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# Adapting substrate formulas used for shiitake for production of brown *Agaricus bisporus*

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**Abstract**

A pasteurized, non-composted substrate (basal mixture) consisting of oak sawdust (28%), millet (29%), rye (8%), peat (8%), alfalfa meal (4%), soybean flour (4%), wheat bran (9%), and $CaCO_3$ (10%) was adapted from shiitake culture to produce the common cultivated mushroom (brown; portabello), *Agaricus bisporus*. Percentage biological efficiency (ratio of fresh mushroom harvested/oven-dry substrate weight, %BE) ranged from a low of 30.1% (when wheat straw was substituted for sawdust) to 77.1% for the basal mixture. Special, high gas-exchange bags were required to optimize mycelial growth during spawn run. Our formula may allow specialty mushroom growers to produce portabello mushrooms on a modified, pasteurized (110°C for 20 min) substrate commonly used for shiitake production without the added expense of compost preparation. 

## 1. Introduction

The first successful demonstration that *Agaricus bisporus* could be produced on non-composted substrates was reported by Till (1962). He obtained yields that were comparable to conventional compost if a five-step procedure was used that involved the grinding, mixing, sterilizing, spawning and filling of a mixture of 69% straw, 12.3% peat, 9.8% $CaCO_3$, 4.1% soybean flour and 4.1% cotton seed meal. Later, Murphy (1972) reported that it was possible to eliminate phase I composting and obtain respectable yields with only phase II composting if mixtures of sawdust, brewers grain, corn cobs and/or spent compost were used. Finally, Mee (1978) reported that a non-composted mixture of cold manure, peat and lime successfully was used to produce good quality *A. bisporus*.

In recent years, production of specialty mushrooms on nutrient-supplemented sawdust has increased at a rate of more than 20% annually in the United States (USDA, 1992, 1999). In 1998, for example, shiitake production increased 31% (USDA, 1999). Most production of shiitake is on synthetic media containing sawdust as the basal ingredient (Royse, 1996). Growers typically produce more than one type of specialty mushroom on their farm, but only mushrooms that are adapted to grow on non-composted substrate are available for production. Thus, the increasingly popular brown form (''portabello'') of *A. bisporus* has not been produced using non-composted substrate. Therefore, we wanted to explore the possibility of producing *A. bisporus* on non-composted substrate using technology developed for shiitake production.

## 2. Methods

### 2.1. Strains

A commercial brown strain (CS800, Sylvan Spawn Laboratories, Kittanning, Pennsylvania) of *A. bisporus* for the production of ''portabello'' (Fig. 1) was used.

### 2.2. Substrate for mycelial growth

In order to measure the linear extension rate of strain CS800, two experiments were conducted. The first experiment consisted of a mixture of different organic

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Fig. 1. *Agaricus bisporus* (''portabello'') grown on pasteurized, non-composted hardwood oak sawdust (28%) supplemented with millet (29%), rye (8%), peat (8%), alfalfa meal (4%), wheat bran (9%), and $CaCO_3$ (10%).

by-products and supplements as stated in Table 1. A phase II wheat straw-based compost (freshly prepared according to the short method of composting by Sinden and Heuser, 1953) was used as a control. The second experiment consisted of different mixtures of peat/limestone supplemented with various nitrogen-rich compounds as shown in Table 2. Sterilization was completed in an autoclave at 121°C for 1 h. After cooling, six grains of rye spawn were placed in the bottom of a sterile 50-ml centrifuge tube (Beckman) and 25 g of wet sterile substrate was added. The tubes then were incubated at 25°C for 20–25 days. Mycelial growth was observed and measured every two days. At least five tubes per treatment were evaluated.

### 2.3. Substrate for fruiting

A basic mixture (BM) was defined with ingredients as follows (% based on weight of oven dry ingredients): oak sawdust (28%), millet (29%), rye (8%), peat (8%), alfalfa meal (4%), soybean flour (4%), wheat bran (9%), and $CaCO_3$ (10%). Substituting ingredients such as straw, brewers grain, and corncobs for rye and sawdust modified the BM as shown in Table 3.

### 2.4. Cultivation methods

Standard cultivation methods for *A. bisporus* were used (Wuest and Bengston, 1982), except that a non-composted substrate was used. The ingredients were mixed, moistened to 48–55%, thermally treated (110°C, 20 min), cooled and spawned with 0.8–1% rye grain

Table 1
Linear mycelial growth rate of *Agaricus bisporus* (''portabello'', CS800) on five formulas of synthetic substrate and phase II compost (control)

| Trt. no. | Ingredients | | | | | | | | Mycelial growth (mm/day)[a] |
|---|---|---|---|---|---|---|---|---|---|
| | Wheat straw (%) | Cottonseed hulls (%) | Sawdust (%) | Wheat bran (%) | Peat (%) | Ground soybean (%) | Charcoal (%) | $CaCO_3$ (%) | |
| 1 | 0 | 32 | 32 | 10 | 12 | 4 | 0 | 10 | 2.91b |
| 2 | 0 | 37 | 37 | 0 | 12 | 4 | 0 | 10 | 3.22b |
| 3 | 37 | 37 | 0 | 0 | 12 | 4 | 0 | 10 | 2.22c |
| 4 | 66 | 0 | 0 | 0 | 12 | 8 | 4 | 10 | 1.88c |
| 5 | 70 | 0 | 0 | 0 | 12 | 8 | 0 | 10 | 1.94c |
| Control[a] | | | | | | | | | 6.18a |

[a] Means followed by the same letter are not significantly different ($\alpha = 0.05$ after Bonferroni bound).
[a] Standard wheat straw-bedded horse manure phase II compost.

Table 2
Linear mycelial growth rate of *Agaricus bisporus* (''portabello'' CS800) on mixture of peat/lime supplemented with various protein sources

| Trt. No. | Ingredients | | | | | | | | Mycelial growth (mm/day)[a] |
|---|---|---|---|---|---|---|---|---|---|
| | Yeast culture (%) | Skimmed milk (%) | Rye (%) | Wheat bran (%) | Peat (%) | Ground soybean (%) | Corn (%) | $CaCO_3$ (%) | |
| 1 | 0 | 0 | 0 | 0 | 25 | 25 | 0 | 50 | 2.76a |
| 2 | 0 | 0 | 0 | 25 | 25 | 0 | 0 | 50 | 2.17b |
| 3 | 0 | 0 | 0 | 0 | 25 | 0 | 25 | 50 | 1.45c |
| 4 | 0 | 0 | 25 | 0 | 25 | 0 | 0 | 50 | 0.38d |
| 5 | 0 | 25 | 0 | 0 | 25 | 0 | 0 | 50 | 0.26d |
| 6 | 25 | 0 | 0 | 0 | 25 | 0 | 0 | 50 | 0.01e |
| Control | 0 | 0 | 0 | 0 | 50 | 0 | 0 | 50 | 1.63bc |

[a] Means followed by the same letter are not significantly different ($\alpha = 0.05$ after Bonferroni bound).

Table 3
Yield ($kg/m^2$) and percentage biological efficiencies (%BE) of *Agaricus bisporus* grown (three crops) on basal ingredients and basal ingredients plus or minus various supplements

| Crop No. | Treatment description | Yield ($kg/m^2$) | BE (%) |
|---|---|---|---|
| 1[a] | Basal ingredients[b] | 31.4a[c] | 77.1 |
| | Basal ingredients (minus type) | | |
| | +4% brewer's grain+4% rye | 20.9b | 51.3 |
| | +8% brewer's grain | 19.0b | 46.5 |
| 2[a] | Basal ingredients | 18.7a | 47.7 |
| | Basal ingredients (minus sawdust) | | |
| | +14% wheat straw+14% sawdust | 15.7ab | 40.0 |
| | +28% wheat straw | 11.0b | 30.1 |
| 3[d] | Basal ingredients | 61.5a | 69.2 |
| | Basal ingredients (minus rye) | | |
| | +8% corncobs | 60.2a | 66.4 |

[a] Wet (53% moisture) substrate weight = 2.65 kg/bag.
[b] Basal ingredients were as follows: Oak sawdust (28%), millet (29%), rye (8%), peat (8%), alfalfa meal (4%), ground soybean (4%), wheat bran (9%), and $CaCO_3$ (10%).
[c] Means followed by the same letter within a crop are not significantly different according to the Waller-Duncan *k*-ratio *t*-test ($P = 0.05$).
[d] Wet (53% moisture) substrate weight = 6 kg/bag.

spawn using a paddle mixer previously described (Royse, 1997). After spawning, the substrate was bagged in 2.65 or 6.0 kg sterile bags with a patch filter (Unicorn Import and Manufacturing, Commerce, TX) and then heat-sealed. Temperature during spawn run was maintained at 18–19°C for 2–3 weeks. Bags were opened and 2.5 cm of casing (neutralized peat moss inoculated with mycelium-colonized substrate) was overlaid on the substrate surface (Tschierpe, 1990). Case hold lasted 2–3 weeks at 18–19°C. During case hold, water was applied daily as outlined by Schisler and Wuest (1978).

### 2.5. Parameters evaluated

Mycelial growth measurements were plotted against time and the regression line for each treatment was calculated (Statistica, StatsSoft, USA, version 5.5). The slope of each line was defined as the linear extension rate (LER). Total weight of mushrooms per bag (TWM), weight of mushrooms >20 g/mushroom (= total portabellos or TPM), biological efficiency (BE – ratio of fresh mushroom harvested (kg)/kg dry substrate, expressed as a percentage) and the yield in $kg/m^2$ were determined after the third break.

### 2.6. Experimental design and statistical analysis

For LER a multivariate comparison of slopes was made (Kleinbaum et al., 1998) applying a correction of the significance level by the Bonferroni procedure (Simes, 1986). For fruiting parameters, a completely randomized design with 10 replications was used. The ANOVA and means separation was performed using the GLM procedure (SAS Institute, 1998).

## 3. Results

### 3.1. Mycelial growth

The effect of various mixtures on LER of CS800 is shown in Table 1. The LER was 6.18 mm/day on standard phase II compost (control). Treatments one and two, containing a mixture of cotton seed hulls and sawdust had LERs of 2.91 and 3.22 mm/day, respectively. The three treatments containing straw were considered group C containing the lowest LERs (2.22, 1.88 and 1.94 mm/day).

The LERs of different peat/lime mixtures supplemented with various proteinaceous materials are shown in Table 2. The highest value (2.76 mm/day) was obtained when grown soybean was used as a supplement. This treatment formed group A that was significantly different ($P = 0.05$) from the rest of the treatments. A second group (B) was formed by both the control and the treatment, where wheat bran was used as a supplement (1.63 and 2.17 mm/day, respectively). Although no statistical difference ($P = 0.05$) was observed in LERs between these two treatments, a difference in mycelial growth pattern was observed. For example, the mycelium in the peat/lime mixture was thin and dispersed while in the wheat bran/peat mixture, and in the soy mixture the mycelium was whiter and denser. The corn-supplemented mixture formed group C with a LER of 1.45 mm/day. The last treatment contained yeast culture, where no growth was observed.

### 3.2. Biological efficiency

The results obtained when brewer's grain was substituted for rye grain (crop 1) in the BM are shown in Table 3. The highest % BE was obtained with BM

(77.1%) and was significantly higher ($P = 0.05$) than the other two treatments containing brewer's grain (51.3% and 46.5%, respectively).

The effect of increasing levels of wheat straw substituted for sawdust (crop 2) in the BM is shown in Table 3. The highest yield (18.7 kg/m$^2$) was obtained with BM (without wheat straw). There was no significant difference ($P = 0.05$) between the BM and the treatment where 50% of the sawdust was replaced by wheat straw. Lower mushroom yield was obtained from the treatment where wheat straw completely replaced the sawdust. Mycelial growth was more uniform and more rapid in BM than in the other two treatments.

The effect of substituting corncobs for rye is shown in Table 3 (crop 3). Overall, yields were higher for crop 3 than the other two crops. This was due to the increased amount (more than 2X) of substrate (6 kg/bag) compared with crops 1 and 2 (2.65 kg/bag). There was no significant ($P = 0.05$) difference in yield or BE between treatments when corncobs were substituted for rye in the BM.

## 4. Discussion

In the United States, production and consumption of brown *A. bisporus* (portabello) increased 7% from 1998 (21,220 tons) to 1999 (22,771 tons; USDA, 1999). The value of the crop increased 12% (USDA, 1999). The increasing popularity of these mushrooms has paralleled the increasing sales (up 35% last year) of specialty mushrooms in the United States. Some consumers consider the brown varieties as specialty mushrooms. Thus specialty growers would like to cultivate portabellos but may not have compost available. The ability to economically produce portabello mushrooms on non-composted substrate would open the brown market to specialty mushroom growers. It also may benefit certified organic growers since no fungicides or additives are used.

We have demonstrated that it is possible to obtain good yields of the brown *A. bisporus* on non-composted substrates. According to Schisler (1982) BEs of 50–70% are considered average for the white form of *A. bisporus*, while BEs of 70–90% are considered good. Our yields were as good or better than those obtained from commercial cropping houses in the United States where average yields were approximately 25.7 kg/m$^2$ (USDA, 1999).

Our observation that yeast culture did not support growth is not well understood since yeast culture contains considerable protein and many essential vitamins and minerals. Also, the reduction in yield when brewer's grain was added to the formula was unexpected since this ingredient is normally used as a nitrogen source in *A. bisporus* compost (Murphy, 1972). Brewer's grain contains more nitrogen than rye (Stamets, 1993), thus it was expected to produce more mushrooms. Some factor(s) other than nitrogen content may have been responsible for decreased yields since nitrogen contents were 1.45% in BM, and 1.61 and 1.76% in mixtures containing 4% and 8% brewer's grain, respectively. Brewer's grain may be more important in feeding the microbial population during composting than in supplying nutrients to the developing *A. bisporus* mycelium.

It is not surprising that *A. bisporus* grew relatively well on sawdust since Block and Rao (1962) reported that composted sawdust was suitable for producing *A. bisporus*. Murphy (1972) also used it for producing mushrooms with only phase II composting. The fact that soybean flour supported faster LER in all the peat/limestone mixtures tested agrees with Sinden and Schisler (1962) who demonstrated that soybean improved mushroom yields. It is interesting to note that although the growth rate observed in SBF/peat/limestone mixture was very low (2.76 mm/day) when compared to that obtained with phase II compost (6.18 mm/day; Table 1), the peat-limestone mixture was selective for *A. bisporus*. It may be worth exploring the possibility that supplementing peat moss directly with delayed release supplements would be useful for mushroom production.

During the course of our work, we observed that special care must be taken to ensure optimum substrate moisture and to provide optimum aeration inside the bag during spawn run. It was noticed that the greater the amount of substrate contained in the bag, the greater the yield. However, biological efficiency did not increase with increasing substrate quantities. Although we did not conduct specific experiments to determine the optimal size of bags, it was noticed that mycelium did not grow as well in substrate at the bottom of large (6 kg, 25 cm deep) bags (microporus filter patches were present near the top). Thus, it is important that adequate air exchange be provided through appropriate-sized filters. Bags made specifically for this purpose provided sufficient gas exchange and uniform growth from the top to the bottom of the bag.

We also observed that optimum moisture contents in the substrate ranged from 50% to 53%. These levels are considerably below the desired level for phase II compost (around 72%). Thus, additional work regarding substrate moisture contents may improve yields on non-composted substrate.

While our results are promising, substantially more work is needed. Less expensive and more readily available ingredients may reduce the cost of production and make this technology more competitive with compost-produced mushrooms. Such a development would benefit specialty mushroom growers wishing to diversify their product line.

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