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Cattle Bedding Waste Used as a Substrate in the Cultivation of *Agaricus blazei* Murill

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The potential use of beef cattle bedding compost as a substrate for the production of the biomedical mushroom *Agaricus blazei* Murrill was tested and evaluated on various compost concentrations with fortified supplements. All tested concentrations (100% with equal supplement), 75, 50 and 25%) were found to be suitable for mycelial growth and fruit body development. Biological efficiency (BE) ranged from 28.6% to 70.9% in the two harvests of mushrooms. Compost with 100% and 75% were far superior to all other compost concentrations. The yield was greatest on 100% and 75% compost, with BE of 70.9% and 63.4%, respectively. Compost with 50% and 25% yielded significantly less with BE of 45.1% and 28.6%, respectively. Compost with 75% influenced faster mycelial extension, earlier spawn run, primordial initiation, earlier fruiting with bigger mushroom size, whereas higher supplement (100%) achieved a higher yield with smaller mushroom size. Our results showed that beef cattle bedding compost is a potential substrate in *A. blazei* cultivation; however, compost concentration selection is important to improve biological efficiency and mushroom yield.

INTRODUCTION

Agaricus blazei Murrill, an edible mushroom belonging to the *Agaricaceae* family, is native to southern Brazil. It is popularly known as "Himematsutake" in Japan. *A. blazei* Murrill, is an attractive brown mushroom with sparkling gill caps and snow–white stalks, which are often enlarged at the base. It naturally occurs in fields and mountainous regions in warm and humid weather, in soil–rich organic matter associated with mixed wood and grasslands rich in cattle manure. It favors high temperatures and light. This mushroom is the secondary decomposer, which grows on material already partially degraded by microorganisms, and requires fermented compost as a substrate, unlike other primary saprophytes such as *Lentinus edodes*, *Grifola frondosa* and *Ganoderma lucidum*. Cultivation of *A. blazei* is well established and pioneered throughout Japan in indoor cultivation and is the most popular in consumption as well.

At present, *A. blazei* Murrill is widely used as a medicinal and functional food rather than for its nutritional purposes because of its potent medicinal properties (Kuroiwa *et al*., 2005). Brazilian mushroom (*A. blazei*) was reported to possess antitumour and immunomodulating activities (Kawagishi *et al*., 1998). Its isolated polysaccharides could stimulate lymphocyte T–cells in mice (Mizuno *et al*., 1998). *A. blazei* Murill is particularly rich in polysaccharides and has shown particularly strong results in the treatment and the prevention of cancer (Fujimiya *et al*., 1998; Mizuno *et al*., 1998; Ebina and Fujiyama, 1998). The polysaccharides contained in this mushroom vitalized production of interferon and interleukin (Nakajima *et al*., 2002). In addition, many experiments proved that *A. blazei* could also prevent viruses and other external factors from entering the tissue (Nakajima, *et al*., 2002).

This mushroom is usually cultivated in naturally fermented substrates. The main substrate used in the cultivation of *A. blazei* are rice straw, wheat straw and sugar cane bagasse. However, we hypothesized that fermented livestock bedding materials as a substrate in the cultivation of this mushroom have endless possibilities. This substrate can be said to be economical and environmentally friendly, too.

Therefore, the conversion of cattle bedding compost into the biomedical fungus is a low cost initiative and an easily available substrate which can be a most profitable agri–business as it helps in its disposal in an environmentally friendly manner. To the best of our knowledge, beef cattle bedding compost has not yet been tested as a substrate for the cultivation of *A. blazei*. The aim of the study was to determine the effect of beef cattle bedding compost on the productivity of *Agaricus blazei*.

MATERIALS AND METHODS

Microorganism

The strain of *Agaricus blazei* Murrill KS–72 was used in the experiment which originated from Kyushu University. It was cultured on Potato Dextrose Agar (PDA) at 25˚C, and sub–cultured every three months.

Source of compost

One year fermented beef cattle bedding compost was used as a basal ingredient for substrate preparation. It was directly obtained from a local livestock company (Susuki Co. Ltd. Fukuoka, Japan). The Compost con-

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tained a mixture of chips from *Cryptomeria japonica* (L. f.) D. Don [Sugi], *Picea glauca* (Moench) Voss [White spruce, imported], *Pseudotsuga menziesii* (Mirb.) Franco [Douglas fir], *Pinus thunbergii* Parl. [Japanese black Pine], *Pinus densiflora* Sieb. & Zucc. [Japanese red pine], *Pinus ponderosa* P. & C. Lawson [Western yellow pine, imported], *Tsuga sieboldii* Carr. [Hemlock] and *Abies firma* Sieb. & Zucc. [Momi fir]. The bedding had been about 10 cm thick in pens of beef cattle for a week. During the composting process, the material had been tilled once each month.

Experimental preparation and conditions

Collected cattle bedding compost was mixed with sawdust (*Quercus* sp) in the ratio of 25, 50, 75 and 100% (by weight) (compost/sawdust 1:3, 1:1, 3:1 and 1:0) and each mixture was supplemented by 20% (w/w) while 100% was supplemented by equal ratio of supplement (1:1) and mixed thoroughly. A mixture of wheat, rice and barley bran was used as supplements. The completed substrates were adjusted to 65% moisture with tap water and the pH of substrate was measured and observed between pH 6.5 to 7.4. Seven hundred grams of wet substrate were placed in polypropylene bags (100 g was set into the Petri dish for mycelial growth measurement) and sterilized at 121 ˚C for 45 min, then allowed to cool to the desired inoculation temperature and then 3% sawdust spawn was inoculated (A 5 mm disc was inoculated for mycelium diameter measurement). The inoculated substrate was incubated at 25 ˚C in darkness. Mycelial growth (diameter) was measured every week at intervals for three weeks.

After complete colonization, bags were cased with sterilized soil (2–2.5 cm, pH 6.5–7.5), and transferred into the mushroom house where the temperature was maintained at 23±1 ˚C, the humidity was maintained at 90–95%, CO_2 concentration was maintained at 1000 ppm by automatic aeration, and a light intensity of 500 lux was automatically changed from dark to light (12/12) by way of a fluorescent lamp. The data collection technique pertaining to colonization, primordial initiation, fruit body formation (day and yield), biological efficiency and mushroom size, as well as element analysis methods of substrate ingredients, were the same as the Pokhrel *et al*. (2006). pH and Ec were determined by using the HORIBA pH METER F–21 and CM–60V conductivity meter, respectively. Water holding capacity (WHC) was calculated from the following equation: W= (wet mass ×100/dry mass). The results of various nutrients of substrate and supplements are presented in Table 1.

RESULTS AND DISCUSSION

Effect on vegetative growth

Weekly mycelial extension on differently mixed substrates is given in Table 2. Mycelial extension varied in different ratio of compost during incubation. The mycelial extension was significantly different in the substrate with different concentrations for one to three weeks of incubation. The substrate containing 75% compost was best for mycelial extension followed by 100, 50 and 25%, respectively. Increasing the concentration of compost resulted better for mycelial extension. There are several factors which affect the vegetative growth and increasing concentration of compost showed better results for growth, and this could be due to high mineral nutrients, high moisture accumulation

Table 1. Nutrients contained in different substrate and supplements used

Ingredients	Nutrient elements (%)						
	pH	Ec	Moisture %	WHC %	N	K	P
Substrates							
Compost	8.3	0.658	46	262.9	1.9±0.1	2.0	0.8
Sawdust	–	–	–	–	0.2±0.0	0.2	.1
Supplements							
Barley bran	–	–	–	–	3.0±1.1	0.1	0.3
Rice bran	–	–	–	–	2.1	1.6	1.6
Wheat bran	–	–		–	2.3±0.4	0.7	0.3
Casing soil	7.5	0.272	60	62.9	–	–	–

Values are the mean±SD.

Table 2. Comparison of weekly mycelial extension of *A. blazei* on various concentrations of compost

Compost %	Nutrient elements (%)		
	1	2	3
25	9.8±1.2	23.2±3.2	42.1±1.9
50	12.0±0.9	33.2±1.0	59.8±1.0
75	19.5±1.5	58.9±1.3a	79.2±1.6
100	14.9±1.7	41.5±2.3	73.8±1.3

Values are the mean±SD of mycelial growth measurement (mm).

and rise in temperature inside the cattle bedding compost substrate during fungal growth. It is reported that mycelial growth was best at 69.9% moisture. Moreover, mycelial growth is very vigorous and fast, because it emits heat which is rather suitable in cattle compost. Therefore, increasing concentrations of compost promote the rate of mycelial growth.

Effect on spawn run, primordial initiation and fruit body development

Spawn run was completed when vigorous mycelial growth reached the surface. The result of time period to spawn run, primordial initiation and fruit body development are presented in Table 3. The most rapid spawn run (substrate colonization) took place in 38 days with 75% compost, which was the fastest among the different combinations used. It was followed by 41 days with 100% compost, and 44 days and 48 days with 50% and 25% compost, respectively. Mycelial extension was positively correlated to spawn run, which corresponds with the result of day to primordial initiation and day to yield.

Table 3. Total day to spawn run, primordial initiation and fruit body development of *A. blazei*

Compost %	Spawn run	Primordial initiation	Fruit body development	Total day to first crop
25	48	24	29	77
50	44	19	23	67
75	38	13	16	54
100	41	14	18	59

The results of primordial initiation and fruit body development (first crop) were recorded after the colonized bags were transferred into the growing house. Primordial initiation on compost containing 75% was observed at 13 days and a first crop was obtained at 16 days (Fig. 1). In the case of 100% compost, primordial initiation appeared on day 14 and first crop was obtained on day 18 (Fig. 2). Similarly, primordial initiation was recorded from 50% and 25% compost on day 19 and 24, respectively, whereas first crop was obtained on days 23 and 29, in respective order. A higher concentration of compost (75%) accelerates the mycelial extension and spawn run, primordial initiation and early fruiting; however, a higher concentration of supplement (100% compost with equal supplement) activated a higher yield and an enhanced mushroom number. Supplements serve as nutrients to provide a more optimum growth medium (Royse, 1997). There were no significant differences in primordial initiation and fruiting period in between 75 and 100% compost. Tan (1981) reported that *Pleurotus osteratus* and other species on cotton waste took 2–3 weeks for fruit body formation after spawn running, with a similar result also reported by Baysal *et al.* (2003) on oyster mushrooms. This study concluded that the first flush of mushrooms harvested from two to four weeks after transferred into mushroom house were better, which is consist with several reports.

Fig. 1. Fruit body formation on 75% compost.

Fig. 2. Fruit body formation on 100% compost.

Effect on yields

The effects of compost concentration on productivity were determined. Mushrooms were harvested before the pileus (cap) was fully extended. The button stage with intact veil membrane enclosing the gill is the most desirable stage. Average mushroom yield of two flushes from the four replicates are given in Table 4. The two flushes crops gave a maximum yield of 173.9 g on 100% compost followed by 155.4 g on 75% compost with a corresponding biological efficiency of 70.9% and 63.4%, respectively. The lowest yield 70.0 g was obtained from 25%, whereas moderate yield of 110.4 g on 50% compost with biological efficiency of 28.6 and 45.1, respectively. Compost with 100 and 75% were superior to all the compost concentrations. Our results indicated that the addition of higher supplements increased the mushroom yield, whereas mushroom size was better from 75% compost. Furthermore, both higher amounts of compost and supplements are able to improve the growth and yield of this mushroom.

Slower mycelial extension, spawn running, fruit body formation and yield were observed where the concentration of compost was low. The first flush was observed between 54 to 77 days of cultivation. The second flush of mushrooms was obtained after a month

Table 4. Cumulative mushroom yield (fresh wt. (g)/700 g wet substrate), biological efficiency and mushroom size of *A. blazei* on various concentrations of compost

Compost %	Fresh weight of mushrooms by flushes (g)			BE (%)	Size g/mushroom
	First	Second	Total fresh weight (g)		
25	30.9±4.0	39.1±9.2	70.0±8.6	28.6	30.1±1.3
50	39.9±12.6	70.5±9.1	110.4±5.6	45.1	41.1±1.7
75	75.5±3.6	79.9±5.4	155.4±8.3	63.4	59.3±18.6
100	110.5±7.1	63.4±6.0	173.9±11.5	70.9	34.26±3.6

Values are the mean±SD.

of first flush. Interestingly, the maximum yield was harvested during the first flush (about double) when compost was enriched with higher amounts of supplements. This result is not consist with reports from Stamets, (2000). Likewise, more or less equal mushroom yield was obtained from both flushes with 75% compost, whereas a higher amount of yield was harvested in the second flush with 50% and 25% compost in the respective order. This lower concentration of compost to the substrate significantly decreased mushroom yield probably due to the low nutrient content in substrate. Therefore, fresh sawdust or non–fermented substrates are not very appropriate in the cultivation of this fungus, as it cannot easily degrade complex lignocelluloses components. The increasing concentration of cattle bedding compost is better for its growth, and development showed that this fungus easily thrives on sole cattle bedding compost. Generally, higher concentrations of livestock manure did not produce better results due to the presence of elevated nitrogen concentrations already present in it, but this statement did not apply to this mushroom. Our result was not consistent with the result of Baysal, *et al*. (2003) and Laborde *et al*. (1984). The selection of substrate for the cultivation of mushroom is largely determined by the abundance and cost of the substrate. Two factors are required of substrate for economic mushroom production: low cost and good yield (Pokhrel *et al*., 2006). Thus, the substrate used in the experiment was made relatively economically and was an easily available material. The yield with the 100 and 75% compost were quite acceptable.

CONCLUSIONS

In conclusion, *A. blazei*, a choice edible mushroom of biomedical importance, is a second stage decomposer with simple carbohydrate metabolism. It grows in soil rich in lignicolous debris, in mixed woods, well–composted soil, and along forest edges. This mushroom is a complex saprophyte and prefers composting soil rich in plant debris. It also grows in well–manured grasslands. Cultivation of this species, primarly based on fermented compost. In this study, we successfully found a great possibility for its cultivation in beef cattle bedding compost–a refractory waste, with massive potential.

Appropriate concentration and supplements can enhance mushroom quality and yield while creating a shorter production time. High compost concentration gave us better results for mycelial extension and spawn run, earlier primodial initiation and fruit body development with bigger sized mushrooms; however, a higher percentage of supplement stimulated higher yield and number of fruit bodies.

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