El Colegio de la Frontera Sur

Caracterización de residuos de agave comiteco durante el crecimiento de *Pleurotus ostreatus* y su potencial como biomasa energética

TESIS
Presentada como requisito parcial para optar al grado de
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Con Orientación en Biotecnología Ambiental

Por

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para obtener el grado de **Maestro en Ciencias en Recursos Naturales y Desarrollo Rural.**

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Tabla de Contenido

Resumen

En la Meseta Comiteca-Tojolabal de Chiapas (México), se produce “El comiteco” una bebida espirituosa, obtenida de un agave nativo que genera un residuo agroindustrial con alto contenido lignocelulósico y potencial biotecnológico. Los hongos *Pleurotus* producen enzimas que les permiten crecer y aprovechar esta clase de residuos y hacerlo a través de un método biológico con el menor impacto ambiental, optimizando costos y tiempos, además son el escenario ideal para producir bioprocesos y bioproductos útiles y sustentables. Por lo que el objetivo fue evaluar el efecto de tres métodos de protección al sustrato esterilización, inmersión alcalina y autocalentamiento sobre el residuo de *Agave* en función de propiciar el crecimiento de *Pleurotus* como pretratamiento de biomasa energética. A través de la caracterización fisicoquímica: microscopía del diámetro de la fibra, humedad, análisis bromatológico, contenido de polisacáridos estructurales: lignina, hemicelulosa y celulosa. Para la caracterización biológica se evaluaron las enzimas lignolíticas: Lacasa, Manganeso peroxidasa y Fenol oxidasa en espectrofotómetro y los parámetros de eficiencia biológica %, rendimiento, tasa de producción %, peso promedio de los hongos en gramos y bioconversión %. El método autocalentamiento v demostró ser el mejor método al obtener los mayores valores de EB% (57.4), R (0.21), TP% (0.96), PPH (11.9) y B% (17.9) y por el mayor efecto de degradación posterior a los tratamientos de protección al sustrato y al crecimiento de *Pleurotus*.

Palabras claves: bagazo lignocelulósico, setas comestibles, autocalentamiento, biodegradación, Eficiencia biológica, desarrollo sustentable.

Capítulo 1. Introducción

En la Meseta Comiteca-Tojolabal del estado de Chiapas, México, crece un agave nativo de la región (*Agave americana*), poco estudiado, cuya identidad taxonómica aún se encuentra en discusión, por su fenotipo local (Reynoso-Santos et al. 2012). Este agave, como en otras partes de México, se ofrece como una valiosa fuente de materia prima de varios procesos biotecnológicos, debido a su alto contenido fibroso y complejo de azúcares tanto de sus hojas como del interior de sus piñas (Narváez y Sánchez 2010) y es utilizado para elaborar una bebida artesanal espirituosa bajo un proceso particular de obtención de aguamiel y destilado del mismo -denominado “comiteco”- que consolida una importante industria de bebidas en la región, por su rol en la cultura, la historia, la economía y la ecología del lugar (Lara-Hidalgo et al. 2017; Valdivieso 2018). Esta pequeña y artesanal industria genera un subproducto agroindustrial, el bagazo que posee un alto contenido lignocelulósico, que actualmente ha dejado de ser un desecho problema para convertirse en materia prima de procesos comerciales e industriales que se requieren ser explorados con fines biotecnológicos. (Baena 2005; Rodríguez-Macías et al. 2010; Solís et al. 2018).

En el caso de otras especies del género *Agave*, se ha reportado el cultivo de hongos comestibles sobre el bagazo como una estrategia factible de aprovechamiento (Álvarez-Navarrete 2019; Cruz-Moreno 2020; Guzmán-Dávalos et al. 1987; Muthangya et al. 2013; Muthangya. et al. 2019 y Soto-Velazco et al.1989. Además, para el aprovechamiento de subproductos de agave se ha reportado también, la obtención de enzimas ligninolíticas (Cabrera-Soto et al. 2011; Castillejos-Márquez. et al. 2015; Heredia-Solís et al. 2014; Muthangya et al. 2013) y por su contenido nutricional rico en polisacáridos, azúcares fermentables, enzimas, etc. Se posiciona como una alternativa viable de pretratamiento de biomasa energética a través de métodos biológicos (Mora y Martínez-Carrera 2007, Saval 2012). Tales investigaciones demuestran el potencial biotecnológico del cultivo de *P. ostreatus* en bagazo de agave y sus posibles aplicaciones.

Actualmente, la producción mundial de *Pleurotus* spp. se incrementa y destaca por las múltiples cualidades que ofrece como cultivo (impacto social, económico y ecológico), y

como alimento (cualidades nutritivas, organolépticas, nutracéuticas, medicinales y biotecnológicas), en períodos relativamente cortos de tiempo (Sánchez et al. 2017). Se hace énfasis en la importancia ecológica, ya que se ha reportado el uso y reciclaje de más de 474, 000 toneladas anuales de subproductos agroindustriales (Morales 2009). Lo cual lo posiciona como una alternativa viable de pretratamiento para biomasa energética a través de un método biológico. Se prefiere hacerlo a través de un método biológico con el menor impacto ambiental, optimizando costos y tiempos como escenario ideal para producir biotecnologías útiles y sustentables. Si bien, el género *Pleurotus* destaca por su capacidad para producir enzimas degradadoras de lignocelulosa, presentar un crecimiento rápido, y tener relativamente pocos competidores en el medio donde crece (Cruz-López 2014), es fundamental promover cierta selectividad física, química y biológica en el sustrato para que el hongo cultivado tome los nutrientes necesarios para su desarrollo, propiciando que la técnica de cultivo garantice la eliminación o inhibición de los competidores y contaminantes; y al mismo tiempo optimice la producción, los costos, el rendimiento, y la calidad de los hongos cosechados con el máximo ahorro energético y aporte socioambiental (Sánchez 2002; Sánchez et al. 2017; Stölzer y Grabbe 1991).

Dado que se ha reportado que no todos los métodos de tratamiento funcionan con todos los sustratos posibles (Avendaño y Sánchez 2013, Barrios-Espinosa et al. 2009, Contreras et al. 2004) y que es necesario hacer estudios previos para determinar el método más adecuado para cada caso; por lo que el presente estudio tuvo como objetivo caracterizar el residuo de agave comiteco mediante la respuesta del agave comiteco en el cultivo de hongos comestibles al aplicarle el efecto de tres métodos de protección del sustrato (autocalentamiento, inmersión alcalina y esterilización) sobre el bagazo a través de la determinación de sus características físicas, químicas y biológicas.

Además, se evaluó la capacidad del bagazo para la producción de basidiomas *de Pleurotus ostreatus* así como su capacidad ligninolítica, a través de su actividad enzimática. Con el propósito de contribuir a la producción y aprovechamiento de un recurso nativo de la región y ampliar los beneficios de esta agroindustria como alternativa de soberanía alimentaria y desarrollo sustentable para los productores de la región de Chiapas en México.

Posterior del Capítulo 1 (Introducción), el trabajo comprende dos capítulos adicionales. El capítulo 2 consiste en un artículo científico titulado “Caracterización de residuos de agave comiteco durante el crecimiento de *Pleurotus ostreatus* bajo tres métodos de protección al sustrato como pretratamiento de biomasa energética”; finalmente, el capítulo 3 integra las conclusiones del estudio.

Capítulo 2. Artículo: Caracterización de residuos de agave comiteco durante el crecimiento de *Pleurotus ostreatus* bajo tres métodos de protección al sustrato como pretratamiento de biomasa energética

Characterization of agave comiteco residues during the growth of *Pleurotus ostreatus* under three substrate protection methods as pretreatment of energetic biomass

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CHARACTERIZATION OF AGAVE COMITECO RESIDUES DURING THE GROWTH OF *PLEUROTUS OSTREATUS* UNDER THREE SUBSTRATE PROTECTION METHODS AS A PRETREATMENT FOR ENERGETIC BIOMASS

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Running title: Characterization of agave residues during the growth of *Pleurotus ostreatus* under three substrate protection methods

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Authors' contributions: All authors contributed to the study conception and design, material preparation, data collection and analysis were performed by Mayra Lagunes Reyes, José E Sánchez, René Humberto Andrade Gallegos and Rubén Fernando Gutiérrez Hernández.

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Cover letter

Tapachula, Chiapas, Mexico, June 30, 2021

Dr. Bharat Patel Executive
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3 Biotech
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University of Technology
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Dear Dr. Patel,

Enclosed please find the manuscript entitled **Characterization of agave comiteco residues during the growth of *Pleurotus ostreatus* under three substrate protection methods as a pretreatment for energetic biomass**, authored by Mayra Lagunes-Reyes, José E Sánchez, René H Andrade-Gallegos and Rubén F Gutiérrez-Hernández**.** We would like to submit this manuscript for publication in *3 Biotech*, as an original research article.
The paper reports the interesting results obtained when evaluating the use of three substrate protection treatments prior to the cultivation of the edible mushroom *Pleurotus ostreatus* on a by-product of the mezcal agroindustry, the bagasse of *Agave americana*, and characterizing the residue obtained. We demonstrate that it is possible to pasteurize the bagasse by self-heating, that good mushroom production is obtained, that an important laccase activity is observed and that the residue has still potential use as energetic biomass because of its polysaccharide final content.

We manifest that the authors elaborated, thoroughly discussed the results, and approved the content in its present form. This document is original and is not under review in any other scientific journal for publication.
We hope this MS will meet all the requirements and high standards demanded by *3 Biotech* for publishing scientific articles.

We look forward to your kind reply

Sincerely

José E. Sánchez
Tropical Mushrooms
El Colegio de la Frontera Sur

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Abstract

The comiteco is a spirit beverage obtained from an agave plant native to the Comiteca-Tojolabal Meseta of Chiapas (Mexico). This plant generates bagasse as an agroindustrial byproduct that has a high lignocellosic content and biotechnological potential. Mushrooms of the Pleurotus genus produce enzymes that allow them to grow and exploit this kind of residue. The objective of this work was to evaluate the effect of three substrate protection methods, namely, sterilization, alkaline immersion and self-heating, on agave bagasse to promote the growth of Pleurotus ostreatus as a pretreatment for energetic biomass. Polysaccharide degradation (lignin, hemicellulose and cellulose), ligninolytic activity (laccase, manganese peroxidase and phenol oxidase) and basidiome production were evaluated. Self-heating pasteurization proved to be the best method for obtaining the highest values (%) of biological efficiency (57.4), yield (0.21), production rate (0.96), mean mushroom size (11.9 g) and bioconversion (17.9) and the highest degradation effect following substrate protection treatments and P. ostreatus growth. The main ligninolytic activity was laccase ($0.5x10^5$ to $5.32x10^5$ U mL^{-1}).

Keywords: biodegradation, biological efficiency, edible mushrooms, lignocellulosic bagasse, self-heating, sustainable development.

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Keywords: biodegradation, biological efficiency, edible mushrooms, lignocellulosic bagasse, self-heating, sustainable development.

Introduction

This study focused on an *Agave americana* L. plant (agave comiteco) native to the Meseta Comiteca-Tojolabal region of the state of Chiapas, Mexico, whose taxonomic identity is still under discussion due to its local phenotype (Reynoso-Santos et al. 2012). This agave has a high content of fiber and sugars (Narváez and Sánchez 2010) and is used to produce an artisanal spirit under a particular process in which mead is obtained and distilled. This distilled product is called *comiteco*, and its production represents an important beverage industry in the region due to its role in the culture, history, economy and ecology of the place (Lara-Hidalgo et al. 2017). This small and artisanal industry generates an agroindustrial byproduct, bagasse, which has been little used to date. In the case of other *Agave* species, the cultivation of edible mushrooms on bagasse has been reported as a feasible utilization strategy (Álvarez 2019; Cruz-Moreno 2020; Guzmán-Dávalos et al. 1987; Muthangya et al. 2013; 2019; Soto-Velazco et al.1989). In addition, for the utilization of agave byproducts, ligninolytic enzymes have been obtained (Cabrera-Soto et al. 2011; Castillejos-Márquez 2015; Heredia-Solís et al. 2014; Muthangya et al. 2013), and because of the nutritional content of agave, which is rich in polysaccharides, fermentable sugars, enzymes, etc., this method represents a viable alternative for obtaining biomass energy by biological methods (Mora and Martínez-Carrera 2007, Saval 2012).

For this reason, the capacity of the bagasse generated during the production of agave comiteco was assessed for the production of *Pleurotus ostreatus* basidiomes and the bagasse was assessed in terms of its ligninolytic capacity. Currently, the world production of *Pleurotus* spp. is increasing and stands out because of the multiple qualities it offers as a crop (social, economic and ecological impact) and a food (nutritional, organoleptic, nutraceutical, medicinal and biotechnological qualities) in relatively short periods of time (Sánchez et al. 2017). Although the genus *Pleurotus* stands out for its ability to produce lignocellulose-degrading enzymes and grow rapidly and because of its relatively few competitors in the environment where it grows (Mata et al. 2017)), it is essential to promote certain physical, chemical and biological selectivity in the substrate so that the cultivated fungus uptakes the necessary nutrients for its development and ensure that the cultivation technique eliminates or inhibits competitors and pollutants. Moreover, the production,

costs, yield, and quality of harvested mushrooms must be optimized to achieve maximum energy savings and socioenvironmental benefits (Sánchez 2002; Sánchez et al. 2017; Stölzer and Grabbe 1991). Not all treatment methods work with all possible substrates (Avendaño and Sánchez 2013; Barrios-Espinoza et al. 2009; Contreras et al. 2004), and the most appropriate method for each case must be determined. Thus, the present study aimed to evaluate the effect of three substrate protection methods (self-heating, alkaline immersion and sterilization) on agave comiteco bagasse by assessing its physical, chemical and biological charact eristics, with the purpose of contributing to the production and use of a native resource of the region and expanding the benefits of this industry.

Materials and Methods

Strain used and inoculum preparation

Pleurotus ostreatus strain ECS-1123, deposited in the mycological collection of El Colegio de la Frontera Sur (ECOSUR), Tapachula, Chiapas, Mexico, was used for the study. The strain was spread on potato dextrose agar (PDA) at pH 6.5. The inoculum was prepared with sorghum (*Sorghum bicolor*) grains sterilized at 1.05 kg/cm^2 (121°C) for 30 min, according to the method of Quimio (2001). Subsequently, 5-cm diameter implants of the five-day-old strain were transferred to the sterilized sorghum, the material was incubated at 24°C in the dark for 15 days and subsequently kept refrigerated at 5°C.

Substrate used

Agave comiteco bagasse was collected in San José de las Rosas and San Rafael Jocom, which belong to the municipality of Comitán de Domínguez, Chiapas, Mexico. Agave comiteco stalks were used, and they represent waste from pruning, and the residue was collected after dismembering the quiote to obtain wort (pulque). The collected leaves were manually defibrated, the cuticle was removed and as many fibers as possible were removed to facilitate drying (in direct sunlight for 3 weeks). The material was then ground in a forage mill.

Substrate treatment

For comparative purposes, three methods of substrate protection treatment were used: 1) steam sterilization, 2) disinfection by alkaline immersion and 3) pasteurization by self-heating.

For the steam sterilization method, 5 kg of agave bagasse with 70% moisture and 4% hydrated lime were used. This mixture was processed in an autoclave (Felisa R.) at 1.05 kg/cm^2 (121°C) for 0.5 hours

For alkaline immersion, 0.5 kg of $Ca(OH)_2$ was added to 50 liters of water and mixed at room temperature, and then 10 kg of dry bagasse was immersed and left to stand overnight. The next day, the water was removed and drained until a humidity of 70% was reached (Contreras et al. 2004).

For self-heating pasteurization, the procedure described by Avendaño and Sánchez (2013) and Morales and Sánchez (2017) was used, with modifications: in a wooden box (1 m^3), 300 kg of agave comiteco bagasse (at 60% moisture and 4% lime) was stacked, which was removed at 46 hours of processing. At removal, the whole substrate was relocated so that the upper part (L_1) passed to the bottom, the lower part (L_3) passed to the center of the crate and the part that started in the middle (L_2) passed to the upper part of the crate. At 67 hours, the treatment was stopped by emptying the crate and letting the substrate cool to room temperature (25.5°C). The substrate temperature was measured using 30 cm bimetallic thermometers. During self-heating, temperature measurements were performed every three hours throughout the whole process. The temperature was recorded in three places at the front side of the crate: a) 15 cm below the upper surface of the substrate, b) at the center of the crate and c) 15 cm above the bottom of the crate.

Cultivation and obtaining basidiomata

One kilogram of treated bagasse was aseptically placed in polypropylene bags and mixed with 50 g of mushroom spawn. This material was incubated at 24°C in the dark for 2-3 weeks. Subsequently, primordium formation was induced by modifying the environmental parameters (light, temperature, relative humidity and ventilation) according to Zadrazil and Kurtzman (1982). Two harvests of the mushrooms were obtained.

Physicochemical characterization of agave comiteco bagasse

The physical characteristics of the bagasse related to fungal growth and delignification in the substrate were determined; pH was measured with a potentiometer Science Med. Model 25cw and particle size (Sánchez et al. 2017), fiber diameter and moisture were also determined (NOM-116-SSA1-1994). In addition, a proximate analysis was performed by the Bromatological Analysis Laboratory of El Colegio de la Frontera Sur (ECOSUR) for the content of crude protein (NMX-F-608-NORMEX-2011), Ash (NMX-F-607-NOMRMEX-2002), crude fiber (NMX-F-613-NORMEX-2003), fat (NMX-F-615-NORMEX-2004), carbohydrates and reducing sugars (Miller 1959). The lignin, hemicellulose and cellulose contents were also determined by the Van Soest method (1982). All chemical parameters were determined prior to substrate protection treatments and spawning of *P. ostreatus*, as well as at the end of two harvests.

Biological characterization

For the biological characterization of the substrate and to determine the degradation efficiency of the peroxidase enzymes (lignocellulolytic), the laccase activity was measured with ABTS at 436 nm (according to Tien and Kirk (1988), manganese peroxidase (Mn peroxidase) activity was measured at 270 nm with H_2O_2 according to Wariishi et al. (1992) and phenol oxidase activity was measured with catechol at 420 nm according to Ögel et al. (2006) using a spectrophotometer (L6S UV-VS spectrophotometer).

To determine the productive capacity of the substrate in the production of *P. ostreatus* basidiomata, the following variables were determined (Sánchez et al. 2002): biological efficiency (BE) was determined by dividing the weight of the fresh fruiting bodies by the weight of the dry substrate used and multiplying by 100 (Royse 1985); yield (Y) was determined by dividing the weight of the dry carpophores by the weight of the dry substrate used; production rate (PR) was determined by dividing the BE by the time elapsed from the moment of inoculation until the last mushroom cutting (Royse 1989); and mean mushroom size (MMS) was determined by dividing the weight of the fresh fruiting bodies in grams by the number of mushrooms harvested. The percentage of bioconversion (B) was determined by the following formula:

$$\%B = \frac{Iw - Fw}{Iw} \times 100$$

Iw: Initial dry weight

Fw: Final dry weight

Experimental design and statistical analysis

To determination of laccase, Mn peroxidase and phenol oxidase enzyme activity and the production variables BE, Y, PR, MMS and B, a completely randomized experimental design was used, and it included one factor (protection treatment) with three different levels, namely, alkaline disinfection, pasteurization by self-heating and steam sterilization, with five replicates for each treatment (15 experimental units).

For the statistical analysis, an analysis of variance (ANOVA) of the lignolytic activity and production variables was performed. Significant differences were determined by Tukey's HSD mean comparison (α=0.05) with R software version 4.0.2, with graphs produced using Statistica 7.0 software.

Results

Temperature profile by self-heating

The temperature development of the agave comiteco bagasse showed a steady increase in the first 46 hours of treatment and reached 49, 59 and 58°C for levels L_1, L_2 and L_3, respectively. Subsequently, after removal and relocation of the substrate batches inside the wood box, the temperature continued to increase, and at 67 hours, temperatures of 64°C (L_1 and L_2) and 58°C (L_3) has been reached (Fig. 1).

Physicochemical characterization of bagasse

The physicochemical parameters were obtained on dry agave comiteco bagasse without any treatment, which indicated a particle size of 5 ± 2 cm, a fiber diameter of 0.36 mm, a moisture content of 9.4 g/100 g, a pH of 8.8, and contents (g/100 g) of crude protein of 3.57, crude fiber of 16.81, fat of 0.59, carbohydrates of 42.34 and reducing sugars of 9.6.

Lignin, hemicellulose and cellulose content

Figure 2 shows the content of structural polysaccharides before and after fungal cultivation in agave comiteco bagasse for each substrate protection treatment. For sterilization, a percentage increase in the content of the three carbohydrates was observed (8.3% lignin, 305% hemicellulose, 126% cellulose); in the case of alkaline immersion, a decrease in lignin and hemicellulose (3.8% lignin and 57. 9% hemicellulose) and an increase in cellulose (302%) were observed. In pasteurization by self-heating, a decrease in lignin (26.5%), hemicellulose (9.42%) and cellulose (70.8%) was observed until values of 14.1% lignin, 14.04% hemicellulose and 1.65% cellulose were obtained.

Ligninolytic activity

Laccase, Mn peroxidase and phenol oxidase enzyme activities

The analysis of variance recorded no significant differences in laccase activity among the three different substrate protection methods used. A range of activity from $0.5x10^5$ to $5.32x10^5$ U mL^{-1} was observed, and higher laccase activity was recorded relative to the other enzymes tested ($p<0.05$) (Fig. 3A).

In the case of manganese peroxidase activity, the values varied between 2.19 $x10^{-3}$ and 7.84 x 10^{-3} U mL^{-1}, with no significant differences between the three protection methods ($p>0.05$) (Fig. 3B).

Finally, the analysis of variance of phenol oxidase activity showed the highest significant activity with the sterilization and alkaline immersion method at $6.55x10^{-3}$ U mL^{-1} ($p<0.05$), while the lowest significant activity was recorded by the self-heating method with $1.22x10^{-3}$ U mL^{-1} ($p<0.05$) (Fig. 3C).

Production parameters of *Pleurotus ostreatus* cultivation in agave comiteco bagasse.

The method that registered the highest significant BE% was self-heating (57.4 ± 4.9) ($p<0.05$), followed by the sterilization method (32.7 ± 6.2). In turn, the lowest significant BE% was recorded for the alkaline immersion method (20.5 ± 11.9) ($p<0.05$) (Table 1). In the case of yield (Y) the most significant value was recorded by the self-heating method (0.214 ± 0.02) ($p<0.05$), followed by the sterilization method (0.114 ± 0.01). The lowest value was recorded with the alkaline immersion method with 0.090 ±0.009 (Table 1).

The PR also produced a higher percentage by the self-heating method (0.96 ± 0.08) ($p<0.05$), followed by the sterilization method with 0.36 ± 0.07 and finally by the alkaline immersion method 0.22 ± 0.09 ($p<0.05$). In the case of the mean mushroom size (MMS), no significant differences were recorded between the different substrate protection methods, and a range of 11.9 ± 2.8 - 13.4 ± 4.6 g ($p>0.05$) was recorded. Finally, the analysis of variance showed no significant differences in the percentage of bioconversion (B%) among the three substrate protection methods, with a percentage of 16.6 ± 1.5 to 17.9 ± 1.1 ($p>0.05$) recorded (Table 1).

Discussion

This research compares the use of three methods for the protection of agave comiteco bagasse and the cultivation of *P. ostreatus* as a means to promote the growth of *P. ostreatus* as a pretreatment for energetic biomass. The results demonstrated that the agave comiteco bagasse substrate allows for the growth and fructification of the mushroom species but also the production of ligninolytic enzymes (mainly laccases) that are useful in other processes or products. This finding is associated with the physical and chemical characteristics of the initial substrate, which had a content of 19-24% lignin, 1-15% hemicellulose and 5-12% cellulose. The chemical contents of the substrate degraded during cultivation is interesting because of the usable properties, such as the contents of reducing sugars (7.0%), cellulose (1.65-11.44%), hemicellulose (1.58-14.4), and even various enzymes. The energy biomass produced shows the potential of agave bagasse to be used in other bioprocesses and products that can be generated from this byproduct (Kang 2019; Rinker 2002; Shitole et al. 2014; Ünal 2015; Zhu et al. 2012).

Of the three methods studied, the one that showed the highest BE% (57.4±4.9), Y (0.21±0.02), PR% (0.96±0.08), MMS (11.9±2.8) and B% (17.9±1.1) was self-heating. This method stands out not only because of its production parameters but also because its energy consumption and water consumption were low compared to the other methods used. Moreover, this situation highlights the importance of a beneficial microbiota present in the mycosphere that developed during the self-heating process (Díaz-Martínez et al. 2019; Torres-Ruíz et al. 2016).

The temperature profile observed with the self-heating method in this study was different from that reported in previous studies because temperatures above 50°C were reached only 46 hours after starting and pasteurization treatment was achieved after 67 hours of processing. Colmenares-Cruz et al. (2017) reached the same temperatures at 30 hours of processing. Sánchez et al. (2011) also reported the same temperatures at 48 hours, and Barrios-Espinoza et al. (2009) reported reached 70°C in 48 hours. Such differences may be related to the sugar content that is available to the microbiota of the substrate, which is responsible for producing the necessary heat and preserving it in a controlled manner to reach pasteurization (Barrios-Espinoza et al. 2009). Indeed, Morales and Sánchez (2017) indicated that a substrate with a low content of fermentable sugars can be severely limited in temperature development such that pasteurization may not be possible since the microbiota present in the substrate develops with the degradation of carbon sources, mainly sugars and lipids (Sánchez et al. 2017). The fact that agave plants have a low lipid content and store fructans as the main reserve carbohydrate indicates their reduced caloric power (Mellado-Mojica and López-Pérez 2013, Mitmesser and Combs 2017) and thus may explain in part the delayed time to reach pasteurization temperatures (>55°C) by self-heating. Regarding the nitrogen content, agave bagasse seems to have sources that promote certain microbial groups that generate caloric activity in the pasteurization process (Vieira et al. 2018), and at low concentrations, contributes to the optimal production of carpophores (Picornell et al. 2017).

The biological efficiency values reported (50-112%) by previous authors for other substrates (Avendaño-Hernández and Sánchez 2013; Barrios-Espinoza et al. 2009; Sánchez et al. 2011) are higher than those reported in this study, which could be due to the type of substrate and the supplementation they performed to increase yields. Such factors should be taken into account to increase the production of *P. ostreatus* on this type of substrate.

On the other hand, this study obtained similar BE values as other studies in which alkaline immersion was used, such as in the case of Batz-Patal (2010) with *P. ostreatus* and *P. levis* (10-48% EB). In turn, other investigations have reported biological efficiency values higher than those recorded in this research (40-120%) (Bernabé et al. 2004; Contreras et al. 2004; De León-Monzón et al. 2004: Iossi et al. 2008). In our study, alkaline immersion presented the lowest parameters of BE% (20.5±11.9), Y (0.90±0.009), PR% (0.22±0.09), MMS (12.1±5.8), and B% (16.6±1.5).

The sterilization method has been described as the easiest to control and simplest since with sterilization, all organisms living on the substrate are exterminated and fungal growth is pure and fast and can be achieved accurately and in the shortest time (Muéz-Ororbia and Pardo-Nuñez 2002). In this study, the values of BE% (32.7±6.2) and PR (0.36±0.07) were lower than those obtained with self-heating protection. On the other hand, the lignin, cellulose and hemicellulose contents were recorded after the substrate protection methods changed in composition. In the case of sterilization, the polysaccharide profile recorded after the treatment is likely the result of temperature and pressure (121°C at 1.05 kg/cm^2). Since high pressures and temperatures have a hydrolytic effect on these structural polysaccharides, mainly lignin, hydrolysis that takes place and generates compounds of low molecular weight and changes their structure after certain exposure (Holladay et al. 2007). The apparent increase in the polysaccharide content after mushroom growth in sterilized bagasse is mostly an artifact due to the loss of weight of the bagasse through the development/respiration of the mushroom. If the polysaccharide content varies little during the development of the mushroom, its percentage will ultimately increase due to the decreasing weight of the substrate. In the case of alkaline immersion, the reduction of lignin and hemicellulose may be related to the alkaline hydrolysis carried out by hydrated lime. It has been reported that alkaline hydrolysis is an efficient process to remove lignin that does not promote selective separation; therefore, it can degrade other carbohydrates, mainly hemicellulose (Mussatto et al. 2006). With respect to the self-heating method, the greatest decrease in lignin of the three different methods was recorded with this method, and hemicellulose and cellulose also decreased, which could indicate that the self-heating method promoted the degradation of lignins due to the presence of microorganisms present during the process. *Bacillus* spp. have been reported in this type of procedure (Cruz-Guillén 2015; Torres-Ruíz et al. 2016), and these organisms have the ability to generate organic acids (Tejera-Hernández et al. 2011), which can induce acid hydrolysis at the same time. However, thermolysis of different sugars occurs during the pasteurization process. In turn, this significant reduction in lignin allowed the fungus access to hemicellulose and cellulose sugars, and a reduction in both polysaccharides was observed. These changes allowed for the establishment of the fungi through the utilization of the sugars present in the hemicellulose and cellulose, which was reflected in higher BE%, Y PR%, MMS and B%.

The decrease in lignin content was explained by the presence of three ligninolytic enzymes that participate in this biodegradation (laccase, Mn peroxidase and phenol oxidase). From this enzymatic profile, significant laccase activity was recorded, with high ranges of activity obtained ($0.5x10^5$ to $5.32x10^5$ U mL^{-1}) compared with the other enzymes determined ($2.19\ x10^{-3}$ a $7.84\ x\ 10^{-3}$ U mL^{-1} and $6.55x10^{-3}$ a $1.22x10^{-3}$ U mL^{-1}). The predominance of laccase activity was explained by Reinhammar (1984), who mentioned that these enzymes have low substrate specificity and are capable of oxidizing monophenols, diphenols, polyphenols, aminophenols and diamines, i.e., a variety of substrates.

The amount of laccases reported in this study was higher than that presented in other investigations that used lignocellulose residues to obtain laccases from *Pleurotus* spp. e.g. Manjarrés et al. (2010) with banana peel and sugarcane bagasse, Morales-Campos et al. (2019) with corn byproducts, Reddy et al. (2003) with banana residues and Velázquez-Cedeño et al. (2002) with coffee pulp. The values obtained for phenol oxidase were lower than those reported for *Pleurotus* spp. (Palmeiri et al. 1993), and this low phenol oxidase production may be related to the role of these enzymes in the detoxification of low molecular weight phenols during lignin degradation; therefore, their role may be more limited or dependent based on the activity of the enzymes that are participating in the degradation (laccase and Mn peroxidase) (Ander and Eriksson 1976).

Conclusion

Of the three substrate protection methods studied for the cultivation of *P. ostreatus* on agave comiteco bagasse, the self-heating method proved to be the best and obtained the highest values of BE% (57.4), Y (0.21), PR% (0.96), MMS (11.9) and B% (17.9).

In turn, a notable decrease in structural polysaccharides due to *P. ostreatus* cultivation was observed, and it varied according to the substrate protection treatment used and was higher in the self-heating and alkaline immersion methods.

The self-heating method applied to agave comiteco bagasse promoted laccase activity ($0.5x10^5$ to $5.32x10^5$ U mL^{-1}) and Mn peroxidase ($2.19\ x10^{-3}$ to $7.84\ x\ 10^{-3}$ U mL^{-1}) and phenol oxidase ($6.55x10^{-3}$ to $1.22x10^{-3}$ U mL^{-1}) activity to lesser extents. These enzymes are potentially useful for obtaining energetic biomass and other biotechnologies.

Finally, the present characterization demonstrated that agave comiteco bagasse is an agro-industrial byproduct that possesses the necessary nutrients to produce edible basidiomes and can be used to generate ligninolytic enzymes and bioprocesses for the biotransformation of usable energy biomass.

References

Álvarez LJN (2019) Crecimiento de hongo *Pleurotus* spp. a partir de desecho de agave. Dissertation, Universidad autónoma de Querétaro

Ander P, Eriksson KE (1976) The importance of phenol oxidase activity in lignin degradation by the white-rot fungus *Sporotrichum pulverulentum*. Arch Microbiol 109(1):1-8. https://doi.org/10.1007/BF00425105

Avendaño-Hernandez RJ, Sánchez JE (2013) Self-pasteurized substrate for growing oyster mushrooms (*Pleurotus* spp.). Afr J Microbiol Res 7(3):220-226. https://doi.org/10.5897/AJMR12.1832

Barrios-Espinoza BM, Moreno-Ruiz L, Sánchez JE (2009). Composteo en cajones de madera como pretratamiento del sustrato para cultivar *Pleurotus ostreatus*. Rev Mex Mic 29:19-25.

Batz-Patal EL (2010) Producción de cuerpos fructíferos de cepas nativas de *Pleurotus* en sustratos desinfectados por inmersión en agua alcalina. Dissertation, Universidad de San Carlos de Guatemala

Bernabé-González T, Cayetano CM, Adán DA, Torres PMA (2004) Cultivo de *Pleurotus pulmonarius* sobre diversos subproductos agrícolas de Guerrero, México. Rev Mex Mic 18: 77-80.

Cabrera-Soto ML (2011) Producción de lacasa de *Pleurotus ostreatus* utilizando los residuos de *Agave tequilana* Weber como sustrato. Dissertation, Instituto Politécnico Nacional

Castillejos-Márquez S (2015) Producción de lacasa a partir de hongos ligninolíticos utilizando vinazas y bagazo de origen mezcalero. Dissertation, Universidad tecnológica de la mixteca

Colmenares-Cruz S, Sánchez JE, Valle-Mora J (2017) *Agaricus bisporus* production on substrates pasteurized by self-heating. AMB Express 7(1):1-9. https://doi.org/10.1186/s13568-017-0438-6

Contreras EP, Sokolov M, Mejía G, Sánchez JE (2004) Soaking of substrate in alkaline water as a pre-treatment for the cultivation of *Pleurotus ostreatus*. J Hortic Sci Biotechnol 79(2):234-240. https://doi.org/10.1080/14620316.2004.11511754

Cruz-Guillén YI (2015) Aislamiento e identificación de microorganismos fijadores de nitrógeno de la micosfera de *Pleurotus ostreatus*. Dissertation, Universidad Autónoma de Chiapas

Cruz-Moreno BA (2020) Cultivo de Hongos comestibles *Pleurotus djamor* y *Pleurotus ostreatus* sobre sustrato de bagazo de *Agave tequilana*. Dissertation, Universidad Autónoma de Querétaro

De León-Monzón JH, Sánchez JE, Nahed-Toral J (2004) *Pleurotus ostreatus* cultivation in Los Altos de Chiapas México. Rev Mex Mic 18:31-38

Díaz-Martínez YC, Guillén GK, Sánchez JE (2019) Growth-promoting thermophilic microorganisms in self-heating pasteurized substrate improve *Agaricus bisporus* mycelial growth.

Scientia Fungorum 49:e1261. https://doi.org/10.33885/sf.2019.49.1261
Elisashvili V, Kachlishvili E, Penninckx M (2008) Effect of growth substrate, of fermentation, and nitrogen source on lignocellulose-degrading enzyme production by white-rot basidiomycetes. J Microbiol Biotech 35:1531-1538. https://doi.org/10.1007/s11274-008-9897-x
Guzman-Davalos L, Martinez-Carrera D, Morales P, Soto C (1987) El cultivo de hongos comestibles (*Pleurotus*) sobre el bagazo del maguey de la industria tequilera. Rev Mex Mic 3:47-49.
Heredia-Solís A, Esparza IE, Romero BL, Cabral AF, Bañuelos VR (2014) Bagazos de *Agave salmiana* y *Agave weberi* utilizados como sustrato para producir *Pleurotus ostreatus*. ReIbCi 1(5):103-110.
Holladay JE, Bozell JJ, White JF, Johnson D (2007) Top value-added chemicals from biomass volume II—results of screening for potential candidates from biorefinery lignin. Oak Ridge, TN, USA: Pacific Northwest National Laboratory
Iossi MR, Cobos JD, Alegria VFJG, Zied DC (2018) *Pleurotus* spp. cultivation on *Brachiaria* sp. straw treatment with alkaline water. Braz J Microbiol 49:64-67. https://doi.org/10.1016/j.bjm.2018.06.003
Kang H W (2019) Industrial utilization of spent mushroom substrate. J Mushrooms 17(3):85-92. https://doi.org/10.14480/JM.2019.17.3.85
Lara-Hidalgo C, Grajales-Lagunes A, Ruiz-Cabrera MA, Ventura-Canseco C, Gutiérrez-Miceli FA, Ruiz-Valdiviezo VM, Archila MA (2017) *Agave americana* honey fermentation by *Kluyveromyces marxianus* strain for "comiteco" production, a spirit from mexican southeast. Rev Mex Ing Quim 16(3):771-779
Manjarrés K, Castro A, Sandoval ER (2010) Producción de lacasa utilizando *Pleurotus ostreatus* sobre cáscaras de plátano y bagazo de caña. Revista Lasallista de Investigación 7(2):9-15.
Mellado-Mojica E, López-Pérez MG (2013) Análisis comparativo entre jarabe de agave azul (*Agave tequilana* Weber var. azul) y otros jarabes naturales. Agrociencia 47(3):233-244.
Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31(3):426-428. https://doi.org/10.1021/ac60147a030
Mitmesser S. Combs M. (2017) Prebiotics: Inulin and other oligosaccharides. In Floch MH, Ringel Y, Allan-Walker W (eds) The Microbiota in gastrointestinal pathophysiology. Academic Press, pp 201–208. https://doi.org/10.1016/B978-0-12-804024-9.00023-9
Mora VM, Martínez-Carrera D (2007) Investigaciones básicas, aplicadas y socioeconómicas sobre el cultivo de setas (*Pleurotus*) en México. In: Sánchez JE, Martínez-Carrera D, Mata G, Leal H (eds) El cultivo de setas *Pleurotus* spp. en México, 1st edn. El Colegio de la Frontera Sur, Tapachula, pp 69-110.
Morales V, Sánchez JE (2017) Self-heating pasteurization of substrates for culinary-medicinal mushrooms cultivation in Mexico. Int J Med Mushrooms 19(5):477-484. https://doi.org/10.1615/IntJMedMushrooms.v19.i5.90
Morales-Campos BK, González-Melo M, Sanchez-Santillan P, Torres-Salado N, Rojas-García AR, Maldonado-Peralta MDLA (2019) Producción de celulasas y lacasas de *Pleurotus ostreatus* por fermentación sólida usando subproductos de maíz como sustratos. Rev mex agroecosistemas 6(2):1327-1332.
Muez-Ororbia MA, Pardo-Núñez J (2002) La preparación del sustrato. In: Sánchez JE, Royse DJ (eds) La Biología y el cultivo de *Pleurotus* spp. 1 st (edn) El Colegio de la Frontera Sur, Limusa, S.A. de C.V, Distrito Federal, pp 157-186.
Mussatto S, Dragone G, Fernandes M, Rochan G, Robert I (2006) Efecto de los tratamientos de hidrólisis ácida e hidrólisis alcalina en la estructura del bagazo de malta para liberación de fibras

de celulosa. XXII Interamerican Confederation of Chemical Engineering. V. Asociación Argentina de Ingenieros Químicos. Resúmen en extenso, 357
Muthangya M, Amana MJ, Hashim SO, Mshandete AM, Kivaisi AK (2019) Proximate Nutrient Composition and Antioxidant Properties of *Pleurotus sapidus* 969 Cultivated on *Agave sisalana* Saline Solid Waste. J Appl Life Sci Int 20(2):1-13. https://doi.org/10.9734/JALSI/2019/v20i230081
Muthangya M, Hashim SO, Amana JM, Mshandete AM, Kivaisi AK (2013) Optimization of *Pleurotus* mushroom cultivation on saline sisal solid waste. World Appl Sci J 23(9):1146-1150. https://doi.org/10.5829/idosi.wasj.2013.23.09.912
Narváez-Zapata JA, Sánchez-Teyer LF (2010) Agaves as a raw material: recent technologies and applications. Recent Pat Biotechnol 3(3):185-191. https://doi.org/10.2174/187220809789389144
NMX-F-607-NOMRMEX-2002. Alimentos- Determinación de cenizas en alimentos. Diario oficial de la Federación, 27 de Agosto de 2013. México, DF.
NMX-F-608-NORMEX-2011. Alimentos-Determinación de Proteínas en Alimentos. Diario oficial de la Federación, 18 de septiembre de 2014. México, DF.
NMX-F-613-NORMEX-2003. Alimentos. Determinación de fibra cruda en alimentos.
NMX-F-615-NORMEX-2004. Alimentos-Determinación de extracto etéreo (Método Soxhlet) en Alimentos. Diario oficial de la Federación, 05 de Julio de 2019. México, DF.
NOM-116-SSA1-1994. Determinación de humedad en alimentos por tratamiento térmico, mediante método por arena o gasa. Norma Oficial Mexicana. Diario oficial de la Federación, 30 de Octubre de 2017. México, DF.
Ögel ZB, Yüzügüllü Y, Mete S, Bakir U, Kaptan Y, Sutay D, Demir AS (2006) Production, properties and application to biocatalysis of a novel extracellular alkaline phenol oxidase from the thermophilic fungus *Scytalidium thermophilum*. Appl Microbiol Biotechnol 71(6):853-862. https://doi.org/10.1007/s00253-005-0216-2
Palmeiri G, Giardina P, Marzullo L, Desiderio B, Nittii G, Cannio R, Sannia G (1993) Stability and activity of a phenol oxidase from the ligninolytic fungus *Pleurotus ostreatus*. Appl Microbiol Biotechnol 39(4):632-636. https://doi.org/10.1007/BF00205066
Picornell MR, Pardo-Giménez A, Navarro MJ, Gea FJ (2017) Updates on substrate preparation for the cultivation of oyster mushroom *Pleurotus* spp. In: Sánchez JE, Royse DJ (eds) The biology, cultivation and nutritional properties of *Pleurotus* spp. Mushrooms, 1st edn. El Colegio de la Frontera Sur, Tapachula, pp 63-83
Quimio T (2001) Preparación de la semilla. In: Sánchez JE, Royse DJ (eds) La Biología y el Cultivo de *Pleurotus* spp, 1st edn. El Colegio de la Frontera Sur, San Cristobal de las Casas, pp 125-137
Reddy GV, Babu PR, Komaraiah P, Roy KRRM, Kothari IL (2003) Utilization of banana waste for the production of lignolytic and cellulolytic enzymes by solid substrate fermentation using two *Pleurotus* species (*P. ostreatus* and *P. sajor-caju*). Process Biochem 38(10):1457-1462.
Reinhammar B (1984) Laccase. In: Lontie R (ed) Copper proteins and copper enzymes, Vol 3. CRC Press, Boca Raton, pp 1–35
Reynoso-Santos R, García MAJ, López BW, López LA, Iñíguez PC, Pérez FMA, Gutiérrez MD (2012) Identificación taxonómica de agaves (*Agave* spp.): utilizados para la elaboración del licor comiteco en Chiapas, México. Agro productividad 5(4):9-18
Rinker DL (2002) Handling and using “spent” mushroom substrate around the world. In: Sánchez JE, Huerta G, Montiel E (eds) Mushroom biology and mushroom products. Impresos Júpiter, Cuernavaca, pp 43–60
Royse DJ (1985) Effect of Spawn Run Time and Substrate Nutrition on Yield and Size of the Shiitake Mushroom. Mycol 77(5):756-762. https://doi.org/10.1080/00275514.1985.12025163

Royse DJ (1989) Factors influencing the production rate of shiitake. Mushroom J Trop 9:127–138.
Sanchez A, Ysunza F, Beltrán-García MJ, Esqueda M (2002) Biodegradation of viticulture wastes by *Pleurotus*: a source of microbial and human food and its potential use in animal feeding. J Agric Food Chem 50(9): 2537-2542. https://doi.org/10.1021/jf011308s
Sánchez JE (2002) Crecimiento y fructificación. In: Sánchez JE, Royse DJ (eds) La Biología y el cultivo de *Pleurotus* spp. 1 st (edn) El Colegio de la Frontera Sur, Limusa, S.A. de C.V, Distrito Federal, pp 51-67
Sánchez JE, Moreno L, Andrade R (2011) Pasteurization of substrate for growing *Pleurotus ostreatus* by self-heating. In: Savoie JM, Foulogne-Oriol M, Largeteau M, Barroso G (eds) Proceed. 7th Int. Conf. Mush. Biol. Mush. Prod. Institut Nationale de la Recherche Agronomique-World Society for Mushroom Biology and Mushroom Products. Arcachon, France, pp 403-410
Sánchez JE, Moreno L, Andrade R (2017) Substrate protection for the cultivation of oyster mushrooms and other edible mushrooms. In: Sánchez JE, Royse DJ (eds) The biology, cultivation and nutritional properties of *Pleurotus* spp. Mushrooms, 1st edn. El Colegio de la Frontera Sur, Tapachula, pp 107-125
Saval S (2012) Aprovechamiento de residuos agroindustriales: pasado, presente y futuro. BioTecnología 16(2):14-46
Shitole AV, Gade RM, Bandgar MS, Wavare SH, Belkar YK (2014) Utilization of spent mushroom substrate as carrier for biocontrol agent and biofertilizer. Bioscan 9:271-275
Soto-Velazco C, Guzmán-Dávalos L, Rodríguez O (1989) Cultivo del hongo comestible *Pleurotus ostreatus* sobre bagazo de maguey tequilero fermentado y mezclado con paja de trigo. Rev Mex Mic 5:97-101.
Stölzer S, Grabbe K (1991) Mechanisms of substrate selectivity in the cultivation of edible fungi. Mushroom Sci 13:141-146
Tejera-Hernández B, Rojas-Badía MM, Heydrich-Pérez M (2011) Potencialidades del género *Bacillus* en la promoción del crecimiento vegetal y el control biológico de hongos fitopatógenos. Rev CENIC Cienc Biol 42(3):131-138.
Tien M, Kirk TK (1988) Lignin peroxidase of *Phanerochaete chrysosporium*. Meth Enzymol 161:238–249. https://doi.org/10.1016/0076-6879(88)61025-1
Torres-Ruíz E, Sánchez JE, Guillen-Navarro GK, Ramos-Pérez DG, Royse DJ (2016) Microbial promoters of mycelial growth, fruiting and production of *Pleurotus ostreatus*. Sydowia 68:151-161. https://dx.doi.org/10.12905/0380.sydowia 68-2016-0151
Ünal M (2015) The utilization of spent mushroom compost applied at different rates in tomato (*Lycopersicon esculentum* Mill.) seedling production. Emir J Food Agric 27(9):692-697. https://doi.org/10.9755/ejfa.2015-05-206
Van Soest PJ (1982) Nutritional Ecology of the Ruminant. OB Books, Corvallis, pp 1–373
Velázquez-Cedeño MA, Mata G, Savoie JM (2002) Waste-reducing cultivation of *Pleurotus ostreatus* and *Pleurotus pulmonarius* on coffee pulp: changes in the production of some lignocellulolytic enzymes. World J Microbiol Biotechnol 18(3):201-207. https://doi.org/10.1023/A:1014999616381
Vieira FR, Pecchia JA, Segato F, Polikarpov I (2018) Exploring oyster mushroom (*Pleurotus ostreatus*) substrate preparation by varying phase I composting time: changes in bacterial communities and physicochemical composition of biomass impacting mushroom yields. Appl Microbiol 126(3):931-944. https://doi.org/10.1111/jam.14168
Wariishi H, Valli K, Gold MH (1992) Manganese (II) oxidation by manganese peroxidase from the basidiomycete *Phanerochaete chrysosporium*. Kinetic mechanism and role of chelators. J Biol Chem 267(33):23688-23695. https://doi.org/10.1016/S0021-9258(18)35893-9

Zadrazil F, Kurtzman RH (1982) The biology of *Pleurotus* cultivation in the tropics. In: Chang ST, Quimio TH (eds) Tropical Mushrooms Biological Nature and Cultivation Methods, 1st edn. The Chinese University Press, Hong Kong, pp 277-298

Zhu H, Sheng K, Yan E, Qiao J, Lv F (2012) Extraction, purification and antibacterial activities of a polysaccharide from spent mushroom substrate. Int J Biol Macromol 50(3):840-843. https://doi.org/10.1016/j.ijbiomac.2011.11.016

Table

Table 1. Biological efficiency (BE), yield (Y), production rate (PR), mean mushroom size (MMS) and bioconversion (B) of *P. ostreatus* ECS-1123 grown on agave comiteco bagasse after two harvests.

Method	**BE (%)**	**Y**	**PR (%)**	**MMS (g)**	**B (%)**
Sterilization	32.7 ± 6.2^{b}	0.114 ± 0.01^{b}	0.36 ± 0.07^{b}	13.4 ± 4.6^{a}	17.6 ± 2.1^{a}
Alkaline immersion	20.5 ± 11.9^{c}	0.090 ± 0.009^{c}	0.22 ± 0.09^{c}	12.1 ± 5.8^{a}	16.6 ± 1.5^{a}
Self-heating	57.4 ± 4.9^{a}	0.214 ± 0.02^{a}	0.96 ± 0.08^{a}	11.9 ± 2.8^{a}	17.9 ± 1.1^{a}

Different letters in the same column indicate significant differences between treatments $\alpha=0.05$ (Tukey HSD multiple comparisons test).

Figures

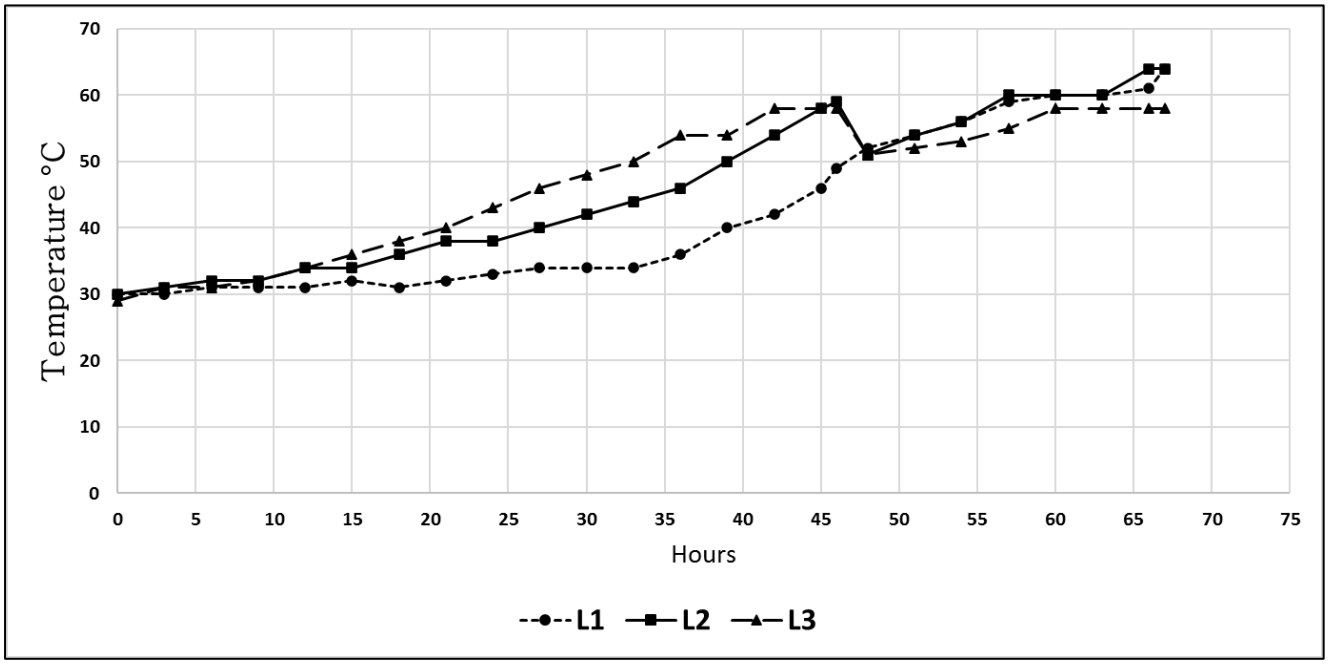


Fig. 1

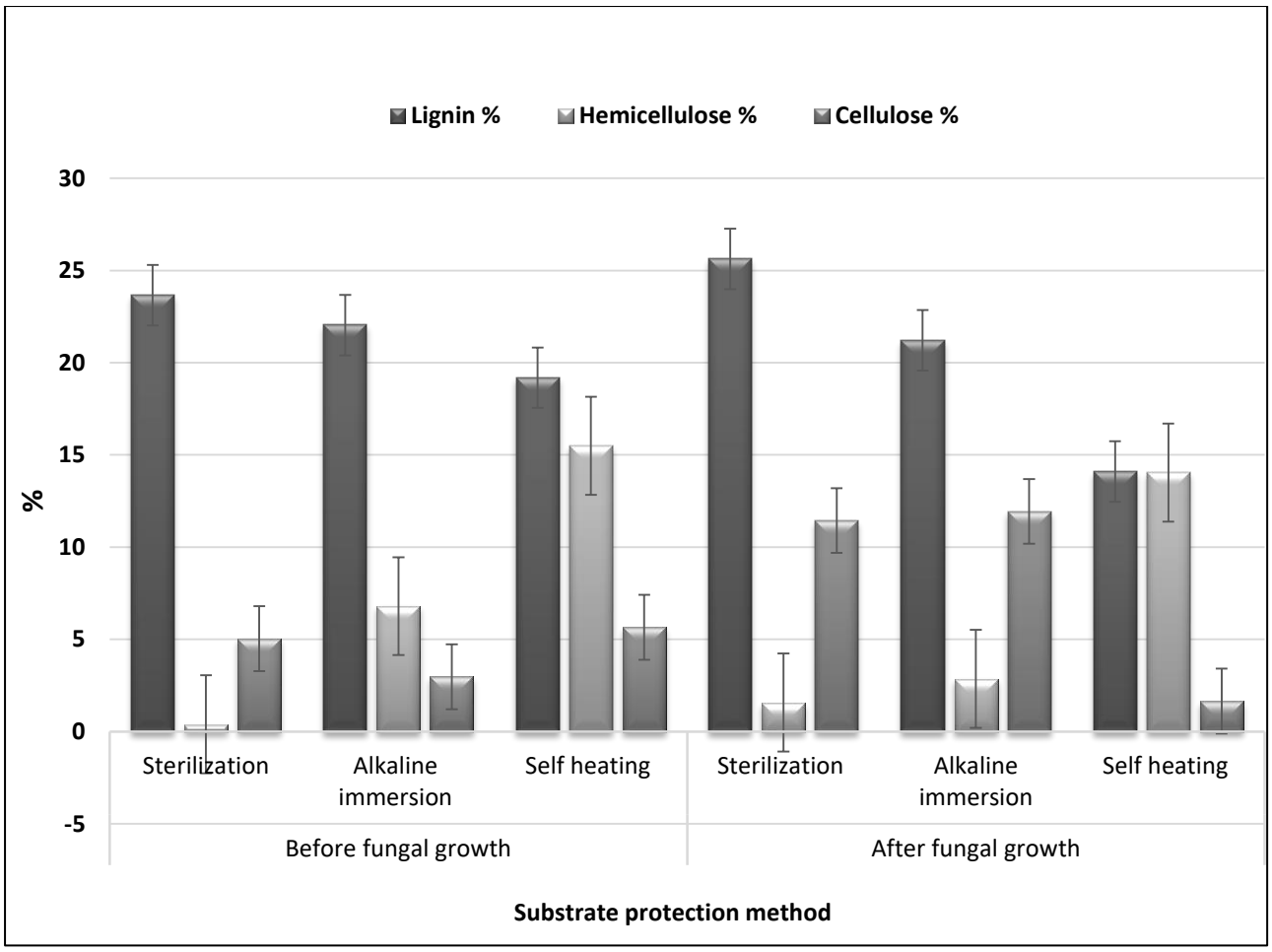

Lignin %
Hemicellulose %
Cellulose %
30
25
20
15
10
5
0
-5
%
Sterilization
Alkaline immersion
Self heating
Sterilization
Alkaline immersion
Self heating
Before fungal growth
After fungal growth
Substrate protection method

Fig. 2

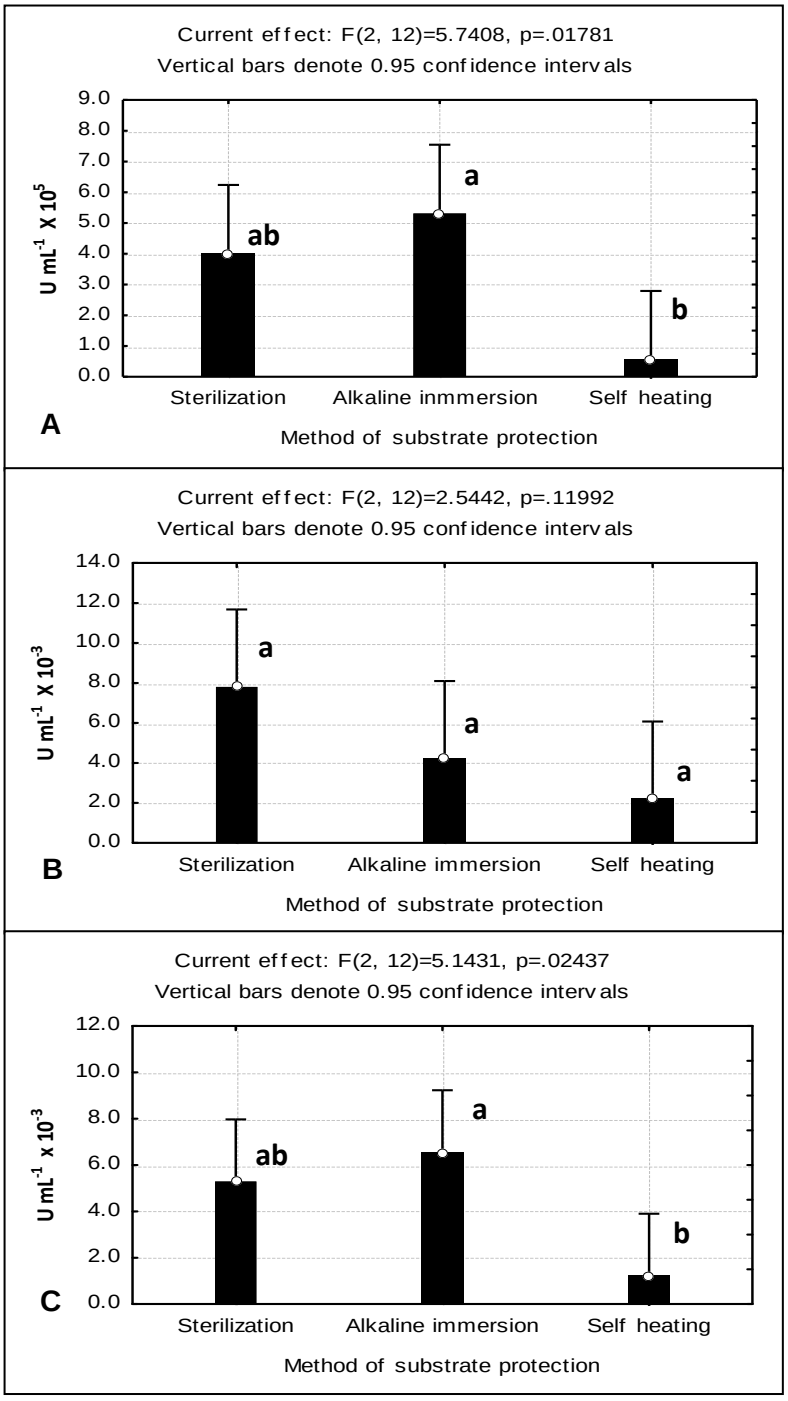

Current effect: F(2, 12)=5.7408, p=.01781
Vertical bars denote 0.95 confidence intervals
U mL-1 X 10^5
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ab
a
b
Sterilization
Alkaline inmmersion
Self heating
A
Method of substrate protection
Current effect: F(2, 12)=2.5442, p=.11992
Vertical bars denote 0.95 confidence intervals
U mL-1 X 10-3
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
a
a
a
Sterilization
Alkaline immersion
Self heating
B
Method of substrate protection
Current effect: F(2, 12)=5.1431, p=.02437
Vertical bars denote 0.95 confidence intervals
U mL-1 x 10-3
12.0
10.0
8.0
6.0
4.0
2.0
0.0
ab
a
b
Sterilization
Alkaline immersion
Self heating
C
Method of substrate protection

Fig. 3

Legend to Figures

Figure 1. Temperature profile at three levels of the substrate (agave comiteco bagasse) during self-heating in a 1 m^3 wooden box for 67 hours, with removal of the contents at 46 hours. Symbols: L_1, 15 cm below the substrate surface; L_2, at the center; and L_3, 15 cm above the bottom of the crate

Figure 2. Content (%) of lignin, hemicellulose and cellulose in the agave comiteco bagasse after applying three substrate protection treatments and after two harvests of *Pleurotus ostreatus* ECS-1123

Figure 3. Laccase (A), Mn peroxidase (B) and Phenol oxidase (C) activity detected in agave comiteco substrate after the second harvest of *Pleurotus ostreatus*. Letters indicate significant differences by Tukey's HSD multiple comparisons, with means of α=0.05

Capítulo 3. Conclusiones

El estudio realizado demostró que *P. ostreatus* crece en el bagazo de agave comiteco sin suplementación, después de promover cierta selectividad al sustrato; es decir, al aplicar algún método de tratamiento con el fin de eliminar microorganismos competidores de los nutrientes que ofrece el bagazo. A su vez durante el proceso de desarrollo del hongo se corroboró a través del análisis del contenido de nutrientes del bagazo de agave comiteco; que dicho subproducto agroindustrial es rico en polisacáridos aprovechables por *P. ostreatus*, principalmente hemicelulosa y celulosa, fuente de glucosa para el hongo. Y se determinó que *P. ostreatus* utiliza las enzimas de la degradación (lacasa, Mn peroxidasa y fenol oxidasa) para poder acceder a la glucosa; siendo la lacasa las más activa, dado que se caracteriza por tener poca selectividad al sustrato. De los tres métodos de protección del sustrato estudiados para el cultivo de *P. ostreatus* sobre el agave comiteco, el de autocalentamiento resultó ser el mejor dado que obtuvo los mayores valores de EB% (57,4), R (0,21), TP% (0,96), PPH (11,9) y B% (17,9).

También se observó una notable disminución de los polisacáridos estructurales debido al cultivo de *P. ostreatus*, que varió en función del tratamiento de protección del sustrato utilizado y fue mayor en los métodos de autocalentamiento e inmersión alcalina. El método de autocalentamiento aplicado al bagazo de agave comiteco destacó del resto de los tratamientos no solo por promover la mayor productividad en el cultivo de *P. ostreatus* y por ser un método ecológico de esterilización; si no también por que promovió la actividad lacasa ($0{,}5x10^{5}$ a $5{,}32x10^{5}$ U mL^{-1}) Mn peroxidasa ($2{,}19\ x10^{-3}$ a $7{,}84\ x\ 10^{-3}$ U mL^{-1}) y fenol oxidasa ($6{,}55x10^{-3}$ a $1{,}22x10^{-3}$ U mL^{-1}) en menor medida. Estas enzimas son potencialmente útiles para la obtención de biomasa energética y otras biotecnologías.

Finalmente, el estudio realizado propone generar alimentos nutracéuticos, así como enzimas ligninolíticas y bioprocesos para la biotransformación de biomasa energética utilizable a partir de residuos de la agroindustria, lo cuales generalmente se encuentran siendo un problema de contaminación; pues se conoce poco sobre su mejor disposición final. Por lo que generar bioprocesos, bioproductos y tecnologías que sean afines al desarrollo sustentable resultan alternativas interesantes de mitigación del cambio climático necesarias para establecer formas de vida limpia para todos los seres vivos.

Literatura Citada

Álvarez LJN. (2019). Crecimiento de hongo *Pleurotus* spp. a partir de desecho de agave. [Tesis de maestría] Universidad autónoma de Querétaro, 106 p.

Avendaño-Hernandez RJ, Sánchez JE. (2013). Self-pasteurised substrate for growing oyster mushrooms (*Pleurotus* spp.). African Journal of Microbiology Research 7(3):220-226.

Baena GA. (2005). Aprovechamiento del bagazo de maguey verde (*Agave Salmiana*) de la agroindustria del mezcal en San Luis Potosí para la producción de hongo ostra (*Pleurotus ostreatus*). [Tesis de maestría] Instituto Potosino de Investigación Científica y Tecnológica, 102 p.

Barrios-Espinoza BM, Moreno-Ruíz L, Sánchez JE. (2009). Composteo en cajones de madera como pretratamiento del sustrato para cultivar *Pleurotus ostreatus*. Revista Mexicana de Micología 29:19-25.

Cabrera-Soto ML. (2011). Producción de lacasa de *Pleurotus ostreatus* utilizando los residuos de *Agave tequilana* Weber como sustrato. [Tesis Doctoral] Instituto Politécnico Nacional, 135 p.

Castillejos Márquez, S. (2015). Producción de lacasa a partir de hongos ligninolíticos utilizando vinazas y bagazo de origen mezcalero. [Tesis de maestría] Universidad tecnológica de la mixteca, 102 p.

Contreras EP, Sokolov M, Mejía G, Sánchez JE. (2004). Soaking of substrate in alkaline water as a pretreatment for the cultivation of *Pleurotus ostreatus*. Journal of Horticultural Science & Biotechnology 79(2): 234-240.

Cruz-López M. (2014). Cultivo de setas: (*Pleurotus ostreatus*) en desperdicios de la destilación alcohólica del sotol (*Dasylirion cedrosanum*). [Tesis de licenciatura] Universidad Autónoma Agraria Antonio Narro, 64 p.

Cruz-Moreno, B. A. (2020). Cultivo de Hongos comestibles *Pleurotus djamor* y *Pleurotus ostreatus* sobre sustrato de bagazo de *Agave tequilana*. [Tesis de maestría] Universidad autónoma de Querétaro, 100 p.

Guzmán-Dávalos L, Martínez-Carrera D, Morales P, Soto C. (1987). El cultivo de hongos comestibles (*Pleurotus*) sobre el bagazo del maguey de la industria tequilera. Revista Mexicana de Micología 3:47-49.

Heredia-Solís A, Esparza IE, Romero BL, Cabral AF, Bañuelos VR. (2014). Bagazos de *Agave salmiana* y *Agave weberi* utilizados como sustrato para producir *Pleurotus ostreatus*. Revista Iberoamericana de Ciencias 1(5):103-110.

Lara-Hidalgo C, Grajales LA, Ruiz CMA, Ventura CC, Gutiérrez MFA, Ruiz VM, Archila MA. (2017). *Agave americana* Honey fermentation by *Kluyveromyces marxianus* strain for “Comiteco” production, a spirit from mexican southeast. Revista Mexicana de Ingeniería Química 16(3):771-779.

Mora VM, Martínez-Carrera D. (2007). Investigaciones básicas, aplicadas y socioeconómicas sobre el cultivo de setas (*Pleurotus*) en México. En: Sánchez JE, Martínez-Carrera D, Mata G, Leal H. eds. El Cultivo de Setas *Pleurotus* spp. en México. México DF: ECOSUR-CONACYT, p. 69-110.

Morales BV. (2009). Determinación de patrones genéticos de cepas parentales e híbridas de *Pleurotus* spp. [Tesis de maestría] Unidad Profesional Interdisciplinaria de Biotecnología, 68 p.

Muthangya M, Amana MJ, Hashim SO, Mshandete AM, Kivaisi, AK. (2019). Proximate Nutrient Composition and Antioxidant Properties of *Pleurotus sapidus* 969 Cultivated on *Agave sisalana* Saline Solid Waste. Journal of Applied Life Sciences International 20(2):1-13.

Muthangya M, Hashim SO, Amana JM, Mshandete AM, Kivaisi AK. (2013). Optimization of *Pleurotus* mushroom cultivation on saline sisal solid waste. World Applied Sciences Journal 23(9):1146-1150.

Narváez-Zapata JA, Sánchez-Teyer LF. (2010). Agaves as a raw material: recent technologies and applications. Recent Patents on Biotechnology 3(3):185-191.

Reynoso-Santos R, García MAJ, López BW, López LA, Iñíguez PC, Pérez FMA, Gutiérrez MD. (2012). Identificación taxonómica de agaves (*Agave* spp.): utilizados para la elaboración del licor comiteco en Chiapas, México. Agroproductividad 5(4):9-18.

Rodríguez-Macías R, Alcantar GEG, Iñiguez CG, Zamora NF, García LPM, Ruiz LMA, Salcedo PE. (2010). Caracterización física y química de sustratos agrícolas a partir de bagazo de agave tequilero. Interciencia 35(7):515-520.

Sánchez JE, Moreno L, Andrade R. (2017). La protección del sustrato para el cultivo de Pleurotus spp. y otros hongos comestibles. En: Sánchez JE, Royse DJ. eds. La biología, el cultivo y las propiedades nutricionales y medicinales de las setas *Pleurotus* spp. Tapachula: El Colegio de la Frontera Sur, p. 107-125.

Sánchez VJE. (2002). Crecimiento y fructificación. En: Sánchez JE y Royse D (eds), La Biología y el Cultivo de *Pleurotus* spp. México DF: ECOSUR-LIMUSA, p. 51-67.

Saval S. (2012). Aprovechamiento de residuos agroindustriales: pasado, presente y futuro. BioTecnología 16(2):14-46.

Solís AH, Valenzuela RB, Ibarra ELE. (2018). Producción de *Pleurotus ostreatus* sobre sustratos de *Agave salmiana* Y *Agave weberi*. Biotecnología y Sustentabilidad 2(1):31-37.

Soto-Velazco C, Guzmán-Dávalos L, Rodríguez O. (1989). Cultivo del hongo comestible *Pleurotus ostreatus* sobre bagazo de maguey tequilero fermentado y mezclado con paja de trigo. Revista Mexicana de Micología 5:97-101.

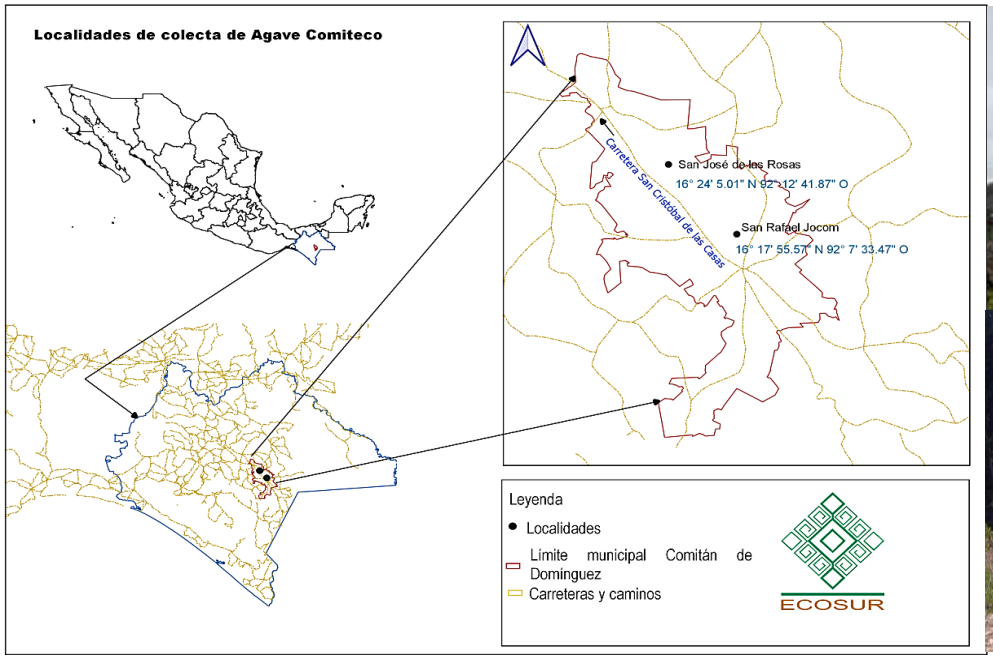
Stölzer S, Grabbe K. (1991). Mechanisms of substrate selectivity in the cultivation of edible fungi. Mushroom Science 13:141-146.

Valdivieso-Solís DG. (2019). Dinámica de comunidades de levaduras asociadas al microambiente de producción de comiteco. [Tesis de Maestría]. Instituto de ciencias biológicas. Universidad de ciencias y Artes de Chiapas, 154p.

Anexos

Metodología

Área de estudio



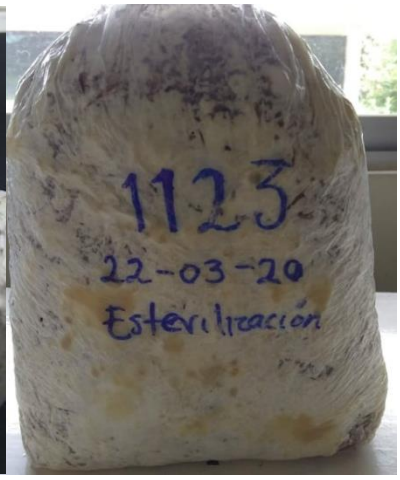
Cepas utilizadas y preparación del inoculo

Sustrato utilizado

Tratamiento del sustrato de bagazo de agave comité

Esterilización





Inmersión alcalina



Autocalentamiento

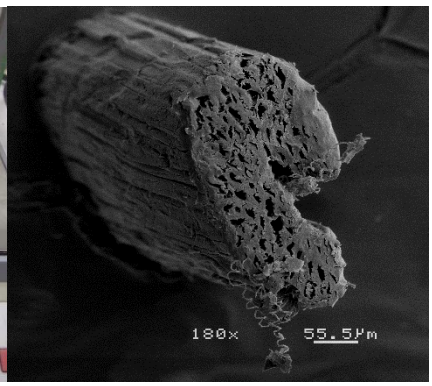


Cultivo y obtención de basidiomas



Parámetros físico-químicos del bagazo de agave comiteco





Caracterización biológica: actividad enzimática


Parámetros de productividad