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MORPHOLOGY AND MYCELIAL GROWTH RATE *OF Pleurotus* spp. STRAINS FROM THE MEXICAN MIXTEC REGION

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ABSTRACT

Two native *Pleurotus* spp. strains (white LB-050 and pale pink LB-051) were isolated from rotten tree trunks of cazahuate (*Ipomoea murucoides*) from the Mexican Mixtec Region. Both strains were chemically dedikaryotized to obtain their symmetrical monokaryotic components (neohaplonts). This was achieved employing homogenization time periods from 60 to 65 s, and 3 day incubation at 28°C in a peptone-glucose solution (PGS). Pairing of compatible neohaplonts resulted in 56 hybrid strains which were classified into the four following hybrid types: ($R_{1-n}xB_{1-n}$, $R_{1-n}xB_{2-1}$, $R_{2-n}xB_{1-n}$ and $R_{2-n}xB_{2-1}$). The mycelial growth of *Pleurotus* spp. monokaryotic and dikaryotic strains showed differences in texture (cottony or floccose), growth (scarce, regular or abundant), density (high, regular or low), and pigmentation (off-white, white or pale pink). To determine the rate and the amount of mycelium growth in malt extract agar at 28°C, the diameter of the colony was measured every 24 hours until the Petri dish was completely colonized. A linear model had the best fit to the mycelial growth kinetics. A direct relationship between mycelial morphology and growth rate was observed. Cottony mycelium presented significantly higher growth rates ($p<0.01$) in comparison with floccose mycelium. Thus, mycelial morphology can be used as criterion to select which pairs must be used for optimizing compatible-mating studies. Hybrids resulting from cottony neohaplonts maintained the characteristically high growth rates of their parental strains with the hybrid $R_{1-n}xB_{1-n}$ being faster than the latter.

Key words: *Pleurotus*, mycelial growth, mycelial morphology, hybrid strains, monokaryotization.

Introduction

Production of edible fungi is a biotechnological industry which is undergoing a continuous worldwide expansion. The four more important species based on their production and demand, are: 1) *Agaricus bisporus* (mushroom), in Europe, North America and Mexico; 2) *Lentinula edodes* (Shiitake) in Japan; 3) *Pleurotus* spp. (seta) in México and South America, and 4) *Volvariella volvacea* in Asian countries (Martínez-Carrera and López-Martínez 2010).

The *Pleurotus* genus (Pleurotaceae, higher Basidiomycetes) includes a diverse group of aromatic edible fungi which have been praised for their culinary and high nutritional value due to the fact that they are rich in protein, fiber, vitamins and minerals (Chang and Miles 2004, Reis *et al.*, 2012). Several *Pleurotus* species constitutes a very valuable protein source, especially in the rural areas of developing countries (Aguilar *et al.*, 2002, Mata and Salmones 2003, Mayet *et al.*, 2004). In addition to their nutritional value, these fungi produce important biomolecules (Papaspyridi *et al.*, 2011), that include lectins, proteins, enzymes, organic acids, polysaccharides and glycoproteins with a number of biologic activities such as antitumoral (Lindequist *et al.*, 2005), antimutagenic (Jose *et al.*, 2002), antiinflamatory (Lull *et al.*, 2005), antiviral (Ng and Wang 2004), antioxidant (Gregori *et al.*, 2007) and which are capable to reduce the level of cholesterol in blood (Nuhu *et al.*, 2011). Also, some *Pleurotus* species have been reported to be able to degrade and decolorize various dyes and aromatic organic compounds that are important environmental pollutants in the dyestuff industries (Novotny *et al*., 1999, Novotny *et al*, 2001, Vyas and Molitoris 1995). This property has generally been attributed to lignin-degrading enzymes such as manganese-dependent peroxidase and laccase (Vyas and Molitoris 1995).

In 2009 the annual *Pleurotus* production in Mexico was estimated as 2920 tons, which represented the 6.3% of that year´s total edible fungi production (Martínez-Carrera and López-Martínez 2010). Despite the growing interest in *Pleurotus* production, most of the small-scale producing companies do not last for a long time due to a number of factors which include: product contamination, difficulty to obtain good quality spawns and poor adaptability of commercial strains to the local climatic conditions (De León-Monzón 2004). Therefore, the cultivation of *Pleurotus* in tropical and subtropical climates requires the search of new strains capable to grow and produce fruit under diverse region-specific climatic conditions (Kashangura *et al*., 2006). In this regard, the development of a genetic improvement program should take advantage of the natural *Pleurotus* germplasm. Such program needs to consider: a) accurate species identification, b) characterization of the sexual compatibility between *Pleurotus* species lines, and c) collection and study of strains from different geographical locations to ensure diversity (Martínez-Carrera 2002, Petersen 1995a, Petersen 1995b, Vigalys *et al*., 1996, Vigalys *et al*., 1994, Vigalys *et al*., 1993).

The main aim of this research is to collect and characterize the germplasm of two native wild strains from the Mexican Mixtec region. With this study, the authors also intend to promote the production of *Pleurotus* spp. fungi in Mexico. A chemical dedikaryotization process was used for the recovery of symmetrical monokaryotic components, where the compatible neohaplonts produced different types of hybrid strains. Morphology and mycelium growth rate characteristics were compared between native, neohaplonts and hybrid strains.

MATERIALS AND METHODS

Voucher material

The carpophores of the *Pleurotus* ssp., native strains denoted as LB-051 and LB-050 were isolated from rotten tree trunks of cazahuate (*Ipomoea murucoides*) located in the Universidad Tecnológica de la Mixteca in the city of Huajuapan de León, Oaxaca (17°49´40´´ N, 97°48´23´´ W, 1785 m asl.), during August and September 2010. The strains of *Pleurotus* spp. are stored in the culture collection of the Bioconversion Laboratory of the Department of Bioprocess, Unidad Profesional Interdisciplinaria de Biotechnología (UPIBI-IPN), México. Under the accession numbers: LB-050 and LB-051. The strains were collected by Paula Cecilia Guadarrama-Mendoza and Carlos Guillermo Hernández. The germplasm was obtained from vegetative cultures by performing consecutive mycelium inoculations on malt extract agar until an axenic culture was attained.

Culture media

The malt extract agar medium (MEA) was prepared by dissolving 9 g of malt extract and 12 g of Bacteriological Agar (Bioxon) in 600 mL of distilled water. The medium was autoclaved at 1.1 kg_f/cm^2 (121ºC) for 20 min. Subsequently, 10 mL of the sterile medium were poured into Petri dishes (90 x 15 mm), solidified and incubated at 28ºC for 24 hours for sterility testing. Petri dishes without contamination were used for isolation, propagation and storage of mycelium.

Dedikaryotization solution (Peptone-Glucose Solution PGS)

A dedikaryotization solution was prepared by dissolving 20 g of anhydrous glucose and 20 g of peptone P (Oxoid LP0037) in 1 L of distilled water. Aliquots of 50 mL were poured into glass jars and autoclaved at 1 kg_f/cm^2 (121ºC) for 20 min. After cooling, the jars were incubated at 28ºC for 24 hours for sterility testing. Sterile media were used for the chemical dedikaryotization tests.

Recovery and identification of monokaryotic components (neohaplonts)

The chemical dedikaryotization method proposed by Leal-Lara and Eger-Hummel (1982) was used for obtaining the neohaplonts. A PGS solution was employed as the dedikaryotization medium and the following parameters were varied: homogenization time (Ht), volume of inoculum (Vi) and incubation time (It).

The procedure consists of the following stages: 1) A small section of 0.8 cm of diameter was cut from the edge of a growing colony in MEA and re-cultured in petri dishes (90 x 15 mm) with 10 mL of MEA. After 10 days of incubation at 28°C, the colonies covered the plate´s surface completely until they reached a diameter of 8.5 cm. Afterwards, the dish´s mycelial growth was divided in fourths and three of them were homogenized in a Waring Blender (8010S) homogenizer using an sterile jar and 50 mL of sterile water. Different homogenization times were used (Ht: 55, 60 and 65 seconds) at 22,000 rpm.

2) Aliquots of the homogenized inoculum (Vi: 50 and 100 µL) were pipetted into jars that contained 50 mL of PGS, and incubated at 28°C until the new colonies formed small conglomerates. 3) The liquid culture was further homogenized with 50 mL of sterile distilled water for 60 sec. 4) Aliquots of the homogenized culture (30, 60 and 90 µL) were spread on the MEA Petri dishes and incubated at 28°C until colonies were formed (It: 3 to 7 days). Following this, the plates were observed under the microscope. The colonies that developed hyphae without clamp connections (neohaplonts) were isolated and propagated individually on Petri dishes with MEA medium.

To identify the two types of monokaryotic components, the neohaplonts from the same strain were paired with each other in all possible combinations. Fragments of each monokaryon (0.5 cm) were placed on Petri dishes with 10 mL of MEA and incubated at 28°C. Following this, the plates were regularly inspected under the microscope to determine the presence or absence of clamp connections. The presence of clamp connections between the two mated monokaryotic colonies indicated that their nucleous were of different type, whereas clamp connections absence indicated that the colonies had the same type of nucleus. According to this, neohaplonts were classified as R_{1-n} and R_{2-n} for the strain LB-051 and B_{1-n} and B_{2-n} for the strain LB-050. R_{1-n} refers to all neohaplonts from the pale pink strain (LB-051) that belong to the nh1 type: R_{1-1}, R_{1-2}, R_{1-3}, R_{1-4} and R_{1-5}. The R_{2-n} term refers to all neohaplonts from the pinkish strain (LB-051) that belong to the nh2 type: R_{2-1}, R_{2-2} y R_{2-3}. On the other hand, the B_{1-n} term refers to all neohaplonts from the white strain (LB-050) that belong to the nh1 type: B_{1-1}, B_{1-2}, B_{1-3}, B_{1-4}, B_{1-5} y B_{1-6}. The B_{2-n} refers to the neohaplont B_{2-1} from the white strain (LB-050).

The reconstituted strains were obtained by performing an intra-specimen pairing (nh1 and nh2) of each parental strain: $R_{1-n}xR_{2-n}$ (LB-051-r) and $B_{1-n}xB_{2-n}$ (LB-050-r).

Production of hybrids strains of *Pleurotus* spp. by pairing compatible neohaplonts

The two neohaplonts (nh1 and nh2) obtained from each native strain were paired in all possible combinations: ($R_{1-n}xB_{1-n}$, $R_{1-n}xB_{2-1}$, $R_{2-n}xB_{1-n}$ and $R_{2-n}xB_{2-1}$) following the procedure described above. Clamp connections presence on three equidistant points of the colony periphery indicated that the pair was compatible, thus yielding a hybrid strain (Valencia del Toro 2002). Each new hybrid was inoculated individually on Petri dishes using 20 mL of MEA medium.

Mycelia morphology

The main characteristics of mycelia morphology as texture (cottony or floccose), density (high, regular or low), color (off-white, white or pale pink) and growth (scarce, regular or abundant) were identified by visual observation after the complete colonization of the Petri dishes using 20 mL of the MEA medium (Sobal *et al*., 2007).

Growth monitoring of native, neohaplonts and hybrids mycelium of *Pleurotus* spp.

Mycelium fragments (0.8 cm diameter) of the strains under study were transferred to Petri dishes using 20 mL of MEA and incubated in the darkness at 28ºC. Fungal growth was determined by daily measuring the diameter of the colony with a vernier until the Petri dish was completely covered. Seven replicates were measured per each strain.

Area and mycelial growth rate determination

The mycelial growth area was calculated considering that the colonies grew in a circular regular manner. The mycelial growth area was plotted as a function of time and the curve was fitted with a linear model. The mycelial growth rate (mm^2/day) was obtained from the slope of the linear function considering the time interval from 4 to 8 days. The average growth rate values of seven replicates were reported for each sample.

Statistical methods

The statistics analyses were conducted using the IBM SPSS Statistics software v. 18. A chi-square test (χ^2, p <0.01) was used to evaluate the symmetric recovery of neohaplonts. A one-way ANOVA test and Duncan post hoc analysis (p <0.01) were used to determine the effects of the different treatments on the mycelia growth rate.

RESULTS AND DISCUSSION

Pleurotus spp. native germplasm recovery

LB-051 and LB-050 strains have no stipe and their carpophores have the appearance of a flower petal, with a smooth pileus and a firm leathery consistency. The pileus size varied from 3 to 6 cm of diameter. Both strains presented a mycelial morphology of cottony texture with differences in density, growth and pigmentation when growing in MEA. The LB-050 strain was characterized by an abundant growth of high density and white color, whereas the LB-051 showed regular growth and density and a pale pink color.

Pleurotus djamor studies have shown that this inter-sterile group, that is commonly found in Mexico (Valencia del Toro 2002), is formed by three different phenotypic carpophores varieties: white, grey or pink; denominated as *djamor*, *opuntiae* and *salmoneostramineus*, respectively (Petersen 1995a, Petersen and Hughes1999, Petersen and Ridley 1996). A taxonomic determination of the species was made in the Herbarium of the Faculty of Sciences - Universidad Nacional Autónoma de México (UNAM). The taxonomic study resulted in the following classification: LB-050 belongs to the *Pleurotus djamor* var.

djamor (Rumph. Ex. Fr.) Boedijn. egistration number: 26234. On the other hand, LB-051 belongs to the *Pleurotus djamor* var. *roseus* Corner. Registration number: 26233.

Mycelial recovery and characterization of neohaplonts through chemical dedikaryotization

Chemical dedikaryotization

The two *Pleurotus* spp. strains showed different mechanical resistance to the chemical homogenization treatment. The LB-050 strain resisted homogenization times of up to 65 s, whereas LB-051 strains only resisted times up to 60 s. Table 1 lists the conditions required to obtain the monokaryotic components of the strains as well as the total neohaplonts obtained from each type (nh1 and nh2). In agreement with other authors (Guerrero *et al*., 2007, Maldonado 2007, Morales 2009, Valencia del Toro 2002), the use of peptone-glucose solution (PGS) as a dedikaryotizing medium reduced the incubation time to 72h, compared to the traditional method that requires more than 120 h (Leal-Lara 1980). In addition, with this method it was possible to attain symmetrical recovery of both neohaplonts for each strain. Arias-García (1998) and Arteaga-Santillas *et al*. (1996) observed that the results of the chemical homogenization treatment depend on the susceptibility of the strain against the toxicity of the chemicals used and on the extent of the modification of the cell wall. Thus, the monokaryotization conditions such as homogenization time, inoculum volume and incubation time must be individually adjusted for each strain, in order to establish the optimum treatment for the symmetric recovery of neohaplonts.

Table 1. Conditions used for the recovery of neohaplonts of LB-050 and LB-051 strains by chemical dedikaryotized process.

Dikaryotic strain	Ht* (s)	Vi* (μl)	It* (days)	Recovered neohaplonts			χ^2 Test for symmetric recovery (nh1:nh2 = 1:1)*•
				Total	Type nh1	Type nh2	
LB-050	60	100	3	7	6	1	3.57
LB-051	65	100	3	8	5	3	0.5

* Ht is the time of blending; Vi is the volume of inoculum; It is the time of incubation in PGS. *• Test reference value χ^2 = 6.635 (p <0.01). Smaller χ^2 values indicate no significant differences

Mycelial morphology, color and growth rate

Tables 2 and 3 show the main mycelial morphologic characteristics and the growth rates for the neohaplonts obtained from both strains LB-050 (B_{1-n} and B_{2-n}) and LB-051 (R_{1-n} and R_{2-n}). Neohaplonts showed two types of mycelium texture: cottony and floccose. The floccose mycelium developed a low density and scarce growth. The cottony mycelium resulted in two different types of growth: cottony with high density and abundant growth, and cottony with regular density and regular growth. Thus, the mycelial morphology of the monokaryotic components can be classified as follows: cottony with high density and abundant growth (C-high); cottony with regular density and regular growth (C-reg) and floccose with low density and scarce growth (F-low) (Figure 1). Neohaplonts presented predominantly a C-reg mycelial morphology, which accounted for 50% and 43% of the LB-051 and LB-050 strains, respectively.

Table 2. Colony morphology and growth rate of LB-050 neohaplonts grown on MEA at 28°C.

Neohaplont (Code)	Type of neohaplont	Mycelial characteristics				
		Texture	Density	Growth	Color	Growth Rate (mm^2/day)*
B_{1-1}	nh1	Cottony	High	Abundant	White	245.160 ± 9.168^{e}
B_{1-2}	nh1	Cottony	Regular	Regular	White	154.207 ± 3.601^{c}
B_{1-3}	nh1	Floccose	Low	Scarce	Off-white	18.046 ± 1.975^{a}
B_{1-4}	nh1	Cottony	High	Abundant	White	189.510 ± 16.763^{d}
B_{1-5}	nh1	Cottony	Regular	Regular	White	55.281 ± 0.611^{b}
B_{1-6}	nh1	Floccose	Low	Scarce	Off-white	11.725 ± 0.958^{a}
B_{2-1}	nh2	Cottony	Regular	Regular	White	44.361 ± 3.748^{b}

* Growth rate (Value ± SD). Different letters indicated significant difference among growth rate values of neohaplonts at level $p<0.01$, according to Duncan test.

Table 3. Colony morphology and growth rate of LB-051 neohaplonts grown on MEA at 28°C.

Neohaplont (Code)	Type of neohaplont	Mycelial characteristics				
		Texture	Density	Growth	Color	Growth Rate (mm^2/day)*
R_{1-1}	nh1	Cottony	High	Abundant	White	117.996±3.293[d]
R_{1-2}	nh1	Cottony	Regular	Regular	White	81.243±3.211[c]
R_{1-3}	nh1	Cottony	High	Abundant	White	136.928±5.822[e]
R_{1-4}	nh1	Floccose	Low	Scarce	Off-white	55.692 ±5.906[b]
R_{1-5}	nh1	Floccose	Low	Scarce	Off-white	42.516± 2.546[a]
R_{2-1}	nh2	Cottony	Regular	Regular	Pale pink	86.517±0.914[c]
R_{2-2}	nh2	Cottony	Regular	Regular	Pale pink	84.748±2.017[c]
R_{2-3}	nh2	Cottony	Regular	Regular	Pale pink	63.435±5.271[b]

* Growth rate (Value ± SD). Different letters indicated significant difference among growth rate values of neohaplonts at level $p<0.01$, according to Duncan test.

a b c

Figure 1. Mycelium morphology type for *Pleurotus* spp. strains: (a) cottony texture- high density-abundant growth (C-high); (b) cottony texture-regular density-regular growth (C-reg) and (c) floccose texture-low density-scarce growth (F-low).

The color of the LB-050 cottony mycelium was white, while the LB-051 cottony mycelium presented two different colors: white and pale pink for the nh1 and nh2 neohaplonts, respectively. The floccose mycelium was off-white regardless of its parental strain. These results are consistent to those reported elsewhere (Eichlerová and Homolka 1999, Maldonado 2007), in which the morphology of monokaryotic colonies of *Pleurotus* spp. strains (neohaplonts and monosporic) presented differences with respect to their parental dikaryotic strains.

The mycelial growth in solid medium has been measured in different basidiomycetes such as *Schizophyllum commune* (Clark and Anderson 2004), *Coprinus cinereus* and *Pleurotus* ssp. (Larraya *et al*., 2001). Although there is not a unified criterion to report it (Baumer *et al*., 2008, Castro *et al*., 2006, Larraya *et al*., 2001, Larraya *et al*., 2002), mycelial growth is a characteristic property of each strain. In addition, this parameter can be used as a selection criterion for the edible-fungi cultivation programs considering that fast growing strains have the potential to achieve high production yields because they colonize the substrate much faster and develop better fruiting characteristics compared to the slower strains (Clark and Anderson 2004). Tables 2 and 3, present the mycelial growth rates of the 15 recovered neohaplonts. Neohaplont B_{1-1} resulted in the fastest growth rate at 245.160±9.168 mm^2/day; whereas neohaplont B_{1-6} had the slowest growth rate (11.725±0.958 mm^2/day).

A study of the relationship between the mycelium morphology of the neohaplonts (C-high, C-reg and F-low) and their growth rate was performed. In Figure 2, it can be observed that the C-high mycelia were significantly faster than the C-reg and F-low mycelia for both LB-050 and LB-051 strains [F (5,27) =308.968; $p<0.01$]. Eichlerová and Homolka (1999) reported that monosporic mycelia morphology was neither correlated with the growth rate, nor with the enzymatic activity. Nevertheless, Simchen (1996) and Clark and Anderson (2004) observed a correlation between morphology and growth rate for the monosporic components of the *Schizophyllum commune* fungi.

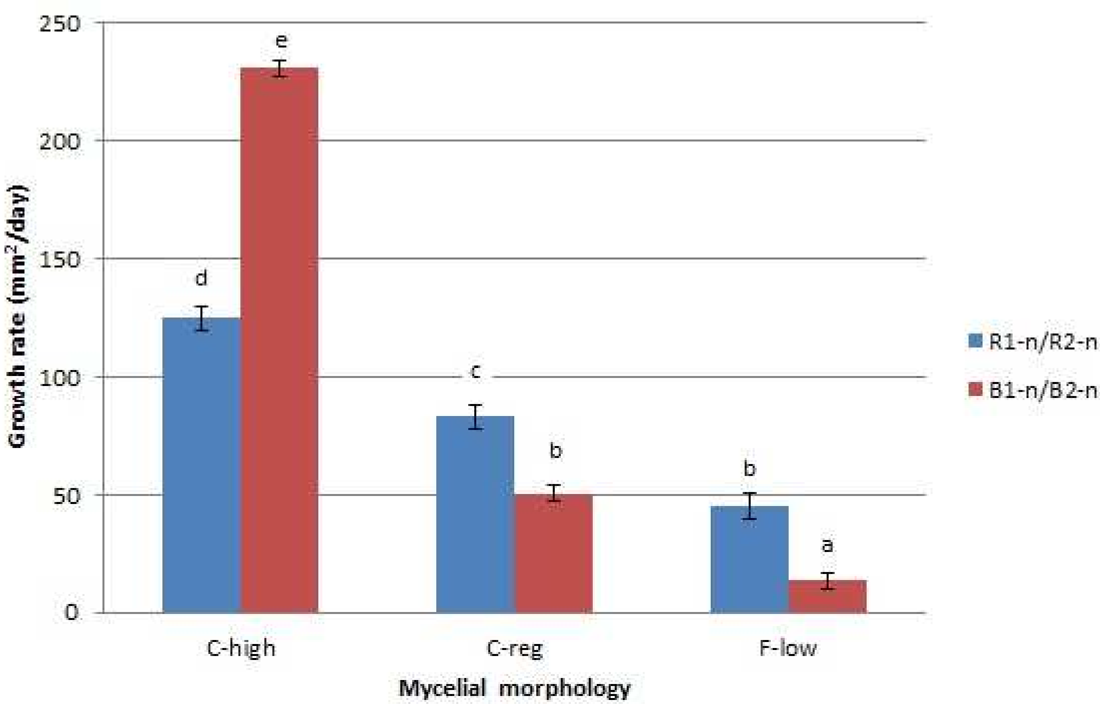

Figure 2. Comparison of mycelium growth rate based on the morphology type for LB-050 (B_{1-n}/B_{2-n}) and LB-051 (R_{1-n}/R_{2-n}) neohaplonts. Cottony texture- high density-abundant growth (C-high); cottony texture-regular density-regular growth (C-reg) and floccose

texture-low density-scarce growth (F-low). Different letters indicated significant difference among growth rate values of the neohaplont's morphology at level $p<0.01$, according to Duncan test.

Production and characterization of mycelial hybrid strains

Mycelial morphology

Pairing of the 15 recovered neohaplonts yielded 56 hybrid strains that were classified into the following four groups: $R_{1-n}xB_{1-n}$ (30 hybrids), $R_{1-n}xB_{2-1}$ (5 hybrids), $R_{2-n}xB_{1-n}$ (18 hybrids) and $R_{2-n}xB_{2-1}$ (3 hybrids). The mycelial morphologies of these strains corresponded to the three types of mycelial morphology previously established for the monokaryotic components. The C-high mycelial morphology was predominant (57.10%), followed by the F-low morphology (28.57%), whereas the C-reg was the less frequent morphology (14.29%) (Table 4). Hybrid strains with C-high morphology resulted from pairing the LB-051 neohaplonts with the LB-050 neohaplonts of mycelial morphologies C-high (B_{1-1} and B_{1-4}) and C-reg (B_{1-5} and B_{2-1}). The hybrids obtained from the neohaplont B_{1-2} maintained a C-reg morphology, whereas those obtained from neohaplonts B_{1-3} and B_{1-6} kept the F-low mycelial morphology independently of the LB-051 neohaplont used for pairing. Therefore, the mycelial morphology of the hybrid strains primarily depended on the dominant genetic characteristics inherited from the LB-050 neohaplonts. Hybrid strains with C-high mycelial characteristics could not be obtained from F-low neohaplonts pairs, since these can only inherit F-low or C-reg morphology types.

Table 4. Compatibility of the neohaplont strains LB-050 (B_{1-n} and B_{2-n}) and LB-051 (R_{1-n} and R_{2-n}) neohaplonts and mycelium morphology of hybrid strains.

LB-050 neohaplonts

LB-051 neohaplonts		B_{1-1}	B_{1-2}	B_{1-3}	B_{1-4}	B_{1-5}	B_{1-6}	B_{2-1}
	R_{1-1}	+[1,1]	+[2,1]	+[3,1]	+[1,1]	+[1,1]	+[3,1]	+[1,1]
	R_{1-2}	+[1,1]	+[2,1]	+[3,1]	+[1,1]	+[1,1]	+[3,1]	+[1,1]
	R_{1-3}	+[1,1]	+[2,1]	+[3,1]	+[1,1]	+[1,1]	+[3,1]	+[1,1]
	R_{1-4}	+[1,1]	+[2,1]	+[3,1]	+[1,1]	+[1,1]	+[3,1]	+[1,1]
	R_{1-5}	+[1,1]	+[2,1]	+[3,1]	+[1,1]	+[1,1]	+[3,1]	+[1,1]
	R_{2-1}	+[1,2]	+[2,2]	+[3,1]	+[1,2]	+[1,2]	+[3,1]	+[1,2]
	R_{2-2}	+[1,2]	+[2,2]	+[3,1]	+[1,2]	+[1,2]	+[3,1]	+[1,2]
	R_{2-3}	+[1,2]	+[2,2]	+[3,1]	+[1,2]	+[1,2]	+[3,1]	+[1,2]

(+) indicates clamp formation (i.e. compatible pairing = hybrid strain). [1,1] indicates cottony texture with high density, abundant growth and white mycelium; [2,1] indicates cottony texture, regular density, regular growth and white mycelium; [3,1] indicates floccose texture with low density, scarce growth and off-white mycelium; [1,2] indicates cottony texture with high density, abundant growth and pale pink mycelium and [2,2] indicates cottony texture, regular density, regular growth and pale pink mycelium.

Mycelial color

Resulting from the hybridization, 44.6% of the hybrid strains were white as the LB-050 strain and 26.80% of the hybrid strains inherited the pink pale color of the LB-051 strain (resulted from pairing the neohaplonts R_{2-1}, R_{2-2} and R_{2-3} with the neohaplonts B_{1-1}, $B_{1-2,}$ $B_{1-4,}$ B_{1-5} and B_{2-1}). The remaining 28.60% of hybrids presented an off-white color resulting from pairing any LB-051 neohaplont with neohaplonts of F-low mycelium (B_{1-3} and B_{1-6}). It was observed that pairing between white neohaplonts always produced white mycelia; whereas pink pale neohaplonts paired with white ones produced pale pink mycelia. Finally, pairing of off-white neohaplonts always resulted in off-white mycelia regardless of the other neohaplont color. These results agree with Mendel´s Laws, in which the pink pale neohaplonts are probably the dominant alleles and the white and off-white neohaplonts the recessive ones.

Morphology and growth rate.

In order to study the relationship between the neohaplonts mycelial morphology and the growth rate of the resulting hybrid, the four hybrid types were subdivided into 16 subgroups, and these were regrouped depending on the native neohaplont morphology type (Table 5).

Table 5. Classification of the hybrid strains based on the neohaplonts mycelium morphology used for pairing.

Hybrid type	Pairing of:		Subgroup*	Hybrids
	R_{x-y}	$B_{x'-y'}$	$R_{x-y\,x}B_{x'-y'}$	
$R_{1-n}xB_{1-n}$	nh1	nh1	C-high $_x$ C-high	$R_{1-1}xB_{1-1}$ $R_{1-1}xB_{1-4}$ $R_{1-3}xB_{1-1}$ $R_{1-3}xB_{1-4}$
	nh1	nh1	C-reg $_x$ C-high	$R_{1-2}xB_{1-1}$ $R_{1-2}xB_{1-4}$
	nh1	nh1	F-low $_x$ C-high	$R_{1-4}xB_{1-1}$ $R_{1-4}xB_{1-4}$ $R_{1-5}xB_{1-1}$ $R_{1-5}xB_{1-4}$
	nh1	nh1	C-high $_x$ C-reg	$R_{1-1}xB_{1-2}$ $R_{1-1}xB_{1-5}$ $R_{1-3}xB_{1-2}$ $R_{1-3}xB_{1-5}$
	nh1	nh1	C-reg $_x$ C-reg	$R_{1-2}xB_{1-2}$ $R_{1-2}xB_{1-5}$
	nh1	nh1	F-low $_x$ C-reg	$R_{1-4}xB_{1-2}$ $R_{1-4}xB_{1-5}$ $R_{1-5}xB_{1-2}$ $R_{1-5}xB_{1-5}$
	nh1	nh1	C-high $_x$ F-low	$R_{1-1}xB_{1-3}$ $R_{1-1}xB_{1-6}$ $R_{1-3}xB_{1-3}$ $R_{1-3}xB_{1-6}$
	nh1	nh1	C-reg $_x$ F-low	$R_{1-2}xB_{1-3}$ $R_{1-2}xB_{1-6}$
	nh1	nh1	F-low $_x$ F-low	$R_{1-4}xB_{1-3}$ $R_{1-4}xB_{1-6}$ $R_{1-5}xB_{1-3}$ $R_{1-5}xB_{1-6}$
$R_{1-n}xB_{2-n}$	nh1	nh2	C-high $_x$ C-reg	$R_{1-1}xB_{2-1}$ $R_{1-3}xB_{2-1}$
	nh1	nh2	C-reg $_x$ C-reg	$R_{1-2}xB_{2-1}$
	nh1	nh2	F-low $_x$ C-reg	$R_{1-4}xB_{2-1}$ $R_{1-5}xB_{2-1}$

$R_{2\text{-}n}xB_{1\text{-}n}$	nh2	nh1	C-reg $_x$ C-high	$R_{2\text{-}1}xB_{1\text{-}1}$ $R_{2\text{-}1}xB_{1\text{-}4}$ $R_{2\text{-}2}xB_{1\text{-}1}$ $R_{2\text{-}2}xB_{1\text{-}4}$ $R_{2\text{-}3}xB_{1\text{-}1}$ $R_{2\text{-}3}xB_{1\text{-}4}$
	nh2	nh1	C-reg $_x$ C-reg	$R_{2\text{-}1}xB_{1\text{-}2}$ $R_{2\text{-}1}xB_{1\text{-}5}$ $R_{2\text{-}2}xB_{1\text{-}2}$ $R_{2\text{-}2}xB_{1\text{-}5}$ $R_{2\text{-}3}xB_{1\text{-}2}$ $R_{2\text{-}3}xB_{1\text{-}5}$
	nh2	nh1	C-reg $_x$ F-low	$R_{2\text{-}1}xB_{1\text{-}3}$ $R_{2\text{-}1}xB_{1\text{-}6}$ $R_{2\text{-}2}xB_{1\text{-}3}$ $R_{2\text{-}2}xB_{1\text{-}6}$ $R_{2\text{-}3}xB_{1\text{-}3}$ $R_{2\text{-}3}xB_{1\text{-}6}$
$R_{2\text{-}n}xB_{2\text{-}n}$	nh2	nh2	C-reg $_x$ C-reg	$R_{2\text{-}1}xB_{2\text{-}1}$ $R_{2\text{-}2}xB_{2\text{-}1}$ $R_{2\text{-}3}xB_{2\text{-}1}$

* C-high means cottony texture- high density-abundant growth; C-reg means cottony texture-regular density-regular growth and F-low means floccose texture-low density-scarce growth.

In general, the hybrids obtained from F-low neohaplonts, such as $R_{1\text{-}5}xB_{1\text{-}6}$, had lower growth rates (19.011± 0.902 mm^2/day) than those obtained from cottony neohaplonts, such as $R_{1\text{-}3}xB_{1\text{-}4}$ (937.453 ± 24.043 mm^2/day) [F (15,39) =630.381; $p<0.01$] (Table 6). These results demonstrate that the mycelial growth rate of the hybrid strains is directly dependent on the mycelial morphology of the neohaplont. Clark and Anderson (2004) postulated that fast growing monosporic strains, have better colonization capability, fruiting and spore production, as compared to the slower strains. Considering this, the morphology of the neohaplonts can be used as a selection parameter to define the neohaplont pairs for obtaining the most productive dicaryotic strains.

Table 6. Mycelium growth rate of hybrid strains selected according to the neohaplonts mycelia morphology.

Type of hybrid	Subgroup* R_{x-y} x $B_{x'-y'}$	Hybrid selected	Growth rate (mm^2/day) **	R^{***}
$R_{1-n}xB_{1-n}$	C-high x C-high	$R_{1-3}xB_{1-4}$	937.453±24.043[g]	0.9924
	C-reg x C-high	$R_{1-2}xB_{1-4}$	1059.170±17.815[h]	0.9977
	F-low x C-high	$R_{1-5}xB_{1-4}$	945.270±17.401[g]	0.9940
	C-high x C-reg	$R_{1-3}xB_{1-5}$	488.060±51.127[d]	0.9912
	C-reg x C-reg	$R_{1-2}xB_{1-2}$	204.390±3.658[c]	0.9922
	F-low x C-reg	$R_{1-4}xB_{1-2}$	51.545±2.225[a]	0.9932
	C-high x F-low	$R_{1-1}xB_{1-6}$	196.360± 7.695[c]	0.9960
	C-reg x F-low	$R_{1-2}xB_{1-3}$	196.090±11.135[c]	0.9958
	F-low x F-low	$R_{1-5}xB_{1-6}$	19.011±.902[a]	0.9858
$R_{1-n}xB_{2-1}$	C-high x C-reg	$R_{1-3}xB_{2-1}$	682.010±13.311[f]	0.9956
	C-reg x C-reg	$R_{1-2}xB_{2-1}$	478.003±5.869[d]	0.9902
	F-low x C-reg	$R_{1-5}xB_{2-1}$	564.553±24.532[e]	0.9780
$R_{2-n}xB_{1-n}$	C-reg x C-high	$R_{2-3}xB_{1-4}$	518.663±30.646[e]	0.9958
	C-reg x C-reg	$R_{2-1}xB_{1-5}$	557.183±18.917[e]	0.9960
	C-reg x F-low	$R_{2-1}xB_{1-6}$	139.906±5.602[b]	0.9817
$R_{2-n}xB_{2-1}$	C-reg x C-reg	$R_{2-3}xB_{2-1}$	477.107±2.949[d]	0.9964

* C-high means cottony texture- high density-abundant growth; C-reg means cottony texture-regular density-regular growth and F-low means floccose texture-low density-scarce growth.

** Growth rate (Value ± SD). Different letters indicated significant difference among growth rate values of the hybrids at level $p<0.01$, according to Duncan test.

*** R is the correlation coefficient for the linear model.

Growth rate kinetic model

The fastest strains were selected for plotting from each mycelium group: parental (LB-050 and LB-051), reconstituted ($R_{1-3}xB_{2-1}$ and $B_{1-1}xB_{2-1}$) and hybrids ($R_{1-2}xB_{1-4}$, $R_{1-3}xB_{2-1}$, $R_{2-3}xB_{1-4}$ and $R_{2-3}xB_{2-1}$).

In general, *Pleurotus* ssp. strains growth presented a short lag phase (<72 h), while the exponential phase lasted just about 4 days. According to this, the kinetics studies were stopped before the stationary phase was reached (8 days). Figure 3 depicts the mycelial growth curves (colony area vs. time) of selected *Pleurotus* ssp. dikaryotic strains grown in MEA medium for the period of time from 4 to 8 days of incubation, which correspond to the log phase. So, the mycelial growth kinetics curves of neohaplonts and dikaryons were adjusted to the linear model : $Y = mx + b$, where Y is the accumulated mycelial growth area (mm^2); x is the time (days), m is the slope that represents the growth rate (mm^2/day) and b is the y-intercept. These results were similar to those reported by Sánchez (2001) for the *Pleurotus ostreatus* strains in which the growth curve was linear, too. Authors elsewhere reported that the mycelial growths of other fungi such as *Lentinula edodes* (Castro *et al*., 2006), *Pycnoporus sanguineus* (Baumer *et al*., 2008) and *Monascus purpureus* (Pacífico *et al*., 2005) were better fitted with an exponential model. The differences in the growth curve models are due to the fact that each strain has a distinctive enzymatic adaptability to the culturing solid medium. In addition, the models consider different growing time intervals and method to quantify mycelial growth such as: radio, area and rate.

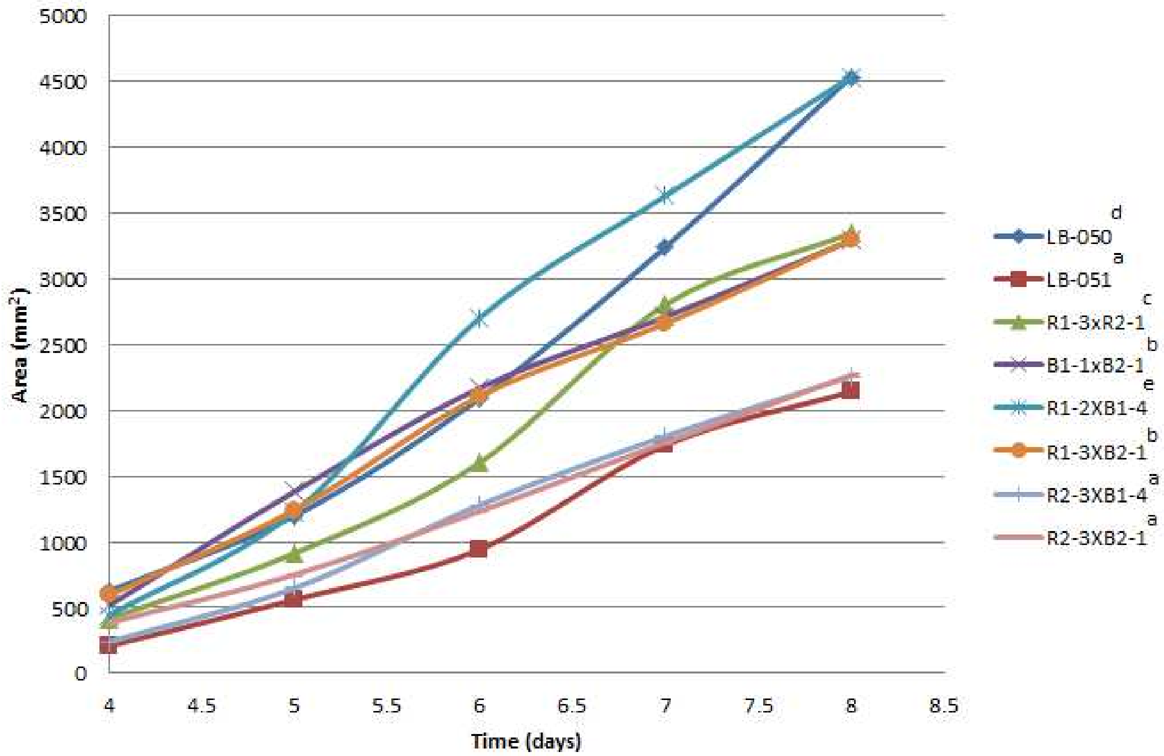

Figure 3. Mycelium growth curves of *Pleurotus* spp. Strains (variance does not exceed 5% of the means). Native strains (LB-050 and LB-051); reconstituted strains ($R_{1-3}xR_{2-1}$ and $B_{1-1}xB_{2-1}$) and hybrid strains ($R_{1-2}xB_{1-4}$, $R_{1-3}xB_{2-1}$, $R_{2-3}xB_{1-4}$ and $R_{2-3}xB_{2-1}$). Different letters indicated significant difference among mycelium growth curve of the dikaryotic strains at level $p<0.01$, according to Duncan test.

Growth rate assessment of selected strains

The one-way ANOVA showed statistically significant differences in the growth rates of the *Pleurotus* spp. strains that were compared [F (7, 22) = 442.810; $p<0.01$]. The hybrid strain $R_{1-2}xB_{1-4}$ from the $R_{1-n}xB_{1-n}$ type had the highest growth rate (1059.170 ± 17.815

mm^2/day), followed by the LB-050 parental strain (986.388 ± 6.017 mm^2/day), whereas the hybrid strain $R_{2-3}xB_{2-1}$ from the $R_{2-n}xB_{2-n}$ type had the lowest rate (477.107 ± 2.949 mm^2/day) (Table 7). Hence, pairing of compatible neohaplonts resulted in hybrid strains which can improved mycelial growth with respect to the parental strains. On the other hand, there was a reduction on the mycelial growth rate for the LB-050 reconstituted strain (688.492± 34.187 mm^2/day) compared to the parental one (986.388 ± 6.017 mm^2/day). In contrast, the mycelial growth rate of the LB-051 reconstituted strain (775.322 ± 5.554 mm^2/day) was higher than that of the native strain (504.340 ± 9.723 mm^2/day). This might be due to the large variability of the neohaplonts nuclei which may have prompted that the phenotypical characteristics that were not present in the parental dikaryon could be expressed by the reconstituted strain.

Table 7. Mycelium morphology and growth rate for native, reconstituted and hybrid *Pleurotus* spp. strains on MEA at 28 °C.

Dikaryotic strain	Type of strain	Mycelium					
		Texture	Density	Growth	Color	Growth rate (mm^2/day)*	R**
LB-050	Native	Cottony	High	Abundant	White	986.390 ± 6.018^{d}	.9880
$B_{1-1}xB_{2-1}$	Reconstituted	Cottony	Regular	Regular	White	688.490 ± 34.187^{b}	.9933
LB-051	Native	Cottony	Regular	Regular	Pale pink	504.340 ± 9.723^{a}	.9881
$R_{1-3}xR_{2-1}$	Reconstituted	Cottony	Regular	Regular	Pale pink	775.320 ± 5.554^{c}	.9891
$R_{1-2}xB_{1-4}$	Hybrid type $R_{1-n}xB_{1-n}$	Cottony	High	Abundant	White	1059.170 ± 17.815^{e}	.9947
$R_{1-3}xB_{2-1}$	Hybrid type $R_{1-n}xB_{2-n}$	Cottony	High	Abundant	White	682.010 ± 13.311^{b}	.9956

$R_{2-3}xB_{1-4}$	Hybrid type $R_{2-n}xB_{1-n}$	Cottony	High	Abundant	Pale pink	518.663 ± 30.646^{a}	.9958
$R_{2-3}xB_{2-1}$	Hybrid type $R_{2-n}xB_{2-n}$	Cottony	High	Abundant	Pale pink	477.107 ± 2.949^{a}	.9964

* Growth rate (Value ± SD). Different letters indicated significant difference among dikaryotic strains growth rate values at level $p<0.01$, according to Duncan test.

** R is the correlation coefficient for the linear model.

Conclusions

The germplasm of two *Pleurotus* ssp. native strains from the Mexican Mixtec region was isolated, which constitutes one of the first reports of these fungi in the region. Chemical dedikaryotization using a peptone-glucose solution allowed the symmetrical recovery of both monokaryotic components (nh1:nh2) from the native strains. The mycelium morphologic characteristics and growth rates of the strains varied remarkably, in which both properties are directly related. The cottony strains with high density and exuberant growth had higher growth rates compared to those that developed a floccose texture, low density and scarce growth. The neohaplonts morphology and growth rate determined the mycelial growth characteristics of the hybrid strains; therefore, selection of the fast growing cottony neohaplonts promoted the production of fast growing hybrid strains. A linear model had a good fit to the mycelial growth kinetics of both monokaryotic and dikaryotic strains. The use of neohaplonts compatible pairing promoted the production of strains with higher mycelial growth rates in comparison with those of the native strains. This might result in the production of strains with a high production and commercial potential.

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