Asian Journal of Biological and Life Sciences | Short Communication

Preliminary studies of white rot fungi, brown rot fungi and soft rot fungi for coffee pulp degradation

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Submitted : 15.01.2012 Accepted :12.3.2012 Published : 10.5.2012

Abstract

Out of six species of *Pleurotus* showed wide variation in their linear downward growth and mycelial density. *P. flabellatus* and *P. sajor-caju* showed fast growth (linear downward growth 12-13 days). *P.florida* grew slower than these two and produced moderate (diffuse) biomass (linear downward growth-16days) *P. djamor* and *P.citrinopileatus* required 17 and 20 days respectively to colonize coffee pulp and produced sparse biomass. *P. eous* (linear downward growth 10days) resembled *P.chrysosporium* in its fast colonization potential and moderate (diffuse) mycelial biomass. The three brown rot fungal isolates *viz*., *Fomes badius, Ganoderma lucidum* and *Polyporus biformis* showed slower colonization of coffee pulp requiring 17-20 days but produced dense mycelial biomass. Among the *Pleurotus spp., P. eous* and *P. flabellatus* recorded 21.4% and 28.9% decrease in radial growth respectively on CA medium compared to their growth on MEA medium, but they showed a moderate radial growth rate between 9.0 and 12.8 mm/day on CA medium like *Fomes badius* ,*Aspergillus terreus* and *Chaetomium globosum*. *Aspergillus terreus* which produced low biomass showed the highest tannin degradation, while the high biomass producer *Pleurotus florida* caused only less than 10% tannin degradation in coffee pulp.

INTRODUCTION

Coffee pulp is one of the most abundant agroindustrial wastes and pollutants generated by the coffee industry. The utilization of coffee pulp as food, animal feed and compost has been investigated for several years[1,2,3] but its chemical composition is a great limiting factor due to the presence of anti-physiological factors such as caffeine, polyphenolic compounds and tannins [4] which include tannic acid in high concentrations (3-4%).

The use of raw coffee pulp has been suggested to be lower than 20% in ruminant diets [5] due to the presence of caffeine, tannins and other polyphenols. Solid- state fermentation technique was adopted earlier for protein enrichment of coffee-pulp using different strains of *Aspergillus sp.* [6]. Later it was proposed that desirable biochemical changes in terms of higher nitrogen content and degradation of cellulose, hemicellulose, lignin and other toxic components can be effected in these substrates through mushroom growth[7,8,9].

White rot basidiomycetous fungi or mushrooms are mainly used for producing protein rich food from various agro wastes through their cellulolytic and ligninolytic activities [10,11,12, 13,14,15,16,17,18,19,20]. They can degrade wood [21,22,23], chemicals related to lignin degradation products [24,25] and other recalcitrant chemicals like phenolic derivatives, tannins and caffeine [26,27,28]. Hence , an attempt was made to study on Isolation, Screening and Selection of Tannin degrading fungi on Coffee pulp.

MATERIALS AND METHODS

Substrate

Coffee pulp, the solid waste of coffee industry, processing the coffee beans by wet processing method was used as the substrate for biodegradation studies. It was sun dried, coarsely ground to uniform size (2mm) and was stored in gunny bags. The material was used within three months after procurement.

Organisms

Pure cultures of six species of the oyster mushrooms *viz., Pleurotus sajor-caju* (Fr.) Singer (M2), *Pleurotus djamor* (Fr.) Boedijn (MDU 1), *Pleurotus citrinopileatus* Singer (CO1), *Pleurotus eous* (Berk.) Sacc. (APK-1) , *Pleurotus flabellatus* (Berk and Br.) Sacc. (MDU 2), *Pleurotus florida* Fovose (M 1) were obtained from Tamil nadu Agricultural College, Coimbatore, India. Pure cultures of *Phanerochaete chrysosporium* Burdsall (NCIM 1197) was procured from National Chemical Laboratory, Pune, India. Sporophores of the brown rot fungi, growing on wood logs were collected from the coffee estate. Four soft rot fungi were isolated from a sample of coffee pulp -dumped soil by serial dilution and pour plate technique. The pure cultures were made on PDA plates and they were identified by their morphological and colony characteristics [29]. The organisms were maintained on PDA slants at 4C and were sub cultured once a month. *Chaetomium globosum* a known cellulolytic fungus, was a laboratory isolate and had been maintained on PDA slants. It was earlier recovered from paper mill effluent by primary selection through enrichment culture method.

Linear downward growth

The linear downward growth of white rot and brown rot fungi and their mycelial proliferation on coffee pulp were studied following the procedure[30]. Coffee pulp (6g) moistened with water to have 60% moisture content was filled in boiling tubes to a depth of 10 cm. The boiling tubes were plugged with cotton, autoclaved at 121C and 15 lb pressure for 15min. Agar blocks (8mm) of seven - days old mycelia of the chosen white rot fungi and brown rot fungi were aseptically inoculated into individual tubes. The boiling tubes were incubated at 28 2C in the culture room. The linear downward growth in days required by the different fungi to reach the bottom of the tube was measured, and the extent of mycelial proliferation was noted in terms of mycelial biomass as good, moderate or dense.

Radial growth on coffee medium

Coffee medium for solid state culture was prepared as described [31]. Coffee pulp (200g) was boiled in one litre of hot water (90C) for 15 min. The supernatant of coffee pulp was used to prepare one litre of coffee agar (CA) medium by adding 15g of agar. This medium was sterilized at 121C for 15min and dispensed into Petri plates (20ml/plate). Cultures were inoculated at the centre with a mycelial agar block taken from the margin of 7 days - old fungal colony growing on PDA medium. The plates were incubated at 28 2C in the dark for 6 to 8 days. The radial growth of white rot, brown rot and soft rot fungi was measured on malt extract medium (control) and coffee agar medium on the 6th day after inoculation. The radial growth rate was calculated by noting the days required by the fungi for completely covering the plate. The bleaching of the colour at the reverse of the individual plates was observed on the 21st day after inoculation.

RESULTS AND DISCUSSION

Coffee pulp is one of the most abundantly available agro industrial waste produced, during the pulping operation of the coffee cherries to obtain coffee beans in many coffee- producing areas of the tropics [32]. Owing to the presence of the antinutritional factors, its use as an animal feed has been restricted to a large extent. The presence of proteins, sugars and minerals in coffee pulp and its high humidity favours the rapid growth of microorganisms and if it is not utilized immediately, it causes environmental pollution [32].

The morphological characters, their pore size, colour and dimensions of spores of the three brown rot fungal sporophores collected from the coffee estate are given in Table 1. The sporophores were identified as those of *Fomes badius* (Berk .) Cooke , *Ganoderma lucidum* (Leyss.) Karst. [Plate II] and *Polyporus biformis (* Fries).

The morphological and colony characteristics of four soft rot fungi isolated from coffee pulp - dumped soil are given in Table 2. The organisms were identified as *Aspergillus terreus* Thom., *Aspergillus niger* van Tieghem, *Rhizopus stolonifer* (Ehrenberg ex Fr.) Linder and *Penicillium funiculosum* Thom. [32] reported earlier that most of the microflora native to coffee growing areas and isolated from coffee plants and coffee cherries belonged to *Aspergillus* and *Penicillium* genera.

Linear Downward Growth

Table 3 shows that *Phanerochaete chrysosporium* was the fastest colonizer of coffee pulp requiring 7 days to cover a 10 cm length of coffee pulp. *Phanerochaete chrysosporium* had been used extensively as a model organism to study lignocellulosic biodegradation [33,34,35]. As in the present study, it had been commonly used as a control species to compare with new strains with potential ligninolytic capacities [36].

The six species of *Pleurotus* showed wide variation in their linear downward growth and mycelial density. *P. flabellatus* and *P. sajor-caju* showed fast growth (linear downward growth 12-13 days). *P.florida* grew slower than these two and produced moderate (diffuse) biomass (linear downward growth-16days) *P. djamor* and *P.citrinopileatus* required 17 and 20 days respectively to colonize coffee pulp and produced sparse biomass. *P. eous* (linear downward growth 10 days) resembled *P.chrysosporium* in its fast colonization potential and moderate (diffuse) mycelial biomass. *Pleurotus spp*., have been shown to have increased interspecific genetic variability which might explain the variation in their growth and colonization on coffee pulp [37].

The ability of *Pleurotus sp*. to colonise coffee pulp as assessed in the present study, by their vegetative growth and mycelial

Table: 1 Identification of brown rot fungi isolated from coffee estate.

S.No	Morphological characters	Pore and spore characteristics	Identification
1	Sporophore : Stipitate, tough, heavy, leathery to woody Sporophore size: 10-12 x 10-12 x 3-4 cm Stalk : Central, varnished and encrusted Upper surface : Smooth, lacquered appearance, reddish-brown, 2-10mm thick. Hymenial surface: Whitish, turning brown later	Pores: Small, brown, 90-250µm dia Basidiospores: Brown, thick-walled, truncate at end, Spore size: 8.3-10 x 5.8 – 6.7µ	*Ganoderma lucidum*
2	Sporophore: Sessile, resupinate, soft, coriaceous when fresh, corky when dry, Sporophore size: 1.5-7 x 1-4 x 0.2 – 0.8 cm Upper surface: white to light yellow, Margin :Brown when dry, tomentose when fresh Context : Fibrous, white to pale yellow up to 0.5cm thick Hymenial surface: white, on drying biscuit colour	Pores: Angular, 1-2 per mm, Basidiospores : hyaline, smooth, cylindrical, Spore size: 5.5-7.8 x 2.0-2.5µm	*Polyporus biformis*
3	Sporophore: sessile, attached by a broad base, hoof shaped, hard, woody, Sporophore size: 6-8 x 3-4. 5 x 3cm Upper surface: yellow-brown in current year's growth, black when old glabrous, rough. Context: Yellowish brown Hymenial surface: Dull brown,	Pores: regular round, 3-4 per mm Basidiospores: yellow-brown, round Spore size: 5.8-7.5 x 4.1 – 5.8µm	*Fomes badius*

Table: 2 Identification of soft rot fungi isolated from coffee pulp dumped soil.

S. No	Morphological characters	Colony characters	Identification
1	Colony growth : Fast Colony texture : Fluffy Colour : White Reverse of medium : Colourless.	Sporangiophores : Erect, arising in groups opposite the rhizoids Length : 2.5 mm Sporangia : Globose Spores : Ellipsoid; 10-15 µm (long axis); black.	*Rhizopus stolonifer*
2	Colony growth : Slow Colony texture : Velvetty Colour : Sand brown Reverse of medium : Yellow.	Conidiophores : Smooth, hyaline Length : 500 µm Conidial heads : Columnar Vesicles : Dome shaped Conidia : Globose; smooth;small,2µm dia;brown.	Aspergillus terreus
3	Colony growth : Fast Colony texture : Fluffy Colour : White at first frequently developing areas of bright yellow Reverse of medium : Colourless.	Conidiophores : Smooth; colourless Length : 800 µm Conidial heads : Globose; radiate Vesicle : Globose; rough; 5 µm dia , Conidia : Rough; globose; 4-5 µm dia, black.	*Aspergillus niger*
4	Colony growth : Slow,spreading Colony texture : Felt like Reverse of medium: Colourless.	Conidiophores : Short; arise from funicles Conidia : Green; elliptical; 2.5-3.5x 2-2.5 µm.	*Penicillium funiculosum*

Table: 3 Linear downward growth (days) and mycelial density of chosen white rot, brown rot and soft rot on coffee pulp biodegradation. Values are mean ± SE of three replicates.

S. No	Organisms	Linear downward growth (days)	Mycelial proliferation
1	Phanerochaete chrysosporium	7 ± 0.6	+++
2	*Pleurotus sajor – caju*	13 ± 0.7	++++
3	*Pleurotus djamor*	20 ± 0.8	++
4	*Pleurotus citrinopileatus*	17 ± 1.4	++
5	*Pleurotus eous*	10 ± 0.5	+++
6	*Pleurotus flabellatus*	12 ± 0.3	++++
7	*Pleurotus florida*	16 ± 0.4	+++
8	*Fomes badius*	18 ± 1.1	++++
9	*Ganoderma lucidum*	17 ± 1.4	++++
10	*Polyporus biformis*	20 ± 0.5	++++

(++) = Sparse; (+++) = Moderate; (++++) = Dense

extension was supported by *[38]* who reported that *Pleurotus spp.* were able to utilize a wide array of biomass wastes due to their extreme plasticity. The three brown rot fungal isolates *viz*., *Fomes badius, Ganoderma lucidum* and *Polyporus biformis* showed slower colonization of coffee pulp requiring 17-20 days but produced dense mycelial biomass.

Radial growth and Tannin degradation

Ganoderma lucidum showed the same spread and rate of growth on both the malt extact agar medium and coffee agar medium (30mm radial growth on the 6th day; 10 mm/day radial growth rate). Even though *P. chrysosporium* and *Rhizopus stolonifer* grew equally well (45mm radial growth) on both the media, the former showed higher radial growth rate (22.5, 18mm/day and 18, 15 mm/day in MEA and CA media respectively). Among the *Pleurotus spp., P. eous* and *P. flabellatus* recorded 21.4% and 28.9% decrease in radial growth respectively on CA medium compared to their growth on MEA medium, but they showed a moderate radial growth rate between 9.0 and 12.8 mm/day on CA medium like *Fomes badius*

Table: 4 Radial growth, radial growth rate, biomass of chosen white rot, brown rot and soft rot fungi and coffee pulp residual tannins during biodegradation. Values are mean ± SE of three replicates.

S. No	Organisms	Radial growth (mm)		Radial growth rate (mm/day)		Residual tannins (% Dry wt)	Biomass in CAM (mg/dish)
		MEA	CAM	MEA	CAM		
1	*Phanerochaete chrysosporium*	45 ± 0	45 ± 0	18.0 ± 0.2	15.0 ± 0	6.7 ± 0.11	220 ± 1.2
2	*Pleurotus sajor – caju*	42 ± 1.4	33 ± 1.7	15.0 ± 1.1	7.5 ± 0.5	6.9 ± 0.1	300 ± 1.7
3	*Pleurotus djamor*	38 ± 1.1	25 ± 1.0	11.2 ± 0.8	6.4 ± 0.1	7.7 ± 0.1	190 ± 0.6
4	*Pleurotus citrinopileatus*	38 ± 1.5	25 ± 1.0	11.2 ± 0.8	7.5 ± 0.5	7.7 ± 0.13	170 ± 0.9
5	*Pleurotus eous*	42 ± 1.2	33 ± 1.7	15.0 ± 1.0	12.8 ± 0.4	6.3 ± 0.05	250 ± 0.6
6	*Pleurotus flabellatus*	38 ± 1.1	27 ± 1.7	12.8 ± 1.0	9.0 ± 0.3	6.9 ± 0.14	370 ± 1.4
7	*Pleurotus florida*	38 ± 1.6	30 ± 1.2	11.2 ± 1.0	7.5 ± 0.5	7.2 ± 0.05	330 ± 1.7
8	*Fomes badius*	35 ± 0.6	30 ± 1.0	11.2 ± 1.0	9.0 ± 0.3	6.7 ± 0.06	350 ± 0.9
9	*Ganoderma lucidum*	31 ± 1.7	30 ± 1.0	10.0 ± 0.5	10.0 ± 0.5	6.7 ± 0.10	390 ± 1.2
10	*Polyporus biformis*	30 ± 1.7	22 ± 1.0	10.0 ± 0.5	6.4 ± 0.1	6.9 ± 0.06	330 ± 1.3
11	*Aspergillus niger*	34 ± 0.8	30 ± 1.5	11.2 ± 0.8	10.0 ± 0.3	7.1 ± 0.10	60 ± 0.4
12	*Aspergillus tereus*	41 ± 1.0	30 ± 1.3	15.0 ± 1.0	10.0 ± 0.3	6.8 ± 0.06	100 ± 1.2
13	*Chaetomium globosum*	42 ± 1.4	35 ± 0.7	15.0 ± 1.0	11.2 ± 0.3	6.6 ±0.17	120 ± 1.1
14	*Penicillium funciculosum*	34 ± 0.8	30 ± 1.2	11.2 ± 0.8	10.0 ± 0.5	7.1 ± 0.10	50 ± 0.6
15	*Rhizopus Stolonifer*	45	45	22.5 ± 0	18.0 ± 1.3	7.7 ± 0.05	40 ± 0.2

,*Aspergillus terreus* and *Chaetomium globosum* (Table 4).

Pleurotus sajor-caju, P. djamor, P. citrinopileatus, P. florida and *P . biformis* varied in their biomass production but showed less than 7.5 mm/day growth on coffee agar medium and hence were not selected for further study . Even though *Aspergillus niger, Rhizopus stolonifer* and *Penicillium funiculosum* showed high to moderate radial growth and radial growth rate, they were not selected for further study as they showed less than 10% tannin degradation in 21 days and low biomass production.

The variation in coffee pulp colonization among *Pleurotus* sp., may be due to the difference in their sensitivity to coffee pulp caffeine and tannin decided by the interspecific genetic variability found in the genus. The anti-physiological and anti-nutritional factors of coffee pulp *i.e*., caffeine, tannins and polyphenols reportedly restricted or inhibited mushroom fungal colonization and fruiting at different levels [31,32,39,40,]. Similar to our observations, considerable variation among different *Pleurotus sp*., in colonizing different spawn bases had been reported [30]. The best *Lentinus edodes strain* growing on coffee pulp based on similar parameters used in this study [41].

The high biomass production by *A.terreus* on olive oil black liquor was positively correlated with high bioconversion efficiency [42] .Similarly *P.florida* showed high biomass production on sugarcane bagasse and it correlated with high loss in organic matter, cellulose and hemicellulose [43]. Contradictory to these findings, *Aspergillus terreus* which produced low biomass showed the highest tannin degradation, while the high biomass producer *Pleurotus florida* caused only less than 10% tannin degradation in coffee pulp.

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