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## Cultivation of shiitake mushroom, *Lentinula edodes*, in several lignocellulosic materials originating from the subtropics

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**Abstract** – Mycelial growth of two strains of the mushroom *Lentinula edodes* (IE-40 and IE-105) was evaluated on barley straw, corn cob, corn stover, rice bran, bracts of pineapple crown, coffee husk, sugarcane bagasse and sugarcane leaves. The last four substrates were chosen for mushroom production essays. A mixture of coffee husks, coffee pulp and sorghum grains was used as spawn. The substrates were sterilised and inoculated, under aseptic conditions, in plastic bags. The bags were incubated at 25 °C in darkness. There was no development of primordia in the coffee husks. In the other substrates, primordia were observed between 30 and 62 days. Both strains, IE-40 and IE-105, showed their highest production in sugarcane bagasse, with biological efficiencies of 130–133 %, followed by 83–98 % in sugarcane leaves and 36–37 % in bracts of pineapple crown. (© Inra/Elsevier, Paris.)

*Lentinula edodes* / shiitake / mushroom cultivation / alternative substrates / Mexico

**Résumé.** La culture du shiitake, *Lentinula edodes*, sur quelques matériaux lignocellulosiques originaires des sous-tropiques. La croissance mycélienne de deux souches du champignon comestible *Lentinula edodes* (IE 40 et IE 105) a été testée sur divers substrats : paille d'orge, rafles et paille de maïs, son de riz, bractées de la couronne d'ananas, cosse du grain de café, bagasse et feuilles de la canne à sucre. La meilleure croissance a été obtenue avec les quatre derniers substrats et en conséquence ces substrats ont été utilisés pour des essais de production de champignons. L'inoculum a été produit dans un mélange de cosse du grain café, de pulpe de café et de grains de sorgho. Le substrat, conditionné dans des poches plastiques, a été stérilisé puis ensemencé en conditions aseptiques. Les souches ont été incubées à 25 °C à l'obscurité. Selon les souches, l'apparition des primordia a varié entre 30 et 62 j. Dans le substrat de cosse du grain de café, aucune souche, n'a produit de fructifications. C'est pour la bagasse de canne à sucre que les deux souches ont montré la productivité la plus élevée avec une efficacité biologique de 130–133 %; pour les feuilles de canne à sucre, elle était de 83–98 % et pour les bractées de la couronne d'ananas, de 36–37 %. (© Inra/Elsevier, Paris.)

*Lentinula edodes* / shiitake / culture de champignons / substrat alternatif / Mexique

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## 1. INTRODUCTION

The popular Japanese shiitake, *Lentinula edodes* (Berk.) Pegler, is the second most important mushroom among the species industrially cultivated, with about 17 % of total production, and showing an increase of 110 % between 1990 and 1994 [3]. Traditionally, shiitake has been cultivated on oak logs [4, 13], but recently there is a trend to cultivate it on sterilised or pasteurised substrates in order to increase yield and reduce the time of its culture cycle [7, 17, 27, 28]. Shiitake is produced mainly in China and Japan (more than 93 %) on oak logs or sawdust but other countries are also initiating production or experimenting with *L. edodes* farming [4]. Interest in shiitake cultivation has recently augmented due mainly to 1) high values on the international market, 2) an increase in importation of dried mushrooms by Japan, its main consumer (in 1990 over 2 000 metric tons), and 3) antitumoral effects, attributed by various authors to this mushroom [5, 9, 25].

Mushroom cultivation has received comparatively little attention in most developing countries especially in tropical regions, where millions of tons of lignocellulose-rich wastes are unused [26]. In Mexico most of the studies about shiitake have been directed towards finding alternative substrates, and they include: sawdust from different tree species [21–24], tequila plant bagasse and sugarcane bagasse [8] and coffee pulp [19]. The aim of the present study was to evaluate for shiitake cultivation some abundant lignocellulosics by-products in Mexico and other subtropical regions.

## 2. MATERIALS AND METHODS

### 2.1. Strains

The strains of *L. edodes* are deposited in the Fungi Strain Collection of the Institute of Ecology (Xalapa, Mexico). Strain IE-40 (= LC-35) was donated by Dr S.T. Chang, University of Hong Kong and strain IE-105 (= CS-2) was acquired in Fungi Perfecti (Washington,

USA). The strains were maintained on potato dextrose agar at 25 °C.

### 2.2. Mycelial growth on the substrates

Eight agroindustrial wastes were tested: barley straw, bracts of pineapple crown, coffee husk, corn cob, corn stover, rice bran, sugarcane bagasse and sugarcane leaves. These lignocellulosic materials were chosen due to their easy availability in the tropical and subtropical regions (in Mexico). All materials were fragmented into small pieces of 5 mm approximately and oven dried (65 °C) for 24 h. Prior to mycelial growth evaluation, samples of 5 g (by quintuplicate) of dry substrates were hot water-soaked for 12 h, drained and weighed in order to determine their capacity of water absorption. By differences between dry and wet weight, percentage of humidity in the substrates was adjusted to 80 %. Then, samples of substrates equivalent to 7 g of dry weight were placed on 90 × 15 mm Petri dishes. Five replicates were prepared by each substrate and sterilised at 121 °C for 1 h. When the samples cooled down they were inoculated at the centre of Petri dish with an inoculum disc (0.5 cm) taken from cultures on malt agar. The inoculated substrates were incubated at 25 °C in darkness. The mycelial size was measured by two diameters of the colony, one taken perpendicularly from the other after 14 days of incubation. The four best substrates for mycelial growth of the strains were chosen for mushroom production essays at pilot plant level.

### 2.3. Mushroom production

#### 2.3.1. Spawn preparation

A mixture of coffee husks, coffee pulp and sorghum grains, equivalent in dry weight of 15:15:70 % was used as spawn. Each material was soaked separately in water for 12 h, allowed to drain and then thoroughly mixed. Samples of 150 g (wet weight) of the mixture were put into plastic bags and sterilised at 121 °C for 1 h, cotton plugs were sterilised separately. After cooling, the mixture was inoculated with a piece of cultures on malt agar ( $\pm 1 \text{ cm}^2$ ) and then the bags with cotton plugs were incubated at 25 °C for approximately 3 weeks.

#### 2.3.2. Culture conditions

According to the results of mycelial growth on different substrates, coffee husks, bracts of pineapple crown,

sugarcane bagasse and leaves were used in order to obtain sporocarps. Sugarcane leaves and bracts of pineapple crown were cut into small lengths of 5 cm and dried, the former under the sun and the latter in an oven at 50 °C. Sugarcane bagasse and coffee husks did not require fragmentation and they were only dried in the sun. Seven samples (200 g) of each dry substrate were rehydrated with hot water until reaching 80 % humidity, placed in plastic bags and sterilised at 121 °C for 1 h, following the method of Auetragul [1], adapted by Guzmán et al. [10]. The bags were cooled down and then inoculated with 150 g of spawn and incubated at 25 °C in darkness. When the mycelium had completely covered the substrates, forming a dark coffee coloured crust, the plastic bags were removed and the samples placed in the following environmental conditions: temperature 16–30 °C, relative humidity 82–86 % and natural light 12 h per day. Fruit body production was expressed as a percentage of biological efficiency (fresh weight mushrooms/dry weight substrates). The fruit bodies were classified into three groups by pileus diameter, following the method of Mata and Guzmán [20]. The groups were, G1: less than 5 cm, G2: 5–9.9 cm and G3: 10 cm and above.

Proximate analysis of substrates before colonisation by mycelia and sporophores collected during the first flush from each substrate, were determined according to the method described by Calvo-Carrillo and Morales de León [2]. Samples were tested by triplicate and the analyses included moisture, ash, crude protein, crude fat, carbohydrates and crude fibre contents.

## 2.4. Experimental design and statistical analysis

The mean and standard deviation of mycelial size at 14 days and harvested sporophores data were calculated, significant differences were estimated using the Tukey's multiple range test with  $P < 0.05$ . During incubation and fruiting, the samples were arranged in a randomised complete plot design.

## 3. RESULTS AND DISCUSSION

### 3.1. Mycelial growth on the substrates

The results of mycelial growth of strains on different substrates are presented in *table I*. Fourteen

**Table I.** Comparison of mycelial growth of *Lentinula edodes* strains (IE-40 and IE-105) on different substrates.

Substrates	Diameter (mm) of mycelia <sup>1</sup> 14 days after inoculation	
	IE-40	IE-105
Barley straw	5.5 ± 0.57 e <sup>2</sup>	5.2 ± 0.57 c
Bracts of pineapple crown	74.3 ± 8.45 ab	50.1 ± 27.55 b
Coffee husk	61.6 ± 17.55 b	56.3 ± 5.35 b
Corn cob	41.2 ± 3.76 cd	22.4 ± 3.50 c
Corn stover	24.3 ± 6.50 de	26.6 ± 7.27 c
Rice bran	8.3 ± 1.76 e	10.4 ± 3.62 c
Sugarcane bagasse	89.6 ± 0.69 a	89.4 ± 0.84 a
Sugarcane leaves	59.6 ± 3.71 bc	51.8 ± 4.14 b

<sup>1</sup> Mean of ten replications.

<sup>2</sup> Different letters in the same column indicate significant differences ( $P < 0.05$ ) according to Tukey's multiple range test.

days after the inoculation, sugarcane bagasse supported the fastest mycelial growth for both strains, the mycelia covered the total diameter of Petri dishes in less time than registered by *L. edodes* growing in *Quercus* and *Carpinus*, the common sawdusts utilised for commercial production of shiitake mushroom [11, 18]. Bracts of pineapple crown, coffee husks and sugarcane leaves also supported good growth. On the contrary, mycelial growth was low on rice bran and barley straw. In general, higher mycelial size was registered for IE-40 than for IE-105. According to the statistical analysis, sugarcane bagasse, bracts of pineapple crown, coffee husks and sugarcane leaves were chosen for mushroom production.

### 3.2. Mushroom production

Both strains did not develop mushrooms on coffee husk substrate, although the mycelium showed good growth. Therefore, only the results of three substrates are presented.

Strain IE-40 required 27–32 days of incubation and the primordia appeared between 30 and 59 days, while strain IE-105 required 31–38 days for

incubation and primordia developed between 47 and 73 days. In both strains, the best substrate colonisation and earliest primordia formation were observed on the sugarcane bagasse (*table II*). With the exception of the strain IE-105 on sugarcane leaves, the time of primordia appearance was less than those previous studies reported from Mexico [21–23]. However, taking account of the high rate of spawn used in this study (between 12.5 and 15 % depending on the substrate) which was higher than the recommended rate for sterile substrates [14, 15],

mycelia colonisation and precocity in primordia formation could be related to number of inoculation points into the substrate.

The number of flushes varied with strains and substrates, the strain IE-40 produced 2–4 flushes and strain IE-105 3–4 flushes; the highest number of flushes was obtained in sugarcane bagasse. Both strains showed their highest biological efficiency on sugarcane bagasse, followed by sugarcane leaves and pineapple crown bracts (*table III*). There were

**Table II.** Spawn run and sporophores development time (days after spawning) for two strains of *L. edodes* on three different substrates.

Strains	Substrates <sup>1</sup>	Time of first development	Time of flushes			
			1st	2nd	3rd	4th
IE-40	SB	30–32	38–41 <sup>2</sup>	64–66	87–108	117–121
	SL	54–57	61–64	79–110	105–133	–
	BP	47–59	54–75	95–97	–	–
IE-105	SB	47–57	54–66	86–91	113–135	156–163
	SL	69–73	75–79	97–106	113–135	146
	BP	49–60	61–78	77–79	93–96	–

<sup>1</sup> SB: sugarcane bagasse; SL: sugarcane leaves; BP: bracts of pineapple crown.

<sup>2</sup> Days of spawn running required for collecting the sporophores.

**Table III.** Cumulated mushrooms yield and biological efficiency (from seven samples) of two *Lentinula edodes* strains cultivated on different substrates.

Strains	Substrates <sup>1</sup>	Fresh weight (total) of mushrooms by flush (g)				Total fresh weight (g)	BE <sup>2</sup>
		1 <sup>st</sup>	2nd	3rd	4th		
IE-40	SB	442.4	1 112.9	241.2	26.8	1 823.3	130.2 a <sup>3</sup>
	SL	424.9	498.8	235.4	–	1 159.1	82.7 b
	BP	470.6	55.0	–	–	525.6	37.5 c
IE-105	SB	602.6	1 031.6	164.2	69.2	1867.6	133.4 a
	SL	765.9	492.9	86.2	24.3	1367.3	97.8 b
	BP	328.1	37.4	8.7	–	374.2	36.3 c

<sup>1</sup> SB: sugarcane bagasse; SL: sugarcane leaves; BP: bracts of pineapple crown.

<sup>2</sup> Biological efficiency average (%) of seven blocks.

<sup>3</sup> Different letters in the same column indicate significative differences ( $P < 0.05$ ) according to Tukey's multiple range test.

**Table IV.** Proximate composition of *Lentinula edodes* sporophores cultivated on different substrates.

Composition	Strain IE-40			Strain IE-105		
	SB	SL	BP	SB	SL	BP
Moisture	89.2 <sup>1,2</sup>	87.3	83.8	92.3	88.3	83.9
Protein crude (N x 4.38)	13.8	14.4	14.0	13.1	14.4	14.0
Fat crude	1.0	0.9	0.5	0.9	0.7	0.5
Carbohydrates	73.0	78.2	78.8	78.9	78.5	78.5
Ash	6.2	6.5	6.7	7.1	6.4	7.0

<sup>1</sup> All data are presented as percentage of dry weight, except moisture (percentage of fresh weight).

<sup>2</sup> Mean of three replicates.

SB: sugarcane bagasse; SL: sugarcane leaves; BP: bracts of pineapple crown.

significant differences in mushroom production between substrates, but there was no difference between the strains. It is important to state that the biological efficiencies registered on sugarcane bagasse and leaves were higher than those previously reported for *Carpinus* and *Quercus* sawdust, tequila maguey bagasse and coffee pulp [8, 19, 21–24].

With reference to basidiome size, the predominant group in the strain IE-40 grown on sugarcane bagasse and sugarcane leaves was G<sub>2</sub> (57 and 63 % of total fruiting bodies harvested, respectively) and G<sub>3</sub> in pineapple crown bracts (45 %). In strain IE-105, G<sub>2</sub> was the main group in sugarcane bagasse and pineapple crown bracts (41 and 53 %) and G<sub>3</sub> in sugarcane leaves. Therefore, nearly 75 % of the mushroom production presented pileus with a diameter of 5–10 cm.

The average values of proximate composition of sporophores growing on different substrates were similar and fluctuated between: 83.8 and 92.3 % of moisture, 13.1 and 14.4 % (dry weight) of crude protein, 0.5 and 1 % of crude fat, 73 and 78.9 % of carbohydrates and 7.4 and 12 % of crude fibre (table IV); therefore it confirmed that *L. edodes* is an appropriate source of protein that can be used as complementary in the daily diet. The values registered agree with Crisan and Sands [6]; however, the fluctuations in the percentages depend more on the analytical process and precision of tests, than the proximate composition of the fruiting bodies.

**Table V.** Proximate and chemical composition of lignocellulose materials used.

	Proximate composition			
	CH	BP	SL	SB
Moisture	9.3 <sup>1,2</sup>	11.1	8.6	5.6
Crude protein	5.6	8.7	2.1	2.0
Carbohydrates	92.6	81.3	85.5	93.4
Crude fat	0.8	2	0.3	1.2
Crude fiber	59	25.2	39	36
Ash	1	8	12.1	3.4
	Chemical composition			
	Nitrogen	Carbon	C/N ratio	pH
Nitrogen	0.9 <sup>3</sup>	1.4	0.3	0.3
Carbon	53.5 <sup>3</sup>	51.3	44.6	51.1
C/N ratio	59.4	36.6	131.1	159.6
pH	5.6	6.4	6.0	4.5

<sup>1</sup> All data are presented as percentage of dry weight, except moisture (percentage of fresh weight).

<sup>2</sup> Mean of three samples.

<sup>3</sup> Percentage of dry weight.

CH: coffee huks; BP: bracts of pineapple crown; SL: sugarcane leaves; SB: sugarcane bagasse.

On the other hand, by comparing the proximal composition and pH of the substrates with the yield of the strains (table V), sugarcane bagasse and sugarcane leaves appeared as the most adequate for shiitake cultivation, since their C/N ratios agree with the recommended values [16]. The bracts of pineapple crown registered a high content of nitrogen and

pH near neutrality. In addition, the open physical structure of this residues, cannot provide support for the mycelium and affect the normal development of the fructification. In reference to coffee husks, although the chemical composition was appropriate for *L. edodes* cultivation, the presence of some phenolic compounds (not determined in this study) could be detrimental to the formation and development of the fructifications. On coffee husks both strains showed a good mycelial colonisation; however, this substrate was very susceptible to drying, which affected sporophore formation.

The present data show that both sugarcane bagasse and sugarcane leaves are potential substrates for shiitake cultivation, since the strains studied presented the highest production of sporophores in these materials. In addition, the utilisation of these residues offers the following advantages: 1) alternative uses of abundant lignocellulosic wastes in Mexico where approximately 18 million tons are produced annually [12], and also in other tropical or subtropical areas; 2) increased profits from sugarcane cultivation, since two of its by-products would have additional uses; and 3) reduction in the cropping cycle of shiitake in comparison to other forest wastes products. However, it is necessary to carry out a specific study to compare shiitake production on sugar cane wastes and wood sawdust, using the same culture conditions.

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