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Original article

Cultivation of oyster mushroom (*Pleurotus ostreatus*) on fermented moso bamboo sawdust

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ABSTRACT

In this study, the potential of Moso bamboo sawdust as an alternative substrate for the cultivation of oyster mushroom (*Pleurotus ostreatus*) was investigated. Oyster mushroom was cultivated on 2-months fermented bamboo sawdust (BS) and mixed with rice bran (RB) and sweet potato *schochu lees* (SPSL) as additional nutrition. The growth condition, morphological properties, nutritional, mineral contents and free amino acid content of mushroom cultivated were evaluated. Based on the results, the total growth days on the bamboo media were between 3 and 7 days shorter than the conventional media. The bamboo media mixed with RB had better yield and fruiting bodies at 97.9 ± 3.9 g/bottle and 33.6 ± 4.2 no/bottle, respectively. Furthermore, the addition of SPSL to BS increased the protein content and decreased the carbohydrate contents of fruit bodies. In addition, the free-amino acids in the fruit bodies from the bamboo media were 1.5 times higher than the conventional media, which potentially added the higher value to usual mushrooms. Hence, oyster mushroom cultivation can be an alternative method to reduce bamboos wastes in Japan and would promote sustainable growth in agricultural industry.

1. Introduction

Moso bamboo (*Phyllostachys pubescens*) is a large woody bamboo that has ecological, economic and cultural value in Asia and accounts about 70% of the total bamboo growth area (Peng et al., 2013). In western Japan, Moso bamboo forests have been expanding which can raises concerns about possible changes in terrestrial water and carbon cycles (Komatsu et al., 2012). As reported by Tanaka et al. (2013), bamboo shoot skin is used as a preservative container to maintain the quality of tea leaves in China and has been used to wrap rice balls and meats in Japan. Fukuoka prefecture is the major cultivation area of bamboo shoots in Japan. Efficient and sustainable utilization of 5 years old bamboos is a serious concern. Although, the old bamboos are used in traditional craftworks or as pulp, charcoal or livestock feed, but the demand has not exceeded the supply. Bamboo contain cellulose, hemicellulose, and lignin which are similar to conifer sawdust and can be used as media for edible mushroom cultivation. Based on the previous finding, oyster mushroom can be cultivated on variety of substrates that contain lignin and cellulose. Therefore, it have a significant role in managing agricultural wastes which have become a critical issue for disposal (Marlina et al., 2015).

Oyster mushroom is widely studied as it has flavour and contains nutritional and medicinal proprieties. As nutrient source of protein, carbohydrates, vitamins, calcium and iron, it can be used in a variety of applications (Corrêa et al., 2016). For instance, oyster mushroom can be used for medical purposes which can increase the immune power of our body against diseases. Therefore, it can be used as a dietary supplement (Khatun et al., 2015).

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Recently, oyster mushroom cultivation on various lignocellulosic materials has been investigated by a number of researchers (Pereira et al., 2017; Postemsky et al., 2017; Sardar et al., 2017). This species is rich in protein sources and minerals such as phosphorus, calcium, iron, potassium and sodium (Szabová et al., 2013). Furthermore, proteins in oyster mushroom has the nutritional requirements of all essential amino acids for adults (Carrasco-González et al., 2017). Oyster mushroom has been cultivated using various agro wastes such as rice straw and wheat straw (Yang et al., 2013; Rezania et al., 2017), date-palm leaves (Alananbeh et al., 2014), empty fruit bunch (Marlina et al., 2015), olive cake (Ananbeh and Almomany, 2005), tomato tuff (Ananbeh and Almomany, 2008), banana leaves and pine needles (Ananbeh, 2003) and sugarcane bagasse (Hasan et al., 2015). Mushroom cultivation on Moso bamboo is an economic approach in agro-industry as the residues is readily available. In mushroom cultivation, typical commercial industry focused on profits gain in terms of most effective, low cost and locally available mushroom substrates materials (Fatriasari et al., 2016). As Moso bamboo can be one of attractive substrates for oyster mushroom cultivation, therefore the aim of this study was to investigate the feasibility of using Moso bamboo as a medium substrate for oyster mushroom cultivation.

2. Materials and methods

2.1. Proximate analysis of medium materials

The components of base materials and nutrient supplements have been evaluated for the following parameters: the moisture content using normal pressure heating and watering method as described by (Alam et al., 2008). Crude protein was determined using Kjeldahl method with a nitrogen/protein conversion factor of 6.25 (Shumaila and Mahpara, 2009). The ash content was measured by method (No. 924.05), and ether extract (EE) using Soxhlet diethyl ether sampling method (No. 920.39), while and crude fiber was calculated by filtration method) (No. 978.10) (AOAC, 1995). Nitrogen-free extract was calculated as described by (Segato et al., 2008).

$$100 - (\text{Moisture content } \% + \text{crude fiber } \% + \text{crude protein } \% + \text{ether extract } \% + \% \text{ ash}) \quad (1)$$

Minerals composition of phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) were determined by atomic absorption spectrometry (AAS) (No. 968.08) (AOAC, 1995). The cedar sawdust was watered and kept for 6 months. Moso bamboo sawdust was fermented for 2 months indoors before media preparation.

2.2. Cultivation test of oyster mushroom

2.2.1. Tested mycelia

Oyster mushroom (*Pleurotus ostreatus*) mycelia (H67th) which provided by Kinox cooperation, (Sendai Japan) was used for the cultivation. Their outgrowth term was relatively short and they were endowed with antibacterial characteristics (Katya et al., 2016).

2.2.2. Media preparation and inoculation

To determine the effectiveness of bamboo (*Phyllostachys pubescencs*) sawdust (BS) as an alternative substrate for oyster mushroom cultivation, various combinations of BS, rice bran (RB) Sweet Potato Schochu Lees (SPSL) were used (Table 1). BS was prepared with rice bran/*shochu lees* as experimental groups, and conifer sawdust (Japanese cedar, *Cryptomeria japonica*) with rice bran/*shochu lees* as control groups. The media materials and nutrient supplements were prepared at the rate of 46%: 50% (dry weight) for each group. Then the shell fossil (uncoagulated shell grit aragonite lime) was added at 4% (dry weight) in order to reach to pH 5.5. Media were prepared in a mixer by filling with the base materials and the nutrient supplements and were stirred for 10 min. Then, shell fossil were added to the mixtures and the substrates were humidified with water which resulted in 63% water content in the medium. Water content was calculated after media substrates preparation. Then, the substrates were stirred for 20 min. The prepared media were packed into polypropylene culture pots (capacity: 850 ml, diameter: 58 mm with total weight of 600 g). The packed media were autoclaved at 121 °C for three hours, and cooled at room temperature. Each pot was inoculated with 10 g which is equivalent to 6% (dry weight) oyster mushroom mycelium H67th in a clean room and experiments were conducted with three replication. In this study, CS + RB treatment was considered as a control.

2.2.3. Culturing conditions

The inoculated culture pots were maintained at 22 ± 2 °C temperature and 65 ± 5% humidity in incubation room for 28 days. After the scraping and treatment with tap water (*known as Kinkaki treatment*) for 3 h, the pots were transferred to a another room and maintained at 14 ± 1 °C and 90 ± 5% humidity to allow for the primordial formation of fruiting bodies. The spawn percentage was maintained at 6% (dry weight). The room was lightened for 8 h/day with 100 lux fluorescent lights.

2.3. Growth conditions

2.3.1. Yield of fruit bodies

The fruiting bodies were harvested when reached to a diameter between 40–50 mm. Various parameters such as the *Kinkaki* treatment to harvest, total culturing days, yield (raw weights), number of fruit bodies and the biological properties were evaluated.

2.3.2. Analysis of the components of the fruiting bodies

Main components, minerals and heavy metals in the fruiting bodies were evaluated in the same analysis as the media materials mentioned in Section 2.1. Amino acids in fruiting bodies were determined by post-column derivative high performance liquid chromatography (HPLC) (Kim et al., 2009).

2.3.3. Statistical analysis

Completely randomized design was used to arrange the treatments. Three agro-wastes bamboo sawdust (BS), conifer sawdust, sweet potato *schochu lees* (SPSL) and rice bran (RB), two ratios (46:4%, 50%), 6% spawning rate (of total dry weight in each pot) two replicates were used. The experiment was repeated twice and the data were analysed using Minitab16 Statistical Software.

3. Results and discussion

3.1. Components of raw materials used in mushroom media

Evaluation of component properties is essential to observe the phenomenon of media composition before and after cultivation. Table 2 shows the component properties of raw materials derived from bamboo sawdust (BS), conifer sawdust, sweet potato *schochu lees* (SPSL) and rice bran (RB). As expected, BS as the base material had more nitrogen-free extract (NFE) (45.1 g/100 g) and less crude fiber (50.8 g/100 g) when compared to conifer sawdust. BS had high amount of NFE which consists plenty of single oligosaccharides and starch that are easily decomposed by mycelia (Zullaikah et al., 2015). The crude protein content of conifer saw-

Table 1
Media composition for cultivation.

Test group	Media composition (dry weight%)					Packed weight (g)	Water content (%)
	Base materials		Nutrition		Other		
	CS	BS	SPSL	RB	SF		
1 CS + RB (BL)	46			50	4	600	63.8
2 BS + RB		46					63.0
3 CS + SPSL			50				62.8
4 BS + SPSL			50				63.9

CS: Conifer Sawdust, BS: Bamboo Sawdust, SPSL: Sweet Potato Schochu Lees (dry); RB: Rice Bran, SF: Shell Fossil.

Table 2
The main components and minerals in each source material.

Materials	CP	EE	CF	CA	NFE	P	K	Ca	Mg
	(g/100 g dry weight)					(mg/100 g dry weight)			
Bamboo Sawdust	1.5	0.9	50.8	1.7	45.1	24	508	24	39
Conifer Sawdust	1.1	1.1	75.9	2.5	19.4	8.6	5.3	265	32
Sweet Potato Shochu Lees (dry)	24.7	3.9	11.8	4.7	54.9	364	1390	717	98
Rice Bran	15.8	21.8	8.3	10.5	43.6	860	1697	40	950

dust grew only 1.1 g/100 g compared to BS which is 1.5 g/100 g. Compared to bamboo sawdust, component properties of conifer sawdust were quite low amount except for ether extract (1.1 g/100 g), crude fiber (75.9 g/100 g), crude ash (2.5 g/100 g), and calcium (Ca) (265 g/100 g). In addition, BS comprised significant amount of potassium (K) 50.8 mg/100 g and lower amount of Ca (24 g/100 g).

In order to obtain optimized composition for oyster mushroom cultivation, nutrient supplements is needed to stabilize the macro and micronutrients of base materials. In terms of nutrient supplements, SPSL had high level of crude protein (24.7 g/100 g), crude fiber (11.8 g/100 g), NFE (54.9 g/100 g), and Ca (717 g/100 g). *Shochu* is a traditional Japanese distilled liquor, made by rice *koji (Aspergillus kawachii)*, which is *koji* mould grown on rice grain. Rice *koji* is an essential ingredient of Japanese liquors such as *shochu* and plays as a source of enzyme to degrade starch (Shiraishi et al., 2016). In contrast, RB contains higher amount of ether extract (21.8 g/100 g), crude ash (10.5 g/100 g), phosphorus (P) (860 g/100 g), K (1,697 g/100 g) and magnesium (Mg) (950 g/100 g) compared to SPSL. Although, both materials contain enough amount of K as to allow for the formation of fruit bodies, the quantity of other mineral elements are much different.

3.2. Cultivation of oyster mushrooms

Table 3 demonstrates the growth conditions for different test groups. The growth days for mycelial were more in the *shochu lees*/bamboo sawdust groups (Group 4) compared to the control group. This is due to the presence of some mycelial growth inhibitors such as tannin and phenol in bamboos (Nirmala et al., 2014), and fatty acid ester in *shochu lees* (Shiraishi et al., 2016). However, after the incubation, both the days from *Kinkaki* treatment to harvest and the total growth days were shortened to 3–7 days compared to the control group. Mycelia growth successfully accelerated with the presence of the nucleic acid-related substances (Ohga et al., 2003). SPSL contain 1.4 times the nucleic acid-related substances of RB since it contains *koji* from *shochu lees* and yeast. BS which have been fermented for 2 months, contains microbe-derived nucleic acid substances, which seem to have shortened the growth. The mycelial growth inhibitors in Group 4 must have been decomposed by mycelia during the incubation.

Table 4 shows the morphological properties, the number, and the yield (raw) of mushrooms from each group. In test group 2, the samples showed high yield of mushroom compared to other mixtures where the yield was 97.9 ± 3.9 g and number of fruiting bodies were 33.6 ± 4.2. Morphological properties did not show any remarkable differences from the control group except the top layers were slightly thinner (9.1 ± 1.2 mm). SPSL, one of Japanese local food wastes was as a qualified nutrient supplement for growing edible mushrooms with a high yield (Yamauchi et al., 2011, 2013). Present of SPSL in the media had significant yield for mushrooms which test group 3 and 4 obtained higher yield compared to control group (test group 1) at 91.5 ± 7.4 g and 94.2 ± 3.9 g, respectively.

Table 5 shows the analysis of the main components and the minerals of fruit bodies from each test group. In test groups 3 and 4 with SPSL as a nutrient supplement, the protein content in the fruit bodies was higher than RB as a nutrient supplement by 43.8 g and 31.1 g, respectively. Meanwhile, the carbohydrate content slightly lower than media supplemented by RB (Group 1 and 2). Protein content in the oyster mushroom's fruit bodies increased when they were grown with high-protein nutrient supplements compared to control groups with relatively less protein nutrients (Kawai et al., 1994). Protein content in SPSL was 24.7 g/100 g

Table 3
Growth conditions for different test groups.

Test group	Days for mycelial growth	Incubation days	Days from Kinkaki treatment to harvest	Total growth days
1 CS + RB (BL)	15.4 ± 0.4^{a}	28	20.2 ± 2.4^{c}	48.2 ± 2.4^{c}
2 BS + RB	18.0 ± 0.7^{b}		16.8 ± 1.6^{bc}	44.8 ± 1.6^{bc}
3 CS + SPSL	17.6 ± 0.5^{b}		12.0 ± 0.7^{a}	40.0 ± 0.7^{a}
4 BS + SPSL	18.2 ± 0.4^{b}		13.2 ± 0.8^{ab}	41.2 ± 0.8^{ab}

Values are mean ± standard deviation. **Abbreviations (CS: Conifer Sawdust, BS: Bamboo Sawdust, SPSL: Sweet Potato *Schochu Lees* (dry), RB: Rice Bran Significant difference between the values with different alphabets at the 5% level (Tukey's test) (Tukey, 1949).

Table 4
Mean of the biological properties different measurements conducted for the test groups.

Test groups	Quality				
	Maximum diameter of Pileus (mm)	Maximum thickness of Pileus (mm)	Maximum diameter of Stalk (mm)	Fruiting bodies (No./bottle)	Yield (raw) (g/bottle)
1 CS + RB (BL)	45.5 ± 1.4	11.7 ± 1.2^{ab}	14.2 ± 1.0^{b}	30.8 ± 7.0	90.3 ± 6.8
2 BS + RB	44.1 ± 0.8	9.1 ± 1.2^{a}	10.4 ± 2.5^{a}	33.6 ± 4.2	97.9 ± 3.9
3 CS + SPSL	48.4 ± 4.1	13.6 ± 2.1^{bc}	10.8 ± 0.6^{ab}	29.4 ± 4.0	91.5 ± 7.4
4 BS + SPSL	49.1 ± 4.4	16.0 ± 1.6^{c}	12.1 ± 0.9^{ab}	27.0 ± 2.4	94.2 ± 3.9

Significant difference between the values with different alphabets at the 5% level (Tukey's test).
Values are mean ± standard deviation.

Table 5
Main components and the minerals composition of fruit bodies from each test group.

Test group	Protein	Lipids	Carbohydrate	Ash	P	K	Ca	Mg
	(g/100 g dry weight)				(mg/100 g dry weight)			
1 CS + RB (BL)	35.1	1.8	56.8	6.3	1171	2523	N.D	117
2 BS + RB	33.6	1.8	57.5	7.1	1150	2655	N.D	106
3 CS + SPSL	41.1	3.2	48.3	7.4	1474	2842	N.D	168
4 BS + SPSL	43.8	3.4	43.8	9.0	1573	3707	N.D	146
- Seventh Revised Edition*	31.1	2.8	58.6	7.5	943	3208	9.4	142

* Standard tables of food composition in Japan. All the experiments were conducted in duplicate.

Table 6
Free-amino acid contents of the fruiting bodies from each test group.

Test groups	Essential							Semi essential		Non-essential								Total
	Leu	Met	Ile	Val	Thr	Phe	Lys	His	Arg	Gly	Ser	Glu	Pro	Tyr	Gln	Ala	Asp	
	(mg/100 g dry weight)																	
1CS + RB(BL)	316	84	155	201	234	272	398	138	458	219	269	387	216	248	351	1051	39	5035
2 BS + RB	378	169	349	481	435	463	401	211	442	219	504	513	565	435	754	1036	145	7500
3 CS + SPSL	252	181	135	180	189	216	171	225	1036	171	243	955	126	279	1048	946	54	6407
4 BS + SPSL	453	98	301	355	398	402	491	218	599	370	406	894	301	407	602	1126	111	7532

*All the experiments were conducted in duplicate.

(dry) while in RB was 15.8 g/100 g (dry). Lower amount of carbohydrate content might be caused by the absorption of nitrogen compounds in SPSL.

The minerals in the fruit bodies for all test groups were K > P > Mg in decreasing order of abundance. The value of K was 38–41% of the ash. One of characteristics of mushrooms is possess high amount of potassium and have been distributed unevenly within cap, stipe, spore-forming part and spore (Kalac, 2012). Ca was not found in all test groups of the fruit bodies. The use of BS as a replacement for the conifer sawdust did not affect the main components nor the minerals of fruit bodies.

Amino acids are generally known as protein regulation metabolism which effectively repairs human body metabolisms (Murakami et al., 2012). All test groups were analyzed for free-amino acids contents in the fruit bodies of oyster mushrooms (Table 6). The amount of free-amino acids in the samples from test groups 2 and 4 was 1.5 times higher than the control group (Group 1). The details on the free-amino acids were as follows: Alanine (Ala) and Glutamic acid (Glu), Arginine (Arg) and Glutamine (Gln) were higher than other amino acids, since they are generated by transamination at an early stage. In the test groups 2 and 4, Isoleucine (Ile), Valine (Val), Threonine (Thr), Phenylalanine (Phe) appeared to be the essential amino acids; Serine (Ser), Glutamic acid (Glu), Proline (Pro), Tyrosine (Tyr), Glutamine (Gln), Aspartic acid (Asp) are non-essential amino acids and are higher than Group 1. Optimizing the amino acid supply required for body protein growth with limited impacts on the environment is a generic imperative in all production systems (Kaushik and Seiliez, 2010). Based on the results it is concluded that BS promotes the production of free amino acids in oyster mushrooms compared to conifer sawdust substrates.

4. Conclusion

Conifer sawdust or broad-leaved tree sawdust is generally used for mushroom cultivations. Bamboo sawdust contains more nitrogen-free extracts (NFE) and less crude fiber compared to conifer sawdust. Based on the obtained results, bamboo sawdust with sweet potato *shochu lees* or mixture of rice bran shortened the total growth days by 3–7 days compared to the control group. Bamboo sawdust as the substitution for conifer sawdust did not affect the main components or the mineral compositions in the fruit bodies. The oyster mushrooms grown in the media with bamboo sawdust contained higher amount of free-amino acids than those from the control group of conifer sawdust. From these results, we concluded that bamboo sawdust can be used as the base material for the oyster mushroom cultivation. In future, the investigation of Moso bamboo sawdust could be applied in Malaysia, Thailand and Indonesia with their subtropical climate in order to extend their usage.

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