African Journal of Biotechnology Vol. 9(27), pp. 4211-4216, 5 July, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Screening of some *Zygomycetes* for cellulase activity

Abolfazl Lotfi[2], Mohammad Ali Tajick Ghanbary[1]*, Gholam Ali Ranjbar[2] and Ahmad Asgharzadeh[3]

[1]Department of Plant Protection, Sari Agricultural and Natural Resources University Sari, Iran.
[2]Department of Biotechnology, Sari Agricultural and Natural Resources University Sari, Iran.
[3]Department of Soil Biology, Soil and Water Researches Institute, Tehran, Iran.

Accepted 30 December, 2009

This study was aimed to screen the cellulytic ability of some genera of *Zygomycetes* under laboratory conditions. Carboxy methyl cellulose (CMC) and Wheat straw (WS) were used as the only carbon source in a minimal culture medium. Four days after inoculation, released proteins and sugars were assayed with related reagents and repeated each, 3 days up to the 31th day. Statistical analysis showed significant variation in released sugars but no significant variation in released proteins among tested genera. *Mucor hiemalis* f. *corticola* had highest and *Mucor circinelloides* f. *circinelloides* had lowest sugar levels. Glucose levels for *M. hiemalis* f. *corticola* increased until 16 days after inoculation, then decreased until 25th day, but had no variation until 30th day. These results showed that isolates belonging to the same forms had no significant difference in cellulase activity, but the ability of different genera and species were noticeable. This study also showed that WS medium can be effectively used for cellulase production by fungi.

Key words: Cellulase activiy, carboxy methyl cellulose, wheat straw, protein assay, sugar assay.

INTRODUCTION

Plant cell walls are the most abundant renewable source of fermentable sugars on earth (Himmel et al., 1999; Saleem et al., 2008) and are the major reservoir of fixed carbon in nature (Yang et al., 2007). The major components of plant cell walls are cellulose, hemi-cellulose and lignin, with cellulose being the most abundant component (Han et al., 2003). Cellulose consists mainly of long polymers of β 1-4, linked glucose units and forms a crystalline structure (Shallom and Shoham, 2003). Cellulase enzymes, which can hydrolyze cellulose forming glucose and other commodity chemicals, can be divided into three types: endoglucanase (endo-1, 4-β-D-glucanase, EG, EC 3.2.1.4); cellobiohydrolase or exoglucanase (exo-1, 4-β-D-glucanase, CBH, EC 3.2.1.91) and β-glucosidase (1, 4-β- D-glucosidase, BG, EC 3.2.1.21) (Li et al., 2006; Gao et al., 2008). Cellulases are important industrial enzymes and find applications in several industrial processes (Hanif et al., 2004).

*Corresponding author. E-mail: m.tajick@sanru.ac.ir or m.tajick @gmail.com. Tel: +98 151 3822574. Fax: +98 151 3822578.

Researchers have strong interests in cellulases because of their applications in industries for starch processing, grain alcohol fermentation, malting and brewing, extraction of fruit and vegetable juices, pulp and paper industry and textile industry (Gao et al., 2008; Zhou et al., 2008). One of the potential applications of cellulases is the production of fuel ethanol from lignocellulosic biomass (Duff and Murray, 1996), which is a good substitute for gasoline in internal combustion engines. The most promising technology for the conversion of the lignocellulosic biomass to ethanol biofuel is based on the enzymatic breakdown of cellulose using cellulase enzymes (Holker et al., 2004; Ahamed and Vermette, 2008). Many fungal strains secrete higher amounts of cellulases than bacterial ones, with *Trichoderma* as the leading one (Amouri and Gargouri, 2006). Most commercial cellulases are mesophilic enzymes produced by the filamentous fungus *Trichoderma reesei* and *Aspergillus niger*. This process reflects well the fact that filamentous fungi are naturally excellent protein secretors and can produce industrial enzymes in feasible amounts (Bergquist et al., 2002). Considering the importance and application of the cellulases, this study was aimed to screen the indigenous fungal isolates of

Table 1. Analysis of variance of protein and sugar assay on CMC and wheat straw media.

Source variations	DF	Mean Square	
		Protein assay (mg/l)	Sugar assay (g/l)
Isolate	15	0.000223^{ns}	0.001917**
Culture medium	1	0.000776^{ns}	0.000195^{ns}
Isolate* Culture medium	15	0.000287^{ns}	0.001617**
Error total	926	0.000287	0.0001834

** = Significant at 1% probability; ns = non-significant at 5% probability.

some genera of zygomycetes for cellulase activity in laboratory condition.

MATERIALS AND METHODS

Fungal isolates and maintenance

Sixteen isolates of *Zygomycetes* belonging to *Mucor circinelloides* f. *circinelloides, Mucor circinelloides* f. *griseocyanus, Mucor circinelloides* f. *janssenii, Mucor circinelloides* f. *lusitanicus, Mucor hiemalis* f. *corticola, Mucor hiemalis* f. *luteus, Mucor hiemalis* f. *silvaticus, Mucor genevensis, Mucor strictus, Mucor plumbeus, Cunninghamella echinulata, Rhizomucor pusillus* and *Rhizopus stolonifer* which were isolated from cultural soils of different regions of Iran were selected for the experiments. The isolates were grown on PDA slants and stored at 4°C.

Culture medium

Wheat straw (WS) medium containing 1 g wheat straw that were cut to 1 cm pieces per 50 ml distilled water and carboxy methyl cellulose (CMC) medium containing 0.05 g $FeSO_4$ $7H_2O$, 0.25 g $MnSO_4$ H_2O, 0.25 g $CoCl_2$, 0.25 g $ZnSO_4$, 0.25 g $(NH_4)_2SO_4$, 2 g KH_2PO_4, 0.25 g $MgSO_4$ 7 H_2O, 0.4 g $CaCl_2$, 0.3 g urea, 0.2 ml Tween 80 and 10 g carboxy methyl cellulose per liter were prepared for cellulose degradation experiments. 50 ml of broth were distributed in 250 ml erlenmeyers and then both media were autoclaved at 120°C for 20 min.

Inoculation and sampling

Each flask was inoculated with 1 ml spore suspension in three replicates for each species. The flasks were treated at 25°C for 31 days. Sampling was started four days after inoculation and repeated every two days for protein and sugar assays.

Protein and sugar

Five hundred µl of broth medium in each clean test tube were subjected to protein and released sugars assays. Released fungal extracellular proteins and produced sugars concentrations were determined using Bradford method and Arsenate-Molybdate reagent, respectively (Bradford, 1976; Kossem and Nannipieri, 1995).

Statistical analysis

The results obtained in the present study were analysed by SAS (v. 9,1) software.

RESULTS

The growth process started at least 12 h after inoculation. Table 1 shows the significant differences for measurements of sugar assays. No statistical variations were detectable between released proteins of different isolates. No significant differences for protein and sugar assays were observed between both CMC and WS media.

Sugar assay

Released extracellulare enzymes of different species caused some increase and decreases in sugar levels produced from cellulose degradation. *M. hiemalis* f. *corticola* showed the highest and *M. circinelloides* f. *circinelloides* and *R. pusillus* showed the lowest potential of glucose production (Table 2). *M. hiemalis* f. *corticola* showed the highest released sugar content in the CMC medium and *M. plumbeus* and *M. circinelloides* f. *lusitanicus* showed the highest released sugar content in the wheat straw medium (Figure 1). Nevertheless, these several superior isolates (*M. hiemalis* f. *corticola, M. plumbeus* and *M. circinelloides* f. *lusitanicus*) produced the highest amount of released sugar in 12, 9 and 18 days after inoculation, respectively, however, the trend dropped down until days 15th and 21th (Figure 2). There was a significant difference in the released sugar content between different genera and species in this study but between CMC and wheat straw media no significant statistical variation in sugar production was observed (Table 1).

Protein assay

Protein assays during the experiments showed some gradual changes in released proteins concentration. According to Tables 1 and 2, there were no significant statistical variations between different isolates also between CMC and wheat straw media in released protein content. Levels of released protein of tested isolates in CMC and wheat straw are shown in Figure 3. *M. hiemalis* f. *corticola* , *M. plumbeus* and *M. circinelloides* f. *lusitanicus* which showed the highest amount of released sugar content, had the highest levels of released protein in 12

Table 2. Means comparison of sugar and protein assay in different tested isolates.

Isolate	Protein assay (mg/l)	Sugar assay (g/l)
Mucor hiemalis f. *corticola*	0.021a	0.0329a
M. hiemalis f. *corticola*	0.02a	0.0323a
M. plumbeus	0.0171a	0.0237b
M. circinelloides f. *lusitanicus*	0.0148a	0.0234b
Cunninghamella echinulata	0.0137a	0.0232b
M. circinelloides f. *janssenii*	0.0225a	0.0230b
M. circinelloides f. *griseocyanus*	0.0164a	0.0227bc
Rhizopus stolonifer	0.0169a	0.0216bc
M. genevensis	0.0192a	0.0207bc
C. echinulata	0.0132a	0.0202bc
M. hiemalis f. *luteus*	0.0176a	0.0201bc
M. strictus	0.0160a	0.0184bc
M. hiemalis f. *silvaticus*	0.0202a	0.0173cd
Rhizomucor pusillus	0.0217a	0.0145de
M. circinelloides f. *circinelloides*	0.0179a	0.0137de
M. circinelloides f. *circinelloides*	0.0178a	0.0125e

Means with the same letter are not significantly different (p = 0.01).

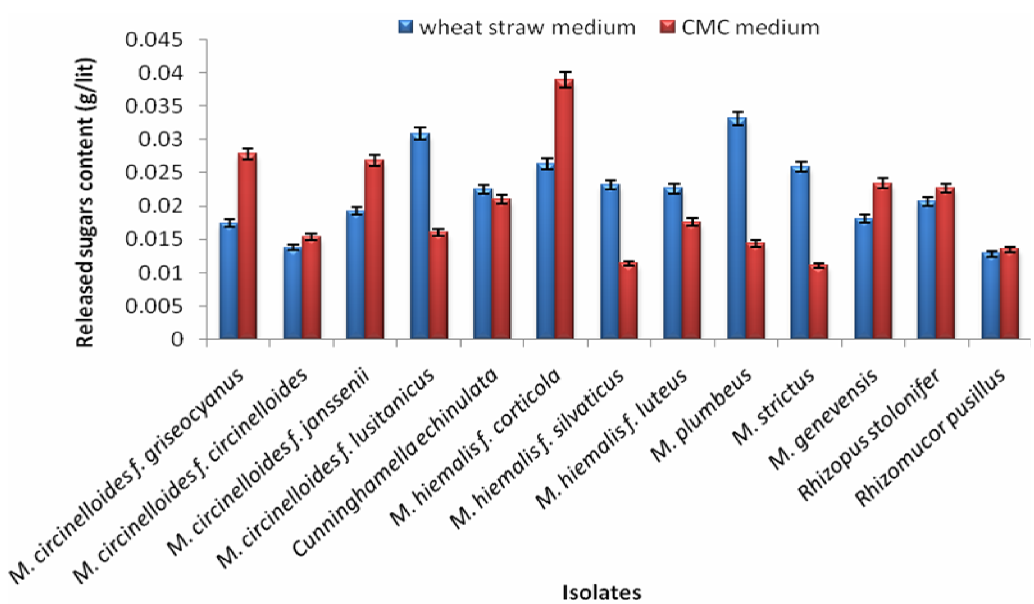

Figure 1. Variations of released sugar in different isolates in CMC and wheat straw media.

and 15 days after inoculation, respectively. However, these levels gradually decreased by day 18th (Figure 4).

DISCUSSION

Cellulase complex enzymes have a series of industrial applications that increase their commercial importance. The fungi have been described as the best sources of cellulase extraction. Different fungi belonging to *Zygomycetes*, *Ascomycetes*, *Basidiomycetes* and *Deutromycetes* can degrade cellulose and hemicelluloses (Schulein, 2000). Screening of different genera, species and even isolates is the first step for finding acceptable enzyme producer isolates. Macris (1983) in a survey on some genera like *Trichoderma*, *Fusarium*, *Aspergillus*, *Phanerocheate*, *Chrysosporium* and *Sclerotium* showed some differences in their cellulase activity. Sazci et al. (1986) in surveys on cellulase activity of different genera reported that *Trichoderma harzianum* and *A. niger* showed highest

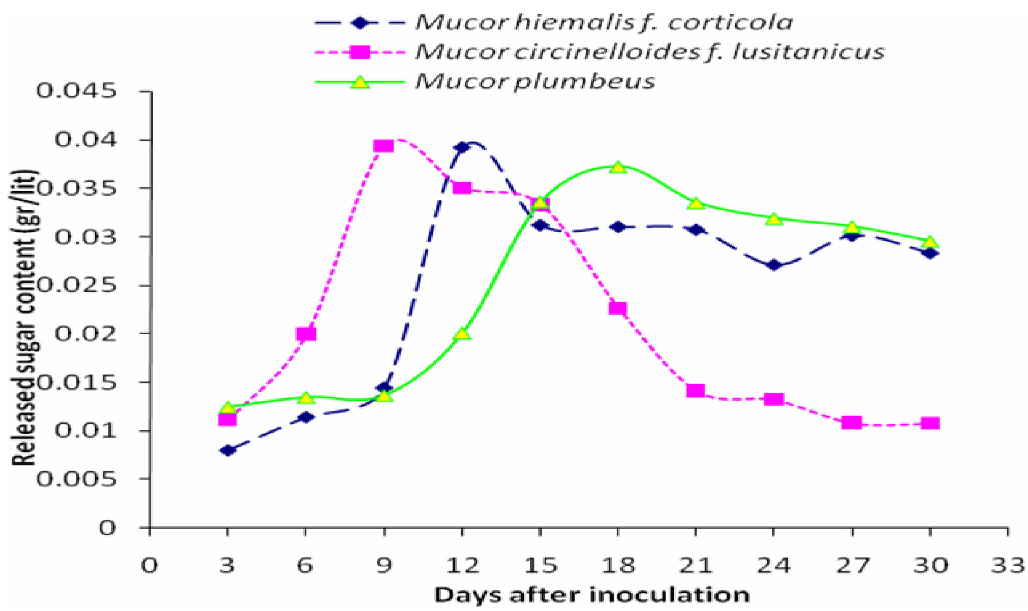

Figure 2. Variations of released sugar in three isolates with highest sugar production in different days after inoculation.

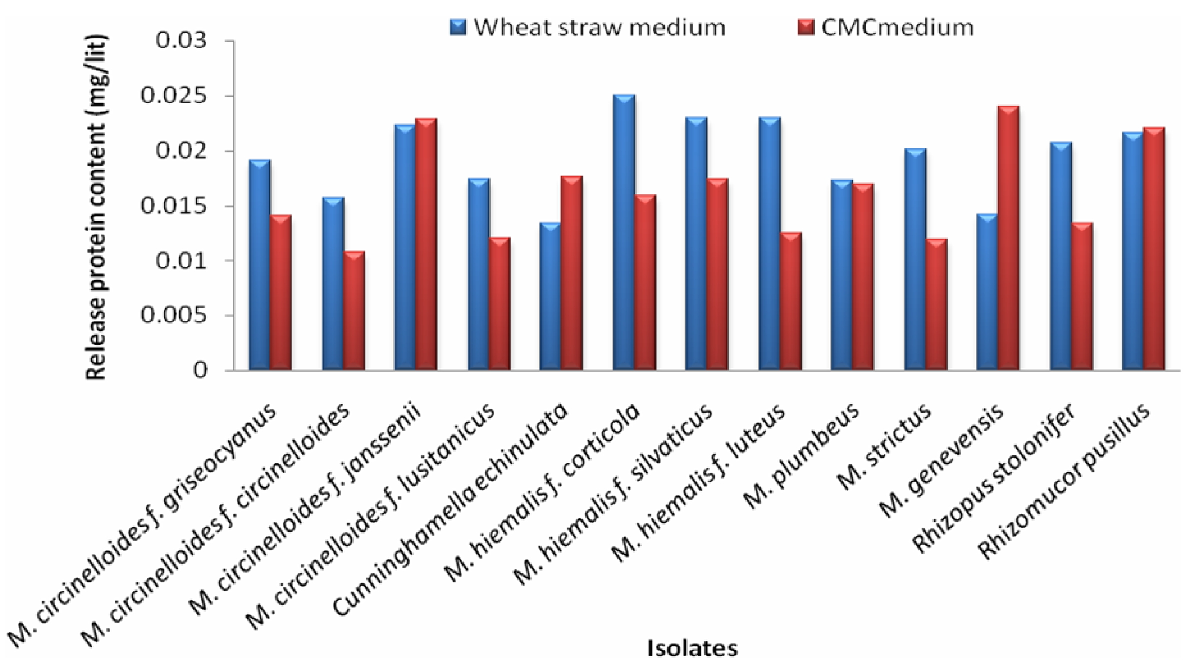

Figure 3. Variations of released protein in different isolates in CMC and wheat straw media.

and *Trichothecium roseum, T. reesei*, *Aspergillus ochraceus* and *Penicillium italicum* exhibited lower activity against CMC. In another research Jahangeer et al. (2005) on the measurement of cellulase activity among 115 isolated fungal strains showed that *Trichoderma, Aspergillus* and *Fusarium* strains had the highest activity. Oyeleke et al. (2008) reported cellulose degradation ability in *Mucor* species. Endoglucanase production were reported by Boyce and walsh (2007) for *Rhizomucor mehei* and Moria et al. (2004) in *Rhizopus oryzea*. Results of Yeoh et al. (1984) showed that Cuninnghamella sp. did not show any cellulolytic activity when cultured on medium containing cellulose.

The results showed that there were detectable significant statistical variations in released sugar between tested genera and species but no significant difference between

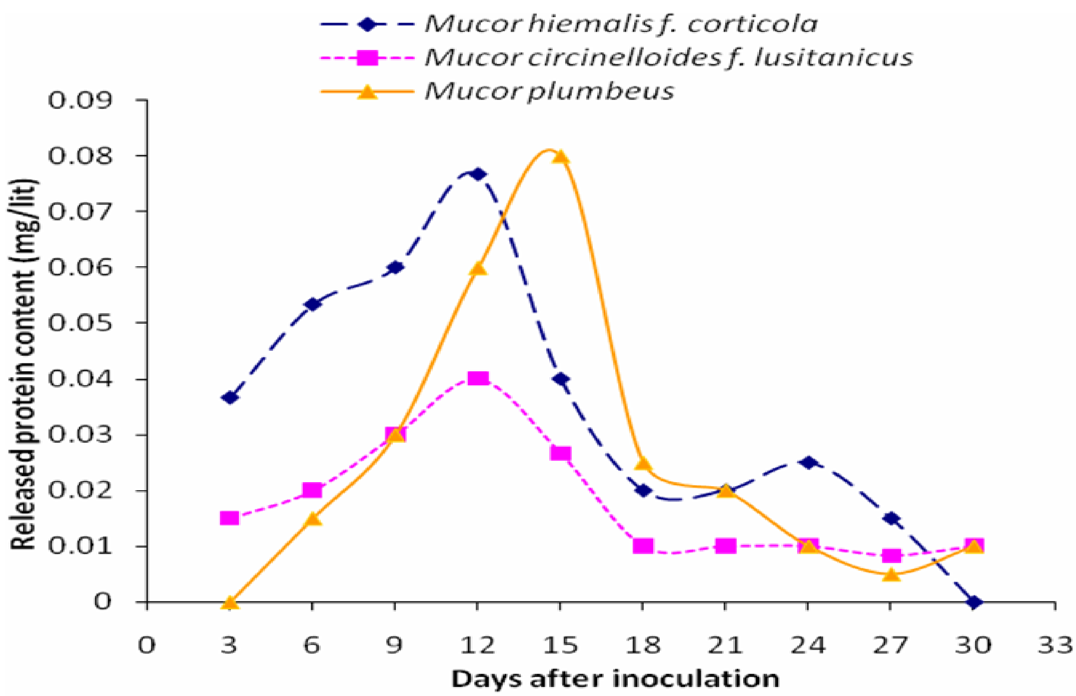

Figure 4. Variations of released protein in three isolates with highest sugar production in different days after inoculation.

M. circinelloides and *M. hiemalis* was shown. Results on protein assay showed that there was no significant difference in released protein between tested genera and species. Results of sugar assays had significant difference together but there was no significant difference between results of protein assays. Consequently, cellulase production of the isolates was similar but the type and amount of enzyme was different. According to the results, among treated isolates, *M. hiemalis* f. *corticola* had the highest released sugars and *M. circinelloides* f. *circinelloides* and *R. pusillus* showed the lowest cellulase activity. A relatively high protein production took place after CMC inoculation, probably because of the initial fungal growth (Jahangeer et al., 2005). Decrease in protein levels after initial growth might be due to the feedback process of CMC degradation and some protease secretion that reduced the amount of released sugars (Rad et al., 2005; Yeoh et al., 1984). Finally, different *Zygomycetes* had unsimilar behavior in cellulose degradation and their rate was variable. In cellulose rich soils and *in situ*, there are some factors which affected the process. Other sugar consuming microorganisms specially soil bacteria may change *in vitro* results. Soil microbiota may compete with *Zygomycota* with an increase in cellulase production until the substrate is present. Interaction between soil inhabitant micro-organisms is an interesting subject for more researches. In the present study, only one highlighted biochemical process from a few species of *Zygomycetes* were tested using laboratory conditions.

REFERENCES

Ahamed A, Vermette P (2008). Culture-based strategies to enhance cellulase enzyme production from *Trichoderma reesei* RUT-C30 in bioreactor culture conditions. Biochem. Eng. J. 40: 399-407.

Amouri B, Gargouri A (2006). Characterization of a novel β-glucosidase from a *Stachybotrys* strain. Biochem. Eng. J. 32: 191-197.

Bergquist P, Teo V, Gibbs M (2002). Expression of xylanase enzymes from thermophilic microorganisms in fungal host. Extermophiles. 6: 177-184.

Boyce A, Walsh G (2007). Production, purification and application-relevant characterisation of an endo-1,3(4)-β-glucanase from *Rhizomucor miehei*. Appl. Microbiol. Biotechnol. 76: 835-841.

Bradford MM (1976). A rapid and sensitive method for quantification of microgram quantities of protein of utilizing the principle dye binding. Anal. Biochem. 72: 680-685.

Duff SJB, Murray WD (1996). Bioconversion of forest products industry waste cellulosics to fuel ethanol: a review. Bioresour. Technol. 55: 1-33.

Gao J, Weng H, Zhu D, Yuan M, Guan F, Xi Yu (2008). Production and characterization of cellulolytic enzymes from the thermoacidophilic fungal *Aspergillus terreus* M11 under solidstate cultivation of corn stover. Bioresour. Technol. 99: 7623-7629.

Han SO, Yukawa H, Inui M, Doi RH (2003). Regulation of expression of cellulosomal cellulase and hemicellulase genes in *Clostridium cellulovorans*. J. Bacteriol. 185: 6067-6075.

Hanif A, Yasmin A, Rajoka MI (2004). Induction, production, repression and de-repression of exoglucanase synthesis in *Aspergillus niger*. Bioresour. Technol. 94: 311-319.

Himmel ME, Ruth MF, Wyman CE (1999). Cellulase for commodity products from cellulosic biomass. Curr. Opin. Biotechnol. 10: 358-364.

Holker U, Hofer M, Lenz J (2004). Biotechnological advantages of laboratory scale solid-state fermentation with fungi. Appl. Microbiol. Biotechnol. 64: 175-186.

Jahangeer S, Khan N, Jahangeer S, Sohail M, Shahzad S, Ahmad A, Ahmed Khan S (2005). Screening and characterization of fungal cellulases isolated from the native environmental source. Pak. J. Bot.

37: 739-748.
Kossem A, Nannipieri P (1995). Soil cellulase activity methods. In: Kossem A, Nannipieri P (eds) Applied Soil Microbiology and Biochemistry. Academic Press: San Diego, pp. 345-350.
Li YH, Ding M, Wang J, Xu GJ, Zhao F (2006). A novel thermoacidophilic endoglucanase, Ba-EGA, from a new cellulose degrading bacterium, *Bacillus* sp. AC-1. Appl. Microbiol. Biotechnol. 70: 430-436.
Macris BJ (1983). Production and characterization of cellulose and ß–glucosidase from a mutant of *Alternaria alternate*. Appl. Environ. Microbiol. 47: 560-565.
Oyeleke SB, Okusanmi TA (2008). Isolation and characterization of cellulose hydrolyzing microorganism from the rumen of ruminants. Afri. J. Biotechnol. 7: 1503-1504.
Rad BL, Yazdanparast R (1998). Desorption of cellulose systems of *Trichoderma ressei* and a *Botrytis* sp. from Avicel. Biotechnol. Technol. 12: 693-696.
Saleem F, Ahmed S, Jamil A (2008). Isolation of a xylan degrading gene from genomic DNA library of a thermophilic fungus *Chaetomium thermophile* ATCC 28076. Pak. J. Bot. 40: 1225-1230.
Sazci A, Radford A, Erenle K (1986). Detection of cellulolytic fungi by using Congo red as an indicator: a comparative study with the dinitrosalicyclic acid reagent method. Appl. Bocteriol. 61: 559-562.
Schulein M (2000). Protein engineering of cellulose. Biochem. Biophys. Acta. 1543: 239-252.
Shallom D, Shoham Y (2003). Microbial Hemicellulases. Curr. Opin. Microiol. 6: 219-228.
Yang CH, Yang SF, Liu WH (2007). Produciton of xylooligosaccharides from xylans by extracellular xylanases from *Thermobida fusca*. J. Agric. Food Chem. 55: 3955-3959.
Yeoh HY, Tan TK, Tian KE, (1984). Cellulolytic enzymes of fungi isolated from wood materials. Mycopathologia. 87: 51-55.
Zhou J, Wang YH, Chu J, Zhuang YP, Zhang SL, Yin P (2008). Identification and purification of the main components of cellulases from a mutant strain of *Trichoderma viride* T 100-14. Bioresour. Technol. 99: 6826-6833