

## Favorable Culture Conditions for Mycelial Growth of Korean Wild Strains in *Ganoderma lucidum*

Chandana Jayasinghe<sup>1</sup>, Ahmed Imtiaj<sup>1</sup>, Hyun Hur<sup>2</sup>, Geon Woo Lee<sup>1</sup>, Tae Soo Lee<sup>1</sup> and U Youn Lee<sup>1\*</sup>

<sup>1</sup>Department of Biology, University of Incheon, Incheon 402-749, Korea

<sup>2</sup>Department of Biology, Dongguk University, Seoul 100-715, Korea

(Received February 19, 2008. Accepted March 15, 2008)

*Ganoderma lucidum* (Fr.) Karst (Polyporaceae), belonging to basidiomycota, is one of the most famous medicinal mushrooms. This study was carried out to investigate favorable mycelial growth conditions, such as pH, temperature, growth media, carbon sources and nitrogen sources of Korean strains in *G. lucidum*. The most suitable temperature for the mycelial growth was obtained at 30°C. In general, optimal temperature range for the mycelial growth was found at 25–30°C. This Mushroom has a broad pH range (5–9) for its mycelial growth and mostly favorable growth was found at pH 5. Generally, Hamada, Glucose peptone, YM, Mushroom complete and Lilly media were the most suitable for the mycelial growth of *G. lucidum*. Among 10 different carbon sources, dextrin, galactose and fructose were best but the rest of other carbon sources also facilitated the growth of mycelia. The most suitable nitrogen sources were ammonium acetate, glycine, arginine and calcium nitrate, but to a certain extent, all of the supplemented nitrogen sources also stimulated the mycelial growth.

**KEYWORDS :** Culture condition, *Ganoderma lucidum*, Media, Mycelial growth, Nutrition

*Ganoderma lucidum* (Fr.) Karst (Polyporaceae), a basidiomycota, is one of the most famous medicinal mushrooms in Asian countries. Its fruiting body is called 'Bulnocho' in Korea, 'Lingzhi' in China, and 'Reishi' in Japan. In Korea, China and Japan, *G. lucidum* has been a popular medicinal mushroom used for treating many diseases, such as hepatitis, hypertension, hypercholesterolemia and gastric cancer (Mizuno *et al.*, 1995). Several biological active triterpenes and sterols have been isolated from this mushroom and showed cytotoxic, (Toth *et al.*, 1983; Kohda *et al.*, 1985) antiviral (Lindequist *et al.*, 1989) and anti inflammatory activities (Tasaka *et al.*, 1988). Polysaccharides and glycoproteins possessing hypoglycemic (Hikino and Mizuno 1989) and immunostimulant (Kino *et al.*, 1989) activities have also been found from the water extract of the fruiting body in Taiwan. In recent years, submerged cultivation of *G. lucidum* has been developed to obtain mycelial biomass, ganoderic acid and polysaccharides which can be used to produce medicinal products (Yang and Liao, 1998; Yang *et al.*, 2000). To accelerate mycelial growth and metabolite production, the major concerns were to find environmentally good and economically feasible compounds that stimulate mycelial growth and metabolite production of *G. lucidum* (Yang *et al.*, 2000). Therefore, the submerged culture of *G. lucidum* has received great attention as a promising alternative for efficient production of its valuable metabolites, especially polysaccharide (Lee *et al.*, 1999a; Yang and Liao, 1998)

and ganoderic acid (Tsujikura *et al.*, 1992).

Thus, it is necessary to find optimal nutritional and environmental conditions for culturing mycelia in the liquid and solid media. Because of that, this study was conducted to find out important parameters that affect mycelia growth of Korean strains in *G. lucidum*.

### Materials and Methods

**Collection, identification and isolation.** The fruiting bodies of 10 strains of *Ganoderma lucidum* were collected from different parts of Korea (Table 1). After identification, mycelia were isolated using standard potato dextrose agar (PDA) method and incubated for 10 days at 25°C. After isolation, pure culture of the mycelia was stored at 4°C for further study.

**Effect of temperature.** To find out the optimum temperature for the favorable mycelial growth of *G. lucidum*,

**Table 1.** List of *G. lucidum* strains used in this study

Strain No.	Geographical origin, Korea
IUM 0037	Jangneung, Gyeonggido
IUM 0047	Jongmyo, Seoul
IUM 0637	Yungunneung, Gyeonggido
IUM 0751	Jangneung, Gyeonggido
IUM 0757	Cheolmasan, Incheon
IUM 0805	Dong gureung, Gyeonggido
IUM 0938	Cheolmasan, Incheon
IUM 1027	Cheolmasan, Incheon

\*Corresponding author <E-mail : uylee@incheon.ac.kr>

15 ml of molten PDA was dispensed into each of 9 cm sterile Petri dish. Disc (5 mm in diameter) taken from advancing margin of 10 day old cultures of the isolates of each strain by the aid of a cork borer were separately placed each at the centre of the dish. The inoculated dishes were incubated at 15–35°C (5°C intervals) for 10 days under dark condition. Mycelial growths on agar plates were measured on the basis of mean colony diameter and density. Each test was replicated 4 times.

**Effect of pH.** To find out optimum pH for the favorable mycelial growth of *G. lucidum*, 15ml of molten PDA was dispensed into each of 9 cm sterile Petri dish. Dishes (5 mm in diameter) was taken from 10 day old PDA culture and placed on the center of the plate similarly. The medium was adjusted to pH of 5, 6, 7, 8 and 9 with the addition of 1 N NaOH or HCl and incubated for 10 days at 25°C under dark condition. The measurement of mycelial growth and density was performed as described earlier.

**Favorable culture media.** Disc (5 mm in diameter) was taken and placed on the center of each plate separately filled with 10 different solid culture media namely Czapek dox, Hamada, Hennerberg, Hoppkins, Glucose peptone, Glucose tryptone, Lilly, Mushroom complete, PDA and YM (Yeast-malt extract) (Table 2). The inoculated

dishes were replicated four times and incubated at 25°C for 10 days under dark condition. Mycelial growth on agar plate was measured following same manner described previously.

**Effect of carbon and nitrogen sources.** Tests were performed in the basal medium (Sung *et al.*, 1993) to screen carbon and nitrogen sources favorable for the mycelial growth of selected *G. lucidum* strains, supplemented with each of 10 carbon and 10 nitrogen sources. The basal medium was composed of MgSO<sub>4</sub> (0.05 g), KH<sub>2</sub>PO<sub>4</sub> (0.46 g), K<sub>2</sub>HPO<sub>4</sub> (1 g), Thiamine-HCl (120 µg), Agar (20 g) and distilled water (1000 ml). To screen for the best carbon source favorable for the mycelial growth, each carbon source supplemented with 5 g of peptone to the basal medium at the concentration of 0.1 M per 1000 ml and mixed thoroughly (Shim *et al.*, 1997). The basal medium which was used for screening the favorable nitrogen source was made of same additives as those described by Sung *et al.* (1993). Each nitrogen source with 20 g of glucose was added to the basal medium at the concentration of 0.02 M (Shim *et al.*, 1997). In both cases, the basal medium was adjusted to pH 6 and autoclaved for 15 minutes at 121°C, poured into plates. The inoculated dishes were replicated four times and incubated at 25°C for 10 days under dark condition. Mycelial growth was measured as describe before.

**Table 2.** Media and their compositions used in this study

Composition	Media (g/l)									
	Cza	Ham	Hen	Hop	GP	GT	Lil	MC	PDA	YM
Agar	20	20	20	20	20	20	20	20	20	20
Asparagine							2			
Dextrose		10							20	10
Ebiose		5								
Hyponex		3								
Glucose			50	10	10	5				
Malt-extract					15			20		3
Maltose							10			
Peptone					10			2		5
Potatoes									200	
Sucrose	30									
Tryptone						10				
Yeast-extract		3			10	3		2		3
NaNO <sub>3</sub>	3		2							
K <sub>2</sub> HPO <sub>4</sub>	1							1		
MgSO <sub>4</sub>	0.5		0.5	0.5			0.5	0.5		
KCl	0.5									
FeSO <sub>4</sub>	0.01									
CaCl <sub>2</sub>			0.1							
KH <sub>2</sub> PO <sub>4</sub>			1	0.1			1	0.5		
KNO <sub>3</sub>			2	2						

Cza: Czapek dox, Ham: Hamada, Hen: Hennerberg, Hop: Hoppkins, GP: glucose peptone, GT: glucose tryptone, Lil: Lilly, MC: mushroom complete, PDA: potato dextrose agar and YM: yeast-malt extract

**Table 3.** Effect of temperatures on the mycelial growth and density of different strains of *G. lucidum*

Strain No.	Mycelial growth (mm) <sup>a</sup> and density				
	15°C	20°C	25°C	30°C	35°C
IUM 0037	17.0 ± 1c	64.7 ± 1c	68.0 ± 3c	87.0 ± 0c	34.0 ± 1c
IUM 0047	13.7 ± 3c	60.7 ± 3c	75.7 ± 4c	87.0 ± 0c	35.3 ± 5c
IUM 0637	28.0 ± 4c	50.3 ± 6c	87.0 ± 0c	87.0 ± 0c	11.7 ± 2c
IUM 0751	26.0 ± 0c	41.0 ± 5c	87.0 ± 0c	87.0 ± 0c	–
IUM 0757	17.3 ± 1c	40.0 ± 8c	87.0 ± 0	87.0 ± 0c	–
IUM 0805	–	23.7 ± 1c	34.3 ± 2c	23.0 ± 6c	14.0 ± 2c
IUM 0938	–	22.7 ± 2c	34.3 ± 1c	26.3 ± 1c	13.7 ± 1c
IUM 1027	14.3 ± 2c	38.0 ± 4c	87.0 ± 0c	87.0 ± 0c	–
Mean	14.53 ± 1	42.6 ± 4	70.0 ± 1	71.4 ± 1	13.5 ± 1

<sup>a</sup>Mean of four replications. Temperature and pH effects were conducted in potato dextrose agar medium (PDA). c: Compact, sc: Somewhat compact, t: Thin and st: Somewhat thin.

## Results and Discussion

**Effect of temperature.** In general, the minimum and maximum cardinal temperatures for the mycelial growth and density of *G. lucidum* were 15 and 35°C, respectively. The best mycelial growth was (87 mm) obtained at 30°C followed by 35°C (Table 3). Nevertheless, some of strains (IUM 637, 751, 757 and 1027) showed optimum growth at 25°C. However, IUM 938 always showed slow growth at all temperatures. No mycelial growth was found at 15°C (IUM805 and 938) and 35°C (IUM721, 757 and 1027). But, incubation at 20°C showed considerable mycelial growth for all strains of *G. lucidum*. Therefore, experimental results indicated that, optimum temperature range was 25–30°C for the mycelial growth of 8 strains of *G. lucidum*. Lee *et al.* (1999b) and Shim *et al.* (2003) reported that the mycelial growth of *Paecilomyces fumosoroseus* had been expedited gradually in proportion to the rise of temperature and was the most suitable at 25°C. Even though the mycelial growth of *P. fumosoroseus* was favorable at the range of 20–25°C and had been expedited in proportion to the rise of temperature, the mycelial growth appeared to be sup-

pressed at the temperature higher than 30°C. Similarly, there were slow growth at 15 and 35°C. This could be due to the denaturation and inactivation of important enzymes which catalyze metabolic processes of tested mushroom strains. Jonathan (2002) also reported that the growth of *S. commune* was inhibited at 45 and 50°C. Jonathan and Fasidi (2003) found that *Psathyrella atroumbonatai* grew fairly well within the temperature range of 25–35°C.

**Effect of pH.** Favorable mycelial growth of *G. lucidum* was obtained at the pH range of 5–9 (Table 4). Among the eight strains, optimal mycelial growth was found at pH 5 (IUM 37, 47, 805 and 938), 8 (IUM637) and 9 (IUM751, 757 and 1027). But, 8 strains of *G. lucidum* also showed good mycelial growth and density in the rest of pH. Therefore, the results indicated that *G. lucidum* strains can grow at broad pH range, such as pH 5–9. This result also implies that different *G. lucidum* strains prefer different pH values tending toward neutrality. Likewise, Chandra and Purkayastha (1977) and Jonathan *et al.* (2004) obtained very good mycelial growth of *Agaricus campestris* and *Volvariella esculenta* at pH 6. Fasola *et al.* (2007) also reported that *V. speciosa* grew over a wide

**Table 4.** Effect of pH on the mycelial growth and density of different strains of *G. lucidum*

Strain No.	Mycelial growth (mm) <sup>a</sup> and density				
	pH 5	pH 6	pH 7	pH 8	pH 9
IUM 0037	87.0 ± 0c	78.0 ± 3c	73.7 ± 4c	71.3 ± 1c	66.7 ± 4c
IUM 0047	87.0 ± 0c	73.3 ± 1c	65.7 ± 2c	59.0 ± 1c	49.3 ± 1c
IUM 0637	40.3 ± 1c	42.3 ± 3c	54.7 ± 2c	58.0 ± 7c	57.0 ± 3c
IUM 0751	16.0 ± 2c	16.7 ± 3c	20.3 ± 1c	20.7 ± 1c	25.3 ± 3c
IUM 0757	27.7 ± 3c	30.3 ± 3c	36.7 ± 2c	42.7 ± 7c	46.0 ± 8c
IUM 0805	87.0 ± 0c	87.0 ± 0c	84.7 ± 3c	54.3 ± 1c	16.7 ± 1c
IUM 0938	87.0 ± 0c	78.7 ± 1c	71.7 ± 1c	71.3 ± 1c	68.7 ± 1c
IUM1027	21.3 ± 2c	22.3 ± 3c	24.7 ± 1c	30.7 ± 3c	31.7 ± 3c
Mean	56.6 ± 1	53.5 ± 2	54.0 ± 2	51.0 ± 3	45.2 ± 3

<sup>a</sup>Mean of four replications. Temperature and pH effects were conducted in potato dextrose agar medium (PDA). c: Compact, sc: Somewhat compact, t: Thin and st: Somewhat thin.

range of pH but optimum growth was obtained at pH 6. Adejaye *et al.* (2007) also reported *S. commune* showed favorable growth at pH 5.5. Akinyele and Adetuyi (2005) reported that pH range for the mycelial growth of *V. volvacea* was 5.5–8.5, while Kuforiji and Fasidi (1998) obtained an optimal pH for mycelial growth of *Pleurotus tuberregium*, *Phellinus japonica* and *P. linteus* at 5–7, 6–7 and 7, respectively. They suggested that mushrooms may have a broad pH range for their favorable mycelial growth.

**Screening for favorable culture media.** Ten different culture media were used to screen the optimal mycelial growth of 8 different strains of *G. lucidum*. The result showed that Hamada, Glucose peptone, YM, Mushroom complete and Lilly media were the most suitable and Hopkins was the most unfavorable for mycelial growth of *G. lucidum*. But remaining media were also showed comparatively good mycelial growth for *G. lucidum* (Table 5). This result is corresponded with that of *P. sinclairii* and *P. fumosoroseus* which had been studied by Shim *et al.* (2003) where mycelial growth was optimal on

Hamada medium. Shim *et al.* (2005) also reported that PDA, YM, Mushroom complete and Hamada were the most suitable, where Czapek dox and Glucose peptone were unfavorable to mycelial growth of *Macrolepiota procera*.

**Effect of carbon sources.** It was found that dextrin was the best carbon source for mycelial growth of *G. lucidum* (Table 6). This was closely followed by galactose and fructose which were not considerably different each other. On the other hand, mannose, maltose, sucrose and xylose showed moderate mycelial growth, while sorbitol lactose and glucose showed slow level of mycelial growth in *G. lucidum*. But in case of IUM 751, 757 and 1027, optimum mycelial growth was obtained at galactose, while IUM 938, optimum mycelial growth was found on fructose. However, Chandra and Purkayastha (1977) reported that most of the tropical edible macrofungi were in favor of utilizing glucose than other carbon sources. The preference of glucose over other carbon compounds may be due to the fast metabolization of glucose by the fungi to produce cellular energy easily (Garraway and Evans, 1984).

**Table 5.** Effect of media on the mycelial growth and density of different strains of *G. lucidum*

Strain No.	Mycelial growth (mm) <sup>a</sup> and density									
	MC	Lil	Hop	Cza	Ham	GT	YM	GP	Hen	PDA
IUM 0037	87.0 ± 0c	70.0 ± 1c	32.0 ± 1t	51.7 ± 2t	87.0 ± 0c	25.7 ± 1sc	87.0 ± 0c	87.0 ± 0.c	87.0 ± 0c	68.0 ± 3c
IUM 0047	87.0 ± 0c	87.0 ± 0c	75.0 ± 4t	47.7 ± 8t	87.0 ± 0c	87.0 ± 0c	87.0 ± 0c	75.7 ± 4.c	87.0 ± 0c	75.7 ± 4c
IUM 0637	87.0 ± 0c	87.0 ± 0sc	74.7 ± 9st	77.0 ± 0st	87.0 ± 0c	87.0 ± 0c	87.0 ± 0c	87.0 ± 0c	87.0 ± 0c	87.0 ± 0c
IUM 0751	86.0 ± 1c	87.0 ± 0sc	70.3 ± 1st	66.3 ± 6sc	87.0 ± 0c	74.0 ± 6c	87.0 ± 0c	83.0 ± 4c	87.0 ± 0c	87.0 ± 0c
IUM 0757	87.0 ± 0c	83.0 ± 3sc	29.7 ± 3st	59.7 ± 4sc	87.0 ± 0c	78.7 ± 1c	87.0 ± 0c	87.0 ± 0c	87.0 ± 0c	87.0 ± 0c
IUM 0805	52.3 ± 2c	57.3 ± 1c	41.7 ± 2t	30.7 ± 2t	87.0 ± 0c	55.3 ± 2c	70.0 ± 3c	83.7 ± 2c	87.0 ± 0c	34.3 ± 2c
IUM 0938	17.7 ± 1sc	23.7 ± 2t	14.7 ± 1t	23.0 ± 1sc	19.7 ± 2sc	20.0 ± 2c	23.0 ± 1c	30.3 ± 2c	31.0 ± 1t	34.3 ± 1c
IUM 1027	87.0 ± 0c	87.0 ± 0sc	38.0 ± 3st	57.7 ± 4sc	87.0 ± 0c	79.0 ± 3c	87.0 ± 0c	87.0 ± 0c	–	87.0 ± 0c
Mean	73.8 ± 1	72.8 ± 1	47.1 ± 3	51.7 ± 3	78.6 ± 0	63.3 ± 2	76.8 ± 1	76.3 ± 2	69.1 ± 0	70.0 ± 1

<sup>a</sup>Mean of four replications. Cza: Czapek dox, Ham: Hamada, Hen: Hennerberg, Hop: Hopkins, GP: glucose peptone, GT: glucose tryptone, Lil: Lilly, MC: mushroom complete, PDA: potato dextrose agar and YM: yeast-malt extract. c: Compact, sc: Somewhat compact, t: Thin and st: Somewhat thin.

**Table 6.** Effect of carbon sources on the mycelial growth and density of different strains of *G. lucidum*

Strain No.	Mycelial growth (mm) <sup>a</sup> and density									
	Suc	Sor	Lac	Xy	Fru	Glu	Gal	Dex	Mal	Man
IUM 0037	42.0 ± 1c	30.7 ± 3c	33.0 ± 1c	51.7 ± 1c	52.0 ± 1c	35.3 ± 1c	57.0 ± 2c	64.3 ± 1c	41.3 ± 1c	35.7 ± 1c
IUM 0047	41.0 ± 1c	31.3 ± 3c	33.7 ± 1c	46.7 ± 9c	52.7 ± 1c	36.0 ± 1c	56.3 ± 2c	63.3 ± 2c	41.0 ± 0c	36.7 ± 1c
IUM 0637	16.0 ± 5c	16.3 ± 2c	12.3 ± 1c	18.7 ± 3c	19.7 ± 2c	13.7 ± 2c	17.7 ± 1c	32.0 ± 2c	17.7 ± 3c	24.3 ± 7c
IUM 0751	27.7 ± 2c	26.0 ± 1c	22.0 ± 4c	27.7 ± 1c	9.0 ± 2c	25.3 ± 3c	28.3 ± 3c	27.3 ± 2c	26.7 ± 1c	30.0 ± 2c
IUM 0757	20.0 ± 1c	10.0 ± 0c	13.7 ± 2c	27.0 ± 0c	15.7 ± 1c	13.0 ± 0sc	28.7 ± 3c	20.3 ± 5c	12.0 ± 0c	15.7 ± 1c
IUM 0805	41.0 ± 1c	31.3 ± 3c	33.7 ± 1c	46.7 ± 9c	52.7 ± 1c	36.0 ± 1c	56.3 ± 2c	63.3 ± 2c	41.0 ± 0c	36.7 ± 1c
IUM 0938	71.3 ± 2c	56.7 ± 1c	42.0 ± 1t	30.3 ± 1t	87.0 ± 0c	53.3 ± 1c	70.7 ± 1c	84.7 ± 1c	87.0 ± 0c	72.3 ± 2sc
IUM 1027	22.3 ± 1c	10.3 ± 1c	13.0 ± 1c	24.7 ± 1c	17.7 ± 1c	12.3 ± 1c	25.3 ± 1c	20.7 ± 1c	14.3 ± 2c	15.0 ± 2c
Mean	35.2 ± 2	26.6 ± 2	25.4 ± 2	34.2 ± 3	40.8 ± 1	28.1 ± 1	42.5 ± 2	46.9 ± 2	35.1 ± 1	33.3 ± 2

<sup>a</sup>Mean of four replications. Dex: dextrin, Fr: fructose, Ga: galactose, Gl: glucose, Lac: lactose, Mal: maltose, Man: mannose, Sor: sorbitol, Suc: sucrose and Xy: xylose. Each carbon source was added to the basal medium at the concentration of 0.1 M. c: Compact, sc: Somewhat compact, t: Thin and st: Somewhat thin

**Table 7.** Effect of nitrogen sources on the mycelial growth and density of different strains of *G. lucidum*

Strain No.	Mycelial growth (mm) <sup>a</sup> and density									
	Met	PN	Ala	Arg	His	Ur	Gly	AP	CN	AA
IUM 0037	49.7 ± 3st	48.7 ± 2st	61.3 ± 1t	82.7 ± 1sc	28.3 ± 1st	61.7 ± 1sc	67.3 ± 2sc	45.3 ± 2sc	69.0 ± 2st	70.7 ± 2sc
IUM 0047	59.0 ± 2st	77.3 ± 2sc	51.0 ± 1t	87.0 ± 0c	64.3 ± 1st	87.0 ± 0c	87.0 ± 0c	54.7 ± 3st	87.0 ± 0c	87.0 ± 0c
IUM 0637	19.0 ± 3st	21.0 ± 2sc	42.0 ± 10sc	35.0 ± 10c	8.3 ± 8st	36.7 ± 3c	30.7 ± 6c	23.0 ± 10c	35.7 ± 1c	41.7 ± 10c
IUM 0751	24.0 ± 0sc	21.7 ± 10c	23.7 ± 2sc	28.7 ± 2c	–	33.7 ± 6c	27.7 ± 8c	36.3 ± 4c	34.0 ± 11sc	48.3 ± 8c
IUM 0757	10.0 ± 0st	8.7 ± 1st	33.0 ± 3st	9.0 ± 1st	–	–	10.0 ± 0st	–	10.0 ± 0st	25.0 ± 0st
IUM 0805	41.0 ± 1c	31.3 ± 3c	33.7 ± 1c	46.7 ± 9c	52.7 ± 1c	36.0 ± 1c	56.3 ± 2c	63.3 ± 2c	41.0 ± 0c	36.7 ± 10c
IUM 0938	59.0 ± 2st	77.3 ± 2sc	51.0 ± 1t	87.0 ± 0c	64.3 ± 1st	87.0 ± 0c	87.0 ± 0c	54.7 ± 3st	87.0 ± 0c	87.0 ± 0c
IUM 1027	10.7 ± 2st	21.0 ± 2sc	18.3 ± 2sc	35.0 ± 10c	–	–	9.70 ± 6st	–	10.3 ± 1st	22.7 ± 2st
Mean	34.05 ± 2	38.4 ± 3	39.3 ± 3	51.4 ± 4	29.9 ± 2	42.8 ± 1	52.3 ± 1	34.7 ± 3	46.8 ± 2	52.4 ± 4

<sup>a</sup>Mean of four replications. Ala: alanine, AA: ammonium acetate, AP: ammonium phosphate, Arg: arginine, CN: calcium nitrate, Gly: glycine, His: histidine, Met: methionine, PN: potassium nitrate and Ur: urea. Each nitrogen source was added to the basal medium at the concentration of 0.02 M. c: Compact, sc: Somewhat compact, t: Thin and st: Somewhat thin.

*dum* strains, is an isomer of glucose which can be easily transformed to glucose during metabolic pathway (Morrisson and Boyd, 1992). Griffin (1994) suggested that mannose and fructose are the most commonly utilized sugars after glucose. Shim *et al.* (2005) proved that maltose, dextrin, sucrose and mannose were effective where lactose was highly negative for mycelial growth of *M. procera*. Shim *et al.* (1997) reported that mycelial growth of *Gri-fola umbellata* was favorable to all tested carbon sources except salicin, cellobiose and lactose. Shim *et al.* (2003) revealed that dextrin was suitable for mycelial growth of *P. fumosoroseus*. Those results are partially similar to our findings, but they showed that mycelial density in all carbon sources is thin where our result is opposite.

**Effect of nitrogen sources.** According to the experimental results, eight strains of *G. lucidum* showed optimum mycelial growth on ammonium acetate, glycine, arginine and calcium nitrate. However, remaining other nitrogen sources also facilitated considerable mycelial growth of *G. lucidum* (Table 7). In case of IUM 757 and 1027, these strains showed no mycelial growth on histidine, urea and ammonium phosphate. The strain of IUM 751 also showed no mycelial growth on histidine. Shim *et al.* (2005) clarified that glycine was the most favorable and histidine, arginine and ammonium oxalate were the most unfavorable nitrogen sources for the mycelial growth of *M. procera*. In general, organic nitrogen sources are more effective than inorganic nitrogen source for mycelial growth. But from this experiment, it was observed that inorganic nitrogen sources also enhanced the mycelial growth of *G. lucidum*. Therefore, the present findings are very similar to the observations for *Lentinus lepideus* (Kim *et al.*, 1994) and *V. esculenta* (Jonathan *et al.*, 2004). They reported that mycelial growth of these 2 species was more favorable on the culture media containing organic nitrogen sources than inorganic nitrogen sources.

## Acknowledgements

This research was supported by research grant from University of Incheon, Korea (2006).

## References

- Adejoye, O. D., Adebayo-Tayo, B. C., Ogunjobi, A. A. and Afolabi, O. O. 2007. Physicochemical studies on *Schizophyllum commune* (Fries), a Nigerian edible fungus. *World Appl. Sci. J.* 2:73-76.
- Akinyele, B. J. and Adetuyi, F. C. 2005. Effect of agrowastes, pH and temperature variation on the growth of *Volvariella volvacea*. *Afr. J. Biotech.* 4:1390-1395.
- Chandra, A. and Purkayastha, R. P. 1977. Physiological studies on Indian mushrooms. *Trans. Br. Mycol. Soc.* 69:63-70.
- Fasola, T. R., Gbolagade, J. S. and Fasidi, I. O. 2007. Nutritional requirements of *Volvariella speciosa* (Fr. Ex. Fr.) Singer, a Nigerian edible mushroom. *Food Chem.* 100:904-908.
- Griffin, D. H. 1994. Chemical requirement for growth. In: *Fungal Physiology*, 2nd edition, pp. 130-157. Wiley Liss. New York.
- Garraway, O. M. and Evans, C. R. 1984. *Fungal Nutrition and Physiology*. John Wiley and Sons, New York.
- Hikino, H. and Mizuno, T. 1989. Hypoglycemic actions of some heteroglycans of *Ganoderma lucidum* fruit bodies. *Planta Medica* 55:385-389.
- Jonathan, S. G., Fasidi, I. O. and Ajayi, E. J. 2004. Physico-chemical studies on *Volvariella esculenta* Mass (Singer), a Nigerian edible fungus. *Food Chem.* 85:339-342.
- Jonathan, S. G. and Fasidi, I. O. 2003. Studies on *Psathyrella atroumbonatai* (Pegler), a Nigerian edible fungus. *Food Chem.* 81:481-484.
- Jonathan, S. G. 2002. Vegetative growth requirements and antimicrobial activities of some higher fungi in Nigeria. Ph. D. Thesis. University of Ibadan, Ibadan, Nigeria.
- 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.). *Kor. J. Mycol.* 22:145-149.
- Kino, K., Yamashita, A., Yamaoka, K., Watanabe, J., Tanaka, S., Ko, K., Shimizu, K. and Tsuno, H. 1989. Isolation and characterization of a new immunomodulatory protein, Ling Zhi-8 (LZ-8),

- from *Ganoderma lucidum*. *J. Biol. Chem.* 264:472-478.
- Kohda, H., Tokumoto, W., Sakamoto, K., Fujii, M., Hirai, Y., Yamasaki, K., Komoda, Y., Nakamura, H., Ishihara, S. and Uchida, M. 1985. The biologically active constituents of *Ganoderma lucidum* (Fr.) Karst. histamine release-inhibitory triterpenes. *Chem. Pharm. Bull.* 33:1367-1374.
- Kuforiji, O. O. and Fasidi, I. O. 1998. Growth requirement of *Pleurotus tuberregium*, a Nigerian mushroom. *Proc. Nigerian Soci. Micro.* 1:73-78.
- Lee, K. M., Lee, S. Y. and Lee, H. Y. 1999a. Bistage control of pH for improving exopolysaccharide production from mycelia of *Ganoderma lucidum* in an air-lift fermentor. *J. Biosci. Bioeng.* 88:646-650.
- Lee, I. K., Shim, H. J., Woo, S. D., Je, Y. H., Yang, Z. and Kang, S. K. 1999b. Variation in growth and pathogenicity of *Beauveria barssiana* and *Paecilomyces fumosoroseus* pathogenic to the pine gall midge, *Thecodiplosis japonensis*. *Kor. J. Appl. Micro. Biotech.* 27:415-418.
- Lindequist, U., Lesnau, A., Teuscher, E. and Pilgrim, H. 1989. Untersuchungen zur antiviralen Wirksamkeit von Ergosterolperoxid. *Pharmazie* 44:579-580.
- Mizuno, T., Wang, G., Zhang, J., Kawagishi, H., Nishitoba, T. and Li, J. 1995. Reishi, *Ganoderma lucidum* and *Ganoderma tsugae*: bioactive substances and medicinal effects. *Food Rev. Intl.* 11:151-166.
- Morrison, R. and Boyd, R. N. 1992. Carbohydrates I. Monosaccharides. In: *Organic Chemistry*, pp. 1144-1146. Englewood Cliffs, Prentice Hall. New Jersey.
- 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 culture conditions affecting the mycelial growth of *Grifola umbellata*. *Kor. J. Mycol.* 25:209-218.
- Shim, S. M., Lee, K. R., Kim, S. H., Im, K. H., Kim, J. W., Lee, U. Y., Shim, J. O., Lee, M. W. and Lee, T. S. 2003. The optimal culture conditions affecting the mycelial growth and fruiting body formation of *Paecilomyces fumosoroseus*. *Mycobiology* 31:214-220.
- Shim, S. M., Oh, Y. H., Lee, K. R., Kim, S. H., Im, K. H., Kim, J. W., Lee, U. Y., Shim, J. O., Shim, M. J., Lee, M. W., Ro, H. S., Lee, H. S. and Lee, T. S. 2005. The characteristics of culture conditions for the mycelial growth of *Macrolepiota procera*. *Mycobiology* 33:15-18.
- Sung, J. M., Kim, C. H., Yang, K. J., Lee, H. K. and Kim, Y. S. 1993. Studies on distribution and utilization of *Cordyceps militaris* and *C. nutans*. *Kor. J. Mycol.* 21:94-105.
- Tasaka, K., Akagi, M., Miyoshi, K., Mio, M. and Makino, T. 1988. Antiallergic constituents in the culture medium of *Ganoderma lucidum*. (I) Inhibitory effect of cyclooctasulfur on histamine release. *Agents Actions* 23:153-156.
- Toth, J. O., Luu, B. and Ourisson, G. 1983. Ganoderic acid T and Z: cytotoxic triterpenes from *Ganoderma lucidum*. *Tetrahedron Lett.* 24:1081-1084.
- Tsujikura, Y., Higuchi, T., Miyamoto, Y. and Sato, S. 1992. Manufacture of ganoderic acid by fermentation of *Ganoderma lucidum*. *Jpn. Kokai Tokkyo Koho.* JP04304890.
- Yang, F. C. and Liao, C. B. 1998. The influence of environmental conditions on polysaccharide formation by *Ganoderma lucidum* in submerged fermentations. *Process. Biochem.* 33:547-553.
- Yang, F. C., Ke, Y. F. and Kuo, S. S. 2000. Effect of fatty acids on the mycelia growth and polysaccharide formation by *Ganoderma lucidum* in shake flask cultures. *Enz. Microb. Technol.* 27:295-301.