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TECHNOLOGY OF MUSHROOM CULTIVATION

Quyen Ho Bao Thuy[1, *], Akira Suzuki[2, 3]

[1]*Faculty of Biotechnology, Ho Chi Minh City Open University, 700000, Viet Nam*
[2]*Department of Natural Sciences, Faculty of Knowledge Engineering, Tokyo City University, 158-8577, Japan*
[3]*Laboratory of Mycology, Agricultural Hi-Tech Park of Ho Chi Minh City, 700000, Viet Nam*

[*]Email: *quyen.hbt@ou.edu.vn*

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Abstract. Mushroom cultivation has been developed by two ways, *i. e*., one based on cultivation by wood-log and another based on cultivation by non-wood-log. Non-wood-log cultivation would be categorized into two aspects. Mushroom cultivation under non-aseptic condition, *i. e*., one characterized by the processes of fermentation (composting) with/without casing, and another characterized by using sterilized media followed by cultivation process associated with strict control of environmental factors such as light, temperature, humidity, and CO_2 concentration. The former has been developed in the cultivation of weed community fungi such as *Agaricus bisporus* and *Volvariella volvacea*, etc. The latter has been introduced to cultivation of wood rotting fungi such as *Flammulina velutipes*, *Pholiota microspora*, *Hypsizygus marmreus*, and *Grifola frondosa*, etc. and various kinds of modern technology for the induction of fruit body primordium formation such as Kinkai (scratching) and flooding have been developed. Casing is often introduced to the mushroom cultivation by herbaceous materials. There are two trends for spawning, namely one using solid spawn and another using liquid spawn. For mushroom production, fruit body induction and its synchronization are important for achievement of higher biological efficiency in the production. Currently, all cultivation processes of indoor mushroom cultivation without fermentation process are manipulated automatically by machines although automated cropping has not yet spread. Recently, irradiation by LED light during fruiting (budding and growth) is gradually introduced instead of irradiation by fluorescent lamps to save energy for the mushroom production under strict environmental control.

Keywords: automated cultivation, casing, energy saving, environmental control, Kinkaki.

Classification numbers: 1.3.1, 1.3.2.

1. INTRODUCTION

Mushroom cultivation has been developed by two ways, *i. e*., one by wood-log cultivation and another by non-wood-log cultivation. The progress in mushroom cultivation technology mainly has been done by mushroom companies after the development of the technology of

mushroom production by farmers based on their experiences. The improvement of the technology for mushroom production of the existing cultivated mushroom species and exploration of the cultivation technology for new mushroom species have been done mainly by researchers and experts of mushroom growers. The scientific analyses for each process of mushroom production have been mostly conducted by the cultures grown on agar media and those grown in liquid media. Many physiological, biochemical, and genetic experiments for vegetative growth and fruit body formation and development of mushrooms have been progressed by several model mushroom species such as a coprophilous fungus *Coprinopsis cinerea* (syn.: *Coprinus cinereus*), wood rotting fungi *Schizophyllum commune* and *Polyporus arcularius* (syn.: *Favolus arcularius*), and a facultative ectomycorrhizal fungus *Hebeloma vinosophyllum*, etc. as well as by easy fruiting isolates of the cultivated mushroom species such as wood rotting fungi Oyster mushroom (*Pleurotus ostreatus*) and Winter Mushroom [Enokitake; *Flammulina velutipes* (syn.: *Collybia velutipes*)] [1, 2]. They are easy to fruit on nutrient agar media and in nutrient liquid media which are more suitable for physiological, biochemical, and genetic researches in mushroom morphogenesis.

Cultivation technology of several edible mushrooms is basic and of standard technology for most mushrooms, including medicinal mushrooms. Namely, the cultivation procedures of *Ganoderma* spp. are similar as those of edible mushrooms [3]. Other famous medicinal mushrooms including entomopathogenic fungi are also cultivated by very classical and simple technology based on the cultivation technology of edible mushrooms, except for the modification of substrates constituents [4, 5]. In the following, we would like to introduce each process of mushroom cultivation and their physiological background, based on edible mushroom models, in order to find new idea to improve the mushroom production.

2. VARIOUS KINDS OF MUSHROOM CULTIVATION

2.1. Mushroom cultivation by wood-log (log, log wood)

Wood-log cultivation (log cultivation, log culture) has been developed in Japan and China, especially for the cultivation of Shiitake [*Lentinula edodes* (syn.: *Lentinus edodes*)] [6]. The wood-log cultivation was introduced to the production of *Pl. ostreatus*, Nameko [*Pholiota microspora* (syn.: *Pholiota nameko*)], Wood ear(s) (Ear Fungus; *Auricularia polytricha*), and Rinzi (Reishi; *Ganoderma lucidum*), etc. Short wood-logs have been also used for the cultivations of the latter four species and other mushroom species, but not for the cultivation of *L. edodes*. Sometimes, the short wood-log is packed in a P.P. (polypropylene) cultivation bag and sterilized before inoculation [cf. Fig. 8B]. The short bed logs are embedded in the soils or stood on the soil for fruiting.

2.1.1. Outdoor wood-log cultivation (Open field wood-log cultivation)

Outdoor wood-log cultivation, namely mushroom non-aseptic cultivation composed of seven or more processes: 1. Felling and cross cutting of tree, 2. Spawning, 3. Laying, 4. Raising, 5. Cropping, 6. Drying, and 7. Packing.

1. Felling and cross cutting of trees: In four seasoned countries, trees for mushroom cultivation should be felled on the forest floor in autumn right after the fall of leaves. The broadleaved trees such as *Quercus* spp., *Castanopsis* spp., *Carpinus* spp., and *Platycarya* spp. etc. are suitable for wood-logs for mushroom cultivation [7]. It is advantageous for mushroom

cultivation when wood-log was felled at the season of increasing sugars in a trunk and twigs for the next year budding. The trunks stored on the forest floor after the trees are fallen until just before inoculation. They are cut into 1-1.5 m long just before inoculation of spawn [8]. The logs in diameter 5 - 15 cm are the most suitable for the operation of mushroom cultivation, but sometimes thick logs are used for the cultivation [9].

2. Spawning (Inoculation): Drilling inoculation (Fig. 1) is standard for the spawning for the cultivation of *Le. edodes* whereas log-end sandwich inoculation is often used for other mushrooms such as *Pl. ostreatus* and *Ph. microspora*. Cutting logs (15 - 40 cm long) are used for the log-end sandwich inoculation. Plug spawns [wedge shaped spawn, round bar type spawn, and round wedge shaped spawn (Fig. 2)] and sawdust spawn have been used for drilling inoculation. The latter is modified to molding spawn (mold spawn: bullet shape spawn made of sawdust with mycelium) for easy-to-operate for inoculation.

Figure 1. Drilling of wood-log for the inoculation of plug spawns. (Photo by Mr. Nakazawa).

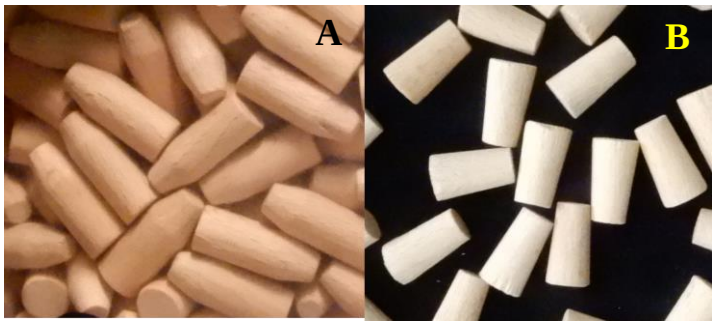

Figure 2. Different types of plug spawns. A: Round bar type spawn; B: Round wedge shaped spawn.

3. Laying (Log stacking): The bed logs are placed together siding or standing in laying yard. The piles of the cluster of bed logs are covered with plastic nets or straw mats to avoid drying during spawn run. The mycelium growth in the bed log is accelerated by a rise in temperature and CO_2 concentration caused by own respiration since the vegetative growth of lignicolous species, in general, is markedly stimulated at around 10 % CO_2 and tolerated up to around 20 - 30 % CO_2 [10].

4. Raising: After the vegetative mycelium colonized whole area of the bed log, the logs are transferred to another place which is called raising yard [cf. Fig. 4].

5. Cropping (Picking, Harvest): The logs are kept on the forest floor. The moisture content of the bed logs is controlled for keeping suitable concentration for fruiting as well as that for vegetative growth by covering them by various kinds of sheets or by watering. Fruit bodies are harvested one by one by hand labor when they reach to suitable developmental stage [fruit bodies reach the stages of basidiospore formation, *i. e.*, equivalent to 60 % - 80 % full development of pileus (cap)].

6. Drying: Post-harvest fruit bodies are dried under sun irradiation (sun drying) or artificial heating, for enhancing flavor and taste for foodstuffs. Nowadays, most fruit bodies are dried by artificial heating to obtain standard quality level of dried food. The changing in drying procedures for *Le. edodes* from sun drying to artificial heating caused the decrease of Vitamin D

content in the dried fruit bodies. It introduced the idea of increasing Vitamin D content in the dried fruit bodies of *Le. edodes* by UV irradiation during the drying process [11].

Light is essential for the fruiting of many mushrooms [1], including *Le. edodes* [12]. The light intensity transmitted to the mycelium grown under the bark would be sufficient for the fruit body initiation of *Le. edodes*. The shape of fruit body is affected by environmental factors, such as light, temperature, humidity, and CO_2 concentration during fruit body development [13, 14]. For example, fruit bodies of *Le. edodes* become shorter stipe and larger pileus at lower temperature [15], namely, harvest in winter is suitable for the production of high quality of dried fruit bodies called Donko (dried Shiitake with incurved pileus and thick flesh).

Figure 3. Laying of wood-log cultivation of *Lentinula edodes* in a greenhouse (semi-indoor type). (Photo by Mr. Nakazawa).

Figure 4. Cross piling of wood-log cultivation of *Lentinula edodes* in a greenhouse (semi-indoor type). (Photo by Mr. Nakazawa).

7. Packing (Packaging, Wrapping): Most post-harvested fresh or dried fruit bodies are sold loose, but some dried post-harvest fruit bodies are packed in a plastic box, especially high quality dried fruit bodies are packed in a gorgeous paper box or a wooden box for a gift.

2.1.2. Indoor wood-log cultivation

Nowadays many wood-log cultivations are conducted indoors such as in greenhouses made of plastic films and mushroom houses (Figs. 3, 4) since it is an advantage for year-round cultivation. The indoor cultivation processes for bed logs of *Le. edodes* are always accompanied by soaking (Dipping, Flooding, Submerging) for 12 - 24 hours followed by draining for 2-5 days for synchronizing fruiting. This procedure is sometimes conducted for outdoor wood-log cultivation for controlling the cropping schedule. Induction of primordium formation by the flooding is confirmed by sawdust cultivation of *Le. edodes* under light irradiation [16], but not by wood-log cultivation. The stimulatory wavelengths of light are dependent on the concentration of calcium in the medium. Namely, red light (620 - 680 nm) stimulates whereas blue light (400 - 500 nm) inhibits fruiting when *Le. edodes* is cultured on the medium containing calcium less than 40 ppm. In contrast, the blue light stimulates the fruiting when it is cultured on the medium containing calcium higher than 130 ppm. Light exposure elicits pigmentation of primordia and the pigmentation is essential for the pileus expansion. Even a relatively short light exposure long before fruiting is effective for fruiting (a kind of memory effect) [17].

Various mechanized equipment such as a chain saw, an electric drill, and a fork lift, etc. have been used for reduction of labor through wood-log cultivation. Wood-log cultivation is not, however, suitable for the introduction of automated manipulation for all cultivation processes

2.2. Mushroom cultivation by non-wood-log

Mushroom cultivation by non-wood-log would be categorized in two trends, one based on preparation of media by fermentation (composting) followed by non-aseptic cultivation and another based on that of media by sterilization associated with aseptic cultivation at least during vegetative growth (the process of spawn run). The former technology has been developed in the production of weed community fungi Button Mushroom (*Agaricus bisporus*) mostly cultivated in European and American countries and Straw Mushroom (Fukurotake; *Volvariella volvacea*)

Figure 5. Occurrence of a brown rot fungus *Sparassis crispa* on the forest floor near a red pine (*Pinus densiflora*) in Fujimi-cho, Nagano Prefecture, Japan.

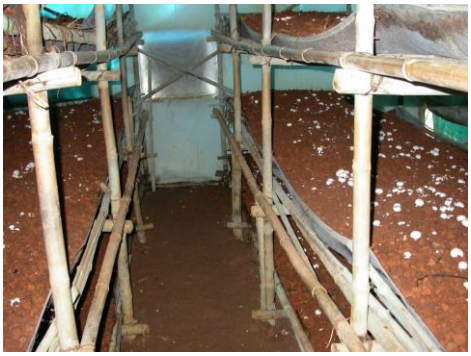

Figure 6. Flush (appearance of fruit bodies) of *Agaricus bisporus* on a casing soil. Shelf beds are composed of bamboo culms. The mushroom cultivation in Da Lat, Viet Nam.

cultivated mainly in East Asian countries. The latter has been developed in cultivation of wood rotting fungus *Pleurotus* spp. mostly in Asian and European countries [18] and many other wood rotting fungi such as *Fl. velutipes*, *Ph. microspora*, Beech Mushroom (Bunashimeji; *Hypsizygus marmoreus*), and Hen-of-the-Wood (Maitake; *Grifola frondosa*), etc. mostly in East Asian countries, especially in Japan [18]. Most cultivated wood rotting fungi including mushroom species described above are white rot fungi. In Japan, only one mushroom species of brown rot fungi *Sparassis crispa* (Fig. 5) is produced at commercial level. The mushroom cultivation under aseptic condition associated with environmental control has been mainly developed in Japan although later the technology was quickly followed by The Republic of Korea, Taiwan and China. Nowadays mushroom production under aseptic condition in China is the largest in the world. In Japan, even an ectomycorrhizal fungus Hon-Shimeji (*Lyophyllum shimeji*) succeeded in commercial scale of production on artificial media under aseptic condition [19].

2.2.1. Mushroom non-aseptic cultivation

The mushroom non-aseptic cultivation is composed of ten or more processes, namely, 1. Composting; 2. Filling; 3. Post-fermentation; 4. Cooling; 5. Spawning; 6. Casing; 7. Maintenance of the mushroom house [Sprinkling (Watering) and ventilation, etc.]; 8. Flush; 9. Cropping; 10. Packing.

Wheat straw or rye straw combined with manure, and gypsum, etc. is used for the production of *Agaricus* spp. [20 - 22] whereas paddy straw is used in combination with cotton-waste, or guinea grass, and sugar-cane bagasse, etc. for the production of *V. volvacea* [23]. Light irradiation is inhibitive for fruiting of *Ag. bisporus* [24]. In contrast, light irradiation is essential for fruiting of most mushrooms and the effective wavelength for fruiting is 300 nm to 520 nm [1, 14]. Casing is crucial for abundant fruit body primordium formation of *Agaricus* spp. [25] (Fig. 6), whereas not necessary for the fruit body initiation of *V. volvacea*. Automated cropping has been tried to introduce into the cultivation of *Ag. bisporus* [25, 26] although it is more difficult than that of bottle cultivation of mushrooms.

2.2.2. Mushroom aseptic cultivation

The mushroom aseptic cultivation is composed of twelve or more processes, namely, 1. Preparation of media; 2. Filling; 3. Sterilization; 4. Cooling; 5. Spawning; 6. Spawn run; 7. Budding; 8. Growth; 9. Cropping; 10. Drying; 11. Packing, and 12. Kakidashi (Exhausting of waste substrate).

1. Preparation of media: Various kinds of sawdust, bagasse, and corn comb, etc. are major components for media with supplements such as rice bran, wheat bran, and Okara (simmered soybean pulp). In general, sawdust of broadleaved trees is more suitable for the mushroom cultivation than that of coniferous trees. However, the sawdust of coniferous trees widely used for media for the cultivation of wood rotting fungi in spite of an unsuitable substrate in the field. The sawdust is generally deposited sometimes with turning to keep aerobic condition. The heap is left in the rain for a while to remove phenolic substances (mostly come from heartwood) which inhibit the vegetative growth. The components of each medium and water are blended with a mixer (mixing machine) to adjust the moisture content to around 65 – 70 % (water content; W/W at wet weight basis) which is suitable for the cultivation of most mushroom species.

Figure 7. Filling machine for P.P. bottle cultivation. (✶: P.P. cap, ○: empty P.P cultivation bottle, ●: medium filling).

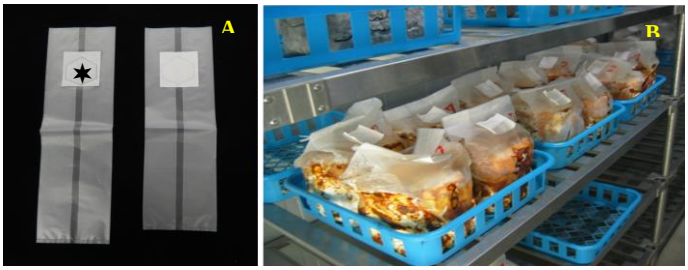

Figure 8. P.P. cultivation bags. A: Small P.P. cultivation bags with a membrane filter (✶) for *Lentinula edodes.* B: *Grifola frondosa* cultured on sterilized short wood-log of *Quercus serrata* in a P.P. cultivation bag with a membrane filter (✶). (B: Photo by Ibaraki Prefectural Forestry Research Institute, Japan).

2. Filling (Bottling): Filling of medium into a plastic bag or a plastic bottle is done by filling machine (filler, bottling machine; Fig. 7). A spawn hole (spawning hole) about 1 cm in diameter is bored at the center of the pressed medium [cf. Fig. 15]. The spawn hole is effective to avoid the cracking of the medium during autoclaving as well as to achieve high efficiency of aeration by “chimney effect” [27]. The idea was introduced by the aeration observed in compost placed in the container [28]. In Japan, the plastic bag with a small hole covered by membrane

filter (Fig. 8) is sometimes used for the commercial mushroom production in order to accelerate the aeration although it costs a lot.

3. Sterilization: The medium in a P.P. cultivation bottle or a P.P. cultivation bag is sterilized by autoclaving. Sometimes its sterilization is done by steam-heat without application of pressure.

4. Cooling: The medium should be cooled down gently after the sterilization since the sterilized medium is easy to be contaminated by the microbes in returning current.

5. Spawning (Inoculation): There are two types of spawning, i. e., one by solid spawn (Fig. 9A) and another by liquid spawn (Fig. 9B-D). The former technology has been advanced in Japan and the latter mainly developed in The Republic of Korea. The liquid spawn contributes to quick growth, but has higher risk for the contamination comparing with the solid spawn. Nowadays, the spawning is done by the inoculation machine (Fig. 10).

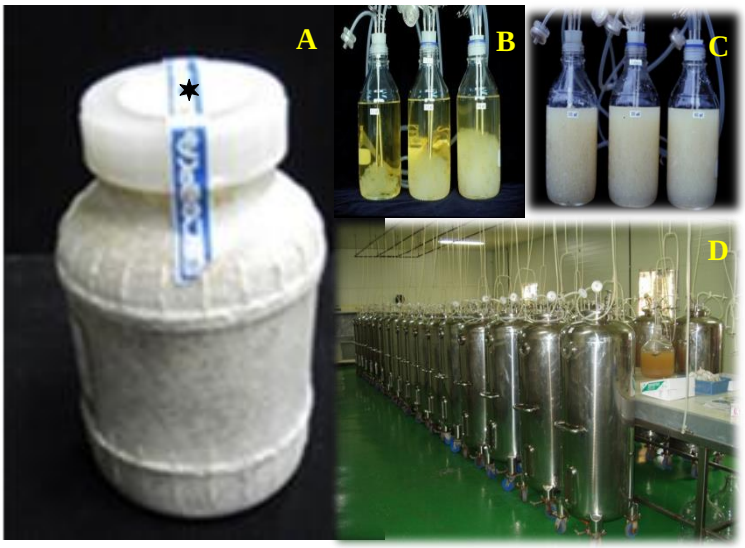

Figure 9. Spawn. A: Solid spawn with membrane filter (✶); B: Liquid spawns 3 days after inoculation; C: Liquid spawns 7 days after inoculation; D: Fermenters for the liquid spawn. (B-D: Photos by Prof. Geon-Sik Seo, Korea National College of Agriculture and Fisheries, The Republic of Korea).

Figure 10: Inoculation machine for solid spawn. (✶: solid spawn, ○: stainless slide, ●: sterilized medium in P.P. cultivation bottle) (Photo by Dr. K. Yamanaka).

6. Spawn run (Spawn running): Spawn run room is adjusted 2-3°C lower than the optimum for vegetative growth of each strain of mushroom species to avoid a rise in the culture temperature by own respiration. In other words, a rise in the temperature around each culture would be affected by density of cultures, wind direction, types of wind stream and wind velocity around cultures, and volume of culture. The spawn run is normally completed at 60-70% air relative humidity, in darkness. Oxygen and carbon dioxide concentrations of spawn room are adjusted to optimum range for each strain by manipulation of ventilation equipment(s). Spawn run usually continues even after the mycelium spreading over the whole medium. The suitable additional incubation is called mycelial maturation and its suitable period is specific to mushroom species. P.P. cultivation bags are used just once for mushroom production. In contrast, P.P. cultivation bottles can be used more than 100 times before discarding when they are used indoors, namely used under the condition without UV irradiation.

7. Budding (Pinning, Sprouting): Budding usually initiates after lowering of incubation temperature subsequent to spawn run because temperature ranges for fruiting is narrower than those for vegetative growth (Table 1) [1]. Sometimes, the upper limit of temperature for fruiting is lower than the optimum temperature for vegetative growth in each isolate (Table 1) [1]. Based on the character, temperature subsequent to spawn run is shifted to lower than 5°C or more. Most mushrooms require light irradiation at low intensity [1, 13, 14] and effective light spectrum for fruiting of mushrooms in Basidiomycota is NUV-Blue as described above [1, 14]. Thus, in indoor mushroom cultivation, plant growth florescent lamps with spectra including NUV-Blue range are used for the cultivation processes from Budding to Growth (Development) [Fig. 11]. Injury, flooding, and casing would be effective for fruit body primordium induction. Therefore, either of them is used for the budding associated with lowering the cultivation temperature [1, 17, 29]. For P.P. bag cultivation of most mushrooms, the upper part of the bag is opened for the achievement of a suitable condition for fruit-body primordium formation through the supply of O_2 and decreasing of CO_2 concentration (Fig. 12). In P.P. bag cultivation of *Le. edodes*, the bag is often stripped off absolutely after the whole surface of the culture comes to browning as a symptom of mycelial maturation for fruiting (Fig. 13). It is called artificial bed log since the mycelial block looks like short bed log.

Table 1. Temperature (°C) ranges for vegetative growth and fruit body initiation.

Mushroom species	Vegetative growth			Fruit body initiation			References
	minimum	optimum	maximum	minimum	optimum	maximum	
Agaricus bisporus	3	24 - 25	30				30
				8	15 - 18	20	31
Flammulina velutipes	3 - 4	22 - 26	33 - 34	5	10 - 15	20	32
Lentinula edodes	5	25	35				12
				10 - 15	20	25 - 30	Tokimoto*
				(cold adapted strain)			
				10 - 15	20 - 25	25 - 30	Tokimoto*
				(warm adapted strain)			
Pholiota microspora	8	24 - 26	32	5		15	33
				(cold adapted strain)			
				8		20	33
				(warm adapted strain)			
Volvariela volvacea	20	30 - 35	40	20<	28 - 32	<34	23

*Personal information from Dr. Keisuke Tokimoto.

Figure 11. A flush of *Hypsizygus marmoreus* under the illumination of growth lamps in a growth room. (Photo by Dr. K. Yamanaka).

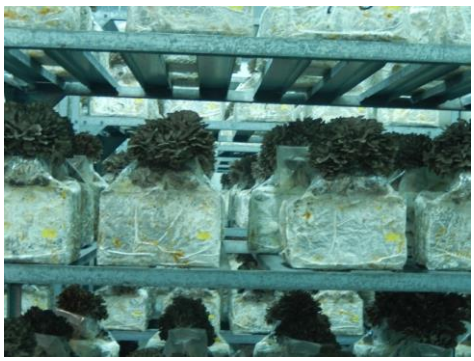

Figure 12. P.P. bag cultivation of *Grifola frondosa*. Fruit body development after opening top part of the bag.

Figure 13. P.P. bag cultivation of *Lentinula edodes*. Fruit bodies appeared after browning of mycelial block.

7.1-A. Injury: In P.P. bag cultivation of *Auricularia* spp., the bag is slit by a knife to induce fruit body primordium induction (Fig. 14). It may contribute to supply enough amount of O_2 for fruiting and avoid the inhibitive effect of fruiting by the concentrated CO_2 as well as the direct effect of injury itself described above.

Kinkaki (Scratching) [34] is a characteristic process not only for the induction of fruit body primordium formation but also for synchronizing fruiting on the upper surface area of the medium in the bottle. For mushroom production, synchronized fruit body formation is important for achievement of higher "biological efficiency". It is the treatment for the removal of whole or outer edge parts of the old hyphae covering the upper surface of the mycelium. In Japanese, the former type of Kinkaki is called "Hiragaki" (Flat scratching; Plain scratching) and the latter type of Kinkaki is called "Manju-Kinkaki" (Convex scratching) [35]. Either type of Kinkaki has been selected for each mushroom species (Table 2, Figs. 15, 16). Kinkaki should be performed right after the respiration rate of each mushroom reaches the minimum [36]. Kinkaki is the procedure invented for bottle culture by Japanese mushroom growers and developed its technology by Japanese mushroom companies. The mechanism of Kinkaki for the fruiting is not completely understood, but it may be direct induction of fruit body initiation which was reported in monokaryotic isolate of *Schizophyllum commune* by injury itself [37] and/or rejuvenation of the mycelium by the injury of the old hyphae.

Table 2. Types of Kinkaki (scratching).

Mushroom species	Convex type	Plain type
Flammulina velutipes		○
Hypsizygus marmoreus	○	
Pholiota microspora		○
Pleurotus eryngii		○
Pleurotus ostreatus		○

Figure 14. Appearance of fruit bodies in *Auricularia polytricha* on a slit of P.P. cultivation bag after an incision by a knife on the bag. The incision was conducted subsequent to spawn run by sugar cane bagasse culture.

Figure 15. Right after Hiragaki in P.P. bottle cultivation of *Flammulina velutipes*. (✶: spawn hole).

Figure 16. Right after Manju-Kinkaki in P.P. bottle cultivation of *Hypsizygus marmoreus*.

7.1-B. Flooding (Dipping, Soaking, Submerging, Watering): Kinkaki is always accompanied by flooding for the induction of fruit body primordium formation. Flooding is a treatment by an addition of water at the bottle top (the part between the top of the bottle mouth

and the upper surface of the culture in the bottle) for an hour or just spraying water on the scratched surface. A polyethylene sheet with small pores is sometime used to cover the bottle surface after flooding. This acts to avoid subsequent drying of the upper surface of the medium with the mycelium until appearance of fruit body primordia.

In sawdust cultivation of *Le. edodes*, the cultures are dipped in water for 2-4 hours in each for the second and more flushes [7].

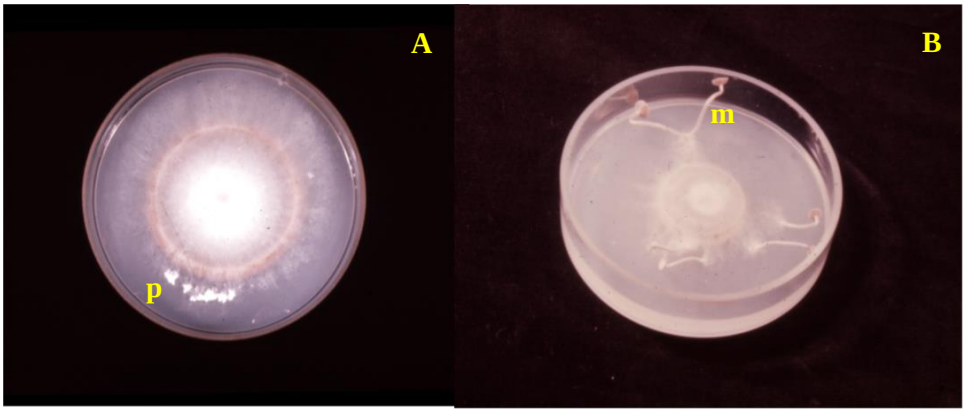

Figure 17. Fruit body formation of *Hebeloma vinosophyllum* (NBRC107913) on plain agar media placed on Petri dishes of 143 mm in diameter, surrounding MY agar media in diameters of 76 mm (A) and 54 mm (B), respectively. The depths of the media were adjusted at 6 mm. The dikaryotic mycelia were incubated at 26.0 ± 0.5 ^{o}C in darkness. After 30 days of cultivation, primordia were formed by the former (A) and mature fruit bodies associating with basidiospore discharge were formed by the latter (B). In contrast, the dikaryotic mycelium cultured on MY agar medium 143 mm in diameter (without plain agar) did not fruit by 30 days of incubation at 26 ^{o}C in dark (Photo is not shown here). p: primordia, m: mature fruit bodies which initiate basidiospore discharge.

7.1-C. Casing: Casing firstly introduced for fruit body induction of *Agaricus* spp. [38, 39] as described above. The casing is then introduced for fruit body primordium induction of non-wood rotting saprobic fungi such as *Lyophyllum decastes* [40] and *Mycena chlorophos* [41], and an ectomycorrhizal fungus *Ly. shimeji*) [19]. The mechanism of primordium formation by casing has not yet fully elucidated. One possibility is induction and/or stimulation of fruiting through changing the inhibitive/inductive fruiting activities of bacteria in the compost [42, 43]. Another possibility is induction and/or stimulation of fruiting by depletion of nutrients for growing hyphae which colonized into casing layer, namely, quickly exposed to severe declining of nutritional level from rich to poor around newly growing hyphae [44]. This kind of fruiting was demonstrated by a facultative ectomycorrhizal fungus *He. vinosophyllum* grown on agar media. The fruit body primordium formation of the fungus is induced by less than a few minutes of light irradiation when its mycelium is grown on MY agar medium more than 3 days at 25 ^{o}C in darkness. It does not fruit in total darkness until the nutrient level of the medium reaching less than its threshold level. However, fruiting of the fungus is induced even in total darkness before the nutrient level of the whole medium becomes less than the threshold level. Namely, fruiting of the fungus is induced even in darkness when the peripheral region of the mycelium is exposed to a sudden change in nutrient condition from rich (nutrient agar medium) to poor (plain agar medium) after a certain amount of nutrients were deposited in the mycelium (Fig. 17).

7.2. Growth control under low temperature (Yokusei): Yokusei [45] is the cultivation process for synchronizing the fruit body development by low temperature used only for the cultivation of *Fl. velutipes* [46] after pre-treatment process (acclimation to low temperature) for Yokusei.

8. Growth: The cluster of fruit bodies reached to suitable sizes for Development are placed in a growing room where temperature, humidity, and gaseous conditions are adjusted to the optimum conditions of each mushroom strain. Usually, gaseous conditions are controlled by the frequency of ventilation (or by ventilation velocity). During the fruiting (during Budding and Growth processes), CO_2 concentration is sustaining less than 1000 to 2000 ppm since the stage of fruit body development is generally more sensitive to concentrated CO_2 comparing with the stages of primordium formation and vegetative growth [47, 48]. Concentration of CO_2 affects not only the form of fruit body (Figs. 18, 19), but also the quality of fruit body (Table 3) [49, 50]. Relative humidity should be controlled strictly by regulation in operation time of humidifier (humidistat) since it severely affects the development of fruit body [13]. The growth room is irradiated normally with growing lamps (a kind of fluorescent lamp) to regulate the form and color of fruit bodies [14, 51, 52]. Only for the cultivation of *Fl. velutipes*, a sheet of plastic film (originally, wax paper) is rolled around the cluster of fruit bodies after Yokusei (Fig. 20), and it is called in Japanese “Kamimaki” (paper wrapping) [46]. The procedure associated with acceleration of an increase in air humidity and CO_2 concentration has been introduced for stimulation of stipe elongation of *Fl. velutipes* since etiolated fruit bodies have been evaluated as desirable for marketing in Japan and accepted as global standard for the production of *Fl. velutipes*.

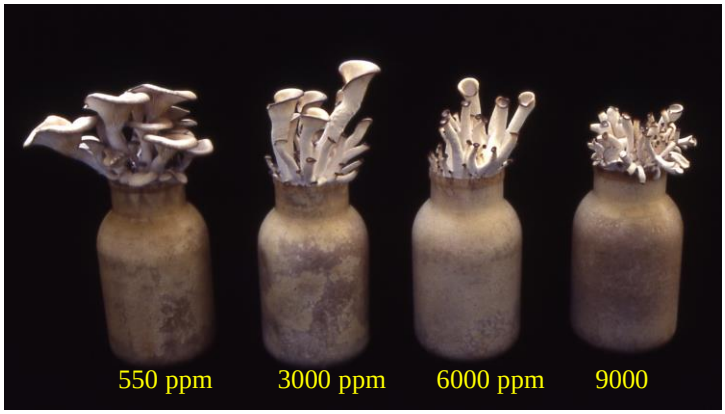

Figure 18. Effect of different concentrations of CO_2 exposure on the shape of fruit bodies in *Pleurotus ostreatus* (after Fig. 13 in Kinugawa *et al.* [45]).

Figure 19. Abnormal fruit bodies of *Flammulina velutipes* appeared by the exposure of 9000 ppm CO_2. (✶: hypertrophic stipe, →: abnormal gill).

9. Cropping: Fruit bodies are pulled out of the mycelium when the cluster of fruit bodies reaches a suitable stage for harvest or cut off 2-3 cm above the medium surface (equivalent to the bottle mouth in bottle cultivation). The former is used for cropping of most cultivated mushrooms in Japan, such as *Fl. velutipes*, *Gr. frondosa*, *Hy. marmoreus*, *Le. edodes*, and *Pl. ostreatus*, etc. and the latter used for *Ph. microspora*. Recently, mushroom farms adopting P.P.

bottle cultivation have gradually introduced automatic harvest machines for the mushrooms used for the former type of cropping in Japan (Fig. 21).

Figure 20. Paper wrapping (Kamimaki) in P.P. bottle cultivation of *Flammulina velutipes*.
A: Fruit body cluster, right after low temperature treatment (Yokusei) for Kamimaki;
B: Fruit body cluster, wrapped with a sheet of plastic film, just before cropping.

Table 3. Effect of concentrated CO_2 on the chemical composition of fruit bodies in cultivated mushrooms.

Composition	*Hypsizygus marmoreus* [49]		*Pleurotus ostreatus* [50]	
	CO_2 concentration (ppm)		CO_2 concentration (ppm)	
	350	6000	350	6000
Moisture content (%)	81.7	87.5	77.9	83.8
Protein*	40.0	40.3	39.7	39.9
Lipid*	4.8	5.2	4.6	5.8
Carbohydrate*	47.3	46.4	47.0	45.5
Ash*	7.9	8.1	8.8	8.8
Ca**	21.3	36.0	5.9	8.6
Fe**	9.6	10.8	9.4	7.9
Na**	14.4	14.4	13.1	12.1
K**	3230.0	3850.0	2920.0	3560.0
Zn**	7.0	7.0	10.9	10.9
Thiamin (Vitamin B_1)**	1.9	1.8	21.7	27.7
Riboflavin (Vitamin B_2)**	2.6	3.0	2.6	2.5
Ascorbic acid (Vitamin C)**	30.2	44.0	32.1	31.1
O/L ratio***	0.13	0.11	-	-
P/S ratio****	3.42	4.01	-	-

*Dry weight basis %; **Dry weight basis mg%; -: No data; ***Oleic acid/Linoleic acid;
****Polyunsaturated fatty acids/Saturated fatty acids

Ph. microspora is normally harvested at the stage equivalent to the button stage of *Ag. bisporus*. *Fl. velutipes* is always harvested at the early stage of pileus development. Most cultivated mushrooms in Japan are harvested at 60 - 80 % of full development of pileus. The suitable stage for harvest for each mushroom has been gradually fixed by the demand of the wholescale markets and the preference of consumers of each province in Japan as well as by the standpoint of prolonged storage of the post-harvest fruit bodies.

Fruit bodies of some mushrooms, such as *Ph. microspora* and *Le. edodes*, etc. are normally harvested from the first flushes to the second and the third flushes, whereas fruit bodies of other major cultivated mushrooms, such as *Fl. velutipes*, *Hy. marmoreus*, and *Gr. frondosa*, etc., are harvested only once.

10. Drying: Post-harvest fruit bodies of *Auricularia* spp., *Le. edodes*, and *Ga. lucidum*, etc. are intended for dried use by sun irradiation or heating machines to enhance flavor, texture, and taste as well as long-term storage [53, 54].

Figure 21. Automated cropping of *Hypsizygus marmoreus.* (Photo by Dr. K. Yamanaka).

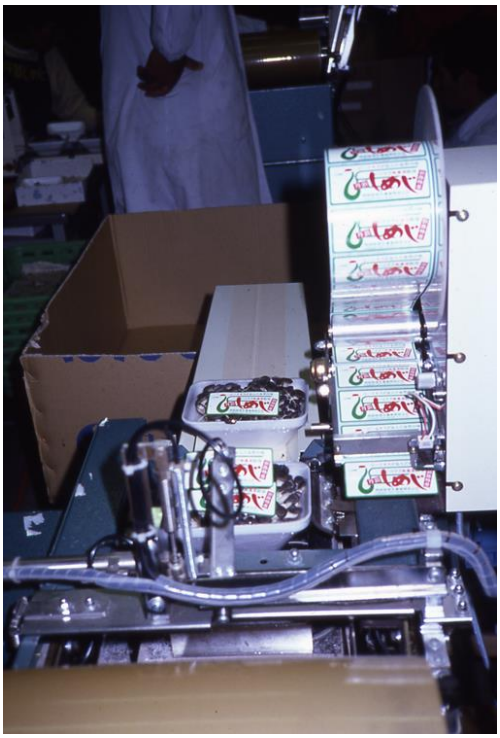

Figure 22. Wrapping machine for *Pleurotus ostreatus*. (Photo by Dr. K. Yamanaka).

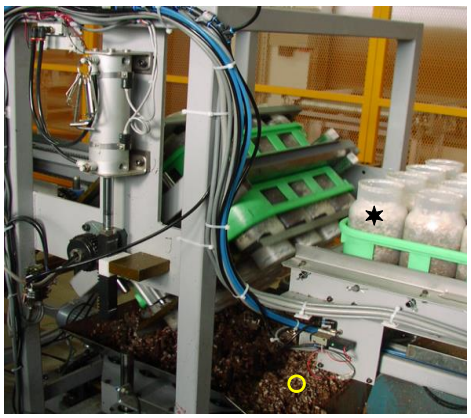

Figure 23. Exhausting machine for the spent substrate of P.P. bottle cultivation. (Photo by Dr. K. Yamanaka). ✶: P.P. cultivation bottle containing the mycelium grown in substrate after cropping, ○: spent substrate exhausted from the P.P. bottles.

11. Packing: Post-harvest fruit bodies of several mushrooms such as *Gr. frondosa*, *Hy. marmoreus*, and *Pl. eryngii*, etc. are placed in a plastic tray, respectively, after they are adjusted to the designated weight for selling. Thereafter, the fruit bodies in each plastic tray is covered by a sheet of thin plastic film affixed with the label indicating the mushroom company's name or mushroom growers' name (Fig. 22), while post-harvest fruit bodies of a few mushrooms such as *Fl. velutipes* and *Ph. microspora* are sometimes covered directly by a sheet of thin plastic film. To avoid the procedure for weighing, usually mushroom growers choose suitable volume of medium for the designated weight of production in each mushroom.

In Japan, the packaged fruit bodies are usually delivered to supermarkets or fruit and vegetable shops within one day after harvest. The delivered mushrooms are normally kept at 10 ℃ to 15 ℃ for display in the supermarket, but are placed at room temperature for display in fruit and vegetable shops and smaller markets.

Senescence of the post-harvest fruit body of *Le. edodes* has been elucidated by gene expression level [55]. It would be useful for the quality control of post-harvest fruit bodies by gene manipulation in future. However, we have not enough information about the changing qualities of fruit bodies during display except for some mushrooms [56-59].

12. Exhausting of waste substrate: The spent substrate (waste substrate) is scraped from P.P. cultivation bottles (Fig. 23) and bags are sometimes used for the compost for farming or the litter for cattle breeding instead of straw because it contains relatively high amount of nutrients even after cropping and has warming ability by itself through the respiration by the mycelium. Spent mushroom substrates are recycled for the cultivation of the same or another species of mushrooms [60, 61]. The storage of spent substrate is also used for the source of ethanol conversion (resource for so-called bioethanol) [62] and for the source of bioremediation [63].

3. CURRENT MUSHROOM CULTIVATION UNDER ASEPTIC CONDITION

Currently all cultivation processes of P.P. bottle cultivation and those of P.P. bag cultivation are automated by machines although automated cropping has not yet widely spread. The former cultivation technology is more suitable for rapid manipulation (Table 4). For example, Japanese mushroom companies have spent more money to develop new excellent isolates by breeding, and registered it to the Ministry of Agriculture, Forestry and Fisheries (MAFF), Japan, based on International Convention for the Protection of New Variety of Plants, but not much to survey new mushroom species for the production. This is derived from the problem for mushroom production in Japan. Namely total consumption of mushrooms in Japan has not increased associated with declining of the population. Total consumption of mushrooms is now at a plateau and increase in the production of new mushroom species correlated to decrease in the total production of existing mushroom species.

For advances in mushroom breeding, the classical hybridization breeding has given us successful results comparing with the breeding by modern technology such as protoplast fusion [64] and gene manipulation. The creation of a new strain of each mushroom species is, however, time and labour consuming work. Therefore, mushroom companies and those affiliated institutes are keen to develop the technology for simple and easy strain-typing at a low price [65, 66] in order to take a countermeasures against the larceny of the strain. Unfortunately, those kinds of strain-typing technology has not yet been completely established. Recently, Japanese mushroom companies are also very eager to energy saving for the mushroom cultivation. They now try to use LED lamps for illumination instead of plant growth lamp (a kind of fluorescent lamp) during

Budding and Growth. For example, blue LED is effective for the production of *Hy. marmoreus* and *Le. edodes* (Fig. 24), but not red LED [67, 68].

Table 4. Performance of automatic cultivation machines for mushrooms in 2019.

Filling machine for		Inoculation machine for		Scratching machine* (Kinkaki machine)	Exhausting machine (Kakidashi-ki)
P.P. cultivation bottle	P.P. cultivation bag	*Hypsizygus marmoreus*	*Flammulina velutipes***		
8000 bottles/hour	1200 - 1500 bags/hour (1.2 kg - 1.5 kg medium)	8000 bottles/hour	9500 - 10000 bottles/hour	9000 bottle/hour	5000 - 6000 bottles/hour

* No differences in performance speed between plain scratching and convex scratching

** Liquid spawn: 6000 bottles/hour

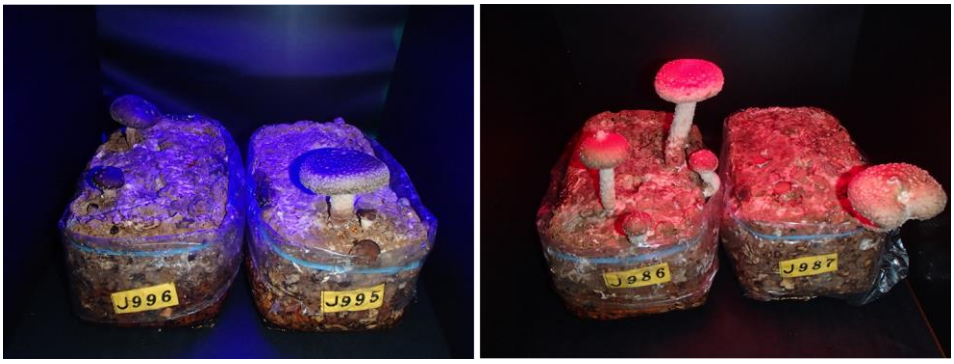

Figure 24. Sawdust cultivation of *Lentinula edodes* under LED illumination at research level. A: Blue LED illumination during fruit body development (cultivation process of Growth), B: Red LED illumination during fruit body development (cultivation process of Growth). (Photo by Miyazaki Pref. Forestry Center, Japan).

4. MUSHROOM CULTURE COLLECTION

Nowadays, the preservation of isolates keeping enough fruiting abilities and the recognition of isolate (races) are important activities for mushroom spawn company. Moreover, many researches have been focused on mushroom genome, especially the gene organization and functions in developing markers for selection breeding systems [69]. There are many culture collection Centers (Biological Resource Center) such as The American Type Culture Collection (ATCC), Westerdijk Fungal Biodiversity Institute (The former name: The CBS Fungal Biodiversity Centre; Centraalbureau voor Schimmelcultures; Central Bureau of Fungal Cultures), and NBRC Biological Resource Center, NITE (NBRC National Institute of Technology and Evaluation), etc. They pay attention how to keep alive cultures, but not fruiting abilities of mushroom isolates (stock cultures, strains). Most of the stored isolates are dikaryotic mycelia in case of mushrooms in Basidiomycota. In contrast, mushroom spawn companies try to store monokaryotic mycelia of Basidiomycota as well as dikaryotic mycelia of Basidiomycota since the former are essential for the breeding. They are also keen to keep fruiting abilities of

dikaryotic isolates, but not yet found perfect storage method(s) for keeping fruiting abilities of fungal isolates. Thus, the spawn companies have introduced several storage methods for each isolate. Namely, they subculture the isolates on different kinds of media including wood-log of various tree species on which the mushroom species frequently occur in the fields, and continuously follow up the fruiting ability in each sub-culture, and confirm fruiting abilities of the isolates stored under ultralow temperature by deep freezer (below -80 °C), and/or by liquid nitrogen, etc. The isolates stored under deep freezing are usually treated with cryoprotectants such as 10 % glycerol and 5 % dimethyl sulfoxide (DMSO) for protecting harmful effect of freezing of hyphal cells. Fruiting abilities of wood rotting fungal isolates are kept better when sawdust cultures treated with 10 % glycerol are stored in a deep freezer. Moreover, the storage method is simple and convenient since it is able to place the isolates directly in a deep freezer without using programmed freezer [70].

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