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The culture conditions for mycelial growth and sclerotial formation of *Polyporus umbellatus*

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ABSTRACT – *Polyporus umbellatus* (Syn. *Grifola umbellata*) is a sclerotium forming mushroom belongs to family Polyporaceae of Polyphorales, Basidiomycota. The sclerotia of *P. umbellatus* have long been used for traditional medicines in China, Korea and Japan. This study was initiated to obtain the basic data for artificial sclerotial production of *P. umbellatus*. Here, we investigated the favorable conditions for mycelial growth of *P. umbellatus* and its symbiotic fungus *Armillaria mellea*. We also evaluate the favorable carbon and nitrogen sources for sclerotial formation in dual culture between *P. umbellatus* and *A. mellea*. The favorable conditions for mycelial growth of *P. umbellatus* were 20°C and pH 4, while optimal conditions for mycelial growth of *A. mellea* were 25°C and pH 6. The carbon sources for optimal mycelial growth of *P. umbellatus* were fructose and glucose, while carbon sources for favorable mycelial growth of *A. mellea* were also fructose and glucose. The nitrogen sources for favorable mycelial growth *P. umbellatus* were peptone and yeast extract, while optimal mycelial growth of *A. mellea* were obtained in peptone and yeast extract. When *P. umbellatus* and *A. mellea* were dual cultured on carbon sources, sclerotia were induced on basal media supplemented with glucose, fructose and maltose at pH 4~6, while nitrogen sources inducing sclerotia were basal media supplemented with peptone and yeast extract for 60 days at 20°C under dark condition.

KEYWORDS – *Armillaria mellea, Polyporus umbellatus*, Sclerotium, Symbiosis

Introduction

The *Polyporus umbellatus* belongs to Polyporaceae of Polyphorales, Basidiomycota, considered as wood rotting fungi. This fungus forms tuber-like sclerotium in underground, while the fruiting bodies were produced on the ground (Cheng *et al.* 2006). The mycelia can turn into sclerotia under extreme environmental conditions such as cold, drought and nutritional depletion (Liu and Guo, 2009). In nature, sclerotia of *P. umbellatus* could not produce new sclerotia without symbiotic relationship with *A. mellea* (Guo and Xu, 1991, Kikuchi and Yamaji, 2010). *A. mellea* can provide nutrients to sclerotia of *P. umbellatus* after forming symbiosis with *A. mellea* (Guo and Xu, 1991). However, the full life cycles of *P. umbellatus* remain unclear. *A. mellea*, one of edible and medicinal mushroom, belongs to Tricholomataceae of Agaricales, Basidiomycota has been known to be saprophyte or parasite on many different woody plants, and also have symbiotic relationships with *P. umbellatus*. and *Gastrodia elata*. The sclerotium of *P. umbellatus* has been used for medicinal purposes in Korea, China and Japan. The sclerotium of this fungus contains substances promoting a diuretic activity (Lu *et al.*, 1985), hair growth (Inaoka *et al.*, 1994), anti-cancer (You *et al.*, 1994), immuno-modulating (Oh *et al.*, 2004) and suppressing cytotoxicity induced by leukemia (Ohsawa *et al.*, 1992). Since sclerotia of *P. umbellatus* have not been produced in Korea and all sclerotia

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Table 1. List of *Polyporus umbellatus* and *Armillaria mellea* used in this study

IUM strain	Scientific name	Geographical origin	IUM strain	Scientific name	Geographical origin
IUM 4958	*P. umbellatus*	China	IUM 0955	*A. mellea*	Korea
IUM 4959	*P. umbellatus*	China	IUM 0957	*A. mellea*	Czech
IUM 4960	*P. umbellatus*	China	IUM 1238	*A. mellea*	China
IUM 4961	*P. umbellatus*	China	IUM 1239	*A. mellea*	China
IUM 4962	*P. umbellatus*	China	IUM 4585	*A. mellea*	Korea

used in Korea were imported from China, the price of the sclerotia has been increased year after year because of the demand for the sclerotia have been increased. Thus, it is necessary to produce sclerotia directly from mycelia of *P. umbellatus* under laboratory conditions. In these regards, we tried to elucidate the sclerotial formation of *P. umbellatus* in conjunction with *A. mellea*. Therefore, this study will not only reveal the sclerotial development of *P. umbellatus* under artificial condition, but also contribute to initiation of mass production of *P. umbellatus* sclerotia.

Materials and Methods

Fungal strains

Two fungal strains, P. *umbellatus* and *A. mellea*, used for this study, were obtained from Culture Collection and DNA Bank of Mushrooms in Division of Life Sciences, Incheon National University (Table 1). The fungal strains were transferred to Potato Dextrose Agar (PDA) plates and incubated for 2 weeks at 25°C in the dark and kept on PDA at 4°C. Unless otherwise stated, all experiments were done at least four times.

Culture conditions for mycelial growth of *P. umbellatus* and *A. mellea.*

Effects of temperature:

To screen the optimum temperature for mycelial growth of *P. umbellatus,* and *A. mellea,* 15°C, 20°C, 25°C, 30°C and 35°C were used. A 5 mm diameter agar plug was removed from 15 day-old culture of *P. umbellatus* and *A. mellea* and placed in the center of a Petri plate contained 20 mL of solidified PDA and adjusted to pH 4 for *P. umbellatus* and pH 6 for *A mellea*. Then, Petri plates were incubated for 30 days at 5 different temperatures under dark condition. The radial growth of mycelia was measured by the method described by Shim *et al*. (1997).

Effects of pH:

PDA medium was used to screen pH value for suitable growth of *P. umbellatus* and *A. mellea*. A 5 mm diameter agar plug was removed from 15 day-old culture of *P. umbellatus* and *A. mellea* and placed on the center of a Petri plate contained 20 mL of solidified PDA, which was adjusted to pH 4, 5, 6, 7, 8 or 9 with 1 N NaOH or HCL, and incubated for 30 days at 25°C under dark condition. The mycelial growth was measured according to the method described by Shim *et al*. (1997).

Effect of nutrient source

Carbon sources:

Basal medium (0.5 g $MgSO_4$, 0.46 g KH_2PO_4, 1.0 g K_2HPO_4, 120 g thiamine-HCl, and 15 g agar in 1,000 mL of distilled water) was prepared (Sung *et al*., 1999) and supplemented with each six carbon source (dextrin, fructose, glucose, maltose, mannose and sucrose). To screen favorable carbon sources for mycelial growth, each carbon source with 5 g of peptone was added to the basal medium separately at the concentration of 0.1 M and mixed thoroughly (Shim *et al*., 1997). The basal medium was adjusted to pH 4.0 for *P. umbellatus* and pH 6 for *A. mellea* before high-pressured steam sterilization for 15 minutes at 121°C. To measure the colony diameter, all Petri plates were incubated for 30 days at 20°C for *P. umbellatus* and 25°C for *A. mellea*. The radial growth of mycelia was measured as described above.

Nitrogen sources:

To screen nitrogen sources for suitable mycelial growth of *P. umbellatus* and *A. mellea*, the basal medium was supplemented with 6 nitrogen sources (ammonium acetate, ammonium phosphate, arginine, peptone, urea and yeast extract) at a concentration of 0.02 M. The basal medium contained each nitrogen source was supplemented with 20 g of glucose per 1 L of distilled water and adjusted to pH 4.0 for *P. umbellatus* and pH 6 for *A. mellea* before sterilization for 15 minutes at 121°C. To measure the colony diameter, all Petri plates were incubated for 30 days at 20°C for *P. umbellatus* and 25°C for *A. mellea*. The radial growth of mycelia was measured by the method described by Shim *et al*. (1997).

Effect of nutrient source for sclerotium formation

Carbon sources:

For the screening of carbon sources for promoting sclerotial formation in the selected carbon media, two fungal strains (*P. umbellatus*; IUM 4958 and *A. mellea*; IUM 4585) were selected by their good mycelial growth in the media which contained various carbon and nitrogen sources from previous experiments. The medium used for promoting sclerotia was the same as basal medium used in the previous experiment except supplementation of different carbon sources.. To screen the sclerotial formation in different pH values, the pH of the media were adjusted to 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 before autoclave. After sterilization, 5 mm of mycelial disc of *P. umbellatus* and *A. mellea* were placed in the middle of each Petri plate about 2 cm apart and incubated for 60 days at 20°C under dark condition. The formation of sclerotia on the medium was observed at 60 days after inoculation.

Nitrogen sources:

To screen for nitrogen source for promoting formation of sclerotia was the same as above experimental method with only different nitrogen sources such as ammonium acetate, ammonium phosphate, arginine, peptone, urea and yeast extract were supplemented to the basal medium at a concentration of 0.02 M. The basal medium which contained each nitrogen source was supplemented with 20 g of glucose and adjusted to pH values 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 before sterilization for 15 minutes at 121°C. After high pressured steam sterilization, 5 mm of mycelial disc of *P. umbellatus* and *A. mellea* were placed in the middle of each Petri plate about 2 cm apart and incubated for 60 days at 20°C under dark condition. The formation of sclerotia on the medium was observed from 60 days after inoculation.

Statistical analysis

The results are expressed as mean values and standard deviation (SD) and were analyzed by using one-way analysis of variance (ANOVA) followed by Duncan's new multiple-range test with $p = 0.05$. The analysis was carried out using SPSS V. 13 program(SPSS Inc., Chicago, IL, USA).

Results and Discussion

Culture conditions for mycelial growth of *P. umellatus* and *A. mellea*

Effects of temperature:

The mycelia of *P. umbellatus* and *A. mellea* were cultured for 30 days at 4 different temperatures. The best mycelial growth of *P. umbellatus* was obtained in the temperature of 20°C (Table 2). Sato *et al.* (1984) reported that good mycelial growth of *Grifola frondosa* was observed in the temperature range of 24~27°C. However, the mycelial growth of *P. umbellatus* was optimal at 20°C. The optimum mycelial growth of *A. mellea* appeared to be 25°C, which is a little higher than that of *P. umbellatus*.

Effects of pH:

To screen pH for favorable mycelial growth of *P. umbellatus* and *A. mellea*, pH of the PDA was adjusted to the range of pH 4~9. The favorable mycelial growth of *P. umbellatus* was observed at pH 4, and the poorest mycelial growth was found at pH 9 (Table 3). Xing *et al.* (2011) found that pH for favorable mycelial growth of *P. umbellatus* were in the range of 4.2~5.8 which were very similar to our

Table 2. Effect of temperature on mycelial growth of *Polyporus umbellatus* and *Armillaria mellea*

Strain No	Scientific name	Temperature				
		15°C	20°C	25°C	30°C	35°C
		Mycelial growth (diameter in mm)				
IUM 4958	*P. umbellatus*	33.1±1.45[b]	37.7±2.41[a]	30.1±1.34[c]	27.0±1.65[d]	17.3±2.25[e]
IUM 4959	*P. umbellatus*	34.5±2.10[b]	36.6±2.65[a]	32.5±2.33[c]	31.3±2.82[c]	18.4±1.39[e]
IUM 4960	*P. umbellatus*	29.3±1.54[c]	39.5±1.80[a]	30.3±3.12[c]	28.0±3.15[c]	16.2±1.52[e]
IUM 4961	*P. umbellatus*	30.2±1.84[b]	37.4±1.27[a]	27.4±2.65[c]	26.6±2.19[c]	15.5±1.74[e]
IUM 4962	*P. umbellatus*	29.9±1.55[c]	40.3±2.62[a]	25.5±2.26[d]	24.8±2.41[d]	19.7±2.67[e]
IUM 0955	*A. mellea*	35.7±2.15[f]	42.5±1.27[e]	60.3±2.45[a]	54.8±3.26[b]	50.3±3.27[c]
IUM 0957	*A. mellea*	36.8±2.95[e]	42.3±2.75[d]	64.1±2..95[a]	55.7±2.87[b]	48.1±3.15[c]
IUM 1238	*A. mellea*	39.6±2.74[e]	45.2±3.12[d]	61.7±3.16[a]	58.9±3.17a	53.2±2.83[b]
IUM 1239	*A. mellea*	37.9±1.95[e]	44.1±2.54[d]	63.5±2.47[a]	56.0±2.98[b]	51.1±2.73[c]
IUM 4585	*A. mellea*	40.5±3.15[e]	47.6±2.36[c]	65.5±1.93[a]	59.8±2.48[b]	49.9±2.69[d]

[a]Values in the same row followed by the same letter are not significantly different ($P < 0.05$).
Temperature effect on mycelial growth was assessed using PDA

Table 3. Effect of pH on mycelial growth of *Polyporus umbellatus* and *Armillaria mellea*

Strain No	Scientific name	pH					
		4	5	6	7	8	9
		Mycelial growth (diameter in mm)					
IUM 4958	*P. umbellatus*	38.7±2.36[a]	37.1±2.64[a]	35.6±2.31[b]	31.2±2.65[c]	21.4±2.55[e]	14.9±2.45[f]
IUM 4959	*P. umbellatus*	37.5±1.63[a]	35.6±1.43[a]	33.3±2.42[b]	30.1±1.75[c]	20.8±1.96[d]	15.1±1.69[e]
IUM 4960	*P. umbellatus*	39.3±2.13[a]	35.3±2.97[b]	33.7±2.12[b]	29.9±2.35[c]	18.5±1.73[d]	12.7±1.62[e]
IUM 4961	*P. umbellatus*	39.2±2.46[a]	34.5±2.28[b]	32.9±2.84[b]	28.7±2.35[c]	19.6±1.47[d]	13.2±2.12[e]
IUM 4962	*P. umbellatus*	39.8±1.78[a]	36.4±1.05[b]	34.5±1.21[b]	30.5±0,89[c]	20.5±1.85[e]	13.4±2.21[f]
IUM 0955	*A. mellea*	40.1±1.98[c]	48.1±2.55[b]	53.3±2.87[a]	33.3±1.91[d]	24.3±1.57[e]	22.7±1.85[e]
IUM 0957	*A. mellea*	39.7±2.57[c]	47.4±2.21[b]	54.7±2.38[a]	35.1±1.65[d]	25.5±2.27[e]	21.9±1.78[f]
IUM 1238	*A. mellea*	40.3±1.65[c]	48.3±1.91[b]	55.5±3.15[a]	36.7±1.73[d]	26.7±1.63[e]	23.5±1.49[f]
IUM 1239	*A. mellea*	39.9±0.93[c]	49.5±2.15[b]	56.1±2.81[a]	34.2±2.17[d]	27.2±0.79[e]	24.7±0.85[f]
IUM 4585	*A. mellea*	43.2±2.67[c]	50.2±3.28[b]	57.2±2.57[a]	37.6±2.48[d]	30.5±1.76[e]	27.2±2.35[f]

[a]Values in the same row followed by the same letter are not significantly different ($P < 0.05$).
Effect of pH on mycelial growth was assessed using PDA.

findings in this experiment. The optimum mycelial growth of *A. mellea* was found at pH 6, whereas poor mycelial growth was observed at pH 9. Therefore, the pH value for optimum mycelial growth of *P. umbellatus* is lower than that of *A. mellea*.

Effect of nutrient source:

Carbon sources

The mycelial growth of *P. umbellatus* was good in the fructose and glucose supplemented media, while relatively poor mycelial growth was observed in maltose and sucrose added medium. The favorable mycelial growth of *A. mellea* was found in fructose and glucose, whereas other 4 carbon sources such as dextrin, maltose, mannose and sucrose showed a moderate mycelial growth (Table 4). Hong *et al.* (1986) reported that cellobiose, one of disaccharide produced from degradation of cellulose, stimulated good mycelial growth of *Ganoderma lucidum*. However, Chang *et al*., (1995) found that cellobiose showed unfavorable mycelial growth for *Fomitella fraxinea*. Therefore, the favorable carbon sources for

Table 4. Effect of carbon sources on mycelial growth of *Polyporus umbellatus* and *Armillaria mellea*

Strain No	Scientific name	Carbon sources[x]					
		Dex	Fru	Glu	Mal	Man	Suc
		Mycelial growth (diameter in mm)					
IUM 4958	*P. umbellatus*	47.1±1.57[b]	51.2±1.65[a]	50.3±2.13[a]	42.5±2.35[d]	52.6±2.11[a]	40.2±.2.24[e]
IUM 4959	*P. umbellatus*	45.3±2.12[b]	51.5±1.12[a]	51.4±2.48[a]	43.3±1.24[c]	50.4±1.65[a]	41.1±1.62[c]
IUM 4960	*P. umbellatus*	47.6±1.68[b]	51.7±2.32[a]	50.5±0.89[a]	43.7±2.15[c]	48.6±1.55[b]	43.5±1.42[c]
IUM 4961	*P. umbellatus*	43.3±2.48[c]	51.6±1.47[a]	52.1±1.59[a]	41.1±0.95[c]	51.7±0.87a	38.7±2.43[d]
IUM 4962	*P. umbellatus*	46.8±2.36[c]	53.5±1.65[a]	53.6±1.98[a]	39.9±1.81[f]	49.8±2.12[b]	43.5±1.15[e]
IUM 0955	*A. mellea*	63.1±2.17[b]	67.1±2.56[a]	66.9±1.55[a]	64.8±1.59[a]	65.5±1.48[a]	63.2±1.78[b]
IUM 0957	*A. mellea*	64.6±2.39[b]	67.9±3.28[a]	68.4±2.63[a]	62.3±2.48[b]	63.1±0.93[b]	63.2±3.12[b]
IUM 1238	*A. mellea*	61.7±1.48[c]	68.5±3.12[a]	67.6±3.42[a]	60.1±3.21[c]	65.3±2.41[b]	63.2±1.68[b]
IUM 1239	*A. mellea*	59.3±3.27[d]	71.4±2.47[a]	70.5±2.24[a]	63.7±2.57[c]	61.8±2.38[c]	63.2±2.57[c]
IUM 4585	*A. mellea*	64.5±3.18[b]	72.1±2.89[a]	71.7±1.68[a]	65.9±2.85[b]	65.4±3.21[b]	63.2±2.62[c]

[a]Values in the same row followed by the same letter are not significantly different ($P < 0.05$).
[x]Dex: Dextrin, Fru: Fructose, Glu: Glucose, Mal: Maltose, Man: Mannose, and Sucrose. Each carbon source was added to the basal medium at the concentration of 0.1M.

Table 5. Effect of nitrogen sources on mycelial growth of *Polyporus umbellatus* and *Armillaria mellea*

Strain No	Scientific name	Nitrogen sources[x]					
		AA	AP	Arg	Pep	Ure	YE
		Mycedlial growth (diameter in mm)					
IUM 4958	*P. umbellatus*	18.2±1.25[f]	29.6±2.52[e]	39.2±1.15[c]	50.3±2.85[a]	34.2±2.65[d]	49.4±2.26[a]
IUM 4959	*P. umbellatus*	17.3±0.85[f]	32.2±2.63[d]	40.4±1.45[c]	52.7±1.46[a]	29.8±1.58[d]	50.1±1.63[a]
IUM 4960	*P. umbellatus*	19.8±2.32[f]	33.7±1.80[d]	41.8±2.57[c]	53.1±0.87[a]	35.6±1.67[d]	52.6±1.43[a]
IUM 4961	*P. umbellatus*	20.5±1.35[f]	35.4±1.40[d]	39.7±2.60[c]	49.9±1.68[a]	36.5±1.86[c]	51.3±1.86[a]
IUM 4962	*P. umbellatus*	21.0±1.24[f]	37.1±2.71[d]	42.5±2.80[c]	54.5±2.36[a]	38.7±1.95[c]	52.8±2.58[a]
IUM 0955	*A. mellea*	34.9±1.87[f]	52.7±2.82[d]	59.8±2.95[c]	69.7±2.60[a]	49.4±2.35[e]	66.4±2.47[a]
IUM 0957	*A. mellea*	37.3±1.63[f]	50.3±2.12[d]	57.6±2.74[c]	68.2±2.15[a]	45.7±2.45[e]	66.4±1.14[a]
IUM 1238	*A. mellea*	35.4±0.91[f]	49.2±1.79[b]	56.1±1.84[c]	70.1±1.58[a]	49.1±2.14[e]	66.4±2.47[a]
IUM 1239	*A. mellea*	36.6±1.55[f]	52.6±1.24[c]	60.5±1.49b	68.4±1.85[a]	47.3±0.87[e]	66.4±1.92[a]
IUM 4585	*A. mellea*	39.7±2.43[f]	53.4±1.78[c]	61.3±1.53[c]	70.3±2.33[a]	50.6±2.64c	66.4±2.38[a]

[a]Values in the same row followed by the same letter are not significantly different ($P < 0.05$).
[x]AA: Ammonium acetate, AP: Ammonium phosphate, Arg: Arginine, Pep: Peptone, Ure, Urea and YE; yeast extract. Each nitrogen source was added to the basal medium at the concentration of 0.02 M.

good mycelial growth are different in many fungi. The experimental results suggested that *A. mellea* utilize wide range of carbon sources for its metabolic pathway than *P. umbellatus*.

Nitrogen sources

It was observed that mycelia of *P. umbellatus* grew well on organic nitrogen such as peptone and yeast extract. The mycelia of *A. mellea* also showed good growth on peptone and yeast extract. However, mycelial growth on ammonium acetate and ammonium phosphate which belong to inorganic nitrogen source exhibited moderately poor mycelial growth compared with amino or organic nitrogen sources such as arginine, peptone and yeast extract (Table 5). Kim *et al*. (1994) reported that the mycelial growth of *Lentinus lepideus* was better in the organic nitrogen sources than that of inorganic nitrogen source. Therefore, our experimental results suggested that mycelial growth of *P. umbellatus* and

Table 6. Carbon sources and pH on sclerotial formation by dual culture between *Polyporus umbellatus* and *Armillaria mellea*

Carbon source	pH 4.0	pH 5.0	pH 6.0	pH 7.0	pH 8.0
Glucose	+	+	+	-	-
Fructose	+	+	+	-	-
Maltose	+	+	+	-	-
Mannose	-	-	-	-	-
Sucrose	-	-	-	-	-
Dextrin	-	-	-	-	-

+, sclerotial formation; -, no sclerotial formation

Table 7. Nitrogen sources and pH on sclerotial formation by dual culture between *Polyporus umbellatus* and *Armillaria mellea*

Carbon source	pH 4.0	pH 5.0	pH 6.0	pH 7.0	pH 8.0
Ammonium acetate	-	-	-	-	-
Ammonium phosphate	-	-	-	-	-
Arginine	-	-	-	-	-
Peptone	+	+	+	-	-
Urea	-	-	-	-	-
Yeast extract	+	+	+	-	-

+, sclerotial formation; -, no sclerotial formation

Fig. 1. Sclerotium formed by dual culture of *Polyporus umbellatus* and *Armillaria mellea* (A) after 40 days of incubation; (B) after 60 days of incubation. S1 ; Young sclerotium formation from dual culture of *P. umbellatus* and *A. mellea*. RM ; Rhizomorph of *A. mellea*

A. mellea were good in organic nitrogen sources than those of inorganic nitrogen sources.

Effect of nutrient sources and pH values for sclerotium formation

Effect of carbon sources and pH value

To screen favorable carbon sources and pH values for sclerotial formation in the media under dual culture of *P. umbellatus* (IUM 4958) and *A. mellea* (IUM 4585), 6 different carbon sources and 5 g peptone as a sole nitrogen nutrient were used. Out of 6 carbon sources, 3 carbon sources such as glucose, fructose and maltose produced sclerotia in the media at pH 4, 5 and 6 (Table 6, Fig. 1). However, *P. umbellatus* could not produce sclerotia at pH 7, 8 and 9. These results suggested that sclerotia of *P. umbellatus* were produced in organic carbon sources at acidic condition under symbiotic relationship with *A. mellea*.

Nitrogen sources:

To screen favorable nitrogen sources and pH values for sclerotial formation, we used the basal medium supplemented with 6 different nitrogen sources and 20 g of fructose as a sole carbon nutrient source. Under the dual culture of *P. umbellatus* and *A. mellea* for 60 days incubation at 20℃ in dark condition, a media contained each peptone and yeast extract produced sclerotia only at pH 4, 5 and 6. However the fungus could not produce sclerotia at pH 7, 8 and 9 (Table 7). These results indicated that *P. umbellatus* only produce sclerotia on organic nitrogen sources and at low pH values in the artificial condition. From these experimental results, we are now searching for new organic nitrogen sources to improve the efficiency of sclerotial production in the laboratory condition.

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References

Cheng, X. H., Guo, S. X. and Wang, C. L. 2006. Factors

influencing formation of sclerotia in *Grifola umbellate* (Pers.) Pilt under artificial conditions. J Integr Plant Biol **48**(11) : 1312-1317.

Liu, Y. Y. and Guo, S. X. 2009. Nutritional fators determining sclerotial formation of *Polyporus umbellatus*. Lett Appl Microbiol **49**(2) : 283-288.

Guo, S. X., Xu, J. T. 1991. Nutrient source of sclerotia of *Grifola umbellata* and its relationship to *Armillaria mellea*. Acta Bot Sin **34** : 576-580.

Kikuchi, G., Yamaji, H. 2010. Identificantion of *Armillaria* species associated with *Polyporus umbellatus* using ITS sequences of nuclear ribosomal DNA. Mycoscience **51** : 366-372.

Lu, W., Adachi, I., Kand, K., Yasuta, A., Toriizuka, K., Ueno, M. and Gorikoshi, I. 1985. Platelet aggregation potentiators from Cho-Rei. Chem. Pharm. Bull. **33**(11) : 5083-5087.

Inaoka, Y., Shakuya, A., Fukazawa, H., Ishida, H., Nukaya, H., Tsuji, K., Kuroda, H., Okada, M., Fukushima, M. and Kosuge, T. 1994. Studies on active substances in herbs used for hair treatment. I. Effects of herb extracts on hair growth and isolation of an active substance from *Polyporus umbellatus* F. Chem. Pharm. Bull. **42**(3) : 530-533.

You, J. S., Hau, D. M., Chen, K. T. and Huang, H. F. 1994. Combined effects of Chuling (*Polyporus umbellatus*) extract and mitomycin C on experimental liver cancer. Amer. J. Chinese Medicine **22**(1) : 19-28.

Oh, M. H., Houghton, P. J., Whang, W. K. and Cho, J. H. 2004. Screening of Korean herbal medicines used to improve cognitive function for anti-cholinesterase activity. Phytomedicine **11** : 544-548.

Ohsawa, T., Yukawa, M., Takao, C., Murayama, M. and Bando, H. 1992. Studies on constituents of fruit body of *Polyporus umbellatus* and their cytotoxic activity. Chem. Pharm. Bull. **40**(1) : 143-147.

Shim, J. O., Son, S. G., Kim, Y. H., Lee, Y. S., Lee, J. Y., Lee, T. S., Lee, S. S., and Lee, M. W. 1997. The Cultural Conditions Affecting the Mycelial Growth of *Grifola umbellata*. Kor. J. Mycol. **25**(3) : 209-218.

Sung, J. M., Moon, H. W. and Park, D. S. 1999. Growth condition of liquid culture by *Pleurotus ostreatus*. Kor. J. Mycol. **27(1)** : 1-9.

Sato, K., Osawa, M., Suzuki, Y. and Oikawa, S. 1984. Difference in fruiting capability of stocks in Grifola frondosa and its allied species. Trans. Mycol. Soc. Japan. **25** : 205-209.

Xing, Y. M., Chen, J., Lv, Y. L., Liang, H. Q. and Guo, S. X. 2011. Determination of optimal carbon source and pH value for sclerotial formation of *Polyporus umbellatus* under artificial conditions. Mycol Progress. **10** : 121-125.

Hong, J. S., Choi, Y. H. and Yun, S. E. 1986. Studies on the Cellulolytic Enzymes Produced by *Ganoderma lucidum* in Synthetic Media. Kor. J. Mycol. **14** : 121-130.

Chang, H. Y., Cha, D. Y., Kang, A. S., Hong, I. P., Kim, K. P., Seok, S. J., Ryu, Y. J. and Sung, J. M. 1995. Cultural Characteristics of *Fomitella fraxinea* (Fr.) Imaz. Kor. J. Mycol. **23**(3) : 238-245.

Kim, H. K., Park, J. S., Cha, D. Y., Kim, Y. S. and Moon B. J. 1994. Study on the artificial cultivation of *Lentinus lepideus*(Fr. ex Fr.) Fr. -investigation of mycelial growth conditions- Kor. J. Mycol. **22**(2) : 145-152.