Domestication of wild strain of *Pleurotus giganteus*

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Pleurotus giganteus has relatively large fruiting bodies and is a saprobe in heavily rotting underground wood in forests; it is collected and widely consumed in many tropical countries including Thailand. Although *P. giganteus* is a popular edible mushroom, it is not cultivated in Thailand or Sri Lanka as a commercial mushroom. Recently a method for the cultivation of *P. giganteus* at the experimental level using saw dust as a substrate has been developed. The strain was isolated from a fresh fruiting body of *P. giganteus* (MFLU10 0154) using a piece of cap tissue and cultivated on Potato Dextrose Agar (PDA). Spawn was grown in sorghum (*Sorghum bicolor*) seeds. The cultivation method involves two steps, inoculating on a saw dust substrate in polypropylene bags as the preliminary step and transferring to the soil as a second step, which is very important for fruiting. The developed method of growing *P. giganteus* is fully described with all necessary steps.

Keywords: mushroom, polypropylene bags, saw dust substrate, tropical countries, underground wood.

Recently *Pleurotus giganteus* was transferred from *Lentinus* based on morphological and molecular evidences (Karunarathna *et al.* 2012). This species was previously named as *Lentinus giganteus* Berk. and was first described from Sri Lanka locally referred to as "Uru Paha" and classified in 'Decades of Fungi' (Berkeley 1847). *Pleurotus giganteus* has been treated as a special food since ancient times as mentioned in Buddhist literature (Berkeley 1847, Udugama & Wickramaratna 1991). When fully grown, the basidioma is typically infundibuliform measuring up to 35 cm in diameter and 28 cm high (Berkeley 1847, Udugama & Wickramaratna 1991). The mushroom may be solitary but often forms in groups on the ground. *Pleurotus giganteus* has a thick, radicant stipe and subdistant broad lamellae which is typical of *P. giganteus* (Pegler 1983, Karunarathna *et al.* 2012). It is a very popular mushroom because of its high protein content, excellent taste, bioactive components and the health-related functions (Udugama & Wickramaratna 1991, Huang 2005).

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Mushrooms have been domesticated for cultivation since early times, however most commonly grown strains are temperate species (e.g. Stamets 2000, Muruke *et al.* 2002, Fereira 2010). Tropical mushrooms are, however, numerous and recent studies on various genera have shown them to be specious with numerous new species being described (Le *et al.* 2007 a, b; Sanmee et al. 2008; Zhao 2008; Kerekes & Desjardin 2009; Wannathes *et al.* 2009 a, b; Sysouphanthong *et al.* 2010; Zhao *et al.* 2010, 2011 a, b; Karunarathna *et al.* 2011, 2012). Domesticating tropical mushrooms therefore provides a huge opportunity for tropical and subtropical countries and progress has already been made (Boa 2004, Vostrovský & Jablonská 2007, Marshall & Nair 2009). Tropical mushrooms grow rapidly and produce fruiting bodies at 25 °C or higher and thus can be produced more quickly than temperate species. They can also be produced on readily available waste products such as saw dust, corn cobs, rice straw, sugarcane bagasse, and other forest and agricultural waste. Growing mushrooms will therefore help recycle agricultural and forest waste products, provide income to various entrepreneurs, provide nutritional and medicinal foods and prevent pollution through less dumping and burning of agricultural waste (Vostrovský & Jablonská 2007, Kwon & Thatithatgoon 2004).

Only a small number of mushrooms are presently commonly cultivated in Thailand and include the Oyster Mushroom, *Pleurotus ostreatus* (Jacq.) P. Kumm., Hed Nanglom Khao in Thai, Straw mushroom, *Volvariella volvacea* (Bull.) Singer, Hed Fang in Thai, and Wood ear, *Auricularia polytricha* (Mont.) Sacc., Hed Hoo-Nu (Hanko 2001, Boa 2004, Karunarathna *et al.* 2011). Other less commonly produced species are King oyster mushroom, *Pleurotus eryngii* (DC.) Quél., Heh Nanglom Luang in Thai, Abalone mushroom, *Pleurotus cystidiosus* O. K. Mill., Hed Pao-hue in Thai, Golden oyster mushroom, *Pleurotus citrinopileatus* Singer, Black poplar mushroom, *Agrocybe cylindracea* (DC.: Fr.) Maire, Hed yanagi in Thai, Enokitake, *Flammulina velutipes* (Curt.: Fr.) Karst., Hed Khemthong in Thai, Reishi, *Ganoderma lucidum* (Leyss.: Fr.) Karst., Hed Lin Juer in Thai, Shiitake, *Lentinula edodes* (Berk.) Pegler, Hed Hom in Thai, Button mushroom, *Agaricus bisporus* (J. E. Lange) Imbach, Hed Kradum in Thai, while Inky cap, *Coprinus atramentarius* (Bull.) Fr., Hed Muerk in Thai, Lion's mane, *Hericium erinaceus* (Bull.) Pers., Hed Hua Ling in Thai, Silver ear, *Tremella fuciformis* Berk., Hed Hu-nu-Khao in Thai, and Parasol mushroom, *Macrolepiota gracilenta* (Krombh.) Wasser, Hed Nok Yoong in Thai (Kwon & Thatithatgoon 2004) may be rarely produced. Most of the mushrooms presently produced are imported strains and as far as we are aware little use is made of Thai strains. According to Boa (2004) there are over 1100 species of edible and medicinal fungi from over 80 countries and when this list is compared with the edible mushroom species that are presently commercially harvested in Thailand, many opportunities are clearly available for other species domestication (Jones *et al.* 2004, Kwon & Thatithatgoon 2004, Berch *et al.* 2007). Studies centered around the Mushroom Research Centre in Chiang Mai have resulted in records and descriptions of numerous mushroom species (Le *et al.* 2007 a, b; Sanmee *et al.* 2008;

Zhao 2008; Kerekes & Desjardin 2009; Wannathes *et al.* 2009 a, b; Sysouphanthong *et al.* 2010; Karunarathna *et al.* 2011, 2012; Zhao *et al.* 2011 a, b), many of which are edible and have the potential to be domesticated (Zhao *et al.* 2011 a, b; Karunarathna *et al.* 2011, 2012; Chen *et al.*, unpubl.). Thai people like to eat mushrooms and there is extensive cultivation of common mushrooms in Thailand. Mushrooms are also used in traditional Thai medicines and have been shown to contain various bioactive components and are used in cosmetics (Kwon & Thatithatgoon 2004, Hyde *et al.* 2010), cancer treatments (Wisitrassameewong *et al.* 2012 a, b) and have anti-diabetic properties (De Silva *et al.* 2012). The Thai government and Royal project also encourages rural farmers to grow mushrooms because of the large income from the mushroom growing using low cost agricultural wastes (Kwon & Thatithatgoon 2004). As Thai's appear to like eating mushrooms, the potential for introducing new mushrooms to the Thailand market is great.

During studies of the genus *Lentinus* in northern Thailand we collected several species of *Lentinus* including three species new to science (Karunarathna *et al.* 2011). We also collected *Lentinus giganteus* and following molecular study found this taxon to be more closely related to *Pleurotus* (Karunarathna *et al.* 2012) showing how molecular methods have revolutionized the study of taxonomy, systematics, phylogeny, biogeography, population and microevolutionary processes in basidiomycetes in the last two decades (Yang 2011). Species of the genus *Pleurotus* are the best known of edible higher basidiomycetes as producers of the pharmacologic agent lovastatin (mevinolin) (Gunde-Cimerman *et al.* 1993 a, b; Gunde-Cimerman & Cimerman 1995). The presence of lovastatin was determined in four species: *P. ostreatus*, *P. cornucopiae*, *P. eryngii*, and *P. sapidus* (Wasser & Weis 1999).

Pleurotus giganteus is one of the largest edible mushrooms in the world and can be grown on saw dust medium with supplements (Udugama & Wickramaratna 1991). Saw dust from a mixture of wood species or Jak wood is preferred as the main substrate for *P. giganteus* growing (Udugama & Wickramaratna 1991). *Pleurotus giganteus* is cultivated in China (Huang 2005) and Taiwan (Peng 2006), but even though it has a very good taste (Udugama & Wickramaratna 1991), it is not yet cultivated in Thailand or Sri Lanka as a commercial mushroom (Karunarathna *et al.* 2012). The present experiment was undertaken to investigate the best conditions for domestication of wild *P. giganteus* using saw dust as a locally available substrate.

Materials and methods

Isolation of pure cultures

Pure cultures were isolated from the sterile internal fungal tissues. About 5 ml of PDA medium was poured into 50 ml culture tubes followed by tight capping with sterile cotton wool, sterilization, and kept as slants. Fresh juvenile fruiting bodies of *Pleurotus giganteus* were collected from the Mushroom Research Center, Chiang Mai, Thailand (MFLU10 0154) and used

for tissue culture. Small pieces of the internal tissue of the broken mushroom was cut and removed with a flamed needle.

The needle with the tissue was then transferred into a culture-tube slant and the tissue was laid on the agar surface, and incubated at 30 °C. After 3 to 4 days, the agar surface was covered with a white mycelium as pure culture.

Mycelial growth tests

Effect of raw materials: *Vigna angularis* (Willd.) Ohwi & Ohashi. (red bean), *Phaseolus vulgaris* L. (black bean), *Vigna radiata* (L.) R. Wilczek Steve Hurst. (mung bean), and *Glycine max* (L.) Merr. (soybean) were obtained from Tesco-Lotus (Mae Chan) and *Sorghum bicolor* (L.) Moench (sorghum) was bought from Chiang Rai local market in Thailand. Malt extract agar (DIFCO) and potato dextrose agar (CRITERION) were used as synthetic media. Fifty grams of each grain type was soaked in 250 ml of distilled water (Gbolagade *et al.* 2006) for 12 h and boiled. Each grain type was then ground using a pestle and mortar and filtered through a clean white cloth. Twenty grams of agar (UNION SCIENCE) and distilled water were added to obtain the final volume of 1000 milliliters and autoclaved. The media were poured into 10 cm Petri dishes and allowed to solidify. The mycelia were subcultured into semi-synthetic media and incubated at 30±2 °C (Gbolagade *et al.* 2006). The radial colony diameter was measured after two days incubation and daily until mycelia reached the edge of the plates. The Soybean raw material prepared to support the growth of *P. giganteus* mycelia and used to determine the effect of pH and temperature for the growth and reproduction.

Effect of pH: The pH of the medium prepared from soybean, was adjusted to 5-8 (5, 5.5, 6, 6.5, 7, 7.5, and 8). The best pH for the growth of *P. giganteus* was determined by measuring the colony diameter following the method described below.

Effect of temperature: The growth of *P. giganteus* mycelium was compared using different temperatures (20, 25, 30, and 35 °C) to determine the optimal temperature requirement for this mushroom. The growth of the colony diameter was measured and compared to establish the optimum temperature for mycelia growth.

Grain spawns development and production

Red bean, black bean, soy bean, mung bean and sorghum were used as substrates for spawn production. Materials were washed and soaked in distilled water for 12 hours, boiled for 5-10 minutes (almost cooked) and filtered through a clean white cloth. All materials were dried at room temperature. Forty grams of each substrate were placed in Erlenmeyer flasks and loosely covered with a cotton plug and aluminium foil on top and autoclaved. The mycelia from pure culture of *P. giganteus* were cut and inoculated into each grain material and incubated at the optimal temperature of 30 ºC. The myce-

lial growth of each grain was recorded in order to choose the best raw material for spawn production.

Spawn was prepared as above using *Sorghum bicolor*. After sterilization the bottles filled with *Sorghum* were allowed to cool under room temperature, transferred with the mycelial disks of *P. giganteus* under aseptic conditions, plugged with cotton plug and incubated at 30 ºC, in the spawn room, in the dark. Observations were recorded on the diameter of mycelium running through the substrate. After 15–20 days the bottles became white due to complete colonizing by mycelia. The spawn was then ready for transferring to substrate bags.

Bag preparation, cultivation and fructification

Saw dust from a mixture of wood species was used as the main substrate. For 1 kg of clean sawdust, 50 g of rice bran, 10 g of brewer's waste, 10 g of *Leucaena* leaf, 10 g of pumice sulfate, 10 g of calcium carbonate, and 10 g of flour were added to prepare the substrate. The components were mixed well and water gradually added until the moisture content was around 65–70 %. Polypropylene bags (25 × 8 cm) were filled with 800 g prepared substrate and packed tightly. A hole (about 5 cm) was made with a PVC pipe at the centre for space to place the mycelial plugs. A plastic ring was used to make a "bottle neck" for easy handling. Plastic rings were used on the bags end, the bags end was pulled out through the ring, the pulled out part was folded down, tied with a rubber band and the hole plugged with cotton plug. The substrate bags were autoclaved at 15 psi for 15 min at 121 °C or by using a steamer at 90–100 °C for 3 h. After sterilization the substrate bags were allowed to cool to room temperature, transferred with the spawn, normally using about 80 g for a substrate bag which was about 10 % of the weight of a substrate bag, with *P. giganteus* under aseptic conditions. The bags were kept in a dark incubation room at 25±1 °C under 70–80 % relative humidity and opened when the mycelia had completely colonized the substrate.

After the mycelia had completely grown in the substrate, the upper portions of the bags were opened. The opened surface of the substrate was scraped slightly with a sterile teaspoon to remove the thin whitish mycelia. The substrate bags were then placed on the shelf and covered with black cloth to give appropriate ventilation. To maintain 80–85 % relative humidity in the culture house, water was sprinkled on the open end of the growing bags. Water spraying was carried out daily until pin heads developed.

When the pin heads had started to develop the polypropylene bags were completely removed and the contents transferred to the soil and buried or the top part of the growing bags was covered with soil and transferred to the growing house. The experiment was composed of 30 growing bags, ten bags were transferred to the soil, ten bags were covered with soil at the top, and ten bags were kept without soil as controls. Water spraying was carried out daily until the fruiting bodies had fully developed.

Data collection and statistical analysis

A Completely Randomized Design (CRD) with five replications was used in the experiment. Data were collected for mycelium growth rate in culture plates and growing bags, time required for completion of mycelium running, optimum temperature and pH for mycelium growth, duration from stimulation to primordia initiation, duration from stimulation to harvest, number of mature fruiting bodies, stalk length and diameter, pileus diameter and thickness. The data were analyzed statistically in terms of variance and mean showing statistical significance using Duncan's multiple range tests by SPSS-16 program (Brosius 2008).

Results

Mycelial growth tests

Effect of raw materials: All five types of raw materials used enhanced mycelia growth and radial mycelia extension of *P. giganteus* at 30 °C, being the optimal temperature. The best mycelia growth occurred on soybean media (Tab. 1, Fig. 1).

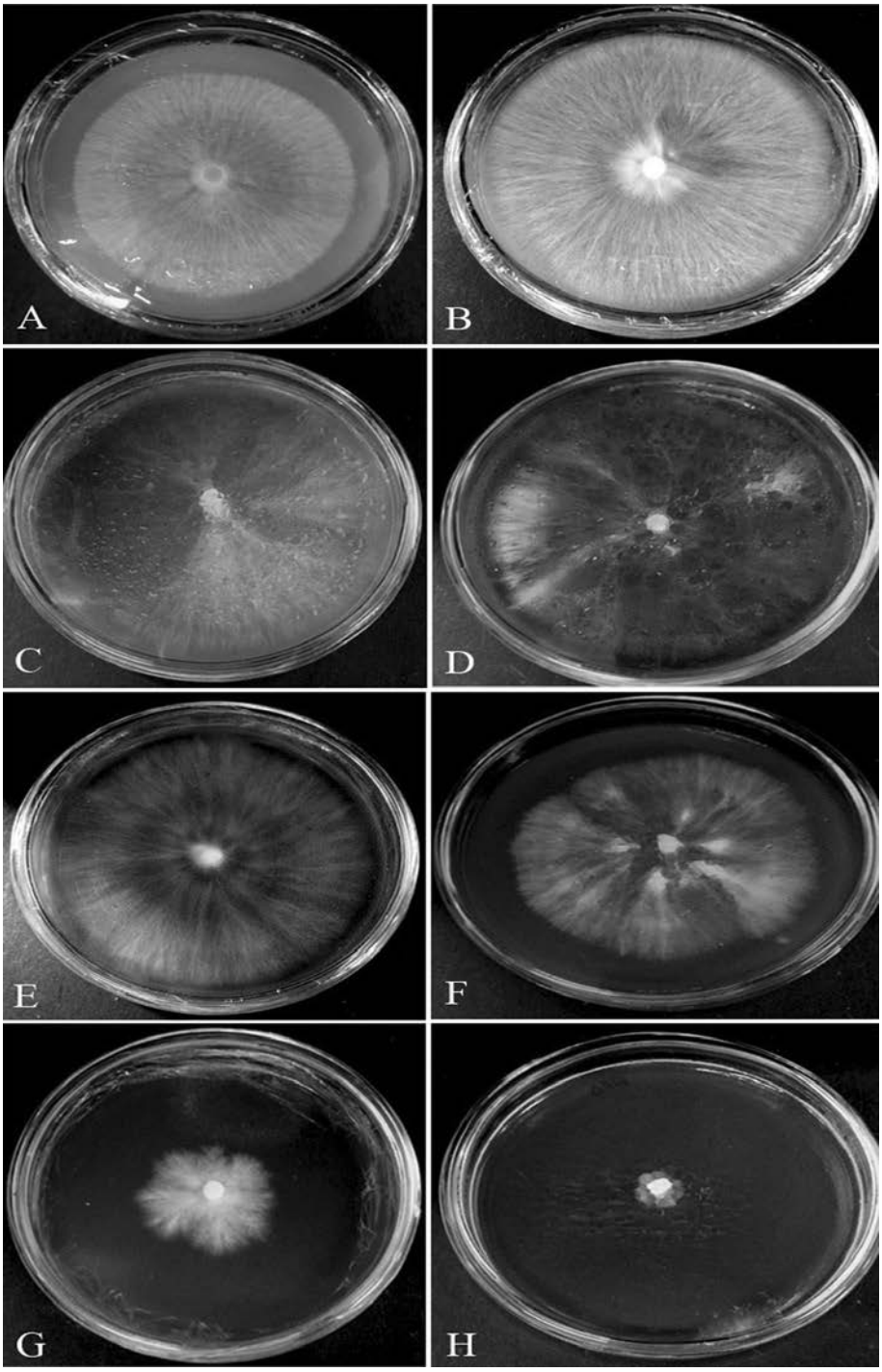
Tab. 1. – Effect of raw materials on mycelia growth of *Pleurotus giganteus* at 30 °C. Each value represents a mean of five replicates. Means followed by the same letters are not significantly different by Duncan's multiple range test (P<0.05)

Raw materials	Days				
	Radial mycelia extension (mm)				
	2	4	6	8	10
Mung bean	7.6^{cd}	16.4^{c}	37^{c}	60.4^{c}	74.4^{c}
Black bean	7^{bc}	19.4^{d}	43^{d}	66.6^{d}	80.4^{d}
Red bean	8.8^{e}	27.8^{f}	49.4^{e}	70.2^{e}	82.8^{e}
Sorghum	6.8^{b}	20.4^{d}	44.6^{d}	71.8^{f}	85^{f}
Soy bean	7.8^{d}	24.2^{e}	51^{f}	73^{f}	90^{g}
MEA	6.4^{ab}	7.4^{a}	19^{a}	24^{a}	31.2^{a}
PDA	6^{a}	11.8^{b}	22.2^{b}	27^{b}	33.2

Effect of pH: Mycelia growth of *P. giganteus* was observed at a pH of 5–8. Mycelia of *P. giganteus* can grow in acidic, neutral and alkaline conditions (Tab. 2), but the maximum growth was obtained at pH 5–6.5.

Effect of temperature: Radial mycelia growth at 20, 25, 30 and 35 °C is shown in Tab. 3, with an optimum at 25 °C followed by 30 °C and 20 °C. No growth occurred at 35 °C.

Fig. 1. Effect of raw materials on mycelia growth of *Pleurotus giganteus*. **A, B** Soybean medium (best growth), **C** *Sorghum* medium, **D** red bean medium, **E** black bean medium, **F** mung bean medium, **G** PDA, **H** MEA (poorest growth).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Tab. 2. – Effect of pH on mycelia growth of *Pleurotus giganteus*. Each value represents a mean of five replicates. Means followed by the same letters are not significantly different by Duncan's multiple range test (P<0.05)

pH	Days					
	Radial mycelia extension (mm)					
	2	4	6	8	10	12
5	11.4^{c}	21.6^{b}	33.6^{b}	52^{b}	76.4^{c}	90^{b}
5.5	12.2^{c}	24.4^{d}	40.6^{d}	58.8^{c}	78.6^{d}	90^{b}
6	11.6^{c}	22.8^{c}	35.8^{c}	52.4^{b}	79.2^{d}	90^{b}
6.5	10.2^{c}	21^{b}	35^{c}	51.8^{b}	76^{c}	90^{b}
7	9.8^{b}	19.8^{a}	35.2^{c}	49.2^{a}	70.6^{b}	81^{a}
7.5	8.2^{a}	18.6^{a}	31.4^{a}	48.6^{a}	67.6^{a}	80.8^{a}
8	8.6^{a}	19^{a}	33.6^{b}	48.4^{a}	67^{a}	80.4^{a}

Tab. 3. – Effect of temperature on mycelia growth of *Pleurotus giganteus*. Each value represents a mean of five replicates. Means followed by the same letters are not significantly different by Duncan's multiple range test (P<0.05)

Temperature (°C)	Days				
	Radial mycelia extension (mm)				
	2	4	6	8	10
20	10^{a}	18.2^{a}	27.8^{a}	41^{a}	53.8^{a}
25	13.4^{b}	35.8^{c}	35.8^{c}	77.8^{c}	90^{c}
30	12.6^{b}	25.8^{b}	25.8^{b}	52.8^{b}	72.2^{b}
35	5	5	5	5	5

Grain spawn development and production

Mycelia of *P. giganteus* could grow in all five grain types to a varying extent. The best growth in terms of mycelial extension was obtained on soybean (12 days after inoculation) followed by black bean, red bean, mung bean and the poorest mycelia growth occurred in *Sorghum* (Fig. 2).

Fructification

We observed that the mycelia of *P. giganteus* took 30-32 days to run from the top to the bottom of the substrate bags until pin heads developed. After 31 days of encasing, *P. giganteus* produced fruiting bodies in two bags and after 53 days in eight bags. In control bags, the fruiting was observed only in two bags after 33 days and until 53 days it was still only in two bags. The results of fruiting body development are shown in Tab. 4 and Fig. 3.

Fig. 2. *Pleurotus giganteus* mycelial growth on different grain types. **A** Soy bean (best growth), **B** black bean, **C** red bean, **D** mung bean, and **E** *Sorghum* (poorest growth).


A
B
C
D
E

Tab. 4. – Fruiting body production of *Pleurotus giganteus* in substrate bags. * Total number of fruiting bodies after 53 days. **Total number of bags completed with mycelia running from the top to the bottom of the substrate bags after 32 days

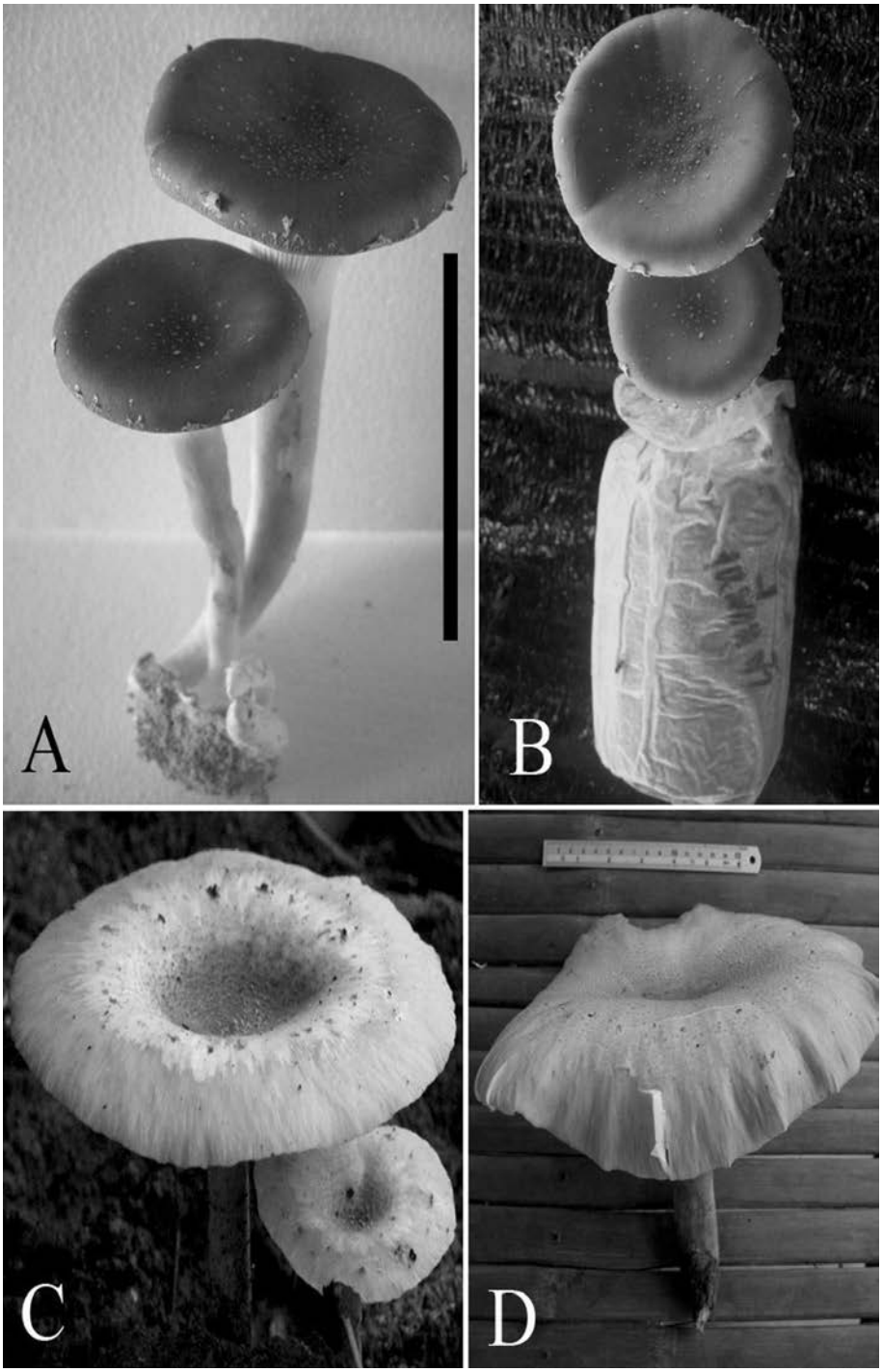
Mycelia running		Fruiting bodies development		
Days	Bags	Days	Fruiting bodies No.	
			Casing	Non casing
30	5	31	2	0
31	11	33	1	2
32	4	36	1	2
	20**	42	2	2
		48	1	2
		53	3	2
			10*	2*

Discussion

Huge quantities of waste are freely available from the agro-forest and timber industries in Thailand (Kwon & Thatithatgoon 2004). Mushroom yields of 317 million metric tons (317 billion kg) of fresh mushrooms per year could be achieved only using 25 % of the yearly volume of burned cereal straws in the world (Chang & Miles 1989). In fact, we could simply grow 360 billion kg of fresh mushrooms on the total of 600 billion kg of dry waste, using the annual available world waste in agriculture (500 billion kg) and forestry (100 billion kg). A yearly mushroom yield of 60 kg per head per year could be achieved, all containing the 4 % protein of fresh mushrooms. Recent analysis has shown that 200 g of mushrooms can efficiently replace 100 g of meat as a protein source which could solve 30 % protein deficiency in their diet of the world population (Souci *et al.* 1975–1989). The fast mycelial growth and multilateral enzyme system of *Pleurotus ostreatus* (oyster mushroom) which is very special among mushrooms could be used to biodegrade nearly all types of different available wastes (Kwon & Thatithatgoon 2004). Usually, sawdust is used as a substrate for mushroom cultivation (Stamets 2000). The sawdust used in composting does not have sufficient nitrogen and other components required for the fermentation process therefore, the compounding mixture is supplemented with nitrogen and carbohydrate sources, in our case rice bran and meal concentrate, to enhance this process (Pathak *et al.* 1998).

The comparative mycelia growth rate of *P. giganteus* on culture media of different substrates, pH and temperature showed varying responses. The mycelia growth on saw dust substrate was best at 25 °C (Tab. 3) with a pH of 5–6.5 (Tab. 2) and the superior raw material for mycelia growth in culture at

Fig. 3. Fruiting bodies of *Pleurotus giganteus*. **A, B** Developing young fruiting bodies. **C, D** fully grown fruiting bodies. Bar **A** = 20 cm.


A
B
C
D

30 °C was soybean which could easily be available in local markets (Tab. 1, Fig. 1). On the other hand the best material for spawn run was also soybean whereas black bean and red bean are also good according to the results obtained (Fig. 2). In Thailand, mushroom growers normally use *Sorghum* seeds as the spawn carrier (Kwon 2004). *Sorghum* was the least suitable substrate according to our results (Fig. 2), when compared to soy bean, black bean, red bean and mung bean. The reason why mushroom growers use *Sorghum*, could be its readily availability and low cost.

There was a significant difference of biological yield between the control group and the experimental group. Biological yield of *P. giganteus* grown on control bags was poor. According to Moorthy (1993), 25–28 °C were found to be the optimum for *P. sajor-caju in vitro* studies. Cartwright & Findlay (1934) had observed that most of the fungi prefer a temperature range of 25–30 °C for mushroom production.

The total biological yield of the experimental group was 10 fruiting bodies/10 growing bags after 53 days (Tab. 4). The control group showed a very poor yield of only two fruiting bodies/10 bags even after 53 days (Tab. 4). Saw dust bags are mainly used for *Pleurotus* growing especially with *P. ostreatus*, whereas for *P. giganteus* growing it is essential to use soil casing in order to obtain a better yield.

Pleurotus giganteus has been shown to have medicinal properties (Huang 2005), and the protein content of *P. giganteus* in dry weight basis reported as 37.8 %, which was the highest compared to most other cultivated popular mushrooms (Udugama & Wickramaratna 1991). Our effort is to introduce *P. giganteus* to Thai markets as a new member, like Yanagi matsutake in Thailand (*Agrocybe cylindracea*) growing on saw dust substrate, which has a high demand and brings a handsome income to mushroom farmers (Kwon & Thatithatgoon 2004).

The low yield and long time for *Pleurotus giganteus* to produce fruiting bodies would possibly make it an expensive mushroom. It is now important to develop better protocols for growing it at high yields which are produced quickly. It is also important to isolate other strains and find better strains for mushroom production. It may also be possible to breed hybrid strains using the methods described in Callac (1995).

The study showed that it is possible to domesticate local strains of *P. giganteus* that can grow at a temperature consistent with Thailand farm productions. In China it was successfully domesticated in the 1980's and strains are now extensively grown there (Chen & Hu 2002; Wang-Qiu *et al.* 2006).

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