

Full Length Research Paper

## The cultivation of *Agaricus bisporus* on the spent substrate of *Flammulina velutipes*

Wenming Zhang, Xin Niu, Wen Zhang, Zhonghua Liu and Sheng Yuan\*

Jiangsu Key Laboratory for Microbes and Functional Genomics, Jiangsu Engineering and Technology Research Center for Industrialization of Microbial Resources, College of Life Science, Nanjing Normal University, Nanjing 210023, PR China.

Accepted 16 September, 2013

A large amount of spent substrate produced from the commercial culturing of edible mushrooms is becoming an environmental pollution issue. Here spent *Flammulina velutipes* substrate, supplemented with unfermented cow dung, gypsum, and calcium superphosphate, as the main compost constituent for the cultivation of *Agaricus bisporus* were tested. Compared to the conventional culturing method using rice straw as the primary compost constituent, the reuse of spent *F. velutipes* substrate reduces the cost, shortens the production cycle, and increases the economic yield of the *A. bisporus* culture. Therefore, such recycling is not only practical, but less environmentally damaging as well.

**Key words:** Spent mushroom substrate, *Flammulina velutipes*, compost, *Agaricus bisporus*.

### INTRODUCTION

The increased demand for edible mushrooms, due to their food and medicinal value, has stimulated large-scale culturing of certain strains (Gern et al., 2010), such as *Agaricus bisporus*, *Flammulina velutipes*, *Lentinula edodes*, and *Coprinus comatus*. Although large-scale production helps to satisfy food demand and creates huge economic benefits, it also imposes an environmental burden by generating a large amount of spent substrate during the industrial culturing of edible mushrooms. If not handled properly, the spent mushroom substrate (SMS) may impede the culturing of edible mushrooms and result in environmental pollution (Rinker, 2002). However, there are still high levels of residual nutrients and enzymes left in the SMS (Ball and Jackson, 1995; Chiu et al., 1998; Semple et al., 2001). When used rationally, it can be turned into a valuable resource. Poppe (1995) demonstrated how spent *Pleurotus* substrate can be used to cultivate *Stropharia rugosoannulata*, which is an agaric of the family Strophariaceae found in Europe and

North America. In this study, we mixed ground spent *F. velutipes* substrate from an industrial culture with supporting materials to serve as a culture for *A. bisporus*.

### MATERIALS AND METHODS

*A. bisporus*, also known as mushroom, and white mushroom, is delicate in texture and tasty in flavor (Chiu et al., 2000). It is rich in nutritional components, such as tyrosinase, which is very effective in decreasing blood pressure (Shi et al., 2002; Didukh et al., 2003). *A. bisporus* also has great potential for the development of fungus-derived drugs, especially anti-oncogenic drugs (Toth and Erickson 1986; Chen et al., 2006; Adams et al., 2008). Because of these benefits, *A. bisporus* is being mass-produced in China. The current culture medium for *A. bisporus* in China is composed primarily of rice straw, fermented cow dung, lime, gypsum, calcium superphosphate, urea, and compound fertilizer (Zhang et al., 2002). Nevertheless, despite its wide use, this culturing method has several shortcomings. Our research indicates that *F. velutipes* SMS, in comparison with the aforementioned conventional culture

\*Corresponding author. E-mail: [yuansheng@njnu.edu.cn](mailto:yuansheng@njnu.edu.cn) Tel: 86-25-85891067(O). Fax: 86-25-85891067 (O).

**Table 1.** *Agaricus bisporus* cultivation formulas.

Variable	Control group	Experimental group
Rice straw	66.96% (sdm)	-
<i>F. velutipes</i> SMS	-	56.61% (sdm)
Fermented cow dung	22.32% (sdm)	-
Unfermented cow dung	-	37.73% (sdm)
Compound fertilizer	2.23% (sdm)	-
Urea	0.90% (sdm)	-
Ammonium bicarbonate	0.90% (sdm)	-
Lime	2.23% (sdm)	-
Gypsum	2.23% (sdm)	2.83% (sdm)
Calcium superphosphate	2.23% (sdm)	2.83% (sdm)

\*sdm, Substrate dry mass. The control culture medium was prepared as follows: Rice straw was cut into 2 to 3 cm pieces and hydrated with lime water or distilled water for 2 days (Derikx et al., 1990). After removing the water, other supporting nutrients were added, mixed evenly and adjusted to humidity 70% before being stored in plastic boxes. The medium was first piled and fermented for 25 days at 37°C, then the medium was fermented again at 60°C for 7 hours, 50°C for 6 days, during which time the stacks of medium were turned 6 times (Fermor et al., 1985; Gerrits 1988). The experimental culture medium was prepared as follows: Mildew-free spent *F. velutipes* substrate was ground and mixed evenly with water and other ingredients in fixed proportions. After mixing, the medium was stored in plastic boxes and kept at room temperature (25°C) for 2 days, then at 65°C for 24 h and finally at 50°C for 3 additional days. The stacks of medium were turned 2 to 3 times during fermentation.

**Table 2.** The fermentation and pinning time of the culture media and their effects on mycelium growth.

Variable	Fermentation time of culture medium (days)	Growth of mycelium	Pinning time (days)
Control group	32	Active mycelium growth, white and thick, quickly colonizes the whole culture substrate	42
Experimental group	7	Same as control group	40

The fermented media, which had been adjusted to 8.0-pH, were moved into the phytotron. The size of culture stack was 50 cm \* 32 cm \* 13 cm (length \* width \* height). And each stack has three repeats. The grain spawn was mixed into the medium by grasping the medium gently by hand and softly patting the culture surface. When the substrate was completely colonized by fungal mycelium 15 to 20 days after sowing, vermicompost was put on top of the substrate. To promote pinning, sprayed enough water on the soil as needed to regulate the water content. To condition the culture, the temperature and humidity of the room were maintained at 23°C and 70%, respectively.

medium, not only is more readily available and cheaper than rice straw, but also simplifies composition of culture medium and can be mixed directly with raw cow dung for *A. bisporus* production. Therefore, we recommend the use of *F. velutipes* SMS as the primary constituent of the culture medium for *A. bisporus* production, which effectively recycles the waste substrate and reduces the fermentation period and the consequent environmental pollution.

## RESULTS AND DISCUSSION

The formulas and preparation of experimental culture medium containing *F. velutipes* SMS and the control culture medium containing rice straw for cultivation of *A. bisporus* are shown in Table 1. Experiments showed that

there was no difference observed in the mycelium growth rate or in the length of time from sowing to pinning appearance between the control and experimental groups (Table 2). However, there was a significant difference in the time required for fermentation of the culture medium between the two groups. In the control group, the culture medium was fermented for approximately 32 days, during which it was turned 6 times. Conversely, in the experimental group, it took only 7 days to ferment, and the culture medium needed to be turned only 3 times and it was easier to turn the stack each time. Therefore, the culture formula that was applied in the experimental group saved time and labor in comparison to the formula used in the control group.

Table 3 shows that in the experimental group, the

**Table 3.** Yield of *Agaricus bisporus* production and biological efficiency using different culture formulas.

Variable	Dry weight of culture medium (g)	Total weight of produced mushroom (g)	Average yield (kg/m <sup>2</sup> )	Biological efficiency (%)
Control group	3073.50	879±38.14 <sup>a</sup>	5.78±0.25 <sup>a</sup>	28.60±1.24 <sup>a</sup>
Experimental group	11925.00	3462.28±44.88 <sup>b</sup>	21.64±0.28 <sup>b</sup>	29.03±0.37 <sup>a</sup>

The same letter in the same column does not differ significantly from each other according to T-test at 5% probability. The harvest was carried out when the pileus of *A. bisporus* grew to 3 to 5 cm, ensuring that the pellicle remained unbroken and unopened. For all the test runs, three flushes of mushrooms were harvested. The interval between flushes was about 7 to 10 days.

**Table 4.** The ratio of yield to cost using different culture medium formulas.

Group	Cost (Yuan per kg culture medium)	Fresh mushroom weight (g) per kg culture medium	Earning (Yuan per kg culture medium)	Yield versus cost ratio
Control	0.61	285.99±12.41 <sup>a</sup>	4.58±0.20 <sup>a</sup>	1:7.50
Experimental	0.29	290.34±3.76 <sup>a</sup>	4.65±0.06 <sup>a</sup>	1:16.00

The same letter in the same column does not differ significantly from each other according to T-test at 5% probability.

average yield per unit of culture area reached 21.64 kg/m<sup>2</sup>, which was 4 times that of the control group. However, as shown in Table 3, an increase of dry weight of the experimental culture medium to appr 4-fold of the control group in a pile of the same size. This relates to a appr 4-fold increase of density of the *F. velutipes* spent substrate compared to that of rice straws substrate, so more *F. velutipes* spent substrate was needed to pile a culture bed of the same size. Apparently, the greater production yield is due to the higher density culture substrate in the same culture size rather than a significant improvement in the biological efficiency achieved using the new culture formula. However, although the biological efficiency is not increased by using *F. velutipes* spent substrate to culture *A. bisporus*, there is a significant increase in the average yield per unit of culture area. Therefore, cultivation of *A. bisporus* using the experimental culture medium saves space in the mushroom house by increasing the efficient use of the growth area and improves the economic benefits.

The cost of culture medium prepared with different formulas was calculated at the market price of the raw materials, as shown in Table 4. The yield of fresh mushrooms, using equal amounts of culture medium in the experimental group and in the control group, was unchanged. However, the total cost of the substrate in the experimental group was approximately 50% lower, when compared to that in the control group. Thus, our novel culturing method provides a significant economic advantage for large-scale production of *A. bisporus*. Because the market price of *A. bisporus* fluctuates greatly in different seasons and years, the earnings as well as the yield versus cost ratios of the two medium formulas were calculated based on the average local market price of 16 Yuan per kilogram in the last three years. As indicated in Table 4, the ratio of the experimental group is 2-fold higher than that of the control group;

therefore, the experimental culture medium is more economically beneficial than that of the control.

## Conclusion

To summarize, it is feasible to cultivate *A. bisporus* with the spent substrate of *F. velutipes* as a substitute for the conventional culture method of using rice straw. Our method not only offers a solution to help prevent environmental pollution from the improper disposal of spent mushroom substrate, but also reduces the cost and increases the economic benefits of the cultivation of *A. bisporus*. To further validate the efficacy of our *A. bisporus* culturing method, we expanded our small lab-scale method to an industrialized scale via collaboration with Nanjing Gaogu Edible Fungi Science and Trade Co., Ltd. We obtained similar results to our laboratory experiments (data not shown), demonstrating that our method significantly decreases the overall cost of and time for fermentation of the culture medium while significantly increasing the yield of *A. bisporus*.

## ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (No. 31170028), Nanjing Science and Technology Council (No.200903044, 201105058) and the Priority Academic Program Development of Jiangsu Higher Education Institutions.

## REFERENCES

- Adams LS, Chen S, Phung S, Wu X, Ki L (2008). White button mushroom (*Agaricus bisporus*) exhibits antiproliferative and proapoptotic properties and inhibits prostate tumor growth in athymic

- mice. *Nutr. Cancer*. 60(6):744-756.
- Ball AS, Jackson AM (1995). The recovery of lignocellulose-degrading enzymes from spent mushroom compost. *Bioresour. Technol.* 54(3):311-314.
- Chen S, Oh SR, Phung S, Hur G, Ye JJ, Kwok SL, Shrode GE, Belury M, Adams LS, Williams D (2006). Anti-aromatase activity of phytochemicals in white button mushrooms (*Agaricus bisporus*). *Cancer Res.* 66(24):12026-12034.
- Chiu S, Ching M, Fong K, Moore D (1998). Spent oyster mushroom substrate performs better than many mushroom mycelia in removing the biocide pentachlorophenol. *Myc. Res.* 102(12):1553-1562.
- Chiu SW, Law SC, Ching ML, Cheung KW, Chen MJ (2000). Themes for mushroom exploitation in the 21st century: Sustainability, waste management, and conservation. *J. Gen. Appl. Microbiol.* 46(6):269-282.
- Derikx PJJ, Den Camp HJMO, Van Der Drift C, Van Griensven LJLD, Vogels GD (1990). Biomass and biological activity during the production of compost used as a substrate in mushroom cultivation. *Appl. Environ. Microb.* 56(10):3029-3034.
- Didukh MY, Wasser SP, Nevo E (2003). Medicinal value of species of the family Agaricaceae Cohn (higher Basidiomycetes) current stage of knowledge and future perspectives. *Int. J. Med. Mushrooms* 5:133-152.
- Fermor T, Randle P, Smith J (1985). Compost as a substrate and its preparation. In Flegg PB et al. (eds) *The Biology and Technology of the Cultivated Mushroom*, New York. Wiley, pp. 81-109.
- Gern RMM, Libardi N, Patricio GN, Wisbeck E, Chaves MB, Furlan SA (2010). Cultivation of *Agaricus blazei* on *Pleurotus* spp. Spent Substrate. *Braz. Arch. Biol. Technol.* 53(4):939-944.
- Gerrits J (1988). Nutrition and compost. In Van Griensven (ed) *The cultivation of mushrooms: Rustington, Darlington Mushroom Laboratories*, pp. 29-72.
- Poppe J (1995). *Cultivation of Edible mushrooms on tropical agricultural wastes*. 1st ed. Gent: University Gent.
- Rinker DL (2002). Handling and using "spent" mushroom substrate around the world. In Sanchez JE (ed) *Proceedings of the Fourth International Conference on Mushroom Biology and Mushroom Products*, Mexico. Cuernavaca, pp. 43-60.
- Semple KT, Reid BJ, Fermor TR (2001). Impact of composting strategies on the treatment of soils contaminated with organic pollutants. *Environ. Pollut.* 112 (2):269-283.
- Shi Y L, Benzie I F F, Buswell J (2002). A. Role of tyrosinase in the genoprotective effect of the edible mushroom, *Agaricus bisporus*. *Life Sci*, 70:1595-1608.
- Toth B, Erickson J (1986). Cancer induction in mice by feeding of the uncooked cultivated mushroom of commerce *Agaricus bisporus*. *Cancer Res.* 46 (8):4007-4011.
- Zhang R, Li X, Fadel JG (2002). Oyster mushroom cultivation with rice and wheat straw. *Bioresour. Technol.* 82(3):277-284.