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Cellulase Production by *Aspergillus fumigatus* Grown on Mixed Substrate of Rice Straw and Wheat Bran

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Abstract: This study aimed to the production of some active cellulases from some local fungi. Twelve *Aspergillus* species were isolated from some local soil samples. On the basis of cellulolytic activity, *Aspergillus fumigatus* was selected and used for production of exoglucanase (EXG; EC: 3.2.1.91), endoglucanase (EG; EC: 3.2.1.4), CMCase, β-glucosidase (BG; EC: 3.2.1.21) and xylanase (Xy; EC: 3.2.1.8) by adopting SSF condition using mixed substrate of rice straw amended with wheat bran. Effect of Culture conditions including; incubation period, initial pH, incubation temperature, moisture level, different nitrogen sources, different lignocelluloses as carbon source and different ratios of mixed rice straw and wheat bran (9:1-1:9) were evaluated. The fungus expressed high enzyme production after 4.0 days incubation at moisture level 75%, initial pH 5-6, at 40°C in presence of $NaNO_3$ as an inorganic nitrogen source. The recorded activities were 14.71, 8.51, 0.93, 0.68 and 42.7 IU g^{-1} for CMCase, β-glucosidase, exoglucanase, endoglucanase and xylanase, respectively.

Key words: *Aspergillus fumigatus*, cellulases, β-glucosidase, xylanase, SSF, rice straw degradation

INTRODUCTION

Enzymatic hydrolysis of celluloses, the most abundant renewable resource on the earth, offers an attractive alternative for the generation of sugars which can serve as the raw material for the production of various products of commercial interest such as bio-ethanol (Carere *et al*., 2008; Dashtban *et al*., 2009). A number of approaches have been adopted, aiming towards reducing the cost of enzyme production, these have included the use of different lingo-cellulosic wastes including sawdust (Lo *et al*., 2005), corn cob (Betini *et al*., 2009), bagasse (Guerra *et al*., 2006), wheat straw (Norma and Guillermo, 2003; Yang *et al*., 2006), rice straw (Gaind and Nain, 2007) and wheat bran (Betini *et al*., 2009) as examples of low-cost materials which have been successfully used as substrates for cellulase fermentation by fungi mainly *Aspergillus* sp. and *Trichoderma* sp. Annually, about 573 million tons of rice straw residues are available in the world (FAO, 1997), It generally composed of 30-56% cellulose, 10-27% or more hemicelluloses, 3.0-30% lignin and 3.6-7.2% protein; while, rice straw also contain a quantity (9.0%) of silica. Cellulases which catalyze the hydrolysis of homo-polymer cellulose forming glucose are complex working in synergistic manner contain three types of enzymes; endoglucanase (EG; EC 3.2.1.4), exoglucanase (EXG; EC 3.2.1.91) and β-glucosidase (BG; EC 3.2.1.21) (Hong *et al*., 2001; Li *et al*., 2006). However, xylan as the main hemicellulose content in different lignocelluloses is hydrolyzed

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to xylose by two main enzymes, endo-xylanase (XYL; EC 3.2.1.8), β-xylosidase (EC 3.2.1.37) and other helping enzymes depending on hemicellulose structure and origin (Coughlan and Hazlewood, 1993).

Solid-State Fermentation (SSF) is an attractive process to produce cellulase and hemicellulase economically due to its lower capital investment and lower operating expenses (Xia and Cen, 1999). Another approach to reduce the cost of cellulase production is use of the cheap and easily available substrates. Several studies indicate that the carbon source used in cultivations is one of most important factors affecting the cost and yield of enzyme production. Therefore, for reducing the cost of enzyme production, selection of a cheap and easily available substrate appears to be essential (Beg *et al*., 2000; Senthilkumar *et al*., 2005).

Much work has been directed to find suitable cellulase-producing fungi through strain selection and development. Since, *T. reesei* produces very high titers of cellulase system with very little β-glucosidase, which is a practical disadvantage. As β-glucosidase plays an important role in the hydrolysis of cellulose by converting cellobiose to glucose. Otherwise, *Aspergillus* species are the most efficient producer of β-glucosidase compared with *Trichoderma* sp. (Wen *et al*., 2005). Cellulases production has been described for many *Aspergillus* species (Lockington *et al*., 2002; Kang *et al*., 2004; Wang *et al*., 2006; Gao *et al*., 2008). But only few reports available on the production of cellulases and hemicellulase from *Aspergillus funigatus* (Stewart *et al*., 1984; Ximenes *et al*., 1996; Grigorevski-Lima *et al*., 2009; Peixoto-Nogueira *et al*., 2009). The present research aimed to study the factors affecting cellulolytic enzymes production by a local *Aspergillus fumigatus* strain under SSF using mixed substrate of rice straw and wheat bran.

MATERIALS AND METHODS

Fungi

The fungi used in this study were isolated locally from some soil samples collected from Mansoura district in May 2007 according to the procedures adopted by Johnson *et al*. (1960). Fungal strains were subjected to full identification using the most recent sophisticated facilities; an Imaging analysis system using soft-imaging GbH software (analy SIS Pro ver.3.0) at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, EGYPT. The stock cultures were maintained routinely on PDA slants. The freshly grown slants at 28 °C subsequently used for further work or stored at 4°C. The slants were subcultured routinely every 4-5 weeks interval.

Substrates

Air-dried and milled Rice Straw (RS) and Wheat Bran (WB) were utilized as natural substrates for solid state fermentation.

Culture Media

The composition of mineral culture medium was (g L^{-1}): 3.0 g $NaNO_3$; 0.1 g KH_2PO_4; 0.5 g $MgSO_4.7H_2O$ and 0.5 g $KCl.2H_2O$. Rice straw and wheat bran in equal proportions (1:1) and controls (WS and WB) were dispersed. Medium was inoculated with 2.0 mL fungal spores suspended in sterile buffered basal solution (0.1 M acetate buffer; pH 5.5).

Growth Conditions

A set of flasks containing 1.0 g rice straw and wheat bran (1:1) with 3.0 mL moisture level (0.1 M acetate buffer; pH 5.5) were incubated at 30°C. Three flasks were harvested daily along

8.0 days intervals. The cultures were incubated at different temperatures: 25; 30; 35; 40; 45 and 50°C. Static cultivation was performed at various initial pH values: 4.5; 5.0; 5.5; 6.0; 7.0 and 8.0. The moisture content was varied by adding buffered solution (0.1 M acetate buffer; pH 5.5) to obtain a humidity range of 33-83% (w:w). The incubation time was limited to optimum fermentation period after which moulds substrates were extracted.

Effect of Different Agricultural Residues on Enzyme Production

Lignocelluloses of some local agricultural residues and wastes namely; rice straw, wheat straw; wheat bran, Alfa alfa, corncob and sawdust were used. In addition effect of mixed substrate containing rice straw and wheat bran in different proportions (1:9; to 9:1) were used.

Effect of Different Nitrogen Sources

Sodium nitrate in basal medium was replaced by nitrogen equivalent of different nitrogen sources including NH_4NO_3, NH_4Cl, $CaNO_3$, KNO_3, yeast extract, peptone, urea and L-asparagine. Culture medium without nitrogen was used as control.

Enzyme Extraction

Fresh moulds substrates in each culture flask were soaked in 50 mL buffered solution (0.1 M acetate buffer; pH 5.5); the mixtures were allowed to stand for 1.0 h at room temperature (24±2 °C). The extracts were obtained by filtering the mixtures through nylon cloth; then, centrifuged for 10 min at 5000 rpm to remove fungal and substrate residues (Shamala and Sreekantiah, 1986). The clarified filtrates were checked for exoglucanase, endoglucanase, β-glucosidase, CMCase and xylanase activity.

Enzyme Activity

Cellulases Assay

Cellulases activity was determined according to Mandels *et al.* (1976), using 1.0% amorphous cellulose (for endoglucanase), CMC (for CMCase) or Avicel PH 101; microcrystalline cellulose (for avicellase; exoglucanase) dissolved in 0.1 M acetate buffers (pH 5.5). The amount of reducing sugars released was determined by Nelson (1944) and Somogyi (1952) method against boiled enzyme as control with D-glucose as standard. The activity was expressed in IU g^{-1} substrate, defined as the amount of enzyme required to produce one μmol of glucose per min per ug protein for each used substrate.

Xylanase Assay

Xylanase was assayed by measuring the reducing sugars liberated from oat xylan (Sigma chemicals) dissolved in buffer solution (0.1 M acetate buffer; pH 5.5). The reaction mixture containing 0.1 mL of 1.0% (w/v) xylan solution and 0.1 mL enzyme was incubated at 40°C for 30 min. The reducing sugars were measured by Nelson (1944) and Somogyi (1952) method against boiled enzyme as control with D-xylose as standard. One unit of xylanase was defined as the amount of enzyme that released one μmol xylose from oat xylan per minute per ug protein under assay conditions. Xylanase production was expressed as IU g^{-1} substrate.

β-Glucosidase Assay

According to Christakopoulos *et al.* (1994); β-glucosidase activity was determined by measuring the increase in absorbance at 420 nm, after 30 min incubation of enzyme with

0.0136 M *p*-nitrophenyl-β-D-glucopyranoside (Sigma-Aldrich chem.) using standard curve of *p*-nitrophenol. The reaction was stopped by the addition of sodium carbonate solution. One unit of β-glucosidase is defined as the amount of enzyme which releases one μmole of p-nitrophenol per minute per μg protein under culture conditions. The unit of β-glucosidase was expressed as IU g^{-1} substrate.

Protein Determination

Protein was determined according to Bradford (1976) method by measuring optical density of developed color at 595 nm using Spectro UV-VIS RS spectrophotometer (Serial number: UV-VIS 0478; Labomed Inc. USA). The ug of protein was estimated using ug standard of bovine serum albumin (BSA).

RESULTS

Results in Table 1 showed that *Aspergillus fumigatus*, *A. terreus* and *Emericella niveus* were the most active cellulolytic species based upon their high yield of exoglucanase, endoglucanase, CMCase, β-glucosidase and xylanase activities. However, the results also indicate that no significant relation between enzyme activities and both amounts of total protein and released free sugars in all tested fungi.

Effect of Time Course on Enzyme Production

Aspergillus fumigatus was the most active cellulolytic species followed by *A. terreus*, *E. niveus* as shown in Table 2, along different incubation periods. However, maximum yield of CMCase (9.65 IU g^{-1}), exoglucanase (0.54 IU g^{-1}) and xylanase (25.36 IU g^{-1}) activities were obtained after 4 days. However, maximum endoglucanase (0.33 IU g^{-1}) and β-glucosidase (8.71 IU g^{-1}) activities were shown after 2-4 days.

Effect of Temperature on Enzyme Production

The results of the test made at different temperature values showed that the optimum temperature for exoglucanase (0.53-0.56 IU g^{-1}), endoglucanase (0.36 IU g^{-}), CMCase (10.8-14.3 IU g^{-1}), β-glucosidase (7.6-8.2 IU g^{-1}) and xylanase (24.4-29.4 IU g^{-1}) production by *A. fumigatus* was showed between 35-45°C (Fig. 1); however, 40°C appeared to be the optimum for *A. fumigatus* enzyme production. Due to thermal deactivation, the results also showed higher decreases in enzymes activity at 50°C. Generally, the temperature did not affect strongly the enzyme production, this behavior being favorable for SSF.

Table 1: Screening for the most active cellulases producing *Aspergillus* strain

			Enzyme activity (IU g^{-1})				
Aspergillus species	Free sugars (mM g^{-1})	Protein (ug mL^{-1})	EXG	EG	CMCase	BG	Xy
Aspergillus aculeatus	22.05	35.97	0.40	0.09	4.91	3.51	16.92
Aspergillus awamori	21.70	41.69	0.35	0.14	5.14	3.81	15.57
Aspergillus flavus	18.55	24.53	0.21	0.08	3.72	3.82	12.64
Aspergillus fumigatus	26.78	24.12	0.51	0.22	8.39	7.87	19.35
Aspergillus glaucus	21.88	31.47	0.22	0.13	3.77	4.33	11.93
Aspergillus japonicus	19.08	23.30	0.13	0.03	2.44	2.72	07.21
Aspergillus ochraceus	22.05	24.12	0.21	0.08	3.85	3.70	11.15
Aspergillus ornatus	19.78	33.93	0.17	0.04	3.24	2.37	10.64
Aspergillus terreus	24.15	39.24	0.43	0.14	5.23	4.43	18.42
Aspergillus terreus ------	19.95	46.60	0.31	0.17	4.35	4.07	14.91
Aspergillus viridi-nutans	20.30	32.29	0.48	0.18	4.60	5.37	16.73
Emericella niveus	24.33	24.53	0.26	0.25	7.30	7.49	18.27

Table 2: Time course for some isolated strains grown on mixed culture of RS and WB (1:1)

	Enzyme activity (IU g^{-1})				
Incubation time (days)	EXG	EG	CMCase	BG	Xy
A. terreus					
1	0.12	0.06	2.41	1.68	05.19
2	0.29	0.25	4.40	3.43	12.74
3	0.44	0.27	5.15	5.65	19.69
4	0.50	0.28	5.97	4.96	18.23
5	0.43	0.14	5.20	4.40	18.40
E. niveus					
1	0.07	0.06	3.79	2.85	07.65
2	0.16	0.19	5.37	5.94	12.61
3	0.37	0.24	7.61	7.63	14.37
4	0.32	0.29	7.94	8.21	22.03
5	0.27	0.25	7.32	7.50	18.20
A. fumigatus					
1	0.09	0.08	03.16	2.45	11.91
2	0.20	0.24	05.63	6.43	15.42
3	0.46	0.33	07.55	8.71	18.37
4	0.54	0.29	09.65	7.93	25.36
5	0.50	0.29	08.96	7.88	19.56

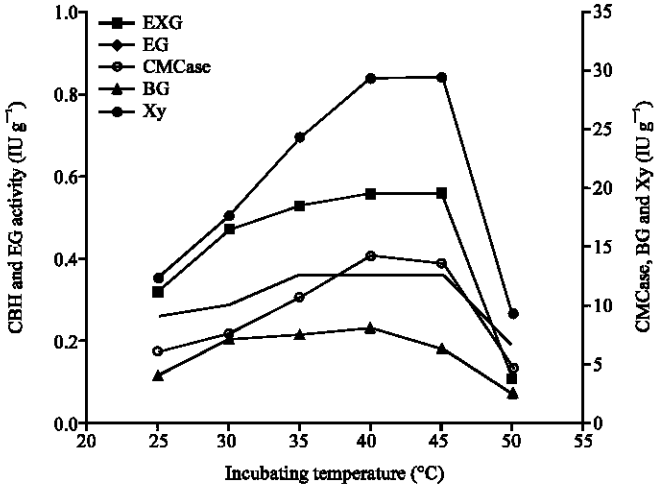

Fig. 1: Effect of different temperatures on enzyme production by *A. fumigatus*

Effect of Initial pH on Enzyme Production

The effect of initial pH levels on cellulases production was investigated at the optimum incubating temperature (40°C) determined above. The results shown in Fig. 2 showed that the production of cellulases did not vary appreciably within an initial pH range of 5.0-7.0. However, pH 6.0 was the optimum for maximal production of *A. fumigatus* enzyme.

Effect of Different Initial Moisture Levels on Enzyme Production

The highest A. *fumigatus* cellulases were obtained at moderate (75%) moisture levels (Fig. 3); while, it was decreased at lower (33-66%) and higher (78-80%) values of initial moisture.

Effect of Different Nitrogen Sources on Enzyme Production

The results in Table 3 showed that nitrogen sources showed variable effects on each enzyme activity. However, maximal exoglucanase (1.54 IU g^{-1}), endoglucanase, CMCase

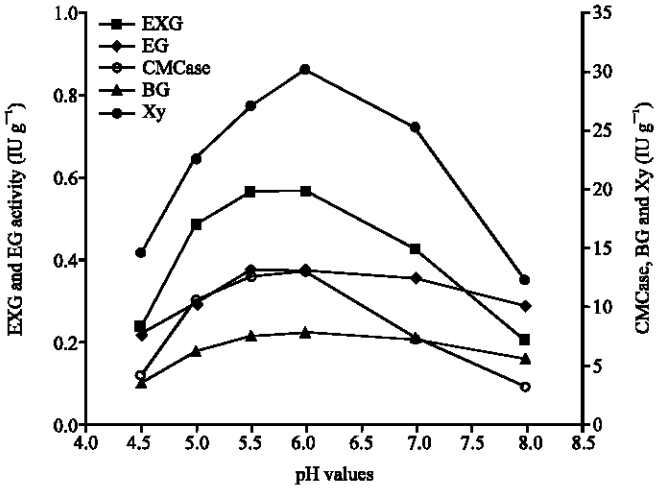

Fig. 2: Effect of different initial pH levels on enzyme production by *A. fumigatus*

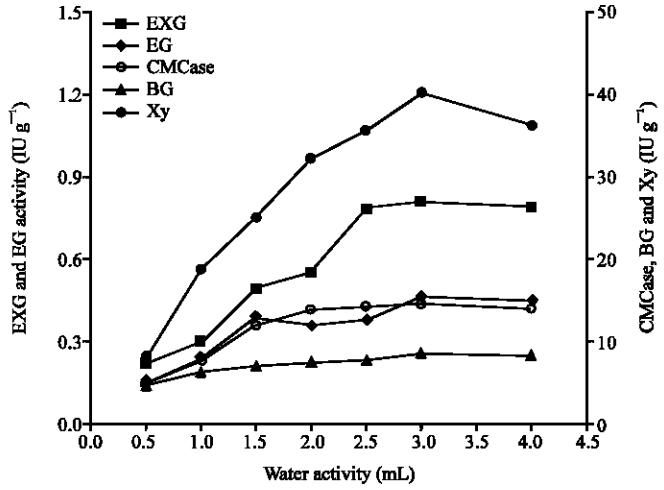

Fig. 3: Effect of different moisture levels on enzyme production by *A. fumigatus*

Table 3: Effect of different nitrogen sources on enzyme production by *A. fumigatus*

	Enzyme activity (IU g^{-1})				
Nitrogen source	EXG	EG	CMCase	BG	Xy
KNO_3	0.80	0.44	14.05	9.31	33.79
NH_4Cl	0.93	0.54	15.09	10.07	48.36
NH_4NO_3	0.72	0.43	14.26	8.46	44.36
Ca $(NO_3)_2$	0.63	0.35	15.54	8.01	38.85
$NaNO_3$	1.54	0.92	16.78	10.93	56.81
Aspargine	0.56	0.24	10.59	8.51	36.66
Urea	0.59	0.24	11.23	8.88	55.46
Peptone	0.64	0.41	13.56	10.76	39.97
Yeast	0.75	0.32	11.44	9.40	33.54
Control	0.37	0.28	12.90	8.15	31.43

Table 4: Effect of different lignocelluloses residues on enzyme production by *A. fumigatus*

	Enzyme activity (IU g^{-1})				
Carbon source	EXG	EG	CMCase	BG	Xy
Alfa alfa	0.43	0.34	05.67	6.69	19.09
Corn cob	0.11	0.24	05.31	4.33	28.62
Sawdust	0.67	0.07	04.68	2.67	25.51
Wheat straw	1.64	0.98	16.86	11.83	56.37
Rice straw	0.43	0.28	08.51	6.77	35.92
Wheat bran	0.69	0.14	10.26	6.10	32.70

Table 5: Effect of different ratios of rice straw and wheat bran on enzymes production by *A. fumigatus*

	Enzyme activity (IU g^{-1})				
Ratios of RS:WB	EXG	EG	CMCase	BG	Xy
RS	0.43	0.28	08.51	6.77	35.92
9:1	0.31	0.35	07.63	7.21	40.91
7:3	0.93	0.34	09.24	8.51	39.39
1:1	0.85	0.68	14.71	6.92	49.33
3:7	0.73	0.27	09.96	6.90	42.70
1:9	0.60	0.20	09.70	4.34	38.90
WB	0.69	0.14	10.26	6.10	32.70

(16.8 IU g^{-1}), (0.92 IU g^{-1}), β-glucosidase (10.9 IU g^{-1}) and xylanase (56.8 IU g^{-1}) were recorded in presence of $NaNO_3$. The used rice straw and wheat bran may have low nitrogen to support fungal growth and cellulases activity, so additive nitrogen; $NaNO_3$ may enhance cellulases and hemicellulases production.

Effect of Different Lignocelluloses Residues on Enzyme Production

The results indicated that the levels of *A. fumigatus* enzyme after optimum fermentation period were significantly varied on different lignocelluloses materials as shown in Table 4. However, maximum xylanase (56.4 IU g^{-1}), CMCase (16.9 IU g^{-1}), β-glucosidase (11.8 IU g^{-1}), exoglucanase (1.7 IU g^{-1}) and endoglucanase (0.98 IU g^{-1}) were detected on wheat straw as compared with the other. This variation may be attributed to the chemical nature and nutrient availability of the used substrate.

On sawdust, Alfa alfa, corncob and rice straw; more mineral solution has to be added to support the initial growth of the fungi, after which it can attack the substrate and produce enzymes.

Effect of Different Ratios of Rice Straw and Wheat Bran on Enzyme Production

The ratio of RS and WB in 1:9 to 9:1 ratios was tested for their effect on *A. fumigatus* enzymes production as shown in Table 5. Maximum xylanase (49.3 IU g^{-1}), CMCase (14.7 IU g^{-1}) and endoglucanase (0.68 IU g^{-1}) activities were markedly higher in the mixed culture of rice straw and wheat bran (1:1), while higher β-glucosidase (8.5 IU g^{-1}) and exoglucanase (0.93 IU g^{-1}) were detected in rice straw and wheat bran ratio 7 RS : 3 WB. In contrast, xylanase, CMCase, β-glucosidase, avicellase and endoglucanase were reduced about 27.2, 42.1, 20.4, 53.8 and 58.8%, respectively, when rice straw was used alone; these means that wheat bran stimulates *A. fumigatus* for cellulolytic and hemicelluolytic production.

Aspergillus fumigatus xylanase, CMCase, β-glucosidase, exoglucanase and endoglucanase at different culture conditions revealed that it become around 2.6, 1.8, 1.1, 1.8 and 3.5 folds increase after optimization culture conditions (temperatures, initial pH value, moisture content, nitrogen source) on mixed rice straw and wheat bran (1:1), respectively.

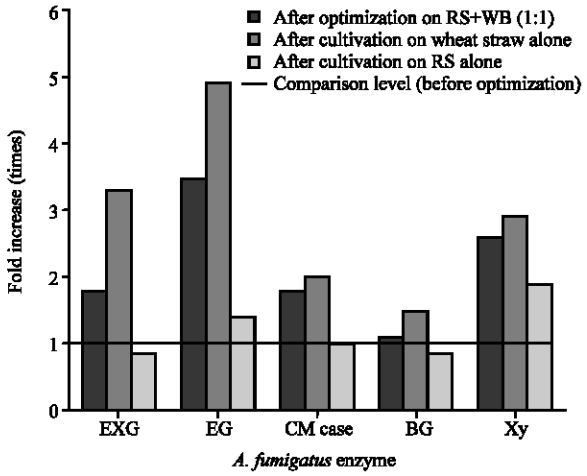

Fig. 4: Fold increase of *A. fumigatus* enzyme after optimization

However, fold increase results after optimization was also compared with that obtained in case of using wheat straw and rice straw (Fig. 4). The above results show that rice straw amended with wheat bran serves as a good substrate for cellulase and hemicellulase production.

DISCUSSION

Due to the wide distribution of rice straw not only in Egypt, but all over the world as agriculture residues; increases its availability to use as substrate for healthy conversion and industrially for fungal cellulases production. The crude enzyme was consequently studied in this study due to the expensive demand of enzyme purification; as well as, purified cellulases of several *Aspergilli* show little capacity to cleave highly ordered forms of cellulose (Berka *et al*., 1992).

Taking count of the advantages of the solid state fermentation, we have investigated some factors affecting cellulases and xylanase production during degradation of rice straw, such as: fermentation periods, water moisture contents, growth temperature, initial pH, different nitrogen sources and other lignocellulosic substrates. Moreover we have investigated the optimal composition for cellulase production of the mixed substrates. Generally, production of fungal cellulases and hemicellulases has been shown to be inducible and was affected by the nature of used substrate (Ojumu *et al*., 2003). Therefore, choice of an appropriate inducing substrate is importance. Mixture of rice straw and wheat bran showed better results in SmF (Kocher *et al*., 2008) and SSF (Shamala and Sreekantiah, 1985; Park *et al*., 2002; Kang *et al*., 2004) for the production of fungal hemicellulases and cellulases. Experiments in this study were conducted using mixed rice straw and wheat bran for screening and selection of the most active stains.

Screening for the most active fungi in cellulases production was recorded after 5.0 days incubation at 30°C on 1.0 g mixed RS and WB culture. The results showed that *Aspergillus terreus*, *Emericella niveus* and *A. fumigatus* showed to be the most active cellulolytic strains based upon their high yield of exoglucanase, endoglucanase, CMCase, β-glucosidase and xylanase activities. However, the results also indicate that no significant relation between

enzyme activities and both amounts of total protein and released free sugars in all tested *Aspergilli*.

Effect of Time Course on Enzyme Production

Aspergillus fumigatus showed the most active cellulolytic species along different incubation periods. The maximum yield of CMCase, exoglucanase and xylanase activities were detected at the fourth day incubation; while, maximum endoglucanase and β-glucosidase activities were shown after 2-4 days. Behavior patterns of results are in agreement with those obtained by *A. niger* KK2 grown on rice straw (Kang *et al*., 2004). Since comparisons of the results obtained in this study with those obtained by other researchers are difficult, because the yields of other cellulases were expressed in U mL^{-1} culture extract or U g^{-1} fermented substrate; as well they used other fungal strains and different lignocelluloses substrates such as sugarcane straw (Guerra *et al*., 2006), groundnut fodder, wheat bran, rice bran and sawdust (Chandra *et al*., 2007).

Effect of Temperature on Enzyme Production

The results showed that 35-45°C were suitable for significant production of different *A. fumigatus* enzyme; however, 40°C was appeared to be the optimum. Due to temperature deactivation, results showed higher decreases in enzymes activity at 50°C. Similar results were reported in case of *A. niger* (Szewczyk and Myszka, 1994; Jecu, 2000) above 34°C. Furthermore, temperature; 30-45°C was the optimum for maximal cellulases and xylanases production by fungi cultivated on other wastes; *Aspergillus* sp. (Asquieri and Park, 1992) and *A. sulphureus* (Lu *et al*., 2003).

Effect of Initial pH on Enzyme Production

The results indicated that the production of cellulases did not vary appreciably within an initial pH range of 5.0-7.0. However, pH 6.0 was the optimum for maximal production of *A. fumigatus* enzymes. These results are in agreement with those reported for other fungal cellulases and xylanases produced by *Aspergillus aculeatus* (Murao *et al*., 1988) and *A. niger* (Jecu, 2000).

Effect of Different Initial Moisture Levels on Enzyme Production

Moisture level is the most determined factors for SSF. Increase in moisture level is believed to decrease enzyme production due to decreasing substrate porosity, alteration in substrate particle structure, lowering oxygen transfer, higher soluble protein and enhance aerial mycelial growth. On the other hand, low moisture reduces nutrients and protein solubility as well as effect on swelling of used substrate (Gervais and Molin, 2003). The highest *A. fumigatus* cellulases were obtained at moderate (75%) moisture levels; while, it was decreased at lower and higher values of initial moisture. These results are similar with other fungal cellulase and xylanase obtained by *A. fumigatus* (Gupte and Madamwar, 1997), *A. niger* (Jecu, 2000), *A. terreus* (Gervais and Molin, 2003) and *A. foetidus* (Shah and Madamwar, 2005 a,b) by using other lignocellulosic substrates in SSF. Furthermore, Botella *et al*. (2007) showed that 65% moisture level was the optimum for xylanase production when *A. awamori* grown on grape pomace.

Effect of Different Nitrogen Sources on Enzyme Production

The results showed that the nitrogen sources showed variable effects on enzyme activity. Maximum exoglucanase, endoglucanase, CMCase, β-glucosidase and xylanase

activities were recorded in presence of $NaNO_3$. The mixture of rice straw and wheat bran used may have low nitrogen to support fungal growth and cellulases activity, so NaNO3 may enhance cellulases and hemicellulases production. These results are in parallel with those reported additive nitrogen to rice straw for higher enzymes productivity (Haltrich *et al*., 1996). Furthermore, (Ghanem *et al*., 2000) recorded that additive yeast extract lead to fold increase cellulases and hemicellulases by *A. terreus* and *Paecilomyces themophila* (Yang *et al*., 2006) when grown on wheat straw. On the other hand, due to the increasing values of nitrogen in many natural fermentable substrates, Fadel (2000) showed inhibition of CMCase with high additive levels of inorganic nitrogen when *A. niger* grown on radicle waste.

Effect of Different Lignocellulose Residues on Enzyme Production

The results indicate that the growth and the level of *A. fumigatus* enzymes after optimum fermentation period were significantly varied on different lignocelluloses as shown in Table 4. However, maximum xylanase (56.4 IU g^{-1}), CMCase (16.9 IU g^{-1}), β-glucosidase (11.8 IU g^{-1}), exoglucanase (1.7 IU g^{-1}) and endoglucanase (0.98 IU g^{-1}) was detected on wheat straw compared with the other. This variation may be attributed to the chemical nature and nutrient availability of used substrate. On sawdust, Alfa alfa, corncob and rice straw; more mineral solution has to be added to support the initial growth of the fungi, after which it can attack the substrate and produce enzymes. In this connection more or less similar results were obtained by Ghanem *et al.* (2000) showing the effect of different lignocellulosic substrates as carbon source on *A. terreus* cellulases production along different fermentation periods.

Effect of Different Ratios of Rice Straw and Wheat Bran on Enzyme Production

The ratios of RS and WB in 1:9 to 9:1 ratios were tested for their effect on *A. fumigatus* enzymes production as shown in Table 5. Maximum xylanase (49.3 IU g^{-1}), CMCase (14.7 IU g^{-1}) and endoglucanase (0.68 IU g^{-1}) activities appeared markedly higher in the mixed substrates of rice straw and wheat bran (1:1). However higher β-glucosidase (8.5 IU g^{-1}) and exoglucanase (0.93 IU g^{-1}) were recorded at rice straw and wheat bran at ratio (7 RS : 3 WB). On the other hand, xylanase, CMCase, β-glucosidase, avicellase and endoglucanase were reduced by a rate of about 27.2, 42.1, 20.4, 53.8 and 58.8%, respectively, when rice straw was used alone. This means that wheat bran stimulates *A. fumigatus* for cellulolytic and hemicelluolytic enzymes production. Similar results obtained by Fadel (2000) indicated that enhancement of cellulases production requires little free sugars in initial fermentation culture. This result is opposite to those obtained with *A. niger* KK2 FPase and xylanase that highly produced when rice straw was used alone; and similar for its β-glucosidase with 36% reduction in absence of amendment wheat bran (Kang *et al*., 2004).

Aspergillus fumigatus endoglucanase, exoglucanase, CMCase, β-glucosidase and xylanase, at different culture conditions factors revealed that it become around 3.5, 1.8, 1.8, 1.1and 2.6 folds increase after optimization culture conditions (culture temperatures, initial pH value, moisture content, nitrogen source) on mixed rice straw and wheat bran (1:1), respectively (Fig. 4).

The results obtained in case of using rice straw alone showed that *A. fumigatus* xylanase, CMCase, β-glucosidase, endoglucanase and exoglucanase were considerably acceptable compared with other fungal cellulases as reported for *A. glaucus* XC9 (Chang *et al*., 2006), *A. niger* (Chandra *et al*., 2007) and *A. fumigatus* (Grigorevski-Lima *et al*., 2009). Present results showed that rice straw amended with wheat bran could serve as a good substrate for cellulase and hemicellulase production concurrently.

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