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ORIGINAL PAPER

# *Polyporus tenuiculus*: a new naturally occurring mushroom that can be industrially cultivated on agricultural waste

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**Abstract** *Polyporus tenuiculus* is a naturally occurring species from Central and South America that is consumed by different ethnic groups in the region. To determine the optimal conditions for fruiting body production, two strains were assayed on wheat straw and sawdust with or without supplements. Sixty days of incubation at 25°C were needed to produce a solid block. The highest yield was obtained with strain ICFC 383/00 grown on supplemented willow sawdust. In a second experiment the strain ICFC 383/00 and different supplements were used to improve the biological efficiency (BE) and to determine the quality traits and its biodegradation capacity. The highest yields were obtained on sawdust with 25% of supplements reaching 82.7% of BE. Supplements raised the number of flushes, generally from four to five, contributing to increased yields. The type of substrate had a significant effect on fruiting body diameters of *P. tenuiculus*, and the largest mushrooms were harvested on supplemented substrate with the highest BE coinciding with the highest dry matter loss in substrates. *P. tenuiculus* showed a capacity to degrade sawdust, causing a decrease of 67.2–74.5% in cellulose, 80.4–85.7% in hemicellulose, and 60.6–66.2% in lignin content at the end of the cultivation cycle. The decrease in hemicellulose was relatively greater than that of cellulose and lignin on supplemented substrates. This is the first report of the cultivation of *P. tenuiculus* on lignocellulosic waste, and it is a promising species both for commercial production and for its potential use in the degradation of other biowastes.

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**Keywords** *Polyporus tenuiculus* · Cultivation · Lignocellulosic wastes · Substrate biodegradation · Naturally occurring strains

## Introduction

Worldwide commercial mushroom production has progressively improved during the last decade, increasing 35.9% from 1995 to 2005. World production comprises about $5 \times 10^6$ tons fresh weight year$^{-1}$, although, at present, only a few genera of basidiomycetes (*Agaricus*, *Lentinula*, *Pleurotus*, *Auricularia*, *Volvariella*, *Flammulina*, *Tremella* and a few others) are industrially cultivated [9].

Mushroom cultivation not only reduces the environmental impact of wastes used as substrate but also provides an economically acceptable alternative for the production of food with high nutritional quality and taste [2, 3, 12, 18]. Various abundantly available lignocellulosic wastes may be used as cheap substrates for mushroom production, such as wheat, rice, paddy straw, sawdust, cotton waste, corn-cobs, coffee pulp, viticulture residues, olive oil mill waste, and switch grass [13, 18, 22, 25, 33–35]. At present, several species of edible mushrooms are cultivated worldwide, such as *Pleurotus ostreatus*, *P. sajor-caju*, *P. pulmonarius*, *P. cornucopiae*, *Lentinula edodes*, *Agrocybe aegerita*, *Volvariella volvacea*, and *Polyporus umbellatus*, that use different lignocellulosic wastes for their cultivation with a high bioconversion rate [16, 22, 26, 35].

*Polyporus tenuiculus* (P. Beauv.) Fr (Polyporales, Basidiomycetes) is a member of the family Polyporaceae [8]. The fruiting bodies are stipited with a poroid himenium. The pileus is whitish to yellowish when fresh and becomes darker when dry, the surface is fibrillose to smooth, and they can be found growing on woods in the northern rain

forest of Argentina and southern Brazil [2]. This species is characterized by poroid hymenophore with large pores (3–5 per cm) and soft flesh. It is collected and harvested jointly with other edible mushrooms by different ethnic groups in the forest. Ruán-Soto et al. [23, 24] reported that it is sold in Mexico, mainly in markets in the coastal plain of the Gulf of Mexico. This species is also consumed in Guatemala and in Peruvian and Brazilian Amazonia. Nevertheless, nothing is known about the performance of this fungus on different waste substrates and its bioconversion capacity.

Many studies focus on the search of new wild species of edible mushrooms to enlarge the number of available species for human consumption. Moreover, the cultivation of these naturally occurring species could permit the continuous availability of the product in markets. There are no reports of the cultivation of *Polyporus* species, apparently they were not studied. *P. umbellatus* is the only one that was commercially cultivated, but this species is tough and is consumed mainly for medicinal purposes as dry powder in tablets [5, 26, 29]. It is known that the genus *Polyporus* grows naturally on dead trunks and different lignocellulosic substrates [23], so it is interesting to evaluate if naturally occurring strains of *P. tenuiculus* could be cultivated on lignocellulosic wastes.

The goal of this paper is to determine the optimal conditions to cultivate the edible mushroom *P. tenuiculus* on lignocellulosic wastes and to evaluate its biological efficiency and biodegradation.

## Materials and methods

### Strains

Cultures used in this study are maintained in the IIB-INTECH Collection of Fungal Cultures (ICFC) reference in the WDCM database: WDCM 826. *P. tenuiculus* ICFC 383/00, Brazil, Río Grande do Soul, Porto Alegre, Parque Faroupilla (cut banana tree) and *P. tenuiculus* ICFC 233/00, Brazil, Río Grande do Soul, Viamão, Parque St. Hilaire (on dead indeterminate wood).

### Optimal temperature for mycelium growth

Cultures were inoculated with a 7 mm diameter cylinder of agar with mycelia of strains ICFC 233/00 and 383/00 in 90 mm Petri dishes containing potato dextrose agar (PDA) and incubated in darkness at 20, 25 and 30°C. Diameters of the colonies were measured in triplicate with a ruler (0.5 mm scale).

### Culture media and spawn preparation

Potato dextrose agar (PDA, Britania, Argentina, 39 g/l) culture medium was used for routine culture and storage purposes. Spawn was prepared from boiled wheat seeds (*Triticum* sp.) supplemented with 2% w/w calcium carbonate ($CaCO_3$), placed in polypropylene bags and then sterilized at 121°C for 2 h [10]. Once cooled, each bag was inoculated with mycelia grown on PDA and incubated in darkness at 25°C until mycelia had completely covered the wheat grains.

### Substrate formulation

Dried willow tree (*Salix* sp.) sawdust and wheat (*Triticum* sp.) straw were chopped to a size particle of 1.5–3 mm and of 30–50 mm long, respectively. These substrates were used with and without supplements (Table 1). Distilled water was added to all formulas and left overnight to moisturize completely to obtain 70% w/w of water content. Polypropylene bags (25 × 45 cm, 30 μm thick) were filled with 1 kg of wet substrate and stoppered with cotton plugs held by PVC (polyvinyl chloride) cylinders before they were sterilized twice at 120°C for 2.5 h. After cooling, the bags were inoculated with 5% w/w spawn and incubated in the dark at 25°C until the mycelium completely colonized the substrate. Optimal incubation time was determined.

### Experimental design and cultivation conditions for mushroom production

In the first experiment, two different non-supplemented (WS and S1) and supplemented substrates (WSS and S2)

**Table 1** Substrate formulation

| Substrates | Main component (%) | Supplements (%) | | | |
|---|---|---|---|---|---|
| | | Wheat brand | Soybean flour | Millet | $CaCO_3$ |
| WS | Wheat straw (98) | 0 | 0 | 0 | 2 |
| WSS | Wheat straw (78) | 15 | 5 | 0 | 2 |
| S1 | Willow sawdust (98) | 0 | 0 | 0 | 2 |
| S2 | Willow sawdust (78) | 15 | 5 | 0 | 2 |
| S3 | Willow sawdust (73) | 15 | 5 | 5 | 2 |

(Table 1) and two strains of *P. tenuiculus* (ICFC 233/00 and 383/00) were evaluated. Six replicates per substrate and strain were used.

In the second experiment, one selected strain was cultivated using three different formulations: S1, S2 and S3 (Table 1). To determine if this species needs any induction for primordia formation, two treatments were assayed before transferring substrates to the fruiting room: (i) light exposure (LE): polypropylene bags were exposed to 12 h light/12 h dark photoperiod under a fluorescent light (40 W) for 12 days at 15°C ± 3°C and then removed; (ii) cold soaking (CS): polypropylene bags were first removed and then the substrate soaked in cold water (10°C) for 12 h. Samples from the three different formulations were removed after the incubation period and directly transferred to the fruiting room. Eight replicates per formulation and treatment were performed. An exception was made for samples that were induced to fruit without any induction treatment (WI). In this case, 20 replicates were carried out. Cropping conditions to induce fruiting body formation for all treatments and substrates were 22 ± 2°C, 9 h light/15 h dark photoperiod (20 W fluorescent light), 75% to 85% humidity levels, and watering by spray (fog type) for 5 min every 8 h, which was automatically provided.

### Analysis of compositional changes of substrate

From the second experiment, three bags per substrate (S1, S2 and S3) without any induction treatment were selected randomly and removed at each of the four growth stages defined as: (1) at zero time (0 day, sterilized bags), (2) 40 days of incubation, (3) after the first mushroom harvest (68–74 days after inoculation) and (4) spent substrate after the last mushroom harvest (180 days after inoculation). Each bag was dried immediately at 60°C, milled and sieved. The dry matter content of each substrate at different stages was determined by drying samples to a constant mass in a 60°C oven [35]. The fiber component of all substrates was determined using the detergent method [4], and both the neutral detergent fiber (NDF) and the acid detergent fiber (ADF) were extracted. Lignin fractions were determined with sulphuric acid (72%), and the cellulose content was estimated directly from ADF–lignin. Hemicellulose was arithmetically calculated as NDF–ADF. All results are presented on a dry matter basis (percentages).

### Crop yield and quality traits assessment

Mature fruiting bodies were harvested daily, and production and quality traits were registered as: (i) primordia initiation (days): time until appearance of primordia under fruiting conditions; (ii) biological efficiency (BE): fresh fruiting bodies weight (total yield)/dry substrate weight, expressed as a percentage; (iii) diameter of fruiting bodies; (vi) flush number and percentages of BE per flush. Fruiting bodies were measured using a ruler (0.5 mm scale) and grouped according to their diameters as follows: group 1 (G1), diameter < 5.4 cm; group 2 (G2), diameter 5.5–9.4 cm; and group 3 (G3), diameter > 9.5 cm. In the first experiment, only BE was recorded, in the second experiments, BE and all other traits were measured.

### Statistical analyses

Prior to analysis, Kolmogorov–Smirnov test (with Lilliefors' correction) and Levene median test were applied to test data for normality and equal variation. Data for fruiting body diameter were previously log-transformed. Differences between mean values of treatments were analyzed with a two-way ANOVA analysis, followed by all pairwise multiple comparison procedures (post hoc testing). The Holm–Sidak method was used to detect significant differences ($P < 0.05$) among mean values of factors and interactions. Statistic analyses were conducted with the SigmaStat® program for windows, version 3.1 (Systat software Inc., Chicago, USA).

## Results

### Optimal temperature for mycelium growth

Strain ICFC 383/00 reached maximum growth rate at 30°C (6.21 mm/day) and was significantly higher ($P < 0.05$) than the same strain at 20°C and the strain ICFC 233/00 for 20°C and 25°C (Fig. 1). No significant differences between both strains were found at 20°C and 25°C ($P > 0.05$). Strain ICFC 233/00 reached the maximum growth rate at 30°C. There were no significant differences between 25°C and 30°C; the only differences were found between 20 and 25°C and, 20°C and 30°C. We determined that this species cannot grow at 37°C (data not shown).

### Strain and substrate selection

Two strains were cultivated on supplemented and non-supplemented sawdust or wheat straw. Spawn of high quality was obtained after 21 days of incubation. Strains colonized the substrate after 45 days of spawning run, but a total of 60 days were needed to obtain a mycelium matrix which permitted the formation of a compact block that did not disaggregate after bag removal.

Strain ICFC 383/00 obtained the highest BE average value on S2 (82.3%, Fig. 2), showing significant differences with other substrates and with strain ICFC 233/00 ($P < 0.05$).

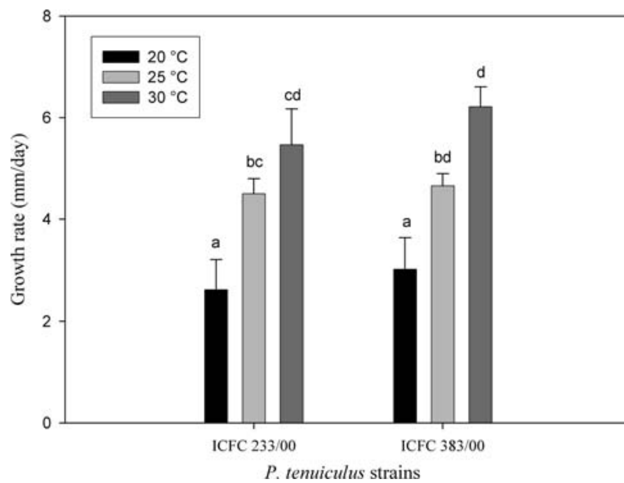


**Fig. 1** Effect of different temperatures on mycelium relative growth rate of two strains of *P. tenuiculus* (ICFC 233/00 and 383/00). Three replicates per temperature and strain were used. Values (means of three replicates) not sharing common letters are significantly different at $P = 0.05$

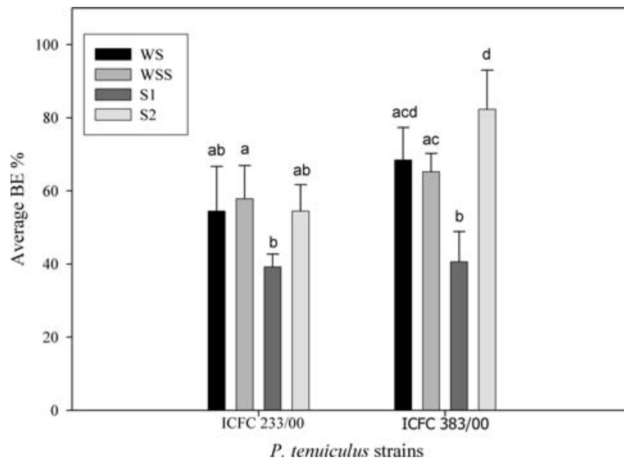


**Fig. 2** BE % of two strains of *P. tenuiculus*, ICFC 233/00 and 383/00, cultivated on supplemented and non-supplemented wheat straw and willow sawdust. Six replicates per substrate and strain were used. Values (means of six replicates) not sharing common letters are significantly different at $P = 0.05$

The lowest BE values for both strains were obtained on S1 and ranged from 39.23 to 40.6%, without significant differences between strains (Fig. 2). A significant difference in yield was obtained between strains when S2 was used; ICFC 383/00 showed the highest values.

These results justified the use of the willow sawdust and strain ICFC 383/00 for the second experiment.

## Effect of supplement and induction treatments on production and quality traits

Time for primordia initiation varied with the induction treatment. *P. tenuiculus* required, under fruiting conditions, 8–10 days without any treatment and 11–14 days when receiving light exposure or cold soaking treatments (Table 2).

The highest BE was obtained with S3 (Table 2). No significant differences were observed between S3 and S2, but S1 was significantly ($P < 0.05$) lower (0.3–2.6 fold). Induction treatments did not affect the yields significantly, although values obtained in S3 with LE were 19.1% superior to the blocks without treatments.

The number of flushes obtained along 90 days of the cropping period varied from 4 to 6 (Table 2). In S1, the number of flushes was mainly 4, while, in supplemented substrates, the flushes were mainly 5. Yield distribution among flushes displayed a similar pattern independent of supplements or induction treatments. More than 65% of the total yield was obtained from the first two flushes.

The morphology and fruiting body development were normal compared with specimens collected in nature. The diameters of fruiting bodies varied between $5.9 \pm 0.6$ and $8.1 \pm 1.4$ cm, showing significant differences ($P < 0.05$) between substrates (Table 2). Mushrooms harvested on S3 had the biggest diameter showing significant differences ($P < 0.05$) with S2 and S1 (Table 2). An induction treatments did not modify mushroom diameter significantly (Table 3); nevertheless, the use of cold socking in S3 (CS) caused an increase of 11.1% in G3 compared with the same substrate without treatment (WI). The predominant fruiting body size corresponded to G1 and G2 for all substrates and induction treatments (Table 3).

## Biodegradation of substrates during cultivation process

The trends of compositional dry matter loss and fiber contents on substrates during stages of *P. tenuiculus* growth are shown in Table 4. There was an increase in the percentage of dry matter loss in all substrates and stages sampled, showing significant differences in the mean values ($P < 0.05$); the maximum value was obtained in stage 4. The cellulose content showed significant differences at substrates and stages ($P < 0.05$) during the cultivation cycle. Cellulose content was reduced during stage 2 in S2 and S3, reaching nearly 21.44 to 26.83%. In S1, the decrease was 17.55% less ($P < 0.05$). At the end of the cultivation cycle, cellulose content had a great decrease among 67.26 to 74.52% in all substrates, being more evident in S2 and S3 ($P < 0.05$).

The hemicellulose content showed significant differences only along the cultivation cycle ($P < 0.05$) but not among substrates. After 40 days of incubation, this component had important decreases in S2 and S3, being 22.04 and 28.45%, respectively. In S1, however, it decreased only 5.75% ($P < 0.05$). The hemicellulose content decreased 80.42% to 86.03 from the first to fourth stage in the three substrates ($P < 0.05$).

**Table 2** Production and quality traits of *P. tenuiculus* ICFC 383/00 on three substrates with willow sawdust as the main component

| Substrate | Treatment | Primordia initiation (days) | Percentage of BE by flushes | | | | | | Total BE % | Fruiting bodies diam. (cm) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | First | Second | Third | Fourth | Fifth | Sixth | | |
| S1 | WI | 8 | 51.06 | 22.98 | 15.04 | 9.70 | 8.00 | – | 45 ± 19a | 6.0 ± 0.6a |
| S1 | LE | 13 | 42.03 | 35.13 | 18.72 | 14.47 | – | – | 23 ± 13a | 6.1 ± 0.6a |
| S1 | CS | 14 | 50.52 | 33.12 | 18.10 | 11.70 | – | – | 31 ± 14a | 6.7 ± 1.1a |
| S2 | WI | 8 | 41.19 | 22.21 | 14.22 | 9.70 | 7.85 | 8.79 | 71 ± 16b | 6.1 ± 0.4a |
| S2 | LE | 11 | 51.14 | 22.41 | 14.48 | 11.97 | – | – | 70 ± 13b | 5.9 ± 0.6a |
| S2 | CS | 13 | 47.93 | 17.51 | 15.43 | 13.91 | 13.35 | – | 63 ± 16b | 6.2 ± 0.2a |
| S3 | WI | 10 | 40.13 | 25.90 | 18.00 | 13.08 | 10.66 | – | 69 ± 16b | 7.3 ± 0.5b |
| S3 | LE | 14 | 34.31 | 26.61 | 18.00 | 13.31 | 10.65 | – | 83 ± 17b | 6.3 ± 0.6b |
| S3 | CS | 13 | 54.96 | 21.10 | 13.74 | 6.13 | 4.07 | – | 61 ± 23b | 8.1 ± 1.4b |

Values (means of eight replicates) in the same trait not sharing common letters are significantly different at $P = 0.05$. Eight replicates per substrate and treatment were performed. Comparisons were made between treatments on each substrate and between substrates on each treatment

Substrates: *S1* willow sawdust, *S2* willow sawdust + wheat bran + soybean flour, *S3* willow sawdust + wheat bran + soybean flour + millet

Treatments: *WI* without induction, *CS* cold soaking, *LE* light exposure

**Table 3** Mushroom size of *P. tenuiculus* harvested from the different combination of willow sawdust and treatments

| Substrate | Treatment | Production of each size group (%)[a] | | |
|---|---|---|---|---|
| | | G1 | G2 | G3 |
| S1 | WI | 49.6 | 42.3 | 8.1 |
| S1 | LE | 52.7 | 39.9 | 7.4 |
| S1 | CS | 43.9 | 41.4 | 14.6 |
| S2 | WI | 47.7 | 46.5 | 6.7 |
| S2 | LE | 53.6 | 38.4 | 7.1 |
| S2 | CS | 47.3 | 44.3 | 8.3 |
| S3 | WI | 41.6 | 48 | 10.4 |
| S3 | LE | 56.6 | 30.1 | 13.3 |
| S3 | CS | 38.5 | 39.9 | 21.5 |

[a] Groups of pileus size according to diameter: G1 < 5.4 cm; G2 5.5–9.5 cm, G3 > 9.5 cm

Substrates: *S1* willow sawdust, *S2* willow sawdust + wheat bran + soybean flour, *S3* willow sawdust + wheat bran + soybean flour + millet

Treatments: *WI* without induction, *CS* cold soaking, *LE* light exposure

Lignin values showed significant differences between substrates and stages ($P < 0.05$). Lignin decreased from 10.77 to 14.98% from stage 1 to 2 and 60.62% to 66.19% from stage 1 to 4 in all substrates ($P < 0.05$), indicating less consumption of lignin.

**Table 4** Changes in the composition of the three substrates during mushroom growth

| Substrate | Time | Dry matter loss (%) | % on dry weight basis | | |
|---|---|---|---|---|---|
| | | | Cellulose | Hemicellulose | Lignin |
| S1 | 1 | 0.00 | 50 ± 2 | 14 ± 0.4 | 23 ± 0.4 |
| | 2 | 9 ± 2 | 41 ± 1 | 13 ± 2 | 21 ± 0.9 |
| | 3 | 25 ± 4 | 35 ± 1 | 6 ± 2 | 16 ± 2 |
| | 4 | 61 ± 2 | 16 ± 1 | 3 ± 0.7 | 9 ± 1 |
| S2 | 1 | 0.00 | 44 ± 2 | 16 ± 0.4 | 22 ± 1 |
| | 2 | 18 ± 2 | 32 ± 0.6 | 12 ± 0 | 18 ± 1 |
| | 3 | 22 ± 2 | 32 ± 2 | 9 ± 3 | 17 ± 1 |
| | 4 | 69 ± 2 | 11 ± 0.6 | 2 ± 2 | 7 ± 0.7 |
| S3 | 1 | 0.00 | 45 ± 2 | 16 ± 1 | 21 ± 0 |
| | 2 | 12 ± 4 | 35 ± 1 | 11 ± 2 | 19 ± 1 |
| | 3 | 32 ± 1 | 27 ± 1 | 9 ± 0.5 | 15 ± 0.2 |
| | 4 | 66 ± 1 | 12 ± 0.2 | 3 ± 0.2 | 8 ± 0.4 |

All values are means ± standard deviation of triplicate measurements

*S1* willow sawdust, *S2* willow sawdust + wheat bran + soybean flour, *S3* willow sawdust + wheat bran + soybean flour + millet

Stage: 1, zero time, previous mushroom inoculation; 2, 40 days of incubation; 3, after the first flush (68–74 days after inoculation); and 4, after the last flush (180 days after inoculation)

## Discussion

Mycelial growth measurements were performed to investigate the optimal temperature of incubation of *P. tenuiculus* strains. Generally, worldwide cultivated species, such as *Agaricus bisporus*, *Pleurotus ostreatus*, and *Agrocybe cylindracea* have an optimal temperature of growth at 25°C [29, 30]. *P. tenuiculus* has a tropical and subtropical distribution; the strain ICFC 383/00 had an optimal temperature of growth at 30°C, which is coincident with the median temperature of the region where this species naturally fruits. Lechner and Albertó [10]. reported that three strains of *Lentinus tigrinus*, another subtropical species found in the region, also had the same optimal temperature of growth.

In the first experiment, it was possible to determine the combination of strain and substrate that produced highest yields; in conclusion, supplemented willow sawdust and strain ICFC 383/00 were selected. Wheat straw is generally a very good substrate for mushroom cultivation, and it has been used to cultivate *Pleurotus ostreatus*, *P. pulmonarius*, *P. djamor* [24], *P. eryngii*, *P. ostreatus*, *Volvariella volvacea* [16], *Agrocybe cylindracea (=A. aegerita)* [16, 30], and *Lentinula edodes* [3]. Supplements normally provide nitrogen, phosphorous, potassium and carbohydrates that are useful for mushrooms to increase yields and reduce primordium formation time [3, 18, 20, 33].

We observed that this species forms substrate blocks light brown color. After 45 days of incubation, brown spots covered 5–70% of the block surface (data not shown). These spots resembled the browning phenomenon of substrate blocks of *Lentinula edodes*, in which the brown mycelium forms a protector skin over the outside of the substrate. In this species, the skin acts as a moisture barrier and defense against invading organisms [19]. In *P. tenuiculus* this phenomenon was not described before and there is no information about it.

In the second experiment, the time required for primordia formation was influenced by induction treatments. Independent of substrate composition, the blocks that were transferred directly (WI) from the incubation to the fruiting room developed fruiting bodies earlier than those that received treatment. Soaking treatment of blocks produced a major synchronization of the first flush similar to what is seen with *L. edodes* [19]. This means that primordial initiation was trigged at the same time in all blocks. The synchronization of the blocks is usually a desired trait for mushroom farmers because it facilitates their cultivation management. When an induction treatment was applied, no correlation between treatment and spots formation was found. Moreover, no correlation between BE and spot formation could be found, and the maximum percentages of spots was observed in S1 (data not shown).

Supplemented willow tree sawdust produced the highest yields attaining 83% of BE on S3.

The use of supplement sawdust is in agreement with previous studies in others mushrooms species in which it was reported to be a suitable substrate for cultivation [10, 21, 28, 33].

Supplements raised the number of flushes, generally from four to five, contributing to increased yields. This effect was also found by Uhart et al. [30] in three strains of *Agrocybe cylindracea*, which raised the number of flushes from two to three when supplements were used.

Regarding the percentage of mushrooms harvested per flush, *P. tenuiculus* had a similar behavior than *L. edodes* [3, 18], *P. ostreatus*, *P. pulmonarius* and *P. eryngii* [16], obtaining a first flush with 45.92% and a second with 25.55% (average values).

Substrate had a significant effect on fruiting bodies' diameters of *P. tenuiculus*; the largest mushrooms were harvested on supplemented substrate (S3). Yildiz et al. [33], however, found that *P. ostreatus* produced biggest diameters on non-supplemented sawdust. We found that the diameter of fruiting bodies can be increased in *P. tenuiculus* with supplement addition. Philippoussis et al. [18] found significant differences in fruiting bodies' diameters in *L. edodes* harvested in different supplemented substrates. The strain ICFC 383/00 was characterized by the diameters of fruiting bodies lower than 9.5 cm, with G1 and G2 being the most represented size groups with more than 91% of production on S1 and S2 and 78% on S3. The G values, not the mean of the diameters, could be employed to characterize strain diameters of fruiting bodies to give information about size distribution.

The use of supplemented substrates in *P. tenuiculus* improved the BE and, with a 25% of supplements, like in S3, the diameter of fruiting bodies was also increased [20].

The use of induction treatments did not affect the BE but CS increased the production of fruiting bodies belonging to the G3 group.

Detailed analyses of sawdust biodegradation during *P. tenuiculus* growth were not reported before. Dry matter loss of the substrate was in agreement with BE increase. According to BE, bioconversion was more efficient on S2 and S3. Presumably, the dry matter lost was partly assimilated into mushroom fruiting bodies and partly lost into the atmosphere as carbon dioxide due to mushroom respiration [35].

*P. tenuiculus* showed the capacity to degrade sawdust, causing a decrease of 67.2–74.5% in cellulose content, 80.4–85.7% in hemicellulose and 60.6–66.2% in lignin at the end of the cultivation cycle (5 months). These values are high when compared with other related mushrooms. In a previous study, Nazareth and Sampy [15] reported that the decrease in lignin and cellulose content of hardwood sawdust by *Panus tigrinus* (=*Lentinus tigrinus*) after a 4-month incubation period was 56% and 64%, respectively, while, in Lechner and Papinutti [11], the decrease of lignin and cellulose content in wheat straw was 21.49% and 53.26%, respectively, in a similar period of time for the same species.

After solid-state fermentation (SSF) process (stage 4), the decrease in hemicellulose was relatively greater than cellulose and lignin, implying that *P. tenuiculus* can utilize hemicellulose as an easily digestible energy source. Similar patterns of utilization of lignocellulosic components were reported by Mukherjee and Nandi [15] during degradation of water hyacinth biomass by *Pleurotus citrinopileatus* after 48 days of SSF.

In lignocellulosic materials, lignin acts as a barrier to microorganisms. These microorganisms cannot use this complex molecule as the sole carbon and energy source [7]. White rot fungi such as *P. tenuiculus* can degrade lignin to reach other more readily available carbon sources present in the wood (cellulose and hemicellulose). Shashirekta and Rajarathnam [27] found higher lignin degradation in a period from incubation until the formation of pinheads, coinciding with a laccase peak activity. On the contrary, we found more lignin degradation during the fruiting state [27]. At the end of the cropping period, the substrate turned dark brown, with the production of fluid that could possibly be due to the dissolution of lignin, as was reported by Wainright [32]. Cellulose and hemicellulose degradation pattern were similar to the results obtained with *Pleurotus florida* in a substrate formulated with a mixture of 80% of coir pith and 20% of rice straw [27]. It is important to state that, in our experiments, supplemented substrates showed higher decreases in cellulose, hemicelluloses and lignin content than S1 along the cultivation cycle. This result is in accordance with Philippoussis et al. [17], who reported that this behavior could be attributed to the existence of easily metabolized carbohydrates (deriving from the organic supplements of the substrates, wheat bran and millet). It could explain the higher yield and biodegradation rates of *P. tenuiculus* obtained on S2 and S3. Myoson and Verachtert [14] have demonstrated that substrate decomposition by *L. edodes* is initially associated with its hemicellulose content.

The variability observed in the degradation capacity of lignocellulosic components in the substrates is influenced by the substrate's nature, by environmental factors and, most importantly, by genetic factors among species or even among strains of the same species [1].

These results indicate that this fungus has a lignocellulolytic capacity that could potentially be used in the degradation of other natural lignocellulosic waste as has been demonstrated for *Pleurotus ostreatus* [6], *Trametes versicolor* [31] and *Lentinus tigrinus* [11], and can also be used in the recycling of the treated waste to use as fertilizer.

Apart from reducing the environment impact of the wastes, this bioconversion process would represent an economically sound strategy to convert agro-residues into nutritional food.

In conclusion, it was possible to determine some optimal conditions for the cultivation of *P. tenuiculus*, and this is the first report of the cultivation of this species on lignocellulosic wastes. Also, it was possible to observe that the lignocelluloses components in substrates decreased considerably during the *P. tenuiculus* growing period. The BE obtained on sawdust, the relatively high temperature of fruiting, and easy cultivation techniques indicates that *P. tenuiculus* could be an interesting species for commercial production, especially for tropical and subtropical areas. Other uses, such as a recycling of agroindustrial organic waste for the production of substrates and organic fertilizers or its use as bioremediation in soil pollution should also be investigated.

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