Microbial inoculation during composting improves productivity of sun mushroom (Agaricus subrufescens Peck)

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The aim of this research was to evaluate the application of different microbial additives during composting, on some parameters of the production of Agaricus subrufescens. Compost was prepared over two weeks with ammonia assimilating bacterial and a thermophillic fungus as microbiological additives. These additives were introduced during two week composting to promote greater selectivity of the substrate cultivation and provide increased productivity of mushrooms. The data shows that the microbiological additives used in composting had a significantly higher productivity, when compared to treatments without additives. These species can be used as microbiological additives in A. subrufescens cultivation.

Key words: Agaricus subrufescens, microbial additives, productivity, composting, mushroom sun.

INTRODUCTION

The conventional composting process for cultivation of Agaricus subrufescens (also known as A. blazei or A. brasiliensis) (Wasser et al., 2002, 2005; Kerrigan, 2005, 2007; Wasser, 2007; Dias et al., 2008) is derived from the method developed by Sinden and Hauser (1950) used for the cultivation of Agaricus bisporus. This method has two phases. The first one is called phase I or “outdoor”, in which the raw materials are wetted and stacked in piles, and turned over a few times, for approximately two weeks. The second stage, called phase II or “indoor” is carried out in an appropriate tunnel to control temperature and aeration. Phase II has two objectives: Pasteurization (to eliminate pests, diseases and competitors) and conditioning (to create adequate conditions for mushroom growth and suppressive conditions for undesirable microorganisms). Therefore, the substrate must be pasteurized at 60°C for 6 h and conditioned at 45°C up to 14 days or until ammonia dissipation (Straatsma et al., 2000; Eira, 2003; Oei, 2003; Chang and Miles, 2004; Salar and Aneja, 2007).

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According to Siqueira et al. (2011), the initial nitrogen concentration can also influence the A. subrufescens productivity, because the high initial ammonia concentration at the end of Phase I is favorable for thermophilic microorganisms’ nutrition, which aids in substrate degradation. However, on the other hand, at the end of Phase II, ammonia must be almost completely dissipated, due to its toxicity to the mycelial growth of the Agaricus mushrooms. However, there are evidences for different behaviors between these two species (A. subrufescens and A. bisporus) for the initial C/N ratio (Matute et al., 2011; Dias, 2010). In spite of using the same strategy for the compost production, these are two different species (A. subrufescens and A. bisporus), which obviously present different requirements regarding the compost quality, casing layer, temperature among others (Dias, 2010).

Considering that thermophilic microorganisms convert free ammonia into microbial protein during the compost conditioning process, the performance of these microorganisms play a crucial role in the preparation of the compost, making it more appropriate for mycelial growth (Chang and Miles, 2004; Bechara, 2007). Among different species of thermophilic fungi found in the mushroom compost, the fungus *Scytalidium thermophilum* is reported as being the dominant species during Phase II (Straatsma et al., 1994), although alterations occur in the population dynamics during this process (Vajna et al., 2010).

Besides the thermophilic fungi, prokaryotes are present and probably play an important role during the composting process. The actinobacteria stands out for producing a white-grayish mass throughout the compost, at the end of Phase II, evidencing its colonization on the compost (Silva et al., 2009). In agreement with Ahlawat and Vijay (2010), *Staphylococcus* and *Bacillus* species can be used as microbial inoculants in the composting process seeking the cultivation of *A. bisporus*. According to the authors, the use of those bacteria in the mushroom compost provided an earlier harvest and an increase in productivity. According to the same authors, the extracellular enzymes produced by the bacteria can convert agroindustrial residues into a more selective and appropriate compost for the mushroom production.

However, conventional phase II requires a tunnel in which the temperature is properly controlled for pasteurization and the conditioning stages of the compost. Considering the investment and cost of this process, the small producers pay a high price to purchase the compost, which makes this activity not economically viable for small mushroom growers in developing countries. An alternative could be the production of the compost by the small producers, using a simpler, less expensive process which is the long composting, followed by steam pasteurization. However, that strategy has a great disadvantage of low compost quality obtained without phase II.

Based on the above, the objective of this work was to evaluate the use of microbial inoculants during composting seeking the production of compost for cultivation of the A. subrufescens mushroom, using the composting system and steam pasteurization.

**MATERIALS AND METHODS**

In this work, the species of microorganisms isolated directly from the A. subrufescens cultivation compost during Phase II of the composting were used as microbial inoculants.

**Isolates, culture media and microbiological inoculants**

The CS10 (A. subrufescens) strain was used, belonging to the mycological collection of the Edible Mushrooms Laboratory of the University of Lavras (UFLA). The strain was reactivated and maintained in wheat agar (WA) medium until the production of the *spawn*. For the medium preparation, 1 Kg of wheat grain was used, which was cooked in boiling water for 15 min. Subsequently, 1 L of the broth was used, to which 15 g of agar and 15 g of yeast extract were added. The medium was autoclaved at 121°C/30 min. After cooling, the medium was poured into Petri dishes (20 mL plate - )

Right after, the strain were inoculated on the plates and incubated at the 25°C ±1/10 days or until complete colonization. For spawn production, 6 mm discs from colonized plates were used to inoculate sterilized 500 mL glass flasks containing wheat grains, 2% calcium carbonate and 1% gypsum. The inoculated flasks were incubated at 25°C ±1/30 days, or until complete colonization.

The following microbial inoculants were used: *S. thermophilum* (isolate UFLA 17/536), *Bacillus megaterium* (isolate UFLA 84/4 A3.5), *Bacillus cereus* (isolate UFLA 83/4 A4.4), all of them from the Laboratory of Edible Mushrooms, Department of Biology, The University of Lavras, Minas Gerais, Brazil. For the cultivation of *S. thermophilum*, oat meal agar was used, enriched with sorbose and kept at 40°C for seven days. After the incubation period, the spores were resuspended in saline solution (0.9%) and counted in a Neubauer chamber. For the cultivation of the bacteria, the isolates were reactivated in nutrient broth, (0.2% Na2HPO4; 0.3%NaCl; 0.3% meat extract; 0.5% peptone, pH 7.5) and later cultivated in the same medium at 45°C for two days.

**Composting**

The compost was prepared according to the methodology described by Siqueira et al. (2009), using the following ingredients: Sugarcane bagasse (45 kg), coacass cross hay (45 kg), wheat straw (10 kg), lime (2 kg), gypsum (2 kg) and ammonium sulfate (1 kg). The treatments were divided into five groups: Treatment 1, Without microorganism addition (control); Treatment 2, *B. megaterium*; Treatment 3, *B. cereus*; Treatment 4, *S. thermophilum*; and Treatment 5, *B. megaterium, B. cereus* and *S. thermophilum* (Table 1). All inoculants were used at a concentration of 10⁶ CFU g⁻¹ of dry compost. This concentration was chosen in function of the results obtained by Silva et al. (2009), who reported a total microbial population during composting Phase I between 10^7 and 10^9 CFU g⁻¹. The cultures were initially diluted in 10 L of water and followed by its addition to the compost on the 8th day of the composting process.

At the end of the composting, the substrate was pasteurized at 60°C/12 h of continuous steam as described by Siqueira et al. (2009). After cooling to room temperature, the compost was inocu-
Table 1. Treatments used in the cultivation of *A. subrufescens*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Microbiological additive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Without microorganism addition</td>
</tr>
<tr>
<td>2</td>
<td><em>Bacillus megaterium</em></td>
</tr>
<tr>
<td>3</td>
<td><em>Bacillus cereus</em></td>
</tr>
<tr>
<td>4</td>
<td><em>Scytalidium termophyllum</em></td>
</tr>
<tr>
<td>5</td>
<td><em>Bacillus megaterium, B. cereus, Scytalidium termophyllum</em></td>
</tr>
</tbody>
</table>

The productivity of *A. subrufescens* can vary from 33.8 to 58.7%, depending on the cultivation conditions. The highest dry matter content was obtained in Treatment 5 (89.78 and 29.64%, respectively). It is important to have edible mushrooms with high dry matter content: the higher the dry matter content, the lower the water content, and, consequently, higher will be the content of protein and other nutrient values, such as vitamins, minerals, carbohydrates, fibers, fats and amino acids (Chang and Miles, 2004). Therefore, in the present work, mushrooms with higher dry matter content showed higher protein content. In this way, we can suggest that the tested inoculants affected both agronomic parameters as well the food quality, since the lowest values for the dry matter and protein content were observed when no inoculants were used.

Considering the inoculants tested separately, the worst results were obtained with *B. megaterium*, followed by *S. termophyllum* and, then, by *B. cereus*. It is interesting because two species from the same genus have showed such differences (10.25 and 14.5% of productivity for *B. megaterium* and *B. cereus*, respectively). These results are an evidence for the potential to seek and test new inoculants for a better quality of the mushroom production.
species as microbial inoculants for the composting process aiming *A. subrufescens* cultivation. The productivity obtained in the treatments with inoculants separately ranged between 10.25 and 14.5%, while the productivity reached in the control was 8.52%. Siqueira et al. (2011) reported productivity values for *A. subrufescens* between 10.36 and 13.28% due to the initial content of nitrogen in the compost. Zied et al. (2010) obtained an average of 15.5%, when compared different casing layers, while Colauto et al. (2010) reported an average productivity of 11.8% using alternatives to peat as casing layers. It is important to note that in all cited works, compost was used from conventional system of phases I and II, and a similar period of cycle cultivation. Therefore, the utilization of *B. cereus* as the only inoculant was sufficient to get a compost of similar quality to that produced in Brazil using the conventional process.

On the other hand, the best results were observed in the treatment 5, characterized by the addition of the all microorganisms (*B. megaterium, B. cereus* and *S. thermophilum*) to the composting process, with a productivity of 18.75%, which is more than twice as high as that in Treatment 1 (without inoculants). Not only the agronomic parameters, but also the protein content was superior in Treatment 5, when compared with other treatments. Probably, the utilization of a mixture of bacteria and fungus has contributed to obtain a compost of higher nutritional quality in comparison to the control. We must emphasize that even in the control there was a microbial succession, but we did not use the conventional Phase II, where thermophilic microorganisms may grow uniformly in all compost, due to the uniform and constant temperature. Therefore, the addition of inoculants to the compost had a compensation effect for not using composting Phase II.

According to Coello-Castillo et al. (2009), substrates pre-colonized by *S. thermophilum* stimulated the colonization of *A. bisporus*. However, the productivity was negatively affected when the period of pre-colonization was longer than three days. That reason still remains without explanation, but it can be due to competition effects, since only one specie was used, resulting in the production of inhibitory secondary metabolites. In the present work, the utilization of a combination of microorganisms, besides the natural microbiota, probably resulted in equilibrium between different species during the composting process.

The positive effect of bacteria isolated from compost or casing layer on the mushroom growing and fruiting has been reported previously for the button mushroom *A. bisporus* (Reddy and Patrick, 1990; Zarenejad et al., 2012). Gill et al. (2005) reported a new automated system for button mushroom production in three phases, carried out inside a container, reducing the cycle cultivation from 109 days to 69 days, when compared to conventional system. According to the same authors, among other factors, these results were only possible because a mixture of bacteria and *S. thermophilum* were added to the compost. Inoculation of *S. thermophilum* during Phase II of the composting has been reported to provide higher productivity, with *A. bisporus* yields of up to three times higher in relation to the control (Vijay et al., 1999; Straatsma et al., 1994).

For *A. subrufescens* cultivation, the use of inoculants on casing layer has been reported (Young et al., 2012) but the present work is the first to report the utilization of inoculants for the composting process aiming a simpler technology which may be appropriate for small mushroom growers in developing countries.

**Conclusions**

The utilization of a combination of bacteria and fungus (*B. megaterium, B. cereus* and *S. thermophilum*) may be used as an alternative to conventional Phase II to produce mushroom compost in a small scale aiming *A. subrufescens* cultivation by small mushroom growers.

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**Table 2. Quantitative and qualitative data for different A. subrufescens (CS10) cultivation treatments.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total mushroom (kg)</th>
<th>Weight (kg)</th>
<th>Unit weight*</th>
<th>BE (%)</th>
<th>Productivity (%)</th>
<th>Dry matter (%)</th>
<th>Protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8d</td>
<td>0.135e</td>
<td>16.88c</td>
<td>25.78e</td>
<td>8.25e</td>
<td>87.33e</td>
<td>22.53e</td>
</tr>
<tr>
<td>2</td>
<td>11c</td>
<td>0.205d</td>
<td>18.63b</td>
<td>32.03d</td>
<td>10.25d</td>
<td>88.05d</td>
<td>23.38d</td>
</tr>
<tr>
<td>3</td>
<td>14b</td>
<td>0.290b</td>
<td>20.71a</td>
<td>45.31b</td>
<td>14.50b</td>
<td>89.29b</td>
<td>27.22b</td>
</tr>
<tr>
<td>4</td>
<td>13b</td>
<td>0.240c</td>
<td>18.46b</td>
<td>37.50c</td>
<td>12.00c</td>
<td>88.80c</td>
<td>25.80c</td>
</tr>
<tr>
<td>5</td>
<td>20a</td>
<td>0.375a</td>
<td>18.75b</td>
<td>58.59a</td>
<td>18.75a</td>
<td>89.78a</td>
<td>29.64a</td>
</tr>
<tr>
<td>Mean</td>
<td>13.2</td>
<td>0.249g</td>
<td>18.69h</td>
<td>39.84i</td>
<td>12.75j</td>
<td>88.65k</td>
<td>25.71l</td>
</tr>
</tbody>
</table>

Means with the same letters in the same column do not differ statistically by the Scott-Knott test (5% of probability). *Average weight of mushrooms harvested from each treatment (g).
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