Biological efficiency and nutritional composition of *Pleurotus djamor* cultivated on bagasse of *Agave salmiana*

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Abstract

The objective of the present study was to use molecular techniques to identify a wild mushroom isolated from *A. salmiana*, and then evaluate its biological efficiency, production rate, and nutritional and morphological characteristics when grown on *A. salmiana* bagasse with various concentrations of urea as a source of nitrogen. Two types of inoculum were employed: in grain (WG) and pellet (WP) form. The substrate was supplemented with total nitrogen concentrations (TN) of 0.77, 0.95, 1.14, 1.32, and 1.5% to evaluate its effect on the biological efficiency (BE), production rate (PR), and morphological and nutritional characteristics of the fruiting bodies. The molecular analysis of the ITS region permitted the amplification of a product of 750 pb. The mushroom was identified as *Pleurotus djamor*. After supplementing the substrate with urea, a BE of 70% was obtained in the sample inoculated with WG at 1.32% TN. Observations found that the TN concentration of 1.5% produced malformations in the fruiting bodies. The analysis of the sporocarps indicated a raw protein content (RP) of 15–26% and that the mushroom's nutritional composition changed according to the inoculum utilized and the percentage of nitrogen in the substrate. This is the first report on the isolation of *P. djamor* on *A. salmiana* as an atypical substrate, and so represents an opportunity for further study and commercialization. To its chemical composition and high availability, *A. salmiana* bagasse is a suitable alternative substrate for cultivating edible mushrooms, specifically *P. djamor*.

Introduction

Cultivation of edible mushrooms has increased worldwide in recent years due to their culinary characteristics, health benefits, biological activity (antioxidant, antihypertensive, anticancerogenic), and multiple biotechnological and environmental applications, among other reasons. The genus *Pleurotus* is the second-most widely cultivated mushroom on the planet with an estimated production of 6.46×10^9 kg (Arango and Nieto 2013; Sardar et al. 2017; Royse et al. 2017). Some 50 species of this genus have been reported. Its cultivation requirements and nutraceutical and biodegradative properties have attracted interest, and it is now a promising agroindustrial product with expanding cultivation in Latin America and other areas of the world (Royse and Sánchez 2001; Barba et al. 2016; Salmones 2017).

In Mexico, the species with the highest production volumes are *Pleurotus ostreatus*, *P. djamor*, and *P. pulmonaris* (Salmones 2017). They are cultivated on a broad range of agroforest by-products: rice chaff, oats, wheat, cotton, palm oil, scrub, sawdust, pulps, fruit peels, jute waste, bagasse, and other kinds of chaff, among others (Bellettini et al. 2019), all of which are rich in the carbon, nitrogen, sulfur, and phosphorus necessary for the development of mushroom biomass (Gregori et al. 2007). *Agave* bagasse has these same properties, and estimates indicate that this material is produced at a rate of 4'709,000 tons per day. Accumulation of this bagasse causes environmental problems and contributes to the proliferation of rodents, insects, bacteria, and fungi (Gallardo 2018). It has been proposed as an alternative substrate for growing mushrooms (Heredia-Solís et al. 2014; Heredia-Solís et al. 2017), but more studies are required to improve cultivation and production conditions.

Due to the genus *Pleurotus*' ability to grow in diverse substrates, it has been found on agave plants where it develops mainly on shoots near the ground around the base of plant, and in the cavity of exhausted magueys. It has been identified as *P. opuntiae*. This genus, however, is subject to controversy because not all the mushrooms that grow on these plants correspond to this species. In these circumstances, molecular studies are highly useful (Granados 1993; Barrales and Mata 2016; Heredia-Solís et al. 2017; Zervakis et al. 2019).

The objective of the present study was to use molecular techniques to identify a wild mushroom isolated from *A. salmiana*, and then evaluate its biological efficiency, production rate, and nutritional and morphological characteristics when grown on *A. salmiana* bagasse with various concentrations of urea as a source of nitrogen.

Materials And Methods

Isolation and cultivation

The wild mushroom was isolated from a fruiting body that developed on *A. salmiana* in the municipality of Chilcuautila, Hidalgo, Mexico (20°20'00"N 99°14'00"Or). The hymenia were cut in 1-cm² fragments, subjected to treatment (5 min in sterile distilled water, 5 min in a hypochlorite solution at 5%, and washing with sterile water), and then seeded on plates in potato dextrose agar (PDA) medium for 7 days at 28°C until the mycelium characteristic of the mushroom was obtained. Re-seedings were done periodically to achieve purification.

Molecular identification

DNA extraction

The mushroom's genomic DNA was obtained from 0.750-g pellets recovered by filtration, frozen, and then lyophilized from a liquid culture in Kirk medium for 7 days, utilizing the protocol proposed and modified by Huanca-Mamani et al. (2014). The concentration and purity of the

DNA were measured in a NANODROP 2000, and integrity was verified by electrophoresis in agarose gel at 1% (30 min, 80V, electrophoresis chamber).

Amplification and sequencing

The ITS1-5.8-ITS2 region of the DNA of the mushroom was amplified by the polymerase chain reaction (PCR) using the universal oligonucleotides ITS1 and ITS4, following Díaz et al. (2014) modified protocol. The product of PCR was analyzed by electrophoresis in agarose gel at 1%. The base pairs were determined by comparison to the 1 Kb marker. The Wizard® S.V. Gel and PCR Clean Up System kit was utilized to purify the PCR products. Sequencing was performed at the Unidad de Síntesis y Secuenciación de ADN at the Instituto de Biotecnología of the National Autonomous University of Mexico (UNAM).

Analysis of the sequence

The sequence was compared to the NCBI's DNA database (www.ncbi.nlm.nih.gov) using the Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990). A total of nine sequences of *Pleurotus* were recovered, along with one sequence of *Fusarium phyllophilum*, to create the phylogenetic tree (Table 1). The evolution model employed was GTR I + G, determined by ModelTest v. 2.3 (Nylander, 2004). The tree was generated on the Phylogeny.fr platform (<https://www.phylogeny.fr/>; Dereeper et al. 2008) using the ClustalW, Gblocks, Mr.Bayes, and TreeDyn programs (Thompson et al. 1994; Huelsenbeck and Ronquist 2001; Chevenet et al. 2006; Dereeper et al. 2008; Dereeper et al. 2010). The phylogenetic tree was visualized in FigTree v1.4.4. (Rambaut 2018). The sequence was deposited in GenBank under access number MW581271. The phylogenetic tree was deposited in TreeBASE (Accession URL:<http://purl.org/phylo/treebase/phyloids/study/TB2:S28312>).

Table 1
Reference ITS sequences of *Pleurotus* and *Fusarium phyllophilum* (external organism) for the Phylogenetic analysis obtained from GenBank.

Species	Geographic Origin	Assession No. GenBank	Reference
Pleurotus opuntiae	India	MN477934	Kantharaja and Krishnappa, not published
Pleurotus djamor	India	MF574728	Suresh and Kalaiselvam, not published.
Pleurotus djamor	Cuba	JN637828	Manzano et al. not published
Pleurotus djamor	India	MG328900	Johney, Radhai Sri, and Ragunathan, 2018
Pleurotus djamor	India	MN398667	Pandey <i>et al.</i> not published
Pleurotus djamor	Cuernavaca, Morelos, México	GU722273	Huerta et al. 2010
Pleurotus djamor	Mérida, Yucatán, México	GU722271	Huerta et al. 2010
Pleurotus djamor	Talquian, Union Juarez, Chiapas, México	GU722265	Huerta et al. 2010
Pleurotus ostreatoroseus	South Korea	MG282434	Yan, not published
Pleurotus opuntiae	Tetela de Ocampo, Puebla, México	MK757594	Portilla-Segura et al. 2019
Pleurotus opuntiae	India	KY214255	Krishnapriya et al. 2017
Fusarium phyllophilum	—	KR909430	Al-Hatmi et al. 2016

Cultivation and production parameters of *Pleurotus* spp.

Preparation of the primary inoculum

The primary inoculum was obtained in two forms: wheat grains (WG) and pellets (WP). The former was prepared with wheat seeds hydrated for 12 h. Excess water was drained and 250 g of the wheat were placed in polypaper bags. These were sterilized for 15 min and then inoculated with 15, 1-cm² squares of the mushroom's mycelium. Incubation was performed at 28°C under total darkness until the mycelium completely invaded the seeds. The WP were prepared in 250-mL Erlenmeyer flasks containing 100 mL of potato dextrose broth. They were sterilized and inoculated with 10 mL of the mushroom's mycelium suspended in sterile water, and incubated at 28°C under stirring at 160 rpm until uniform pellets formed.

Selection and characterization of the substrate

The *A. salmiana* bagasse employed consisted of residues from the pulque, inulin, and honey industries. It was provided by the Cooperativo Magueyero San Isidro, S.A. de C.V. in the municipality of Nanacamilpa, Tlaxcala, Mexico (19°29'00"N 98°32'00"O). Selecting the residue required physicochemical analysis of the bagasse from the shoots, the pineapple, and a 50:50 mixture of shoots:pineapple. The following determinations were made: moisture (M) by the AOAC 930.15 method, ash (C) by the AOAC 942.05 method, organic material (OM) by difference of ash, crude protein (CP) by the adapted A.O.A.C. 955.04 method, total organic carbon (TOC) by calculation (OM/1.74) (Gouleke 1977), and the C/N ratio. pH was measured in extracts of distilled water in a weight/volume ratio of 1:5 (Crespo et al. 2018).

Inoculation of the substrate

Bagasse from the shoots of *A. salmiana* was washed in distilled water and pasteurized at 65°C for 60 min. Excess water was drained and the material was left to cool at ambient temperature. Once cooled, 27 bags, each containing 150 g (dry weight) of *A. salmiana* bagasse, were inoculated for each experiment (WG, WP) and distributed in 5 treatments for later supplementation with urea. The procedure followed the methodology proposed by López-Rodríguez et al. (2008) for inoculation with WG, while for inoculation with WP, we took the total content of the pellets from each flask, filtered the culture broth and deposited the pellets in the center of the polypaper bags that contained the previously sterilized substrate, according to Abdullah et al.'s modified protocol (2013).

Supplementation with urea

During supplementation of the substrate, the total nitrogen content (TN) of the bagasse of the *A. salmiana* shoots was taken as the initial parameter. The experimental design for WG and WP consisted in 9 treatments (T1: 0.77% TN, T2: 0.95% TN, T3: 1.14% TN, T4: 1.32% TN, T5: 1.5% TN) and 3 repeats in which the minimum, maximum, and central values for total N in the *A. salmiana* bagasse were 0.95, 1.5, and 1.32% TN, respectively. Nitrogen was calculated in percentage amounts equivalent to the weight in grams of the urea. The solutions were prepared with distilled water and sterilized with a 0.2-µm cellulose membrane. A potentiometer was used to measure pH.

Supplementation was performed 3 days after inoculation using a sterile syringe to cover all the bags. The amount of solution administered was calculated on the basis of the moisture of the inoculated bags, adjusted to 80%. Once all the bags were supplemented, they were kept under darkness until primordia appeared, protocol modified by Monterroso (2009).

Fructification and harvesting

After the appearance of the primordia, the bagasse was exposed to light at a temperature of 18–20°C and a relative humidity of 80–90% until mature fruiting bodies were obtained (López-Rodríguez et al. 2008). The mushrooms were gathered manually with a sterile scalpel and measured. This procedure was repeated for each harvest (Fernández 2004).

Evaluation of productive parameters

The following parameters were recorded: running time of the mycelium on the substrate, precociousness (time of appearance of primordia), and fructification. The productive parameters determined were biological efficiency (BE) and production rate (PR) (Bernabé-González et al. 2004):

$$\text{Biological efficiency (BE)} = \frac{\text{Weight of the fresh mushrooms (g)}}{\text{Weight of the dry substrate (g)}} * 100 \quad \text{Eq. 1}$$

$$\text{Production rate (PR)} = \frac{\text{Biological efficiency}}{\text{Days from seeding to the end of production}} \quad \text{Eq. 2}$$

Morphological and proximal chemical characterization of the fruiting bodies

The fruiting bodies from each treatment were characterized morphologically by texture, growth, color, form of the laminae, and pileus. For the proximal chemical composition, moisture (M) was determined by method 930.15, crude protein (CP) by the adapted 955.04 method, ethereal extract (EE) by method 920.39, and ash (C) by method 942.05, following the methodologies established by the AOAC (Association of Official Analytical Chemists).

Statistical analyses

Data analyses were performed using the Minitab 19 statistical program. A one-way ANOVA was applied with a Tukey test for multiple comparisons. The confidence level was 95%.

Results

Molecular identification

The molecular study of the fungus isolated from *A. salmiana* allowed us to obtain one amplification product with a size of 750 pb. It was identified as *Pleurotus djamor*. The sequence was deposited in GenBank under access number MW581271, and a phylogenetic tree was elaborated (Fig. 1 Accession URL:<http://purl.org/phylo/treebase/phyloids/study/TB2:S28312>) using the sequences of *Pleurotus* available at GenBank.

Culture and productive parameters of *P. djamor*

Table 2 shows the results of the characterization of various sections of the bagasse of *A. salmiana*. The residue of the pineapples is rich in crude protein (CP) at 11.51 %, compared to just 4.8% in bagasse from the shoots.

Table 2
Chemical composition of *Agave salmiana* bagasse.

	Shoots	Pineapples	Pineapples- shoots (50:50)
Moisture (%)	10.61 ± 0.4	10.14 ± 0.3	11.92 ± 0.3
Ash (%)	10.51 ± 0.4	10.91 ± 0.8	11.33 ± 0.3
Organic matter (%)	89.57 ± 0.4	89.09 ± 0.8	88.67 ± 0.3
Crude protein (%)	4.80 ± 0	11.51 ± 0.5	8.75 ± 0.8
Nitrogen (%)	0.77 ± 0	1.84 ± 0.1	1.40 ± 0.1
TOC (%)	49.76 ± 0.2	49.49 ± 0.4	49.26 ± 0.2
C/N ratio	64.62	26.90	35.19
pH	5.14 ± 0.6	5.90 ± 0.2	5.77 ± 0.03
± Standard deviation			
TOC: Total organic carbón			

During cultivation, we analyzed two types of primary inoculum: a traditional one that utilizes wheat grains as support, and a second made from pellets of the mushroom itself. Observations showed that the type of primary inoculum used did not affect the development of *P. djamor*, and that the use of the inoculum had no impact on the mycelial growth of the mushroom on the substrate. The inoculum in pellet form (WP) colonized the *A. salmiana* bagasse in 13 days, while the inoculum in grain form (WG) required 25 days. The seeds that resulted from the WG treatment were homogeneous, especially in the wheat grain, while the WP pellets varied in size, were semi-uniform, and beige in color. Neither product showed signs of contamination.

Table 3 shows the time of appearance of the primordia (precociousness), and the first and second harvests of the experiments inoculated with WG and WP. Table 4 displays the different concentrations of urea and BE and PR. Because the bags inoculated with WG increased the concentration of nitrogen, precociousness decreased. In treatment T1 (substrate not supplemented with nitrogen), 14 days were required for the first primordia to appear, while in T2 and T3 the time of appearance was 11 days, and for treatment T5 it was 10. In contrast, in the bags with WP precociousness increased with higher concentrations of nitrogen, reaching 14 days in T1 and T2, and 18 days in T3, T4, and T5.

Table 3
Precociousness and harvests of *P. djamor* on *A. salmiana* bagasse after the WG and WP treatments.

Treatments	T1			T2			T3			T4			T5		
	P	1ª	2ª	P	1ª	2ª	P	1ª	2ª	P	1ª	2ª	P	1ª	2ª
WG	14	11	13	11	8	26	11	8	25	9	8	25	10	8	23
WP	14	13	0	14	13	0	18	10	0	18	10	0	18	10	0
T1: Substrate not supplemented; T2: 0.95% of nitrogen; T3: 1.14% of nitrogen; T4: 1.32% of nitrogen; T5: 1.5% of nitrogen															
P: Precociousness (d), 1ª: First harvests (d), 2ª: Second harvests (d)															

Table 4
Production parameters of *P. djamor* on *A. salmiana* bagasse using wheat seeds.

Type of inoculum	T1		T2		T3		T4		T5	
	BE	PR	BE	PR	BE	PR	BE	PR	BE	PR
WG	58.33 ± 9 ^a	0.97 ± 0.2 ^a	61.11 ± 10 ^a	1.02 ± 0.2 ^a	63.41 ± 8 ^a	1.06 ± 0.1 ^a	70.00 ± 9 ^a	1.17 ± 0.1 ^a	70.56 ± 10 ^a	1.18 ± 0.2 ^a
WP	20.56 ± 4 ^a	0.34 ± 0.1 ^a	23.33 ± 3 ^a	0.39 ± 0.1 ^a	44.07 ± 7 ^b	0.73 ± 0.1 ^b	42.22 ± 5 ^b	0.70 ± 0.1 ^b	38.11 ± 5 ^b	0.64 ± 0.1 ^b
T1: Substrate not supplemented; T2: 0.95% of nitrogen; T3: 1.14% of nitrogen; T4: 1.32% of nitrogen; T5: 1.5% of nitrogen; BE: Biological efficiency; PR: Production rate. Means not labeled with the letter "a" are significantly different from the mean of the control level in the Tukey Multiple Comparison Test with a significance level ($P \leq 0.05$). ± Standard deviation										

Two harvests were obtained from the bags inoculated with WG. The first (T1) at 25 days from inoculation. For treatments T2 to T5, the first harvest was obtained after an average of 17 ± 1 days. Regarding the second harvest, for T1 it was obtained after 38 days, while for T2 to T5 the time required was 41–45 days. The treatments inoculated with WP only produced one wave of mushrooms at 27 ± 1 days after inoculation. This represents an economic disadvantage for production. It is likely that the WG treatments included nutrients that promoted the growth of the mushroom, while the WP inoculum lacked these compounds that strongly impact the number of harvests achieved. The BE and PR of the treatments inoculated with WG increased with respect to the non-supplemented treatment (T1) and as the concentration of TN in the bagasse increased. The maximum BE achieved was 70%, while the maximum PR was 1.18, both in T5. The BE and PR of the treatments inoculated with WP were lower, with T3 showing the highest values for these two parameters (44% and 0.73). The evidence from this experiment allowed us to determine that the primary inoculum based on pellets (WP) did not generate higher yields in the cultivation of *P. djamor*, as was expected. In contrast, the BE and PR of the bags inoculated with WG and supplemented with urea as a source of nitrogen improved.

The fruiting bodies of the WG and WP experiments are shown in Fig. 2. In both cases, they were of beige color with regular growth, a cottony texture, and smooth decurrent laminae. However, the form of the pileus of the mushrooms inoculated with WG was flabelliform, while that of the mushrooms inoculated with WP was infundibuliform. In the WG treatments, 31% of the fruiting bodies from T1 reached a size of 5–8 cm, 39% measured 8–13 cm, but only 4% achieved a size of 13–16 cm. In T2 with WG, 54% of the mushrooms measured 8–13 cm. For T3 the figures were 62 for 8–13 cm and 24% between 5 and 8 cm. For T4, 39% of the carpophores reached a size of 8–13 cm. For T5, 48% of the fruiting bodies measured 8–13 cm. Although some mushrooms from T4 and T5 achieved average size, some carpophores showed malformations in the clusters and caps. In addition, almost 20% of the fruiting bodies in T4 detained their development after day 14 (Fig. 3).

Regarding the WP experiment, 45.5% of the fruiting bodies in T1 measured 5–8 cm, but only 6% reached 10–13 cm. In T2, 35% of the carpophores were classified in a size of 5–8 cm. In contrast, observations of T3 showed that 16% of the mushrooms achieved a size ≥ 16 cm with a predominance of carpophores between 10 and 13 cm. The average size in T4 was 10–13 cm (37%), while in T5, 21% of the carpophores detained their growth.

Nutritional composition of the fruiting bodies

The effect of the type of inoculum and the substrate supplemented with urea on the morphology and nutritional composition of the fruiting bodies are shown in Tables 5 and 6, respectively.

Table 5
Morphological characterization of the fruiting bodies during the WG and WP experiments.

WG						WP				
	Texture	Growth	Color	Laminae	Pileus	Textura	Growth	Color	Laminae	Pileus
T1	Cottony	Regular	White	Decurrent	Flabelliform	Cottony	Regular	Cream	Decurrent	Infundibuliform
T2	Cottony	Regular	Cream	Decurrent	Flabelliform	Cottony	Regular	Cream	Decurrent	Infundibuliform
T3	Cottony	Regular	Cream	Decurrent	Flabelliform	Cottony	Regular	Cream	Decurrent	Infundibuliform
T4	Cottony	Exuberant	Cream	Decurrent	Flabelliform/ Malformations	Cottony	Regular	Cream	Decurrent	Infundibuliform
T5	Cottony	Exuberant	Cream	Decurrent	Flabelliform/ Malformations	Cottony	Regular	Cream	Decurrent	Infundibuliform

T1: Substrate not supplemented; T2: 0.95% of nitrogen; T3: 1.14% of nitrogen; T4: 1.32% of nitrogen; T5: 1.5% of nitrogen

Table 6
Proximal chemical analysis of the mushrooms from the WG and WP experiments.

WG					WP			
	M (%)	C (%)	CP (%)	EE (%)	M (%)	C (%)	CP (%)	EE (%)
T1	86.33 ± 2 ^a	8.09 ± 0.2 ^a	26.27 ± 0.7 ^a	6.58 ± 0.1 ^a	85.22 ± 2 ^a	7.37 ± 0.2 ^a	16.03 ± 0.4 ^a	7.76 ± 0.1 ^a
T2	89.58 ± 1 ^b	7.58 ± 0.1 ^a	23.22 ± 0.7 ^b	6.02 ± 0 ^a	89.58 ± 1 ^b	7.64 ± 0.2 ^a	15.84 ± 0.9 ^a	7.88 ± 0.5 ^a
T3	89.14 ± 2 ^b	7.67 ± 0 ^a	22.73 ± 0.6 ^b	6.50 ± 0.1 ^a	87.85 ± 2 ^a	8.17 ± 0.1 ^b	21.89 ± 0.9 ^b	8.18 ± 0.1 ^a
T4	89.83 ± 1 ^b	7.42 ± 0.6 ^a	21.91 ± 0.5 ^b	7.13 ± 0.1 ^a	89.83 ± 1 ^b	8.21 ± 0.1 ^b	24.86 ± 0.6 ^c	7.90 ± 0.1 ^a
T5	88.86 ± 0.7 ^b	8.25 ± 0.3 ^a	15.20 ± 0 ^c	6.83 ± 0 ^a	89.02 ± 1 ^b	8.29 ± 0 ^b	22.94 ± 0.2 ^b	8.36 ± 0 ^b

T1: Substrate not supplemented; T2: 0.95% of nitrogen; T3: 1.14% of nitrogen; T4: 1.32% of nitrogen; T5: 1.5% of nitrogen; M: Moisture; C: Ash; CP: Crude protein; EE: ethereal extract. Means not labeled with the letter "a" are significantly different from the mean of the control level in the Tukey Multiple Comparison Test with a significance level ($P \leq 0.05$). ± Standard deviation

The moisture determined for the fruiting bodies in this study ranged from 85.22–89.83% in the two experiments and showed statistically-significant differences with respect to controls (T1) ($p \leq 0.05$). The highest CP content (26.27%) occurred in T1 of the mushrooms inoculated with WG without added nitrogen. We expected that in addition to improving BE and PR, supplementing the substrate with a source of N (urea) would increase CP content, but as the concentration of urea in the substrate increased in the WG treatments, the CP of the mushrooms decreased significantly. Although the concentration of CP in the mushrooms inoculated with WP achieved a value considered typical for this species, it was below the level of the mushrooms inoculated with WG. Treatment T4 (WP) had the highest protein content. On the other hand, the mushrooms in experiments WG and WP the fat content, ranged from 7.42–8.36%. Regarding ash content, the sporomes obtained from WG and WP in the present study contained 7.3–8.3%.

Discussion

The mushrooms that grow on plants of the genera *Opuntia*, *Yucca*, *Agave*, and *Phytolacca*, among others, are described as *P. opuntiae*, but because worldwide the genus *Pleurotus* includes several taxa and species, controversy exists due to the absence of valid sequencing data and/or phylogenetic reconstructions. This has generated significant ambiguities regarding the exact identity and distribution of *P. opuntiae* (Zervakis et al. 2019). The mushroom *P. djamor* has a cosmopolitan distribution that includes species identified as *P. ostreatoroseus*, *P. parsonsiae*, and *P. salmoneostramineus*. *P. djamor* is a pantropical species found in wild form in several countries in the America, including Mexico, though its distribution is probably much broader (Zervakis et al. 2019). Molecular studies have of 600–800 pb have been reported for *P. abalonus*, *P. cystidiosus*, *P. cystidiosus* var. *Formosensis*, *P. fuscoquamosus*, *P. smithii*, and *P. australis* (Zervakis et al. 2004), *P. djamor*, *P. cornucopiae*, *P. cystidiosus*, and *P. pulmonaris* (Imtiaj et al. 2011), but recently fragments of 700 pb were found for *P. djamor* and *P. ostreatus* (Doroteo et al. 2018).

Mushrooms of the genus *Pleurotus* have the ability to grow on materials with low nitrogen concentrations. The results of our nitrogen analysis indicate that the bagasse of *A. salmiana* is a good candidate for use as an alternative substrate for cultivating edible mushrooms. Nitrogen is essential for protein synthesis, purines, pyridine, and chitin in mushrooms, but high levels can shrink yields (Chang and Miles 2004). Thus, it is extremely important to ensure that the substrates utilized to cultivate edible mushrooms contain an adequate nitrogen content that will allow correct growth. According to Heredia-Solís et al. (2014), the content of CP in *A. salmiana* and *Agave weberi* bagasse does not exceed 4%. In this study the nitrogen content in the bagasse, pineapples, and the mixture (50:50) was measured at 0.77, 1.84, and 1.40%, respectively; figures that concur with those reported for bagasse, which range from 0.1-1% of N (Obodai et al. 2003; Koutrotsios et al. 2014).

During the cultivation of fungi, the type of inoculum WP represents a competitive advantage, against other microorganisms that could compete for the substrate and contaminate the culture. Due to its origin, the inoculum in pellet does not harbor spores of other fungi or microorganisms because it exerts greater control (Rosado et al. 2002; Abdullah et al. 2013). According to Khanna et al. (1992), the precociousness in *Pleurotus* spp. ranges from 24–30 days, though Kalmis and Sargin (2004) and Naraian et al. (2009) reported the appearance of primordia at 22 and 20 days, respectively. For *P. opuntiae*, the appearance of primordia occurred at 30 days (Barrales and Mata 2016). In the present study, the time of appearance of the primordia of *P. djamor* was lower and similar to observations by Salmones et al. (2005); that is, greater precociousness of *P. djamor* after 11 and 13 days on wheat chaff and coffee pulp, respectively. Supplementing substrates with nitrogenated sources improves the production of mushrooms of the genus *Pleurotus* spp. (Naim et al. 2020). But other observations have shown deficiencies in mycelial growth and yields when nitrogen sources such as ammonium chloride were applied to *P. ostreatus* and *P. cystidiosus* at concentrations above 0.09 and 0.05% (Hoa and Wang, 2015), and with ammonium sulfate and urea at concentrations of 1% and 1.5%, respectively (Naraian et al. 2009).

The growth, development, quality, and quantitative aspects (biological efficiency, productivity) are closely-related to the type of nutrients present in the substrate, an equilibrium in the C/N ratio, and other conditions of cultivation (Mukhopadhyay et al. 2002; Curvetto et al. 2002; Bellettini et al. 2019). In this study the absence of a second harvest could be due to the inoculum in pellet form (WP) since it did not contain the characteristic nutrients of wheat grain that provide energy for mycelial growth and development (Royse et al. 2004). Other possibilities include the nutritional exhaustion of the substrate during mushroom growth, and/or the accumulation of toxic substances that impeded fructification (Upadhyay et al. 2002). The C/N ratio is a determining factor for mushroom production because excess nitrogen can affect the degradation of lignin, thus impeding the development of the mycelium and the formation of fruiting bodies. In the phase of development of the fruiting bodies, a low C/N ratio is favorable. Bellettini et al. (2015) recommend an C/N ratio of 28–30 of carbon and 1% nitrogen for growing mushrooms. Heredia-Solís et al. (2014) reported the same BE with *P. ostreatus* using a substrate made of bagasse of *A. salmiana* and 40% of BE with *A. weberi*. In other results, a BE of 60.2% was obtained with *Agave tequilana* (Soto-Velazco 1989), and of 33.24% using a substrate of bagasse of *Agave angustifolia* mixed with 30% nogal shavings and 5% wheat bran (Heredia-Solís et al. 2016). Deformations of the fruiting bodies –especially elongated stipes and reduced coloration– have been reported due to the effects of cultivating conditions (high luminosity, temperature during fructification, CO₂ levels) (Urban 2004). However, considering that the environmental conditions in the present study were the same for all levels of nitrogen in the substrate, we deduced that higher N concentrations inhibited the growth of the fruiting bodies (Kanhar et al. 2007; Monterroso et al. 2009; Hoa and Wang 2015), and induced deformations in the mushrooms. High N content also produced slow growth of the stems of the fruiting bodies and delays their formation. It is possible, therefore, that the malformations found in this study are related to an imbalance of the C/N ratio.

The nutritional quality and composition of the fruiting bodies depends on both the cultivation conditions (temperature, moisture, pH, etc.) and the substrate, since this is the source of the nutrients and lignocellulosic material that supports growth, development, and fructification. The use of a waste lignocellulosic material as substrate is key to maximizing yields and enhancing product disposition (Chang and Miles 2004; Sardar et al. 2017). For this reason, we opted to cultivate *P. djamor* on the same source from which it was isolated (i.e., *A. salmiana* bagasse). One of the most important parameters for this type of food is the amount of protein content, since it is well-known that species of *Pleurotus* spp. grow on substrates with low nitrogen content yet the fruiting bodies they produce have higher concentrations of N. Protein content, however, is influenced by factors that include the nature and nutritional components of the substrate, the strain, the stage of development, and the timing of post-harvest analysis (Cowling and Merrill 1966; Sardar et al. 2017). The genus *Pleurotus* sp. is characterized by low fat content, high protein content, and a low concentration of lipids in the fruiting bodies (Bellettini et al. 2019).

In this study the CP content of the fruiting bodies in our WP and WG treatments was found to be within the ranges established for the genus *Pleurotus*; that is, 17–25% (Lelley 2017), with 20.3% of CP for *P. ostreatus* (Del Toro et al. 2018), and 20.7–28% for *P. djamor* (Salmones 2017). In studies of *Pleurotus* spp. cultivated in supplemented bagasse, Ortega et al. (1992) described an increase of nitrogen in the mushrooms that was related to the amount of N present in the initial substrate and to the inoculum. Sturion and Oetterer (1995) suggested that mushrooms might be fixing this element. Wheat seeds can provide nutrients to the inoculum that enrich the substrate and define the development of the mycelium in early stages; however, because the inoculum with WP did not contain nutrients from wheat, it may have impeded optimal growth. Therefore, it is probable that the type of substrate and inoculum influence the nutritional composition of the fruiting bodies (Bellettini et al.

2019). Also the percentage of moisture depends on the strain, substrate, and growing environment (temperature, relative humidity) (Guillamón et al. 2010). The moisture percentages for this species indicate 85-90.9% (Sardar et al. 2017).

The results obtained from the chemical composition analysis in this study suggest that *P. djamor* is a promising food for several types of diet due to its nutritional quality (Salmones 2017). Up to now, this mushroom has not been cultivated on *A. salmiana* bagasse, but its high adaptability to cultivation conditions could well make it attractive in international markets as an exotic mushroom that can be cultivated on maguey bagasse.

The suitability of a substrate for cultivating mushrooms depends on its chemical composition, availability, and cost. In this regard, residual *A. salmiana* bagasse from pulque, inulin, and agave honey production is a promising option that can compete on a small scale with other substrates, since its generation is growing daily, with reports of the generation of 0.5 tons of bagasse from inulin production, and 0.16 tons from producing just 0.4 tons of agave honey (Hoz-Zavala et al. 2017). In addition, its availability, though not specific, contains the elements necessary for the developmental and reproductive functions of edible mushrooms (Salmones et al. 2005; Heredia-Solís et al. 2017; Gallardo 2018). Moreover, in comparison to similar substrates, when supplemented with urea it reaches a BE greater than that of bagasse of *A. tequilana*, *A. augustifolia*, and *A. weberi*. In general, then, exploiting *A. salmiana* bagasse represents a sustainable option in ecological, social, and commercial terms (Heredia-Solís et al. 2014; Heredia-Solís et al. 2017).

This is the first report to address the isolation of *P. djamor* of *Agave salmiana* and grown on bagasse of *Agave salmiana* as an atypical substrate. During cultivation on *A. salmiana* bagasse, the growth of the fruiting bodies depended on the type of inoculum and the concentration of urea. Supplementing the substrate with urea (1.32%) to provide nitrogen improved both BE and PR by as much as 70% and 1.17, respectively, in the cultivation of *P. djamor*. At higher concentrations (1.5%), however, this inhibited the growth of the fruiting bodies and produced malformations. *A. salmiana* bagasse is a suitable alternative substrate due to its chemical composition and high availability because it permits the growth of the mushroom without altering the optimal nutritional composition of this genus, thus providing economic and environmental advantages. Results represent an invitation for further study and commercialization.

Declarations

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Competing interests

The authors declare that they have no competing interests

Availability of data and material

Code availability

Not applicable

Authors' contributions

Brianda Susana Velázquez de Lucio: Investigation, formal analysis, writing - original draft.

Edna María Hernández-Dominguez: Resources, conceptualization, supervision, writing - review y editing.

Xóchitl Tovar-Jiménez: Methodology: writing - review and editing

Gerardo Díaz-Godínez: Methodology, writing - review and editing.

Alejandro Téllez Jurado: Conceptualization, methodology, writing - review and editing.

Jorge Álvarez Cervantes: Conceptualization, term, methodology, writing - review and editing, visualization.

Ethics approval

Not applicable

Consent to participate

Not applicable

Consent for publication

Not applicable

Data availability

The datasets generated during and/or analysed during the current study are available in the GenBank repository, under access number MW581271, the phylogenetic tree was deposited in TreeBASE (Reviewer access URL: <http://purl.org/phylo/treebase/phyloids/study/TB2:S28312?x-access-code=288be19013aeff456bd2b892c2905a0e&format=html>); and this are available from the corresponding author on reasonable request.

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Figures

Figure 1

Phylogenetic tree generated from the sequences of the ITS region of strains of *Pleurotus*.

Figure 2

Fruiting bodies from the different treatments of the experiments with WG and WP. The bodies are of beige color, with regular growth, cottony texture, and decurrent laminae.

Figure 3

Malformations in the caps of *Pleurotus djamor* after adding 1.32% of N in the WG experiment: a) perforation in the center of the caps; b) inhibition of the growth of the fruiting body; c) division of the carpophore.

Supplementary Files

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- [abstract.tif](#)