Jour. Agri. Sci.
Chungnam Nat'l Univ., Korea
Vol.35(2): 199~212 (2008)

Characteristic for Growth and Fruit Body Polysaccharide of Caterpillar Fungi, *Cordyceps sobolifera*(Hill.) Berk. et Br.

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ABSTRACT

Mycelial expansion and fruit body development of caterpillar fungi, *Cordyceps sobolifera*(Hill.) Berk. et Br. was examined on various carbon and nitrogen sources, some substrates with various supplements. Best temperature for the mycelial expansion was 25°C, while optimal pH was 7. Glucose and polypeptone were the best sources of carbon and nitrogen, respectively, as well as 1:1 was the best ratio of carbon and nitrogen. The unpolish rice and bean curd lees were used as core substrates combined individually with five various supplements. In substrates based, all the supplements with all combinations were found to exhibit the mycelial growth and fruit body formation. Likewise, yolk was found as a superior supplement and also clarified quite high polysaccharide content in the fruit body.

Key words : *Cordyceps sobolifera*, caterpillar fungi, polysaccharide, fruitbody, medicinal fungi

Ⅰ. Introduction

Caterpillar fungi are complex of fungi and insect which belonging to Ascomycete. Insects were attacked with fungi, then invaded mycelium and fruiting in summer season. Host insect is limited for several fungal spices. *Cordyceps sinensis* is one of the most favorable species for its excellent medicinal capacities (Chang *et al.*, 2003; Kim *et al.*, 2003; Li *et*

Received Nov. 14, 2008; Accepted Dec. 9, 2008
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al., 2004). The fungus *C. sinensis* is parasite on special moth which located only Tibet highland. It is quite expensive because of no methods for artificial cultivation. It is a culinary-medicinal mushroom, which occurs widely in eastern country, is a commercially-promising mushroom because of its great popularity due to its excellent medicinal values. A method for its optimal cultivation has not yet to been established, however, technology for artificial cultivation of C. *militaris* (Vuill.) Fr. on the moth pupa, in rack has been developed. It has been reported that this fungus could easily grow in substrate with supplement at indoor cultivation.

Cordyceps sobolifera (Hill.) Berk. et Br. has been noticed to useful just same for the pharmacological effect of *C sinensis*. The fungus is parasite to cicada larva. The recent majority of studies on mycelial growth of various mushrooms have been carried out using submerged fermentation method (Xu *et al.*, 2003; Xiao *et al.*, 2004; Xiao *et al.*, 2006). Solid state fermentation (SSF) has several advantages over liquid culture (Couto and Sanroman, 2006). The method reflected the mycelial growth condition occurring on solid state culture *i.e.* onvarious media and substrates. The SSF deals with the controlled growth and metabolism of microorganism inside water insoluble materials, in the presence of varying free water amounts (Doelle *et al.*, 1992).

In present works, various nutrients and different wastes were tested to find out the potential substrate or nutrients for mycelial growth under the solid-state culture. Therefore, the aim of the study was to know the suitability of physico-chemical factors, various nutrient supplements and substrates for mycelial growth of *C. sobolifera*. Moreover, also clarify the substrate supplements for fruit body yield and polysaccharide production.

II. Material and Methods

1. Organism

Strain of *C. sobolifera* (KS-80) used in the experiment was originated in KyushuUniversity Forest (**Fig. 1**). The strain was cultured on potato dextrose agar (PDA) plates at 25°C for two weeks.

2. Temperature and pH

Effect of temperature and pH on the mycelial growth was determined by radial growth of colony measurement method. To test the effect of temperature and pH on mycelial growth, *C. sobolifera* was inoculated on PDA medium plates and growth was assayed at a temperature range of 15-31°C with a 2°C graduation and a pH range of 4-10 with 1 graduation. The pH of the medium was adjusted prior to sterilization with 1N NaOH or HCl.

The prepared PDA medium was autoclaved at 120°C for 15 min. About 20 ml of each medium was poured into Petri dishes. After cooling a 5 mm diameter disc of actively

Fig. 1. Fruit body of *Cordycepssobolifera* Scale bar means 1 cm

growing mycelia from two weeks PDA medium was transferred into the medium, and then incubated at 25℃ in darkness. Colony diameters were measured with caliper after 2 weeks of inoculation.

3. Carbon source

A favorable carbon source screening, capable of stimulating mycelial growth of *C. sobolifera* was tested on various carbon sources. The basal medium contained glucose 18 g, yeast extract 3 g, polypeptone 1 g, KH_2PO_4 0.5 g, K_2HPO_4 0.5 g and $MgSO_4.7H_2O$ 0.5 g, agar 20 g and distilled water to make 1,000 ml. Various carbon sources were substituted for glucose. Based on each molecular weight of seven different carbon sources, including glucose, were added, each carbon source was added to the basal medium at the concentration of 0.1 M. Twenty ml of medium of various carbon sources poured into the Petri-dish and cooled to the desired temperature. Each Petri-dish was inoculated with 5 mm disc of the fungi. Petri-dishes were incubated at 25℃ for two weeks after which the radial diameter of colony of mycelia was measured. Petri-dishes of the basal medium lacking a carbon source were also inoculated for control.

4. Nitrogen source

The ability for *C. sobolifera* to utilize the different nitrogen sources for growth was also studied. The basal medium was the same composition as the medium used for the carbon source study. Various nitrogen sources were substituted for yeast extract and polypeptone. Based on each molecular weight of six different inorganic nitrogen sources, each nitrogen source was added to the basal medium at the concentration of 0.02 M and organic nitrogen sources were used at 0.7 %. Sterilization, inoculation and incubation were performed as the same method of carbon source studied. The basal medium without nitrogen source was used as a control.

5. Carbon nitrogen ratio

Equal quantities of best carbon source (glucose)

and nitrogen source (polypeptone) were mixed in distilled water at the ratio of 5 g/l (1:1). Other ratios were also prepared by mixing appropriate quantities of nutrients source. Sterilization, inoculation and incubation were performed as the same method of carbon source studies. The used basal medium was the same as carbon and nitrogen excluding carbon and nitrogen sources.

6. Supplements utilization

1) Agar media

Various ingredients such as agriculture products (bean curd lees, barley bran, rice bran, wheat bran, corn powder and fiber, cotton waste), industrial products (crude nucleic acid from wood xylose with *Torula* yeast, cellulose, syochu wastes and corn step liquor) were tested for mycelial growth with agar medium. All the wastes were milled in a grinder machine. Ten g of each supplement was mixed with 100 ml of distilled water and heated at 80°C in a shaker for 3hr at 100 rpm. The hot water extract was filtered and 20 ml filtrated was mixed with a basal medium. The basal medium used was composed of 0.5 g glucose and 0.25 g peptone dissolved in one liter distilled water, 2 % of agar was mixed with the various medium. The prepared media was autoclaved at 120°C for 15 min. About 20 ml of each medium was poured into Petri dishes. After cooling a 5 mm diameter disc of actively growing mycelia of two weeks from previously culture PDA medium was transferred and incubated at 25°C in darkness. Radial colony diameters were recorded after incubation.

2) Solid substrates

Four basal substrates and 5 supplements were used as shown in **Table 7**. Substrate and freeze dry supplement was mixed in the ratio 5:1(weight). Moisture was adjusted to 65 % with tap water and the pH of mixture was measured (HORIBA pH Meter F-21) and observed between pH 6.5 and 7.0. Fifty g of wet substrate was placed in 200 ml Erlenmeyer flask, sterilized at 120°C for 30 min, and allowed to cool to the desired inoculation temperature and then seed spawn was inoculated. A 5 mm disc was inoculated in to 100 ml medium (medium components: 2 % glucose, 0.7 % yeast extract, 0.3 % polypeptone, 0.05 % KH_2PO_4, 0.1% K_2HPO_4, 0.05% $MgSO_4$. $7H_2O$) and cultured at 25°C for ten days to obtain the seed spawn. The inoculated pots were incubated at 25°C in darkness.

7. Culture maturity measurements

1) Staining with BPB of substrate

Bromophenol blue (BPB, pH reagent) was used for detection for maturation of the medium. This method is quite useful for judging the maturity, which is fundamentally pH measurement on coloration with pH reagents. The BPB solution (1 % water) was directly splayed to the newly cut substrate surfaces. The coloration of various substrates was measured in one week intervals for four

weeks. The surface of colonized slices of various substrates was measured using a Minolta Chroma Meter CR- 200.

2) Ergosterol content

Ergosterol contain was determined from colonized substrates. One g dry sample was mixed with 20 ml methanol, 10 ml ethanol and 4 g KOH incubated at 40^0C for 90 min. The solution was filtered and dilute with 20 ml distilled water and 20 ml hexane mixed for 5 min. The hexane extract was evaporated under vacuum at 30^0C. The dry residue was dissolve in 2 ml of methanol. Ergosterol was measured by high performance liquid chromatography(HPLC) (Jasco, PU-1580) using a reversed-phase system consisting of a packed column(Inertsil ODS-3) and 99.7% HPLC grade methanol. Ergosterol was detected by absorption of 282 nm light (Tosoh, UV-8001). At a 1.0 ml per min flow rate, ergosterol was eluted about at 9 min.

8. Fruit body formation

After four weeks incubation, colonized substrates were transferred into the fruiting chambers. The temperature of mushroom house was adjusted to 25^0C, relative humidity was maintained 90% and CO_2 concentration level was less than 2,000 ppm, and a light intensity of 1,000 lux for 8 hr a day by a cool fluorescent lamps. Fruit body yield was weighted after harvest in raw weight. Biological efficiency (BE) was defined as the percentage ratio of the fresh weight of harvested mushrooms over the dry weight of substrate.

9. Polysaccharide extraction

The extraction procedure of crude inner-polysaccharide (IPS) was slightly modified the method of Chen *et al.* (2005). Two hundred mg of lyophilized fruit body powder was multiply extracted with 20 ml distilled water, heated at 80^0Cfor3hr at 100 rpm in the shaker. The extracted slurry was cooled and filtered with a 0.45 μm membrane filter (Millipore) and concentrated in a vacuum and four volumes of 95 % ethanol was added stirred vigorously and then allow to precipitate overnight at 4^0C. The precipitated IPS collected by centrifuging at 10,000 *g* for 20 min, and rewashed with ethanol and centrifuged again described as above, then lyophilized and the weight of crude IPS was estimated.

10. Statistical analysis

All the obtained data were analyzed by one-way analysis of variance (one way ANOVA) by using SPSS 12.0 software package. A test for significant differences was determined by Tukey-HSD at ($P<0.05$).

III. Results and Discussion

1. Effect of temperature and pH

The mycelial growth of *C. sobolifera* assayed

at temperature range between 15-30°C. The optimum temperature for vegetative growth was 25°C, the second best was recorded at 23°C but growth was not significantly different (P<0.05) between 23-27°C (**Table 1**). The mycelial growth was significantly decreased at 30°C and 15°C, where as 30°C did not response, but 17°C also showed active growth. Temperature above 27°C was more manipulated to sharply decrease growth; this result states that declining temperature was rather in favored of mycelial growth. It has been suggested that at optimum temperature of growth of each fungus, enzyme and metabolic activities will increase and energy will be release and good mycelial growth will be enhanced (Garraway and Evans, 1984). This mushroom might be difficult to cultivate in hot climatic regions, as well as during the summer season under natural conditions.

Table 1. Effect of temperature on mycelial growth of *C. sobolifera*

Temperature (°C)	Colony diameter (mm)
15	20.25 ± 1.03f
17	36.98 ± 0.33d
19	44.69 ± 0.22c
21	57.39 ± 1.24b
23	66.90 ± 0.85a
25	68.75 ± 2.16a
27	65.32 ± 2.16a
29	27.39 ± 0.86e
31	20.15 ± 1.02f

Values are the mean ± SE of mycelial growth measurements and small alphabet letters indicate the same letters are not significant different according to the Tukey HSD (P<0.05).

The mycelial growth was most favorable at pH 7 followed by 6, 5, 8 and 9, respectively; however growth was not significantly different between pH 5-9. The growth was poorly observed at pH 4, where as at pH 10 it also seems to be effectively enhanced. More effective growth was observed in acidic than in alkaline medium. In contrast, mycelial growth range was wide in alkaline medium. At high acidic and alkaline pH, cell walls of plants may corrode and selective permeability function of the cell membrane may be impaired (Hopkins, 1995). There was information that many mushrooms were not able to grow in strong acidic and basic medium such as pH 4 or 10. The pH of the medium is a very important but it is often a neglected environmental factor[8]. This fungus has a wide range of pH for mycelial growth. So it can be established that this fungus favored all kinds of pH medium, although we suggest that suitable pH for its superior growth must be neutral and a slightly acidic or alkaline medium. The radial diameter of colony on different pH is presented in **Table 2.**

2. Effects of carbon, nitrogen and C/N ratio

Carbohydrates are important carbon and energy sources for cultured cells. The mycelial growth of *C. sobolifera* was favorable to six carbon sources as compare with control (**Table 3**). The most favorable carbon sources was

Table 2. Effect of pH on mycelial growth of *C. sobolifera*

PH	Colony diameter (mm)
4	27.94 ±0.33c
5	62.18 ± 4.48a
6	63.18 ± 1.98a
7	65.77 ± 2.52a
8	60.94 ± 4.12a
9	59.72 ± 2.91ab
10	52.32 ± 1.36b

Values are the mean ± SE of mycelial growth measurements same letters are not significant different according to the Tukey HSD ($P<0.05$).

Table 3. Effect of various carbon sources on mycelial growth of *C. sobolifera*

Carbon sources	Colony diameter (mm)
Control	44.89 ± 2.73c
Glucose	64.81 ± 0.83a
Sucrose	51.86 ± 1.81bc
Fructose	63.83 ± 2.66a
Maltose	59.60 ± 2.03ab
Lactose	27.07 ± 0.36d
Sorbitol	50.92 ± 2.01bc
Xylitol	47.35 ± 1.97c

Values are the mean ± SE of mycelial growth measurements and small alphabet letters indicate the same letters are not significant different according to the Tukey HSD ($P<0.05$).

glucose with colony diameter of 64.8 mm, followed by fructose and maltose with colony diameter of 63.8 mm and 59.6 mm, respectively and their growth did not differ significantly. Growth was effectively enhanced in glucose and fructose. These two compounds could undergo molecular rearrangement (isomerism) to one another in cell during metabolism, and glucose supported very good mycelial growth is an isomers of fructose. The result of glucose on growth was similar to that on *Grifola umbellate* and *Pleurotus tuber-regium*, respectively (Shim *etal.*, 1997; Fasidi and Olorunmaiye, 1994). Sucrose, sorbitol and xylitol performed moderate growth, and were not significantly different to control, while lactose showed negative result to growth. The poor growth in lactose may be ascribed to its failure to produce enzymes, which could catalyze the breakdown of lactose in cell. The different growth performed by various carbon sources knows that nutrient requirements of mushrooms may differ.

Similarly, the effect of nitrogen sources on mycelial growth was examined by employing nine various nitrogen sources, and all most all exhibited growth; however, there were growth differences (**Table 4**). Five nitrogen sources were favorable to the mycelial growth as compared with control. Polypeptone (68.0 mm) was most suitable for stimulating a favorable growth of mycelium, followed by yeast extract agar (58.8 mm), ammonium tertarate (54.0 mm), ammonium sulphate (50.3 mm) and peptone (46.9 mm). Growth was less than control in four different inorganic nitrogen sources. It was also observed that mycelial density was higher in organic nitrogen sources than that of inorganic sources. This result says that different nitrogen sources might have

different effect on metabolic activities during fungal growth.

Table 4. Effect of various nitrogen sources on mycelial growth of *C. sobolifera*

Nitrogen source	Colony diameter (mm)
Control	44.73 ± 0.93e
Ammonium citrate	25.26 ± 0.49g
Ammonium tartrate	53.99 ± 1.41bc
Ammonium chloride	35.01 ± 1.93f
Ammonium sulphate	50.34 ± 0.61cd
Ammonium nitrate	43.03 ± 2.23e
Ammonium phosphate	20.71 ± 1.53g
Yeast extract	58.79 ± 0.45b
Polypeptone	68.00 ± 1.55a
Peptone	46.85 ± 1.43de

Values are the mean ± SE of mycelial growth measurements and small alphabet letters indicate the same letters are not significant different according to the Tukey HSD (P<0.05).

Table 5. Effect of various C/N ratio on mycelial growth of *C. sobolifera*

C/N ratios	Colony diameter (mm)
1:1	68.20 ± 0.60a
2:1	66.03 ± 1.31a
3:1	65.98 ± 0.68a
4:1	65.30 ± 0.94a
1:2	63.84 ± 1.43ab
1:3	60.33 ± 0.49b
1:4	55.17 ± 1.14c

Values are the mean ± SE of mycelial growth measurements and small alphabet letters indicate the same letters are not significant different according to the Tukey HSD (P<0.05).

The C/N ratios used in this study promoted growth significantly (Table 5). This fungus grew best in media with the ratio 1:1 (68.2 mm) and least growth occurred on 1:3 (60.3 mm). Growth decreased with increased amounts of carbon and nitrogen; however increasing carbon ratio was fairly better than nitrogen. The results obtained of this fungus are different from those obtained by for *Lentinus subnudus* (Gbolagade *et al*., 2006). The results show that nutrient supplements enhance mycelial growth to a tolerate limit.

3. Effect of ingredient agar on mycelial growth

The various wastes with agar adopt the growth of mycelium; however growth was varied from substance to substance. Mycelial growth of *C. sobolifera* on hot water extract agar of various wastes is presented in Table 6. All tested agriculture wastes agar, rice bran, wheat bran and corn fiber improved mycelial growth. Bean curd lees, barley bran and corn powder were better for growth, whereas cotton waste depressed the growth. Bean curd lees (59.4 mm) was much faster, followed by barley bran (52.7 mm) and corn powder (52.6 mm), respectively and were not significantly different (P<0.05). Wheat bran and rice bran also performed positively but were not statically different to the control.

Similarly, among the tested industrial wastes agar manipulated mycelial growth more than control, except for syochu waste. Mycelial growth on cellulose (48.2 mm) was best, followed by nucleic acid (45.0 mm) and did

not differ significantly ($P<0.05$). Syochu waste and corn steep liquid were also in favor of mycelial growth. Among the industrial wastes, cellulose and nucleic acid adopt the mycelial growth significantly, whereas syochu waste suppressed growth. These results obviously that various industrial wastes and chemical could be applied as a supplement in the growth of this fungus.

All tested agar based media of used wastes supported growth of *C. sobolifera*. Although all kinds of tested composts showed the most excellent mycelial growth, there were no significant growth differences among the composts as wellas PDA, and fresh sawdust ($P<0.05$), whereas cotton waste and syochu waste illustrated negative effects on growth. Mycelial growth on different composts was noticeably better than PDA along with all wastes. The results of this fungus growth are a new disclosure in the mycelial growth of *C. sobolifera*. Among the all agar testes group, substrate group showed better performance, followed by agriculture by-products and industrial wastes.

4. Effect of supplements on colonization

The durations required for spawn running, pinning, primordial initiation on different crops are given in **Table 7.** Rice was excellent substrate for *C. sobolifera* mycelial growth, especially unpolish rice was good results. Bean curd lees was also among the varying supplements, colonization days were significantly accelerated when yolk was supplemented followed by barley bran and rice bran. Inhibition of mycelial growth by soybean residue was similar to the findings of Akamatsu (1998). Similarly, fruiting was inhibited on both crops when substrate was supplemented by nucleic

Table 6. Effects of various wastes agar media on mycelial growth of *C. sobolifera*

Agriculture products	Colony diameter (mm)
Rice	57.57 ± 1.98b
Wheat bean	45.64 ± 1.34bc
Rice bran	47.63 ± 1.38c
Bean curd lees	59.44 ± 1.42a
Corn fiber	40.84 ± 0.69e
Barley bran	58.68 ± 1.70b
Cotton waste	34.78 ± 1.57f
Industrial products	
Nucleic acid	45.03 ± 0.75b
Syochu wastes	42.93 ± 2.26g
Cellulose	48.19 ± 0.64bc
Corn steep liquid	43.90 ± 1.19gh

Values are the mean ± SE of mycelial growth measurements and the same letters are not significant different according to the Tukey HSD ($^{P}<0.05$).

acid (chemical supplement) which may be due to a high concentration of nitrogen. A highly proteineous medium may also be toxic to the fungal cell wall, thereby preventing growth. Fungi are also known to bio-accumulate metals. Therefore, using a variety of substrates and supplements is essential (Gbolagade *et. al.*, 2006).

We presented the same environmental condition but used different nutritional supplements for each crop, the difference in time taken for pinning and primordial formation, and fruiting period with varying supplements. Likewise, different substrates figure into the variance in growth and production. Our results showed that yolk is suitable for the growth of this fungus. This is resulted to higher ergosterol production to fruiting period.

5. Effect of supplements on culture maturity

Culture maturity of various supplemented substrates is shown in **Table 8.** The b* value indicate yellowish of the surface color (L*, a*, b* values in the Colorimeter), which sprayed with BPB pH reagent. Acidic substrates with larger b* value means well mature culture of *C. sobolifera* mycelia. Yolk, bean curd lees and barley bran resulted excellent supplements.

Ergosterol content is the suitable indicator for judging the substrate maturity (Seitz *et al*, 1979; Ohga and Wood, 2000). Yolk supplemented substrate was the most effective for ergosterol assimilation, followed by bean curd lees and barley bran. Significantly less ergosterol was displayed by cotton waste supplement. This result demonstrates that primary substrates used are responsible for fungal growth. Also, supplements used can play an important role to increase fungal bio-mass. Ergosterol production and yields corresponded closely in this study of *C. sobolifera*. This result showed that early colonization (spawn running) and higher ergosterol are accompanied by higher yield.

6. Effect of supplements on fruit body yield

Fruit bodies flushed spontaneously on the supplemented substrates at 25℃, RH 90 % in

Table 7. Mycelial growth with various supplements on the substrate.

Substrates \ Supplements	Rice bran	Corn bran	Wheat bran	Yolk	Nucleic acid
Bean curd lees	39.82 ± 0.94a	37.78 ± 0.70ab	39.25 ± 0.42b	44.58 ± 1.86c	33.98 ± 3.02cd
Barley bran	37.80 ± 1.91d	31.86 ± 1.90de	33.89 ± 0.26ab	45.06 ± 0.26bc	26.24 ± 0.10cde
Rice (polish)	41.82 ± 2.08f	33.69 ± 1.96cb	40.04 ± 0.86ef	51.89 ± 0.17def	29.61 ± 0.12 ae
Rice (unpolish)	47.79 ± 1.44af	37.22 ± 0.63ab	41.51 ± 1.18af	53.78 ± 0.88a	35.49 ± 1.21ad

Values are the mean ± SE of mycelial growth measurements (mm) and small alphabet letters indicate the same letters are not significant different according to the Tukey HSD ($P<0.05$).

the chamber. Color was yellowish white with specific shapes (**Fig. 2**).

Yield and BE of various supplemented substrates are shown in **Table 9.** For each crop of varying supplements, only one flush of mushrooms was harvested. Similarly, as different growth mechanism on different substrate and supplement, yield between two crops were relatively unlike. The yield was highest when substrate was supplemented by yolk with BE of 79.7 %, followed by barley bran and bean curd lees, with BE 67.8 and 59.1 %, respectively. Minimum yield was obtained from cotton fiber (31.8 %).

Different sources of compost yielded the different result but it is surprising that there should be such a difference between those grown under similar conditions. Each compost differed only by the supplement, comprising of 20 % of the substrate. Therefore, selection of supplement is also important factor for mushroom growth and development.

Our desire was find a good substrate and supplement combination for mushroom production. Three things are required for economic production, low cost, easily available and good yields. The substrates used in these

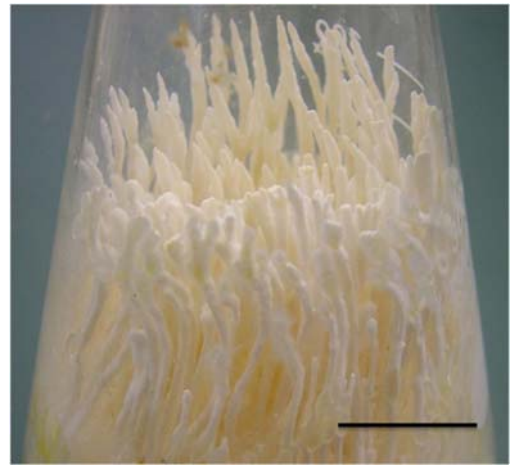

Fig. 2. Fruit body formation of *Cordyceps sobolifera* on the supplemented substrates. Scale bar means 2 cm

Table 8. Culture maturity of several substrates

Supplements	b* value	Ergosterol (μg/g)
Corn powder	9.31±2.12f	684.7±2.47f
Wheat bean	11.13±1.12e	821.4±1.15e
Rice bran	12.31±3.21d	989.1±3.31d
Bean curd lees	17.42±2.35b	1029.1±1.59b
Corn fiber	1.36±3.21hi	468.1±0.35g
Barley bran	17.62±1.34d	1021.3±2.11b
Cotton waste	2.15±2.31h	435.6±1.18h
Yolk	19.61±3.12a	1213.5±1.71a
Nucleic acid	13.73±1.12c	981.3±3.21cd
Corn steep liquid	4.23±1.16g	1011.1±2.11bc

Values are the mean ± SE of b* values and ergosterol content and small alphabet letters indicate the same letters are not significant different according to the Tukey HSD ($P<0.05$).

experiments, were made relatively inexpensive materials and yield with the supplements were quite acceptable. This study found better result for yield than our previous studies with sawdust combination. This experimented substrate will allow better production than current practice, but it also opens a starting place for further studies to attain even greater yields.

7. Polysaccharide content

The IPS production ranged from 207-490 mg/g in dry fruit body. The highest polysaccharide was achieved with 490 mg/g of dry fruit body (Table 10). Yolk was found to be the best supplement to produce a significant increase in the IPS followed by corn steep liquor and bean crud lees. Rice bran and wheat bran were also well for IPS production in the fruit body.

Ikegawa *et al.* (1968) reported that the extract obtained by hot water from fruit bodies of some mushrooms belonging to the family *Polyporaceae* showed host-medication antitumor activity against cancer(Sarcoma 180). Hot water extract of mushroom has been used medicinally by the Chinese for a very long time (Fan *et al.*,2007). Most of the active polysaccharides from mushrooms form complexes with protein (Wasser, 2002). The polysaccharopeptides is a hot water extract of mushrooms and appears in light or dark brown powder which is soluble and stable in hot water. The powder extracted typically contains 34-35 % soluble carbohydrate (91-93 % β -glucan), 28-35 % protein, 1-7 % moisture, 6-7 % ash and the remainder is free sugars and amino acid (Ueno *et al.*, 1980).

Supplements can influence the chemical composition and nutritional value of the *Pleurotus*

Table 9. Fruit body yield of supplemented substrates

Supplements	g	BE* (%)
Corn powder	3.26±1.21g	31.0
Wheat bean	4.63±2.11e	44.1
Rice bran	4.65±1.22e	44.3
Bean curd lees	6.21±1.56c	59.1
Corn fiber	3.34±3.21f	31.8
Barley bran	7.12±1.35b	67.8
Cotton waste	3.34±2.34f	31.8
Yolk	8.37±2.78a	79.7
Nucleic acid	4.78±1.23d	45.5
Corn steep liquid	4.78±2.31d	45.5

* Biological efficiency (BE)

Values are the mean ± SE of fruit body yield (g, raw) and small alphabet letters indicate the same letters are not significant different according to the Tukey HSD ($P<0.05$).

mushroom (Tshinyangu, 1996; Krisnamoorthy, 1997). As the main carbon and energy source for most fungi, carbohydrate may play an important role in cell growth and polysaccharides production, and using it in proper nutrient source addition in cultivation, both in intracellular and extracellular polysaccharides production were greatly enhance (Hsieh *et al*., 2005).

Table 10. Polysaccharide of fruit body grown in various substrates

Supplements	IPS*(mg/g)
Corn powder	207±3.13f
Wheat bran	220±4.12de
Rice bran	240±1.34d
Bean curd lees	347±2.63c
Yolk	490±1.52a
Corn steep liquor	394±2.93b

* Crude inner-polysaccharide (IPS)

Values are the mean ± SD, and alphabet letters indicate the same letters in the same column are not statistically significantly different according to Tukey-HSD (P<0.05).

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