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USE OF SOME AGRO-INDUSTRIAL LIGNOCELLULOSE BY-PRODUCTS FOR EDIBLE MUSHROOM *Volvariella volvacea* CULTIVATION

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ABSTRACT

The mycelial growth of two Mexican strains of *Volvariella volvacea* (Bull.: Fr.) Sing., in 13 agroindustrial wastes is reported. The following substrates were used: banana leaves, bracts of pineapple crown, coconut fibre, coffee bran, coffee pulp, corn cob, corn stover, orange peel, rice bran, rice straw, sisal bagasse, sugarcane bagasse and wheat straw. Mycelial growth, mycelial thickness and pinhead formations were the parameters evaluated. Fruiting bodies were obtained only from one strain growing in bracts of pineapple crown, coffee pulp, rice straw and sisal bagasse. Primordia were developed between 13 and 15 days. The highest biological efficiency was achieved on rice straw, 33.8%, while the results obtained for coffee pulp, sisal bagasse and bracts of pineapple crown were 15, 7.8 and 6.2%, respectively. Chemical analyses of the substrates registered C/N ratios of 33:1 to 80:1. The results demonstrate the possible use of rice straw and coffee pulp for mushrooom cultivation in Mexico, which would provide a source of protein rich food as well as encouroge the biological conversion processess of agro-industrial wastes.

RESUMEN

Se estudió el cultivo de dos cepas mexicanas de *Volvariella volvacea* (Bull.:Fr.) Sing., tanto en su desarrollo micelial, como en la obtención de fructificaciones sobre residuos lignocelulósicos. Los sustratos empleados, considerados como contaminantes, fueron: brácteas de la corona de la piña, bagazos de caña de azúcar y de henequén, cáscaras de naranja, cascarillas de arroz y de café, fibra de coco, hojas de plátano, olote de maíz, pajas de arroz y de trigo, pulpa de café y rastrojo de maíz. Los parámetros evaluados en el laboratorio fueron densidad y velocidad de crecimiento de los micelios, así como la formación de agregaciones hifales. De los resultados obtenidos en esta etapa se eligieron las brácteas de la corona de la piña, el bagazo de henequén, la paja de arroz y la pulpa de café para evaluar la producción de los cuerpos fructíferos con una de las cepas. Los primordios se desarrollaron entre 13 y 15 días. En la paja de arroz se logró una eficiencia biológica promedio de 33.8%, mientras que en la pulpa de café, en el bagazo de henequén y en las brácteas de la corona de la piña fue de 15, 7.8 y 6.2%, respectivamente. La composición química de los sustratos determinó que las relaciones de C/N variaron de 33:1 a 80:1. Los resultados demuestran la factibilidad de utilizar la paja de arroz y la pulpa de café para el cultivo del hongo en México, obteniendo un alimento proteico y favoreciendo la biodegradación de desechos agroindustriales.

INTRODUCTION

The continuously increasing demand for food products by the growing human population has created a need for an agricultural by-products upgrading. Approximately 40% of the biomass produced yearly by photosynthesis is regarded as lignocellulose fibrous rich products, such as straw, bagasse, stalks, wood and sawdust, etc. Large quantities of these crop residues are currently utilized as a potential animal feed source because they are of low feeding value and restricted intake due to strong physical and chemical bonds between carbohydrates and indigestible lignin (Zelenak 1990). It is estimated that, only in Mexico, 70 million tons of crop residues are produced every year. Therefore, the development of processes for lignocellulose bioconversion, such as the cultivation of edible fungi, could be an alternative for human food production and upgrading fiber rich by-products for animal feeding (Smith *et al.* 1988, Rajarathnam and Bano 1991). *Volvariella volvacea* is appropriate for cultivation in tropical and subtropical regions in Mexico, since it is very common (Guzmán 1977) and its optimal temperature for growth is around 30°C (Chang 1978, Martínez-Carrera *et al.* 1991,

Guzmán *et al.* 1993). Moreover this species is the most common edible mushroom in southeast Asia and the fourth most produced in the world (Chang and Miles 1991). *V. volvacea* is principally cultivated on cotton wastes and rice straw (Chang and Miles 1989) and was tested on other lignocellulosic materials, such as banana leaves, wheat straw, tea leaves waste, oil palm wastes and fresh water hyacinth (Kwang and Chang 1981, Quimio 1986, Li *et al.* 1988, Petcharat and Koewthong 1990). However, there are scarce studies in Mexico on *V. volvacea* cultivation (Martínez-Carrera *et al.* 1986, Salmones *et al.* 1988, Vela and Martínez-Carrera 1989).

MATERIALS AND METHODS

Fungi

Two Mexican strains of *Volvariella volvacea* from the Strains Collection of Instituto de Ecología at Xalapa, Mexico were tested. For preservation, a medium of malt extract agar (Bioxon) at 30°C was used.

Substrates and preparation

The following 13 agroindustrial wastes were used: banana leaves, bracts of pineapple crown, coconut fibre, coffee hulls, coffee pulp, corn cob, corn stover, orange pulp, rice bran, rice straw, sisal bagasse, sugarcane bagasse and wheat straw. These substrates were selected due to their high abundance and availability in tropical and subtropical regions of Mexico. The materials were dried and divided into small lengths of 5 mm. The percentage of water retained by the substrates was determined through differences with dry weight, according to Han *et al.* (1981) and Mata (1992). Samples of 4 g (dry weight), with a moisture content adjusted to 70%, were deposited on 90 x 15 mm Petri dishes. Five replicates were prepared of each substrate and were sterilized at 121°C for 60 min. The inoculation was with small discs (0.5 cm) of agar with the mycelium. The incubation was at 30°C in darkness. The mycelial growth was measured by means of mycelial diameter, mycelial thickness and primordia initiation. Results of mycelial growth were subjected to analysis of variance, and the Tukey's Multiple Range Test was used to determine significant differences between the means by a Minitab computing programme.

Spawn preparation

The inoculum was prepared only with one strain (IE-158), on rice straw, which was fermented for 3 days. The material was placed in glass flaks and sterilized at 121°C for 60 min. A small portion of mycelium was deposited on the substrate for inoculation. The samples were incubated in darkness at 32°C.

Substrate preparation and fruit body cultivation

Bracts of pineapple crown, coffee pulp, rice straw and sisal bagasse were used. The materials were fermented for five days, following the technique of Guzmán *et al.* (1993). Except for rice straw and sisal bagasse, the substrates were mixed with $CaCO_3$. Samples equivalent to 300 g of dry weight were pasteurized in water at 80°C for 60 min. The spawn was mixed with substrates in a plastic container of 35 x 29 x 8 cm and incubated in darkness at 30°C. Five samples of each substrate were evaluated.

For fructification, the containers were removed from the plastic bags and placed in a chamber which was built according to Li (1984), in order to develop fruit bodies with controlled light, aeration, temperature and air humidity. Temperature and relative humidity during the experiment were 27-35 °C and 85-95%, respectively. Biological efficiency was determined by percentage of yield of fresh mushroom in relation to dry weight of substrate, according to Tchierpe and Hartman (1977). Prior to spawning, the nitrogen content of the different substrates was determined by the Kjeldahl method, and the total carbon content by differences between dry weight of such materials and their ashes. These analyses were conducted in the Instituto Cubano de Investigaciones de los Derivados de la Caña de Azúcar, La Habana, Cuba.

RESULTS

The strains grew well on all substrates, except orange peel and coffee bran. Mycelial thickness was almost the same in both strains, thicker on coffee pulp, bracts of pineapple crown and corn stover, and thinner on sugarcane bagasse, banana leaves and rice bran. Pinhead formations were observed only on sisal bagasse and coffee pulp. The average number of days taken by the strains to cover the Petri dish diameter are presented in **table I**. Strain IE-106 grew faster on coffee pulp, rice straw, rice bran, wheat straw and banana leaves, while strain IE-158 performed better on bracts of pineapple crown, banana leaves and sisal bagasse. Both strains had the lowest growth on corn stover and sugarcane bagasse.

The best substrates for mycelial growth of the strains were coffee pulp, rice straw, bracts of pineapple crown, sisal bagasse and rice bran. These results were comparable for mycelial thickness and the first four substrates were selected for yield determination.

In the evaluation of fruiting bodies yield, the substrates were covered by the mycelium of strain IE-158 in 3 to 4 days, pinhead formations were observed beteween 11 to 16 days, and the first flush was reached between 18 to 35 days. The fastest flush was observed in sisal bagasse and the slowest in bracts of pineapple crown. Two total flushes were produced in 38 to 50 days. The highest yield was obtained on rice straw, with a biological efficiency average of 33.8%, while on coffee pulp the average reached was 15%. The biological efficiencies on sisal bagasse and bracts of pineapple crown were of 7.8 and 6.2%, respectively (**Table II**). Fruiting bodies were morphologically and organoleptically normal and similar to

TABLE I. MYCELIAL GROWTH OF *Volvariella volvacea* STRAINS ON DIFFERENT SUBSTRATES

Code	Substrate	Mycelial thickness	Average number of days to cover the Petri dish	
			IE-106	IE-158
CP	Coffee pulp[1,2]	Thick	6.2	5.2
BP	Bracts of pineapple crown	Thick	8.8	5.8
CS	Corn stover	Thick	11.4	11.6
RS	Rice straw	Medium	7.0	5.2
SB	Sisal bagasse[2]	Medium	9.0	6.2
WS	Wheat straw	Medium	7.0	8.6
CC	Corn cob	Medium	10.6	7.6
CF	Coconut fibre[3]	Medium	-	-
BL	Banana leaves	Thin	7.4	8.4
RB	Rice bran	Thin	7.4	6.4
SC	Sugarcane bagasse	Thin	14.2	8.6

[1] Pinheads formation with strain IE-106 [2] with strain IE-158
[3] The mycelia did not totally cover the Petri dish diameter

those in the wild, with pilei of (26) 40-75(-95) mm diameter and stipes of (24-)30-80(-100) mm long (**Fig. 1**).

The results of chemical analyses of substrates are presented in **table III**. The highest contents of nitrogen and carbon were registered in sisal bagasse and bracts of pineapple crown. The highest ratio in the C/N relation was 79.9 for rice straw and the lowest (33.2) for the sissal bagasse.

DISCUSSION

Five days after inoculation, the strains reached different mycelium sizes according to the substrate (**Fig. 2**). The quickest growth for strains was observed in coffee pulp and rice straw, and the slowest growth development on sugarcane bagasse and coconut fibre. The latter substrate probably had a high content of lignin, since *V. volvacea* does not break down this polysaccharide and depends on hemicellulose and cellulose as sources of energy.

With respect to the basidiomata yield, except with the bracts of pineapple crown, the substrates presented appropriate physical characteristics for mushroom cultivation, as there was good humidity retention. Thus, it supported pinheads formation and reduced damage in basidiomata development. The biological efficiency average in rice straw achieved 33.8% for strain IE-158, which was higher than that recorded by Martínez-Carrera *et al.* (1985), Chua (1976) and Chang (1978), who reported a yield of 11.2 to 28.3%, respectively. In coffee pulp, the biological efficiency average was 15%, comparable to *V. volvacea* cultivated on rice straw (Chang and Miles 1989). This was probably due to the ambient conditions for fructification, which were controlled, particularly temperature and relative humidity. However, in this study the productivity was based on the mature fungus, while in the mentioned bibliography it seemed to be based on young stages of the fungi.

In sisal bagasse and bracts of pineapple crown, the strain only reached biological efficiencies of 7.8 and 6.2% , respectively. The former material registered the lowest C/N ratio (33.2), which seem to indicate that low ratios may affect the mushroom yield.

Contamination was due only to *Trichoderma* and *Neurospora,* as previously cited by Yee and Ho (1980) and Pitakpaivan *et al.* (1991). *Trichoderma* is the most damaging, since its development inhibits the growth of *Volvariella*, because it produces antagonistic metabolites (Rivera-Vargas

TABLE II. FRUCTIFICATIONS RESULTS ON DIFFERENT SUBSTRATES

Substrate	Primordia initation	Flushes		Mushroom fresh weight g	Biological efficiency %
		1st	2st		
Bracts of pineapple crown	15*	27.4*	48.2	18.5	6.2c**
Coffee pulp	14.8	21.6	37	45.3	15.0b
Rice straw	13.4	23	37.4	101.5	33.8a
Sisal bagasse	13	20.8	36.2	23.6	7.8c

* days average
** The letters indicate significant differences to 95%, in accordance with Tukey's Multiple Range Test.

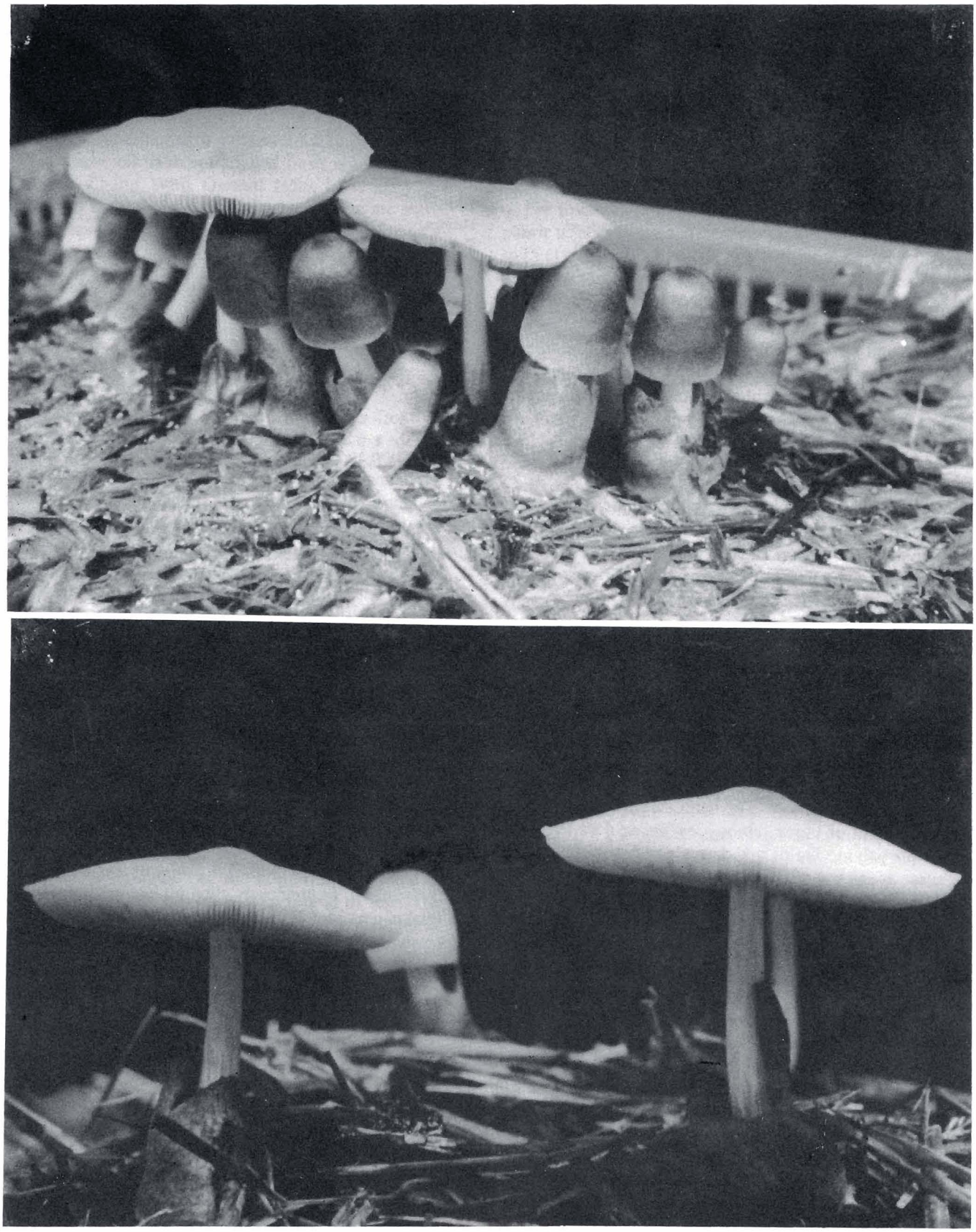

Fig. 1. *Volvariella volvacea* fruiting bodies growing in different substrates. Rice straw and coffee pulp (top and bottom, respectively)

TABLE III. CHEMICAL COMPOSITION OF SUBSTRATES EMPLOYED FOR FRUCTIFICATION STUDY

Substrate	Dry matter g	N g/100	C g/100	C/N g/100
Bracts of pineapple crown	94.54	1.06	52.93	49.93
Coffee pulp	93.22	0.89	43.73	49.13
Rice straw	91.77	0.65	51.95	79.92
Sisal bagasse	90.71	1.58	52.53	33.24

and Hepperly 1977). In this study, coffee pulp was the substrate most affected by contaminants.

The results obtained suggest that the differences in strain yield in the substrates depend on their physical conditions and chemical composition. It is conclusive that *V. volvacea* is a cellulolitic mushroom (Kwang and Chang 1981, Ho 1985), because it required high C/N ratios (Chang-Ho and Yee 1977).

Finally, with regard to the integral use of agricultural by-products and taking rice straw as an example, Mexico has 100,000 ha under rice cultivation which produce about 400,000 tons of fresh straw. According to our results on biological efficiency, 38 tons of fresh mushrooms would be harvested from such a resource. Apart from making rice cultivation more profitable, it would allow biological degradation of the substrate, increasing its digestibility and consequently favouring its reutilization in other biological processess.

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REFERENCES

Chang S.T. (1978). *Volvariella volvacea*. In: *The biology and cultivation of edible mushrooms* (S.T. Chang and W.A. Hayes, Eds.). Academic Press, New York, pp. 573-603.

Chang S.T. and Miles P.G. (1989). *Edible mushrooms and their cultivation*. CRC Press, Boca Ratón, 345 p.

Chang S.T. and Miles P.G. (1991). Recent trends in world production of cultivated mushrooms. Mushroom J. *504*, 15-18.

Chang-Ho Y. and Yee N.T. (1977). Comparative study of the physiology of *Volvariella volvacea* and *Coprinus cinereus*. Trans. Brit. Mycol. Soc. *68*, 167-172.

Chua S.E. (1976). Cultivation of straw mushroom (*Volvariella volvacea*) using different substrates in multi-tiered racks. Mush. Sci. *9*, 701-706.

Guzmán G. (1977). *Identificación de los hongos: comestibles, venenosos y alucinantes*. Limusa, México, 452 p.

Guzmán G., Mata G., Salmones D., Soto C. and Guzmán-Dávalos L. (1993). *El cultivo de los hongos comestibles: con especial atención a especies tropicales y subtropicales en esquilmos y residuos agro-industriales*. Instituto Politécnico Nacional, México, 252 p.

Han Y.H., Ueng W.T., Chen L.C. and Cheng S. (1981). Physiology and ecology of *Lentinus edodes* (Berk) Sing. Mush. Sci. *11*, 623-658.

Ho K.Y. (1985). Indoor cultivation of straw mushroom in Hong Kong. Mush. Newsletter for the Tropics *6*, 4-9.

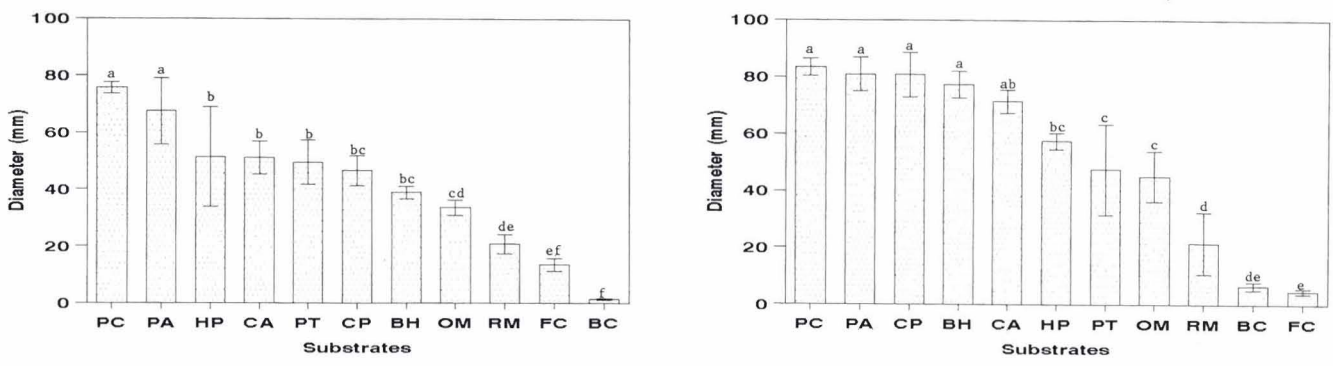

Fig. 2. Average size of the mycelia after five days of the inoculation. Strains IE-106 and IE-158 (left and right, respectively). The substrates names are cited in the **Table I**. The same letter above the columns indicates not significant differences at 0.05 probability level based on Tukey's Multiple Range Test

Kwang H.S. and Chang S.T. (1981). Biochemical studies on cotton waste compost during the cultivation of *Volvariella volvacea*. Mush. Sci. *11*, 585-593.

Li G.S.F. (1984). A chamber for fruiting of *Volvariella volvacea* in laboratories. Mush. Newsletter for the Tropics *4*, 11-14.

Li Y.Y., Wang L., Jia Q.Y., Li J. and Wu Y. (1988). A study on the cultivation of straw mushrooms with wheat straw. Mush. J. Tropics *8*, 67-72.

Martínez-Carrera D., Chang S.T. and Mok S.N. (1985). Cultivation of the edible mushroom *Volvariella volvacea* on the three different composts in Hong Kong. Rev. Mex. Mic. *1*, 227-238.

Martínez-Carrera D., Quirarte M., Sobal M. and Guzmán G. (1986). Estudio comparativo entre cepas mexicanas de *Volvariella bakeri* y una extranjera de *Volvariella volvacea*. Rev. Mex. Mic. *2*, 145-155.

Martínez-Carrera D., Morales P., Sobal M., Chang S.T. and Larqué-Saavedra A. (1991). Edible mushroom cultivation for rural development in tropical America. Mush. Sci. *13*, 805-811.

Mata G. (1992). The optimum percentage of water content in the mycelial growth of *Lentinus boryanus* (Fungi, Basidiomycotina) in five different woods. Crypt. Bot. *2*, 387-390.

Quimio T.H. (1986). Cultivation of *Volvariella volvacea,* the straw mushroom. In: *Guide to low cost mushroom cultivation in the tropics*. University of the Philippines at Los Baños, Laguna, pp. 26-36.

Petcharat V. and Koewthong S. (1990). Fresh water hyacinth and sawdust of rubber logs as substrates for straw mushroom cultivation. Mush. J. Tropics *10*, 65-68.

Pitakpaivan P., Choobamroong W., Sontirat P. and Tontyaporn S. (1991). Study of the species of fungi associated with the cultivation of straw mushroom *Volvariella volvacea* (Bull. ex Fr.) Sing. in Thailand. Mush. Sci. *13*, 385-388.

Rajarathnam S. and Bano Z. (1991). Biological utilization of edible fruiting fungi. In: *Handbook of applied mycology* (D.K. Arora, K.G. Mukerji and E.H. Marth, Eds.). Marcel Dekker, New York, Vol. 3, pp. 241-292.

Rivera-Vargas L.I. and Hepperly P.R. (1987). Assessment of Chinese straw mushroom (*Volvariella volvacea*): fungal competitors on sugarcane bagasse. In: *Cultivating edible fungi, developments in crop science 10* (P.J. West, D.J. Royse, R.B. Beelman, Eds.). Elsevier, Amsterdam, pp. 341-347.

Salmones D., Martínez-Carrera D. and Guzmán G. (1988). Estudio comparativo sobre el cultivo de *Volvariella bakeri* y *Volvariella bombycina* en diferentes desechos agroindustriales. Biótica *13*, 7-16.

Smith J.F., Fermor T.R. and Zadrazil F. (1988). Pretreatment of lignocellulosics for edible fungi. In: *Treatment of lignocellulosics with white rot fungi* (F. Zadrazil and P. Reiniger, Eds). Elsevier, London, pp. 3-13.

Tchierpe M.J. and Hartman K. (1977). A comparison of different growing methods. Mush. J. *60*, 404-416.

Vela R. and Martínez-Carrera D. (1989). Cultivation of *Volvariella bakeri* and *V. volvacea* in Mexico: a comparative study. Mush. J. Tropics *9*, 99-108.

Yee N.T. and Ho T.C. (1980). Interaction between *Volvariella volvacea* and some weed fungi. Trans. Brit. Mycol. Soc. *75*, 498-501.

Zelenak I. (1990). Lignocellulose materials. *In*: *Nonconventional feedstuffs in the nutrition of farm animals* (K. Boda, Ed.). Developments in animal and veterinary sciences, *23*, Bratislava.