BIOTECHNOLOGICAL POTENTIAL OF TEN *PLEUROTUS DJAMOR* STRAINS

LILIA MORENO RUIZ, RENÉ H ANDRADE, JOSÉ E SÁNCHEZ* AND EL COLEGIO DE LA FRONTERA SUR
Apdo.Postal 36.Tapachula, Chiapas, México
esanchez@ecosur.mx

ABSTRACT

With the aim of knowing some capabilities of biotechnological interest of the edible mushroom *Pleurotus djamor*, ten strains of this species were characterized. Besides mycelial growth and sporophore production, laccase (on ABTS), Mn-peroxidase (on dimethoxyphenol), aryl alcohol oxidase (on veratryl alcohol) and phenol oxidase activities (on catechol) were determined. Also the antioxidant capacity was determined. Reported radial extension rate varied between 1.12 mm/d (*P. djamor* ECS-0150) and 3.9 mm/d (*P. djamor* ECS-0143). Biological efficiency, production rate, performance in two harvests varied between 36.4 and 71.4%, 1.14 and 2.1% and 0.033 and 0.069, respectively. All the strains tested have at a certain degree, laccase, Mn-Peroxidase and phenol oxidase, except for aryl alcohol oxidase. The strain ECS-0123 showed important ligninolytic activity, good antioxidant capacity, good growth and an average capacity to produce sporocarps (mushrooms).

Keywords: oyster mushroom, ligninolytic enzymes, antioxidants, edible mushrooms

INTRODUCTION

Among the species in the genus *Pleurotus*, which includes edible mushroom species, *P. djamor* is one of the most commonly found mushroom species in the tropical areas of the world. *P. djamor* is reported more frequently in the wild in Mexico than any other species of this genus [1, 2]. Although *P. djamor* is recognised as edible, harvested for family consumption and sold in local markets, this species is not used in commercial cultivation, which is in contrast to the other commercially exploited species in the genus, such as *P. ostreatus* and *P. pulmonarius*. The cumulative production of these two mushroom species is the second highest in the country after *Agaricus bisporus* [3].

There is little information on tropical macrofungi, although the tropics are one of the most biologically diverse biomes. Therefore, studying strains that originate from the Soconusco region of southeastern Mexico is important. *P. djamor* specimens are commonly found in the wild in this under studied region, which has a high biodiversity [4].

White rot fungi are becoming more important because of their enzymatic potential. These mushrooms are known to degrade lignin and have recently been investigated for their antioxidant capacity. Fungi such as *P. sajor-caju* and *P. salmoneostramineus* have been reported to have a significant antioxidant capacity [5, 6]. These reports have increased the interest in the genus and in *P. djamor* in particular, which has a wide distribution, is able to degrade lignin and has the potential for use in the degradation of difficult compounds. The present work studies characteristics of ten *P. djamor* strains originating from Chiapas, Mexico that are of biotechnological interest. This characterisation includes the quantification of ligninolytic and antioxidant activities and production of sporocarp. These strains are maintained in the collection of macrofungi of Ecosur and have been deposited at the National Centre for Genetic Resources (CNRG in Spanish) of Mexico. The CNRG is an organisation that preserves microbial germplasm and native macrofungi of interest and has been in operation since 2012.

MATERIALS AND METHODS

Strains and preservation media

The *Pleurotus djamor* strains ECS-0122, ECS-0123, ECS-0127, ECS-0130, ECS-0139, ECS-0141, ECS-0143, ECS-0144, ECS-0150 and ECS-0151 originating from the state of Chiapas, Mexico were used in this study. Two *P. ostreatus* commercial strains (ECS-0152 and ECS-1123) from the collection of macrofungi of the College of the Southern Border (Ecosur) were used for comparison purposes. Strains that were preserved in distilled water at 4 °C were reactivated in malt extract liquid medium (Bioxon).

Radial extension rate

The radial extension rate (RER) was measured in a Petri dish with malt extract agar (Bioxon) at pH 6. Agar fragments of 0.5 cm in diameter were colonised by each strain and seeded in the centre of a 15 x 100 mm Petri dish with a sterile medium. The dishes were then incubated for 10 days at 25 °C. The daily growth radius attained by the colony was marked under each dish in two perpendicular directions. The experiment was repeated five times for each strain, and the RER was calculated using the formula of Sinclair and Cantero [7] in which RER = [(ln X3 – ln X1)/2] [X2].

Ligninolytic activity

The ligninolytic activity was determined for the mycelium and sporocarp. For the mycelium, 10 g of sorghum seeds were colonised by each strain, and an extract was prepared. This material was macerated in a mortar with 20 ml of a specific buffer for each enzymatic reaction and filtered through a cloth. The extract was then filtered through Whatman filter paper No. 6 and centrifuged at 5,000 rpm for 10 minutes. To measure the ligninolytic activity of the sporocarp of each strain, 5 g of pileus were freshly harvested, macerated in 10 ml of buffer and extracted as described above. The phenol oxidase activity was measured with catechol (δ_{420nm} = 3.45 $mM^{-1}cm^{-1}$) in a phosphate buffer at pH 7 [8]. The laccase activity was determined with an ABTS (2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt) substrate (δ_{436} = 29.3 $mM^{-1}cm^{-1}$) in sodium acetate at pH 4.5 [9]. The manganese-peroxidase (MnP) activity was measured with dimethoxyphenol (δ_{470} = 49.6 $mM^{-1}cm^{-1}$) in the presence of H_2O_2 in a succinate buffer at pH 5 [10]. Finally, the aryl alcohol oxidase activity of veratryl alcohol (δ_{310} = 9.3 mM^{-1} cm^{-1}) was determined in a sodium phosphate buffer at pH 6 [11]. A unit was defined as the amount of enzyme forming 1 ìmol of the product in one minute under these conditions.

Antioxidant activity

The antioxidant activity was estimated in the harvested mycelium and sporocarp by determining the total phenols using the method described by Folin Ciocalteu. The activity was expressed as the equivalent of gallic acid [12]. The free radical scavenging activity was also determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as described by Fukumoto and Mazza [13].

Finally, the taxonomic characterisation was performed on the harvested fruit bodies according to descriptions from the literature [1, 14-16].

Production assays

Fruiting assays were performed by seeding an inoculum prepared on sorghum grain [17] (2.5 wt%) in batches of one kilogram of *Digitaria decumbens* Pangola grass with 2% lime and 70% humidity. This substrate was pasteurised at 90 °C for one hour using steam. Production (P) was determined as the weight in grams of mushrooms harvested in two waves, biological efficiency (BE) was calculated as BE = (fresh mushrooms harvested / dry substrate used) x 100 and the production rate (PR) was calculated as PR = (BE / days from planting to harvest) x 100 for the 10 strains studied. The yield or Performance (P) was calculated as the ratio of the grams of dry fungi obtained for the two crops to the dry weight of the substrate used.

Statistical analysis

In all of the studies, a random model with five repetitions was used. Variance was analysed and the mean separation was calculated using Tukey's test at $\alpha = 0.05$. Analyses were performed using the JMP program version 4.0 from SAS Institute (Campus Drive Cary, NC 27513 USA 1998).

RESULTS

Radial extension rate

Figure 1 shows the rate of radial expansion of the strains studied. The values ranged between 1.12 and 3.9 mm/d for the strains ECS-0150 and ECS-0143, respectively. Group A was formed by the fastest strains and consisted of *P. ostreatus* strains ECS-0152 (control, 4.15 mm/d) and *P. djamor* strains ECS-0143 and ECS-0123. Radial extension values for ECS-0143 and ECS-0123 ranged between 3.69 and 4.15 mm/d, respectively.

Ligninolytic activity

Table 1 shows the results of the ligninolytic activity tests performed on the extracts of the harvested mycelium and sporocarp. These tests determined the phenoloxidase, MnP, laccase and aryl alcohol oxidase activities for the two extracts of each strain.

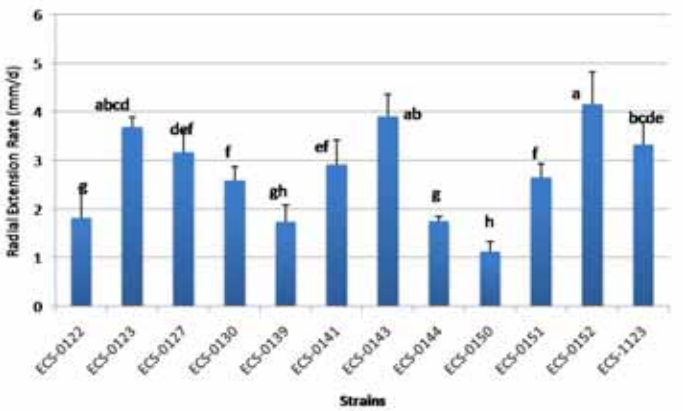

Figure 1. Radial extension rate (mm/d) of 10 *Pleurotus djamor* strains compared with two control strains (*P. ostreatus* ECS-0152 and ECS-1123) on MEA at 25 ºC, pH 6. Mean of five repetitions. Different letters indicate significant statistical differences between strains (Tukey's test, α=0.05).

Table 1. Ligninolytic activity (phenol oxidase, Mn-peroxidase, laccase and aryl alcohol oxidase) in mycelium and sporocarp extracts of 10 *Pleurotus djamor* strains and two control strains (*P. ostreatus* ECS-1123 and ECS-0152). Substrate (and pH) of each test: catechol (7), DMF (5), ABTS (4.5) and veratryl alcohol (6), respectively, at 25 ºC (U/ml). Mean of three repetitions

Strain	Phenol oxidase		Mn-Peroxidase		Laccase		AAO
	Mycelium	Sporocarp	Mycelium	Sporocarp	Mycelium	Sporocarp	Sporocarp
ECS-0122	6.42±0.27 CD	70.92±0.61 D	0.23±0.09 F	0.16±0.03 D	99.48±1.38 DE	41.75±0.35 G	1.63±0.02 DE
ECS-0123	7.08±0.79 BC	7.78±0.16 F	13.19±1.8 B	0.67±0.01 D	546.2±4.5 A	91.30±2.25 B	2.65±0.01 D
ECS-0127	0.65±0.07 G	0.38±0.10 G	8.23±1.04 C	0.06±0.00 D	105.49±0.83 D	28.33±0.78 I	1.16±0.02 DEF
ECS-0130	2.74±0.33 F	1.01±0.37 G	6.37±0.19 CD	9.87±0.77 B	79.54±17.6 FG	47.10±3.11 F	0.12±0.02 F
ECS-0139	4.76±0.50 DE	130.18±2.89 B	17.10±1.7 A	4.89±0.16 C	80.04±1.91 EF	76.68±1.72 C	4.44±0 C
ECS-0141	7.74±0.16 BC	0.59±0.06 G	6.81±1.3 C	9.04±0.95 B	60.19±1.73 G	93.94±2.50 B	0.01±0 F
ECS-0143	4.95±0.14 DE	1.48±0.18 G	2.81±0.31 EF	11.23±0.13 B	101.66±5.79 D	33.14±0.84 H	0.25±0 F
ECS-0144	22.69±1.43 A	96.55±0.46 C	14.79±0.79 AB	1.53±0.09 D	106.27±1.46 D	56.84±0.27 E	18.8±0.9 A
ECS-0150	8.88±1.28 B	93.57±0.74 C	3.58±0.06 DE	0.28±0.05 D	259.5±11.3 B	55.08±0.43 E	7.47±0.53 B
ECS-0151	4.95±0.71 DE	183.31±0.67 A	6.70±0.38 C	4.52±0.09 C	231.8±3.9 C	107.92±0.39 A	8.31±0.01 EB
ECS-0152	3.54±0.19 EF	48.63±1.46 E	1.91±0.65 EF	15.60±2.52 A	35.69±3.18 H	28.62±0.85 I	0.34±0.02 EF
ECS-01123	3.69±0.14 EF	0.57±0.23 G	2.87±0.41 EF	1.12±1.02 D	25.76±0.05 H	8.25±0.43 J	0.10±0.05 F
p	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Same letter in the same column indicate significant statistical differences in enzymatic activity between strains after Tukey's test (α= 0.05).

The assessment of phenoloxidase activity in the extract from the fungal mycelium showed levels of activity between 0.65 and 22.6 U/ml. The statistical analysis showed significant differences between the strains ($p = 0.0001$) and separated the strains in seven groups. Strain ECS-0144 had the highest phenoloxidase activity (22.7U/ml) and formed group A. The two *P. ostreatus* strains used as the control were placed in the groups with the lowest activities (groups E and F with activities ranging from 3.54 to 3.69U/ml). *P. djamor* ECS-0127 was the strain with the lowest activity (0.65U/ml). The phenol oxidase activity measured in the harvested sporocarp varied between 0.38 U/ml (strain ECS-0127) and 183.3U/ml (strain ECS-0151). The statistical analysis showed significant differences between the strains ($p = 0.0001$) and separated the strains in seven groups. Six strains (ECS-0151, ECS-0139, ECS-0144, ECS-0150, ECS-0122 and ECS-0152) included in the statistical groups A to E had activity levels higher than 48.6 U/ml in the sporocarp extracts. These values were considerably higher in the sporocarp extract than in mycelium extract.

The MnP activity observed in the mycelium extract ranged from 0.23 U/ml (strain ECS-0122) to 17.1 U/ml (ECS-0139 strain). The statistical analysis found significant differences ($p = 0.0001$) and formed six groups. ECS-0139 was the most active strain and different from the other strains. The MnP activity in the pileus extracts ranged from 0.06 U/ml (strain ECS-0127) to 15.6 U/ml (control strain *P. ostreatus* ECS-0152). The statistical analysis found significant differences between the strains and formed four groups ($p = 0.0001$). In this case, the activity of the control strain *P. ostreatus* ECS-0152 was statistically superior to all of the *P. djamor* strains.

The laccase activity determined in the mycelium extracts ranged from 25.7 U/ml (control strain *P. ostreatus* ECS-1123) to 546.2 U/ml (strain ECS-0123, group A). The statistical analysis separated the strains into eight groups ($p = 0.0001$) in which groups B and C (strains *P. djamor* ECS-0150 and ECS-0151) showed activities ranging between 259.5 and 231.8 U/ml, respectively. In the sporocarp extract, differences between the strains were significant ($p = 0.0001$), but the values were lower and ranged from 8.25 U/ml (strain ECS-1123, group J) to 107.92 U/ml (strain ECS-0151).

The aryl alcohol oxidase activity detected in the mycelium extract was almost equal to zero, and the analysis found no significant differences between the different extracts ($p = 0.4238$). The aryl alcohol oxidase activity in the sporocarp extract showed values that ranged from 0.01 U/ml (strain ECS-0141, statistical group F) to 18.8 U/ml (ECS-0144 strain, group A) ($p = 0.0001$).

Antioxidant activity

The total phenolic content and antioxidant activity measured by the DPPH test are shown in Table 2. Strain ECS-0141 had the lowest total phenolic content in the mycelium extract (229 eq. gal. ac/g, group D). Four statistical groups were defined according to Tukey's test ($p = 0.0001$). Strain ECS-0123 had the highest value at 517.8 eq. gal. ac/g, which was significantly different from the other groups. Strain ECS-0143 had the lowest content of antioxidant in the sporocarp extract (54.1 eq. gal. ac/g). Group A had the highest antioxidant values, with contents ranging between 214.9 and 231 eq gal. ac/g. Group A was formed by *P. djamor* strains ECS-122, ECS-0139, ECS-0144, ECS-0151 and the *P. ostreatus* control strains ECS-0152 and ECS-1123.

The free radical scavenging activity of the mycelium extract was measured using DPPH and varied between 3.73 and 38.11%. The statistical analysis showed significant differences ($p = 0.0001$) and identified four groups. Group A had the highest values and was comprised solely of the extract of *P. ostreatus* strain ECS-1123 (38.1% activity). The other control strain (ECS-0152) formed the second group with the highest values (group B, 20.75%). The *P. djamor* strain ECS-0139 formed the third group (group C, 10.72%). The three groups were significantly different from each other, and the remaining strains were classified in group D with values between 3.73 and 5.82%. The free radical scavenging activity of the sporocarp extract varied between 8.85 and 41.4%. The statistical analysis identified three groups ($p = 0.0003$). Strains ECS-0151, ECS-0144, ECS-0143, ECS-0139, ECS-0123 and ECS-0122 formed group A and had the highest values. The two control strains had the lowest values and belonged to group C. All of the *P. djamor* strains had average activities that were substantially higher in the sporocarp extract than in the mycelium extract. However, *P. ostreatus* had similar or even lower activities in the sporocarp extract than in the mycelium extract.

Table 2. Antioxidant activity (measured as total phenols and by the DPPH reaction) of mycelium and sporocarp extracts of 10 *P. djamor* strains, compared with two commercial strains *P. ostreatus* ECS-0152 and ECS-1123. Mean of 3 repetitions

Strains	Total phenols (eq. gal. ac/g)		DPPH(%)	
	Mycelium	Sporocarp	Mycelium	Sporocarp
ECS-0122	341± 12.0 C	223.1 ±1.0 AB	3.84±1.15 D	34.4±12.1 AB
ECS-0123	517.8± 15.3 A	199.5±5.9 D	4.54±0.16 D	34.7±0.32 AB
ECS-0127	426.4± 31.4 B	208.8±7.4 CD	5.82± 0.33 D	23.4±4.1 BC
ECS-0130	309.6± 24.0 CD	73.0±2.0 E	4.43± 0.32 D	18.8±1.6 BC
ECS-0139	252±4.9 D	219 ±2.0 ABC	10.72± 0.98 C	34.9±4.9 AB
ECS-0141	229.0±29.5 D	56.5±0.7 F	4.43±1.31 D	19.3±1.6 BC
ECS-0143	351.6±32.3 BC	54.1±3.1 F	4.19±0.33 D	25.8± 0 AB
ECS-0144	351.6±38.3 BC	214.9±4.6 ABC	3.96±1.97 D	26.8± 0.32 AB
ECS-0150	348.3 ± 46.7 BC	210.2±9.0 BCD	5.59±0 D	24.24± 1.9 BC
ECS-0151	273± 18.8 CD	219.2±2.3 ABC	3.73±0.66 D	41.0± 3.2 A
ECS-0152	ND	231.3±4.2 A	20.75±0.98 B	18.41± 0 BC
ECS-1123	ND	219.8±1.3 ABC	38.11±0.82 A	8.85± 0.32 C
p	0.0001	0.0001	0.0001	0.0003

ND: non determined
Different letters in the same column indicate significant statistical differences (Tukey's test, α= 0.05)

Sporocarp production

Table 3 presents the production of sporocarp (S) in one kilogram of Pangola grass with 70% humidity for two crops as well as the biological efficiency (BE), production rate (PR) and performance (P) of the strains tested. The values varied between a minimum of S = 108 g, BE = 36.4%, PR = 1.14% and P = 0.0336 for the strain ECS-0151 and a maximum of S = 212.2 g, BE = 71.4%, PR = 2.06% and P = 0.069 for the strain ECS-0130. The statistical analysis defined three statistical groups for S, BE and P values. Strain ECS-0151 had the lowest values and was significantly different from the other

Table 3. Production variables after two flushes (Sporocarp production, Biological efficiency, Production rate and Performance) of 10 *Pleurotus djamor* strains, compared with two *P. ostreatus* strains ECS-0152 and ECS-1123, grown in 1 kg bags of Pangola grass with 70% moisture and 2% calcium hydroxide, at 22 ºC. Mean of five repetitions

Strain	Sg/bag	BE (%)	PR(%)	P(10^{-2})
ECS-0122	125.0±38.9BC	42.1±13.1 BC	1.36±0.39 AB	04.6±1.5 AB
ECS-0123	192.1±45.7ABC	64.7±15.3 ABC	1.94±0.32 A	6.0±1.5 AB
ECS-0127	191±32.2ABC	64.3±10.8 ABC	1.62±0.44 AB	6.02±0.8 AB
ECS-0130	212.2±65.9 A	71.4±22.1 A	2.06±0.18 A	6.96±1.7 A
ECS-0139	149.2±41.6ABC	50.2±14.0 ABC	2.1±0.25 A	6.5±0.17 A
ECS-0141	159.8±51.6ABC	53.8±17.3 ABC	1.64±0.55 AB	5.16±1.6 AB
ECS-0143	174.6±24.8ABC	58.8±8.3 ABC	2.04±0.30 A	6.6±1.1 A
ECS-0144	127.8±47.6ABC	43.0±16.0 ABC	1.48±0.56 AB	4.76±1.1 AB
ECS-0150	188.6±15.8ABC	63.5±5.3 ABC	1.92±0.16 A	5.9±0.7 Ab
ECS-0151	108.0±32.6 C	36.4±11.0 C	1.14±0.35 AB	3.36±1.1 B
ECS-0152	208.4±22.8ABC	69.4±7.6 AB	1.74±0.20 AB	6.52±0.9 A
ECS-1123	186.6±28.2ABC	62.2±9.4 ABC	1.38±0.22 AB	6.06±0.4 AB
p	0.0007	0.0008	0.0004	0.0018

Different letters in the same column indicate significant statistical differences between strains (Tukey's test, α= 0.05).

groups. Group A had the highest values and included the two control strains and strains ECS-0130, ECS-0123, ECS-0127, ECS-0139, ECS-0141, ECS-0143, ECS-0144 and ECS-0150. The PR analysis defined two groups, with group A formed by the previously mentioned strains and group B formed by the strains ECS-0122, ECS-0127, ECS-0141, ECS-0144, ECS-0151 and the two control strains.

Table 4 shows the morphological characteristics of the sporocarps cultivated on the Pangola grass at 25 °C. The colour varied between pink (strains ECS-0130 and ECS-0127) and white (remaining strains). A typical mushroom smell was detected in seven strains, and three of them had a floury smell (ECS-0130, ECS-0143 and ECS-0150). The taste varied from typical (ECS-0139 and ECS-0144) to sweet (ECS-0123, ECS-130 and ECS-141), floury (ECS-0150), oily (ECS-0144 and ECS-0151) or oily and sweet (ECS-0122 and ECS-0127). The texture was usually subcoriaceous to leathery except for strain ECS-0139, which had a smooth texture. The size of the sporocarps varied between 2 and 16 cm, and they had a firm and imbricated consistency. Overall, these variations are consistent with the description given for the species [14].

DISCUSSION

In this work, we studied various characteristics of ten *P. djamor* strains that are of biotechnological interest, and compared them to two commercial *P. ostreatus* control strains. Strains showed significant variability in mycelium growth speed in the malt extract agar (between 1.12 and 4.15 mm/d). Strain ECS-0143 was the fastest and statistically similar to the *P. ostreatus* EC-0152 control strain. However, none of the tested strains had a mycelium growth speed superior to the two strains used as the control. Other studies have reported higher growth speeds for *P. djamor* strains, including that of Benitez Camilo *et al*. [28], who reported rates of 3.1 to 10.1 mm/d, and Hernández-Ibarra *et al*. [18], who documented rates of 5.9 to 7 mm/d. Moreover, in a study by Ahuatzi-Sastre *et al*. [19], five *P. ostreatus* strains showed values between 171 and 324 ìm/h (4.1 to 7.7 mm/d).

In the study of ligninolytic activity, we observed that the strains had high laccase and phenoloxidase activity, decreased MnP activity and little to no aril alcohol oxidase activity. The *P. djamor* strains had an overall higher oxidase and laccase activity than the *P. ostreatus* strains. The *P. ostreatus* strains belonged to the group with the lowest activity. These results are consistent with a study from Salmones and Mata [20] that compared the laccase production in liquid medium and determined that *P. djamor* strains had a higher activity than *P. ostreatus* and *P. pulmonarius* strains. These authors reported that the activities from strains grown in a liquid medium ranged between 3.5 and 4.9 U/ml, which were highly inferior to those found in a solid substrate in this study. A greater laccase activity was observed in the fungal mycelium extract compared to the sporocarps extract. However, a higher phenoloxidase activity was observed in the sporocarp extract than in the mycelium extract in seven of the strains tested. According to previous studies, this phenomenon is likely a result of the role of phenoloxidases and laccases, in particular, in macrofungi morphogenesis [21, 22].

The MnP activity was lower than the laccase and phenoloxidase activity but was detected with a higher intensity in the mycelium extract than in the pileus (in 8 of 12 strains). The MnP activity was statistically higher in the *P. ostreatus* control strain ECS-0152 than in the other strains. The highest laccase activity was shown by strain ECS-0123 (546.2 ± 4.5 U / ml), which was particularly noteworthy. This strain was located in the second group with the highest mycelial extract MnP(13.19 U/ml) and phenoloxidase activities (7.08), and it was ranked among the highest for mycelial growth and production of intermediate fruiting bodies. Strains ECS-0150, ECS-0151 and ECS-0144 had higher enzymatic activities than the average and were considered outstanding and of high study interest. Ramirez *et al*. [23] obtained a laccase activity of 20 U/ml from a crude extract of a *P. ostreatus* strain grown on bran. Similarly, Salmones and Mata [20] found that *P. djamor* strains from different parts of the world usually had a higher laccase capacity when cultured in agar than *P. ostreatus* and *P. pulmonarius.* Because the activity was determined in a crude extract, an analysis with purified extracts must be performed to more accurately determine the potential and characteristics of the enzymes involved. Many enzymes have been shown to be involved in the degradation of toxic compounds, and this mode of bioremediation is considered inexpensive and environmentally friendly [24].

Table 4. Morphological characterization of *Pleurotusdjamor* strains studied and compared to control strains *P. ostreatus* ECS-0152 and ECS-1123

Strain	Colour when ripe*	Odor	Flavor	Texture	Size (cm)	Form	Consistency	Habit	Stipe	Sporeprintcolour*
ECS-0122	White (N00, M00, C00)	Fungus	Oily, Sweet	Subcoriaceous	2-11.5	Flabellate	Firm	Imbricate	NF	Gray (N10, M00, C00)
ECS-0123	White (N00, M00, C00)	Fungus	Sweet	Coriaceous	3.5-16.5	Flabellate	Firm	Imbricate	NF	Gray (N10, M00, C00)
ECS-0127	Rose (N00, M10, C00)	Fungus	Oily, Sweet	Coriaceous	3.5-10.5	Flabellate	Firm	Imbricate	NF	Rose (N00, Y20, M20)
ECS-0130	Rose (N00, M10, C00)	Farinaceous	Sweet	Subcoriaceous	2-9.5	Flabellate	Firm	Imbricate	NF	Rose (N00, Y30, M20)
ECS-0139	White (N00, M00, C00)	Fungus	Fungus	Soft	2-7.5	Flabellate-Recurved	Firm	Gregarious	0.5 cm long	White (N00, M00, C00)
ECS-0141	White (N00, M00, C00)	Fungus	Sweet	Coriaceous	2-9.5	Conchate -	Firm	Imbricate	NF	White (N00, M00, C00)
ECS-0143	White (N00, M00, C00)	Farinaceous	Oily	Subcoriaceous	2-8.5	Conchate	Firm	Imbricate	NF	Gray (N10, M00, C00)
ECS-0144	White (N00, M00, C00)	Fungus	Fungus	Coriaceous	2-10.5	Flabellate	Firm	Imbricate	NF	Gray (N10, M00, C00)
ECS-0150	White (N00, M00, C00)	Farinaceous	Farinaceous	Subcoriaceous	2-8.5	Conchate	Firm	Imbricate	NF	Gray (N10, M00, C00)
ECS-0151	White (N00, M00, C00)	Fungus	Oily	Coriaceous	2-10.5	Flabellate-Recurved	Firm	Gregarious	0.5 cm long	White (N00, M00, C00)
ECS-0152	White (N00, M00, C00)	Fungus	Fungus	Soft	4-12.5	Conchate	Firm	Imbricate	0.5-4 cm long	Gray (N10, Y10, M0)
ECS-1123	Sand (N50, Y10, M10)	Fungus	Fungus	Chalky	4.5-16	Conchate	Firm	Imbricate	0.5-4 cm long	Gray (N10, Y10, M00)

*Color determination based on Küppers 2002
NF: Without stipe

The determination of antioxidant activity using the Folin Ciocalteu test estimated that the total phenol content in the samples varied from 229 to 517.8 eq. gal. ac/g (strains ECS-0141 and ECS-123, respectively) in the mycelium extract and from 54.1 to 231.3 eq. gal. ac/g (strains ECS-0143 and ECS-0152, respectively) in the pileus extract. These values were higher than those reported by Kim *et al*. [6] for methanol extracts from three types of fungi of this genus that have grey, pink and yellow pileus; these authors reported values ranging from 21.2 to 39.3 mg/g. In all cases, the phenol content was higher in the extract from the mycelium than in the sporocarp. According to Gutfinger [12], a high polyphenol concentration was associated with a high resistance to oxidation, and there was a linear relationship between the polyphenol content and oxidative stability. DPPH is one of the most commonly used tests to determine antioxidant properties. The mycelium extracts had antioxidant values ranging between 3.7 and 38% for ECS-0151 and ECS-1123, respectively, and between 8.8% and 41% in the pileus. Thus far, *P. djamor* antioxidant activity has not been determined using DPPH. A high antioxidant activity has been reported for other species of this genus, such as *P. sajor-caju*, *P. ostreatus*, *P. salmoneostramineus* and *P. cornucopiae* [5, 6, 25]. Vamanu [26] found a 30 to 40% antioxidant capacity using the DPPH assay in samples containing 4 mg/ml of *P. ostreatus* exopolysaccharides and endopolysaccharides. A growing number of findings have evaluated the role of oxygen and free radicals on cell damage and their effects on disease and aging. For this reason, antioxidants are becoming more relevant not only as food supplements but also as food for direct consumption [27].

The strains evaluated in this study showed significant morphological and organoleptic (colour, flavour, odour and texture) variability. Variations in colour between pink and white were observed, and leathery and subcoriaceous textures were the most frequent textures. However, the strain ECS-0139 had a smooth texture. The production of fruiting bodies did not provide for a statistical differentiation of the strains because all of the strains had a BE value that ranged between 36.4 and 71.4%. These values were low compared to other results reported for this species, which were 52-92% [18] and 49-125% [28]. The authors of these two studies used a coffee pulp substrate, and they calculated the production using more than two crops. Huerta *et al*. [15] reported even higher BE values for *P. djamor* strains from other parts of Mexico that were grown with two crops of coffee pulp (138 to 178%). However, the *P. djamor* strain ECS-0150 studied here and tested by Huerta *et al*. [15] had a BE value of 63%, which was similar to what was produced in this study. It is likely that the substrate used in this study (Pangola grass) is not optimal for this species and does not allow the expression of their full productive potential. The production rate ranged from 1.36 to 2.1%, which was similar to the results obtained Hernández Ibarra *et al*. [18] (1.18 to 2.21%).

ACKNOWLEDGEMENTS

This work was financially supported by the National Subsystem of Microbial Genetic Resources (SUBNARGEM in Spanish) dependent of the National System of Genetic Resource of Mexico.

REFERENCES

[1] Guzmán G *et al*. (1993). Studies of the Genus *Pleurotus* (Basidiomycotina), II. *P. djamor* in Mexico and other Latin–American countries, taxonomic confusions, distribution and semi–industrial culture. *Criptogamic Botany.* 3: 213–220.

[2] Huerta Palacios *et al*. (2010). Genetic relationships between Mexican species of *Pleurotus* analyzing the ITS-region from rDNA. *Micologia Aplicada International.* 22(1):15-25.

[3] Andrade Gallegos RH *et al*. (2012). La producciónіberoamericana de hongos comestibles en el contextointernacional. In: Sánchez JE, Mata G (eds.) Hongos comestibles y medicinales en Iberoamerica. Investigación y desarrollo en unentorno cultural. Ecosur-Inecol. Tapachula, Mexico. 9-16.

[4] Conabio. (2013). La biodiversidad en Chiapas: Estudio de Estado. Comision Nacionalpara el Conocimiento y Uso de la Biodiversidad/Gobierno del Estado de Chiapas. Mexico. Vols. 1 y 2.

[5] Khan MA *et al*. (2011). *Pleurotus sajor-caju* and *Pleurotus florida* mushrooms improve some extent of the antioxidant systems in the liver of hypercholesterolemic rats. *The Open Nutraceuticals Journal.* 4: 20-24.

[6] Kim JH *et al*. (2009). The different antioxidant and anticancer activities depending on the color of oyster mushrooms. *Journal of Medicinal Plants Research.* 3(12): 1016-1020.

[7] Sinclair CG and Cantero D. (1990). Fermentation modelling. In: Fermentation. A practical approach. Mc Neil B, Harvey LM (Eds.). IRL Press. 65-112.

[8] Ögel ZB *et al*. (2006). Production, properties and application to biocatalysis of a novel extracellular alkaline phenol oxidase from the thermophilic fungus *Scytalidium thermophilum*. *Appl Microbiol Biotechnol.* 71: 853–862.

[9] Li K, Xu F and Eriksson KEL. (1999). Comparison of Fungal Laccases and Redox Mediators in Oxidation of a Nonphenolic Lignin Model Compound. *Appl Env Microbiol.* 65(6): 2654–2660.

[10] Urra J *et al*. (2006). Screening of static culture and comparison of batch and continuous culture for the textile dye biological decolorization by *Phanerochaete chrysosporium. Braz J Chem Eng.* 23: 281-290.

[11] Muñoz G *et al*. (1997). Laccaseiso enzymes of *Pleurotus eryngii*: characterization, catalytic properties, and participation in activation of molecular oxygen and Mn oxidation. *Appl Environ Microbiol.* 63:2166-2174.

[12] Gutfinger T. (1981). Polyphenols in olive oils. *J Am Oil Chem Soc.* 58: 966-968.

[13] Fukumoto L and Mazza G.. (2000). Assessing antioxidant and prooxidant activities of phenolic compounds. *Journal of Agricultural and Food Chemistry.* 48: 3597-3604.

[14] Corner EJH. (1981). The agaric genera *Lentinus, Panus* and *Pleurotus* with particular reference to Malaysian species. *Beiheftezur Nova Hedwigia.* 69: 1-169.

[15] Huerta G *et al*. (2009). Grupos de interesterilidad y productividad de *Pleurotus* de regiones tropicales y subtropicales de México. Rev Mex Mic 30: 31-42.

[16] Vilgalys R *et al*. (1996). Recent advances in molecu-lar systematics of the genus *Pleurotus*. En: Mushroom Biology and Mushroom Products. Royse DJ (Ed.). The Pennsylvania State University, University Park 91-101.

[17] Chang ST. (1982). Mushroom spawn. In: Chang ST, Quimio TH (Eds.) Tropical mushrooms. Biological nature and cultivation methods. The Chinese University Press. 31-46.

[18] Hernández-Ibarra H *et al*. (1995). Evaluación de 5 cepasnativas de *Pleurotus* spp. de la región de Tapachula. *Rev Mex Mic.* 11: 29-38.

[19] Sastre-Ahuatzi M *et al*. (2007). Mycelial growth of strains of *Pleurotus ostreatus* developed on agar and its correlation with the productivity in pilot production farm. *Brazilian Journal of Microbiology.* 38: 568-572.

[20] Salmones D and Mata G.. (2005). Efecto de la presencia de compuestossolubles de lignina y fenolessobre la producción de lacasa y biomasa en cultivos de *Pleurotus* spp. *Rev Mex Mic.* 21: 63-69.

[21] Das N *et al*. (1997). Importance of laccase in vegetative growth of *Pleurotus florida*. *Appl Env Microbiol.* 63(10): 4120.

[22] Leatham GF and Stahmann MA. (1981. Studies on the Laccase of *Lentinus edodes:* Specificity, Localization and Association with the Development of Fruiting Bodies. *J Gen Microbiol.* 125: 147-157.

[23] Ramírez NE *et al*. (2003). Characterising laccase obtained by two production methods using *Pleurotus ostreatus*. *Revista colombiana de biotecnología.* 2: 64-72.

[24] Karigar CS and Rao SS. (2011). Role of microbial enzymes in the bioremediation of pollutants: A Review. Enzyme Research. Article ID 805187, 11 p. http://dx.doi.org/10.4061/2011/805187.

[25] Jayakumar T *et al*. (2009). *Invitro* antioxidant activities of an ethanolic extract of the oyster mushroom, *Pleurotus ostreatus*. *Innovative Food Science and Emerging Technologies.* 10: 228-234.

[26] Vamanu E. (2012). Biological activities of the polysaccharides produced in submerged culture of two edible *Pleurotus ostreatus* mushrooms. *J Biomed Biotechnol*.,Article ID 565974, doi:10.1155/2012/565974. 8 p.

[27] Rao AV and Balachandran B. (2002). Role of Oxidative Stress and Antioxidants in Neurodegenerative Diseases. *Nutritional Neuroscience.* 5(5): 291-309.

[28] Benítez-Camilo FA, Huerta-Palacios G and Sánchez-Vázquez JE. (1998). Producción de 18 cepas de *Pleurotus djamor* del Soconusco, Chiapas. Quehacercientífico en Chiapas. UNACH. 1(2): 25-36.