

MORPHOMOLECULAR CHARACTERIZATION OF *Pleurotus ostreatus* (JACQ. FR.) KUMMER STRAINS IN RELATION TO LUMINOSITY AND TEMPERATURE OF FRUTIFICATION

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ABSTRACT: Temperature is one of the main factors affecting mushrooms development and introduction in new areas. Effects of temperature (15°C and 28°C) and luminosity (120 and 900 lux) were evaluated for eight *P. ostreatus* strains in relation to precocity, yield, pileus area, stalk formation pattern, coloration and handling resistance. Genetic variability of strains was analysed by the Random Amplified Polymorphic DNA (RAPD) method. The Pos 98/37 strain was the only to yield white pileus at 28°C – 900 lux, and grey ones at 15°C and 120 lux. The Pos 96/05 strain, the latest, produced lead-coloured pileus at 15°C, as did the remaining strains at this temperature. Strains cultivated at 15°C did not differ in relation to handling resistance. At 28°C mushrooms were less resistant. In relation to yield, the Pos 98/38 strain was significantly more efficient. The Pos 98/37 strain, at 28°C, as compared to the same strain at 15°C, was more efficient and had an asymmetric stalk formation pattern. Among strains cultivated at 15°C, the stalk formation pattern was symmetric, except for the Pos 97/15 and Pos 97/17 strains. Molecular characterization of the Pos 98/37 strain was 30% similar to the remaining strains. The temperature of fructification and luminosity influence the induction and development of the isolates.

Key words: mushrooms, temperature, morphology of basidiocarps, yield and marked RAPD

CARACTERIZAÇÃO MORFOMOLECULAR DE ISOLADOS DE *Pleurotus ostreatus* (JACQ. FR.) KUMMER EM RELAÇÃO À LUMINOSIDADE E TEMPERATURA DE FRUTIFICAÇÃO

RESUMO: A temperatura é um dos principais fatores que influenciam o desenvolvimento e a introdução de cogumelos em nova áreas. O efeito da temperatura (15°C e 28°C) e a luminosidade (120 e 900 lux) foram avaliados em oito isolados de *P. ostreatus* quanto à precocidade, eficiência biológica, área do pileus, padrão de formação das pencas, coloração e resistência ao manuseio. A variabilidade genética dos isolados foi analisada pelo método “Random Amplified Polymorphic DNA” ou DNA polimórfico amplificado ao acaso (RAPD). O isolado Pos 98/37 foi o único a produzir a 28°C e 900 lux, apresentando píleo branco nessa temperatura e cinza a 15°C e 120 lux. O isolado Pos 96/05, o mais tardio, apresentou píleo chumbo a 15°C a 120 lux, assim como os demais isolados nesta temperatura. Os isolados cultivados a 15°C não diferiram quanto à maior resistência ao manuseio, enquanto a 28°C, os cogumelos obtidos foram mais frágeis. Quanto à eficiência biológica, o isolado Pos 98/38 foi mais eficiente. O isolado 98/37 a 28°C, comparado com o mesmo isolado a 15°C, foi mais eficiente e apresentou padrão de formação de pencas assimétrico. Entre os isolados cultivados a 15°C, o padrão de formação de pencas foi similar exceto nos isolados Pos 97/15 e Pos 97/17. Na caracterização molecular, o isolado Pos 98/37 apresentou 30% de similaridade com os demais. A temperatura de frutificação e a intensidade luminosa influenciaram a indução e o desenvolvimento dos isolados.

Palavras-chave: cogumelos, temperatura de produção, morfologia dos basidiocarpos, eficiência biológica e marcador RAPD

INTRODUCTION

Pleurotus ostreatus is an edible fungus of great biotechnological interest, not only for its ability to grow on numerous agricultural residues to produce mushrooms of high organoleptical quality (Rajarithnam & Bano, 1987), but also because this fungus produces secondary metabolites with pharmaceutical applications and some

proteins of industrial use - amino acids, vitamins, etc. These properties significantly increased its commercial value in the last years (Bunyard et al., 1996; Zervakis & Balis, 1996).

P. ostreatus is extensively produced out in localities with average temperature of 15°C (Zadrazil, 1978). There is, however, a variety of *P. ostreatus* from Thailand, which grows in temperatures above 25°C and is

considered to be *P. ostreatus* var. Florida, described by Eger et al. (1976) and Eger (1978), since it presents cream-coloured to white pileus, and sexual compatibility with the Japanese isolate of *P. ostreatus* (Kinugawa et al., 1997).

The coloration of the pileus is probably related to luminous intensity (Durand, 1976). The coloration of "Shiitake" mushrooms (*Lentinula edodes*) is a characteristic associated to the interactive effect of the temperature and luminous intensity (Przybylowicz & Donoghue, 1990).

The objective of this work was to describe the morphomolecular characteristics of *P. ostreatus* isolates in relation to luminosity and temperature in axenic culture, evaluating its precocity, biological efficiency, pileus area, stalk formation pattern, coloration, handling resistance and molecular characteristics of the isolates, using Random Amplified Polymorphic DNA (RAPD) method.

MATERIAL AND METHODS

Eight isolates of *P. ostreatus* (cultures covered with mineral oil) were used in this experiment carried out in Botucatu, SP, Brazil (22°53'09"S and 48°26'42"W) (Table 1). They were obtained from growers and from re-isolates of the based context of basidiocarp and, under aseptic conditions multiplied in culture media based on sawdust-dextrose-agar (SDA) and incubated at 25°C for seven days (Eira & Minihoni, 1997).

The substrate consisted of sawdust (49.8%), wheat bran (20%), rice bran (20%), sugarcane bagasse (10%) and lime (0.2%). The mixture was conditioned in bottles, with the standardization of 400 and autoclaved at 120°C during 4 hours. Inoculation was achieved through the transference of 0.5 cm diameter circle of culture medium (Eira & Minihoni, 1997) colonized in the bottles with the sterilized sawdust-based substrate. Inoculation was performed in an aseptic chamber and at the temperature of 25°C for 30 days, without light.

After incubation, primordium induction was made by taking off the coverage and adding 2 to 3 cm of water during 4 hours, without thermal shock. Treatments

consisted of, (1) 15 ± 5°C, 120 lux (photoperiod of 12h) and 85 to 90% relative humidity and (2) 28 ± 5°C, 900 lux (photoperiod of 24h) and 85 to 90% relative humidity, set in a completely randomized design (n = 10) (bottles with 400 of humid substrate). Parameters for analyses were: primordium formation and harvest (days), biological efficiency (grams of fresh mushrooms/grams of dry substrate x 100), coloration of the pileus at harvest (visual analysis), size of the harvested basidiocarps (photographic analysis) and handling resistance evaluated by the presence of breakable pileus (easily broken or firm).

Each isolate was photographed after the harvest with a 640 x 480 pixel resolution digital camera. Digital analysis evaluated pileus area, which was related to temperature of fructification, trying to establish a stalk formation pattern and the intensity of coloration of the pileus given by the number of pixels in the grey scale (0 - black to 250 - white), using the Image Tool program (Celso, 1999). The fructification pattern was studied through the analysis of frequency, asymmetry and curvature, crimping to separate the isolates of greater similarity.

The process of differentiation of the genetic material of the isolates followed the RAPD method described by Williams et al. (1990). The genomic DNA was extracted as suggested by Sadowsky et al. (1987) and Kuramae-Izioka (1997). Amplification reactions were performed using a DNA thermal cycler (MJ Research), programmed according to Sambrook et al. (1989). The samples and the Ladder 1 Kb (Gibco BRL Life Technologies, Inc.) molecular marker were applied to 1.5% agarose gel using the TBE buffer (0.1 mol L⁻¹ Tris-HCl; 0.1 mol boric acid L⁻¹; 0.02 mol L⁻¹ EDTA; pH 8.0). The electrophoresis ran on approximately 120 V for 3 hours. The gel was photographed using ultraviolet light. The genetic similarity between the isolates was calculated using program Numerical Taxonomy and Multivariate Analysis System (NTSYS), with the coefficient "simple matching" (SM) and the construction of the dendrogram for the Unweighted Pair-Group method Arithmetic Average (UPGMA) method.

Table 1 - Origin of *P. ostreatus* isolates used in the experiments.

Collection code	Origin	Geographic coordinates		Temperature of fructification	Color of pileus
Pos 96/05	Arujá - SP	23°23'46"S	46°19'15"W	10 to 15°C	dark grey
Pos 97/12	Moji das Cruzes - SP	23°31'22"S	46°11'18"W	10 to 15°C	dark grey
Pos 97/14	São Roque - SP	23°31'45"S	47°08'07"W	10 to 15°C	dark grey
Pos 97/15	São Paulo - SP	23°32'51"S	46°38'10"W	10 to 15°C	dark grey
Pos 97/17	Brasília - DF	15°46'47"S	47°55'47"W	10 to 15°C	dark grey
Pos 98/37	Moji das Cruzes - SP	23°31'22"S	46°11'18"W	n	white
Pos 98/38	São Paulo - SP	23°32'51"S	46°38'10"W	10 to 15°C	dark grey
Pos 98/40	Moji das Cruzes - SP	23°31'22"S	46°11'18"W	10 to 15°C	dark grey

n - not known

RESULTS AND DISCUSSION

The isolate of *P. ostreatus* cultivated at 15°C was later in relation to the period of formation of primordium and harvest. Isolate Pos 98/37 was the only isolate that fruited at 28°C (Table 2). The reduction of the metabolism of microorganisms under low temperature was already expected, as mentioned for Zadrazil (1978). The use of isolate of *L. edodes* not adapted to tropical regions does not form primordia, similarly to what occurred at 28°C with isolates of *P. ostreatus*, except for Pos 98/37 (Chang et al., 1995). This behavior is credited to the use of isolates introduced by Japanese immigrants for the commercial production of *P. ostreatus* (Eira & Minhoni, 1997) mushrooms. Isolates from Japan are commercially cultivated at an average 15°C (Kinugawa et al., 1997). Isolates of *P. ostreatus* var. Florida are adapted to an average 25°C, and produce pileus cream to white coloured (Eger et al., 1976). Kinugawa et al. (1997) also recorded an isolate similar the *P. ostreatus* var. Florida in Thailand with these characteristics. In this work, isolate Pos 98/37 at 28°C and 900 lux presented white pileus, as mentioned by Kinugawa et al. (1997) for isolate of *P. ostreatus* of Thailand and by Eger et al. (1976) for *P. ostreatus* var. Florida. At 15°C and 120 lux (Table 2), all isolates presented pileus with colorations changing from grey to lead, same as observed by Eger et al. (1976) and Kinugawa et al. (1997) in studies with *P. ostreatus* at 15°C.

Producing mushroom “Shiitake” at low temperature needs less luminosity than at high temperature (Przybylowicz & Donoghue, 1990). This behavior was also observed for isolate of *P. ostreatus* at 15°C, when a luminous intensity of 120 lux was enough to induce the coloration of the pileus of all isolates. On the other hand, only the isolate that fruited at 28°C (Pos 98/37), with the luminous intensity of 900 lux, did not present coloration of the pileus, remaining white (Table 2 and Figure 1).

Similarly, in parallel experiments, *P. ostreatus* activated at with luminous intensity of 120 lux and 28°C did not present pileus coloration. But at 15°C with luminous intensity of 900 lux, the eight isolates of *P. ostreatus* presented reduction of coloration with the increase of the luminosity (results not presented), evidencing the importance of the interaction temperature vs. isolate vs. luminous intensity. This color variation is very frequent in the species and depends on environmental conditions, as reported by Rajarathnam & Bano (1987) and Eger et al. (1974).

Edible mushrooms cultivated between 15-20°C present better quality and durability than those at 25°C (Eger et al., 1976; Przybylowicz & Donoghue, 1990). An example was observed in this work in relation to the handling resistance after harvest (Table 2).

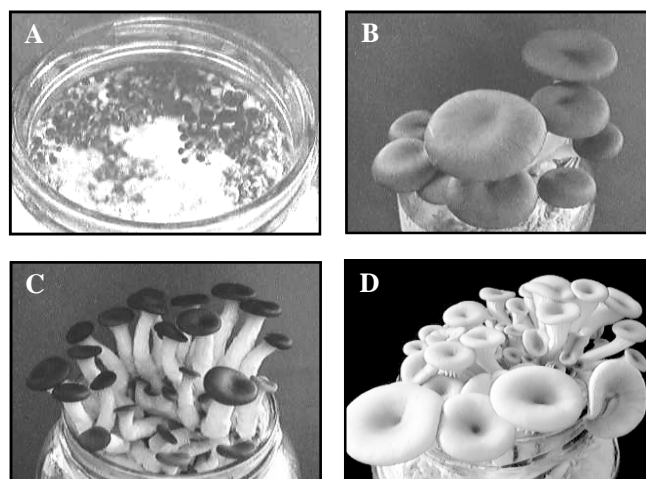


Figure 1 - *P. ostreatus* basidiocarps in production conditions. A - Isolated Pos 96/05 15°C, 120 lux, after 11 days of the hydration. B - Isolated Pos 98/38 15°C, 120 lux, after 7 days of the hydration. C - Isolated Pos 98/37 15°C, 120 lux, after 11 days of hydration. D - Isolated 98/37 Pos 28°C, 900 lux, after 7 day of the hydration

Table 2 - Period of formation of primordium, harvest, biological efficiency, coloration of the pileus and handling resistance of *P. ostreatus* mushrooms in relation to temperature and luminosity.

Temp ¹ / Luminosity ²	Isolate	Primordium	Harvest	EB4	Color of pileus	Handling resistance
		----- days ³ -----		%		
28°C/ 900 Lux	Pos 98/37	2.0 b	7.0 b	35.8 b	white	breakable
	Pos 96/05	10.5 a	14.5 a	11.6 c	lead-grey	firm
	Pos 97/12	9.0 a	14.0 a	17.7 c	lead-grey	firm
	Pos 97/14	9.0 a	13.0 a	17.2 c	lead-grey	firm
15°C/120 Lux	Pos 97/15	9.0 a	13.0 a	11.4 c	lead-grey	firm
	Pos 97/17	8.0 a	13.0 a	16.2 c	lead-grey	firm
	Pos 98/37	8.0 a	13.0 a	22.3 c	grey	firm
	Pos 98/38	8.0 a	13.0 a	43.1 a	dark grey	firm
	Pos 98/40	8.0 a	13.0 a	24.2 c	lead-grey	firm

¹Average temperature; ²Average luminous intensity; ³Medians in the same column followed by distinct letters differ by Dunn Test ($P < 0.05$); ⁴EB: biological efficiency.

Isolate Pos 98/37 presented greater biological efficiency (EB) at 28°C than at 15°C. The isolates Pos 97/15 and Pos 98/38 presented, at 15°C, respectively, the lowest and the highest values of EB. However, isolate Pos 98/38 was harvested after the established commercial pattern what, in part, can explain the high values of EB (Table 2). The commercial pattern of mushrooms with pileus area up to 150 mm² increases their value. In this experiment, isolates Pos 98/37, Pos 98/38 and Pos 98/40 at 15°C and Pos 98/37 at 28°C were harvested after reaching the commercial pattern (Figure 2).

The fructification temperature influenced the pattern of stalk formation of mushrooms, therefore isolate Pos 98/37 cultivated at 28°C presented greater formation of primordium as compared to 15°C. Isolates Pos 97/15, Pos 97/17 and Pos 98/40 also presented high concentration of primordium, with uniform distribution of mushrooms in relation to stalk formation pattern and area. In a similar way, isolate Pos 98/38, harvested after reaching the commercial pattern, presented uniform stalk formation patterns (Figure 2). Pos 98/37 of *P. ostreatus* (white variety and the only to fructify at 28°C), presented polymorphism with 30% of genetic similarity in relation to isolated adapted to 15°C for production (Figure 3). Isolate Pos 98/37 also was certified as *P. ostreatus* (Meijer¹, 2000).

The presence of polymorphism and minor similarity between isolates Pos 98/37 and all other, is probably related to the existence of genes important to the tolerance to high temperatures, as observed by Li (1980) and cited by Müller (1988) for some isolates of *P. ostreatus*, which have seven dominant genes, inherited from gen-

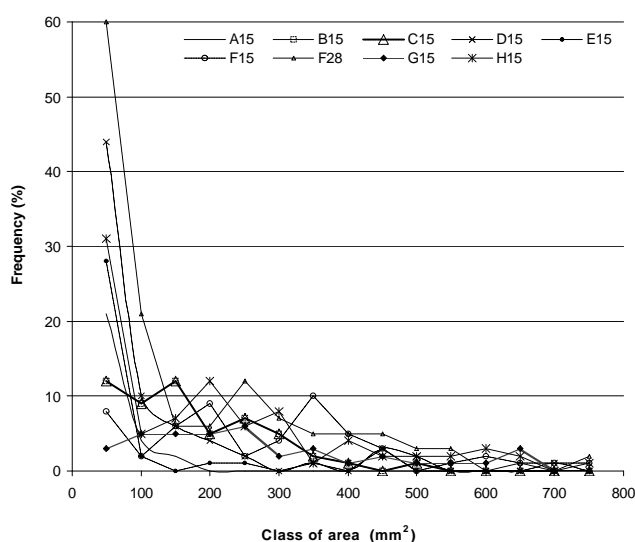


Figure 2 - Basidiocarp distribution according to the pileus area of eight isolates of *P. ostreatus*, at the fructification (15°C) and isolated (28°C).

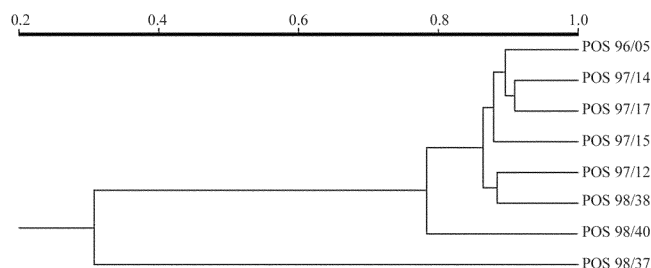


Figure 3 - Dendrogram generated to group UPGMA, based on the Coefficient of "Simple Matching", from the bands for RAPD with primers OPA-13, OPA-18, OPG-15, OPP-07 and OPG-14, in isolates analyses of *P. ostreatus*.

eration to generation, related to the resistance to heat (Guinberteau et al., 1991; Callac et al., 1998) report the presence of genes responsible for the induction of primordium and other genes related to the coloration of the basidiocarps. These authors indicated that the genetic control of the pileus coloration is given by a monogenic, dominant factor, while the white color or without coloration is related to recessive alleles. However, considering pileus coloration, the temperature effect has also to be taken into consideration since isolate Pos 98/37, cultivated at 28°C had white pileus, but at 15°C became grey. Thus, the genetic manifestation of each isolate is influenced by temperature and luminous intensity (qualitative evaluation of the isolates), leaving aside other factors not studied herein.

On the other hand, as observed by Larraya et al. (1999), the other isolates of *P. ostreatus* were similar, indicating low genetic variability, which can result in a production risk, specially, in cases of biotic or abiotic stress. Therefore, among the isolates used in this experiment, Pos 98/37 have great importance for genetic improvement programs which aim precocious and heat tolerant isolates, behaving with commercially desirable morphologic characteristics, such as those described by Pahil et al. (1991), for an isolate of *Agaricus bitorquis* that, in addition to fructifying at 25°C, presented good commercial quality.

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