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Extraction and characterization of cellulase from forest and compost soil fungal isolates for the application of straw degradation

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***Abstract*---**Cellulases are complex hydrolytic enzymes working synergistically on the hydrolysis of cellulolytic materials for the production of simple sugars. These enzymes have tremendous environmental, industrial and agricultural applications including enhancement of the degradability of lignocellulosic materials for cattle's feed. The aim of this study was to isolate fungus and extract cellulose enzyme from forest and compost soil samples and examine the extent to which these enzymes enhance the degradability of finger millet and oat straw for making palatable cattle's feed. Accordingly, a total of 53 fungal isolates were isolated from forest and compost soil. On 1% CMC media resulted 40% were cellulolytic fungal species. Six were selected based on their clear zone. These isolates belonged to the genera Trichoderma, Aspergillus and Penicillium based on their morphological characteristics. Cultivation of fungal isolates for cellulase production using submerged fermentation and Solid-state fermentation was undertaken through make the variation on their levels of different growth conditions such as temperature, pH, minerals and substrates. The results showed that the highest

cellulase production i.e CMCase 83.12 ± 3.18 U/dL and FPase 44.51± 0.391FPU/dL were obtained from FSI6 in FMS supplemented SSF at 280C and a pH of 6. CSI3 showed a maximum CMCase stability (48.37 ± 0.27U/dL) at 600C and a pH of 5. Partial characterization; more than 50% residual activity of cellulase was obtained at temperatures 50 and 60°C incubated for 30min for all six isolates. The effect of metallic ions Mg2+, Co2+ and Ca2+ decreased the activity while Fe2+ enhanced the same as the concentration increased. Generally, the findings suggest that the compost soil isolates had high potential for cellulase production and its stability at higher temperature and at low pH. The percentage saccharification on finger millet (28.9%) was higher than that of oat straw (28.9%). Which indicates that, these cellulases as they can be produced in different ranges of growth factors as we mentioned above they showed different intensities on the improvement of degradation of cellulosic biomasses depends on the isolates type.

***Keywords*---**cellulase, fungi, extraction, degradability, soil.

Introduction

Cellulose is the world's most abundant naturally occurring renewable resource available to man and it's the most promising biomass for energy source to the current as well as to the future world (Czaja *et al.*, 2006). Agricultural crops are a cellulosic biomass sources too; this cellulosic biomass can serve as low cost feed stocks, for production of fuel (Parimala *et al.,* 2007). The enzymatic degradation of them forms reducing sugars, which can be used as a source of human food and animal feed as well as a substrate and a raw material for industrial fermentation (Mariamma, 1995). Applications of exogenous enzymes (cellulases and hemicellulases) in the feed industry have also received considerable attention because of their potential to improve feed value, to increase the digestibility and absorption of nutrients, to remove anti-nutritional factors from feeds (Arora *et al.*, 2011; Kholif *et al.,* 2015).

In tropical and subtropical farming systems, cereal crops are the major sources of energy for livestock's. However, it has low energy and low digestibility potential (Azzaz *et al.*, 2012). The complex and dynamic structures of cellulose can be a barrier against enzymatic digestion and limit their access to the digestible cell wall networks of plants (McDonald *et al.*, 2011, sampathkumar *et al.*, 2021 and Leta *et al.*, 2021). This study was conducted to screen forest and compost soil fungi, characterize their cellulase and apply to enhance the degradability of cellulolytic materials (straw) and the specific objective was to find out best cellulase producing fungi isolates from forest and compost soil as well as characterize or assaying the effect of physiochemical parameters then evaluates their degradation ability against natural cellulosic substrates (Finger millet and Oat straw).

Materials and Methods

Description of study area

The study was conducted at the National Agricultural Biotechnology Research Center (NABRC), Microbial Biotechnology Laboratory. NABRC is located 34 km to the west of the capital city of Ethiopia, Addis Ababa. The geographical location of NABRC is 9°4'N Latitude, 38°30'E Longitude at an Altitude of 2390 meters above sea level. The area has an average annual rainfall of 1100mm and maximum and minimum temperature of 22.2°C and 6.13°C, respectively (EIAR, 2013).

Research Design

The research was accomplished using CRD with two replications. The experiment included five factors as treatments, i.e. six isolates, three levels of temperature, three levels of pH, two types of carbon source, and two types of fermentation techniques (Gomez and Gomez, 1984).

Soil Samples Collection

Forest soil sample were brought from Menagesha Suba forest whereas compost soil samples were collected from Holeta Agricultural Research Center Horticulture department compost site using sterile polyethylene bag from a depth of 15-20cm and brought to National Agricultural Biotechnology Research Center (NABRC) laboratory.

Preparation of Growth Substrates

Oat straw (OS) and Finger Millet straw (FMS) were collected from Holeta Agricultural Research Center Livestock department forage site and from local farm land in Dangila district, respectively. These substrates were then ground from 1.0 up to 2mm (Shrivastava *et al.*, 2011).

Isolation and Screening of Cellulolytic Filamentous Fungi

Fungal isolates were isolated through spread plate technique on to sterile Malt Extract Agar (MEA) medium and incubated at 20-40°C for 7 days. About 53 Fungi isolates were isolated and sub-cultured on MEA to maintain the purity of the cultures through streaking techniques.

Screening for cellulolytic fungal isolates

The isolated fungal cultures were screened for their ability to produce cellulase enzyme following the protocol developed by Mandels (1974). Screening was done through detection and measurement of the cellulase activities of each fungal isolate on Mandels-Weber medium containing Mandels' mineral salt in grams per litter (g/L) of solution: Urea, 0.3; $(NH_4)_2SO_4$, 1.4, KH_2PO_4, 2.0; $CaCl_2$, 0.3; $MgSO_4$, 0.3; yeast extract, 0.25; peptone, 0.75; carboxymethyl cellulose (CMC), 10; and agar, 15 (Mandels, 1974). The plates were incubated at 28ºC for seven days. Then the plates were then examined for cellulolytic activity using Congo red test.

Congo red test

Cellulolytic fungal species were selected on the basis of the diameter of the hydrolysis zone in the plates. This were done through such operations primarily plates were flooded with 0.1% Congo red aqueous solution and shaken at 80 rpm for 30mins then poured off and destained with 1 M NaCl solution at 80 rpm for 20 mins (Khokhar *et al.*, 2012). Clear zones were observed around colonies and the diameters measured. Cellulase activity on CMC agar was then recorded as an Index of Relative Enzyme Activity (I_{CMC}) by calculating the ratio of clear zone diameter and colony diameter (Pečiulytė, 2007).

$$\text{Index of Relative Enzyme Activity } (I_{CMC}) = \frac{\text{Clear zone diameter}}{\text{Colony diameter}}$$

Identification of the Isolated Fungi

Identification of the isolated fungi was done through their macroscopic (obverse-reverse pigmentation on the plates) and microscopic (conidia, conidiophore and philaid shapes) appearances; and especially shape of their spore structure. Microscopic observation was done under the 40x objective (Domsch *et al.*, 1980). Spores, phialides and conidia were observed(Sivaramanan, 2014).

Preparation of Fungal Inoculum

Cells or mycelia of the fungal isolates were collected from the maintenance cultures (PDA slants) and inoculated into fresh PDA slants and the cultures were incubated at 28^{o}C for seven days. Then, about two milliliters of physiological saline solution was introduced into the slant culture and shaken for few minutes. From the resulting spore suspension, the OD was measured and adjusted through addition of sterile saline or incubation at 28^{o}C for 72 hrs. Finally, the OD were measured and resulted in 1 x 10^{7} colonies/ml was used as inoculum (Korish, 2003).

Pretreatment of Lignocellulosic Substrates

Pretreatment of the substrates was undertaken to enhancing the rate of hydrolysis and increasing the amount of cellulase. Oat straw (OS) and Finger Millet straw (FMS) were, therefore, pretreated using physical methods such as chopping, milling and steam explosion (100^{o}C for 15 mins) (Sharmila,2014; Dashtban *et al.,* 2009).

Production and Extraction of Cellulases
Cellulase production and extraction in SmF

The six-efficient cellulolytic fungal isolates were subjected for cellulase production done through a mixing of an amount of 200 ml of Mandels-Weber medium amended with 1% substrate (OS or FMS) in a separated 250 ml conical flasks

(CFs) under liquid state fermentation. The pH 5, 5.5 and 6 were sets too. The flasks were incubated at 20, 28 and 38 °C on a rotary shaker at 120 rpm for 5 days. After 5 days of incubation, the fermented biomass of the flasks were filtered using Whatman No.1 filter paper to separate culture filtrates after centrifuged using falcon in centrifuge of model Herareus MulltifugeX3R (Miller, 1959). Then the culture filtrate was centrifuged at 10,000 rpm for 15 mins. The crude enzyme was stored in sterilized test tube at 4^{O}C until used for cellulase assay (Linko *et al.*, 2012; Bagewadi *et al.*, 2015).

Cellulase production and extraction in SSF

Ten grams of substrate (OS or FMS) was added into separate 250 ml CFs and amended using Mandels-Weber medium adjusted to a moisture content of 65% and was optimized to the pH of 5, 5.5 or 6 and then subjected to incubation of 20, 28 and 38 °C for 12 days. After 12 days of incubation, 100ml of 0.5M extraction buffer (sodium acetate with a pH of 5) was added, shaken the CFs for five minutes. Then distributed in to falcon tubes and centrifuged at 10,000 rpm at 4^{o}C for 10 minutes using Herareus MulltifugeX3R centrifuge. The culture filtrate was centrifuged at 5,000 rpm for 15 min at 4^{o}C; and the crude enzyme stored in sterilized test tube at 4^{O}C until used for cellulase assay (Miller, 1959).

Glucose Standard Curve

A standard curve was used to find the unknown concentrations of reducing sugars in all samples. The optical density readings were plotted against the concentration of glucose. The amount of reducing sugar obtained from the hydrolysis of the cellulosic substrates was estimated from extraplotations made using the standard graph plotted for known glucose concentrations following the methods used by Liming and Xeliang (2004)

Enzyme Assay
Assay for CMCase activity

According to Agarwal *et al.* (2014), a reaction mixture containing 1 ml of 1 % carboxymethyl cellulose (CMC) in 1ml of 0.5M acetate buffer (pH 5.0) and 1ml of crude enzyme was prepared and incubated at a mixture at 50^{O}C for 30 minutes. A blank was also prepared in the same way and incubated without enzyme. Then, the activity of the enzyme was bans by 3ml of DNS-reagent. At the end of incubation, boiled the test tubes for 5min and then withdraw up to room temperature. Then 4ml of distilled water was added into the cooled reaction mixture and finally the OD was immediately measured at 540nm using a spectrophotometer model ELx800.

Assay for Fpase activity

According to Mandels *et al.* (1976), one milliliter of culture supernatant, 1ml of 0.2M acetate buffer (pH 5) and Whatman #1 filter paper strip was mixed in the test tube (Lee and Kim, 1999). Then vortexed the tubes and incubated at 50^{o}C for one hour and 3ml of DNS reagent was added. Then Tubes were boiled for 5 minutes and then in an ice-bath, followed by the addition of 6 ml distilled water

to each tube. Absorbance was noted at 540nm of spectrophotometer model ELx800. Cellulase activity was indicated in term of filter paper unit (FPU) per dL of undiluted crude enzyme.

Effect of the type of lignocellulosic on the production of cellulose

As cellulases are inducible enzyme (Koo, 2001), in this experiment, two types of inducin substrates (OS and FMS) were prepared in separate 250ml capacity CFs. Inoculation of the fungal isolates in to the media was done and then incubating it for 5 and 12 days (for SMF and SSF, respectively). The supernatants obtained after centrifugation were saved and used for cellulase assay.

Partial Characterization of Cellulase
Cellulase activity and stability on different level of temperature

The impact of temperature on cellulase activity was measured and incubated from 20 up to 90^{O}C with the intervals of 10°C. The reaction mixture consisted of 1ml of crude enzyme, 1ml of acetate buffer (pH 5.0) and 1ml of 1% CMC as a substrate. Then cellulase activity was measured as according to Ghosh (1987). Cellulase stability was evaluated through subjected the crude enzyme to temperatures of 20, 30, 40, 50, 60, 70, 80 & 90 ^{O}C for 24hrs in the incubator. Then after, the reaction mixtures were prepared as described above and their activities were measured as an indicator of enzyme stability.

Cellulase activity and stability on different level of pH

The impact of pH was evaluated from 4.5 up to 9.0 with the interval of 0.5. The reaction mixture consists of 1ml of crude enzyme and 1ml of 1% CMC as a substrate and the reaction was allowed to proceed at different pH conditions 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5 & 9.0. Then the mixture was incubated at 50^{O}C for 30mins for all pH conditions. Cellulase stability over different pH level was also evaluated after withdraw the mixtures of crude enzyme with buffer overnight at different pH and their activities were measured as an indicator of enzyme stability.

Cellulase activity and stability on different metallic ions

To determine the impact of metal ions, the following metal ions or salt solutions were used with final concentration of 10mM Na^{+} (NaCl), Ca^{2+} ($CaCl_2$), Mg^{2+} ($MgSO_4$), Fe^{2+} ($FeSO_4$) and Co^{2+} ($CoCl_2$) (Zeng *et al.*, 2016). The crude enzyme, buffer solution and metal ion solutions were incubated overnight in separate test tubes. The blanks were also incubated without the addition of metal ion solution.
Treatment Lignocellulosic Substrates with Crude enzyme

FMS and OS were oven dried at 70^{O}C for overnight. 500 mg of each of the dried agricultural residues (lignocellulosic substrates) was taken and subjected to pre-treatments as described by Shankar *et al.* (2014). FMS and OS were ground with a grinder (Coffee grinding machine) and sieved to give particles ranging from 1-2 mm. Then, 5 ml of distilled water was added to the ground lignocellulosic materials separately and the resulting suspension was subjected to autoclaving at

121°C (15 psi) for 15 minutes. The physically pre-treated suspensions were then subjected to crude enzyme treatment for 16hrs at 50°C. Percentage of saccharification was then calculated using the following equation (Kaniz and Manchur, 2015).

$$\text{Saccharification}\,\% = \frac{\text{Glucose released in mg/ml} * \text{TVH}}{\text{Cellulose content}} \times 100$$

Where, TVH = Total volume of hydrolysate

Data Analysis

Data were statistically analyzed using SAS Software version 9.2 and ANOVA and Tukey's multiple-range test was used to compare treatment means at $P < 0.001$.

Results and Discussion

Isolation and Screening of Cellulolytic Fungi

In this study, 53 fungal isolates were collected from forest and compost soil samples. Screening of cellulolytic fungal isolates was carried out using Congo red test (Figure 1). The result indicated that 21 of the total isolates were positive for cellulase production (Table 1).

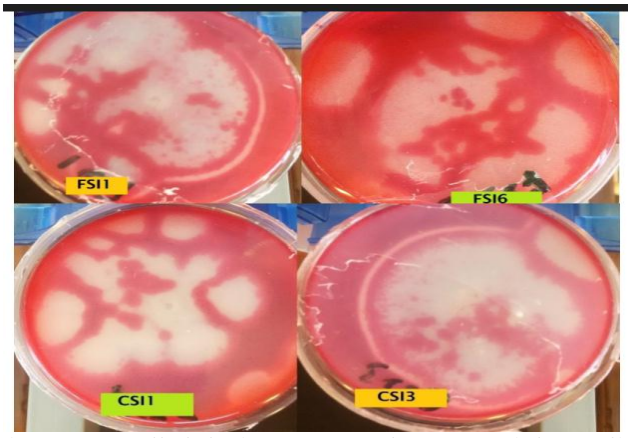

Figure 1. Cellulolytic zone on the CMC rich media

Clear zones were measured around colonies of the active fungal strains as indicated in Table-1 for analysis. Cellulase activity on carboxymethyl cellulose agar media was expressed as Pečiulytė (2007).

Table 1
Results of the screening test for cellulolytic fungal isolates

S.N.	Isolate's Code Number	Colony diameter (cm)	Diameter of clear zone (cm)	I_{CMC}
1	FSI1	6.7	8	1.19
2	FSI2	5.3	6	1.13
3	FSI5	7.06	6.5	0.92
4	FSI6	4.52	9.5	2.10
5	FSI10	4.6	4.3	0.93
6	FSI11	3.73	3.1	0.83
7	FSI17	1.01	2.9	0.73
8	FSI19	7	6.3	0.97
9	FSI25	10.1	9	0.89
10	FSI27	8.3	7.5	0.90
11	FSI32	6.12	4.9	0.82
12	CSI1	4.34	10	2.30
13	CSI2	3.87	5.5	1.42
14	CSI3	3.38	6.9	2.04
15	CSI7	4.34	4	0.92
16	CSI8	3.5	2	0.57
17	CSI9	3.8	1.8	0.47
18	CSI12	3.5	1.1	0.31
19	CSI13	3	3	1.00
20	CSI15	5	4	0.85
21	CSI17	6.3	1.4	0.22

The screening in this experiment allowed the selection of six potential isolates viz. FSI1, FSI2 and FSI6 from forest soil, and CSI1, CSI2 and CSI3 from compost soil.

Morphological Characterization of the Isolates

On the basis of morphological features, isolates FSI1, FSI2 and CSI2 were tentatively identified as members of the genus *Aspergillus* while isolates FSI6 & CSI1 and CSI3 were identified that they are genera of *Trichoderma* and *Penicillium*, respectively. The isolates FSI6 and CSI1 showed branched, smooth-walled and colorless hyphae in common with members of the genus *Trichoderma*. Moreover, the conidiophores of these isolates were bearing aerial hyphae. Their conidiophores are terminated with two or more phialides. In the genus *Aspergillus*, FSI1, FSI2 and CSI2 had their own morphs as indicated biseriate conodia, Globose to subglobose Phialides. On the other hand, isolate CSI3 had dark green obverse pigmentation color with yellowish reverse pigmentation; which

are all characteristics shared by members of the genus *Penicillium* which was similar to the study of Tiwari *et al.*, (2007).

Effect of Fermentation Techniques on Cellulase Production

Cellulose production seems to be greatly affected by the type of fermentation technique used. All isolates except FSI1 were a higher level of cellulase record in SSF than in SmF throughout the experiments (Table 2). In line with this result, higher efficiency of enzymatic production in SSF was reported previously by Sharma *et al.*, (2005). This may due to SSF provides the fungus with an environment closer to its natural habitat to give more cellulolytic enzymes (Silva *et al.*, 2005).

Table 2
Comparison between SmF Vs SSF

	Treatments					
parameters	T20	T21	T22	T23	T24	T25
SmF	33.71±0.69^{b}	33.44±0.33^{b}	33.77±1.29^{b}	30.47±0.24^{a}	32.99±1.23^{b}	35.5±0.069^{b}
SSF	41.28±1.44^{a}	45.01±0.59^{a}	47.29±1.65^{a}	37.06±0.05^{a}	44.19±1.02^{a}	55.9±0.25^{a}
SEM	0.99	1.23	2.11	0.22	0.89	0.07
SL	**	***	***	ns	**	***

a, b = means with different superscripts in a raw are significantly different. *** =($p<0.001$), **=($p<0.01$)*=($p<0.05$; SEM =standard error of the mean, SL=significance level, ns= non-significant,T20 = CSI1 production at(20°C+28°C+38°C), F21= CSI2 production at(20°C+28°C+38°C), F22= CSI3 production at(20°C+28°C+38°C), T23=FSI1 production at(20°C+28°C+38°C), F24= FSI2 production at(20°C+28°C+38°C) and F25= FSI6 production at(20°C+28°C+38°C).

Effect of the Type of Lignocellulosic Substrate on the Production of Cellulase

Finger millet straw was observed to induce the highest production of CMCase (44.93U/dL) and FPase (38.8FPU/dL) in submerged fermentation and CMCase (83.12U/dL) and Fpase (44.69FPU/dL) in solid state fermentation. On the other hand, Oat straw resulted in the production of 46.52U/dL and 41.57FPU/dL

CMCase and Fpase respectively in submerged fermentation and 82.98U/dL and 44.69 FPU/dL CMCase and Fpase respectively in solid state fermentation as shown in Figures 2 a& b.

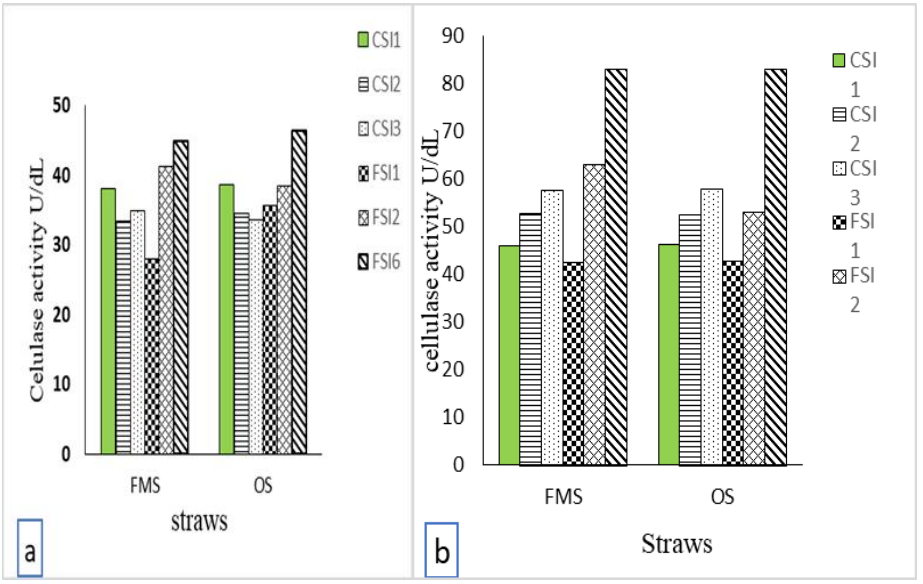

Figure 2A-B. The impact of substrate type on cellulase production in a) SmF and b) SSF

This indicates that FMS is more suitable substrate for cellulase production than OS. This might be due to the Cellulose Crystallinity difference between them. Which means finger millet (*Eleusine coracana*) had low crystallinity index than oat (*Avena sativa*) as described by Rambo and Ferreira, (2015).

Partial Characterization of Cellulase Produced by the Fungal Isolates
Activity of cellulase at varying temperatures

The crude enzyme and the substrate were incubated together from 20 to 90°C for 30mins and maximum cellulase activity were recorded for isolates CSI1, CSI2, FSI1, FSI2 and FSI6 at 50°C, whereas the highest activity was obtained for CSI3 at 60°C. However, in all cases the activity generally decreased significantly at temperatures below 40°C and above 70°C (Figure 3).

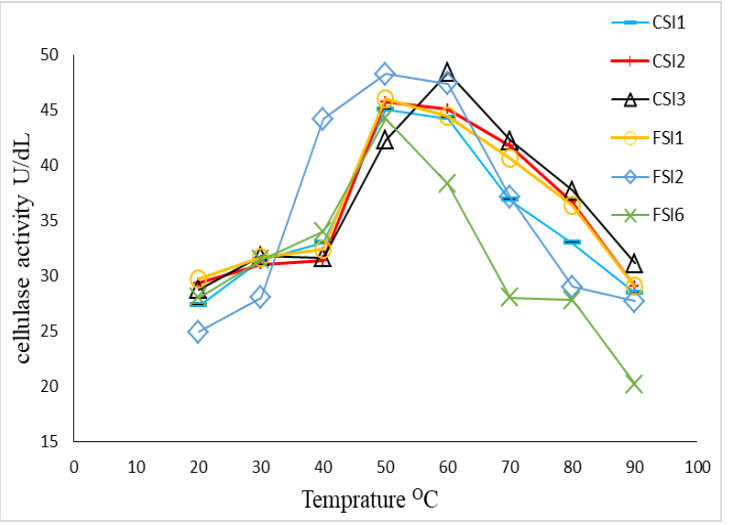

Figure 3. Impact of temperature on cellulase activity

Stability of cellulase at varying temperatures

The crude enzyme and the substrate were incubated together from 20°C– 90°C for 24hrs. Then Cellulase activity was determined under the standard enzyme (endo-1,4-β-D-glucanase) assay conditions. The control (activity at optimum temperature) was taken as having 100% activity. The stability of cellulase slightly decreased with increase in incubation temperature (table 3). The stability of cellulase enzyme could be due to the organisms' genetic adaptability (Al-Shehri and Mostafa, 2004).

Table 3
Impact of temperature on cellulase stability

Source of cellulase	Activity at optimum temperature	% Residual activity at different temperatures treatments						
		20°C	30°C	40°C	50°C	60°C	80°C	90°C
CFI 1	50°C	35.48	44.99	48.63	100	74.59	57.47	48.56
CFI 2	50°C	40.33	44.04	44.88	100	76.54	68.93	57.18
CFI 3	60°C	38.95	45.88	45.57	70.11	100	69.98	59.60
FSI 1	50°C	40.96	45.50	47.38	100	75.09	66.32	56.58
FSI 2	50°C	30.02	37.15	74.49	100	81.95	58.22	39.49
FSI 6	50°C	37.21	45.07	51.02	100	60.95	37.23	36.65

Activity of cellulase at different pH values

The pH optima for the activity of the cellulase determined through incubated the enzyme from 4.5 to 9.0 levels (with 0.5 intervals). The highest cellulase activity in CSI1 and FSI2 was shown at pH 5.5. In contrary CSI2, CSI3, FSI1, and FSI6's highest activity were recorded at pH 7.0, 5.0, 8.0 and 6.0 respectively (Figure 4). The results were in line with cellulase produced from *A. niger* and *Trichoderma species* at wide range of pH and maximum activity at pH 9.0 (Gautam *et al.*, 2011; Salwa *et al.,* 2012).

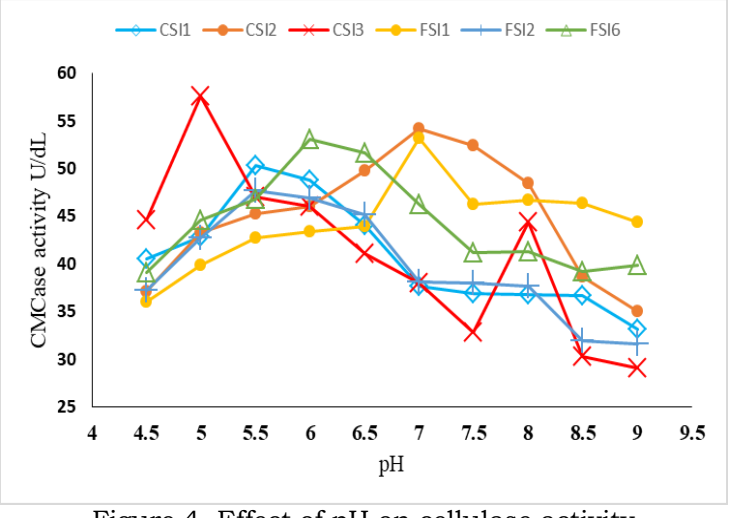

Figure 4. Effect of pH on cellulase activity

Stability of cellulase at different pH values

The impact of pH on the enzyme stability was determined by pre-exposing the enzyme from 4.5 to 9.0 levels for 24hrs. The control (activity at optimum pH) was taken as having 100% activity.

Table 4
Stability of cellulase at different pH values

Source of cellulase	Activity at optimum pH	% Residual activity for different pH treatments									
		4.5	5	5.5	6	6.5	7	7.5	8	8.5	9
CFI 1	5.5	40.5	42.8	100	48.7	44.0	37.6	36.9	36.8	36.7	33.1
CFI 2	7	37.1	43.3	45.3	46.0	49.8	100	52.4	48.4	38.7	35.0
CFI 3	5	44.6	100	47.0	46.1	41.1	38.0	32.8	44.4	30.3	29.1
FSI 1	7	36.0	39.8	42.7	43.4	43.9	100	46.3	46.7	46.3	44.3
FSI 2	5.5	37.2	42.7	100	46.9	45.1	38.1	37.9	37.7	31.9	31.5
FSI 6	6	39.1	44.5	46.8	100	51.6	46.3	41.1	41.3	39.2	39.9

Impacts of metallic ions on cellulase activity

The impact of metal ions on the work of cellulase of FSI1, FSI2, FSI6, CSI1, CSI2 and CSI3 indicated in figure 5. The presence of Fe^{2+} enhances activity of cellulase of CSI1 and CSI2. Whereas, Mg^{2+} and Co^{2+} were highly inhibitory effect on cellulase activity of FSI6 and CSI1 respectively. Among them Mg^{2+} was significantly inhibited the cellulase activity of all studied fungi. Na^{+} showed slightly and similar inhibitory effect to all isolates cellulase followed by Co^{2+}. The experiment was Contrary result to Wen *et al.* (2005) not all metallic ions were inhibitory effect to cellulase enzyme activity. As evidence even at concentration 10mM Fe^{2+} cannot inhibit the activity.

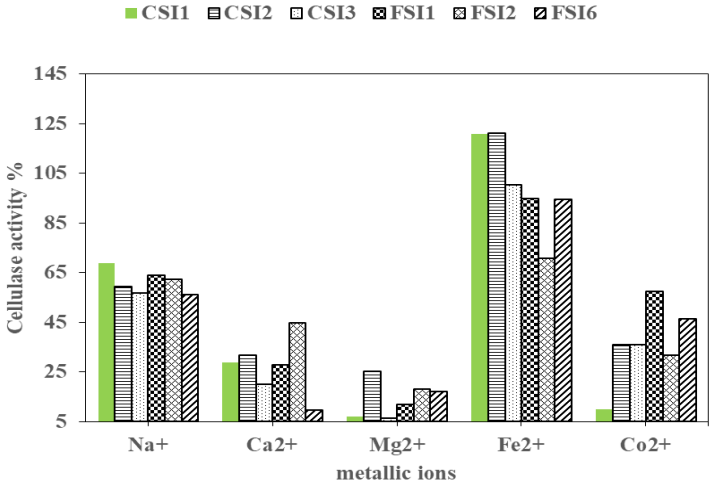

Figure 5. Impact of metallic ions on cellulase activity

Enzymatic Treatment of Cellulosic Substrates

FMS and OS were pre-treated and subjected to crude enzyme for estimating the saccharification of cellulosic substrates. Followed by physical treatment, enzymatic action was undertaken and the results presented in table 5. Maximum conversion of 22.76 % and 28.9 % were obtained from FMS and OS respectively by using crude enzyme of FSI6 fungal isolates.

Table 5
Percentage Conversion of cellulosic residues into reducing sugars

substrates	Cellulose content	Physical pre-treatment	% of saccharification after enzymatic treatment
OS	35%	Grinding and steaming	28.9%
FMS	29%	Grinding and steaming	29.2%

Percentage of saccharification after enzymatic treatment of straw was relatively higher for finger millet. This variation might be due to crystallinity index of the finger millet is higher as compared to oat straw.

Conclusion and Recommendations

Conclusion

The present study primarily shows that it is possible to isolate diversified cellulase producing fungi from forest and compost soil for multi-purpose forms. The study indicates that the forest and compost soil are good sources of filamentous fungi that produced the cellulolytic enzyme. These enzymes depolymerize the cellulose biomass and converted into smaller subunits viz. oligosaccharides, glucans, cellobiose, and glucose. Fermentations by using agro-residual substrates OS and FMS by *Aspergillus, Trichoderma* and *penicillium spps* proved possibility of the enzyme production in economical range. The major physiochemical properties of the cellulase were studied. The effect of metallic ions (Ca^{2+}, Na^{+}, Co^{2+}, Mg^{2+} and Fe^{2+}) in the activity of cellulase by fungal strain using SSF and SMF was determined. Among all the selected metallic ions the minimum enzyme activities were occurred in the presence of Mg^{2+}, Ca^{2+} and Co^{2+} ions and subsequently increase in their activity as the iron ion concentration increases. The isolates were produced maximum yield in SSF than SmF and the optimum temperature were recorded at 28^{O}C with in a pH of 6. Perhaps, in SSF isolates gave the maximum production of cellulase. Cellulolytic nature of the enzyme helps to convert cellulose (straws) into smaller sugar units and then for animal consumption and other energy sources. All isolates enzyme had highly active at 50^{o}C except CSI3 at 60^{O}C. They are also active in wide ranges of pH. Iron (II) ion is an inducer for the activity of cellulases. Among the six isolates FSI6 has a higher saccharification potential of lignocellulosic biomasses as compared to other isolates. Saccharification of the finger millet straw was resulted higher percentage than oat biomass in all six experimental fungi isolates. This difference might be due to crystallinity index of the biomasses and the inherent nature of the fungi.

Recommendations

These fungal isolates can be used for the production of physiochemically diversified cellulases using abundant cellulosic substrates; which implies that cellulase can be desirable in animal feed and various industries too.Additionally, further research should be conducted characterization of CSI1, CSI2, CSI3, FSI1, FSI2 and FSI6 at molecular level and cellulase enzymes shall be studied for industrial purpose such as textile industry, food industry, animal feed (with in vivo experiment) and bioethanol production.

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