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High-yield cellulase and LiP production after SSF of agricultural wastes by *Pleurotus ostreatus* using different surfactants

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ABSTRACT

White rot fungi are well known for their ability to degrade the structures of the plant cell walls and to produce of a wide range of extracellular enzymes using lignocellulosic biomass as substrate. In an attempt to reduce the costs and obtain high-yield of lignocellulolytic enzymes such as cellulases and lignin peroxidase, their production has been studied by solid-state fermentation using low-cost agroindustrial residues as carbon sources and surfactants. In this context, this work aimed the produce cellulases and lignin peroxidase from the fungus *Pleurotus ostreatus* PLO 9 using banana pseudostem, jatropha and coconut fiber as the substrates supplemented with the surfactants tween 80, sodium dodecyl sulfate and glycerol. The results showed the maximum lignin peroxidase activity was obtained after 15 days of fermentation using jatropha supplemented with sodium dodecyl sulfate (49916 ± 541 U g^{-1} of substrate). The highest cellulase production was reached after 5 days of fermentation using banana pseudostem supplemented with glycerol as the substrate (19.5 ± 0.2 FPU g^{-1} of substrate). The results indicate that it was possible to reach significant activities of cellulase and LiP using the white rot *Pleurotus ostreatus* after solid state fermentation of lignocellulosic biomass using surfactants as exogenous inducers.

1. Introduction

Lignocellulosic biomasses are considered the most abundant sustainable renewable energy sources (Lazim and Hadibarata, 2016), and are mainly composed of the polymers cellulose, hemicelluloses, and lignin. The development of biotechnological applications using lignocellulosic biomass requires an understanding of its biodegradation. Even in smaller amounts, the lignin fraction involves the polysaccharide portion affecting the porosity of the cell wall, forming a barrier to the biodegradation process (Huijgen et al., 2014; Singh et al., 2014). This protective effect caused by lignin is one of the main economic and technical obstacles for the production of biofuels and other chemical inputs based on lignocellulosic materials (Guan et al., 2015).

There is a large spectrum of microorganisms capable of degrading lignocellulosic materials, of which two groups of filamentous fungi belonging to the class Basidiomycetes are distinguished: white rot fungi and brown rot fungi. The fungi known as white rot have the ability to degrade all structures of the plant cell walls (Bari et al., 2015), unlike brown rot fungi, which selectively degrade the carbohydrates, with limited ability to degrade lignin (Pandey, 2003). *Pleurotus ostreatus* (Jacq.: Fr.) Kumm. (Dikarya, Basidiomycota, Agaricomycotina, Agaricales), a white rot basidiomycete fungus industrially cultivated in a variety of lignocellulosic materials, is an edible mushroom with high nutritional value (Reis et al., 2012). *P. ostreatus* produces a wide range of extracellular enzymes and can be used to produce lignocellulolytic enzymes (Talebnia et al., 2010).

These enzymes have been used extensively in different industries. Cellulases are among the enzymes with the greatest number of uses in industrial scale processes, such as in pulp and paper production stages, in the textile industry and in the bio-polymerization of jeans. In addition, they can be applied in the wine and brewery industries, in the fermentation of polysaccharide to produce alcoholic beverages.

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Cellulases are used in the extraction of fruit juices, and in the feed industry, by improving the quality, digestibility and nutritional value of animal feed. In recent years the cellulases has been extensively studied in the production of second-generation ethanol (Manisha and Yadav, 2017).

Lignin peroxidase (LiP) is an important member of the ligninolytic fungal enzymes, along with manganese peroxidase and laccase. LiP can disrupt the alpha and beta carbon bonds, by opening the aromatic rings of the dye structures and cause catalytic degradation of phenolic and non-phenolic compounds. A wide range of applications of LiP include the delignification of lignocellulosic materials for the production of biofuel, the conversion of coal into low molecular weight elements, the use as feedstock to produce chemicals, usage in the paper and polymer industry causing bio bonding and enzymatic polymerization, respectively, and removal of undesirable toxic organic pollutants, making it a biotechnologically important enzyme (Shaheen et al., 2017).

Due to the diverse applications of these enzymes in industrial processes, it is necessary to obtain enzymatic cocktails with high activity and stability, improving enzyme performance and consequently reducing the production costs of these biocatalysts (Lee et al., 2014). The production of lignocellulosic enzymes can be stimulated by the presence of a wide variety of inducers and may be represented by carbon sources for cell growth or inducers of enzymatic synthesis (Usha et al., 2014). The surfactants can act as inducers of enzymatic synthesis, stimulating spore growth, improving the permeability of the cell membrane leading to a greater secretion of the extracellular enzymes, enhancing enzyme stability, increasing the bioavailability of less soluble substrates to the fungus, and finally, contributing to an increase in enzyme production (Zheng and Obbard, 2001).

Solid state fermentation (SSF) has gained significant credibility in recent years in producing enzymes, where a fermented product can be directly used as an enzyme source (Castro and Sato, 2015). The SSF resembles the natural habitat of the fungi, resulting in improvements in growth and secretion of a wide range of enzymes when compared to the submerged fermentation (SmF). Other advantages include a low risk of contamination, a high yield, lower production costs, a reduction in environmental problems, making the SSF more attractive to be used in industrial processes for the production of enzymes (Lizardi-Jiménez and Hernández-Martínez, 2017). Many studies have reported the use of surfactants using lignocellulosic biomass to produce enzymes through SSF processes, pointing out the enormous potential of their use with SSF to increase enzymes production (Bharti et al., 2018; El-batal et al., 2015; Gautam et al., 2018; Irfan et al., 2014; Jain and Naik, 2018; Postemsky et al., 2017; Saratale et al., 2017; Singh and Singh, 2017; Szabo et al., 2015). However, this induction process was not demonstrated for agroindustrial residues and fungal strains used in our study. There is also no standardization of surfactant concentration and the comparison between the actions of these surfactants in different agroindustrial residues has been only slightly reported. Furthermore, our study groups performed enzyme production processes and edible mushrooms using enzyme synthesis inducers.

Considering the biotechnological relevance of these enzymes, especially cellulases and lignin peroxidase (LiP), the present study aims to evaluate their production using jatropha, banana pseudostem and coconut fiber as substrates and the surfactants tween 80, glycerol and sodium dodecyl sulfate (SDS) by *P. Ostreatus* through solid state fermentation (SSF).

2. Methods

2.1. Fungal strain and maintenance

The production of LiP and cellulases was performed using the fungus from the species *Pleurotus ostreatus* PLO 9, acquired from the fungi collection of the Laboratory of Mycorrhizal Associations of the Federal University of Viçosa (UFV). The fungus was grown and maintained in plates containing potato dextrose agar (PDA) at 25 °C, at a pH of 4.5, and stored at 4 °C.

2.2. Culture media

The inoculum was prepared by growing the fungus in petri dishes containing BDA at 25 °C for 7 days. After the growth, the disks of mycelia were removed and transferred to the culture media.

Twenty grams of each substrate (~70% moisture), previously autoclaved (1 h at 121 °C), were transferred into vials with the addition of 2 mL of Kirk's culture medium (pH 4.5) (Tien and Kent Kirk, 1983) and 1 mL of each surfactant tested (0.3 mM tween, 0.1 mM, SDS or glycerol P.A.). Four mycelial discs (1 cm) obtained after 7 days of growth, were added into each vial containing the culture media.

The vials were incubated for 5, 10 and 20 days at room temperature (around 25 °C). The crude enzyme extracts were collected by adding 100 mL of tartrate buffer to each flask (100 mmol L^{-1}, pH 3.5) containing EDTA (5 mM). Afterwards, the vials were centrifuged at 4000 rpm for 30 min and the supernatant was filtered on filter paper (Whatman N°. 1 filter paper). The filtrates were collected to determine the enzymatic activities.

All the experiments were performed in triplicate, including the control groups (substrates without the addition of surfactants).

2.3. Chemical composition of the substrates

The banana pseudostem, "Prata Anã" variety, was provided by UNIMONTES (State University of Montes Claros) from plantations in Janaúba, Minas Gerais state, Brazil. The coconut fiber (*cocos nucifera* L.) was collected in Acaiaca, Minas Gerais state, Brazil. The jatropha was donated from Fusermann biocombustível, located in Barbacena, Minas Gerais state, Brazil.

To perform the chemical composition analyses, the substrates were first converted into sawdust (40/60 mesh) using a Wiley mill, then dried at room temperature (23 ± 1 °C) and kept in closed bags for further analyses. The moisture contents were determined according to TAPPI 204 cm-97. The contents of Ash, extractive, carbohydrate (glucan, xylan, galactans, mannans and arabinans) and lignin (soluble, insoluble) were also determined.

The Ash content was analysed using the gravimetric method. Samples (0.5 g) were incinerated at 575 °C for 3 h. After calcination, the samples were cooled in a desiccator until they reached room temperature, and then weighed. The incineration was repeated until constant mass (TAPPI 211 om-02).

The extractive content was determined by submitting the samples to Soxhlet extraction with acetone for 5 h. Extractive-free biomasses (0.3 g) were submitted to acid hydrolysis with 72 wt% H_2SO_4 at 30 °C for 1 h with occasional mixing. The resulted hydrolysates were used to determine carbohydrates and lignin contents. The Klason lignin (insoluble lignin) was determined gravimetrically (Gomide and Demuner, 1986) and the soluble lignin was measured by ultraviolet spectroscopy using an equation, according to Goldschmid, (1971):

$$C\ (g\ L^{-1}) = 4.53\ (Al - Ac) / 300 \quad (1)$$

Where *Al* and *Ac* correspond to the absorbance at a wavelength of 215 nm and 280 nm, respectively.

The sugar contents (arabinose, galactose, glucose, xylose, and mannose) were determined using the supernatant after acid hydrolysis according to Wallis et al. (1996). This was carried out using a high-performance liquid chromatography (HPLC) system Dionex ICS3000 (Dionex Co. – Sunnyvale, CA, USA), equipped with a pulsed amperometric detector (PAD), a gold electrode, a CarboPac PA1 column (ThermoScientific, USA), an injection volume of 25 μL and a flow rate

of 1 mL min^{-1}. The external sugar standards used for the calibration were glucose (Merck, Germany), xylose (Merck, Germany), galactose (Sigma-Aldrich, Germany), mannose (Merck, Germany) and arabinose (Sigma, USA). Fucose (Sigma, Slovakia) was used as an internal standard.

2.3.1. Quantification of minerals content

Initially, the materials were digested using 65 vol.% of nitric acid in a digester block at a temperature of ±95 °C. Later, a second digestion was performed by using 70 vol.% of perchloric acid at a gradual increase in temperature up to ±150 °C until the samples were cleared in the digester tube (Skoog et al., 2007). The determination of the minerals Cu, Mn, Mg, Fe, Ca, P, S and Z was performed using a plasma optical emission spectroscopy (Perkin Elmer, Optima 3300 DV). All the samples were digested in triplicates.

2.4. Lignocellulolytic enzymes assays

2.4.1. Lignolytic activity

Lignin peroxidase (LiP) activity was determined by spectrophotometry based on a change in absorbance at 310 nm at 30 °C (Multiskan ™ FC Microplate Photometer), according to the method of Tien and Kirk (1984). The enzyme assay contained 100 μL of sodium tartrate buffer (100 mmol L^{-1}, pH 3.5), 100 μL of veratryl alcohol (4 mmol L^{-1}), 50 μL of hydrogen peroxide (0.2 mol L^{-1}) and 10 μL of the enzyme extract. LiP activity was expressed in unit (U) per gram of dry substrate. The assay was performed in triplicate. A unit (U) of LiP is defined as the amount of enzyme required to oxidize 1 μmol veratryl alcohol in 1 min, at a pH 3.5 at 30 °C.

Veratryl alcohol and Remazol brilliant blue R (RBBR) were purchased from Sigma-Aldrich (St. Louis, MO-USA).

2.4.2. Cellulase activity

Filter paper activity (FPase) was estimated based on the dinitrosalicylic acid (DNS) method through the measurement of the released reducing sugars (Miller, 1959). The enzyme assay composed of a Whatman No. 1 paper strip with dimensions of 1 × 6 cm (50 mg), 1.0 mL of sodium citrate buffer at pH 5.0, and 500 μL of the enzyme extract. The enzyme activity was carried at 50 °C for 1 h, followed by the addition of 3 mL of DNS and an incubation in boiling water for 5 min. The assay was performed in triplicate. FPase is expressed as FPU (filter paper units) per gram of dry substrate. According to Ghose (1987), 2.0 mg of reducing sugar as glucose from 50 mg of filter paper in 60 min, has been designated as the intercept for calculating filter paper cellulase units (FPU).

2.5. Statistical analysis

One–way ANOVAs analysis and Tukey's test were performed using GraphPad Prism software (Version 5.0, GraphPad Inc., USA), considering a confidence level of 95% ($p > 0.05$).

3. Results

3.1. Chemical characterization of the substrates

Table 1 shows the main chemical composition of the substrates tested. Banana pseudostem presented higher cellulose and smaller content of lignin when compared to the other substrates. This could favour the production of cellulases, as the lignin can limit the access of the cellulose portion and also attract the cellulase, leading to non-productive binding and as a result, enzyme yield losses (Ellilä et al., 2017; Lu et al., 2016). On the other hand, the higher amount of lignin present in the substrates jatropha and coconut fiber could induce the production of ligninases, such as LiP.

The content of ashes represents the minerals present in the materials. Banana pseudostem presented the highest amount of minerals when compared to jatropha and coconut fiber. A balance between the sources of carbon, nitrogen, phosphorus, vitamins and micronutrients (Mg, Mn, Ca, S, Fe, Zn) in the culture medium is crucial to microbial growth and consequently the production of enzymes. Different strains and species of fungi differ in their sensitivity to metals during their growth on lignocellulosic substrates. White rot fungi require important Mg, Ca, Mn, Zn, Cu ions in microbial growth. These ions are also important as cofactors for the production of enzymes (Sathiya et al., 2007). Regarding the *Pleurotus ostreatus*, minerals such as sulfur ions, phosphorus, potassium and magnesium are important in stimulating its development (Bellettini et al., 2019). However, the excess of these metals in the culture medium can be toxic, causing a decrease in the fungal growth rate and a prolonged latency phase (Sathiya et al., 2007).

Table 2 shows the composition of the minerals of each substrate tested. Copper and iron presented higher amounts in the jatropha substrate when compared to the other substrates (0.022 ± 0.003 g Kg^{-1} and 0.35 ± 0.002 g Kg^{-1} respectively), which presented the highest LiP activities. In addition, this substrate also presented higher amounts of phosphorus (4.6 ± 0.04 g Kg^{-1}). Ions such as iron, manganese and copper can favour the formation of free radicals in the medium. These formed radicals, can significantly help the production of enzymes such as LiP which use peroxidic radicals as a cofactor. Earlier studies (Liang et al., 2012; Manavalan et al., 2015) have

Table 1
Chemical composition of the substrates tested for the production of cellulases and lignin peroxidase (% dry mass).

	Cellulose	Hemicellulose	Total Lignin	Ashes	Extractives
Banana pseudostem	40.7 ± 1.0a	14.1 ± 0.3a	13.1 ± 0.1a	10.2 ± 0.0a	3.8 ± 0.5a
Coconut fiber	31.6 ± 2.0b	13.5 ± 1.0a	33.1 ± 1.5b	0.9 ± 0.1b	3.3 ± 0.6a
Jatropha	25.7 ± 1.2b	21.7 ± 1.5b	20.9 ± 1.3c	6.7 ± 0.9c	-

Data are the means ± standard deviations of triplicate experiments. Values within a column bearing the same letter are not significantly different ($p > 0.05$) according to Tukey's test.

Table 2
Mineral composition of the substrates used in the production of cellulases and lignin peroxidase (% dry mass).

Minerals (g Kg^{-1})	Cu	Mg	Mn	Fe	Ca	P	S	Z
Banana pseudostem	0.002 ± 0.0a	2.5 ± 0.2a	0.11 ± 0.0a	0.25 ± 0.01a	7.1 ± 1.8a	2.5 ± 0.1a	1.2 ± 0.1a	-
Coconut fiber	0.003 ± 0.0a	0.3 ± 0.0b	0.008 ± 0.0b	0.14 ± 0.0b	0.6 ± 0.1b	0.42 ± 0.1b	0.85 ± 0.1a	-
Jatropha	0.022 ± 0.003b	-	0.044 ± 0.001c	0.35 ± 0.002c	-	4.6 ± 0.04c	-	0.04 ± 0.001

Data are the means ± standard deviations of triplicate experiments. Values within a column bearing the same letter are not significantly different ($p > 0.05$) according to Tukey's test.

shown that phosphorus has a significant impact on white-rot fungus growth and in the production of ligninolytic enzymes such as LiP.

Regarding the cellulase production, Bhavsar et al. (2015) reported an increase in the cellulase activity when ions such as Mn^{2+} and Ca^{2+} were present in higher amounts in the banana pseudostem against any other substrates tested (0.11 ± 0.0 g Kg^{-1} and 7.1 ± 1.8 g Kg^{-1}). In general, the coconut fiber presented lower contents of metals among the other substrates.

The presence of copper ions in cultures of *Pleurotus ostreatus* decreases the activity of extracellular proteases (Palmieri et al., 2000), which can lead to more stability in the enzymes cellulases and LiP. An increase in LiP production in the presence of 500 μM Mn^{2+} and 1.0 mM Cu^{2+} using the fungus *Pleurotus eryngii* was observed by Akpinar and Urek (2012) in solid culture media composed by grape residues. Levin et al. (2008) observed an increase of cellulase activity in the presence of 3 mM Cu^{2+} in the culture medium.

3.2. Effect of the surfactants on the production of cellulase and LiP

Figs. 1 and 2 show the activities of lignin peroxidase and cellulase, respectively, after 5, 10, 15, and 20 days of SSF.

From Fig. 1 it can be observed that LiP activities presented the lowest values on day 5, increasing their activities after 10 days of SSF. Apart from jatropha supplemented with SDS, all the other substrates containing surfactants showed a decrease or similar values in their LiP activities from the 10th to the 15th day of SSF. Besides this substrate, the jatropha supplemented with tween 80 and coconut fiber with SDS also presented significant difference LiP activities between the evaluated days of fermentation ($p < 0.05$). The substrate banana pseudostem with tween 80 and glycerol, as well as the coconut fiber supplemented with tween 80 and jatropha supplemented with glycerol showed significant increases in their LiP activity from the 5th to the 10th day of fermentation ($p < 0.05$). The substrate banana pseudostem supplemented with SDS also presented an increase in LiP activity at these same days, however they were not statistically significant ($p > 0.05$). All the LiP activities declined gradually after 15 days of cultivation, not presenting any activity on the 20th day.

The highest activity of LiP was obtained using jatropha supplemented with SDS (J-SDS) (49916 ± 541 U g^{-1} of substrate) after 15 days of fermentation. The second highest LiP activity was found using jatropha supplemented with tween-80 (J-TW) (37622 ± 2154 U g^{-1} of substrate) after 10 days of fermentation (Fig. 1). These activities

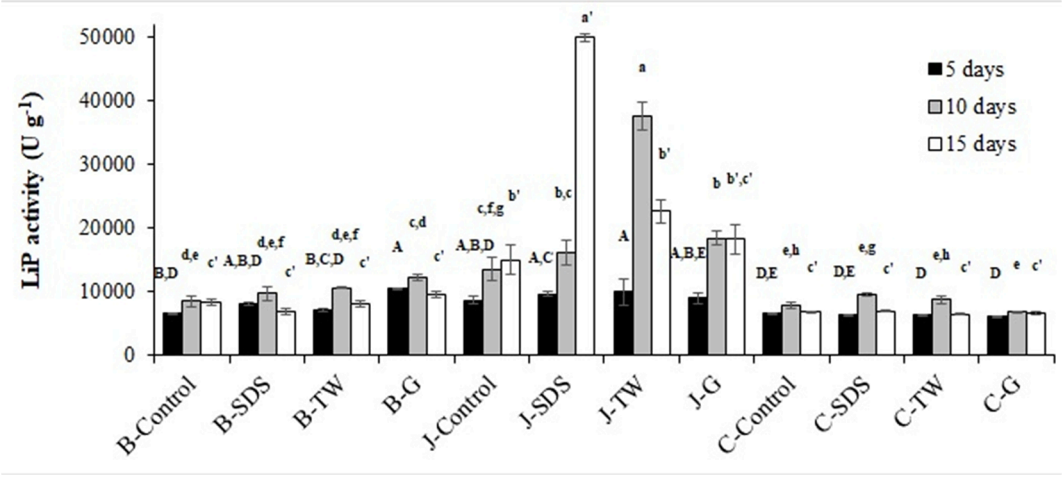

Fig. 1. Lignin peroxidase production by SSF of *Pleurotus ostreatus* PLO9 using agroindustry residues (J = jatropha, B = banana pseudostem, C = coconut fiber) supplemented with surfactants (SDS = sodium dodecyl sulfate, TW = tween 80, G = glycerol) and without surfactants (B-Control, J-Control, C-Control) after 5, 10 and 15 days of incubation. Mean values followed by the same letter in the columns within the same day of incubation are not significantly different ($p > 0.05$) according to Tukey's test.

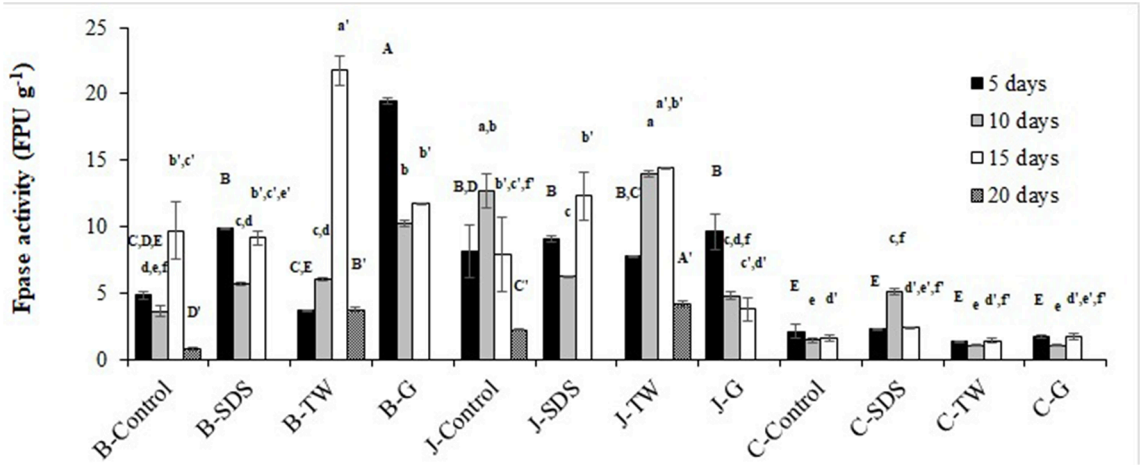

Fig. 2. Cellulase production by SSF of *Pleurotus ostreatus* PLO9 using agroindustry residues (J = jatropha, b = banana pseudostem, c = coconut fiber) supplemented with surfactants (SDS = sodium dodecyl sulfate, TW = tween 80, G = glycerol) and without surfactants (B-Control, J-Control, C-Control) after 5, 10 and 15 days of incubation. Mean values followed by the same letter in the columns within the same day of incubation are not significantly different ($p > 0.05$) according to Tukey's test.

showed to be 3 times higher when compared to the LiP activities obtained without the surfactants (14978 ± 2397 U g^{-1} and 13422 ± 1827 U g^{-1}, respectively). Apart from these two activities, only jatropha supplemented with SDS and glycerol after 10 days of SSF (16111 ± 2031 U g^{-1} and 18375 ± 1055 U g^{-1}, respectively) and banana pseudostem supplemented with glycerol after 5 days of SSF (10376 ± 215 U g^{-1}) presented significant values of LiP activities when compared to the controls performed without the surfactants ($p > 0.05$) (Fig. 1).

Fig. 2 showed that the FPase activity can increase or decrease after 5 days of SSF depending on the composition of the substrates. Banana pseudostem supplemented with tween 80 and jatropha with SDS presented maximum cellulase production on the 15th day, showing significant differences in their FPase activities between all the days evaluated ($p < 0.05$). Jatropha supplemented with tween 80 and coconut fiber with SDS also presented significant increases in their FPase activities after 5 days of SSF, although no significant differences were observed on the FPase activities between 10th and 15th days ($p < 0.05$). The substrate banana pseudostem supplemented with SDS and glycerol, as well as the jatropha with glycerol presented maximum FPase activities on the 5th day of SSF, meaning the fermentation process did not need to be longer than 5 days. The substrates coconut supplemented with tween 80 and glycerol presented very similar and small values between the days of fermentations evaluated ($p > 0.05$). Similar to LiP activities, all the FPase values declined after 15 days of SSF. Only the substrates jatropha and banana pseudostem supplemented with tween 80 presented FPase activities on the 20th day.

The maximum cellulase activities were obtained using banana pseudostem supplemented with tween 80 (B-TW) (21.8 ± 1.1 FPU g^{-1}) after 15 days of fermentation, and banana pseudostem with glycerol (19.5 ± 0.2 FPU g^{-1}) after 5 days of fermentation (Fig. 2). The cultivation of banana pseudostem with tween 80 reached the maximum FPase activity after 15 days of SSF (0.061 FPU g^{-1} h^{-1}), compared to only 5 days of SSF when using the same substrate supplemented with glycerol (0.16 FPU g^{-1} h^{-1}), therefore, the latter was considered the best media composition for cellulase production as it required less SSF time to reach a significant FPase activity. These FPase activities showed to be 2 and 4 times higher respectively, when compared to the FPase obtained without surfactants (9.7 ± 2.1 U g^{-1} and 4.8 ± 0.3 U g^{-1}, respectively). After 20 days of fermentation (Fig. 2), only jatropha and banana pseudostem both supplemented with tween 80 showed FPase activities (4.1 ± 0.2 FPU g^{-1} and 3.7 ± 0.2 FPU g^{-1}, respectively).

From the substrates tested, the coconut fiber was the one that presented the lowest values of LiP and FPase activities (Figs. 1 and 2). The high lignin content of the coconut fiber (33.1 ± 1.5%), which explains its high durability and strength, could induce the production of ligninases, but it can also make the direct contact between the fungus *P. ostreatus* PLO 9 and the substrate harder, limiting the production of enzymes. Considering the composition of the coconut fiber, the production of enzymes using this substrate could be more effective by making some changes, such as submitting this substrate to mechanical or chemical changes, altering the physicochemical characteristics; porosity, volumetric specific surface, crystallinity of solid-state substrate, as well as increasing the moisture of the process, and agitating the flasks during the SSF (Pandey, 1991). These changes in the SSF could favour the contact surface between the fungus and media, and also improve aeration, enhancing the accessibility of the fungus to the nutrients and leading to an improvement in the production of enzymes (El-Mansi et al., 2006).

This study showed higher LiP and cellulase activities (49916 ± 541 U g-1 and 19.5 ± 0.2 FPU g^{-1} respectively) when compared to several studies present in literature. Saratale et al. (2017) reported 0.89 ± 0.1 U mL-1 of LiP activity after 8 days of SSF at 30 °C using waste-house wood supplemented with surfactants (PEG-4000; PEG-6000, tween-20, tween-80, Triton X-100) by *Streptomyces* sp. MDS. Szabo et al. (2015) achieved 22 U g-1 after SSF at 30 °C by Trichoderma vireus TUBF – 498, using fibrous flax supplemented with tween 80.92.5 ± 3.6 U g-1 and 11.8 ± 0.3 U g-1 of LiP were achieved after SSF by Pleurotus ostreatus and Pleurotus sapidus respectively (Bilal and Asgher, 2016; Ergun and Urek, 2017).

Yoon et al. (2019) performed an SSF using Pleurotus sajor-caju and achieved 0.0045 FPU g^{-1} at room temperature (30 °C) using pretreated sugarcane as a substrate. Trivedi et al. (2015) and Hu et al. (2018) also produced cellulases, reaching 9.60 FPU g^{-1} and 1.56 FPU g^{-1} after SSF using green algae and textile waste scrap as substrates respectively.

According to the results presented, the surfactant tween-80 can be considered a good exogenous inducer for the production of cellulases and LiP. Several authors have shown improvements in the secretion of lignocellulolytic enzymes in the presence of surfactants such as tween 80 in solid and submerged cultures (Babič et al., 2012). It has been suggested that surfactants increase the production of extracellular enzymes in filamentous fungi, improving the output of compounds from the cells by altering the permeability of the plasma membrane (Ding et al., 2008).

Holmberg (2018) observed that the nonionic surfactants such as tween 80 and glycerol have a beneficial effect on cellulases activities, when a lignocellulosic material is used as a substrate. The improved efficiency of the cellulases activities is probably due to the adsorption of the amphiphile on hydrophobic lignin patches, preventing the non-specific adsorption of the enzyme on these patches. This corroborates with the results from this work, which presented the highest cellulases activities after using the nonionic surfactants tween 80 and glycerol.

Lazim and Hadibarata (2016) reported an increase in the biomass production and ligninases activities in the presence of tween 80 after fermentation with a white-rot fungus *Polyporus* sp. S133. The tween 80 with jatropha presented the second highest LiP activity (37622 ± 2154 U g^{-1}) after 10 days of SSF. The highest LiP activity was reached after cultivation using jatropha supplemented with SDS. Earlier studies reported increases in enzyme activities after using SDS in cultivation media (Gebicka, 2001; Hahn et al., 2017; Mitsou et al., 2017). These could be down to changes in the pH values induced by the surfactant ions, strongly affecting the catalytic activity of the enzyme (Mitsou et al., 2017).

4. Conclusion

The strain *P. ostreatus* PLO 9 was considered an excellent producer of cellulase and lignin peroxidase using banana pseudostem and jatropha respectively, under SSF conditions. The higher LiP activities were achieved after 15 days of SSF using jatropha supplemented with SDS (49916 ± 541 U g^{-1}) and 10 days of SSF using jatropha supplemented with tween (37622 ± 2154 U g^{-1}). The maximum cellulase activities were achieved after 5 days of SSF using banana supplemented with glycerol (19.5 ± 0.2 FPU g^{-1}) and 15 days of SSF using banana pseudostem supplemented with tween 80 (21.8 ± 1.1 FPU g^{-1}). These high enzyme activities were enhanced by the presence of the surfactants tween 80, SDS and glycerol on the culture media.

Among the substrates tested, banana pseudostem was the best for cellulase production and jatropha was more desirable for LiP production. The coconut fiber did not present a good production of the enzymes.

The results achieved in this study were higher than several studies presented in literature, showing the effectiveness of the SSF process in using the surfactants tween 80, glycerol and SDS for the production of lignocellulosic enzymes by the white rot fungus *Pleurotus ostreatus* PLO 9. Furthermore, further studies on the increase in mushroom production and the potential of this food by humans and animals after

the addition of surfactants in the cultivation substrate can be performed.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101428.

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