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Colony Interaction Between White Rotters and Their Fungal Associations

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Abstract: Interaction among and between mushroom / white rot and brown rot fungi or soft rot fungi, studied by the dual culture plate assays showed that the outcome of a specific interaction was strongly dependent on the two organisms concerned. *Phanerochate chrysosporium* as one of the partner in dual cultures, invariably scored grade 2 for colony interaction as it overgrew and covered the colony of other partner, due to its higher ability to spread and colonize the coffee pulp. *Pleurotus flabellatus* and *Pleurotus eous* showed synergistic mutual intermingling growth without any antagonistic interaction. Exceptionally *Pleurotus eous* and *Fomes badius* dual culture showed a clear antagonistic interaction seen as the inhibition zone between the two cultures.

Key words: Interaction studies · *Phanerochate chrysosporium* · *Pleurotus flabellatus* · *Pleurotus eous* · *Fomes badius*

INTRODUCTION

Coffee pulp is one of the most abundant agro-industrial 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] but its chemical composition is a great limiting factor due to the presence of anti-physiological factors such as caffeine, polyphenolic compounds and tannins [3] which include tannic acid in high concentrations (3-4%). With a view of increasing the rate of biodegradation and to improve the kinetics of the process to suit the demands at industrial level, several scientists have studied the effect of cocultures of amylolytic [4], cellulolytic [5] and lignocellulolytic organisms [6, 7] on bioconversion of agricultural residues in SSF. Hence, we attempt a study on colony interaction between white rotters and their fungal associations.

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.

Organisms: The selected white rot fungi were *Phanerochaete chrysosporium, Pleurotus eous, Pleurotus flabellatus*; brown rot fungi namely *Ganoderma luciderm* and *Fomes badius*; soft rot fungi namely *Chaetomium globosum* and *Aspergillus terreus* are used for biodegradation of coffee pulp. These fungal cultures were maintained on malt extract (2%) agar medium.

Colony Interaction Studies: Colony interactions between the mushroom / white rot fungi and the brown rot and soft rot fungi were studied *in vitro* in coculture experiments. White rot (WR), brown rot (BR) and soft rot (SR) fungi were grown separately on PDA media. The agar blocks cut from the margin of the actively growing 7 days-old colonies of BR and SR fungi and test WR organisms were inoculated juxtaposed to each other approximately 3 cm apart, on coffee agar medium Petri plates. Three replicates for each set were maintained. Controls were set as single agar block inoculated monocultures of the corresponding fungi. Assessments were made when the fungi had achieved an equilibrium after which there was no further alteration in the growth. The colony interactions between the test fungi and their fungal associations were assessed

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following the model with slight modification [8]. The five types of interaction grades used were as follows:

- Mutual intermingling growth without any macroscopic signs of interaction-Grade 1.
- Intermingling growth where the fungus under observation is growing into the opposed fungus either above or below-Grade 2.
- Mutual intermingling growth where the growth of the fungus is ceased and is being overgrown by the opposed fungus-Grade 3.
- Slight inhibition of both the interacting fungi with a narrow demarcation line (1-2mm)-Grade 4.
- Mutual inhibition of growth at a distance of >2mm-Grade 5.

RESULTS AND DISCUSSION

Interaction among and between mushroom/ WR and BR fungi or SR fungi, studied by the dual culture plate assays showed that the outcome of a specific interaction was strongly dependent on the two organisms concerned [8]. Table 1 show the different grades of interaction between the three WR fungi as opposed to the other members of their group or to the selected BR or SR fungi. Except in *Pleurotus eous* + *Fomes badius* coculture, all the other cocultures showed no sign of inhibition. *Phanerochaete chrysosporium* as one of the partner in dual cultures, invariably scored grade 2 for colony interaction as it overgrew and covered the colony of other partner, due to its higher ability to spread and colonize the coffee pulp. *Pleurotus flabellatus* and *Pleurotus eous* showed synergistic mutual intermingling growth without any antagonistic interaction. Both these fungi, during the initial colonization were overgrown by the opposing fungus when grown on plates in the presence of *Fomes badius* / *Ganoderma lucidum* / *Aspergillus terreus* / *Chaetomium globosum*. Exceptionally *Pleurotus eous* and *Fomes badius* dual culture showed a clear antagonistic interaction seen as the inhibition zone between the two cultures. Thus different species of the same genus, *Pleurotus* were found to differ in the success with which they could compete with another organism [9-11].

Phanerochaete chrysosporium, Trametes versicolor and *Pleurotus sajor-caju* were able to grow on sawdust with crossing of hyphae showing no signs of antagonism and theorized that ligninolytic fungi could form synergistic associations as they differed in their physiology [12]. Synergistic associations were reportedly highly successful in biodegradation of lignocellulosic and recalcitrant biomolecules as they ensured elevated levels of lignin peroxidase and laccase and achieved novel combinations of lignin attacking enzymes [12-14]. The inhibition zone observed between the colonies of *Pleurotus eous* and *Fomes badius* indicated the production of antibiotic substances by either of the two partners in the coculture [8]. Similarly, among cellulolytic microorganisms in cocultures, those which showed no inhibition zone were compatible and active [15]. Our results of the coculture interaction studies in plates and flasks were corroborated by this report. *Trichoderma harizianum* and *Chaetomium globosum* to be strong competitors to *Pleurotus sajor-caju* on agar and paddy straw [16]. The growth of *Chaetomium* and that of the mushroom was found to have ceased after contact, while *Trichoderma harizianum* overgrew *Pleurotus sajor-caju*

Table 1: Colony interactions between the test white rot fungi and their fungal associations

	Colony Interaction Grades		
	Test fungi		
Fungal associates	*Phanerochaete chrysosporium*	*Pleurotus eous*	*Pleurotus flabellatus*
Phanerochaete chrysosporium	-	3	3
Pleurotus eous	2	-	1
Pleurotus flabellatus	2	1	-
Fomes badius	2	4	3
Ganoderma lucidum	2	3	3
Aspergillus terreus	2	3	3
Chaetomium globosum	2	3	3

Grade 1: Mutual intermingling growth without any macroscopic signs of interaction.

Grade 2: Intermingling growth where the fungus under observation is growing into the opposed fungus either above or below.

Grade 3: Mutual intermingling growth where the growth of the fungus is ceased and is being overgrown by the opposed fungus.

Grade 4: Slight inhibition of both the interacting fungi with a narrow demarcation line (1-2mm).

mycelium within 72hrs after inoculation. Our observations in the interaction studies showed that *Chaetomium* overgrew the colonies of *Pleurotus eous* and *Pleurotus flabellatus* and thus restricted and inhibited the mushroom mycelial colonization without producing any inhibition zone [17].

Many fungi isolated from *Pleurotus* beds [18] were reported to be nutritional competitors, antagonists or just chance contaminants to the mushroom. Similarly, the different fungal associations observed as cocultures in this study could be segregated into various groups based on their crop duration, colonization ability and the degree of competition. Mycelia of fungal isolates in the same mycelial compatibility group showed the compatible reaction of intermingling and formation of a white ridge of mycelia [19]. Similar observations were made in plates containing *Phaenochaete chrysosporium* + *Pleurotus eous* and *Phanerochaete chrysosporium* + *Aspergillus terreus* cocultures indicating the mutual compatibility between these organisms.

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