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REGULAR ARTICLE

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

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ABSTRACT

Three inter-generic hybrid strains were obtained by pairing compatible neohaplonts recovered by dedikaryotization of four parental strains, i.e. *Pleurotus ostreatus* (CC060), *Pleurotus djamor* (CC051) and *Lentinula edodes* (CC003 and CC004). Fifteen neohaplonts were recovered employing homogenization time periods for *Pleurotus* spp. since 60 to 90 s, and for *Lentinula edodes* strains time periods ranged from 5 to 20 s, and incubation at 28 °C in a peptone-glucose solution (PGS). The mycelial growth of the parental and hybrid strains was determined on malt extract agar (MEA) and wheat grain by calculating the parameters A, B and C of nonlinear regression models. For the parameter A, the mycelial growth on MEA showed values ranged from 1.08 to 1.28, and for the parameter B since 5.65 to 20.85. On the other hand, the A values by mycelium growth on wheat grain ranged from 1.57 to 16.13, for B since 28.36 to 86.53 and for C were in an interval from 11.89 to 44.24. Parental and hybrid strains presented instantaneous velocity values on MEA since 2.88 to 7.26 $cm^2 \bullet day^{-1}$, whereas on wheat grain were in an interval from 5.45 to 10.05 $cm^3 \bullet day^{-1}$. Furthemore, the μ_{max} values on MEA and wheat grain were calculated by using the Gompertz model and the λ values on both medium were estimated by using the Hill model, the μ_{max} values on MEA ranged from 0.26 to 1.69 day^{-1} and λ values in an interval from 0.41 to 2.74, whereas the μ_{max} values on wheat grain ranged from 0.05 to 0.43 day^{-1} and λ since 1.82 to 28 h. Moreover, the estimated equations based on nonlinear models were used to calculate the μ_{max} values on MEA and wheat grain of the strains and the λ values were obtained by using the μ_{max} values of the proposed equations on the Hill model, the μ_{max} values on MEA ranged from 0.44 to 1.27 day^{-1} andthe λ values between 0.57 to 1.72 h, while the μ_{max} values on wheat grain ranged from 0.04 to 0.64 day^{-1} and λ values in an interval from 1.26 to 20.11 h. The results evidenced that the hybrid strains of *PleurotusxLentinula* presented highest rate of growth in comparison with the parental, encouraging the production of hybrid strain and their use in the industrial field.

Keywords: Dedikaryotization; Mycelium growth; Mushroom; Neohaplonts; Strains

INTRODUCTION

The production of new edible mushroom strains is restricted due to incompatibility barrier, in some cases hybrid strains are produced through mating compatible neohaplonts recovered by chemical dedikaryotization. This process allows the recovery of the two monokaryotic components from a dikaryon (Leal-Lara and Eger-Hummel, 1982) by using toxic substances such as sodium taurocholate, colic acid, peptone or glucose (Miles and Raper, 1956).

The mushrooms with more production in the world are: 1) *Agaricus* (30%); 2) *Pleurotus* spp. (27%); *Lentinula edodes*

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(17%); *Auricularia* (6%) and *Flamulina* (5%) (Royse, 2014). In Mexico the mushrooms with highest production and demand are *Agaricus bisporus*, *Pleurotus* spp. and *Lentinula edodes* (Ramírez-Carrillo, 2011); in 2011 the annual production of *Pleurotus* spp. was 3000 tons and of *Lentinula edodes* was 25 tons; both species represented the 4.90 % of total edible fungi production (Martínez-Carrera et al., 2012).

Pleurotus spp. and *Lentinula edodes* are characterized for their high nutritional value (Manzi et al., 2001; Reis et al., 2012; Zengin et al., 2015) and can be taken as an important source of proteins, vitamins and minerals (Manzi et al., 1999). However, in mushrooms the different species needs different conditions to grow and produce fruit body; *Pleurotus* spp. requires tropical and subtropical climates (Mori et al., 1974; Fultz, 1988; Kashangura et al., 2006), while *Lentinula edodes* needs long incubation times, specific substrates to produce fruits body and low temperature (Imbernon et al., 1983; Gaitán-Hernández et al., 2006; Sánchez-Hernández et al., 2014; Sharma et al., 2015).

The presented study aimed to obtain new strains of the genera *Pleurotus*x*Lentinula*; and evaluate the mycelium growth kinetics on malt extract agar and wheat grain in comparison with the parental strains.

MATERIALS AND METHODS

Biological material

In this research was used the following mushroom strains: two parental strains i.e. *Pleurotus ostreatus* (CC060) and *Pleurotus djamor* (CC051); two parental strains of *Lentinula edodes* CC003 (supplied from Faculty of Chemistry, UNAM) and CC004 (commercial strain); three hybrid strains PO_2xLC_2, PO_5xLC_2, PD_4xLC_3 obtained by mating compatible neohaplonts recovered by dedikaryotization of parental strains. Stocks of all strains are deposited at the fungal collection of the Cellular Cultures of the Biotechnology Interdisciplinary Professional Unit (UPIBI-IPN).

Culture media

The malt extract agar (MEA) was prepared by dissolving 18 g of malt extract and 15 g of bacteriological agar in 1 L of distilled water using an Erlenmeyer flask. The flask was sterilized in autoclave at 15 psi (121 °C) for 15 min, subsequent, 10 mL of sterile medium were poured into sterile Petri dishes. The dishes with the medium solidified were wrapped in plastic bags and incubated at 28 °C for 24 h to check the sterility. Then, the Petri dishes without contamination were used for propagation of mycelium and storage of strains (Eger et al., 1976).

Dedikaryotization solution (Peptone-Glucose Solution PGS)

The dedikaryotization solution was prepared by dissolving in 1 L of distilled water with 20% of anhydrous glucose and 20% of peptone P (Oxoid LP0037). Thereafter, 50 mL were poured into glass jars and sterilized at 15 psi (121 °C) for 15 min. Then, the glass jars with the dedikaryotization solution were incubated at 28 °C for 24 h to check the sterility (Leal-Lara and Eger-Hummel, 1982).

Recovery of neohaplonts by dedikaryotization

The mycelium in the Petri dish were divided in fourth parts and were put in a Blender (Model N.4237, Mark: Marnie) and were homogenized with 50 mL of sterile water. Different periods of time were used depending of the genera, by *Pleurotus* spp. strains were employed blending times of 60 and 90 s, and for *Lentinula edodes* strains were blended with times of 5 and 20 s. Then, the jars contained 50 mL of PGS were inoculated with 50 and 100 µL of the homogenized inoculum, and incubated at 28 °C until mycelium growth was noticeable. Thereafter, the liquid was homogenized with 50 mL of sterile distilled water for 20 s to both strain types. Subsequently MEA plates were inoculated with 15 and 20 µL of the homogenized and incubated at 28 °C until colonies were formed. After that, the Petri dishes were observed under the microscope 10(x) to identify the micelyum without clamp connections (neohaplonts). Finally the monokaryotic components were cultivated individually on Petri dishes with 10 mL of MEA to verify under the microscope 10(x) the absence of clamp connections (Guadarrama-Mendoza et al., 2014).

Identification of two types of neohaplonts (compatibility types)

To identify the two types of neohaplonts for each parental strain, was necessary paired all the monokaryotic components of the same strain among them on MEA dishes. Subsequently the Petri dishes were incubated at 28 °C and inspected under the microscope 10(x) every day to determine the mycelium with presence of clamp connections. Then the dikaryon was cultivated on MEA plates to verify presence of clamp connections (Valencia del Toro and Leal-Lara, 1999; Valencia del Toro and Leal-Lara, 2002).

Production of hybrid strains of *Pleurotus*x*Lentinula* by pairing compatible neohaplonts

The neohaplonts obtained from the *Pleurotus* spp. were paired with the monokaryotic components of *Lentinula edodes* on Petri dishes using 10 mL of MEA in all possible combinations. Then, the plates were incubated at 28 °C and inspected under the microscope 10(x) each 24 h to determine the strain with presence of clamp connections. Finally to check presence of clamp connections the new strain was cultivated on MEA plates (Valencia del Toro and Leal-Lara, 2002).

Mycelial growth kinetics on MEA with no linear model

The mycelial growth rate on Petri dish with MEA was estimated by the diameter of the colony, and the growth velocity was determinate used the following nonlinear regression model (Regina, 2001):

$f'(x) = B*A^{x}*\ln(A)$, where:

A, B = parameters of the model
x= days
f'(x)= growth velocity

Based on this math model was predictable the next equation to determinate the maximum growth (μ_{max}) on MEA and the lag time (λ) was calculated by using the μ_{max} value on the Hill model.

$\mu_{max} = M/(A*B)$, where: M= day with maximum growth rate

$\lambda = [\ln(1+(\mu_{max}/v)]/\mu_{max}$, where: $v= \mu_{max}$

Mycelial growth kinetics on grain wheat with no linear model

Mycelial growth was evaluated daily by measurement the diameter of the colony when filled the bag with wheat grain, and the velocity was calculated by using with the next nonlinear regression model (Regina, 2001):

$f'(x) = -[(2A+C)*B]/(x+A)*(-A-C+x)$, where:

A, B, C= parameters of the model
x= days
f'(x)= growth velocity

To determinate the μ_{max} values of the strains on wheat grain based on this nonlinear model was proposed the following formula and the lag phase (λ) was calculated by using the μ_{max} value on the Hill model.

$\mu_{max} = M/(A*C)$, where: M= day with maximum growth rate

$\lambda = [\ln(1+(\mu_{max}/v)]/\mu_{max}$, where: $v= \mu_{max}$

Mycelial growth kinetics with Gompertz model

The modified Gompertz model consists of three phases: lag, exponential, and stationary phases and was used to determinate the kinetics of mycelium growth on MEA and wheat grain (Gibson et al., 1987; Liu et al., 2017):

$\log N = A+C*\exp\{-\exp[-B(t-M)]\}$, where:

A, B, C= parameters of the model
t= days of mycelial colonization
M=day with maximum growth rate
log N= growth kinetics

By using the parameters of the model was calculated the maximum growth (μ_{max}) and the lag time (λ) (Gibson et al., 1987; Hills and Wright, 1994):

$\mu_{max} = (B*C)/e$, where: e= The Euler constant

$\lambda = [\ln(1+(\mu_{max}/v)]/\mu_{max}$, where: $v= \mu_{max}$

Statistical analysis

The results were examined using one-way analysis of variance (ANOVA) to determine the significance of individual differences at $p<0.05$ level, of A, B or C parameters of the models and for comparing the velocity growth in different days of growth, the maximum growth specific speed (μ_{max}) and the lag phase (λ), when statistical differences were found, the Duncan Test with $\alpha = 0.05$ was applied. Also the analysis of variance of measure repeated was used to compare the behavior of each strain in the time. The χ^2 test was applied to determinate the symmetry in the recovery of neohaplonts of parental strain. The analyses were carried out using Statgraphic ver. 16 statistical software.

RESULTS AND DISCUSSION

Chemical dedikaryotization

Parental strains were dedikariotized and fifteen monokaryotic components were recovered by using different conditions such as: time of blending between 60 to 90 s for *Pleurotus* and for *Lentinula edodes* times of blending ranged from 5 to 20 s, and volume of inoculation in in the dedikaryotization solution between 50 to 100 µL and volume of inoculation on MEA since 15 to 20 µL (Table 1).

Valencia del Toro and Leal-Lara (1999) used homogenization times 150 s and incubation time from 48 to 120 h in the dedikaryotization solution to recover 32 monokaryotic components of three *Pleurotus* spp., whereas Guadarrama-Mendoza et al. (2014) proved blending times among 40 and 70 s and incubation time of 72 h in peptone-glucose solution (PGS) to recover 15 neohaplonts of two *Pleurotus* spp.

On the other hand, Ramírez-Carrillo and Leal-Lara (2002) used homogenization time from 5 to 150 s and incubation time of 72 h in the dedikaryotization solution (PGS) to recover 23 monokaryons of seven *Lentinula edodes* strains.

The authors presented symmetrical recovery of neohaplonts, include the CC003 strain studied by Ramírez-Carrillo and Leal-Lara (2002) but needed volume of inoculum of 50 µL in petri dishes with MEA.

The reduced number of neohaplonts obtained and the symmetric recover is directly related with the mechanic

Table 1: Conditions to recover monokaryotic components of the parental strains by chemical dedikaryotization process

Strains	Time of blending (s)	Volume of inoculum (μL)		Neohaplonts			χ^2 test for symmetric recovery (nh1:nh2=1:1)*
		Dedikaryotization solution	Malt extract agar	Total	Type nh1	Type nh2	
CC060	60	50	15	1	3	2	0.20 (0.66)
	60	50	20	1			
	60	100	20	2			
	90	50	20	1			
CC051	60	50	15	1	4	1	1.8 (0.18)
	60	50	20	1			
	90	50	15	1			
	90	100	15	2			
CC003	5	50	20	2	2	0	-
CC004	5	50	15	1	2	1	0.33 (0.56)
	20	50	15	2			

*Test reference value χ^2 ($p < 0.05$). Smaller χ^2 values indicate no significant differences

sensitivity of the strains, volume of inoculum, blending times and incubation time used (Kawasumi et al., 1987; Ramírez-Carrillo and Leal-Lara, 2002) in comparison with the authors. Moreover, the neohaplonts presented symmetrical recovery to difference of the CC003 strain that only was possible to get one type, because were used volume of inoculum between 15 - 20 μL in petri dishes with MEA.

Formation of inter-generic strains

The neohaplonts recovered from the four parental strains formed 3 hybrid strains by mating of 10 *Pleurotus* neohaplonts with 5 monokaryons of *Lentinula edodes*. Table 2 shows the pairing of neohaplonts to formation of inter-generic hybrid. Some authors have published about formation of inter-generic hybrid with consistent results, Ramírez-Carrillo et al. (2011) reported the formation of 11 hybrid inter-generic strains through pairing 11 neohaplonts of *Pleurotus eryngii* with 6 monokaryons of *Lentinula edodes*, whereas Sánchez-Hernández et al. (2014) produced 12 inter-generic hybrid by mating 6 neohaplonts of *Lentinula edodes* with 2 monokaryotic components of *Pleurotus ostreatus*.

Production protoplast is other way to the formation of inter-generic strains, Chakraborty and Sikdar (2008) presented the formation of 12 somatic hybrids between *Volvariella volvacea* and *Pleurotus florida* through inter-generic protoplast fusion, while Mallik and Sikdar (2014) presented 9 inter-generic strains produced of through protoplast fusion between *Pleurotus florida* and *Lentinula edodes*.

To pair monokaryons of different strains is necessary break down the compatibility barrier of the species; only in that moment will be possible obtain hybrid stains with characteristic of the parental strains (Eichlerová and Homolka, 1999; Eichlerová et al., 2000).

Table 2: Formation of hybrid strains by mating compatible neohaplonts inter-generic

Formation of hybrid strains by mating compatible neohaplonts inter-generic						
	Strains	CC003		CC004		
Strains	Neohaplonts	1	2	1	2	3
CC060	1	-	-	-	-	-
2	-	-	-	+	-	
3	-	-	-	-	-	
4	-	-	-	-	-	
5	-	-	-	+	-	
CC051	1	-	-	-	-	-
2	-	-	-	-	-	
3	-	-	-	-	-	
4	-	-	-	-	+	
5	-	-	-	-	-	
+	Positive pairing=dikaryon					
-	Negative pairing=incompatibility neohaplonts					

Mycelial growth speed of parental and hybrid strains on MEA and wheat grain

The instantaneous velocities of parental and hybrid strains on MEA were compared showing speed values ranged from 3.44 to 6.45$cm^2 \bullet day^{-1}$ for parental strains, whereas the hybrid strains presented velocity values since 2.88 to 7.26 $cm^2 \bullet day^{-1}$ on the 9th day. Similarly results have been presented by Regina (2001), this author evaluated growth rate for two strains of *Lentinula edodes* in different culture media showing instantaneous velocity values since 3.69 to 10.70 $cm^2 \bullet day^{-1}$ on the 10th day, while Castro et al. (2006) introduced growth rate for four strains of *Lentinula edodes* under different agar medium compositions showing instantaneous speed values since 6.0 to 8.5 $cm^2 \bullet day^{-1}$ on the 9th day. The different values of instantaneous velocity with these authors depend of the different culture media used to the mycelium growth.

Figure 1 shows the increasing growth speed on MEA, due to the rich content of nutrients in the culture media, Straatsma et al. (1991) indicates that nutritive medium

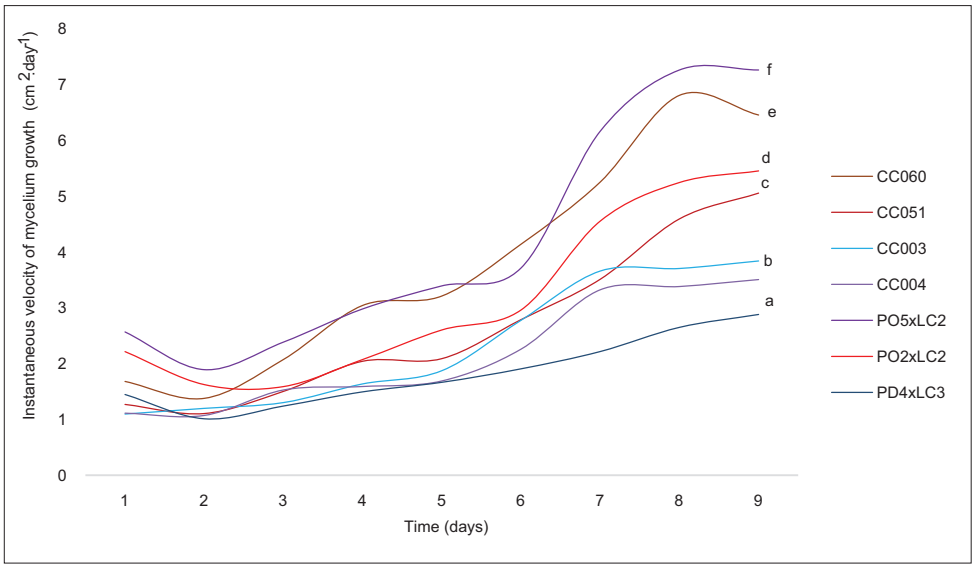

Fig 1. Mycelium growth curves of the parental and hybrid strains on MEA. Different letters indicated significant difference among of the instantaneous velocity values of the dikaryotic strains at level $p < 0.05$, according to Duncan test, n=10.

promotes the vigor of the mycelium. According to the Duncan test the hybrid strain PO_5xLC_2 presented the highest instantaneous speed 7.26 $cm^2 \cdot day^{-1}$ on MEA even better than the parent strains (CC060 and CC004), the first one showed the second one highest speed 6.45 $cm^2 \cdot day^{-1}$, while the second presented a low speed 3.44 $cm^2 \cdot day^{-1}$, these results suggest that the hybrid showed a metabolic capacity and growth rate closer to *Pleurotus* spp.

The mycelial growth rate on MEA was determined according to the nonlinear model, which allows determinate the parameters A and B from the growth velocity of the strains on area ($cm^2 \cdot day^{-1}$). The A values for the parental and hybrid strains ranged from 1.08 to 1.28 and B values in an interval from 5.65 to 20.85. These results are similar to some authors, Regina (2001) measured the mycelial growth for two strains of *Lentinula edodes* in different culture media showing A values since 1.09 to 1.29 and B values ranged from 3.58 to 38.04.

A is the parameter that indicates the maximum potential of the growth rate, while B is the biological variable that related the growth speed and the incubation time (Fekedulegn et al., 1999). Table 3 shows that hybrid PO_5xLC_2 presents the highest A values in comparison with the other strains, and this confirm the previous results that this hybrid submitted the highest speed.

Instantaneous velocities of dikaryotic strains on wheat grain were compared showing instantaneous velocity values since 5.45 to 7.53 $cm^3 \cdot day^{-1}$ for parental strains, whereas the hybrid strains presented speed values were in an interval from 8.70 to 10.05 $cm^3 \cdot day^{-1}$ on the 9th day. Regina (2001) reported growth rate for two strains of *Lentinula edodes* in different substrates showing instantaneous speed values since 0.24 to 0.32 $cm^3 \cdot day^{-1}$ on the 10th day.

Figure 2 shows the decreasing growth speed on wheat grain, which is probably induced by the low aeration of the substrate (Leatham and Stahmann, 1987; Edwards, 1993) indicated that the low gas exchange is an inhibitory factor and causes that big part of ATP is used in other processes different to the mycelium growth. Multiple range test indicated that the hybrid strain PO_2xLC_2 presented the highest instantaneous speed 10.05 $cm^3 \cdot day^{-1}$ on wheat grain even than the parental (CC060 and CC004),

The first strain showed a speed 6.37 $cm^3 \cdot day^{-1}$, while the second presented the lowest speed 5.62 $cm^3 \cdot day^{-1}$, so this is possible to infer that PO_2xLC_2 can absorb of a better way the nutrients of the medium and growth rate closer to *Pleurotus* spp.

The determination of mycelial growth rate on wheat grain was calculated by using a nonlinear regression model, that permits determinate the parameters A, B and C from the mycelial growth speed on volume ($cm^3 \cdot day^{-1}$). The A values estimated ranged from 1.57 to 16.13, for B since 28.36 to 86.53 and for C values in an interval from 11.89 to 44.24. Regina (2001) calculated the mycelial speed on different substrates for two strains of *Lentinula edodes* reporting A values since 0.84 to 1.20, B values ranged from 0.96 to 1.40 and for C values in an interval from 18.1 to 27.9.

B is the variable that indicate the maximum growth rate of the strain in correlation with A, whereas C is the biological constant that correlated the incubation time and the instantaneous velocity (Fekedulegn et al., 1999). Table 4

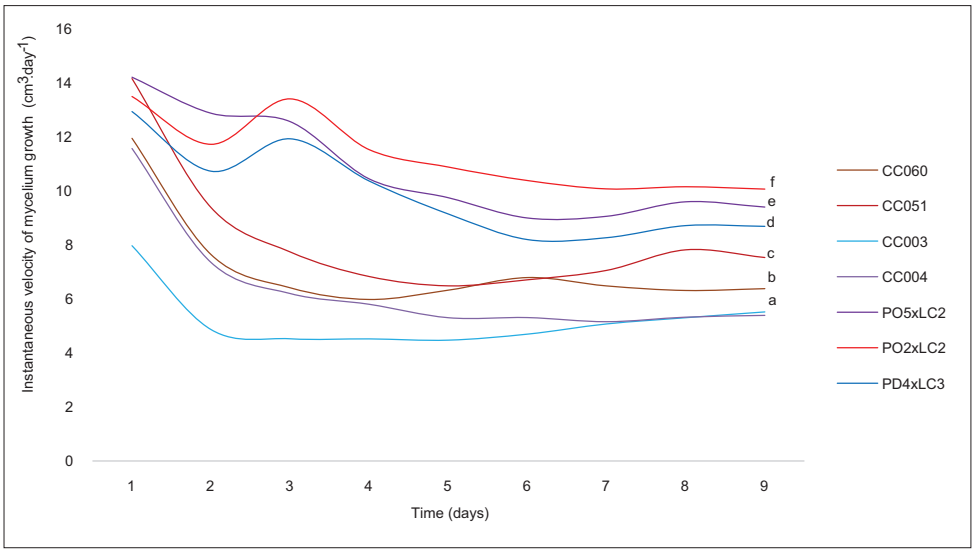

Fig 2. Mycelium growth curves of the parental and hybrid strains on wheat grain. Different letters indicated significant difference among of the instantaneous speed values of the dikaryotic strains at level p < 0.05, according to Duncan test, n=10.

Table 3: Comparation of the parameters A and B used in the determination of mycelial growth on MEA of parental and hybrid strains

Strains	A*	B*
CC060	1.21±0.03^{e}	7.39±4.46^{a}
CC051	1.21±0.04^{e}	7.15±2.41^{a}
CC003	1.11±0.09^{b}	19.59±6.38^{c}
CC004	1.08±0.01^{a}	20.85±6.32^{c}
PO_2xLC_2	1.13±0.01^{c}	12.68±2.37^{b}
PO_5xLC_2	1.28±0.16^{f}	5.89±1.49^{a}
PD_4xLC_3	1.17±0.11^{d}	12.65±1.59^{b}

*Different letters in each column indicated significant difference among the parameters values at level P < 0.05, according to Duncan test, n = 10

Table 4: Comparation of the parameters A, B and C used in the determination of mycelial growth on wheat grain for the parental and hybrid strains

Strains	A*	B*	C*
CC060	3.38±2.20^{b}	33.93±5.59^{a}	15.85±1.47^{a}
CC051	1.57±0.74^{a}	28.36±2.76^{a}	14.21±0.68^{a}
CC003	5.23±2.08^{c}	48.93±11.10^{b}	31.31±2.90^{c}
CC004	16.13±4.49^{e}	86.14±18.60^{d}	44.24±9.02^{d}
PO_2xLC_2	7.70±2.67^{d}	86.53±23.27^{e}	20.62±6.82^{b}
PO_5xLC_2	4.62±1.57^{c}	65.13±10.72^{c}	11.89±1.65^{a}
PD_4xLC_3	7.82±2.21^{d}	85.83±16.92^{d}	27.81±6.18^{c}

*Different letters in each column indicated significant difference among the parameters values at level P < 0.05, according to Duncan test, n = 10

presents a statistical relation between the parameters A and B, so confirm the results obtained that the hybrid PO_2xLC_2 submitted the highest instantaneous velocity.

Mycelial growth kinetics of dikaryotic strains on MEA and wheat grain

The Gompertz and Hill models describe the best growth tendencies both in terms of statistical accuracy and simplicity (McDonald and Sun, 1999) and their parameters A, B and C were 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.

Dikaryotic strains showed μ_{max} values on MEA by using the estimated equation based on the nonlinear regression model ranged from 0.44 to 1.27 day^{-1} and with the modified Gompertz model since 0.26 to 1.69 day^{-1}. On the other hand, the strains showed μ_{max} values on wheat grain with the proposed equation estimated by the nonlinear model between 0.04 to 0.64 day^{-1} and with the Gompertz model ranged from 0.05 to 0.43 day^{-1} (Table 5). López-Peña et al. (2013) obtained μmax values of 0.05 and 0.13 day^{-1} for mycelial growth kinetics of three *Lentinula edodes* strains on liquid medium supplemented with vine wood extracts. For otherwise, Guillén-Navarro et al. (1998) reported μ_{max} values for growth kinetics of *Pleurotus ostreatus* on agar DLA by calculating the value of the slope of 0.86 day^{-1} on 12th day, also presented μ_{max} value of 0.65 day^{-1} of the same strain on synthetic medium with yeast extract and glucose concentration of 2.5 (g/L). The μ_{max} indicates the better capacity of the strain to absorb the nutrients on both medium (Liu et al., 2017), the hybrid PO_5xLC_2 presented the highest instantaneous velocity values (μ_{max}), therefore this strain can adapt easily on MEA and wheat grain even better than the parental strains.

All the strains presented λ values on MEA by using the Gompertz model since 0.41 to 2.74 hand with the estimated formula based on a nonlinear model ranged from 0.57 to 1.72 day^{-1}. For otherwise, with the proposed equation the dikaryotic strains showed λ values on wheat strain between 1.26 to 20.11 h and with the Gompertz model ranged from 1.82 to 28 h (Table 6). These results

Table 5: Comparation of μ_{max} values of the dikaryotic strains on MEA and wheat grain

Strains	Gompertz Model	Nonlinear Model	Gompertz Model	Nonlinear Model
	μ_{max} (MEA) (day-1)*	μ_{max} (MEA) (day-1)*	μ_{max} (wheat grain) (day-1)*	μ_{max} (wheat grain) (day-1)*
CC060	1.01±0.21^{d}	1.18±0.37^{b}	0.11±0.07^{b}	0.34±0.18^{b}
CC051	0.86±019^{c}	1.12±0.32^{b}	0.22±011^{c}	0.61±0.19^{c}
CC003	0.32±0.05^{a}	0.46±0.17^{a}	0.23±0.20^{c}	0.16±0.07^{a}
CC004	0.26±0.03^{a}	0.44±0.16^{a}	0.05±0.03^{a}	0.04±0.02^{a}
PO_2xLC_2	0.57±0.05^{b}	0.64±0.10^{a}	0.36±0.09^{e}	0.09±0.06^{a}
PO_5xLC_2	1.69±0.23^{e}	1.27±0.32^{b}	0.43±0.13^{f}	0.64±0.28^{c}
PD_4xLC_3	0.46±0.04^{b}	0.61±0.06^{a}	0.31±0.12^{d}	0.07±0.03^{a}

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

Table 6: Comparation of λ values of the dikaryotic strains on MEA and wheat grain

Strains	Gompertz Model	Nonlinear Model	Gompertz Model	Nonlinear Model
	λ (MEA) (h)*	λ (MEA) (h)*	λ (wheat grain) (h)*	λ (wheat grain) (h)*
CC060	0.71±0.16^{b}	0.68±0.37^{a}	13.86±17.19^{b}	2.52±1.32^{a}
CC051	0.84±0.21^{b}	0.66±0.20^{a}	7.17±11.52^{a}	1.27±0.52^{a}
CC003	2.22±0.32^{e}	1.68±0.55^{c}	4.27±1.91^{a}	5.04±2.38^{b}
CC004	2.74±0.58^{f}	1.72±0.51^{c}	28.00±20.55^{c}	20.11±7.36^{d}
PO_2xLC_2	1.22±0.12^{c}	1.10±0.19^{b}	2.04±0.53^{a}	10.37±5.39^{c}
PO_5xLC_2	0.41±0.05^{a}	0.57±0.11^{a}	1.82±0.72^{a}	1.26±0.36^{a}
PD_4xLC_3	1.52±0.16^{d}	1.13±0.11^{b}	2.65±1.17^{a}	10.90±4.38^{c}

Different letters in each column indicated significant difference among the λ values on MEA and wheat grain of the parental and hybrid strains at level $P < 0.05$, according to Duncan test, n = 10

are similar to other authors using different math models, Straatsma et al. (1991) reported λ values for *A. bisporus* since 0.46 to 0.71 h by using logistic functions. The lag phase (λ) indicates the capacity of the strain to adapt to new environmental condition (Chatterjee et al., 2015), in relation with this the hybrid PO_5xLC_2 presented the shorter lag time (λ), hence this strain can get to the exponential phase faster to the other strains even to the parental strain.

CONCLUSIONS

The nonlinear regression models showed that the hybrid PO_5xLC_2 and PO_2xLC_2 presented the highest instantaneous velocity values on MEA and wheat grain, these results are according to the Gompertz and Hill models that confirmed that the hybrid PO_5xLC_2 presented the maximum growth specific speed (μ_{max}) and shorter phase duration (λ) on MEA and wheat grain.

The estimated equation calculated similar μ_{max} values to the indicated by the modified Gompertz model, also can be used the values of the μ_{max} to calculate the λ by using the Hill model.

The results obtained of the mycelial growth rate of the new strains represent a positive predictor to increase the development of hybrid strains to raise the commercial production at industrial level.

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