Science and Cultivation of Edible Fungi, Van Griensven (ed.)
© 2000 Balkema, Rotterdam, ISBN 90 5809 143 0

Cultivation techniques for *Dictyophom, Polyporus umbellata,* and *Coprinus comatus*

M.-M.Chen
Forest Products Laboratory, Richmond, & University of California andJepson Herbarium, Berkeley, Calif., USA

ABSTRACT: Results of research on the bamboo long shirt mushroom *(Dictyophora indusiata),* Zhu Ling *(Polyporus umbellata)* and drumstick mushroom *(Coprinus comatus)* are presented. The conditions for growing these three edible and medicinal species are best in China and California where the weather conditions are optimal for all three species. Detailed information on cultivation strains, spawn production and substrates, pH, temperature, humidity, and nutrition requirements for each species will be presented. Research has shown that *Dictyophora* cultivation requires special techniques: (1) inoculation of the tough mycelium with a "sharper applicable tool," and (2) careful management of the fruiting stage during which the basidiocarp (egg) breaks and cast skirts. Zhu Ling cultivation requires selected high-quality sclerotia and cultivated rhizomorph logs. New data show that *Coprinus comatus mycelium* grown on corn media, rapidly produces spawn. Liquefaction of fruiting bodies at outdoor production can be prevented by effective growing measures.

1 *DICTYOPHORA* AND ITS CULTIVATION

Bamboo skirt mushrooms, *Dictyophora,* are basidiomycetes belonging to the family Phallaceae. There are many nicknames for these mushrooms in China, such as "ghost holding an umbrella," "mushroom Ginseng," "the veil mushroom," "the king of mushrooms," "the queen of mushrooms," "the flower mushroom," and "the king of medicinal mushrooms." In China, it has been used as a tonic to treat high blood pressure, tumors, and diseases of the kidneys, eyes, and lungs (Liu 1978, Ying et al.1987). An interesting characteristic of the bamboo mushroom is that it is a natural preservative for other foods. It also contains many proteins, about 15-18% of dry weight; different kinds of amino acids, about 16 types in all, including glutamic acid (Huang 1993) and high concentrations of riboflavin (vitamin B2) (Huang 1993). Therefore, its nutritional value is very high. Bamboo skirt mushrooms are highly valued in Chinese cuisine for their flavor and fragrance, and they are often used for banquets in high-class restaurants.

In nature, *Dictyophora* species are found in the Sub-Alpine zone at elevations of 1000-2000 meters in *Pleioblastus* and *Sinocalamus* bamboo forests in Yunnan, Sichuan, and Guizhou. Cultivation began in 1968. Currently, *Dictyophora* species are grown in Fujian, Guizhou, Sichuan, Shandong and Liaoning (Liu 1978, Huang 1993, Fang & Yu 1996, Yao & Huang 1993,Luetal. 1992).

1.1 *Dictyophora Desv.*

According to the Dictionary of Fungi and Names (Yu et al. 1986, Teng 1996), Dictyophora comprises nine species, four of which are known to be edible: *D. indusiata* (Vent.) Fischer, *D. duplicata* (Bose) Fischer, *D. echinovolvata* Zang and *D. merulina* Berk. Of these, *D. indusiata* and *D. echinovolvata* are frequently cultivated (The Edible Mushrooms Institute, The Academy

of Shanghai Agriculture. 1991, Huang 1993, Chen 1987, Fang & Yu 1996), although *only D. indusiata* is available in quantity.

1.2 Dictyophora indusiata *(Vent.) Fischer. Morphological description.*

Receptacle 12-20 cm. high, sheathed at the base by a whitish volva 3-5 cm. in diameter. Pileus campanulate, 3-5 cm. long and broad, strongly reticulate, covered with olive-citrine slimy gleba of slightly unpleasant odor, apex truncate and perforate. Veil white, extended below the cap for more than 10 cm., made up of slender tubular threads, with polygonal meshes 5-10 mm in diameter. Stem white, hollow, with cellular-spongy walls, tapering gradually upward, 2-3 cm thick near the base. Spores 3-3.5 X 1.5-2 mm. Distributed in Yunnan, Hainan, Jiangxi, Guangdong. (Teng 1996).

1.3 *Long Skirt mushroom cultivation* (Dictyophora indusiataj

Spawn culture production goes through three steps: 1) isolation of the mother culture, 2) original culture expansion, and 3) spawn grain propagation. Keeping the culture sterile is very important; everything should be kept clean, including the laboratory, clothes, tools, and especially the cultivator.

1.3.1 *Mother culture*

Use the spore isolation with general guidelines for spores collection; place the receptive body (basidiocarp bud) hand-up in the spore collection glass container; incubate at 22°C until the sheathed volvo develops and breaks, the pileus becomes campanulate, long and broad, and the central stem becomes covered with slimy gleba. Put a drop of glutinous slimy gleba into sterile water, make a spore suspension, then inoculate in the special PDA medium*. When the spores germinate into mycelium, they are ready for the mother culture.

Since the entire long skirt bamboo mushroom is composed of compressed mycelia, the basidiocarp buds represent the best locations for tissue isolation. Select a few clean living basidiocarp bud specimens, split the bud into two parts to expose interior tissue by cutting tough mycelium with "Lu's sharper applicable tool" (Lu et al. 1992); excising a piece of tissue for transfer into the special PDA dish as mother culture.

*Special PDA media menu: bamboo root pieces lOOg, fern roots lOOg, pine needles 5-10g, potato flourlOOg, dextrose 20g, and agar 18-20g, and water, 1,000ml (Huang 1993).

1.3.2 *Original culture and spawn cultivation*

Inoculation of sterilized grain from original PDA dish culture and inoculation of grain from grain master jars. Use the same cultivation method and a grain ingredient: 75% bamboo substrates, some times use reed instead of bamboo (Chen 1997, Liu et al. 1997), 18% bran, 3 % soy bean powder, 1-% limestone, 1% sugar. The mixture should have a 65% water content, and a pH of 6.5 (Mei et al. 1997). Mix the compost then add the supplement; the compost at filling should release some moisture when firmly squeezed. Fill the mason jars with spawn grain with the lids making an imperfect seal to allow some air exchange during commercial spawn makers' autoclave sterilization. 2-3 original special PDA dishes for per 500 mi-grain master mason jar inoculation. After that, place jars at 18°C during the first week, at 20°C for the second week, and at 23°C for the third and fourth weeks. After this, incubate at 15-29°C for 60 days cultivation (Fang & Yu 1996).

1.3.3 *Preparation ofspawn*

Recently research has shown (Mei et al. 1997) that a high-quality spawn culture can be produced using the following method: A substrate of 68% mixed hardwood sawdust, 5 % bamboo leaves (or reeds, *Phragmites communis)* and 5% needles (add 2 ml boiling water), 18% bran, 3% soybean litters, 2% sugar, and 1% limestone is autoclaved in plastic bags (240 x!20 x 0.4 mm) and inoculated with the contents of one spawn culture mason jar per bag. The bags are incubated at 15-29°C for 80 days.

1.3.4 *Outdoor "Mushroom Qi Bed"production*

This is a popular field cultivation method used by Chinese Farmers. A "Mushroom Qi Bed" consists of well-drained, rectangular areas (100 x 400 x 30 cm) on which the mushrooms are grown. These plots are separated by ridges for growing mushrooms, 0.5% limestone water is used on a substrate of bamboo litter or reeds or wood chips that has been sterilized for 48 hours. The substrate should be well drained but retain 65% moisture content. For each square meter use 25 kg substrate; pile it 80-100 cm. wide with 10 cm.-high ridges on the Qi bed. Spread one layer of spawn culture, then cover with 5 cm. of substrate. The ratio of substrate to spawn is 15:1. Then spread another layer of spawn. Repeat the process until there are three layers of spawn. The final, top layer should consist of a 5 cm.-thick layer of composted bamboo (or reed) litter. Maintain consistent humidity and aeration with plastic covers in the outdoor bed.

1.3.5 *The management techniques*

To achieve good fruiting with this method, the fruiting should occur 40-60 days after spawning. The temperature for mycelial growth is 28-33°C. An additional fifteen days are needed at 20-24°C for the mycelium to reach physical maturation. Then the surface of the substrate is full of mycelium, in mass primordium formation. At this time the relative humidity should be 85%-90%, and the water content of the substrate should be 60%-65%. During the period of about 30 days between breaking -basidiocarp (egg) to skirts casting, air circulation is required and a temperature of 18-25°C. (no higher than 25°C). Basidiocarp buds (the eggs) gradually develop into an ovaliform basidiocarp. As soon as the top membrane becomes thin, the mushrooms will all break the basidiocarps for the next 1-2 days. Then, in several hours the fruiting bodies mature and the long shirts spread, to be harvested immediately (Mei et al. 1997).

After harvesting, only the stem (not the skirt or the receptacle) is suitable for eating.

2 CULTIVATION OF POLYPORUS UMBELLATA

Zhu Ling, *Polyporus umbellata* (Pers.) Fr., is a basidiomycete fungus belonging to the Polyporaceae family. The sclerotium of Zhu ling is medicinally unique. In traditional Chinese medicine, it has been used mainly as a diuretic, but recently it has been shown to be beneficial in treating lung cancer and leukemia. (Liu 1978, Ying 1987)

Zhu ling grows on honey fungi *(Armelliaria mellea)* rhizomorph for nutrients, leading to a unique cultivation process (Huang 1993, Yao & Huang 1993).

2.1 *Selected high-quality sclerotia*

Zhu ling cultivation uses sclerotium as spawn, the exposed fruiting body borne out of underground sclerotia. Sclerotium is irregularly shaped while the fruiting bodies are rounded, clarinet-shaped, the surface having a swollen appearance and bearing small scales and fine strands. The exterior is colored black to brown with internal color being near white to light yellow. After drying, the tissue is firm, woody, and cork-like when squeezed. The tissue side of fruiting body is white but after exposure to air, it becomes light brown. Zhu ling sclerotia have three varieties of shapes which Chinese farmers describe, graphically, as pig scats, chicken scat and horse scat. It is better to select the pig and horse scat appearing sclerotia for sclerotia spawn. Use young sclerotia, which are 1 to 2 years old. The color should be yellow-green and the consistency soft when squeezed. (Tian & Jian 1998)

2.2 *Honey fungi logs cultivation*

The spawn plug method is used for the cultivation of *Armellaria mellea* rhizomorph in logs, in the similar way, as traditional Shiitake are cultivated on logs. Alder logs are appropriate for rhizomorph substrate material. 1 x 2 cm sized chip sections are chopped for chip cultures, and then inoculated and incubated at 25°C for one month. When the *Armelliaria mellea* mycelium is fully-grown on hardwood chips, it is inoculated into each "scale of fish" spot of log. Each log is about 50 cm long and 8 - 10cm.in diameters with water content of 70 % and receives 10 spots

inoculation. The logs are laid in a sand-lined pit 50 cm. deep, 70 cm. high and 70 cm. wide, with sufficient moisture and minimal air exchange. After 2 months of incubation at normal room temperature, the *Armelliaria mellea* rhizomorph will have fully colonized the exterior of the log providing excellent nutrients for Zhu Ling production. (Liu 1978, Tain & Jian 1998)

2.3 *Zhu Ling cultivation*

First a humus rich cultivation bed of 50 cm wide and 50 cm deep is prepared (in China this bed is referred to as a "den"). A 5 cm layer of tree leaves lines the bottom of the den and on this is placed a layer of fully colonized Honey Fungus logs which have been prepared by the above method. Above and in contact with this layer is another layer of new un-inoculated hardwood logs and the spaces in between are filled up with fresh tree leaves and chips and conditions are kept moist. The important point is that the sclerotia must reach to Honey Fungus logs in exposed positions during inoculation and cultivation process. Because the strong *Armelliaria mellea* mycelia rhizomorph sustained providing nutrients for new sclerotia growth on logs under the wood chips under the humus soil, keep conditions moist. After four or five more log layers are added, a 10 cm mixed layer of wood chips and humus soil are placed on top of the den. For cultivation of sclerotia from germination to growth until production, the new sclerotia require a process of 2 - 3 years. (Tain & Jian 1998, Yao & Huang 1993)

3 CULTIVATION OF THE SHAGGY MANE MUSHROOM FOR FOOD AND MEDICINE

Coprinus is a genus of the basiodiomycete fungi belonging to the family Coprinaceae.

3.1 *Nutritional value*

A variety of *Coprinus comatus* (Muell. Ex Fr.) Gray var. *ovatus*, also known as the "White Chicken leg mushroom," is an edible wild mushroom which is common in many parts of China, appearing often after rain from late spring through autumn. It has long been prized as a food with fine color, texture and flavor and as a valuable medicinal resource. Traditionally, the white chicken leg mushroom has been used by Chinese physicians to calm the mind and to treat the spleen, stomach and digestive system. Modern chemical analysis (Ying et al. 1987) revealed that white Chicken leg mushroom contains 20 kinds of amino acids (among them 8 kinds of human being). The mushroom cap contains aspartyl, asparagine and glutamine. The stem is a source of glutamine, glyceric acid, threonine, B-aminobutyric acid, isoleucine, and lysine. The Chinese Medicinal Fungi Illustrated Monograph reports that compounds extracted from white Chicken leg mushroom were used to treat mice with malignant tumors and appeared to have a significant effect in restraining the development of the cancers. Results also suggests that white Chicken leg mushroom contains compound which can lower blood sugar in mice and may have a potential for the treatment of diabetes. Many generations of Chinese farmers have cultivated the white Chicken leg mushroom and in recent decades growers in the USA, the Netherlands, France, Germany, Italy, and Japan have been successful in the commercial production of *Coprinus* from which a variety of food and medicinal products are made. (Liu 1978, Ying et al. 1987)

3.2 Coprinus comatus *var.* ovatus *cultivation process*

The ground-arch roof mushroom room is recommended as the best environment for cultivation of winter crops. A suitable temperature should be kept during harvesting winter fruiting-bodies and should be maintained inside the growing chamber while cold outdoor temperature provides the best conditions for preserving fresh mushrooms while they are being transported to market.

3.2.1 *Mother culture*

Research shows (Lin et al.1998) that the best mother culture media is multiple PDA with wheat. It is composed of potato flour 200 g, glucose 20 g 1% KH_2PO_4, 0.5% K_2HPO_4, 0.5%$MgSO_4$,

peptone 3 g and agar 20 g. The standard method requires 25- 27 °C for seven to nine days. Currently, the Shan Dong Chicken leg 9653, Gui Zhou, CCIOO, Jiang Shu 963, Hunan Ccl68 are the most productive strains (Luo & Qian 1999).

3.2.2 *Original and spawn culture cultivation*

Use PDA to expand the mother culture on the petri dish. The spawn grain ingredients consist of cottonseed casings (75%), sugar (1%), limestone (2%), and water to about 65% approximately. The pH level should be neutral after inoculation onto the spawn media into 500-ml mason jars. Place the sterilized grain-filled jars in the sterile room until ready for inoculation ((Luo & Qian 1999, Luo 1997). Also research suggests (Luo 1997) that when using a large size grain such as corn as inoculation grain, a hole should be made at the center of the jar's cover with a woody pencil, because this gives a much easier inoculation process, when filling the jar from top to bottom.

3.2.3 *Cultivation Techniques*

Use the standard method to inoculate. Incubation requires 23 to 25°C for 20 to 30 days. Then mycelium will colonize the entire medium. Plastic Bag of 18 x 35cm are used. Cottonseed meal (100 kg) is considered the best filling substrate with a supplement of Urea (0.5 kg), phosphorus fertilizer (2.0 kg), limestone (1-2 kg), and water (150 kg). Use a bag filling machine. Seal bags by tying up both ends. Standard sterilization: autoclave for 10-12 hours. Inoculate both sides. Each jar of spawn grain will be enough for forty bags. Incubate twenty-five to thirty days in the temperature range of 24-26°C for spawn run. (Zhu 1998, Mei 1997). Once white mycelium has thoroughly filled the bags, they are ready for ground cultivation.

Inside a ground arch mushroom cover; prepare a den of 30 cm. deep in sandy rich soil. After removing the plastic bags, distribute the mycelium "logs" 30 per square meter leaving a 2-cm. space between each one. Completely fill and cover the den with the clean sandy soil. This species grows well under soil. Maintain temperature within 16-22°C and 85-95% humidity (Liu et al. 1999). Chicken leg mushroom fruiting body requires both moisture and oxygen. Ground level windows built into the ground-arch-mushroom-cover aid ventilation. Mushroom fruiting buds will then emerge from the ground soil. Fruiting bodies quickly form within 7-10 days (Liu et al. 1999), depending on moisture and air circulation. Mushrooms are ready for harvest at 70-80 % maturation. If they are left to mature further, "ink" will start leaking from the fruit body and the mushroom will decompose rapidly. This process of cultivation is a prevent- liquefaction technique.

REFERENCES

Chen Hui. 1997. The Utilization of Reeds *(Phragmites communis)* for *Dictyophora indusiata* cultivation. *Edible Fungi 5:* 26.

Chen Shi Yu. 1987. *Encyclopedia of Edible Mushrooms Cultivation.* Beijing: Chinese Agricultural Publisher House, pp. 450-459.

Fang Shui Ci & Yu Pei Shen. 1996. *The Dictyophora High Yield Integrate Cultivation with Orchard Field. Edible Fungi:* 29.

Huang Jian Ping. 1993. *Mushroom Cultivation.* Hunan: Hunan Sciences and Technology Publisher House, pp. 206-221.

Lin xuan, Zhou Xuan Wei & Tao Yu Feng. 1998. The Preliminary Report on Selection of stock Culture Medium *of Coprinus comatus. Edible Fungi of China 2:* 22-24

Liu *Bo.\918.Medicinal Fungi of China.* Tai Yuan, Shan Xi the People's Publish House pp. 252, pp. 111-114,pp.l77-182.

Liu Rui Bi, Wei Chang Yong & Xie Luo Guarig. 1997. The Model of integrates Cultivation of Bamboo mushroom and Soybean Crops. *Edible Fungi* 1: 33.

Liu Wen Bi, Hu Zhen Mao & Wang Chun Hui. 1999. The Study on *commercial Cultivation Techniques of Coprinus comatus. Edible Fungi of China* 2: 32-33

Lu Zuo Zhou, Chen Li Guo, & Xie Bao Gui. 1992. *The 200 questions of Edible Mushroom on Cultivation.* Beijing. Chinese Agricultural Publisher House.

Luo Tai Xun. 1997. The CCIOO *Coprinus comatus* Characters and The Techniques of Cultivation spawn rapidly. *Edible Fungi* 4: 16.

Luo Tai Xun & Qian Zuo Mei. 1999. The Key Techniques of the CC100 *Coprinus coma!us* Cultivation. *Edible Fungi* 4: 14-15.

Mei Li Ping, Wu Min Fang & Wu Chao Ming. 1997. *The Outdoor cultivation Techniques ofDictyophora Edible Fungi* 5: *21*'-28.

Teng, S.C. 1996. *Fungi ofChina.* Ithaca, New York: Mycotaxon LTD. p. 489.

The Edible Mushrooms Institute, The Academy of Shanghai Agriculture. 1991 *the Flora of Edible Mushrooms ofChina.* Beijing: Chinese Forestry Publisher House, pp. 271-274.

Tian Mao Lin & Jiang Jin Chi. 1998. The Techniques Cultivation of *Polyporus umbellata.* Edible Fungi of China 1:22.

Yao Zhong Fan & Huang Ying. 1993. *The Cultivation Technique ofCommon Chinese Medicinal Herbs.* 1989.Beijing: Jing Duan Publisher House, pp.545-550.

Ying Jian Zhe et al.1987. *Icons ofmedicinalfungifrom China.* Beijing. Science Press, pp. 572-575

Yu Yong Nian & Zhen Ru Yong et al. 1986. *The Dictionary ofFungi and names.* Sciences Press. Beijing. p. 210.

Zhu Jian Biao. 1998. The Cultivation Techniques *of Coprinus comatus* on Shanghai Nan-Hui County. *199% Edible Fungi 3: 32.*