Pangola grass colonized with *Scytalidium thermophilum* for production of *Agaricus bisporus*

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Abstract

This work had the dual objective of selecting a substrate for rapid mycelial growth of *Scytalidium thermophilum* and then comparing the growth and production of a brown variety of *Agaricus bisporus* on substrate non-colonized and colonized with *S. thermophilum*. Mycelial growth of *S. thermophilum* at 45 °C was significantly greater on potato dextrose yeast extract agar (0.58 mm/h) as compared to malt extract glucose agar (0.24 mm/h) and yeast extract glucose agar (0.44 mm/h). On cereal grain, *S. thermophilum* grew significantly faster on rice (0.31 mm/h) compared to sorghum (0.22 mm/h) and millet (0.18 mm/h). It also grew faster on Pangola grass (0.49 mm/h) compared to corncobs (0.30 mm/h) and sawdust (0.18 mm/h). Colonization of Pangola grass with *S. thermophilum* was influenced by the addition of calcium salts in the form of gypsum, hydrated lime and ground limestone. For production of *A. bisporus*, biological efficiency (BE) on pasteurized Pangola grass pre-colonized by *S. thermophilum* for 4 days at 45 °C was more than twice (26.4%) that on grass non-colonized by *S. thermophilum* (11.0%). The addition of 2% hydrated lime to Pangola grass prior to colonization by *S. thermophilum* resulted in an additional doubling of BE of mushroom production (48.1%). These results show the possibility of developing a non-composted substrate method for producing *A. bisporus* without autoclaving the substrate.

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Keywords: Portobello cultivation; *Digitaria decumbens*; Non composted substrate; Edible mushrooms; Mushroom production

1. Introduction

Commercial production of *Agaricus bisporus* is on a composted substrate prepared using a traditional two-phase composting methodology with a mixture of straw-beded horse or poultry manure, hay, corncobs and other supplemental raw materials. In Phase I, an aerobic, microbial digestion of organic matter is achieved. This phase takes place outdoors or under cover in aerated bunkers (Beyer, 2005) and may last 5–15 days depending on the type and extent of mechanical processing of raw materials and weather conditions. Phase II is an indoor process lasting approximately 5–7 days at 45–60 °C. In Phase II, the compost is pasteurized and ammonia is reduced to levels that are non-toxic to *A. bisporus* (Laborde et al., 1993).

Because growers desire more rapid substrate preparation without odor generation during composting, alternative methods of cultivation on non-composted substrates have been studied and developed (Till, 1962; San Antonio, 1971; Castle et al., 1988; Sanchez and Royse, 2001; Sanchez et al., 2002; García et al., 2005; Bechara et al., 2005a,b, 2006a,b). With these alternative methods it is possible to obtain biological efficiencies as high as 90–200% in three flushes – as good or much better than traditional two-phase substrates. However, the major drawbacks of these methods are the use of high cost ingredients such as grain and the use of high temperatures to pasteurize/sterilize the substrate. Therefore, at present, these methods are not eco-
nomically competitive with the traditional two-phase method of substrate preparation.

In a search for improvements to the two-phase method of substrate preparation, researchers have shown that Phase I is not a prerequisite for Phase II and that a compost-colonizing, thermophilic fungus, *Scytalidium thermophilum* (St) is important for stimulating growth, development and yield of *A. bisporus* (Ab) (Wiegant, 1992; Straatsma et al., 1991). Thus, several studies have focused on the use of thermophilic fungi, mainly St, to shorten the compost production cycle, maintain better control of the composting process and obtain a more homogenous substrate. However, most of these studies have concluded that inoculation of Phase I compost prior to Phase II with St is not beneficial because sufficient inoculum of the fungus already is present. The exact effects of St on growth and development of Ab is unclear. On the other hand, attempts to eliminate Phase I composting through direct inoculation of Phase II compost with St is not economically viable either, because Phase I composting still is needed to help soften straw and other raw materials. The bulk density of raw materials is increased both biologically (bacterial activity) and chemically (ammonia) with Phase I composting and a higher compost dry fill wt may facilitate (bacterial activity) and chemically (ammonia) with Phase I composting and a higher compost dry fill wt may be achieved for filling maximum quantities of substrate per m² (Straatsma et al., 1995; Gerrits et al., 1995).

Thus, we sought to develop a selective substrate for Ab in a shorter time than the traditional process. We selected Pangola grass as the major ingredient in the substrate because the traditional wheat straw and horse manure are not readily available in southeast Mexico. We eliminated Phase I composting by grinding and moistening the substrate followed by pasteurization and then inoculation with St. After a relatively short colonization period (4 days) with St, we determined the mushroom yield capacity of the substrate.

### 2. Methods

#### 2.1. Strains and conservation medium

*Scytalidium thermophilum* ATCC 66938 and the brown variety of *A. bisporus* (Portobello) ECS-0305 from the mycological collection of El Colegio de la Frontera Sur (ECOSUR) were used (Sanchez and Royse, 2001; Sanchez et al., 2002). The medium for maintenance of both strains was (w/w) glucose (0.5%), yeast extract (0.5%) and agar (2%) in distilled water.

#### 2.2. Spawn preparation and rate of use

*Scytalidium thermophilum* spawn was prepared using rice grain autoclaved at 110 °C for 15 min; a spawn rate of 0.5% (w/w basis) was used. *A. bisporus* spawn was prepared using sorghum grains sterilized by autoclaving for 30 min at 120 °C. The spawn rate was 5% (w/w basis).

#### 2.3. Substrate preparation

Substrates and ingredients were used as follows: Pangola grass, sorghum grains, sawdust (*Cybitax donneli*), corncobs, hydrated lime (commercial lime with 81.5-82% calcium hydroxide, from Calhidra Balun, Canan Inc., Mexico), pulverized limestone or agstone (97% CaCO₃, Pulverizadora Agroindustrial, Guatemala) and CaSO₄ (Meyer). For mycelial growth tests (Table 1), substrates were rehydrated prior to utilization at 50-80% moisture as stated, then glass Petri dishes were filled with ca. 20 g substrate or mixtures thereof and then autoclaved at 120 °C for 20 min.

#### 2.4. Chemical analysis of pangola grass

Three samples (1 kg each) were taken from the ground Pangola grass *Digitaria decumbens* just before preparing the substrate for mushroom production. The samples were sent to the laboratory of Bromatology of Ecosur (Carretera Panamericana y Periférico Sur S/N, San Cristobal de las Casas, Chiapas, CP 29290, Mexico) for chemical analysis as follows: crude fiber, ash and fat (Williams, 1984), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, cellulose and hemicellulose (Van Soest, 1994) and soluble carbohydrates (Tejada de Hernández, 1992). Carbon, nitrogen and hydrogen were determined with an auto analyzer Leco (Leco Corporation, 1988). Protein was estimated by multiplying the nitrogen content by factor 6.25.

#### 2.5. Treatments

To determine the effect of medium type (2% agar) on growth of St, media were used as follows: malt extract (3%) glucose (0.5%) (MEGA), yeast extract (0.5%) glucose (0.5%) (YEGA) and potato dextrose agar (Bioxon) plus...

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**Table 1**

Mean (10 replications) radial growth rate (RGR) of *Scytalidium thermophilum* ATCC 66938 on synthetic media, cereal grains and agricultural by-products (45 °C)

<table>
<thead>
<tr>
<th>Synthetic media</th>
<th>RGR (mm/h)</th>
<th>± Std. dev. (10⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEGA³</td>
<td>0.235a⁵</td>
<td>0.082</td>
</tr>
<tr>
<td>YEGA⁴</td>
<td>0.44b</td>
<td>0.053</td>
</tr>
<tr>
<td>PDYA³</td>
<td>0.582a</td>
<td>0.041</td>
</tr>
<tr>
<td>Cereal grain⁵</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sorghum</td>
<td>0.220b</td>
<td>8.4</td>
</tr>
<tr>
<td>Rice</td>
<td>0.305a</td>
<td>2.0</td>
</tr>
<tr>
<td>Millet</td>
<td>0.184c</td>
<td>0.4</td>
</tr>
<tr>
<td>Agricultural by-products</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pangola grass</td>
<td>0.486a</td>
<td>2.4</td>
</tr>
<tr>
<td>Corncobs</td>
<td>0.298b</td>
<td>0.5</td>
</tr>
<tr>
<td>Sawdust (<em>Cybitax donneli</em>)</td>
<td>0.176c</td>
<td>0.1</td>
</tr>
</tbody>
</table>

³ Malt extract glucose agar.
⁴ Yeast extract glucose agar.
⁵ Potato dextrose yeast extract agar.
⁶ Means followed by the same letter in the same column and group is not significantly different, according to Tukey’s test (α = 0.05).
⁷ Varieties unknown. Grain samples were collected from the market in Tapachula, Mexico.
were made every five days. The radial growth rate (RGR) measurement of the colony radius of 2.7. Parameters evaluated
molds and diseases were made. No attempts to control weed
carry out the veil broken. No attempts to control weed
mushrooms were harvested when the pileus was
opened and then it was inoculated (spawned) with
St
sured on substrate in Petri dishes containing ground and
sieved (16 mesh) D. decumbens (70% moisture), with and
without 2% hydrated lime and previously colonized or
non-colonized by St.

2.6. Cultivation methods
Substrates consisting of ground and sieved Pangola
grass without and with hydrated lime or limestone and
mixtures thereof were used for the cultivation of Ab. Water
was added to raw substrates prior to filling (70% moisture)
into polyethylene bags (30 x 40 cm; 950 g substrate/bag).
The bagged substrate then was pasteurized at 60
°C for
6 h inside a non-agitated shaker (Lab-Line Instruments,
Mod 3525). The substrate was allowed to cool to 45
°C and then it was inoculated (spawned) with St and
incubated (45°C) in the same shaker until complete colonization
of substrate was achieved (4 days). To allow gas
exchange inside the bags, a hole (Φ = 4 cm), covered with
a clean white absorbent paper (paper towel) was made by
introducing the upper end of the plastic bag into a plastic
cylinder. After complete colonization of the substrate by
St the substrate was allowed to cool to 28
°C, then it was
through-spawned with Ab and incubated at 45
°C for
three weeks at 25°C. The same plastic cup used previously was again
positioned for better aeration. The cultivation method for
Portobello on non-composted substrate was followed (Sánchez
and Royse, 2001). Casing inoculum (fully colonized
mushroom substrate) was mixed with the casing layer (peat
moss/lime:water 1:1:4) and the incubation period (case
hold) lasted 3 weeks at 18–20
°C and 85% relative humidity. Mushrooms were harvested when the pileus
was opened and the veil broken. No attempts to control weed
molds and diseases were made.

2.7. Parameters evaluated
Mycelial growth was determined after the daily
measurement of the colony radius of St. For Ab, measurements
were made every five days. The radial growth rate (RGR)
was calculated as: \( \text{RGR} = \frac{(X_2 - X_1) / (t_2 - t_1)}{t_2} \), where \( X \),

1. Biological efficiency (BE, ratio of fresh mushrooms
harvested (kg/kg dry substrate, expressed as percentage) was
determined after the third harvest or break. Mushroom size
(average mushroom weight) was determined by dividing
the total weight of harvested fresh mushrooms (per replicate)
by the total number of mushrooms harvested (per replicate).

2.8. Experimental design and statistical analysis
For the determination of RGR in Petri dishes and for
the determination of biological efficiency (BE), 10 replicates
per treatment were used in a completely randomized
design (Steel et al., 1997). Analyses of variance and mean
separations were conducted using Tukey’s test (SAS
Institute, 2000).

3. Results
3.1. Mycelial growth of Scytalidium thermophilum
The RGRs of St on three agar media (45
°C), three
different grain types and three agricultural by-products are
shown in Table 1. Values ranged from a low of
0.176 mm/h (sawdust) to a high of 0.582 mm/h (PDYA).
On cereal grain, St reached maximum growth on rice
(0.305 mm/h) significantly faster than on sorghum and millet
(\( p = 0.05 \)). Besides the fastest growth on rice, the form
of the colony on this grain was denser. Among the agricul-
tural by-products, Pangola grass allowed the fastest
growth, also significantly different from the other two
(\( p = 0.05 \)). No growth was observed on sugar cane bagasse
and coffee husk.

The influence of substrate moisture content on mycelial
growth of St is shown in Table 2. Maximum growth was
obtained on substrate with 70–80% moisture content
(\( p < 0.0001 \)). The most suitable temperature for growth
was 45°C (\( p < 0.0001 \)) and it was observed that at 25
°C,
St had a RGR of 0.207 mm/h (Table 3).

The influence of pH on the mycelial growth of St is
shown in Fig. 1. We observed that the fungus grew over
a wide range of pH values, although it grew faster at pH
values above 7 (0.425–0.443 mm/h). No growth was
observed at pH 12. The colony morphology changed with
pH, i.e., at pH 7–8, a denser and more cotton-like colony

<table>
<thead>
<tr>
<th>Moisture (%)</th>
<th>RGR (mm/h)</th>
<th>± Std. dev. (10⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0.25b</td>
<td>0.27</td>
</tr>
<tr>
<td>60</td>
<td>0.26b</td>
<td>0.13</td>
</tr>
<tr>
<td>70</td>
<td>0.41c</td>
<td>0.14</td>
</tr>
<tr>
<td>80</td>
<td>0.42a</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Substrate: Pangola grass ±2% hydrated lime.

* Means followed by the same letter in the same column are not
significantly different, according to Tukey’s test (\( z = 0.05 \)).
form was observed while at other pH values colony morphology was more velvet-like in appearance.

Hydrated lime increased or had no effect on the mycelial growth of *St* when applied at rates of 0–4%, but above this concentration it became inhibitory, up to 14% where fungal growth ceased (*R² = 0.93*) (Fig. 2). This was not unexpected, as hydrated lime is known to be toxic at higher levels. Alternatively, when limestone or CaSO₄ was used, growth was stimulated (*R² = 0.95, p = 0.0001; *R² = 0.92, p = 0.03*, respectively) even at a level of up to 15% in the substrate.

The chemical composition of Pangola grass is shown in Table 4. Nitrogen content was 0.53 ± 0.01% while cellulose, hemicellulose and lignin were 34.5 ± 0.7%, 30.4 ± 0.4% and 10.3 ± 0.1%, respectively.

Some physicochemical characteristics of the substrate before and after mycelial growth of *St* are show in Table 5. Moisture content decreased due to high temperatures in the environment (45°C) while the pH became more alkaline (from 7.6 to 8.2) while the C/N ratio decreased (76.5–36.3) due to the aerobic metabolism of *St* on carbohydrates. A decrease of 10% in dry matter also was observed.

### 3.2. Growth and fruiting of Agaricus bisporus

*Agaricus bisporus* was able to grow on sterile Pangola grass in Petri dishes (RGR = 2.84 mm/day) or with a 2% hydrated lime addition (2.83 mm/day). When *Ab* was inoculated in this substrate previously colonized by *St*, it grew at a RGR of 2.75 mm/day without lime and 2.5 mm/day with lime. No statistical difference was found between these treatments (*p = 0.1134*). Upon completion of colonization of Pangola grass by *Ab*, *St* was barely visible with the

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**Table 3**

<table>
<thead>
<tr>
<th>Incubation temperature (°C)</th>
<th>RGR (mm/h)</th>
<th>± Std. dev. (10⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>0.20d</td>
<td>0.86</td>
</tr>
<tr>
<td>35</td>
<td>0.44b</td>
<td>0.87</td>
</tr>
<tr>
<td>45</td>
<td>0.59a</td>
<td>0.78</td>
</tr>
<tr>
<td>50</td>
<td>0.25c</td>
<td>1.02</td>
</tr>
</tbody>
</table>

*Means followed by the same letter in the same column are not significantly different, according to Tukey’s test (α = 0.05).*

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**Fig. 1.** Radial growth rate (RGR) at 45°C of *Scytalidium thermophilum* ATCC 66938 at various pH levels on Pangola grass.

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**Fig. 2.** Influence of the concentration of three calcium sources on mycelial growth (45°C) of *Scytalidium thermophilum*. Substrate: *Digitaria decumbens* 70% moisture. ▲ Hydrated lime; ■ CaSO₄; ○ Limestone.
Table 4
Chemical composition of Pangola grass

<table>
<thead>
<tr>
<th>Component</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash</td>
<td>9.2 ± 0.09</td>
</tr>
<tr>
<td>Fat</td>
<td>1.3 ± 0.02</td>
</tr>
<tr>
<td>Fiber</td>
<td>31.7 ± 0.9</td>
</tr>
<tr>
<td>Protein</td>
<td>3.5 ± 0.07</td>
</tr>
<tr>
<td>Soluble carbohydrates</td>
<td>54.1 ± 0.5</td>
</tr>
<tr>
<td>Neutral detergent fiber</td>
<td>77.7 ± 1.5</td>
</tr>
<tr>
<td>Acid detergent fiber</td>
<td>47.2 ± 0.4</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>30.4 ± 0.4</td>
</tr>
<tr>
<td>Cellulose</td>
<td>34.5 ± 0.7</td>
</tr>
<tr>
<td>Lignin</td>
<td>10.3 ± 0.1</td>
</tr>
<tr>
<td>Carbon</td>
<td>43.1 ± 0.8</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>5.6 ± 0.1</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>0.53 ± 0.01</td>
</tr>
</tbody>
</table>

Mean of three replications ± standard deviation.

Table 5
Some physicochemical properties of substrate (Pangola grass + 2% hydrated lime) before and after colonization by Scytalidium thermophilum ATCC 66938 incubated in Petri dishes (3 days at 45 °C)

<table>
<thead>
<tr>
<th>Property</th>
<th>Before growth</th>
<th>After growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>67.3 ± 0.5</td>
<td>64.9 ± 1.0</td>
</tr>
<tr>
<td>pH</td>
<td>7.6 ± 0.1</td>
<td>8.2 ± 0.1</td>
</tr>
<tr>
<td>C/N</td>
<td>76.5 ± 6.5</td>
<td>36.3 ± 10.0</td>
</tr>
<tr>
<td>Dry matter (%)</td>
<td>100</td>
<td>90.0 ± 2.0</td>
</tr>
</tbody>
</table>

Mean of 10 repetitions ± standard deviation.

Table 6
Biological efficiency (BE), yield and mean size of mushrooms of Agaricus bisporus ECS-0305 produced on a substrate (Pangola grass + different sources of calcium) pre-grown with Scytalidium thermophilum at 45 °C for 4 days

<table>
<thead>
<tr>
<th>Trt. no.</th>
<th>Treatment</th>
<th>BE (%)</th>
<th>Std. dev.</th>
<th>Mushroom size (g)</th>
<th>Std. dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>Control (Grass, no St)</td>
<td>11.0c</td>
<td>4.1</td>
<td>25</td>
<td>5.2</td>
</tr>
<tr>
<td>T1</td>
<td>Control (Grass + St, no calcium salt)</td>
<td>26.4b</td>
<td>11.2</td>
<td>23.9</td>
<td>3.4</td>
</tr>
<tr>
<td>T2</td>
<td>Grass + St + 2% hydrated lime</td>
<td>48.1a</td>
<td>6.3</td>
<td>33.1</td>
<td>14</td>
</tr>
<tr>
<td>T3</td>
<td>Grass + St + 10% limestone</td>
<td>32.7b</td>
<td>7.9</td>
<td>37.8</td>
<td>29</td>
</tr>
<tr>
<td>T4</td>
<td>Grass + St + 10% limestone + 1% hydrated lime</td>
<td>37.3ab</td>
<td>5.2</td>
<td>45.2</td>
<td>9</td>
</tr>
<tr>
<td>T5</td>
<td>Grass + St + 10% limestone + 2% hydrated lime</td>
<td>39.9ab</td>
<td>5.7</td>
<td>49.8</td>
<td>14</td>
</tr>
</tbody>
</table>

Mean of 10 repetitions ± standard deviation.
Hydrated lime has been used successfully to stimulate the growth of *Pleurotus ostreatus* (Hernandez et al., 2003; Contreras et al., 2004).

Chemical analysis of Pangola grass revealed a low nitrogen content (0.53%) and a high fiber content. In fact, wheat straw, the main ingredient in mushroom composts, has a nitrogen content of ca. 0.47% and a C/N ratio of 115.2 (AFRIS, 2005; Muez and Pardo, 2002). With such a low nitrogen content and high C/N ratio for Pangola grass (C/N = 76.5 ± 6.5), we did not expect to have crop production because regular Phase II composts have ca. 2-2.2% nitrogen content and a C/N ratio of 14–19 (Wuest, 1977; Laborde et al., 1993; Pardo, 1993), however, after 4 days colonization by *St*, the C/N ratio of the substrate decreased to 36.3 which was suitable for growth and fruiting of *Ab*. The maximum BE obtained was 48% (without nitrogen supplementation) revealing that this alternative is feasible and promising.

Renard and Cailleux (1973) and Straatsma et al. (1989) reported the stimulation effect of mycelial growth of *Ab* by *St*. The reason for this stimulation remains unexplained; however, several hypotheses have been proposed: reduction of ammonia levels, increased selectivity of the compost (Ross and Harris, 1983), respiratory CO₂ (Wiegant et al., 1992) and immobilization of nutrients (Fermor and Wood, 1981). This stimulating effect on growth has been demonstrated only when cultivation was on compost/glucose agar, while in other synthetic media no growth stimulation was observed (Renard and Cailleux, 1973; Op den Camp et al., 1990). *Ab* grew on grass at 70% moisture, at a similar RGR as that on the same substrate already colonized by *St* indicating no stimulatory effect on mycelial growth. However, the importance of inoculating *St* was demonstrated by the fact that the treatment spawned with *Ab* alone (no *St*) obtained the lowest BE (11%). This fact confirms the results reported by Op den Camp et al. (1990) and Lyons et al. (1999), who suggested that by colonizing the substrate to a high density and producing metabolites that are inhibitory for other fungi, *St* plays an important role in determining the selectivity of compost for *Ab*. The fact that one replicate in T3 had bacterial contamination would not necessarily indicate lack of selectivity, although specific studies should be conducted to evaluate the selectivity of substrate in regard to contaminant bacteria and molds.

The RGR reported here is low when compared to the growth observed for *Ab* by other authors (3–9 mm/day, Olivier and Guillaume, 1979; Straatsma et al., 1989, 1991, 1994) on Phase II compost type. This explains why the mushroom took three weeks to colonize the raw substrate.

Two main changes were observed in the substrate after colonization by *St*: lowering of the C/N ratio due to carbohydrate catabolism and an increase in pH, probably due to the excretion of metabolites. These changes may help to partially explain the fruiting increase of *Ab*. More chemical analysis should be done to investigate substrate conversions: a more complete chemical analysis of the Pangola grass at the end of incubation with *St* and at the end of colonization (spawn run) by *Ab*, the production of fungal metabolites and enzymes, and the contribution of sporulation of *St* to growth stimulation of *Ab*.

This new alternative of growing button mushrooms by using *St* has an advantage over previous work using non-composted substrates (Hunhke and Von Sengbush, 1969; Sanchez and Royse, 2001; Sanchez et al., 2002; Garcia et al., 2005; Bechara et al., 2005a,b, 2006a,b) because high autoclave temperatures are not required. In fact, a treatment of 60 °C for 6 h to pasteurize the substrate as was used here, is done regularly on mushroom farms (Wuest et al., 1970). In addition, a 90 °C treatment for 1 h also gave good results. The method proposed here differs from the traditional two-phase method in that substrate was not composted outdoors (Phase I). The conditions for colonization of Pangola grass by *St* were similar to those used for Phase II (45 °C, 70% moisture, 4–5 days). Inoculation of Pangola grass with *St* may be necessary for the process reported here, but this should be examined further in regular Phase II rooms (tunnels) where the fungal inoculum already is present in walls, floors, and roofs and in the substrate where appropriate temperature management favors the growth of *St*. Inoculation may not be necessary, in this case, or it may require a lower inoculum level. The use of *St* in this way implies several reductions in processing operations meaning less cost because of less space, less investment and less labor. The loss of substrate dry matter, estimated from the traditional composting as 10% after Phase I composting plus an additional 26% after Phase II (Straatsma et al., 1995), should be decreased, i.e., at the end of colonization by *St* dry matter loss (under lab conditions) is estimated only in the 10% range. Moreover, as Perrin and Gaze (1989) mentioned, offensive odors are not produced. A more detailed study should be conducted in order to compare the beneficial effects of Phase I in traditional composts compared to this method using raw materials, mainly softening of substrate and better filling of rooms due to a higher bulk density of Phase II compost (Straatsma et al., 1995).

The maximum BE obtained in this work (48.1%) was substantially lower than that obtained with other composted and non-composted substrates (Schisler, 1982; Bechara et al., 2005a,b, 2006a,b; Garcia et al., 2005) where more than 96% BE was possible. On the other hand, with a grass known to be low in nitrogen (0.53%), a BE of 48% may be obtained. In order to increase nitrogen content of the substrate, a thorough investigation of the effects of delayed release supplements and micronutrients should be completed. This would include supplementation at spawning and at casing.

Our results are in agreement with Perrin and Gaze (1989) who found that a wetted and blended raw material is capable of producing mushrooms without a period of conventional uncontrolled Phase I. They used a typical recipe for conventional compost with wheat straw, chicken manure, supplements and gypsum giving a C/N ratio of...
21.5 at filling and (after 10 days composting at 45–50 °C) 16.4 at spawning.

The spawn rate used here may be considered high (5%) because in previous studies a range of 0.8–1% spawning rate was used (Sanchez and Royse, 2001). The influence of spawn rate on mushroom yield using a grass substrate has been studied with the idea of selective pre-growth of the fungus on raw substrates then using this colonized substrate in traditional composting. However, this organism has not been studied from both rice and sorghum spawn.

Scytalidium thermophilum is an important organism for traditional composting. However, this organism has not been studied with the idea of selective pre-growth of the fungus on raw substrates then using this colonized substrate as a medium to produce Ab. Refining this process could lead to the development of a new alternative substrate for small growers that do not have access to large composting machinery for traditional composting or to autoclaves for sterilizing non-composted substrates.

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References


