

## Full Length Research Paper

# Initiation of fruiting body development of the medicinal mushroom *Phellinus linteus* from Cambodia

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The purpose of this study was to determine the mating type system and initiation of fruiting body development of a medicinal mushroom *Phellinus linteus* from Cambodia. The evidence of fungal crossing analysis revealed that Cambodian *P. linteus* was a heterothallic basidiomycete and had a bipolar mating system. Clamp connection and peg cells formed on the dikaryons were possibly regulated by *A* and *B* genes. Initiation of the fruiting body was observed first on the Malt Extract Agar (MEA). In addition, we also found that fruiting bodies rapidly initiated on wood blocks. Unfortunately, their fruiting body initials were arrested before the stage of fruiting body maturation. Hence, a mushroom cultivation approach was chosen for observing the proper fruiting body development. The results showed that most mushroom spawns inoculated by dikaryons had initiated the small size (1.5 to 4.0 cm in diameter) fruiting bodies with a semicircular-shape. Fruiting body was not form in the mushroom spawns inoculated by monokaryons.

**Key words:** *Phellinus linteus*, fruiting body, mating system, heterothallic basidiomycete.

## INTRODUCTION

Mushroom production worldwide exceeds more than 10 million metric tons per year. Some mushrooms are medicinally important because they produce substances that express potential medicinal benefits. These medicinal mushrooms are now particularly interesting because they indeed have been shown to have profound health promoting benefits. Several articles have discussed the medicinal efficacy and active biological compounds (Chang, 1999; Wasser, 2002; Zhang et al., 2007; Firenzuoli et al., 2008; Song and Van Griensven, 2008). Most researchers have focused on any existing therapeutic effects of extracted medicinal mushrooms. The biological components such as polysaccharides, peptides and anti-oxidants have been determined and docu-

mented (Shon and Nam, 2001; Wasser, 2002; Zhang et al., 2007; Song and Van Griensven, 2008). Most medicinal mushrooms are collected from forests. Medicinal mushrooms have not been successfully cultivated on farms because they have high host specificity (Fisher and Binder, 2004; Hiroto et al., 2006).

*Phellinus linteus* (Berk et W.Curt.) is among the most important medicinal mushrooms. It is called Sangwhang in Korea but is unlike *Ganoderma lucidum*. The biological compounds in *P. linteus* have been used for medical purposes such as antitumor, anticancer, arthritis, anti-liver damage, antiviral and antibacterial activities (Han et al., 1999; Li et al., 2004; Kim et al., 2004; Matsuba 2007; Ohno et al., 2007; Jung et al., 2008; Song and Van

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Griensven 2008; Shnyreva et al., 2010). *P. linteus* is distributed throughout North America, Europe, Japan, China, Korea and Southeast Asia (Dai and Xu, 1998). DNA sequence analysis of this species has been determined, and 99% of the DNA sequences were homologous to another *P. linteus* type (Lee et al., 2005). Species classification of *Phellinus* spp. was elucidated by Internal Transcription Spacer (ITS), nuclear large subunit rDNA (nuc-18S-rDNA), small subunit mitochondrial ribosomal locus (mt-SSU), RAPD, and RFLP (Park et al., 2001; Wagner and Fischer, 2002; Fischer and Binder, 2004; Rizzo et al., 2003; Lee et al., 2006). Fischer and Binder (2004) focused on species recognition, geographic distribution, host-pathogen relationships and genetic relationship of various *Phellinus* species; litter details of fruiting body development, mating type system and cultivation of *Phellinus* spp. are available (Hansen, 1979; Fisher, 1995; Mallett and Myrholm, 1995). The mating type gene is a crucial gene for fruiting and basidiomycete development, particularly in the excellent basidiomycetous models; *Coprinopsis cinerea*, *Shizophyllum commune*, *Lentinus endodes*, *Ustilago maydis* and *Plerotus ostreatus* (Raper, 1866; Kües, 2000; Kothe, 2001). Kothe (2001) described that mating type genes are traits for any breeding program and more understanding of mating type genes can be used in mushroom breeding programs. Based on the mating system, an estimated 85 to 90% of all basidiomycetous species is heterothallic and requires mating. The heterothallic species are controlled by one or two mating type loci, whereas 10 to 15% of the basidiomycete species are assumed to be homothallic, namely, fruiting body formation without mating (Whitehouse, 1949; Quintanilha and Pinto-Lopes, 1950). Mallett and Myrholm (1995) reported the mating type system of *Phellinus tremulae*, and found that it was a heterothallic and had a tetrapolar mating system while, another white rot fungus (*Ganoderma boninense*) had a tetrapolar mating system with multiple alleles determined by a mating test (Piotti et al., 2002). Currently, Cambodian *P. linteus* has a high risk of extinction due to several factors such as deforestation, poor forest management, forest fire, indiscriminate harvesting and climate change. Moreover, there is no report on the achievements of Cambodian *P. linteus* cultivation.

In this study, we determined the mating type system, clamp cell formation, initiation of fruiting body on artificial medium and natural wood blocks which provided host parasite-specificity and mushroom cultivation.

## MATERIALS AND METHODS

### Study samples

Cambodian *P. linteus* samples were kindly provided by Dr. Frankie Chan from the Thai-Korea *Phellinus* Research Center. Culture and maintenance of the Cambodian *P. linteus* strain was performed at the Department of Biology, Faculty of Science, Mahasarakham University, Thailand.

### Culture and isolation

The grained fruiting bodies of Cambodian *P. linteus* were placed over aluminum foil to collect the dark brown basidiospores. The basidiospores were scraped from the aluminum foil into H<sub>2</sub>O, and a series of dilutions were placed on malt extracted with 1% agar (MEA). An antibiotic (Ampicillin) was required. Following 1 to 2 days of growth at 37°C and 12 h/dark and 12 h/light rhythm, colonies were inspected under a stereo microscope. A small agar piece of each single germinated spore (ca.1x2 mm<sup>2</sup>) was transferred to a new MEA plate for full growth, and each monokaryons was named as M1, M2, M3, M4, M5 and M6 and preserved for further study.

### Mating type compatibility test

Monokaryons (M1, M2, M3, M4, M5 and M6) were crossed among population samples. All crosses were conducted on MEA plates with ~ 1 cm distance between monokaryons. Inoculated MEA plates were kept in a black box with moisture tissue papers and incubated at 37°C for three days. The dikaryotic formation in a genetic cross could be observed by vigorous and full growth of mycelium. Complete and incomplete dikaryotic formation was examined under compound microscopy for the presence of clamp cells. The clamp cells (dikaryons) were represented as positive (+) and no clamp cells (heterokaryons) as a negative (-). Monokaryotic and dikaryotic mycelium were also observed by scanning electron microscopy (SEM).

For SEM, samples were fixed in 2.5% glutaraldehyde: 1% Osmiumtetroxide: 0.2 M phosphate buffer, (pH 7.2), at room temperature for 2 to 4 h, and then washed with the same buffer three times, and dehydrated by an acetone series (20%, 40, 60, 80 and 100) and then left to air dry. Gold coated sample films were determined by a SEM (JSM 6460 LV).

### Initiation of fruiting body test on MEA plate and wood blocks

A plug (~1.0 x1.0 cm<sup>2</sup>) of dikaryotic and monokaryotic mycelium were taken from dikaryotic or monokaryotic plates and transferred to new MEA plates. The experiment was designed as three replicates, (6 monokaryons X 3 = 18 MEA plates and 5 dikaryons X 3 = 15 MEA plates). Thirty three inoculated MEA plates were incubated at room temperature (28 to 30°C), for 12 h/dark and 12 h/light rhythm or until fruiting initially appeared. ~1.5 x1.5 cm<sup>2</sup> of a plug of dikaryotic and monokaryotic mycelium taken from the same preserved culture plates, were inoculated in each of the 250 ml flasks containing the MEA medium. Three *Shorea* (*Shorea obtusa*) wood blocks (1x1x2 cm) were placed on and close to the edge of each 250 ml flasks and stainless steel nets were used to support wood blocks. The experimental design of wood blocks was the same as the MEA experiment. All inoculated flasks were also incubated at room temperature. Fruiting body initials were observed and photographed by digital camera.

### Cultivation of fruiting body

Monokaryons (M1, M2, M3, M4, M5 and M6) and dikaryon (Di2) were chosen for making the starters by inoculating 5 mycelial plugs on sough grain contained in sterilized bottles and then incubating at 37°C for 2 weeks. The monokaryotic and dikaryotic mycelium were homogenously grown with sorghum grains or until the sorghum grains became white. They were then transferred into the 500 sterilized mushroom spawns (1.0 liter of cylindrical plastic bags), containing 93% rubber tree powders, 3.2% rice bran, 2.6% molasses, 0.2% MgSO<sub>4</sub> and 1% CaCO<sub>3</sub>. 300 sterilized mushroom spawns were inoculated by dikaryon (Di2) and another 200

sterilized mushroom spawns were inoculated by monokaryons as controls. They were then closed with cotton plugs and incubated at room temperature until the mycelium fully grew into mushroom spawns. The cotton plugs were removed, and all inoculated mushroom spawns were developed in a mushroom house with controlled environmental conditions (temperature; 24 to 28 °C, humidity; 80 to 90%).

## RESULTS

### Mating type system and initial fruiting body formation tests

Six monokaryons were cultured and the morphological characters were observed by Compound microscopy and SEM. The size of the monokaryotic hyphae varied from 1.2 to 1.8  $\mu\text{m}$  diameter (Figure 1B, C and D). Septum formation appeared but without clamp cells. It was found that the monokaryons grew well on Malt Extract Agar (MEA) medium and fully grew on agar plates within 5 to 6 days (Figure 1A). Monokaryons were intra-crossed on MEA plates (Table 1). Under compound microscopy each cross was determined by the presence of clamp cells. It was found that many clamp cells formed on crosses (M1x M2), (M2 x M3), (M2 x M4), (M2 x M5) and (M2 x M6), and were assigned as dikaryons Di1, Di2, Di3, Di4 and Di5, respectively. The clamp and peg cells were formed at the septum area. The tips of the clamp cells were fused with peg cells that emerged from lateral hyphae. The results of SEM showed that a bulge was generated from the hyphal surface indicating clamp cell formation initiation, whereas, arches were not placed directly on the level surface of hyphae but were placed somewhat above the hyphal surface and septum formation was apparent. From observations, indicating that the peg cells were generated prior to clamp cell fusion, there were unfused clamp cells numerously distributed on hyphae, while, the fused clamp cells were rarely present (Figure 1F, G, H, J, K and L). 14 other crosses (M1 x M3), (M1 x M4), (M1 x M5), (M1 x M6), (M3 x M1), (M3 x M4), (M3 x M5), (M3 x M6), (M4 x M1), (M4 x M3), (M4 x M5), (M4 x M6), (M5 x M1) and (M5 x M6) showed failed clamp cell formation (Table 1).

These crosses were observed at the initiation of fruiting bodies on MEA medium plates but they could not initiate the fruiting body. Five crosses (Di1, Di2, Di3, Di4 and Di5) formed the fruiting body initials after 2 weeks of inoculation (Figure 1E). Morphological characters of fruiting body initials were sponge-like and always initially formed on the edge of plates (Figure 1I). Fruiting body was not formed in 14 crosses. Genetic crossing analysis suggested that this mushroom was heterothallic, thereby requiring the mating of another compatible strains and had a bipolar mating type system or unifactorial.

### Initiation of fruiting body on wood blocks

Mycelium fully grew and covered the medium surface and

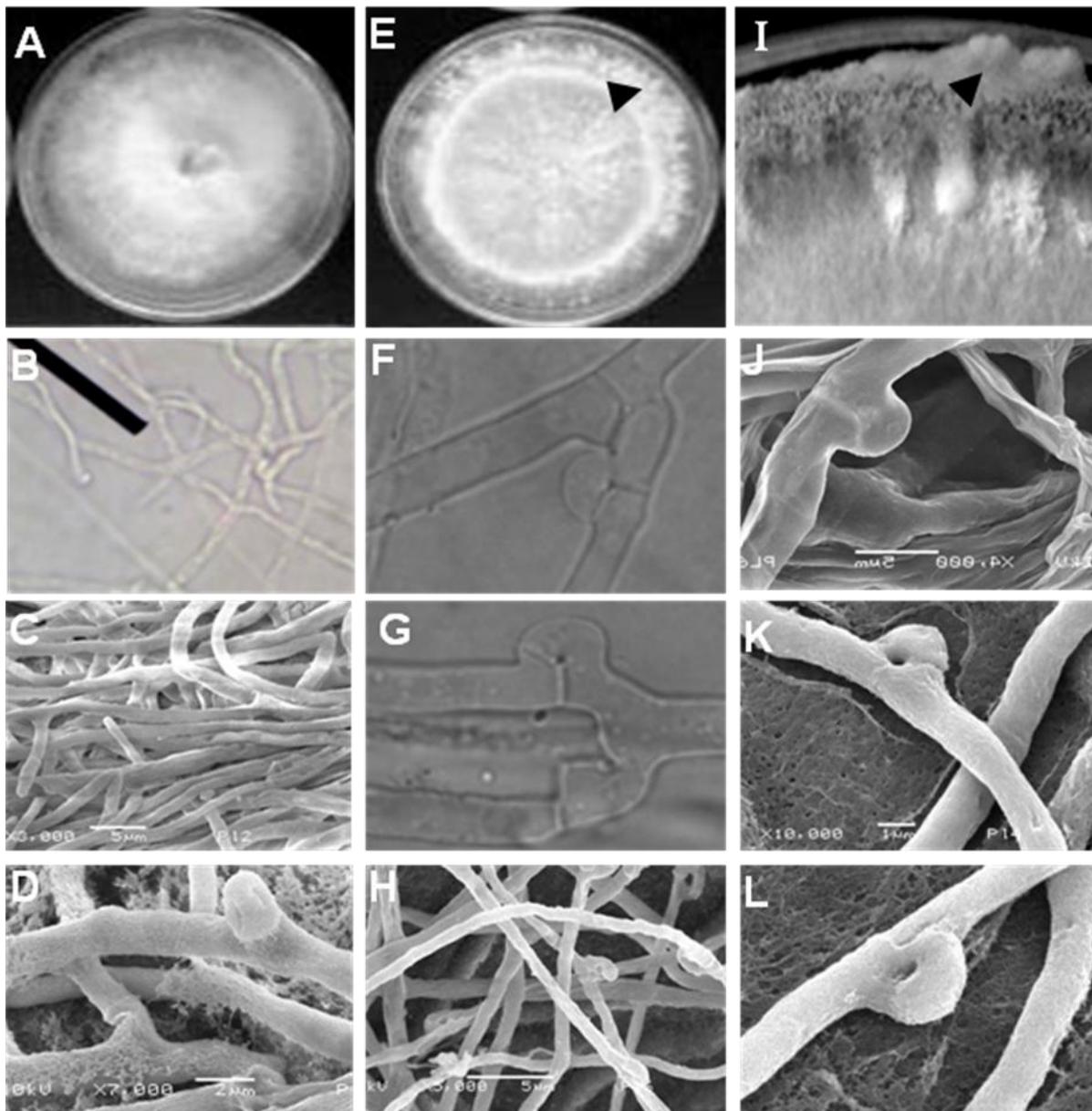
the wood blocks within a week. 15 flasks were inoculated by dikaryons, where they formed the fruiting body initials on wood blocks and inoculums after two weeks (Figure 2D, E and F). No fruiting body was formed on the wood blocks in 18 flasks inoculated by monokaryons (Figure 2A, B and C). The fruiting body initials that formed on MEA plates were slightly different from the fruiting body initials that developed on the wood blocks. However, they did not successfully form any mature fruiting body on either the MEA plates or the wood blocks. Therefore, initiation of mature fruiting body development needs to be elucidated. Subsequently, a mushroom cultivation approach was provided to observe the mature fruiting body development.

### Development of fruiting body on mushroom spawns

Monokaryons were also inoculated on mushroom spawns as controls. Dikaryon (Di2) was chosen as the first priority for mushroom cultivation because numerous fruiting body initials were developed on the MEA plate and wood block experiments. We found that 97% (291/300) of inoculated mushroom spawns had fruiting body initials present. 3% (9/300) of inoculated mushroom spawns were contaminated during fruiting body development. 94% (188/200) of controls did not have the initiation of fruiting body while other mushroom spawns were infected by unusual fungus. Fruiting bodies varied in size, the size of the basidiocarps was 1.5 to 4.0 cm in diameter, and many fruiting bodies were formed at the bottom of the mushroom spawn (Figure 3D). Unfortunately, the fruiting bodies were arrested before fruiting body maturation, even though, those mushroom spawns had prolonged incubation for more than three months, but no fruiting body appeared.

## DISCUSSION

It is generally known that most medicinal mushrooms are collected from nature. Several reports have also mentioned that medicinal mushrooms cannot be cultivated or form the fruiting body on the artificial substrates because fungi display host specificity or host-parasite specificity (Chang, 1991; Kües and Lui, 2000, Hiroto et al., 2006). However, several fungi easily grow on artificial substrates, for example, *Agaricus bisporus*, *Agarius subrufescens*, *Asterophora lycoperdoides*, *Favolus arcularius* (Fr.) Ames *Pleurotus* spp, *L. edodes* and *C. cinerea* (Chang and Miles, 1989; Kües and Lui, 2000; Kerrigan, 2005). Additionally, several plant pathogenic fungi (*G. lucidum*, *L. endodes*, *Lentinus* spp.) are successfully cultivated on mushroom farms (Chang and Miles, 1989). Most studies of the *Phellinus* mushrooms were focused on the biologically active compounds and pharmacodynamic effects under animal and cellular experiments (Han et al., 1999; Li et al., 2004; Kim et al.,



**Figure 1.** Dikaryotic hyphae and various phenotypes of the clamp cells. A fully grown monokaryotic mycelium is shown on a MEA plate (A). Monokaryotic mycelium was photographed by compound microscopy, 100 X (B). A high resolution of monokaryotic mycelium was observed by SEM; (C) and (D). Dikaryotic mycelium formed the fruiting body initials on MEA plate (a head arrow) (E and I). Clamp cells observed by compound microscopy (F and G). Clamp cells were present (H). Clamp cells were determined by SEM (J, K and L).

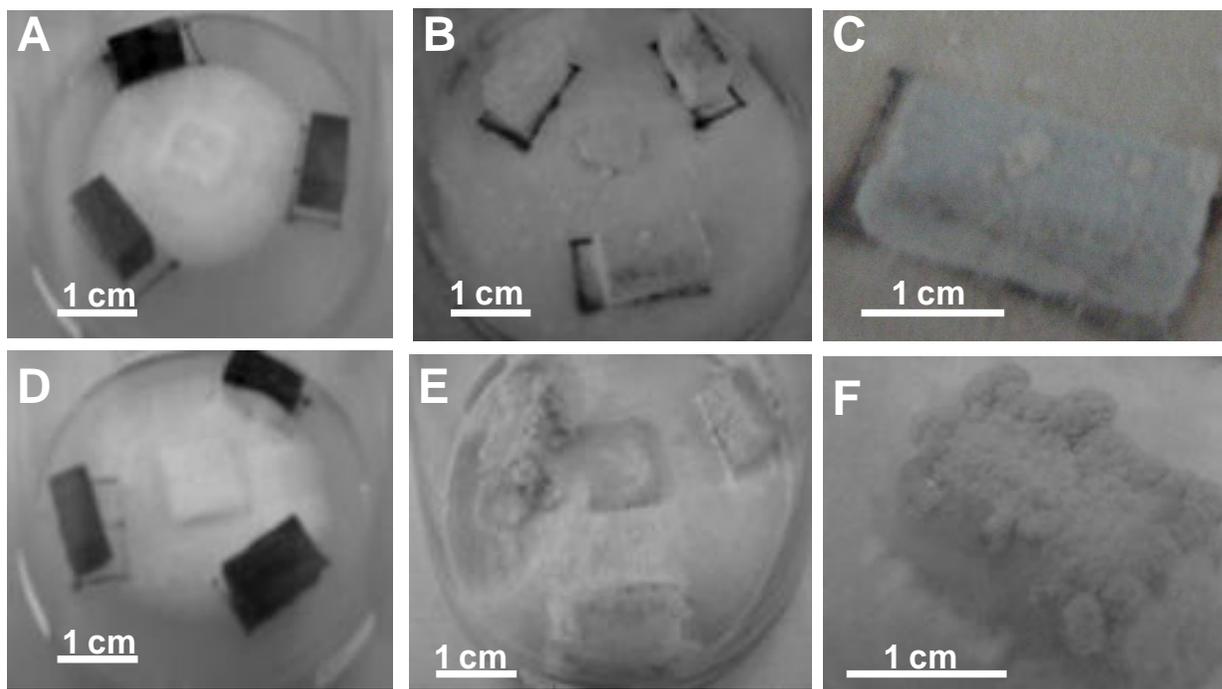
2004; Matsuba, 2007; Ohno et al., 2007; Jung et al., 2008; Song and Van Griensven, 2008; Shnyreva et al., 2010). The vegetative compatibility, mating type system and development are the basic information required for supporting mushroom cultivation but they have to date received little attention. Here, we determined the mating type system, and also observed fruiting body development of Cambodian *P. lintues*. From our genetic analysis, results suggested that Cambodian *P. lintues*

was a heterothallic basidiomycete, and it functioned as a bipolar mating type system because monokaryotic crosses (M1xM3), (M1xM4), (M1xM5), (M1xM6), (M3xM4), (M3xM5), (M3xM6), (M4xM5), (M4xM6) and (M5xM6) did not form clamp connection, indicating that they have the same mating type genes. Most basidiomycetes required mating or were heterothallic, and found that most *Phellinus* mushroom were unifactorial or had a bipolar mating type system and only few

**Table 1.** Intra-crossed monokaryons among population. Clamp cell formation was determined on six crosses.

Monokaryotic clone number	M1	M2	M3	M4	M5	M6
M1	=	+	-	-	-	-
M2	+	=	+	+	+	+
M3	-	+	=	-	-	-
M4	-	+	-	=	-	-
M5	-	+	-	-	=	-
M6	-	+	-	-	-	=

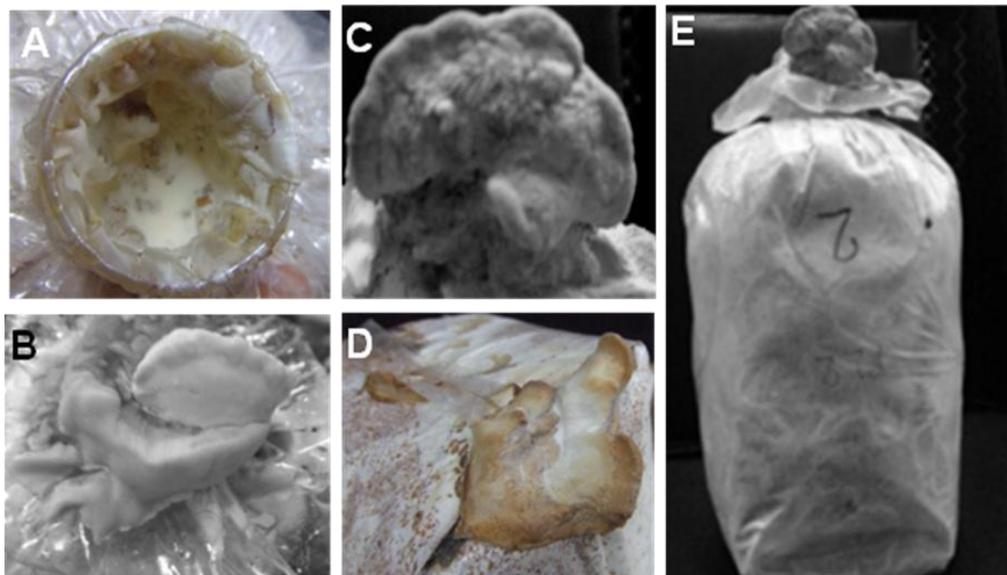
+, Clamp cell formation represents a positive; -, failure clamp cell formation represents a negative; =, no crossed.



**Figure 2.** Monokaryon grown on *Shorea* wood blocks in 250 ml flask, containing MEA. No fruiting body initial was formed (A, B and C). Dikaryotic mycelium inoculated on the surface of the MEA medium, prepared in 250 ml flasks and fruiting body initials were formed on the wood blocks and inoculums (D, E and F).

taxa of *Phellinus* were homothallic (Hensen, 1979; Fisher, 1994,1995). For example, *P. tremulae* was heterothallic and had a tetrapolar mating system (Mallett and Myrholm, 1999). The clamp connection and sub-apical peg cells which appeared on the hyphae during dikaryotic formation were possibly controlled by *A* and *B* genes which have been known as *A*- and *B*-factors (Rapper, 1966). Clamp cell formation specifically occurred in both typical mushrooms *C. cinerea* or *C. lagopus* and *S. commune* are regulated by *A* and *B* mating type genes (Pardo, 1996; Casselton and Olesnicky, 1998; Kothe, 2001; Kües, 2002; James et al., 2006). Since the development of molecular techniques, the function of *A* and *B* mating type genes of basidiomycetes, such as *C.*

*cinerea*, *S. commune*, *Coprinus disseminatus* and *Pholiota nameko*, have become clearer (Pardo, 1996; Fowler, 2001; Aimi et al., 2005; James et al., 2006). Mating type *A* genes regulate clamp cell formation, septation and nuclear synchronization, while mating type *B* genes control fused clamp cells and peg cell formation (Pardo, 1996; Kües, 2002; Balayan, 2006; James et al., 2006). Our study reveals that Cambodian *P. linteus* carries mating type *A* genes and functions in compatibility systems, whereas *B* genes may also exist, but it was not implicated in compatibility system of this fungus, similar to the mating type system of *C. disseminatus* (James et al., 2006). During peg cells formation on the hyphal surface near the septum positions indicate that peg cells



**Figure 3.** Fruiting body grown on mushroom spawns. Fruiting bodies have various shapes and size. A fruiting body developed on mushroom spawns after 45 days (A) and fruiting body developed on the top of mushroom spawns (B and C). A fruiting body was formed on the bottom of mushroom spawn (D) and fruiting body grew on an opened area of a mushroom spawn (E).

are individually regulated by the *B* gene, according to the function of mating type *B* genes in model mushrooms *C. cinerea* and *S. commune* (Fowler, 2001; Kothe, 2001; Kües et al., 2002; Balayan, 2006; James et al., 2006). We also have additional data that confirmed that mating type *A* gene is located on the chromosomal DNA of Cambodian *P. linteus*. Mitochondrial Intermediate Peptidase gene (*mip*) can be amplified from the DNA of Cambodian *P. linteus* by degenerated primers (MIPF1 and MIPR2); a partial 300 bp of *mip* gene was reproduced (Prayook et al unpublished; James et al., 2004a). A *mip* gene is linked to *A* mating type locus, ~ 1 kb distant between *mip* gene and *A* mating type locus of various basidiomycetes (James et al., 2004a). Initiation of fruiting body was demonstrated in both MEA and wood blocks. A wood block experiment was assigned based on the knowledge of fungal host specificity and also the understanding that most medicinal mushrooms are plant pathogenic fungi. Commonly, *Phellinus* spp. specifically attaches the hard wood trees. Our results revealed that fruiting body initials were developed on wood blocks, and showed that its morphological characters were different from the fruiting bodies grown on MEA plates. We found that their fruiting body initials were stopped at early fruiting body maturation, even though both experiments were prolonged in incubation, meanwhile, mushroom cultivation was provided to observe the manner of fruiting body development. Methodology of *G. lucidum* and *L. edodes* cultivation was used as guidance in this study (Chang, 1991). *G. lucidum* and *L. edodes* are white rotting fungi and behave as plant pathogenic fungus, like *Phellinus*. For Cambodian *P. linteus* cultivation herein, we

found that the mycelium fully grew on the mushroom spawns for a month, in contrast to *G. lucidum* mycelium which fully grew on its mushroom spawns within 10 to 20 days, indicating the speed of mycelial growth depending on a unique species. We also found that monokaryotic mycelium had slow growth compare to dikaryotic mycelium. The fruiting body initials were formed on opened mushroom spawns (Figure 3A, B, C, D) and on the bottom of the mushroom spawns (Figure 3D). The fruiting bodies were various forms in shape. Some fruiting bodies were similar to native basidiocarps of Cambodian *P. linteus*. However, the cultivation of Cambodian *P. linteus* was difficult due to rapidly decreasing moisture and nutrition involving the mushroom spawns, even though, 80 to 90 % of moisture content was given daily, and temperatures from 24 to 28°C were used in this cultivation which are relatively similar to the optimal temperatures (21 to 27°C) of *G. lucidum* (Adaskaveg, 1986). Stopping of fruiting body development on mushroom spawns may be due to limited water, minerals and nutrition in contrast with behavior of *Phellinus* mushrooms which are tree parasite in nature. However, this study is valuable in providing guidance for commercial medicinal mushroom production for further study.

### Conclusion

Cambodian *P. linteus* is a popular medicinal mushroom and is found in Cambodia where it commonly grows on hard wood, and to date there has been no report on the achievement of cultivation. Here, we studied the biologi-

cal information of this fungal species for supporting the success of cultivation. Cambodian *P. linteus* was a heterothallic mushroom that requires mating with other compatible strains and also had a bipolar mating type system or unifactorial system. Clamp and peg cell formation may be influenced by *A* and *B* genes. Fruiting body initials were developed on MEA plates and on wood blocks. Interestingly, the fruiting bodies were able to form on the mushroom spawns; unfortunately, their fruiting bodies stopped at early stage of fruiting body development.

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## REFERENCES

- Adaskaveg JE, Gilbertson RL (1986). Cultural studies and genetics of sexuality of *Ganoderma lucidum* and *Ganoderma tsugae* in relation to the taxonomy of the *Ganoderma lucidum* complex. *Mycologia*. 78:694-705.
- Aimi T, Yoshida R, Ishikawa M, Bao D, Kitamoto Y (2005). Identification and linkage mapping of the genes for the putative homeodomain protein (*hox1*) and the putative pheromone receptor protein homologue (*rcb1*) in a bipolar basidiomycete, *Pholiota nameko*. *Curr. Genet.* 48:184-194. doi:10.1007/s00294-005-0012-7.
- Badalyan SM, Polak E, Aebi M, Kües U (2004). Role of peg formation in clamp cell fusion of homobasidiomycete fungi. *J. Basic Microbiol.* 44:167-177. doi: 10.1002/jobm.200310361.
- Casselton LA, Olesnicky N (1998). Molecular genetics of mating recognition in basidiomycete fungi. *Microbiol. Mol. Biol.* 62(1):55-70.
- Chang ST (1991). Mushroom and mushroom biology, in *Genetics and Breeding of Edible Mushrooms*, edited by ST Chang, Buswell JA, P. G. Miles. Gordon and Breach Science Publishers, the Netherlands. pp. 1-13.
- Chang ST (1999). Global impact of edible and medicinal mushrooms on human welfare in the 21<sup>st</sup> century: Nongreen revolution. *Int. J. Med. Mushrooms.* 1:1-7.
- Chang ST, Miles PG (1989). *Edible Mushrooms and Their Cultivation*. CRC Press, Boca Raton, Florida USA.
- Dai YC, Xu MQ (1998). Studies on the medicinal polypore, *Phellinus baumii*, and its kin, *Phellinus linteus*. *Mycotaxon.* 67:191-200.
- Firenzuoli F, Gori L, Combardo G (2008). The medicinal mushroom *Agaricus blazei* Murrill: Review of literature of phamaco-toxicological problems. *eCAM.* 5(1):3-15. doi:10./ecam/nem007
- Fischer M (1994). Pairing tests in the *Phellinus pini* group. *Mycologia.* 86:524-539.
- Fischer M and Binder M (2004). Species recognition, geographic distribution and host-pathogen relationships: a case study in a group of lignicolous basidiomycetes, *Phellinus* s.l. *Mycologia.* 96(4):799-811.
- Fisher M (1995). *Phellinus igniarius* and its closest relatives in Europe. *Mycol. Res.* 99(6):735-744.
- Fowler TJ, Mitton MF, Vaillancourt LJ, Raper CA (2001). Changes in mate recognition through alterations of pheromones and receptors in the multisexual mushroom fungus *Schizophyllum commune*. *Genetics* 158:1491-1503.
- Han SB, Lee CW, Joen YJ, Hong ND, Yoo ID, Yang KH, Kim HM (1999). The inhibitory effect of polysaccharides isolated from *Phellinus linteus* on tumor growth and metastasis. *Immunopharmacology* 41:157-164.
- Hansen FM (1979) Sexual and vegetative incompatibility reactions in *Phellinus weirii*. *Canad. J. Bot.* 57:1573-1578.
- Hiroto H, Hirofumi S, Mutsumi T, Seigo A, Toshimitsu F, Takaaki F (2006). Fruiting-body formation, cultivation properties, and host specificity of a fungicolous fungus, *Asterophora lycoperdoides*. *Mycoscience.* 47(5):263-268. doi.org/10.1007/s10267-006-0305-9
- James TY, Kües U, Rehner SA, Vilgalys R (2004a). Evolution of the gene encoding mitochondrial intermediate peptidase and its cosegregation with the *A* mating-type locus of mushroom fungi. *Fungal Genet. Biol.* 41:81-390.
- James TY, Srivilai P, Kües U, Vilgalys R (2006). Evolution of the bipolar mating system of the mushroom *Coprinellus disseminatus* from its tetrapolar ancestors involves loss of mating-type-specific pheromone receptor function. *Genetics.* 172:1877-1891. doi: 10.1534/genetics.105.051128
- Jung JY, Lee IK, Seok SJ, Lee HJ, Kim YH, Yun BS (2008). Antioxidant polyphenols from the mycelia culture of the medicinal fungi *Inonotus xeranticus* and *Phellinus linteus*. *J. Appl. Microbiol.* 104(6):1824-1832. doi: 10.1111/jam.2008.104.issue-6
- Kerrigan RW (2005). *Agaricus subrufescens*, a cultivated edible and medicinal mushroom and its synonyms. *Mycologia.* 97(1):12-24. doi: 10.3852/mycologia.97.1.12
- Kim GY, Oh WK, Shin BC, Shin YI, Park YC, Ahn SC, Lee JD, Bae YS (2004). Proteoglycan isolated from *Phellinus linteus* inhibits tumor growth through mechanisms leading to an activation of CD11c<sup>+</sup>CD8<sup>+</sup>DC and type I helper T cell-dominant immune state. *FEBS Letters.* 576:391-400
- Kothe E (2001). Mating-type genes for basidiomycete strain improvement in mushroom farming. *Appl. Microbiol. Biotechnol.* 56:602-612.
- Kües U (2000). Life history and developmental processes in the basidiomycete *Coprinus cinereus*. *Microbiology.* 64:316-353.
- Kües U, Liu Y (2000). Fruiting body production in Basidiomycetes. *Appl. Microbiol. Biotechnol.* 54:141-152.
- Kües U, Walse PJ, Klaus MJ and Aebi M (2002). Influence of activated *A* and *B* mating type pathways on developmental processes in the basidiomycete *Coprinus cinereus*. *Mol. Gen. Genom.* 268:262-271.
- Lee HJ, Lee HJ, Lim ES, Ahn KS, Shim BS, Kim HM, Gong SJ, Kim DK, Kim SH (2005). Cambodian *Phellinus linteus* inhibits experimental metastasis of melanoma cells in mice via regulation of urokinase type plasminogen activator. *Biol. Pharm. Bull.* 28(1):27-31.
- Lee SJ, Lim OM, Cho YK, Cho HJ, Chang YS, Nam HD (2006). Identification of medicinal mushroom species based on nuclear large subunit rDNA sequence Korea. *J. Microbiol.* 44(1):29-34.
- Li G, Kim DH, Kim TD, Park BJ, Park HD, Park JI (2004). Protein-bound polysaccharide from *Phellinus linteus* induces G2/M phase arrest and apoptosis in SW480 human colon cancer cells. *Cancer Lett.* 28(216):175-181.
- Mallett RI, Myrholm CI (1995). The mating system of *Phellinus tremulae*. *Mycologia.* 87(5):597-603.
- Matsuba S, Matsuno H, Sakuma M, Komatsu Y (2007). *Phellinus linteus* Extract Augments the Immune Response in Mitomycin C-Induced Immunodeficient Mice. *eCAM.* 11:1-6. doi: 10.1093/ecam/nem001.
- Ohno T, Takahashi Y, Hiroki T, Hirayama H, Mizukami H, Ogihara Y, Inoue M (2007). Inhibitory effect of oral intake of natural *Phellinus linteus* fruit body growth pulmonary metastasis of B16/BL6 melanoma. *Nat. Med.* doi 10.1007/s11418-007-0163-6 61:438-442.
- Pardo EH, O'Shea SF, Casselton LA (1996). Multiple version of the a mating type locus of *Coprinopsis cinerea* are generated by three paralogous pairs of multiallelic homeobox genes. *Genetics* 144:87-94.
- Park DS, Kang HW, Kim KT, Cho SM, Park YJ, Shin HS, Lee BM, Go SJ (2001). PCR-based sensitive detection of wood decaying fungus *Phellinus linteus* by specific primers from rDNA ITS regions. *Mycobiology* 29(1):7-10.
- Pilotti CA, Sanderson FR, Aitken EAB (2002). Sexuality and interactions of monokaryotic and dikaryotic mycelia of ganoderma boninense. *Mycol.Res.*106(11):1315-1322.

- Quintanilha, Pinot-Lopes (1950). Aperçu sur l'état actuel de nos connaissances concernant la 'conduite sexuelle' des espèces d'Hymenomyces. Bol. Soc. Broteriana. 24:115-290.
- Raper JR (1966). Genetics of Sexuality in Higher Fungi. Ronald Press, New York.
- Rizzo DM, Gieser PT, Jr Burdsall HH (2003). *Phellinus coronadensis*: a new species from southern Arizona, USA. Mycologia 95(1):74-79.
- Shnyreva AV, Song W, Van Griensven LLJLD (2010). Extracts of medicinal mushrooms *Agaricus bisporus* and *Phellinus linteus* induce proapoptotic effects in the human leukemia cell line KA562. Int. J. Med. Mushrooms 12(2):167-175.
- Shon YH, Nam NS (2001). Antimutagenicity and induction of anticarcinogenic phase II enzymes by basidiomycetes. J. Ethnopharmacol. 77:103-109.
- Song W, Van Griensven LLJLD (2008). Pro-and antioxidative properties of medicinal mushroom extracts. Int. J. Med. Mushrooms. 10(4):315-324.
- Wagner T, Fischer M (2002). Proceeding towards a natural classification of the worldwide taxa *Phellinus* s.l. and *Inonotus* s.l., and phylogenetic relationships of allied genera. Mycologia 94:998-1016.
- Wasser SP (2002). Medicinal mushrooms as a source of anti-tumor and immunomodulating polysaccharides. Appl. Microbiol. Biotechnol. 60:258-74.
- Whitehouse HLK (1949). Heterothallism and sex in the fungi. Biological reviews. 21:1469-185.
- Zhang M, Cui SW, Cheung PCK, Wang Q (2007). Antitumor polysaccharides from mushrooms: a review on their isolation process, structural characteristics and antitumor activity. Trends Food sci. Technol.18(1):4-19. doi.org/10.1016/j.tifs.2006.07.013.