Plant Environment Development 4(2):01-06, 2015 (July)
©Department of Botany, University of Rajshahi
e-mail: plant.environ.dev@ru.ac.bd

Print ISSN 1994-1501
Online ISSN 2311-3529

Research Paper

Performance of Biomass Production of *Antrodia camphorata*, an Industrial Important Mushroom

Ahmed Imtiaj*, M. Monirul Islam[1], Jun-Ichi Ikeda[2] and Shoji Ohga[2]

Department of Botany, University of Rajshahi, Rajshahi 6205, Bangladesh
[1]Institute of Environmental Sciences, University of Rajshahi, Rajshahi 6205, Bangladesh
[2]Department of Agro-Environmental Sciences, Kyushu University, Fukuoka 811-2415, Japan

*Corresponding author e-mail: aimtiajbot@ru.ac.bd

Abstract

This study was conducted to observe the performance of biomass production of *Antrodia camphorate* under different factors. The growth, color and thickness of mycelial mat were affected by different treatments. *A. camphorata* prefers acidic to alkaline condition of culture media for its proper phenotypic exposure. The highest and lowest mycelial growth was found 68.6 and 24.6 mm at pH 5 and 9, respectively. As C-source; dextrose, glucose, fructose, sucrose and host substrates were tested (g/v) to find out the optimal farming condition, and 2% dextrose showed the highest mycelial proliferation (52.8 mm). As ready C-source, dextrose was needed to scale up the biomass production in/on the culture media. The most suitable N-source was $NH_4H_2PO_4$, and concentration of 0.5% was the best for supporting sufficient vegetative propagation. The best filamentous growth was observed when camphor oil (CO) and sawdust of host tree (SHT) were added to PDA at different concentrations. Nowadays, effort is devoted to improve the biomass yield of *A. camphorata* with natural quality through a new innovative experimental method by changing physicochemical factors, substrates and additives of the growing media. Ultimately, a large volume of biomass of *A. camphorata* could be produced for industrial feasible uses. 25 -30 ^{0}C temperature was found optimum or suitable filamentous growth of studied mushroom.

Keywords: *Antrodia camphorata*, Biomass, *Cinnamomum camphora*, Mycelial mat, Physicochemical factors

INTRODUCTION

Antrodia camphorata (Syn. *A. cinnamomea*) is a highly valuable polypore mushroom native to Taiwan and hosts only one species of rainforest known as Chang tree or Camphor tree (*Cinnamomum camphora* and *C. kanehirai* Hay*)*. This orange-red to brown-red colored lichen-shaped mushroom is very bitter in taste and rich with camphor aroma. It grows usually in hollow areas of the trunks of mature trees. The host tree contains highly aromatic oils rich in polysaccharides, triterpenoids and sterols, and these are commonly used as anti-cancer, anti-itching, anti-allergy and liver protective drug (Chen *et al*., 1995; Lee *et al*., 2002; Geethangili and Tzeng, 2011). Scientific research also focused that the mushroom can be useful in the treatment of diabetes, cardiovascular diseases, hypertension, hepatitis and many types of cancers including liver, lung, cervical, colon, breast cancers and also leukemia and adenoma (Huang *et al*., 2012; Song and Yen, 2002). Research from Taiwan showed that crude extract of *A. camphorata* does indeed have strong activity against prostate, breast and bladder cancer (Chen *et al*., 2007). It inhibits prostate cancer cells and concluded that *A. camphorata* due to its nontoxicity, might be used as a

good adjuvant anticancer therapy for prostate cancers (Hsiao *et al*., 2003). The crude extract of *Antrodia* sp. showed rather significant inhibitory effects on the growth and proliferation of invasive transitional bladder carcinomas cells (Peng *et al*., 2007). *Antrodia* sp. exerts growth inhibition on highly-invasive estrogen-nonresponsive human breast cancer cells through apoptosis induction associated with COX-2 inhibition (Song *et al*., 2005).

Regardless to say that fruit bodies of *Antrodia* sp. are very rare even in the native forest and eventually its price has dramatically increased. However, there is no credible method developed to form artificial fruit bodies of the fungus to date. Therefore, mycelial biomass is only reliable natural source to harvest industrial and commercially important compounds since both the fruit bodies and mycelia have been proven to contain similar active properties. To meet up this limitation, though scientists recently developed some techniques to cultivate mycelia in submerged liquid and solid culture (Ma *et al*., 2014) but success rate is still unsatisfied.

In these circumstances, present research attempts to build up newer feasible method than the existing techniques to propagate bulky volume of biomass of concern with thicker mycelial mat of *A. camphorata*.

MATERIALS AND METHODS

Mushroom sample: *Antrodia camphorata* was collected from Taiwan and brought to Kyushu University, Japan. The mushroom was cultured and subcultured repeatedly on PDA untill mycelial pure culture was obtained. After occurrence pure culture, molecular identification was also done to confirm the biological species following the method described by Imtiaj *et al*. (2011). The mycelial pure culture was deposited to Kyushu University Mushroom Bank (KUMB), acquired accession number (KUMB1211) and used in this experiment. To facilitate the study, the culture of *A. camphorata* was preseved at 15°C for further use. Unless otherwise stated, the tests were performed at least 5 times for every experiment.

Effects of temperature and pH: The experiment was conducted to screen the suitable temperature and pH for the vegetative growth of *A. camphorata*. Five different temperatures (15, 20, 25, 30 and 35ºC) were used. A 5 mm diameter inoculum of agar plug was removed from 20 day old cultures grown on PDA and was placed in the centre of each plate filled with 20ml of sterelized virgin PDA (pH 6) and incubated for 20 days at 15, 20, 25, 30 and 35ºC separately. For pH effect, the medium was adjusted to pH 5, 6, 7, 8 and 9 with the addition of 1N NaOH or HCl before autoclaving. A 5 mm diameter agar plug of an inoculum was removed and placed similarly and incubated for 20 days at 25ºC. In every case, radial growth of mycelia on each Petri dish was measured at 3 directions and average value was calculated out of the 3 measurements. To calculate final mean value of mycelial growth, 5 replications were used.

Effects of carbon and nitrogen sources: To screen carbon and nitrogen sources favorable for the vegetative growth of selected mushroom, the tests were performed on potato agar (PA) medium following the modified technique used by Sung *et al.* (1993). Each of 4 C-sources (dextrose, fructose, glucose and sucrose) were supplemented at the concentrations of 0.5, 1.0, 1.5, 2.0 and 3.0 g/100 ml to PA medium separately. The powder of sawdust of host tree (SHT) was added to PDA medium at the same concentrations of C-source used. In case of each N-source, $NaNO_3$, KNO_3, $NH_4H_2PO_4$, NH_4Cl and Urea were supplemented separately at the concentration of 0.5, 0.75, 1.0, 1.25 and 1.5 g to 100ml to PA medium with 2% (v/g) dextrose. In both cases, the medium was adjusted to pH 6 before autoclaving. To measure the colony diameter, all the plates were incubated for 20 days at 25ºC. Radial growth of mycelial mat was measured following the same method described earlier.

Effects of sawdust/products of host tree: To determine suitable additives for the mycelial development, sawdust of host tree (*Cinnamomum camphora*) and its commercial products were used as additives. The culture media (PDA and PA) were prepared separately supplimented (v/g) with 0.6, 0.8, 1.0, 2.0 and 3.0% of camphor powder (CP), camphor oil (CO) and sawdust of host tree (SHT). The medium was adjusted to pH 6 with the addition of 1N NaOH or HCl and autoclaved for 30 minutes at 121°C. After autoclaving, media were poured into Petri dishes and waited for coagulation. Thereafter, all the plates were inoculated with mother culture of *A. camphorata* grown on PDA and incubated for 20 days at 25ºC. When incubation was ended, mycelial growth was measured following the same technique described before to screen suitable substrate for propagating mycelia.

RESULTS AND DISCUSSION

Effect of temperature: To screen the environmental requirements for the artificial vegetative growth of *A. camphorata*, 5 different temperatures (15, 20, 25, 30 and 35ºC) were studied (**Table 1**). The optimum temperature

for the filamentous growth of the studied mushroom was obtained at 25-30ºC. The best mycelial growth of *A. camphorata* was recorded 67.5 mm followed by 67.4 mm found at temperature 30 and 25ºC, respectively. The lowest mycelial growth was recorded 39.2 mm at 15ºC. Shim *et al*. (2005) and Jo *et al*. (2006) stated that the favorable mycelial growth of *Macrolepiota procera* and *Phellinus* spp. was at 30ºC. Hur *et al*. (2008) observed the temperature effect on the mycelial growth of *Phellinus linteus* and found that the temperature of 30ºC was the best which does not fully comply with our result. Jayasinghe *et al.* (2008) studied the temperature effect on the mycelial growth of 8 strains belonging to *Ganoderma lucidum* and found suitable growth at 25ºC with extension range of 20~30ºC. Chang *et al*. (2006) indicated that when a submerged culture of *A. cinnamomea* in shake flasks was operated at 28°C, initial pH 5.5, and rotation speed 105 rpm, the biomass and triterpenoid content in dry weight basis could be increased to 3.20% (w/w) and 31.8 mg/g, respectively. Therefore, their results are corresponded with that of our findings.

Table 1. Effect of temperature and pH on radial mycelial growth of *A. camphorata*

Temperature effect		pH effect	
ºC	Mycelial growth (mm)	pH	Mycelial growth (mm)
15	39.2	5	68.6
20	61.3	6	67.6
25	67.4	7	59.0
30	67.5	8	39.4
35	48.9	9	24.6
Mean	**56.9**	**Mean**	**51.9**

Mycelial growth was calculated (n=5) after 20 days of incubation on PDA and effect of pH on mycelial growth was recorded at 25ºC.

Effect of pH: Hydrogen ion concentration of the culture medium has a profound influence upon overall metabolic activities of fungi. Considering this, a research work was conducted to screen the suitable pH for mycelial growth of *A. camphorata*. The highest and lowest mycelial growth was found 68.6 mm and 24.6 mm at pH 5 and 9, respectively (**Table 1 & Fig. 1**). It was found that *A. camphorata* prefered acidic to alkaline condition of culture media. Imtiaj *et al*. (2008a) observed the pH requirement for the vegetative growth of 10 strains of *Schizophyllum commune* and reported that pH 5 was the best. Shim *et al.* (2005) revealed that pH 7 is the most suitable for the optimal growth of *M. procera*. Choi *et al*. (1999) and Chi *et al*. (1996) reported that mycelial growth of *Phellinus japonica* and *P. linteus* was optimal at pH 7 and 6-7, respectively. Shim *et al*. (2003) reported that optimal pH was 8 for *Paecilomyces sinclairii*. This result suggested that mushrooms may have a broad pH range for their optimal mycelial growth in nature. The results of this study is completely similar to Imtiaj *et al*. (2008a).

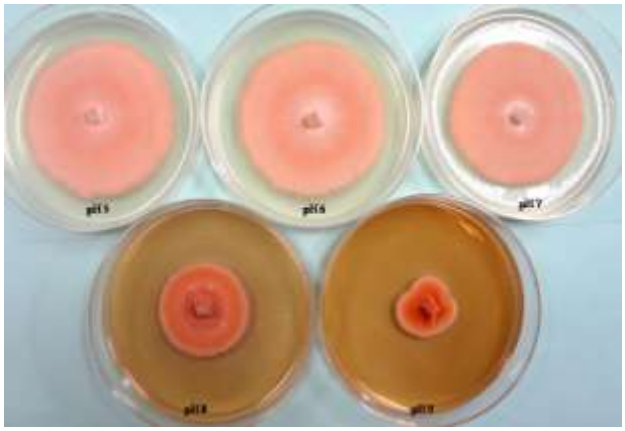

Fig. 1. Effect of pH on mycelial growth (mm) of *A. camphorata*. Mycelial growth was calculated (n=5) after 20 days of incubation on PDA at 25ºC.

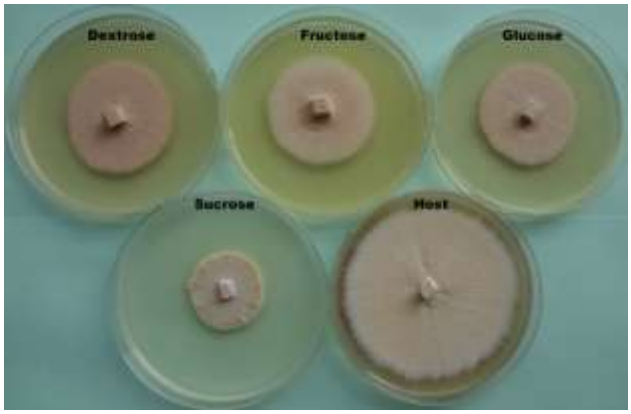

Fig. 2. Effect of C-source and host substrate on mycelial growth (mm) of *A. camphorata*. Mycelial growth was calculated (n=5) after 20 days of incubation on PDA at 25ºC.

Effect of C-sources: Carbon is the most required nutrient for vegetative growth of fungi. Four different C-sources (dextrose, glucose, fructose and sucrose) and host substrate were used to find out the optimal farming condition. Among them 2% dextrose (g/v) showed the highest mycelial proliferation (52.8 mm). Fructose and glucose of 2-3% showed average mycelial growth of *A. camphorata*. However, mycelial growth was found (74.6 mm) to be enhanced when sawdust powder of host plant was supplimented with PDA. The concentration of both C-sources and host substrate were found to be good ranging 1.5 to 3% (**Table 2 & Fig. 2**). Shim *et al.* (1997) reported that *Grifola umbellata* was favorable to maximum C-sources except salicin, cellobiose and lactose. Shim *et al.* (2005) proved that sucrose, maltose,

dextrin and mannose were effective where lactose was highly negative upon mycelial growth of *M. procera*. Therefore, this result is partially similar to Shim *et al.* (1997) and Shim *et at.* (2005).

Table 2. Effect of C-sources and host substrate on the radial mycelial growth (mm) of *A. camphorata*

Source	% of C-sources /host substrate (g/100ml)					Mean
	0.5	1.0	1.5	2.0	3.0	
Dextrose	41.4±5.1	47.8±2.6	50.8±4.0	52.8±3.6	51.0±3.2	48.8±3.7
Fructose	40.4±1.1	48.6±1.3	50.2±2.6	50.4±1.7	50.6±2.3	48.0±1.8
Glucose	35.8±2.8	47.6±1.8	50.0±2.8	48.2±1.9	48.0±1.4	45.9±2.1
Sucrose	22.6±0.9	23.2±1.9	24.0±1.2	25.2±3.3	27.4±1.8	24.5±1.8
Host*	64.4±3.4	68.2±1.1	70.4±0.9	72.6±1.3	74.6±1.8	70.0±1.7
Mean	40.9±2.7	47.1±1.7	49.1±2.3	49.8±2.4	50.3±2.1	47.4±2.2

*Sawdust of host plant was supplimented to PDA at 0.5, 1.0, 1.5. 2.0 and 3.0 percent (g/ml). Mycelial growth was calculated (n=5) after 30 days of incubation at 25ºC.

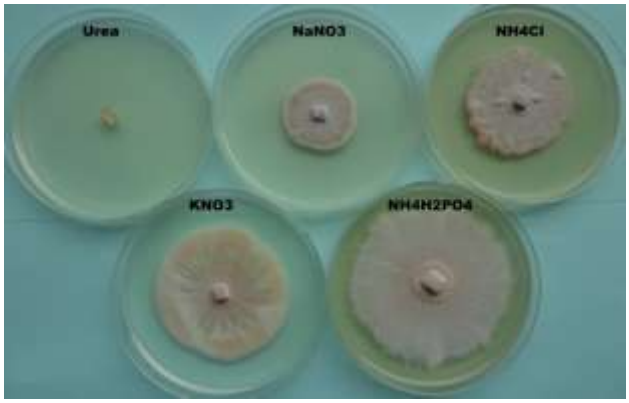

Fig. 3. Effect of N-source on mycelial growth (mm) of *A. camphorata*. Mycelial growth was calculated (n=5) after 20 days of incubation at 25ºC on PDA supplimented with N-source.

Effect of N-sources: To find out the effects of N-source for mycelial growth, five N-sources were used in this study. The most suitable N-sources was $NH_4H_2PO_4$ and 0.5% was the best concentration for supporting vigorus mycelial propagation. Mentionalble that urea showed absolutely negative result and no mycelial growth was obseved on culture media (**Table 3 & Fig. 3**). Shim *et al.* (2005) clarified that glycine was the most favorable and histidine, arginine and ammonium oxalate were the most unfavorable for the mycelial growth of *M. procera* on the culture media. Lee *et al.* (2005) showed that soytone, malt extract, yeast extract and bacto-peptone were the most favorable but $NaNO_3$ and urea were the most unfavorable for the mycelial growth of *Ramaria botrytis*. Therefore, present study fully matched with the result stated by Lee *et al.* (2005).

Table 3. Effect of N-sources on the radial growth (mm) of *A. camphorata.*

Source	% of N-sources (g/ml)					Mean
	0.50	0.75	1.00	1.25	1.50	
$NaNO_3$	35.8±2.2	32.0±1.4	27.0±5.8	25.8±0.8	23.4±0.5	28.8±2.2
KNO_3	55.2±3.1	48.6±1.3	40.6±2.3	34.4±1.7	31.8±4.0	42.1±2.5
$NH_4H_2PO_4$	70.0±1.4	69.6±0.9	69.0±2.3	68.2±3.3	65.2±1.1	68.4±1.8
NH_4Cl	53.8±5.1	45.4±2.9	40.2±2.9	31.8±3.3	27.6±0.5	39.8±2.9
Urea	-	-	-	-	-	-
Mean	53.7±3.0	48.9±1.6	44.2±3.3	40.1±2.3	37.0±1.6	44.8±2.4

Each N-source was added to PDA separately at 0.5, 0.75, 1.00, 1.25 and 1.50 percent (g/ml). Mycelial growth was measured (n=5) after 30 days of incubation at 25ºC.

Effect of camphor products on vegetative growth: The experiment was conducted to reveal the effect of different substrates of host plant on the vegetative growth and yield of *A. camphorata*. The best vegetative growth was observed when CO and SHT were added to PDA at different concentrations. As ready C-source, dextrose was needed to scale up the biomass production in/on the culture media since mycelial growth was not supported

by PA medium with similar ingredients other than dextrose (**Table 4**). Imtiaj *et al*. (2008b) reported that 10% rice bran was better supplement with sawdust to produce fruiting bodies of *Hericium erinaceus*. Shim *et al*. (2006) and Semerdzieva *et al*. (1988) also reported that fruiting bodies of *O. radicata* were formed very well in the oak

Table 4. Effect of host substrates/products on the radial mycelial growth (mm) of *A. camphorate.*

Camphor substrate		% of camphor substrate					Mean
		0.6	0.8	1.0	2.0	3.0	
CO	PDA	82.2	79.8	79.0	81.0	80.6	80.5
	PA	19.8	21.2	17.0	12.0	10.6	16.1
CP	PDA	75.2	74.8	67.6	59.0	31.2	61.6
	PA	20.0	22.0	18.8	19.4	0.0	16.0
SHT	PDA	73.0	76.6	76.2	74.4	72.6	74.6
	PA	18.6	18.8	15.6	0.0	0.0	10.6
Mean		48.1	48.9	45.7	41.0	32.5	43.2

PDA: Potato dextrose agar; PA: Potato agar; CP: Camphor powder; SHT: Sawdust of host tree; CO: Camphor oil. Mycelial growth was measured (n=5) after 20 days of incubation at 25ºC.

sawdust medium mixed with 10% rice bran. Rew *et al*. (2004) showed that the mycelial growth of *Phellinus baumii* was the best on oak sawdust mixed with 20% of rice bran (v/v). Shim *et al*. (2006) reported that mycelial growth and density of *O. radicata* were also good on oak sawdust mixed with 10% rice bran that might contain some ingredients to facilitate the good mycelial growth of *O. mucida* and the other mushrooms. Semerdzieva *et al*. (1988) also reported that fruiting bodies of *O. radicata* were formed very well in the oak sawdust medium mixed with 10% rice bran. Therefore, rice/wheat bran might contain some ingredients to facilitate the good mycelial growth of mushrooms and our result correnspond with many evidences.

REFERENCES

Chang, C.Y., C.L. Lee and T.M. Pan. 2006. Statistical optimization of medium components for the production of *Antrodia cinnamomea* AC0623 in submerged cultures. Appl. Microbiol. Biotechnol. 72:654–661.

Chen, C.H., S.W. Yang and Y.C. Shen. 1995. New steroid acids from *Antrodia cinnamomea*, a fungal parasite of *Cinnamomum micranthum*. J. Nat. Prod. 58(11):1655–1661.

Chen, K.C., C.C. Peng and R.Y. Peng. 2007. Unique formosan mushroom *Antrodia camphorata* differentially inhibits androgen-responsive LNCaP and independent PC-3 prostate cancer cells. Nutrition and Cancer 57:111–121.

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

Choi, Y.J., H.K. Hwang and W.H. Lee. 1999. The production of artificial fruiting body of *Paecilomyces japonica.* Korean J. Mycol. 27:87-93.

Geethangili, M. and Y.M. Tzeng. 2011. Review of pharmacological effects of *Antrodia camphorata* and its bioactive compounds. Evidence-Based Complementary and Alternative Medicine 2011:01-17.

Hsiao, G, M.Y. Shen, K.H. Lin, M.H. Lan, L.Y. Wu, D.S. Chou, C.H. Lin, C.H. Su and J.R. Sheu. 2003. Antioxidative and hepatoprotective effects of *Antrodia camphorata* Extract. J. Agric Food Chem. 51:3302–3308.

Huang, C.C., M.C. Hsu, W.C. Huang, H.R. Yang and C.C. Hou. 2012. Triterpenoid-rich extract from *Antrodia camphorata* improves physical fFatigue and exercise performance in mice. Evidence-Based Complementary and Alternative Medicine 2012:01-08.

Hur, H., A. Imtiaj, M.W. Lee and T.S. Lee. 2008. Suitable conditions for mycelial growth of *Phellinus* spp. Mycobiology 36:152-156.

Imtiaj, A., C. Jayasinghe, G.W. Lee, H.Y. Kim, M.J. Shim, H.S. Rho, H.S. Lee, H. Hur, M.W. Lee, U.Y. Lee, and T.S. Lee. 2008a. Physicochemical requirement for the vegetative growth of *Schizophyllum commune* collected from different ecological origins. Mycobiology 36:34-39.

Imtiaj, A., C. Jayasinghe, G.W. Lee, M.J. Shim, H.S. Rho, H.S. Lee, H. Hur, M.W. Lee, U.Y. Lee and T.S. Lee. 2008b. Vegetative growth of four strains of *Hericium erinaceus* collected from different habitats. Mycobiology 36:88-92.

Imtiaj, A., T.S. Lee and S. Ohga. 2011. Sequence variation of *Pleurotus* species collected from Eastern Asia. Micologia Aplicada International 23:01-10.

Jayasinghe, C., A. Imtiaj, H. Hur, G.W. Lee, T.S. Lee and U.Y. Lee. 2008. Favorable culture conditions for mycelial growth of Korean wild strains in *Ganoderma lucidum*. Mycobiology 36:28-33.

Jo, W.S., Y.H. Rew, S.G. Choi, G.K. Seo, J.M. Sung and J.Y. Uhm. 2006. The culture conditions for the mycelial growth of *Phellinus* spp. Mycobiology 34:200-205.

Lee I.H., R.L. Huang, C.T. Chen, H.C. Chen, W.C. Hsu and M.K. Lu, 2002. *Antrodia camphorata* polysaccharides exhibit anti-hepatitis B virus effects. FEMS Microbiol Lett. 209:63–67.

Lee, T. H. and H.H. Han. 2005. Cultural characteristics for the enhanced mycelial growth of *Ramaria botrytis*. Mycobiology 33:12-14.

Ma, T.W., Y. Lai and F.C. Yang. 2014. Enhanced production of triterpenoid in submerged cultures of *Antrodia cinnamomea* with the addition of citrus peel extract. Bioprocess Biosyst. Eng. 37:2251–2261.

Peng, C.C., K.C. Chen, R.Y. Peng, C.C. Chyau, C.H. Su and H.M. Hsieh-Li. 2007. *Antrodia camphorata* extract induces replicative senescence in superficial TCC, and inhibits the absolute migration capability in invasive bladder carcinoma cells. J. Ethnopharmacology 109:93–103.

Rew, Y.H., W.S. Cho, J.M. Lee and J.K. Kim. 2004. Cultural characteristics of *Phellinus baumii* grown in bottle. Korean J. Mycol. 32:101-104.

Semerdzieva, M., A.S. Buchalo, P. Hobsch, O.A. Zakordonec, S.P. Wasser and V. Musilek. 1988. Comparative study of cultures of four species of the genus *Oudemansiella*. Folia Microbiol. 33:115-120.

Shim, J.O., K.J. Chang, T.H. Kim, Y.S. Lee, U.Y. Lee, T.S. Lee and M.W. Lee. 2006. The fruiting body formation of *Oudemansiell*a *radicata* in the saw dust oak (*Quercus variabilis*) mixed with rice bran. Mycobiology 34:30-33.

Shim, J.O., S.G. Son, Y.H. Kim, Y.S. Lee, J.Y. Lee, T.S. Lee, S.S. Lee and M.W. Lee. 1997. The culture conditions affecting the mycelial growth of *Grifola umbellata.* Korean J. Mycology 25:209-218.

Shim, S.M., K.R. Lee, S.H. Kim, K.H. Im, J.W. Kim, U.Y. Lee, J.O. Shim, M.W. Lee and T.S. Lee. 2003. The optimal culture conditions affecting the mycelial growth and fruiting body formation of *Paecilomyces fumosoroseus.* Mycobiology 31:214-220.

Shim, S.M., Y.H. Oh, K.R. Lee, S.H. Kim, K.H. Im, J.W. Kim, U.Y. Lee, J.O. Shim, M.J. Shim, M.W. Lee, H.S. Ro, H.S. Lee and T.S. Lee. 2005. The characteristics of culture conditions for the mycelial growth of *Macrolepiota procera*. Mycobiology 33:15-18.

Song T.Y., S.L. Hsu, C.T. Yeh and G.C. Yen. 2005. Mycelia from *Antrodia camphorata* in submerged culture induce apoptosis of human hepatoma HepG2 cells possibly through regulation of fas pathway. J. Agric Food Chem. 53:5559–5564.

Song T.Y. and G.C. Yen. 2002. Antioxidant properties of *Antrodia camphorata* in submerged culture. J. Agric Food Chem. 50:3322–3327.

Sung, J.M., C.H. Kim, K.J. Yang, H.K. Lee and Y.S. Kim. 1993. Studies on distribution and utilization of *Cordyceps militaris* and *C. nutans*. Korean J. Mycology 21:94-105.