Production and Optimization of Cellulolytic Enzymes from *Trichoderma* Isolates under Solid State Fermentation

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ABSTRACT

Agricultural wastes were used as sole carbon sources for the production of cellulase by *Trichoderma* isolates in solid state fermentation (SSF). The study aimed to identify and optimize the potential agricultural wastes for cellulase production by *Trichoderma* isolates under SSF. Carboxymethyl cellulose (CMC) and Congo Red were used to screen four isolates that had stronger ability to produce cellulase for further study. Cellulase production was assayed by measuring the amount of glucose liberated in μmol/ml/min by using the dinitrosalisalic acid (DNS) assay method at 540 nm. Maximum cellulase was recorded between 5-11 days incubation. The experiment found that wheat bran, Rice bran and wheat straw were comparatively better for high cellulase production whereas cotton seed, coffee pulp and barely bran relatively showed the least cellulase production in SSF. *Trichoderma* isolates, AUT1 produce the highest carboxymethyl cellulase on wheat straw (5.68 U/g), AUT5 on rice bran (8.15 U/g), AUT2 and AUT4 on wheat bran, their enzymatic activities were 4.92 U/g and 7.01 U/g, respectively. However, the isolate AUT5 gave the highest carboxymethyl cellulase (8.15 U/g) on rice bran whereas isolate AUT2 produce the least this enzyme on cotton seed (1.02 U/g). The maximum amount of cellulase was observed between 55% to 65% moisture contents. It is evident from the present study agricultural wastes were better carbon source for the production of cellulase by *Trichoderma* isolates under solid state fermentation.

Keywords: Agricultural wastes, cellulase, production and optimization, solid state fermentation, *Trichoderma* isolates.

INTRODUCTION

The agricultural wastes and industrial residues adversely affect human and animal health and also pollute the environment (Belewu and Babalola, 2009). The increasing expansion of agro-industrial activity has led to the accumulation of a large quantity of lignocellulosic residues all over the world (Albores *et al*., 2006). The main components of agricultural wastes are cellulose, hemicelluloses, pectin and lignin (Tahoun and Ibrahim, 1999). Cellulose is the most abundant biopolymer in nature and constitutes a large pool of carbon source for the microorganisms responsible for the decomposition of organic matter in soil (Shankar *et al.,* 2011 and Shin *et al*., 2000). Lignocelluloses residues are very crucial for the production of cellulolytic, hemicellulolytic and ligninolytic by solid state fermentation (Sanchez, 2009).

Cellulase is a family of O-glycoside enzymes that hyrolyse β,1-4 glycosidic bonds of native cellulose and other related cello-oligosaccharide derivatives (Shafque

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and Bajwa, 2009). They are extracellular, inducible, and hydrolyzes the conversion of cellulose into disaccharides and simpler sugars. The complete degradation of cellulose to glucose requires the action of at least three types of enzymes (Gow and Gadd, 1996): endo-β-1,4 -glucanase, exo-β-1,4-glucanase (cellobiohydrolase) and β-glucosidase (Zahri *et al*., 2005; Aneja, 2005; Miettinen-Oinonen, 2007). Cellulolytic enzymes have been applicable in many industries such as food industries, animal feed industries, brewing and wine making, agriculture biomass refining, pulp and paper industries, textile and laundry industries and ethanol production (Nakari & Pentilla, 1996). However, the cost of cellulase production and optimization profoundly influences the economics of the entire production process (Bhat, 2000). Currently, these enzymes account for approximately 20% of world enzyme market (Murphy and Horgan, 2005; Bhat and Hazlewood, 2003). Cellulases chiefly produced by microorganisms such as fungi, bacteria and actinomycetes. *Trichoderma* species is one of the best-known cellulolytic organisms (Chinedu and Okochi, 2003). Commercially speaking, the main production organisms are strains of *Trichoderma reesei* (Murphy and Horgan, 2005). *Trichoderma* are filamentous fungi belonging to a group of largely asexually reproducing soil fungi that includes a wide spectrum of microorganisms that range from very effective soil colonizers with high biodegradation potential to facultative plant symbionts that colonize the rhizosphere (Chet and Baker, 1981; Kubicek, 2004).

Although the use of cellulases in various industries has been increasing very rapidly, the cellulases used hitherto have mainly been crude mixtures causing unacceptable losses of fabric strength and weight. Furthermore, the un-optimized cellulase composition of commercial preparations and non-optimal dosage of the enzymes has led to low reproducibility of the processes. Furthermore, attempts to use these enzymes in the degradation of cellulosic wastes have not been successful for several reasons such as low enzymatic yields, low specific activities, heterogeneity of wastes and end product inhibition of the enzymes. Therefore, it is a prerequisite to design a set of optimal process operating conditions to achieve high enzyme production (Bhat, 2000).

Enzymes could be produced by submerged state fermentation (SmF) and solid-state fermentation (SSF). SSF is a fermentation process performed on a non-soluble material that acts both as physical support and source of nutrients in absences of free flowing liquid. Generally, SSF holds tremendous potential for the production of enzymes (Pandey, 1992).

The exploitation of agricultural by-products such as wheat straw and bran, rice bran, barley bran, peanut shell, and sawdust, by fermentation is a very interesting biotechnological approach for the production of cellulases due to their high cellulose content. This paper describes screening *Trichoderma* isolates for their cellulolytic activity and then testing the isolates for their ability to produce cellulases in a solid substrate fermentation process using agricultural wastes. The objective of the present study is designed to optimize the production and optimization of cellulase enzyme from *Trichoderma* isolates under solid state fermentation and to find out the potential agricultural wastes for cellulase production in the aforementioned condition.

MATERIALS AND METHODS

Source of Trichoderma *isolates*

Seven isolates of *Trichoderma* were obtained from Mycology Laboratory, Department of Microbial, Cellular and Molecular Biology, College of Natural Sciences, Addis Ababa University. Further studies have been done in Mycology Laboratory, Addis Ababa University. All

Trichoderma isolates used in this study were previously isolated from soil collected from Jimma zone. All the isolates were designated as AUT1 to AUT7 which stands for Addis Ababa University *Trichoderma* isolates.

Preparation of inoculants

Potatoes dextrose agar (PDA) (Oxiod)) was prepared and poured into the Petri dishes. The preserved *Trichoderma* isolates were transferred into PDA (pH 5.6) and incubated at 30^{o}C. Cultures were grown aerobically for 7 days and then after 7 days of cultivation on PDA, the isolates of *Trichoderma* were transferred into CM-cellulose containing media for screening, optimization and evaluation of the potential *Trichoderma* isolates for the production of cellulase enzyme.

Screening and evaluation of* Trichoderma *isolates for the production of cellulase

To screen the potential cellulase producing *Trichoderma* isolates, enrichment procedure was done in minimal CMC medium comprising ($NaNO_3$; 2 g, K_2HPO_4; 1 g, $MgSO_4$ $7H_2HO$; 0.5 g, KCl; 0.5 g, CMC; 5 g and peptone; 2 g with 15 g agar pH 5.5) (Aneja, 2005). After incubation for 3 to 5 days at 30°C, the plates were flooded with 0.1% Congo Red for 15 min. Again the plates were distained with 1M NaCl for 30 min. The *Trichoderma* isolates that showed a clearing zone around the colony were isolated as cellulase producing potential.

Solid state fermentation

Wheat straw (WS), wheat bran (WB), barley bran (BB), rice bran (RB), coffee pulp (CP) and cotton seed (CS) were used for the production of cellulase from *Trichoderma* isolates under SSF. To ten gram of wheat straw in 250ml Erlenmeyer flask capacity, 10ml stock mineral salt solution (K_2HPO_4 0.05 g, $MgSO_4.7H_2O$ 0.02 g, NH_4NO_3 0.1 g, $CaCl_2.2H_2O$ 0.01 g and 1ml of 1% $FeCl_3$) was added and sterilized for 15 min, at 121^{o}C (Ul-Huque, 1992). After 12 days of incubation at 30^{o}C, the culture was extracted by adding 100 ml distilled water, filtering (Whatman No.1 filter paper) and centrifuged at 10,000 rpm for 15 min. The filtrates were used to assay enzyme activity (Ghose, 1987). The same procedure was followed for coffee pulp, rice bran, cotton seed, wheat bran and barley bran.

Extraction of enzyme

The enzyme was extracted by adding 100 ml of distilled water to the fermented substrate in each flask. The flasks were rotated on a rotary shaker at 121 rpm for 1 hr at room temperature (25^{o}C) (Ul-Huque, 1992). The fermented broth was filtered by using Whatman No.1 filter paper and centrifuged at 10,000 rpm for 15 min to remove fungal biomass. The filtrates were used to assay cellulase enzymes activity (Ghose, 1987).

Effect of additives for cellulase enzyme production

WS, RB and WB were supplemented with different glucose, fructose, maltose, lactose and cellulose as carbon source at a concentration of 5% (w/w) and yeast extract, sodium nitrate, ammonium sulphate, peptone as nitrogen sources at a concentration of 1% (w/w) and the effect of these additives on the level of cellulase production were evaluated. The enzyme was extracted on the optimum time of growth and its activity was measured following the standard assay procedure (Ghose, 1987).

Optimization of moisture content of the solid media

The effect of moisture content on enzyme production was studied by varying the percentage of water in the medium from 45% to 80% (with an interval of 10%). All the liquid added into the flask and original moisture content of the WB (6.2%), RB (6.5%) and WS (6.1%) was taken into consideration in calculating the percentage of water in the medium. After 12 days of incubation the enzyme was extracted and assayed

following the standard assay procedure (Ghose, 1987).

Effect of time course for cellulase enzyme production

The optimum time course for cellulase production by *Trichoderma* isolates in SSF was determined by inoculating 10 g of WB, WS and RB with AUT1, AUT2, AUT4 and AUT5 fungal isolates and incubated at room temperature (25^{o}C) over a period of 14 days. An Erlenmeyer flask (250 ml) containing minimal medium using cellulose as sole carbon source, pH 5.5, were inoculated with two plugs (0.5 mm diameter) of *Trichoderma* isolates. Samples were withdrawn from inoculated flasks at 2 days intervals. The samples were extracted by adding 100 ml distilled water and then followed by filtration and centrifugation at 10,000 rpm for 15 min to remove fungal biomass were assayed to determine reducing sugars using DNS method (Ghose, 1987).

Cellulase activity assay

Carboxymethyl cellulase (CMCase) was assayed by using a modified method described by Mandel et al. (1976). The activity was determined by mixing 0.1 ml of enzyme solution with 0.9 ml of 0.5% CMC in 50 mM of sodium acetate buffer in a 14 ml of test tube, at pH 5, vortexed for 1 min, incubated for 30 min at 50^{o}C. The reaction was stopped by adding 2 ml of dinitrosalicyclic acid (DNS) reagent in the above mixture. The mixture was boiled for 15 min (95-100^{o}C) in a boiling water bath and cooled in cold water. The formation of reducing sugars was measured by DNS reagents (Ghose, 1987) spectrophotometerically (JENWAY, 6405UV/Vis. Spectrophotometer, UK) at 540 nm. One unit of enzyme activity in each case was defined as the amount of enzyme which released 1µm of glucose per minute (Ghose, 1987).

Statistical Analysis

All experiments and enzyme assays were performed in duplicates, statistically evaluated by excel and SPSS (version 16). Statistically significant differences between means were tested by analysis of variance and post hoc test by using ANOVA software. The differences between means were considered statistically significant when the test yielded a value $P < 0.05$. The results of the experiment were presented as mean ± SE (standard mean error) (Raghunathan, 2013).

RESULTS

Screening and evaluation of cellulase production from* Trichoderma *isolates

All *Trichoderma* isolates were subjected to CMC agar for isolation of potential cellulase producing isolates. Growth of each test isolate of *Trichoderma* was observed after 3 days of incubation at 30^{o}C. All isolates of *Trichoderma* were positive for CMCase. However, isolates were differing in their ability to produce cellulose degrading enzymes (Table 1). The isolate (AUT5) was showed the highest hallow zone on the CM-cellulose agar media (75 mm) whereas AUT7 showed the least clear zone diameter (9 mm). It is evident from Table 1 that AUT1 (32 mm), AUT2 (30 mm), AUT4 (54 mm) and AUT5 (75 mm) were the most efficient isolates selected for further studies according to their high clear zone diameter on CMC agar. Moreover, this experiment was confirmed again by DNS method (Table 1). From qualitatively assay of AUT5 produced 0.33U/ml cellulase enzyme. It produced small amount of enzyme activity (0.33 U/ml) when compared to other isolates. Similarly, isolates AUT1 and AUT2 were showed the highest enzymatic activity, 0.4 U/ml and 0.41 U/ml, respectively. The isolates AUT3, AUT6 and AUT7 were produced very small amount of cellulolytic activity, 0.12, 0.11 and 0.14 U/ml respectively.

Table 1. Screening of potential cellulase producing ability of *Trichoderma* isolates on cellulose media

Isolates	Clear zone (mm) Mean	Enzyme activity (U/ml)
AUT1	32	0.4
AUT2	30	0.41
AUT3	15	0.12
AUT4	54	0.37
AUT5	75	0.33
AUT6	10	0.11
AUT7	9	0.14

Solid state fermentation for cellulase production

Different agricultural wastes were employed for the production of cellulase from *Trichoderma* isolates. The experiment found that WS, WB and RB were showed maximum cellulase production after 12 days of incubation at 30^{o}C whereas CS, BB and CP were showed the least amount of cellulase production by *Trichoderma* isolates (Table 2). The maximum cellulase production was recorded by AUT1 (5.68±0.06 U/g) on WS, AUT2 (4.92±0.16 U/g) and AUT4 (7.01±0.055 U/g) on WB and AUT5 (8.15±0.065 U/g) on RB. The minimum cellulase production was observed by AUT2 (1.05±0.08 U/g) on CS and by AUT5 (1.26±0.02 U/g) on WS. Therefore, optimization and other experiments were done only on four fungal isolates and four solid wastes that showed maximum cellulase activity. The combination of fungal isolates and substrates were AUT2 (WB), AUT4 (WB), AUT5 (RB) and AUT1 (WS) (Table 3 and 4, Fig. 1).

As indicated in Table 1. (a and b) the production of cellulase from *Trichoderma* isolates were significantly affected by substrates. AUT1, AUT2, AUT4 and AUT5 showed a significant difference for cellulase production on WS, RB, CS and BB whereas the isolates showed no significant difference on WB and CP. AUT1 produced large amount of cellulase on WS, this is significantly different from the cellulase obtained from CS and CP. However, there was no significant difference among WB, RB, and BB. Isolate AUT2 was showed maximum amount of cellulase on WB followed by BB and RB. No significant differences observed among WB, BB, RB, CP and WS; WS and CS but they were significant differed with CS, except WS. Isolate AUT4 produced large amount of cellulase on WB followed by WS and RB. No significant differences were observed among the substrates except BB, and BB was not significantly different from CP. Isolate AUT5 produced maximum amount of cellulase on RB followed by CS and WB. There were no significant differences among RB, CS and WB; BB and WS; CP, CS and WB but significant difference were observe among RB, BB, CP and WS.

Table 2. Evaluation of different solid substrates for the production of cellulase by *Trichoderma* isolates

a. Comparison among isolates on the same substrates/comparison across the raw

Isolates/ substrates	Enzyme Activity U/g			
	AUT1	AUT2	AUT4	AUT5
WB	4.98± 0.17^{a}	4.92±0.16^{a}	7.01±0.055^{a}	5.99±0.055^{a}
WS	5.68±0.06^{a}	2.95±0.065^{b}	6.62±0.105^{a}	1.26±0.02^{b}
RB	5.32±0.17^{a}	4.08±0.065^{b}	5.95±0.1^{a}	8.15±0.065^{b}
CS	2.43±0.065^{b}	1.05±0.08^{b}	5.81±0.015^{s}	6.26±0.105^{a}

Isolates/ substrates	Enzyme Activity U/g			
	AUT1	AUT2	AUT4	AUT5
CP	2.13±0.04 [b]	3.72±0.06[b]	4. 68±0.04[b]	3.81±0.055[b]
BB	3.49±0.055[ac]	4.42±0.045[a]	2.80±0.03[ac]	1.66±0.075[c]

b. Comparison among substrates by inoculating the same fungi/comparison across the column

Isolates	Enzyme Activity U/g			
	AUT1	AUT2	AUT4	AUT5
WB	4.98± 0.17[ac]	4.92±0.16[a]	7.01±0.055[a]	5.99±0.055[ad]
WS	5.68±0.06[a]	2.95±0.065[ac]	6.62±0.105[a]	1.26±0.02[bc]
RB	5.32±0.17[a]	4.08±0.065[a]	5.95±0.1[a]	8.15±0.065[a]
CS	2.43±0.065[b]	1.05±0.08[bc]	5.81±0.015[s]	6.26±0.105[ad]
CP	2.13±0.04 [b]	3.72±0.06[a]	4. 68±0.04[ac]	3.81±0.055[dc]
BB	3.49±0.055[bc]	4.42±0.045[a]	2.80±0.03[bc]	1.66±0.075[bc]

NB: The same letter indicates no significant difference and different letter indicates there is significant difference. ±SE

The effect of additives on cellulase production under SSF

The effect of different additives was evaluated for the production of cellulase by *Trichoderma* isolates (Table 3). Comparatively cellulose (5.95±0.06 U/g) and lactose (5.59±0.07 U/g) showed the highest cellulase production by AUT4, whereas, glucose showed the least amount of cellulase production as compared to the control. Similarly, cellulase was not produced in the presence of fructose and maltose by isolates AUT1, AUT4 and AUT5. However, cellulase production by AUT2 was not significantly affected by the presence of carbon sources except glucose. Therefore, cellulose and lactose increased the activity of cellulase whereas glucose decreased the activity of cellulase as compared to the control.

Similarly, the production of cellulase by *Trichoderma* isolates were also significantly affected by different nitrogen sources (Table 3). The combination of wheat straw and sodium nitrate showed the highest cellulase production by isolate AUT1 (5.54±0.05 U/g) and significantly different from yeast extract and peptone but not Ammonium sulphate. On the other hand, the combination of rice bran and peptone showed the highest cellulase production by isolate AUT5 (8.955±0.135 U/g) and this is significantly different from yeast extract and sodium nitrate but not Ammonium sulphate; and peptone and wheat bran showed highest cellulase production by AUT2 and AUT4, their enzyme activity were 6.46±0.11, 6.795±1.465 U/g, respectively. No significant differences were observed among the nitrogen sources for isolates AUT2 and AUT4.

Table 3. The effect of carbon and nitrogen sources on the production of cellulase under solid state fermentation (SSF) by *Trichoderma* isolates.

Sources of substrates		Enzyme activity U/g of *Trichoderma* isolates			
		AUT1 + WS	**AUT2 + WB**	**AUT4 + WB**	**AUT5 + RB**
Carbon	Control	3.68±0.06^{ac}	4.92±0.16^{ab}	4.01±0.05^{ab}	3.15±0.06^{ab}
	Glucose	1.1±0.03^{a}	3.34±0.01^{a}	2.61±0.03^{a}	1.93±0.04^{a}
	fructose	None	6.42 ± 0.22^{b}	None	None
	Maltose	None	5.82 ±0.05^{ab}	None	None
	Lactose	4 ±0.05^{bc}	5.33 ±0.08^{ab}	5.59 ±0.07^{b}	5.57±0.09^{b}
	Cellulose	3.94 ±0.05^{bc}	4.99 ±0.06^{ab}	5.95 ±0.06^{b}	4.66 ±0.07^{b}
Nitrogen	Yeast extract	2.87±0.07^{ac}	5.00 ± 0.09^{a}	5.92±0.03^{a}	3.72±0.03^{a}
	Sodium nitrate	5.54±0.05^{b}	5.19±0.08^{a}	4.90±0.08^{a}	3.81±0.15^{a}
	Ammonium sulphate	4.36±0.03^{bc}	5.64±0.05^{a}	6.11±0.03^{a}	5.73±0.09^{ab}
	Peptone	2.645±0.05^{ac}	6.46±0.11^{a}	6.79±1.46^{a}	8.95±0.13^{b}

± SE

The effect of moisture levels

The cellulase produced by *Trichoderma* isolates were affected by the moisture content of the substrate as indicated in Fig. 1. The maximum amount of cellulase was recorded between 55% to 65% moisture contents. At 65% moisture content, isolates AUT5 and AUT1 showed maximum enzyme activity being 7.34 U/g and 5.37 U/g , respectively whereas isolates AUT2 and AUT4 showed maximum enzyme activity at 55% moisture content being 5.92 U/g and 4.65 U/g , respectively. Moisture contents < 45% or > 70% were not suitable for high cellulase production. On the other hand isolate AUT1 did not show any enzyme activities at the moisture contents 45% and 80%, isolates AUT4 and AUT5 did not show any enzymatic activities at the moisture level 80%. Isolates AUT2, AUT4 and AUT5 showed no significant differences between 55% and 65% moisture contents but significant difference was showed by isolate AUT1 between 55% and 65% moisture content.

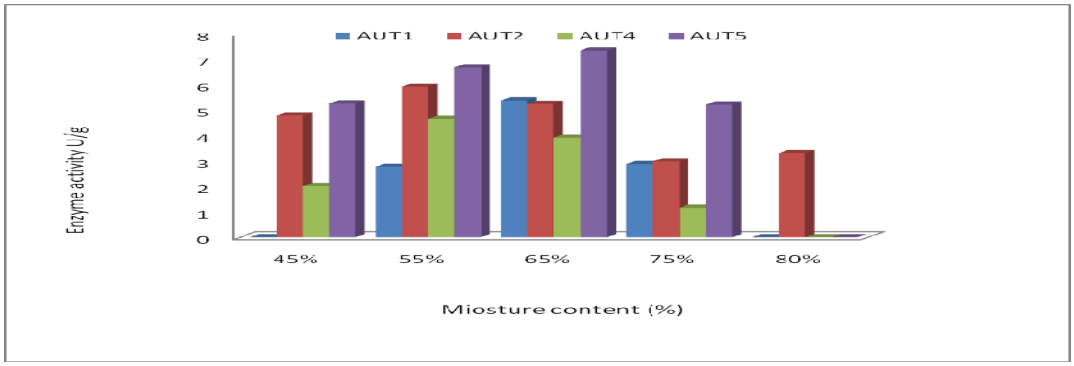

Figure 1. The effect of moisture level on the production cellulase from *Trichoderma* isolates.

The effect of time course on the production of cellulase under SSF

The time course of maximum cellulase production under SSF was varied depend up on the substrates and isolates employed (Table 4). The isolate AUT4 showed maximum enzyme production peak (9.96±0.03 U/g) after 5 days incubation on wheat bran at 30°C; isolate AUT2 showed maximum enzyme production (8.305±0.06 U/g) at 9 days incubation at 30°C; isolates AUT1 and AUT5 showed maximum cellulase enzyme production being 5.47±0.06 U/g and 7.675±0.03 U/g after 11 days incubation on WS and RB, respectively.

Table 4. Time course of cellulase enzyme production under solid state fermentation (SSF)

Isolates/days	Enzyme activity U/g					
	3	**5**	**7**	**9**	**11**	**13**
AUT1+WS	2.745±0.05^{a}	4.19±0.12^{ab}	4.31±0.13^{ab}	3.92±0.03^{ab}	5.47±0.06^{b}	3.73±0.07^{ab}
AUT2 +WB	2.52±0.06^{a}	3.73±0.1^{a}	6.68±0.04^{b}	8.31±0.07^{b}	7.36±0.06^{b}	6.99±0.1^{b}
AUT4 + WB	7.47±0.43^{a}	9.96±0.03^{a}	9.12±0.18^{a}	8.15±0.87^{a}	7.17±0.12^{a}	7.33±0.08^{a}
AUT5+RB	None	None	1.49±0.03^{b}	5.74±0.06^{a}	7.68±0.04^{a}	7.19±0.08^{a}

± SE

It is evident from table 4 cellulase production significantly affected by time course of enzyme production. Cellulase produced by isolate AUT1 at day 3 was significantly different with at day 11. Similarly, cellulase produced by isolate AUT2 at days 3 and 5 were significantly different with days 7, 9, 11and 13. Cellulase produced by AUT5 at day 7 was significantly different from days 9, 11 and 13. However, in the case of isolate AUT4 no significant differences were observed among the days.

DISCUSSION

Agricultural wastes (wheat bran, wheat straw, rice bran, barley bran, cotton seed and coffee pulp) were tested and fermentation parameters were optimized for production of cellulase by *Trichoderma* isolates in SSF medium of wheat bran, wheat straw, rice bran and results have been discussed as under:

The study revealed that all isolates used in this study were able to produce CMCase while cultivated on the agricultural wastes. Wheat straw, wheat bran and rice bran were comparatively better for cellulase production by AUT1, AUT2 and AUT4, and AUT5 isolates, respectively. However, the activity of cellulase was varied in agricultural wastes. The cellulase activity of AUT1 was (5.68±0.06 U/g) on WS, AUT5 was (8.15±0.065 U/g) on RB, AUT2 and AUT4 were (4.92±0.16 U/g and 7.01±0.055 U/g) on WB. There is a significant difference between AUT1 and AUT5; AUT2 and AUT5; and AUT2 and AUT4. However, there is no significant difference between AUT1 and AUT2; and AUT1 and AUT4. This may be due to the adsorption of enzymes and the formation of enzyme-substrate complexes are considered to be critical steps in the enzymatic hydrolysis of cellulose. Cellulose fibers contain both amorphous and crystalline regions. Crystalline regions are considered to be more difficult to be degraded than the amorphous regions (Abo-State *et al.,* 2010). Moreover, Balaraju *et al*., (2010) reported that WB and RB were better for cellulase production by *Oudemansiella radicata* under

SSF. It might be due to the fact that WB contains adequate amount of nutrients like proteins 1.32%, carbohydrates 69%, fats 1.9%, fibers 2.6%, ash 1.8% Ca 0.05%, Mg 0.17%, P 0.35%, K 0.45%, S 0.12%, various amino acids and porosity for oxygen supply. However, this work appeared to contradict with the previous results reported by Ravindran *et al.,* (2010) reported that cotton seed under SSF condition showed maximum enzyme production at high alkaline pH by *Chaetomium* spp.

For studying the effect of nitrogen source, supplementation of the fermentation medium with different nitrogen sources was carried out. A significant increase in the enzyme productivity by the tested isolates was recorded in the presence of 1% peptone and sodium nitrate as compared to the control. A combination of peptone with wheat bran and rice bran were comparatively better for cellulase production by AUT2 and AUT4, and AUT5, respectively. This result well agreement with the study conducted by Mrudula and Murugammal (2011), good cellulase production can be obtained with peptone as the organic nitrogen source in SSF. In the case of isolate AUT1, a combination of wheat straw and sodium nitrate was better for cellulase production.

The results indicated that when moisture level increased beyond a certain limit the enzyme activity started decreasing. This decline may be attributed to poor aeration in SSF and partial adsorption of enzyme to the substrate. Xia *et al*., (1999) studied the cellulase production by solid state fermentation on lignocellulosic waste and reported that water content of solid substrate is one of the key factors in cellulase production experiments. The present study nearly similar to the study conducted by Xia *et al*., (1999) SSF at a water contents ranging between 55-65% was found to be the most suitable for cellulase production.

The experiment found that maximum cellulase production obtained ranging between 5-11 days of incubation. Masbah *et al*. (1983) observed that *T. koningii* the cellulase activity reached to maximum after 16 days of incubation under SSF. Khare and Upadhyay (2011) have reported that the maximum production of cellulases by *T. viride* was observed after 6 days of incubation. Whereas, Sun *et al*. (2010) observed that the enzyme activity from apple pomace by *Trichoderma* spp was maximum at 120 h in SSF. This is probably due to the cease of the growth, the release of simpler sugar and proteases into the medium during the later growth phase (Ishaque and Kluepfel, 1980).

CONCLUSIONS

Trichoderma isolates produced high level of cellulase in solid state fermentation and agricultural wastes were better media for the production of cellulase by the isolates. The activities were comparable with some of the fungal strains reported so far. Wheat straw, wheat bran and rice bran were comparatively better substrate for cellulase production by isolates. The production of cellulase was affected by in the presence of different additives, carbon and nitrogen sources. Cellulose and lactose were the preferred carbon sources whereas peptone was the preferred nitrogen source for cellulase production under solid state fermentation.

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انتاج انزيم التحلل السليلوزي من فطريات ترايكوديرم ياستخدام طريقة SSF على المخلفات الزراعية

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ملخص

استخدمت المخلفات الزراعية كمصدر كربوني وحيد لإنتاج انزيمات النحلل السيليولوزي بوساطة فطر ترايكوديرم المعزول بطريقة تخمرات الحالة الصلبه. تهدف الدراسة الى التحديد الامثل لاستخدام المخلفات الزراعية لإنتاج انزيم التحلل السيليولوزي بطريقة تخمرات الحالة الصلبة. تم استخدام الكاربوكسي ميثل سليسلوز والكونكو رد للكشف عن اربع عزلات والتي اثبتت القدرة على انتاج السليولوز . تم تقدير انتاج انزيم السليسلوز عن طريق قياس كمية الجلوكوز المحرر (مايكروومول/مل/دقيقة) حيث استخدم طريقة دايناينتروسلسالك اسد على طول موجي 540 نانومتر، تم الحصول على اعلى تركيز للانزيم بعد 5-11 يوم من التخمر. اعطت عزلات AUT1 تفوقا ملوحظا لانتاج الكاربكوميثل سليلوز (5.6U/g)في قش القمح ونخالة الرز AUT5 (8.15 U/g) و AUT2 وAUT4 في نخالة القمح حيث كانت الفاعلية الانزيمية 4.92 U/g و 7.01 U/g للعزلات على التوالي. ومع ذلك اعطت عزلات AUT5 اعلى الكاربكوميثل سليلوز (8.15 U/g) في عزلات نخالة الرز بينما اعطت عزلات AUT2 اقل تركيز للانزيم في بذور القطن (1.02 U/g) لوحظ ان اعلى تركيز للانزيم كانت على رطوبة 55% الى 65% . اثبتت الدراسة ان المخلفات الزراعية كانت افضل مصدر كربوني لعزلات الفطر ترايكوديرم باستخدام طريقة SSF على المخلفات الزراعية.

الكلمات الدالة: المخلفات الزراعية، السيليولوزي، انتاج الانزيمات.

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