MAGUEY MUSHROOM: AN EDIBLE SPECIES CULTIVATED FOR THE FIRST TIME

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ABSTRACT

Saprophytically in the pulque maguey, a mushroom called "menanacatl" (metl = maguey, nanacatl = mushroom) grows and produces fruiting bodies in summer with increasing moisture and heat. The aim of this study is to obtain fruiting bodies of the IE-771 strain isolated from pulque maguey in Alchichica Puebla, Mexico, cultivated in a mixture of agave bagasse and barley straw 1:1 (AB-BS 1:1), barley straw (BS) as the control, as well as to identify them morphologically and determine their proximate composition. To achieve this goal, the optimal fermentation time for mycelium growth on pulque maguey bagasse was previously established and the result was 7 d. Based on this data pulque maguey bagasse fermented for 7 d and barley straw for 3 d were mixed 1:1 on a wet basis. After 30 d of incubation, they were moved to the production room for their fructification. The biological efficiency (BE) and production rate (PR) were evaluated using a wild strain (IE-771) on the two substrates. The BE and PR on barley straw reached 44.95 % and 0.270 respectively whereas the BE and PR on agave bagasse-barley straw was 62.45 % and 0.378 respectively. The mushroom obtained was identified as *Pleurotus dryinus* and its approximate analysis is within the parameters recorded for this species except for fat and crude fiber which indicated higher values.

Key words: Agave wastes, *Pleurotus dryinus*, Mexico, edible mushroom cultivation

INTRODUCTION

One of the most notable plants found within the Mexican landscape is the maguey or agave (*Agave* spp.) appearing in arid and semiarid regions of the country. *Agave* spp. are xerophytic plants adapted to live in bad weather conditions with long periods of drought and strong temperature fluctuations between day and night [1]. From around 200 species of the genus *Agave* that exist in America, 150 of them are to be found in Mexico distributed mainly in the central part of the country [2]. Agaves in Mexico have had and still have great economic and cultural importance for many people. They have been used for at least 5000 years, making Mexico the center of domestication and diversification through human selection [3]. Agaves are used as a source of food, drink (tequila, mezcal, pulque), medicine, fuel, shelter, ornament and are a source of tough fibers, extracted from the leaves (ixtle), as fertilizer, building material and in the development of agricultural implements among other uses. Of the drinks made with this plant, pulque is considered the oldest and most traditional and was used by pre-Hispanic priests in ritual ceremonies as witnessed by numerous manuscripts and is still currently produced [2]. "Agua miel" is the liquid sap obtained from the maguey *Agave salminana* which is the ingredient for making pulque that contains a moderate amount of alcohol. Pulque had its heyday in the nineteenth century with the use of rail transport, as due to its perishable nature, it could not be drunk in places remote from centers of production [4]. Its preparation continued and it was considered a profitable industry in Mexico during the first 70 years of the XX s [5]. Later, as a result of a complex interaction of economic, technological and social variables, its

demand decreased by 60% and was replaced by beer that could be stored longer and was economically viable, healthy and hygienic [5,6,7]. This has resulted in a drastic reduction in the cultivation of pulque maguey which, associated with the faults of genetic improvement and overexploitation could in the future lead to its extinction [8]. This would also affect the survival of pulque maguey mushrooms that grow saprophytely between the leaves "pencas" of the plant, feeding on the lignocellulosic residues on dead leaves. On the other hand, the mushroom is also edible and has been collected from the wild for consumption since ancient times. Because of this, the aim of this study is to identify taxonomically the maguey mushroom and obtain fruiting bodies, through their culture using an agave bagasse- barley straw mixture 1:1 ratio with barley straw as the control, and obtain the approximate composition of the cultivated mushroom.

MATERIALS AND METHODS

Strain and spawn preparation. The strain used in this study was obtained from a mushroom collected from pulque maguey in Alchichica Puebla, Mexico (19° 24' N and 97° 23' W) (G. Mata collection No. 728) and maintained in liquid nitrogen in the Strain Collection of the Institute of Ecology AC Xalapa, Mexico, registered as IE-771. Agar inoculum was made on potato dextrose agar medium (PDA), supplemented with wheat extract according to Guzman *et al*. (2002) [9], sterilized for 20 min at 121 °C and 15 psi, placing 25 ml in Petri dishes of 90 mm ø. The medium was inoculated with the strain and incubated at 28 °C for one month and then kept refrigerated at 4 °C. To prepare the primary spawn or master, grain sorghum was used (*Sorghum vulgare* Pers), placing 50 g of sorghum and 40 g of water in glass jars, sterilized for 1 h at 121 °C and 15 psi, reaching a relative humidity of 50%. To make grain spawn, 1 cm^2 of mycelium of IE-771 was transferred in a laminar flow hood and incubated for one month at 28 °C in darkness. The secondary spawn was prepared using sorghum seeds previously soaked in water for 24 h. 175 g of sorghum was placed into plastic bags and sterilized for 1 h at 121 °C and 15 psi. After cooling, 25 g of primary spawn were transferred into new bags with sterilized sorghum seeds. The bags were incubated in a dark room at 28 °C until the mycelium fully covered the grain and then kept stored under refrigeration (5 °C) in dark conditions. To reactivate the spawn before use in the inoculation of the substrate, the bags were incubated at 28 °C for 12 to 24 h [9].

Cultivation. We used the mixture agave bagasse-barley straw 1:1 and barley straw as the control. The barley straw was cut into 5 cm, and then it was soaked for 24 h and fermented for 3 d at room temperature. For use as agave bagasse, maguey leaves were sun-dried, soaked for 24 h and fermented at room temperature for 7 d [10] and cut into pieces of approximately 5 cm. Agave bagasse and barley straw were mixed in proportion 1:1 (w / w). The substrates were pasteurized for 18 h using steam at 65 °C [11] and cooled at room temperature before inoculation. Then the substrates were placed in polyethylene bags (60 x 40 cm) by mixing the spawn 5% (w / w), up to 4 kg. The inoculated bags were incubated at 28 ° C in total darkness. On the second day of incubation the bags were cut with a knife to allow the mycelium to breathe (12 in total). The bags were kept in the incubation area until completely covered with mycelium and primordia appeared. At the end of incubation period, the bags were transferred to the production room with light and dark periods of 12/12 h at 23 °C and a relative humidity of 80-85%, to encourage the development of fruiting bodies. Once collected, the fruiting bodies were cut, weighed and measured manually, 4 crops were evaluated in each substrate. The productivity of the strain was analyzed using as parameters the Biological Efficiency (BE) = g

fresh fruiting bodies /100 g dry weight based substrate [12] and precocious using Rate Production (RP) = BE / total number of days evaluated from the day of incubation.

Statistical analysis. 24 replicates were used with the mixture of agave bagasse, barley straw 1:1 and 13 replicas with barley straw. The data obtained was analyzed using an analysis of variance (ANOVA) to determine significant differences in both substrates and when a significant difference was found, an average comparison Tukey's multiple range test at 0.05% significance was applied, using the Statistica software version 7.0.

Morphological identification. For microscopic observation, radial tangential and transverse hand cuts were made to the basidiomas with a razor. Mounted in 70% alcohol and then in a solution of KOH 5% and 1% phloxine. The size and shape of spores were observed and sections were observed under a microscope using Melzer's reagent [13].

Proximate analysis. The proximate composition of cultured mushroom was determined according to the Association of Official Analytical Chemists methods (AOAC) [14]. The moisture content was determined through oven drying at 105 °C for 24 h. The ash content was determined by incinerating at 600 °C in a muffle furnace for 6 h or until pale gray or white ash was obtained. The total protein content was determined by the Kjeldahl method using a conversion factor of 4.38 [15]. The fat content was determined by the Soxhlet method and dietary fiber by the enzymatic gravimetric method. The carbohydrate content was calculated by difference [16].

RESULTS AND DISCUSSION

Environmental conditions of wild maguey mushroom. The Climate of the town of Alchichica, where the wild mushroom grows (Fig. 1) is semi-dry with summer rains. The annual average temperature reported is 12.9 °C, June being the hottest month with 15.4 °C average temperature and the coldest being January with 9.2 C. Total annual precipitation is 372.0 mm and the rainfall is concentrated in June to 76.5 mm on average, and January is the driest month with 5.0 mm. [17].

Substrate conditioning. For substrate preparation we based ourselves on previous research employing grown *Pleurotus ostreatus* using tequila agave bagasse obtained from the central part of the agave called the "piña" [18]. Continuing with the same study using the same mushroom, it is recommended that the agave bagasse used as substrate is fermented in order to obtain the softening of the fiber and when mixed with wheat straw, it increases the absorption of water, retains moisture better, reduces the presence of contaminants and forms a better consistency [19]. This is confirmed by the results obtained by Bernabe-Gonzalez, *et al*. [20], where they grow *P. pulmonarius* in mezcal maguey bagasse (*Agave cupreata* L.) fermented for 7 days and mixed with rice straw in ratios of 2:1 and 3:1, where they obtain a BE of 111% and 120% respectively. Given these considerations, we measured the mycelial growth of the strain IE-771 in agave bagasse obtained from the leaves, fermented for different times (0, 3, 5 and 7 d), reporting the best growth at 7 d of fermentation (data not shown). Then we proceeded to find the right mix of agave bagasse- barley straw in order to find the best BE with mixtures of agave bagasse-barley straw 1:0, 1:1, 1:3 and 0:1 in a previous study (data not shown). In each of the mixtures we used maguey bagasse fermented for 7 d and barley straw for 3 d at room temperature finding that the BE was the best for mixing agave bagasse-barley straw 1:1 ratio.

Figure 1: Maguey mushroom from Alchichica Puebla, Mexico. It can be observed that the mushroom grows on the dead leaves of the maguey ("pencas").

The production of fruiting bodies. The time between substrate inoculation to the primordia appearance in both AB-BS 1:1 and BS was 30 d, and later in the production room, the appearance of fruiting bodies took between 10 and 12 d. The highest BE recorded was obtained in the mixture AB-BS 1:1 (Table 1). This is consistent with the results obtained by mixing cereal straws with other agro-industrial wastes [19.20]. The PR is also higher in the AB-BS 1:1 mixture, although the values obtained in this research are much lower than those reported using mixtures of straw with agro-industrial waste, where the PR is 0.8 to 3.41 range [20]. With regard to crops, stocking grams obtained in each of the 4 flushes show no significant differences between the two substrates used AB-BS 1:1 and BS (Table 1).

Table 1: Productivity (g) of the strain IE-771 in two substrates

			Crops			
Substrate	BE [1]	PR [2]	1st	2nd	3rd	4th
AB-BS 1:1	64.2[a]	0.378[a]	226.1[a]	176.1[ab]	169.6[ab]	163.3[ab]
BS	45.0[b]	0.270[b]	186.8[ab]	108.3[b]	75.1[b]	130.3[b]

[1] Biological efficiency (%), fresh mushrooms (g) / dry weight of substrate (g). [2] Production Rate (%) (BE / total number of production days, from inoculation). Means in a column with different letters are significantly different ($p < 0.05$, Tukey).

For the analysis of group size, the averages were obtained by dividing the total weight of the mushrooms in a group by the total number of fungi of the same group. The results (Table 2) show no significant differences among the same group size in both substrates. We could not compare the weight of the pileus obtained because research consulted on the size measure only diameter but not the average weight per group. [21]. However, the size-weight data may prove valuable for producers looking to improve the quality of their crops or for their taxonomic identification. In addition, this mushroom has organoleptic qualities very acceptable to the consumer as the color and turgidity can be maintained under refrigeration for at least 2 weeks, its smell is pleasant, unlike those reported for European species [22].

Table 2: Average production (g) of the mushrooms by group size diameter of pileus

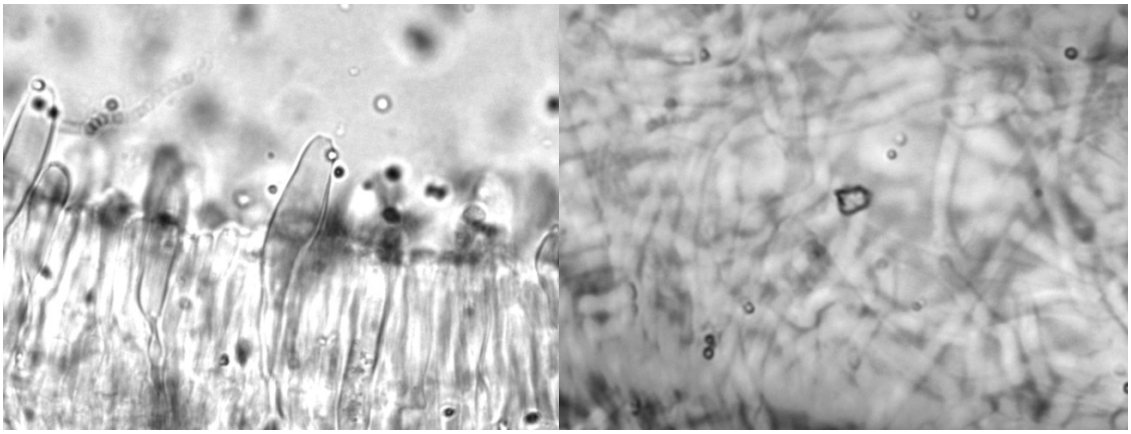
	Production by size group				
Substrate	G1(0-4.9cm) [1]	G2(5-9.9 cm)	G3(10-14.9 cm)	G4(15-19.9 cm)	G5(>20 cm)
AB-BS 1:1 [2]	9.2[d]	25.6[c]	58.5[b]	105.5[a]	181.1
BS [3]	7.5[d]	20.2[cd]	54.4[b]	97.3[a]	-------

[1] G1-G5 correspond at mean diameter of pileus. [2] Agave bagasse-barley straw 1:1. [3] Barley straw. Means in a column with different letters are significantly different ($p < 0.05$, Tukey.

Morphological identification. In the macro-morphological description, fruiting bodies grown from mycelium of the strain IE-771 obtained from a maguey mushroom were observed for the first time. Morphologically, there is a viscid pileus, flat in the center to fibrillar on the periphery, creamy yellow, clear in the center to dark on the periphery with white flakes of 10 cm in diameter. De-current white lamellae. White stipe solid to semi hollow, fleshy to leathery down, covered with fine hairs or hirsute at the base, which is a little wider, measuring 6 to 8 cm long and 1 to 1.5 cm wide (Fig. 2). Veil well developed in the primordial and there remains a delicate ring but it is thick and fibrillar in the apical part. All white (Fig. 3). Micromorphological descrpción spores were found of (12.5-) 13-14 (-15) x 3.5-4 (-5) µm, cylindrical to ellipsoid, smooth thin wall. Basidia of (40-) 42-44 (-46) x 6-7 (-8) µm, cylindrical to slightly clavate, quadrispore of thin wall (Fig.4). Cystidium elements of 42-52 x 7-7.5 µm, clavate, hyaline, thin wall, basidioles protruding slightly. Absent cheilocystidium or similar to basidioles. Hyphal system dimitic with skeletal hyphae (Fig. 5). Generative hyphae of 3.5- µm, thin wall, and occasionally branched. Scarce eskeletic hyphae, of 5 µm, thick wall. The veil has generative hyphae of 3.5-4 (-4.5) µm, hyaline, wall thickened slightly thicker. The above descriptions are consistent with *Pleurotus dryinus* (Pers.:Fr.) P. Kummer [23, 24, 25] with which different authors [23, 25] mention that the stipe is velvety with pubescent villose towards the base. *Pleurotus dryinus* has been identified in Asia (Siberia, Russia), Europe (Norway, Netherlands, Germany, Italy, Spain, Austria, Croatia, Slovakia), Africa (Egypt) and North America [22]. Recently in Georgia, Russia, *P. dryinus* has been distinguished among other white rot basidiomycetes due to the high activity of lygnocellulolytic enzymes identified in its mycelium [26].

Proximate analysis. Edible mushrooms are considered healthy foods because they are rich in protein and vitamins and low in calories and fat [27, 16]. The protein content is higher than for many vegetables, but less than in meat and milk [28]. For the present study, despite the fact that maguey mushrooms have been consumed since ancient times in Mexico collected from the wild, as yet their nutrient composition has not been studied (Table 3). In general, in the case of *Pleurotus* the protein content is reported in a range of 17.8 to 53.3%, the fat content varies from 1.1 to 11.7%, and its ash content is reported in a range from 6.7 to 15.4% [29]. It can be seen that the percentage of protein, fat and crude fiber, is within the ranges reported for this genus, but the ashes reported in this study are below the lower limit. Other authors place the range of proteins in *Pleurotus* between 18 and 35% and fat content from1 to 2.4% [30]. Based on these criteria, the fat content of the mushroom is above the upper limit. In the case of the crude fiber reported for *P. columbinus* and *P. pulmonarius* 7.57% and 11.7% are reported respectively in the fruiting bodies [15], and in our analysis there is a higher fiber content of 23.93% perhaps because we used the full mushroom and stipe was very fibrous.

Figures 2 - 3. Fruiting bodies of the strain IE-771 obtained in barley straw. **2:** Remnants of the veil on the edge of the pileus and the delicate ring on the stipe. **3:** Primordium showing the veil and the ring.



Figures 4 - 5. Microscopic characteristics of *Pleurotus dryinus* obtained from the strain IE-771. **4:** basidia and basidioles. **5:** hymenophoral trama (100 x).

Table 3: Proximal analysis in dry basis of *P. dryinus* grown in AB-BS 1:1

Proximal Analysis	
Crude Protein	27.00 %
Fat	2.45 %
Crude Fiber	23.93 %
Ash	6.13 %
Carbohidrate	40.49 %

CONCLUSIONS

Maguey mushrooms were cultivated for first time using mixtures of maguey leaves bagasse and barley straw and achieved a better BE than using barley straw only. However, PR values show very low precocity. According to the taxonomic study of strain IE-771 it was identified as *Pleurotus dryinus* (Pers.: Fr) P. Kummer, pending confirmation by molecular analysis. An proximate analysis shows that the mushroom has a high content of fiber and fat in

relation to other species of the genus. In agave bagasse-barley-straw 1:1mixture some mushrooms showed pileus of more than 20 cm in diameter.

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