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Production and characterization of reconstituted strains of *Pleurotus* spp. cultivated on different agricultural wastes

Producción y caracterización de cepas reconstituídas de *Pleurotus* spp. cultivadas en diferentes residuos agrícolas

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Abstract

Two reconstituted strains were produced by mating neohaplonts (monokaryons) obtained by chemical dedikaryotization of two parental strains i.e. *Pleurotus ostreatus* (OS) and *Pleurotus djamor* (DS). Five monokaryons were recovered employing homogenization time periods between 60 to 90 s, and incubation at 28 ºC in a peptone-glucose solution (PGS) for *Pleurotus* spp. Mycelial kinetics on MEA and wheat grain were determined using 4 math models, the parental strains on malt extract agar (MEA) showed μ_{max} values between 8.11 and 10.73 mm d^{-1} and on wheat grain presented μ_{max} values since 0.23 to 9.18 cm^3 d^{-1}, while the reconstituted strains on MEA exhibited μ_{max} values ranged from 8.68 to 12.93 mm d^{-1} and on wheat grain showed μ_{max} values between 0.25 and 9.30 cm^3 d^{-1}. The Logistic and Exponential mathematical models presented the best statistical precision in the growth of the strains on MEA and wheat grain respectively. The parental and reconstituted strains were cultivated using two agricultural wastes such as wheat straw (WS) and a mixture of oak sawdust, wheat straw, millet seed, cotton seed hull and CO_3 (AP). The productivity and chemical composition of the mushrooms, and also the chemical composition of the substrates before and after harvest were determined. Reconstituted strain *Pleurotus djamor* (DS_2xDS_4) cultivated on WS showed the highest biological efficiency (125.84%) and production rate (2.79%), and also this strain produced on AP presented the highest productivity parameters: biological efficiency (98.43%) and productivity rate (2.27%). The reconstituted strain of *Pleurotus ostreatus* (OS_4xOS_5) produced on AP presented fruit bodies with highest protein content being of 24.03%, while the reconstituted strain of *Pleurotus djamor* (DS_2xDS_4) cultivated on WS exhibited the highest protein content of 32.94%. The substrate AP used in the cultivation of the reconstituted strain (DS_2xDS_4) presented the highest biodegradation value of lignin (33.60%).

Keywords: Biodegradation, reconstituted strain, *Pleurotus*, production.

Resumen

Se obtuvieron dos cepas reconstituidas por apareamiento de neohaplontes (monocariones) compatibles obtenidos por dedicariotización química de dos cepas parentales: *Pleurotus ostreatus* (OS) y *Pleurotus djamor* (DS). Se recuperaron cinco monocariones empleando tiempos de homogenización entre 60 y 90 s y temperatura de incubación de 28 ºC en una solución de peptona-glucosa (PGS) para las cepas de *Pleurotus*. La cinética micelial en extracto de malta agar (EMA) y en grano de trigo se determinó utilizando 4 modelos matemáticos, las cepas parentales en EMA mostraron valores μ_{max} entre 8.11 y 10.73 mm d^{-1} y en grano de trigo presentaron valores μ_{max} desde 0.23 hasta 9.18 cm^3 d^{-1}, mientras que las cepas reconstituidas en EMA mostraron valores de μ_{max} oscilaron entre 8.68 y 12.93 mm d^{-1} y en grano de trigo mostraron valores de μ_{max} entre 0.25 y 9.30 cm^3 d^{-1}. Los modelos matemáticos Logístico y Exponencial presentaron la mejor precisión estadística en el crecimiento de las cepas en EMA y grano de trigo respectivamente. Las cepas parentales y reconstituidas se cultivaron utilizando dos desechos agrícolas tales como paja de trigo (WS) y una mezcla de aserrín de encino, paja de trigo, semillas de mijo, cáscara de semilla de algodón y CO_3 (AP). Se determinó la productividad y la composición química de los hongos, y también la composición química de los sustratos antes y después de la cosecha. La cepa reconstituida *Pleurotus djamor* (DS_2xDS_4) cultivada en WS mostró la mayor eficiencia biológica (125.84%) y la tasa de producción (2.79%), también esta cepa producida en AP presentó los parámetros de productividad más altos: eficiencia biológica (98.43%) y tasa de productividad (2.27%). La cepa reconstituida de *Pleurotus ostreatus* (OS_4xOS_5) producida en AP presentó hongos con un mayor contenido de proteínas de 24.03%, mientras que la cepa reconstituida de *Pleurotus djamor* (DS_2xDS_4) cultivada en WS exhibió el mayor contenido de proteínas del 32.94%. El sustrato AP utilizado en el cultivo de la cepa reconstituida (DS_2xDS_4) presentó el mayor valor de biodegradación de la lignina (33.60%).

Palabras clave: Biodegradación, cepa reconstituida, *Pleurotus*, producción.

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1 Introduction

Pleurotus spp. are the second genera with the highest production in the world (Adebayo *et al*., 2018), are characterized by their nutritional value and are important source of proteins, vitamins and minerals (Manzi *et al*., 2001; Reis *et al*., 2012). Studies have showed that some characteristics have been improved in the production of reconstituted strains of these fungi such as: kinetics growth, morphological characteristics and nutritional composition (Guadarrama-Mendoza *et al*., 2014; Oropeza-Guerrero *et al*., 2018). This variability is due to the separation of the nuclei during the formation of the monokaryons and their subsequent union during the formation of the dikaryon because there are epistatic interactions, which cause the phenotypic characteristics not present in the parental dikaryon to be expressed in the reconstituted strain (Clark and Anderson, 2004). The production of reconstituted strains in edible fungi is a process that requires the mating of monokaryons previously obtained by chemical dedikaryotization, process that allows to obtain the two monokaryotic components of a dikaryon using chemical substances such as: peptone and glucose (Leal-Lara and Eger-Hummel, 1982; Aguilar-Doroteo *et al*., 2018). Obtaining reconstituted strains is important for the conservation and genetic improvement of edible fungi strains, in some cases reconstituted strains presented high rates of invasion in substrates so these new strains will have higher productivities compared to those with low colonization rates (Clark and Anderson, 2004). The development of reconstituted strains allow to improve commercial attributes, decreasing incubation time, increase the protein content and in some cases can be used different mixtures of agricultural wastes for mushroom cultivation (Valenzuela-Cobos *et al*., 2019a).

The studies that are carried out in the determination of the growth kinetics of edible fungi are in liquid mycelium cultures (Kim *et al*., 2002; Aminuddin *et al*., 2013; Loshchinina and Nikitina, 2016), but in commercial production of these fungi requires mycelial growth in solid substrates, such as different lignocellulosic materials (Barshteyn and Krupodorova 2016; Iossi *et al*., 2018). For this reason, in this study the culture was carried out in two solid media using 4 mathematical models to determine maximum specific growth rate (μ_{max}) and the duration of the latency phase (λ), necessary parameters for characterization of fungal strains (López *et al*., 2004). The aim of this investigation was to obtain reconstituted strains of the genera *Pleurotus*, determine the kinetics mycelial growth of parental and reconstituted strains in two different solid substrates (MEA and wheat grain) using different mathematical models, evaluate the productivity, morphology parameters and the chemical composition of the fruit bodies of the parental and reconstituted strains using two different agricultural wastes (WS and AP) in the cultivation, and compare the chemical composition of the wastes before and after harvest.

2 Materials and methods

2.1 Biological material

In this research the parental strains were used: *Pleurotus ostreatus* (OS, commercial strain), *Pleurotus djamor* (DS, commercial strain), and 2 reconstituted strains: OS_4xOS_5, DS_2xDS_4 obtained by matching compatible monokaryons. All the dikaryotic strains are deposited at the Research and Development Laboratory of Ecuahidrolizados Industry, which open access to other researchers who are interested in continuing the present research.

2.2 Solid culture media

The malt extract agar (MEA) was prepared by dissolving 9 g of malt extract and 7.5 g of bacteriological agar in 0.5 L of distilled water using an Erlenmeyer flask. The flask was sterilized at 15 psi (121 ºC) for 15 min, immediately 10 mL of MEA were poured into 86 mm diameter Petri dishes. The dishes with MEA were incubated at 28 ºC for 48 h to verify sterility.

2.3 Dedikaryotization solution (Peptone-Glucose Solution PGS)

The dedikaryotization solution was prepared by dissolving glucose and peptone in 0.5 L of distilled water with 20% anhydrous glucose and 20% peptone P (Oxoid LP0037) (Leal-Lara and Eger-Hummel 1982). Then, 30 mL were poured into flasks and sterilized at 15 psi (121 ºC) for 15 min. Then, the flasks with the peptone-glucose solution were incubated at 28 ºC for 48 h to verify sterility.

2.4 Recovery of neohaplonts

The mycelium of the parental strains of *Pleurotus* spp. (OS and DS) were homogenized between 60 and 90 s. Then, they were inoculated with 50 μL of the homogenized in 50 mL of dedikaryotization solution (PGS) and incubated at 28 ºC for 72 h. After the dedikaryotization solution (PGS) was homogenized with 50 mL of sterile distilled water for 20 s, 20 μL of the homogenized was inoculated on MEA and incubated at 28 ºC until the formation of colonies. Petri dishes with MEA were observed under the microscope 10(x) to identify the micelyum without clamp connections (neohaplonts) (Valenzuela-Cobos *et al*., 2017).

2.5 Types of neohaplonts compatibility

To identify the two types of neohaplonts obtained by the chemical dedikaryotization process, the mating was done in Petri dishes with MEA among all the monokaryons obtained of the same strain in all the possible combinations. Each mating was revised under a microscope with 10x, to determine the presence of the mycelium with clamp connections, the presence of this mycelial structure indicated the formation of a dikaryon and, therefore, the compatibility of the paired neohaplonts, classifying them as neohaplonts type I and neohaplonts type II. In the case that the clamp connections were not formed, the two neohaplonts were considered of one type (Valencia del Toro and Leal-Lara, 1999).

2.6 Production of reconstituted strains

To produce a reconstituted strain, the neohaplonts of type I and type II of each parental strain were paired.

2.7 Mycelial characterization

The main macroscopic characteristics of the mycelium of the parental and reconstituted strains studied were texture (cottony or floccose), density (high, regular or low) and growth (abundant, regular or scarce). These parameters were obtained by visual observation once the mycelium completely colonized the medium (Sobal *et al*., 2007; Valenzuela-Cobos *et al*., 2020).

2.8 Preparation of wheat grain

The wheat grain was hydrated by immersion in 2 L of water for 24 hours and sterilized at 15 psi (121 ºC) for 45 min, allowed to cool and the wheat grain (150 g of wet weight) was placed in bags plastic cylinder.

2.9 Area and volume of mycelial growth in solid media

The mycelial growth radio of the dikaryotic strains was measured every day on Petri dishes with MEA until total colonization, the mycelial growth area was calculated with the equation of the circumference area, see Eq. (1):

$$A = \pi r^2 \tag{1}$$

where: A = mycelial growth area, r = mycelial growth radio, π = 3.1416 To determine the mycelial growth volume on wheat grain, the mycelium was placed in the end of the bag with wheat grain with cylindrical shape and the height of the mycelial growth of the dikaryotic strains was measured daily until total colonization, the mycelial invasion volume was calculated with the following equation, see Eq. (2):

$$V = h\pi r^2 \tag{2}$$

where: h = height of mycelial growth in bag with wheat grain, r = radio of the cylinder in the wheat bag, V = mycelial growth volume.

2.10 Determination of the kinetic parameters of mycelial growth

To calculate the growth rate (μ_{max}) and the lag phase duration (λ) on MEA, the data obtained from the mycelial growth area were adjusted to one linear model and 3 non-linear models (Baranyi, Exponential and Logistic models). Likewise, to calculate the growth rate (μ_{max}) and the duration of the lag phase (λ) on wheat grain, the data obtained from the mycelial growth volume were adjusted to the math models.

2.11 Mycelial growth kinetics using linear model

The maximum growth rate (μ_{max}) and the latency phase duration (λ) on MEA and wheat grain were calculated with the following equations (López *et al*., 2004), see Eq. (3) and (4):

$$y - y(t_{max} = \mu_{max}(t - t_{max} \tag{3}$$

where μ_{max} = specific maximum growth speed, y = growth rate on the first day, t_{max} = day of maximum speed, and t_{max} = growth rate.

Lag phase duration

$$\lambda = t_{max} - \frac{y(t) - y}{\mu_{max}} \tag{4}$$

where λ = lag phase duration, μ_{max} = maximum specific growth velocity, y = growth velocity on the first day, t_{max} = maximum speed day, and $y(t)$ = growth rate on each day.

2.12 Mycelial growth kinetics using nonlinear models

To determine the maximum specific growth rate (μ_{max}) and the lag phase (λ) were used the models of Baranyi, Exponential and Logistic (Gibson *et al*., 1987; Zwietering *et al*., 1990; Baty and Delignette-Muller, 2004), see Eq. (5) - Eq (7). Baranyi Model

$$y(t_{max}) = y_{max} + \ln\left(\frac{-1 + e^{\mu_{max}\lambda} + e^{\mu_{max}t}}{(-1 + e^{\mu_{max}t}) + e^{(\mu_{max}\lambda + y_{max} - y)}}\right) \tag{5}$$

where λ = lag phase duration, μ_{max} = maximum specific growth velocity, and y_{max} = growth velocity on the last day, y = growth velocity on the first day, t = days, e = 2.7183, and t_{max} = velocity of growth.
Exponential Model

$$y(t_{max} = y_{max} + \mu_{max}(t - \lambda) - \ln\left(e^{(y_{max} - y)} - 1 + e^{\mu_{max}(t-\lambda)}\right) \tag{6}$$

where λ = lag phase duration, μ_{max} = maximum specific growth velocity, and y_{max} = growth velocity on the last day, y = growth velocity on the first day, t = days, e = 2.7183, and t_{max}= velocity of growth.
Logistic Model

$$y(t_{max}) = \frac{A}{1 + \exp\left[\frac{4\mu_{max}}{A}(\lambda - T) + 2\right]} \tag{7}$$

where λ = lag phase duration, μ_{max} = maximum specific growth rate, A = model parameter, T = days, and $y(t_{max})$ = growth velocity.

2.13 Productivity of dikaryotic strains

Bags of the parental and reconstituted strains of *Pleurotus* were cultivated in wheat straw with a humidity of 80% (1 kg of wheat straw was hydrated in a container with 4 liters of water for 24 hours) and also in a mixture of 44% oak sawdust, 30% wheat straw, 16% millet seed, 5% cotton seed hull and 5% CO_3 with a humidity of 65% (1 kg of each ingredient of the mixture was hydrated in a container with 4 liters of water for 6 hours). The experimental design was completely random, the treatments were the strains in the different culture media, with 10 repetitions for each experimentation.

The bags with the wheat straw or the mixture were placed (1 kg on a wet weight) in plastic bags and sterilized 2 h at 15 psi (121 ºC). Then, the bags were cooled and inoculated with 10% (w/w) of wheat grain invaded with mycelium and incubated in a dark room at 28 ºC until the total colonization of the substrate. Once the first harvest was obtained, the production rate (daily average biological efficiency) of the parental and reconstituted strains, was calculated with the following equation (Salmones *et al*., 1997), see Eq (8):

$$\%PR = \frac{\%BE}{T} \tag{8}$$

where % BE = biological efficiency (weight of fresh fungi / weight of dry substrate) x 100, T = total number of production days starting from inoculation.

2.14 Chemical composition of the fruit bodies

Mushrooms were dried at 75 ºC for 12 h and then milled to perform proximal analysis using standard methods. Moisture, ash, crude fiber and fat were determined according to (AOAC, 1997). Total nitrogen was evaluated with the micro-kjeldahl method, protein was calculated from total nitrogen content by employing the converting factor 4.38, total carbohydrates were calculated by the formula: 100 - (%moisture + %protein + %fat + %ash contents), and energy value was estimated according to the equation: energy = 4 x (%protein + %carbohydrate) + 9 x (% fat) (Manzi *et al*., 2004; Valencia del Toro *et al*., 2018). The energy value of edible mushrooms must be estimated based on the content of crude protein (Nx4.38), fat and carbohydrate using specific modified Atwater factors 3.75, 8.37 and 4.2 kcal g^{-1} of each component, respectively (Lau, 1982).

2.15 Biodegradation of the mixture of the agriculture wastes

To determinate the biodegradation of the agricultural wastes was necessary to determinate the chemical composition: acid detergent fiber (ADF), neutral detergent fiber (NDF), hemicellulose, cellulose, and lignin at spawning (control) and after the harvest

(Gaitán-Hernández *et al*., 2006; Valenzuela-Cobos *et al*., 2019a).

2.16 Statistical analysis

The experimental design was completely random, the treatments were the strains in the different culture media, with 10 repetitions for each experimentation and the results were analyzed using one-way analysis of variance (ANOVA) to determine the significance of individual differences at ($p \leq 0.05$) level, of the μ_{max} and λ values obtained from 4 mathematical models, the productivity, the chemical composition of the fruit bodies and the chemical composition of the substrates, when statistical differences were found, the Duncan Test with $\alpha = 0{:}05$ was applied. The analyzes were carried out using Statgraphic ver. 16.

3 Results and discussion

3.1 Recovery of neohaplonts

After the process of chemical dedikaryotization of the parental strains of *Pleurotus* spp. were recovered 2 neohaplonts of the strains of *Pleurotus ostreatus*, 1 of compatibility type I and 1 compatibility type II, and were recovered 4 neohaplonts of the strains of *Pleurotus djamor*, 2 of compatibility type I and 2 compatibility type II. Sánchez-Hernández *et al*. (2019) recovered 11 monokaryotic components of *Pleurotus djamor*, 5 of compatibility types I and 6 to compatibility type 2, while Valenzuela-Cobos *et al*. (2017), obtained 5 neohaplonts of *Pleurotus ostreatus*, 4 of compatibility type I and 1 of compatibility type II. The amount of neohaplonts recovered is directly related to the mechanical sensitivity of the strains, the volume of inoculation, the times of homogenization and incubation used (Kawasumi *et al*., 1987).

3.2 Production of reconstituted strains

The monokaryons obtained by chemical dedikaryotization of the parental strains produced 1 reconstituted strain of *Pleurotus ostreatus* and 4 reconstituted strains of *Pleurotus djamor* pairing the neohaplonts of type I with the monokaryons of the type II of the same strain. It was verified under the microscope 10x that all the strains present clamp connections. Guadarrama-Mendoza *et al*. (2014) reported the production of 2 reconstituted strains of *Pleurotus djamor* obtained by combining compatible neohaplonts obtained by chemical dedikaryotization. However, Alfaro *et al*. (2016) recovered monokaryons by protoplast fusion to produce 1 reconstituted strain of *Pleurotus ostreatus*.

3.3 Morphology of the mycelium of parental and reconstituted strains

The morphology of the mycelium of the dikaryotic strains is indicated in Table 1. The parental strains of *Pleurotus ostreatus* (OS) and *Pleurotus djamor* (DS) showed texture (cottony), density (high), growth (abundant). The reconstituted strains of *Pleurotus ostreatus* (OS_4xOS_5) and *Pleurotus djamor* (DS_2xDS_4) also presented similar morphology to their parental strain of *Pleurotus*. Valenzuela-Cobos *et al*. (2020) reported texture (cottony), density (high), growth (abundant) for two reconstituted strains of *Pleurotus*. The morphology of the mycelium is a characteristic property of each strain (Baumer *et al*., 2008; Castro *et al*., 2006).

Table 1. Morphology of parental and reconstituted strains grown on MEA at 28 ºC.

Strain	Type of strain	Texture	Density	Growth	Color
OS	Parental	Cottony	High	Abundant	White
DS	Parental	Cottony	High	Abundant	Pink
OS_4xOS_5	Reconstituted	Cottony	High	Abundant	White
DS_2xDS_4	Reconstituted	Cottony	High	Abundant	Pale pink

Table 2. Values of μ_{max} and λ for parental and reconstituted strains of *Pleurotus* on MEA.

Strains	Type of Strains	$\mu_{max\ (MEA)}$ (mm d^{-1}) * Models				$\lambda_{(MEA)}$ (d)* Models			
		Logistic	Lineal	Exponential	Baranyi	Logistic	Lineal	Exponential	Baranyi
OS	Parental	8.72±0.72^{a}	9.03±0.45^{b}	8.11± 1.68^{a}	9.52±0.08^{a}	0.98±0.23^{d}	0.93±0.12^{c}	0.97±0.01^{c}	0.85±0.02^{c}
DS	Parental	10.23±0.11^{b}	10.57±0.23^{a}	9.85± 2.03^{a}	10.73±0.01^{b}	0.73±0.15^{b}	0.75±0.04^{b}	0.86±0.06^{b}	0.69±0.06^{b}
OS_4xOS_5	Reconstituted	9.15±1.01^{a}	9.64±0.19^{a}	8.68±0.75^{a}	9.94±0.05^{a}	0.84±0.07^{c}	0.80±0.17^{b}	0.95±0.12^{c}	0.74±0.15^{b}
DS_2xDS_4	Reconstituted	12.93±0.87^{c}	12.01±0.52^{c}	11.75±1.19^{b}	13.29±0.12^{c}	0.52±0.12^{a}	0.69±0.06^{a}	0.73±0.18^{a}	0.45±0.01^{a}

* Different letters in each column indicated significant difference among the μ_{max} and λ values on MEA of the parental and reconstituted strains at level $p < 0.05$ according to Duncan's test, n = 10.

3.4 Mycelial growth kinetics of dikaryotic strains on MEA and wheat grain using a linear model and 3 non-linear

The specific maximum growth rate on MEA for parental and reconstituted strains was calculated using the Baranyi, Exponential and Logistic models. The parental strains using the Logistic model showed μ_{max} between 8.72 and 10.23 mm d^{-1}, while the reconstituted strains presented μ_{max} ranged from 9.15 to 12.93 mm d^{-1}. Using the Baranyi model, the parental strains presented μ_{max} between 9.52 and 10.73 mm d^{-1}, while the reconstituted strains showed μ_{max} between 9.94 and 13.29 mm d^{-1}. On the other hand, by using the Exponential model the parental strains exhibited μ_{max} ranged from 8.11 to 9.85 mm d^{-1}, whereas the reconstituted strains presented μ_{max} since 8.68 to 11.75 mm d^{-1} (Table 2). Valenzuela-Cobos *et al*. (2020) reported for three reconstituted strains of *Pleurotus ostreatus*, *Pleurotus djamor* and Lentinula edodes growth on MEA μ_{max} values between 8.84 and 13.02 mm d^{-1}.

The specific speed of maximum growth on wheat grain was determined using 4 mathematical models. By using the Linear model, the parental strains presented μ_{max} ranged from 6.48 to 8.11 cm^3 d^{-1}, and the reconstituted strains exhibited μ_{max} since 6.93 to 8.54 cm^3 d^{-1}. Parental strains using the Logistic model showed μ_{max} between 0.23 and 0.32 cm^3 d^{-1}, and the reconstituted strains showed μ_{max} since 0.25 to 0.37 cm^3 d^{-1}. The parental strains using the Baranyi model exhibited μ_{max} ranged from 7.31 to 9.18 cm^3 d^{-1}, and the reconstituted strains showed μ_{max} between 8.54 and 9.30 cm^3 d^{-1}. For otherwise using the Exponential model, the parental strains presented μ_{max} ranged from 6.72 to 9.07 cm^3 d^{-1}, and the reconstituted strains exhibited μ_{max} since 7.09 to 9.21 cm^3 d^{-1} (Table 3). Valenzuela-Cobos *et al*. (2017) used the Gompertz model for two strains of *Pleurotus* calculated μ_{max} values on wheat grain since 0.11 to 0.22 d^{-1}, and also reported for two hybrid strains of *PleurotusxLentinula* μ_{max} values on wheat grain between 0.31 and 0.43 d^{-1}. The specific growth rate (μ_{max}) is the ability of the strain to degrade polysaccharides and lignocellulosic materials in substrates (Sánchez, 2009). Based on the results, the reconstituted strain DS_2xDS_4 can absorb the nutrients on MEA and wheat grain respectively in shorter time in comparison with the other strains used in the investigation.

Also the lag phase time (λ) was determined for all dikaryotic strains on MEA, by using the Linear model for parental strains presented values of λ between 0.75 and 0.93 d, and values of λ for reconstituted strains since 0.69 to 0.80 d. The parental strains showed λ values ranged from 0.73 to 0.98 d using the Logistic model, and the reconstituted strains presented λ values since 0.52 to 0.84 d. By using the Baranyi model the parental strains presented λ values ranged from 0.69 to 0.85 d, and the reconstituted strains showed λ values between 0.45 and 0.74 d. On the other hand using the Exponential model, the parental strains presented λ values ranged from 0.86 to 0.97 d, and the reconstituted strains λ values since 0.73 to 0.95 d. Valenzuela-Cobos *et al*. (2017) used the Hill model calculating values of λ on MEA ranged from 0.41 to 1.52 h for two parental strains of *Pleurotus* and three hybrid *PleurotusxLentinula* strains.

The lag phase time (λ) was calculated on wheat grain using Linear model for parental strains presented λ values since 0.47 to 0.68 d, and for reconstituted strains showed λ values between 0.42 and 0.65 d. By using the Logistic model the parental strains showed λ values ranged from 2.73 to 3.84 d, and the reconstituted strains presented λ values between 2.02 and 3.47 d.

Table 3. Values of μ_{max} and λ for parental and reconstituted strains of *Pleurotus* on wheat grain.

Strains	Type of Strains	$\mu_{max\ (wheat\ grain)}$ ($cm^3\ d^{-1}$) * Models				$\lambda_{(wheat\ grain)}$ (d)* Models			
		Logistic	Lineal	Exponential	Baranyi	Logistic	Lineal	Exponential	Baranyi
OS	Parental	0.23± 0.05^{a}	6.48±0.32^{a}	6.72±0.19^{a}	7.31±0.25^{a}	3.84±0.28^{c}	0.68±0.12^{c}	0.10±0.01^{c}	0.09±0.00^{d}
DS	Parental	0.32±0.10^{b}	8.11±0.15^{b}	9.07±0.54^{c}	9.18±0.19^{c}	2.73±0.54^{b}	0.47±0.09^{b}	0.07±0.00^{b}	0.06±0.00^{b}
OS_4xOS_5	Reconstituted	0.25±0.01^{a}	6.93±0.40^{a}	7.09±0.28^{b}	8.54±0.20^{b}	3.47±0.13^{c}	0.65±0.07^{c}	0.09±0.01^{c}	0.07±0.01^{c}
DS_2xDS_4	Reconstituted	0.37± 0.06^{c}	8.54±0.56^{c}	9.21±0.62^{d}	9.30±0.08^{d}	2.02±0.25^{a}	0.42±0.00^{a}	0.04±0.00^{a}	0.04±0.00^{a}

* Different letters in each column indicated significant difference among the μ_{max} and λ values on wheat grain of the parental and reconstituted strains at level $p < 0.05$ according to Duncan's test, n = 10.

Using the Baranyi model the parental strains exhibited λ values ranged from 0.06 to 0.09 d, while the reconstituted strains showed λ values between 0.04 and 0.07 d. On the other hand, using the Exponential model exhibited λ values for the parental strains ranged from 0.07 to 0.10 d, while the reconstituted strains exhibited λ values since 0.04 to 0.09 d. Valenzuela-Cobos *et al*. (2017) used the Hill model calculating λ values for two strains of *Pleurotus* since 7.17 to 13.86 h and for three hybrid *PleurotusxLentinula* strains between 1.82 and 2.65 h. The lag phase indicates the adaptability of the strain to new conditions (Chatterjee *et al*., 2015). Based on the results of our study, the reconstituted strain DS_2xDS_4 can adapt in less time on MEA and wheat grain respectively in comparison with the other strains in the research.

In order to determine the mathematical model that presented the highest statistical precision in the kinetic of mycelial growth on MEA and wheat grain was calculated the Mean Absolute Error (MAE). Smaller values of Mean Absolute Error (MAE) indicated better fit of the model. The lowest value of MAE (2.52%) was presented by the logistic model in the kinetics growth on MEA. On the other hand, the lowest value of MAE (1.03%) was presented by the exponential model in the kinetics of mycelial growth on wheat grain.

3.5 Productivity of the dikaryotic strains

Table 4 shows the productivity parameters of the parental and reconstituted strains that were grown in the mixture of oak sawdust, wheat straw, millet, cotton husk and CO_3.

Table 4. Productivity parameters of the parental and reconstituted strains of *Pleurotus* cultivated on WS and AP.

Strains	Type of strains	Substrates	Total cultivation time (days)	Biological efficiency (%)	Production rate (%)
OS	Parental	WS	49.10±1.75^{b}	95.56±18.11^{a}	1.94±0.25^{b}
		AP	40.20±0.54^{B}	72.15±7.23^{A}	1.79±0.54^{A}
DS	Parental	WS	53.80±1.10^{c}	110.29±12.08^{b}	2.08±0.54^{c}
		AP	42.40±1.83^{C}	86.34±13.75^{B}	2.03±0.32^{B}
OS_4xOS_5	Reconstituted	WS	50.00±2.01^{b}	92.17±12.77^{a}	1.84±0.19^{a}
		AP	38.30±1.57^{A}	78.95±9.32^{A}	2.06±0.48^{B}
DS_2xDS_4	Reconstituted	WS	45.10±2.19^{a}	125.84±14.10^{c}	2.79±0.70^{d}
		AP	43.40±1.16^{D}	98.43±12.84^{C}	2.27±0.31^{C}

*WS= wheat straw, AP= mixture of 44% oak sawdust, 30% wheat straw, 16% millet seed, 5% cotton seed hull and 5% $CaCO_3$.

* All values are means ± standard deviation of ten replicates. Uppercase letters indicate difference between the productivity parameters of the mushrooms obtained on AP, while lowercase letters indicate difference between the productivity parameters of the mushrooms obtained on WS according to Duncan's test ($p < 0.05$), n = 10.

The parental strain of *Pleurotus ostreatus* (OS) and its reconstituted OS_4xOS_5 cultivated in the two substrates presented biological efficiencies between 72.15 and 92.15%, while the parental strain of *Pleurotus djamor* (DS) and its reconstituted DS_2xDS_4 showed biological efficiencies since 86.34 to 125.84%. Similar studies have been reported, Mandeel *et al*. (2005) cultivated a strain of *Pleurotus ostreatus* on four different substrates (paper, cardboard, fiber, sawdust) presenting biological efficiencies between 59.60 and 117.50%, while Valenzuela-Cobos *et al*. (2019a) reported biological efficiency of 98.13% for one reconstituted strain of *Pleurotus ostreatus* cultivated on wheat straw, and also informed biological efficiency of 106.69% for one reconstituted strain of *Pleurotus djamor* cultivated on wheat straw. Parental strain of *Pleurotus ostreatus* (OS) and its reconstituted OS_4xOS_5 showed production rates between 1.79 and 2.06%, whereas the parental strain of *Pleurotus djamor* (DS) and its reconstituted DS_2xDS_4 presented production rates since 2.03 to 2.79%. Salmones *et al*. (2004) presented low values of production rates for three parental strains of *P. djamor* in comparison their hybrid strains obtained by monokaryons mating, while (Valenzuela-Cobos *et al*., 2019a) showed . The productivity rate is an indicator for the cultivation of a commercial strain because it relates the biological efficiency to the production cycle.

Reconstituted strains of *Pleurotus* have the ability to improve the growth mycelial in different substrates, the sensorial characteristics and the protein content (Guadarrama-Mendoza *et al*., 2014; Valenzuela-Cobos *et al*., 2019a).

3.6 Chemical composition of the fruit bodies

Table 5 presents the chemical composition of the mushrooms of the parental and reconstituted strains produced on WS and AP. Valenzuela-Cobos *et al*. (2019) indicated that the nutritional composition of the fruit bodies is influenced by the substrate used in the cultivation of the mushrooms and the strain.

Fruit bodies of the parental strain of *Pleurotus djamor* produced on the two substrates, and the mushrooms of the reconstituted strain (OS_4xOS_5) and the parental strain OS cultivated on AP presented the lowest moisture content since 88.16 to 92.83%. The highest value of moisture was presented for the mushrooms of the reconstituted strain of *Pleurotus djamor* (DS_2xDS_4) cultivated on WS and AP being in a range between 93.27 to 97.19%. The fruit bodies of the reconstituted strain of *Pleurotus djamor* (DS_2xDS_4) produced on the two substrates presented the lowest fat content since 1.05 to 1.19%, whereas the mushrooms of the parental strain OS cultivated on WS and its reconstituted strain OS_4xOS_5 produced on AP showed the highest fat content between 1.74 to 2.54%. The moisture and fat content of the fruit bodies is influenced by the composition of the substrates used in the production of the mushroom (Liu *et al*., 2005; Valencia del Toro *et al*., 2018).

Table 5. Chemical composition of the mushrooms of the parental and reconstituted strains of *Pleurotus* cultivated on WS and AP.

Strains	Type of strains	Substrates	%Moisture	%Fat	%Crude Protein	%Ash	% Carbohydrate	Energy value (kcal/100g dm)
OS	Parental	WS	94.17±0.73^{c}	1.74±0.24^{c}	28.31±0.19^{b}	10.24±0.01^{d}	59.71±1.06^{a}	367.74±3.19^{a}
		AP	92.83±0.18^{A}	1.18±0.07^{A}	21.95±1.34^{B}	8.66±0.79^{D}	68.21±2.17^{B}	371.26±2.74^{A}
DS	Parental	WS	92.09±0.54^{a}	1.59±0.15^{b}	23.84±0.32^{a}	6.93±0.52^{a}	67.63±1.93^{c}	380.19±4.01^{c}
		AP	88.16±0.32^{A}	1.24±0.37^{B}	20.19±0.97^{A}	7.09±0.31^{B}	71.48±1.68^{D}	377.84±3.11^{B}
OS_4xOS_5	Reconstituted	WS	93.19±0.20^{b}	1.32±0.09^{b}	29.17±0.58^{c}	9.19±1.04^{c}	60.32±0.57^{b}	369.84±0.87^{a}
		AP	88.24±0.74^{A}	2.54±0.08^{C}	24.03±0.32^{D}	9.08±0.93^{C}	64.35±0.69^{A}	376.38±1.15^{B}
DS_2xDS_4	Reconstituted	WS	97.19±0.94^{d}	1.05±0.24^{a}	32.94±1.45^{d}	7.11±0.19^{b}	58.90±0.38^{a}	376.81±4.19^{b}
		AP	93.27±0.85^{B}	1.19±0.31^{A}	23.66±0.79^{C}	5.93±0.62^{A}	69.02±1.34^{B}	382.23±2.67^{C}

*WS= wheat straw, AP= mixture of 44% oak sawdust, 30% wheat straw, 16% millet seed, 5% cotton seed hull and 5% $CaCO_3$.

*All values are means ± standard deviation of ten replicates. Uppercase letters indicate difference between chemical composition of the mushrooms obtained on AP, while lowercase letters indicate difference between chemical composition of the mushrooms obtained on WS according to Duncan's test ($p < 0:05$), n = 10.

Fruit bodies of the reconstituted strain OS_4xOS_5 produced on AP presented the highest content of crude protein being of 24.03% and also the lowest carbohydrate content being of 64.35%, whereas the mushrooms of the reconstituted strain of *Pleurotus djamor* (DS_2xDS_4) cultivated on AP showed the highest protein content being of 32.94% and the lowest content of carbohydrate being of 58.90%. Mushrooms of the parental strain of *Pleurotus djamor* cultivated on WS presented the lowest content of ash (6.93%), while the reconstituted strain of *Pleurotus djamor* (DS_2xDS_4) cultivated on AP presented the lowest content of ash being of 5.936%.

3.7 Biodegradation of the agricultural wastes

The biodegradation of the substrates were determined according to the following chemical parameters: detergent fiber (ADF), neutral detergent fiber (NDF), hemicellulose, cellulose and lignin of two substrates (WS and AP) used in the cultivation of parental and reconstituted strains (Table 6). The highest biodegradation of lignin and cellulose was presented by the substrate (AP) used in the mushroom cultivation. However, in a recent work, the crude protein content of *Pleurotus ostreatus* carpophores was not significantly correlated with any of the parameters that define the composition of the substrates (Picornell-Buendía *et al*., 2016). The biodegradation of the cellulose, hemicellulose and lignin was calculated according to the following equation: %Biodegradation = 100-(%Final composition of the substrate*100 / % Initial composition of the substrate (Valenzuela-Cobos *et al*., 2019b).

The substrate AP used in the fructification of the parental strain of *Pleurotus djamor* (DS) presented the highest degradation value of cellulose (49.60%), the substrate AP used in the production of the parental strain of *Pleurotus ostreatus* (OS) showed the highest biodegradation of hemicellulose (57.70%) On the other hand, the substrate WS used in the fructification of the reconstituted strain OS_4xOS_5 exhibited the lowest biodegradation value of lignin (7.40%). Laccase in the main enzyme that produce the lignin biodegradation in basidiomycetes (Durrant *et al*., 1991), white-rot basidiomycetous fungi are the principal organisms that possess remarkable potential to degrade wood lignin (Bilal and Asgher, 2016).

Valenzuela-Cobos *et al*. (2019a) cultivated one reconstituted strain of *Pleurotus djamor* on a mixture of wheat straw with oak sawdust showing the following chemical composition of the substrate after harvest: hemicellulose content of 22.40%, cellulose content of 30.10% and lignin content of 29.30%, and also produced one reconstituted strain of *Pleurotus ostreatus* on wheat straw presenting the following chemical composition of the substrate after harvest: hemicellulose content of 27.60%, cellulose content of 41.20% and lignin content of 12.40%. Bae *et al*. (2006) cultivated *Pleurotus ostreatus*, *Pleurotus eryngii, Flammulina velutupes* using as a substrate (mixture of sawdust, rice bran and corn cob) showing the following chemical composition of the mixture after harvest: neutral detergent fiber of 78.20%, acid detergent fiber of 60.40%, lignin of 20.00%, hemicellulose of 17.80%, cellulose of 40.40%.

Table 6. Chemical composition of WS and AP before and after harvest.

Strains	Type of strains	Substrates	Acid Detergent Fiber (%)	Neutral Detergent Fiber (%)	Lignin (%)	Hemicelullose (%)	Celullose (%)
Control	Without strain	WS	35.42 ± 0.19^{d}	67.13 ± 0.46^{e}	11.10 ± 0.19^{c} (0%)	31.71 ± 0.73^{d} (0%)	56.03 ± 0.29^{e} (0%)
		AP	39.17 ± 0.54^{D}	72.84 ± 0.18^{D}	14.84 ± 0.15^{D} (0%)	33.67 ± 1.02^{D} (0%)	58.00 ± 0.84^{D} (0%)
OS	Parental	WS	28.16 ± 0.97^{b}	52.45 ± 0.51^{d}	10.87 ± 0.03^{a} (2.1%)	24.29 ± 0.62^{b} (23.4%)	41.58 ± 0.17^{c} (25.8%)
		AP	32.84 ± 0.28^{C}	47.09 ± 0.35^{C}	10.53 ± 0.42^{B} (29.0%)	14.25 ± 0.94^{A} (57.7%)	36.56 ± 0.39^{C} (37.0%)
DS	Parental	WS	30.19 ± 1.12^{c}	43.52 ± 0.07^{a}	10.28 ± 0.21^{a} (7.4%)	23.33 ± 1.42^{b} (26.4%)	43.24 ± 0.08^{d} (22.8%)
		AP	24.03 ± 0.76^{A}	39.18 ± 0.70^{A}	9.93 ± 0.19^{A} (33.1%)	15.15 ± 0.29^{A} (55.0%)	29.25 ± 0.35^{A} (49.6%)
OS_4xOS_5	Reconstituted	WS	24.75 ± 0.27^{a}	49.87 ± 0.17^{c}	11.02 ± 0.05^{b} (0.7%)	25.12 ± 0.57^{c} (20.8%)	38.85 ± 0.11^{b} (30.7%)
		AP	29.20 ± 0.58^{B}	45.29 ± 0.86^{B}	12.84 ± 0.51^{C} (13.5%)	16.09 ± 0.28^{B} (52.2%)	32.45 ± 0.34^{B} (44.1%)
DS_2xDS_4	Reconstituted	WS	27.57 ± 0.36^{b}	46.16 ± 0.29^{b}	10.04 ± 0.29^{a} (9.5%)	18.59 ± 0.19^{a} (41.4%)	36.12 ± 0.24^{a} (35.5%)
		AP	22.01 ± 0.84^{A}	45.92 ± 0.78^{B}	9.86 ± 0.46^{A} (33.6%)	23.91 ± 0.42^{C} (29.0%)	36.06 ± 0.15^{C} (37.8%)

*WS= wheat straw, AP= mixture of 44% oak sawdust, 30% wheat straw, 16% millet seed, 5% cotton seed hull and 5% $CaCO_3$.

* All values are means ± standard deviation of triplicate measurements. Uppercase letters indicate difference between the chemical composition of the substrate AP, while lowercase letters indicate difference between chemical composition of the substrate WS according to Duncan's test ($p < 0.05$), n = 10.

*The biodegradation of the cellulose, hemicellulose and lignin was calculated according to the following equation: %Biodegradation = 100-(%Final composition of the substrate*100 / % Initial composition of the substrate). Values in brackets represent the percentage of biodegradation of the substrates.

Conclusions

The reconstituted strain DS_2xDS_4 showed the highest growth rate speed and the lowest lag phase on MEA, while the parental strain of *Pleurotus djamor* (DS) presented the highest growth velocity and the lowest latency phase on wheat grain. The reconstituted strain DS_2xDS_4 showed at great potential in the nutritional composition because its mushrooms presented highest protein content then we could use for this strain for industrial production. The fruit bodies of the reconstituted strains DS_2xDS_4 and OS_4xOS_5 cultivated on WS and AP respectively presented the highest protein content in comparison with their parental strains.

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