PRODUCTION AND MOLECULAR CHARACTERIZATION OF INTERGENERIC *Pleurotus ostreatus* × *Lentinula edodes* HYBRIDS BY MATING OF COMPATIBLE NEOHAPLONTS

PRODUCCIÓN Y CARACTERIZACIÓN MOLECULAR DE HÍBRIDOS INTER-GÉNERO *Pleurotus ostreatus* × *Lentinula edodes* POR APAREAMIENTO DE NEOHAPLONTES COMPATIBLES

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SUMMARY

Mushroom cultivation of *Pleurotus* and *Lentinula* genera has increased in Mexico. The use of germplasm with the best phenotypes is important for commercial production; thus, genetic improvement programs are required. Production of intergeneric hybrids is restricted by compatibility barriers; however, the monokaryotic components obtained by chemical dedikaryotization (neohaplonts) can be mated to produce intergeneric hybrids *Pleurotus* × *Lentinula*. Neohaplonts were recovered from strains of *Pleurotus ostreatus* and *Lentinula edodes*, which mated to generate 12 intergeneric hybrids. The phenotype of all hybrids was *Pleurotus*, with biological efficiencies between 26.72 and 96.55 %. Two hybrid, parental and neohaplont strains were selected for molecular characterization using 7 ISSR markers. The average total of bands was 16.29, with a polymorphism of 91.31 %. Other parameters calculated were PIC with a value of 0.37, MI of 2.94, and RP of 9.51. The dendrogram, constructed from the binary matrix, indicated that the *Lentinula* strains were grouped separately from the *Pleurotus* strains and intergeneric hybrids, which suggests a closer genetic similarity between the hybrids and the *Pleurotus* strains.

Index words: *Pleurotus*, *Lentinula*, intergeneric hybrids, ISSR markers, molecular characterization.

RESUMEN

El cultivo de hongos de los géneros *Pleurotus* y *Lentinula* se ha incrementado en México. El uso de germoplasma con los mejores fenotipo es importante para la producción comercial, por lo que proyectos de mejoramiento genético son necesarios. La producción de híbridos inter-género está restringida por barreras de compatibilidad; sin embargo, los monocariontes obtenidos por dedicariotización química (neohaplontes) de cepas dicarióticas pueden ser apareados para producir híbridos inter-género *Pleurotus* × *Lentinula*. Se obtuvieron neohaplontes de *Pleurotus ostreatus* y *Lentinula edodes*, los cuales se aparearon para generar 12 híbridos intergénero. El fenotipo de todos los híbridos fue de *Pleurotus*, con eficiencias biológicas entre 26.72 y 96.55 %. Se seleccionaron dos cepas híbridas, parentales y neohaplontes para la caracterización molecular mediante 12 marcadores ISSR. El promedio total de bandas fue de 16.29, con un polimorfismo de 91.31 %. Otros parámetros calculados fueron el PIC con un valor de 0.37, MI de 2.94, y RP de 9.51. El dendrograma construido a partir de la matriz binaria indicó que las cepas de *Lentinula* se agruparon de forma separada a las cepas de *Pleurotus* e híbridos inter-género, lo cual sugiere una mayor similitud genética entre los híbridos y las cepas de *Pleurotus*.

Palabras clave: *Pleurotus*, *Lentinula*, caracterización molecular, híbridos inter-género, marcadores ISSR.

INTRODUCTION

Mushroom cultivation has increased worldwide during the last decades, reaching a production of 34 billion kg in 2013. The two most cultivated genera are *Lentinula* (22 %) and *Pleurotus* (19 %) (Royse *et al*., 2017). Due to the increasing production of edible mushrooms, genetic programs should be implemented to obtain strains with more attractive phenotypic characteristics for consumers (Avin *et al*., 2012; 2014; Kumara and Edirimanna, 2009).

Mushroom genetic improvement programs are based on hybridization, *i.e.*, the combination of desirable characteristics from different strains, creating variability within the germplasm (Kumara and Edirimanna, 2009). The basis of genetic improvement by hybridizations is natural variability, mutations (produced either naturally or by mutagenic agents), and meiotic recombination in meiosis (Singh and Kamal, 2017). The interaction within different genotypes by mating monokaryotic strains allows the generation of novel combinations and the expression of a distinct phenotype.

There are two methods of obtaining initial material (monokaryotic mycelium) for genetic improvement programs: 1) isolation and recovery of monosporic mycelia from spore prints and 2) monokaryotization, *i.e.*, obtention of neohaplonts (monokaryotic mycelium) from dikaryotic strains without karyogamy and meiosis (Fries and Aschan, 1952). The monokaryotization of fungi can be performed

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by two different methods: chemical dedikaryotization, using solutions of glucose and peptone (Leal-Lara and Eger-Hummel, 1982), and protoplast production, cell wall degradation of fungi by lytic enzymes (Peberdy, 1980). Both methods allow the obtention of neohaplonts, used for hybrid production (intraspecific, interspecific, intergeneric).

Intra-specific hybrids have been produced by mating neohaplonts or monosporic strains of *Pleurotus ostreatus* (Eger *et al*., 1976; Kumara and Edirimanna, 2009) and *P. pulmonarius* (Avin *et al*., 2016). Intergeneric hybrids have also been reported by mating neohaplonts and protoplast fusion of *P. ostreatus* × *Lentinula edodes* (Mallick and Sidkar, 2014; Ramírez-Carrillo *et al*., 2007; Valenzuela *et al*., 2017), *Volvariella volvacea* × *P. florida (*Chakraborty and Sikdar, 2008), *Calocybe indica* × *P. florida* (Chakraborty and Sikdar, 2010) and *P. florida* × *L. edodes* (Mallick and Sikdar, 2014).

Somatic hybrids and their progeny have been characterized by different methods, *e.g*., gene segregation, morphology, productivity (Ramírez-Carrillo *et al*., 2007), enzymes polymorphism (Anné and Peberdy, 1981) and DNA polymorphism (Avin *et al*., 2016; Chakraborty and Sikdar, 2008; 2010; Mallick and Sikdar, 2014). Hybrid production by protoplast fusion and chemical dedikaryotization allow a molecular characterization of parental strains, hybrids and neohaplonts using RAPD or ISSR markers (Aguilar *et al*., 2018; Chakraborty and Sikdar, 2008; 2010 Mallick and Sikdar, 2014; Sánchez *et al*., 2019). Dendrograms generated allowed to analyze neohaplonts from the parental strains and the resulting hybrid strains, a crucial point. The production of hybrids by mating compatible neohaplonts can be analyzed and compared using molecular markers among parental, hybrid and neohaplont strains.

Evidence supports that dedikaryotization methods affect the phenotypic characteristics of the neohaplonts (Sánchez *et al*., 2019); however, the interspecies hybrids have never been studied with their neohaplonts and parental strains. In this study, compatible neohaplonts of *Pleurotus* and *Lentinula* were generated and mated to produce hybrid strains that were genetically improved. The neohaplonts, parental and reconstituted dikaryons were then characterized and compared regarding their molecular characteristics, morphology, and productivity, allowing a better understanding of the nature of interspecific hybrids and their meiotic progeny.

MATERIALS AND METHODS

Biological material

Three strains of *Lentinula edodes* (L5, L9, L15) and one of *Pleurotus ostreatus* (PAsp14) were studied. All strains produced in this study were stored at Laboratorio de Cultivos Celulares of UPIBI-IPN, Mexico and cultured in malt extract agar (MEA) (35 g L^{-1}).

Genomic DNA Extraction

Mushroom strains were cultivated in liquid media (20 g L^{-1} sucrose and 20 g L^{-1} malt extract), and DNA was extracted by the gDNA Plant Kit (#CS 18000).

Molecular identification of parental strains

The ITS1-5.8r-ITS2 region was amplified with the ITS1 and ITS4 primers (White *et al*., 1990). The PCR reactions consisted in 22 µL PCR SuperMix (Cat. No. 10572-014, Invitrogen), ITS1 and ITS4 primers (0.25 µM), DNA (30-50 ng μL^{-1}) and distilled water. PCR steps were: initial denaturation, 5 min at 94 °C; 35 cycles (denaturation, 60 s 94 °C; annealing primers, 30 s 55 °C; extension, 60 s 72 °C) and final extension 5 min at 72 °C. PCR amplicons were verified in a 1.2 % agarose gel, TAE, and stained with GelRed™. Gels were run at 100 V for 1 h. The agarose gel was observed with a UV Transilluminator (ChemiDOC ™MP Imaging, Biorad® System, Hercules, California, EUA). The PCR products were sequenced from both sides at IBT, UNAM, Mexico.

The ITS1-5.8s-ITS2 sequences obtained were assembled and edited with AliView software (Larsson, 2014), deposited into the NCBI platform and compared with sequences of *Lentinula* and *Pleurotus* from the GenBank using the BLAST-n. Sequences of distinct species of the same genera were downloaded (Table 1). Eight species of *Pleurotus* and four species of *Lentinula* were used in this analysis. The sequences were aligned with MAFFT (Katoh *et al*., 2019) with minor manual adjustments in AliView (Larsson, 2014). The Mesquite software (Maddison and Maddison, 2019) was used to generate compatible files with Bayesian analysis (MrBayes) in CIPRES Science Gateway V 3.3 (Miller *et al*., 2010). The parameters used were GTR+invgamma, 1×10^6 generations, sampling every 1000 generations with a burning fraction of 0.25. The phylogenetic tree generated in CIPRES was visualized and edited in ITOL (Letunic and Bork, 2019).

Chemical dedikaryotization

Fresh cultures on MEA plates with mycelium just reaching the plate edge were blended (Waring Blender, Stamford, Connecticut, USA) 5-30 s for *L. edodes* or 60-120 s for *P. ostreatus*. The homogenized solution (50 µL) was added to flasks containing 50 mL of dedikaryotization solution (DS; 30 g L^{-1} Peptone P, Oxoid ™, and 20 g L^{-1}

Table 1. Accession numbers of sequences.

Accession	Species	Origin Country	Reference
JX429942.1	*Pleurotus eryngii*	China	Avin *et al.* (2014)
HM561986.1	*Pleurotus eryngii*	China	NCBI (1988)
LC149608.1	*Pleurotus ostreatus*	Nepal	NCBI (1988)
MN179421.1	*Pleurotus pulmonarius*	NS	NCBI (1988)
AY450349.1	*Pleurotus pulmonarius*	Sweden	NCBI (1988)
KY962483.1	*Pleurotus calyptratus*	South Korea	NCBI (1988)
AY450338.1	*Pleurotus calyptratus*	Austria	NCBI (1988)
KY886808.1	*Pleurotus djamor*	NS	NCBI (1988)
GU722274.1	*Pleurotus djamor*	Mexico	Huerta *et al.* (2010)
AY540319.1	*Pleurotus citrinopileatus*	NS	NCBI (1988)
AY540318.1	*Pleurotus citrinopileatus*	NS	NCBI (1988)
MK169240.1	*Pleurotus dryinus*	Croatia	NCBI (1988)
JF908617.1	*Pleurotus dryinus*	Italy	NCBI (1988)
AB733141.1	*Pleurotus cystidiosus*	Japan	NCBI (1988)
AY315766.1	*Pleurotus cystidiosus*	USA	Zervakis *et al.* (2003)
MH859080.1	*Agaricus bisporus*	NS	Vu *et al.* (2017)
AB286064.1	*Lentinula edodes*	NS	NCBI (1988)
EU021472.1	*Lentinula edodes*	NS	NCBI (1988)
AF031181.1	*Lentinula lateritia*	NS	Hibbett *et al.* (1998)
AF031192.1	*Lentinula lateritia*	NS	Hibbett *et al.* (1998)
AY016442.1	*Lentinula raphanica*	Puerto Rio	Mata *et al.* (2000)
AF356168.1	*Lentinula raphanica*	NS	Hibbett *et al.* (2001)
AF031178.1	*Lentinula boryana*	NS	Hibbett *et al.* (1998)
MK910772.1	*Schizophyllum commune*	South Korea	NCBI (1988)
KP171570.1	*Saccharomyces cerevisiae*	NS	NCBI (1988)

glucose) and stored at 24 °C for 16 d for *L. edodes* and 2 d for *P. ostreatus*, respectively.

In the case of *P. ostreatus* strains, when the mycelium was visible in the DS, liquid cultures were blended for a second time (1 min), and 25 µL were used to inoculate on MEA plates, which were incubated at 24 °C until mycelial colonies were observed. The same procedure was followed with *L. edodes* strains but skipping homogenization for a second time (Valenzuela *et al*., 2017). The MEA plates were observed daily to identify monokaryotic colonies (lacking clamp connections).

Classification of neohaplonts and production of hybrids

To recognize the compatibility types of each parental

strain, a single neohaplont was paired with all the neohaplonts of the same strain. MEA plates were then stored at 24 °C and observed under the microscope (10X) to identify the presence or absence of clamp connections; then, the dikaryotic strains were transferred into other MEA petri dishes to confirm the presence of clamp connections

ISSR profile

Seven ISSR primers were used to obtain ISSR profiles of parental, reconstituted hybrid strains and neohaplonts (Table 2). The PCR reactions contained 22.5 µL PCR SuperMix (Cat. No. 10572-014, Invitrogen), DNA (30-50 ng μL^{-1}), ISSR primer (20 µM) and distilled water to obtain a final volume of 25 µL. PCR steps were: initial denaturation, 5 min at 94 °C; 35 cycles of denaturation, 60 s at 94 °C; anneal primers, 60 s at 55 to 64 °C; extension, 3 min at 72 °C, and final extension, 10 min at 72 °C. Amplicons were separated in 1.5 % agarose electrophoresis gels using TAE and stained with GelRed™. Gels were run at 100 V for 90 min and visualized with a UV Transilluminator (ChemiDOC™ MP Imaging de Biorad® System, Hercules, California, USA) (Sánchez *et al*., 2019).

Statistical analysis

An χ^2 test was applied to determine the symmetry in the recovery of neohaplonts of parental strain using the SPSS program (Valenzuela *et al.*, 2017). Data generated from seven ISSR primers for all strains were entered into a presence/absence matrix. Euclidean distance was calculated from the matrix, and the R function (fviz_dend) was used to generate a dendrogram in R. To evaluate genetic profiles, marker parameters were recovered from electrophoresis gels: total bands (TB), monomorphic bands (MB) and polymorphic bands (PB) were used to calculate the percentage of polymorphism (PP). To evaluate primer efficacy, other marker parameters were calculated: effective multiplex ratio (EMR), resolving power (RP), marker index (MI) and polymorphic information content (PIC) (Roldán-Ruiz *et al*., 2000; Varshney *et al*., 2007).

Production of wheat grain

Wheat grains were hydrated for 24 h; then, 200 g of hydrated wheat grains were placed in a plastic bag and sterilized for 2 h at 121 ºC and 1.05 kg cm^{-2}. Once the bags were cooled , each bag was inoculated with a fresh culture on an MEA plate (mycelium just reaching the plate edge) of the parental and hybrid strains. Inoculated bags were incubated for two weeks at 25 ºC.

Production of substrates

Two types of substrates were prepared. Substrate M1 for the fruiting of *Lentinula* strains contained 40 % white oak sawdust, 40 % cotton husk, 16 % wheat bran, 3 % $CaCO_3$ and 1 % $CaSO_4$ with a final moisture of 64.5%. Substrate M2 for fruiting of *Pleurotus* strains contained 40 % pepper tree sawdust, 29 % cotton husk, 29 % wheat straw, 9 % millet, 3 % $CaCO_3$ and 1 % $CaSO_4$ with a final moisture of 72 %. Hybrid strains were cultivated on both substrates. Bags were filled with 0.5 kg of each substrate and sterilized for 2 h at 121 ºC and 1.05 kgf cm^{-2}. After that, bags were inoculated with wheat grain spawn fully colonized by the mycelium of the corresponding parental or hybrid strains and incubated for 2 weeks at 25 ºC.

Production of fruiting bodies

Bags with the fully colonized substrates by the mushroom mycelium were transferred into the fructification room at 18-20 ºC and a relative humidity of 80-95 %. Mushrooms were weighted and biological efficiency was calculated [(g of moist mushrooms/g of dry substrate) × 100].

Recovery of meiotic progeny and classification into compatibility types

The spore print of fruiting bodies of *L. edodes* × *P. ostreatus* hybrids was collected by placing the fruiting body on filter paper for 24 h at room temperature and later preserved at 2ºC. A segment of 1 cm^2 of filter paper with spore print was cut and vortexed with 1 mL of distilled water in a tube. Serial dilutions were done (10^{-1} and 10^{-5}) and 50 µL of each dilution was inoculated on EMA Petri dishes; once spores germinated, mycelia were transferred to new AEM Petri dishes and verified for their monokaryotic status. The monosporic colonies were classified into four compatibility types by cross-mating all monokaryotic isolates.

RESULTS AND DISCUSSION

Molecular identification of parental strains

The amplification by PCR of the ITS1-5.8r-ITS2 region produced a 756 bp amplicon for L5, L9 and L15 strains, and 676 bp for PAsp14. The sequences were uploaded to the NCBI platform and accession numbers were generated: PAsp14 (KX683410), L5 (KX683413), L9 (KX683412) and L15 (KX683411).

The phylogenetic tree (Figure 1) grouped the strains of the two genera (*Pleurotus* and *Lentinula*) in different clusters. The sporeless strain PAsp14 of *Pleurotus* clustered with

sequences from species of *P. ostreatus*. The strains of *Lentinula* (L5, L9 and L15) clustered with sequences of *L. edodes*. Different phylogenies based on analysis of the ITS1-5.8r-ITS2 region allowed the identification of strains studied.

Dedikaryotization of parental strains and production of hybrid strains

The recovery of neohaplonts (nh) from three strains of *Lentinula* (L5, L9 and L15) and a *Pleurotus* strain (PAsp14) was successful. Neohaplonts were recovered for all strains: L5 (10 nh), L9 (9 nh), L15 (9 nh) and PAsp14 (16 nh). Matings among neohaplonts of the same strain allowed the identification of the compatibility types (TI and TII, respectively). The χ^2 test determined that recovery of the strains L9 (five neohaplonts of TI and four neohaplonts of TII) and L15 (seven neohaplonts of TI and two neohaplonts of TII) were symmetric, while for strains L5 (nine neohaplonts of TI and one neohaplont of TII) and PAsp14 (two neohaplonts of TI and 14 neohaplonts of TII) were asymmetric. Chemical dedikaryotization was feasible for *L. edodes* using short homogenization time (5 to 20 s) and glucose and peptone concentrations between 20 and 30 g L^{-1}. The effect of homogenization represented an essential factor for the recovery of the neohaplonts.

Fukumasa-Nakai *et al*. (1994) reported recovery of the neohaplonts of *L. edodes* by production of protoplasts, while Valenzuela *et al.* (2017) reported recovery of neohaplonts of *L. edodes* using chemical dedikaryotization with short homogenization times (5 s), the shortest times being more effective. In this research, the shortest homogenization times allowed low damage to mycelia; consequently, the monokaryotic components were recovered. To recover the monokaryotic components of strain PAsp14,

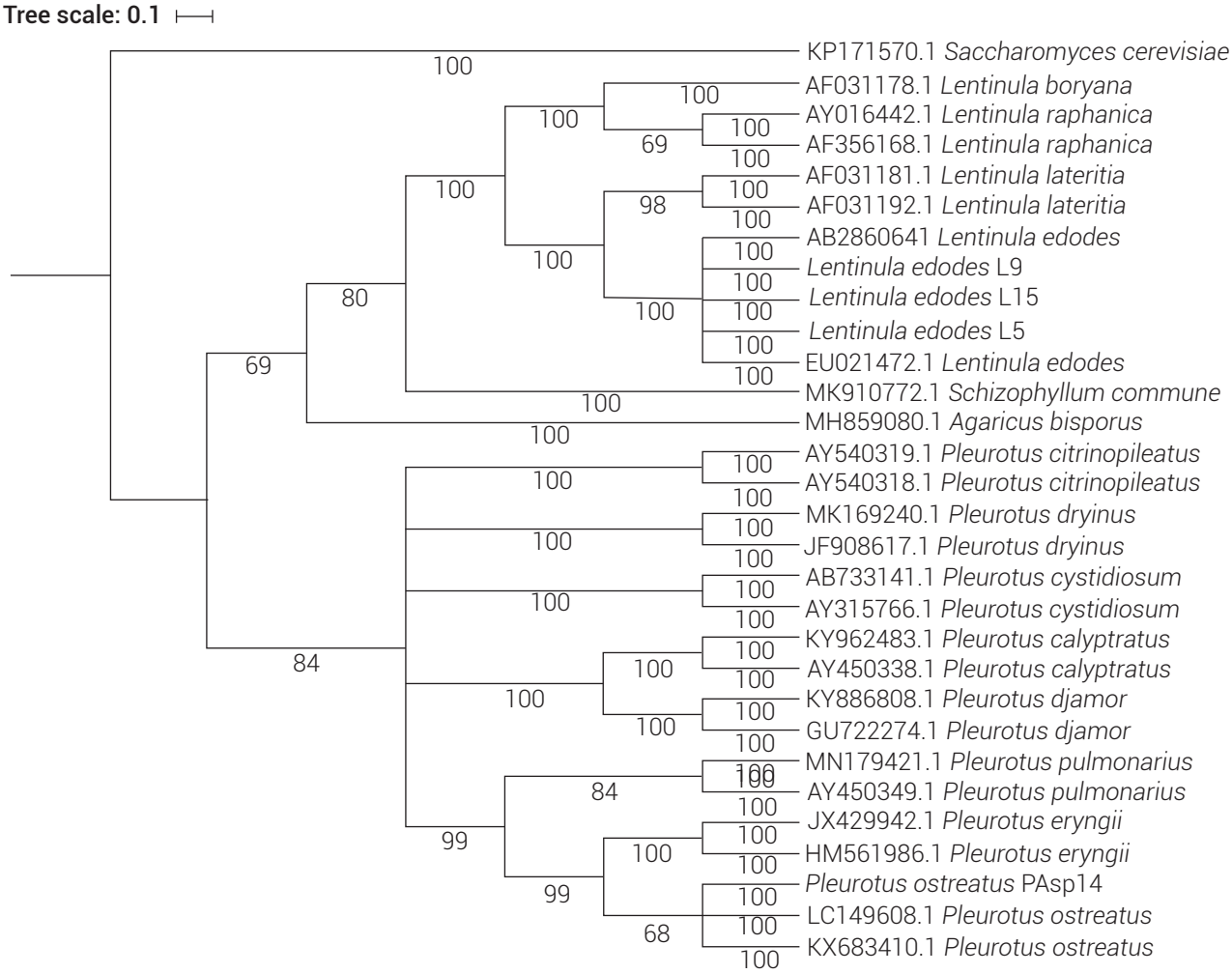


Figure 1. Phylogenetic tree based on the Bayesian inference analysis of the ITS1-5.8r-ITS2 region from strains studied compared to selected sequences from the GenBank.

homogenization for 1 min was necessary, while Valencia and Leal-Lara (1999) reported homogenization times of 2.5 min to recover neohaplonts of *Pleurotus* spp. strains.

To produce intergeneric hybrids of *L. edodes* and *P. ostreatus* strains, one neohaplont was randomly chosen of each compatible type of each *L. edodes* strain (L5, L9 and L15) and mated with all the neohaplonts of PAsp14 in all possible combinations. Twelve intergeneric hybrids were obtained (Table 3).

The production of intergeneric hybrids has been previously reported. Valenzuela *et al*. (2017) produced intergeneric hybrids by mating neohaplonts of *L. edodes* × *P. ostreatus*. Ramírez-Carrillo *et al*. (2007) reported the production of interspecific (*P. eryngii* × *P. ostreatus*) and intergeneric (*P. eryngii* × *L. edodes*) hybrids. Hybrid strains between *P. florida* × *Volvariella volvacea* (Chakraborty and Sikdar, 2008) and *P. florida* × *Calocybe indica* (Chakraborty and Sikdar, 2010). *Pleurotus florida* × *L. edodes* were also obtained by protoplast fusion (Mallick and Sikdar, 2014). Chemical dedikaryotization and protoplast fusion allow the production of hybrids, either intra- or interspecies; however, hybrid production by chemical dedikaryotization offers the advantage of recovering the monokaryotic components that constitute the parental strain. However, producing hybrids by protoplast fusion does not involve the recovery of neohaplonts; thus, molecular characterization is restricted only to parental and hybrid strains (Chakraborty and Sikdar, 2008; Mallik and Sikdar, 2014).

By the aforementioned techniques, interspecies and intergeneric strains can be produced, and thus, some incompatibility barriers are broken among different species. This process cannot be achieved by standard mating of monosporic isolates (Worrall, 1997). Remarkably, Valenzuela *et al*. (2017) reported similar compatibility types among neohaplonts of *L. edodes* and *P. ostreatus*, which suggests a considerable similarity in the compatibility system of these two species.

Interspecific hybridization in fungi has been reported in nature as a mechanism for DNA exchange where two genomes from different species are merged into a single, often highly unstable, polyploid genome that evolves rapidly into stabler derivatives, the resulting interspecies hybrids can show novel combinations of genes and phenotypic variation that enhance fitness and allow colonization of new niches (Steensels *et al*., 2021). In mushrooms, genetic improvement is widespread in producing interspecies hybrids to generate strains with high yields or different phenotypes.

Molecular characterization with ISSR markers

Seven ISSR markers were used to characterize 10 strains (Table 2). Figure 2 shows electrophoresis gel from amplified products using the ISR11 marker. The bands by ISSR markers had reproducibility in triplicated PCR essays. Similar results have been published by Mallick and Sikdar (2014) while Ramírez-Carrillo *et al*. (2007) reported that intergeneric hybrids of *Pleurotus* spp. × *L. edodes* presented *Pleurotus* phenotype. Mallik and Sikdar (2014) generated a band profile with six ISSR markers of *L. edodes* × *P. florida* hybrids and their parental strains. Chakraborty and Sikdar (2008) mentioned that recombinant bands proved that the hybrid lines were not heterokaryons of the two parental nuclei; instead, the dikaryons fused after protoplast fusion to form a synkaryon followed by recombination without meiosis and can be present in a possible elimination of different chromosomes.

Figure 3 shows the dendrogram constructed from Euclidean distance. Strains were separated into two groups. The yellow cluster grouped the *Lentinula* strains L9 (parental), L9R (reconstituted) and neohaplonts of L9 (L9nh5a and L9nh8a), while the blue cluster grouped the *Pleurotus* strains PAsp14 (parental), PAsp14R (reconstituted), neohaplonts (PAsp14nh1a and PAsp14-nh12a) and intergeneric hybrids (L9-nh5a × PAsp14-nh12a and L9-nh8a × Pasp14-nh12a), the whole cluster (dikaryotic strains) expressed *P. ostreatus* phenotype, showing a molecular similarity based on ISSR markers.

Morales and Dujon (2012) reported that the hybrids of natural hybridization of different yeast species show post-hybridization events for genome stabilization. These events included a chromosomal rearrange and modification of genetic contributions from parentals by aneuploidization and partial loss of chromosomes. The elimination of chromosomes in one species can stimulate the phenotypic expression of the other species that retained more chromosomes. For this reason, the dominance of *P. ostreatus* phenotype may be related to a loss of genetic material of *L. edodes* caused by a chromosomal rearrangement for genetic stabilization induced by intergeneric hybrid production.

The number of amplified products (TB) with distinct markers ranged from 12 to 21 different bands (Table 2). The UBC 807 was the primer that produced the largest number of bands (21), while the marker with fewer bands was UBC 811 (12). The seven markers studied generated 114 bands/amplicons with a mean of 16.29 by marker; 104 bands (91.31 %) were polymorphic (with an average of 14.86 PB by marker) and 10 bands (8.69 %) were monomorphic. Polymorphism oscillated from 73.33 % for ISR markers

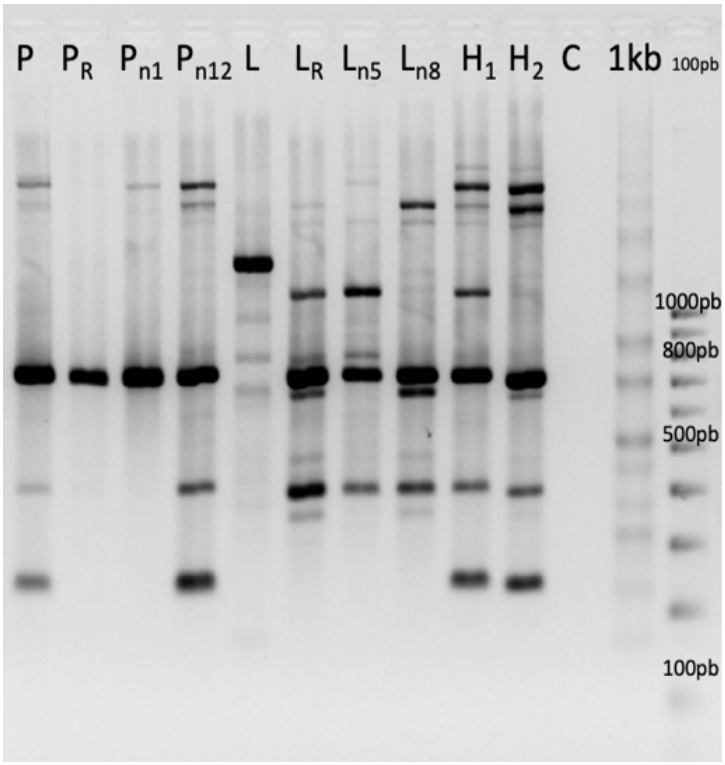


Figure 2. Polymorphism profile of neohaplonts and dikaryotic strains using the ISR 11 marker. Strains: PAsp14 (P), PAsp14R (P_R), PAsp14-nh1a (P_{n1}), PAsp14-nh12a (P_{n12}), L9 (L), L9R (L_R), L9-nh5a (L_{n5}), L9nh8a (L_{n8}), L9-nh5a×PAsp14-nh12 (H_1), L9-h8a×Pasp14nh12a (H_2), Control (C), 100 bp ladder (100pb), 1kb plus ladder (1kb).

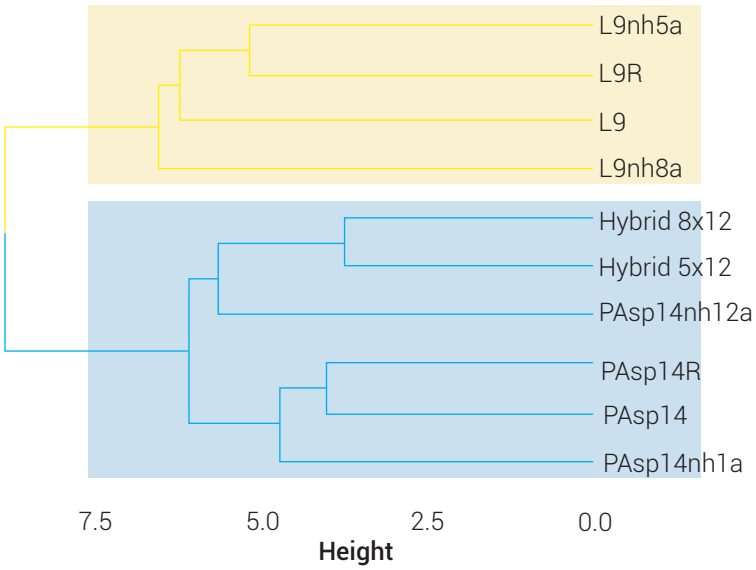


Figure 3. Dendrogram constructed from euclidean distance. *Lentinula* strains: L9, L9R, L9-nh5a, L9-nh8a and *Pleurotus* and hybrid strains: PAsp14, PAsp14R, PAsp14-nh1a, PAsp14-nh12a, L9-nh5a×PAsp14-nh12 (Hybrid 5 × 12) y L9-nh8a×PAsp14-nh12a (Hybrid 8 × 12).

Table 2. Marker parameters calculated for each ISSR primer.

Primer	Sequence	T (°C)	TB	PB	MB	PPB (%)	PIC	EMR	MI	RP
ISR 11	$(CAC)_3GC$	56.3	17	16	1	94.12	0.37	7.62	2.81	9.4
ISR 12	$(GAG)_3GC$	56.3	17	13	4	76.47	0.30	8.56	2.60	8.4
UBC 807	$(AG)_3T$	55.0	21	20	1	95.24	0.41	10.10	4.10	14
UBC 811	$(GA)_3C$	55.0	12	12	0	100	0.41	6.40	2.62	8
ISR 15	$(GCA)_3CT$	53.2	19	19	0	100	0.36	10.60	3.84	10.4
ISR 02	$(CAG)_3$	63.8	13	13	0	100	0.41	5.50	2.28	8.6
ISR	$(AAGAG)_3$	56.9	15	11	4	73.33	0.31	7.55	2.37	7.8
Total			114	104	10					
Avg./ Primer		56.6	16.29	14.86	1.43	91.31	0.37	8.05	2.94	9.51

TB: total bands, PB: polymorphic bands, MB: monomorphic bands, PPB: percentage of polymorphic bands, PIC: polymorphic information content, EMR: effective multiplex ratio, MI: marker index, RP. resolving power.

Table 3. Productivity and phenotype of parental and hybrid strains studied.

Strains	Biological efficiency without stipe (μ ± σ)		Morphologic characteristics of pileus		Phenotype
	Substrate type		Color	Diameter (cm)	
	M1	M2			
PAsp14	76.9 ± 3 .6†	-	Gray	5-7	*Pleurotus*
L5	-	56.36 ± 20.65	Dark brown	5-6	*Lentinula*
L9	-	76.91 ± 8.50	Dark brown	4-5	*Lentinula*
L15	-	29.64 ± 3.6	Dark brown	4-5	*Lentinula*
L5-3 × P-1	56.20 ± 1.70	33.09 ± 25.45	Dark brown	5-6	*Pleurotus*
L5-4 × P-1	60.12 ± 6.15	66.92 ± 19.28	White	5-6	*Pleurotus*
L5-3 × P-12	56.22 ± 5.49	27.83 ± 6.33	Light brown	5	*Pleurotus*
L5-4 × P-12	87.85 ± 5.70	28.67 ± 7.31	White	7-15	*Pleurotus*
L9-5 × P-1	57.70 ± 55.22	56.13 ± 2.43	Light brown	7-9	*Pleurotus*
L9-5 × P-12	48.19 ± 17.01	53.88 ± 40.19	Light brown	5-7	*Pleurotus*
L9-8 × P-1	52.15 ± 6.91	47.91 ± 6.29	Light brown	5-6	*Pleurotus*
L9-8 × P-12	26.72 ± 0.09	36.06 ± 10.17	Light brown	5-7	*Pleurotus*
L15-15 × P-1	56.64 ± 3.92	41.12 ± 26.07	Dark brown	7	*Pleurotus*
L15-8 × P-1	34.18 ± 3.91	96.55 ± 10.40	White	8-10	*Pleurotus*
L15-15 × P-12	74.03 ± 6.53	45.96 ± 14.42	Dark brown	6-7	*Pleurotus*
L15-48 × P-12	51.70 ± 0.04	48.83 ± 19.46	White	7-10	*Pleurotus*

†All values are means ± standard deviation of three replicates.



2A. PAsp14 2B. L9



2C. L9-nh5a × PAsp14-nh12 2D.L9-nh8a × PAsp14nh12a

Figure 4. Phenotipical diversity of parental (A-B) and hybrid strains (C-D).

up to 100 % polymorphism for markers UBC811, ISR 15 and ISR 02. The PPB is linked to genetic variability. Yin *et al*. (2014) reported an average polymorphism with ISSR markers of 74 % for 15 *P. pulmonarius* strains in China. A higher PPB value was observed in this study, *i.e*. 91.31 %, indicating that a high genetic diversity was generated when neohaplonts from different species were mated.

The PIC values indicate that both states (presence/absence) are in different proportions. In this research, the PIC average was 0.37, and ranged from 0.30 (ISR12) to 0.41 (UBC 807, UBC 811 and ISR 02). Mallick and Sikdar (2014) reported an average PIC of 0.475; however, Zhang *et al*. (2015) reported PIC values with SCoT markers ranging from 0.20 to 0.50, while other researchers (Gorji *et al*., 2011; Kumar *et al*., 2014) reported PIC values with ISSR markers ranging from 0.15 to 0.49. The EMR values show the number of non-polymorphic loci analyzed by marker systems (Varshney *et al*., 2007). In this research, the EMR average was 8.05 per primer, the major value (10.60) was observed with ISR 15, and the minimum (5.50) with ISR02. Badfar-Chaleshtori *et al*. (2012) mentioned that MI supplies an approximation of polymorphism generated per essay for a certain primer and they obtained values from 0.019 to 4.7. In this research, an MI average of 2.94 was obtained, the lowest one was 2.60 for ISR 12, while the highest was 4.10 for UBC 807. The EMR and MI parameters were used to calculate the resolving power (RP) for each marker, indicating the discriminatory potential of the primers chosen (Kumar *et al.,* 2014). The highest value of RP was obtained by marker UBC 807 (14) and the lowest one by marker ISSR 15 (7.8). Badfar-Chaleshtori *et al*. (2012) and Kumar *et al*. (2014) reported values of genetic variation in the population from one species ranging from 0.1 to 8.56.

Fructification of hybrid strains of *L. edodes* × *P. ostreatus* and classification of a meiotic progeny

The intergeneric hybrids of *P. ostreatus* × *L. edodes* were fructified on two different substrates: M1 (*Pleurotus* commercial substrate) and M2 (*Lentinula* commercial substrate) (Table 3). All hybrid strains produced fruit bodies with a *P. ostreatus* phenotype (Figure 4) regardless of the substrate type, this agrees with previous studies reporting that *P. ostreatus* phenotype was predominantly expressed by *Lentinula* × *Pleurotus* hybrids (Mallick and Sikdar, 2014; Ramírez-Carrillo *et al*., 2007; Valenzuela *et al.,* 2017).

Noteworthy, although biological efficiencies produced by hybrid strains in this study varied widely depending on the type of substrate, similar biological efficiencies on both types of substrates were the pattern most frequently observed (Table 3). The biological efficiency ranged from 50 to 60 % on both substrates (M1 and M2) by hybrids L5-4 × P-1, L9-5 × P-1, L9-5 × P-12, L9-8 × P-1 and L15-48 × P-12. By contrast, the hybrid L5-4 × P-12 produced the highest yield on the M1 substrate (87.85 ± 5.70) among all hybrids, which was markedly higher than the biological efficiency produced on the M2 substrate by this strain (28.67 ± 7.3). On the other hand, the hybrid L15-8 × P-1 produced the highest yield on the M2 substrate (96.55 ± 10.40) among all hybrids, which was markedly higher than the biological efficiency produced on the M1 substrate by this strain (34.18 ± 3.91). This variation of biological efficiency depending on substrate composition may be related to the prevalence of certain genes due to genetic stabilization after the hybridization process, as Morales and Dujon (2012) observed with the natural hybridization of different yeast species.

Noteworthy, the analysis of the compatibility types in the progenies of interspecies hybrid strains of edible mushrooms has not been reported in previous studies. One hybrid strain (L9-5 × P-12) was selected randomly to classify its meiotic progeny. Monosporic strains were classified by mating tests in four compatibility types. Remarkably, this monokaryotic progeny derives from an intergeneric hybrid where both parental strains have a heterothallic tetrapolar compatibility system. The resulting meiotic progeny presented the same heterothallic tetrapolar compatibility system; 22 monosporic strains were recovered and classified (6 for T I, 3 for T II, 8 for T III, and 5 for T IV). Compatibility types I and II were identified as the parental types, corresponding to parental neohaplonts L9-5 and P-12, respectively, while III and IV were the recombinants. Noticeably, the mating capability in the resulting progeny was maintained as a heterothallic tetrapolar system, which is required for producing fruiting bodies.

This represents an important contribution to this type of studies since Gallone *et al.* (2019) and Morales and Dujon (2012) reported that interspecies hybridization with yeasts is a frequent event, but meiotic fertility is not so common; however, the interspecies hybridization in Basidiomycota has been reported in multiple articles. Chen *et al.* (2016) studied inter- and intra-specific diversity in *Agaricus endoxanthus* and closer species, which revealed a new taxon, *A. punjabensis*, since the ITS sequences from *A. endoxanthus* were highly variable but were separated from *A. punjabensis*. In addition, numerous heteromorphisms and allelic distribution at certain loci indicate that hybridization and recombination have likely occurred throughout the history of certain strains. Moreover, Orihara *et al*. (2021) reported a robust multilocus phylogenetic analysis of the genus *Octaviania* (Boletaceae) suggests that heterogenous sequences are probably the result of previous inter- and intra-specific hybridization and may have promoted the high genetic and species diversity found within the genus.

CONCLUSIONS

The recovery of neohaplonts was successful from three strains of *L. edodes* and one strain of *P. ostreatus*; however, it was symmetric only for two strains (L9 and L15), and a short time of homogenization was the most important factor for the recovery of neohaplonts, particularly for *L. edodes* strains. PAsp14 neohaplonts of *P. ostreatus* were mated with neohaplonts of strains L5, L9 and L15 of *L. edodes*. Twelve intergeneric hybrids were produced, all of them showing a *Pleurotus* phenotype. The dendrogram generated by molecular characterization grouped the strains into two clusters; the *L. edodes* strains were classified in the first group, while the intergeneric hybrids of *Pleurotus* × *Lentinula* were classified together with the parental *Pleurotus* strain PAsp14, both the original and the reconstituted strain. This matched the *Pleurotus* fruit body phenotypes shown by all the intergeneric hybrids. The molecular analysis using ISSR markers indicated a closer genetic relationship of these intergeneric hybrids to *P. ostreatus* neohaplonts, explaining thus the expression of *Pleurotus* phenotype in their fruit bodies. Finally, a successful mating capability was shown in the meiotic progeny of an intergeneric hybrid from two distinct edible mushrooms (*Pleurotus* and *Lentinula*).

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BIBLIOGRAPHY

Aguilar D. L., P. B. Zárate S., R. Villanueva A., J. Yáñez F., M. E. Garín A., P. C. Guadarrama M. and G. Valencia T. (2018) Use of ITS and ISSR markers in the molecular characterization of *Pleurotus djamor* hybrid strains. *Revista Iberoamericana de Micología* 35:49-55, https://doi.org/10.1016/j.riam.2017.06.003

Anné J. and J. F. Peberdy (1981) Characterization of inter-specific hybrids between *Penicillium chrysogenum* and *P. roqueforti* iso-enzyme analysis. *Transactions of the British Mycological Society* 77:401-408, https://doi.org/10.1016/s0007-1536(81)80043-5

Avin F. A., S. Bhassu, T. Y. Shin and V. Sabaratnam (2012) Molecular classification and phylogenetic relationships of selected edible Basidiomycetes species. *Molecular Biology Reports* 39:7355-7364, https://doi.org/10.1007/s11033-012-1567-2

Avin F. A., S. Bhassu, Y. S. Tan, P. Shahbazi and S. Vikineswary (2014) Molecular divergence and species delimitation of the cultivated oyster

mushrooms: integration of IGS1 and ITS. *The Scientific World Journal* 2014:793414, https://doi.org/10.1155/2014/793414

Avin F. A., S. Bhassu, V. Rameeh, Y. S. Tan and S. Vikineswary (2016) Genetics and hybrid breeding of *Pleurotus pulmonarius*: heterosis, heritability and combining ability. *Euphytica* 209:85-102, https://doi.org/10.1007/s10681-016-1638-x

Badfar-Chaleshtori S., B. Shiran, M. Kohgard, H. Mommeni, A. Hafizi, M. Khodambashi, … and K. Sorkheh (2012) Assessment of genetic diversity and structure of Imperial Crown (*Fritillaria imperialis* L.) populations in the Zagros region of Iran using AFLP, ISSR and RAPD markers and implications for its conservation. *Biochemical Systematics and Ecology* 42:35-48, https://doi.org/10.1016/j.bse.2011.12.027

Chakraborty U. and S. R. Sikdar (2008) Production and characterization of somatic hybrids raised through protoplast fusion between edible mushroom strains *Volvariella volvacea* and *Pleurotus florida*. *World Journal of Microbiology and Biotechnology* 24:1481-1492, https://doi.org/10.1007/s11274-007-9630-1

Chakraborty U. and S. R. Sikdar (2010) Intergeneric protoplast fusion between *Calocybe indica* (milky mushroom) and *Pleurotus florida* aids in the qualitative and quantitative improvement of sporophore of the milky mushroom. *World Journal of Microbiology and Biotechnology* 26:213-225, https://doi.org/10.1007/s11274-009-0162-8

Chen J., L. A. Parra, A. de Kesel, A. N. Khalid, T. Qasim, A. Ashraf, … and P. Callac (2016) Inter- and intra-specific diversity in *Agaricus endoxanthus* and allied species reveals a new taxon, *A. punjabensis*. *Phytotaxa* 252:1-16, https://doi.org/10.11646/phytotaxa.252.1.1

Eger G., G. Eden and E. Wissig (1976) *Pleurotus ostreatus* breeding potential of a new cultivated mushroom. *Theoretical and Applied Genetics* 47:155-163, https://doi.org/10.1007/BF00278373

Fries N. and K. Aschan (1952) The physiological heterogeneity of the dikaryotic mycelium of *Polyporus abietinus* investigated with the aid of microsurgical technique. *Svensk Botanisk Tidskrift* 46:429-445.

Fukumasa-Nakai Y., T. Matsumoto and M. Komatsu (1994) Dedikaryotization of the Shiitake mushroom, *Lentinula edodes* by the protoplast regeneration method. *The Journal of General and Applied Microbiology* 40:551-562.

Gallone B., J. Steensels, S. Mertens, M. C. Dzialo, J. L. Gordon, R. Wauters, … and K. J. Verstrepen (2019) Interspecific hybridization facilitates niche adaptation in beer yeast. *Nature Ecology and Evolution* 3:1562-1575, https://doi.org/10.1038/s41559-019-0997-9

Gorji A. M., P. Poczai, Z. Polgar and J. Taller (2011) Efficiency of arbitrarily amplified dominant Markers (SCOT, ISSR and RAPD) for diagnostic fingerprinting in tetraploid potato. *American Journal of Potato Research* 88:226-237, https://doi.org/10.1007/s12230-011-9187-2

Hibbett D. S. (2001) Shiitake mushrooms and molecular clocks: historical biogeography of *Lentinula*. *Journal of Biogeography* 28:231-241, https://doi.org/10.1046/j.1365-2699.2001.00528.x

Hibbett, D. S., K., Hansen and M. J. Donoghue (1998) Phylogeny and biogeography of *Lentinula* inferred from an expanded rDNA dataset. *Mycological Research* 102:1041-1049, https://doi.org/10.1017/S0953756297005996

Huerta G., D. Martínez-Carrera, J. E. Sánchez, H. Leal-Lara and R. Vilgalys (2010) Genetic relationships between Mexican species of *Pleurotus* analyzing the ITS-region from rDNA. *Micología Aplicada International* 22:15-25.

Katoh K., J. Rozewicki and K. D. Yamada (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 20:1160-1166, https://doi.org/10.1093/bib/bbx108

Kumar A., P. Mishra, S. C. Singh and V. Sundaresan (2014) Efficiency of ISSR and RAPD markers in genetic divergence analysis and conservation management of *Justicia adhatoda* L., a medicinal plant. *Plant Systematics and Evolution* 300:1409-1420, https://doi.org/10.1007/s00606-013-0970-z

Kumara K. L. W. and I. C. S. Edirimanna (2009) Improvement of strains of two oyster mushroom cultivars using duel culture technique. *World Applied Sciences Journal* 7:654-660, https://doi.org/10.1155/2014/793414

Larsson A. (2014) AliView: a fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics* 30:3276-3278, https://doi.org/10.1093/bioinformatics/btu531

Leal-Lara H. and G. Eger-Hummel (1982) A monokaryotization method and its use for genetic studies in wood-rotting basidiomycetes. *Theoretical and Applied Genetics* 61:65-68, https://doi.org/10.1007/BF00261513

Letunic I. and P. Bork (2019) Interactive Tree of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Research* 47:2-5, https://doi.org/10.1093/nar/gkz239

Maddison W. P. and D. R. Maddison (2019) Mesquite: a modular system for evolutionary analysis. Version 3.61. Sacramento, California, USA. http://www.mesquiteproject.org (November 2024).

Mallick P. and S. R. Sikdar (2014) Production and molecular characterization of somatic hybrids between *Pleurotus florida* and *Lentinula edodes*. *World Journal of Microbiology and Biotechnology* 30:2283-2293, https://doi.org/10.1007/s11274-014-1652-x

Mata J. L., R. H. Petersen and K. W. Hughes (2001) The genus *Lentinula* in the Americas. *Mycologia* 93:1102-1112, https://doi.org/10.2307/3761671

Miller M. A., W. Pfeiffer and T. Schwartz (2010) Creating the CIPRES science gateway for inference of large phylogenetic trees. *In*: Proceedings of the 2010 Gateway Computing Environments Workshop (GCE). 14 Nov. 2010. IEEE Computer Society. New Orleans, Louisiana, USA. pp:1-8, https://doi.org/10.1109/GCE.2010.5676129.

Morales L. and B. Dujon (2012) Evolutionary role of interspecies hybridization and genetic exchanges in yeasts. *Microbiology and Molecular Biology Reviews* 76:721-739, https://doi.org/10.1128/mmbr.00022-12

NCBI, National Center for Biotechnology Information (1988) National Library of Medicine. National Center for Biotechnology Information. Bethesda, Maryland, USA. https://www.ncbi.nlm.nih.gov/ (Septemper, 2024).

Orihara T., R. Healy, A. Corrales and M. E. Smith (2021) Multilocus phylogenies reveal three new truffle-like taxa and the traces of interspecific hybridization in *Octaviania* (Boletaceae, Boletales). *IMA Fungus* 12:14, https://doi.org/10.1186/s43008-021-00066-y

Peberdy J. F. (1980) Protoplast fusion – a tool for genetic manipulation and breeding in industrial microorganisms. *Enzyme and Microbial Technology* 2:23-29, http://doi.org/10.1016/0141-0229(80)90004-6

Ramírez-Carrillo R., O. Hernández-Vargas, F. Galván-Pallach y H. Leal-Lara (2007) Productividad de cepas híbridas de *Pleurotus* × *Lentinula*. *In*: El Cultivo de Setas *Pleurotus* ssp. en México. J. E. Sánchez, D. Martínez-Carrera, G. Mata y H. Leal (eds.). ECOSUR. Tapachula, Chiapas, México. 236 p.

Roldán-Ruiz I., J. Dendauw, E. Van Bockstaele, A. Depicker and M. De Loose (2000) AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium* spp.). *Molecular Breeding* 6:125-134, https://doi.org/10.1023/A:1009680614564

Royse D., J. Baars and Q. Tan (2017) Current overview of mushroom production in the world. *In*: Edible and Medicinal Mushrooms: Technology and Applications. D. C. Zied and A. Pardo-Giménez (eds.). Wiley-Blackwell. Hoboken, New Jersey, USA. pp:5-12, https://doi. org/10.1002/9781119149446.ch4

Sánchez H. A., J. D. Valenzuela C., J. Herrera M., R. Villanueva A., Y. M. Gómez G., P. B. Zarate S., … and G. Valencia T. (2019) Characterization of *Pleurotus djamor* neohaplonts recovered by production of protoplasts and chemical dedikaryotization. *3 Biotech* 9:24, https://doi.org/10.1007/s13205-018-1532-4

Singh M. and S. Kamal (2017) Genetic aspects and strategies for obtaining hybrids. *In*: Edible and Medicinal Mushrooms: Technology and Applications. D. C. Zied and A. Pardo-Giménez (eds.). Wiley-Blackwell. Hoboken, New Jersey, USA. pp:35-87, https://doi. org/10.1002/9781119149446.ch4

Steensels J., B. Gallone and K. J. Verstrepen (2021) Interspecific hybridization as a driver of fungal evolution and adaptation. *Nature Reviews Microbiology* 19:485-500, https://doi.org/10.1038/s41579-021-00537-4

Valencia T. G. y H. Leal-Lara (1999) Estudios de la compatibilidad de cepas de *Pleurotus* spp. con cuerpos fructíferos de diferentes

colores. *Revista Mexicana de Micología* 15:67-71, https://doi.org/10.33885/sf.1999.3.885

Valenzuela C. J. D., E. Durán P., R. Villanueva A., M. E. Garín A., A. Sánchez H., H. Leal L. and G. Valencia T. (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, https://doi.org/10.9755/ejfa.2017.v29.i12.1563

Varshney R. K., K. Chabane, P. S. Hendre, R. K. Aggarwal and A. Graner (2007) Comparative assessment of EST-SSR, EST-SNP and AFLP markers for evaluation of genetic diversity and conservation of genetic resources using wild, cultivated and elite barleys. *Plant Science* 173:638-649, https://doi.org/10.1016/j.plantsci.2007.08.010

Vu D., M. Groenewald, M. de Vries, T. Gehrmann, B. Stielow, U. Eberhardt, … and G. J. M. Verkley (2019) Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. *Studies in Mycology* 92:135-154, https://doi.org/10.1016/j.simyco.2018.05.001

White T. J., T. Bruns, S. J. W. T. Lee and J. Taylor (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: PCR Protocols: A Guide to Methods and Applications. M. A. Innis, D. H. Gelfand, J. J. Sninsky and T. J. White (eds.). Academic Press. New York, USA. pp:315-322, https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Worrall J. J. (1997) Somatic incompatibility in Basidiomycetes. *Mycologia* 89:24-36, https://doi.org/10.1080/00275514.1997.12026751

Yin Y., Y. Liu, H. Li, S. Zhao, S. Wang, Y. Liu, … and F. Xu (2014) Genetic diversity of *Pleurotus pulmonarius* revealed by RAPD, ISSR, and SRAP fingerprinting. *Current Microbiology* 68:397-403, https://doi.org/10.1007/s00284-013-0489-0

Zhang J., W. Xie, Y. Wang and X. Zhao (2015) Potential of start codon targeted (SCoT) markers to estimate genetic diversity and relationships among Chinese *Elymus sibiricus* accessions. *Molecules* 20:5987-6001, https://doi.org/10.3390/molecules20045987

Zervakis G. I., J.-M. Moncalvo and R. Vilgalys (2004) Molecular phylogeny, biogeography and speciation of the mushroom species *Pleurotus cystidiosus* and allied taxa. *Microbiology* 150:715-726, https://doi.org/10.1099/mic.0.26673-0