韓蠶學誌 44(2), 74~81 (2002)
Korean J. Seric. Sci.
<論 文>

Artificial cultivation of Medicinal Mushroom, *Phellinus linteus* using Mulberry logs

In-Pyo Hong, Gyoo-Byung Sung, In-Mo Chung and Won Chu Lee

Department of Sericulture and Entomology, National Institute of Agricultural Science and Technology, R.D.A., Suwon 441-707, Korea

ABSTRACT

The optimal conditions for mycelial growth of *P. linteus* ASI 26011 were 25-30°C and pH 6.0, respectively. The mycelial growth of *P. linteus* was excellent on MCM medium. In case of carbon sources, the mycelial growth of *P. linteus* was best on the culture media that were contained with sucrose, mannose and glucose. Potassium nitrate and sodium nitrate were good for the mycelial growth of *P.* linteus as a nitrogen source. For comparison of the mycelial colonization of *P. linteus* on logs, several techniques of inoculation were tested; the sterilized short log inoculation, drilling inoculation and log-end sandwich inoculation. The mycelial colonization of *P. linteus* on logs was good in the treatment of sterilized short log inoculation, but poor in the traditional methods such as drilling inoculation and log-end sandwich. The initial mycelial growth and the full mycelial colonization of *P. linteus* were the best on 20 cm logs under the condition of 42% of moisture content in log. Also the initial mycelial growth of *P. linteus* was accelerated over 12 hours of sterilization. Burying method of logs after 5-6 months of incubation was the best for formation of basidiocarp of *P. linteus*. The formation of fruiting body of *P. linteus* was quite good in the cultivation house at the 31-35°C and over 96% of relative humidity.

Key words: Fruiting body formation, Inoculation method, Mycelial growth, *Phellinus linteus*

Introduction

Mushrooms have recently become attractive as functional foods or sources of physiologically beneficial medicine. In addition to *Ganoderma* spp., the species of *Phellinus* are currently popular in Korea. The genus *Phellinus* belongs to the Hymenochaetaceae in the basidiomycetes, and was erected by Quelet (1886). Some species of them, especially *Phellinus linteus* was reported to possess antitumor and immuno-modulating activities; remarkable host-mediatory antitumor activity against grafted cancer in animals as Sarcoma 180 (Ikekawa *et al.*, 1968). This fungus has been well known as "Sanghuang (yellow polyporus)" for hundreds years in traditional Chinese medicine (Chen & Chen, 2000). *P. linteus* was first described as *Polyporus linteus* by Berkeley and Curtis (1860) and later renamed *Phellinus linteus* by Teng (1964). From this specie, numerous researchers have isolated some essential substances, which stimulate the immune system of human (Chung & Kim, 1994; Lee *et al.*, 1996; Song *et al.*, 1998).

The physiological characteristics, chemical composition, and development of the cultivation methods of *P. linteus* including other species of the genus *Phellinus* have been intensively studied (Chi *et al.*, 1996; Jung *et al.*, 1997). Basidiocarps of *P. linteus* (yellow medicinal polyporus) are highly prized for antitumor activity of its bioactive protein-polysaccharide complex and rarity in nature (Oh & Han, 1993). This fungus inhabits mainly on mulberry tree and has perennial forms in Korea. Despite such a great medicinal value, study on artificial cultivation of *P. linteus* was rarely conducted.

The present study was carried out to investigate the physiological characteristics required for mycelial growth and especially tried to elucidate the possibility of the artificial production of *P. linteus* by cultural method using mulberry logs.

Materials and Methods

Physiological Characteristics

Cultures

The *Phellinus linteus* (ASI 26011) strain was obtained from the American Type Culture Collection (USA) and cultured at 25°C on yeast-malt agar (YMA) medium slants. This medium was consisted of 0.5% peptone, 0.3% yeast extract, 0.3% malt extract, 1% dextrose, and 2% agar and adjusted volume to 1,000 ml. Subcultures were made routinely every 30 days.

Inoculation

The YMA medium was autoclaved at 121°C (15 psi) for 15 minutes and poured into a petri-dish. After cooling, a piece of mycelia from the slant was inoculated on this agar medium plate to use as inoculum for the next step.

Table 1. Composition of media used

Ingredient	Medium(g/l)*			
	MCM	MEA	PDA	YMA
$MgSO_47H_2O$	0.5			
KH_2PO_4	0.46			
K_2HPO_4	1.0			
Peptone	1.0	5.0		5.0
Yeast extract	2.0			3.0
Malt extract		20.0		3.0
Dextrose	20.0			10.0
PDA(Difco)			39.0	
Agar	20.0	20.0		20.0

*MCM (Mushroom Complete Medium), MEA (Malt Extract Agar), PDA (Potato Dextrose Agar), YMA (Yeast Malt Agar)

Selection of optimal medium

Four different culture media (Table 1) were used to investigate a favorable growth of *P. linteus*. For inoculum, the fungus was grown for 14 days on the yeast malt agar medium, and then the mycelium of the fungus was inoculated at the center of each medium petri-dish using a sterile cork borer (5 mm dia.). Five inoculated petri-dishes were incubated at 25°C. After 14 days of incubation, the mycelial growth was observed.

Effect of temperature on mycelial growth

The effect of temperature on mycelial growth was carried out using the selected medium as the cultural media study. After autoclaving for 15 minutes at 121°C, the medium was aseptically poured into a petri-dish. The inoculation was performed as the same method described above. For incubation, the five petri-dishes inoculated with the fungus were incubated at 20°C, 25°C, 30°C, 35°C and 40°C, respectively. After 14 days of incubation, the mycelial growth was observed.

Effect of pH on mycelial growth

The effect of pH on mycelial growth was studied by the mycelial dry-weight method. The liquid medium was employed the selected medium as temperature study without agar. The medium was adjusted to the range of pH 4~8 with 1N NaOH or HCl before being dispensed into 250 ml culture flasks at the rate of 50 ml per flask and then autoclaved for 15 minutes at 121°C (15 psi). The inoculum was obtained by growing fungus for 14 days in petri dish on the yeast-malt agar medium. Each flask was inoculated with a mycelial inoculum and incubated at 25°C for 14 days. The mycelia were filtered through filter paper (Whatman No. 2, dia., 9 cm), dried in an oven at 85°C for 24 hours, cooled in a desiccator and weighed.

Effect of carbon source

Carbon source utilization by *P. linteus* was tested by the method described by Lilly & Barnett (1951). The medium used was contained; Glucose 10 g, Asparagine 2 g, KH_2PO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, agar 20 g and distilled water to make 1000 ml. Various carbon sources were substituted for glucose. Namely, based on each molecular weight of 13 different carbon sources, each carbon source was added to the basal medium at the concentration of 0.1 M per liter.

The basal medium was adjusted to pH 6.0 and then autoclaved for 15 minutes at 121°C (15 psi). Cellobiose and xylose were sterilized by millipore filtration to avoid their breakdown to glucose and furfural, respectively. The inoculum was obtained by growing fungus for 14 days in petri dishes on the basal medium. Five petri dishes of each carbon source were inoculated with 5 mm disk of the fungus and five petri dishes of the basal medium lacking a carbon source were also inoculated for the control. The petri-dishes were incubated at 25°C for 14 days after the colony diameter of mycelia were measured as above

Effect of nitrogen source

The ability of *P. linteus* to utilize different nitrogen sources for growth was also studied. Except for the substitution of 1% fructose as carbon source per 1000 ml, the basal medium was the same composition as the medium used for the carbon source requirements study. Various nitrogen sources were substituted for asparagine. Based on each molecular weight of 12 different nitrogen sources, each nitrogen source was added to the basal medium at the concentration of 0.02 M per liter. The basal medium was adjusted to pH 6.0 and then autoclaved for 15 minutes at 121°C (15 psi). All the other processes including the inoculation, incubation and measurement of mycelial growth were performed as the same method of carbon source.

Cultural Characteristics

The process for basidiocarps production of *Phellinus linteus* was divided into two major stages; The first stage included the preparation of the stock culture, mother spawn and planting spawn, and the second entailed the preparation of the growth substrates for mushroom cultivation.

Mother spawn

The sawdust of mulberry tree was mixed with rice bran at a ratio of 4:1 (v/v) and moisture content was adjusted to about 65% with water. Then the mixed medium was put into a 250 ml flask and sterilized with 121°C for 45 minutes. After cooling to 20°C, a piece of mycelia from the agar plate was inoculated on this sawdust medium to use as an inoculum for the planting spawn.

Planting spawn

The planting spawn medium was prepared by the same

method as used for the mother spawn. The medium containing sawdust and rice bran was put into 850 ml polyethylene bottle, sterilized with 121°C for 90 minutes and cooled to 20°C. Two or three spoonfuls of an inoculum of the precultured sawdust medium (mother spawn) in 250 ml flask were inoculated in 850 ml polyethylene bottle of the sawdust culture medium. The inoculated sawdust media were incubated at 25°C for about 45 days until the mycelia spreaded all over the media and then used as an inoculum for the log cultivation.

Log cultivation procedure

The cultivation method for *Phellinus linteus* was modified orderly as follows; selecting and felling of the tree, sawing/cutting the log into short segments, transferring segments to polyethylene bags, sterilization, inoculation, spawn running, burial of logs in soil and tending the fruiting bodies during development from the pinhead stage to maturity.

Selection of logs

To select natural logs suitable for cultivation of *P. linteus* mushroom, C/N reduction rate of sawdust was measured using CHN-1000 elemental analyser (Leco corp., St. Joseph, Mich.). The media were prepared with sawdust of *Morus alba* (mulberry), *Quercus acutissima* (oak), *Populus euamericana* (Suwon-Populus) and *Castanea crenata* (chestnut). The moisture of each sawdust medium was adjusted to about 65% with water. A 50 g of each medium was put into a column (ϕ3.0× 20.0 cm) and sterilized at 121°C for 60 minutes. After cooling to 20°C, one spoonful of inoculum of the precultured sawdust medium was inoculated in column of the sawdust media. The inoculated media were incubated in darkened room under controlled growth parameters. After 40 days of incubation, C/N ratio of sawdust was measured.

Inoculation (seeding) method

The inoculation method for the sterilized short log was conducted as follows : The selected natural logs cut to about 60 cm long were put in the heat-resistant polyethylene bags and covered with a cotton plug. The polyethylene bags entailed the logs were sterilized by autoclaving at 121°C (15 psi) for 2 hours and then cooled to 20°C. Inoculation was made by top spawning method; 0.5 cm (20-30 g) thickness of sawdust mycelial inoculum (planting spawn) was left on the top of cutting log and then plugged with a cotton plug. The inoculated logs were incubated in darkened room under controlled growth parameters. After one month of incubation, the mycelial growth was observed.

Size of logs

The logs were cut to shorten 20 cm, 30 cm, 40 cm, 50 cm and 60 cm, respectively and put into the polyethylene bags. After 2 months of incubation, the mycelial growth was observed. Sterilization and inoculation were made by the same method as described previously.

Content of logs

Moisture content of the short logs was adjusted to 35%, 40% and 42%, respectively. The logs that have 35% of moisture content were prepared by drying for 3-4 months after fellen and were used as a control. Forty percent of log moisture content was made by submerging of the dried logs in water for one day, and 42% was made by adding water to the submerged logs for one day. Inoculation and incubation were made by the same method as used for the sterilized short log inoculation. After 4 months of incubation, the mycelial growth was observed.

Sterilization time of substrates (short log)

The short logs about 15 cm in diameter and approximately 20 cm long were put into polyethylene bags, fitted with ring necks, and plugged with cotton to allow air exchange. The polyethylene bags containing a short logs were sterilized by autoclaving at 121°C (15 psi) for 8 hours, 10 hours, 12 hours and 14 hours, respectively. The colonized sawdust spawns (planting spawns), 0.5 cm thick or more, were inoculated on top of the sterilized short logs by top spawning inoculation method. After 6 months of incubation, the mycelial growth of short logs was observed.

Supplements

Short logs put into polyethylene bags, and various supplements such as mulberry sawdust, sawdust-rice bran (4:1; v/v), mulberry tree leaves and mulberry tree leaves-rice bran (4:1; v/v) were added on top of the short logs prior to plugging. Sterilization was done at 121°C (15 psi) for 14 hours and inoculation was made by top spawning. After 3 months of incubation, the mycelial growth of the short logs was observed.

Incubation time of log

The inoculated logs were placed in darkened room under the control of 22-25°C of temperature and 65-70% of relative humidity, and then were incubated for various periods such as 3 months, 4 months, 5 months and 6 months, respectively. The colonized logs were buried in soil of the mushroom cultivation house, and the formation of fruiting bodies was investigated according to incubation period of logs.

Temperature of mushroom cultivation house

Logs well-colonized with mycelium were separated from

polyethylene bags and buried vertically in soil of the mushroom cultivation house. The temperature of mushroom cultivation house was controlled at 20-25°C, 26-30°C and 31-35°C, respectively. The pinhead formation of mushroom was investigated according to the temperature of cultivation house.

Relative humidity of mushroom cultivation house

The mycelium-colonized logs were buried in soil of mushroom cultivation house at 31-35°C of temperature. The relative humidity of mushroom cultivation house was controlled at 80-90%, 91-95%, and 96-99%, respectively. Formation of fruiting body was investigated according to the relative humidity of mushroom cultivation house.

Burying depth of logs

The well colonized logs were buried vertically a quarter, a half length and three quarters in soil of the mushroom cultivation house at 31-35°C of temperature and 96-99% moisture. The pinhead formation of the fungus was investigated according to the buried depth of logs.

Results and Discussions

Physiological Characteristics

Selection of optimal media

Four different culture media were used to investigate a favorable growth of *P. linteus*. It was observed that the mycelial growth of *P. linteus* was excellent on MCM medium and a diameter of 70.2 mm for 14 days incubation (Fig. 1). Also the colony diameter of 3 different culture media was observed in the range of 40.2-45.7 mm and followed by MEA, YMA and PDA.

Effect of culture temperature

The mycelial growth of *P. linteus* which was cultured on MCM medium for 14 days at 5 different temperature levels was favorable in the range of 25-30°C (Fig. 2). When the temperature was raised above 30, the mycelial growth of *P. linteus* appeared to be suppressed. Chi *et al.* (1996) clarified that a favorable growth of *P. linteus* could be obtained in the range of 25-30°C.

Effect of pH

The effect of pH on mycelial growth of *P. linteus* was studied by the mycelial dry-weight method. The pH range suitable for a favorable growth of *P. linteus* was pH 6.0-7.0. The mycelial growth was best favorable at pH 6.0 and recorded dry weight of 74.2 mg, whereas most unfavorable at pH 4.0 (Fig. 3). As the fungus grows, the pH of the medium tends to decrease and to become more acidic. Chi *et al.* (1996) reported that pH

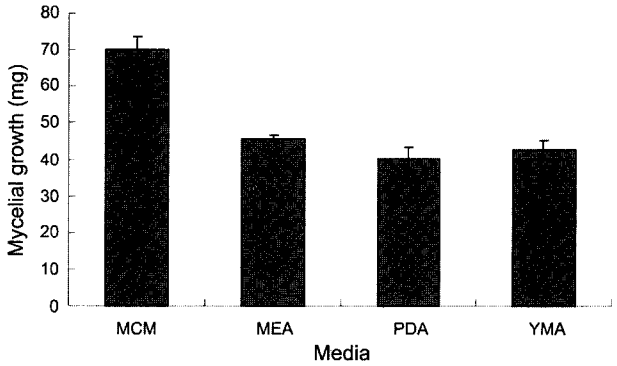

Fig. 1. Mycelial growth of *P. linteus* on different culture media.

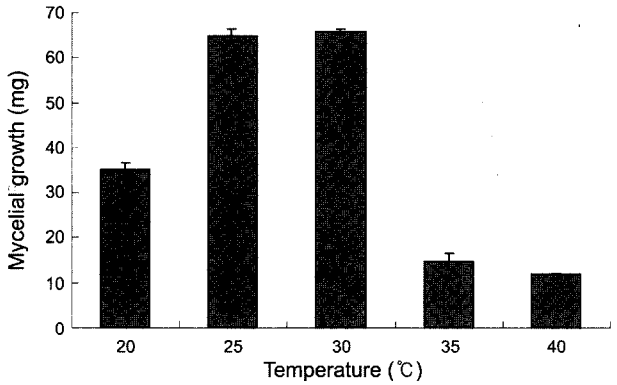

Fig. 2. Effect of temperature on the mycelial growth of *P. linteus*.

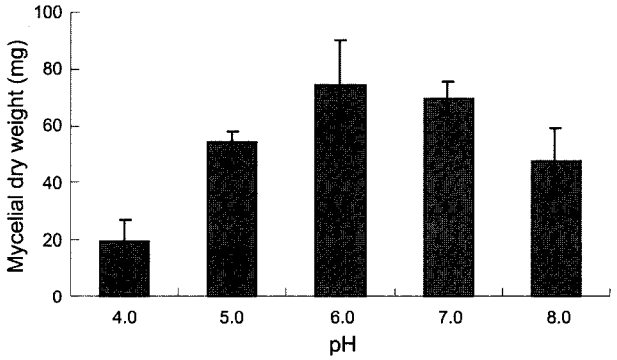

Fig. 3. Effect of pH on the mycelial growth of *P. linteus* in MCM broth medium.

value suitable for a favorable growth of *P. linteus* could be obtained in the range of pH 6.0-7.0.

Effect of carbon source

Carbon source utilization by *P. linteus* was tested by the method described by Lilly & Barnett (1951). Among 13 different carbon sources, 10 carbon sources were favorable to mycelial growth of *P. linteus* as compared with the control (Table 2). The mycelial growth of *P. linteus* was best on the

Table 2. Effect of carbon source on the mycelial growth of *P. linteus* in the basal medium

Carbon source[a]	Colony diameter[b] (mm)	Mycelial density[c]
Arabinose	32.3±0.50	T
Fructose	50.4±1.95	C
Galactose	29.5±0.71	T
Glucose	54.3±2.16	C
Mannose	55.0±1.41	SC
Xylose	45.8±1.60	SC
Cellobiose	42.0±0.00	SC
Lactose	23.6±1.34	T
Maltose	49.2±1.33	SC
Sucrose	57.3±1.50	ST
Dextrin	49.0±2.00	C
Inuline	48.5±3.42	ST
Soluble starch	46.7±0.82	ST
Control	34.5±0.71	T

[a]Each carbon source was added to the basal medium at the concentration of 0.1 M.
[b]The colony diameter was measured after 14 days of incubation.
[c]Mycelial density: C; compact, SC; somewhat compact, ST; somewhat thin, T; thin.

culture media which were contained with sucrose and recorded colony diameter of 57.3, and followed in order by mannose, glucose and fructose. Sucrose and maltose were the only oligosaccharides that supported good growth, but growth on the former was much better. Arabinose, galactose and lactose were unable to support for mycelial growth compared with the control. The three polysaccharides such as dextrin, inulin and soluble starch were good for mycelial growth.

Effect of nitrogen source

Nitrogen source utilization by *P. linteus* was tested by the method described by Lilly & Barnett (1951). Among 9 nitrogen sources, the mycelial growth of *P. linteus* was best on the culture media which were supplemented with potassium nitrate and recorded colony diameter of 82.2, and followed by sodium nitrate and ammonium tartrate (Table 3). There was no mycelial growth on sodium nitrite, while growth was hardly done on ammonium sulfate and urea compared with the control.

Table 3. Effect of nitrogen source on the mycelial growth of *P. linteus* in the basal medium

Nitrogen source[a]	Colony diameter[b] (mm)	Mycelial density[c]
Ammonium chloride	71.2±1.94	SC
Ammonium nitrate	71.4±3.28	SC
Ammonium phosphate	62.3±2.07	TC
Ammonium sulfate	56.7±2.58	TC
Ammonium tartrate	72.5±1.38	SC
Potassium nitrate	82.2±1.33	C
Sodium nitrate	79.7±1.51	C
Sodium nitrite	Non-growth	T
Urea	41.5±2.50	T
Control	58.9±5.51	T

[a]Each nitrogen source was added to the basal medium at the concentration of 0.1 M.
[b]The colony diameter was measured after 14 days of incubation.
[c]Mycelial density: C; compact, SC; somewhat compact, ST; somewhat thin, T; thin.

Cultural Characteristics

Selection of logs

To select of the optimal tree for *P. linteus* mushroom cultivation, C/N ratio of 7 different kinds of sawdust was measured after 40 days of incubation. The C/N ratio was the highest in the sawdust of *Poplus tomentiglandulosa* (suwon-poplus), followed in order by *Pinus sp.*, *Alnus japonica* and *Castanea ctrenata* (chestnut) before inoculation (Table 4). The results of C/N ratio reduction after 40 days incubation were described in Table 4. C/N ratio of the mulberry sawdust was slightly reduced, while that of the *Alnus japonica* and *Poplus tomentiglandulosa* was much reduced. Therefore, the log of mulberry tree was chosen for *P. linteus* mushroom cultivation, since *P. linteus* is a perennial mushroom and inhabits in mulberry tree, and also reduction of C/N ratio of these trees was a little.

Inoculation method

In order to select effective inoculation method for artificial cultivation of *P. linteus* mushroom, three different inoculation method were applied as follows; drilling inoculation method, log-end sandwich inoculation and sterilized short log inoculation method. The results showed that sterilized short logs was the best for mycelial running, while drilling inoculation method and log-end sandwich inoculation were not suitable for *P. linteus* mushroom cultivation because the rate of initial colonization was very so low as below 7% (Table 5). Many inoculation models have been used to cultivate the mushrooms in Korea. The drilling inoculation and log-end sandwich inoculation

Table 4. C/N ratio of various sawdust media after cultivation of *P. linteus*

Sawdust	C/N		C/N reduction
	Original	After incubation[a]	
Alnus japonica	236.9	107.1	54.8
Castanea crenata	195.8	121.3	38.1
Celtis sinensis	124.6	96.4	22.6
Morus alba	184.6	172.5	6.5
Pinus sp.	363.9	302.5	16.8
Poplus tomentiglandulosa	364.2	195.8	46.2
Quercus acutissima	101.2	85.6	15.4

[a]C/N ratio was measured after 40 days of incubation of *P. linteus*.

Table 5. Effect of various inoculation methods on the mycelial colonization in logs

Inoculation method	Rate of initial colonization[a] (%)	
	Oak tree	Mulberry tree
Drilling inoculation	3	3
Log-end sandwich inoculation	7	5
Sterilized short log inoculation	30	20

[a]Rate of initial colonization was measured after 1 month of incubation.

method have been used traditionally for *Lentinus edodes* and *Ganoderma spp.* cultivation, respectively, and the sterilized short log inoculation was recently conducted as a modified procedures for these mushroom cultivation.

Size for logs

To determine the favorable log size for the mycelial colonization, *P. linteus* was cultivated on different sizes of logs prepared with 20 cm, 30 cm, 40 cm, 50 cm and 60 cm, respectively. The rate of initial mycelial colonization was 30-40% after 2 momths of incubation, but that of full mycelial colonization was 5-17%. As the log size was short, the rate of mycelial colonization tended to increase. Therefore, short log sized with approximately 20 cm long was suitable for the cultivation of *P. linteus* mushroom. But rate of full mycelial colonization on the 20 cm sized log was no more than 17% (Table 6).

Content of logs

To screen log moisture content suitable for mycelial colonization of *P. linteus*, the log moisture content of mulberry tree was adjusted to 35%, 40% and 42%, respectively. After 4 months of incubation, the mycelial growth was observed. Rate of full mycelial colonization on 35% and 40% of log moisture content was 20% and 25%, respectively. In case of 42% of moisture content of the log, mycelial colonization of *P. linteus* was best (Table 7). As the moisture content level of log was high, rate of full mycelial colonization tended to increase and mycelia density also tended to compact. But rate of full mycelial

Table 6. Effect of length of logs on the mycelial colonization of *P. linteus*

Item	Log length (cm)				
	20	30	40	50	60
Rate of initial mycelial colonization[a] (%)	40	35	30	30	30
Rate of full mycelial colonization[b] (%)	17	15	10	5	5

[a]Rate of initial mycelial colonization was measured after 2 months of incubation.
[b]Rate of full mycelial colonization was measured after 4 months of incubation.

Table 7. Effect of moisture content in logs on the mycelial growth

Moisture content (%)	Rate of full mycelial colonization[a] (%)	Mycelial density[b]
40	25	++
42	27	++
35 (control)	20	+

[a]Rate of full mycelial colonization was measured after 4 months of incubation.
[b]Mycelial density: +; poor, ++; good.

colonization was below 27%. Rew et al (2000) reported that rate of successful inoculation of *P. pini* was 26%.

Sterilization time of substrates

Using the sterilized short log inoculation, the mycelial colonization of *P. linteus* was observed on logs after 6 months incubation. The rates of initial mycelial colonization and full mycelial colonization of *P. linteus* were the best in 14 hours of sterilization with record of 98% and 85%, respectively (Table 8). Generally liquid media are autoclaved by standardized sterilization at 121°C (15 psi) for 15 minutes and sawdust substrates for 90 minutes. On the other hand, these results showed that polyethylene bags filled with logs for *P. linteus* cultivation needed to be sterilized at 121°C over 12 hours. But depending on the nature and the bulk of the substrate, and

Table 8. Effect of sterilization time on the mycelial colonization of *P. linteus*

Item	Sterilization time (hours)			
	8	10	12	14
Rate of initial mycelial colonization[a] (%)	50	70	93	98
Rate of full mycelial colonization[b] (%)	50	63	80	85

[a]Rate of initial mycelial colonization was measured after 2 months of incubation
[b]Rate of full mycelial colonization was measured after 6 months of incubation.

Table 9. Effect of different supplements to mulberry logs on the mycelial growth

Supplement	Rate of full mycelial colonization[a] (%)	Mycelial density[b]
Mulberry sawdust	90	+++
Mulberry sawdust + rice bran (4:1; v/v)	92	+++
Mulberry tree leaf	93	+++
Mulberry tree leaf + rice bran (4:1; v/v)	95	+++
Non-addition (control)	86	+

[a]Rate of full mycelial colonization was measured after 3 months of incubation.
[b]Mycelial density: +; poor, +++; compact.

fungus activity, sterilization parameters need to be settled.

Supplements

Addition of supplements to short logs resulted in high rate of full mycelial colonization and accelerated the compact mycelial density compared with control (Table 9). Among of supplements used, the rate of full mycelial colonization and the mycelial density were the best on the log substrate which was supplemented with mulberry tree leaves-rice bran (4:1; v/v).

Incubation time of logs

To investigate favorable incubation period of logs for the formation of fruiting body, the logs were incubated for 3 months, 4 months, 5 months and 6 months, respectively.

The logs buried in soil before 4 months of incubation period were infected with undesirable fungi. The pinhead of *P. linteus* was not also formed. These results showed that the inoculated logs for *P. linteus* mushroom cultivation needed to be incubated at least over 5 months (Table 10).

Temperature of mushroom cultivation house

Temperature for the formation of fruiting bodies in the mushroom cultivation house was investigated. The pinhead of fruiting bodies on logs which were transfered to mushroom cultivation house controlled at 31-35°C was formed well, while not formed in the mushroom cultivation house at 21-25°C (Table 11). The results showed that the mushroom house for *P. linteus* cultivation need to be maintained over 26°C.

Relative humidity of mushroom cultivation house

The colonized logs which were buried in the mushroom cultivation house at 96-99% of relative humidity produced fruiting bodies well, while fruiting body was not formed at in the mushroom cultivation house at 81-90% relative humidity (Table 12). These results showed that the mushroom house for *P. linteus* cultivation needed to be maintained at least over 91% relative humidity.

Table 10. Effect of the incubation time of logs on the pinhead formation

Item	Incubation period (months)				
	3	4	5	6	7
Pinhead formation[a]	–	–	+	+	+

[a]Pinhead formation: –; non-formation, +; some formation.

Table 11. Effect of temperature in the cultivation house on the fruiting body formation

Item	Temperature (°C)		
	21-25	26-30	31-35
Fruiting body formation[a]	–	+	++

[a]Fruiting body formation : –; non-formation, +; some formation, ++; good formation.

Table 12. Effect of relative humidity in the cultivation house on the fruiting body formation of *P. linteus*

Item	Relative humidity(%)		
	81-90	91-95	96-100
Fruiting body formation[a]	–	+	++

[a]Fruiting body formation : –; non-formation, +; some formation, ++; good formation.

Table 13. Effect of buried depth of logs on the pinhead of fruiting bodies

Item	Burying depth of log			
	all	3/4	1/2	1/4
Pinhead formation[a]	–	+	++	++

[a]Pinhead formation: –; non-formation, +; some formation, ++; good formation.

Buried depth of logs

The well- colonized logs that were buried vertically a quarter or a half-length in soil was good for the formation of pinhead (Table 13). In case of burial of all or three quarters of logs in soil, fruiting bodies were formed on only small part of logs. The burial depth of logs was closely related with relative humidity in mushroom cultivation house. If the relative humidity of mushroom cultivation house is insufficient, the pinhead of fruiting body is formed on nearby soil, otherwise, if sufficient, the pinhead of fruiting body was formed on upper parts of logs.

Basidiocarps of *Phellinus linteus* ASI 26011

Fruiting bodies of *Phellinus linteus* ASI 26011 were formed

Fig. 4. Fruiting-bodies of *Phellinus linteus* ASI 26011 formed on mulberry logs.

after one year of inoculation. Basidiocarps grown for 2 years on logs were ungulate, sessile, 144×71 mm and hard woody. Upper surface of basidiocarps was concentrically zonate and shallowly sulcate, and dark chestnut (Fig. 4).

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