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Residues from the Amazon Region as Substrates for the Production of Oyster Mushrooms and Laccases

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A B S T R A C T

Mushroom cultivation is an economically viable process for the conversion of various lignocellulosic residues. Recently in order to increase the feasibility of mushroom production, research has been carried out to investigate the use of residues as substrates and the possibility of obtaining two or more products in a single bioprocess. The aim of this study wasto investigate the potential of biomass residues from street markets in the city of Manaus (Amazonas, Brazil) as a substrate for the production of oyster mushroom and laccase. *Pleurotus ostreatus* was cultivated in the following biomass residues: a) peel from the fruit of *Astrocaryum aculeatum* Meyer, b) peel from the fruit of *Bactris gasipaes* Kunth, c) fibers from the endocarp of the fruit of *Euterpe oleracea* Mart. and d) peel from the fruit of *Theobroma grandiflorum* Schumann. The peel from the fruit of *Bactris gasipaes* was the best substrate to produce oyster mushroom (4.8 % biological efficiency -ratio between the fresh weight of the mushroom and the dry weight of the compost), and the peel from the fruit of *Astrocaryum aculeatum* was the best substrate for laccase production (21,766 U/Kg). The present work is important since it allowed us to demonstrate that two biomass residues from Amazonian plants could be utilized to produce mushrooms and laccases.

Introduction

Pleurotus spp., commonly known as the oyster mushroom, is a common primary decomposer of wood and plant residues(Adebayo *et al.*, 2009; Bellettini *et al.*, 2019). This fungus can be naturally found in tropical and subtropical rainforests and can be artificially cultivated. Oyster mushrooms have been intensively studied in many different parts in the world, and are noted for having high

gastronomic and nutritional value since they have high quantities of proteins, carbohydrates, minerals (calcium, phosphorus, iron) and vitamins (thiamin, riboflavin, and niacin) as well as being low in fat (Adebayo *et al.,* 2009; Khatun *et al.,* 2015).

Pleurotus ostreatus can be cultivated on several substrates, such as rice straw, maize stalks/cobs, vegetable residues and bagasse, and require a shorter growth time when compared to other edible mushrooms. In the last decade, several studies have investigated the production of this mushroom using agro-industrial residues and the results obtained have been promising in terms of the use of residues, more economical processes and greater productivity (Alkoaik *et al.,* 2015; Inácio *et al.,* 2015; Parthasarathy *et al.,* 2017).

During the cultivation of oyster mushrooms, the fungi produce several enzymes that include hydrolases (cellulases and hemicellulases) and phenoloxidases that include laccases. Laccases (E.C. 1.10.3.2, p-benzenedial: oxygen oxidoreductase) catalyze the oxidation of various aromatic compounds (particularly phenol) with the concomitant reduction of oxygen to water, which presents several applications, such as color reduction of pigments, fine chemistry, effluent treatment, precipitation reactions in the wine industry, use as biosensors and preservatives(Souza *et al.,* 2011; Inácio *et al.,* 2015; Parthasarathy *et al.,* 2017). Due to the possibility of the simultaneous production of mushrooms and enzymes, research has explored the possibility of obtaining more than one product through this bioprocess (Souza *et al.,* 2010; Inácio *et al.,* 2015; Economou *et al.,* 2017).

Street markets in the city of Manaus produce about 35 tons of biomass residue daily(Clarice Manhã, 2014), and these residues include the peel from the fruit of *Astrocaryum aculeatum*, peel from the fruit of *Bactris gasipaes*, peel from the fruit of *Theobroma grandiflorum* and the endocarp of the fruit of *Euterpe oleracea*. Despite their abundance, few studies have investigated these biomass residues as substrates for biotechnological uses. The aim of the present study was to evaluate different agricultural residues obtained from Amazonian fruits in the production of oyster mushrooms and laccases.

Materials and Methods

Pleurotus ostreatus culture

Pleurotus ostreatus (code 154) was obtained from Cogumelo Hobby (Jundiaí, São Paulo, Brazil, http://www.cogumelohobby.com/). This strain was deposited in the Microbial Collection of the National Institute of Amazonian Research under number LMN154. This strain was maintained on malt extract agar at 26–28°C and 60–65% relative humidity.

Biomass residues

Four types of residue were used: a) peel from the fruit of *Astrocaryum aculeatum*, b) peel from the fruit of *Bactris gasipaes*, c) peel from the fruit of *Theobroma grandiflorum* and d) fibers from the endocarp of the fruit of *Euterpe oleracea* Mart. Theses residues were obtained (March 2015) from a street market in the city of Manaus, Amazonas State, Brazil (Coroado Market 3^{o}.08'36"S, 59^{o}.97'98"W) - Amazonas, Brazil. These residues are produced daily by the merchants during the sales and then discarded. For our study, we collected these residues before they were disposed of by the merchants. Subsequently, they were washed (to remove visible solids), dried (80 ºC for 24 h), ground (size < 3 mm) and then stored at -20 °C. As a control in the experiment, we also included sawdust from *Pinus elliottii, which was* obtained from a local carpenter's workshop.

Chemical materials

All chemical reagents (solvents, culture media, siringaldazine) were of analytical grade, and were obtained from commercial suppliers (SIGMA-ADRICH, MERK, Burlington, MA, United States) and used without further purification.

Substrate preparation and cultivation

Initially, *P. ostreatus* was cultivated on malt extract agar plates (7 days, 25°C). The mycelium disks from the agar plates (10 disks, 5 mm diameter) were used to inoculate the "seed": 30 g of wheat bran (particles of <3mm diameter and 70 % moisture) in 125 mL Erlenmeyer flasks (15 days, 25°C). The entire "seed" was used to inoculate one kilogram of each residue (70% moisture) in polypropylene bags (1 kg). After complete colonization of the substrate in polypropylene bags (approximately 15 days, 25°C), the bags were transferred to the incubation chamber and maintained under controlled conditions (22 °C, 90 % humidity, 12-hour photoperiod, 2000 lux) to induce fructification.

Biological efficiency

After the cultivation period, the mushrooms were harvested and weighed, and then dried in anoven at 70°C with air-circulation to determine their dry weight. Biological efficiency (BE, bioconversion of the substrate into fruiting bodies) was used to assess the effect of each residue on mushroom production: BE = fresh mushroom mass (g)/initial substrate dry mass (g) × 100%.

Chemical analysis

The composition of the biomass residue was determined according to the recommendations of the Association of Official Analytical Chemists(AOAC-Analitycal Association of Official Chemist 1999). Moisture content was determined based on weight loss and volatility by using an oven set at 100 °C until constant weight was reached. The fixed mineral residue (ash) was determined by incinerating the sample in an oven at 550°C. Protein content was determined using the Micro-Kjeldahl method by using nitrogen content as a proxy. A 6.25 factor was used for nitrogen conversion. Lipid content was determined by continuous ether extraction in a Soxhlet apparatus for 6 h, with diethyl ether serving as the solvent, and the samples were further evaporated in an oven at 105°C for 2 h. Total carbohydrate content was calculated as follows: total carbohydrate= 100 - (weight in grams [protein + fat + water + ash + alcohol] in 100 g of food).

Laccase activity

Laccase activity was determined using syringaldazine as the substrate, as described by (Souza *et al.,* 2014). Samples were collected from the bags at 10, 15, 20, 25 and 30 days. Enzyme extraction was carried out by mixing 1g of bioprocess substrate with 9 mL of water under orbital rotation (100 rpm) for 30 min. The mixture was centrifuged at 3000 *g* for 5 min and the supernatant was then collected. The presence of laccases was determined by mixing 0.5 mL of the supernatant with 0.3 mL of citrate–phosphate buffer (0.2 M, pH 5.0), 0.1 mL syringaldazine (1 mM) and 0.1 mL H_2O. The activities were analyzed in a spectrophotometer (UV/visible U-2000 Hitachi-Japan) at 525 nm. One unit of enzyme activity (IU) was defined as the amount of enzyme that oxidized 1 µmol of the syringaldazine per minute.

Statistical analysis

All experiments were performed in triplicate and means and standard deviation were calculated. Parametric t-tests were carried out to assess differences (95% confidence) between the means of the different substrates.

Results and Discussion

The proximate composition of the residues were analyzed and Table 1 shows the proximate composition of the biomass residues used in the present study. Peel from the fruit of *Astrocaryum aculeatum* Meyer presented highest content of protein and the fibers from the endocarp of the fruit of *Euterpe oleracea* Mart. had the highest carbohydrate content.

P. ostreatus produced fruiting bodies using the peel from the fruit of *Bactris gasipaes* and the peel from

the fruit of *Astrocaryum aculeatum* as the substrate (Figure 1).

In order to investigate which biomass residues were most suitable for cultivating and producing *P. ostreatus*, the biological efficiency (BE) values were determined (Table 2). The peel from the fruit of *Bactris gasipaes* Kunth was the substrate that presented the highest BE.

For each residue, the kinetics of laccase production by *P. ostreatus* were investigated (Figure 2). Laccase production was determined after 10, 15, 20, 25 and 30 days of culture. *P. ostreatus* produced the highest levels of laccase when using the peel from the fruit of *Astrocaryum aculeatum* (day 30, 21,766 UI/Kg).

We found that the peel from the fruit of *Bactris gasipaes* was the most adequate substrate for the growth of oyster mushrooms and the peel from the fruit of *Astrocaryum aculeatum* has potential as a substrate for the production of laccases by *Pleurotus ostreatus*. These findings support the discussion regarding the utilization of residues as substrates for fungal growth and the development of simultaneous fermentations to increase the economic viability of bioprocesses.

The results of the experiment demonstrated that*P. ostreatus* produced fruiting bodies using as sole substrate the peel from the fruit of *Bactris gasipaes* and the peel from the fruit of *Astrocaryum aculeatum.* Evaluation of component properties is essential in order to understand the influence of media composition in mushroom cultivation.

In the present study, the peel from the fruit of *Astrocaryum aculeatum* presented the highest protein content (23% w/w) among the residues that were tested. Previous studies have demonstrated the positive influence of nitrogen/protein sources in the growth of oyster mushrooms (Obodai *et al.,* 2003; Silva *et al.,* 2007; Thongklang and Luangharn, 2016; Bellettini *et al.,* 2019). Biological efficiency (BE) is defined as the ratio of the weight (g) of fresh mushrooms harvested to the initial dry weight (kg) of the substrate or as the ratio of the weight of the fresh mushrooms harvested (g) to the initial dry weight (g) of substrate expressed as a percentage (Obodai *et al.,* 2003; Iruoma and Nduka 2013; Bellettini *et al.,* 2019).

Biological efficiency mainly depends on the characteristics of the substrate and the environmental conditions of the growth process (Iruoma and Nduka, 2013; Bellettini *et al.,* 2019).

Our results demonstrated that the highest biological efficiency was obtained by cultivating *P. ostreatus* using the peel from the fruit of *Bactris gasipaes*as the substrate (4.8 %). When comparing our results to those of other studies, we observed that this result is similar to previous works that investigated new sources of substrates; however, our BE value is low when compared to works regarding production optimization (Table 3).

In addition to using residues as substrates, another way to reduce the costs of a bioprocess is to obtain two or more products in a single bioprocess.

In the present study, the culture medium used to produce oyster mushrooms was also investigated for laccase production. The laccases were produced with all the investigated residues. The highest level of laccases obtained was 21,766 U/Kg. This value can be considered similar and sometimes higher in comparison to other works dedicated to the production of laccases by this species (Membrillo *et al.,* 2011; Karp *et al.,* 2012; Inácio *et al.,* 2015; Economou *et al.,* 2017; Parthasarathy *et al.,* 2017).

The perspectives developed with the knowledge produced in the present work include: a) development of studies for optimization of the bioprocess; b) investigation of the influence of the bioprocess in the composition of the mushroom; and c) large scale experiments for studying the economic viability of the process.

Table.1 The proximate analysis of biomass residues collected from a street market in Manaus, Amazonas state, Brazil.

Composition	Ash (%)	Lipids (%)	Proteins (%)	Carbohydrates (%)
Peel from the fruit of *Astrocaryum aculeatum* Meyer	11.4 ± 0.1	52.9 ± 0.4	23.0 ± 0.1	12.7 ± 0.4
Peel from the fruit of *Bactris gasipaes* Kunth	3.9 ± 0.2	54.5 ± 0.4	10.0 ± 0.1	31.4 ± 0.4
Fibers obtained from the endocarp of the fruit of *Euterpe oleracea* Mart.	1.7 ± 0.1	0.2 ± 0.1	5.8 ± 0.4	92.2 ± 0.8
Peel from the fruit of *Theobroma grandiflorum* Schummann	6.5 ± 0.5	2.0 ± 0.1	13.8 ± 0.1	77.7 ± 0.6

Table.2 Biological efficiency (%) from Amazonian biomass residues for mushroom production (*Pleurotus ostreatus*).

Substrate	Biological efficiency % (g/g)
Peel of the fruit of *Bactris gasipaes* Kunth	4.8 ± 0.2%
Peel of the fruit of *Astrocaryum aculeatum*Meyer	1.5 ± 0.1%
Fibers obtained from the endocarp of the fruit of *Euterpe oleracea* Mart.	No fruitification
Peel from the fruit of *Theobroma grandiflorum* Schummann	No fruitification
Sawdust obtained from *Pinus elliottii*	1.2 ± 0.2%

Table.3 Biological efficiency of the production of oyster mushrooms using different substrates.

Authors	Main Substrate	Biological efficiency (%)
Present work	Peel from the fruit of *Bactris gasipaes* Kunth	4.8
Present work	Peel from the fruit of *Astrocaryum aculeatum* Meyer	1.5
(Adebayo *et al*. 2009)	Cotton residues	5.0
(Sales-campos 2010)	*Bactris gasipaes* stem	115
(Alkoaik *et al*. 2015)	Date palm residues	8-20
(Aguiar *et al*. 2013)	Sawdust from *Simarouba amara* and *Ochromapiramidale*	65-94*
(Thongklang and Luangharn 2016)	Corn straw	83.4*
(Tesfay *et al*. 2020)	Waste paper	17,92
(Papadaki *et al*. 2019)	Grape pomace	31,4
(Ritota and Manzi 2019)	Grape pomace + cotton gin residues (1:1)	137

*Studies included bioprocess optimization.

Fig.1 Fruiting bodies produced with biomass residues under the following experimental conditions: a) Sawdust obtained from *Pinus elliottii* (control); b-e) Peel from the fruit of *Bactris gasipaes* Kunth, f) Peel from the fruit of *Astrocaryum aculeatum*

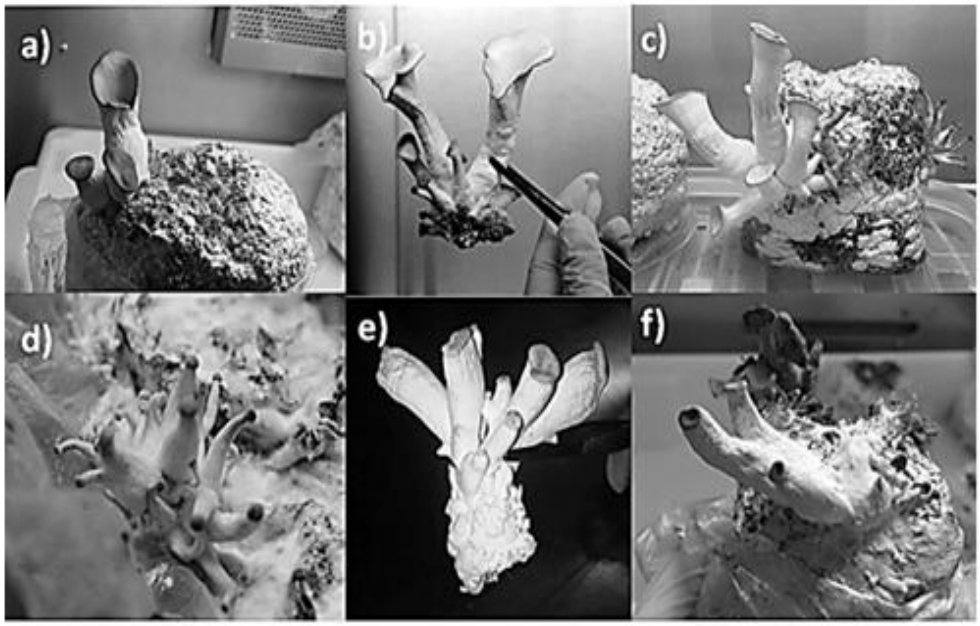
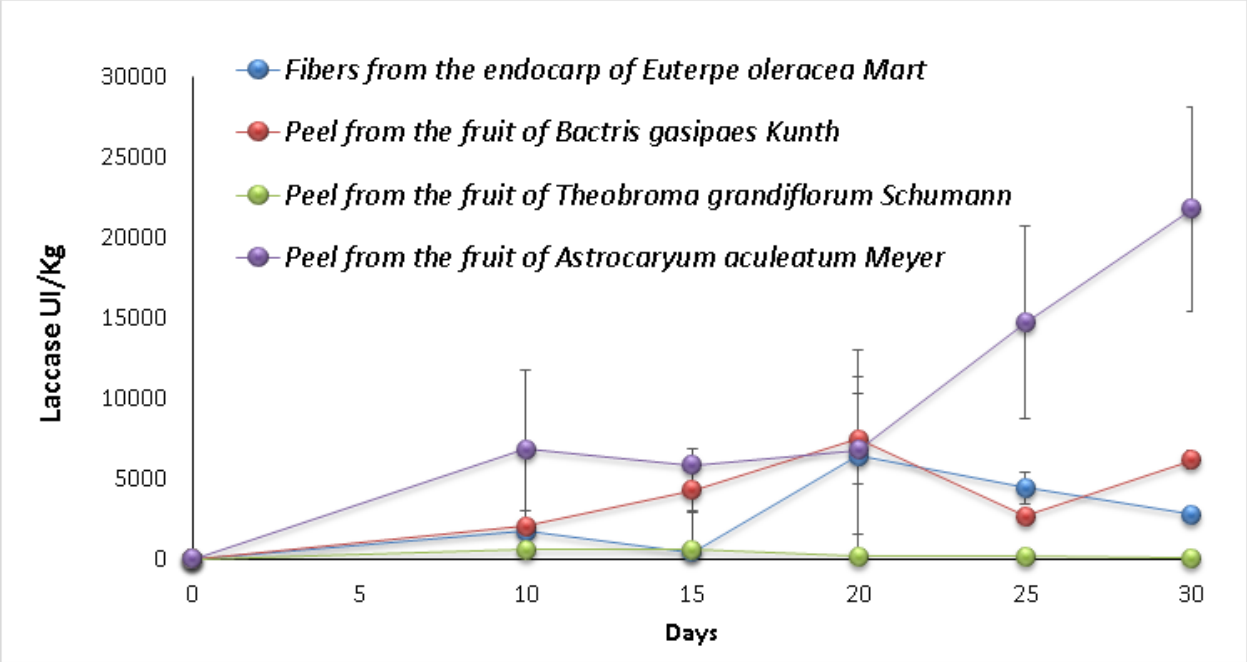


Fig.2 Laccase production by *Pleurotus osteatus* using different residues as substrates (peel from *Astrocaryum aculeatum*, peel from *Theobroma grandiflorum*, peel from *Bactris gasipaes*, and fibers from the endocarp of *Euterpe oleracea*).



Specialists agree that Amazon Rainforest must be preserved, mainly for protection of the climate and the huge biodiversity that is still unstudied (Oliveira and Baccaro, 2008). Around 20 million people live in the Amazon Rainforest and a bio-economy lead by biotechnology has the potential to be an appropriate route for economic development without environmental impacts(Bücker and Bücker-Falcão, 2018). Natives from the Amazon Rainforest have been consuming mushrooms for a very long time; however, in the developed cities of the region, low consumption of mushrooms is observed due to culinary culture but also due to the price of mushrooms (Aguiar *et al.*, 2013). The present work is important since it allowed us to demonstrate that two biomass residues from Amazonian plants could be utilized for the production of mushrooms and laccases. Optimization studies generated from this study have the potential to result in viable bioprocesses suitable for use in the Brazil Amazon.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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