Full Length Research Paper

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 thermophilic 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 specie 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⁻¹). 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 84i4 A3.5), *Bacillus cereus* (isolate UFLA 83i4 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), coastcross 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 10⁷ and 10⁸ 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*.

Treatment	Microbiological additive
1	Without microorganism addition
2	<i>Bacillus megaterium</i>
3	<i>Bacillus cereus</i>
4	<i>Scytalidium termophyllum</i>
5	<i>Bacillus megaterium</i> , <i>B.cereus</i> , <i>Scytalidium termophyllum</i>

lated (10 g spawn kg⁻¹), conditioned in polypropylene bags (2 kg/bag) and incubated at 25±3°C until complete colonization. The addition of the casing layer followed the same procedures described by Siqueira et al. (2009).

The first harvest took place 29 days after the induction of fructification and the total duration of the cultivation cycle was 117 days, with 4 harvesting cycles. On the day of maximum production, in the first flush, samples were collected for determination of the mushroom protein content for each treatment. The protein content was calculated from the total concentration of nitrogen, using a conversion factor of 4.38. For the calculation of the total nitrogen content, the Kjeldahl method was used (Pardo et al., 2004; MAPA, 1994).

The production cycle, from the inoculation to the end of the harvest, was conducted under the temperature, relative humidity and carbon dioxide concentration conditions recommended for the selected species, maintaining the temperature at 25±3°C and the relative humidity at 80±4%. The carbon dioxide concentration was reduced from 3000 ppm during the compost colonization period to 700 ppm for induction of the fructification, maintaining these conditions until the end of the cycle.

Productivity and biological efficiency

The productivity was calculated using the formula: [(Mass of fresh mushrooms/mass of moist compost) x 100], while the biological efficiency was calculated using the formula: [(Mass of fresh mushrooms/mass of dry compost) x 100], both expressed in percentage.

Harvest and production analysis

The mushrooms were picked daily according to their optimum state of commercial development, when the veil was stretched, but before the lamellae were exposed.

Experimental design

A completely randomized design (CRD) was used, in which the treatments corresponded to the variations of the microbial inoculants used for compost production. For each treatment, 10 replicates were used, resulting in a total of 50 bags. The statistical program SISVAR[®]UFLA (Ferreira, 2000) was used, to which the data were submitted for variance analysis and the averages were compared by the Scott-Knott (5%) test (Andrade et al., 2010).

RESULTS AND DISCUSSION

The biological efficiency and the productivity of *A. subrufescens* were influenced by the different treatments

using microbiological inoculants added to the composting process (Table 2). The biological efficiency varied from 25.78 to 58.59% while the productivity reached values between 8.25 and 18.75%. Colauto et al. (2010) reported that environmental characteristics, such as the type of casing layer, can influence the production of strains of *A. subrufescens*, suggesting that suitable conditions depend on the strain used in the cultivation cycle.

The biological efficiency of *A. subrufescens* can vary from 33.8 to 58.7%, depending on the cultivation conditions, when the compost is prepared according to the conventional composting process (Phases I and II) (Eira, 2003). The data obtained in this work demonstrated compatible biological efficiency when any microbial inoculant was used. As reported before, the quality of the compost, besides its chemical composition, is directly related to its microbial community (Peters et al., 2000), mainly if the conventional system is not used.

With regard to the analyses of the quantitative parameters, significant differences were observed in all treatments, with the highest values being found in treatment 5 and the lowest values in Treatment 1 for the analyzed parameters: mushroom mass, biological efficiency and productivity (Table 2).

Significant differences were also observed among the different treatments regarding the dry matter and protein content. The highest dry matter and protein content were 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

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

Treatment	Quantitative data				Qualitative data		
	Total mushroom	Weight (kg)	Unit weight*	BE (%)	Productivity (%)	Dry matter (%)	Protein (%)
1	8d	0.135e	16.88c	25.78e	8.25e	87.33e	22.53e
2	11c	0.205d	18.63b	32.03d	10.25d	88.05d	23.38d
3	14b	0.290b	20.71a	45.31b	14.50b	89.29b	27.22b
4	13b	0.240c	18.46b	37.50c	12.00c	88.80c	25.80c
5	20a	0.375a	18.75b	58.59a	18.75a	89.78a	29.64a
Mean	13.2	0.249	18.69	39.84	12.75	88.65	25.71

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).

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. thermophyllum*) 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. thermophyllum*) 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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