Part II

MUSHROOM CULTIVATION AND BIOCONVERSION TECHNOLOGY

CHAPTER 8

BIOLOGY AND CULTIVATION TECHNOLOGY OF *VOLVARIELLA VOLVACEA*

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1. INTRODUCTION

Although more than 100 species, subspecies and varieties of *Volvariella* have been described throughout the world (Shaffer, 1957), four species, including *V. esculenta* Massee, *V. bakeri* Dennis, *V. displasia* Singer and *V. volvacea* Singer, are reported to be cultivated in Asia and Africa. The most cultivated forms of *Volvariella* in Southeast Asia are probably members of the species *V. volvacea* (Bull. ex Fr.) Sing. *V. volvacea*, commonly known as the straw mushroom, belongs to the family Pluteaceae Kotl. & Pouz. of the Basidiomycetes. It is an edible mushroom of the tropics and subtropics. The cultivation of this fungus can be traced back to 1822 in China (Chang, 1977). It was introduced into the Philippines, Malaysia and other Southeast Asian countries about 1932-35 (Baker, 1934; Benemerito, 1936). The importance of this mushroom is reflected by the steady increase in world production every year (Fig. 1). Among the countries cultivating *V. volvacea*, China is the top producer, followed by Thailand, Indonesia and Vietnam (Table 1).

V. volvacea is also referred to as a "warm mushroom" because it grows at the relatively high temperature range of 30-36°C. The high temperature requirement for cultivation makes it suitable for South Asian countries where it is especially important as a source of protein to the rural populace. However, the inability to enhance its post-harvest life by ordinary refrigeration at 5°C, a temperature at which it autolyzes, is one of the major setbacks to big-scale commercial exploitation except where facilities for a superb marketing network exist.

The natural growth substrate of *V. volvacea* is paddy straw. With rice being the major crop in Southeast Asian countries, the source of substrate for *Volvariella* cultivation is not restricted. Other plant cellulosic residues, including cotton waste, bagasse, banana leaves, oil palm pericarp, sorghum straw and fibre crop wastes, can also be used for growing *V. volvacea*. Growth of this fungus from spawning to harvesting can be completed within 10 days. It is considered to be one of the easiest mushrooms to cultivate, with a short cropping period of 14 days, yet it is also the most difficult where it comes to obtaining high, consistent yields.

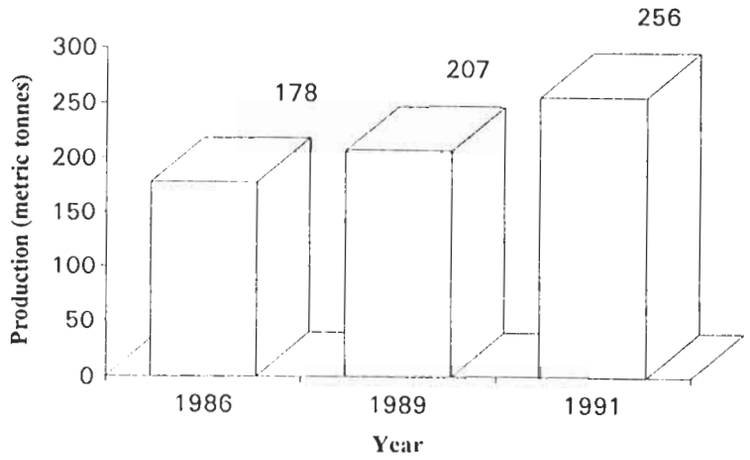

FIGURE 1. Comparison of world production of *V. volvacea* in 1986, 1989 and 1991.

2. PHYSIOLOGICAL NATURE

It is impossible to obtain good crops without good cultures. To obtain and maintain good cultures, an understanding of the biological nature of the mushroom is important. It is clear that mushrooms are fungi which are characterized by their heterotrophic mode of nutrition. Digestive enzymes are liberated from the fungal cells into the immediate environment where organic matter is broken down into simpler molecules which in turn pass into the fungal cells.

The diversity of substrates on which *V. volvacea* grows and its wide distribution may indicate a physiological and nutritional adaptibility within the species. Such attributes are also shown by other cosmopolitan fungi. In terms of nitrogen source, the lignocellulosic materials on which *V. volvacea* is cultivated are usually low in nitrogen. Various studies have shown that *V. volvacea* frequently responds better to organic than inorganic nitrogen. Ofosu-Asiedu *et al.*, (1984) reported that the best yields were obtained on media containing peptone or potassium nitrate. Urea, glutamic acid and

TABLE 1. World production of *V. volvacea* in 1991 (fresh weight, metric tonnes).

Country	Production	%
China	150,000	58.6
Thailand	63,000	24.6
Indonesia	35,000	13.7
Vietnam	3,500	1.4
Taiwan	3,000	1.2
Philippines	800	0.3
India	400	0.2
Others	400	0.2
	256,100	100.0

TABLE 2. Growth of four isolates of *V. volvacea* on different nitrogen sources (maximum mg mycelial dry weight per culture).

Nitrogen source	Isolate			
	V1	42	130	132
Control	32*	36	37	31
$(NH_4)_2SO_4$	55	49	71	46
KNO_3	250	202	253	166
Urea	186	177	157	138
Glutamic acid	156	175	186	198
Asparagine	230	142	191	200
Peptone	193	294	261	229

Source: Ofosu-Asiedu *et al.*, (1984).

* Values presented are biomass harvested on the modified complete medium without nitrogen source (Raper & Miles, 1958) included separately with different nitrogen compounds.

asparagine were also good nitrogen sources. Ammonium sulphate was a poor source (Table 2). At low levels of nitrogen, however, the response to organic and nitrate nitrogen is similar to that reported by Chang-Ho & Ho (1979).

In terms of carbon source, the results obtained by Ofosu-Asiedu *et al.*, (1984) show that *V. volvacea* utilizes starch and glucose better than other carbon sources. None of the isolates tested could grow on lignin (Table 3). On the other hand, Chakravarty & Mallick (1979) obtained considerably more growth with glucose than with starch. Although good growth of *V. volvacea* can be observed on such simpler carbohydrates, the monomeric sugars and starch are available in limited amount in the natural substrates of *V. volvacea*. In general, the different isolates of *V. volvacea* show similar physiological behaviour although some differences have been observed. The discrepancies between two subcultures, V1 and V2, which were derived from the same isolate, may be attributed to the different culture methods used in the laboratories of origin. It should be noted that the growth of mushrooms is based on the hydrolysis of polymeric lignocellulose. That is, mushrooms can produce a range of extracellular hydrolytic and oxidative enzymes including lignocellulolytic and oxidative enzymes. The primary function of hydrolytic enzymes is to break down the macromolecular polymer into more-readily assimilated low molecular weight carbohydrates. For example, the cellulolytic and hemicellulolytic enzymes produced by mushrooms can degrade respectively the polysaccharide components into hexoses (e.g., glucose) and pentoses (e.g., xylose). The simpler sugars resulting from enzymic hydrolysis support the growth of mushrooms. Glucose, which is the basic unit of the cellulose component in the lignocellulosic substrate, was the most favourable carbon source for the growth of *V. volvacea* (Table 4). Xylose also served as a carbon source although to a lesser extent. Therefore, the ability of the mushroom to produce cellulose- and hemicellulose-degrading enzymes is an important factor for fungal growth.

Lignocellulosic substrates that are commonly used in mushroom cultivation include cellulose, hemicellulose and lignin. The proportion of each component varies with different lignocellulosic materials from nearly zero lignin and high cellulose substrate (e.g., cotton waste) to high lignin content substrate (e.g., wood residues). For example, the lignocellulose in paddy straw consists of 36% cellulose, 18% hemicellulose and 8.5% lignin (Dale, 1987). The cellulose and hemicellulose

TABLE 3. Growth of four isolates of *V. volvacea* on different carbon sources (maximum mg mycelial dry weight per culture).

Carbon source	Isolate			
	V1	42	130	132
Control	16	18	15	15
Glucose	160	241	197	137
Galactose	30	33	35	33
Xylose	28	29	27	29
Sucrose	32	36	32	32
Cellulose	47	63	54	52
Xylan	43	59	64	50
Lignin	14	18	16	15
Starch	146	274	343	162

Source: Ofosu-Asiedu *et al.*, (1984). As indicated in Table 2, the modified complete medium without carbohydrates.

components of the plant cell wall are intimately associated with the lignin moiety. The cellulose component is generally enveloped by a lignin-hemicellulose matrix (Kirk, 1985). The heterogeneity and complexity of lignocellulosic materials impart a very stable resistance to microbial attack. For those species possessing ligninolytic ability, such as many white-rot basidiomycetes, the lignin-

TABLE 4. Growth of *V. volvacea* on different reducing sugars and short chain polysaccharides as carbon source.

Carbon source	Growth (%)*
arabinose	34(9)
cellobiose	63(0)
fructose	96(4)
galactose	35(12)
glucose	100
lactose	15(8)
maltose	90(4)
mannose	94(7)
raffinose	72(8)
saccharose	88(7)
xylose	34(26)
soluble starch	88(7)

(Cai, Buswell & Chang, unpublished data)

*Values are expressed as a percentage of the growth recorded over the growth on glucose and represent the mean of three replicates. Figures in parentheses refer to the percentage variation about the mean. The growth rate of *V. volvacea* and the values presented are those recorded after 4 days of incubation. In all cases, the density of fungal colonies was similar when the values shown were taken.

hemicellulose matrix will not be a barrier to fungal growth since the lignin component would be degraded by lignin-transforming enzymes produced by such fungi (e.g., *Pleurotus sajor-caju* and *Lentinus edodes*). Degradation products (e.g., simpler phenolic compounds) would be further modified by a phenol-oxidizing system. However, for those species lacking ligninolytic activity, the lignified complex of lignocellulose would restrict the access of mushroom hyphae to the cellulose component. Consequently, the ability of a mushroom to produce oxidative enzymes, including phenol oxidases and lignin-transforming enzymes, are also important to fungal growth when the fungus is cultured on lignocellulosic substrate.

When cultured on different lignocellulosic substrates, including paddy straw and cellulose, *V. volvacea* exhibited a general inability to synthesize phenol-oxidizing and lignin-transforming enzymes. However, the mushroom produces several of the enzymes necessary for the hydrolysis of cellulose and hemicellulose. These include endo- and exogluconases, β-glucosidase, xylanase and β-xylosidase (Cai *et al.*, to be published). In this respect, *V. volvacea* behaves like a brown-rot fungus. Brown-rot fungi have been defined as being able to degrade cellulose and hemicelluloses, leaving the lignin more or less intact as a brown layer (Deacon, 1980).

Lignin-related components are known to repress fungal growth and the activity of enzymes hydrolyzing cellulose and hemicellulose. In the work of Cai *et al.*, (1993), several lignin-related phenolic monomers and tannin derivatives were reported to inhibit the growth of *V. volvacea* (Tables 5 & 6). Recently, the activity of cellulose-degrading enzymes produced by *V. volvacea* was also found to be inhibited by lignin-related and tannin phenolic compounds (Cai, Buswell & Chang, unpublished data). Tannin was also reported to inhibit the growth of *V. volvacea* by Yu and Chang (1989).

Recent results suggest that the growth of *V. volvacea* is more sensitive to lignified substrates because of the lack of ability to produce phenol-oxidizing and lignin-transforming enzymes. Inhibition of polysaccharide-degrading enzymes (e.g., cellulase) by phenolic compounds present in the straw might explain why the formation of mushrooms on paddy straw is limited, giving a low biological efficiency. The means to produce simpler sugars for growth might be inhibited by the phenolic compounds existing in the straw. It has been shown that cellulase production in cultures of *V. volvacea* grown on rice straw was lower than cultures grown on pure cellulose (Cai, Buswell & Chang, unpublished data). In general results suggest that when *V. volvacea* was cultured on a rich cellulosic substrate such as cotton waste, in which the amount of lignified components is negligible, the cellulose-degrading activity of the fungus was not inhibited and fungal growth was less disturbed. As a result, production yields were increased. It has been shown in the case of some fungi that the production of extracellular enzymes involved in lignocellulose degradation is closely coupled to fungal growth and colonization as well as to the formation of the sporophore (fruiting body).

According to preliminary research results, *V. volvacea* can also tolerate sodium chloride. There are numerous plants that grow in saline and alkaline soils and they are widely distributed throughout the world, especially along seashores and in saline areas in various continents. Many of them are known as "salt bushes" and "salt grasses" and could perhaps be used in the preparation of composted substrate for the growth of *V. volvacea.*

Several fatty acids and sunflower oil were found to enhance the fungal growth of *V. volvacea* (Li *et al.*, 1992). More recently, a complex of fatty acids was found to increase the production of cellulases and hemicellulases in *V. volvacea* cultures (Cai *et al.*, to be published).

TABLE 5. Effect of lignin-related phenolic compounds on the growth of *V. volvacea*.

Phenolic compound	Concentration (mM)	Growth (%)*
4-hydroxybenzoic acid	1	80(3)
	2	76(7)
	5	68(3)
	10	45(3)
Vanillic acid	1	109(2)
	2	115(2)
	5	97(3)
	10	81(5)
Syringic acid	1	92(3)
	2	93(3)
	5	97(3)
	10	94(2)
p-Coumaric acid	1	83(6)
	2	78(3)
	5	53(8)
	10	32(7)
Caffeic acid	1	107(3)
	2	103(4)
	5	94(2)
	10	73(3)
Ferulic acid	1	92(3)
	2	74(9)
	5	45(4)
	10	23(16)
p-Hydroxybenzaldehyde	1	69(1)
	2	48(11)
	5	0
Vanillin	1	34(16)
	2	0

Source: Cai *et al.*, (1993).

* Values are expressed as a percentage of the growth recorded in the absence of lignin-related phenolic compounds and represent the mean of three replicates. Figures in parentheses refer to the percentage variation about the mean. The growth rate of *V. volvacea* and the values presented are those recorded after 42 hours of incubation. In all cases, fungal growth rates were linear with time when the values shown were taken.

3. PROSPECTS FOR GENETIC MANIPULATION IN THE BREEDING OF *VOLVARIELLA* MUSHROOMS

V. volvacea is regarded as a homothallic mushroom species (Chang & Yau, 1971). Each basidiospore is uninucleate when first formed but it gives rise to a multinucleate mycelium. There is no dikaryotic phase, and no clamp connections have been observed. Karyogamy and meiosis take

TABLE 6. Effect of tannin derivatives on the growth of *V. volvacea*.

Phenolic compound	Concentration (%,v.v)	Growth (%)*
Gallic acid	0.01	93(5)
	0.02	88(3)
	0.05	78(6)
	0.10	68(3)
	0.15	64(5)
Tannic acid	0.01	91(6)
	0.02	74(16)
	0.05	20(29)
	0.10	0
Catechin	0.01	90(3)
	0.02	102(7)
	0.05	90(5)
	0.10	77(13)
	0.15	69(13)

Source: Cai *et al.*, (1993).

* Values are expressed as a percentage of the growth recorded in the absence of the tannin derivative and represent the mean of three replicates. Figures in parentheses refer to the percentage variation about the mean. The growth rate and the values presented are those recorded after 42 hours of incubation. In all cases, fungal growth rates were linear with time when the values shown were taken.

place in the basidium and four basidiospores are produced. With reference to that, some single spores can give rise to fruit bodies. The technique of single spore selection might still be the best way to maintain good strains of *V. volvacea*; yet the great variation of many characteristics found among the single spores of one progeny cannot be properly explained. It is hoped that much will soon be clarified since this is a great handicap for the breeding work and strain improvement of the straw mushroom.

The cellulase complex appears to be involved in the utilization of the polymeric substrate for fungal growth and is probably related to the further production of fruit bodies. Therefore, increasing the ability of *V. volvacea* to produce cellulase would be a potential target in strain improvement. Wong *et al.*, (1988) reported that two *Cellulomonas fini* cellulase genes, *cenA* (encoding an endoglucanase) and *cex* (encoding an exoglucanase) have been cloned and transformed into *Saccharomyces cerevisiae* through plasmids. This displays the possibility of using recombinant DNA techniques to engineer co-expression of cellulases. By such molecular biological techniques it would be possible, through enforcing the ability of *V. volvacea* to produce substrate-utilizing enzymes, or introducing the genes encoding lignin- and phenol- transforming enzymes, to obtain improved strain(s) of *V. volvacea* with adaptation to a wider range of substrates and with higher production yields.

Obtaining fusion products between *V. volvacea* and other species by protoplast fusion demonstrates another possibility for achieving the improvement of *Volvariella* strains (Zhao & Chang, unpublished data). This approach of hybridization may open a new channel for breeding programmes.

4. CULTIVATION

The traditional method of cultivating *Volvariella* mushrooms in Southeast Asia is by straw beds in the open field or, more recently, by the use of wooden frames. Although this is an inexpensive method, it is subject to pests and diseases and to the vagaries of nature as environmental conditions for fruiting are difficult to control. The most recent development of *Volvariella* culture in Southeast Asia is indoor cultivation utilizing mushroom houses under a controlled environment. Pasteurization and heating facilities are incorporated. The use of cotton waste in place of rice straw as substrate resulted in a significant increase (two to three fold) of the biological efficiency (B.E. = fresh weight of harvest mushroom over the air dried weight of substrate X 100 expressed as percentage) and more stable production yield. This technique proved to be a turning point in the history of straw mushroom cultivation (Chang, 1974). Although the B.E. of this species was significantly increased when the solid-state culture was grown on cotton waste in place of paddy straw, it is still lower than other cultivated mushroom species (Table 7).

Several techniques have been adopted for the cultivation of *V. volvacea*, which thrives in the temperature range of 28-36°C and a relative humidity of 75-85%. Detailed descriptions of the various methods are given in the Food and Agriculture Organization Plant Production and Protection Paper (FAO) No.106: "Technical Guidelines for Mushroom Growing in the Topics" by Quimio *et al.*, (1990) and will not be repeated here. Choice of technologies usually depends on personal preference and on the availability of substrates and the amount of resources available. While the more sophisticated indoor technique is recommended for the industrial-scale production of this mushroom, most of the other techniques are low-cost and appropriate for rural area development, especially when production is established at the community level.

Four examples of regional variations in cultivation conditions used to grow the straw mushroom are given below:

Mexico: *V. volvacea* culture is grown at 32-38°C on beds of fermented and pasteurized barley straw and cotton waste.

Thailand: *V. volvacea* is cultivated over a temperature range of 25-38°C in beds of pasteurized straw, cotton waste or kapok waste.

Indonesia: *V. volvacea* grows in the 30-35°C region. In 360 growing sheds on 3 locations. The mushroom company P.T. Dieng Djaya is producing approximately 40 tonnes per day on pasteurized straw and cotton waste as a growing medium (Vedder, 1991). In rural areas, the rice straw is still used as a traditional substrate for the cultivation of the mushroom. The main consideration is that the bed have an optimum height in order that sufficient heat, which is conductive to mycelial growth, can be developed.

China: *V. volvacea* has been traditionally cultivated in southern provinces (e.g., Guangdong, Guangxi and Fujian). In recent years, this mushroom has also been grown in other parts of the country during the summer months. In Nanhui (Shanghai), *V. volvacea* is cultivated in early summer. The major substrate is cotton waste, a byproduct of the textile factories, and the resulting B.E. was reported as 34-38%. In Qing Yuan County (Hebei Province), the cultivation technique using plastic shelter trenches (hut trenches) on a compost mixture consisting of spent corn cobs, wheat straw and cotton-seed hulls gave a B.E. of 28-29%. In Jiangxi, the pretreatment of paddy straw by water washing under pressure in a machine resulted in an increase of B.E. up to 20-30% from 6-10%.

TABLE 7. Approximate biological efficiency of edible mushrooms.

Species	Substrate	B.E. (%)
Lentinus edodes	Wood logs (outdoors,sometimes protected)	40
	Sterilized sawdust (polypropylene bags)	60-100
Auricularia spp.	Wood logs (outdoors, sometimes protected)	2-12
	Sterilized sawdust (polypropylene bags)	70-85
Tremella fuciformis	Wood logs (outdoors)	20
	Sterilized cotton-seed hull (polypropylene bags)	100-160
Pleurotus spp.	Pasteurized cereal straw (indoor)	80-100 or more
Agaricus bisporus	Composted cereal straw/animal manure mixtures	65-80
Volvariella volvacea	Straw (outdoor)	6-10
	Composted cotton waste	30-45
Flammulina velutipes	Sterilized sawdust (polypropylene bags)	70-100

Source: Smith *et al.*, (1988); Chang & Miles, (1989).

5. UTILIZATION OF WASTE TEA-LEAVES IN THE PREPARATION OF SPAWN

Chang (1982) and Bisht and Harsh (1984) reported that waste tea-leaves, unavoidable waste materials after decoction of tea in both restaurants and households, can be used with good effect in straw mushroom spawn production when mixed with wheat grains or cotton waste. The selected cotton waste contains short fibre material as well as cotton-seed hulls. A mixture of cotton waste / wheat grain and used tea-leaves in equal ratio has been found suitable for the production of the spawn. The mushroom mycelium can grow both inside and outside the waste tea-leaves which also discourages the development of competing molds under the high temperature and humidity conditions in the mushroom beds. The spread of spawn (spawn running) on mushroom beds was also good and there was no difference in the yield by utilizing this spawn in comparison with chopped straw or grain spawns. A spawning rate of 2% (spawn to substrate, w/w) is sufficient.

6. GENERAL REMARKS

Volvariella volvacea is an edible mushroom of the tropics and subtropics. In traditional cultivation it grows on paddy straw in subtropical and tropical climates. In recent years, it has been cultivated on cotton wastes and cotton-seed hulls which contain a higher proportion of the cellulose component than does paddy straw, and it can be cultivated in temperate climates in summer months. Since *V. volvacea* lacks the capability of producing phenoloxidizing and lignin-transforming enzymes, it is difficult to obtain a reasonably high yield if the substrate contains a high lignin component, as is the case with wood chips and sawdust. On the other hand, if the compost contains a higher proportion of cellulose, as in cotton wastes, and growth is under optimal conditions, a reasonably high and stable yield can be achieved. It has been noted in a review of the literature that untreated paddy straw usually gives low biological conversion rates, in the range of 5-15%. It is recommended that cotton waste/cotton-seed hulls should be mixed with paddy straw or bagasse for

composting for growth of *V. volvacea*. Before spawning, the compost should be pasteurized, or the compost should be presoaked in 2% $CaCO_3$ overnight in order to inhibit or eliminate harmful molds. After spawning, the bed should be covered with plastic sheets for the first 3-4 days in order to create microclimatic conditions in the bed. This exercise can provide conditions of higher temperature, higher moisture and higher concentrations of CO_2 that promote mycelial running in the compost. After removal of the plastic sheets, the bed should be gently sprinkled with water in order to lower the temperature of the bed. In the meantime, the culture room or the surroundings of the bed should be provided with more fresh air and adequate light. It is suggested that these environmental factors can stimulate fructification. Furthermore, some reports suggest that the bed should be covered with a thin layer of casing soil.

Since *V. volvacea* has been regarded as a homothallic species, selection for desirable strains by single spore isolation may still be preferable. However, using protoplast fusion techniques, interspecific somatic hybrids, such as between *V. volvacea* and *V. esculenta* and between *V. volvacea* and *V. bombycina,* may be obtained. This approach to hybridization may open a new channel for breeding programmes. In practice, *V. volvacea* normally produces only one flush per crop (one compost duration). This is quite different from other mushrooms, e.g., *Agaricus bisporus* and *Pleurotus sajor-caju*, which can have more than 4 flushes in each crop. This is an important issue in *V. volvacea* cultivation. Researchers should consider both physiological and nutritive conditions as well as genetic and breeding aspects.

Lack of access to good quality spawn represents a major problem for mushroom growers in developing countries. Quality controls may be non-existent and there is no "in-house" testing of spawn by spawn producers to ensure the character and conditions of their product. Spawn cultures are normally obtained from outside the region concerned. The spawn may be well-suited to climatic conditions and the cultivation procedures adopted in the source regions, but little attention may be given to the particular requirements of local conditions. Furthermore, should the spawn prove defective or inappropriate, the grower is left to carry the burden of an unsuccessful harvest.

The absence of systematic government support for, and promotion of, mushroom growers appears to be responsible in part for the failure of some countries to sustain a viable mushroom cultivation programme. In countries with thriving mushroom cultivation industries, a coordinated government policy allots production quotas for each grower and provides price guarantees for those quotas, thereby reducing some of the financial uncertainly associated with the enterprise. In countries where no such coordinated government involvement exists potential growers are dissuaded from entering into the business.

REFERENCES

BAKER, J.A. (1934). Mushrooms growing in Wellesley and Penang Provinces. *Malaysian Agricultural Journal* **22**, 25-28.

BENEMERITO, A.N. (1936). Mushroom culture in Canton. *Philippine Agriculture* **24**, 624-34.

BISHT, N.S. & HARSH, N.S.K. (1984). Utilization of waste tea-leave - preparation of spawn of *Agaricus* and *Volvariella volvacea*. *Mushroom Journal* **139**, 231-233.

CAI, Y.J., BUSWELL, J.A. & CHANG, S.T. (1993). Effect of lignin-related phenolic monomers and tannin derivatives on the growth of the edible mushrooms *Lentinus edodes, Pleurotus sajor-caju* and *Volvariella volvacea*. *World Journal of Microbiology and Biotechnology* (in press).

CHAKRAVARTY, D.K. & MALLICK, A.K. (1979). Studies on the physiology of mushroom fungi, *Volvariella displasia* and *V. esculenta*. *Indian Journal of Mycology and Plant Pathology* **9**, 9-12.

CHANG, S.T. (1974). Production of the straw mushroom (*Volvariella volvacea*) from cotton wastes. *Mushroom Journal* **21**, 348-53.

CHANG, S.T. (1977). The origin and early development of straw mushroom cultivation. *Economic Botany* **31**, 374-76.

CHANG, S.T. (1982). Mushroom spawn. In *Tropical Mushrooms - Biological Nature and Cultivation Methods*, pp.31-46. Edited by S.T. Chang, & T.H. Quimio. Hong Kong: The Chinese University Press.

CHANG, S.T. & MILES, P.G. (1989). *Edible Mushrooms and Their Cultivation.* Boca Raton, Florida: CRC Press, Inc.

CHANG, S.T. & YAU, C.K. (1971). *Volvariella volvacea* and its life history. *American Journal of Botany* **58**, 552-61.

CHANG-HO, Y., & HO, T.M. (1979). Effect of nitrogen amendment on the growth of *Volvariella volvacea. Mushroom Science* **10**, 619-28.

DALE, B.E. (1987). Lignocellulose conversion and future of fermentation biotechnology. *Trends in Biotechnology* **5**, 287-291.

DEACON, J.W. (1980). *Introduction to Modern Mycology,* pp.61-62. ELBS, UK.

KIRK, T.K. (1985). The discovery and promise of lignin-degrading enzymes. In *New Horizons for Biotechnological Utilization of theForest Resource*, pp.27-42. Marcus Wallenberg Foundation Symposium.

LI, Y., CHO, K.Y., WU, Y.Z. & NAIR, N.G. (1992). The effect of lipids and temperature on the physiology and growth of *Volvariella volvacea. World Journal of Microbiology and Biotechnology* **8**, 621-626.

OFOSU-ASIEDU, A., SCHMIDT, O. & LIESE, W. (1984). Growth studies of *Volvariella volvacea* for cultivation on wood waste. *Material and Organism* **19**, 241-251.

QUIMIO, T.H., CHANG, S.T. & ROYSE, D.J. (1990). *Technical Guidelines for Mushroom Growing in the Tropics.* FAO, Rome.

RAPER, J.R. & MILES, P.G. (1958). The genetics of *Schizophyllum commune*. *Genetics* **43**, 530-546.

SHAFFER, R.L. (1957). *Volvariella* in North America. *Mycologia* **49**, 545-79.

SMITH, J.F., FERMOR, T.R. & ZADRAZIL, F. (1988). Pretreatment of lignocellulosics for edible fungi. In *Treatment of Lignocellulosics with White Rot Fungi*, pp.3-13. Edited by F. Zadrazil & P. Reiniger. London: Elsevier Applied Science.

VEDDER, P.J.C. (1991). The biggest mushroom farm in the world. In *Science and Cultivation of Edible Fungi*, pp.813-819. Edited by M.J. Maher. A.A. Balkema/Rotterdam/Brookfield.

WONG, W.K.R., CURRY, C., PAREKH, R.S., WAYMAN, M., DAVIES, R.W., KILBURN, D.G. & SKIPPER, N. (1988). Wood hydrolysis by *Cellulomonas fini* endoglucanase and exoglucanase coexpressed as secreted enzymes in *Saccharomyces cerevisiae. Biotechnology* **6**, 713-718.

YU, M. & CHANG, S.T. (1989). Tolerance of tannin by the shiitake mushroom, *Lentinus edodes. World Journal of Microbiology and Biotechnology* **5**, 375-378.