compost. Compost was produced from straw-bedded horse manure, wheat straw, chicken manure, and gypsum. The traditional scheme of mixing ingredients, phase I and phase II, was applied, but it was simplified occasionally and horse manure was omitted as an ingredient and/or phase I was omitted in processing (12, 14). Despite differences in formulation (nitrogen source) and processing (duration of phase I), composting processes tend to converge: at the end of phase II parameters like total nitrogen, NH_4-nitrogen and pH are mutually similar (11); the same holds for the presence of *S. thermophilum* (30). Also, phase I does not seem to be important for the processes before cropping, phase II, and the colonization of compost by mushroom mycelium (12, 13). Here, the term "young compost" is used for any substrate prior to phase II. In cropping trials 1 to 4 and 11 to 13, young compost was commercially obtained. It consisted of about half horse manure and half wheat straw and chicken manure. In cropping trials 5 to 10, the composts consisted of wheat straw, chicken manure, gypsum, and water, the mixture being prepared either in 1 day (trials 5 to 7) or in 6 days (trials 8 to 10). Self-heating had occurred in trials 8 to 10 but not in trials 5 to 7. Analytical data of composts are given in Table 1.

Organisms. Nine species of thermophilic fungi, having passed a selection for growth stimulation of *A. bisporus* (30), were studied. Most isolates came from our own collection: *Chaetomium thermophilum* isolates 209.3 and T49.6.5, *Chaetomium* sp. isolates M4.7 and 275.4, *Corynascus sepedonium* CBS 223.81, *Malbranchea sulfurea* T20.1, *Myriococcum thermophilum* 82.2.9 (=CBS 208.89) and T49.6.6, *Stilbella thermophila* 200.3, *Thielavia heterothallica* CBS 117.65, *Thielavia terrestris* T104.1.2, and 18 isolates of *S. thermophilum*. Of *S. thermophilum* isolates, those of types 1 and 2 (29) were used. Type 2 is divided as follows: 2a, the wild type in compost, has only intercalary chains of conidia in the aerial mycelium; 2b has intercalary chains and also short terminal chains of conidia; and 2c consists of slowly growing isolates with an atypical purple color development (type 1, isolates 77.7.8 [=CBS 622.91], M9.5.3, T.49.3.1, CBS 184.64, and IMI 131012; type 2a, isolates 15.8 [=CBS 671.88], 144.4a, 303.2, 304.2, 304.5, and CBS 227.63; type 2b, isolates 15.1 [=CBS 619.91], M7.5.1 [=CBS 623.91], 170.2.2 [=CBS 621.91], 303.1, and 304.1; type 2c, isolates 201.11 [=CBS 618.91] and T49.5.1 [=CBS 620.91]). The nonstimulatory but common compost organisms *Talaromyces thermophilus* isolate 37C1 and *Thermomyces lanuginosus* isolate 122.3 were also studied. In cropping trials, commercial millet grain spawn of *A. bisporus* Horst U1 (Somycel, Langeais, France) was used.

Counting of *S. thermophilum*. Samples taken at the pilot plant were subsampled at 10 g, blended in 100 ml of water, and sieved; an alcohol-disinfected blender and sieve were used under nonsterile conditions. Either compost suspensions and dilutions or washed compost particles were plated onto yeast-glucose agar containing penicillin and streptomycin (both at 50 $\mu g \cdot liter^{-1}$) to count fungal CFU per gram of fresh weight or the percentage of particles showing recovery of *S. thermophilum*, respectively (4, 30). Suspensions were plated with five replicates, each plate receiving 1 ml. The amount of compost in the undiluted suspension was 0.1 $g \cdot ml^{-1}$. The resulting detection limit, one colony per five plates, was CFU = 2 g^{-1}. Plates were incubated at 45°C and were observed for up to 5 days.

Laboratory experiments. (i) Pasteurization. Survival of endogenous *S. thermophilum* in compost was studied as follows. Forty grams of young compost was filled into culture tubes (160 [height] by 25 mm [diameter]), 100 g was filled into pots (120 by 140 mm), and the samples were incubated at 70°C in an air circulation incubator. The temperature inside tubes and pots was read every 10 min. When the temperature rise had become less than 0.5°C, the previous reading time was taken as the start of pasteurization. After pasteurization, samples were taken to count *S. thermophilum* and other fungi. Survival in phase II compost was studied in pots filled with 100 g. Survival of pure cultures of *S. thermophilum* was also studied. Fully colonized yeast-glucose plates were blended in 100 ml of sterile water, and 1 ml, containing at least 10^5 conidia, was pipetted in tubes holding 4 ml of yeast-glucose broth. Tubes were incubated at 65 or 70°C for 0.5, 1, or 4 h and then incubated at 45°C for 2 days to detect survival. Alternatively, isolates were grown for 3 days on 10 g of sterile compost in tubes and then tested; after pasteurization, 15 ml of water was added for easy observation of survival.

(ii) Growth of thermophilic fungi. Culture tubes (160 by 25 mm), each holding a plastic tube of 10-mm diameter, were filled with young compost. The plastic tube was removed to leave a ventilation channel in the substrate. The compost-filled culture tubes were pasteurized for 4 h at 70°C and were inoculated in three replicates with a 5-mm agar disc of the isolate to be tested. The tubes were placed in plastic bags and incubated at 45°C. Growth was recorded daily. As a growth parameter, the colony radius after 5 days of incubation, $r_{t=5}$, was used. The required inoculum densities for colonization of compost were tested. Plates of isolates 144.4a, 304.1, and T49.3.1 were blended in sterile water and sieved through 60-μm nylon mesh, and the number of conidia was counted microscopically. The suspension was diluted to just above 100, 10, and 1 conidium per ml, and 100 g of pasteurized compost in pots was inoculated with 1 ml of suspension. Pots were screened for growth after 3 and 6 days of incubation at 45°C.

Trials in the pilot plant. Trials were done in the pilot plant of our station of four small tunnels, each holding four containers of 1-tonne capacity. The containers have a removable panel wall for inspection and emptying (14). Tunnels were filled on day 0. One tunnel was used for a traditional phase II as follows. The compost was pasteurized at 56°C for 8 h and processed at 45°C for 6 days (33); the compost was left untouched after pasteurization. On day 1, three tunnels were pasteurized at 70°C. Air entering the compost was brought to 70°C with steam. When the temperature of the air leaving the compost reached 70°C, pasteurization started and continued for 8 h. The compost was cooled to 40°C for further handling. Also on day 1, the floor of the tunnel hall and the equipment to be used for inoculation were disinfected by spraying with 2% formaldehyde, and the hall was left closed overnight. On day 2, the tunnels were opened and the panel walls of the containers were removed. Before handling, compost samples were taken at the bottom, in the middle, and at the top along the vertical plane of two containers per tunnel. The samples of one container

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TABLE 2. Design of the 13 inoculation trials in the pilot plant of four tunnels, each holding four containers, each for 1 tonne of compost

Trials	Compost used for filling	Phase II treatment[a]	Replication and placement/placing
1–4	Commercially obtained	Control 1, control 2, Scyt 15.8, traditional phase II	Each treatment in 4 containers in 1 tunnel
5–7, 8–10	Straw based[b]	Control 1, Scyt 15.8, compost inoculum, traditional phase II	Each treatment in 2 containers for trials 5–7 and 2 containers for trials 8–10, in 1 tunnel
11–13	Commercially obtained	Scyt 144.4a, Scyt M7.5.1, Scyt 304.1, Myt 82.29, Myt T49.6.6, compost inoculum, traditional phase II	Each inoculation in 2 containers randomized in 3 tunnels, traditional phase II in 4 containers in 1 tunnel

[a] Treatments were applied at the beginning of phase II. Control 1, pasteurized at 70°C, untouched; control 2, pasteurized and mixed but not inoculated; Scyt, inoculated with *Scytalidium thermophilum*; Myt, inoculated with *M. thermophilum*; traditional phase II, pasteurized at 56°C, untouched. All treatments were subsequently incubated at 45°C until day 6 as in traditional phase II.

[b] Prior to phase II, straw-based compost was produced in 1 day for trials 5 to 7 and in 6 days for trials 8 to 10; trials 5 to 7 and 8 to 10 ran simultaneously.

were analyzed directly, the samples of the other container were bulked. The contents of each container were emptied in a bin for loosening and mixing. The compost was transported over a conveyor belt and filled again in the container. Compost was inoculated at filling; the inoculum did not touch the bin and the conveyor belt. Containers were inoculated with 2 liters of millet grains fully colonized by a thermophilic isolate, almost 10^{10} propagules, or with 15 kg of phase II compost as a source for *S. thermophilum*.

Thirteen trials were done (Table 2). In trials 1 to 4, two controls were applied, each comprising one tunnel. The tunnel holding control 1 was not opened after pasteurization, and the compost was left untouched and was not inoculated. Compost of control 2 was not inoculated but was mixed as a control for the inoculation handling. A third tunnel was inoculated with *S. thermophilum* isolate 15.8. In the next three runs, two different young composts were used. These were considered six trials, 5 to 7 and 8 to 10. Each compost was filled into two containers per tunnel. One tunnel was used as control 1. A second tunnel was inoculated with *S. thermophilum* isolate 15.8. A third tunnel was inoculated with phase II compost. In another three runs, 11 to 13, controls 1 and 2 were not applied. Five isolates of thermophilic fungi as well as phase II compost were each inoculated into two containers. In all experiments, composts were incubated at 45°C until day 6 as in traditional phase II. They were then inoculated with *A. bisporus* spawn and incubated in the tunnels for 15 days at 24°C. After growth of *A. bisporus* mycelium, composts were filled on shelves in cropping rooms at 71.3 $kg \cdot m^{-2}$ on average and mushrooms were cultivated.

Presentation of data. Calculations were done with data from independent experiments, their number indicated by n. Means and pooled standard deviations (SD) were calculated by analysis of variance. Least-significance differences between pairs of means depend on n. Since n varied, numerous least-significance difference values can be calculated from SD values. For convenience, significant differences are indicated where appropriate. The nine treatments in the 13 experiments in the pilot plant resulted in an incomplete block design. Analysis of variance was performed on the nine treatments. Experiments were considered as blocks, and the presented means were corrected for differences between experiments (3). Analyses were performed with GENSTAT 5 (21).

RESULTS

Laboratory experiments. (i) Pasteurization. Heat transfer in compost in tubes depends on conduction, and thus the temperature rose to the plateau of 70°C very slowly. The initial rises in tubes and pots were 2.0 and 1.5°C · min^{-1}, and the times required to rise from 69 to 70°C were 20 and 40 min, respectively. Incubation of tubes and pots for 1 and 2 h, respectively, was required before pasteurization started. After treatment of young compost for 4 h at 70°C, *S. thermophilum* could not be recovered. However, *Talaromyces thermophilus*, *Thermomyces lanuginosus*, and *Thermoascus aurantiacus* were occasionally recovered. Tubes that were subsequently used in growth experiments remained free of the random colonization by *S. thermophilum* or other thermophilic fungi during their incubation for 5 days. A 4-h treatment at 70°C was also sufficient to inactivate *S. thermophilum* in phase II compost. Before pasteurization of phase II compost, *S. thermophilum*'s CFU equaled $10^{6.5}$ g^{-1} (30), and after pasteurization, its density is below the detection limit of CFU = 2 g^{-1}. Thus, the decimal reduction time, D_{70} value (1), of *S. thermophilum* can be estimated as at least 6.2/4 = 1.6 h^{-1}. Full pasteurization of 1 tonne of phase I compost, to be applied in the trials in the pilot plant, required inactivation to one propagule per 10^6 g. Phase I compost has CFU equal to $10^{2.4}$ g^{-1} (30), and a pasteurization time of maximally 8.4/1.6 = 5.3 h is required.

All isolates of *S. thermophilum* cultured in yeast-glucose broth or in sterile compost were inactivated at 70°C. All five type 2a isolates, among them 15.8 and 144.4a, survived for up to 1 h. Four type 1 isolates survived for up to 0.5 h. Five type 2b isolates, among them M7.5.1 and 304.1, did not resist 70°C but resisted 65°C for 1 h. Two type 2c isolates were inactivated at 60°C.

(ii) Growth of thermophilic fungi. Twenty-two isolates of *S. thermophilum* were tested on pasteurized compost, and most, in particular isolates M7.5.1 and 304.1, grew well (Table 3). Of the other species tested, *M. thermophilum* grew best. From growth curves, the lag times for *S. thermophilum* 15.8 and 304.1 and for *M. thermophilum* 82.2.9 were estimated to be 0.6, 0.4, and 1.3 days, respectively. Culture suspensions of *S. thermophilum* isolates 144.4a, 304.1, 77.7.8, and T49.3.1 were diluted and inoculated into 100 g of pasteurized young compost in pots. Inocula consisting of one single conidium colonized the compost. Applied to the trials in the pilot plant, a single propagule may colonize the compost in 5 days as a sphere with a radius of 100 mm, having a volume of 4.2×10^6 mm^3. For 1 tonne of compost of about 1,700 liters (1.7×10^9 mm^3), this means 0.25% (vol/vol) colonization. Thus, 400 propagules could be sufficient for full colonization. The common compost species *Talaromyces thermophilus* and *Thermomyces lanuginosus* grew poorly, indicating low competitive abilities.

Trials in the pilot plant. (i) Pasteurization. In contrast to compost in tubes, heat transfer in compost in tunnels depends largely on convection rather than on conduction because of the forced-air ventilation of the compost. Temperature readings of

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TABLE 3. Growth of thermophilic fungi on pasteurized compost in test tubes after 5 days of incubation

Species	Isolate designation	$r_{t=5}$ (mm)[a]	n
Chaetomium thermophilum	T49.6.5	19	5
	242.2.4	51	5
Chaetomium sp.	M4.7	30	5
Corynascus sepedonium	CBS 223.81	0	2
Malbranchea sulfurea	T20.1	22	1[b]
Myriococcum thermophilum	82.2.9	70	9
	T49.6.6	61	7
Scytalidium thermophilum	CBS 184.64	9	5
	CBS 227.63	20	4
	IMI 131012	28	5
	15.8	68	11
	144.4a	77	6
	15.1	60	5
	M7.5.1	84	6
	304.1	101	7
Stilbella thermophila	200.3	49	5
Thielavia heterothallica	CBS 117.65	18	5
T. terrestris	T104.1.2	15	5

[a] SD of $r_{t=5}$ is 19.7. Compared with *S. thermophilum* isolate 15.8, only isolate 304.1 grew significantly better; growth of *M. thermophilum* isolates was not significantly different.
[b] *M. sulfurea* was also tested in another way, showing poor growth.

TABLE 4. Compost inoculation with thermophilic fungi and cropping of *A. bisporus* in the 13 trials in the pilot plant of tunnels

Treatment	*S. thermophilum* after phase II, (% recovery)[a]	Yield of mushrooms, (kg · tonne^{-1})[b]	n
Control 1, no mixing, no inoculation	2	189	10
Control 2, mixing, no inoculation	55	335	4
S. thermophilum inoculum			
15.8 (=CBS 671.88)	67	339	10
144.4a	100	363	3
M7.5.1 (=CBS 623.91)	99	383	3
304.1	100	381	3
Myriococcum thermophilum inoculum			
82.2.9 (=CBS 208.89)	7/92[c]	349	3
T49.6.6	4/93[c]	360	3
Compost inoculum	100	398	9
Traditional phase II process	86	358	13

[a] SD of recovery of *S. thermophilum* is 16.2. Inoculations, except with isolate 15.8, resulted in significantly higher recoveries of *S. thermophilum* than in control 2.
[b] SD of yield of mushrooms is 53.2. Mushroom yield on control 1 was significantly lower than for the other treatments.
[c] Percent recovery of *M. thermophilum*.

the compost, of the air entering the compost, and of the air leaving it ranged from 70 to 73°C during pasteurization. CFU of *S. thermophilum* were $10^{2.4}$ g^{-1} before and 2 g^{-1} after pasteurization. Total fungal CFU were $10^{3.4}$ and $10^{1.5}$ g^{-1}, respectively. *Talaromyces thermophilus* and *Thermomyces lanuginosus* were found in most samples; *Thermoascus aurantiacus* was found in only a few. CFU counts before and after pasteurization showed low correlation. When samples of 10 g were directly incubated at 45°C, no colonization of *S. thermophilum* occurred within 4 days, indicating CFU of <0.1 g^{-1}. Evaluation of these methods showed that blending the sample in a disinfected, not sterilized blender introduced cross-contamination. We concluded that *S. thermophilum* was killed off by pasteurization in tunnels at 70°C and that *Talaromyces thermophilus*, *Thermomyces lanuginosus*, and *Thermoascus aurantiacus* survived incidentally. Samples taken from the conveyor belt during the handling for inoculation gave higher CFU counts than samples taken immediately after opening the tunnels and containers. Contamination of the bulk of the compost may have occurred by machine handling.

(ii) Growth of thermophilic fungi. After phase II, fungi were counted by plating washed compost particles. Only *S. thermophilum*, or *M. thermophilum* if that organism had been inoculated, was recovered. Compost remaining untouched after pasteurization (control 1) was almost free of *S. thermophilum* (Table 4), indicating that pasteurization had been efficient. Compost handled without deliberate inoculation (control 2) yielded wild-type *S. thermophilum*. Inadvertent introduction of *S. thermophilum* into pasteurized compost could not be avoided. It also occurred in experiments with trays holding 15 kg of compost, with all treatments placed in a single room for phase II (unpublished results). Our standard isolate *S. thermophilum* isolate 15.8 colonized the compost only partially (Table 4). In the cases of isolates 15.8 and 144.4a, reisolations could not be distinguished from wild-type *S. thermophilum*, as expected. With isolates M7.5.1 and 304.1 and with the *M. thermophilum* isolates, almost complete colonization occurred, and reisolated cultures appeared, microscopically, to be the inoculated types rather than wild-type *S. thermophilum*. This was evidence that both pasteurization and inoculation were effective. Compost submitted to traditional phase II was well colonized with *S. thermophilum* (Table 4), as expected. During incubation at 45°C, NH_3 disappeared at least 1 day sooner in all pasteurized treatments than in the traditional phase II process. Inoculated composts had a higher density (weight/volume) than compost submitted to traditional phase II; with inoculation with *M. thermophilum*, the compost was soft and greasy.

(iii) Cropping of *A. bisporus*. After inoculation with *A. bisporus* and incubation for 15 days, the inoculated and traditional phase II composts were fully colonized by *A. bisporus* mycelium. The pH of inoculated composts was 6.5, that of the traditional phase II compost was 0.1 unit higher, and that of controls 1 and 2 was 0.4 unit higher, indicating weaker colonizations (11). In some experiments, controls 1 and 2 were locally colonized with the opportunistic fungi *Oedocephalum* sp. and *Trichoderma* sp. However, *A. bisporus* was present in the bulk of the compost, and the development of the opportunistic fungi stopped during cropping; their development did not cause practical problems. Further analytical data of compost (not shown) did not anticipate differences in mushroom yields. The yield from control 1 compost was clearly below that from traditional phase II compost (Table 4). Yields from composts inoculated with either *S. thermophilum*, *M. thermophilum*, or compost were similar to that on traditional phase II compost.

DISCUSSION

Laboratory experiments. (i) Pasteurization. Cultures of *S. thermophilum* isolates were inactivated at 70°C, corresponding to published data (9, 25). Isolates of wild-type 2a were most resistant to temperature inactivation. This could explain their selection during phase I and their domination after phase II. Wild-type *S. thermophilum* in phase I and phase II compost was inactivated at 70°C, as the cultured isolates, and types resistant to temperature inactivation did not appear.

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(ii) Growth of thermophilic fungi. *S. thermophilum* and *M. thermophilum* grew well in pasteurized compost in test tubes (Table 3) and were selected for trials in the pilot plant. The test probably provides an overestimation of growth under conditions in the pilot plant because the fungistatic volatile NH_3 is likely to disappear more easily from test tubes than from tunnels. *Chaetomium thermophilum*, a fast-growing species on agar media, behaved variably in compost in test tubes; it might be considered for further testing.

Trials in the pilot plant. (i) Pasteurization. Compost samples were easily cross-contaminated during their handling (blending). To prevent contamination, samples could better be shaken vigorously in sterile water in bottles (4). Direct evidence for the effectiveness of pasteurization consisted of the very low densities of *S. thermophilum* in control 1 and the successful colonization by *S. thermophilum* isolates M7.5.1 and 304.1 and by the *M. thermophilum* isolates when the latter were inoculated (Table 4).

(ii) Growth of thermophilic fungi. The presence of *S. thermophilum* isolates M7.5.1 and 304.1 and of the *M. thermophilum* isolates, when inoculated, showed that compost colonization by selected isolates was successful and that microbial manipulation of phase II composting is possible. The standard, nonselected isolate 15.8 performed poorly, similar to previous results with nonselected isolates (15). Control 2 showed that mixing of pasteurized compost without deliberate inoculation resulted in a considerable development of *S. thermophilum*. Propagules must have been introduced from air or machinery, despite disinfection of the tunnel hall and the machinery. The number of propagules of *S. thermophilum* in air in tunnels during handling of compost after phase II is about 1 liter^{-1} (32); after pasteurization, at the beginning of phase II, the number must be far lower. The presence of *S. thermophilum* in control 2 indicated the need for careful evaluation of inoculation experiments on a pilot plant scale.

(iii) Cropping of *A. bisporus*. Mushroom yields from inoculated composts were high, almost twice that from pasteurized control 1 compost (Table 4). This result may be explained as experimental evidence for the effectiveness of *S. thermophilum* in phase II compost preparation; *M. thermophilum* is able to play a similar role. In control 2, *S. thermophilum* was introduced inadvertently by mixing the compost without inoculation. Organisms other than thermophilic fungi could have been introduced simultaneously. The effectiveness of such other species in the composts cannot be excluded.

Prospects for yield improvement in commercial practice by inoculation are not apparent. However, some advantages of pasteurization and inoculation may be gained. The early disappearance of NH_3 from compost by pasteurization is of interest because growth of *A. bisporus* mycelium requires the absence of NH_3. Shortening the phase II process by 1 day by pasteurization should be investigated. A faster rate of colonization by *A. bisporus* may be possible if a better thermophile is used. However, isolates causing a short adaptation period of growth of *A. bisporus*, IMI 131012, CBS 184.64, and CBS 227.63 (30), grew poorly in pasteurized compost and were not tested in the pilot plant. In the trials, a fixed incubation period for growth of *A. bisporus* mycelium of 15 days was used and the colonization rate of *A. bisporus* was not critically evaluated. The increased density of compost in case of *M. thermophilum* inoculation is an important feature of practical value. Density determines the amount of compost that can be filled in the cropping area, and this amount is an important determinant of yield.

On control 1, colonization of *A. bisporus* mycelium was good despite the limited presence of *S. thermophilum* and the occurrence of opportunistic fungi. However, yield was low. Perhaps compost of control 1 was not well suited for the conversion by *A. bisporus* into fruit body biomass. This should be studied because the nature of such a failure is unknown.

Applications. A major aim of phase II composting should be to prepare a substrate fully colonized with *S. thermophilum* (or with another suitable fungus like *M. thermophilum*). Causal relationships between the presence of *S. thermophilum* and the crop yield of mushrooms remain obscure. Fast compost colonization by *A. bisporus* is of obvious importance, and growth stimulation by *S. thermophilum* seems important. Respiratory CO_2 of *S. thermophilum* plays a stimulatory role (35), but under different experimental conditions, neither volatiles nor CO_2 were stimulatory (27). A requirement for some degradation of compost by *S. thermophilum* for fast mycelial growth could not be established. Only the mode of branching, not the specific growth rate, of *A. bisporus* was affected, resulting in fast but less dense colonization in the presence of *S. thermophilum*. Additionally, conditions wherein *S. thermophilum* inhibits growth of *A. bisporus* have been found (27). What remains is the need for the mere presence of *S. thermophilum*. Ross and Harris (23) suspected that a viable but dormant biomass should be present to fill an otherwise biological vacuum. This prevents colonization by unwanted competitors. This view is compatible with results of Till (31), who found that a high yield of mushrooms could be produced on a sterilized, straw-based substrate that had not been submitted to microbial conversion(s). Our work focused on thermophiles promoting mycelial growth of *A. bisporus*, but obtaining a good crop on compost fully grown with a nonstimulating organism may be possible.

Inoculation of phase I compost, prior to phase II, does not seem useful. Traditional phase I compost contains enough *S. thermophilum* propagules for rapid colonization during phase II. A similar conclusion was reached by Wiegant (34). Survival of *S. thermophilum* in phase I is at risk if high temperatures are maintained in the whole compost body. This situation is easily created if phase I is performed in tunnel fermentors. Phase I composting in tunnels is the object of further study. The mechanisms underlying phase I are not understood. The temperatures during this uncontrolled phase exceed the optimum temperature for degradation, 45 to 55°C (7, 14). Chemical reactions may be important (19).

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REFERENCES

1. **Baggerman, W. I., and R. A. Samson.** 1988. Heat resistance of fungal spores, p. 262–267. *In* R. A. Samson and E. S. Van Reenen Hoekstra (ed.), Introduction to food-borne fungi. Centraalbureau voor Schimmelcultures, Baarn, The Netherlands.
2. **Bonnen, A. M., L. H. Anton, and A. B. Orth.** 1994. Lignin degrading enzymes of the commercial button mushroom, *Agaricus bisporus*. Appl. Environ. Microbiol. **60**:960–965.
3. **Burgers, S. L. G. E. (Agricultural Mathematics Group, Wageningen).** Personal communication.
4. **Chang, Y., and H. J. Hudson.** 1967. The fungi of wheat straw compost. I. Ecological studies. Trans. Br. Mycol. Soc. **50**:649–666.
5. **Chapman, E. S.** 1974. Effect of temperature on growth rate of seven thermophilic fungi. Mycologia **66**:542–546.
6. **Cooney, D. G., and R. Emerson.** 1964. Thermophilic fungi. Freeman, San Francisco.
7. **Derikx, P. J. L., H. J. M. Op den Camp, C. Van der Drift, L. J. L. D. Van Griensven, and G. D. Vogels.** 1990. Biomass and biological activity during the production of compost used as a

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substrate in mushroom cultivation. Appl. Environ. Microbiol. **56:**3029–3034.
8. **Evans, H. C.** 1971. Thermophilous fungi of coal spoil tips. II. Occurrence, distribution and temperature relationships. Trans. Br. Mycol. Soc. **57:**255–266.
9. **Fergus, C. L., and R. M. Amelung.** 1971. The heat resistance of some thermophilic fungi on mushroom compost. Mycologia **63:** 675–679.
10. **Fermor, T. R., P. E. Randle, and J. F. Smith.** 1985. Compost as a substrate and its preparation, p. 81–109. *In* P. B. Flegg, D. M. Spencer, and D. A. Wood (ed.), The biology and technology of the cultivated mushroom. John Wiley & Sons, Chichester, United Kingdom.
11. **Gerrits, J. P. G.** 1988. Nutrition and compost, p. 29–72. *In* L. J. L. D. Van Griensven (ed.), The cultivation of mushrooms. Darlington Mushroom Laboratories, Rustington, United Kingdom.
12. **Gerrits, J. P. G.** 1992. Trends in composting. Mushroom J. **508:**46–51.
13. **Gerrits, J. P. G., J. G. M. Amsing, G. Straatsma, and L. J. L. D. Van Griensven.** 1993. Indoor compost: fase I processen van 3 of 6 dagen in tunnels. Champignoncultuur **37:**339–353.
14. **Gerrits, J. P. G., and L. J. L. D. Van Griensven.** 1990. New developments in indoor composting (tunnel process). Mushroom J. **205:**21–29.
15. **Houdeau, G., J. M. Olivier, and B. Chabbert.** 1991. Improvement of indoor short composting. Mushroom Sci. **13(1):**215–220.
16. **Huhnke, W., and R. Von Sengbusch.** 1969. Champignonanbau auf nicht kompostiertem Nährsubstrat. Mushroom Sci. **7:**405–419.
17. **Laborde, J., G. Lanzi, B. Francescutti, and E. Giordani.** 1993. Indoor composting: general principles and large scale developments in Italy, p. 93–113. *In* S. Chang, J. A. Buswell, and S. Chiu (ed.), Mushroom biology and mushroom products. Chinese University Press, Hong Kong.
18. **Laborde, J., N. Loirette-Baldit, P. Delpech, and J. Delmas.** 1984. Le compostage aere rapide pour la culture du champignon de couche, *Agaricus bisporus*: essais preliminaires, p. 50–74. Proc. Int. Symp. Substrates Mushroom Growing Cultivation *Pleurotus* species.
19. **Miller, F. C.** 1993. Conventional composting, p. 1–3. *In* N. G. Nair (ed.), Workshop discussion papers and seminar summaries 2nd AMGA/ISMS international workshop and seminar on *Agaricus* compost. Australian Mushroom Growers' Association, Windsor, New South Wales, Australia.
20. **Overstijns, A.** 1993. Indoor compostering. Champignoncultuur **37:**325–337.
21. **Payne, R. W., P. W. Lane, A. E. Ainsley, K. E. Bicknel, P. G. N. Digsby, S. A. Harding, P. K. Leech, H. R. Simpson, A. D. Todd, P. J. Verrier, R. P. White, J. C. Gower, G. Tunnicliffe Wilson, and L. J. Paterson.** 1987. Genstat 5 reference manual. Clarendon Press, Oxford, United Kingdom.
22. **Rosenberg, S. L.** 1975. Temperature and pH optima for 21 species of thermophilic and thermotolerant fungi. Can. J. Microbiol. **21:**1535–1540.
23. **Ross, R. C., and P. J. Harris.** 1983. An investigation into the selective nature of mushroom compost. Sci. Hortic. (Amst.) **19:**55–64.
24. **Ross, R. C., and P. J. Harris.** 1983. The significance of thermophilic fungi in mushroom compost preparation. Sci. Hortic. (Amst.) **20:**61–70.
25. **Satyanarayana, T., and B. N. Johri.** 1984. Thermophilic fungi of paddy straw compost: their growth, nutrition and temperature relationships. J. Indian Bot. Soc. **63:**165–170.
26. **Straatsma, G., J. P. G. Gerrits, M. P. A. M. Augustijn, H. J. M. Op den Camp, G. D. Vogels, and L. J. L. D. Van Griensven.** 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.
27. **Straatsma, G., G. Di Lena, T. W. Olijnsma, H. J. M. Op den Camp, and L. J. L. D. Van Griensven.** 1993. Laboratory media for measuring growth parameters of *Agaricus bisporus* mycelium as influenced by *Scytalidium thermophilum*. Cultivated Mushroom Res. Newsl. **1:**1–6.
28. **Straatsma, G., J. P. G. Gerrits, T. M. Gerrits, H. J. M. Op den Camp, and L. J. L. D. Van Griensven.** 1991. Growth kinetics of Agaricus bisporus mycelium on solid substrate (mushroom compost). J. Gen. Microbiol. **137:**1471–1477.
29. **Straatsma, G., and R. A. Samson.** 1993. Taxonomy of *Scytalidium thermophilum*, an important thermophilic fungus in mushroom compost. Mycol. Res. **97:**321–328.
30. **Straatsma, G., R. A. Samson, T. W. Olijnsma, H. J. M. Op den Camp, J. P. G. Gerrits, and L. J. L. D. van Griensven.** 1994. Ecology of thermophilic fungi in mushroom compost, with emphasis on *Scytalidium thermophilum* and growth stimulation of *Agaricus bisporus* mycelium. Appl. Environ. Microbiol. **60:**454–458.
31. **Till, O.** 1962. Champignonkultur auf sterilisiertem Nährsubstrat und die Wiederverwendung von abgetragenem Kompost. Mushroom Sci. **5:**127–133.
32. **Van den Bogart, H. G. G., G. Van den Ende, P. C. C. Van Loon, and L. J. L. D. Van Griensven.** 1993. Mushroom worker's lung: serologic reactions to thermophilic actinomycetes present in the air of compost tunnels. Mycopathologia **122:**21–28.
33. **Van Gils, J. J.** 1988. Cultivation, p. 263–308. *In* L. J. L. D. Van Griensven (ed.), The cultivation of mushrooms. Darlington Mushroom Laboratories, Rustington, United Kingdom.
34. **Wiegant, W. M.** 1992. Growth characteristics of the thermophilic fungus *Scytalidium thermophilum* in relation to production of mushroom compost. Appl. Environ. Microbiol. **58:**1301–1307.
35. **Wiegant, W. M., J. Wery, E. T. Buitenhuis, and J. A. M. De Bont.** 1992. Growth-promoting effect of thermophilic fungi on the mycelium of the edible mushroom *Agaricus bisporus*. Appl. Environ. Microbiol. **58:**2654–2659.
36. **Wood, D. A., and C. F. Thurston.** 1991. Progress in the molecular analysis of *Agaricus* enzymes, p. 81–86. *In* L. J. L. D. Van Griensven (ed.), Genetics and breeding of Agaricus. Pudoc, Wageningen, The Netherlands.

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