Efficacy of ligno-cellulolytic fungi on recycling sericultural wastes

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ABSTRACT

The study was undertaken to know the efficacy of various fungi for their ligno-cellulolytic activity for recycling the sericultural waste. About 75 microbes belonging to the genera of *Pleurotus, Agaricus, Calocybe, Phanerochaete, Lentinus, Trichoderma, Verticillium* and *Aspergillus* were isolated from various sources *viz*., waste of paper industry, sugar cane bagasse as well as dead and decayed woods. They were screened for their lignolytic and cellulolytic activities. The lignolytic activity was studied in Coir pith Guaiacol Agar medium and cellulolytic activity using modified Czapek Dox Agar medium. Most of the microbes were found positive for cellulolytic activity while five fungi *viz*., *Pleurotus ananthan, P. ostreatus, P. sajorcaju, P. florida* and *Phanerochaete* spp. were showed positive reaction for both lignolytic and cellulolytic activities. In further screening for enzymes such as laccase, lignin peroxidase and manganese peroxidase, which are associated with lignin degradation, *P. florida* and *P. ostreatus* were found positive to all the three enzymes. The present study therefore suggests that *P. florida* and *P. ostreatus* can be exploited for recycling the sericultural waste for producing quality compost to improve the soil health.

Keywords: ligno-cellulolytic activity, sericultural waste, mulberry, *Pleurotus florida*.

INTRODUCTION

Huge quantity of waste is generated during mulberry cultivation and silkworm rearing, which is a rich source of plant nutrients and can be utilized as alternate to farmyard manure after decomposition. These waste materials mainly consists of mulberry shoots having about 50-60% cellulose, 10-20% lignin and conspicuous quantity of hemicellulose. Among these, lignin is very hard to biodegrade which also reduces the bioavailability of other cell wall constituents. Due to these hard components, it takes more than one year to fully decompose these waste materials in natural condition. Therefore, there is a need to find out ecofriendly and cost effective method for decomposition of these hard materials in a short span of time. Few fungi belonging to the group of Basidiomycetes are having the ability to degrade woody materials [1]. Fungi that cause wood to decay are of increasing biotechnological importance as wood and other ligno-cellulose materials are renewable resources for the production of paper products, fuel, chemicals and manures [2]. Although there is an increasing research emphasis on the fungal degradation of ligno-cellulose materials, work on this aspect in sericultural waste degradation is scarce. Therefore, screening was made to exploit certain fungi for their ligno-cellulolytic degrading potentiality of sericultural waste consisted of left over mulberry shoots.

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MATERIALS AND METHODS

Collection, isolation and identification of lignolytic/cellulolytic fungi

To study the ligno-cellulolytic activities of fungi, 60 samples were collected from paper industry, sugar cane bagasse as well as dead and decayed wood from various places of South India. The fungi were isolated by dilution plate technique [3] and placing the bits of Basidiomycetes fungi on suitable media. After incubation, the fungal colonies grown were transferred in slants and later purified by mono hyphal tip method for further study. The fungi were identified based on their morphological and cultural characters [4,5].

Screening of cellulolytic fungi

The isolated fungi were first inoculated on Potato Dextrose Agar plates and incubated at 28±2ºC for seven days. For detecting the cellulolytic property 2mm disc of 7day old fungus was transferred to a modified Czapek Dox Agar medium in which sucrose is replaced by 1% cellulose. The inoculated Czapek Dox Agar plates were incubated at 28±2ºC for seven days. Then the plates containing fungal colonies were flooded with a mixture of Hydrochloric acid (HCl) and Iodine solution [Mixing 1ml 0.1M HCl with 5ml 1% (w/v) Iodine (I_2) in 2 % (w/v) Potassium iodide (KI)]. The cellulolytic activity was ascertained by presence of a clear yellow zone with reddish background within 3 to 5 minutes [6].

Screening of lignolytic fungi

To know the lignolytic activity, the isolated fungi were inoculated on the Petri Plates seeded with Coir pith Guaiacol Agar medium. The plates were incubated for 7 days at 28±2ºC. The lignolytic activities were ascertained by the appearance of red colour around the colony indicated positive lignolytic activity [6].

Evaluation of lignolytic/cellulolytic enzymes

For evaluation of lignolytic enzymes from fungi, Potato Dextrose Agar medium was supplemented with tannic acid, phenol red, and ABTS (2,2'-azinobis(3-ethylbenz-thiazoline-6-sulfonic acid) [7]. A piece of isolated fungi was inoculated on each selective medium and incubated at 28±2°C in dark condition for 7 days. The lignolytic activities were identified by color formation around the mycelium. The fungi having lignin peroxidase activity showed brown, manganese peroxidase showed lemon yellow and laccase showed green colour around the mycelium. The fungi without these enzymes did not show any colour.

RESULTS AND DISCUSSION

The microbes were isolated from 60 samples collected from various sources such as paper pulp, sugar cane bagasse, dead and decaying wood collected from Karnataka, Tamil Nadu and Kerala. Based on cultural and morphological characters 75 microbes of different genera were isolated (Table 1). These microbes belong to genera, viz., *Pleurotus, Trichoderma, Phanerochaete, Lentinus, Agaricus, Penicillium, Aspergillus, Verticillium* and *Calocybe*.

Lignolytic and cellulolytic activity revealed that out of 75 microbes, 5 fungi were showed positive reaction for lignolytic and cellulolytic activity (Table 2). The lignolytic activity is characterized by the appearance of reddish brown colour pigment around the colony in Coir pith

Guaiacol agar medium, while a formation of clear yellow zone around the reddish back ground for cellulolytic activity in modified Czapek Dox Agar medium. However, remaining 70 microbes were positive only for cellulolytic activity. Five ligno-cellulolytic fungi, were further screened for three enzymes such as laccase, lignin peroxidase and manganese peroxidase associated with lignin degradation. Two isolates, *Pleurotus florida* and *P. ostreatus* showed positive reaction for all the three enzymes (Table 3).

Table 1. Fungi screened for lignolytic and cellulolytic activity.

Place	No.	Sources	Fungal species*
Karnataka	22	Dead and decaying wood	*Pleurotus* (12); *Trichoderma* (6); *Verticillium* (1); *Phanerochaete* (4); *Lentinus* (3); *Agaricus* (2), *Calocybe* (2); *Aspergillus* (3); *Penicillium* (5)
	8	Paper pulp	*Trichoderma (4)*
	7	Sugarcane bagasse	*Trichoderma* (3); *Coprinus* (2)
	4	Cow dung	*Coprophilus* (1); *Cheilymenia* (1); *Panaeolus* (1); *Psilocybe* (1); *Trichoderma* (1); *Rhizopus* (1); *Aspergillus* (3)
Tamil Nadu	10	Dead and decaying wood	*Pleurotus* (4); *Lentinus* (2); *Agaricus* (3)
Kerala	9	Dead and decaying wood	*Pleurotus* (6); *Phanerochaete* (2); *Calocybe* (2)

*Numbers in parenthesis are total number of isolates of a particular genus screened.

Table 2. Fungi showing lignolytic and cellulolytic activities.

Isolate No.	Fungi	Cellulolytic	Lignolytic
1	*Pleurotus ananthan*	+	+
2	*P. ostreatus*	+	+
3	*P. sajorcaju*	+	+
4	*P. florida* spp.	+	+
5	*Phanerochaete* spp.	+	+
6	*Calocybe* spp.	+	-
7	*Lentinus* spp.	+	-
8	*Agaricus* spp.	+	-
9	*Verticillium* spp.	+	-
10	*Trichoderma* spp.	+	-
11	*T. pseudokoningii*	+	-
12	*T. viride*	+	-
13	*T. harzianum*	+	-
14	*T. koningii*	+	-

Perusal of literature revealed that several microorganisms produce lingo-cellulolytic enzymes. Extra cellular lignin and manganese peroxidase produced by white rot fungi mediates decomposition process [8,9]. Oyster mushroom (*Pleurotus* sp.) has the potential to degrade cellulose and lignin on waste substrates such as sawdust and sugarcane bagasse [10]. Similarly, biological efficiency of *P. citrinopileatus* on the degradation of coir waste and paddy straw was also reported [11]. In the present study, among 75 fungi isolated, 5 fungi were positive for ligno-cellulolytic activity and rest were positive to cellulolytic activity only. Further, screening for three major enzymes associated with lignin degradation revealed that fungi *P. florida* and *P. ostreatus* possess all three lignolytic enzymes. It is reported that all the three lignolytic enzymes such as laccase, lignin peroxidase and manganese peroxidase are present in *Pleurotus* spp. [12]. Lignin degradation

and ligno-cellulolytic enzyme activity varies in different substrates and fungal strains [13]. Similar results were reported [14] in decomposition of residues using *Pleurotus* spp.

Table 3. Reaction of various fungi to three major enzymes associated with lignin degradation.

Fungi	Cellulolytic	Lignolytic	Enzymes associated with lignin degradation		
			Laccase	Lignin peroxidase	Manganese peroxidase
Pleurotus ananthan	+	+	+	-	-
P. ostreatus	+	+	+	+	+
P. sajorcaju	+	+	+	-	+
P. florida spp.	+	+	+	+	+
Phanerochaete spp.	+	+	+	+	-
Calocybe spp.	+	-	-	-	-
Lentinus spp.	+	-	-	-	-
Agaricus spp.	+	-	-	-	-
Verticillium spp.	+	-	-	-	-
Trichoderma spp.	+	-	-	-	-
T. pseudokoningii	+	-	-	-	-
T. viride	+	-	-	-	-
T. harzianum	+	-	-	-	-
T. koningii	+	-	-	-	-

Mulberry shoots are having about 50-60% cellulose, 10-20% lignin and conspicuous quantity of hemicellulose. Among these, lignin is very hard to biodegrade which also reduces the bioavailability of other cell wall constituents. The present study shows that *P. florida* and *P. ostreatus* are having the ability to degrade lignin and cellulose which are the major components responsible for slow degradation of mulberry shoots in the natural condition. Therefore, these fungi can be exploited for hastening decomposition of sericulture waste and recycle the organic matter for sustainable sericulture and improve productivity.

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