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CELLULASE AND XYLANASE PRODUCTIONS FROM CELLULOLYTIC AS WELL AS XYLANOLYTIC MICROORGANISMS ISOLATED FROM FOREST SOILS OF NORTHERN HIMALAYAS

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ABSTRACT

Industrial attention towards cellulase and xylanase hyper production has consistently increased over the years, due to its potential use in several biotechnological applications such as use in clarification of juices, wine industries, poultry diets, animal feed, production of ethanol and application in biofuel. The present study was concerned with isolation and screening of cellulolytic and xylanolytic microorganisms from soil. 84 bacterial and 5 fungal isolates were isolated from soils of different districts of Himachal Pradesh (India). Out of 84 bacterial isolates 59 % were tentatively identified as *Bacillus* sp. and 41 % were as *cocci* sp. in which 62% were Gram +ve and 38% were Gram-ve. N_{13} bacterial isolate showed hypercellulase i.e. 2.138 IU and SL_8 showed hyperxylanase i.e. 61.54 IU after screening on the basis of their enzyme activity. The conversion of cellulosic mass to fermentable sugars through biocatalyst cellulase derived from cellulolytic microorganisms has been suggested as a feasible process and offers potential to reduce use of fossil fuels and reduce environmental pollution.

KEYWORDS: Isolation, soil, cellulase, xylanase.

1. INTRODUCTION

Interest in bioenergy has been sharply increasing in recent years due to the necessity of sustainable economies and clean environments.[1] Cellulose and hemicellulose are the most abundant biomasses on earth, and therefore have the greatest potential to resolve both the energetic and environmental demands of bioenergy. The production of ethanol and other biofuels from lignocellulosic biomass has recently received tremendous attention both in industry and in academic communities worldwide, and great progress has been made in the production and use of biofuels.[2] Lignocellulose is the major structural component of woody plants and non-woody plants such as grass and represents a major source of renewable organic matter. Lignocellulose consists of lignin, hemicellulose and cellulose.[3] Cellulose is composed of D-glucose monomers joined together by β- 1, 4-glycosidic bonds could be primarily converted into glucose.[4] Xylan, a major constituent of hemicelluloses, is composed of β 1, 4-linked xylopyranosyl residues which can be substituted with arabinosyl and methylglucuronyl side-chains.[5] Cellulases and xylanases have numerous applications and biotechnological potential for various industries including chemicals, fuel, food, brewery and wine, animal feed, textile and laundry, pulp and paper and agriculture. Cellulases are inducible enzymes which are synthesized by large number of microorganisms either cell-bound or extracellular during their growth on cellulosic materials. Both fungi and bacteria have been heavily exploited for their abilities to produce a wide variety of cellulases and xylanases.[6] Although a large number of microorganisms can degrade cellulose, only a few them produce significant quantities of free enzyme capable of completely hydrolyzing crystalline cellulose.[7] Therefore soil being rich habitat of potential and novel microorganisms has been chosen for isolation of cellulolytic and xylanolytic bacteria and fungi.

2. MATERIALS AND METHODS

2.1 collections of soil samples

Soil samples were collected from different sites of Himachal Pradesh Viz. Solan, Shimla and Kangra district and brought to the laboratory. To 100g of soil sample, 2 % of cellulose powder was added and water was spinkled in the petriplate and mixed thoroughly. The petriplates containing the above mixture were incubated at 35 ±2 °C for one week.

2.2 isolation of cellulolytic and xylanolytic microorganisms

One gram of enriched soil 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 Riviere's medium[8] in a petriplate and evenly spreaded and incubated at 35±2°C for 2-5 days and pure line cultures were obtained by streak plate method and bit method for bacteria and fungus respectively.

2.3 screening of cellulolytic and xylanolytic microorganisms

The microbial isolates i.e. isolated from soil were screened for the production of extracellular enzymes i.e. cellulase - CMCase, FPase and β-glucosidase and xylanase.

2.3.1 production of extracellular enzymes - cellulase and xylanase by microbial isolates

Each bacterial isolate was grown in 100 ml of nutrient broth at 35±2°C for 24 h to make 1.0 O.D. culture. Whereas for fungus, full plate growth of different fungal cultures were scratched and poured in 10 ml distilled water blank. The spore suspension thus formed contained 1×10^7 spores /ml. 5 ml of inoculum was added to each 45 ml of Riviere's broth containing 1% cellulose for cellulase and 1% xylan for xylanase in 250 ml of Erlenmeyer flasks and the flasks were incubated for 5 days at 35±2°C for bacteria and at 28±2°C for fungus at 120 rpm. After incubation, the culture contents were centrifuged at 10,000 rpm for 15 min (4°C). The supernatant was collected. The following quantitative tests were performed.

2.3.2 cellulase assay

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 50^0C for 1 h. After incubation and 3 ml of 3,5 –dinitrosalicylic acid (DNS) 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 50^0C 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.[9] 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 45^0C 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.[10]

2.3.3 xylanase Assay

To 0.5 ml of xylan solution, 0.3 ml citrate buffer was added and 0.2 ml of enzyme. The reaction mixture was incubated at 45°C for 10 min. After the incubation, 3 ml of DNSA reagent was added and the mixture was then heated on boiling water bath for 30 min. After cooling down at room temperature, absorbance of reaction mixture was read at 540 nm.[11]

2.3.4 Protein assay

Protein estimation was done by using Lowry's method.[12]

3. RESULTS AND DISCUSSION

3.1 Isolation of cellulolytic and xylanolytic bacteria from soil and their biochemical characteristics

In total, 84 bacteria isolated from pooled samples of soil in Riviere's medium having pH 6.8. Bacteria isolated from soil showed a great variation in their morphological characteristics i.e. colour, texture and shape and the colour of the colonies also exhibited a variation amongst the different strains, they varied from cream, brown, white, pink, yellow to pale green. Gram staining of all these bacterial isolates differentiated them into gram +ve and gram-ve. Amongst these 84 isolates, 62 % were found to be gram +ve and the rest 38 % were found to be gram -ve. Among all isolates, 59 % were rod shaped while 41 % were spherical in shape. Different biochemical tests were performed such as catalase test, H_2S production test, urease test, citrate utilization test, fermentation of carbohydrates, hydrolysis of gelatin, casein test and indole test for the characterization bacterial isolates. Urea and indole test was found negative for all isolates while gelatin hydrolysis test was positive for all isolates. Out of 84 bacterial isolates, 62 % showed positive H_2S production test, 58 % for citrate utilization test, 67 % for MR, 34 % for VP test, 24 % showed positive test for fermentation of carbohydrates. 30 % isolate showed positive test for casein hydrolysis and for catalase test 73 % isolates was positive. Depending upon their morphological, physiological and biochemical characteristics, these bacteria was tentatively identified as *Bacillus* sp. and *Coccus* sp.

Different types of fungi isolated from soil with their morphological and cultural characteristics have been shown in Table 1. These showed different mycelial characteristics and spore morphology which includes the spore colour and its texture. The fungal isolate NF_1 had septate mycelium, blackish colour and rough, globular and tightly packed conidia and was identified as *Aspergillus niger*. NF_2 due to green colour and feathery mycelium was identified as *Trichoderma harzianum*. NF_3 was having purple coloured, sickle-shaped macroconidium and had been identified as *Fusarium oxysporum*. NF4 have non-septate, abundant cottony mycelium, blackish-brown sporangia and was identified as *Rhizopus* sp. Whereas NF_5 showed green and rough mycelium and was identified as *Penicillium* sp. Nair *et al*., isolated seventy fungal strains from soil collected from different parts of Southern Kerala in India. Most of them were screened and identified as *Aspergillus niger, A. flavis, A. fumigatus* and *A. ochraceous.*[13]

3.2 Cellulase and xylanase productions from hypercellulolytic and hyperxylanolytic bacteria from soil

The production of extracellular enzymes i.e. cellulase and xylanase from different bacterial isolates were depicted in Table 2. Among them, N_{13} showed the highest total cellulase activity of 2.138 IU (CMCase 1.577 IU, FPase 0.088 IU and 0.473 IU β-glucosidase) followed by NS_1 showing enzyme activity of 1.689 IU (CMCase 1.200 IU, FPase 0.068 IU and β-glucosidase 0.421 IU) whereas N_9 showed the lowest cellulase activity i.e. 0.018 IU. Maximum xylanase activity was exhibited by SL_8- 61.54 IU followed by Kd_1 – 31.74 IU and the least xylanase activity was noted in SL_7 – 2.930 IU. Table 3 depicted the screening of hypercellulolytic and hyperxylanolytic fungi from soil. It was found that *Rhizopus spp*.NF4 was the highest cellulase producer (0.623 IU) i.e. CMCase (0.304), FPase (0.154 IU) and β-glucosidase (0.165 IU) followed by *Aspergillus niger* NF1 (0.574 IU) i.e. FPase (0.072 IU), CMCase (0.333 IU) and β-glucosidase (0.169 IU), respectively. The lowest cellulase activity was shown by *Trichoderma harzianum* NF2 i.e. (0.470 IU) while other isolates produced cellulase between these two extremes. As for xylanase enzyme was concerned highest enzyme producers i.e. xylanase activity was also found maximum in *Aspergillus niger* NF1 i.e. (18.05 IU) followed by *Trichoderma harzianum* NF2 (17.20 IU) and the least enzyme activity was shown by NF3 i.e. 5.33 IU. Kar *et al*., isolated a xylanase producing fungi from soil and identified as *Trichoderma reesei* SAF3. Maximum growth of the organism was found at 48 h under submerged condition in xylan containing enriched medium, whereas highest enzyme production (4.75 IU) was recorded at 72 h. No detectable cellulase activity was noted during whole cultivation period.[14] Rana and Kaur isolated three fungal strains showed good enzyme production but out of the three *Penicillium* spp. had been showed maximum activity of 2.037 U/ml/min which is followed by *Aspergillus* spp. which gave an activity of 0.057 U/ml/min and *Stachybotrys* spp. showing 0.023 U/ml/min.[15] Our results indicate that soil is an attractive source for the study of cellulolytic and hemicellulolytic microorganisms and enzymes useful for cellulose degradation.

Table1. Isolation of cellulolytic and xylanolytic fungi from soil and their morphological and cultural characteristics

Fungal Isolate	Source	Mycelium	Colour	Texture	Tentative Identification
NF_1	Solan	Hyphae(long)	Black	Rough	*Aspergillus niger*
NF_2	Shimla	Hyphae (long)	Green	Feathery	*Trichoderma harzianum*
NF_3	Solan	Filamentous	Purple	Feathery	*Fusarium oxysporium*
NF_4	Solan	Short hyphae	Brown	Rough	*Rhizopus spp.*
NF_5	Kangra	Short Hyphae	Green	Rough	*Penicillium* sp.

Table2. Cellulase and xylanase production from hypercellulolytic and hyperxylanolytic bacteria

S. No.	Isolate name	Protein (mg/ml)	CMCase activity(IU)		FPase activity(IU)		β-glucosidase(IU)		Cellulase (CMCase + FPase + β-glucosidase)		Protein (mg/ml)	Xylanase(IU)		Total enzyme (cellulase + xylanase)	Total protein (mg/ml)
			Enzyme activity	Specific activity	Enzyme activity	Specific activity	Enzyme activity	Specific activity	Enzyme activity	Specific activity		Enzyme activity	Specific activity		
1	N_1	1.50	0.388	0.258	0.122	0.081	0.026	0.017	0.536	0.357	1.09	19.98	18.33	20.51	2.59
2	N_2	1.83	0.014	0.007	0.111	0.060	0.526	0.287	0.651	0.355	1.23	17.04	13.85	17.69	3.06
3	N_3	1.52	0.021	0.013	0.044	0.028	0.368	0.242	0.433	0.284	1.08	22.38	20.72	22.81	2.60
4	N_4	1.89	0.148	0.078	0.066	0.034	0.052	0.027	0.266	0.140	1.09	22.30	20.45	22.56	2.98
5	N_5	1.83	0.740	0.404	0.126	0.068	0.637	0.348	1.503	0.821	1.00	15.45	15.45	16.95	2.83
6	N_6	1.24	0.444	0.358	0.124	0.100	0.000	0.079	0.568	0.537	1.34	19.71	14.70	20.37	2.58
7	N_7	1.62	0.555	0.342	0.111	0.068	0.210	0.129	0.876	0.540	1.46	17.31	11.85	18.18	3.08
8	N_8	1.76	0.004	0.002	0.028	0.015	0.131	0.074	0.163	0.092	1.80	11.45	6.36	11.61	3.56
9	N_9	1.40	0.002	0.001	0.016	0.011	0.000	0.053	0.018	0.066	1.67	16.51	9.88	15.60	3.07
10	N_{10}	1.26	0.003	0.002	0.055	0.043	0.052	0.041	0.110	0.087	1.54	17.84	11.58	17.95	2.80
11	N_{11}	2.56	0.005	0.001	0.002	0.0007	0.657	0.256	0.664	0.259	1.98	29.64	14.96	30.30	4.54
12	N_{12}	2.72	1.274	0.468	0.093	0.034	0.109	0.040	1.476	0.542	1.09	17.31	15.88	18.78	3.81
13	**N_{13}**	**3.16**	**1.577**	**0.499**	**0.088**	**0.027**	**0.473**	**0.149**	**2.138**	**0.676**	1.45	14.12	9.73	16.25	4.61
14	NS_1	2.92	1.200	0.410	0.068	0.023	0.421	0.144	1.689	0.578	1.57	11.72	7.46	13.40	4.49

15	NS_2	3.40	0.600	0.176	0.104	0.030	0.236	0.069	0.940	0.276	1.32	15.78	11.95	16.72	4.72
16	NS_3	2.48	0.177	0.071	0.078	0.031	0.105	0.042	0.360	0.145	1.89	12.52	6.62	14.02	4.45
17	NS_4	2.40	0.696	0.290	0.088	0.036	0.004	0.001	0.788	0.328	1.23	13.32	10.82	14.10	3.63
18	NS_5	3.28	0.962	0.293	0.120	0.036	0.000	0.029	1.082	0.329	1.09	16.78	15.39	17.86	4.37
19	NS_6	1.00	0.010	0.010	0.032	0.032	0.007	0.007	0.049	0.049	1.04	16.85	16.20	18.04	2.64
20	Kd_1	3.36	0.148	0.044	0.066	0.019	0.104	0.030	0.318	0.094	2.34	31.72	13.55	32.03	5.70
21	Kd_2	3.12	0.377	0.120	0.120	0.038	0.131	0.041	0.628	0.201	1.89	15.71	8.31	16.33	5.01
22	Kd_3	3.52	0.229	0.065	0.097	0.027	0.001	0.0002	0.327	0.092	1.90	22.11	11.63	22.43	5.42
23	Kd_4	3.24	0.012	0.003	0.055	0.016	0.210	0.064	0.277	0.085	1.98	14.98	7.56	15.25	5.22
24	Kd_5	4.32	0.348	0.080	0.068	0.015	0.215	0.049	0.631	0.146	1.53	12.12	7.92	12.75	5.85
25	Kd_6	2.90	0.296	0.102	0.111	0.038	0.184	0.063	0.591	0.203	0.67	5.461	8.14	4.05	3.57
26	Kd_7	1.85	0.237	0.128	0.115	0.062	0.078	0.042	0.430	0.232	0.98	7.593	7.74	8.02	2.83
27	Kd_8	2.56	0.170	0.066	0.053	0.020	0.010	0.003	0.233	0.091	1.04	14.65	14.08	14.88	3.60
28	Kd_9	2.48	0.177	0.071	0.078	0.031	0.105	0.042	0.360	0.145	1.07	5.328	4.97	5.68	3.55
29	Kd_{10}	2.96	0.181	0.061	0.095	0.032	0.021	0.007	0.297	0.100	0.89	6.194	6.95	6.48	3.85
30	Kd_{11}	1.22	0.006	0.004	0.004	0.003	0.012	0.009	0.022	0.018	0.56	3.263	5.82	3.28	1.78
31	Kd_{12}	1.24	0.007	0.003	0.014	0.011	0.010	0.008	0.031	0.025	2.10	25.57	12.17	25.60	3.34
32	Kd_{13}	1.26	0.004	0.003	0.036	0.028	0.008	0.006	0.048	0.038	2.14	23.97	11.20	24.01	3.40
33	Kd_{14}	1.00	0.008	0.008	0.043	0.043	0.010	0.010	0.061	0.061	0.67	5.72	8.53	5.78	1.67
34	Kd_{15}	1.71	0.036	0.021	0.067	0.039	0.009	0.005	0.112	0.065	1.07	16.51	15.42	16.62	2.78
35	Kd_{16}	1.62	0.048	0.029	0.059	0.036	0.008	0.004	0.115	0.070	1.15	6.128	5.32	6.23	2.77
36	KD1	1.52	0.040	0.026	0.031	0.020	0.026	0.017	0.097	0.063	1.04	12.78	12.28	12.87	2.56
37	KD2	0.70	0.012	0.017	0.008	0.011	0.151	0.215	0.171	0.244	1.04	12.38	11.90	12.55	1.74
38	KD3	1.14	0.044	0.038	0.066	0.057	0.006	0.005	0.116	0.101	1.23	17.78	14.45	17.89	2.37
39	KD4	1.24	0.003	0.002	0.087	0.070	0.009	0.007	0.099	0.079	1.11	16.58	14.93	16.67	2.35
40	KD5	1.52	0.004	0.002	0.012	0.007	0.010	0.006	0.026	0.017	0.97	7.19	7.41	7.21	2.49
41	KD6	1.00	0.010	0.010	0.023	0.023	0.008	0.008	0.041	0.041	2.24	35.96	15.84	36.00	1.24
42	KD7	1.83	0.033	0.018	0.090	0.049	0.009	0.004	0.132	0.072	0.99	8.92	9.01	9.05	2.82
43	KD8	1.36	0.023	0.016	0.015	0.011	0.010	0.007	0.048	0.035	1.30	11.85	9.11	11.89	2.66
44	Kp_1	1.00	0.010	0.010	0.032	0.032	0.007	0.007	0.049	0.049	1.20	12.89	10.74	12.93	2.20
45	Kp_2	1.00	0.004	0.004	0.027	0.027	0.006	0.006	0.037	0.037	0.69	9.87	14.30	9.90	1.69

46	Kp_3	1.26	0.036		0.028	0.042	0.033	0.010	0.007	0.088	0.069		1.06	12.90	12.16	12.98	2.32
47	Kp_4	1.56	0.009	0.005	0.011	0.007	0.012	0.007	0.023		0.014	2.89	23.00	7.95	23.02		4.45
48	KG1	1.40	0.008	0.005	0.042	0.030	0.014	0.010	0.056		0.040	1.45	12.00	8.27	12.05		2.80
49	KG2	1.22	0.003	0.002	0.028	0.022	0.009	0.007	0.040		0.032	1.87	9.080	4.85	9.12		3.09
50	KG3	1.72	0.001	0.005	0.028	0.016	0.010	0.005	0.038		0.022	1.23	13.89	11.29	13.92		2.95
51	KG4	0.82	0.005	0.006	0.064	0.078	0.289	0.352	0.358		0.436	1.67	11.98	7.17	12.33		2.49
52	KG5	0.90	0.007	0.007	0.042	0.046	0.009	0.010	0.051		0.056	1.45	23.78	16.40	23.83		2.35
53	S_1	1.80	1.096	0.608	0.148	0.082	0.395	0.219	1.639		0.910	1.45	7.650	5.27	9.28		3.25
54	S_2	0.94	0.466	0.495	0.082	0.087	0.078	0.082	0.626		0.665	2.00	4.789	2.39	5.40		2.94
55	S_3	1.60	0.004	0.002	0.046	0.028	0.158	0.098	0.208		0.130	2.34	15.87	6.78	16.07		3.94
56	S_4	1.50	0.037	0.024	0.053	0.035	0.009	0.006	0.099		0.066	1.00	16.79	16.79	16.85		2.50
57	S_5	1.34	0.016	0.011	0.084	0.062	0.012	0.008	0.112		0.083	1.90	10.98	5.77	11.09		3.24
58	S_6	2.07	0.037	0.017	0.066	0.031	0.010	0.004	0.113		0.054	0.967	23.09	24.05	23.20		3.03
59	S_7	1.76	0.002	0.001	0.053	0.030	1.080	0.613	1.135		0.644	0.876	21.08	24.22	22.21		2.63
60	S_8	1.72	0.045	0.026	0.057	0.033	0.710	0.412	0.812		0.472	1.02	12.08	11.84	12.89		2.74
61	S_9	1.83	0.017	0.009	0.060	0.032	0.026	0.014	0.103		0.056	1.11	10.09	9.09	10.19		2.94
62	S_{10}	1.66	0.013	0.007	0.053	0.031	0.922	0.555	0.988		0.595	1.34	23.09	17.23	24.07		3.00
63	S_{11}	1.50	0.037	0.024	0.053	0.035	0.009	0.006	0.099		0.066	1.45	22.75	15.68	24.04		3.11
64	S_{12}	1.34	0.012	0.008	0.086	0.064	1.122	0.837	1.220		0.910	1.78	9.76	5.48	10.98		3.12
65	S_{13}	1.86	0.015	0.008	0.053	0.028	0.057	0.030	0.125		0.067	1.23	13.98	11.36	14.10		3.09
66	S_{14}	1.80	0.259	0.143	0.054	0.030	0.009	0.005	0.322		0.178	1.22	14.09	11.54	14.41		3.02
67	S_{15}	2.19	0.027	0.012	0.060	0.027	0.004	0.001	0.091		0.041	1.90	11.54	6.07	11.63		4.09
68	S_{16}	2.19	0.162	0.073	0.057	0.026	0.006	0.002	0.225		0.102	0.256	9.87	38.55	10.09		2.44
69	S_{17}	3.00	0.016		0.005	0.005	0.001	0.009	0.003	0.030	0.010	0.987	7.732	7.88	7.76		3.98
70	S_{18}	1.68	0.036		0.021	0.008	0.004	0.010	0.005	0.054	0.032	0.543	8.759	16.22	8.80		2.22
71	S_{19}	1.74	0.340		0.195	0.113	0.064	0.014	0.008	0.467	0.268	0.654	6.879	10.56	7.33		2.39
72	S_{20}	1.92	0.170		0.088	0.055	0.028	0.210	0.109	0.435	0.226	0.678	8.437	12.58	8.86		2.59
73	S_{21}	2.04	0.011		0.005	0.008	0.003	0.315	0.154	0.334	0.163	0.123	4.422	35.95	4.75		2.16
74	SL_1	2.52	0.281		0.111	0.071	0.028	0.184	0.073	0.536	0.212	0.234	2.959	12.64	3.48		2.75
75	SL_2	1.68	0.150		0.089	0.165	0.098	0.095	0.056	0.410	0.244	0.987	15.65	15.96	16.06		2.66
76	SL_3	1.89	0.222		0.117	0.231	0.122	0.012	0.006	0.465	0.246	1.00	18.98	18.98	19.44		2.89

77	SL$_4$	1.46	0.136	0.093	0.157	0.107	0.034	0.023	0.327	0.223	0.78	4.195	5.37	4.51	2.24
78	SL$_5$	2.19	0.254	0.115	0.240	0.109	0.023	0.010	0.517	0.236	0.67	4.395	6.55	4.90	2.86
79	SL$_6$	2.34	0.354	0.151	0.328	0.140	0.023	0.009	0.705	0.301	0.45	6.059	13.46	6.75	2.79
80	SL$_7$	2.13	0.360	0.169	0.216	0.101	0.047	0.022	0.623	0.292	0.56	2.930	5.23	3.55	2.69
81	**SL$_8$**	1.86	0.234	0.125	0.186	0.101	0.035	0.018	0.455	0.244	2.97	**61.54**	**20.72**	**61.99**	**4.83**
82	SL$_9$	2.10	0.136	0.064	0.145	0.069	0.095	0.045	0.376	0.179	1.98	22.64	11.43	23.01	4.08
83	SL$_{10}$	1.05	0.096	0.091	0.121	0.115	0.191	0.181	0.408	0.388	0.56	3.795	6.76	4.19	1.61
84	SL$_{11}$	1.92	0.384	0.200	0.213	0.110	0.022	0.011	0.619	0.322	0.57	5.694	9.98	6.30	2.49

Enzyme activity (IU): µmoles of reducing sugars released/min/ml of enzyme.

Specific activity: enzyme activity/mg of protein.

Table 3. Screening of fungal isolates for hypercellulase and xylanase production

S. no.	Isolate name	Protein (mg/ml)	CMCase activity(IU)		FPase activity(IU)		β-glucosidase(IU)		Cellulase (CMCase + FPase + β-glucosidase		Protein (mg/ml)	Xylanase(IU)		Total enzyme (cellulase + xylanase)	Total protein (mg/ml)
			Enzyme activity	Specific activity	Enzyme activity	Specific activity	Enzyme activity	Specific activity	Enzyme activity	Specific activity		Enzyme activity	Specific activity		
1.	NF$_1$	2.40	0.333	0.138	0.072	0.030	0.169	0.070	0.574	0.239	1.24	18.05	14.55	18.62	3.64
2.	NF$_2$	1.68	0.241	0.143	0.103	0.061	0.080	0.047	0.424	0.252	1.06	17.20	16.22	17.62	2.74
3.	NF$_3$	1.40	0.206	0.147	0.059	0.042	0.205	0.146	0.470	0.335	1.21	5.33	4.40	5.80	2.61
4.	NF$_4$	1.35	0.304	0.225	0.154	0.114	0.165	0.122	0.623	0.461	1.02	9.57	9.38	10.19	2.37
5.	NF$_5$	1.40	0.208	0.148	0.106	0.075	0.211	0.150	0.525	0.375	1.30	6.79	5.22	7.31	2.70

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5. REFERENCES

1. Sun JZ, Scharf M E. Exploring and integrating cellulolytic systems of insects to advance biofuel technology. Insect Sci, 2010; 17: 163–165.
2. Sanderson K. Us biofuels: A field in ferment, Nature, 2006; 444: 673–676.
3. Charitha MD, Kumar MS. Isolation and screening of lignocellulose hydrolytic saprophytic fungi from manure soil. Annals of Biological Research, 2012; 3(2): 1145-1152.
4. Chheda JN, Huber GW, Dumesic JA. Liquid-phase catalytic processing of biomass-derived oxygenated hydrocarbons to fuels and chemicals. Angrew Chemistry International Edition, 2007; 46: 7164-7183.
5. Kiddinamoorthy, Anceno AJ, Haki GD, Rakshit SK. Production, purification and characterization of Bacillus sp. GRE 7 xylanase and its application in eucalyptus kraft pulp biobleaching. World Journal of Microbiology and Biotechnology, 2007; 24: 605-612.
6. Miranda M, Kam TL, Wensheng Q. The prospects of cellulase producing bacteria for the bioconversion of lignocellulosic biomass. International Journal of Biological Sciences, 2009; 5(5): 500-516.
7. Koomnok C. Selection of cellulase producing thermophilic fungi. 31st Congress on Science and Technology of Thailand of Technology, Suranaree University: Nakhonratchasima Thailand, 2005.
8. Riviere J. Isolement of purification des bacteries cellulolytiques aerobies du sol Annales de L'institut Pasteur, 1961; 253-258.
9. Reese ET, Mandel M. Enzymatic hydrolysis of cellulose and its derivatives. In: Methods Carbohydrate Chemistry (ed Whistler R L) 3rd edn. Academic Press, London, 1963.
10. Berghem LER, Petterson LG. Mechanism of enzymatic cellulose degradation and purification of a cellulolytic enzyme from T.viride active on highly ordered cellulose. J Biochem, 1973; 37: 21-30.
11. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Ann Chem, 1959; 3: 426-428.

12. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. Journal of Biology and Chemistry, 1951; 193: 265-275.
13. Nair SG, Sindhu R, Shashidhar S. Fungal xylanase production under solid state and submerged fermentation conditions. African Journal of Microbiology, 2008; 2: 82-86.
14. Kar S, Mandal A, Mohapatra PKD, Mondal KC, Pati BR. Production of cellulase-free-xylanase by Trichoderma reesei SAF3. Brazilian Journal of Microbiology, 2006; 37: 462-464.
15. Rana S and Kaur M. Isolation and screening of cellulase producing microorganisms from degraded wood. International Journal of Pharmaceutical and Biological Sciences Fundamentals, 2012; 2(1): 10-15.