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Availability of Sikhae Factory Wastewater as a Submerged Culture Medium for *Lentinula edodes*

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Sikhae is a Korean traditional beverage of saccharified rice. Its factory waste (SFW) is usually thrown away instead of being used. We developed a cheap substrate of SFW for use in liquid spawn that is known for its higher fruit body yields than grain spawn in sawdust cultivation. Mycelia of *Lentinula edodes* ASI 3046, which is regarded as the most suitable strain for sawdust cultivation, were cultured on six kinds of previous known media and SFW. As the seven kinds of media were applied, a Sikhae Factory Waste (SFW) was most excellent in growth. The dried mycelial weight in SFW was almost four times as much as that in the other media. In the flask culture, optimum culture conditions for the mycelial growth were obtained after 13 days of cultivation at media volume of 100 ml, 100 rpm, initial pH 4.5, and 25°C. The best mycelial growth was observed when $MgSO_4 \cdot 7H_2O$ and D-sucrose were added as a supplement in SFW. SWM must be a remarkable medium for *L. edodes* because of its simple preparation and low cost.

KEYWORDS: *Lentinula edodes*, Submerged culture, Sikhae factory wastewater

About two thousand tons of factory waste from sikhae, a Korean traditional beverage of saccharified rice, is produced each year and is thrown away instead of being used (Korean Food Annual Analysis, 2000). This wastewater contains the residue of germinating barley and water used in the saccharification steps of sikhae production. We expected that sikhe factory waste (SFW) contained abundant sugars and other nutrients without harmful ingredients. Therefore, we inspected the availability of SFW as a medium for mycelial cultivation of mushroom.

Over the past two decades there has been interest in the optimization of physical, chemical and biological treatment to establish a mushroom cultivation process form the use of abundant food and agricultural industry wastes (Isola, 1996). The general method for the cultivation of *Lentinula edodes* (Shiitake mushroom) was to inoculate broad-leaved deciduous wood logs with the mycelia and harvest the mushrooms for four to five years. The development of an alternative cultivation method, sawdust cultivation, was stimulated by the long fruiting cycle and low yield of mushrooms in wood cultivation. The traditional method involved pretreatment of sawdust substrate, inoculation of spawn, spawn run, and fruiting.

The objective of the present study was to develop a cheap substrate for use of liquid spawn that is known for higher fruit body yield than grain spawn in sawdust cultivation (Genshiro *et al.*, 1996; Lee *et al.*, 1998).

Over the years, interest has been growing in the application of food industry wastewater as a complex medium for mushroom cultivation. Mushrooms are considered to be one of the most efficient producers of food protein from worthless food and agricultural industry wastes (Jo *et al.*, 1995; Jung *et al.*, 1996).

Several past studies have demonstrated the availability and the advantages of using soy milk waste (Cheung, 1997), jute waste (Basak *et al.*, 1996), fruit processing wastes (Zuoxing, 2000), olive oil factory waste (Sandra, 1999), sugar cane waste (Shin *et al.*, 1998; Mohammed *et al.*, 1994), coffee industry residues (Fan *et al.*, 2000), grape waste (Ferrer *et al.*, 2001), and other materials, but none has examined that of SFW. This study will establish information about SFW utilization as a substrate of the submerged culture for mushroom cultivation, especially *L. edodes*.

Materials and Methods

Fungal strain. *L. edodes* ASI 3046 known for good strain of sawdust cultivation was used and maintained at 4°C on potato dextrose agar (PDA) slants with periodic transfers.

Preparation of SFW. A crude SFW was sterilized 121°C for 60 min and cooled. The SFW was incubated at 45°C on a rotary shaker for 2 h with 0.5% viscozyme (Novo-Nordisk, Denmark) and incubated at 90°C for 2 h with 0.5% thermoamylase (Novo-Nordisk, Denmark). Before using the SFW, it was sieved to select smaller particle sizes than 300 by the standard sieve No 50 (KS/ASTM mesh No 50, Daihan science , Korea).

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Culture. A pure mycelial culture of *L. edodes* was maintained on potato dextrose agar (PDA) with periodic transfers. The PDA medium was sterilized in an autoclave at 121°C for 15 min. Then, the PDA was poured into Petri dishes. The dishes were inoculated with an 8 mm (diameter) disc of *L. edodes* mycelium (Bajpai *et al.*, 1993). After seven days, of incubation the mycelial diameter was measured while mycelia density was compared visually. Eight carbon sources such as D-fructose, D-galactose, D-glucose, sucrose, maltose, starch, D-mannose and cellulose were used as additives. 10 g/*l* of carbon source or none (for control) was added to the basal medium which consisted of SFW (26.5 g/*l*) and $MgSO_4 \cdot 7H_2O$ (0.5 g/*l*). For the nitrogen source test, the seven nitrogen sources such as $Ca(NO_3)_2$, KNO_3, NH_4NO_3, $NaNO_3$, tryptone, yeast extract and peptone were tested as additives. 2 g/*l* of nitrogen source or none (for control) was added to the basal medium. For the mineral source test, the seven mineral sources such as $MgSO_4 \cdot 7H_2O$, $ZnSO_4 \cdot 7H_2O$, KCl, NaCl, $CaCl_2$, $CuSO_4$, and $FeSO_4$ were tested as an additive. 0.5 g/*l* of mineral source or none (for control) was added to the basal medium. 100 m*l* of this medium was dispensed into a 250 m*l* flask and adjusted to pH 4.5. This flask was autoclaved at 121°C for 15 min. Each medium was inoculated with seven mycelial discs (each 8 mm diameter) (Bajpai *et al.*, 1993) and incubated at 25°C on a rotary shaker for 13 days with 100 rpm. Results were mean of triplicated samples.

Measuring mycelial growth. Mycelia in liquid media were collected by the standard sieve No 50 (KS/ASTM mesh No50, Daihan science, Korea), rinsed with distilled water three times, dried at 100°C for 24 hours and measured for dry weight (DW). Mycelial growth on agar plate media was measured in four radius lengths of a colony from the outer edge of the mycelia (Yoshie, 1994). The growth of the fungus was determined by the mycelial dry weight method.

Results and Discussion

Mycelium of *Lentinula edodes* ASI 3046, which is regarded as the most suitable strain for sawdust cultivation (Kim *et al.*, 1987), was cultured on six kinds of previous known media and SFW. Out of seven kinds of media were applied, SFW showed the most excellent in growth. The dried mycelial weight in SFW was almost four times as much as that in the other media (Table 1).

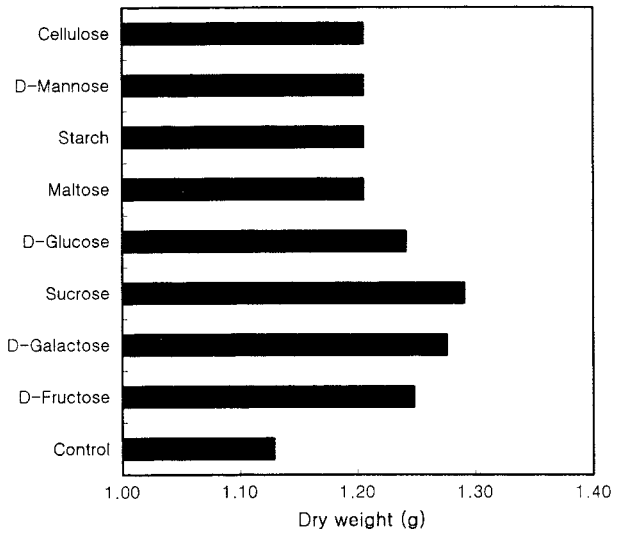

Fig. 1. Effect of carbon source on mycelial dry weight (g) of *Lentinula edodes* after 13 days of incubation in basal media (100 m*l* SFW).

Figure 1 shows the mycelial growth of *L. edodes* on 8 carbon sources. As the carbon sources were added, the disaccharide sucrose showed the best growth (1.291 g dry weight), followed by the D-galactose (1.276 g dry weight) and D-fructose (1.248 g dry weight). With all tested sugars, mycelial growth of these was sufficiently better than that of control.

In Fig. 2, tryptone showed the best growth (1.109 g dry weight), followed by $Ca(NO_3)_2$ (1.092 g dry weight), KNO_3 (1.014 g dry weight), yeast ex. (0.981 g dry weight) and peptone (0.981 g dry weight). All of the seven nitrogen sources showed worse mycelial growth than the control. We decided that no nitrogen source in SFW is needed. Ten of the C/N ratio on element analysis of SFW meant that it might have a sufficient nitrogen source (data not shown). We did choose, as seen in Fig. 7, only the cheap

Table 1. Comparison of selected media for liquid culture of *Lentinula edodes*

	Modified Hamada	PDA/PDB	GPM	MYG	Czapex-dox	OakMC	SFW
1	40	42 PDA	42	42	27	45	35
2	83.5	128 PDB	85.5	86	75	88.5	458.5
Density	+	+	++	++	+	++	+++

1: diameter of mycelium in plate (mm), 2: dried weight of mycelia in submerged culture (mg/100 m*l*). modified Hamada: Glucose 1%, Yeast ex. 0.5%, KH_2PO_4 0.1%, $MgSo_4 \cdot 7H_2O$ 0.05%, pH 5.1, PD: Potato starch 0.4%, Dextrose 2%, pH 6.1, GPM: Glucose 1%, Peptone 0.1%, Malt ext. 2%, pH 5.9, MYG: Malt ext. 0.5%, Yeast ex. 0.5%, Glucose 2%, pH 4.8, Czapex-dox: Sucrose 3%, $NaNO_3$ 0.2%, K_2HPO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.05%, KCl 0.05%, $FeSO_4 \cdot 7H_2O$ 0.001%, pH 5.2, OakMC: Glucose 5%, Peptone 0.25%, Yeast ex. 0.25%, KH_2PO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05%, $CaCl_2 \cdot 2H_2O$ 0.05%, $FeSO_4 \cdot 6H_2O$ 0.001%, $MnCl_2 \cdot 4H_2O$ 0.00072%, $ZnCl_2$ 0.0004%, $CuSO_4 \cdot 5H_2O$ 0.0001%, pH 5.3, -- Add 2 g agar in above media for plate culture.

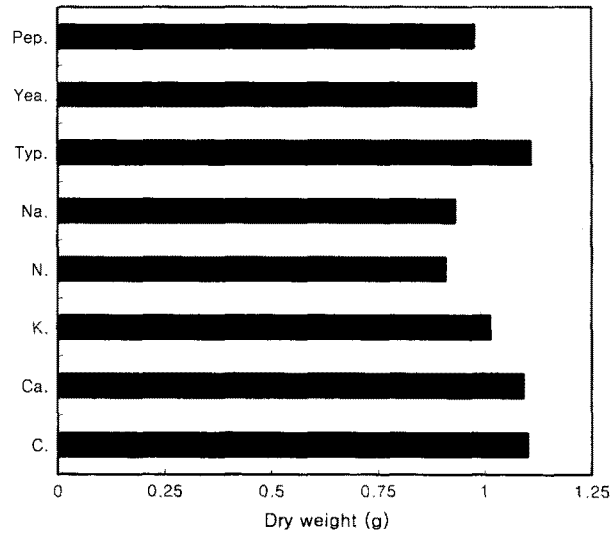

Fig. 2. Effect of nitrogen source on mycelial dry weight (g) of *Lentinula edodes* after 13 days of incubation in basal media (100 m*l* SFW). C: Control, Ca.: $Ca(NO_3)_2$, K.: KNO_3, N.: NH_4NO_3, Na.: $NaNO_3$, Typ.: Tryptone, Yea.: Yeast ex., Pep.: Peptone.

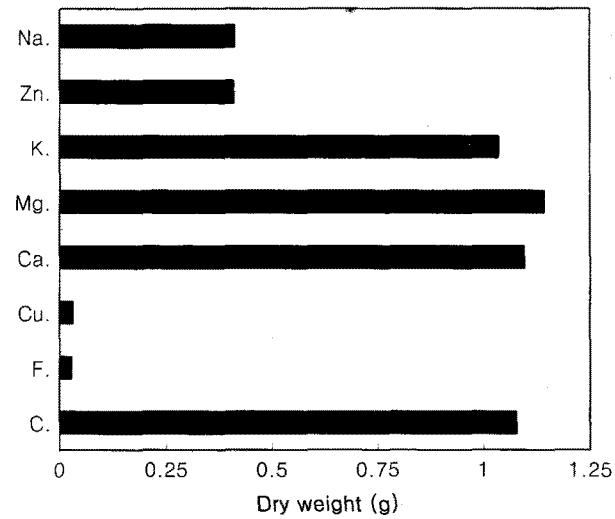

Fig. 3. Effect of mineral salts on mycelial dry weight (g) of *Lentinula edodes* after 13 days of incubation in basal media (100 m*l* SFW). C: Control, Fe.: $FeSO_4$, Cu.: $CuSO_4$, Mg.: $MgSO_4 \cdot 7H_2O$, Ca.: $CaCl_2$, K.: KCl, Zn.: $ZnSO_4 \cdot 7H_2O$, Na.: NaCl.

carbon additives on account of low cost for mushroom farmers.

As shown in Fig. 3, $MgSO_4 \cdot 7H_2O$ in seven mineral sources stimulated the growth of mycelia. Madigan (1997) reported that magnesium functioned to stabilize ribosome, cell membranes, and nucleic acids and was also required for the activity of many enzymes. As described above, $MgSO_4 \cdot 7H_2O$ in SFW stimulated the growth of mycelia more than the others.

The results in Fig. 4 showed that the optimal temperature for the fast mycelial growth of *Lentinula edodes* in SFW was found to be 25°C. The 22°C to 28°C range seemed to be suitable for growth of mycelia.

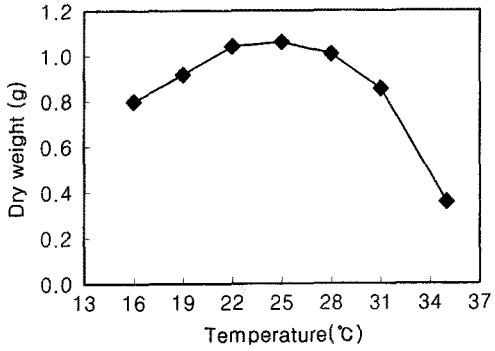

Fig. 4. Effect of temperature (°C) on mycelial dry weight (g) of *Lentinula edodes* after 13 days of incubation in basal me- dia (100 m*l* SFW).

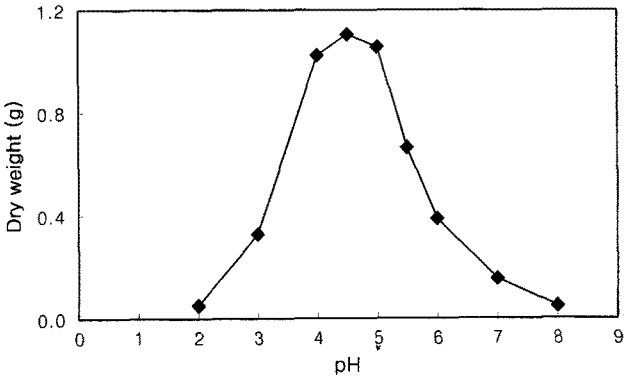

Fig. 5. Effect of pH on mycelial dry weight (g) of *Lentinula edodes* after 13 days of incubation in basal media (100 m*l* SFW).

Growth factors such as vitamins, amino acids, purines and pyrimidines were not applied to this experiment because of the low cost consideration for mushroom farmers.

In the case of effect of initial pH, the highest yield was obtained at an initial pH 4.5, reaching a concentration of 1.105 g dry weight/100 m*l* in 13 days (Fig. 5). At low initial pH values, pH 2 and pH 3, the mycelia growth was very slow, reaching a concentration of 0.328 g dry weight/ 100 m*l* in 13 days. At high initial pH values, pH 7 and pH 8, the concentration dropped sharply in this range (1.55 mg dry weight/m*l*).

The influence of rpm was studied on a rotary shaker in the range of 0~250 rpm for 13 days in a submerged culture. The best yield of mycelia in the 250 m*l* flask was 100 RPM. The yield of mycelia increased sharply at 0 to 100 rpm and decreased slightly from 150 to 250 (Fig. 6).

Between SFW and SFW+C, little difference was observed in mycelia growth until the eight days (Fig. 7). About 20% of the mycelia growth of SFW+C (1.149 g/ 100 m*l*) was more than that of SFW (0.898 g/100 m*l*) after

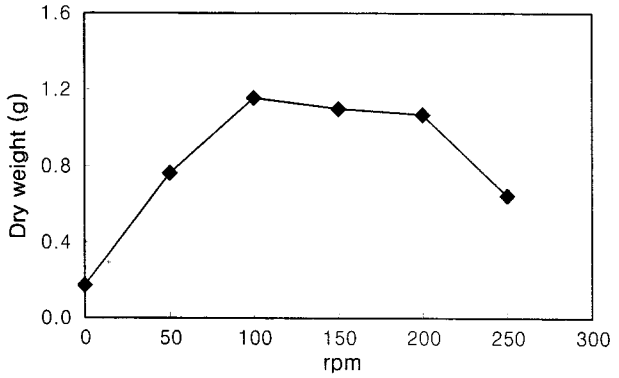

Fig. 6. Effect of rpm on mycelial dry weight (g) of *Lentinula edodes* after 13 days of incubation in basal media (100 m*l* SFW).

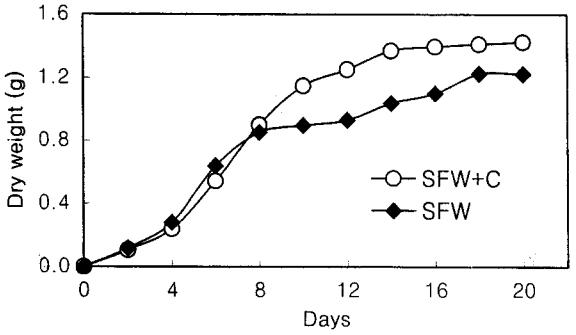

Fig. 7. Mycelial dry weight of *Lentinula edodes* at 100 m*l* in the 250 m*l* flask, incubation at 25°C, 100 rpm, and pH 7. SFW 2.65%, $MgSO_4 \cdot 7H_2O$ 0.05%), SFW+C (SFW 2.65%, sucrose 1%, $MgSO_4 \cdot 7H_2O$ 0.05%).

10 days. In SFW+C, the yield of mycelia increased sharply until the 12th day and increased slightly after 14 days. The yield of mycelia at 14 days was 1.371 g/100 m*l* in SFW+C and 1.039 g/100 m*l* in SFW.

The optimal medium ingredients for *L.edodes* in SFW+C were 2.65% SFW, 1% sucrose, and 0.05% $MgSO_4 \cdot 7H_2O$. The best growth of *L. edodes* in SFW+C was obtained at 100 rpm, pH 4.5, 14 days of incubation time. Further study seems to be necessary to determine the optimal strategy for higher scales of in incubation vessels as our study was carried out in 250 m*l* flask.

With proper pretreatment, sikhae factory waste has the potential to be an excellent medium for plate and submerged culture of *L. edodes*. In Fig. 7, there are little differences between SFW+C and SFW in the dry weight of mycelia. No carbon, nitrogen additive, vitamins, or nutrients could be added to the sikhae waste medium used in the production of mycelia of *L. edodes*. SFW is a valuable resource when used as a low cost - complex medium for *L. edodes* cultivation. Furthermore, the use of SFW could diminish the potential of wastewater pollution by sikhae factory.

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