Chapter 9

SCREENING OF EDIBLE MUSHROOMS FOR POLYPHENOL DEGRADATION AND TANNASE PRODUCTION FROM COFFEE PULP AND COFFEE HUSK

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Abstract

Edible mushrooms capable of degrading condensed tannins were selected in solid-state culture using coffee pulp and coffee husk as substrates. Eight different strains of *Pleurotus*, *Ganoderma*, and *Lentinula* were screened showing positive results. Several parameters, such as morphology of the colony, apical growth, biomass, and the pH were studied. Biochemical analyses of *P. ostreatus*, strain CP-50, growing on coffee-husk agar (CHA) have shown that this mushroom degrades the polyphenols presents in the culture medium. Three strains of *P. ostreatus* (CP-11, CP-37, CP-50), selected for further studies, degraded up to 75.4 % of condensed tannins after growing for 24 days on coffee-husk solid culture medium. This research opens up the possibility to recycle solid by-products from the coffee agro-industry as substrates in solid-state fermentation, using strains of edible mushrooms for degrading hydrolysable tannins by tannases, and for producing fruit bodies as a human food.

Key words: Coffee pulp, coffee husk, edible mushrooms, polyphenols, condensed tannins, tannases.

Introduction

The coffee pulp and coffee husk are the main solid by-products in the coffee agro-industry (**1**). These by-products are: 1) Produced in large amounts causing serious problems of environmental contamination (**2,3**); 2) Highly biodegradable due to high contents of sugars, proteins, and mineral salts (**4**); and 3) A potential animal feed (**5**). Accordingly, we carried out research work in order to stabilize fresh coffee pulp by the lactic acid silage technique during the crop season (**6**). However, recalcitrant and toxic compounds (RTC), such as hydrolysable tannins (caffeine), and condensed tannins, need to be removed as they are a serious drawback for using coffee pulp/husk as a nutritive source for feeding animals. The enzyme tannase catalyzes the breakdown of hydrolysable tannins (**7,8**), and it has many potential

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applications in the food, pharmaceutical, and chemical industries (**9**). At present, many countries are trying to use coffee pulp for the cultivation of edible mushrooms for human consumption, and the production of animal feed through solid-state fermentation (**10,11**).

Coffee pulp and coffee husk are good substrates for the cultivation of edible mushrooms, such as *Pleurotus, Lentinula, Ganoderma,* and *Flammulina* (**12,13**). These mushrooms generally show a good ability to produce fruit bodies, while simultaneously reducing or degrading the toxic substances present in the substrate. Fann *et al.* (**14**) screened several strains of *P. ostreatus*, *L. edodes*, and *F. velutipes* for their ability to grow on a medium containing the extract of coffee husk and agar. Calzada *et al.* (**15**) carried out a similar study using coffee pulp as substrate for the selection of edible mushrooms. In this work, we report the degradation of condensed tannins and tannase activity in solid-state culture (SSC) by eight selected strains of edible mushrooms.

Materials and Methods

Strains

Eight strains of cultivated mushrooms were used in this study (Table 1). All strains are deposited at the culture collection from the College of Postgraduates at Puebla, Mexico (**16**).

Culture media

The mycelium was grown and maintained on potato-dextrose-agar medium (PDA), which was also used as spawn. Coffee medium for solid-state culture was prepared as follows: 200 g of coffee husk were boiled in 1 L of hot water (90°C) for 15 min. The supernatant was used to prepare one litre of coffee-husk-agar (CHA) medium, by adding 15 g of agar. This medium was sterilized at 121°C for 20 min. After sterilization, 10 ml of CHA medium were poured into Petri dishes (90 mm diameter) under aseptic conditions.

Table 1. Strains of edible mushrooms selected for this study.

Species	Code	Origin (Country/State)
Ganoderma lucidum (Leysser)Karsten	CP-158	Mexico (Morelos)
Lentinula lepideus Fr.	CP-6 (ATCC-62610)	Mexico (Veracruz)
Pleurotus ostreatoroseus Singer	CP-34	Mexico (Morelos)
	CP-44	Mexico (Morelos)
P. ostreatus (Jacq. ex Fr.) Kumm.	CP-37 (ATCC-60271)	Germany
	CP-50	Mexico
P. ostreatus f.sp. florida	CP-11	Germany
Pleurotus spp.	CP-91	Mexico (Chiapas)

ATCC= American Type Culture Collection, Manassas, Virginia, U.S.A.

Solid state culture

Substrates. The coffee husk was obtained from Damasco, Curitiba, Brazil, after processing coffee cherries by the dry process. The coffee pulp was obtained from Puebla, Mexico, by the wet process of coffee cherries. Both substrates were milled with an Ultraturrax IKA (Bioblock, USA).

Solid state culture. Coffee pulp (40 g, 70 % moisture) and coffee husk (40 g, 77% moisture) were placed within 250 ml Erlenmeyer flasks, which were sterilized at 110°C for 20 min.

Inoculation and culture conditions

Agar. Culture media were inoculated centrally with a piece of actively growing mycelia from a colony grown on PDA medium for 15 days. All plates were wrapped in Parafilm, and incubated at 25°C in the dark. Strains grown on CHA medium were incubated at 25°C for 24 days. Biomass was estimated using a sterilized cellophane disc placed on the agar surface from Petri dishes (90 mm diameter), as described by De Araujo *et al.* (**17**). The mushroom mycelium was allowed to grow over the cellophane disc, which was then lifted away, and dried at 105°C for 24 h.

Solid state culture. Substrates were also inoculated centrally with a piece of actively growing mycelia from a colony grown on PDA medium for 15 days.

Analyses

The reducing sugars were assessed by the method of Miller (**18**), while total phenols by the method of Folin and Ciocalteau (**19**). The method of Swain and Hillis (**20**) was used for condensed tannins, and the tannase assay was carried out according to Sharma *et al.* (**21**). One unit of enzyme (IU) was equivalent to the amount of enzyme able to release 1 μmole of gallic acid per min.

Results and Discussion

Screening of edible mushrooms

Growth parameters of eight selected strains of edible mushrooms growing on two different culture media are shown in Table 2. *Pleurotus ostreatus* (CP-50) showed an apical growth of 1.74 mm/day in the solid coffee-husk agar surface. A higher apical growth of 3.4 mm/day was recorded in *P. ostreatus* (CP-11).

Table 2. Screening of edible mushrooms for tannin degradation, tannase and biomass production after 21 days of mycelial growth at 25°C on coffee-husk agar medium (CHA) and coffee-husk solid culture medium.

Strains	Code	Culture media			
		Coffee-husk agar		Coffee husk solid substrate	
		Apical growth (mm/day)	Biomass (g/L/day)	Tannins (mg/g)	Tannase (IU/ml)
Ganoderma lucidum	CP-158	1.7	1.9	0	5.8
Lentinula lepideus	CP-6	0.2	0.6	1.2	71.42
Pleurotus ostreatoroseus	CP-34	2.5	1.2	2.4	9.9
	CP-44	1.4	1.2	1.7	7.8
P. ostreatus	CP-37	ND	ND	2.9	24.74
	CP-50	1.74	1.9	1.4	20.46
P. ostreatus f.sp. florida	CP-11	3.4	ND	ND	21.2
Pleurotus spp.	CP-91	1.1	1.2	1.8	16.74

ND= Not determined.

The highest quantity of biomass produced after 21 days of mycelial growth was recorded in *Ganoderma lucidum* (1.9 g/L/day), and *P. ostreatus* (CP-50: 1.9 g/L/day), followed by *P.* spp. (1.2 g/L/day), and *Lentinula lepideus* (0.6 g/L/day). However, a higher biomass production has been reported for *Trichoderma harzianum* (47 g/L/day) growing on coffee pulp juice (**22**). Brand *et al.* (**23**) reported a biomass production of 10.8 mg/20 ml/32 h by *Rhizopus* spp. grown on CHA. The strain CP-50 of *P. ostreatus* was selected for further studies.

Characterization of Pleurotus

The selected strain of *P. ostreatus* (CP-50) was grown on CHA medium for 24 days at 25°C. Colony size, biomass production, pH evolution, the consumption of reducing sugars, and total phenol degradation are shown in Table 3.

Table 3. Evolution of colony size, biomass, pH, reducing sugars and total phenols during mycelial growth of *Pleurotus ostreatus* (CP-50) on coffee-husk agar medium (CHA), after 24 days of incubation at 25°C.

Incubation time (days)	Colony diameter (mm/day)	Biomass (g/L/day)	pH	Reducing sugars (mg/ml)	Total phenols (mg/ml)
0	0	0	5.7	55.5	14.2
3	11	0.723	5.7	53.3	14.2
6	19	0.660	5.7	50.5	13.5
9	31	1.34	5.7	51.0	12.1
12	35	1.206	5.6	50.0	12.4
18	55	1.5	6.0	45.1	10.3
21	73	1.87	6.2	44.7	10.1
24	77	1.9	6.3	41.4	9.5
Utilisation (%)				25.4	33.1

A fast growth rate was recorded for the strain, producing about 1.9 g/L/day of biomass, and degrading around 33.1% of total phenols present in the culture medium (Table 3). The culture medium had an initial black colour, which changed to a yellow colour after 24 days of incubation. This change of colour indicated the action of fungal enzymes on the hydrolysis of phenols during mycelial growth (Fig. 1), as previously described for *Aspergillus* and *Penicillium* by Bradoo *et al.* (**24**) using a culture medium with tannic acid.

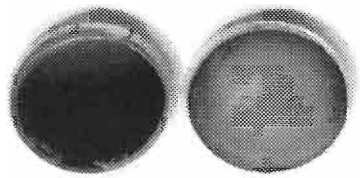

Figure 1. Petri dishes containing coffee-husk agar (CHA) medium after the inoculation of *Pleurotus ostreatus* (CP-50), which showed the hydrolysis of phenols. A: Time zero before inoculation. B: After 11 days of incubation at 25°C (the mycelium was lifted away from the agar surface).

Growth on coffee-husk solid medium

The reducing sugars consumption was 68.3 % for coffee husk in solid substrates, and the mycelial growth of *P. ostreatus* (CP-50) was vigorous. Furthermore, the degradation of phenols and condensed tannins was higher for coffee husk than for coffee pulp. Mycelial development observed on coffee husk was better than that on coffee pulp, as shown in Table 4. Degradation of total phenols and condensed tannins was very high for coffee husk substrate (58.5-75.4 %, respectively), in comparison with coffee pulp (8.3 % for total phenols). Wong and Wang (**25**) also reported degradation of tannins by *P. sajor-caju* and *L. edodes* grown on coffee grounds.

Table 4. Evolution of reducing sugars, total phenols and condensed tannins during the growth of *Pleurotus ostreatus* (CP-50) on coffee husk and coffee pulp in solid culture media after 24 days of incubation at 25°C.

Incubation time (days)	Reducing sugars (mg/g)	Total phenols (mg/g)		Condensed tannins (mg/g)	
	Coffee husk	Coffee pulp	Coffee husk	Coffee pulp	Coffee Husk
0	108.6	9.78	36.84	0.45	5.7
6	75.7	8.85	32.08	0.61	4.0
12	91.2	5.45	31.48	0.47	3.4
18	63.0	5.32	23.8	0.47	1.6
24	34.4	5.9	15.27	0.47	1.4
Utilization (%)	68.3	39.7	58.5	ND	75.4

ND= Not determined.

In general, it was established that the cellophane technique can be used successfully to allow the development of the mushroom mycelium, as well as to measure fungal biomass from edible mushrooms. The cultivation of *Pleurotus, Ganoderma* and *Lentinula* on coffee pulp and coffee husk has been reported for the production of fruit bodies (**11,12,14**). In this work, we showed that strains of these mushrooms are capable of degrading condensed tannins, and producing high levels of tannase.

Conclusions

The screening of eight strains from edible mushrooms (*Pleurotus, Ganoderma* and *Lentinus*), grown on a coffee-husk agar medium using a cellophane membrane, allowed to select *P. ostreatus* (CP-50) on the basis of its apical growth, biomass and tannase production. This strain degraded efficiently polyphenols and condensed tannins from coffee pulp and coffee husk, when used as solid substrates. Further studies are being carried out in solid-state fermentation (SSF) in order to optimise culture conditions, and to study the physiology and the metabolism of edible mushrooms for biomass and enzyme production from coffee pulp and coffee husk.

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