Mycelial growth of two *Lentinula edodes* strains in culture media prepared with sawdust extracts from seven eucalyptus species and three eucalyptus clones

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ABSTRACT. The *in vitro* mycelial growth of *Lentinula edodes* strains LE-95/01 and LE-96/18 were evaluated in solid culture media prepared with sawdust extracts from seven eucalyptus species (*E. saligna*, *E. grandis*, *E. urophylla*, *E. pellita*, *E. paniculata*, *E. citriodora*, and *E. camaldulensis*) and three eucalyptus clones (*E. grandis* × *E. urophylla* hybrids). Evaluations were made every 48 hours by means of colony diameter measurements (mean of four transversely-oriented measurements), during ten days of incubation in the dark at 25ºC ±1°C. The experimental design consisted of randomized blocks, and treatment means were compared by Tukey test. The culture medium prepared from *E. citriodora* sawdust extract was the most promising to grow *L. edodes* strains LE-96/18 and LE-95/01. *L. edodes* strain LE-96/18 presented the fastest mycelial growth after incubation for ten days, regardless of sawdust extract type used in the culture medium.

Key words: shiitake, *in vitro*, mushroom.

RESUMO. Crescimento micelial de duas linhagens de *Lentinula edodes* em meios de cultura à base de extrato de serragem de sete espécies e três clones de eucalipto. Avaliou-se o crescimento micelial *in vitro* das linhagens LE-95/01 e LE-96/18 de *Lentinula edodes*, em meios de cultura sólidos à base de extrato de serragem de sete espécies (*Eucalyptus saligna, E. grandis, E. urophylla, E. pellita, E. paniculata, E. citriodora* e *E. camaldulensis*) e três clones (híbridos de *E. grandis* x *E. urophylla*) de eucalipto. As avaliações foram realizadas por meio de medições do diâmetro das colônias (média de quatro medidas diametralmente opostas), a cada 48 horas, durante dez dias de incubação, no escuro a 25ºC ±1°C. O delineamento experimental foi o de blocos casualizados, com uso do teste de Tukey para a comparação das médias. O meio de cultura à base de extrato de serragem de *E. citriodora* foi o mais promissor no crescimento das linhagens LE-96/18 e LE-95/01 de *L. edodes*. A linhagem LE-96/18 de *L. edodes* foi a que apresentou o crescimento micelial mais rápido após dez dias de incubação, independentemente do tipo de extrato de serragem utilizado no meio de cultura.

Palavras-chave: shiitake, *in vitro*, cogumelo.

Introduction

The mushroom *Lentinula edodes* (Berk.) Pegler, popularly known as Shiitake, is a lignolytic, aerobic basidiomycete (Chen, 2005; Minhoni *et al*., 2007).

In its metabolism, the fungus secretes exoenzymes that degrade compounds to obtain carbon, nitrogen, sulfur, and other nutrients (Donini *et al*., 2005). The *in vitro* cultivation of this mushroom seeks to elucidate the optimal growth conditions for the fungus with regard to culture media, temperatures, and incubation times (Hatvani, 2001). This knowledge is a pre-requisite for commercial cultivation in formulated substrate.

During a given time period, the fungus mycelial growth can be represented as a typical sigmoidal curve, including several stages with distinctive physiological properties (Montini *et al*., 2006). Mycelial growth can be measured in different ways, such as through radial growth, vigor, growth velocity, and mycelial mass (Marino, 1997). Under experimental conditions, the use of solid culture medium to evaluate fungal growth is considered adequate, since in nature fungi normally develop on solid substrates, such as plant and animal residues, or in the soil (Griffin, 1994).

In Brazil, the most frequently used substrate for *L. edodes* commercial cultivation consists of *Eucalyptus* spp. logs or sawdust. Hence, based on *in vitro L. edodes* mycelial growth evaluation in culture media prepared with sawdust extract from different species and clones of eucalyptus, it is possible to determine the most suitable type of eucalyptus for development, by simulating the natural cultivation conditions of the fungus.

Therefore, the objective of this work was to evaluate the *in vitro* mycelial growth of *L. edodes* strains LE-95/01 and LE-96/18 in solid culture media prepared with sawdust extract from seven eucalyptus species (*E. saligna*, *E. grandis*, *E. urophylla*, *E. pellita*, *E. paniculata*, *E. citriodora*, and *E. camaldulensis*) and three eucalyptus clones (*E. grandis* × *E. urophylla* hybrids).

Material and methods

The experiment was conducted at the Microbiology Laboratory of the Departamento de Produção Vegetal (Plant Production Department), Faculdade de Ciências Agronômicas (College of Agronomic Sciences), Unesp, Botucatu, São Paulo State, Brazil.

Lentinula edodes strains

Two *Lentinula edodes* strains, LE-95/01 and LE-96/18 were used. The strains were stored at the Microbiology Laboratory's Fungus Culture Collection (DPV - FCA/Unesp). Strain LE-95/01 was isolated from mushrooms of a producer from Londrina, Paraná State, in 1995. Strain LE-96/18 was isolated from mushrooms of producers from Cooperativa dos Produtores de Cogumelos de Rio Claro (COPCO) in Rio Claro, São Paulo State.

Extracts used in culture media preparation

The extracts used to prepare the culture media consisted of sawdust obtained from eucalyptus species *E. saligna, E. grandis, E. urophylla, E. pellita, E. paniculata, E. citriodora*, and *E. camaldulensis*, and from clones 23, 24, and 25. The clones are *E. grandis* × *E. urophylla* hybrids and were developed by the following companies: VCP, International Paper, and Ripasa. All eucalyptus material was obtained from Estação Experimental de Ciências Florestais (Experimental Station), Esalq - USP, located in the municipality of Itatinga, São Paulo State, whose stands were planted in an area with the same soil type and same planting date (January/1997).

Culture media preparation

The culture media were prepared with sawdust extracts from the seven eucalyptus species and three eucalyptus clones previously cited. In order to prepare each extract, we used 230 g sawdust, 30 g soybean meal, and 10 g lime. The substrate resulting from this mixture was moistened at 60-70%, arranged in HDPE (high density polyethylene) plastic bags, and autoclaved twice at 121°C for one and a half hour each time, with a 24-hour interval between times. Then, 40 g of each substrate were added to 500 mL distilled water and then fully boiled for five minutes. The extract thus obtained was filtered through a fine mesh screen and through cotton fabric and then placed in Duran® flasks, and the volume was completed to 500 mL with distilled water. The extract was then autoclaved at 121ºC for 30 minutes; after 24 hours, 8 g agar were added and the extract was autoclaved again for 30 minutes. The medium thus prepared was poured into Petri dishes.

Inoculation, colonization and variable analyzed

Secondary-parent disks (4 mm in diameter) of strains LE-95/01 and LE-96/18 (grown in Petri dishes containing culture medium prepared with *Eucalyptus* spp. sawdust extract) were inoculated in Petri dishes containing the media previously prepared, comprising the treatments of this experiment. The dishes were distributed in randomized blocks and maintained in an incubator at 25ºC ± 1°C. During this period, colony diameter measurements were made every 48 hours (mean of four transversely-oriented measurements) until the fungus colony in one of the treatments almost reached the edges of the Petri dish, which occurred after ten days of incubation.

Experimental design and statistical analysis

A randomized block experimental design was adopted, organized in a 2 × 10 factorial arrangement, whose treatments corresponded to combinations between the two *L. edodes* strains and seven eucalyptus species and three eucalyptus

clones, totaling 20 treatments. Each treatment consisted of 10 replicates, each corresponding to a Petri dish, totaling 200 plates.

The data were submitted to analysis of variance and the means were compared by Tukey test (5%) (Snedcor and Cochran, 1967).

Results and discussion

The mycelial growth results for *L. edodes* strains after cultivation for ten days in the culture media are presented in Table 1. There were significant differences for the interaction between culture media and *L. edodes* strains. Strain LE-96/18 produced the greatest mycelial growth in all culture media (Table 1). Donini *et al*. (2005) evaluated the mycelial growth velocity of *Pleurotus* spp. strains on different substrates and also observed significant differences for the interaction between strains, substrates, and days of evaluation.

Table 1. *In vitro* mycelial growth (mm) of *L. edodes* strains LE-95/01 and LE-96/18 in solid culture media prepared with sawdust extract from seven eucalyptus species (*E. saligna, E. grandis, E. urophylla, E. pellita, E. paniculata, E. citriodora*, and *E. camaldulensis*) and three eucalyptus clones (*E. grandis* × *E. urophylla* hybrids), after a ten-day development period at 25ºC ± 1°C.

Sawdust Extract	*L. edodes* strains	
	LE-95/01	LE-96/18
E. saligna	50.33 B b[(1)]	58.10 A bc
E. grandis	48.78 B bcd	53.43 A e
Clone 23	46.85 B ef	56.48 A cd
Clone 25	45.13 B f	58.23 A bc
Clone 24	47.30 B de	59.08 A b
E. urophylla	47.33 B de	53.45 A e
E. pellita	46.63 B ef	55.75 A d
E paniculata	49.33 B bc	57.30 A bcd
E. citriodora	53.23 B a	61.40 A a
E. camaldulensis	47.58 B cde	55.48 A d

(1) Means followed by different uppercase letters in each row and lowercase letters in each column are different (Tukey, 1%).

The culture medium prepared from *E. citriodora* sawdust extract provided the fastest mycelial growth after ten days of incubation at 25 ± 1ºC for both strains studied (Table 1). The influence of physical and chemical characteristics of the substrates on the mycelial growth, productivity, and biological efficiency of *L. edodes* strains has been emphasized in recent studies (Dias *et al*., 2003, Donini *et al*., 2006; Kopytowski Filho, 2006; Özçelik and Peksen, 2006). Menin *et al*. (2000) evaluated the effect of *Eucalyptus citriodora* sawdust essential, oil and crude extract on *L. edodes* mycelial growth and observed that the culture medium prepared with *E. citriodora* sawdust extract provided faster mycelial growth than PDA medium (potato-dextrose-agar).

The poorest mycelial growth performances were observed in the culture media from sawdust extracts of clones 23 and 25 and of the species *E. pellita* for strain LE-95/01, and in the culture media from sawdust extract of *E. grandis* and *E. urophylla* for strain LE-96/18. Tarui (1997) and Teixeira (2000) reported that *L. edodes* cultivation in sawdust from different eucalyptus species also conditioned mycelium development differently.

Figure 1 displays the mycelial growth of both *L. edodes* strains (LE-96/18 and LE-95/01), inside each culture medium, during ten days of *in vitro* development. Strain LE-96/18 had significantly higher growth in eight culture media prepared with sawdust extracts from *E. saligna,* clone 23, clone 25, clone 24, *E. pellita*, *E. paniculata*, *E. citriodora* and *E. camaldulensis*, than strain LE-95/01 during the entire evaluation period (Figure 1). These mycelial growth differences between *L. edodes* strains have already been reported by many researchers (Andrade *et al*., 2007; Andrade and Graciolli, 2005; Boyle, 1998; Maki *et al*., 2001; Silva *et al*., 2005).

After 2 days of incubation, strain LE-95/01 produced significantly higher mycelial growth than strain LE-96/18 in the culture medium prepared with *E. grandis* sawdust. However, after 4 and 6 days from inoculation, strains LE-96/18 and LE-95/01 had similar growth periods. Finally, at 8 and 10 days after inoculation, growth of strain LE-96/18 was significantly higher than in strain LE-95/01 (Figure 1). In the culture medium prepared with *E. urophylla* sawdust extract, after 2 and 4 days from inoculation, strains LE-96/18 and LE-95/01 had similar mycelial growths. From then on, at 6, 8, and 10 days after inoculation, strain LE-96/18 had greater mycelial growth (Figure 1). Teixeira (2000) compared the mycelial growth velocity of ten *L. edodes* strains in sawdust from three eucalyptus species and reported that the growth velocities for *L. edodes* strains were not different in the eucalyptus species *E. grandis* and *E. urophylla*, except for strain JAB P.

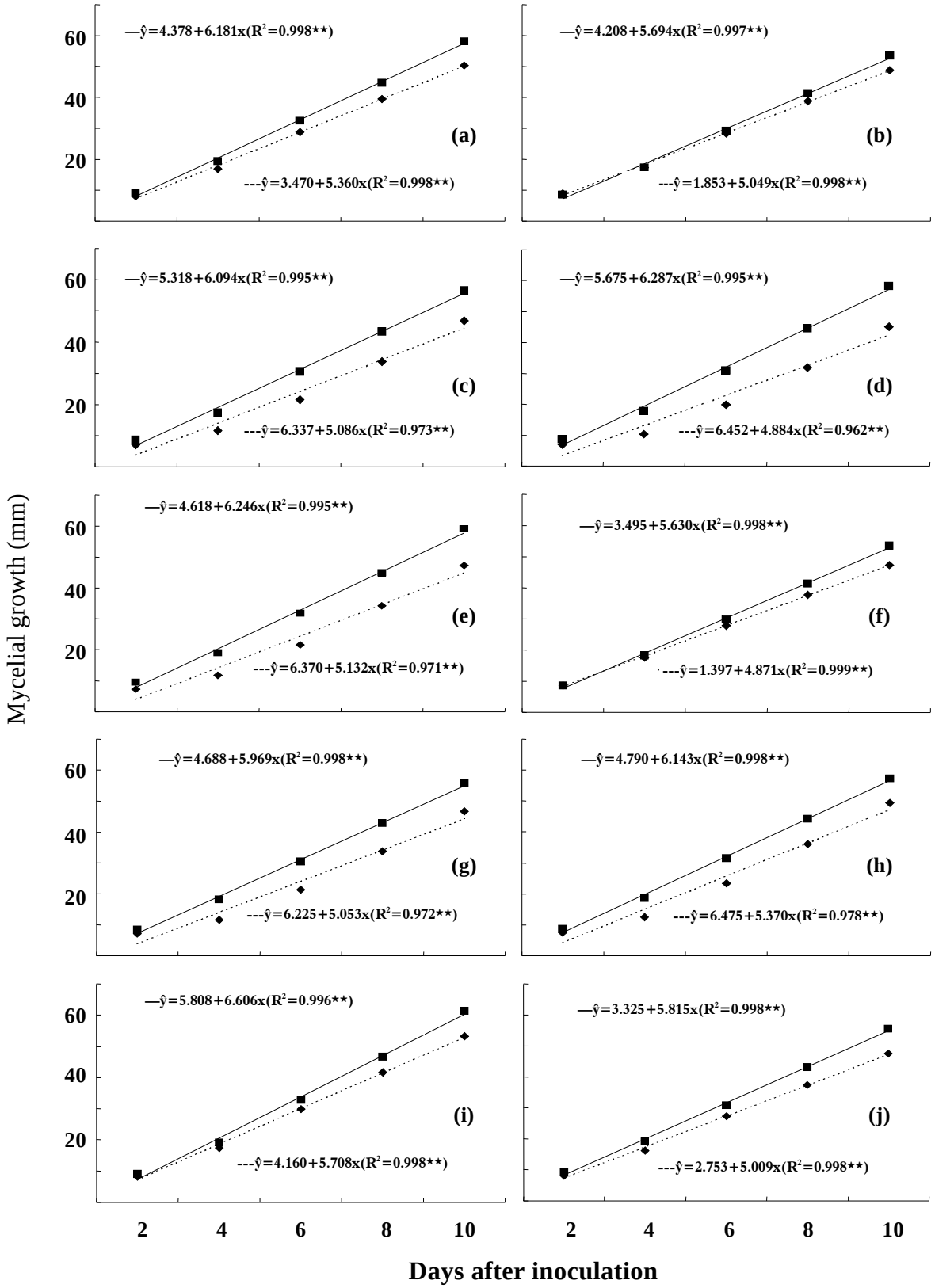

Figure 1. Mycelial growth of two *L. edodes* strains, LE-96/18 (■) and LE-95/01 (♦), in culture media prepared with sawdust extracts from (a) *E. saligna*, (b) *E. grandis*, (c) Clone[(1)] 23, (d) Clone 25, (e) Clone 24, (f) *E. urophylla*, (g) *E. pellita*, (h) *E. paniculata*, (i) *E. citriodora*, (j) *E. camaldulensis*, at 25ºC. ⋆ and ⋆⋆ are significant at 5 and 1%, respectively.

[(1)]Clones 23, 24, and 25 are *E. grandis* × *E. urophylla* hybrids.

Conclusion

The culture medium prepared from *E. citriodora* sawdust extract was the most promising to grow *L. edodes* strains LE-96/18 and LE-95/01.

Strain LE-96/18 presented the fastest mycelial growth after incubation for ten days, regardless of sawdust extract type used in the culture medium.

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