

Biodegradation of agroindustrial wastes by *Pleurotus* spp for its use as ruminant feed

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Abbreviations: ADF: acid detergent fibre
cfg/g: colony forming units per gram
ITS: internal transcribed spacer region
NDF: neutral detergent fibre
PCR: polymerase chain reaction
RFLP: restriction fragment length polymorphism
SSF: solid substrate fermentation

The increasing expansion of agro-industrial activity has led to the accumulation of a large quantity of lignocellulosic residues all over the world. In particular, large quantities of rice straw (300.000 t) and citric bagasse (50.000 t) are annually produced in Uruguay. In

this work we present the study of the bioconversion of these substrates with the edible mushroom *Pleurotus* spp so as to increase nutritional values and digestibility for its use as animal feed. The SSF process was optimized and the products after different periods of

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mushroom growth were evaluated. The microbial counts (cfu/g) for the inoculated substrates 44 days after incubation were 15×10^4 , < 10 and < 10 for aerobic microorganisms, coliforms and *E. coli*, respectively. After 14 days of SSF the percentage of dry matter, ADF and NDF decreased, and the content of protein increased. These results show that vegetal cell-wall components were degraded during the period of mushroom incubation. PCR – RFLP analysis of the ITS region was used to characterize the *Pleurotus* species produced in Uruguay and discriminate between DNAs of *Pleurotus ostreatus* 814 and other fungi from the different substrates.

From the production, processing and consumption of agricultural products, there are a great variety of remainders, which create increasing problems of elimination. In Uruguay, the great majority of the industries that process agricultural products discard their remainders with no treatment, implying a huge aggression to the environment. For example, the citrus processing plants produce 50,000 tons per year of citric bagasse which represents 40-50% in weight of the fresh fruit. Its composition is relatively adequate for the feeding of ruminants, but present palatability problems and is contaminated with normal flora of the rinds, some of which are mycotoxin producers. With respect to rice straw it is obtained at the rate of 2000 kg per harvested hectare (310,000 ton of straw per year); and although it is used in the feeding of ruminants, it presents a very low protein content and low digestibility.

Other authors have shown that some fungi, particularly some species of *Pleurotus* are able to colonize different types of vegetable wastes, increasing their digestibility (Platt et al. 1984; Commanday and Macy, 1985; Rajarathnam and Bano, 1989; Villas-Boas et al. 2002; Zhang et al. 2002; Mukherjee and Nandi, 2004; Salmones et al. 2005). Previous studies have shown the feasibility of using these kind of wastes to produce animal feed (Calzada et al. 1987; Adamovic et al. 1998), and as substrate for mushroom production (Breene, 1990; Sermanni et al. 1994; Kakkar and Dañad, 1998; Yildiz et al. 2002).

In the present work, we study the biodegradation of these wastes by *P. ostreatus* 814 for its use as ruminant feed.

MATERIALS AND METHODS

Determination of the capacity to grow on the wastes under study

The strains used in this study (*P. ostreatus* 814, *P. ostreatus* 816, *P. cornucopiae* and *P. djmour*) were provided by Trinidad Mushrooms. They were grown in malt agar (1,25% malt extract, 1,5% agar, Oxoid) at 28°C. The mycelium was then transferred to bottles with sterilized wheat grains and incubated again at 28°C until colonization of the substrate was observed (approximately 7 days). These fermented grains were used as inocula (10% in weight) for the growth tests in trays at 28°C on 500 g of substrate without sterilizing (citrus bagasse, rice straw and the mixture of both (1:9 w/w)).

From these experiments, *P. ostreatus* 814 was the most promising strain.

Evaluation of the characteristics of the fermentation products

1. Determination of the microbiological quality was performed using Petrifilm (3M). Total aerobes, total coliforms and *E. coli* were determined on the substrates without inoculation and after 44 days of fermentation with *P. ostreatus* 814.

2. Analysis of the chemical composition of the fermentation product consisted in the analysis of dry weight (AOAC, 1990), proteins (AOAC, 1990) and neutral and acid detergent fiber (AOAC, 1996) of the different substrates without inoculating and on different days of fermentation with *P. ostreatus* 814. The results were analyzed using t-Student test with $P < 0.001$.

The significance of the differences were estimated by using the Mann-Whitney U test (Mann and Whitney, 1947), with the limit of significance set at $P < 0,05$. Statistical analyses were performed on SPSS 9.0 Windows.

Molecular characterization

DNA extraction. Genomic DNA was obtained from pure cultures of fungi belonging to the mushroom producers “Trinidad Mushrooms” (*Pleurotus ostreatus* 814, *Pleurotus*

Table 1. Determination of the microbiological quality. The values reported are the mean of two measurements.

	Microbial count (cfu/g)		
	Total aerobic count	Total coliforms	<i>E. coli</i>
Citric bagasse day 0	< 100	< 10	< 10
Fermentation product day 44	15×10^4	< 10	< 10

