Vol. 19, No. 1 (2020) 165-174
Revista Mexicana de Ingeniería Química

MODELING OF MYCELIAL GROWTH OF PARENTAL, HYBRID AND RECONSTITUTED STRAINS OF *Pleurotus* AND *Lentinula*

MODELAMIENTO DE CRECIMIENTO MICELIAL DE CEPAS PARENTALES, HÍBRIDAS Y RECONSTITUIDAS DE *Pleurotus* Y *Lentinula*

J.D. Valenzuela-Cobos[1], R.O. Rodríguez-Grimón[1], M.L. Jara-Bastidas[2], A. Grijalva-Endara[3], D.C. Zied[4], M.E. Garín-Aguilar[5], G. Valencia del Toro[2]*

[1] *Universidad Espíritu Santo - Ecuador.*
[2] *Laboratorio de Cultivos Celulares de la Sección de Estudios de Posgrado e Investigación. UPIBI, Instituto Politécnico Nacional. Barrio la Laguna s/n Ticomán. Ciudad de México CP 07340, México.*
[3] *Facultad de Ciencias Químicas, Universidad de Guayaquil- Ecuador.*
[4] *Universidade Estadual Paulista (UNESP), Faculdade de Ciências Agrárias e Tecnológicas (FCAT), Câmpus de Dracena, 17900-000, Dracena, SP, Brazil.*
[5] *Laboratorio de Farmacobiología de la Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México. Av de los Barrios No.1. Los Reyes Iztacala, Tlalnepantla CP 54090. Edo. de México, México.*

Received: March 2, 2019; Accepted: April 17, 2019

Abstract

The kinetics of mycelial growth of parental strains, hybrid and reconstituted of *Pleurotus* and *Lentinula* was calculated using the mycelial growth diameter for (malt extract agar) MEA. For wheat grain was determinate using the mycelial growth volume. The parameters were adjusted to one Linear Model and 3 nonlinear models (Baranyi, Exponential, Logistic) to determine the specific growth speed (μ_{max}) and lag phase time (λ). The hybrid and reconstituted strains of *Pleurotus* spp. and *Lentinula edodes* showed similar mycelial morphology with exception of the hybrid PD_4xLC_3. The mycelial kinetics of the dikaryotic strains on MEA using 4 mathematical models showed μ_{max} between 6.30 and 23.49 mm day^{-1} and the lag phase ranged from 0.22 to 4.47 h; while on wheat grain presented μ_{max} since 0.08 to 11.41 cm^3 day^{-1} and lag duration between 0.06 and 8.04 h. The hybrid strain PO_5xLC_2 and the reconstituted strain of *Pleurotus djamor* PD_1xPD_4 showed the highest μ_{max} and lowest lag phase on MEA and wheat grain respectively in comparison to the other strains include their parental strains. The results showed that the hybrid and reconstituted strains of *Pleurotus* and *Lentinula* presented highest mycelial growth kinetics on EMA and on wheat grain in relation to their parental strains.

Keywords: hybrid, mycelial growth, morphology, reconstituted strains.

Resumen

Se calculó la cinética del crecimiento micelial de cepas parentales, híbridas y reconstituidas de *Pleurotus* y *Lentinula* utilizando el diámetro de crecimiento micelial en EMA (extracto de malta agar). Para grano de trigo se determinó utilizando el volumen de crecimiento micelial. Los parámetros se ajustaron a un modelo lineal y 3 modelos no lineales (Baranyi, Exponencial, Logístico) para determinar la velocidad de crecimiento específica (μ_{max}) y duración de fase lag (λ). Las cepas híbridas y reconstituidas de *Pleurotus* spp. y *Lentinula edodes* mostraron morfología micelial similar en relación con sus cepas parentales con excepción del híbrido PD_4xLC_3. La cinética micelial de las cepas dicarióticas en EMA con 4 modelos matemáticos mostraron μ_{max} entre 6.30 y 23.48 mm día-1 y la duración de fase lag varió desde 0.22 hasta 4.47 h; mientras que en grano de trigo presentaron μ_{max} desde 0.08 hasta 11.41 cm^3 día-1 y fase lag entre 0.06 y 8.04 h. El híbrido PO_5xLC_2 y la cepa reconstituida de *Pleurotus djamor* PD_1xPD_4 mostraron μ_{max} más altas y menor duración de fase lag en EMA y en grano de trigo respectivamente, en comparación con las otras cepas incluyendo sus cepas parentales. Los resultados evidenciaron que las cepas híbridas y reconstituidas de *Pleurotus* y *Lentinula* presentaron altas cinéticas de crecimiento micelial en EMA y en grano de trigo en relación con sus cepas parentales.

Palabras clave: híbrido, crecimiento micelial, morfología, cepas reconstituidas.

* *Corresponding author. E-mail*: gvovaltor@gmail.com
https://doi.org/10.24275/rmiq/Bio533
issn-e: 2395-8472

1 Introduction

The five genera of edible fungi with more production in the world are: *Lentinula* (22%), *Pleurotus* (19%), *Auricularia* (17%), *Agaricus* (15%) and *Flammulina* (11%) (Royse *et al*., 2017; Sánchez-Hernández *et al*., 2019). *Pleurotus* is the genus with the highest diversity of any other cultivated fungi in different agricultural wastes (Singh and Kamal, 2017; García-Reyes *et al*., 2017; Vásquez-Véliz *et al*., 2018; Iossi *et al*., 2018; Kuss *et al*., 2018), while *Lentinula edodes* mushrooms are characterized for their high nutritional value (Reis *et al*., 2012; Zengin *et al*., 2015). Both genera are considered important source of proteins, vitamins and minerals (Manzi *et al*., 1999; Álvarez-Cervantes *et al*., 2016).

The production of hybrid and reconstituted strains (modified strains by genetic engineering) not only increase the quality of cultivated mushrooms and reduce cultivation costs, but they can also rise farmer's revenue in the short term (Avin *et al*., 2012, Valenzuela-Cobos *et al*., 2019). In some cases these new strains are produced through paring compatible monokaryons recovered by chemical dedikaryotization, this process allows the recovery of the two neohaplonts from the dikaryotic strain (Lara and Eger-Hummel, 1982).

These strains conserve the genetic makeup of the parental strains (Clark and Anderson, 2004). Some researchers have reported studies of mycelial growth in the genus *Pleurotus*, Guadarrama-Mendoza *et al*. (2014) studied reconstituted strains of *Pleurotus djamor* with different morphology in comparison with the parental strains, while (Castro *et al*., 2006 and Valenzuela-Cobos *et al*., 2017) calculated the kinetics growth of fungi strains using instantaneous speeds in the models. However, there are not studies of the morphology of reconstituted strains of *Lentinula edodes*, and also there are not publications until the moment using directly the diameter for MEA and the volume for wheat grain to calculate the kinetic growth of the strains. Thus, it is important to determinate the growth kinetics of parental, hybrid and reconstituted strains of these two genera using the Linear, Baranyi, Exponential and Logistic models that describe the best growth tendencies both in terms of statistical accuracy and simplicity (McDonald and Sun, 1999), the parameters of the models are used to determinate the maximum growth specific speed (μ_{max}) (Gil *et al*., 2011) and the lag time (λ) (Hill and Wright, 1994) of the strains. The purpose of this study was to determinate the morphology of the mycelium and the mycelial growth kinetics of the parental, hybrid and reconstituted strains of *Pleurotus* and *Lentinula* on malt extract agar (MEA) and wheat grain using 4 mathematical models and estimate the model that presents the best adjustment on the growth mycelial of the strains.

2 Materials and methods

2.1 Biological material

For this study was used two parental strains of *Pleurotus*: (PO; *Pleurotus ostreatus*) and (PD; *Pleurotus djamor*); two parental strains of *Lentinula edodes* (LC and L21); three hybrid strains PO_2xLC_2, PO_5xLC_2, PD_4xLC_3 and three reconstituted strains PO_1xPO_2, PD_1xPD_4, LC_2xLC_2. The hybrid and reconstituted strains were obtained by mating compatible monokaryons recovered by dedikaryotization and are maintained on MEA dishes at 28 ºC. Stocks of all strains are deposited at the fungal collection of the Cellular Cultures Laboratory of the Unidad Profesional Interdisciplinaria de Biotecnología (UPIBI-IPN).

2.2 Malt extract agar

The malt extract agar (MEA) was prepared dissolving 18 g of malt extract and 15 g of bacteriological agar in 1 L of distilled water. It was sterilized in an autoclave at 15 psi (121 °C) during 15 min, 10 mL of the medium were poured into sterile Petri dishes. The dishes with the solidified medium were incubated at 28 °C for 24 h to check for sterility. Then, the Petri dishes with MEA were used for the propagation of mycelium and strain storage (Eger *et al*., 1976; Valencia del Toro *et al*., 2018).

2.3 Wheat grain

The wheat grain was hydrated by immersion in 2 L of water for 24 hours. Subsequently, the wheat grain was sterilized in an autoclave at 15 psi (121 °C) for 45 min. After that, the wheat grain cooled (150 g wet weight) was placed into cylindrical plastic bags (low-density polyethylene).

2.4 Mycelial characterization

The main macroscopic characteristics of the mycelium of the parental, hybrid 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).

2.5 Mycelial growth diameter on MEA

The diameter of the colony was measured daily until that the mycelium colonized the Petri dishes with MEA.

2.6 Mycelial growth volume on wheat grain

The sterilized wheat was placed in cylindrical plastic bags (3 cm in diameter x 15 cm in length). The mycelia of the parental, hybrid and reconstituted strains were put in one side of the bags with wheat grain, the colonization height of the mycelium (h) was measured daily until that the mycelium colonized the substrate. The mycelial growth volume on wheat grain was calculated by using the volume of the cylinder equation, see Eq. (1):

$$V = h\pi r^2 \tag{1}$$

where: h = mycelial growth height, r = radius of the cylindrical plastic bag, V = mycelial growth volume.

2.7 Determination of the kinetic parameters of mycelial growth

To calculate the mycelial growth speed (μ_{max}) and the lag time (λ) on MEA and wheat grain, the growth diameter and growth volume were fitted to three Non-linear Models (Models of Baranyi, Exponential, Logistic) and one Linear Model, see Eq. (2)-(6) (Gibson *et al*., 1987; Zwietering *et al*., 1990; Baty and Delignette-Muller, 2004):

(2) Baranyi model

$$y(t_{max}) = y_{max} + \ln((-1 + e^{\mu_{max}\lambda} + e^{\mu_{max}t})/(-1 + e^{\mu_{max}t}) + e^{(\mu_{max}\lambda + y_{max} - y_0)})) \tag{2}$$

where: μ_{max} = specific maximum growth rate, y_{max} = growth rate on the last day, y_0 = growth rate on the first day, t = days, e = 2.7183, $y(t_{max})$ = growth rate, λ = phase lag duration.

(3) Exponential Model

$$y(t_{max}) = y_{max} + \mu_{max}(t - \lambda) - \ln(e^{(y_{max} - y_0)} - 1 + e^{\mu_m ax(t-\lambda)}) \tag{3}$$

where: μ_{max} = specific maximum growth rate, y_{max} = growth rate on the last day, y_0 = growth rate on the first day, t = days, e = 2.7183, $y(t_{max})$ = growth rate, λ = phase lag duration.

(4) Logistic Model

$$y(t_{max}) = \frac{A}{(1 + exp(\frac{4\mu_{max}}{A}(\lambda - t) + 2)} \tag{4}$$

where: A = parameter of the model, μ_{max} = specific maximum growth rate, t = days, e = 2.7183, $y(t_{max})$ = growth rate, λ = phase lag duration.

(5) Specific maximum growth rate (Linear Model)

$$y(t_{max}) = \mu_{max}t + B \tag{5}$$

where: B = model parameter, μ_{max} = specific maximum growth rate, t = days.

(6) Lag phase duration (Linear Model)

$$\lambda = \frac{B}{\mu_{max}} \tag{6}$$

where: B = model parameter, μ_{max} = maximum specific growth rate.

2.8 Statistical analysis

In all experiments a completely randomized design, the treatments were the different substrates, with 10 repetitions for each treatment. The data were analyzed using one-way analysis of variance (ANOVA) to determine the significance of individual differences at $p < 0.05$ level, of the maximum growth specific speed (μ_{max}) and the lag phase (λ) of parental strains, hybrid and reconstituted on MEA and wheat grain, when statistical differences were found, the Duncan Test with $\alpha = 0.05$ was applied. The independent variables were the strains and the dependient variables were the kinetic parameters. The analyses were carried out using statistical software (Statgraphic ver. 16).

Table 1. Colony morphology of parental, hybrid and reconstituted strains grown on MEA at 28 °C.

Strain	Type of strain	Texture	Density	Growth	Color
PO	Parental	Cottony	High	Abundant	White
PD	Parental	Cottony	High	Abundant	Pale pink
L21	Parental	Floccose	Low	Scarce	Off-white
LC	Parental	Floccose	Low	Scarce	Off-white
PO_2xLC_2	Hybrid	Cottony	High	Abundant	White
PO_5xLC_2	Hybrid	Cottony	High	Abundant	Off-white
PD_4xLC_3	Hybrid	Cottony	Regular	Regular	Pale pink
PO_1xPO_2	Reconstituted	Cottony	High	Abundant	White
PD_1xPD_4	Reconstituted	Cottony	High	Abundant	Pale pink
LC_2xLC_2	Reconstituted	Floccose	Low	Scarce	Off-white

3 Results and discussion

3.1 *Morphology of the mycelium of parental, hybrid and reconstituted strains*

The morphology of the mycelium of the dikaryotic strains is indicated in Table 1. The parental strains of *Pleurotus ostreatus* (PO) and djamor (PD) showed texture (cottony), density (high), growth (abundant), and the parental strains of *Lentinula* (L21 and LC) presented texture (floccose), density (low), growth (scarce). The hybrid strains PO_2xLC_2 and PO_5xLC_2 presented similar morphology than its parental PO strain. The hybrid strain PO4xLC3 presented a cottony texture similar to the parental of *Pleurotus djamor*. However, the density (regular) and growth (regular) were different in comparison to its parental strains. The reconstituted strains of *Pleurotus ostreatus* (PO_1xPO_2) and *Pleurotus djamor* (PD_1xPD_4) also presented similar morphology to their parental strain of *Pleurotus*, while the reconstituted strain of *Lentinula* LC_2xLC_2 showed similar morphology to its parental strain. Guadarrama-Mendoza *et al*. (2014) reported texture (cottony), density (high), growth (abundant) for one hybrid strain of *Pleurotus djamor*. The morphology of the mycelium is a characteristic property of each strain (Baumer *et al*., 2008; Castro *et al*., 2006).

3.2 *Mycelial growth kinetics of parental, hybrid and reconstituted strains on MEA*

Table 2 shows the values of μ_{max} and λ of the dikaryotic strains grown on MEA calculated with the growth diameters adjusted to the mathematical models used in this study. The kinetic parameters of the strains on MEA (μ_{max} and λ) obtained from the different models were grouped in statistical groups according to the Duncan test, the groups with different letters showed difference significance between them.

The μ_{max} on MEA was calculated using 4 mathematical models. For the Logistic model using the Duncan test, 5 groups were formed, in the group "a" with the lowest values of μ_{max} (7.62-9.97 mm day^{-1}) were found the parental strains of *Lentinula* (LC and L21), and *Pleurotus djamor* PD, the reconstituted strain of *Lentinula* LC_2xLC_2 and the hybrids PO_2xLC_2 and PD_4xLC_3. In the group "b" with a value of 10.36 mm day^{-1} was found the reconstituted strain PD_1xPD_4. In the "c" was found the parental strain of *Pleurotus ostreatus* PO with μ_{max} value of 11.73 mm day^{-1} and the groups "d" and "e" were formed by the reconstituted strain PO_1xPO_2 and hybrid strain PO_5xLC_2 with values of μ_{max} of 13.02 and 23.49 mm day^{-1} respectively.

According to the Duncan test using the Linear model, 6 groups were formed, the group "a" was formed by the parental strain of *Lentinula* L21 with the lowest value of μ_{max} (5.77 mm day^{-1}). In the group "b" was found the parental strain of *Lentinula* LC, the hybrid PD_4xLC_3 and the reconstituted of *Lentinula* LC_2xLC_2 with values of μ_{max} (6.30-6.32 mm day^{-1}). Group "c" was formed by the parental of *Pleurotus djamor* PD and the hybrid PO_2xLC_2 with μ_{max} values of 8.05 and 8.21 mm day^{-1}. And in the group "d" was found the reconstituted strain of *Pleurotus djamor* PD_1xPD_4 with μ_{max} of 8.71 mm day^{-1}, group "e" was formed by the parental strain of *Pleurotus ostreatus* PO and the hybrid PO_5xLC_2 with values of μ_{max} (9.62-9.69 mm day^{-1}) and in the group "f" was found the reconstituted strain of *Pleurotus ostreatus* with μ_{max} of 10.20 mm day^{-1}.

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

	$\mu_{\max(MEA)}$ **(mm day^{-1})***				$\lambda_{(MEA)}$ **(day)***			
Strains	**Logistic Model**	**Linear Model**	**Exponential Model**	**Baranyi Model**	**Logistic Model**	**Linear Model**	**Exponential Model**	**Baranyi Model**
PO	11.73±0.90^{c}	9.62±0.37^{e}	9.80±0.43^{e}	9.62±0.37^{d}	1.01±0.41^{a}	0.24±0.23^{a}	1.48±0.26^{b}	0.24±0.23^{a}
PD	9.97±0.77^{a}	8.05±0.33^{c}	8.32±0.47^{c}	9.03±0.74^{d}	1.22±0.78^{b}	0.34±0.31^{a}	1.59±0.29^{c}	2.02±0.58^{c}
L21	8.66±1.01^{a}	6.32±0.35^{b}	7.05±0.51^{b}	7.23±0.66^{b}	0.50±0.26^{a}	1.04±0.68^{b}	1.26±0.35^{a}	1.42±0.50^{b}
LC	7.72±0.78^{a}	5.77±0.11^{a}	6.41±0.53^{a}	6.48±0.53^{a}	0.49±0.26^{a}	1.31±0.42^{b}	1.18±0.22^{a}	1.28±0.26^{b}
PO_2xLC_2	9.65±0.81^{a}	8.21±0.57^{c}	8.29±0.64^{c}	9.32±1.15^{d}	0.65±0.30^{a}	0.40±0.33^{a}	1.69±0.17^{c}	2.22±0.40^{c}
PO_5xLC_2	23.49±7.97^{e}	9.69±0.15^{e}	9.68±0.15^{e}	11.05±0.52^{e}	4.47±1.76^{d}	0.24±0.09^{a}	1.61±0.11^{c}	2.15±0.31^{c}
PD_4xLC_3	7.46±0.22^{a}	6.32±0.13^{b}	6.32±0.14^{a}	6.65±0.28^{a}	1.11±0.21^{a}	0.42±0.18^{a}	1.71±0.18^{d}	2.07±0.33^{c}
PO_1xPO_2	13.02±0.54^{d}	10.20±0.44^{f}	10.62±0.63^{f}	12.57±1.02^{f}	1.44±0.37^{c}	0.22±0.12^{a}	1.70±0.05^{d}	2.36±0.10^{d}
PD_1xPD_4	10.36±0.32^{b}	8.71±0.15^{d}	8.91±0.24^{d}	9.62±0.43^{d}	0.92±0.37^{a}	0.22±0.14^{a}	1.52±0.04^{b}	1.90±0.33^{c}
LC_2xLC_2	8.84±0.63^{a}	6.30±0.13^{b}	7.25±0.54^{b}	7.44±0.59^{c}	0.73±0.26^{a}	1.14±0.36^{b}	1.34±0.15^{a}	1.50±0.22^{b}

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

For the Exponential model with the Duncan test, 6 groups were formed, in the group "a" was found the parental strain of *Lentinula edodes* LC with lowest value of μ_{max} (6.41 mm day^{-1}). In the group "b" were formed by the reconstituted strain of *Lentinula edodes* LC_2xLC_2 and the parental strain of *Lentinula edodes* L21 with values of μ_{max} (7.05-7.25 mm day^{-1}). In the group "c" with values of μ_{max} (8.29-8.32 mm day^{-1}) was found the parental strain of *Pleurotus djamor* and the hybrid PO_2xLC_2, in the group "d" with value of μ_{max} of 8.91 mm day^{-1} was found the reconstituted strain of *Pleurotus djamor* PD_1xPD_4. Group "e" was formed by the parental strain of *Pleurotus ostreatus* PO and the hybrid strain PO_5xLC_2 with values of μ_{max} of 9.68 and 9.80 mm day^{-1} respectively, while the group "f" was found the reconstituted of *Pleurotus ostreatus* PO_1xPO_2 with μ_{max} of 10.62 mm day^{-1}.

According to the Duncan test, using the Baranyi model, 6 groups were formed, in the group "a" were found the parental strain of *Lentinula edodes* LC and the hybrid PD_4xLC_3 with the lowest values of μ_{max} (6.48-6.55 mm day^{-1}). In the group "b" with value of μ_{max} (7.23 mm day^{-1}) was found the parental strain of *Lentinula* L21, whereas in the group "c" was found the reconstituted LC_2xLC_2 with value of μ_{max} of 7.44 mm day^{-1}. Group "d" was formed by the parental strains of *Pleurotus ostreatus* and djamor (PO, PD), the hybrid PO_2xLC_2 and the reconstituted of *Pleurotus djamor* PD_1xPD_4 with values of μ_{max} since 9.03 to 9.62 mm day^{-1}. And in the group "e" was found the hybrid strain PO_5xLC_2 with μ_{max} of 11.05 mm day^{-1}, group "f" was formed by the reconstituted strain of *Pleurotus ostreatus* PO_1xPO_2 with value of μ_{max} of 12.57 mm day^{-1}. Marín *et al.* (2008) using the Baranyi model reported μ_{max} between 0.57 and 6.04 mm day^{-1} for fourteen strains of fungi on MEA with different concentrations of potassium sorbate (0.5-1.5%), pH between 5 and 7, aw since 0.85 to 0.95 and temperature ranged from 15 to 30 °C. The specific speed of maximum growth (μ_{max}) indicates the capacity of the strain to absorb nutrients in the medium (Valenzuela-Cobos *et al.*, 2017). The hybrid PO_5xLC_2 presented the highest specific growth rate in comparison to the others strains.

In relation with the lag phase, for the Logistic model using the Duncan test, 4 groups were formed, in the group "a" was found the parental strains of *Pleurotus ostreatus* PO, and *Lentinula* (LC and L21), the reconstituted strains of *Lentinula* LC_2xLC_2 and *Pleurotus djamor* PD_1xPD_4 and the hybrids PO_2xLC_2 and PD_4xLC_3 with the lowest values of λ (0.49-1.11 day). In the group "b" was found the parental strain of P. djamor PD with a value of λ of 1.22 day. In the "c" was found the reconstituted strain of *Pleurotus ostreatus* PO with λ value of 1.44 day and in the group "d" was formed by the hybrid strain PO_5xLC_2 with λ value of 4.47 day.

According to the Duncan test using the Linear model, 2 groups were formed, in the group "a" were found the parental strain of *Pleurotus ostreatus* PO and *Pleurotus djamor* PD, the hybrids PO_2xLC_2, PO_5xLC_2 and PD_4xLC_3, the reconstituted strains of *Pleurotus ostreatus* PO_1xPO_2 and *Pleurotus djamor* PD_1xPD_4 with the lowest values of λ (0.22-0.42 day). In the group "b" with values of λ (1.04-1.31 day) were found the parental strains of *Lentinula edodes* (L21 and LC) and the reconstituted strains of *Lentinula* LC_2xLC_2.

For the Exponential model with the Duncan test, 4 groups were formed, in the group "a" with lowest values of λ (1.18-1.34 day) were found the parental strains of *Lentinula* (L21 and LC) and the reconstituted strain of *Lentinula* LC_2xLC_2. In the group "b" with values of λ (1.48-1.52 day) were found

the parental strain of *Pleurotus ostreatus* PO and the reconstituted *Pleurotus djamor* PD_1xPD_4. In the group "c" were formed by the parental strain of *Pleurotus djamor* PD and the hybrids PO_2xLC_2, PO_5xLC_2 with values of λ (1.59-1.61 day) and in the group "d" with values of λ (1.70-1.71 day) were found the hybrid PD_4xLC_3 and the reconstituted of *Pleurotus ostreatus* PO_1xPO_2.

According to the Duncan test using the Baranyi model, 4 groups were formed, in the group "a" was found the parental strain of *Pleurotus ostreatus* PO with the lowest value of λ (0.24 day). In the group "b" was found the parental strains of *Lentinula* (L21 and LC) and the reconstituted of *Lentinula* LC_2xLC_2 with values of λ between 1.22 and 1.50 day, while in the group "c" was found the parental strain of *Pleurotus djamor* PD and the hybrids PO_2xLC_2, PO_5xLC_2 and PD_4xLC_3 and the reconstituted of *Pleurotus djamor* PD_1xPD_4 with values of λ ranged from 1.90 to 2.22 day. The group "d" was formed by the reconstituted *Pleurotus ostreatus* PO_1xPO_2 with value of λ of 2.36 day. These results are similar to other authors using different mathematical models, Marín *et al*. (2008) using the Baranyi model reported λ values between 0.29 and 1.70 day for fourteen strains of fungi on MEA with different concentrations of potassium sorbate (0.5-1.5%), pH (5-7), aw (0.85-0.95) and temperature (15-30 °C). The lag phase (λ) indicates the capacity of the strain to adapt to the new environmental condition (Chatterjee *et al*., 2015), in relation to this, the hybrid PO_5xLC_2 presented the lowest lag phase time (λ) on MEA, so that this strain can pass to the exponential phase faster than the other strains even than its parental strains.

3.3 Mycelial growth kinetics of parental, hybrid and reconstituted strains on wheat grain

Table 3 presents the values of μ_{max} and λ of the dikaryotic strains grown on wheat grains calculated with the mycelial growth volume adjusted to the different mathematical models used in this study. The growth mycelial of the strains on wheat grain (μ_{max} and λ) obtained from the 4 math models were grouped in statistical groups according to the Duncan test, the statistical groups with different letters showed difference significance between them.

The μ_{max} on wheat grain was calculated using the following mathematical models, for the the Logistic model with the Duncan test, 3 groups were formed, in the group "a" was formed by the two parental strains of *Lentinula* (L21 and LC) and the reconstituted strain of *Lentinula* LC_2xLC_2 with the lowest values of μ_{max} since 0.08 to 0.09 cm^3 day^{-1}. In the group "b" with values of μ_{max} (0.13-0.16 cm^3 day^{-1}) were found the parental strain of PO, the hybrids PO_2xLC_2, PO_5xLC_2, PD_4xLC_3 and the reconstituted of *Pleurotus ostreatus* PO_1xPO_2. In group "c" was formed by the parental strain of *Pleurotus djamor* PD and the reconstituted of *Pleurotus djamor* PD_1xPD_4 with values of μ_{max} between 0.19 and 0.26 cm^3 day^{-1}.

According to the Duncan test using the Linear model, 4 groups were formed, in the group "a" was found the parental strain of *Lentinula* LC and the reconstituted strain of *Lentinula* LC_2xLC_2 with values of μ_{max} since 4.06 to 4.52 cm^3 day^{-1}. The group "b" was formed by the parental strain of *Lentinula* L21 with value of μ_{max} of 4.51 cm^3 day^{-1}.

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

	$\mu_{max\ (wheat\ grain)}$ **(cm^3 day^{-1})***				$\lambda_{(wheat\ grain)}$ **(day)***			
Strains	**Logistic Model**	**Linear Model**	**Exponential Model**	**Baranyi Model**	**Logistic Model**	**Linear Model**	**Exponential Model**	**Baranyi Model**
PO	0.15±0.01^b	7.57±0.18^c	7.59±0.29^b	8.41±0.62^c	4.66±0.33^c	0.51±0.19^a	0.09±0.00^c	0.08±0.00^b
PD	0.26±0.10^c	8.28±0.19^c	8.28±0.45^c	9.58±0.57^d	2.94±0.91^a	0.40±002^a	0.08±0.00^b	0.07±0.00^b
L21	0.09±0.01^a	4.51±0.07^b	4.95±0.36^a	5.04±0.43^a	8.02±0.87f	1.23±0.92^a	0.14±0.18^d	0.14±0.01^d
LC	0.09±0.01^a	4.66±0.02^a	5.19±0.72^a	5.28±0.81^a	7.33±1.12^e	1.35±0.55^b	0.14±0.02^d	0.13±0.02c
PO_2xLC_2	0.16±0.02^b	9.14±0.06^a	9.51±0.92^d	9.54±0.91^d	4.34±0.37^b	0.84±0.54^a	0.07±0.00^a	0.07±0.00^b
PO_5xLC_2	0.14±0.01^b	8.27±0.24^c	8.44±0.70^c	8.44±0.70^c	5.00±0.41^d	1.01±0.72^a	0.08±0.01^b	0.08±0.00^b
PD_4xLC_3	0.13±0.01^b	7.42±0.03^c	7.79±0.46^b	7.80±0.48^b	5.35±0.48^d	1.20±0.16^a	0.09±0.01^c	0.09±0.00c
PO_1xPO_2	0.15±0.00^b	8.84±0.02^c	9.08±0.24^d	9.08±0.23^d	4.68±0.14^c	0.41±0.00^a	0.08±0.00^b	0.08±0.00^b
PD_1xPD_4	0.19±0.01^c	10.26±0.02^d	10.40±0.42^e	11.41±0.88^e	3.71±0.18^a	0.42±0.00^a	0.07±0.00^a	0.06±0.00^a
LC_2xLC_2	0.08±0.01^a	4.52±0.00^a	4.76±0.20^a	4.76±0.20^a	8.04±1.33f	1.39±0.00^a	0.15±0.01^e	0.14±0.00^d

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

The group "c" was formed by the parental strains of *Pleurotus ostreatus* PO and *Pleurotus djamor* PD, the hybrids PO_5xLC_2, PD_4xLC_3 and the reconstituted strain of *Pleurotus ostreatus* PO_1xPO_2 with values of μ_{max} between 7.42 and 8.84 cm^3 day^{-1}. And in the group "d" with μ_{max} values ranged from 9.14 to 10.26 cm^3 day^{-1} were found the reconstituted strain of *Pleurotus djamor* PD_1xPD_4 and the hybrid PO_2xLC_2.

For the Exponential model with the Duncan test, 5 groups were formed, in the group "a" with the lowest values of μ_{max} (4.76-5.19 cm^3 day^{-1}) were found the parental strains of *Lentinula* L21 and LC and the reconstituted of *Lentinula* LC_2xLC_2. The group "b" was formed by the parental strain of *Pleurotus ostreatus* PO and the hybrid PD_4xLC_3 with values of μ_{max} since 7.59 to 7.79 cm^3 day^{-1}. In the group "c" was formed by the parental strain of *Pleurotus djamor* PD and the hybrid PO_5xLC_2 with values of μ_{max} of 8.28 and 8.44 cm^3 day^{-1} respectively, and in the group "d" with values of μ_{max} (9.08-9.51 cm^3 day^{-1}) were found the reconstituted of *Pleurotus ostreatus* PO_1xPO_2 and the hybrid PO_2xLC_2, and the group "e" was formed by the reconstituted *Pleurotus djamor* PD_1xPD_4 with μ_{max} value of 10.40 cm^3 day^{-1}.

According to the Duncan test using the Baranyi model, 5 groups were formed, in the group "a" with the lowest values of μ_{max} (5.04-5.28 cm^3 day^{-1}) were found the parental strains of *Lentinula* (L21 and LC) and the reconstituted of *Lentinula* LC_2xLC_2. The group "b" was formed by the PD_4xLC_3 hybrid with value of μ_{max} (7.80 cm^3 day^{-1}), whereas in the group "c" were found the parental strain of Pleurotus osteatus PO and the hybrid PO_5xLC_2. The group "d" was formed by the parental strains of *Pleurotus djamor* PD and *Lentinula* LC, and the reconstituted of *Pleurotus ostreatus* PO_1xPO_2 with values of μ_{max} between 9.08 and 9.58 cm^3 day^{-1}. In the group "e" was found the reconstituted strain of Pleurous djamor PD_1xPD_4 with μ_{max} value of 11.41 cm^3 day^{-1}. Similar results have been published (Guadarrama-Mendoza, 2013) using the Linear model calculated values of μ_{max} on wheat grain for parental, hybrid and reconstituted strains of *Pleurotus djamor* between 14.60 and 20.79 cm^3 day^{-1}. Nonlinear models are the equations most commonly used to describe growth kinetics (Liu *et al*., 2017). The reconstituted strain of *Pleurotus djamor* PD_1xPD_4 showed the highest specific maximum growth rate on wheat grain, better than the parental strains and the hybrids.

Regarding with the lag phase (λ), for the Logistic model with the Duncan test, 6 groups were formed, in the group "a" with the lowest values of λ (2.94-3.71 day) was found the parental strain PD and the reconstituted strain PD_1xPD_4. In group "b" with a value of λ of 4.34 days was found the hybrid PO_2xLC_2. In the "c" found the parental strain of *Pleurotus ostreatus* PO and the reconstituted of *Pleurotus ostreatus* PO_1xPO_2 with values of λ (4.66-4.68 day), the group "d" was formed by the hybrid strains PO_5xLC_2 and PD_4xLC_3 with values of λ (5-5.35 day), in the group "e" with a value of λ of 7.33 day was found the parental of *Lentinula* LC and in the group "f" was formed by the parental strain of *Lentinula* L21 and the reconstituted of *Lentinula* LC_2xLC_2. According to the Duncan test using the Linear model, 2 groups were formed, in the group "a" with the lowest values of λ (0.41-1.39 day) were found the parental strain of *Pleurotus* (PO and PD) and *Lentinula* L21, hybrids PO_2xLC_2, PO_5xLC_2 and PD_4xLC_3, and the reconstituted strains PO_1xPO_2, PD_1xPD_4 and LC_2xLC_2. In the group "b" with value of λ of 1.35 day was found the parental strain of *Lentinula* LC.

For the Exponential model with the Duncan test, 3 groups were formed, in the group "a" with the lowest values of λ (0.07 day) were found the hybrid strain PO_2xLC_2 and the reconstituted of *Pleurotus djamor* PD_1xPD_4. In the group "b" with values of λ (0.08 day) were found the parental strain of *Pleurotus djamor* PD, the hybrid PO_5xLC_2 and the reconstituted of *Pleurotus ostreatus* PO_1xPO_2. In the group "c" was formed by the parental strain of *Pleurotus ostreatus* PO and the hybrid PD_4xLC_3 with values of λ (0.09 day) and in the group "d" with values of λ (0.14 day) were found the parental strains of *Lentinula* L21 and LC.

According to the Duncan test using the Baranyi model, 4 groups were formed, in the group "a" with the lowest value of λ (0.06 day) was found the reconstituted strain of *Pleurotus djamor* PD_1xPD_4. In the group "b" was formed by the parental strains of *Pleurotus* (PO and PD), the hybrids PO_2xLC_2 and PO_5xLC_2, and the reconstituted of *Pleurotus ostreatus* PO_1xPO_2 with values of λ (0.07-0.08 day), whereas the group "c" was found the parental strain of *Lentinula* LC and the hybrid PD_4xLC_3 with values of λ (0.09-0.13 day). Group "d" was formed by the parental strain of *Lentinula* L21 and the reconstituted of *Lentinula* LC_2xLC_2 with values of λ (0.14 day). These results are similar to other authors using different mathematical models, Yogendrarajah *et al*. (2016) used the Linear model calculated values of λ between 0.62 and 22.04 day for 3 strains of *Aspergillus flavus* grown in black peppercorns with different concentrations of aw (0.826-0.984).

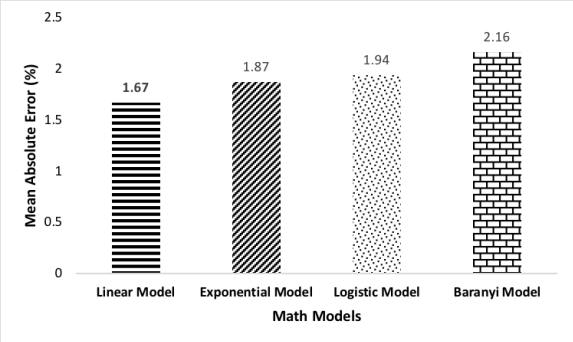

Fig. 1. Values of MAE obtained of the kinetics mycelial parameters of parental, hybrid and reconstituted strains grown on MEA. * Smaller values of the Mean Absolute Error (MAE) indicate better fit of the model.

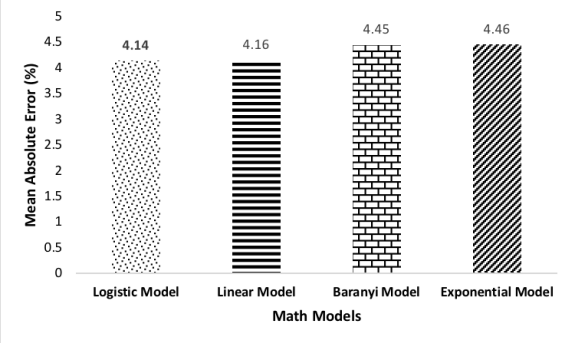

Fig. 2. Values of MAE calculated of the kinetics mycelial parameters of parental, hybrid and reconstituted strains grown on wheat grain. * Smaller values of the Mean Absolute Error (MAE) indicate better fit of the model.

3.4 Statistical fit of the mathematical models

In order to determine the mathematical model that presented the highest statistical precision in the kinetic mycelial growth on MEA and wheat grain the Mean Absolute Error (MAE) was calculated. Figure 1 shows the values of MAE for the 4 models that were used to make the fit of the diameter data of the mycelial growth for the parental, reconstituted and hybrid strains on MEA. It was observed that the model with the better fit was the Linear model with the lowest value of mean absolute error (MAE) of 1.67%, while the model with the worst first was the Baranyi model with the highest value of MAE (2.16%). On the other hand, Figure 2 presents the values of MAE for the 4 predictive models used in the determination of mycelial kinetics of the parental, reconstituted and hybrid strains on wheat grain, the model that showed the better fit was the Logistics model with the lowest value of (MAE) (4.14%), whereas the model that presented the worst fit was the Exponential model with the highest MAE value (4.46%).

Conclusions

The results obtained of the mathematical models showed that the hybrid strain PO_5xLC_2 presented the higher specific growth rate (μ_{max}) and lower lag phase (λ) on MEA in comparison with the others strains include its parental strains. The reconstituted strain of *Pleurotus djamor* PD_1xPD_4 presented the higher growth kinetics (highest growth speed and lowest lag phase) on wheat grain even than the parental strain of *Pleurotus djamor*.

Acknowledgements

We would like to thank to UESS Project, IPN-SIP Project: 20170419 and 20180961, CONACYT Project: CB-2016- 611914.

References

Álvarez-Cervantes, J., Sánchez, C., Díaz, R. and Díaz-Godínez, G. (2016). Characterization of production of laccases, cellulases and xylanases of Pleurotus ostreaus grown on solid-state fermentation using an inert support. *Revista Mexicana de Ingeniería Química 15*, 323-331.

Avin, F.A., Bhassu, S., Shin, T.Y. and Sabaratnam, V. (2012). Molecular classification and phylogenetic relationships of selected edible *Basidiomycetes* species. *Molecular Biology Reports 39*, 7355-7364.

Baumer, J.D., Mas Diego, J.M., Pacheco, S.M.V., Morgado, A.F.M. and Furigo, A.F. (2008). Comparative study of mycelial growth and production of cinnabarin by different strains of *Pycnoporus sanguineus. BIOFAR 2*, 1-5.

Baty, F. and Delignette-Muller, M.L. (2004). Estimating the bacterial lag time: which model, which precision? *International Journal of Food Microbiology 91*, 261-277.

Castro, M.R., Souza, P.J. and Eira, A.F. (2006) Digital monitoring of mycelium growth kinetics and vigor of Shiitake (*Lentinula edodes* Berk. Pegler) on agar medium. *Brazilian Journal of Microbiology 37*, 90-95.

Clark, T.A. and Anderson, J.B. (2004). Dikaryons of the basidiomycete fungus *Schizophylum commune*: evolution in long term culture. *Genetics 167*, 1663-1675.

Chatterjee, T., Chatterjee, B.K., Majumdar, D. and Chakrabarti, P. (2015). Antibacterial effect of silver nanoparticles and the modeling of bacterial growth kinetics using a modified Gompertz model. *Biochimica et Biophysica Acta (BBA)-General Subjects 1850*, 299-306.

Eger, G., Eden, G. and Wissi, E. (1976). *Pleurotus ostreatus*, breeding potential of a new cultivated mushroom. *Theoretical and Applied Genetics 47*, 155-163.

García-Reyes, M., Beltrán-Hernández, R.I., Vázquez-Rodríguez, G.A., Coronel-Olivares, C., Medina-Moreno, S.A., Júarez-Santillán, L.F. and Lucho-Constantino, C.A. (2017). Formation, morphology and biotechnological applications of filamentous fungal pellets: A review. *Revista Mexicana de Ingeniería Química 16*, 703-720.

Guadarrama-Mendoza, P.C. (2013). *Productividad y caracterización de cepas parentales e híbridas provenientes de neohaplontes compatibles de Pleurotus spp. de la región mixteca*. Tesis de Doctorado en Ciencias en Bioprocesos, Instituto Politécnico Nacional, México.

Guadarrama-Mendoza, P.C., Valencia del Toro, G., Ramírez-Carrillo, R., Robles-Martinez, F., Yáñez-Fernández, J., Garín-Aguilar, M.E. and Bravo-Villa, G. (2014). Morphology and mycelial growth rate of *Pleurotus* spp. strains from the Mexican mixtec region. *Brazilian Journal of Microbiology 45*, 861-872.

Gibson, A.M., Bratchell, N. and Roberts, T.A. (1987). The effect of sodium chloride and temperature on the rate and extent of growth of *Clostridium botulinum* type A in pasteurized pork slurry. *Journal of Applied Bacteriology 62*, 479-490.

Gil, M.M., Miller, F.A., Brandao, T.R. and Silva, C.L. (2011). On the use of the Gompertz model to predict microbial thermal inactivation under isothermal and non-isothermal conditions. *Food Engineering Reviews 3*, 17-25.

Hills, B.P. and Wright, K.W. (1994). A new model for bacterial growth in heterogeneous systems. *Journal of Theoretical Biology 168*, 31-41.

Iossi, M., Valenzuela Cobos, J.D., Gea, F.J., and Zied, D. (2018). *Pleurotus* spp. cultivation on Brachiaria sp. straw treatment with alkaline water. *Brazilian Journal of Microbiology 49*, 64-67.

Kuss, K., Alano, C.G., Arbigaus, A., Rampinelli, J.R., Silveira, M.L.L., Bonatti-Chaves, M. and Furlan, S.A. (2018). Study of the removal of bisphenol-a by *Pleurotus sajor-caju* crude enzyme broth. *Revista Mexicana de Ingeniería Química 17*, 989-998.

Leal-Lara, H. and Eger-Hummel, G. (1982). A monokaryotization method its used for genetic studies in wood-rooting basidiomycetes. *Theoretical and Applied Genetics 61*, 65-68.

Liu, H., Chen, N., Feng, C., Tong, S. and Li, R. (2017). Impact of electrostimulation on denitrifying bacterial growth and analysis of bacterial growth kinetics using a modified Gompertz model in a bio-electrochemical denitrification reactor. *Bioresource Technology 232*, 344-353.

Manzi, P., Gambelli, L., Marconi, S., Vivanti, V. and Pizzoferrato, L. (1999). Nutrients in edible mushrooms: An inter-species comparative study. *Food Chemistry 65*, 477-482.

Marín, S., Cuevas, D., Ramos, A.J. and Sanchis, V. (2008). Fitting of colony diameter and ergosterol as indicators of food borne mould growth to known growth models in solid medium. *International Journal of Food Microbiology 121*, 139-149.

McDonald, K. and Sun, D.W. (1999). Predictive food microbiology for the meat industry: A review. *International Journal of Food Microbiology 52*, 1-27.

Reis, F.S., Barros, L., Martins, A. and Ferreira, I. (2012). Chemical composition and nutritional

value of the most widely appreciated cultivated mushrooms: An inter-species comparative study. *Food and Chemistry Toxicology 50*, 191-197.

Royse, D. J., Baars, J. and Tan, Q. (2017). Current Overview of Mushroom Production in the World. In: *Edible and Medicinal Mushrooms: Technology and Applications* (D. Cunha Zied, & A. Pardo-Giménez, eds.). Wiley, Chichester.

Sánchez Hernández, A., Valenzuela Cobos, J.D., Herrera Martínez, J., Villanueva Arce, R., Gómez y Gómez, Y.M., Zarate Segura, P.B., Garín Aguilar, M.E., Leal Lara, H. and Valencia del Toro, G. (2019). Characterization of *Pleurotus djamor* neohaplonts recovered by production of protoplasts and chemical dedikaryotization. *3 Biotech 9*, 24.

Singh, M. and Kamal, S. (2017). Genetic Aspects and Strategies for Obtaining Hybrids. In: *Edible and Medicinal Mushrooms: Technology and Applications* (D. Cunha Zied, & A. Pardo-Giménez, eds.). Wiley, Chichester.

Sobal, M., Martínez-Carrera, D., Morales, P. and Roussos, S. (2007). Classical characterization of mushroom genetic resources from temperate and tropical regions of Mexico. *Micología Aplicada Internacional 19*, 15-23.

Valencia del Toro, G., Ramírez-Ortiz, M. E., Flores-Ramírez, G., Costa-Manzano, M. R., Robles-Martínez, F., Garín Aguilar, M.E. and Leal-Lara, H. (2018). Effect of *Yucca schidigera* bagasse as substrate for Oyster mushroom on cultivation parameters and fruit body quality. *Revista Mexicana de Ingeniería Química 17*, 835-846.

Valenzuela-Cobos, J.D., Durán Páramo, E., Villanueva Arce, R., Sánchez Hernández, A., Garín Aguilar, M. E., Leal Lara, H. and Valencia del Toro, G. (2017). Production of hybrid strains among Pleutorus and *Lentinula* and evaluation of their mycelial growth kinetics on malt extract agar and wheat grain using the Gompertz and Hill models. *Emirates Journal of Food and Agriculture 29*, 927-935.

Valenzuela-Cobos, J.D., Vásquez-Véliz, G.K., Zied, D.C., Franco-Hernández, O.M., Sánchez Hernández, A., Garín Aguilar, M.E., Leal Lara, H. and Valencia del Toro, G. (2019). Bioconversion of agricultural wastes using parental, hybrid and reconstituted strains of *Pleurotus* and *Lentinula*. *Revista Mexicana de Ingeniería Química 18*, 647-657.

Vásquez-Véliz, G., Vargas-Farías, C., Garín-Aguilar, M., Valencia del Toro, G., Jara-Bastidas, L. and Valenzuela-Cobos, J. (2018). Ethanol production using lignocellulosic wastes. *Italian Journal of Food Sciences 31*, 198-203.

Yogendrarajah, P., Vermeulen, A., Jacxsens, L., Mavromichali, E., De Saeger, S., De Meulenaer, B. and Devlieghere, F. (2016). Mycotoxin production and predictive modelling kinetics on the growth of *Aspergillus flavus* and *Aspergillus parasiticus* isolates in whole black peppercorns (*Piper nigrum* L). *International Journal of Food Microbiology 228*, 44-57.

Zengin, G., Sarikurkcu, C., Aktumsek, A., Uysal, S., Ceylan, R., Anwar, F. and Solak, H. (2015). A comparative fatty acid compositional analysis of different wild species of mushrooms from Turkey. *Emirates Journal of Food and Agriculture 27*, 532-536.

Zwietering, M.H., Jongenburger, I., Rombouts, F.M. and Van'triet, K. (1990). Modeling of the bacterial growth curve. *Applied Microbiology and Biotechnology 56*, 1875-1881.