Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioresource Technology 96 (2005) 537–544
BIORESOURCE TECHNOLOGY

Comparative culturing of *Pleurotus* spp. on coffee pulp and wheat straw: biomass production and substrate biodegradation

Dulce Salmones [a,*], Gerardo Mata [a], Krzysztof N. Waliszewski [b]

[a] *Laboratorio de Hongos Comestibles, Departamento de Hongos, Instituto de Ecología, P.O. Box 63, 91070 Xalapa, México*
[b] *Departamento de Ingeniería Química y Bioquímica, Instituto Tecnológico de Veracruz, P.O. Box 1420, 91808 Veracruz, México*

Received 16 September 2003; received in revised form 16 June 2004; accepted 17 June 2004
Available online 15 September 2004

Abstract

The results of the cultivation of six strains of *Pleurotus* (*P. djamor* (2), *P. ostreatus* (2) and *P. pulmonarius* (2)) on coffee pulp and wheat straw are presented. Metabolic activity associated with biomass of each strain was determined, as well as changes in lignin and polysaccharides (cellulose and hemicellulose), phenolic and caffeine contents in substrate samples colonized for a period of up to 36 days. Analysis were made of changes during the mycelium incubation period (16 days) and throughout different stages of fructification. Greater metabolic activity was observed in the wheat straw samples, with a significant increase between 4 and 12 days of incubation. The degradation of polysaccharide compounds was associated with the fruiting stage, while the reduction in phenolic contents was detected in both substrates samples during the first eight days of incubation. A decrease was observed in caffeine content of the coffee pulp samples during fruiting stage, which could mean that some caffeine accumulates in the fruiting bodies.

Keywords: *Pleurotus*; Coffee pulp; Wheat straw; Biomass; Biodegradation

1. Introduction

Coffee pulp, one of the principal byproducts of wet-processed coffee (*Coffea arabica* L.), which constitutes almost 40% of the wet weight of the coffee berry, is rich in carbohydrates, proteins, minerals, and appreciable quantities of tannins, caffeine and potassium (Bresanni, 1979). Approximately 100,000 tons of coffee pulp are generated each year in Mexico, and the majority of this waste has no further economic use; instead, coffee growers generally spread it in the field where it is allowed to decompose.

Diverse technologies have been proposed for utilizing the byproducts generated by the coffee industry (Pandey et al., 2000). Culturing edible mushrooms on coffee pulp seems especially attractive, since it represents a direct conversion of an agricultural waste to human food. Among edible mushrooms evaluated for this commercial activity, *Pleurotus* strains appear promising, primarily because their biological efficiencies can exceed 100% (wet base) (Martínez-Carrera et al., 1985; Martınez-Carrera, 1989).

In the present study, two strains each of *Pleurotus ostreatus*, *Pleurotus pulmonarius* and *Pleurotus djamor* were cultured on coffee pulp and wheat straw. The purpose was to characterize differences in biomass production by the vegetative stage and fruiting bodies production of the strains, as well as changes in lignin and polysaccharide contents (cellulose and hemicellulose) of the substrates attributable to differential use by strains. The degradation of toxic components, particularly phenols and caffeine, was also studied. The practical goal of this investigation was to identify possible advantages for substituting the commercially popular wheat straw substrate with the agroindustrial waste,

* Corresponding author: Fax: +52 228 8187809.
E-mail address: dulce@ecologia.edu.mx (D. Salmones).

0960-8524/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2004.06.019

coffee pulp. In addition, this study permitted the identification of those strains most capable of developing in coffee pulp. This research is part of a series of investigations that are focused on optimizing the use of coffee pulp as a medium for mushroom cultures.

2. Methods

2.1. Strains

The following six strains of *Pleurotus* were studied: IE-38 and IE-49 of *P. ostreatus* (Jacq.: Fr.) Kumm and IE-137 of *P. pulmonarius* (Fr.) Quél. were obtained from commercial strains available in Europe and Asia; IE-121 of *P. djamor* (Fr.) Boedjin was isolated from a wild specimen in Mexico and finally, IE-218 of *P. djamor* and IE-225 of *P. pulmonarius* were obtained from genetic crosses of monosporic cultures in our laboratory. All cultures were deposited in the culture collection of the Instituto de Ecología (Xalapa, Mexico).

2.2. Culture conditions

Spawn was prepared from sorghum seeds that had been hydrated to a moisture content of 55% by weight. Two hundred grams of hydrated grains (wet weight) were placed in polypropylene bags, and then sterilized at 121 °C for 1 h (Gaitán-Hernández et al., 2002). Once cooled, each bag of sorghum seeds was inoculated with mycelia from one of the six pre-cultured *Pleurotus* strains. Inoculated bags were incubated in darkness at 27 °C until mycelia had completely covered the sorghum grains. This process took approximately 2–3 weeks to complete.

Coffee pulp was collected at a coffee processing plant (located in Coatepec, Veracruz, Mexico), air-dried to a moisture content of 18–20%, and then stored at ambient temperature. Dry wheat straw was broken into 1–3 cm fragments with an electric thresher and also stored under identical conditions as the coffee pulp. In all experiments, each of these substrates was rehydrated in water for 12 h, and then excess moisture was allowed to run off until a moisture content of 60% (±5%) was reached. Following this treatment, substrates were placed in polypropylene bags and sterilized at 121 °C for 1 h. Two hundred gram samples (wet weight) of wheat straw or coffee pulp were established for (1) estimating biomass in the vegetative stage, (2) analyzing fiber components, (3) measuring phenolic contents, and (4) assessing caffeine concentrations.

Each 200 g sample was seeded with 10 g (5% inoculation) of spawn and incubated at 27 °C in total darkness. Eight sample replicates × 6 strains × 2 substrates were prepared ($N = 96$ total samples) in order to undertake the analyses. In parallel, three samples per substrate, without mycelia, were prepared and these served as control groups. The time required for the complete colonization of samples of either substrate was established as 16 days of incubation at 28 ± 1 °C, in total darkness (Velázquez-Cedeño et al., 2002). Later, samples colonized were placed, without their plastic bags, in a controlled environment and under conditions favorable for the induction of fruiting; i.e., with an average ambient temperature of 24 ± 5 °C, an relative humidity of 84 ± 6%, 400 m^3 of air circulating per hour, and 11 ± 1 h of natural illumination daily. Samples were cultured under these conditions for 36 days. During the experiment, the samples in the following stages (S) were collected and analyzed: 4, 8, 12, and 16 days of incubation (S2, S3, S4, and S5, respectively); first appearance of primordia (S6); beginning of first harvest (S7); and one week after finishing first harvest (S8). Control samples were collected at 0, 16, and 36 days of incubation (S1, S5, and S9, respectively). All collections were frozen at −20 °C, lyophilized, pulverized in a domestic blender, and then stored at 4 °C.

2.3. Estimation of biomass production during the vegetative stage of the culture

Biomass production was estimated from mycelial metabolism during the substrate colonization phase and was determined using the method of fluorescein diacetate hydrolysis (FDA; Sigma) (Inbar et al., 1991; Mata et al., 2002). This determination requires sterile culture conditions, and for this reason, only sample stages under active incubation and covered with plastic lids were analyzed (i.e., S1–S5, inclusive). This experiment was undertaken by placing 0.2 g of lyophilized material per strain, per substrate, per collection stages (i.e., S2–S5) in each of five, sterile test tubes ($N = 6$ strains × 2 substrates × 5 tubes × 4 collection stages = 300 total tubes). In three tubes, 3 ml of FDA (10 mg/l) were added, and in the two remaining tubes (considered as controls) 3 ml of phosphate buffer solution (60 mM, pH 7.6) were added. Test tubes were incubated at 30 °C for 10 min, after which time the reaction was halted by the addition of 3 ml of acetone. The suspension was filtered (Wattman filter, No. 1) and the remaining solution was detected having a spectrophotometric absorbency of 490 nm (Spectronic Genesys 5, Macedar, NY). One unit of metabolic activity was defined as 1 μmol of FDA hydrolyzed $min^{-1} g^{-1}$ (dry weight). Control samples were analyzed for the stages S1 and S5 ($N = 2$ substrates × 5 tubes × 2 collection stages = 20 tubes).

2.4. Analysis of fiber components

The fiber component of all colonized substrates was determined with a fiber analyzer (Ankom, Model 200/220, Macedar, NY). Using the detergent analysis