Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

European Journal of Experimental Biology, 2014, 4(5):90-94

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Screening and characterization lignin degrading fungi from decayed sawdust

Mahalingam P. U. and Maruthamalai Rasi R. P.*

Department of Biology, Gandhigram Rural Institute-Deemed University, Gandhigram, Dindigul, Tamil Nadu, India

---

ABSTRACT

In this study an attempt was made to characterize the lignolytic fungi against various growth conditions. The decayed sawdust sample was collected and screened for lignolytic fungi using Malt extract medium containing of tannic acid as lignin equivalent. Seven fungal isolates such as and Trichoderma spp, Penicillium spp, Aspergillus niger, Botrytis spp, Rhizopus spp, Aspergillus fumigatus and Cladosporium spp2 were selected based on their solubilization index against tannic acid. All these isolates were further characterized for their activity to degrade lignin analogue i.e., tannic acid with various growth conditions such as concentration of tannic acid (0.25, 0.50, 0.75, 1.0 and 1.25%) different temperature (28°C, 37°C and 45°C) and various pH (acidic, neutral and alkaline) conditions. The bacterial isolates such as Penicillium spp, Aspergillus niger, Aspergillus fumigatus and Trichoderma spp were showed the better lignolytic activity at a concentration of 1.0 percent tannic acid in the acidic (pH 6.0) and neutral (pH 7.0) conditions at 37°C.

Key words: Decayed sawdust, Lignolytic fungi, Malt extract medium, Tannic acid

---

INTRODUCTION

Lignin is the second most abundant biological material on the planet, exceeded only by cellulose and hemicellulose, and comprises 15-25% of the dry weight of woody plants [1,2]. The percentage of lignin content in common agricultural residues such as hardwood stems (18-25%), softwood stems (25-35%), nut shells (30-40%), grasses (10-30 %) and corn cobs (15%) was studied by [3]. In nature, many diverse group of microorganism are able to degrade lignin rich biomass. Cellulolytic and lignolytic fungus are mainly involved to convert agricultural waste, kitchen wastes and all other organic wastes into nutrient rich compost by the activity of their degrading enzymes like laccase [4]. Some genus of fungi such as *Chaetomium, Fusarium, Rhizoctonia, Cylindrocarpon, Trichoderma and Candida* are able to degrade tannic compounds [5]. Hence in the present study, an attempt was made to screen and characterize lignolytic fungi isolated from decayed sawdust.

MATERIALS AND METHODS

The decayed sawdust sample was collected from the dumping yard near sawdust industry, Thenkarai Periyakulam, Theni (District), Tamil Nadu, India. The samples were collected at four different spots randomly and blended uniformly. All the samples were transported aseptically to the laboratory, Department of Biology, Gandhigram Rural Institute – Deemed University, Gandhigram for enumerating total microbial population using standard procedures [6,7].

Screening for lignolytic activity

Thirteen fungal isolates were further screened for lignolytic activity by standard procedures [8]. All the thirteen fungal strains were separately inoculated in the malt extract medium supplemented with tannic acid as source of lignin and as selective agent to check the organism's secreat polyphenol peroxidase. All the inoculated plates were incubated at 28°C for 10 d and observed for zone of clearance with brown color development around the colony as a positive indication for polyphenol peroxidase activity. Based on the results exhibited by the isolates on the selective medium, only seven fungal isolates were selected for further characterization.

The results were expressed in terms of Solubilization Index (SI) and they were measured using the following formula [9]:

SI = Colony diameter + Halozone diameter / Colony diameter

Identification of lignolytic fungal isolates

Seven fungal isolates were selected based on their solubilization index for lignolytic activity and identified based on their macroscopic culture characteristics, i.e., fungal colony growth in Rose Bengal agar medium and microscopic characteristics such as hyphal and reproductive structures after staining with lactophenol cotton blue [10,11,12].

Characterization of selected lignolytic fungi

The seven selected lignolytic fungal isolates were characterized by growing them in media with different environmental conditions by the standard procedure [13]. Malt extract medium was prepared with various buffered solutions, using acetate buffer (pH 6.0) and phosphate buffer (pH 7.0) and tris-Hcl buffer (pH 8.0). The medium of each pH was supplemented with increasing concentrations of tannic acid (source of lignin) i.e., 0.25, 0.50, 0.75, 1.0 and 1.25 percent. The media were sterilized and the seven selected fungal were separately inoculated. Then the inoculated plates were incubated in different sets at various temperature such as 28°C, 37°C and 45°C. The growth performances of the seven fungal strains were observed in all the growth conditions on 7d and the results were recorded.

RESULTS

Screening of lignolytic activity

Thirteen different fungal isolates were selected from the culture medium based on their abundance growth and followed by all these isolates were screened for lignolytic activity using Malt extract medium supplemented with tannic acid. The fungal isolates shown better lignolytic activity through the formation of brown color around the colonies and their solubilization index are presented in Table 1.

Table 1: List of fungal isolates and their Solubilization Index (SI) in malt extract agar supplemented with tannic acid (1%) on 10d

Isolate code	Name of the Fungal Isolates	Solubilization Index (SI) (%)
LFIS – 1	*Trichoderma* spp	49.18 ± 0.03
LFIS – 2	*Penicillium* spp	55.33 ± 0.01
LFIS – 3	*Aspergillus niger*	52.55 ± 0.02
LFIS – 4	*Botrytis* spp	43.23 ± 0.10
LFIS – 5	*Rhizopus* spp	46.70 ± 0.07
LFIS – 6	*Aspergillus fumigatus*	57.96 ± 0.02
LFIS – 7	*Cladosporium* spp2	41.80 ± 0.05

Values are mean of three replicates ± Standard error
LFIS = Lignolytic Fungal Isolates

Identification of efficient lignolytic fungi

Based on the lignolytic activity and their solubilization index seven fungal isolates were selected and identified through as shown in Table 2.

Table 2: The Morphological characteristics of seven lignolytic fungal isolates

Isolate code	Macroscopic (Culture characteristics)	Microscopic characteristics (Morphological and Reproductive features)	Name of the Fungal isolates
LFIS – 1	Dark green color colonies with needle shaped structures	Septate hyaline hyphae. Conidiophores which are hyaline, branched phialides are hyaline, flask - shaped and inflated at the base.	*Trichoderma* spp
LFIS – 2	Greenish colonies with radiated ring	Repeatedly branched conidiospores of long chains on conidia.	*Penicillium* spp
LFIS – 3	Large cottony colony with scattered black spores	Conidia with spherical spores	*Aspergillus niger*
LFIS – 4	Grey, fluffy and greenish colonies	Septate hyphae. Irregularly branched conidiosphores, clusters of grape – like conidial head.	*Botrytis* spp
LFIS – 5	White cottony colonies	Sporangiospores within spherical shaped sporangium	*Rhizopus* spp
LFIS – 6	Greenish colony	Septate mycelium with conidium and elongated spores	*Aspergillus fumigates*
LFIS – 7	Olive green to brown	Septate, conidiospore, conidia mostly globose to subglobose.	*Cladosporium* spp2

LFIS- Lignolytic Fungal Isolates

Characterization of selected lignolytic fungi

The observations on the growth performance of the seven selected lignolytic fungi in the acidic (pH 6.0), neutral (pH 7.0) and alkaline (pH 8.0) conditions with various concentrations of tannic acid at three different temperatures are given in Tables 5, 6 and 7. The growth performance varied for various bacteria in different concentrations of tannic acid, temperatures and pH. Among the fungal isolates, *Penicillium* spp, *Aspergillus niger*, *Aspergillus fumigatus* and *Trichoderma* spp showed good growth performance at 28^{o}C with 1.0 percent tannic acid in the acidic (pH 6.0) and in the neutral (pH 7.0) conditions (Tables 3 and 4).

Table 3: Growth performance of the seven selected lignolytic fungi grown in Malt extract agar medium containing various concentrations of tannic acid at three different temperatures in acidic condition (pH 6.0) on 10d

Growth temperature (^{0}C)	Tannic acid concentration (%)	Diameter of fungal growth (cm)						
		Trichoderma spp	*Penicillium* spp	*Aspergillus niger*	*Botrytis* spp	*Rhizopus* spp	*Aspergillus fumigatus*	*Cladosporium* spp2
28	0.25	1.4	1.8	1.6	1.3	1.4	1.9	1.1
	0.50	1.7	2.0	1.8	1.5	1.5	2.1	1.3
	0.75	1.8	2.1	1.9	1.7	1.6	2.3	1.4
	1.00	1.9	2.3	2.1	1.8	1.7	2.4	1.7
	1.25	1.7	1.9	1.7	1.6	1.6	2.0	1.2
37	0.25	1.2	1.3	1.3	1.0	1.2	1.4	0.9
	0.50	1.3	1.5	1.4	1.2	1.3	1.6	1.0
	0.75	1.4	1.6	1.6	1.3	1.5	1.7	1.2
	1.00	1.5	1.9	1.7	1.5	1.6	1.8	1.1
	1.25	1.2	1.5	1.4	1.3	1.4	1.6	I.0
47	0.25	NG	NG	NG	NG	NG	NG	NG
	0.50	NG	NG	NG	NG	NG	NG	NG
	0.75	NG	NG	NG	NG	NG	NG	NG
	1.00	NG	NG	NG	NG	NG	NG	NG
	1.25	NG	NG	NG	NG	NG	NG	NG

NG - No Growth

Table 4: Growth performance of the seven selected lignolytic fungi grown in Malt extract agar medium containing various concentrations of tannic acid at three different temperatures in neutral condition (pH 7.0) on 10d

Growth temperature (^{0}C)	Tannic acid concentration (%)	Diameter of fungal growth (cm)						
		Trichoderma spp	*Penicillium* spp	*Aspergillus niger*	*Botrytis* spp	*Rhizopus* spp	*Aspergillus fumigatus*	*Cladosporium* spp2
28	0.25	1.3	1.6	1.4	1.2	1.3	1.7	0.8
	0.50	1.5	1.8	1.6	1.3	1.4	1.9	1.0
	0.75	1.6	2.0	1.7	1.4	1.5	2.0	1.2
	1.00	1.7	2.1	1.9	1.6	1.6	2.2	1.4
	1.25	1.5	1.7	1.5	1.4	1.4	1.8	1.1
37	0.25	1.0	1.2	1.2	0.9	1.1	1.2	0.6
	0.50	1.1	1.4	1.3	1.1	1.2	1.4	0.7
	0.75	1.3	1.5	1.4	1.2	1.3	1.5	0.9
	1.00	1.4	1.7	1.6	1.3	1.5	1.6	1.0
	1.25	1.1	1.4	1.3	1.2	1.3	1.3	1.1
47	0.25	NG	NG	NG	NG	NG	NG	NG
	0.50	NG	NG	NG	NG	NG	NG	NG
	0.75	NG	NG	NG	NG	NG	NG	NG
	1.00	NG	NG	NG	NG	NG	NG	NG
	1.25	NG	NG	NG	NG	NG	NG	NG

NG - No Growth

Table 5: Growth performance of the seven selected lignolytic fungi grown in Malt extract agar medium containing various concentrations of tannic acid at three different temperatures in alkaline condition (pH 8.0) on 10d

Growth temperature (^{0}C)	Tannic acid concentration (%)	Diameter of fungal growth (cm)						
		Trichoderma spp	*Penicillium* spp	*Aspergillus niger*	*Botrytis* spp	*Rhizopus* spp	*Aspergillus fumigatus*	*Cladosporium* spp2
28	0.25	1.1	1.4	1.2	0.9	1.0	1.5	0.9
	0.50	1.3	1.6	1.4	1.1	1.2	1.7	1.0
	0.75	1.5	1.8	1.6	1.3	1.4	1.9	1.1
	1.00	1.6	1.9	1.7	1.4	1.5	2.0	1.3
	1.25	1.4	1.5	1.3	1.1	1.2	1.6	1.0
37	0.25	0.9	1.1	1.1	0.9	1.0	1.2	0.8
	0.50	1.0	1.3	1.2	1.0	1.1	1.4	1.0
	0.75	1.2	1.4	1.3	1.1	1.2	1.5	0.9
	1.00	1.3	1.6	1.5	1.3	1.4	1.6	1.2
	1.25	1.1	1.3	1.2	1.1	1.2	1.4	1.1
47	0.25	NG	NG	NG	NG	NG	NG	NG
	0.50	NG	NG	NG	NG	NG	NG	NG
	0.75	NG	NG	NG	NG	NG	NG	NG
	1.00	NG	NG	NG	NG	NG	NG	NG
	1.25	NG	NG	NG	NG	NG	NG	NG

NG - No Growth

DISCUSSION

In general diverse fungal groups in the environment are capable of degrading lingo-cellulosic rich organic substrate into a simplest nutrient form. Hence an attempt was made to screen and characterize of efficient lignolytic fungi isolated from decayed sawdust. Thirteen fungal strains were isolated from decayed sawdust and all of them were

screened for lignolytic activity using Malt extract medium supplemented with tannic acid. The wild type of fungal strains which have lignolytic activity were isolated from different animal dung manure soils [14]. Among thirteen fungal strains screened only seven bacterial isolates showed lignolytic activity and their solubilization index was calculated (Table 1). All the seven lignolytic fungal isolates were identified based on microscopic and macroscopic characteristics (Table 2). The efficacy of various fungi for their ligno-cellulolytic activity for recycling the sericultural waste [15]. In the present study, various growth conditions such as pH, substrate concentration and temperature for the growth of lignin degrading microorganisms were optimized and the results are shown in Tables 3, 4 and 5. [13] isolated lignin degrading fungi such as *Penicillium* spp, *Fusarium* spp *and Aspergillus* spp from termite gut and partially characterized their growth parameter against different pH condition such as pH 4, 7 and 8. In this study the results revealed that the growth performance differ from among the different isolates. From among the selected fungi *Penicillium* spp, *Aspergillus niger, Aspergillus fumigatus* and *Trichoderma* spp showed good growth performance at 28^{o}C with 1.0 percent tannic acid in the acidic (pH 5.0) and neutral (pH 7.0) conditions (Tables 3 and 4) . Similar results were observed in microfungi such as *Penicillium chrysogenum, Fusarium oxysporum* and *Fusarium solani* [16].

CONCLUSION

Seven fungal strains such as *Trichoderma* spp, *Penicillium* spp, *Aspergillus niger*, *Botrytis* spp, *Rhizopus* spp, *Aspergillus fumigatus* and *Cladosporium* spp2 isolated from decayed sawdust showed better lignolytic activity against different growth conditions such as temperature, pH and substrate concentration. Hence these organisms could be used commercially in the rapid degradation of lignolytic rich waste material into nutrient rich biomanure for agricultural practice in Rural India.

REFERENCES

[1] Acharya CN. *Biochem. J*, **1935**, 29:1459-1467.
[2] Babel U. In A. Jonerius,ed.,Soil micromorphology. *Elsevier Publishing Co*. Amsterdam, **1964**, 15-22.
[3] Jorgensen H., Kristensen JB., Felby C. *Biofuels, Bioprod. Bioref*, **2007**, 1: 119 –134.
[4] Rahman MM., Begum MF., Khan MSI. *Journal of Applied Science Research*, **2009**, 5: 1652-1662.
[5] Saxena RK., Sharmila P., Singh VP. *Microbiol.***1995**, 32, 259-270.
[6] Rao NS. Organic matter and composting. *In: Biofertilizer in agriculture and forestry*. III ed., Oxford and IBH publishing co. New Delhi, **1993**,157-159.
[7] Kannan N. *Laboratory manual in general microbiology*, Palani paramount publication, Palani, India, **1996**.
[8] Bavendamm M. *In: Bio fertilizers in agriculture and foretry. (Ed.).* Rao, oxford and IPH Publishing Co.(P) Ltd. New Delhi. **1927**, 153.
[9] Edi-Premono M., Moawad AM., Vlek PLG. *Pakistan Journal of Biological Sciences*, **1996**, 7(2):187-196.
[10] Domsch KH., Gams W., Andarson TH. *Compendium of soil fungi.*1.Academic press, London, **1980**.
[11] Alexopoulos CJ., Mims CW. *Text Book on Introductory Mycology*. Wiley Eastern Ltd., New Delhi, **1983**.
[12] Aneja KR. Experiments in Microbiology, plant pathology, tissue culture and mushroom production technology. *New age International publisher*, New Delhi, 2001, 282-291.
[13] Mahalingam PU., Daniel T. *Journal of Pure and Applied Microbiology*, **2007**, 1(2): 327-330.
[14] Charitha Devi M., Sunil Kumar M. *Annals of Biological Research*, **2012**, 3 (2):1145-1152.
[15] Vorkady Nishitha Naik, Dinesh Dutta Sharma, Punathil Meethal Pratheesh Kumar, Ramakrishna Dayakar Yadav. *Acta Biologica Indica*, **2012**, 1(1): 47-50.
[16] Rodriguez A., Perestelo F., Carnicero A., Regalado V., Perez R., De la Fuente G., Falcon MA. *Microbiol. Ecol.* **1996-a**, 21:213-219.