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Sawdust and fruit residues of Central Amazonian for *Panus strigellus* spawn's production

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Abstract - The objective of this work was to perform a screening of residues of forest species of the Central Amazon to prepare spawn of the edible mushroom *Panus strigellus*. Sawdust substrates from 11 forest species were tested. Then supplementation with beer yeast, cereal bran and regional fruit residues in sawdust:supplementation relation (5:1 and 10:1) were evaluated. Mycelial growth of *P. strigellus* occurred in all the substrates composed of the Amazonian forests species, suggesting that all have potential for use in spawn formulation and/or cultivation of this edible mushroom. Among these species the substrate formulated with *Simarouba amara* sawdust promoted higher mycelial growth ($P<0.05$). The formulation of *S. amara* supplemented with *Astrocaryum aculeatum* fruit shell bran (10:1) presented the best supplementation alternative among regional fruit residues. Three types of packaging for spawn preparation were evaluated, and the polypropylene sack (32×45 cm) was considered the most appropriate. *Simarouba amara* sawdust and *A. aculeatum* fruit shell are readily available in the North region, and the results demonstrating that these residues might substitute *Eucalyptus* sp. sawdust and rice bran, commonly used in the South and Southeast of Brazil for mushroom spawn production.

Serragem e resíduos de frutos da Amazônia Central para produção de semente-inóculo de *Panus strigellus*

Resumo - Neste trabalho objetivou-se realizar uma triagem de resíduos de espécies florestais da Amazônia Central para o preparo da semente-inóculo do cogumelo comestível *Panus strigellus*. Foram testados substratos de serragem de 11 espécies florestais. Em seguida, suplementação com levedura de cerveja, farelos de cereais e resíduos de frutas regionais foram avaliados na relação serragem:suplemento (5:1 e 10:1). O crescimento micelial de *P. strigellus* ocorreu em todos os substratos formulados com espécies florestais da Amazônia, apresentando potencial de uso na formulação da semente-inóculo e/ou cultivo deste fungo comestível. Entre estes, o substrato formulado com serragem de *Simarouba amara* promoveu maior crescimento micelial ($P < 0,05$). A formulação de *S. amara* suplementado com farelo da casca de *Astrocaryum aculeatum* (10:1) apresentou a melhor alternativa de suplementação entre os resíduos de frutos regionais. Três tipos de embalagens para o preparo da semente-inóculo foram avaliados e o saco de polipropileno (32×45 cm) foi considerado a embalagem mais adequada. Serragem de *S. amara* e casca de *A. aculeatum* são de fácil disponibilidade na região Norte e os resultados demonstram que estes resíduos podem substituir a serragem de *Eucalyptus* sp. e farelo de arroz comumente utilizado no Sul e Sudeste de Brasil para a produção de semente-inóculo de cogumelos.

Introduction

The most cultivated mushrooms species worldwide are *Agaricus bisporus* (J.E. Lange) Imbach, *Lentinula edodes* (Berk.) Pegler, *Pleurotus* spp., *Auricularia auricula-judae* (Bull.) Quél., *Flammulina velutipes* (Curt. ex Fr.) Sing. and *Volvariella volvacea* (Bull.) Singer (Sánchez, 2004). The diversity of species used for cultivation of fungi is influenced by the consumption preferences of the producing countries. In Brazil, the main edible mushrooms produced in the South and Southeast are *A. bisporus*, *L. edodes* and *Pleurotus* spp. These are originated from temperate climates places. Since the 90's in Brazil began using agroforestry waste as a substrate for *L. edodes* mushroom production, added to *Eucalyptus* spp. sawdust supplemented with agricultural residues easily found in the region. The Amazon Region has interesting potential for the development of mushroom cultivation, having abundance and diversity of native edible mushrooms species as well as agroforest residues that might be used as lignicolous substrates to produce organic products of high nutritional, medicinal, and gastronomic value.

Ethnomycological studies of indigenous groups such as the Yanomami in Brazil (Fidalgo & Prance, 1976; Fidalgo & Hirata, 1979; Prance, 1984) and the Uitoto, Muinane and Andoke in Colombia (Vasco-Palacios et al., 2008) have described the edibility of various mushrooms. In 2008, we publish the thermophilic characteristic of the INPACM 1464 isolated (Coleção de Microrganismos de Interesse Agrosilvicultura of the Instituto Nacional de Pesquisas da Amazônia-INPA) collected in a lignicolous substrate in the Central Amazon (Vargas-Isla & Ishikawa, 2008). At that time, the isolated was identified as *Lentinus strigosus* (Schwein.) Fr. (*Panus lecomtei* (Fr.) Corner, current name), however after re-examination of exsiccate, the microscopic characteristics and molecular analyses demonstrated that the species is *Panus strigellus* (Berk.) Overh. (= *L. strigellus* Berk.).

The specimens presented mycelial growth from 25 to 45 ºC, with the optimum temperature being 35 ºC. The broad temperature range suitable for mycelial growth of this species is an advantage for its cultivation in the Amazon region that has average annual temperatures of 30 to 33.4 ºC in the shade year-round. In the sun (the condition in which the mushroom was collected), temperatures can reach 40–45 °C. For edible mushrooms production, the search to substrates formulation for spawn production is an important step. The objective was to carry out a substrate screening using sawdust of Amazon forest species and search supplements options available in the region for *P. strigellus* spawn preparation.

Material and methods

Microorganism

The INPACM 1464 isolated of *P. strigellus* utilized in this study was collected in a lignicolous substrate on Campus III of the INPA, Manaus, AM, Brazil. The stock culture was maintained on Sabouraud Dextrose Agar (SDA; Becton Dickinson) slants at 25 °C, in dark. Mycelia of the stock culture were cultivated at 35 °C in Petri plates (90 mm diameter) containing SDA medium. After five days of growth, disks of the mycelia (10 mm diameter) were removed and used as the inoculum for the experiments.

Sawdust screening

The sawdust type were selected from the main Amazon forest species harvested for timber in Manaus, AM, Brazil, as described in a technical report by Vianez & Barbosa (2002). Two exotic species, *Eucalyptus* sp. and *Quercus acutissima* Carr., were also included because *Eucalyptus* sp. sawdust is commonly used to cultivate edible mushrooms such as *L. edodes* in Southern and Southeastern Brazil (Paula et al., 2001; Queiroz et al., 2004; Silva et al., 2005; Shiomi et al., 2007; Ishikawa, 2008) and *Q. acutissima* is used for mushroom cultivation in Asia (Przybylowicz & Donoghue, 1990; Quimio et al., 1990).

The substrates were formulated separately from sawdust from each of the following trees: *Aniba rosaeodora* Ducke, *Astronium lecointei* Ducke, *Bertholletia excelsa* H.B.K., *Bombacopsis quinata* (Jacq.) Dugand., *Caryocar* sp., *Cedrela odorata* L., *Eucalyptus* sp., *Hymenaea courbaril* L., *Hymenolobium petraeum* Ducke, *Hura crepitans* L., *Ocotea cymbarum* Kunth, *Q. acutissima*, and *Simarouba amara* Aubl. The sawdust was sifted at 3 mm mesh sieve from different timbers was screened, then dried in an oven with air circulation at 65 °C until constant weight and stored in plastic bags at room temperature. The sawdust samples were mixed with rice (*Oryza sativa* L.) bran (sawdust:supplement = 5:1; w/w) and with distilled water until approximately 60% hydration (w/v).

Two additional experiments were conducted. The first one was to exam how supplements commonly used in mushroom cultivation affected *P. strigellus* mycelial development. Rice bran, soy (*Glycine max* (L.) Merrill) fiber, soy extract, wheat (*Triticum aestivum* (L.) Thell.) fiber, wheat germ, and beer yeast were added to *S. amara* (sawdust:supplement = 5:1; w/w), selected in the sawdust screening. Pure *S. amara* sawdust was used as a control.

In the second additional experiment, *Astrocaryum aculeatum* Meyer (common name = tucumã) fruit shell, *Carapa guianensis* Aubl. (common name = andiroba) seed shell, *Euterpe oleracea* Mart. (common name = açaí) seed, and *Passiflora edulis* Sims fruit shell were examined. Each residue was dried at 65 °C, crushed and sifted. These materials were separately supplemented to *S. amara* sawdust in weight ratios of 5:1 and 10:1 (sawdust:supplement).

The formulation preparation (sawdust:supplement) was then distributed in five Petri plates (15 ± 1 g/plate) and sterilized twice an hour with interval of 24 h at 121 °C. Following sterilization, one mycelial disk was deposited in the center of the plate containing the formulation and incubated at 35 °C without light. Mycelial growth was evaluated by the index of mycelial growth rates (IMGR), calculated as $\sum$ (D – Da)/N, where D is the diameter of the colony on the observation day (measurement in cm), Da is the diameter of the colony on the previous day (measurement in cm), and N is the number of days after inoculation.

In addition to the IMGR, the colony vigor was visually evaluated and classified under three vigor levels as (+) thin, (++) medium, and (+++) dense (see details on Figure 1).

Spawn production and substrate inoculation

Three kinds of polypropylene packing: (1) flask, 15 cm height × 9 cm diameter, with the capacity to hold 600 g of wet substrate with screw cap. Two holes of 1 cm in diameter were made in the cap for gas exchange, and they were covered with adhesive tape (microporous filters), (2) transparent plastic bags, 23 cm wide × 36 cm height, and holding capacity of 800 g of wet substrate. For gas exchange it was necessary to create a respirator using a ring of PVC tubing with 3 cm height × 5 cm diameter and hydrophobic cotton, and (3) transparent bag, 32 cm wide × 45 cm height with 1200 g of substrate handing capacity and a hole of 4.5 cm diameter covered with filter paper for gas exchange (see details on Figure 2).

As a first step, mycelia were placed on wheat grains to multiply. The grain was washed and immersed in water for 24 h. Soon after, 250 g of wheat grain was put in each of ten 500 ml glass flasks and sterilized in an autoclave at 121 °C for 1 h following the methodology described by Stamets (1993). Ten mycelial disks of *P. strigellus* was then transferred into each flask and incubated at 35 °C for 15 days.

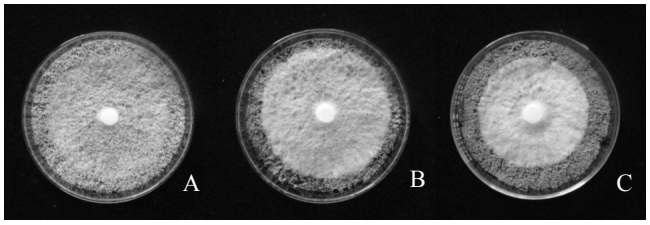

Figure 1. Classification of three colony vigor levels of *Panus strigellus*. (A) thin (+); (B) medium (++); and (C) dense (+++)·

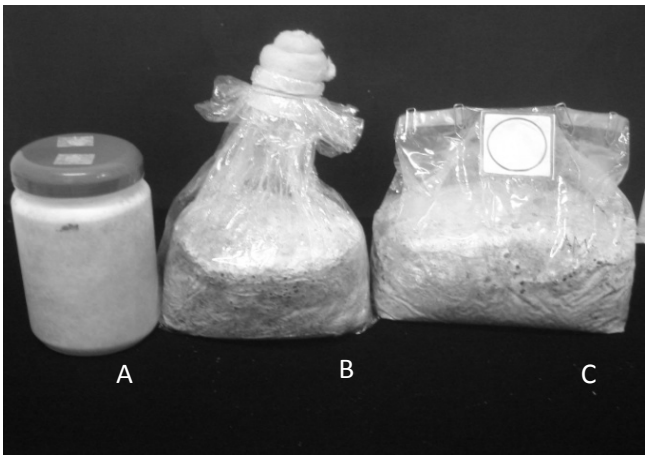

Figure 2. *Panus strigellus* spawn in sawdust of *Simarouba amara*. The polypropylene packing material: (A) 15 × 9 cm flask; (B) 23 × 36 cm bag with an added respirator; and (C) 32 × 46 cm bag with a filter.

Next, sawdust from *S. amara*, *H. petraeum*, and *A. lecointei*, which had shown good results for mycelial growth, was separately evaluated for spawn growth. The sawdust supplemented with rice bran (5:1) was placed in the polypropylene packing. The substrates were sterilized twice an hour with interval of 24 h at 121 °C. For each 100 g of substrate, we added 3.5 g of colonized wheat grains and incubated the material at 35 °C for 25 days in the absence of light in Biologic

Oxygen Demand (BOD – TE-390/TECNAL). After this period, the colonized substrates were taken from the packing materials and cut obliquely into blocks for visual observation of the colonization. Each type of packing material was tested in five replications.

The experiments in Petri plates were tested in five replications and two repetitions. We used analysis of variance (ANOVA) to examine the results of the experiments and compared the averages using the Scott–Knott test at the 5% level of significance.

Results and discussion

The substrates formulated with *B. quinata* and *S. amara* provided the highest *P. strigellus* IMGR values ($P < 0.05$; Table 1), with the colonies reaching the border of the Petri plates (90 mm diameter) in five days after inoculation. This growth was fast compared to that of other edible mushrooms such as *L. edodes* and *F. velutipes* (Ishikawa, 2001). Substrates composed of *C. odorata*, *H. crepitans*, and *O. cymbarum* sawdust showed the lowest IMGR values ($P < 0.05$), with colonies reaching the Petri plate borders in 10 days. However, this growth is considered common for other edible mushrooms. Mycelial growth of *P. strigellus* occurred in all the substrates composed of the Amazonian forests species as well as *Eucalyptus* sp. and *Q. acutissima*, suggesting that all have potential for use in spawn formulation and/or cultivation of this edible mushroom. *Simarouba amara* sawdust was chosen as substrate for the supplementation experiments for its rate of mycelial growth and also because, the specie is frequently used for lumber making and its sawdust is readily available.

Rice bran, soybean fiber, wheat fiber and germ supplementations in spawn production produced the highest *P. strigellus* IMGR values ($P < 0.05$) and improved the colony vigor compared to the control (Table 2). Beer yeast and soybean extract presented lower IMGR values but also improved the colony vigor. Sales-Campos et al. (2008) obtained the highest mycelial growth to *Pleurotus ostreatus* (Jacq.) P. Kumm. using *S. amara* sawdust supplemented with soybean bran. Also *S. amara* residue, rice and wheat bran, and $CaCO_3$ formulation was used for *P. lecomtei* mushroom production (Sales-Campos & Andrade, 2011).

However, while these supplements are generally inexpensive in cereal-producing areas of Brazil, rice, wheat, and soybean cultivation is scarce to nonexistent in the Central Amazon. Thus acquiring large amounts of these supplements would elevate the costs of the substrate in the Amazon region.

Other regional agroforestry residues, however, are produced in large amounts and rarely used. For example, the pulp of *A. aculeatum* fruit is widely consumed in regional dishes throughout the year; the fruit shell residue is generally not used for other purposes and is easy to acquire. Likewise, the shells of *C. guianensis* are an unused residue of oil extraction for cosmetic and therapeutic products, while *E. oleracea* seed shells and *P. edulis* fruit shells are left over from the production of açaí and passion fruit juices.

Regarding supplementation with regional agroforest residues, all of the supplements improved the IMGR and/or colony vigor of *P. strigellus* in *S. amara* sawdust compared to the control (Table 3). Considering both IMGR and colony vigor, the 10:1 mixture of *S. amara* sawdust with *A. aculeatum* presented the best alternative.

For spawn production: *S. amara*, *H. petraeum*, and *A. lecointei* substrates had been totally colonized by *P. strigellus* after 25 days of incubation at 35 ºC in the three types of packing materials tested (Figure 2). However, several aspects should be considered, including the costs of the packing materials, the time required for the spawn run, the transportation viability, and the feasibility of mycelial inoculation of the substrate. From packing materials examined here, type 1 (the flask) was more feasible for inoculation and easier to transport. The flask, however, was also the most expensive packing material and visually checking whether colonization had occurred was difficult because the flask was opaque (Figure 2A), and it could prevent observation of contaminants during the incubation. Packing material 2 (the bag without a filter) was of intermediate cost and allowed for good visibility of colonization. Nevertheless, it was also fragile and required the use of two bags; furthermore, it was necessary to create a respirator using a ring of PVC tubing and hydrophobic cotton (Figure 2B). Packing material 3 (the bag with a filter) held a larger amount of substrate, resulting in lower cost. Produced specifically for the production of *L. edodes* spawn, the bag contains a filter for gaseous change, is resistant enough for transport, and allows for visual checking of colonization (Figure 2C). This polypropylene sack was considered the most appropriate packing by representing smaller cost for the spawn production. However, few distributors of this material operate in Brazil.

Table 1. Effect of forest species sawdust substrates on *Panus strigellus* mycelial growth.

Substrate[1]	Mean[2]	Standard deviation
Bombacopsis quinata	1.197 a	± 0.019
Simarouba amara	1.175 a	± 0.018
Quercus acutissima	1.084 b	± 0.016
Astronium lecointei	1.050 b	± 0.026
Hymenaea courbaril	1.038 b	± 0.042
Hymenolobium petraeum	1.036 b	± 0.017
Eucalyptus sp.	0.923 c	± 0.045
Aniba rosaeodora	0.898 c	± 0.010
Bertholletia excelsa	0.891 c	± 0.084
Caryocar sp.	0.837 d	± 0.019
Cedrela odorata	0.774 e	± 0.014
Hura crepitans	0.756 e	± 0.019
Ocotea cymbarum	0.724 e	± 0.021

(1) All substrates were composed of a five-to-one (w/w) mixture of sawdust and rice bran. (2) Average of five replications and two repetitions of index of mycelial growth rates values-IMGR (cm/day). Means with the same letter(s) are not significantly different ($P < 0.05$) by the Scott–Knott test.

Table 2. Effect of supplementing *Simarouba amara* sawdust substrate with beer yeast and cereal bran on *Panus strigellus* mycelial growth.

Supplement[1]	Mean[2]	Standard deviation	Colony vigor[3]
Wheat fiber	1.22 a	± 0.02	+++
Rice bran	1.21 a	± 0.02	+++
Soy fiber	1.21 a	± 0.02	+++
Wheat germ	1.19 a	± 0.09	+++
Soy extract	1.15 b	± 0.07	+++
Beer yeast	1.13 b	± 0.05	+++
Control (without supplementation)	1.13 b	± 0.02	+

(1)The substrate was composed of a five-to-one (w/w) mixture of *Simarouba amara* sawdust and the supplement. (2)Average of five replications and two repetitions of index of mycelial growth rates values-IMGR (cm/day). Means with the same letter(s) are not significantly different ($P < 0.05$) by the Scott–Knott test. (3) Colony vigor levels: (+) thin, (++) medium, and (+++) dense.

Table 3. Effect of supplementing *Simarouba amara* sawdust substrate with Central Amazon fruit residues on *Panus strigellus* mycelial growth

Substrate	Amount	Mean[1]	Standard deviation	Colony vigor[2]
Euterpe oleracea seed	5:1	1.15 a	± 0.04	+
Carapa guianensis seed shell	10:1	1.14 a	± 0.03	+
Euterpe oleracea seed	10:1	1.13 a	± 0.05	+
Astrocaryum aculeatum fruit shell	10:1	1.13 a	± 0.03	++
Simarouba amara (control)	10:0	1.08 b	± 0.04	+
Carapa guianensis seed shell	5:1	1.08 b	± 0.04	++
Astrocaryum aculeatum fruit shell	5:1	1.08 b	± 0.06	++
Passiflora edulis fruit shell	10:1	1.04 c	± 0.02	++
Passiflora edulis fruit shell	5:1	0.97 d	± 0.02	+++

(1)Average of five replications of index of mycelial growth rates values-IMGR (cm/day).Means with the same letter(s) are not significantly different ($P < 0.05$) by the Scott–Knott test. (2) Colony vigor levels: (+) thin, (++) medium, and (+++) dense.

Conclusion

Simarouba amara sawdust and *A. aculeatum* fruit shell are readily available in the North region and these residues showed potential as substitute of *Eucalyptus* sp. sawdust and rice bran, commonly used in the South and Southeast of Brazil, for *P. strigellus* spawn production.

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