한국버섯학회지 Journal of Mushrooms Research Article

The culture conditions for the mycelial growth of *Auricularia auricula-judae*

Woo-Sik Jo*, Dong-Geun Kim, Soon-Ja Seok[1], Hee-Young Jung[2] and Seung-Chun Park[3]

Gyeongbuk Province Agricultural Technology Administration, Daegu 702-320, Korea Korea
[1]Rural Resources Development Institute, National Academy of Agricultural Science, RDA, Suwon 404-707, Korea
[2]Department of Agricultural Biology, Kyeongbuk National University, Daegu 702-701, Korea
[3]College of Veterinary medicine Kyugpook National University, Daegu 702-701, Korea

ABSTRACT: *Auricularia auricula-judae* is an edible mushroom, which is known as wood ear, free ear, black ear mushroom, and free jelly fish. This study was carried out to obtain the basic information for mycelial culture conditions of *Auricularia auricula-judae*. According to colony diameter and mycelial density, the media for suitable mycelial growth were PDA and MCM. The optimum temperature for mycelial growth was 25~30°C. Carbon and nitrogen sources were mannose and malt extract, respectively. The optimum C/N ratio was in the range of 10 to 1 with 2% glucose. Other minor components for the optimal growth were thiamine-HCl and biotin as vitamins, succinic acid and lactic acid as organic acids, and $MgSO_4 \cdot 7H_2O$ and KH_2PO_4 as mineral salts.

KEYWORDS: *Auricularia auricula-judae,* Culture condition, Edible mushroom

Introduction

The number of mushrooms on Earth is estimated at 140,000 species, yet only 10% of them are known (Kirk *et al*., 2001). For a long time, mushrooms have been valued as an edible and medicinal resource. *Auricularia auricula-judae* known as the Jew's ear, wood ear, jelly ear or by a number of other common names, is a species of edible Auriculariales fungus found worldwide (Sung *et al*., 2000). *Auricularia auricula-judae* is an edible mushroom that is cultivated in China, Taiwan, Thailand, and Indonesia. The mushroom is reddish brown, gelatinous, usually wrinkled or veined surface and without distinctive smell (Chang, 1996; Royse, 1997). It grows singly or usually in clusters on coniferous and deciduous wood, and so is classified as a wood-decaying fungus (Abraham *et al*., 1998; Shin *et al*., 1991). The fruiting body is distinguished by its noticeably ear-like shape and brown colouration; it grows upon wood, especially elder. Fruiting bodies with a diameter 3~12 cm, is a Bell-shaped and ear-shaped (Park and Lee, 1991). Moreover, it was reported anti-cancer effects, cardiovascular therapy, and anti-cholesterol effect (Lee *et al*., 1981; Chen, 1989; Cheung, 1996; Misaki and Kakuta, 1995). This study has been conducted to development of mass culture system and species improvement, food pharmacological action and food materials of *Auricularia auricula-judae.* This study was focused on culture conditions affecting the optimal mycelial growth of *Auricularia auricula-judae.*

J. Mushrooms 2014 June, 12(2):88-95
http://dx.doi.org/10.14480/JM.2014.12.2.88
Print ISSN 1738-0294, Online ISSN 2288-8853

*Corresponding author
E-mail : jws67@korea.kr
Tel : +82-54-832-9669, Fax : +82-54-833-1359
Received June 10, 2014
Revised June 27, 2014
Accepted June 28, 2014

Materials and Methods

Fungal isolates

The isolates of *A. auricula-judae* species used in this study were listed in Table 1. *A. auricula-judae* ASI 6033 and *A. polytricha* ASI 6009 were obtained from Rural Development Administration. *A. auricula-judae* GBAA-01, GBAA-02 and GBAA-03 were collected in the wild. All isolates were maintained on Potato Dextrose Agar medium (PDA).

Culture media: Twelve different culture media were prepared to screen suitable culture media to mycelial growth of *A. auricula-judae* (Table 2). The culture media were sterilized for 20 minutes at 121°C and aseptically poured into plastic petridish. An inoculum was removed from five days old cultures of *A. auricula-judae* grown on PDA at 25°C. Mycelial disk (5 mm in diameter) from the cultures was placed in the center of each 85 mm plastic petridish containing about 20 ml of 12 different media. The fungi were incubated under the dark condition for 10 days at 25°C. Thereafter the mycelial growth, density and color of the colony were examined.

Temperature: The growth of mushroom was investigated at different temperatures in the ranges of 10°C~35°C. The fungi were cultured on PDA for 8 days and the mycelial growth was determined as described above.

Effect of pH: To screen pH value necessary for a favorable growth of *A. auricula-judae* species a 5 mm diameter plug of an inoculum was removed with cork borer from 5 days old cultures of *A. auricula-judae* grown on PDA, placed in the center of PDA adjusted to the range of pH 4~9 with 1 N NaOH or HCl and incubated under the dark condition for 10 days at 25°C.

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

Scientific name	Korean common name	Origin culture	Organ
A. auricula-judae	Mokibeoseot	GBAA-01	Gyeongbuk Agricultural Technology Administration, Korea
A. auricula-judae	Mokibeoseot	GBAA-02	Gyeongbuk Agricultural Technology Administration, Korea
A. auricula-judae	Mokibeoseot	GBAA-03	Gyeongbuk Agricultural Technology Administration, Korea
A. polytricha	Teolmokibeoseot	ASI 6009	Rural Development Administration, Korea
A. auricula-judae	Mokibeoseot	ASI 6033	Rural Development Administration, 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

The measurement of mycelial growth was performed according to the method described by Shim e*t al*. (1997).

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, distilled water 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 14 days at 25°C. Thereafter we examined the mycelial growth, density and color of the colony.

Nitrogen sources: To screen nitrogen source suitable to the mycelial growth of *A. auricula-judae* 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 *A. auricula-judae* cultures placed in the center of petridish and incubated under the dark condition for 10 days at 25°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 *A. auricula-judae* was examined. 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 15 days at 25°C. Thereafter the mycelial growth, density and color of the colony were examined.

Vitamin: On the sterilized mushroom minimal media which were mixed with thiamine-HCl 0.1 mg/l, riboflavin 0.5 mg/l, biotin 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 12 days at 25°C. Thereafter the mycelial growth, density and color of the colony were examined.

Table 3. Effect of culture medium on mycelial growth of *Auricularia* spp. at 25°C

Culture media	Colony diameter(mm/10 days)					Mycelial density[a)]					Color[b)]				
	GBAA-01	GBAA-02	GBAA-03	ASI No.		GBAA-01	GBAA-02	GBAA-03	ASI No.		GBAA-01	GBAA-02	GBAA-03	ASI No.	
				6009	6033				6009	6033				6009	6033
PDA	46.0±1.0^{abc}	45.3±1.5^{bc}	42.7±1.2^{c}	38.0±1.0^{de}	32.3±1.5^{e}	SC	SC	C	C	C	W	W	W	W	W
MEA	52.3±1.5^{a}	52.7±1.5^{ab}	54.3±1.5^{a}	50.0±1.0^{a}	62.0±1.0^{ab}	SC	C	SC	SC	SC	W	W	W	W	W
YEA	40.0±2.0^{c}	50.7±0.6^{ab}	49.0±1.0^{ab}	34.3±1.5^{ef}	52.7±2.5^{cd}	C	C	C	C	C	W	W	W	W	W
Czapek dox	40.0±1.0^{c}	37.0±1.0^{d}	31.0±3.6^{ef}	43.7±3.5^{bcd}	62.0±2.0^{ab}	T	T	T	T	T	W	W	W	W	W
Glucose peptone	42.7±4.9^{bc}	41.3±1.5^{cd}	35.3±1.5^{de}	41.3±1.5^{bcd}	64.3±1.5^{a}	C	C	C	ST	C	W	W	W	W	W
YMA	47.3±3.1^{ab}	42.7±3.2^{cd}	36.7±1.5^{d}	47.0±1.0^{ab}	63.0±1.0^{ab}	SC	C	C	ST	C	W	W	W	W	W
Malt Yeast extract	43.3±1.5^{bc}	47.0±2.6^{abc}	43.7±1.5^{bc}	39.0±1.0^{cde}	65.3±2.1^{a}	C	C	C	C	C	W	W	W	W	W
Leonian	42.3±1.5^{bc}	22.7±2.5^{e}	25.7±3.1^{f}	13.7±3.5^{h}	31.7±4.2^{e}	T	T	T	T	T	W	W	W	W	W
MCM	46.7±3.1^{ab}	53.0±2.6^{a}	52.7±1.5^{a}	44.3±3.1^{abc}	68.7±3.2^{a}	SC	SC	ST	SC	C	W	W	W	W	W
Henner-berg	27.7±1.5^{d}	9.7±0.6^{f}	9.7±1.2^{h}	8.7±0.6^{h}	17.7±0.6^{f}	T	T	T	T	T	W	W	W	W	W
Lilly	13.0±1.0^{e}	16.3±2.1^{ef}	17.0±2.0^{g}	21.3±2.5^{abc}	56.3±1.5^{bc}	ST	T	T	T	ST	W	W	W	W	W
Hoppkins	52.3±1.5^{a}	13.3±1.5^{f}	17.3±2.5^{g}	31.3±3.2^{h}	47.0±6.6^{d}	T	T	T	T	T	W	W	W	W	W

[a)]: C; compact, SC; somewhat compact, ST; somewhat thin, T; thin

[b)]: Br; brownish, SY; somewhat Yellowish, Y; Yellowish, W; Whitish

[a-g)]: Values in the same line with different literal differ at Duncan's multiple range test ($P<0.05$) and results are mean ± standard deviation of three replicates.

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 medium incubated under the dark condition for 13 days at 25°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 *A. auricula-judae*, 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) 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 10 days at 25°C. Thereafter the mycelial growth, density and color of the colony were examined.

Results and Discussion

Screening of the suitable culture media

The mycelial growth of *A. auricula-judae* was favorable in PDA and MCM whereas was poor in Czapek dox, Leonian, Hennerberg and Hoppkins medium (Table 3). The mycelial growth of *A. polytricha* isolate ASI 6009 was less than *A. auricula-judae* GBAA-01, GBAA-02, GBAA-03, and ASI 6033. Colony's color was that *A. auricula-judae* four isolates and *A. polytricha* isolate ASI 6009 were white. Shim *et al.* (2005) also reported that PDA, YM, Mushroom complete and Hamada were the most suitable, whereas Czapex dox and Glucose peptone were unfavorable to mycelial growth of *Macrolepiota procera*.

Effect of the temperature: The suitable temperature for the mycelial growth of *A. auricula-judae* was obtained at 25~30°C (Fig. 1). Their mycelial growth was suppressed rapidly at the temperature higher than 30°C and lower than 20°C. Yu *et al.* (2013) reported that the optimum culture temperature of *A. auricula-judae* was 25~30°C. It was concluded that the above results were similar with this study.

Effect of pH: The pH value suitable for a favorable growth of *A. auricula-judae* was obtained in the range of pH 6~9. However, the mycelial growth and density of *A. auricula-judae* was almost identical in the range of pH 6~9 (Table 4). It is generally known that optimum pH of *A. auricula-judae* was pH 6~7 (Yu *et al.*, 2013). The results suggest that *A. auricula-judae* may have a broad pH range for its favorable mycelial growth in the nature.

Effect of favorable nutrient sources

Carbon sources: The carbon sources promoting a

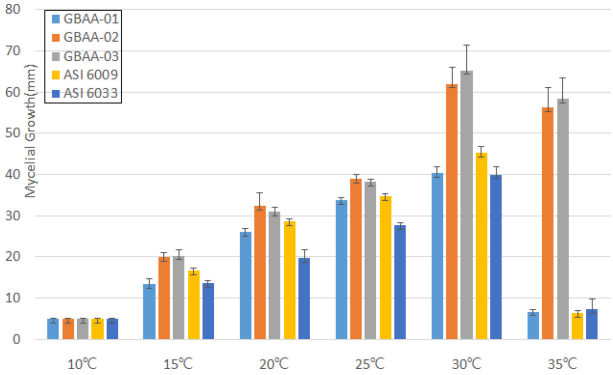

Fig. 1. Mycelial growth of *Auricularia* spp. on PDA for 8 days at different temperatures.

Table 4. Effect of pH on the mycelial growth of *Auricularia* spp. at 25°C

pH	Colony diameter (mm/10 days)					Mycelial density[a)]				
	GBAA-01	GBAA-02	GBAA-03	ASI No.		GBAA-01	GBAA-02	GBAA-03	ASI No.	
				6009	6033				6009	6033
4	45.3±5.5[a]	57.3±1.5[a]	55.7±0.6[a]	24.7±0.6[e]	34.7±0.6[b]	SC	SC	SC	SC	SC
5	46.7±2.1[a]	54.3±2.5[ab]	55.3±1.5[a]	35.0±1.0[d]	38.3±0.6[a]	C	C	SC	C	SC
6	53.3±2.1[a]	50.3±1.5[bc]	48.7±1.5[b]	39.0±1.0[c]	39.3±1.5[a]	C	C	SC	C	SC
7	48.0±4.6[a]	45.7±1.5[cd]	44.0±2.6[b]	45.3±0.6[a]	39.7±0.6[a]	SC	SC	SC	C	SC
8	51.7±4.9[a]	47.3±2.5[c]	47.0±2.0[b]	57.7±2.5[a]	40.0±1.0[a]	SC	SC	SC	C	SC
9	49.3±0.6[a]	41.0±2.6[d]	45.7±4.0[b]	60.0±1.0[a]	50.0±1.0[a]	SC	SC	SC	C	SC

[a)]: C; compact, SC; somewhat compact, ST; somewhat thin, T; thin
[a-d)]: Values in the same line with different literal differ at Duncan's multiple range test ($P<0.05$) and results are mean ± standard deviation of three replicates.

Table 5. Effect of carbon source on the mycelial growth of *Auricularia* spp. at 25°C

Culture media	Colony diameter(mm/14 days)					Mycelial density[a)]				
	GBAA-01	GBAA-02	GBAA-03	ASI No.		GBAA-01	GBAA-02	GBAA-03	ASI No.	
				6009	6033				6009	6033
Sucrose	67.7±2.1^{ab}	27.3±5.2^{ab}	21.7±2.5^{bc}	58.7±4.2^{bcd}	71.0±1.0^{a}	ST	T	T	ST	T
Lactose	45.3±9.5^{c}	25.3±4.6^{ab}	21.0±3.7^{bc}	66.3±2.6^{abc}	80.3±1.2^{a}	ST	T	T	T	ST
Dextrin	54.0±6.5^{bc}	50.7±7.3^{a}	22.0±0.8^{bc}	68.7±2.6^{ab}	80.0±0.8^{a}	ST	T	T	ST	SC
Mannitol	66.3±2.6^{ab}	29.0±0.8^{ab}	20.7±2.9^{bc}	69.0±0.8^{a}	82.0±0.8^{a}	ST	T	T	ST	ST
Maltose	61.3±1.9^{abc}	25.3±1.2^{ab}	19.3±1.2^{bc}	57.7±2.1^{cd}	79.7±0.5^{a}	ST	T	T	ST	T
Glucose	44.7±2.5^{c}	20.0±0.8^{b}	16.3±2.1^{c}	45.3±2.9^{e}	51.3±2.5^{b}	SC	T	T	T	ST
Fructose	64.3±4.8^{ab}	27.0±7.0^{ab}	24.0±3.7^{bc}	57.7±4.8^{cd}	67.7±3.8^{a}	T	T	T	T	ST
Sorbitol	66.7±2.6^{ab}	47.7±20.3^{a}	42.7±8.7^{a}	60.3±2.6^{abc}	81.7±0.5^{a}	ST	ST	T	T	ST
Mannose	73.0±2.4^{a}	29.3±2.5^{ab}	21.0±1.6^{bc}	50.0±0.8^{de}	79.0±2.9^{a}	ST	T	ST	T	ST
Starch	61.3±8.7^{abc}	35.7±4.5^{ab}	30.3±0.5^{ab}	60.3±3.7^{abc}	69.0±12.2^{a}	SC	T	T	ST	SC

[a)]: C; compact, SC; somewhat compact, ST; somewhat thin, T; thin
[a-e)]: Values in the same line with different literal differ at Duncan's multiple range test ($P<0.05$) and results are mean ± standard deviation of three replicates.

Table 6. Effect of nitrogen source on the mycelial growth of *Auricularia* spp. at 25°C

Culture media	Colony diameter(mm/10 days)					Mycelial density[a)]				
	GBAA-01	GBAA-01	GBAA-01	ASI No.		GBAA-01	GBAA-01	GBAA-01	ASI No.	
				6009	6033				6009	6033
Yeast extract	46.3±7.6^{a}	67.0±2.0^{a}	69.0±4.6^{a}	46.3±1.5^{a}	75.7±2.1^{a}	C	SC	SC	SC	C
Malt extract	42.0±4.0^{ab}	43.7±1.5^{b}	35.0±7.1^{b}	41.3±1.5^{ab}	75.7±0.6^{a}	ST	T	ST	T	T
Peptone	36.0±2.6^{bc}	22.3±4.2^{c}	21.3±1.5^{bcd}	24.7±1.5^{def}	60.3±7.5^{c}	SC	T	ST	C	ST
Urea	14.0±1.0^{e}	13.3±1.5^{cd}	14.3±2.1^{d}	15.0±1.0^{fg}	32.3±1.5^{d}	T	T	T	T	T
Ammonium nitrate	14.0±1.0^{e}	9.7±2.1^{d}	14.0±3.0^{d}	37.3±3.1^{abc}	69.0±1.0^{abc}	T	T	T	T	T
Ammonium chloride	21.3±2.1^{de}	10.3±2.3^{d}	15.0±1.0^{d}	29.3±4.9^{cde}	70.7±1.5^{ab}	T	T	T	T	ST
Ammonium acetate	27.0±1.0^{cd}	18.7±8.0^{cd}	19.0±2.0^{cd}	22.7±2.1^{ef}	60.3±1.5^{c}	T	T	T	T	ST
Ammonium sulpate	24.7±3.8^{d}	10.0±1.0^{d}	10.0±1.0^{d}	29.3±2.1^{cde}	60.7±2.5^{c}	T	T	T	T	ST
Potassium nitrate	41.0±3.6^{ab}	14.7±2.1^{cd}	15.3±3.5^{d}	34.3±5.5^{bcd}	63.7±5.5^{bc}	ST	T	T	T	ST
Sodium nitrate	40.3±2.5^{ab}	14.3±2.5^{cd}	16.0±1.0^{d}	31.0±9.6^{b-e}	61.0±3.6^{c}	ST	T	T	T	ST
Calcium nitrate	25.7±4.9^{d}	11.0±1.0^{d}	12.7±0.6^{d}	9.3±0.6^{g}	24.7±0.6^{de}	ST	T	T	T	ST
L-glutamic acid	21.7±4.7^{de}	16.7±0.6^{cd}	17.7±6.4^{cd}	21.7±1.5^{ef}	20.0±2.6^{e}	ST	T	ST	T	ST
L-arginine	41.0±2.0^{ab}	21.0±6.6^{c}	31.5±13.4^{bc}	40.0±2.0^{ab}	71.0±1.0^{ab}	T	T	T	ST	ST

[a)]: C; compact, SC; somewhat compact, ST; somewhat thin, T; thin
[a-g)]: Values in the same line with different literal differ at Duncan's multiple range test ($P<0.05$) and results are mean ± standard deviation of three replicates.

mycelial growth and mycelial density of *A. auricula-judae* were mannose (Table 5). Among 10 carbon sources, mannose showed colony diameter of *A. auricula-judae* GBAA-01 was 73 mm. Griffin (1994) suggested that mannose and fructose are the most commonly utilized sugars after glucose.

Nitrogen sources: The nitrogen sources promoting a mycelial growth of *A. auricula-judae* were malt extract and yeast extract (Table 6). The mycelial density of *A. auricula-judae* GBAA-01 was compact in yeast extract. Among 13 nitrogen sources, yeast extract showed colony diameter of *A. auricula-judae* GBAA-01 was 46.3 mm and peptone showed colony diameter of *A. auricula-judae* ASI 6033 was 60.3 mm. Jeong *et al*. (2005) reported that the optimum culture nitrogen

sources of *G. applanatum* was corn steep power (10%). Kim *et al*. (2014) reported that the optimum culture nitrogen sources of *A. auricula-judae* was peptone. It was concluded that the above results were similar with this study.

C/N ratio: The C/N ratios promoting a mycelial growth of *A. auricula-judae* were 1:1, 2:1, 5:1 and 10:1 (Table 7, Fig. 2). The mycelial density of *A. auricula-judae* GBAA-01 was compact in C/N ratio 10:1, 5:1, 2:1 and 1:1. Among 8 C/N ratios, C/N ratio 2:1 and 5:1 showed colony diameter of *A. auricula-judae* GBAA-01 was 61.7 mm and 52.3 mm. C/N ratio 10:1 and 5:1 showed colony diameter of *A. auricula-judae* ASI 6033 was 77.7 and 78.0 mm. Jo *et al*. (2006) reported that the optimum culture C/N ratio of *Phellinus* spp. was 10:1 and 5:1. Kim *et al*. (2014) reported that the optimum culture C/N ratio of *A. auricula-judae* was 10:1.

Vitamin: When various vitamins were added to the MMM medium, thiamine-HCl and biotin were very excellent for a mycelial growth of *A. auricula-judae* (Table 8). After 12 days cultivation, colony diameter of *A. auricula-judae* GBAA-01 for biotin was 52.3 mm.

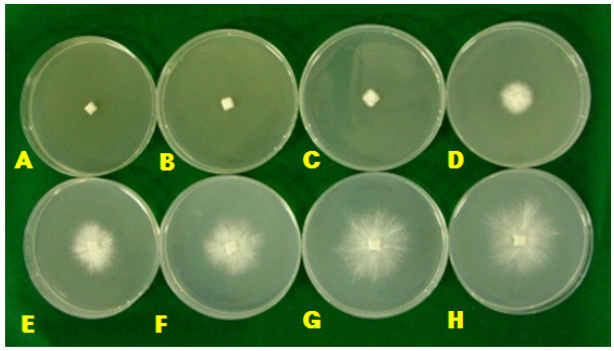

Fig. 2. Mycelial growth of *A. auricula-judae* GBAA-01 on PDA containing different C/N ratios
A: 50, B: 40, C: 30, D: 20, E: 10, F: 5, G: 2, H: 1

Table 7. Effect of C/N ratio on the mycelial growth of *Auricularia* spp. at 25°C

C/N ratio	Colony diameter(mm/15 days)					Mycelial density[a)]				
	GBAA-01	GBAA-02	GBAA-03	ASI No.		GBAA-01	GBAA-02	GBAA-03	ASI No.	
				6009	6033				6009	6033
50 : 1	9.0±1.0^{d}	10.0±1.0^{d}	10.7±0.6^{d}	7.0±0.0^{e}	8.7±0.6^{c}	T	ST	T	T	T
40 : 1	9.3±0.6^{d}	12.7±0.6^{d}	13.3±2.3^{cd}	11.0±1.0^{de}	10.7±2.5^{c}	ST	ST	T	T	T
30 : 1	13.3±0.6^{cd}	15.3±0.6^{d}	16.3±0.6^{bcd}	20.0±1.0^{cd}	14.0±1.7^{c}	ST	ST	T	C	ST
20 : 1	23.3±1.5^{c}	29.7±9.0^{bc}	18.7±2.1^{bc}	28.7±4.0^{c}	32.7±11.1^{b}	SC	ST	T	SC	SC
10 : 1	39.0±1.0^{b}	22.3±4.0^{bcd}	21.3±1.2^{ab}	53.7±2.5^{b}	77.7±2.5^{a}	SC	ST	ST	C	ST
5 : 1	52.3±7.8^{a}	44.3±7.6^{a}	26.7±3.1^{a}	62.7±0.6^{ab}	78.0±3.0^{a}	ST	ST	ST	C	ST
2 : 1	61.7±1.5^{a}	34.7±5.1^{ab}	26.7±3.5^{a}	69.7±2.5^{a}	80.3±0.6^{a}	ST	ST	ST	C	ST
1 : 1	61.7±5.9^{a}	19.3±4.0^{cd}	21.0±0.0^{ab}	61.3±9.1^{ab}	81.0±1.0^{a}	ST	ST	ST	SC	ST

a): C; compact, SC; somewhat compact, ST; somewhat thin, T; thin
a-d): Values in the same line with different literal differ at Duncan's multiple range test (P<0.05) and results are mean±standard deviation of three replicates.

Table 8. Effect of vitamins on the mycelial growth of *Auricularia* spp. at 25°C

Culture media	Colony diameter(ßÆ / 12 days)					Mycelial density[a)]				
	GBAA-01	GBAA-02	GBAA-03	ASI No.		GBAA-01	GBAA-02	GBAA-03	ASI No.	
				6009	6033				6009	6033
Thiamine-HCl	36.7±13.7^{a}	49.3±4.9^{ab}	52.7±11.0^{a}	49.0±3.0^{ab}	73.0±2.0^{c}	SC	ST	T	SC	SC
Riboflavin	44.7±6.7^{a}	41.3±2.1^{b}	57.0±2.0^{a}	42.7±3.1^{b}	77.0±1.0^{bc}	SC	T	T	ST	ST
Biotin	52.3±1.5^{a}	47.0±1.0^{ab}	53.0±4.6^{a}	50.7±2.5^{ab}	80.0±2.0^{ab}	SC	ST	T	SC	SC
Pyridoxine	60.3±4.5^{a}	53.7±5.7^{ab}	58.7±1.5^{a}	54.3±2.9^{a}	81.0±1.0^{ab}	SC	T	T	ST	SC
Nicotinamide	42.5±10.4^{a}	59.0±7.8^{a}	65.3±5.1^{a}	53.0±4.6^{a}	82.0±1.0^{a}	SC	ST	T	ST	SC

a): C; compact, SC; somewhat compact, ST; somewhat thin, T; thin
a-c): Values in the same line with different literal differ at Duncan's multiple range test (P<0.05) and results are mean ± standard deviation of three replicates.

Table 9. Effect of organic acid on the mycelial growth of *Auricularia* spp. at 25°C

Culture media	Colony diameter(mm / 13 days)					Mycelial density[a)]				
	GBAA-01	GBAA-01	GBAA-01	ASI No.		GBAA-01	GBAA-01	GBAA-01	ASI No.	
				6009	6033				6009	6033
Acetic acid	5.0±0.0[d]	24.7±17.2[bc]	62.7±12.4[a]	5.0±0.0[d]	5.0±0.0[c]	T	ST	ST	T	T
Citric acid	47.0±1.0[b]	45.3±5.7[ab]	46.3±35.9[a]	21.0±3.0[b]	57.3±12.4[ab]	ST	ST	ST	C	C
Maleic acid	56.0±4.6[a]	42.0±4.4[abc]	30.0±34.9[a]	5.0±0.0[d]	6.7±2.9[c]	SC	ST	SC	T	C
Lactic acid	48.7±2.1[b]	51.7±6.5[a]	68.7±7.8[a]	57.0±1.0[a]	52.7±41.3[abc]	SC	ST	ST	C	SC
Succinic acid	57.7±2.5[a]	62.7±10.2[a]	62.3±19.4[a]	59.3±3.1[a]	76.3±1.5[a]	SC	ST	ST	C	C
Fumaric acid	26.3±0.6[c]	17.7±0.6[c]	15.7±1.5[a]	12.7±1.2[c]	10.7±0.6[bc]	SC	ST	ST	C	SC

[a)]: C; compact, SC; somewhat compact, ST; somewhat thin, T; thin
[a-d)]: Values in the same line with different literal differ at Duncan's multiple range test ($P<0.05$) and results are mean ± standard deviation of three replicates.

Table 10. Effect of mineral salt on the mycelial growth of *Auricularia* spp. at 25°C

Culture media	Colony diameter(mm / 10 days)					Mycelial density[a)]				
	GBAA-01	GBAA-02	GBAA-03	ASI No.		GBAA-01	GBAA-02	GBAA-03	ASI No.	
				6009	6033				6009	6033
$MgSO_4 \cdot 7H_2O$	44.0±11.4[a]	51.0±1.0[ab]	54.0±1.0[ab]	51.0±2.6[a]	77.3±1.5[a]	SC	SC	ST	C	SC
KCl	40.0±8.0[a]	45.7±3.8[b]	47.0±1.0[b]	49.7±5.9[a]	75.0±1.0[ab]	ST	SC	ST	C	SC
KH_2PO_4	46.7±2.5[a]	46.3±2.5[b]	51.7±4.2[b]	47.7±1.5[ab]	71.0±1.0[b]	SC	SC	ST	C	SC
K_2HPO_4	44.0±4.0[a]	56.3±1.5[a]	60.0±1.0[a]	40.3±2.5[b]	77.0±1.0[a]	ST	C	SC	SC	ST
NaCl	47.7±1.5[a]	48.7±6.1[b]	46.7±3.8[b]	47.7±5.0[ab]	78.0±1.0[a]	ST	SC	ST	SC	SC
$ZnSO_4 \cdot 7H_2O$	5.0±0.0[c]	5.0±0.0[c]	5.0±0.0[c]	5.0±0.0[c]	5.0±0.0[e]	T	T	T	T	T
$FeSO_4 \cdot 7H_2O$	15.0±2.6[bc]	10.0±1.0[c]	11.3±2.1[c]	6.3±2.3[c]	12.0±1.0[d]	SC	ST	T	ST	ST
$CuSO_4 \cdot 5H_2O$	21.7±3.2[b]	11.0±1.0[c]	10.7±1.5[c]	5.7±0.6[c]	19.3±3.1[c]	SC	T	T	T	SC
Control	43.3±3.5[a]	53.0±1.0[ab]	50.3±4.9[b]	46.0±2.6[ab]	72.3±1.5[b]	SC	C	C	SC	ST

[a)]: C; compact, SC; somewhat compact, ST; somewhat thin, T; thin
[a-d)]: Values in the same line with different literal differ at Duncan's multiple range test ($P<0.05$) and results are mean ± standard deviation of three replicates.

Organic acid: When various organic acids were added to the maleic acid, and succinic acid were very excellent for a mycelial growth of *A. auricula-judae* (Table 9). After 12 days cultivation, colony diameter of *A. auricula-judae* GBAA-01 for succinic acid was 57.7 mm.

Mineral salt: When various mineral salts were added to the $MgSO_4 \cdot 7H_2O$, KH_2PO_4 and NaCl were very excellent for a mycelial growth of *A. auricula-judae* whereas $ZnSO_4 7H_2O$ was almost no growth of *A. auricula-judae* isolates (Table 10). 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 the Technology Development Program for Agriculture and Forestry, Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea.

References

Abraham L, Hoffman B, Gao Y, Breuil C. 1998. Action of *Ophiostoma piceae* proteinase and lipase on wood nutrients. *Can J Microbiol.* 44: 698-701.

Chang ST. Mushroom research and development equality and mutual benefit. In: Royse DJ, editor. Mushroom biology and mushroom products. Philadelphia: Pennsylvania State University Press;1996. pp.1-10.

Chen Q. 1989. Antilipemic effect of polysaccharides from *Auricularia auricula, Tremella fuciformis,* and *Tremella fuciformis* spores. *Zhongguo Yaoke Daxue Xuebae.* 20:344-347.

Cheung PCK. 1996. The hypocholesterolemic effect of two edible mushrooms: *Auricularia auricular* (tree-ear) and *Tremella*

fuciformis (white jelly-leaf) in hypercholesterolemic rats. *Nutr Res.* 16:1721-1725.

Chi JH, Ha TM, Kim YH. and Rho YD. 1996. Studies on the Main Factors Affecting the mycelial Growth of *Phellinus linteus*. *Kor. J. mycol*. 24(3): 214-222. (in Korean)

Griffin DH. 1994. Chemical requirement for growth. In: Fungal Physiology, 2nd edition, pp. 130-157. Wiley Liss. New York.

Jeong YT, Yang BK, Jeong SC, Gu YA, Kim GN, Jeong H and Song CH. 2005. Optimum conditions for the mycelial growth and exo-biopolymer production by a submerged culture of *Elfvingia applanata*. *Kor. J. Mycol. News Letter.* 17(1): 97

Jo WS, Rew YH, Choi SG, Seo GS, Sung JM and Uhm JY. 2006. The Culture Conditions for the Mycelial Growth of *Phellinus* spp. *Kor. J. Mycol.* 34(4): 200-205. (in Korean)

Kim JH, Lee YH, Jang MJ, Won SY and Ju YC. 2014. Selection of Superior Strains from Collected Ear Mushrooms for Artificial Cultivation and Their Optimal Condition of Mycelial Growth. *Kor. J. Mycol.* 42(1): 57-63. (in Korean)

Kirk PM, Cannon PF, David JC and Stalpers JA. 2001. Ainsworth and Bisby's dictionary of the fungi. 9th edn., CAB International, Wallingford.

Lee SA, Chung KS, Shim MJ, Choi EC, Kim BK. 1981. Studies on the Antitumor Components of Korean Basidiomycetes(II), Antitumor Components of *Schizophyllum commune* and *Auricularia auricula-judae*. *Kor. J. Mycol.* 9(1): 25-29. (in Korean)

Misaki A and Kakuta M. 1995. Kikurage(tree-ear) and shirokikurage(white jelly-leaf): *Auricularia auricular and Tremella fuciformis. Food Rev Int.* 11(1):219-224.

Park WH and Lee HD. 1991. WILD FUNGI OF KOREA. Kyo-Hak Publishing Co. 504p.

Royse DJ. Specialty mushrooms and their cultivation. *Hortic Rev* 1997;19:59-97

Shim JO, Son SG, Kim YH, Lee YS, Lee JY, Lee TS, Lee SS and Lee MW. 1997. The culture conditions affecting the mycelial growth of Grifola umbellata. *Kor. J. Mycol.* 25(3): 209-218. (in Korean)

Shim SM, Oh YH, Lee KR, Kim SH, Im KH, Kim JW, Lee UY, Shim JO, Shim MJ, Lee MW, Ro HS, Lee HS and Lee TS. 2005. The characteristics of culture conditions for the mycelial growth of *Macrolepiota procera*. *Mycobiology.* 33:15-18.

Shin DS, Lee HH, Lim KP, Cho NS, Cho BM. Chemistry of forest products. Seoul: HaengMunSa; 1991. p.121-2.

Sung JM, Yoo YB, Cha DY. 2000. MUSHROOM SCIENCE. Kyo-Hak Publishing Co. 614p.

Yu YJ, Choi KH, Jeong JS, Lee GK, and Choi SR. 2013. Study on characteristic of mycelial in ear mushroom. *Journal of Mushroom Science and Production*. 11(1): 15-20.