THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF BIOLOGY

GROWTH OF *AGARICUS BISPORUS* ON CORN STOVER COLONIZED BY THERMOPHYLLIC FUNGI (*SCYTALIDIUM THERMOPHILUM* AND *MYRIOCCUM THERMOPHILUM*) AND THEIR INFLUENCE ON SUBSTRATE SELECTIVITY

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Abstract

Traditional commercial mushroom (*A. bisporus*) production involves a two-phase composting process that may result in malodorous emissions and may take three weeks or more to complete. In recent years, researchers have been searching for cost-effective alternative substrates for mushroom cultivation that have a lesser impact on the environment. One proposed alternative is a substrate prepared from corn stover (CS) that is colonized with thermophyllic fungi such as *S. thermophilum* or *M. thermophilum*. Here we report on the growth of *A. bisporus* on *S. thermophilum*- and *M. thermophilum*- colonized CS and the influence of a potential contaminant (*P. chrysogenum*) on growth of *A. bisporus*. Mycelial growth was more rapid on *St*-colonized CS compared to Mt-, *St*- and *Mt*-colonized CS, non-colonized CS and phase II compost. Mycelial growth in non-contaminated substrates was significantly greater compared to mycelial growth in substrates contaminated with *P. chrysogenum* and *St*-colonized substrate conferred selectivity to the substrate by reducing the growth of the inoculated contaminant.

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Introduction

Traditional mushroom compost is composed of a mixture of raw materials that may include straw-bedded horse manure, wheat straw, chicken manure, cottonseed hulls, meals and gypsum (Op den Camp et al., 1990). This mixture is subjected to two phases of composting. In phase I, ingredients are exposed to the open air for uncontrolled self-heating for one to two weeks with temperatures ranging from ambient to 80˚C (Straatsma et al., 1994). After 2 weeks, the compost is maintained at 45˚C for six days in shallow layers in mushroom houses or in bulk in tunnels (Straatsma et al., 1994). Phase II allows for an aerobic process to be carried out, so that toxic ammonia can be converted into non-toxic nitrogen compounds (Straatsma et al., 1994). When the toxic ammonia is changed into an alternative non-toxic form, the compost becomes selective for the growth of *Agaricus bisporus* mycelium. Following phase II, the compost may be spawned.

The production of phase II compost takes about 6 to 14 days depending on the system used (Coello-Castillo et al., 2009). Phase II compost, upon completion, is highly selective for growth and development of mushrooms. However, the preparation of this compost is relatively expensive and may have a negative environmental impact. Environmental problems associated with traditional composting include generation of malodorous emissions and run-off that may pollute surface and ground water (Straatsma et al., 1994; Bechara et al., 2006). Thus, growers are seeking alternative mushroom composts that are less costly and more environmentally friendly than traditional mushroom compost.

Research on alternative substrates has demonstrated that it is possible to obtain biological efficiencies ranging from 90% to 200% using non-composted substrates (Coello-Castillo et al., 2009; Bechara et al., 2006)). However, in order for non-composted substrates to replace phase II

compost, the biological efficiencies and cost of these non-composted substrates must be as good as or better than the biological efficiency of phase II compost. One proposed solution for a non-composted substrate is a substrate prepared from corn stover (CS), which is composed of corn stalks, leaves and corn cobs.

In order for CS to be used as an alternative substrate, it would need to have a potential productivity as good as or better than traditional mushroom compost (Sanchez, Mejia and Royse, 2008). Productivity of an alternative substrate could be affected by both abiotic and biotic factors. One factor that has been noted by several researchers working on non-composted substrates is the occurrence of contaminants in some of their substrate treatments (Sanchez et al., 2008; Sanchez and Royse, 2009; Straatsma et. al., 1994; Bechara et al., 2006). However, no research exists on how the presence of common contaminants affects the growth of *A. bisporus*. *P. chrysogenum* was chosen as the contaminant to explore in this paper, because Sanchez and Royse (2009) reported that *Penicillium* spp. were the most frequent weed molds encountered on non-composted substrates like colonized grain, corncobs, and chopped wheat straw.

Another factor that could affect the productivity of the CS substrate is the presence of thermophyllic fungi. Thermophyllic fungi have been isolated directly from mushroom composts and from materials commonly used in composting (Ross and Harris, 1983). Ross and Harris (1983) found that thermophyllic fungi improve the selectivity of the compost for the cultivation of mushrooms by reducing the concentration of ammonia in the substrate and reducing the amount of competitor fungi in the substrate. In this experiment, two thermophyllic fungi species, *Scytalidium thermophilum (St)* and *Myriococcum thermophilum (Mt)*, were used to explore how thermophyllic fungi affect substrate selectivity for mushroom cultivation. *S. thermophilium* and

M. thermophilum were chosen, because previous experiments in the lab revealed that *St*- and *Mt*-colonized CS promoted the growth of *A. bisporus*.

The objectives of this work were to: 1) determine growth of *A. bisporus* on *Scytalidium thermophilum*- and *Myriococcum thermophilum*-colonized CS, and 2) determine how potential contaminants such as *Penicillium chrysogenum* affect growth of *A. bisporus* on *St*- and *Mt*-colonized CS.

Materials and Methods

Strains and Culture Medium. The thermophyllic fungi *S. thermophilum* DC-295 CBS # 15.8 and *M. thermophilum* DC-327 82.2.9 were obtained from The Pennsylvania State University Mushroom Culture Collection (PSUMCC). Maintenance of both strains was on potato dextrose yeast extract agar (PDYA) with periodic (3 months) transfer. Sylvan Spawn 140 of *A. bisporus* was used.

Spawn Preparation. *S. thermophilum* and *M. thermophilum* inocula were prepared using 200 g millet that was boiled in water (1.3 L) for 20 minutes. The millet was strained using a 0.8 cm screen to remove excess water, and 1.5% hydrated lime was mixed by hand into the millet to raise pH to near neutral. The millet was autoclaved for 20 minutes and then cooled to room temperature. The millet was then inoculated with 5 plugs (0.5 mm diameter) *S. thermophilum* or *M. thermophilum* cultured on PDYA. Millet inoculum then was shaken and incubated at 38˚ C for 5 days.

Substrate preparation. Hydrated lime (1.5%) was added to 600 g of dry CS and thoroughly mixed. The milled CS then was rehydrated to 70% moisture content prior to use. The mixture was subjected to a phase II-like thermal treatment. In the first part of the phase II thermal treatment, CS was heated in a covered hot water bath to 65˚C and maintained for two hours in

order to kill any harmful bacteria or fungi already living in the CS. Then, the water temperature was reduced to 40˚C, and the substrate was inoculated with 1.2 g of either *S. thermophilum* or *M thermophilum* spawn, or both. The inoculated CS was incubated at 36˚C until colonization of the stover was achieved (about 5 days).

Inoculum preparation. The spray contained 25 ml of distilled water with 1.0 x 10^6 spores of *P. chrysogenum* per ml. *P. chrysogenum* was obtained by exposing a Petri dish containing PDYA in various locations in the laboratory for a day. The fallout-captured spores were allowed to grow and then the fungus was sub cultured to fresh PDYA. The *P. chrysogenum* cultures were maintained by periodic transfer to fresh media.

Experimental design and analysis. The experiment was designed as a 4 X 2 factorial experiment plus two controls (non-contaminated phase II compost and contaminated phase II compost). The first factor, thermophyllic fungi, had four levels (ST, MT, ST + MT, and None), and the second factor, contamination, had two levels (contaminated and non-contaminated). A total of ten treatments with ten replicates each were used. Replicates of each treatment were prepared in plastic bags containing 17.5 g of substrate. One-half of the treatments received two sprays (each spray equaled 0.25 ml) each of an aqueous suspension of *P. chrysogenum* while the other half of the treatments received 2 sprays (each spray equal 0.25 ml) each of distilled water only. The CS and contaminant were mixed in a plastic bag, so that the *P. chrysogenum* spores were mixed evenly throughout the CS. The non-inoculated treatments were also mixed in a plastic bag. Then, three spawn grains of *A. bisporus* were placed in the middle of a Petri plate and covered with 15 g treated substrate (Figure 1). The mycelium of *A. bisporus* was allowed to grow for 11 days and radial growth measurements were taken on days 5, 8 and 11. JMP software

was used for statistical analysis. Least-square analysis was used to examine interactions among factors. The Tukey-Kramer HSD was to separate treatment means.

Figure 1. Photo of substrate contained in Petri dish showing placement of 3 grains of spawn of *Agaricus bisporus* in center.

Results

Significant sources of variation for the growth of *A. bisporus* on CS substrate included thermophile and *P. chrysogenum* (Table 1). There was no significant interaction between the thermophyllic fungi and contaminant, *P. chrysogenum*, on mycelial growth of *A. bisporus* for each day examined (Table 1).

Radial growth of *A. bisporus* was greatest on CS that was colonized by *S. thermophilum* (ST) on day 11 (Table 2). However, there was no significant difference in radial growth of *A. bisporus* between the CS only and CS colonized by ST. On day 11, mycelial growth of *A. bisporus* on *St*-colonized CS was higher than growth on *Mt*-, *Mt*- and *St*-, and phase II compost (Table 2). *St*-colonized CS appeared to reduce the effect of the contaminant, *P. chrysogenum*, on mycelial growth of *A. bisporus*. For example, mycelial growth of *A. bisporus* was 19.1 mm by day 11 on ST-colonized CS compared to 7.3 mm on CS-only substrate inoculated with *P.*

chrysogenum. The contaminant also severely restriced growth (0.5 mm vs. 12.3 mm) of *A. bisporus* on phase II control compost.

Means and groupings from analysis of variance of thermophile for radial growth of *A. bisporus* for days 5, 8 and 11 are presented in Table 3. On day 11, mean radial growth ranged from a high of 24.0 mm on ST-colonized substrate to a low of 6.4 mm on ST & MT-colonized substrate. Mycelial growth of *A. bisporus* on non-colonized CS fell in middle of the range for observed growth.

Effect of contaminant *P. chrysogenum*, on mycelial growth of *A. bisporus*. Means and groupings from analysis of variance of substrate contamination for radial growth of *A. bisporus* for days 5, 8 and 11 are presented in Table 4. Radial mycelial growth of *A. bisporus* was approximately twice as fast on non-contaminated CS compared to contaminated CS regardless of day.

Table 1. Probabilities > F for radial mycelial growth (days 5, 8, and 11) of *Agaricus bisporus* on substrates colonized by thermophiles and non-inoculated or inoculated with *P. chrysogenum*.

Source	DF	Prob > F day 5	Prob > F day 8	Prob > F day 11
Thermophile[1]	3	<0.0001	< 0.0001	< 0.0001
Penicillium chrysogenum	1	0.0002	0.0002	< 0.0001
Thermophile**P. chrysogenum*	3	0.5846	0.4121	0.3829

[1]Includes *Scytalidium thermophilum*, *Myriococcum thermophilum*, *Scytalidium thermophilum* and *Myriococcum thermophilum*, and no thermophiles.

Table 2. Mean radial growth of *A. bisporus* on corn stover substrate colonized by thermophyllic fungi and non-inoculated or inoculated with *Penicillium chrysogenum* (+ two phase II substrate controls).

Thermophyllic fungus inoculated in substrate[1]	Mean radial growth of *Agaricus bisporus*[2]		
	Day 5 (mm)	Day 8 (mm)	Day 11 (mm)
ST	10.0a	14.6a	28.8a
MT	5.8abc	9.4abc	13.6bcd
ST & MT	4.2bcd	7.5abcde	9.6bcd
CS	4.7bcd	8.8abcd	22.1ab
PH II	1.4cd	7.7abcde	12.3bcd
ST (inoculated with *P. chrysogenum*)	6.4ab	11.8ab	19.1abc
MT (Inoculated with *P. chrysogenum*)	1.2cd	1.7de	3.4d
ST & MT (Inoculated with *P. chrysogenum*)	2.5bcd	4.6bcde	5.7cd
CS (Inoculated with *P. chrysogenum*)	2.2bcd	3.8cde	7.3cd
PH II (Inoculated with *P. chrysogenum*)	0d	0.5e	0.5d

[1]Treatments as follows: ST: inoculated with *S. thermophilum*; MT: inoculated with *M. thermophilum*; ST+MT: inoculated with *S. thermophilum* and *M. thermophilum;* CS: non-colonized*;* PHII: Phase II compost.

[2] Means within a column followed by the same letter are not significantly different according to the Turkey Kramer HSD test (P= 0.05).

Table 3. Means and groupings from analysis of variance for radial growth of *A. bisporus* on corn stover substrate colonized by thermophiles on days 5, 8 and 11 after inoculation.

Thermophile[1]	Day 5[2]	Day 8[2]	Day 11[2]
ST	8.2a	13.2a	24.0a
MT	3.5b	5.6b	8.5b
None	3.4b	6.3b	14.7ab
ST & MT	3.3b	6.1b	6.4b

[1]ST: inoculated with *S. thermophilum*; None: non-colonized; MT: inoculated with *M. thermophilum*; ST+MT: inoculated with *S. thermophilum* and *M. thermophilum*.

[2] Means within a column followed by the same letter are not significantly different according to the Turkey Kramer HSD test (P= 0.05).

Table 4. Means and groupings from analysis of variance for radial growth of *A. bisporus* on corn stover substrate colonized by thermophyllic fungi and non-inoculated or inoculated with Penicillium chrysogenum on days 5, 8, and 11 after inoculation.

Inoculated with *P. chrysogenum*	Day 5[1]	Day 8[1]	Day 11[1]
Non-Inoculated	6.2a	10.1a	18.5a
Inoculated	3.1b	5.5b	8.9b

[1] Means within a column followed by the same letter are not significantly different according to the Turkey Kramer HSD test (P= 0.05)

Discussion

The feasibility of cultivating *A. bisporus* on a CS substrate pre-colonized by thermophyllic fungi in the presence or absence of *P. chrysogenum*, a common contaminant, was examined. The focus was on the comparison of four combinations of thermophiles including: *S. thermophilum*, *M. thermophilum*, *S. thermophilum/M. thermophilum*, and no thermophyllic fungi.

The mycelium of *A. bisporus* grew relatively well on CS substrate colonized by thermophyllic fungi. Mycelial growth on *St*-colonized CS showed the greatest growth of the four levels of CS substrates colonized by thermophyllic fungi. Sanchez and Royse (2009) also found *St*-colonized substrates to promote the growth of *A. bisporus*; however, they reported a higher mean radial growth rate of 12.2 mm/day for *St*-colonized corn cobs compared to our report of a radial growth rate of 2.62 mm/day for St-colonized CS.

Another member of the lab, Stephanie Loehr, also explored using a phase II-like thermal treatment to make *St*-colonized CS. Loehr explored composting *St*-colonized CS using a larger scale technique for composting than what was used in this experiment. She found that the mycelium of *A. bisporus* grew relatively well in *St*-colonized CS; however, her technique was not viable due to problems with inoculation of *S. thermophilum* and contamination when supplements were added to the CS. She also found that CS already contained *S. thermophilum* in

the medium, and non-inoculated CS exposed to phase II composting only gave higher yields of *A. bisporus* (Loehr, personal communication).

Based on the results of this experiment it would appear that inoculation of CS with *S. thermophilum* may be a promising avenue for research, because *St* provided significant protection from the contaminant compared to non-treated CS. In order for CS to be used as an alternative substrate for mushroom production, further research would be needed to explore use of *St*,to increase the rate of mycelial growth and to increase selectivity for *A. bisporus* growth.

Mycelial growth of *A. bisporus* in phase II compost was significantly lower in this experiment than in previous experiments. In addition, mycelial growth of *A. bisporus* on phase II compost was significantly lower than mycelial growth of *A. bisporus* on *St*-colonized CS. We suspect that the phase II compost was drier than normal and was, in fact too dry. Previous experiments using phase II compost have shown that *A. bisporus* grows as well or better on phase II compost than *St*-colonized- and non-colonized CS.

Inoculation of St-colonized CS with *P. chrysogenum* did not significantly reduce growth of *A. bisporus*. Future research should compare the radial growth of mycelium in contaminated CS to the radial growth of mycelium in contaminated phase II compost since the dryness of the compost probably affected the results of the experiment.

Our finding that *St*-colonized CS significantly reduced the negative effect of the contaminant on the growth of *A. bisporus* is in agreement with the findings of Ross and Harris (1983) who stated that thermophyllic fungi help prevent the growth of competing fungi, like *P. chrysogenum*. The mechanism of the protection effect is unknown but may be related to metabolites produced by the thermophile. Metabolites may selectively reduce growth of the contaminant while increasing growth of mushroom mycelium.

P. chrysogenum is also only one of the contaminants found in alternative substrates. Sanchez and Royse (2009) also reported severe contamination of some types of substrates (primarily containing high levels of millet) with *Aspergillus* spp., *Trichoderma* spp., and others that affected mushroom yield. Future research should determine if spore concentration of the contaminant and the contaminant species plays a role in the growth of *A. bisporus* since all of the treatments had the same concentration of spores ($1.0 \text{ X } 10^6$ spores/ml) and the same contaminant.

In conclusion, *St*-colonized CS allows for the growth of *A. bisporus*, but needs additional work for mushroom cultivation since it was not a significant improvement over non-colonized substrate. Loehr (personal communication) found that non-inoculated CS, exposed to phase II-only composting, may be a promising substrate for mushroom cultivation. The results of this experiment support that finding. Thus, with further research it may be possible to develop a substrate that does not require phase I composting. This could be advantageous to both consumers and mushroom growers by reducing the cost of production.

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Teaching:

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  - Outreach Programs: Coastal Field Studies
  - Classroom teaching: Development of Marine Science Curriculum
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Community Activities (Work):

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- Office Secretary: The Undergraduate Biology Advising Office

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    - Responsibilities: Answers phones, greet students as they come in, and complete small projects.
    - Summer of 2008: helped with Freshmen Testing Counseling and Advising Program (FTCAP) and the open house for potential and incoming students.
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