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Cultural Characteristics of A Medicinal Mushroom, *Phellinus linteus*

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For comparison of mycelial colonization of *Phellinus linteus* on logs, several techniques of inoculation were tested; 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 inoculation. The initial mycelial growth and the full mycelial colonization of *P. linteus* in logs were the best in case of 20 cm logs under the condition of 42% moisture content. 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 basidiocarps of *P. linteus*. The formation of fruiting body of *P. linteus* was quite good in the cultivation house at 31~35°C and over 96% of relative humidity.

KEYWORDS: Formation of fruiting body, Incubation period, Inoculation method, *Phellinus linteus*

Mushrooms have recently become attractive as functional foods or sources of beneficial medicine. With medicinal usage of *Ganoderma* species, the species of *Phellinus* are currently popular in Korea. 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 and Chen, 2000). *P. linteus* was first described as *Polyporus linteus* by Berkeley and Curtis (1860) and later renamed as *Phellinus linteus* by Teng (1964). From the basidiocarps of these species, several researchers have isolated some essential substances, which stimulate the immune system of human (Chung and 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 *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 bioactive protein-polysaccharide complex and rarity in nature (Oh and Han, 1993). This fungus inhabits mainly on mulberry (*Morus* sp.) tree and has perennial forms in Korea (Kim *et al.*, 1999). Despite such a great medicinal value, study on artificial cultivation of *P. linteus* has been rarely conducted.

The present study was especially tried to elucidate the possibility of the artificial production of *P. linteus* by cultural method using tree logs.

Materials and Methods

The process for basidiocarp 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 stage entailed the preparation of the growth substrates for mushroom cultivation.

Cultures. The *Phellinus linteus* (ASI 26099) was obtained from the National Institute of Agricultural Science and Technology, RDA. 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 m*l*. Subcultures were made routinely every 30 days. The original strain of ASI 26099 was isolated from basidiocarp collected in Kwangwon Province and identified as *Phellinus linteus* (Park *et al.*, 2001), and named as Corea sanghuang.

Inoculation. The YMA medium was autoclaved at 121°C (15 psi) for 20 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 an inoculum for the next step.

Mother spawn. The sawdust of tree was mixed with

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rice bran at a ratio of 4 : 1 (v/v) and moisture content was adjusted to about 65% by adding water. Then the mixed medium was put into a 250 m*l*-flask and was sterilized at 121°C for 45 minutes. After cooling to 20°C, a piece of mycelia from the agar plate was inoculated on the sawdust medium to use as 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 m*l*-polyethylene bottles and sterilized at 121°C for 90 minutes and cooled to 20°C. Two or three spoonfuls of inoculum of the precultured sawdust medium (mother spawn) in a 250 m*l*-flask were inoculated to the sawdust culture medium in 850 m*l*-polyethylene bottle. The inoculated sawdust media were incubated at 25°C for about 45 days until 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; selection and felling of the tree, sawing/cutting the log into short segments, transfer segments to polyethylene bags, sterilization, inoculation, spawn running, burial of log 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 albab* (mulberry), *Quercus acutissima* (oak), *Poplus euamericana* (Suwon-Poplus) 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 columm (Ø 3.0× 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 a columm of the sawdust media. The inoculated media were incubated in a darkened room under controlled growth parameters. After 40 days of incubation, C/N ratio of sawdust was measured.

Inoculation (seeding) method. 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 top of a cut log and then plugged with a cotton plug. The inoculated logs were incubated in a darkened room under controlled growth parameters. After one month of incubation, mycelial growth was observed.

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

Contents of logs. Moisture content of the short logs was adjusted to 35%, 40% and 42%, respectively. The log of which moisture content was 35% was prepared by drying for 3~4 months after felling and used as a control. Forty percent of log moisture content was made by submerging 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, 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. The polyethylene bags containing 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, mycelial growth of short logs was observed.

Supplements. Short logs were put into polyethylene bags, and various supplements such as oak sawdust, sawdust-rice bran (4 : 1; v/v), oak tree leaves and oak 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, mycelial growth of the short logs was observed.

Incubation time of logs. 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 incubated for 3 months, 4 months, 5 months and 6 months, respectively. The colonized logs were buried in soil in the mushroom cultivation house, and 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. 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 mushroom cultivation house.

Relative humidity of mushroom cultivation house. The mycelium-colonized logs were buried in soil in the 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 bodies was investigated according to the relative humidity of mushroom cultivation house.

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

Results and Discussion

Selection of logs. The C/N ratio was the highest in the sawdust of *Poplus euamericana* (suwon-poplus) sawdust, followed in order by *Castanea ctrenata* (chestnut), *Morus alba* (mulberry) and *Quercus acutissima* (oak) before inoculation (Table 1). The results of C/N ratio reduction after 40 days of incubation was described in Table 1. C/N ratio of mulberry sawdust and oak sawdust was slightly reduced, while that of Suwon-poplus sawdust and chestnut sawdust was much reduced. Mulberry tree and oak tree were chosen for *P. linteus* mushroom cultivation, since *P. linteus* is a perennial mushroom and reduction of C/N ratio of these trese was a little.

Table 1. C/N ratio of various sawdust media after cultivation of *Phellinus linteus*

Sawdust	C/N		
	Original	After incubation[a]	Reduction
Castanea ctrenata	195.8	109.3	86.6
Morus alba	184.6	155.5	29.2
Poplus euamericana	364.2	166.7	197.5
Quercus acutissima	101.2	86.9	14.3

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

Table 2. Effect of various inoculation methods on the mycelial colonization in log

Inoculation method	Rate of initial colonization[a] (%)	
	Oak logs	Morus logs
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.

Inoculation method. Sterilized short logs inoculation was the best for mycelial running on oak tree and mulberry tree, 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 low as below 7% (Table 2). Many inoculation models have been used to cultivate the mushrooms in Korea. The drilling inoculation and log-end sandwich inoculation 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 of 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 months 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 log was no more than 17% (Table 3).

Contents of logs. To screen log moisture content suitable for mycelial colonization of *P. linteus*, the log moisture contents of oak tree were adjusted to 35%, 40% and 42%, respectively. After 4 months of incubation, the

Table 3. Effect of length of logs on the mycelial colonization of *Phellinus linteus*

Item	Length of logs (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 4. Effect of moisture content in logs on mycelial growth of *Phellinus linteus*

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.

Table 5. Effect of sterilization time on mycelial colonization of *Phellinus 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.

mycelial growth was observed.

Rate of full mycelial colonization of *P. linteus* on 35% and 40% of log moisture content were 20% and 25%, respectively. In case of 42% of moisture content of the log, mycelial colonization of *P. linteus* was the best (Table 4). As the moisture content level of log was high, rate of full mycelial colonization tended to increase and mycelial density also tended to compact. But rate of full mycelial 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, mycelial colonization of *P. linteus* was observed on logs after 6 months of 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 5). 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 fungus activity, sterilization parameters need to be settled.

Supplements. Addition of supplements to short logs resulted in high rate of full mycelial colonization of *P. linteus* and accelerated the compact mycelial density compared with control (Table 6). Among the supplements used, the rates of full mycelial colonization and mycelial density were best on the log substrate which was supplemented with oak-tree-leaves-rice bran (4 : 1; v/v).

Table 6. Effect of different supplements to oak log on the mycelial growth of *Phellinus linteus*

Supplement	Rate of full mycelial colonization[a] (%)	Mycelial density[b]
Oak sawdust	90	+++
Oak sawdust + rice bran (4 : 1; v/v)	92	+++
Oak tree leaf	93	+++
Oak 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.

Table 7. Effect of 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.

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 7).

Effect of 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 transferred to the mushroom cultivation house controlled at 31~35°C was formed well, while not formed in the the mushroom cultivation house at 21~25°C (Table 8). The results showed that the mushroom house for *P. linteus* cultivation needed 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% relative humidity produced fruiting bodies well, while fruiting body was not formed in the mushroom cultivation house at 81~90% relative humidity (Table 8). These results showed that the mushroom house for *P. linteus* cultivation needed to be main-

Table 8. Conditions for fruiting bodies formation of *Phellinus linteus* on logs

Item	Condition		
Temperature (°C)	21~25	26~30	31~35
Fruiting body formation[a]	-	+	++
Relative humidity (%)	81~90	91~95	96~100
Fruiting body formation[a]	-	+	++
Buried depth of logs	3/4	1/2	1/4
Pinhead formation[a]	+	++	++

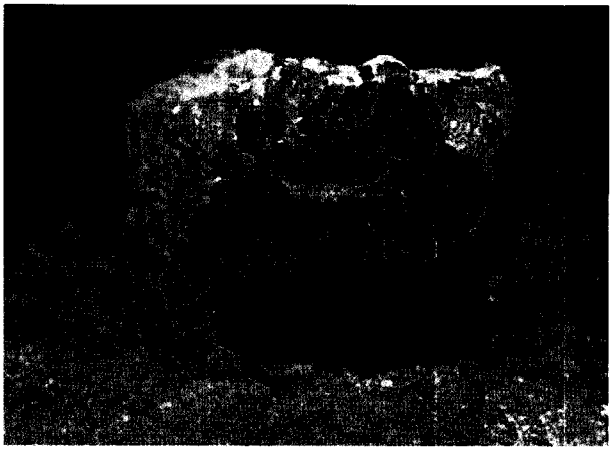

Fig. 1. Fruiting-body of *Phellinus linteus* ASI 26099 (Corea sanghuang).

tained at least over 91% relative humidity.

Buried depth of logs. The well-colonized logs that were buried vertically a quarter or a half length in soil were good for the formation of pinhead (Table 8). In case of burial of all or three quarters of logs in soil, fruiting bodies were formed on only small part of logs. The buring depth of logs was closely related with relative humidity in the mushroom cultivation house. If the relative humidity in the mushroom cultivation house isinsufficient, 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.

Basidiocarp of *Phellinus linteus* (ASI 26099). Fruiting bodies of *Phellinus linteus* ASI 26099 were formed after 1 year of inoculation. Basidiocarps grown for 2 years on logs were ungulate, sessile, 124×85 mm and hard woody. Upper surface of basidiocarps was concentrically zonate and shallowly sulcate, and dark chestnut (Fig. 1).

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