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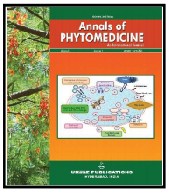

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Isolation and screening of fungi from rotten wood for various hydrolytic enzymes production

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Abstract

In the present study, rotten wood samples were collected from different sites of Solan, Shimla, Sirmour, Kangra and Chamba districts of Himachal Pradesh and 52 fungal isolates were isolated. Out of 52 fungal strains, 25 isolates were found to be positive for laccase activity, 31 isolates showed cellulase activity, out of which maximum zone size was observed. Ligninolytic fungal isolates exhibited multiple hydrolytic enzyme activity of laccase, cellulase and xylanase and different strains were selected on the basis of highest zone of hydrolysis surrounding fungal colonies. In case of quantitative screening, maximum laccase production was observed in S7 (1.22 IU/ml) where as S5 obtained maximum cellulase of 0.793 IU/ml and SH5 produced appreciable amount of xylanase (8.09 IU/ml).

1. Introduction

Lignocellulosic biomass is a promising renewable resource for attaining value added products. Lignocellulosic biomass is mainly composed of cellulose, hemicellulose and lignin distributed within the cell walls. Structural component of these cellulose, hemicelluloses and lignin differ with species, tissues and maturity of the plant cell wall. Many micro-organisms are capable of producing a variety of lignocellulolytic enzymes, *i.e.*, laccase, cellulase and xylanase which are responsible for the biodegradation of lignocellulosic wastes in nature (Acharya *et al*., 2010). Recently, the environment friendly and cost effective approach of microbial pretreatment has received attention of researchers for enhancing enzymatic saccharification of lignocellulosic biomass. This method employs micro-organisms which degrade lignin, the most recalcitrant polymer in biomass through the action of lignin degrading enzymes (Barakat *et al*., 2013). Generally, micro-organisms especially fungi have capability to produce or excrete the hydrolytic enzyme in the nature. The degradation of wood by fungi occurs through different biochemical mechanisms.

Lignolytic fungi overall help in complete decomposition of lignocellulosic waste and convert it into value added products by synergetic action of three groups of enzymes, *i.e*., cellulase, xylanase and laccase (Dwivedi *et al*., 2011). The filamentous fungi are richer in the production of extracellular lignocellulolytic enzymes as compared to bacteria and yeast. Since, today's world demand for more constant, active and specific enzymes, wood decaying fungi serve as an ideal candidate for the management of lignocellulosic wastes. The degradation of biomass and its subsequent bioconversion into ethanol may be attributed to the synergistic and complementary action of many enzymes including hydrolytic enzymes laccase, cellulase and xylanase as well as enzymes from yeasts for fermentation (Nagraj *et al*., 2014). Ligninolytic microbial systems and their enzymes were more applicable to improve the digestibility and nutritive value of animal feeds, degradation of toxic pollutants and bioconversion of lignin into useful organic compounds and to increase the fermentability of lignocellulosic residues and also for the commercial production of biofuels from feed stock, paper pulp. So, the present study was initiated with the aim of isolation and screening of potential ligninolytic fungi from rotten wood samples collected from different forests.

2. Materials and Methods

2.1 Collection and enrichment of samples

Rotten wood samples were collected from different sites rich in pine forests of Himachal Pradesh, *i.e.*, Solan, Shimla, Kangra, Chamba and Sirmour districts. To the 5 g of rotten wood sample, 2% of cellulose powder was added and water was sprinkled in the petriplates to maintain an adequate level of moisture. The petriplates containing the above mixture were incubated at 28 ± 2°C for 4-5 days.

2.2 Isolation of ligninolytic, cellulolytic and xylanolytic fungi

One gram of enriched sample was serially diluted from 10^{-2} to 10^{-8} times using sterilized 9 ml dilution blanks. Each of diluent (0.1 ml) was placed on the surface of Potato Dextrose Agar (PDA) medium and incubated at 28 ± 2°C for 3 days. The pure cultures were obtained by bit method respectively.

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2.3 Qualitative screening of ligninolytic, cellulolytic and xylanolytic fungi

An extensive screening of fungal isolates capable of exhibiting appreciable levels of ligninolytic cellulolytic and xylanolytic, activity was done. The plates showing growth after 48 h of incubation were subjected to qualitative screening for visualizing corresponding zones of hydrolysis of different isolates for assessing their potential to produce multiple carbohydrate, *i.e.*, laccase, cellulase and xylanase.

2.3.1 Laccase activity (Kalra *et al*., 2013)

Fungal isolates were cultured on petriplates containing potato dextrose agar (PDA) supplemented with 0.04% guaiacol. In addition, 0.01% (w/v) chloramphenicol was also added to the media to avoid bacterial growth and the pH of the medium was adjusted to 5.5 with 1N HCl. The plates were incubated at 28 ± 2°C for 5 days. Clear reddish brown colored zones could be observed only around colonies of the active fungal strains.

2.3.2 Cellulase and xylanase activity (Sreedevi *et al.,* 2013)

Detection of cellulase and xylanase activity was done on the culture plate of cellulose hydrolysis (containing 1% cellulose powder) as well as xylan hydrolysis (1% xylan powder) media, the plates were flooded with iodine solution (1% iodine crystals and 2% potassium iodide) and allowed to stand for 15 min at room temperature, a bluish black complex with cellulase and xylanase demarcated a clear zone around the colonies.

2.4 Quantitative screening of ligninolytic, cellulolytic and xylanolytic fungi

Enzymes production: Different fungal cultures were grown in Potato dextrose agar media containing petriplates at 28 ± 2°C. As soon as full plate of fungal isolates was observed, 10 ml of autoclaved distilled water was poured and scratched. The spore suspension thus formed was containing $1x10^7$ spores/ml, five ml of fungal inoculum was added to each 100 ml of laccase production broth amended with 0.04% guaiacol for laccase, Riviere's broth containing 1% cellulose for cellulase and 1% xylan for xylanase in 250 ml of Erlenmeyer flasks and incubated for 7 days at 28 ± 2°C at 120 rpm. After incubation, the culture contents were centrifuged at 10,000 rpm for 15 min (4°C) and the supernatant was collected and enzyme assays were performed.

2.4.1 Enzyme assays

2.4.1.1 Laccase assay (Rehan *et al*., 2016)

The reaction mixture contained 1 ml of 2 mM of guaicol in 3 ml sodium acetate buffer (pH 5.5) and 1ml of enzyme supernatant was added. The blank was also prepared with all components except the enzyme. The reaction mixture was incubated at 30°C for 15 min. The absorbance was read at 450 nm.

2.4.1.2 Cellulase assay (Reese and Mandel, 1963)

The sub-enzymes of cellulase were measured by following standard assays. CMCase activity was determined by incubating 0.5 ml of culture supernatant with 0.5 ml of 1.1% CMC in citrate buffer (0.05M, pH 5.0) at 500C or 1 h. After incubation and 3 ml of 3, 5-dinitrosalicylic acid (DNSA) reagent was added. The tubes were immersed in boiling water bath and removed after 15 min. The optical density was read at 540 nm. FPase activity was measured by Reese and Mandel method. The reaction containing 0.5 ml of culture supernatant, 50 mg strips of filter paper (Whatmann no.1) and 0.5 ml of citrate buffer (0.05 M, pH 5.0) was incubated at 50°C for 1 h. After incubation and 3 ml of DNS reagent was added. The tubes were boiled in boiling water bath and removed after 15 min. The OD was read at 540 nm. For β-glucosidase activity the reaction mixture containing 1 ml of 1mM p-nitrophenol β-D-glucopyranoside in 0.05 M acetate buffer (pH 5.0) and 100 µl of enzyme solution was incubated at 450C for 10 min. After incubation, 2 ml of 1 M Na_2CO_3 was added and the mixture was heated in boiling water bath for 15 min and OD was read at 400 nm.

2.4.1.3 Xylanase assay (Miller, 1959)

To 0.5 ml of xylan, 0.3 ml of citrate buffer (pH 5) and 0.2 ml of enzyme was added. The reaction mixture was incubated at 45°C for 10 min and then 3 ml of DNSA reagent was added and the mixture was then heated on boiling water bath for 15 min, after cooling down at room temperature absorbance of reaction mixture was read at 540 nm.

2.4.1.4 Reducing sugars (Miller, 1959)

To 1.0 ml of culture supernatant, 3 ml of DNSA reagent was added and the mixture was then heated on boiling water bath for 15 min, after cooling down at room temperature, absorbance of the reaction mixture was read at 540 nm.

2.4.1.5 Protein assay (Lowry *et al*., 1951)

To 0.1 ml of culture supernatant, 2.5 ml of Lowry's alkaline reagent was added and allowed to stand for 10 min. After incubation diluted (1N) Folin Ciocalteau's reagent (0.25 ml) was added. The contents were shaken quickly and allowed to stand for 30 min for maximum colour development. Absorbance of reaction mixture was read at 670 nm.

2.5 Statistical analysis

The data recorded for different parameters were subjected to Completely Randomized Design (Gomez and Gomez, 1976). The statistical analysis based on mean value of replications used for each treatment was made using analysis of variance (ANOVA) for Completely Randomized Design.

3. Results

3.1 Isolation of hydrolytic enzymes producing fungi

Different sources, *viz*., rotten wood samples with a high probability of enzymes, *i.e.*, laccase, cellulase, xylanase producing fungi were collected from different sites of Solan, Shimla, Sirmour, Kangra and Chamba districts of Himachal Pradesh (Figure 1). In total, 52 fungal strains were isolated from rotten wood samples in PDA medium, pH 5.6 enlisted in Figure 2 (a-d) along with their morphological properties. Out of 52 fungal strains, 26 were isolated from Solan district, 6 from Shimla district, 11 from Sirmour district and 1 fungal strain was isolated from Kangra and 1 from Chamba district of Himachal Pradesh. These isolates were tentatively

identified on the basis of their mycelium appearance, colour and texture as *Trichoderma* sp., *Fusarium* sp., *Penicillum* sp., *Rhizopus* sp., and *Aspergillus* sp. As depicted in Figure 2 (a), the sites of collection of rotten wood samples showed a great variation of fungal population amongst different sites, *i.e.*, 14% collected from Rajgarh, 8% from Giripul, 14% from Nauni, 12% from Shoghi, 14% from Sultanpur, 10% from Arki, 14% from Kasauli, 2% from Chamba and Kangra. Figure 2(b) showed variation in the colour of different fungal strains and varied from 40% white, 25% green, 26% black, 3% light brown and light black in colour. Figure 2(c) depicted the mycelium of the isolates, out of total, 54% were filamentous, 34% short hyphae, 8% long hyphae and 4% network of hyphae. Figure 2(d) showed the texture of isolated fungi, where 64% were rough, 5% feathery, 19% mucilaginous, 6% spreading type and slightly slimy in texture. Rotten wood is a rich habitat of potential lignocellulolytic enzymes producing fungi as these enzymes help in the degradation of wood. Thus, isolation of hyperligninolytic, hypercellulolytic and hyperxylanolytic fungi had been done in the present study from the rotten wood considering it a valuable niche of fungi for biodegradation. Mahmood *et al.* (2016) isolated *Coniphora puteana* and *Daedalea dickinsii* on the dead trunk of *Ficus carica* collected from Rawalpindi, Islamabad, Murree and Bagh Azad Kashmir. Ferdes *et al*. (2018) isolated five fungal strains namely *Pleurotus ostreates*, *Polyporus squamosus*, *Lentinula ododes*, *Hypsizygus terrulatus* and *Flammulina velutipies* known for the production of wood degrading enzymes. Thirty four mesophilic as well as thermophilic fungal species were isolated from different sources like decaying wood, compost and soil (Kaur and Gupta, 2016). Chayal and Agarwal (2016) isolated 6 endophytic fungi from leaves of *Cupressus torulosa*. Alsohaili and Hasan (2018) isolated filamentous fungi from soil samples of different geographical locations in the north eastern desert in Jordan.

Figure 1: Collection of rotten wood samples from different sites.

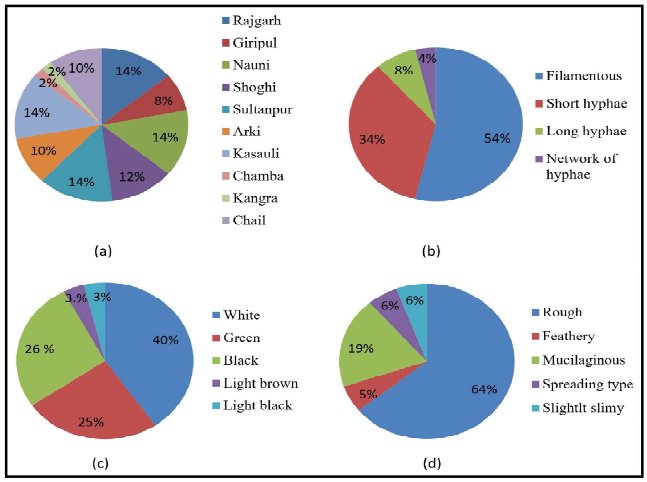

Figure 2: Morphological Characteristics of fungal Isolates (a) sites, (b) mycelium, (c) colour and (d) texture.

4. Discussion

4.1 Qualitative screening of ligninolytic, cellulolytic and xylanolytic fungal isolates

An extensive screening of fungal isolates capable of exhibiting appreciable levels of lignolytic, cellulolytic and xylanolytic activities was done (Table 1 and Figure 3). Figure 3 (a) showed positive activity of laccase on PDA medium where guaicol was used as an indicator. A reddish brown coloured zone surrounding fungal colonies was observed after 5 days of incubation. Out of 52 isolates, 25 isolates were found to be positive for laccase activity. As the results depicted in Table 1, maximum zone size was shown by five isolates, *i.e.*, S7 (20 mm), R4 (19 mm), SH2 (18 mm), K3 (16 mm) and S5 (15 mm) and minimum zone was observed in SH1, *i.e.*, 2 mm. Figure 3 b and 3 c showed the activity of cellulase and xylanase on Reverie's medium containing cellulose/xylan as a substrate. The plates showing growth after 48 h of incubation were subjected to qualitative screening for visualizing corresponding zones of hydrolysis of different isolates showing their potential to produce multiple carbohydrate, *i.e.*, laccase, cellulase and xylanase. 31 isolates showed cellulase activity, out of which maximum zone size was observed in six isolates, *i.e.*, R4 (29 mm), S7 (28 mm), K3 (28 mm), R4 (26 mm), SH5 (26 mm), S5 (24 mm) and SH2 (22 mm) and minimum zone was observed by N7, *i.e.*, 4 mm. On the other hand, 31 isolates showed xylanase activity, out of which maximum zone size was observed in six isolates, *i.e.*, S7 (32 mm), K3 (30 mm), S5 (28 mm), SH5 (28 mm), SH5 (26 mm), R4 and A4 (24 mm). Minimum zone, *i.e.*, 12 mm was observed in N7 and R1 isolate (Table 2). Ligninolytic fungal isolates exhibited multiple hydrolytic enzyme activity of laccase, cellulase and xylanase and different strains were selected on the basis of highest zone of hydrolysis surrounding fungal colonies. Nayak *et al.* (2017) screened 23 lignocellulolytic fungi qualitatively by guaiacol plate assay on potato dextrose agar medium. Out of 23 fungal strains, five fungal isolates showed maximum qualitative laccase production. Vantamuri and Kaliwal (2015) isolated 150 fungal strains and only 8 isolates had the ability to produce laccase. Out of which, one isolate, *i.e.*, FS6 was presumed to be potent, another (FS1) showed medium potency and six isolates (FS2, FS3, FS4, FS5, FS7 and FS8) showed weak laccase producing ability. 56 fungal isolates were screened for

ligninolytic activities. Strains showing significant colored zone around the growing mycelia were particularly considered as potential ligninolytic enzyme producers and selected for quantitative determination of the laccase enzyme (Megersa *et al.,* 2017). Pingli *et al*. (2017) screened 84 fungal strains for their ability to produce cellulase, out of which, 12 isolates were identified and selected for quantitative screening. Ramanjaneyuleo *et al.* (2015) studied xylanase activity in the form of zone of hydrolysis in the range of 20 mm to 90 mm and the isolates with greater than 50 mm of hydrolytic zone were further assessed for the production of xylanase enzyme.

Table 1: Qualitative Screening of fungal isolates isolated from rotten wood samples for hydrolytic enzyme production

Sr. No.	Isolate Name	Laccase enzyme		Cellulase enzyme		Xylanase enzyme	
		Activity	Zone size (mm)	Activity	Zone size (mm)	Activity	Zone size (mm)
1	R1	+ve	9.00	-ve	0.00	+ve	12.00
2	R2	-ve	0.00	+ve	28.00	+ve	0.00
3	R3	-ve	0.00	+ve	22.00	+ve	10.00
4	R4	+ve	19.00	+ve	20.00	+ve	28.00
5	R5	+ve	6.00	-ve	0.00	-ve	0.00
6	R6	+ve	4.00	+ve	28.00	+ve	0.00
7	R7	+ve	6.00	-ve	0.00	-ve	0.00
8	SH1	+ve	2.00	-ve	0.00	+ve	18.00
9	SH2	+ve	16.00	+ve	22.00	+ve	19.00
10	SH3	-ve	0.00	-ve	0.00	-ve	0.00
11	SH4	-ve	0.00	+ve	20.00	+ve	13.00
12	SH5	+ve	10.00	+ve	24.00	+ve	24.00
13	SH6	-ve	0.00	-ve	0.00	-ve	0.00
14	C1	-ve	0.00	-ve	0.00	+ve	18.00
15	C2	-ve	0.00	-ve	0.00	-ve	0.00
16	C3	-ve	0.00	-ve	0.00	-ve	0.00
17	C4	-ve	0.00	+ve	12.00	-ve	0.00
18	C5	+ve	8.00	-ve	0.00	-ve	0.00
19	K1	-ve	0.00	+ve	18.00	+ve	20.00
20	K2	-ve	0.00	-ve	0.00	-ve	0.00
21	K3	+ve	18.00	+ve	28.00	+ve	23.00
22	K4	-ve	0.00	-ve	0.00	-ve	0.00
23	K5	+ve	6.00	-ve	0.00	-ve	0.00
24	K6	-ve	0.00	-ve	0.00	-ve	0.00
25	K7	-ve	0.00	-ve	0.00	-ve	0.00
26	S1	-ve	0.00	+ve	16.00	-ve	0.00
27	S2	-ve	0.00	-ve	0.00	-ve	0.00
28	S3	+ve	2.00	+ve	12.00	-ve	0.00
29	S4	-ve	0.00	+ve	14.00	+ve	18.00
30	S5	+ve	15.00	+ve	26.00	+ve	28.00
31	S6	-ve	0.00	-ve	0.00	-ve	0.00
32	S7	+ve	19.00	+ve	27.00	+ve	32.00
33	Ch	+ve	4.00	-ve	0.00	-ve	0.00
34	Ka	-ve	0.00	+ve	14.00	+ve	16.00
35	N1	+ve	9.00	+ve	10.00	+ve	24.00
36	N2	-ve	0.00	+ve	12.00	+ve	20.00
37	N3	-ve	0.00	+ve	11.00	+ve	20.00
38	N4	+ve	4.00	+ve	18.00	+ve	22.00
39	N5(G)	-ve	0.00	+ve	13.00	+ve	20.00
40	N5(W)	+ve	6.00	+ve	16.00	+ve	24.00
41	N6	-ve	0.00	+ve	6.00	+ve	20.00
42	N7	-ve	0.00	+ve	4.00	+ve	12.00
43	G1(G)	-ve	0.00	+ve	8.00	+ve	18.00
44	G1(W)	-ve	0.00	+ve	6.00	+ve	20.00
45	G2	+ve	8.00	+ve	11.00	+ve	16.00
46	G3	-ve	0.00	+ve	18.00	+ve	20.00
47	G4	+ve	4.00	+ve	12.00	+ve	18.00
48	A1	+ve	5.00	+ve	20.00	+ve	16.00
49	A2	+ve	8.00	+ve	24.00	+ve	22.00
50	A3	+ve	2.00	+ve	20.00	+ve	16.00
51	A4	+ve	10.00	+ve	20.00	+ve	24.00
52	A5	+ve	8.00	+ve	22.00	+ve	20.00

The fungal isolates were grown on their respective agar plates containing indicator at 28 ± 2°C
(+)=Positive for lignolytic potential, (-)=Negative for lignolytic potential.

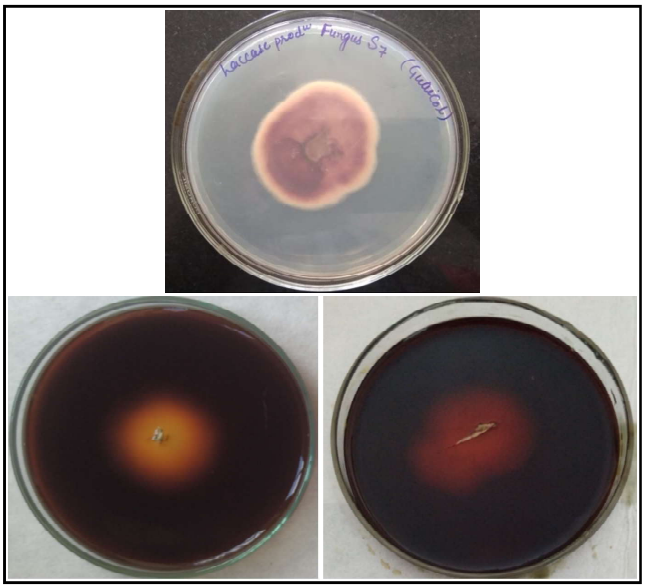

Figure 3: Qualitative screening for (a) laccase production (b) cellulase

Table 2: Quantitative screening of fungal isolates for hyperlaccase production

Sr. No.	Isolate name	Protein (mg/ml)	Laccase enzyme	
			Enzyme activity*(IU)	Specific activity**(IU/mg)
1	R1	0.64	0.02	0.03
2	R5	0.77	0.16	0.21
3	R6	0.78	0.01	0.02
4	R4	0.74	0.98	1.32
5	SH1	0.63	0.01	0.02
6	SH2	0.88	0.72	0.82
7	C5	0.57	0.01	0.02
8	K3	0.52	0.18	0.35
9	K5	0.58	0.10	0.18
10	S3	0.61	0.14	0.02
11	S5	0.75	0.90	1.19
12	S7	0.79	1.22	1.53
13	CH1	0.75	0.01	0.01
14	N1	0.82	0.02	0.03
15	N4	1.07	0.02	0.02
16	N5(W)	0.49	0.02	0.46
17	G2	0.49	0.01	0.03
18	A1	0.92	0.01	0.01
19	A2	0.66	0.00	0.07
20	A3	1.14	0.02	0.01
21	A4	1.25	0.01	0.00
22	A5	1.02	0.02	0.02

*Enzyme activity (IU): µ moles of reducing sugar released/min/ml of enzyme
**Specific activity: Enzyme activity/mg of protein.

4.2 Quantitative screening of fungal isolates for laccase, cellulase and xylanase production under submerged fermentation

The screening of hyperligninolytic fungi was exhibited in Table 2. Total 22 fungal isolates were screened for hyperlaccase production. Out of 22 isolates, four isolates showed maximum laccase activity in submerged fermentation (Figure 4). Four isolates, *viz.,* R4, SH2, S5 and S7 showed highest laccase activity, *i.e.*, 0.984 IU with 1.32 U/mg specific activity, 0.727 IU with 0.822 U/mg specific activity, 0.909 IU with 1.19 U/mg specific activity and 1.226 IU/ml with 1.53 U/mg specific activity respectively and minimum enzyme activity was shown by A2 isolate, *i.e.*, 0.009 IU/ml (0.073 U/mg specific activity) (Figure 5). Bisht *et al*. (2017) screened 15 fungal isolates quantitatively for laccase production and the highest laccase activity was shown by isolate ANF238, *i.e.*, 3.42 U/ml. An another study revealed that, twelve isolates were selected for enzyme production, out of which F6 isolate showed maximum laccase activity of 0.81 U/ml (Sakpetch *et al*., 2017).

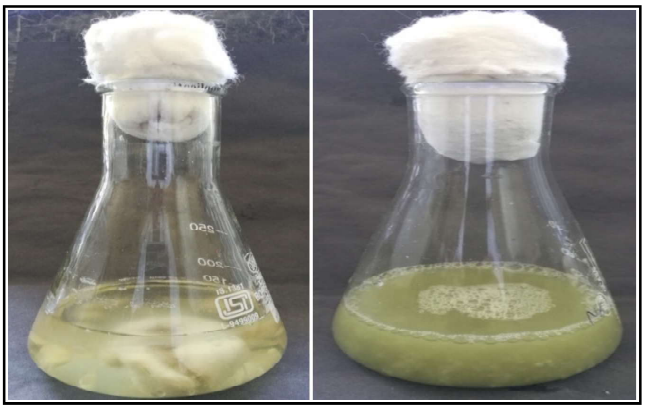

Figure 4: Hydrolytic enzyme production under submerged fermentation.

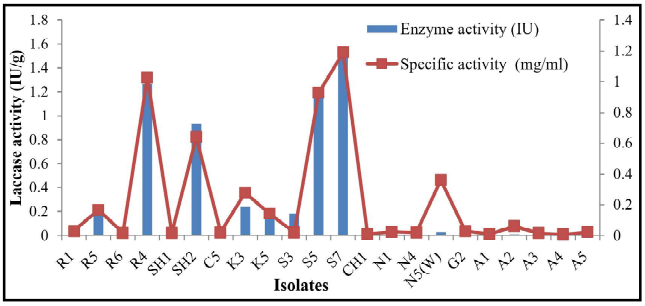

Figure 5: Quantitative screening of fungal isolates for hyperlaccase production.

Among 52 isolates, 13 fungal isolates were screened for hypercellulase production (Table 3). Out of which, four isolates showed the maximum cellulase activity. R4 showed total cellulase activity, *i.e.*, 0.737 IU/ml with specific activity 20.47 U/mg (CMCase 0.297, FPase 0.440 IU, β-glucosidase 0.007 IU), SH2 showed total cellulase activity, *i.e.*, 0.381 IU/ml with specific activity 3.22 U/mg, (CMCase 0.099 IU/ml, FPase 0.28 IU/ml, β-glucosidase 0.002 IU/ml), SH5 gave total cellulase activity, *i.e.*, 0.267 IU/ml with specific activity 2.32 U/mg, (endoglucanase 0.067 IU/ml, exoglucanase 0.18 IU/ml, β-glucosidase 0.026 IU/ml) and S5 showed total cellulase activity, *i.e.*, 0.793 IU with specific activity 5.74/ml (CMCase 0.438 IU/ml, FPase 0.350 IU/ml, β-glucosidase 0.005 IU) whereas minimum cellulase activity was observed by S4, *i.e.*, 0.099 IU/ml with 0.38 mg/ml specific activity (Figure 6). Sakpetch *et al*. (2017) screened three isolates, *i.e.*, F6, A2 and B15 for production of cellulase enzyme and observed CMCase activity of 0.61, 0.33 and 0.21 U/ml, respectively. The isolate NAB37 showed highest level of CMCase (0.948 ±0.01 U/ml) as well as FPase (0.125 ±0.005 U/ml) activity reported by Saini *et al*. (2017). Another study revealed that isolate HST16 showed maximum activity of FPase and CMCase, *i.e.*, 0.026 IU/ml and 0.006 IU/ml, respectively (Tanvi *et al*., 2018).

Table 3: Quantitative screening of fungal isolates for hypercellulase production under submerged fermentation

Sr. No.	Isolate name	Protein (mg/ml)	CMCase		FPase		β-glucosidase		Total Cellulase	
			Enzyme activity* (IU)	Specific activity** (IU/mg)	Enzyme activity (IU)	Specific activity (IU/mg)	Enzyme activity (IU)	Specific activity (IU/mg)	Enzyme activity (IU)	Specific activity (IU/mg)
1	R2	0.09	0.058	0.604	0.10	1.04	0.008	0.08	0.166	1.72
2	R3	0.042	0.072	1.71	0.14	3.50	0.008	0.20	0.218	5.19
3	R4	0.036	0.297	8.25	0.44	14.66	0.007	0.20	0.737	20.47
4	R6	0.029	0.077	2.65	0.15	7.50	0.003	0.11	0.230	7.93
5	SH2	0.118	0.099	0.90	0.28	2.54	0.002	0.02	0.381	3.22
6	SH4	0.031	0.054	1.80	0.15	5.00	0.007	0.23	0.211	6.80
7	SH5	0.115	0.067	0.60	0.18	1.63	0.026	0.22	0.267	2.32
8	C4	0.102	0.031	0.31	0.13	1.30	0.008	0.08	0.169	1.65
9	K1	0.110	0.091	0.82	0.24	2.18	0.002	0.02	0.333	3.00
10	S1	0.102	0.046	0.46	0.13	1.30	0.008	0.08	0.184	1.80
11	S3	0.107	0.048	0.48	0.11	1.10	0.010	0.098	0.168	1.57
12	S4	0.254	0.054	0.21	0.038	0.15	0.007	0.145	0.099	0.38
13	S5	0.138	0.438	3.17	0.35	2.69	0.005	0.036	0.793	5.74

*Enzyme activity (IU): Moles of reducing sugar released/min/ml of enzyme.
**Specific activity: Enzyme activity/mg of protein.

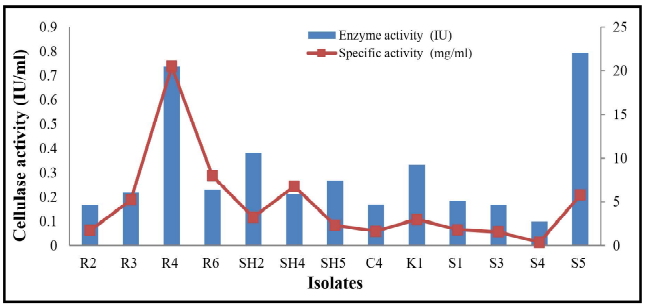

Figure 6: Quantitative screening of fungal isolates for hypercellulase production.

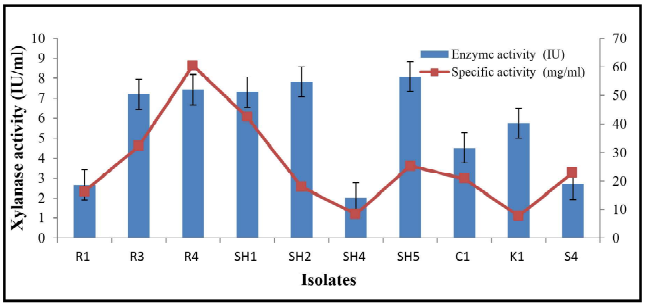

Figure 7: Quantitative screening of fungal isolates for hyperlaccase production.

10 fungal isolates were screened for hyperxylanase activity, out of which, five isolates, *viz*., R3, R4, S5, SH2 and SH5, showed highest xylanase activity, *i.e.*, 7.20, 7.44, 7.31, 7.83, 8.09 IU/ml with 32.24, 60.48, 42.40, 18.01, 25.53 U/mg specific activities, respectively (Table 4). Minimum xylanase activity was observed by SH4, *i.e.*, 2.03 IU/ml with 8.38 U/mg specific activity (Figure 7). *Aspergillus terrus* showed highest production of xylanase enzyme, *i.e.*, 3.4 U/ml (Olanbiwoninu and Odunfa, 2016). Bisht *et al.* (2017) selected twelve isolates for enzyme production in LBM containing decomposed rubber bark as a carbon source. Out of 12 isolates, 3 isolates, *viz*., F6, A2 and B15 showed the highest enzyme activity, *i.e.*, 0.61, 0.36 and 0.32 IU/ml, respectively.

Table 4: Quantitative screening of fungal isolates for hyperxylanase production under submerged fermentation

Sr. No.	Isolate name	Protein (mg/ml)	Xylanase enzyme	
			Enzyme activity*(IU)	Specific activity**(IU/mg)
1	R1	0.163	2.67	16.37
2	R3	0.223	7.20	32.24
3	R4	0.213	7.44	60.48
4	SH1	0.172	7.31	42.40
5	SH2	0.435	7.83	18.01
6	SH4	0.243	2.03	8.38
7	SH5	0.319	8.09	25.33
8	C1	0.217	4.53	20.85
9	K1	0.753	5.76	7.68
10	S4	0.118	2.70	22.95

*Enzyme activity (IU): Moles of reducing sugar released/min/ml of enzyme
**Specific activity: Enzyme activity/mg of protein.

5. Conclusion

In the present study, an attempt has been made for the isolation of most efficient ligninolytic, cellulolytic and xylanolytic fungi from forests of Himachal Pradesh, their screening to select hyperenzyme producing strains for industrially important enzyme production. In total, 52 fungi were isolated out of which 32 isolates were from Solan, 12 from Sirmour, 6 from Shimla and 1 each from Chamba and Kangra proceed in and around Monsoon season of Himachal Pradesh. Under submerged fermentation, these screened isolates produced laccase in the range of 0.01 to 1.22, cellulase 0.099 to 0.793 and Xylanase 2.03 to 8.09 U/ml. The maximum laccase, cellulase and xylanase activity was observed in R4, S5, SH5, and SH2 and found potential producer of hydrolytic enzymes which fulfill our aim of the study.

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Conflict of interest

The authors declare that there are no conflicts of interest in the course of conducting the research. All the authors had final decision regarding the manuscript and decision to submit the findings for publication.

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