Mycelial Growth and Fruit Body Nutritional Composition of *Pleurotus* Species Grown on Different Lignocellulosic Waste-based Media

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Pleurotus species are edible mushrooms that possess the ability to degrade lignocellulose and are cultivated on lignocellulosic substrates to produce fruiting bodies that are appreciated for their nutritional and culinary values. Radial growth rate (u_r), mycelial biomass, and intracellular glycogen and protein contents were evaluated in the colonies of two strains of *P. ostreatus* (strains 26 and 50) and one strain of *P. pulmonarius* (strain 35) grown on barley straw extract agar, corn stover extract agar (CSEA), wheat straw extract agar, and glucose-yeast extract agar (GYEA). The nutritional composition of the *Pleurotus* fruit bodies cultivated on barley straw, corn stover, and wheat straw were also determined. Of all of the strains, *P. ostreatus* 50 showed the highest u_r on the CSEA and GYEA, and *P. ostreatus* 26 showed the greatest mycelial biomass production on the lignocellulosic waste-based agar media (LWAM). All of the *Pleurotus* strains showed the greatest and lowest glycogen contents on the CSEA and GYEA, respectively. These results showed that the LWAM are more suitable for mycelial growth than a glucose-based agar medium, and that *Pleurotus* fruit bodies cultivated on corn stover had the highest nutritional value compared with fruit bodies grown on the wheat and barley substrates.

Keywords: Fruit body; Lignocellulosic wastes; Mycelium; Pleurotus ostreatus; Pleurotus pulmonarius

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INTRODUCTION

Pleurotus species are edible mushrooms that are appreciated for their exquisite flavor and economic and ecological values. *Pleurotus ostreatus* (oyster mushroom) is the second most cultivated edible mushroom worldwide after *Agaricus bisporus* (Sánchez 2010). *Pleurotus ostreatus* is cultivated in colder places (≈ 20 °C) than *P. pulmonarius*, which is a tropical species of edible oyster fungi. These organisms are basidiomycetes and have two phases of growth (vegetative and reproductive), each with specific characteristics and requirements. The vegetative phase or mycelial growth is seen as microscopic filaments or hyphae that absorb digestive products and colonize the substrate (*e.g.* agar or lignocellulosic substrates) (Philippoussis 2009). The reproductive phase corresponds to the fruit body and it is represented by the mushroom itself (oyster shaped), which is formed after the fusion of two primary compatible mycelia with reciprocal nuclei exchange

(Nieuwenhuis *et al*. 2011). *Pleurotus* species have the ability to degrade several lignocellulosic substrates because of their highly efficient enzymatic machinery (Bilal *et al.* 2017; Hernández-Dominguez *et al.* 2017). These species can be cultivated on a wide variety of substrates, such as sugar cane bagasse, corn stover, wheat straw, rice straw, and barley straw, which are some of the largest accumulated lignocellulosic wastes because of agricultural activity (Sánchez 2010; Jeznabadi *et al.* 2016; Sadh *et al*. 2018). Lignocellulosic residues are generated as agricultural by-products, which are disposed of by burning in fields (Saini *et al.* 2015). Therefore, cultivation of edible mushrooms might be the only current process that combines the production of protein-rich food with the reduction of environmental pollution (Sánchez 2010). It represents one of the most efficient biotechnological processes for lignocellulosic residue recycling (Mandeel *et al*. 2005). Substrate and fungal strains are very important for mushroom cultivation because an appropriate substrate and fast-growing strain promote a rapid mycelial invasion of the substrate, which avoids the risk of contamination in the cultivation process. *Pleurotus* species are a rich source of protein, minerals (P, Ca, Fe, K, and Na), and vitamins (thiamine and riboflavin) (Sánchez 2010; Deepalakshmi and Mirunalini 2014). However, the nutritional composition of the fruit body depends on the substrate on which the mushroom is cultivated (Sánchez 2010). *Pleurotus* species have high nutritional values and contain various bioactive compounds that have been isolated and identified from both the fruit body and mycelium (Lavi *et al*. 2012; Maftoun *et al*. 2013; Ganeshpurkar *et al*. 2015). Additionally, the fungal cell wall contains β-glucans, which have antioxidant and immunomodulatory properties (Maftoun *et al*. 2013; Ganeshpurkar *et al*. 2015).

In this work, the radial growth rate, mycelial biomass production, intracellular glycogen content, and intracellular protein content were evaluated in the colonies of two strains of *P. ostreatus* and one strain of *P. pulmonarius* grown on lignocellulosic waste-based agar media and a glucose-yeast extract agar medium. The nutritional composition of the fruit bodies of the three *Pleurotus* strains cultivated on barley straw, corn stover, and wheat straw were also determined.

EXPERIMENTAL

Strains

Three strains of *Pleurotus* were used: *P. ostreatus* NRRL 3526 (Po 26) from the NRRL (now ARS) culture collection (Peoria, USA), *P. ostreatus* 50 from the COLPOS-Puebla culture collection (Puebla, Mexico), and *P. pulmonarius* 35 (PL35) from the culture collection at the Chinese University of Hong Kong (Hong Kong, China). Stock cultures were grown on malt extract agar in the dark at 25 °C in petri dishes for 7 days, which were then stored at 4 °C. Cultures were periodically transferred to fresh culture media.

Culture Media and Culture Conditions

Three lignocellulosic waste-based agar media were prepared: barley straw extract agar (BSEA), corn stover extract agar (CSEA), and wheat straw extract agar (WSEA). The media were prepared as follows: 100 g of dry chopped lignocellulosic material (bought from local farmer's markets) were boiled in 1 L of tap water for 1 h. The lignocellulosic material was filtered through a double layer of muslin and discarded. The liquor was placed in glass containers and the volume was made up to 1 L, and then it was autoclaved at 120 °C for 30 min. Prior to autoclaving, a final concentration of 20 g/L agar was added. The

pH was adjusted to 6.5. Glucose-yeast extract agar (GYEA) was used as the control medium. It contained glucose (10 g/L), yeast extract (5 g/L), KH_2PO_4 (0.6 g/L), $MgSO_4$-$7H_2O$ (0.5 g/L), K_2HPO_4 (0.4 g/L), $CuSO_4$-$5H_2O$ (0.25 g/L), $FeSO_4$-$7H_2O$ (0.05 g/L), $MnSO_4$ (0.05 g/L), and $ZnSO_4$-$7H_2O$ (0.001 g/L). All of the media were poured into petri dishes, which were inoculated in the center with an inoculum (4 mm in diameter) taken from the periphery of a 7-d old colony grown on the malt extract agar. The agar plates were incubated at 25 °C for 7 day.

Radial Growth Rate and Mycelial Biomass

The colony diameters were measured from the second to seventh day of incubation. Radius *versus* time plots were analyzed by linear regression, and the slope for each treatment was recorded as the radial growth rate (u_r) (González-Márquez *et al*. 2015). The mycelial biomass was determined from the 7-day old colonies. Mycelium was separated from the culture medium and filtered using a boiling water bath, as previously reported by González-Márquez *et al*. (2015).

Determination of the Intracellular Glycogen and Intracellular Protein Contents

The contents of the intracellular glycogen and protein were determined in the mycelia of the colonies. The intracellular mycelial extract was obtained from the mycelium scraped from the agar and ground in 1 mL of distilled water for each 0.1 mg of fresh weight biomass using a tissue grinder (Pyrex glass, Corning Inc., Corning, NY, USA). The resulting samples were centrifuged at 20000 × *g* for 10 min at 2 °C. The supernatant was collected as the intracellular extract and the sediment was the cell wall. In the intracellular extract, the total intracellular protein content (mg/g of dry weight (DW)) was measured by the method by Bradford (1976), using bovine serum albumin as the standard. The glycogen content (mg/g of DW) was the difference between the total sugars and reducing sugars (Sánchez *et al*. 2004).

Fruit Body Production

Spawn preparation

The *P. ostreatus* spawn (*i.e.* the seed required for growing the mushrooms) were prepared using wheat grains, which were previously soaked in water over night and then drained. Plastic bags (30 cm x 20 cm) were filled with 500 mg of soft and moist wheat grains and autoclaved at 121 °C for 30 min. After cooling down to room temperature, the bags were inoculated with three mycelial agar fragments (10 mm in diameter) taken from a fully-grown colony of *P. ostreatus*. The inoculated bags were incubated at 25 °C for 7 days (Sánchez 2010).

Substrate preparation, inoculation, incubation, and harvest

Lignocellulosic residues (with lengths of about 2 cm to 6 cm) were milled, immersed in tap water, and then pasteurized at 85 °C for 1 h. The pasteurized lignocellulosic substrate was placed on a wire mesh to drain off the excess water. The lignocellulosic substrate moisture content was adjusted to 65%. Next, 5 kg of pasteurized substrate were packed into polythene bags, inoculated with 10% spawn, and then incubated at 25 °C for 2 weeks. Completely mycelial-invaded bags were moved to a cropping room, where the relative humidity was 85%, the temperature was 25 °C, and the light intensity

was about 100 lux. The cropping room was watered to maintain the moisture content during the cropping time. Mushroom harvest was performed from each of the substrate bags after 7 days of being in the cropping room (Sánchez 2010).

Nutritional Composition of the Fruit Bodies

The harvested mushrooms were dried in an oven at 60 °C to a constant weight to calculate the moisture content, and then the dried mushrooms were ground into a powder with a mortar and pestle for other analyses. Mushroom samples were analyzed to determine their nutritional composition. The ash, protein, carbohydrate, fat, and fiber contents were determined using the procedures from AOAC (1995). The ash content was measured by incineration at 600 °C using a muffle furnace. The protein content (N × 6.25) was evaluated by the macro-Kjeldahl method. The phenol-sulfuric acid method was used to calculate the total carbohydrates content. The fat content was estimated by extracting a known weight of a powdered sample with ethyl ether using a Soxhlet apparatus (FC20, SEV PRENDO, Mexico City, Mexico).

Statistical Analysis

Each treatment was performed in triplicate. The data was evaluated with a one-way analysis of variance (ANOVA) and Tukey post-test using the Graph Pad Prism® program (San Diego, CA, USA).

RESULTS AND DISCUSSION

Radial Growth Rate and Mycelial Biomass

Table 1 shows the u_r of the *Pleurotus* sp. grown on the lignocellulosic waste-based agar media and GYEA. The *P. ostreatus* 26 strain showed the greatest u_r on the BSEA, CSEA, and WSEA media, and the lowest u_r on the GYEA medium. The *P. ostreatus* 50 strain had the highest u_r on the CSEA medium, followed by the WSEA, BSEA, and GYEA media. The *P. pulmonarius* 35 strain showed the highest u_r on the CSEA and WSEA media, followed by the GYEA and BSEA media. Of all of the strains, *P. ostreatus* 26 showed the greatest u_r (0.29 mm/h to 0.3 mm/h) on the lignocellulosic waste-based agar media. Sánchez and Viniegra-González (1996) reported that the u_r of a highly productive strain of *P. ostreatus* grown on a starch agar medium was 0.298 mm/h. Téllez-Téllez *et al*. (2003) reported that the u_r for *P. pulmonarius* grown on a potato dextrose agar (PDA) medium was 0.099 mm/h. The results of this study showed that the lignocellulosic waste-based agar media promoted rapid mycelial growth.

The mycelial biomass production by the *Pleurotus* strains is shown in Table 2. The *P. ostreatus* 26 strain had the highest mycelial biomass production on both the CSEA and WSEA media, and the lowest mycelial biomass production on the BSEA and GYEA media. The *P. ostreatus* 50 strain showed the highest mycelial biomass on the CSEA and GYEA media, followed by the WSEA and BSEA media. The *P. pulmonarius* 35 strain had the highest mycelial biomass production on the CSEA medium, followed by the WSEA and GYEA media, and the lowest mycelial biomass was produced on the BSEA medium. Of the three strains, *P. ostreatus* 50 showed the highest mycelial biomass production on the CSEA and GYEA media (100 mg/cm^2). González-Márquez *et al*. (2015) reported that the mycelial biomass production by *Lentinula edodes* (a slow-growing mushroom) grown on a GYEA medium was 0.020 mg/cm^2. Téllez-Téllez *et al*. (2003) reported that the mycelial

biomass production for *P. pulmonarius* on a PDA medium was 0.26 mg/cm^2. In general, it was shown that the lignocellulosic waste-based agar media were more suitable for growth of the *Pleurotus* sp. than the GYEA.

Table 1. Radial Growth Rate of the *Pleurotus* sp. Colonies Grown on the Lignocellulosic Waste-based Agar Media

Strain	Culture Medium			
	BSEA	CSEA	WSEA	GYEA
P. ostreatus 26	0.29 ± 0.01^a	0.3 ± 0.01^a	0.29 ± 0.01^a	0.22 ± 0.02^b
P. ostreatus 50	0.22 ± 0.02^c	0.28 ± 0.005^a	0.26 ± 0.02^b	0.24 ± 0.01^c
P. pulmonarius 35	0.22 ± 0.01^c	0.28 ± 0.01^a	0.28 ± 0.005^a	0.26 ± 0.01^b

Values are in mm/h and expressed as the mean ± SD (n = 3); means within the same column not sharing common superscript letters (a to c) differ significantly at a 5% level

Table 2. Mycelial Biomass Production by the *Pleurotus* sp. Colonies Grown on the Lignocellulosic Waste-based Agar Media

Strain	Culture Medium			
	BSEA	CSEA	WSEA	GYEA
P. ostreatus 26	50 ± 2^b	60 ± 8^a	60 ± 7^a	50 ± 6^b
P. ostreatus 50	10 ± 2^c	100 ± 15^a	90 ± 3^b	100 ± 6^a
P. pulmonarius 35	10 ± 1^c	70 ± 2^a	60 ± 3^b	60 ± 5^b

Values are in mg/cm^2 and expressed as the mean ± SD (n = 3); means within the same column not sharing common superscript letters (a to c) differ significantly at a 5% level

Intracellular Protein and Glycogen Contents

The strains *P. ostreatus* 26 and *P. ostreatus* 50 had the greatest intracellular protein content in the CSEA medium, and their lowest intracellular protein content was seen on the BSEA, WSEA, and GYEA media. The *P. pulmonarius* strain showed the highest intracellular protein content on the lignocellulosic waste-based agar media, and the lowest intracellular protein content on the GYEA medium. The protein intracellular content was around 2- and 3-fold higher for *P. pulmonarius* and *P. ostreatus*, respectively, on the CSEA medium compared with the GYEA medium (Table 3). González-Márquez *et al*. (2015) reported that the intracellular protein content for *L. edodes* was 0.04 mg/g of DW. In the present research, the highest intracellular protein content (0.19 mg/g of DW) was measured with *P. ostreatus* 50 grown on the CSEA medium.

Table 3. Intracellular Protein Content of the *Pleurotus* sp. Colonies Grown on the Lignocellulosic Waste-based Agar Media

Strain	Culture Medium			
	BSEA	CSEA	WSEA	GYEA
P. ostreatus 26	0.1 ± 0.002^b	0.16 ± 0.002^a	0.09 ± 0.001^b	0.05 ± 0.001^c
P. ostreatus 50	0.13 ± 0.002^b	0.19 ± 0.001^a	0.09 ± 0.001^c	0.07 ± 0.001^c
P. pulmonarius 35	0.07 ± 0.001^a	0.08 ± 0.001^a	0.07 ± 0.001^a	0.04 ± 0.002^b

Values are in mg/g of DW and expressed as the mean ± SD (n = 3); means within the same column not sharing common superscript letters (a to c) differ significantly at a 5% level.

The intracellular glycogen content of the *Pleurotus* sp. colonies is shown in Table 4. All of the *Pleurotus* sp. showed the greatest and lowest glycogen contents on the CSEA and GYEA media, respectively. Of all of the strains, *Pleurotus* 50 had the highest intracellular glycogen content on the lignocellulosic waste-based agar media (91.9 mg/g of DW to 106.9 mg/g of DW) and *P. pulmonarius* had the lowest glycogen content on the GYEA medium (51.3 mg/g of DW). *Lentinula edodes* produced an intracellular glycogen content around 30 mg/g of DW on the GYEA medium (González-Márquez *et al*. 2015). These results showed that the CSEA medium increased both the mycelial growth and mycelial density for all of the strains tested. Furthermore, the media prepared using the lignocellulosic substrates are cheaper than commercial culture media (*i.e.* glucose-based media).

Table 4. Intracellular Glycogen Content of the *Pleurotus* sp. Colonies Grown on the Lignocellulosic Waste-based Agar Media

Strain	Culture Medium			
	BSEA	CSEA	WSEA	GYEA
P. ostreatus 26	84.3 ± 1.2^{c}	94.2 ± 0.98^{a}	89.5 ± 2.5^{b}	58.6 ± 1^{d}
P. ostreatus 50	92.4 ± 2.1^{b}	106.9 ± 3.4^{a}	91.9 ± 0.98^{b}	55.7 ± 2.4^{c}
P. pulmonarius 35	83.8 ± 2.2^{c}	89.9 ± 1.4^{a}	85.3 ± 0.5^{b}	51.3 ± 2^{d}

Values are in mg/g of DW and expressed as the mean ± SD (n = 3); means within the same column not sharing common superscript letters (a to d) differ significantly at a 5% level

Nutritional Composition of the Fruit Bodies

Table 5 shows the nutritional composition on a dry matter basis for the fruit bodies cultivated on the barley straw, wheat straw, and corn stover. The fruit bodies from both strains of *P. ostreatus* cultivated on the barley straw achieved higher moisture contents than those cultivated on the wheat and corn substrates. The *P. pulmonarius* fruit bodies formed on the wheat straw had higher moisture contents than those grown on the barley and corn substrates. In general, the fruit bodies from both strains of *P. ostreatus* grown on the wheat straw showed greater ash (mineral salts) and fiber contents than those grown on the barley and corn substrates. The *P. pulmonarius* fruit bodies cultivated on the barley straw had the highest ash and fiber contents, followed by those formed on the wheat straw and corn stover. The *Pleurotus* sp. fruit bodies cultivated on the barley straw had the highest fat content compared with those grown on the wheat and corn substrates. For all of the strains, the highest protein and carbohydrate contents were recorded from the fruit bodies cultivated on the corn stover. The selection of the substrate during mushroom growth is crucial to promote rapid mycelial growth, avoid the risk of contamination, and enhance mushroom production (Royse 2002).

These results showed that the mushroom fruit bodies grown on the corn stover had the highest nutritional value compared with those grown on the barley and wheat substrates, and that the *Pleurotus* fruit bodies are rich in proteins, carbohydrates, and fiber, and low in fat. The *Pleurotus* nutritional properties determined in the present research were similar to those reported previously for *Pleurotus* sp. grown on different lignocellulosic substrates (Hoa *et al*. 2015). The nature and nutrient composition of the substrate affected the mycelial growth and fruit body nutritional properties (Philippoussis 2009). Obodai *et al*. (2003) studied the growth of *P. ostreatus* on different substrates and reported that rice husk resulted in the fastest mycelial growth rate. However, it was not a suitable substrate

for fruit body production. The cultivation of *P. ostreatus* on maize stover results in higher fruit body yields than on rice husk (Obodai *et al*. 2003). In the present research, corn stover was a better substrate than barley and wheat straw in terms of the nutritive value for mushroom mycelial growth and fruit body cultivation. Corn stover is the above-ground portions of a corn plant, including the stalks, cobs, leaves, and husks (excluding the corn kernels themselves) (Wyman 2003). The glucan content of corn stover is around 40%, which could release up to 44.4 lb of glucose after hydrolysis for every 100 lb of dry feedstock (Wyman 2003). It also contains xylan, arabinan, galactan, and mannan. The glucan content of wheat straw and barley straw is 30.2% and 33.1%, respectively. Wheat straw and barley straw also contain xylan, galactan, and arabinan (Ballesteros *et al*. 2006; Garcia-Aparicio *et al*. 2006). This showed that corn stover has the best nutritional composition of all of the substrates tested. The selection of substrate for mushroom production is mainly determined by the abundance and cost of the substrate (Obodai 2003). Corn stover represents a good alternative for mushroom cultivation. It is one of the most abundant lignocellulosic substrates in the world (Sánchez 2009) and the most abundant in Mexico (Serna-Saldivar 2016).

Table 5. Nutritional Composition of the *Pleurotus* sp. Strains Cultivated on the Different Lignocellulosic Substrates

Component	Lignocellulosic Substrate		
	Barley Straw	Wheat Straw	Corn Stover
P. ostreatus 26			
Moisture	91.23 ± 0.31^{a}	91.16 ± 1.03^{b}	90.08 ± 0.74^{c}
Ash	0.70 ± 0.07^{b}	0.79 ± 0.04^{a}	0.72 ± 0.07^{b}
Protein	4.06 ± 1.05^{c}	4.14 ± 0.81^{b}	5.07 ± 0.93^{a}
Carbohydrate	2.8 ± 0.35^{b}	2.47 ± 0.29^{c}	3.13 ± 0.31^{a}
Fat	0.55 ± 0.02^{a}	0.49 ± 0.01^{b}	0.41 ± 0.02^{c}
Fiber	0.66 ± 0.007^{b}	0.95 ± 0.005^{a}	0.59 ± 0.01^{c}
P. ostreatus 50			
Moisture	91.37 ± 0.53^{a}	91.05 ± 1.31^{b}	90.17 ± 0.31^{c}
Ash	0.61 ± 0.02^{b}	0.77 ± 0.06^{a}	0.73 ± 0.07^{a}
Protein	4.24 ± 0.25^{b}	4.12 ± 1.09^{c}	5.23 ± 1.05^{a}
Carbohydrate	2.67 ± 0.16^{c}	2.91 ± 0.33^{b}	3.02 ± 0.35^{a}
Fat	0.51 ± 0.01^{a}	0.43 ± 0.03^{b}	0.4 ± 0.02^{b}
Fiber	0.6 ± 0.01^{b}	0.72 ± 0.005^{a}	0.45 ± 0.005^{c}
P. pulmonarius 35			
Moisture	91.04 ± 0.47^{b}	91.3 ± 1.24^{a}	90.39 ± 1.45^{c}
Ash	0.87 ± 0.05^{a}	0.68 ± 0.03^{b}	0.63 ± 0.09^{b}
Protein	4.73 ± 0.15^{b}	4.02 ± 0.22^{c}	5.06 ± 0.65^{a}
Carbohydrate	2.03 ± 0.28^{c}	2.9 ± 0.25^{b}	3.13 ± 0.13^{a}
Fat	0.56 ± 0.01^{a}	0.5 ± 0.02^{b}	0.44 ± 0.03^{c}
Fiber	0.77 ± 0.002^{a}	0.6 ± 0.01^{b}	0.35 ± 0.02^{c}

Values are in g/100 g of dry matter and expressed as the mean ± SD (n = 3); means within the same column for each oyster mushroom not sharing common superscript letters (a to c) differ significantly at a 5% level

CONCLUSIONS

1. Lignocellulosic waste-based agar media are more suitable for mycelial growth than a glucose-based agar medium.
2. The CSEA medium increased both the mycelial growth and mycelial density for all of the strains tested.
3. The *Pleurotus* fruit bodies cultivated on the corn stover had the highest nutritional value compared with the fruit bodies grown on the wheat and barley substrates.
4. Corn stover was the best lignocellulosic substrate for culture maintenance and fruit body cultivation of the tested substrates.

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