Prospect for production of *Pleurotus sajor - caju* from cassava fibrous waste

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SUMMARY

Cassava fibrous waste, alone or in combination with sugar cane bagasse, was studied for its potential for production of *Pleurotus sajor-caju* LPB 19 in solid state fermentation. Various physico-chemical parameters such as fermentation temperature, initial pH of the medium, enrichment of the medium with glucose and the level of NaCl in the medium were studied, along with production of fruit bodies. The data indicated high potential of cassava fibrous waste in the production of *Pleurotus* mushroom, as the average production of fresh fruit bodies and the biological efficiency of the process involving 80 : 20 ratio of cassava bagasses : sugar cane bagasse medium are excellent. The work provides a novel approach for upgradation of cassava fibrous waste.

Keywords : *Pleurotus sajor-caju*, solid state fermentation, cassava fibrous waste, sugar cane bagasse, fermentation temperature, initial pH of medium, glucose enrichment, sodium chloride, fruit bodies, biological efficiency.

RESUME

Prospectives de production de *Pleurotus sajor-caju* sur des résidus fibreux de manioc.

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La bagasse de manioc, un produit fibreux, utilisée seule ou mélangée avec de la bagasse de canne à sucre, est étudiée dans le but de connaître sa possibilité d'être employée comme support pour la production de *Pleurotus sajor-caju* LPB 19 par fermentation en milieu solide. De nombreux facteurs sont ainsi évalués pour optimiser la production de carpophores : la température de fermentation, le pH initial et la composition du milieu d'imprégnation (glucose et NaCl). Les résultats obtenus indiquent que la bagasse de manioc est un excellent support pour la production de carpophores de *Pleurotus sajor-caju*. Les meilleurs résultats sont obtenus avec un milieu contenant un mélange de bagasse de manioc et de canne à sucre avec un rapport respectivement de 80% et 20%. Ce travail décrit une nouvelle approche pour la production de ce type de champignons avec ce support.

Mots clés : *Pleurotus sajor-caju*, fermentation en milieu solide, écarts fibreux de manioc, bagasse de manioc, bagasse de canne à sucre, température de fermentation, pH initial de fermentation, enrichissement eu milieu en glucose, addition de NaCl, carpophores, efficacité biologique.

INTRODUCTION

Microbial conversion of cassava fibrous wastes has been extensively worked out in the past. But, the digestibility of cellulose and hemicellulose was considerably reduced due to the presence of lignin (Fan *et al*, 1987). *Pleurotus* is one of the most efficient lignin degrading edible fungi (Bisaria and Madan, 1983). According to several authors, *Pleurotus* species actively degraded lignin of agricultural wastes by secretion of laccase and peroxidase (Bisaria and Madan, 1983; Gujral *et al*, 1987; Hadar *et al*, 1993; Kerem *et al*, 1992). Consequently, agro-industrial wastes could be converted into sugars, fuels (alcohol, methane), solvents (acetone, butanol), animal feeds and organic fertilizers. The cassava fibrous solid residue and especially, the starch present in it, could be used for cultivation and production of these edible mushrooms (Soccol, 1994). Thus, the cultivation of this fungus on cassava fibrous wastes could be a excellent biotechnological aternative to upgrade this byproduct produced by the various milling companies of Parana in Brazil.

Pleurotus can be considered as a good alternative for production of protein-rich food in tropical countries (Bisaria and Madan, 1983; Martínez-Carrera *et al*, 1992). These edible mushrooms are rich in proteins, carbohydrates, lipids, vitamins, organic acids and minerals (Bisaria and Madan, 1983). In addition, it has a therapeutical anti-tumoral value (Martínez-Carrera *et al*, 1992 ; Soccol, 1994). According to Macaya - Lizano (1988), *Pleurotus* mushrooms are resistant to diseases and can be cultivated in short time and at low costs.

The objective of the present work was to define the factors influencing growth of *Pleurotus* on cassava solid wastes. The results described could generate some innovative biotechnological alternatives and better utilization of cassava solid wastes.

MATERIALS AND METHODS

MICROORGANISM

Pleurotus sajor-caju LPB 19 was obtained from Laborat6rio de Processos Biotecnol6gicos (U.P.R., Curitiba, Brasil). The strain is maintained on rye-yeast agar medium at 5°C.

SUBSTRATES

The substrates used were cassava (*Manihot esculenta* Crantz) fibrous waste and sugarcane (*Saccharum officinarum L.*) bagasse. Table 1 summarizes the composition of cassava waste (Cereda, 1994). Solid residues were mixed. Five different compositions of mixture were tested (cassava fibrous waste : sugar cane bagasse) : 20 : 80 ; 40 : 60 ; 60 : 40 ; 80 : 20 and 100 : 0

Table 1. Composition of cassava fibrous waste (on basis of dry substrate).

Component	Concentration (% weight/weight)
Moisture	9.52
Ash	0.66
Starch	63.85
Fat	0.83
Nitrogen (x 6.25)	0.32
Fibers	14.88

GROWTH OF *PLEUROTUS SAJOR -CAJU* LPB 19 ON CASSAVA FIBROUS WASTE AGAR MEDIUM

Incubation of fungi was carried out on cassava fibrous waste agar medium, which contained (g / L) cassava waste, 40.0 and agar 15.0 in Petri plates (8.5 cm diam). The pH was adjusted to 7.0 with 1.0 N $CaCO_3$. Disks (5 mm diam) were cut from the central zone of *Pleurotus sajor-caju* LPB 19 cultivated on rye - yeast medium. These disks were placed in the centre of the test plate, containing the appropriate medium. Test plates were incubated at 25°C for 6 days.

Radial growth measurements were made daily during the entire incubation period (Soccol *et al*, 1994). In some cases, dry biomass was weighted. For this, the medium in the plates was heated and the mycelium was separated from the melted culture medium in a Buchner funnel equipped with a 8 mm filter paper, which was previously weighted. Mycelial cells were washed several times with boiling water for the total elimination of medium that could remain on the paper filter. Then, the filter was dried at 105°C for 24 h and the mycelial biomass was calculated by the difference in the weights. Each determination was carried out in triplicate.

SPAWN PRODUCTION OF *PLEUROTUS SAJOR-CAJU*

Spawn production was made in polyethylene bags containing 100 g wheat grains, supplemented with 1% $CaCO_3$ at the moisture content of 50 %. The bags were sterilized by autoclaving for 1 h at 121°C. After cooling to 25°C, the bags were inoculated with 5 disks of rye-yeast agar and incubated at 25°C for 15 days (Soccol, 1994).

SPAWN RUN AND FRUITING IN CASSAVA FIBROUS WASTE AND SUGARCANE BAGASSE

Substrate mixtures were prepared in polyethylene bags, with addition of 1% $CaCO_3$, moistened to 75 % with distilled water and autoclaved at 121°C for 1 h, according to Manu-Tawiah and Martin (1986) and Royse (1992). After sterilization and cooling, the bags were inoculated with 10 % spawn and incubated in the dark at 24°C until the mycelium has completely covered the medium (Gujral *et al*, 1987). After completion of the spawn run, the environmental conditions were changed to induce fruiting body formation. The bags were perforated and incubated in a chamber with 70 % relative humidity and ambiant temperature of 24 - 28°C for 60 days.

RESULTS AND DISCUSSION

PHYSIOLOGICAL STUDIES OF *PLEUROTUS SAJOR - CAJU* GROWTH IN CASSAVA WASTE AGAR

Influence of temperature

Cultures were carried out at different temperatures, i.e., 5, 22, 24, 28, 32, 35 and 37°C. For each temperature, the growth rate was measured in the exponential phase of growth. As shown in Fig. 1, the growth was highest between 24 and 28°C. There was a sharp reduction in growth at higher temperatures. The metabolism of fungus generates heat. Unless this heat is removed, it may raise the temperature of the fermenting material and leads to growth inhibition or even killing of the fungus at higher temperatures.

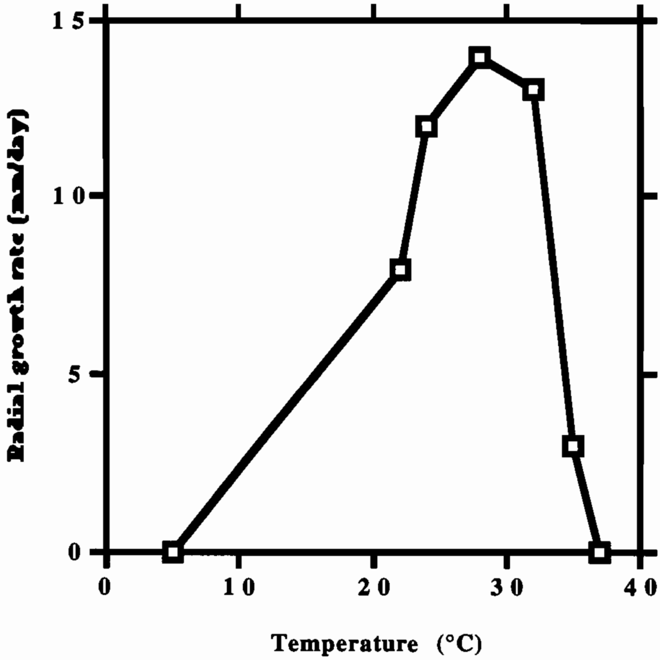

Fig. 1 . Temperature effect on the radial growth of *Pleurotus sajor-caju* LPB 19.

The fungal growth was evaluated by the measurement of colony diam, after six days cultivation at 25°C.

Influence of pH

Data showed that the initial pH of the medium strongly influences the rate and extent of fungal growth (Fig. 2).

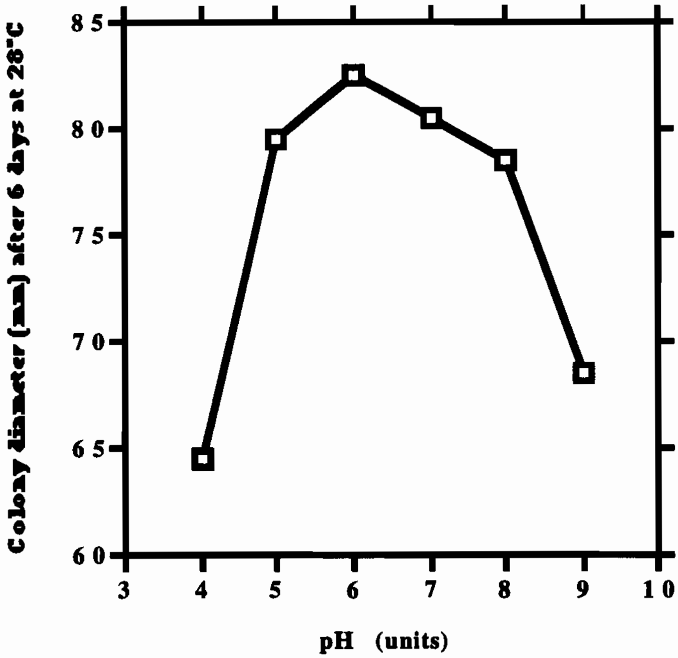

Fig. 2. Effect of initial pH of the medium on the radial growth of *Pleurotus sajor-caju* LPB 19.

The maximal radial growth was observed at the initial pH values in the range of 5.0-8.0, the best being at initial pH of 6.0. It was lower when the medium was too acidic (4.0) or too alkaline (9.0). Most of white-rot fungi grow best at slightly acidic pH values between 4 and 5. Nevertheless, some alkaliphilic species are also reported to grow on such media.

An initial pH of the medium at 6.0 was, therefore, selected for further work.

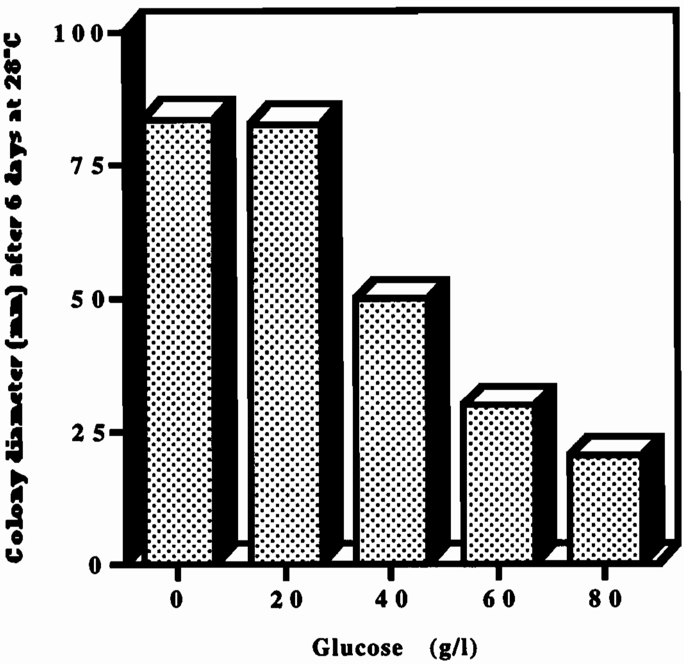

Fig. 3. Effect of glucose addition to the medium on the radial growth of *Pleurotus sajor-caju* LPB 19.

Influence of glucose

Fungal growth was not significantly altered with an increase in the concentration of glucose up to 20 g/L in the medium (Fig. 3). However, glucose concentration over 20 g/L inhibited the growth. This effect could be due to an elevation of osmotic pressure of the medium. Other explanation could be reported in relation with the fungal metabolism of glucose. The catabolism repression induced by the presence of glucose was often reported.

Influence of NaCl

Fig. 4 illustrates the effect of the addition of NaCl on the radial growth of *Pleurotus sajor - caju* under the conditions as described for the above experiments. In the range of concentrations tested, the effect of NaCl was significant.

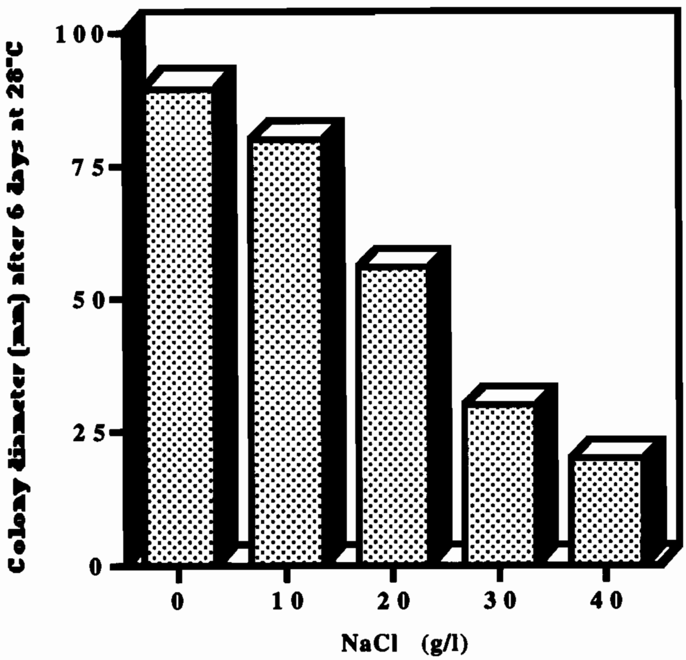

Fig. 4. Effect of NaCl on the radial growth of Pleurotus sajor-caju LPB 19.

The decrease in fungal growth was proportional to the increase in the concentration of NaCl in the medium. With the use of NaCl at 40 g/L, the fungal growth decreased by 76.27 %. Such effect could be related to the sensitivity of membrane translocators to Cl^-. This is the case of the plasmalemma ATPase. The role of Na^+ could also be suggested since this ion modifies the electrical properties of the plasmalemma and, consequently, strongly influences the plasmalemma electrochemical potential.

Influence of addition of yeast extract addition

Addition of yeast extract to the cassava fibrous waste medium showed a positive effect on the fungal growth, until a concentration of 0.8 g/L (Table 2). When supplemented, the production of fungal biomass was significantly higher than the control one (more than 51.4 % of the control). Yeast extract stimulated growth probably, because of its proteins, amino-acids and vitamin content (Manu-Tawiah *et al*, 1990; El-Kattan *et al*, 1990; Fasidi and Olorunmaiye, 1994).

Production of fruit bodies of *Pleurotus sajor-caju* on cassava waste and sugarcane bagasse media

The first crop was harvested on 36 days, after the inoculation, and the second on, 49 days, after the opening of bags. Major yield of mushrooms was in the first flush.

Table 2. Effect of addition of yeast extract on the growth of *Pleurotus sajor-caju.*

Yeast extract (g/L)	Colony diameter (mm)	Biomass produced in Petri dishes (mg)
0.0	9.6 ± 0.4	87.2 ± 19.3
0.4	10.2 ± 0.6	103.0 ± 5.9
0.8	10.4 ± 0.6	132.0 ± 22.8
1.2	10.2 ± 0.4	106.0 ± 0.6
1.4	10.4 ± 0.4	73.6 ± 9.4
2.0	9.7 ± 0.1	73.6 ± 9.4

The best results were obtained with the use of solid support containing 80 % (w/w) cassava bagasse and 20 % (w/w) sugarcane bagasse (Table 3). Parallely, the biological efficiency of *Pleurotus sajor-caju* was estimated to be 30.6 %, as per the methodology reported by Gujral *et al.* (1987) and Ortega *et al* (1992).

Table 3. Average production of fresh fruit bodies of *Pleurotus sajor-caju* LPB 19 and its biological efficiency on cassava and sugar cane wastes.

Substrates					
Cassava bagasse (g)	Sugarcane bagasse (g)	Spawn (g)	Starch (g/100 g)	Crude fiber (g/100 g)	B . E. (%)[a*]
20	80	10	12.77	30.15	22.89 ± 1.56
40	60	10	25.54	26.33	24.31 ± 3.67
60	40	10	38.31	22.50	27.78 ± 6.85
80	20	10	51.08	18.69	30.60 ± 2.82
100	-	10	63.85	14.88	25.89 ± 6.38

a) B.E.% = Biological efficiency % = weight fresh mushrooms /dry substrate x 100

* Average of three determinations

The cassava and sugar cane bagasse mixture were characterized by their specific nutritional equilibrium in starch, and crude fibres. The results are shown in Table 3. Consequently, these substrates permitted the production of fruit bodies of *Pleurotus sajor - caju*. (Fig. 5).

Fig. 5 . Fruit bodies of *Pleurotus sajor-caju* cultivated on cassava and sugar cane bagasse media.

CONCLUSION

The ability of *Pleurotus sajor-caju* to grow on cassava fibrous wastes is an important result. It grew well at a temperature between 24 to 32°C, and a pH range between 5.0 to 8.0. The radial growth was not significantly affected when addition of glucose was not excessive (up to 20 g/L). When the medium was supplemented by yeast extract, up to 0.8 g/L, fungi grew with higher production of biomass.

These results are encouraging and demonstrate the feasibility of the use of cassava fibrous waste as a substrate in solid state fermentation substrate for the production of *Pleurotus*.

Thus, the residues of the starch milling process constitutes a good alternative for the production of edible mushrooms. Nevertheless, further researches is needed in order to improve the biological efficiency of the process, which could be more economically attractive.

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