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Optimal conditions of in vitro mycelial growth of *Lentinus strigosus*, an edible mushroom isolated in the Brazilian Amazon

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Abstract The protocols of in vitro cultivation described in the literature for mushrooms are usually correlated with temperate climate habitat, but it is necessary to study protocols for species of tropical climates. In this article, we collected, isolated, and evaluated the conditions of in vitro mycelial growth of *Lentinus strigosus* and correlated these with the characteristics of its habitat. These results indicate, as optimal conditions of in vitro mycelial growth for *L. strigosus*, the use of 35°C for incubation, initial pH from 5 to 7, without illumination, Sabouraud dextrose agar medium, and agitation for culture in liquid medium.

Key words Basidiomycetes · High temperature · *Lentinus strigosus* · Thermophiles fungi

There are more than 200 genera of macrofungi that contain species of use to people, mostly because of their edible properties. These are distinguished between those recorded as simply "edible" and those that are actually eaten ("food"). To include all edible species as "food" would greatly overstate the number of species consumed by people around the world (Boa 2004). There are nearly 100 species of fungi that can be cultivated (Boa 2004). Commercial markets are dominated by *Agaricus bisporus* (J.E. Lange) Pilát, *Pleurotus* spp. (Fr.) P. Kumm., *Lentinula edodes* (Berk.) Pegler, *Volvariella volvacea* (Bull.) Singer, *Flammulina velutipes* (Curtis) Singer, and *Pholiota nameko* (T. Ito) S. Ito and S. Imai (Kendrick 2000; Sánchez 2004). On the other hand, the wild mushrooms that are still not cultivated commercially have great ethnomycological importance because they constitute a much appreciated food (Herrera and Ulloa 1990; Boa 2004). The potential of the diversity of mushrooms in the tropical ecosystems has been much discussed, but little explored scientifically (Hawksworth 2001; Mueller and Schmit 2007; Mueller et al. 2007).

The genus *Lentinus* Fr. (Polyporaceae tribe Lentineae Fayod) has a worldwide distribution but with species occurring more abundantly in tropical regions. The basidiocarps are often leathery to almost woody in consistency and are therefore more persistent than typical members of the Agaricales. It is because of their persistent nature that they frequently represent the dominant group of agaricoid fungi in the tropical rainforests, being resistant to adverse periods of drought (Pegler 1975). Ethnomycology studies have identified different species of edible mushrooms of the *Lentinus* genus consumed by indigenous groups such as the Yanomami in the Brazilian Amazon: *Lentinus crinitus* (L.) Fr., *L. velutinus* Fr., *L. glabratus* Mont., *L. cubensis* Berk. and M.A. Curtis, *L. strigosus* (Schwein.) Fr. (Fidalgo and Prance 1976; Fidalgo and Hirata 1979; Prance 1984). In addition, the Uitoto natives of the area of Araracuara in the Colombian Amazon consume *L. strigosus*, *L. concavus* (Berk.) Corner, *L. crinitus*, and *L. scleropus* (Pers.) Fr. (Vasco 2002). Chang and Mao (1995) related that this species can be cultivated. We tasted this mushroom sautéed with margarine and some salt and thought it had a good flavor, a high *umami* taste, and was slightly fibrous.

Most of the cultivated mushrooms originated in countries of temperate climate, and the protocols of in vitro cultivation described in the literature are correlated with the habitat of these species. As environmental conditions in the tropics are different, however, it is necessary to study protocols for the tropical climate species. As commented by Mswaka and Magan (1999), the optimum temperature for growth of most wood-decay fungi from temperate regions is between 25°C and 30°C. Very few if any detailed studies have been carried out on the temperature profiles of tropical wood-decay fungi. In Brazil, the species of cultivated edible mushrooms are mainly originally of Europe and Asia, as *A. bisporus*, *L. edodes*, and *Pleurotus* spp. Mushroom cultivation is more developed in the south and southeast of Brazil where the subtropical climate is more

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appropriate for the production of these species. In the north region of tropical climate, the production costs for these species are very high, making cultivation at the highest scales not feasible. Therefore, we began research on domestication and cultivation of species adapted to the tropical climate using as base agroforestry residues of the Amazonian to reduce the costs of production.

The domestication of naturally occurring edible fungi in Brazil will allow their production and commercial exploitation so that the country would have a national product adapted to our climate and atmosphere, competing on the external market (Maki and Paccola-Meirelles 2002).

In the present article, we collected, isolated, and evaluated the conditions of in vitro mycelial growth of *Lentinus strigosus* and correlated the results with the characteristics of the habitat of this mushroom.

The basidiocarps of *L. strigosus* were collected in lignicolous substratum on the campus III of the Instituto Nacional de Pesquisas da Amazônia (INPA) (3°5′31.6″ S, 59°59′36.4″ W), Manaus–AM, Brazil. The mycelium was isolated with inoculation of fragments of the basidiocarp context in potato dextrose agar (PDA; Acumedia) culture medium, incubated at 25°C, without light. Part of the collection was made exsiccate, to be deposited in the Herbarium of INPA. The species was identified with the help of available literature (Stankovicová 1973; Pegler 1975, 1983).

The mycelial growth of the *L. strigosus* isolate was evaluated at temperatures of 25°, 30°, 35°, 40°, and 45°C, on PDA medium. Each Petri dish (90 mm diameter) was inoculated with one disk (10 mm diameter) of the mycelia freshly grown on PDA plates and cultured for 5 days at 25°C. The mycelial growth was evaluated by measuring the diameter of the colony and the mycelial dry weight on the 5th and on the 6th day, respectively. The two common measures of growth rate are (1) increase in radius of colony over time and (2) increase in dry mass of colony over time. The first has the advantage that sequential records can be obtained from each colony. The second is a more absolute measurement, but can be performed only once for each colony (Kendrick 2000). In this study, we adopted a methodology that allowed evaluation of the colony diameter and dry weight, cultivated in solid medium. For evaluation of mycelial dry weight, after the growth of the colony in the respective culture media, the Petri dish was placed in a microwave oven for 20 s to melt the medium. Soon after, the mycelium was separated from the medium for filtration and washed with distilled water, at an approximate temperature of 60°C. This preparation was placed in an oven at 105°C until constant in weight.

The effect of agitation of the liquid culture was evaluated by measuring *L. strigosus* mycelial growth, using potato dextrose broth (PDB): an infusion of 200 g potato, 20 g glucose, and distilled water to complete the volume of 1000 ml (pH = 6.0), sterilized at 121°C for 15 min. Erlenmeyer flasks (250 ml) containing 100 ml PDB were inoculated with five disks of the mycelia. For the agitation treatment, the flasks were placed in a table agitator (TECNAL TE-140) at 75 rpm. As control, other flasks were maintained in static condition. Both treatments were incubated in an atmosphere conditioned to 25° ± 2°C and natural illumination. After 15 days of mycelial growth, the mycelium was separated for filtration. The obtained mass was placed in an oven at 105°C until constant in weight. To measure the effect of initial culture media pH, Erlenmeyer flasks (250 ml) containing 100 ml PDB with respective pH (4, 5, 6, and 7) were inoculated with five disks of mycelia. The pH was adjusted with HCl and NaOH solutions before sterilization. Flasks were incubated at 35°C and with agitation, the optimal conditions observed in the previous experiments. In this experiment the dry weight was obtained after 10 days of mycelium growth.

The *L. strigosus* mycelial growth was evaluated in solid culture media: PDA (Acumedia), malt extract and peptone agar (MEPA) [3% malt extract (Becton Dickinson); 0.3% soy peptone (Acumedia); 1.5% agar (Becton Dickinson)], Sabouraud dextrose agar (SDA) (Becton Dickinson), V8 medium (V8) [200 ml V8 juice (Campbell's); 1.5% agar, 800 ml H_2O], and minimum culture medium (MM) (Pontecorvo et al. 1953). The flasks with the respective culture media were sterilized at 121°C for 15 min, and the media were poured into Petri dishes (90 mm diameter) and inoculated with one disk of the mycelia. This experiment was also incubated at 35°C. Mycelial growth was evaluated by measuring the colony diameter on the 5th day. The dry weight of the mycelium was evaluated according to the methodology described in the evaluation of mycelial dry weight in solid medium.

To evaluate the effect of the influence of light, Petri dishes with PDA medium were inoculated with one disk of the mycelia and incubated at 35°C for 24 h light (1250 lx). Light emission spectra were taken by Illuminance meter IM-5 (Topcon, Japan). Dark treatments were carried out in a box made lightproof with a dark cloth. Dark-grown cultures were viewed and scored for development only after the termination of the experiments. The mycelial growth was evaluated by measuring the diameter of the colony on the 5th day, and mycelial dry weight was measured using the temperature evaluation procedure. To evaluate the mycelial growth in liquid medium, five disks of the mycelia were placed in Erlenmeyer flasks (250 ml) containing 100 ml PDB medium and incubated at 35°C and 75 rpm for 10 days. Light treatments were 24 h light; dark treatments were carried out in flasks covered with aluminum foil. After this period, the mycelium was separated by filtration, obtaining the dry weight.

The experiments were tested in five replications and two repetitions. For statistical evaluation, the experiments were submitted to analysis of variance (ANOVA) and the averages were compared by the Tukey test at the 5% level of significance.

The basidiocarps (Fig. 1) presented pileus 4–7 cm diameter, convex, subinfundibuliform to infundibuliform, or laterally attached and spathulate; surface pale, whitish to pallid ochraceous, more brownish toward the center, at first often with violaceous or purplish tints especially toward the margin, densely villose to hispid-tomentose; margin curved, thin, undulate; context thick at the disk, white. Lamellae deeply decurrent, white to ochraceous buff, or with

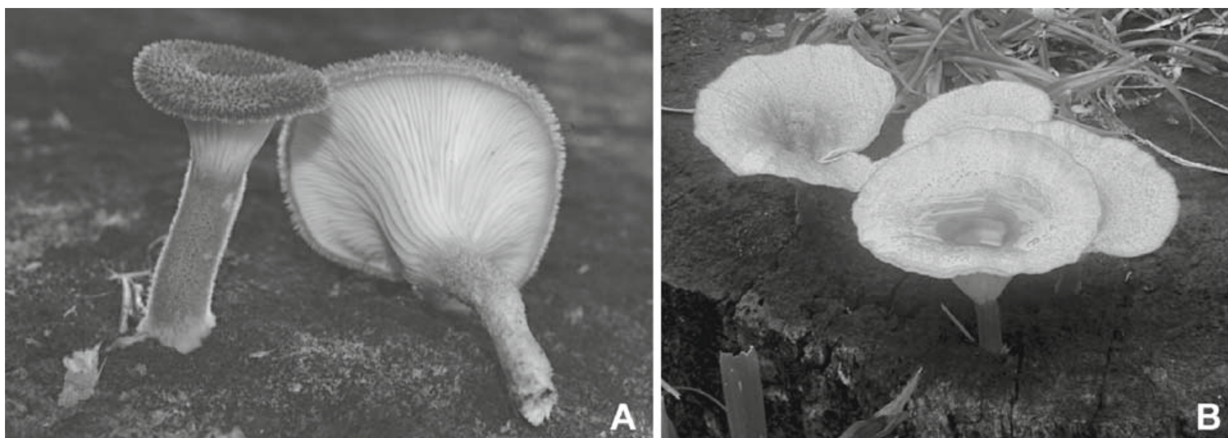

Fig. 1. Basidiocarps of *Lentinus strigosus* collected in lignicolous substratum. **A** Young state. **B** Mature state of basidiocarps

violaceous tints especially on the edge, 1–2 mm wide, crowded, with lamellulae of four lengths. Stipe excentric to lateral, more rarely central, short, cylindrical or with subbulbous base; colorless surface with the pileus but not violaceous, tomentoso, with fibrillose hairs extending to the apex, these characteristics led us to identify this mushroom as *L. strigosus*, considering the descriptions by Pegler (1983). *L. strigosus* is encountered under dense vegetation as well as in semiopen habitat and is exposed to large temperature variations (Castillo et al. 2004). The isolate used in this work was collected in November 2006 in open habitats on lignicolous substratum.

The effects of temperature on *L. strigosus* grown on PDA over a range of temperatures from 25° to 45°C are shown in Fig. 2. Statistical analysis of the data of the diameter of the colonies showed that 35°C and 40°C were the best temperatures for mycelial growth, not differing at 5% significance (Fig. 2A). However, the results of the mycelial mass dry weight showed a significant difference between 35°C and 40°C (Fig. 2B); this measure of mycelial growth indicated that the temperature of 35°C provided a colony with larger mass. This experiment demonstrated the advantage of evaluating colony dry mass in solid medium for determining the size and the density of the colony without the need of cultivation in liquid medium. Mswaka and Magan (1999) studied basidiomycetes and divided them into three groups based on their optimum and maximum temperatures for growth: (1) *low-temperature group* with optimal temperatures lying between 25°C and 30°C and no growth above 37°C; (2) *intermediate group* with optimal temperature range of 30°–37°C with no growth at 45°C; and (3) *high-temperature group* with growth in the range 37°–40°C and growth ceasing at 55°C. Kendrick (2000) defined thermophile fungi with minima temperature above 20°C, maxima above 50°C, and optima between 35°C and 50°C. Once there was growth to 45°C, although it was smaller, the colony reached 2.5 cm diameter in 5 days at 45°C; thus, we considered this mushroom to be of the high-temperature group or a mushroom thermophile. This result is easily understood, because when measuring the internal temperature of the log where the mushrooms were collected, a temperature from 35° ± 3°C was usually registered at midday, which explains the correlation with the optimum temperature obtained in the laboratory. Castillo et al. (2004) studied correlation between the in vitro growth response to temperature and the habitat of some lignicolous fungi from Papua New Guinea coastal forests and obtained similar results for *L. strigosus*.

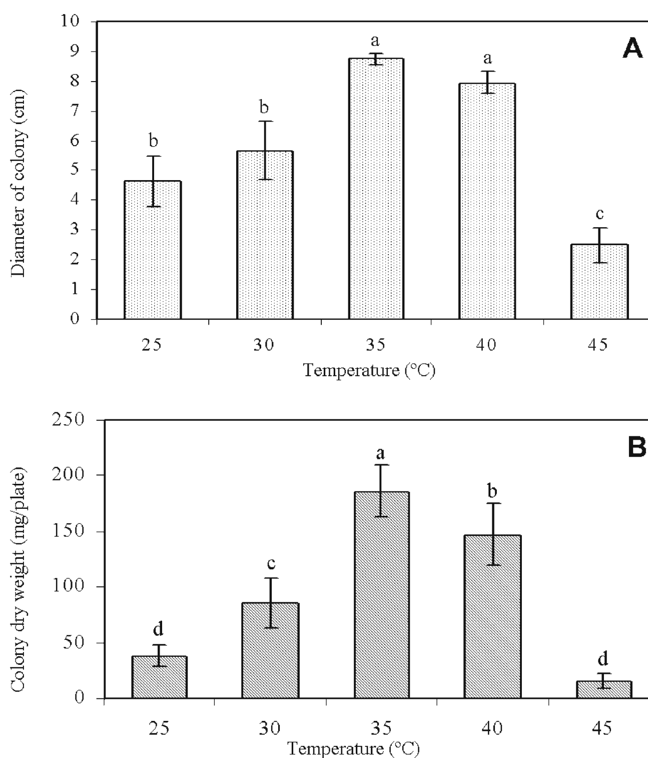

Fig. 2. *Lentinus strigosus* mycelial growth on potato dextrose agar incubated at different temperatures. Means with the same letter (s) are not significantly different ($P < 0.05$) by the Tukey test. Average of five replications. **A** Diameter of colony (cm) on the 5th day. **B** Colony dry weight (mg/plate) on the 6th day

For aerobes, aeration is one of the most important cultivation conditions. Usually, wood-rotting basidiomycetes

Table 1. Effect of agitation on *Lentinus strigosus* mycelial growth on potato dextrose broth medium

Treatment	Colony dry weight (mg/flask)
With agitation	413.4 ± 22.9[a]
Without agitation	286.9 ± 54.7[b]

Colony dry weight on the 15th day incubated at 25° ± 2°C and 75 rpm of agitation
Means with the same superscript letter(s) are not significantly different ($P < 0.05$) by the Tukey test; average of five replications

Table 2. Effect of initial pH of culture media on *Lentinus strigosus* mycelial growth

pH	Colony dry weight (mg/flask)
4	246.5 ± 21.2[b]
5	324.6 ± 25.9[a]
6	283.9 ± 31.3[ab]
7	332.8 ± 39.1[a]

Colony dry weight on the 10th day on potato dextrose broth medium incubated at 35°C and 75 rpm agitation
Means with the same superscript letter(s) are not significantly different ($P < 0.05$) by the Tukey test; average of five replications

grow only on wood where the moisture content is not greater than 60%. Therefore, they are more sensitive to oxygen deficiency than the fungi imperfecti (Emelyanova 2005). Furthermore, biomass production, the intensity of aeration, and agitation conditions also influenced the form of the mycelium growth (diffused filamentous mycelium or agglomerated mycelium: pellets) and the form of the pellets (Emelyanova 2005). These aspects were observed in this experiment (Table 1); the treatment with agitation presented greater mycelial growth compared with stationary condition. Agglomerated mycelium was observed in the treatment with agitation. There was no significant difference ($P < 0.05$) between pH 5, 6, and 7; however, pH 4 presented the lowest mycelial growth (Table 2).

There are many studies of the effect of light on the development of basidiomycetes, and this is an essential factor for the fruit-body formation (Chang and Hayes 1978; Leatham and Stahmann 1987; Matsumoto and Kitamoto 1987; Kitamoto et al. 1999; Sakamoto et al. 2004). Considering that the place of the collection of this mushroom was quite well illuminated (average, 61120 lx at midday), this suggests that light has a strong influence on its fructification. However, in the evaluation of the influence of light, in the mycelial growth in solid medium a significant difference was not present, and in the liquid medium biomass production was higher in the absence of light (Table 3).

As to the effect of culture medium, considering the measurement of the diameter of the colony on the 5th day of incubation, the PDA medium showed greater diameter. However, in the evaluation of colony dry weight, the highest biomass production was obtained in SDA medium (Fig. 3).

Thus, we suggest the use of SDA medium with pH 5, temperature of 35°C for incubation, without illumination, and with agitation for cultivation in liquid medium as optimal conditions of in vitro *L. strigosus* mycelial growth.

Table 3. Effect of light on *Lentinus strigosus* mycelial growth on solid and liquid potato dextrose medium

Treatment	Diameter of colony* (cm)	Colony dry weight (mg/plate or flask)	
		Solid medium**	Liquid medium***
With light	7.49 ± 0.749[a]	228.9 ± 13.9[a]	369.44 ± 1.8[b]
Without light	7.91 ± 0.252[a]	203.5 ± 7.2[a]	397.04 ± 1.1[a]

*Diameter of colony in solid medium on the 5th day of incubation; **colony dry weight on the 6th day of incubation; ***colony dry weight on the 10th day of incubation with 75 rpm of agitation; incubation at 35°C
Means with the same superscript letter(s) are not significantly different ($P < 0.05$) by the Tukey test; average of five replications

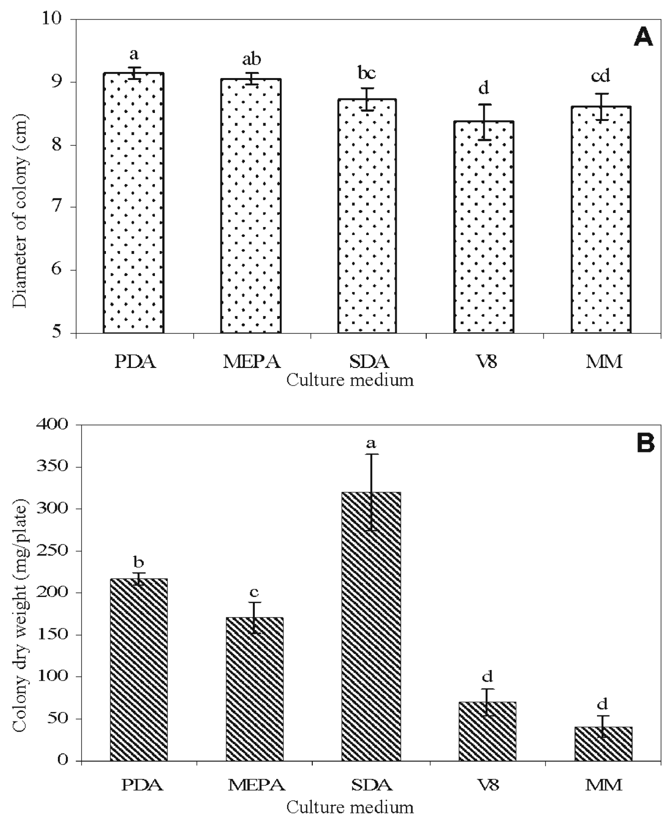

Fig. 3. *Lentinus strigosus* mycelial growth on different culture media incubated at 35°C. *PDA*, potato dextrose agar; *MEPA*, malt extract and peptone agar; *SDA*, Sabouraud dextrose agar; *V8*, V8 juice medium; *MM*, minimum medium. Means with the same letter(s) are not significantly different ($P < 0.05$) by the Tukey test. Average of five replications. **A** Diameter of colony (cm) on the 5th day. **B** Colony dry weight (mg/plate) on the 6th day

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