Mycobiology 34(4): 200-205 (2006)
Copyright © 2006 by The Korean Society of Mycology

The Culture Conditions for the Mycelial Growth of *Phellinus* spp.

Woo-Sik Jo*, Young-Hyun Rew, Sung-Guk Choi, Geon-Sik Seo[1], Jae-Mo Sung[2] and Jae-Youl Uhm[3]

Department of Agricultural Environment, Gyeongbuk Agricultural Technology Administration, Daegu 702-320, Korea
[1]Department of Industrial Crops, Korea National Agricultural College, Hwasung 441-893, Korea
[2]Department of Applied Bioloty, Kangwon National University, Chuncheon 200-701, Korea
[3]Department of Agricultural Biology, Kyeongbuk National University, Daegu 702-701, Korea

(Received October 25, 2006)

***Phellinus* genus belonged to Hymenochaetaceae of Basidiomycetes and has been well known as one of the most popular medicinal mushrooms due to high antitumor activity. This study was carried out to obtain the basic information for mycelial culture conditions of *Phellinus linteus*, *P. baumii*, and *P. gilvus*. According to colony diameter and mycelial density, the media for suitable mycelial growth of them were shown in MEA, glucose peptone, and MCM. The optimum temperature for mycelial growth was 30°C. Carbon and nitrogen sources were mannose and malt extract, respectively. The optimum C/N ratio was 10 : 1 to 5 : 1 with 2% glucose concentration, vitamin was thiamine-HCl, organic acid was succinic acid, and mineral salt was $MgSO_4 \cdot 7H_2O$.**

KEYWORDS: Culture condition, Medicinal mushroom, *Phellinus baumii*, *Phellinus gilvus*, *Phellinus linteus*

The number of mushrooms on Earth is estimated at 140,000, yet maybe only 10% (approximately 14,000 named species) are known (Kirk *et al.*, 2001). For a long time, mushrooms have been valued as an edible and medicinal resource. *Phellinus* genus is known about 220 species and is found mainly in tropical America and Africa (Dai *et al.*, 1998). The genus is distributed into 7 species and commonly referred to as Sangwhang in Korea (Lee, 1993; Hong, 2000). Many kinds of *Phellinus* spp. (e.g. *P. linteus*, *P. igniarius*, *P. gilvus*, *P. pini* and *P. hartigii*) are known and they have a variety of medicinal effects (Lee *et al.*, 1996). Among them *P. linteus*, *P. baumii* and *P. gilvus* have been known as ones of the most popular medicinal mushrooms due to their high antitumor activity in east Asia (Ikekawa *et al.*, 1968; Han *et al.*, 1999; Bae *et al.*, 2004), and safety of acute oral toxicity test (Han *et al.*, 2001). *P. linteus*, *P. baumii* and *P. gilvus* have been cultivated by mushroom farmers in Korea. *P. gilvus* is cheaper than *P. linteus* and *P. baumii* because of very short cultivating period. Therefore, it has a possibility which can be developed as a functional food and livestock for industrial application in near future. This study was focused on culture conditions affecting the optimal mycelial growth of *P. linteus*, *P. baumii* and *P. gilvus*.

Materials and Methods

Fungal isolates. The isolates of *Phellinus* species used in this study were listed in Table 1. *P. linteus* ASI 26099, *P. baumii* Nongong and *P. gilvus* KCTC 6653 were presented by Rural Development Administration, Nongong Agricultural Product Company, and Biological Resources Center of Korea, respectively. All isolates were maintained on Potato Dextrose Agar medium (PDA).

Culture media and temperature: Twelve different culture media were prepared to screen suitable culture media to mycelial growth of *P. linteus*, *P. baumii* and *P. gilvus* (Table 2). The culture media were sterilized for 20 minutes at 121°C and aseptically poured into plastic petridish. An inoculum was removed from seven days old cultures of *Phellinus* spp. grown on PDA at 30°C. Mycelial disk (5 mm in diameter) from the cultures was placed in the center of each 85 mm plastic petridishs containing about 20 *ml* of 12 different media. The fungi were incubated under the dark condition for 9 days at 30°C. Thereafter the mycelial growth, density and color of the colony were examined. To screen temperature condition for a suitable growth of *Phellinus* spp. The ranges of temperature were 10°C, 15°C, 20°C, 25°C, 30°C and 35°C, respectively. The fungi cultured on Malt Extract Agar (MEA) for 10 days by the same method above. Then the measurement of mycelial growth was performed.

Effect of favorable nutrient sources.

Carbon sources: The experiment was performed on the mushroom minimal media (MMM: dextrose 20 g, $MgSO_4$ 0.5 g, KH_2PO_4 0.46 g, K_2HPO_4 1 g, asparagine 2 g, thiamine-HCl 120 μg, agar 20 g, DW 1,000 *ml*) supplemented with each of 10 carbon sources. Each carbon source was added to mushroom minimal media at the concentration of 2%. The fungi were incubated under the dark condition for 10 days at 30°C. Thereafter we exam-

*Corresponding author <E-mail: jowoosik@paran.com>

Table 1. List of *Phellinus* spp. strains used in this study

Scientific name	Strain name	Korean common name	Origin culture	Organ
Phellinus linteus	Koryosanghwang	목질진흙버섯	ASI 26099	Rural Development Administration, Korea
Phellinus baumii	Jangsusanghwang	장수상황버섯	Nongong	Nongong Agricultural Product Co., Korea
Phellinus gilvus	Hwanggeumsanghwang	마른진흙버섯	KCTC 6653	Biological Resources Center, Korea

Table 2. Composition of media used in this study

Nutritional reagents	Media and composition (g/*l*)											
	PDA	MEA	YEA	Czapek dox	Glucose peptone	YMA	Malt yeast extract	Leonian	MCM	Henner-berg	Lilly	Hoppkins
Glucose			10		10		10	25	20	50		10
Sucrose				30								
Maltose											10	
Peptone		3			10	5			2			
Yeast extract			5		10	3	5		2			
Malt extract		30			15	3	3					
Potato extract	4											
DL-Asparagine											2	
Dextrose	20					10						
$NaNO_3$				3						2		
$MgSO_4 \cdot 7H_2O$				0.5				0.5	0.5	0.5	0.5	0.5
KCl				0.5								
$FeSO_4 \cdot 7H_2O$				0.01				0.02				
$CaCl_2 \cdot 2H_2O$										0.1		
$ZnSO_4 \cdot 7H_2O$												
$MnSO_4 \cdot 5H_2O$								0.01				
K_2HPO_4				1					1			
KH_2PO_4				1				1	0.5	1	1	0.1
KNO_3										2		2
Agar	15	15	20	20	20	20	20	20	20	20	20	20

ined the mycelial growth, density and color of the colony.

Nitrogen sources: To screen nitrogen source suitable to the mycelial growth of *Phellinus* spp., the experiment was performed on the mushroom minimal media supplemented with each of 12 nitrogen sources. Each nitrogen source was added to mushroom minimal media at the concentration of 0.2%. A 5 mm diameter plug an inoculum of *Phellinus* spp. cultures placed in the centure of petridish and incubated under the dark condition for 10 days at 30°C. Thereafter the mycelial growth, density and color of the colony were examined.

C/N ratio: On the mushroom minimal media which were mixed with 10, 8, 6, 4, 2, 1, 0.4 and 0.2% glucose as a carbon source and then mixed continually with 0.2% $NaNO_3$ as a nitrogen source, the mycelial growth of *Phellinus* spp. was exammined. The C/N ratio was adjusted to 50 : 1, 40 : 1, 30 : 1, 20 : 1, 10 : 1, 5 : 1, 2 : 1 and 1 : 1 in each medium. Inoculated each media incubated under the dark condition for 9 days at 30°C. Thereafter the mycelial growth, density and color of the colony were examined.

Vitamin: On the sterialized mushroom minimal media which were mixed with thiamine-HCl 0.1 mg/*l*, riboflavin 0.5 mg/*l*, biotine 0.005 mg/*l*, pyridoxine 0.5 mg/*l* and nicotinamide 2.0 mg/*l* those were filtrated by metrical membrane filter (0.2 μm). Inoculated each media incubated under the dark condition for 9 days at 30°C. Thereafter the mycelial growth, density and color of the colony were examined.

Organic acid: On the mushroom minimal media which were mixed with acetic acid, citric acid, maleic acid, lactic acid, succinic acid and fumaric acid at the concentration of 0.1%, respectively. Inoculated each media incubated under the dark condition for 9 days at 30°C. Thereafter the mycelial growth, density and color of the colony were examined.

Mineral salt: To screen mineral salts suitable to the mycelial growth of *Phellinus* spp., the experiment was performed on the YM solid media (peptone 5 g, yeast extract 3 g, malt extract 3 g, dextrose 10 g, agar 20 g and DW 1,000 *ml*) eleminated mineral salt which was supplemented with each of 9 mineral salts. Each mineral salt was added to YM solid media at the concentration of 0.1%. Inoculated each media incubated under the dark condition for 9 days at 30°C. Thereafter the mycelial growth, density and color of the colony were examined.

Table 3. Effect of culture medium on mycelial growth of *Phellinus* spp. at 30°C

Culture media	Colony diameter (mm/9 days)			Mycelial density[a)]			Color[b)]		
	P. l	*P. b*	*P. g*	*P. l*	*P. b*	*P. g*	*P. l*	*P. b*	*P. g*[c)]
PDA	7.7 ± 1.53[f(z)]	68.7 ± 1.15[de]	57.3 ± 2.51[b]	ST	C	SC	SY	Y	Br
MEA	35.0 ± 0[c]	77.0 ± 1.73[ab]	45.7 ± 3.51[cd]	SC	C	SC	SY	Y	Br
YEA	27.3 ± 0.58[d]	55.0 ± 1.0[g]	59.7 ± 1.53[b]	ST	SC	ST	SY	Y	Br
Czapek dox	21.7 ± 1.53[e]	38.7 ± 3.1[h]	12.7 ± 2.51[e]	T	T	T	W	W	W
Glucose peptone	47.0 ± 1.73[b]	73.7 ± 1.15[bcd]	39.3 ± 2.08[d]	SC	C	ST	SY	Y	Br
YMA	46.0 ± 1.0[b]	70.0 ± 2.0[cd]	56.3 ± 6.02[bc]	SC	C	ST	SY	Y	Br
Malt yeast extract	47.3 ± 0.58[b]	62.7 ± 2.51[f]	58.3 ± 2.88[b]	SC	C	SC	SY	Y	Br
Leonian	52.0 ± 1.0[a]	80.3 ± 0.57[a]	39.3 ± 1.15[d]	T	SC	T	W	Y	W
MCM	52.0 ± 1.0[a]	64.3 ± 1.53[ef]	65.7 ± 2.98[ab]	SC	C	SC	SY	Y	Br
Hennerberg	46.0 ± 3.46[b]	73.0 ± 1.0[bcd]	40.7 ± 1.15[d]	T	SC	T	W	Y	W
Lilly	49.9 ± 1.53[ab]	72.7 ± 2.51[bcd]	72.3 ± 2.51[a]	SC	C	T	SY	Y	W
Hoppkins	52.7 ± 1.15[a]	74.0 ± 1.73[bc]	60.3 ± 4.04[b]	T	ST	T	W	Y	W

a): C; compact, SC; somewhat compact, ST; somewhat thin, T; thin
b): Br; brownish, SY; somewhat Yellowish, Y; Yellowish, W; Whitish
c): *P. l*; *P. linteus* ASI 26099, *P. b*; *P. baumii* Nongong, *P. g*; *P. gilvus* KCTC 6653
z): Values in the same line with different literal differ at Duncan's multiple range test ($P<0.05$) and results are mean ± standard error of three replicates.

Results and Discussion

Screening of the suitable culture media. The mycelial growth of *Phellinus* spp. was favorable in MEA, glucose peptone, and MCM whereas was poor in Czapek dox, Leonian, Hennerberg and Hoppkins medium (Table 3). Lee *et al.* (2004) reported that the mycelial growth of *P. linteus* was favorable in MYA and SMS medium. Chi *et al.* (1996) reported that the mycelial growth of *P. linteus* was favorable in YM, malt yeast extract, and MCM medium whereas was poor in Czapek dox, Leonian, Lilly, Modified Lutz and Hoppkins medium. We concluded that the above results were similar with this study. The mycelial growth of *P. linteus* isolate ASI 26099 was less than *P. baumii* isolate Nongong and *P. gilvus* isolate KCTC 6653. Colony's color was that *P. linteus* isolate ASI 26099 was light yellow, *P. baumii* isolate Nongong was yellow and *P. gilvus* isolate KCTC 6653 was brown.

Effect of the temperature: The suitable temperature for the mycelial growth of *Phellinus* spp. was obtained at 30°C (Fig. 1). Their mycelial growth was suppressed rapidly at the temperature higher than 30°C and lower than 20°C. Heo *et al.* (2004) reported that the optimum culture temperature of *P. baumii* and *P. igniarius* was 25~30°C, Chi *et al.* (1996) reported the optimum culture temperature of *P. linteus* was 25~30°C, Rew *et al.* (2000) reported that the optimum culture temperature of *P. gilvus* was 25~30°C. It was concluded that the above results were similar with this study.

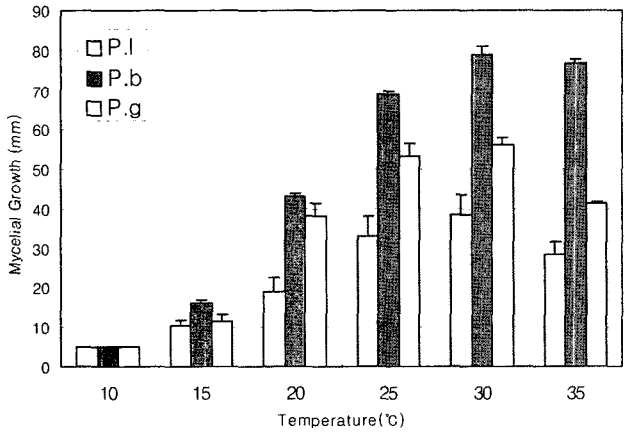

Fig. 1. Mycelial growth of *P. linteus* ASI 26099 (*P. l*), *P. baumii* Nongong (*P. b*), and *P. gilvus* KCTC 6653 (*P. g*) on MEA for 10 days at different temperatures. Vertical bars show standard errors (n = 3).

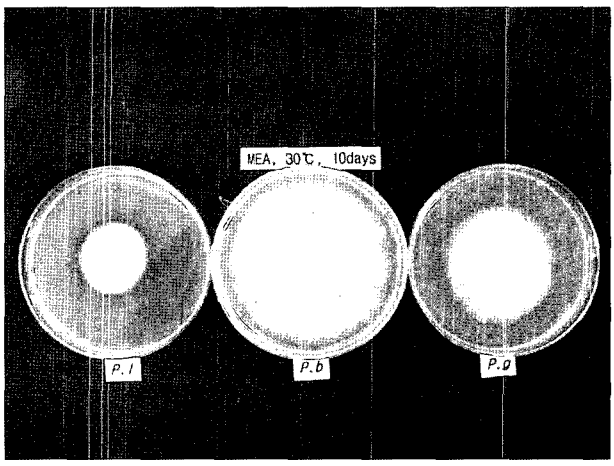

Fig. 2. Colonies of *P. linteus* ASI 26099 (*P. l*), *P. baumii* Nongong (*P. b*), and *P. gilvus* KCTC 6653 (*P. g*) grown on MEA medium for 10 days at 30°C.

Table 4. Effect of carbon source on the mycelial growth of *Phellinus* spp.

Culture media	Colony diameter (mm/10 days)			Mycelial density[a)]			Color[b)]		
	P. l	*P. b*	*P. g*	*P. l*	*P. b*	*P. g*	*P. l*	*P. b*	*P. g*[c)]
Sucrose	55.3 ± 5.03$^{bc(z)}$	72.3 ± 2.52^{b-e}	83.7 ± 1.53^{a}	ST	SC	ST	W	Y	Br
Lactose	51.3 ± 1.53^{c}	66.7 ± 2.89^{e}	84.7 ± 0.58^{a}	ST	SC	ST	W	Y	W
Dextrin	63.0 ± 2.65^{ab}	72.7 ± 3.06^{bcd}	85.3 ± 0.58^{a}	ST	SC	ST	W	Y	Y
Mannitol	64.0 ± 3.04^{ab}	76.3 ± 1.53^{abc}	82.3 ± 2.08^{a}	ST	C	SC	W	Y	Br
Maltose	61.3 ± 3.51^{abc}	79.3 ± 1.15^{de}	82.7 ± 3.21^{a}	SC	C	SC	W	Y	Br
Glucose	57.7 ± 3.21^{abc}	74.0 ± 1.73^{bcd}	79.7 ± 1.53^{a}	SC	C	C	W	Y	Br
Fructose	63.3 ± 1.53^{ab}	81.7 ± 1.53^{a}	84.7 ± 0.58^{a}	SC	C	C	W	Y	Br
Sorbitol	57.3 ± 2.52^{abc}	77.3 ± 2.08^{ab}	63.0 ± 4.09^{b}	SC	C	SC	W	Y	Br
Mannose	65.7 ± 1.53^{a}	77.7 ± 1.53^{ab}	84.3 ± 1.15^{a}	C	C	C	W	Y	Br
Starch	66.0 ± 1.73^{a}	71.0 ± 1.73^{cde}	84.0 ± 1.73^{a}	SC	C	C	W	Y	Br

a): C; compact, SC; somewhat compact, ST; somewhat thin, T; thin
b): Br; brownish, SY; somewhat Yellowish, Y; Yellowish, W; Whitish
c): *P. l*; *P. linteus* ASI 26099, *P. b*; *P. baumii* Nongong, *P. g*; *P. gilvus* KCTC 6653
z): Values in the same line with different literal differ at Duncan's multiple range test ($P < 0.05$) and results are mean ± standard error of three replicates.

Table 5. Effect of nitrogen source on the mycelial growth of *Phellinus* spp.

Culture media	Colony diameter (mm/10 days)			Mycelial density[a)]			Color[b)]		
	P. l	*P. b*	*P. g*	*P. l*	*P. b*	*P. g*	*P. l*	*P. b*	*P. g*[c)]
Yeast extract	57.3 ± 2.08$^{ab(z)}$	59.0 ± 3.61^{c}	75.0 ± 4.35^{bc}	SC	SC	SC	W	SY	Br
Malt extract	62.3 ± 2.52^{a}	77.7 ± 0.58^{a}	83.3 ± 2.89^{a}	SC	C	C	W	Y	Br
Peptone	61.3 ± 1.53^{ab}	78.7 ± 1.53^{a}	84.7 ± 0.58^{a}	SC	C	C	SY	Y	Br
Urea	14.7 ± 2.51^{g}	48.3 ± 5.03^{d}	15.3 ± 0.58^{f}	T	ST	T	W	SY	W
Ammonium nitrate	55.3 ± 1.15^{bc}	76.7 ± 1.53^{ab}	79.3 ± 1.53^{ab}	SC	C	SC	SY	Y	W
Ammonium chloride	49.0 ± 2.00^{d}	77.3 ± 1.15^{a}	64.7 ± 5.03^{d}	SC	C	SC	SY	Y	Br
Ammonium acetate	42.3 ± 3.21^{e}	69.7 ± 3.21^{b}	33.3 ± 2.08^{e}	ST	SC	ST	W	SY	Br
Ammonium sulpate	49.3 ± 2.08^{cd}	78.7 ± 1.15^{a}	67.3 ± 4.93^{cd}	SC	C	SC	W	Y	Br
Potassium nitrate	59.7 ± 1.53^{ab}	75.3 ± 1.53^{ab}	82.3 ± 2.52^{ab}	SC	C	C	W	Y	Br
Sodium nitrate	56.3 ± 1.15^{ab}	79.3 ± 1.15^{a}	81.7 ± 3.21^{ab}	SC	C	C	W	Y	Br
Calcium nitrate	48.3 ± 2.08^{de}	78.7 ± 2.31^{a}	81.3 ± 1.53^{ab}	SC	C	SC	Y	Y	Br
L-glutamic acid	29.7 ± 1.53^{f}	74.0 ± 3.61^{ab}	79.7 ± 0.58^{ab}	SC	C	C	Y	Y	Br
L-arginine	62.0 ± 3.61^{a}	80.0 ± 2.00^{a}	79.3 ± 1.15^{ab}	SC	C	C	W	Y	Br

a): C; compact, SC; somewhat compact, ST; somewhat thin, T; thin
b): Br; brownish, SY; somewhat Yellowish, Y; Yellowish, W; Whitish
c): *P. l*; *P. linteus* ASI 26099, *P. b*; *P. baumii* Nongong, *P. g*; *P. gilvus* KCTC 6653
z): Values in the same line with different literal differ at Duncan's multiple range test ($P < 0.05$) and results are mean ± standard error of three replicates.

Effect of favorable nutrient sources.

Carbon sources: The carbon source promoting a mycelial growth and mycelial density of *Phellinus* spp. was glucose and mannose (Table 4). Among 10 carbon sources, mannose showed colony diameter of *P. gilvus* isolate KCTC 6653 was 84 mm. The mycelial density of *P. gilvus* isolate KCTC 6653 was compact in glucose. Chi *et al.* (1996) reported that the optimum culture carbon sources of *P. linteus* were glucose, mannose and dextrose.

Nitrogen sources: The nitrogen source promoting a mycelial growth of *Phellinus* spp. was malt extract, peptone and potassium nitrate (Table 5). The mycelial density of *P. baumii* isolate Nongong was compact in malt extract. Among 13 nitrogen sources, malt extract showed colony diameter of *P. baumii* isolate Nongong was 78 mm. Chi *et al.* (1996) reported that the optimum culture nitrogen sources of *P. linteus* were cassamino acid, alanine and glutamic acid.

C/N ratio: On the culture media which were mixed with 2% glucose as carbon source and then adjusted to the C/N ratio of 10 : 1 and 5 : 1, *Phellinus* spp. showed the most favorable mycelial growth (Table 6). Lee *et al.* (2004) reported that the optimum culture C/N ratio of *P. linteus* was 10 : 1. Also our results were similar to those of Chi *et al.* (1996).

Vitamin: When various vitamins were added to the MMM medium, thiamine-HCl and biotine were very

Table 6. Effect of C/N ratio on the mycelial growth of *Phellinus* spp.

Culture media	Colony diameter (mm/9 days)			Mycelial density[a]			Color[b]		
	P. l	*P. b*	*P. g*	*P. l*	*P. b*	*P. g*	*P. l*	*P. b*	*P. g*[c]
50 : 1	41.7 ± 0.58$^{e(z)}$	45.7 ± 1.53^{e}	25.7 ± 1.53^{d}	SC	SC	ST	SY	Y	Br
40 : 1	49.0 ± 1.00^{ab}	54.7 ± 3.51^{d}	38.7 ± 3.06^{c}	C	C	ST	SY	Y	Br
30 : 1	47.3 ± 1.53^{b}	58.7 ± 1.53^{cd}	54.7 ± 3.06^{b}	C	C	SC	SY	Y	Br
20 : 1	50.7 ± 0.58^{ab}	62.3 ± 4.04^{bc}	55.3 ± 2.52^{b}	SC	C	SC	SY	Y	Br
10 : 1	52.7 ± 2.52^{a}	72.3 ± 2.08^{a}	63.3 ± 3.51^{ab}	SC	C	SC	SY	Y	Br
5 : 1	51.0 ± 2.00^{ab}	72.0 ± 2.00^{a}	66.3 ± 1.53^{a}	SC	C	SC	SY	Y	Br
2 : 1	48.7 ± 1.53^{ab}	68.7 ± 2.31^{ab}	68.6 ± 2.52^{a}	SC	SC	ST	SY	Y	Br
1 : 1	42.7 ± 2.52^{c}	62.3 ± 3.06^{bc}	64.7 ± 1.53^{ab}	ST	SC	ST	SY	Y	Br

a): C; compact, SC; somewhat compact, ST; somewhat thin, T; thin
b): Br; brownish, SY; somewhat Yellowish, Y; Yellowish, W; Whitish
c): *P. l*; *P. linteus* ASI 26099, *P. b*; *P. baumii* Nongong, *P. g*; *P. gilvus* KCTC 6653
z): Values in the same line with different literal differ at Duncan's multiple range test ($P < 0.05$) and results are mean ± standard error of three replicates.

Table 7. Effect of vitamins on the mycelial growth of *Phellinus* spp.

Culture media	Colony diameter (mm/9 days)			Mycelial density[a]			Color[b]		
	P. l	*P. b*	*P. g*	*P. l*	*P. b*	*P. g*	*P. l*	*P. b*	*P. g*[c]
Thiamine-HCl	54.3 ± 2.08$^{a(z)}$	61.7 ± 1.15^{a}	76.7 ± 3.06^{ab}	C	C	C	W	Y	Br
Riboflavin	45.3 ± 1.53^{c}	48.3 ± 3.51^{b}	69.3 ± 1.15^{c}	SC	SC	SC	W	SY	Br
Biotin	51.3 ± 2.31^{ab}	66.0 ± 2.65^{a}	82.3 ± 0.58^{a}	SC	C	C	W	Y	Br
Pyridoxine	47.7 ± 2.08^{bc}	53.7 ± 2.89^{b}	70.7 ± 0.58^{bc}	SC	SC	SC	W	SY	Br
Nicotinamide	38.7 ± 1.53^{d}	48.0 ± 2.65^{b}	60.7 ± 3.06^{d}	ST	SC	SC	W	SY	Br

a): C; compact, SC; somewhat compact, ST; somewhat thin, T; thin
b): Br; brownish, SY; somewhat Yellowish, Y; Yellowish, W; Whitish
c): *P. l*; *P. linteus* ASI 26099, *P. b*; *P. baumii* Nongong, *P. g*; *P. gilvus* KCTC 6653
z): Values in the same line with different literal differ at Duncan's multiple range test ($P < 0.05$) and results are mean ± standard error of three replicates.

Table 8. Effect of organic acid on the mycelial growth of *Phellinus* spp.

Culture media	Colony diameter (mm/9 days)			Mycelial density[a]			Color[b]		
	P. l	*P. b*	*P. g*	*P. l*	*P. b*	*P. g*	*P. l*	*P. b*	*P. g*[c]
Acetic acid	5.0 ± 0.0$^{e(z)}$	5.0 ± 0.0^{e}	5.0 ± 0.0^{e}	–	–	–	–	–	–
Citric acid	29.3 ± 0.58^{bc}	71.7 ± 2.89^{b}	53.7 ± 3.06^{c}	SC	C	C	Y	Y	Br
Maleic acid	16.7 ± 1.53^{d}	45.7 ± 1.53^{d}	30.7 ± 1.53^{d}	ST	SC	ST	Y	Y	Br
Lactic acid	33.7 ± 2.08^{b}	79.3 ± 1.15^{a}	63.3 ± 4.16^{b}	SC	C	SC	Y	Y	Br
Succinic acid	48.7 ± 1.15^{a}	64.3 ± 2.08^{c}	79.3 ± 0.58^{a}	C	SC	C	SY	Y	Br
Fumaric acid	24.3 ± 4.16^{c}	68.3 ± 1.53^{bc}	51.7 ± 2.89^{c}	SC	C	SC	Y	Y	Br

a): C; compact, SC; somewhat compact, ST; somewhat thin, T; thin
b): Br; brownish, SY; somewhat Yellowish, Y; Yellowish, W; Whitish
c): *P. l*; *P. linteus* ASI 26099, *P. b*; *P. baumii* Nongong, *P. g*; *P. gilvus* KCTC 6653
z): Values in the same line with different literal differ at Duncan's multiple range test ($P < 0.05$) and results are mean ± standard error of three replicates.

excellent for a mycelial growth of *Phellinus* spp. (Table 7). After 9 days cultivation, colony diameter of *P. gilvus* isolate KCTC 6653 for thiamine-HCl and biotine were 77 mm and 82 mm, respectively. Chi *et al.* (1996) reported that the optimum culture vitamins of *P. linteus* were biotin and Ca-pantothenic.

Organic acid: When various organic acids were added to the MMM medium, succinic acid and lactic acid were very excellent for a mycelial growth of *Phellinus* spp., whereas acetic acid was no growth of *Phellinus* spp. (Table 8, Fig. 3). After 9 days cultivation, colony diameter of *P. baumii* isolate Nongong for succinic acid and lactic acid were 64 mm and 79 mm, respectively.

Mineral salt: When various mineral salts were added to the YM medium, $MgSO_4 \cdot 7H_2O$ and KH_2PO_4 were very excellent for a mycelial growth of *Phellinus* spp., whereas

Table 9. Effect of mineral salt on the mycelial growth of *Phellinus* spp.

Culture media	Colony diameter (mm/9 days)			Mycelial density[a)]			Color[b)]		
	P. l	*P. b*	*P. g*	*P. l*	*P. b*	*P. g*	*P. l*	*P. b*	*P. g*[c)]
$MgSO_4 \cdot 7H_2O$	52.3 ± 2.08[a(z)]	76.3 ± 1.15[a]	71.3 ± 1.53[a]	SC	C	SC	SY	Y	Br
KCl	45.3 ± 1.15[bc]	67.7 ± 4.62[b]	52.7 ± 1.15[bc]	SC	C	SC	SY	Y	Br
KH_2PO_4	45.7 ± 2.52[b]	75.3 ± 0.58[a]	58.7 ± 2.08[b]	SC	C	SC	SY	Y	Br
K_2HPO_4	48.3 ± 2.08[ab]	60.3 ± 2.52[c]	43.3 ± 1.53[d]	SC	SC	SC	SY	Y	Br
NaCl	41.3 ± 2.31[c]	75.3 ± 0.58[a]	45.7 ± 2.08[cd]	ST	C	SC	SY	Y	Br
$ZnSO_4 \cdot 7H_2O$	6.7 ± 1.53[d]	7.3 ± 1.53[f]	9.7 ± 1.53[f]	T	T	T	SY	Y	Br
$FeSO_4 \cdot 7H_2O$	7.3 ± 0.58[d]	13.7 ± 1.15[e]	21.7 ± 1.15[e]	T	ST	ST	SY	Y	Br
$CuSO_4 \cdot 5H_2O$	6.0 ± 1.00[d]	32.7 ± 2.52[d]	23.3 ± 3.51[e]	T	ST	ST	SY	Y	Br
Control	44.7 ± 0.58[bc]	64.3 ± 2.08[bc]	48.3 ± 3.21[cd]	SC	SC	SC	Y	Y	Br

a): C; compact, SC; somewhat compact, ST; somewhat thin, T; thin
b): Br; brownish, SY; somewhat Yellowish, Y; Yellowish, W; Whitish
c): *P. l*; *P. linteus* ASI 26099, *P. b*; *P. baumii* Nongong, *P. g*; *P. gilvus* KCTC 6653
z): Values in the same line with different literal differ at Duncan's multiple range test ($P < 0.05$) and results are mean ± standard error of three replicates.

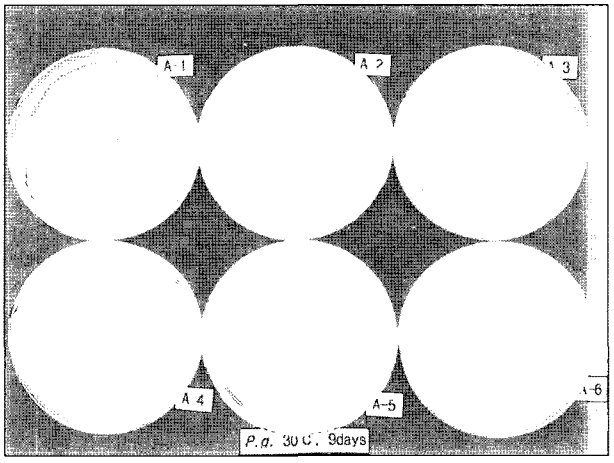

Fig. 3. Mycelial growth of *P. gilvus* KCTC 6653 on the mushroom minimal medium with different organic acid source A-1: Acetic acid, A-2: Citric acid, A-3: Maleic acid, A-4: Lactic acid, A-5: Succinic acid, A-6: Fumaric acid.

$ZnSO_4 \cdot 7H_2O$ was almost no growth of *Phellinus* spp. (Table 9). Chi *et al.* (1996) reported that the optimum culture mineral salt of *P. linteus* was $MgSO_4 \cdot 7H_2O$.

Acknowledgement

This study was supported by Technology Development Program for Agricultural and Forestry, Ministry of Agricultural and Forestry, Republic of Korea.

References

Bae, J. S., Hwang, M. H., Jang, K. H., Rhee, M. H., Lee, K. W., Jo, W. S., Choi, S. K., Yun, H. I., Lim, J. H., Kim, J. C. and Park, S. C. 2004. Comparative antitumor activity of water extracts from fruiting body of *Phellinus linteus*, *Phellinus baumii* and *Phellinus gilvus*. *J. Toxicol. Pub. Health.* **20**: 37-42.

Chi, J. H., Ha, T. M., Kim, Y. H. and Rho, Y. D. 1996. Studies on the main factors affecting the mycelial growth of *Phellinus linteus*. *Kor. J. Mycol.* **24**: 214-222.

Dai, Y. C. and Xu, M. Q. 1998. Studies on the medicinal polypore, *Phellinus baumii* and its kin, *P. linteus*. *Mycotaxon.* **67**: 191-200.

Han, S. B., Lee, C. W., Jeon, Y. J., Hong, N. D., Yoo, I. D., Yang, K. H. and Kim, H. M. 1999. The inhibitory effect of Polysaccharides isolated from *Phellinus linteus* on tumor growth and metastasis. *Immunopharmacology* **41**: 157-164.

Han, Y. S., Park, S. Y., Choi, B. K. and Choung, S. Y. 2001. Acute oral toxicity studies of extract of sanghwang mushroom (*Phellinus linteus*). *J. Applied Pharmaco.* **9**: 46-50.

Heo, B. S., Lee, K. S., Park, S. C. and Lee, Y. S. 2004. Cultural Conditions for the Mycelial Growth of *Phellinus* spp. *Kor. J. Mycol.* **32**: 134-137.

Hong, I. P. 2000. Character of *Phellinus* spp. & Production of *Phellinus* spp. Fruitbody. *Korea Mushroom Research Society.* **4**: 1-15.

Ikekawa, T., Nakanishi, M., Uehara, N., Chihara, G. and Fukuoka, F. 1968. Antitumor action of some basidiomycetes, especially *Phellinus linteus*. *Gann.* **59**: 155-157.

Kirk, P. M., Cannon, P. F., David, J. C. and Stalpers, J. A. 2001. Ainsworth and Bisby's dictionary of the fungi. 9th edn., CAB International, Wallingford.

Lee, J. Y. 1993. Colured Korean Mushrooms. Academy Press, Seoul, Korea.

Lee, J. H., Cho, S. M., Kim, H. M., Hong, N. D. and Yoo, I. D. 1996. Immunostimulating activity of polysaccharides from mycelia of *Phellinus linteus* grown under different culture conditions. *J. Microbiol.* **6**: 52-55.

Lee, W. H., Kim, S. Y., Park, Y. J., Kim, T. W., Kim, H. K. and Sung, J. M. 2004. Favorable Conditions for Myelial Growth of *Phellinus linteus*. *Kor. J. Mycol.* **32**: 95-100.

Rew, Y. H., Jo, W. S., Jeong, K. C., Yoon, J. T. and Choi, B. S. 2000. Cultural characteristics and fruitbody formation of *Phellinus gilvus*. *Kor. J. Mycol.* **28**: 6-10.