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Cellulolytic activities of some filamentous fungi from soil

ABSTRACT:

Twenty fungal species were isolated and identified from soil collected from Tanta city, Gharbia Governorate, Egypt. The fungal species belonging to five genera i.e. *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma* and *Stachybotrys*. The Cellulolytic activities of the isolated fungi were evaluated in solid medium by staining the fungal colonies with 0.1% Congo red. Out of the twenty-fungal species only sixteen species recorded cellulolytic activity. The highest cellulase activity on carboxy methyl cellulose (CMC) agar plates were achieved with *A. terreus*, *A. flavipes* and *A. sydawi*. The cellulolytic fungi were selected to estimate the Carboxy methyl cellulase (CMCase), Filter paper cellulase (FPase) and β-glucosidase (βGase) in liquid medium. All tested fungi showed considerable activity for the three enzymes. The highest CMCase and βGase activities were recorded with *A. terreus* (43.30 and 33.20 u/ml respectively) while *Penicillium lanosum* showed the highest activity (29.20 u/ml). The results indicated that the maximum total cellulolytic activity was recorded with *A. terreus* followed by *A. flavipes* and *A. sydawi* (91.30, 83.50, and 74.8 u/ml, respectively).

KEY WORDS:

Screening, Soil fungi, cellulolytic activities, Congo red.

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INTRODUCTION:

Fungi constitute a group of microorganisms that are widely distributed in environment especially in soil (Boer *et al*., 2005). Since they produce wide variety of hydrolytic enzymes and hence exist in nature in saprophytic mode (Ng, 2004).

Fungi are one of the dominant groups present in soil which strongly influence ecosystem structure and function and thus playing a key role in many ecological services (Orgiazzi *et al*., 2012). Therefore, there is a growing interest in assessing soil biodiversity and its biological function (Barrios, 2007). At the ecosystem scale, extracellular enzyme activity is influenced by organic matter abundance and composition (Sinsabaugh *et al*., 2008).

Cellulose is the world's most abundant natural biopolymer and a potentially important source to produce industrially useful materials such as fuels and chemicals. Degradation of the cellulosic materials is achieved chemically, enzymatically or by the combination of both chemical and enzymatic methods (Christov *et al*., 1999; Xia and Cen, 1999).

Cellulases bring about the hydrolysis of cellulose, a homopolymer of -1,4 linked glucose units that comprises amorphous and crystalline regions, by synergistic action of its constituent enzymes (Bhat and Bhat, 1997; Sari *et al*., 2016). Cellulases are group of extracellular enzymes commonly employed in many industries for the hydrolysis of cellulolytic material (Mahmood *et al*., 2013; De Souza *et al*., 2015; Madadi *et al*., 2017).

β-glucosidase is generally responsible for the regulation of the whole cellulolytic process and is a rate-limiting factor during enzymatic hydrolysis of cellulose (Harhangi *et al*., 2002). Klyosov (1990) described three major types of cellulases viz., *endoglucanase* (endo-cellulase), which breaks down internal bonds to disrupt the crystalline structure of cellulose and expose individual cellulose polysaccharide chains; cellobiohydrolase (exo-cellulase) which cleaves 2-4 units from the ends of the exposed chains produced by endocellulase and cellobiase (Beta-extracellulase) which either hydrolyses cellobiose into individual monosaccharides such as glucose or depolymerizes cellulose by radical reactions.

Adverse spectrum of extensively studied mesophilic fungi degrades organic material aerobically (Falcon *et al*., 1995). Nearly, all the fungi that have been reported to produce cellulases are mesophilic fungi and the best known cellulase producers include *Trichoderma* sp., *Aspergillus* sp., *Acremonium* sp., *Penicillium* sp., *Rhizopus* sp., *F. solani* and *Chaetomium* sp., among other mesophiles (Kuzmanova *et al*., 1991; Teeri and Koivula, 1995; Schülein, 1997; Hildebrand *et al*., 2015). Cellulase enzymes have a wide range of applications, economic importance in agriculture, biotechnology, and bioenergy (Miettinen-Oinonen *et al.,* 2004; Herculano *et al*., 2011; Phitsuwan *et al*., 2012; Nathan *et al*., 2014). Cellulases have been hailed as candidates for the production of pharmaceutically relevant proteins for therapeutic use (Nevalainen and Peterson, 2014). Thus, the present research aimed at isolating and screening of new filamentous fungi producing cellulase enzymes with higher activities and efficiency.

MATERIAL AND METHODS:

Collection of soil samples:

Soil samples were collected from four different localities in Gharbiya Governorate at depth 5 cm from top, sieved through two mm sieve constituted the soil sample. The samples were dispensed into bags and were brought to the laboratory. The four soil samples were mixed thoroughly and used as one sample. The mixed soil sample was air dried and used in vitro for isolation of the fungi (Johnson *et al*., 1959).

Isolation of fungi from the collected soil samples:

Soil fungi were isolated from the collected soil samples using the dilution plate method as described by (Johnson *et al*., 1959). In a sterile 100 ml, conical flask one gram of soil sample was dissolved in 9 ml of sterilized distilled water. The flask containing soil suspension shaken well in vortex mixer for 30 mins. at 160 r.p.m. one ml of the suspension was pipetted into sterilized 100 ml Erlenmeyer flask containing 9 ml sterile distilled water and shaken few minutes. By the same way consecutive dilutions were prepared until 10^{-3} dilution which was found suitable for plating. One ml of 10^{-3} dilution was transferred and distributed over the surface of plate containing appropriate agar medium (Czapek's -Dox agar medium mixed with rose Bengal 25 µg/ml). Three replica of agar plates were prepared, and all plates were incubated at 28^{o}C for 7 days. After the incubation period, the resulting fungal colonies were purified, identified and maintained as stock cultures in sterile Czapek's-Dox agar slants at 4 ± 1^{o}C.

Identification of fungi:

Pure cultures of fungal isolates were identified according to the basis of cultural and morphological features according to consult keys given in standard books on mycology**:** Domesch *et al*. (1980) for fungi in general; Kitch and Pitti (1992) for *Aspergillus niger*; Raper and Fennel (1977) for the genus *Aspergillus*; Gilman (1957) for soil fungi; Booth (1971) for the identification of the genus *Fusarium*; Moubasher (1993) for soil fungi.

Screening for cellulolytic activity of the isolated fungi using solid media:

The isolated fungi were screened based on their ability to hydrolyse cellulose by forming diameter zone of clearance around of the active cellulolytic fungal colony according to the method of Teather and Wood (1988). Czapek's-Dox medium used in this method contained (g/l): $NaNO_3$, 2; K_2HPO_4, 1; $MgSO_4$, 0.5; KCl, 0.5; $FeSO_4$, 0.01; carboxy methyl cellulose, 1%; agar agar, 20. pH of the medium was adjusted to 4.8. After autoclaving at 121°C and 1.5 atm. pressure, the medium was poured into Petri plates and allowed to solidify and inoculated with 5 mm disc of the isolated fungus separately where three replica were used for each fungus. The plates were incubated at 28°C for seven days. After incubation, 20 ml of Congo red solution (0.1%) was added to each plate, and after 5min the Congo red solution was discarded, the plates were washed with 1 N NaCl solution and allowed to stand for 15 - 20 minutes. The clear zone around each colony for each fungus was measured.

Screening for cellulolytic activity of the isolated fungi using liquid media:

Fungal isolates that showed cellulolytic activity in screening on solid media were selected and cultured separately in 250 ml Erlenmeyer flasks each containing 100 mL of Mandel's liquid medium. The medium was prepared with the following composition (g/l): 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 and protease peptone, 0.75 with 10 g L-1 of carboxy methyl cellulose (CMC) and trace elements (mg/l): $FeSO_4.7H_2O$, 5; $MnSO_4.4H_2O$, 1.6; $ZnSO_4.7H_2O$, 1.4, and $CoCl_2.6H_2O$, 20.0 (Mandels *et al*., 1976). pH of the medium was adjusted to 4.8. After autoclaving, each flask inoculated with 5 mm disc of the selected fungus separately. All experiments for quantitative screening were done in triplicate for each selected fungus and data expressed as average values. After incubation of culture flasks at 28^{0}C for 5 days, the cultures were filtered separately using Whatman No. 1 filter paper. Each culture filtrate was used for enzyme assay.

Cellulase activity assay:

The activity of β-glucosidase (βG), filter paper cellulase (FPase) and carboxy methyl cellulase (CMCase) were studied as cellulolytic activity. The cellulase activity of each culture was measured by determining the amount of reducing sugars liberated by using a dinitrosalicylic acid (DNS) method (Mandels *et al*., 1976). One unit of filter paper cellulase

(FPase), glucosidase (βG), and carboxy methyl cellulase (CMCase) was defined as the amount of enzyme, which released µmole of reducing sugar measured as glucose per min under the assay conditions (Gilna and khaleel, 2011).

Filter paper cellulase assay:

Filter paper cellulase (FPase) activity in the culture filtrate was determined according to the (Mandels *et al*., 1976). All reaction mixtures were measured separately using spectrophotometer at 540 nm in triplicate. Filter paper cellulase (FPase) activity was measured using 0.5 ml of each culture filtrate as enzyme source was added to test tube containing Whatman No. 1 filter paper strip (1 x 6 cm; 50 mg) immersed in 1ml of 0.05 M sodium citrate buffer of pH 4.8. The reaction mixture was incubated at 45°C for 45 min. After the incubation, filter paper strips were carefully removed from the tubes using a glass rod. Then, 1 ml of dinitrosalicylic acid reagent was pipetted into each tube. This terminated all enzymatic reactions occurred in the tubes. Then, the lids of tubes were tightly closed and placed in a water bath at 95-100°C for 10 min. After this, the tubes were immediately transferred into an ice old bath and kept for few minutes. Colour change in each tube was measured by using UV spectrophotometer at 540 nm (Labomed, INC spectrophotometer).

Enzyme blank (1.0 ml of sodium citrate buffer added to 0.5 ml of culture filtrate), reagent blank (1.5 ml of sodium citrate buffer), and glucose standard solution were treated in the same way of enzyme assay. Finally, the optical absorbance readings after subtraction of enzyme and reagent blanks were compared and plotted with the standard glucose curve to find the glucose (product) concentrations **(**Miller, 1959).

Carboxy methyl cellulase assay:

Carboxy methyl cellulase (CMCase) activity was assayed using dinitrosalicylic acid (DNS) method (Mandels *et al*., 1976). All reaction mixtures were measured separately using spectrophotometer at 540 nm in triplicate. Carboxy methyl cellulase (CMCase) activity was measured using 0.5 ml of each culture filtrate was added to tube containing 0.5 ml of 1% carboxy methyl cellulose (CMC) in 0.05 M citrate buffer of pH 4.8. The reaction mixture is incubated at 45°C for 45 min. After the incubation, 1 ml of DNS reagent was pipetted into each tube. This terminated all enzymatic reactions occurred in the tube. Then, the lids of tubes were tightly closed and placed in a water bath at 95-100°C for 10 min. After this, the tubes were immediately transferred into an ice-cold bath and kept for few minutes. The color formed is read at 540 nm (Labomed, INC spectrophotometer). Enzyme blank (0.5 ml of sodium citrate buffer added to 0.5 ml of culture filtrate), reagent blank (1.0 ml of sodium citrate buffer), and glucose standard solutions were treated in exactly the same way of enzyme assay.

β- glucosidase assay:

β-glucosidase (βGase) activity was assayed using dinitrosalicylic acid (DNS) method (Mandels *et al*., 1976). All reaction mixtures were measured separately using spectrophotometer at 540 nm in triplicate. β-glucosidase (βGase) activity was measured using 0.5 ml of culture filtrate was added to 0.5 ml of 1% D-salicin in 0.05 M citrate buffer of pH 4.8. The reaction mixture is incubated at 45°C for 45 min. After the incubation, 1ml of DNS reagent was pipetted into each tube. This terminated all enzymatic reactions occurred in the tube. Then, the lids of tubes were tightly closed and placed in a water bath at 95-100°C for 10 min. These tubes were transferred immediately into an ice-cold bath and kept for few minutes. The colour formed is read at 540 nm (Labomed, INC spectrophotometer). Enzyme blank (0.5 ml of sodium citrate buffer added to 0.5 ml of culture filtrate), reagent blank (1.0 ml of sodium citrate buffer), and glucose standard solutions were treated in the same way of enzyme assay.

RESULTS:

Isolation of fungi from the collected soil samples:

Twenty fungal species belonging to five genera i.e. *Aspergillus, Penicillium, Fusarium*, *Trichoderma* and *Stachybotrys* were isolated from mixed soil samples and identified based on cultural, morphological and microscopic features of hyphae and spore's structures according to consult keys given in standard books on mycology (Table 1).

Table1.The Isolated fungi from the collected soil samples.

Isolated fungal species
Aspergillus candidus (Link)
*A. flavipes (*Bainier&Sartory) Thom&Church
*A. flavus (*Link)
*A. fumigatous (*Fresenius)
A. nidulus (Apnis)
*A. niger (*Van Tieghem)
*A. ochraceous (*K. Wilhelm)
A. sydawi (Thom and Church)
A. terreus (Thom)
A. terricola (*E.J.Marchal)*
*Fusarium nivale (*Fries*) Sorauer*
F. oxysporium (Schlechtendal)
*Pencillium biforme (*Thom*)*
P. digitatum (Saccard)
*P. funiculosom (*Thom*)*
*P. lanata (*Link)
*P. lanosum (*Westling*)*
*P. palitans (*Westling*)*
Stachybotrys atra (Link*)*
*Trichoderma harizanium (*Rifai*)*

Screening for cellulolytic activity of the isolated fungi using solid media:

The isolated fungi were screened and compared for their ability to degrade cellulose. Table 2 show that among of 20 fungal species, 16 fungal species were identified as cellulase

producer. The maximum diameter of clear zone was observed with *Aspergillus terreus* (9 cm), *A. flavipes* (8.6 cm) and *A. sydawi* (8 cm) followed by *Fusarium nivale* (7.7 cm), *P. funiculosom* (7.4 cm), *A. niger* (7 cm) and *Trichoderma harizanium* (6.9 cm). The lowest diameter of clear zone was recorded with *A. ochraceous* (3.7 cm) and *A. terricola* (3 cm).

Table 2. Screening for cellulolytic activity of the isolated fungi using solid media.

Fungal species	Clear zone diameter(cm)
Aspergillus candidus	6.00 ± 0.50
A. flavipes	8.60 ± 1.00
A. flavus	0.00 ± 0.00
A. fumigatous	0 .00 ± 0.00
A. nidulus	0 .00 ± 0.00
A. niger	7.00 ± 0.50
A. ochraceous	3.70 ± 0.30
A. sydawi	8.00 ± 1.00
A. terreus	9.00 ± 0.60
A. terricola	3.00 ± 0.50
Fusarium nivale	7.70 ± 0.70
F. oxysporium	5.30 ± 0.30
Pencillium biforme	4.60 ± 4.60
P. digitatum	5.00 ± 0.50
P. funiculosom	7.40 ± 0.40
P. lanata	4.90 ± 0.90
P. lanosum	6.40 ± 0.40
P. palitans	0.00 ± 0.00
Stachybotrys atra	4.16 ± 0.20
Trichoderma harizanium	6.90 ± 0.90

Screening for cellulolytic activities of the isolated fungi using liquid media:

The results represented in table 3 and figures 1 & 2 illustrate that the maximum activities of CMCase and βGase were recorded with *A. terreus* where CMCase activity was 43.3 u/ml and βGase activity was 33.2 u/ml followed by *A. flavipes* and *A. sydawi* where CMCase activity and βGase activity were 38.7, 29.13 u/ml and 28.4, 24.4 u/ml respectively. The other remaining isolated fungi showed various levels of CMCase activity ranging between 3.3 to 20.5 u/ml and various levels of βGase activity ranging between 4.2 to 16.5 u/ml. Also, results illustrated that the maximum activity of FPase was recorded with *P.lanosum* where FPase activity was 29.2 u/ml followed by *P. digitatum* and *A.sydawi* which were 27.95, 21.3 u/ml respectively. The other remaining isolated fungi showed various levels of FPase activity ranging between 4.06 to 19.7 u/ml. The previous results indicated the maximum total activities of CMCase, FPase and βGase were recorded with *A. terreus* where total activity was 91.3 u/ml followed by *A. flavipes* and *A. sydawi* where total activity were 83.5, 74.8 u/ml, respectively.

Table 3. cellulolytic activities of the isolated fungi using liquid media.

Fungal species	CMCase activity (u/ml)	β Gase activity (u/ml)	FPase activity (u/ml)	Total cellulolytic activities (u/ml)
Aspergillus candidus	14.20 ± 0.20	13.20 ± 0.30	21.10 ± 0.20	48.50 ± 0.70
A. flavipes	38.70 ± 0.30	28.40 ± 0.20	16.40 ± 0.50	83.50 ± 1.00
A. niger	15.90 ± 0.40	14.90 ± 0.40	07.60 ± 0.40	38.40 ± 1.20
A. ochraceous	03.90 ± 0.30	10.80 ± 0.50	04.06 ± 0.30	18.76± 1.30
A. sydawi	29.10 ± 0.40	24.40 ± 0.40	21.30 ± 0.60	74.80 ± 1.40
A. terreus	43.30 ± 0.30	33.20 ± 0.10	14.80 ± 0.40	91.30 ± 0.80
A. terricola	03.30 ± 0.30	04.20 ± 0.20	05.30 ± 0.10	12.80 ± 0.60
Fusarium nivale	25.80 ± 0.20	08.00 ± 0.30	13.60 ± 0.70	48.00 ± 1.20
F. oxysporium	13.90 ± 0.30	08.87 ± 0.40	08.86 ± 0.80	31.63 ± 1.50
Pencillium biforme	11.60 ± 0.40	16.50 ± 0.60	06.64 ± 1.00	34.74 ± 2.00
P. digitatum	11.71 ± 0.15	11.10 ± 0.70	27.95 ± 0.60	50.76 ± 1.45
P. funiculosom	20.50 ± 0.50	15.40 ± 1.00	19.70 ± 0.30	55.60 ± 1.80
P. lanata	11.70 ± 0.40	05.90 ± 0.30	15.50 ± 0.20	33.10 ± 0.90
P. lanosum	14.90 ± 0.30	09.90 ± 0.40	29.20 ± 0.50	54.00 ± 1.20
Stachybotrys atra	06.40 ± 0.20	04.60 ± 0.20	04.30 ± 0.60	15.30 ± 1.00
Trichoderma harizanium	15.40 ± 0.40	14.70 ± 0.10	15.90 ± 0.30	46.00 ± 0.80

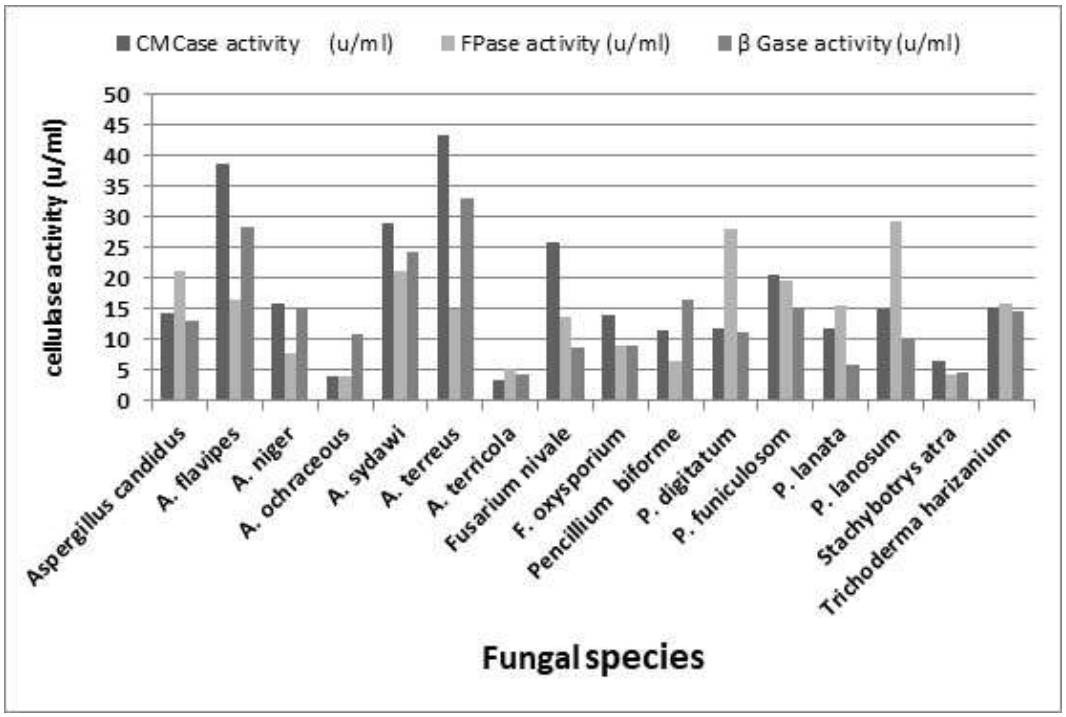

Fig. 1. Cellulolytic activities of the isolated fungi using liquid media.

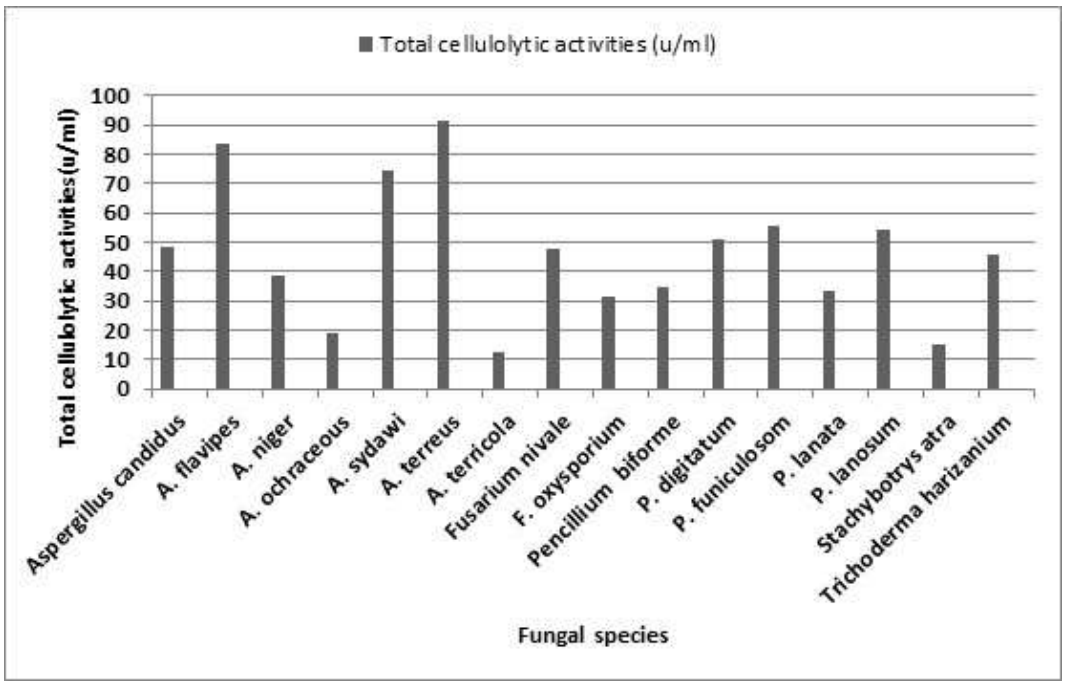

Fig. 2. Total cellulolytic activities of the isolated fungi using liquid media.

DISCUSSION:

Cellulose is world's most abundant organic substance (Ruttloff, 1987) and comprises a major storage form of photosynthesized glucose. Cellulose annual biosynthesis by both land plants and marine algae occurs at a rate of 0.85×10^{11} tons per annum. It is the major component of biomass energy (Scott *et al*., 1987).

Cellulases are a group of hydrolytic enzymes capable of hydrolysing the most abundant organic polymer. Cellulolytic enzymes also play an important role in natural biodegradation processes in which plant lignocellulosic materials are efficiently degraded by cellulolytic fungi, bacteria, actinomycetes and protozoa (Pečiulytė, 2007). However, fungi are well known agents of decomposition of organic matter, in general, and of cellulases have enormous potential in industries and are used in food, beverages, textile, laundry, paper and pulp industries etc. (Walsh, 2002; Cavaco-Paulo and Gübitz, 2003; Jahangeer *et al*., 2005).

Lytic enzymes are a major importance is the protoplast production (Davis, 1985; Bhat, 2000) for tissue culture and plant metabolites production. The demand for more thermostable, highly active and specific

cellulases is on the increase; therefore, cellulase systems of local fungi should be investigated keeping in view the importance and application of the cellulases, this study was designed to screen the native fungal isolates as hyper producers of cellulase. As fungi can utilize wide range of cellulosic waste, therefore, interest in the search for cellulase producing novel fungal species is increasing cellulosic substrate in particular (Lynd *et al*., 2002). The present study was carried out for isolating, screening and identification of efficient cellulase producing fungi from soil. Twenty fungal isolates were identified on the basis of morphological characteristics, i.e. colony coloration, pigmentation on the reverse side of plate, colony morphology, spore structure and arrangement, etc. (Cooney and Emerson, 1964). The same result was obtained by Khokhar *et al*. (2012) who reported that fungal species belonging to three genera i.e. *Trichoderm*, *Aspergillus* and *Penicillium* were isolated from soil.

The screenings of cellulase producing fungi were performed on CMC agar plates flooded with Congo red and washed with NaCl. Depending on the diameter of clear zone around the colony, sixteen fungal stains were identified as cellulase producing fungi. The results recorded that the highest cellulase producing fungal species were *A. terreus*, *A. flavipes* and *A. sydawi* followed by *F. nivale*, *P. Funiculosom*, A*. niger* and *Trichoderma harizanium.* The same result was obtained by Updegraff (2004) and Kuczek-Turpeinen *et al*. (2005) who reported that cellulolytic activity of tested *Aspergillus Trichoderma,* and *Penicillium* species were found relatively higher. *Trichoderma* spp. and *Aspergillus* spp. are two potential cellulase producers as reported by *Lynd et al.* (2002).

In screening of cellulases activity by the isolated fungi using liquid media, the results illustrated that the maximum activities of CMCase and βGase were recorded with *Aspergillus terreus* where CMCase activity was 43.3 u/ml and βGase activity was 33.2 u/ml followed by *A. flavipes* and *A. sydawi* where CMCase activity and βGase activity were 38.7, 29.13 u/ml and 28.4, 24.4 u/ml respectively. Also, results illustrated that the maximum activity of FPase was recorded with *P. lanosum* where FPase activity was 29.2 u/ml followed by *P. digitatum* and *A. sydawi* were 27.95, 21.3 u/ml respectively. Our results agreed with Li *et al.* (2010) who reported that *Trichoderma* spp. and *Aspergillus* spp. are thought to be cellulase producers, and crude enzymes produced by these microorganisms are commercially available for agricultural use. Yalpani (1987) reported that the most common and most effective cellulase producers are *Trichoderm spp, Fusarium* sp, *Aspergillus sp* and *Penicillium* sp.

Cellulose was abundant in nature and microorganisms capable of converting cellulose into simple carbohydrates had been discovered for awaited to be converted into more valuable products used for mankind several decades. However, needs for newly isolated celluloytic microbes were still remained. *Aspergillus terreus*, *A. flavipes* and *A. sydawi* were the most active cellulase producer which could be readily used in many applications such as animal foods and a feed stock for production of valuable organic compounds (Nishida *et al.,* 2007).

CONCLUSION:

The present study concluded that *Aspergillus terreus*, A*. flavipes* and A*. sydawi* were the highest producer to cellulase enzyme compared to the other isolates. So, these cellulase positive isolates may be an integral part of future work to develop good cellulases or produce efficient cellulase producing systems such as microbial consortia which can be used for industry.

REFERENCES:

Barrios E. 2007. Soil biota, ecosystem services and land productivity. Ecol. Econ., 64(2): 269- 285.

Bhat M, Bhat S. 1997. Cellulose degrading enzymes and potential industrial applications. Biotechnol. Adv., 15(3-4): 583-620.

Bhat MK. 2000. Cellulases and related enzymes in biotechnology. Biotechnol. Adv., 18(5): 355–383.

Boer W, Folman LB, Summerbell RC, Boddy L. 2005. Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiol. Rev., 29(4): 79-811.

Booth C. 1971. The genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, UK, pp. 237.

Cavaco-Paulo A, Gübitz G. 2003. Catalysis and processing. In: "Textile Processing with Enzymes. (Cavaco-Paulo A., Gübitz G. (ed.)". England, Woodhead Publishing Ltd., pp. 240.

Christov LP, Sazkacs G, Balakrishnan H. 1999. Production, partial characterization and use of fungal cellulase free xylanases in pulp bleaching. Process Biochem., 34(5): 511- 517.

Cooney DC, Emerson R. 1964. Thermophilic fungi: an account of their biology, activities and classification. San Francisco; London: W.H. Freeman and Co., pp. 200.

Davis B. 1985. Factors influencing protoplas isolation. In: "Fungal protoplasts: applications in biochemistry and genetics. (Peberdy JF, Ferenizy L. eds.)". New York: Marcel Dekker, pp. 45-71.

De Souza PM, Bittencourt ML, Caprara CC, De Freitas M, De Almeida RP, Silveira D, Fonseca YM, Ferreira Filho EX, Pessoa Junior A, Magalhães PO. 2015. A

biotechnology perspective of fungal proteases. Braz. J. Microbiol., 46(2): 337-346.

Domesch KH, Gams W, Traute-Heidi A. 1980. Compendium of soil fungi. I. Academic Press, London, pp. 66-420.

Falcon MA, Rodriguez A, Carnicero A. 1995. Isolation of microorganism with lignintransformation potential from soil of Tenerife Island. Soil Biol. Biochem., 27(2): 121-126.

Gilman JC. 1957. A manual of soil fungi. Ames, Iowa: Iowa State University Press, USA, pp. 450.

Gilna VV, Khaleel KM. 2011. Cellulase enzyme activity of *Aspergillus fumigatus* from mangrove soil on lignocellulosic substrate. Recent Res. Sci. Technol., 3(1):132- 134.

Harhangi HR, Steenbakkers PJ, Akhmanova A, Jetten MS, van der Drift C, Op den Camp HJ. 2002. A highly expressed family 1-glucosidase with transglycosylation capacity from the anaerobic fungus Piromyces sp. E2. Biochim. Biophys. Acta, 1574(3): 293-303.

Herculano PN, Porto TS, Moreira KA, Pinto GAS, Souza-Motta CM, Porto AL. 2011. Cellulase production by Aspergillus japonicus URM5620 using waste from castor bean (*Ricinus communis* L.) under solid-state fermentation. Appl. Biochem. Biotechnol.,165(3-4): 1057-1067.

Hildebrand A, Szewczyk E, Lin H, Kasuga T, Fan Z. 2015. Engineering *Neurospora crassa* for improved cellobiose and cellobionate production. Appl. Environ. Microbiol., 81(2): 597-603.

Jahangeer S, Khan N, Sohail M, Shahzad S, Ahmad A, Khan, SA. 2005. Screening and characterization of fungal cellulases isolated from the native environmental source. Pak. J. Bot., 37(3): 739-748.

Johnson LE, Bond CJ, Fribourg H. 1959. Methods for studying soil microflora-plant disease relationships. Minneapolis: Burgess Pub. Co., pp.178.

Khokhar I, Saleem MH, Mushtaq S, Mukhtar I. 2012. Isolation and screening of highly cellulolytic filamentous fungi. J. Appl. Sci. Environ. Mange, 16(3) 223–226.

Kitch MA, Pitt JI. 1992. A laboratory guide to the common *Aspergillus species* and their teleomorphs. CSIRO, Sydney. Common Wealth Scientific and Industrial Research Organisation, Division of Food Processing, pp. 116.

Klyosov AA. 1990. Trends in biochemistry and enzymology of cellulose degradation. Biochemistry, 29(47): 10577-10585.

Kuczek-Turpeinen B, Maijala P, Tuomela M, Hofrichter M, Hatakka A. 2005. Endoclucanase activity of compost-dweling fungus *Paecilomyces inflatus* is stimulated by humic acids and other low molecular mass aromatics. World J. Microbiol. Biotechnol., 21: 1603–1609.

Kuzmanova S, Vandeska E, Dimitrovski A. 1991. Production of mycelial protein and cellulolytic enzymes from food waste. J. Ind. Microbiol., 7(4): 257-261.

Li XH, Yang HJ, Roy B, Park EY, Jiang LJ, Wang D, Miao YG. 2010. Enhanced cellulase production of the *Trichoderma viride* mutated by microwave and ultraviolet. Microbiol. Res., 165(3): 190-198.

Lynd LR, Weimer PJ, van-Zyl WH, Pretorius IS. 2002. Microbial cellulose utilization: fundamentals and biotechnology. Microbiol. Molecul. Biol. Rev., 66(3): 506-577.

Madadi M, Tu Y, Abbas A. 2017. Recent status on enzymatic saccharification of lignocellulosic biomass for bioethanol production. Electron. J. Biol., 13(2): 135-143.

Mahmood RT, Asad MJ, Mehboob N, Mushtaq M, Gulfraz M, Asgher M, Minhas NM, Hadri SH. 2013. Production, purification, and characterization of exoglucanase by *Aspergillus fumigatus*. Appl. Biochem. Biotechnol., 170(4): 895-908.

Mandels M, Andreotti R, Roche C. 1976. Measurement of saccharifying cellulase. Biotechnol. Bioeng. Symp., 6: 21-33.

Miettinen-Oinonen A, Londesborough J, Joutsjoki V, Lantto R, Vehmaanperä J. 2004. Three cellulases from *Melanocarpus albomyces* with applications in the textile industry treatment at neutral pH. Enzyme Microb. Technol., 34(3-4): 332-341.

Miller GL. 1959. Use of Dinitrosalicylic acid for determination of reducing sugar. Anal. Chem., 31(3): 426-429.

Moubasher AH. 1993. Soil fungi of Qatar and other Arab countries. Centre for Scientific and Applied Research, University of Qatar, pp. 566.

Nathan VK, Rani ME, Rathinasamy G, Dhiraviam KN, Jayavel S. 2014. Process optimization and production kinetics for cellulase production by *Trichoderma viride* VKF3. Springerplus, 3: 92.

Nevalainen H, Peterson R. 2014. Making recombinant proteins in filamentous fungi - are we expecting too much? Front. Microbiol., 5(57): 75.

Ng TB. 2004. Peptides and proteins from fungi. Peptides, 25(6): 1055-1073.

Nishida Y, Suzuki KI, Kumagai Y, Tanaka H, Inoue A, Ojima T. 2007. Isolation and primary structure of a cellulase from the Japanese sea urchin *Strongylocentrotus nudus*. Biochemie, 89(8): 1002–1011.

Orgiazzi A, Lumini E, Nilsson RH, Girlanda M, Vizzini A. 2012. Unravelling soil fungal communities from different Mediterranean land use backgrounds. PLoS One, 7(4): e34847.

Pečiulytė D. 2007. Isolation of cellulolytic fungi from waste paper gradual recycling materials. Ekologija, 53(4): 11–18.

Phitsuwan P, Laohakunjit N, Kerdchoechuen O, Kyu KL, Ratanakhanokchai K. 2013. Potential applications of cellulases in agriculture, biotechnology, and bioenergy. Folia Microbiol. (Praha), 58(2): 163-176.

Raper KB, Fennell DI. 1977. The genus *Aspergillus* Robert EK. Publish, Co., Huntington, New York, USA.

Ruttloff H. 1987. Impact of biotechnology on food and nutrition. In: “Food Biotechnology. (Knorr D. Eds)”. Marcel Dekker, Inc.

Sari SA, Pangstuti A, Susilowati A, Purwoko TJ, Mahajoeno E, Hidayat W, Mardhena I, Panuntun DF, Kurniawati D, Anitasari R. 2016.

Cellulolytic and hemicellulolytic bacteria from the gut of *Oryctes rhinoceros* larvae. Biodiversitas, 17(1): 70-75.

Schülein M. 1997. Enzymatic properties of cellulases from *Humicola insolens*. J. Biotechnol., 57(1-3): 71- 81.

Scott D, Hammer FE, Sczalkucki TJ. 1987. Bioconversion; Enzyme technology. In: "Food Biotechnology. (Knorr D. Eds)". Marcel Dekker, Inc.

Sinsabaugh RL, Lauber CL, Weintraub MN, Ahmed B, Allison SD, Crenshaw C, Contosta AR, Cusack D, Frey S, Gallo ME, Gartner TB, Hobbie SE, Holland K, Keeler BL, Powers JS, Stursova M, Takacs-Vesbach C, Waldrop MP, Wallenstein MD, Zak DR, Zeglin LH. 2008. Stoichiometry of soil enzyme activity at global scale. Ecol. Lett., 11(11): 1252- 1264.

Teather RM, Wood PJ. 1982. Use of Congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. Appl. Environ Microbiol., 43(4): 777-780.

Teeri T, Koivula A. 1995. Cellulose degradation by native and engineered fungal cellulases. Carbohydr. Eur., 12: 28-33.

Updegraff DM. 2004. Utilization of cellulose from waste paper by *Myrothecium verrucaria*. Biotechnol. Bioeng., 13(1): 77–97.

Walsh G. 2002. Industrial enzymes: proteases and carbohydrases. In: Proteins; Biochem. Biotechnol. John Wiley and Sons. Ltd.

Xia L, Cen P. 1999. Cellulase production by solid fermentation on lignocellulosic waste from the xylose industry. Process Biochem., 34(9): 909- 912.

Yalpani M. 1987. Development and prospect in enzymatic biopolymer modification. In: Industrial Polysaccharide Elsevier, (Vol. 3).

الأنشطة السليلولازية لبعض الفطريات الخيطية من التربة

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فى الدراسة الحالية تم عزل وتعريف 20 نوعا من الفطريات المعزولة من عينات تربه مختلفة مجمعة من مدينه طنطا، محافظة الغربية، مصر. وتلك الفطريات تنتمي إلى خمسة أجناس هي اسبرجيللس، بنسليوم، فيوزاريوم، ترايكوديرما وستاكيبتر وتم اختبار قدرة هذه الأنواع على انتاج إنزيم السليلولاز فى الوسط الصلب بواسطة صبغ أحمر الكونجو تركيز 0.1٪ وذلك بإضافته الى الاطباق المزروعة بالفطريات بعد 7 ايام من التحضين. وتم قياس قطر منطقة التحلل الظاهرة حول مستعمرة نمو الفطريات بكل طبق والتي أوضحت نشاط كل فطرة لإنتاج الإنزيم. وسجلت النتائج ستة عشر نوعا من الفطريات إنتاجًا لإنزيم السليولاز وقد تم اختبار نشاط هذه الفطريات المختارة لإنزيمات الكربوكسى مثيل سليلولاز، بيتا جليكوسيداز وسليولاز المحلل لورقة الترشيح وذلك باستخدام الوسط السائل. وقد أظهرت جميع الفطريات المختبرة نشاط كبير للإنزيمات الثلاثة. وقد سجل أعلى نشاط لإنزيمين الكربوكسى مثيل سليلولاز وبيتا جليكوسيداز مع فطرة اسبرجيللس ترييس في حين أظهرت فطرة بنيسيليوم لانوسوم أعلى نشاط لإنزيم السليولاز المحلل لورقة الترشيح. وسجلت النتائج أن الحد الأقصى للنشاط الكلى السليلوزي كان مع أسبرجيللس تيريوس تليها اسبرجيللس الفلافيبس واسبرجيلليس سيداوي.