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Short Communication

Optimal conditions for the fruit body production of natural occurring strains of *Lentinus tigrinus*

Bernardo E. Lechner [a], Edgardo Albertó [b,*]

[a] *PRHIDEB-CONICET, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires (1428), Buenos Aires, Argentina and Instituto Fitotécnico de Santa Catalina, Universidad Nacional de La Plata, Facultad de Ciencias Agrarias y Forestales, Llavallol, Buenos Aires, Argentina*

[b] *Instituto de Investigaciones Biotecnológicas IIB-INTECH (CONICET-UNSAM), Lab. of Mycology and Mushroom Cultivation, C. C. 164, 7130 Chascomús, Buenos Aires, Argentina*

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Abstract

Lentinus tigrinus is a species with a fleshy pileus, strong odor and agreeable taste. In order to determine the optimal conditions for the production of this species, three substrates based on *Salix* sp. sawdust, wheat straw and supplements were tested in 500 g dry weight bags at two different fruiting temperatures. Naturally occurring strains of this species were incubated at 30 °C. Primordium initiation could be observed 11–16 days after induction conditions began. This species produced highest yields with biological efficiency (BE) of 62% with supplemented sawdust at 25 °C. When bags were reduced to 100 g dry weight, spawning run time was reduced from 28 to 30 to 10 to 14 days and BE increased more than 100%. *L. tigrinus* is a promising species with possibilities for commercial production.

Keywords: Natural occurring species; *Lentinus tigrinus*; Mushroom cultivation; Wheat straw; *Salix* sawdust; Agricultural waste

1. Introduction

Edible fungi are an important source of proteins and the number of cultivated species in the world is on the increase (Chang, 1989, 1991, 1999; Rajarathnam and Bano, 1991; Stamets, 1993). During a comprehensive study of the genus *Pleurotus* in Argentina, which included fresh, dry material and strains deposited under this genus, we found a strain in the culture collection of the Biological Department of the Faculty of Exact and Biological Sciences, University of Buenos Aires labeled *Pleurotus lindquistii* Singer. Singer (1960) described this species as growing on trunks of *Salix humboldtiana* in the marginal rain forest of Río de la Plata (Buenos Aires, Argentina). Lechner and Albertó (2000) described this species again and transferred it to the genus *Lentinus* since they found microscopical characters, such as hyphal pegs, which are absent in *Pleurotus*. Later, Grand et al. (2002) renamed it to *L. tigrinus* based on the sexual intercompatibility between single basidiospores isolates of *L. tigrinus* and a tester strain of *Lentinus lindquistii*. In the latter work, Grand et al. (2002) suggested, based on the analysis of a sequence ITS of DNA, that widely scattered geographical populations of *L. tigrinus* complex are genetically divergent.

The dimitic or trimitic hyphal system of the genus *Lentinus* (Pegler, 1983; Corner, 1981) determines a coriaceous consistency for the majority of species. But on the contrary, *L. tigrinus* has a soft flesh, strong odor and agreeable to slightly farinaceous taste that makes this species suitable and attractive for consumption (Lechner and Albertó, 2000).

At present, only a few species of genus *Lentinus* are cultivated (Stamets, 1993). Sobal et al. (1997) reported the culture of *Lentinus levis* (Berk. and Curtis) Singer in Mexico;

* Corresponding author. Tel.: +54 2241 424049; fax: +54 2241 424048.
E-mail address: ealberto@intech.gov.ar (E. Albertó).

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Kurtzman and Zadrazil (1989) pointed out the culture of *L. sajor-caju* (Fr.) Fr. Kadiri and Arzai (2004) obtained fruiting bodies of *L. subnudus* Berk on woodlogs of various hardwood trees. Khurana et al. (1983) cited nutritional values of several wild edible mushrooms, including *L. tigrinus* which contains high quantities of fibers, few sugars and low calories and, a high quantity of the amino acids phenylalanine, threonine and tyrosine. In the present study naturally occurring strains of *L. tigrinus* were cultivated on three different substrates, at two fruiting temperatures and in two block sizes in order to determine yields and optimal conditions for production. Results were compared with those obtained from commercial strains of *Pleurotus ostreatus* used as control.

2. Methods

2.1. Strains used

L. lindquistii (LT), (BAFC 2102), Argentina, Buenos Aires, Pereyra Iraola park, on foot of *Populus* sp., VI-2-1962, Col. R. Singer; (BAFC 197), Ramallo, on trunk of *Salix humboldtiana*, XI-22-1975, Col. J. R. Deschamps; (BAFC 2044), La Plata, Los Hornos, on *Salix* sp., IX-5-1971, Col. J. R. Deschamps and *P. ostreatus* (PO), (BAFC 2067), ITALIA, Commercial strain, IX-93. A culture of each of these strains is also deposited in the IIB-INTECH collection of fungal cultures (ICFC).

2.2. Optimal temperature for mycelium growth

Cultures were inoculated with a 0.7 mm diam. cylinder in 90 mm Petri dishes containing Noble's medium (Nobles, 1948) and incubated in darkness at 25, 30 and 35 °C; temperatures of 20 and 40 °C were also assayed when maximum growth was obtained at 25 or 35 °C. Measurements were taken with a 0.5 mm rule.

2.3. Determination of optimal substrate and fruiting temperature

Traditional methods for fruiting species of *Pleurotus* were used (Zadrazil, 1974; Stamets, 1993). Three substrates were used: sawdust of *Salix* sp., wheat straw, and wheat straw with supplements (Table 1). Six polypropylene bags were filled with 500 g (dry weight) for each formulation; humidity was adjusted in the substrate (w/w) to 74% according to the initial humidity content of components. Bags were stoppered with cotton plugs held by PVC (polyvinyl chloride) cylinders and autoclaved at 123 °C, 1.2 kg/cm^2, for 2 h. After cooling they were inoculated with 5% (wet weight) of spawn of strains BAFC 2102 of *L. tigrinus* and BAFC 2067 of *P. ostreatus*. Bags were incubated in the dark at 25 °C for 30 days.

Table 1
Substrates formulation (%)

Substrate 1		Substrate 2		Substrate 3	
Sawdust	77	Wheat straw	77	Wheat straw	97
Wheat meal	15	Wheat meal	15	$CaCO_3$	3
Oatmeal	5	Oatmeal	5		
$CaCO_3$	3	$CaCO_3$	3		

2.4. Strain screening and block sizes

Strains BAFC 2102, 197 and 2044 of *L. tigrinus* were assayed. Six polypropylene bags were filled with 100 and 500 g (dry weight) of substrates determined in the step before as the most productive. Humidity was adjusted to 74%. Bags were sterilized, spawned and incubated as was indicated in the previous section. Spawning run time and time of primordia initiation were recorded.

2.5. Fruiting conditions

After bags were completely colonized by the mycelium they were moved to the fruiting rooms. Two temperatures were assayed for inducing fruit bodies: 20 ± 1 °C and 25 ± 1 °C; photoperiod of 9 h light/15 h darkness was given; the air in the cultivation room was renewed 6 times per hour. Watering by spray (fog type, pressure = 2 pounds/sq.in.) for 5 min every 3 h was automatically provided. Bags were not removed; eight cuts/bag were made to induce fruit bodies formation. Fruit bodies were cut when mature, fresh weight was recorded during 70 days following induction. Biological efficiency (BE) was calculated as [fresh weight of harvested mushrooms/dry matter content of the substrate] × 100 and compared among treatments.

2.6. Experimental design and statistical treatments

Six bags were used for treatment; number of replications was confirmed by Rabinovich's test (Rabinovich, 1980). Tukey HSD (honest significant difference) test was used to determine the significant differences between groups in an ANOVA (analysis of variance) of two factors with strain and substrate as independent variables and BE as the dependent. Normality and homogeneity assumptions were confirmed by KS and Bartlett tests respectively ($p > 0.05$) for the validity of ANOVA method.

3. Results and discussion

3.1. Optimal temperature for mycelium growth

It was determined as 30 °C for all strains tested (Fig. 1). The highest level of growth was obtained with BAFC 197 with 15 mm/day. This strain nearly doubled the rate of growth of BAFC 2044 which reached 8.18 mm/day. The comparison between 30 and 35 °C for BAFC 2102 and 197 showed that growth rate decreased only 7.8% and 5.5% respectively, meanwhile the comparison between 30 and 25 °C for both strains showed that it decreased 8.6% and 20% respectively (Fig. 1). Although strain 197 was more

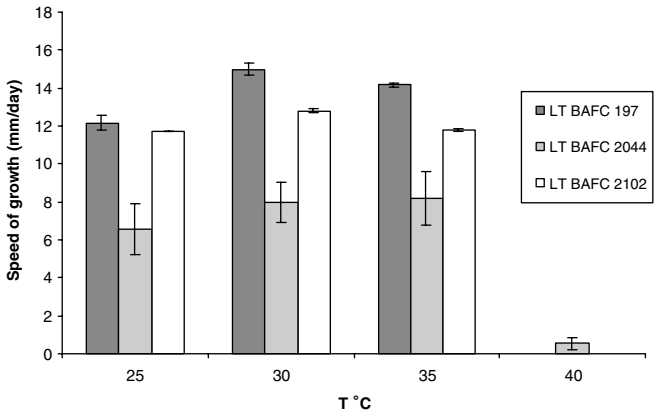

Fig. 1. Effect of temperature on mycelium growth of *L. tigrinus* (BAFC 197, 2044 and 2102).

sensitive to temperature reduction results indicate that these strains had a wide range of growth. While growth was drastically affected at 40 °C (Fig. 1), strains did not die at this temperature, as the growth was reinitiated when they were again incubated at 30 °C.

3.2. Optimal substrate and fruiting temperature

Best yields of *L. tigrinus* were obtained with substrate 1 (S1) with a BE mean of 61.93% which was statistically different from S2 and S3. Both S2 and S3 produced the lowest yields (Table 2). Results indicate that wheat straw is not an optimal substrate for *L. tigrinus* production. When BEs of *L. tigrinus* were compared with yields produced by *P. ostreatus*, we observed that the latter produced similar yields in S1 but higher BEs on wheat straw reaching 101–113% mean (S3 and S2 respectively). The preference of *L. tigrinus* for *Salix* sawdust can be related to its natural habitat since this mushroom is frequently found growing on dead wood of *Salix* (Lechner and Albertó, 2000). Nazareth and Sampy (2003) demonstrated the high capacity of this fungus to degrade sawdust, decreasing its lignin and cellulose content. Regarding fruiting temperatures of *L. tigrinus*, the highest BE were obtained at 25 °C but there were no significant differences ($p > 0.05$) with the results obtained at 20 °C (Table 2). In Argentina, this species has a temperate to subtropical distribution where temperatures fluctuate from 20 to 35 °C during fruiting period. This indicates that the strains behaved according to natural habitat conditions.

Both *L. sajor-caju* and *L. levis* were successfully cultivated on various lignocellulosic materials (Metha et al., 1990; Chang and Miles, 1991; Sangvan and Saini, 1995; Ragunathan et al., 1996; Kues and Liu, 2000; Zhang et al., 2002; Ragunathan and Swaminathan, 2003; Sobal et al., 1997). The taxonomic position of *L. sajor-caju* is controversial since many authors consider it as a *Pleurotus*. The species was firstly transferred to *Pleurotus* by Singer (1951). Pegler (1975) returned it to its original position in the genus *Lentinus* which he later confirmed (Pegler, 1983). However, compared to *L. tigrinus*, *L. sajor-caju* produces high yields on wheat straw reaching from 70.2% (Kalmiş and Sargin, 2004) to 97% of BE mean (Zhang et al., 2002) while *L. levis* reaches BE of 53.8% when cultivated using barley straw as substrate Sobal et al. (1997).

3.3. Strain screening and block sizes

Three flushes were recorded within a period of 70 days. BE obtained showed no significant differences ($p > 0.05$) between the assayed strains. Yields obtained in 100 g dry weight blocks were remarkably higher with BE of 118–140%, than those obtained with 500 g (Table 3) and were significantly different ($p < 0.05$). Differences could be due to a better diffusion of gases when smaller bags were used but this remains to be proven.

As regards spawning run time (Table 3), a remarkable reduction of time was observed when 100 g dry weight blocks were used. While 500 g dry weight blocks required 28–30 days, the smaller blocks only needed 12–13 days. Consequently, the reduction of the volume of the bag not only produced higher yields but also reduced spawning run time. However, times of primordium formation were similar for both treatments (Table 3). Pin-heads could be observed 12–16 days after induction conditions began. Primordium development was characterised by a small spherical dark brown pileus and a relatively long stem densely covered with brown fibrils. Later, the pileus expanded and as a consequence, the fibrils became more distant at the pileus margin and appressed and dense at the centre. Fibrils colour was very variable, from dark brown to cream, depending on light regimes. Fruit bodies can be preserved

Table 2
Yields and biological efficiency (BE) of *L. tigrinus* BAFC 2102 and *P. ostreatus* using three substrates of 500 g dry weight blocks and two temperatures

		L. tigrinus 25 °C	*L. tigrinus* 20 °C	*P. ostreatus* 20 °C
Substrate 1	Yield means (g)	309.67	286.67	253.30
	BE means	61.93 ± 14.93a	57.33 ± 4.73a	50.57 ± 10.26a
Substrate 2	Yield means (g)	29.33	ND	567.30
	BE means	5.87 ± 2.32b	ND	113.47 ± 18.67c
Substrate 3	Yield means (g)	53.33	ND	508.30
	BE means	10.67 ± 1.97b	ND	101.67 ± 8.39c

Means in the same row followed by the same letter are not significantly different according to Tukey HSD test.
ND = Not determined.

Table 3
Yield, BE, mycelial colonization and primordium initiation of *L. tigrinus* growing on supplemented *Salix* sp. at 25 °C

	500 g block			100 g block		
	BE means	Mycelial colonization (days)	Primordium initiation (days)	BE means	Mycelial colonization (days)	Primordium initiation (days)
L. tigrinus BAFC 197	60.67 ± 3.06a	28–29	12–14	141.00 ± 14.52b	12	12
L. tigrinus BAFC 2044	60.93 ± 13.78a	29–30	11–14	118.30 ± 13.58b	12	10
L. tigrinus BAFC 2102	61.93 ± 14.93a	28–29	12–16	127.00 ± 14b	12–13	12–14

Means in the same row followed by the same letter are not significantly different according to Tukey HSD test.

at 2–5 °C for 9–12 days, which can be considered an acceptable shelf life.

4. Conclusions

The strains of *L. tigrinus* grew optimally at 30 °C and fruited at 20–25 °C. Supplemented *Salix* sawdust produced highest yields. Small bags with 100 g dried weight reduced the spawning run time to 12 days and duplicated yields which suggested that artificial logs of 10 cm diam. could be used for cultivation. The high BE obtained on sawdust, the wide range of temperature of growth, the relatively high temperature of fruiting, and an easy cultivation technique indicate that *L. tigrinus* could be an interesting species for commercial production, specially for temperate areas of developing countries where cooling equipment is expensive and not available for farmers. Other uses such as a recycler of agroindustrial organic wastes for the production of substrates and organic fertilizers or its use as bioremediation in soil pollution should also be investigated.

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