Cultivation of New Mushroom Species in East Asia

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Abstract: East and Southeast Asia production of cultivated mushrooms except for *Agaricus* was estimated to account for 99% of the total world production of specialty mushrooms in 2002. Total production of specialty mushrooms in East Asia has reached approximately more than three-fold of the world total production of *Agaricus*. Particularly, many new species of edible and medicinal mushrooms, e.g., *Hypsizygus marmoreus, Grifola frondosa, Pleurotus eryngii, Pleurotus nebrodensis* (Bailinggu in Chinese), *Hericium erinaceum, Sparasis crispa, Phellinus linteus, Agaricus blazei, Lyophyllum decastes* and also the ectomycorrhizal fungus, *Lyophyllum shimeji*, have been cultivated in recent years in East Asia. In the case of some of these mushroom species, new strains with desirable characteristics such as pure white *F. velutipes,* white *H. marmoreus,* and large fruit body Shiitake (Jumbo Shiitake) were improved using appropriate breeding techniques. We have also succeeded in the commercial cultivation of *L. shimeji*, the most delicious mushroom in Japan, by pure culture. In Japan, *H. marmoreus, F. velutipes* and *G. frondosa* are produced in factories on a large scale by a few mushroom companies using high-technology production facilities that include automatic harvest-packing machines and fully-automatic conveyor systems. Bottle cultivation of *F. velutipes* and *P. eryngii* using liquid spawn has been popular in Korea. Year-round cultivation of *F. velutipes* and *H. marmoreus* in plastic bottles in mechanized factories has also increased in China and Thailand.

Key words: New mushroom species, cultivation technology, breeding, bottle culture, East Asia

1 World Production of Cultivated Mushrooms Except for *Agaricus*

Recently, the percentage of world total production of *Agaricus* (*A.bisporus/bitorquis*) has decreased in comparison to the world production of all cultivated mushrooms. On the other hand, world production of cultivated edible and medicinal mushrooms except for *Agaricus* has rapidly increased from 4,302,337 tons in 1997[1] to 8, 209,951 tons in 2002 (Table 1). The production of specialty mushrooms in 2004 is estimated to total at least 10 million tons.

Table 1. World production of the main cultivated edible and medicinal mushrooms except for *Agaricus* and wild mushrooms in 1997 and 2002 (metric tons fresh weight)

Countries and areas	1997	2002 (% increase)
China	3,588,300 (1)	7,283,616 (203%) (2)
Japan	369,537 (3)	389,832 (105%) (3)
Korea	115,000 (4, 5)	157,503 (137%) (5)
Thailand	90,000 (4)	121,000 (134%) (6)
Rest of Asia	120,000 (1, 4)	200,000 (167%) (4)
Other countries	19,500 (1)	58,000 (297%) (4)
Total	4,302,337	8,209,951 (191%)

1) See Ref. [1]. 2) China Government Statistics
3) The Ministry of Agriculture, Forestry and Fisheries, Japan 4) Production estimated by K. Yamanaka
5) The Ministry of Agriculture and Forestry, Korea Forest Service
6) During 2003/2004; Department of Agricultural Experiment Service, Thailand-2003

Total production of cultivated edible and medicinal mushrooms except *Agaricus* in East Asia including Southeast Asia in 2002 was 8,151,951 tons, which amounted to 99.3% of total world output of specialty mushrooms. Particularly, the production in China in 2002 is estimated to have accounted for 89.3% of the specialty mushroom production in East and Southeast Asia. Total world production of cultivated mushrooms except for *Agaricus* will reach more than four-fold of the world production of *Agaricus* mushrooms in the near future.

2 Recent Trends in Specialty Mushroom Production in East Asia

2.1 New mushroom species and consumption in East and Southeast Asian countries

Mushroom cultivation in East Asian countries has been diversified to include many new species in addition to the more conventional species, e.g., *Lentinula edodes, Pleurotus ostreatus, F. velutipes, Pholiota nameko, Auricularia spp. Tremella fuciformis and Volvariella volvacea.*

In China, new mushroom species such as *H. marmoreus, G. frondosa, P. eryngii, Pleurotus abalonus, Pleurotus geesteranus, Pleurotus tuber-regium, Stropharia rugosoannulata, Clitocybe maxima, Agrocybe aegerita, Dictyophora indusiata, Coprinus comatus, Ganoderma lucidum, Wolfiporia cocos, A. blazei, H. erinaceum* and also *P. nebrodensis* (Bailinggu in Chinese) have all been cultivated. In particular, the newest species, *P. nebrodensis,* is preferred by Chinese in terms of taste and texture, and production of this mushroom will increase dramatically in the near future. New edible and medicinal mushroom species in China provide mainly for the domestic markets. Therefore, demand for new species is expected to increase further because China has a huge domestic market for edible mushrooms.

Table 2. Production of Edible Mushrooms in Japan. (metric tons fresh weight)

Species	1960	1970	1980	1985	1990	1995	1997	1999	2001	2003
Dried shiitake	27,448	63,976	108,632	96,552	89,904	64,560	46,288	44,656	39,720	32,864
Fresh shiitake	6,634	38,064	79,855	74,706	79,134	74,495	74,782	70,511	66,128	65,363
F. velutipes		10,914	52,565	69,530	92,255	105,752	109,324	113,713	108,444	110,185
P. ostreatus			12,060	26,211	33,475	17,166	13,243	9,944	6,796	5,210
P. nameko	2,267	8,448	16,776	19,793	22,083	22,858	24,522	25,771	23,775	25,068
H. marmoreus			1,600	9,157	29,757	59,760	72,024	84,330	86,551	84,356
G. frondosa				1,501	7,712	22,757	31,135	39,996	44,042	45,805
P .eryngii						*60	*1150	5,515	10,070	29,882
T. matsutake	3,509	1,974	457	820	513	211	272	147	79	80
Others						822	1,766	684	1,262	1,821
Total	39,858	123,376	271,945	298,270	354,833	368,381	374,506	395,267	386,867	400,634

Source: The Ministry of Agriculture, Forestry and Fisheries, Japan
*Production estimated by K. Yamanaka.

Total production of edible mushrooms in Japan has remained largely unchanged in recent years although traditional categories of mushroom production such as *L. edodes* and *P. ostreatus* have actually fallen (Table 2). However, output of certain new mushroom species such as *H. marmoreus, G. frondosa* and *P. eryngii*, produced in the large-scale mechanized facilities, has increased rapidly. Most Japanese production of specialty mushrooms is characterized by year-round cultivation using polypropylene bottles or bags containing sawdust substrate. Japanese mushroom growers have generally used production machines without regard to whether the scale of production was large or small. Lately, production of other edible and medicinal mushrooms, e.g., *L. decastes, L. shimeji, P. nebrodensis, A. blazei, H. erinaceum* and *S. crispa,* has started, although not on a big scale.

P. ostreatus, L. edodes and *F. velutipes* are the most popular edible mushrooms in Korea and production of *P. eryngii* has also rapidly increased in recent years. Most Korean mushroom growers also use production machines to cultivate mushrooms year-round. On the other hand, in recent years Korea has seen great increases in the production of medicinal mushrooms such as *G. lucidum, Phellinus linteus* and *Cordyceps militalis*. Production of new edible mushroom species in Japan and Korea is mainly for domestic consumption.

In other Southeast Asian countries such as Thailand and Vietnam, there is also a growing tendency to produce new mushroom species such as *F. velutipes* and *P. eryngii* in addition to *Agaricus, L. edodes, Pleurotus* spp., and *V. volvacea.*

2.2 Raw materials used as substrates for mushroom cultivation in Asian countries

The cultivation of *Agaricus* uses waste materials such as wheat straw and horse manure for the substrate. On the other hand, the cultivation of specialty mushrooms in Asia has always involved the utilization of various waste raw materials or by-products from the agricultural, forestry, food, brewing and paper manufacturing industries. Waste materials used as substrates for mushroom cultivation in China have generally included cottonseed hulls or sawdust as the basal ingredient together with wheat bran as a nutritional supplement. More recently, Chinese growers have also used corncob meal, rice bran and corn bran in the case of polypropylene bottle culture.

In Japan, growers have used more varied industrial by-products, e.g., sawdust (broad-leaf sawdust for *L. edodes, P. nameko* and *G. frondosa*, sawdust from conifers for *F. velutipes, H. marmoreus, P. eryngii* and *P. ostreatus*), corncob meal, rice bran, wheat bran, corn bran (hominy feed), corn fiber, soybean hull, sorghum seed flour, beer spent grains, beet pulp and sugarcane bagasse. Dried soybean roughage (Okara, tofu refuse) and soybean hull are extremely effective materials in order to improve the water maintaining capability of the substrate. Sawdust from the rubber tree is also used as an available substrate material for cultivating *Auricularia* spp., *P. ostreatus* and *F. velutipes* in Thailand, Vietnam and Indonesia.

3 Research on Mushroom Production in East Asia

3.1 Genetics and breeding for mushroom production

Research and development on the cultivation technologies of edible mushrooms, especially of new mushrooms species, are indispensable at all times for producing stable, high quality and high-yield fruit bodies. In particular, R&D on the genetic and physiological stability of spawn is the most important assignment to ensure the quality control and stabilization of production.

Genetics and breeding of edible mushrooms in East Asia began with efforts aimed at obtaining high-quality and high-yielding strains of *L. edodes*. Thereafter, improved quality, increased yields and shortening of the harvesting period in *F. velutipes, H. marmoreus* and *P. eryngii* have been achieved mainly by breeding based on mating. Ohta et al.[2] obtained a gill-less mutant fruit body of *L. edodes* by mating homokaryon strains derived from a commercial strain. The gill-less fruit bodies of *L. edodes* obtained by mutation had a prolonged shelf life, although the strain did not show good commercial productivity.

In 1985, Kitamoto et al. successfully produced a novel white strain, M-50, which was the first breed of pure white fruit body forming strains of *F. velutipes*[3-5]. As a result of this accomplishment of breeding white strains of *F. velutipes*, growers could use light illumination to control fruit body development, while growers using conventional colored strains had to work in dark growing rooms in order to produce the white fruit bodies. Since the development of the new M-50 strain by breeding, white strains of *F. velutipes* have taken the place of conventional colored strains and have been used exclusively for most of the cultivation in Japan and Korea.

In the cultivation of *P. nameko, H. marmoreus* and *P. eryngii,* desirable new strains with favorable properties such as high quality and high yield have been adopted for commercial production. As a result of the use of undesirable spawn, that frequently did not form primordia, the production of *P. nameko* in Japan was unstable for many years. Commercial spawns of *P. nameko* may have had unstable genetic characteristics that lead to the formation of monokaryotic hyphal cells from dikaryotic mycelia. Masuda et al.[6, 7] demonstrated that stocks of *P. nameko* mated with a particular group of monokaryons produced wide-ranging monokaryotization at higher rates, and the growth rates of the monokaryotized mycelia exceeded rates achieved by the corresponding parental dikaryons. Moreover, these authors suggested that a relative dominance was active in the selection of one of the two nuclei of the dikaryotic cells during monokaryotization. The hierarchy of relative dominance among nuclei of 18 parental monokaryotic stocks in the monokaryotization of their reciprocal crossing products was estimated It was proposed that a cascade process was involved in dikaryotic cell division in which the first dividing nucleus (to be found in the monokaryotized cell) acted as the "leading nucleus" and the other one as the "following nucleus." Meanwhile, Cao et al.[8, 9] also showed the morphological/cytological aspects of oidium formation and nuclear selection in monokaryotic oidium formation from dikaryotic mycelia in *P. nameko.* Thus, a sequence of genetic research involving *P. nameko* has contributed to the stable production of this mushroom in Japan.

In recent years, white strains of *H. marmoreus* and high-quality/high-quantity strains of *L. shimeji* have been bred by crossing, and the excellent fruit bodies are sold in markets. Breeding and genetic research on *P. nebrodensis* has already started in Japan and China.

Nowadays, excellent Japanese strains of *L. edodes, F. velutipes, H. marmoreus* and *P. eryngii* are used widely in East Asia as well as in Japan. The rights of breeders in relation to these new mushroom strains (varieties) have been protected in Japan by the seeds and seedling law. However, the new Japanese mushroom strains are not protected in many Asian countries although some countries have joined UPOV (International Union for the Protection of New Varieties of Plants). Cultivation of new strains of *P. nebrodensis* developed in China has also spread to other countries. Asian countries rapidly need to protect the intellectual property rights of breeders of new mushroom strains under the UPOV treaty. Such protection will encourage the development of novel strains of new mushroom species in the near future.

3.2 Physiological researches for mushroom production

The innovative development of cultivation technologies based on physiological and morphogenetic research has contributed to stable mushroom production. Certain environmental factors such as temperature, humidity, light and carbon dioxide concentration decide the results of the cultivation. Especially, light exposure is an important environmental factor that controls the growth and development of fruit bodies. The results of some physiological and morphogenetic research on the effects of light exposure for improving the quality and quantity of edible and medicinal fungi have been reported for *F. velutipes,*[10-12] *P. nameko,*[13] *P. ostreatus,*[14, 15] *H. marmoreus,*[16, 17] and *Isaria japonica* (*Paechilomyces tenuipes*).[18-20] The effects of carbon dioxide concentration as a physiological factor favoring morphogenesis has also been investigated in order to obtain desirable forms of synnema of *I. japonica.*[18, 19, 21] Data obtained from this morphogenetic research on the effects of light on fruit body formation have been put to practical use in the development of cultivation technologies relating to quality control and increased efficiency of commercial mushroom production.

3.3 Medicinal researches on mushroom

Since Ikekawa et al.[22, 23] reported on the anti-tumor activities of aqueous extracts of edible and medicinal mushrooms, Lentinan (LEM) from *L. edodes*, Krestin (PSK) or PSP (in China) from *Trametes* (*Coriolus*) *versicolor* and Schizophyllan (Soniphyllan, SPG) from *Schizophyllum commune* were commercially developed, mainly in Japan. Several anti-cancer polysaccharides derived from mushrooms have been used as clinical medicines for the cancer patients.

Recently, various medicinal effects attributable to new species of mushrooms cultivated in Asia, including *H. marmoreus,*[24, 25] *P. eryngii,*[26] *G. frondosa,*[27] *H. erinaceum,*[28-30] *P. linteus,*[31] and also *P. nebrodensis,*[32-34] have been reported. Recently, consumers have shown an increased preference for edible mushrooms in anticipation of reported protective effects against diseases associated with modern life-styles as well as anti-cancer activity. This research on the medicinal effects of mushrooms and the development of medicinal mushroom products have encouraged the consumption of edible mushrooms and have also promoted the production of the new species mushrooms in East Asia.

3.4 Cultivation of mycorrhizal mushroom

The ectomycorrhizal fungus, *L.* shimeji, has been considered the most delicious edible mushroom by Japanese who say that "good smell is the best in Matsutake and the taste is the best in Shimeji among Japanese edible mushrooms." *L. shimeji* have been artificially induced to fruit in pure culture without colonizing the roots of a host plant.[35] Recently, we have used crossing to successfully generate a new breed of *L. shimeji* that, when appropriate cultivation technology involving optimization of the substrate is applied, produces excellent quality fruit bodies (unpublished). Currently, fruit bodies of *L. shimeji* are produced commercially on a small scale by bottle culture and are sold in markets. Furthermore, we have produced young fruit bodies of *Boletus reticulates* in pure culture.[36]

Nowadays, the production of extracellular amylase by *T. matsutake* is well known,[37, 38] and Hur et al.[39] reported that amylase activities of this fungus were variable among isolated strains. In addition, α-amylase from the fungus was purified.[40] Although *T. matsutake* has not yet been induced to fruit in pure culture, fruit bodies of this fungus may be produced in pure culture in the near future by the screening of wild strains, genetic improvement of these strains, and the use of suitable nutritional substrates under optimum physical conditions.

4 Mushroom Cultivation Technologies in East Asia

4.1 Cultivation of new mushroom species in East Asia

In China, a huge number of new mushrooms species and varieties have been cultivated in recent years. For instance, the newest species of edible mushrooms are *P. nebrodensis, P. eryngii, P. geesteranus, P. tuberregium, P. abalonus, P. cornucopiae, H. marmoreus, C. maxima, Tricholoma giganteum, Pholiota adiposa, A. aegirita and Lentinus giganteus.* Chinese mushroom scientists and growers have developed the original cultivation methods and techniques through the screening of wild strains and improvements in the selection of suitable substrate and cultivation conditions. Indeed, the cultivation of *P. nebrodensis* in China has developed year by year through the screening of commercial strains and improvements in cultivation techniques.

In Japan, it is the current trend for most of the newest cultivated edible mushrooms, such as *H. erinaceum* and *S. crispa,* to be grown for the physiological activities they possess that protect against modern life-style related diseases even though production levels are currently small.

4.2 Mushroom cultivation systems in East Asia

In Asia, edible and medicinal mushrooms are mostly produced in low-cost and small-scale facilities without expensive equipment. On the other hand, a few mushroom companies in Japan, Korea and Taiwan have produced *F. velutipes, H. marmoreus* and *G. frondosa* using polypropylene bottle cultivation in large-scale facilities equipped with automatic conveyer systems and harvesting/packing machines.[41] For instance, the biggest Japanese facilities for *G. frondosa* and *H. marmoreus* produce 40 tons or more every day, and the annual mushroom production amounts to 12,000 tons a facility. Liquid spawn inoculation systems for the cultivation of *F. velutipes* and *P. eryngii* have become popular in Korea. Year-round cultivation of *F. velutipes* and *H. marmoreus* in highly mechanized facilities using the plastic bottle technique has increased also in China and Thailand.

Hereafter, enormous quantities of specialty mushrooms will be produced in Asia, especially in China and mostly by small-scale growers or farmers even though innovative production using mechanized facilities has increased in Asian countries.

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