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Identification and Screening of wood rot fungi from Western Ghats area of South India

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Abstract : Fifty six samples were collected from Western Ghats area, of Tamilnadu and Karnataka, South India. The collected fungi were isolated and identified based on the key provided previously. *Phanerochaete chrysosporium*-787 was obtained from Microbial Type Culture Collection, Chandigarh, India and was used as the reference fungus. The fungi were screened for their ligninolytic activity based on their ability to oxidize dyes, poly R-478 and remazol brilliant blue, to degrade native lignin and further confirmation was done by the liberation of ethylene from KTBA (2-keto-4-thiomethyl butyric acid). The color removals in 57 samples were in the range of 11.5 to 38.4% in poly- R dye and 11.1 to 72.0 % in remazol brilliant blue. The mycelial growth rates were in the range of 1.24 to 3.67 mg/day and percent degradation of lignin were found to be in the range 20.4 to 68.8. The ligninolytic activity of the fungi were further confirmed by their ability to release ethylene from KTBA and the results were found to be in the range of 1.210 to 3.121 ppm. From the above screening results three best wood rot fungi *Polyporus hirsutus, Daedalea flavida, Phellinus sp1* were selected for further studies.
Keywords: basidomycetes, *Daedalea flavida,* remazol brilliant blue, poly-R, *Polyporus hirsutus, Phellinus sp1*.

Introduction

Wood is formed of three main constituents, cellulose, hemicelluloses and lignin. Lignins constitute the second most abundant group of biopolymers in the biosphere.[1] Lignin is highly resistant towards chemical and biological degradation, and confers mechanical resistance to wood.[2] The plant material contains 25-30% of lignin, which gives plants their structural integrity and provides protection from pests and pathogens.[3] Lignins are polymers consisting of phenylpropane units in which the aromatic rings are substituted with guaiacyl propane units, syringylpropane units or para hydroxyphenylpropane units.[4] Wood rotting fungi are an important component of forest ecosystems.[5] White rot fungi belong to the order basidomycetes that participates in the biodegradation of lignin in nature, which is essential for global carbon recycling.[6] White rot fungi can degrade lignin and a range of environmental pollutants by many of their extra cellular ligninolytic enzymes.[7] White rot fungi possess a number of advantages that can be exploited in bioremediation systems, since the key components of their lignin-degrading system are extracellular, these fungi can degrade an extremely diverse range of very persistent or toxic environmental pollutants.[8] The ability of fungi to degrade lignocellulosic materials is due to their highly efficient enzymatic system.[9] Microbial degradation of lignin has received considerable attention in recent years. Fungi that selectively remove lignin without loss of appreciable amounts of cellulose are extremely attractive for use in biological pulping processes, to improve the digestibility of highly lignified plant

residues, and for bioconversion of lignocellulosics into industrial products.[10] White rot fungi emerged as a promising group for biotechnological applications, especially in bioremediation.[11] The lignin degrading system of white rot fungi is exploitable for dye decolorization.[12] The possibility of using ligninolytic fungi to remove synthetic dyes have attracted considerable attention, this is due to their production of ligninolytic enzymes that enable these microorganisms to oxidize a broad range of substrates, including synthetic dyes.[13] [14]reported that most of the enzymes produced by white-rot fungi degrade lignin. These ligninolytic enzymes predominantly are lignin peroxidase (LiP), manganese dependant peroxidase (MnP) and laccase. They are non specific enzyme system, enabling fungi to degrade natural complex aromatic polymers of lignin as well as complex aromatic polymers, such as pesticides, polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs). Laccase can oxidize nonphenolic compounds with a relatively low ionization potential, while nonphenolics with high ionization potential are readily oxidized by lignin peroxidases and mangenese dependent peroxidases.[15] Some wood degrading fungi contain all three classes of the lignin modifying enzymes, while others contain only one or two of these enzymes.[16,17,18] showed that the white rot basidiomycetes *Phanerochaete chrysosporium, Pleurotus ostreatus, and Trametes versicolor* were able to biodegrade lignin styrene copolymerization products, since the molecular structure of phenol resin is very similar to that
of lignin. Ligninolytic activity have been found in more than 600 species of wood rot fungi.[19]
[5]reported the collection of nearly 600 specimens of wood rotting fungi from Jiangxi province of eastern china. [20]reported the isolation of 600 basidiomycetes from indigenous forests and commercial *Eucalyptus* and *Pinus* plantations in South Africa. [21]screened 129 isolates of natural wood-rotting fungi and three were found extremely effective to degrade the dye remazol brilliant blue R. The fungi *Phanerochaete chrysosporium, Pycnoporus sanguineus, Phlebia radiata* and *Pleurotus sajor-caju*, were screened for their ligninolytic enzyme activities using guaiacol and remazol brilliant blue-R (RBB-R) as screening reagents. The screening were conducted at the optimal growth temperature of each fungus at 35°C. All the fungi except *Pleurotus radiata*, showed positive remazol brilliant blue-R decolourization activities. [22]Forty fungi isolated from diverse environments in Western Australia were screened for ligninolytic activity based on decolourization of the polymeric dye Poly R- 478 and three isolates were identified as *Botryosphaeria* and *Coniochaeta s*pecies and selected for further studies.[23] Screening of white rot fungi *Pleurotus eous* was carried out based on their ability to degrade the dyes remazol brillant blue and Poly –R 478.[24] Studies of the physiology of lignin degradation by white rot fungi, *Phanerochaete chrysosporium* led to the development of submerged cultivation at which these fungi exhibited ligninolytic activity under laboratory conditions.[25] *Phanerochaete chrysosporium* is the most widely studied organism from the point of view of physiology and enzymology of lignin biodegradation. The lignin peroxidase was first discovered in the extracellular fluid of ligninolytic cultures of *Phanerochaete chrysosporium.*[26] In the present study identification of 56 samples and screening of 57 samples of wood rot fungi have been carried.

Materials and Methods

Collection and Isolation

Fifty six samples of wood rot fungi were collected from decayed wood and living trees of *Tectona grandis, Tarmarindus indica, Borasus flabelliform* of Western Ghats Area of Tamilnadu and Karnataka, SouthIndia. The fruit bodies were collected along with supporting wood. The samples were marked with information such as collection number with names, procurement location and date of collection. Fruit bodies were wrapped in paper bags and brought to the laboratory. The collection sight is situated in the latitude of 11.58°S and longitude of 76.93° East at 420 ± 50 M MSL. It receives rainfall of about 300mm per year with high humidity and even temperature. The fungal growth was cut sterilized with 1% mercuric chloride solution, repeatedly washed with sterile distilled water [27] and inoculated on 2 % malt agar medium in petriplates. The fungal growth which occurred on the plates were subcultured and maintained in malt agar slants.

Identification of the isolated fungi

The isolated samples were identified based on the key provided previously [28.]

Preparation of spore suspension

The Malt agar medium was prepared by dissolving 20g of malt extract and 20g of agar in 1000ml of distilled water. The pH was maintained as 4.5. The fungi were grown on malt agar plates for 6 days at 37°C. Then the plates were flooded with sterile distilled water and brushed with camel brush smoothly without disturbing the mycelia growth and filtered through sterile filter. The concentration of the filtrate was adjusted to 10^5 spores/ml and used as inoculums for further studies immediately.

Screening for ligninolytic activity

The fungi were screened for their ligninolytic activity based on their ability to oxidize dyes, poly R [29] and remazol brilliant blue[30] to degrade native lignin [31] and to liberate ethylene from KTBA (2-keto-4-thiomethyl butyric acid).

Oxidation of dyes

The ability of the fungi to oxidize poly-R and remazol brilliant blue was tested in the C-limited medium of[30]. The experiments were conducted with 10ml medium in 125 ml Erlenmeyer flasks inoculated with 10^5 spores/ml of medium (inoculums volume -10% v/v). At the beginning of carbon limited phase (after 6 days), veratryl alcohol (1.8mm final concentration) and remazol brilliant blue (3mg/l) poly R dye (200 mg/l) were added aseptically to the culture flasks. The decolurization of the dye indicated the presence of ligninolytic system. At the time intervals, the cultures were removed, filtered and the colour intensity of the culture filtrates were measured at 600nm for remazol Brilliant Blue amended medium and at 513nm/360nm for poly R dye amended medium.

Preparation of straw lignin

Alkaline extracted straw lignin was used as substrate for lignin degradation studies[32]. The wheat straw was ground to about 250mm mesh size and suspended in 4 % sodium hydroxide (10g in 250ml). It was heated for 1h at 121°C and filtered. The filtrate was diluted with methanol (1:1v/v) and kept overnight. The precipitate formed was discarded by centrifugation at 5000rpm for 20 min and the supernatant was collected. The pH of the supernatant was reduced to 3.0 with HCl and left overnight. The precipitate formed was collected by centrifugation at 20,000 rpm for 10 min, washed twice with distilled water of pH 3.0, dried under vacuum and stored in glass bottles in desiccators.

Lignin degradation assay

The ability of the fungi to degrade lignin was assayed in the basal medium[25]. The basal medium consists of solution A and Solution B. solution A consists of D-Glucose-10.0g, H_3PO_4 (2N) -10.9, NH_4NO_3 -46.9, L-Aspargine-89.0, $MgSO_4.7H_2O$ -0.5g, $CaCl_2.2H_2O$-0.1g, $FeSO_4$-10.0mg and vitamin solution -1.0 ml dissolved in 500 ml of distilled water. Solution B consists of lignin-1.0g, Potassium hydroxide-0.86g dissolved in 450 ml of distilled water.

The solutions were autoclaved separately. After cooling, solution A was slowly added with mixing to B. To the final solution, 50 ml of previously warmed 20mM acetate buffer (pH adjusted to 4.5 with NaOH) was added via a sterile membrane filter. The medium was distributed in 500ml quantities into 250ml Erlenmeyer flasks fitted with rubber stoppers with glass tubes for gas-flushing. The flasks were inoculated with spore suspension ($X10^5$ spores/ml) and incubated at 30° C without shaking (inoculums volume -10%v/v). Pure oxygen was flushed into the flasks on third day of incubation. After six days, the fungal biomass was removed by filtration wand washed with distilled water. Biomass was determined by drying at 105° C to a constant weight. Residual lignin was determined in the filtrate combined with biomass washing. To the combined filtrate, sodium hydroxide was added to a final concentration of 0.005N and absorbance was recorded at 280nm after appropriate dilution with 0.05N NaOH (about 20x, the original culture volume). Uninoculated medium served as control.

Production of ethylene from 2-keto-4-thiomethyl butyric acid (KTBA)

Enzyme preparation

C-limited medium was distributed as 50 ml aliquots in 250ml Erlenmeyer flasks and inoculated with 10^5 spores/ml (inoculums volume -10% v/v). The flasks were incubated at 30°C for 6 days. After the incubation period, the fungal biomass was removed by filtration and the culture was centrifuged at 18000Xg for 30 min at 4°C and used as enzyme. Boiled culture filtrate was the enzyme blank. To 2 ml of enzyme, 3mM glucose and 1mM KTBA were added in 10ml serum bottled fitted with rubber stoppers and sealed. The bottles were incubated at 37° C. After one hour 1 to 2.5 ml of gas was removed from the head space and analyzed for ethylene content by gas chromatography.[26]

Results and Discussion

Collection of the Samples

In the present study fifty six samples were collected from various location of Western Ghats area of Tamilnadu and Karnataka in South India. General macrocharacters of the fruit body including colour of different tissues and type of rot were noted in the field.

Identification

The samples were isolated and identified (**Table 1)** based on key provided previously[28].

Table 1: Sources and location of the Sample Collection

S.No	Name of the fungi and its sources of sample collection
1	*Polyporus sp1* -- Decayed wood, Kovai Kutralam
2	*Daldenia sp1* -- Decayed wood, Kovai Kutralam
3	*Ganoderma lucidum* -- Decayed wood, Kovai Kutralam
4	*Schizophyllum sp1* -- Decayed wood, Kovai Kutralam
5	*Polyporus hirsutus* -- Decayed wood, Kovai Kutralam
6	*Daedalea flavida* -- Decayed wood, Kovai Kutralam
7	*Schizophyllum commune* -- Decayed wood, Kovai Kutralam
8	*Phellinus sp.1* -- Decayed wood, Kovai Kutralam
9	*Ganoderma sp1* -- Decayed wood, Kovai Kutralam
10	*Lenzites eximia1* -- Decayed wood, Kovai Kutralam
11	*Ganoderma sp2* -- Decayed wood, Kovai Kutralam
12	*Ganoderma sp3* -- Decayed wood, Kovai Kutralam
13	*Polyporus sp2* - Decayed wood,Aliyar
14	*Trametes sp* - Decayed wood,Aliyar
15	*Poria sp1* - Decayed wood,Aliyar
16	*Ganoderma sp4 - Tamaridus indica*, Aliyar
17	*Polyporus grammocephallus*- Decayed wood, Aliyar
18	*Phellinus sp2*- Decayed wood, Aliyar
19	*Phellinus sp3*- Decayed wood, Aliyar
21	*Lenzites sp2*- Decayed wood, Aliyar
22	*Ganoderma sp5*- Decayed wood,Aliyar
23	*Fomes sp*- Decayed wood, Kallar
24	*Tricholomopsis sp*- Borasus flabellifor,Kallar
25	*Ganoderma sp 4a*-Decayed wood, Kallar
26	*Phellinus sp 4a* -Decayed wood, Kallar
27	*Ganoderma sp6*--Decayed wood, Ooty
28	*Daldinea sp2- Tarmaindus indica,* Ooty
29	*Ganoderma sp7*- Decayed wood, Ooty
30	*Schizophyllum sp3*- Decayed wood, Ooty
31	*Ganoderma sp8*- Decayed wood, Ooty
32	*Phellinus sp4* - Decayed wood, Ooty
33	*Phellinus sp5*- Decayed wood, Ooty
34	*Phellinus sp6* - Decayed wood, Ooty
35	*Schizophyllum sp4* - Decayed wood, Ooty
36	*Phellinus sp7* - Decayed wood, Ooty
37	*Daldenia concentrica* - Decayed wood, Ooty
38	*Lenzites sp4* - Decayed wood, Coonor
39	*Ganoderma sp9* - Decayed wood, Coonor
40	*Lenzites sp5* - Decayed wood, Coonor
41	*Stereum hirsutum* - Decayed wood, Coorg
42	*Trametus gibbosa* - Decayed wood, Coorg
43	*Lepiota sp* - Decayed wood, Coorg
44	*Crepidotus sp* - Decayed wood, Coorg
45	*Trametes serialis* - Decayed wood, Coorg
46	*Microporus sp* - Decayed wood, Coorg

47	*Ganoderma applanatum* - Decayed wood, Coorg
48	*Lenzites sp5* - Decayed wood, Coorg
49	*Poria sp2* - Decayed wood, Coorg
50	*Poria sp3* - Decayed wood, Coorg
51	*Irpex sp* - Decayed wood, Coorg
52	*Phellinus sp8* - Decayed wood, Coorg
53	*Phellinus sp8* - Decayed wood, Coorg
54	*Gymnopilus sp - Tectona grandis,* Coorg
55	*Daldenia sp3* - Decayed wood, Gudallur
56	*Daldenia sp4* - Decayed wood, Gudallur
57	Reference fungus *Phanerochaete chrysosporium* -787, MTCC

Table 2: Screening of Ligninolytic activity in wood rot fungi by dye reduction method.

S.No	Name of the Fungi	Poly-R Dye		Remazol Brilliant Blue	
		Degradation/hour	Percent degradation	Degradation/ hour	Percent degradation
1	*Polyporus sp1*	0.040	11.5	0.100	44.4
2	*Daldenia sp1*	0.044	11.5	0.045	20.0
3	*Ganoderma lucidum*	0.101	29.0	0.085	37.7
4	*Schizophyllum sp1*	0.112	32.46	0.095	42.2
5	*Polyporus hirsutus*	0.126	36.4	0.161	71.5
6	*Daedalea flavida*	0.122	34.6	0.159	70.6
7	*Schizophyllum commune*	0.078	22.5	0.041	18.2
8	*Phellinus sp.1*	0.123	35.5	0.140	62.2
9	*Ganoderma sp1*	0.096	27.7	1.155	68.8
10	*Lenzites eximia1*	0.114	32.9	0.125	55.5
11	*Ganoderma sp2*	0.101	34.6	0.152	67.5
12	*Ganoderma sp3*	0.117	33.8	0.147	65.3
13	*Polyporus sp2*	0.041	11.8	0.020	8.8
14	*Trametes sp*	0.101	29.1	0.080	35.5
15	*Poria sp1*	0.0855	24.5	0.105	46.6
16	*Ganoderma sp4*	0.055	15.8	0.025	11.1
17	*Polyporus grammocephallus*	0.111	31.7	0.159	70.6
18	*Phellinus sp2*	0.036	10.4	0.020	8.80
19	*Phellinus sp3*	0.048	13.8	0.037	16.4
20	*Lenzites sp2*	0.112	32.3	0.148	64.4
21	*Ganoderma sp5*	0.106	30.6	0.135	60.0
22	*Fomes sp*	0.061	17.6	0.040	17.7
23	*Tricholomopsis sp-*	0.096	27.7	0.105	47.2
24	*Ganoderma sp 4a*	0.098	28.3	0.142	63.0
25	*Phellinus sp 4a*	0.099	28.6	0.150	66.6
26	*Ganoderma sp6*	0.066	17.3	0.033	15.5
27	*Daldinea sp2*	0.086	24.8	0.157	69
28	*Ganoderma sp7*	0.114	32.9	0.154	68.4
29	*Schizophyllum sp3*	0.077	20.2	0.042	18.6
30	*Ganoderma sp8*	0.122	34.6	0.105	47.2

31	*Phellinus sp4*	0.045	13.0	0.027	12
32	*Phellinus sp5*	0.111	32.0	0.125	55.5
33	*Phellinus sp6*	0.106	21.3	0.040	17.7
34	*Schizophyllum sp4*	0.061	29.1	0.140	62.2
35	*Phellinus sp7*	0.096	19.9	0.055	22.2
36	*Daldenia concentrica*	0.098	34.6	0.105	46.6
37	*Lenzites sp4*	0.099	18.7	0.027	16.4
38	*Ganoderma sp9*	0.066	27.7	0.125	38.2
39	*Lenzites sp*	0.086	15.6	0.040	18.2
40	*Daedalea sp*	0.114	29.1	0.055	62.0
41	*Stereum hirsutum*	0.088	23.1	0.140	37.7
42	*Trametus gibbosa*	0.104	30.05	0.122	54.2
43	*Lepiota sp*	0.101	29.1	0.118	52.4
44	*Crepidotus sp*	0.122	35.2	0.150	66.6
45	*Trametes serialis*	0.108	31.2	0.100	44.4
46	*Microporus sp*	0.119	34.3	0.115	51.1
47	*Ganoderma applanatum*	0.133	38.4	0.140	62.2
48	*Lenzites sp5*	0.115	33.2	0.106	47.1
49	*Poria sp2*	0.108	31.2	0.092	40.8
50	*Poria sp3*	0.133	38.4	0.116	51.5
51	*Irpex sp*	0.124	38.8	0.149	66.2
52	*Phellinus sp8*	0.127	36.7	0.162	72.0
53	*Gymnopilus sp*	0.085	24.5	0.095	42.2
54	*Daldenia sp3*	0.119	34.3	0.125	44.0
55	*Daldenia sp4*	0.144	32.9	0.145	58.0
56	*Reference fungus Phanerochaete chrysosporium-787* MTCC	0.108	31.2	0.138	55.0

Table 3: Ligninolytic activity of wood rot fungi by Lignin degradation and ethylene production as confirmatory test.

S.No.	Name of the Fungi	Lignin Degradation method		Production of Ethylene from KTBA (ppm)
		Growth on Lignin (mg/day)	Percent degradation	
1	*Polyporus sp1*	2.79	34.9	1.621
2	*Daldenia sp1*	2.60	30.5	1.210
3	*Ganoderma lucidum*	2.15	29.2	1.700
4	*Schizophyllum sp1*	2.80	40.6	1.825
5	*Polyporus hirsutus*	2.90	68.8	2.832
6	*Daedalea flavida*	2.58	34.9	2.421
7	*Schizophyllum commune*	3.20	30.5	1.986
8	*Phellinus sp.1*	3.67	29.2	2.381
9	*Ganoderma sp1*	2.58.2	40.6	1.961
10	*Lenzites eximia*	2.69	68.8	2.539
11	*Ganoderma sp2*	2.81.	67.4	2.422
12	*Ganoderma sp3*	1.47	26.0	1.421

13	*Polyporus sp2*	1.48	63.3	1.211
14	*Trametes sp*	1.56	62.6	2.225
15	*Poria sp1*	2.45	38.6	1.831
16	*Ganoderma sp4*	2.34	62.6	2.225
17	*Polyporus grammocephallus*	2.91	26.3	1.426
18	*Phellinus sp2*	1.34	30.6	1.568
19	*Phellinus sp3*	1.91	20.4	1.325
20	*Schizophyllum sp2*	2.38	23.6	1.228
21	*Lenzites sp2*	1..86.	52.2	2.252
22	*Ganoderma sp5*	2.98	55.4	2.621
23	*Fomes sp*	2.76	33.4	1.258
24	*Tricholomopsis sp*	2.32	36.5	1.361
25	*Ganoderma sp 4a*	2.56	57.7	2.623
26	*Phellinus sp 4a*	2.67	55.9	2.521
27	*Ganoderma sp6*	2.87	25.2	1.725
28	*Daldinea sp2*	2.93	52.8	2.624
29	*Ganoderma sp7*	1.24	47.4	1.729
30	*Schizophyllum sp3*	1.67	31.8	2.624
31	*Ganoderma sp8*	1.89	38.6	1.729
32	*Phellinus sp4*	2.34	20.7	1.631
33	*Phellinus sp5*	2.67	43.9	1.832
34	*Phellinus sp6*	2.54	23.4	1.344
35	*Schizophyllum sp4*	1.89	52.2	2.421
36	*Phellinus sp7*	1.90	26.0	1.302
37	*Daldenia concentrica*	2.09	44.2	1.928
38	*Lenzites sp3*	2.45	20.7	1.627
39	*Ganoderma sp9*	2.56	45.6	2.062
40	*Lenzites sp4*	2.78	51.1	2.233
41	*Daedalea sp1*	2.65	48.9	2.129
42	*Stereum hirsutum*	2.89	46.1	1.921
43	*Trametus gibbosa*	2.90	55.08	2.821
44	*Lepiota sp*	2.13	58.30	2.628
45	*Crepidotus sp*	2.34	46..1	2.112
46	*Trametes serialis*	2.67	55.08	2.455
47	*Microporus sp*	2.14	26	2.138
48	*Ganoderma applanatum*	2.45	44.2	`1.981
49	*Lenzites sp5*	2.65	20.7	2.138
50	*Poria sp2*	2.87	46.6	1.981
51	*Poria sp3*	2.35	51.1	3.121
53	*Phellinus sp8*	2.56	36.81	1.762
54	*Gymnopilus sp*	2.68	53.49	1.952
55	*Daldenia sp2*	2.45	51.45	1.821
56	*Daldenia sp2*	2.32	49.0	1.721
57	*Phanerochaete chrysosporium-787* MTCC	2.12	42.0	1.822

Screening for ligninolytic activity

Screenings were carried out by poly R dye (0.02%) and remazol brilliant blue were used as test dyes. The colour removal of 56 samples were in the range of 11.5 to 38.4 in Poly R and 11.1 to 72.0 in remazol brilliant blue (Table 2).[29] attempted to correlate dye decolorization and the ligninolytic activity by *Phanerochaete chrysosporium* by comparing the effect of various physiological parameters, mutations, and inhibitors on both processes. Dye decolorization, appears to be a secondary metabolic process, It was repressed by nutrient nitrogen and only occurred after the nitrogen in the cultures had been consumed**.** [33]Reported the screening of white rot fungi to degrade the polymeric dyes blue dextran and Poly R-478. The most significant rates of Poly R-478 decolorization in liquid cultures were found with the isolates, *Trametes cingulata, Trametes versicolor, Trametes pocas, DSPM95, Datronia concentrica* and *Pycnoporus sanguineus***.** [34]observed 19% decolorization of Poly R-478, after 15 min of dye incubation. [35]Reported, higher than 95% decolourization of poly-R 478 in semi-solid-state cultures of *Phanerochaete chrysosporium.* [36]Observed high percentages of decolorization between 65% and 80% of poly R-478 dye under optimal conditions. [22]Reported that *sajor-caju* may be a suitable fungus in bioconversion of lignocellulose because it maintained guaiacol oxidation and remazol brilliant blue-R decolourization activities at 35°C. A novel strain of *Bjerkandera* sp. (B33/3), with particularly high decoluorization activities of poly R-478 and remazol brilliant blue-R dyes, was isolated. [37]*Pleurotus ostreatus* is able to decolorize remazol brilliant blue-R on agar plate, when grown in liquid media supplemented with veratryl alcohol, the fungus completely decolorizes remazol brilliant blue-R in 3 days.[38] [39]reported the decolourization of two reactive dyes, remazol brilliant blue R (RBBR) and reactive black 5 (RB5), by selected white-rot fungus *Datronia* sp. KAPI0039.

To confirm the ligninolytic activity of the fungi, the ability to grow on and degrade lignin was studied in lignin amended basal medium.[40] The growth was measured in terms of increase in mycelia dry weight (mg/day). The mycelial growth rate were in the range of 1.24 mg/day to 3.67 mg/day and percent degradation of lignin were found to be in the range 20.4 to 68.8 (Table 3). [41]reported that the screening of wood rot fungi capable of degrading lignocellulose from Plantation forests. [42]reported a direct correlation between dye decolorization and ligninolytic activity, measured by degradation of Klason lignin.

The ligninolytic activity of the fungi was further confirmed by their ability to release ethylene from KTBA. The results were found to be in the range of 1.210 to 3.121 ppm (Table 3). Based on the above screening results three species *Polyporus hirsutus, Daedalea flavida, Phellinus sp1* were selected for further study. [43]reported two H_2O_2 dependent oxidases in extracellular medium of white rot fungi *Phanerochaete chrysosporium,* these enzymes catalyze the oxidation of phenol red, O-dianisidine, poly R and a variety of other dyes, the enzyme fractions generated ethylene from KTBA in the presence of alcohol. *Pleurotus eryngii* has the ability to degrade wheat lignin and selectively oxidizes KTBA to ethylene when it is grown in peptone-containing media, as well as during treatment of straw under the Single state fermentation conditions used for biomechanical pulping.[44] [45]reported that a highly significant correlation was found between two ligninolytic indicators, ethylene formation from α-keto-γ-methylthiolbutyric acid and the decolorization of a polymeric dye, poly R. An H_2O_2-requiring enzyme system was found in the extracellular medium of ligninolytic cultures of *Phanerochaete chrysosporium*. The enzyme system generated ethylene from 2-keto-4-thiomethyl butyric acid (KTBA), and oxidized a variety of lignin model compounds.[26] [46]reported the oxidation of KTBA followed by the evolution of ethylene by the white rot fungus *Panus tigrinus* .This study is the first report to screen 56 samples of wood rot fungi from Western Ghats of South India. From the above screening results three best white rot fungi *Polyporus hirsutus, Daedalea flavida, Phellinus sp1* were selected. This newly isolated high potential lignin degrading fungi were used to carry out novel biotechnological applications.

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