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Research Article

Cellulolytic activity of cellulose-decomposing fungi isolated from Aswan hot desert soil, Egypt

Mohamed M. Ahmed[a], Soad A. El-Zayat[a] and Magdi A. El-Sayed[a,b*]

[a] Botany Department, Faculty of Sciences, Aswan University, Aswan 81528, Egypt

[b]Unit of Environmental Studies and Development, Aswan university, Aswan 81528, Egypt

***Corresponding authors:** magdiel_sayed@aswu.edu.eg (M. El-Sayed)

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Abstract

Fungi are well known agents for decomposition of organic matter in common and of cellulosic material, in particular; therefore they considered as the main cellulose producing microorganisms. The present study was aimed to isolate and screen the ability of cellulolytic fungal strains from desert soil living under environmental stress to produce cellulolytic enzymes. Forty-three fungal strains in addition to two varieties were isolated from different sites at Aswan University campus and were able to degrade the cellulose with variable extents. Fungal species were grouped as high, moderate and low cellulolytic activity. The cellulase activity was assayed by carboxymethyl-cellulose "CMase" assay (endoglucanases).The highest cellulase producing isolates were *Fusarium dimerum* and *Rhizopus oryzae*. The optimum parameters for high activity of cellulase enzyme are pH 5, 35°C at 9th, 11 thdays for *Fusarium dimerum* and *Rhizopus oryzae*, respectively.

Keywords: Desert soil, fungal isolates, cellulolytic activity chloroiodid of zinc, CMC, DNS

Introduction

Fungi are a group of microorganisms that are widespread in the environment, especially in the soil (Boer et al. 2005). Fungi are one of the dominant groups that have strong influencing structure and function of the ecosystem, thus they playing an important role in several ecological services (Orgiazzi et al. 2012). Cellulose is the most abundant renewable carbon source on earth (Andersen, 2007). It is a potential raw material for the production of food, fuels and chemicals (Spano et al.1976). Cellulosic material degradation can be achieved by Chemical or enzymatic method or by the combination of both methods (Bailey & Poutanen, 1987; Christov et al. 1999; Spreint & Antranikian, 1990; Xia & Cen, 1999). There are varieties of microorganisms in nature used to increase the productivity of cellulolytic activities and to develop more efficient enzymes (Andersen, 2007). These organisms produce cellobiohydrolaseexo-glucanase (C1) which responsible for the initial attack of native cellulose, endo-glucanase (Cx) which complete the

degradation to short–chain, cellobiose and β-glucosidase which act mainly on cellobiose to produce glucose (Klyosov, 1990). These activities act synergistically to hydrolyse crystalline cellulose (Reese, 1976). Fungi are known agents for degrading cellulosic material as a part of organic matter (Lynd et al. 2002). Several fungi such as members of *Aspergillus*, *Penicillium*, *Trichoderma* and some other moulds of *Mucors* and hyphomycetes produced cellulolytic enzymes (Miller, 1972; Mandels & Reese, 1985; Lakshmikant & Mathur, 1990).
The present study aims to isolate and screen the cellulolytic fungal strains from hot desert soil and explore their ability to produce a high amount of sugar for further potential industrial uses such as bioehtanol production.

Material and Methods

Collection of soil samples

Ten sites were chosen for collection of soil samples, from the desert location of Aswan University campus (Figure 1). The climatic environments in this region ranged between moderately cold dry winter to very hot dry summer (Ali et al. 2018). Soil collected in clean plastic bags at a depth of 5-20 cm from different sites of the various habitats including clay and sandy soil.

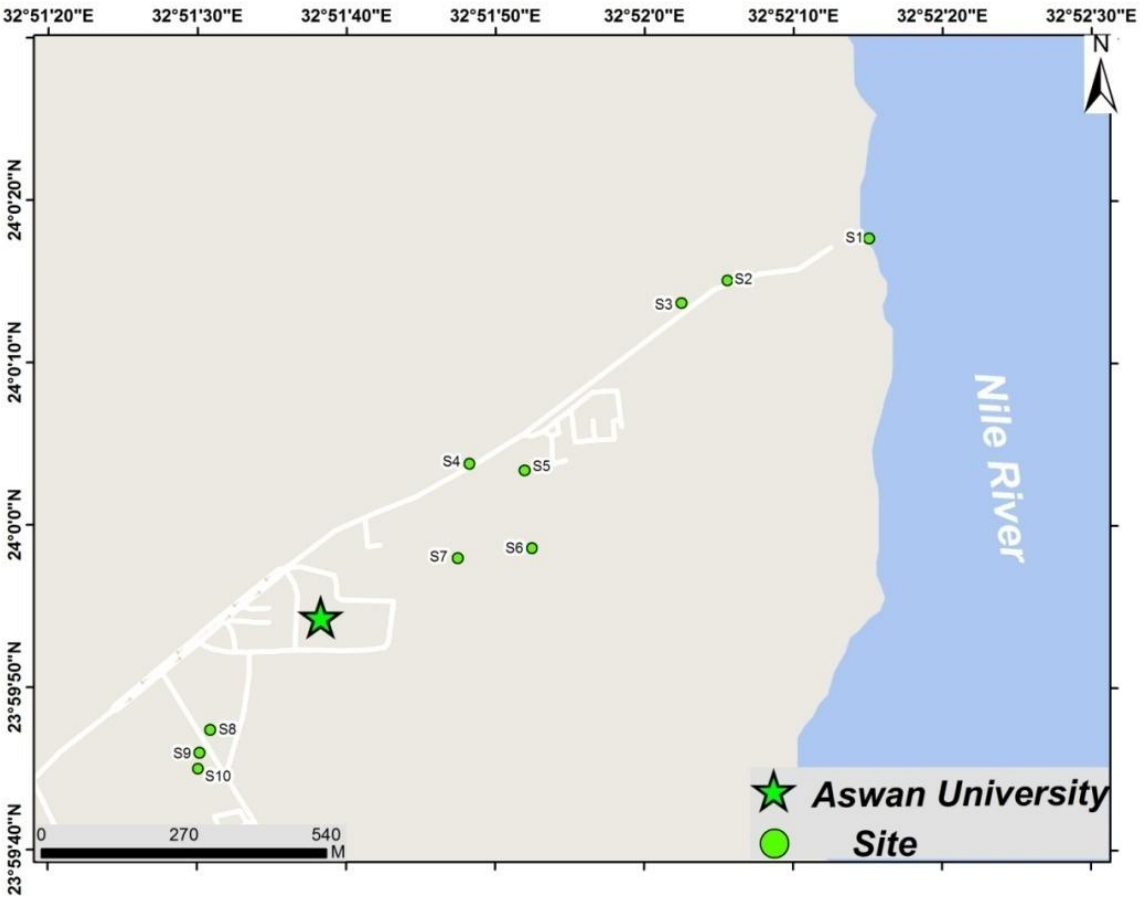

Figure 1. Map showing the sample sites in Aswan University campus

Isolation of cellulose decomposing fungi

The dilution plate method was used for the estimation of cellulose decomposing fungi for soil samples, as described by (Johnson et al. 1959). Modified Czapek's Medium was employed in which cellulose (20g/l) replaced glucose (10g/l). Cellulose is an easily utilized carbohydrate, recommended for the isolation of cellulose decomposing fungi. Rose Bengal (1/15000) and chloramphenicol were used as bacteriostatic agents (Smith & Dawson,1944; Al-Doory, 1980). This medium is probably as good as any or better than most others for isolating cellulose-decomposing fungi by dilution plate method. Three plates were used for each sample. The samples were incubated at 28±1 ̊C for seven days. Pure culture was transferred on to the PDA (potato dextrose agar) slants for further studies.

Morphological identification of isolates

Fungal isolates were identified to the species on the basis of morphological characteristics (Pitt, 1979; Domschet al. 1980; Samson et al. 2000) using microscopic examination.

Screening of cellulolytic fungi

Utilization of soluble cellulose by the tested isolates was detected by growing 45 species were isolated on the modified medium of (Prasad& Verma,1979), which contained (g/l):$(NH_4)_2SO_4$ 2.1 g; carboxymethyle cellulose(CMC) 10g, KH_2PO_4,1.0g; $MgSO_4.7H_2O$,0.5g and agar, 20g. The acetate buffer was used for adjusted pH to 7.0. The reagent was chloroiodide of zinc. The development of clear zones around colonies indicates the production of C_x cellulase enzyme.

Reducing sugars assay

The amount of reducing sugars released by the enzymatic hydrolysis was estimated by dinitrosalicylic acid (DNS) method (Miller, 1959). The sample (1.0 mL) was mixed with 2 mL of DNS reagent. The tubes were then heated in a boiling water bathfor 5 min, after cooling at room temperature; the absorbance was measured at 540 nm. The amount of the released reducing sugar was calculated by using a standard curve of glucose, and expressed as mg/ml.

Improving cellulase production

Since *Fusarium dimerum* 29F3f and *Rhizopus oryzae* 49b8r headed the list of the most active cellulase producing in this work, they were chosen for further studies to achieve the most favorable environmental conditions for CMase production. The studied pH range was 4-7, the temperature range was 25-45 °C, and cultivation time studied was 1-11 days. The experiments were performed in triplicate.

Statistical analysis

Data presented on the average of three replicates as means ± standard error obtained from independent experiments and Data of environmental conditions represented as a heatmap on R program (R i386 3.4.3).

Results

In the present study, 43 fungal strains belonging to 15 genera in addition to two varieties were isolated from desert soil at ten different sites in Aswan University campus as cellulose decomposing fungi according to their ability to grown on czapek'e medium supplemented with 20g/l cellulose as the only carbon source. The isolated fungal cultures were identified up to species based on their morphological and microscopic features, and the result represented in the table, 1. In preliminary screening, all isolated cellulose-decomposing fungal strains from different soil samples were screened for their cellulolytic activity by plating them on carboxymethyl cellulose agar medium and looking for CMC clearing zones. The appearance of the clear zone around the colony when chloroiodide of zinc solution was added was strong evidence that fungi produced cellulase to degrade cellulose (Fig.2). Table 2, showed that selected strains were able to produce cellulase with variable extents. The highest cellulolytic activity was detected in twelve isolates namely: *Rhizopus oryzae* 49b8r (4.50±0.00cm), *Fusarium oxysporum* 28a2 (3.90±0.10cm), *Cochliobolus lunatus* 42I4 (3.85±0.15cm), *Emericella nidulans var. lata* 20c4 (3.75±0.15cm), *Alternaria alternata* 29F3a (3.75±0.05cm), *Emericella nidulans var. lata* 22c2 (3.73±0.08cm), *Monodictys levies* 12c1 (3.70±0.00cm), *Humicola grisea* 17b13 (3.70±0.10cm), *Fusarium dimerum* 29F3f (3.70±0.00), *Trichoderma hamatum* 45a3 (3.70±0.00cm), *Aspergillus carbonarius* 18c5 (3.50±0.00cm) and *Aspergillus terreus var. africanus* 36h2 (3.50±0.00cm), (Fig3). Twelve fungal strains were also selected for quantification assay accordingly their highest cellulolytic activity obtained during plate screening.

The results in (Table 2), (Fig. 4) showed that *Rhizopus oryzae* and *Fusarium dimerum* had the highest activity for cellulase enzyme. They were chosen for further studies to achieve the most favorable environmental conditions for CMase production. The two organisms were cultured under different temperature (25, 35 and 45°C) for 1-11 days interval pH (4-7), the culture medium was supplemented with carbroxymethyl cellulose as the only carbon source. The optimum cultivation period for *Fuarium dimerum* 29F3f was 9 days and the optimum cultivation period for *Rhizopus oryzae* 49b8r was 11 days. *Fusarium dimerum* 29F3f and *Rhizopus oryzae* 49b8r (Figure, 5) exhibited their best activity when cultivation was carried out at 35°C where the amount of reducing sugars released by the enzyme fluctuated between 1.87 mg/l and 1.94 mg/l for the *Fusarium dimerum* 29F3f and *Rhizopus oryzae* 49b8r respectively. Strains also exhibited moderate activity at 25°C, in contrast, the two fungi strains studied exhibited very low amounts of cellulase or absent at 45°C at all incubation periods. The two strains were able to produce cellulase in a better pH range for pH 4 to pH 7, but their highest activity were observed at pH 5(Figure, 5).

Table1. Fungal strains isolated from soil samples at 10 selected sites in Aswan University campus.

Fungi	Strain code	Sites									
		S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Acremoniumfurcatum(F.et V. Moreau) ex W. Gams 1969	27b2		+								
Acremoniumfurcatum(F.et V. Moreau) ex W. Gams 1969	26b3		+								
Alternariaalternata(Fries) Keissler 1912	29F3a						+				
*Aspergillusaculeatus*Iizuka 1953	10e3					+					
*Aspergillusawamori*Nakazawa 1907	30F2						+				
Aspergilluscarbonarius(Bainier) Thom 1916	18c5			+							
*Aspergillusterreus var. africanus*Fennel, Raper 1955	35I2									+	
*Aspergillusterreus var. africanus*Fennel, Raper 1955	41K2										+
*Aspergillusterreus var. africanus*Fennel, Raper 1955	36h2								+		
Aspergillusoryzae(Ahlburg) Cohn 1884	43b10		+								
Aspergillusoryzae (Ahlburg) Cohn 1884	48a1	+									
*Aspergillusterreus*Thom 1918 var. terreus	50b11		+								
Aspergillususstus(Bainier) Thom & Church 1926	34h1								+		
Aspergillusversicolor(Vuillemin) Tiraboschi 1926	37k4										+
Aspergillusversicolor(Vuillemin) Tiraboschi 1926	13b4		+								
*Chaetomiumglobosum*Kunze 1817	21g1							+			
*Chaetomiumglobosum*Kunze 1817	44b9		+								
*Cochlioboluslunatus*Nelson&Haasis 1964	42I4									+	
Emericellanidulans(Eidam) Vuillemin 1927 *var. lata*Subramanian 1972	25F1						+				
Emericellanidulans (Eidam) Vuillemin 1927 var. lata Subramanian 1972	24e2					+					
Emericellanidulans (Eidam) Vuillemin 1927 var. lata Subramanian 1972	22c2			+							
Emericellanidulans (Eidam) Vuillemin 1927 var. lata Subramanian 1972	20c4			+							
Emericellanidulans(Eidam) Vuillemin 1927 var. nidulans	39h2								+		
Emericellanidulans(Eidam) Vuillemin 1927 var. nidulans	40K5										+
Emericellanidulans(Eidam) Vuillemin 1927 var. nidulans	19c3			+							
Emericellaquadrilineata(Thom &Raper) Benjamin 1955	38I3									+	

Emericellaquadrilineata(Thom &Raper) Benjamin 1955	32k2										+
Emericellaviolacea(Fennell &Raper) Malloch& Cain 1972	23e1					+					
Emericellaviolacea(Fennell &Raper) Malloch& Cain 1972	9e4					+					
*Fusariumdimerum*Penzig 1882	29F3f						+				
*Fusariumoxysporum*Schlechtendal 1824	28a2	+									
*Gliocladiumvirens*Miller, Giddens& Foster 1957	11d1				+						
*Humicolagrisea*Traaen 1914	17b13		+								
Monodictys levies (Wiltshire) S. Hughes 1958	12c1			+							
Myrotheciumverrucaria(Albertini&Schweinitz) Ditmar 1813	16b6		+								
*Phomaeubrina*Sacc. 1879	31I1									+	
*Phomaexigua*Desmazieres(1849)	15b1		+								
*Phomamedicaginis*Malbr.&Roum. 1886	49b8w		+								
*Rhizopusoryzae*Went&Prinsen-Geerligs 1895	49b8r		+								
Stachybotryschartarum(Ehrenberg)Hughes 1958	14b7		+								
Stachybotryschartarum(Ehrenberg)Hughes 1958	49b8s		+								
Trichodermahamatum(Bonorden) Bainier 1906	45a3	+									
Trichodermahamatum(Bonorden) Bainier 1906	51x1							+			
*Trichodermaharzianum*Rifai 1969	52a4	+									
*Trichodermaharzianum*Rifai 1969	46a5	+									

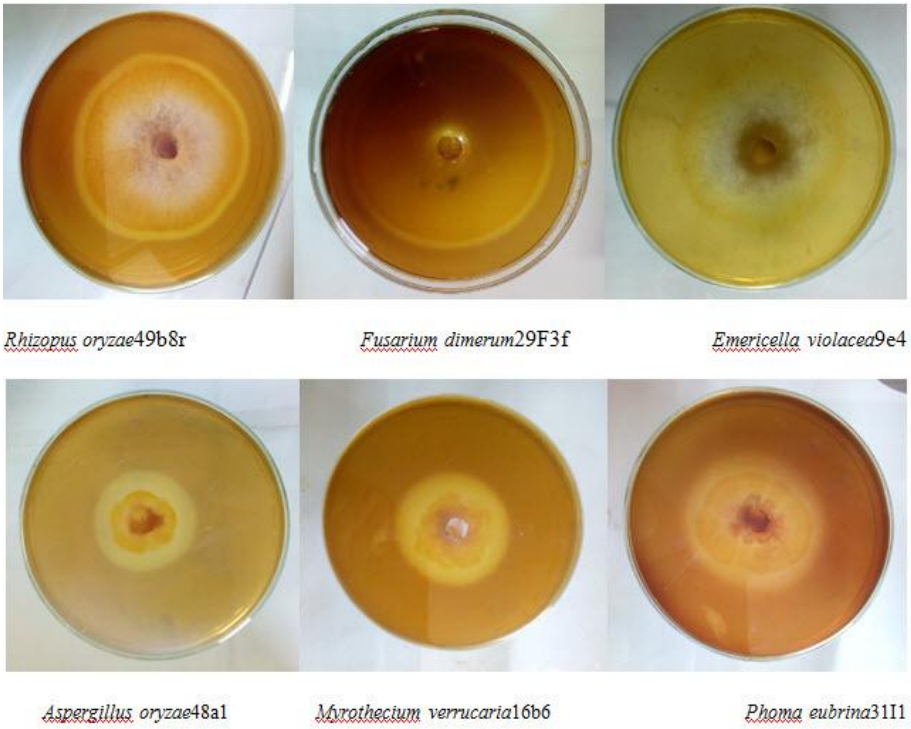

Figure 2. Cellulose hydrolysis of some isolated fungi

Table 2. cellulolytic activity of selected fungal strains

Strain no	diameter of zone(mm)	Cellulase activity(mg/ml)
49b8r*Rhizopusoryzae*	4.50±0.00	1.61±0.04
28a2*Fusarium oxysporum*	3.90±0.10	1.52±0.05
42I4*Cochlioboluslunatus*	3.85±0.15	0.40±0.05
20c4*Emericellanidulans* var. lata	3.75±0.15	0.69±0.11
29F3a*Alternariaalternate*	3.75±0.05	0.77±0.05
22c2*Emericellanidulans* var. lata	3.73±0.08	0.78±0.01
12c1*Monodictys levies*	3.70±0.00	0.56±0.13
17b13*Humicolagrisea*	3.70±0.10	1.11±0.06
29F3f*Fusariumdimerum*	3.70±0.00	1.65±0.06
45a3*Trichodermahamatum*	3.70±0.00	1.40±0.10
18c5*Aspergilluscarbonarius*	3.50±0.00	0.04±0.00
36h2*Aspergillusterreus var. africanus*	3.50±0.00	0.50±0.06

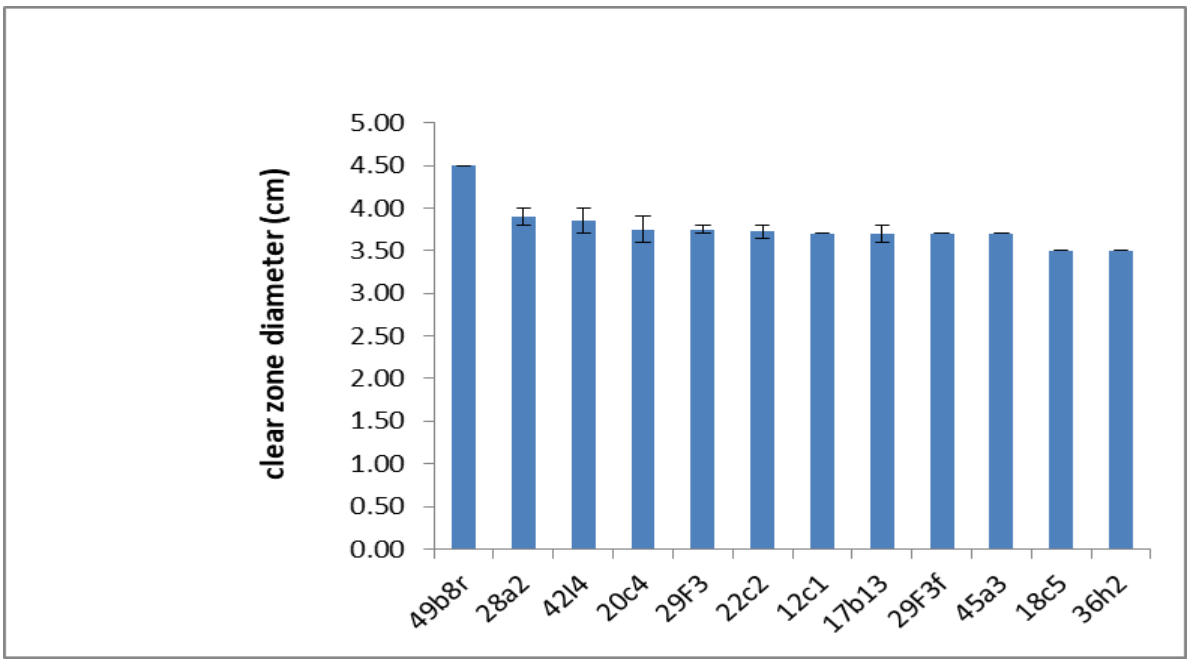

Figure 3. The highest cellulolytic activity by fungal strains isolated from soil in Aswan University campus.

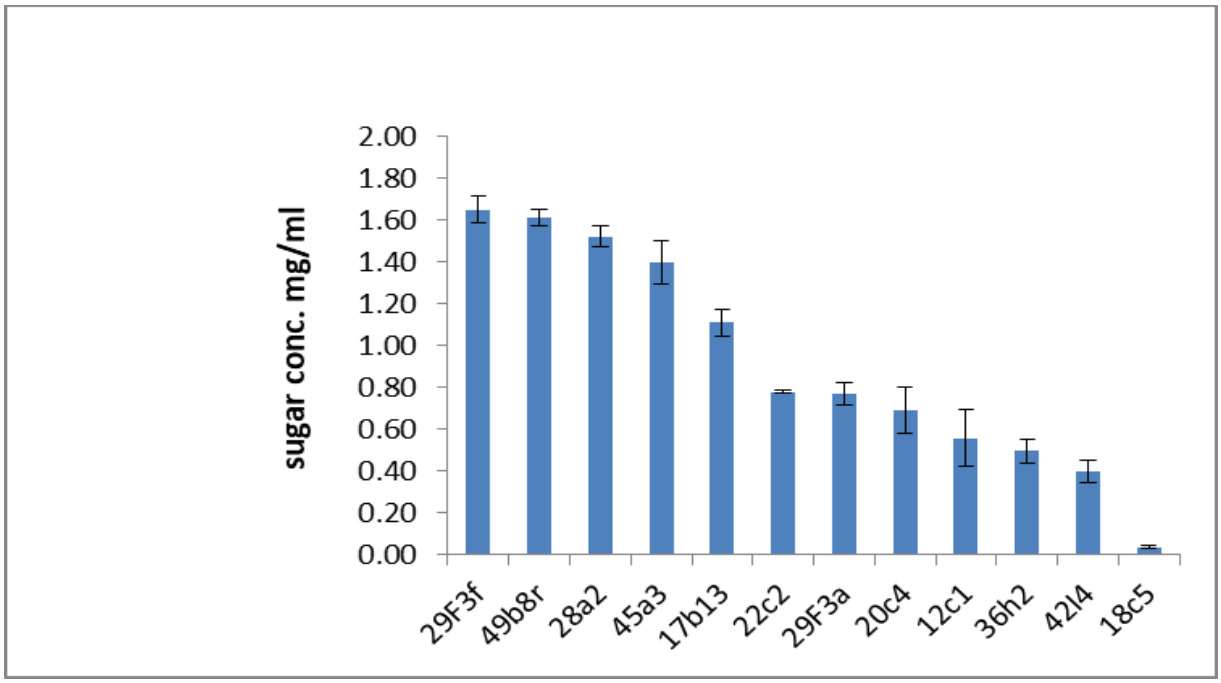

Figure 4. Cellulolytic activity of selected fungal strains

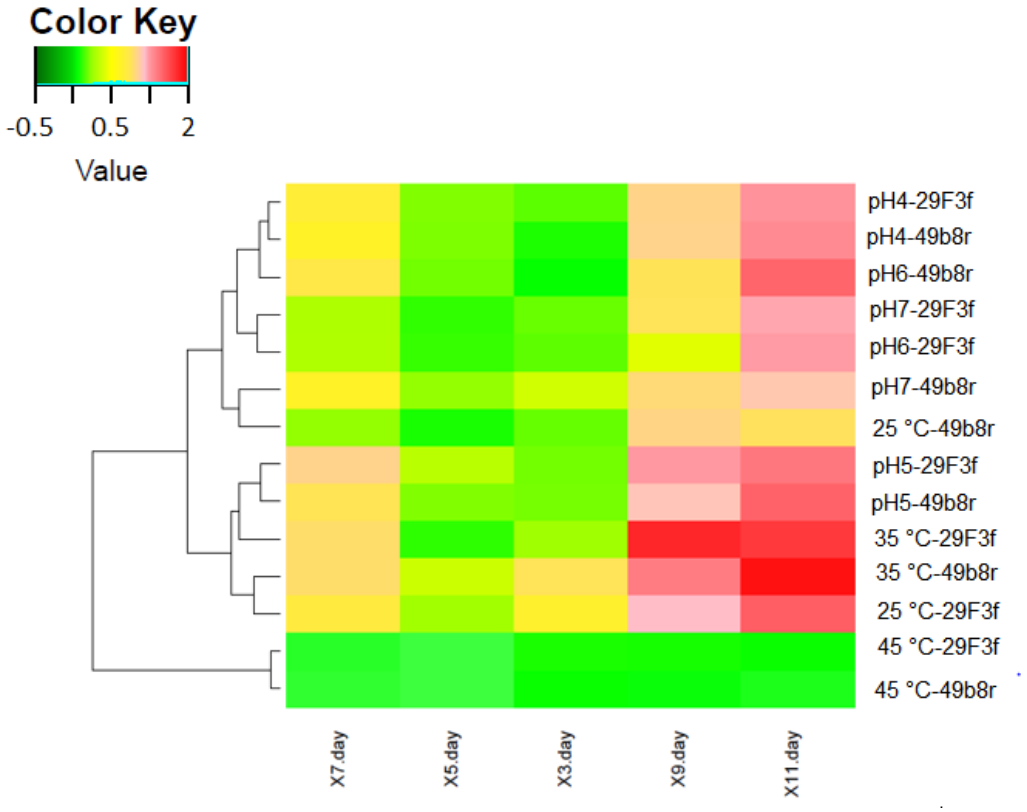

Figure 5. Heatmap illustrates the effect of pH value and temperature with incubation period on *Fusariumdimerum* 29F3f and *Rhizopusoryzae* 49b8r.

Discussion

This study mainly concentrates on isolation and screening the cellulolytic fungal strains from different sites in Aswan university campus and to evaluate their hydrolytic potential for their possible future applications. Forty-three strains and two species varieties representing 15 genera were collected from the soil of ten sites on plates of cellulose- Czapek's agar at 28°C. Most of species recovered on the plate of cellulose agar (Table, 1) were reported to be cellulose decomposing but different degrees (Macris, 1984; Moubasher&mazen, 1991; Naveenkumar & Thippeswamy, 2013; Reddyet al. 2014). The cellulose activity of fungal strains was confirmed by chloroiodide of zinc decoloration and also quantitatively with dinitro-salicylic acid reagent method. Cellulases are a group of hydrolytic enzymes capable of hydrolysis of cellulose to smaller sugar component like glucose units. Lynd et al. (2002) reported that the screened fungal strains would be useful for further studies by enzyme producers. In our study clearly represents that *Rhizopus oryzae* 49b8r (4.50±0.00cm), *Fusarium oxysporum* 28a2 (3.90±0.10cm), *Cochliobolus lunatus* 42I4 (3.85±0.15cm), *Emericella nidulans var. lata* 20c4 (3.75±0.15cm), *Alternaria alternata* 29F3a (3.75±0.05cm), *Emericella nidulans var. lata* 22c2 (3.73±0.08cm), *Monodictys levies* 12c1 (3.70±0.00cm), *Humicola grisea* 17b13 (3.70±0.10cm), *Fusarium*

dimerum 29F3f (3.70±0.00), *Trichoderma hamatum* 45a3 (3.70±0.00cm), *Aspergillus carbonarius* 18c5 (3.50±0.00cm) and *Aspergillus terreus var. africanus* 36h2 (3.50±0.00cm) were shown to be possessing higher cellulose degrading ability. Moderate and low cellulose producer were also recorded among other fungi. Deyab (2006) investigating the ability of 48 isolates to produce extracellular cellulases (C_1, C_x) on solid media and found that *Apergillus oryzae, A. flavus, Peacilomyces variotii, fusarium moniliforme, Eurotium repens* and *Trichoderma hamatum* were high producers. Similarly, reports were made by other authors; (Sadaf Jahangeer et al. 2005) reported that majority of *Aspergillus* and *Penicillium* spp. were found to possess cellulolytic activity . *Trichoderma* and *Aspergillus* were thought to be Cellulase producers and crude enzyme produced by these organisms is commercially available for agricultural use (Li X et al.2010). Gautamet al. (2010) have been reported that *Aspergillus fumigates* was the highest cellulolytic activity, and very low cellulase activity showed by *Humicola* sp., *Torula* sp., these fungi isolated from municipal soil waste. Reddyet al. (2014) isolated 23 fungal strains from different soil samples collected in selected sites and among which nine isolates, found to have cellulolytic activity. The highest cellulase producing isolates was *Aspergillus niger, A. flavus* and the least was *Trichoderma* sp. Cellulase is a complex of three types of enzymatic complexes namely endoglucanase also called carboxymethylcellulase, exoglucanase and β-glucosidase (Iqbal HMN et al.2011). Cellulase activity of the enzyme was measured by cellulose assay. Cellulase assay was done by DNS method (3,5 dinitrosalicylic acid). Besides *Fusarium dimerum* (29F3f) and *Rhizopus oryzae* (49b8r), the other organisms like *Fusarium oxysporum* (28a2), *Trichderma hamatum* (45a3) and *Humicola grisea* (17b13) head the list of most active cellulase production fungi in this study (Table 2). Highly significant values of correlation were found exo-glucanase and endoglucanase activities suggesting that these enzymes may be co-regulated in the above organisms. A wide range of *Aspergillus* sp. has been identified to possess all components of cellulases complex (vries & visser 2001) which is in agreement with the present study. Production of cellulase by fungal isolates requires an optimal condition for their growth which leads to the release of enzymes. During optimization studies, the enzyme activity was analysed only after 3th day of incubation to allow the optimal fungal growth to be achieved. It was reported earlier that the enzyme production by the fungi started after a lag period of 24 hours or more, and the activities reached to maximal levels within 5-7 Days of incubation (Gomes, et al. 2006).

The optimum cultivation period for *Fusarium dimerum* 29F3f was 9 days and the optimum cultivation period for *Rhizopus oryzae* 49b8r was 11 days, that agreement with many literatures (i.e., 2-21 days; Dhillonet al. 2011; Dhillonet al. 2012; Faten & El Atyabeer, 2013). Santos et al. (2017) found that the best results, of incubation time for four marine fungi strains were three days, this incubation period is shorter than our period and shorter than some periods found in many reports. Cultivation time has an important effect on cellulase production; even comparing a wild strain and its mutants they may have different periods for biosynthizing and secreting cellulases according to the type of solid nutrients used and incubation conditions (Raghuwanshiet al.2014).

For the temperature effect on cellulase production by *Fusarium dimerum* 29F3f and *Rhizopus oryzae* 49b8r showed relative activity at 25°C and 35°C but it was best suited to 35°C (Figure 5). Our results were nearly similar with the results of (Hung et al. 2005) in that these fungi strains are mesophyll microorganisms with better growth between 20°C and 40°C. The most important factor among all the physical variables affecting the production of enzymes and

metabolites is usually the incubation temperature because the enzymatic activity is sensitive to temperature (Krishna, 2005)

The cellulase produced by *Fusarium dimerum* 29F3f and *Rhizopus oryzae* 49b8r showed small change in the activity along all the pH range studied, but still has a maximum release of reducing sugar at pH 5 (Figure 5); therefore our study showed that the two tested strains can biosynthesize cellulases in both acidic and neutral conditions. *Aspergillus sydowii* CBMAI935 has the maximum release of reducing sugars at pH 4.8 (Santos et al. 2017) and can be named as acidic while *Bacillus licheniformis* AUOI with optimum pH 8-11 and can be named as alkaline (Annamalaiet al. 2012, 2014). The optimization of the enzyme production clearly demonstrated the impact of the process parameters on the yield of cellulolytic enzymes and beneficial to be utilized for industrial application.

Conclusion

In the present study, it could be that the fungal strains isolated from desert soil in Aswan University campus possess different degrees of cellulolytic activity. Among these fungal strains twelve strains were noticed to show the maximum zone of hydrolysis (4.5 cm) of carboxymethyl cellulose. *Rhizopusoryzae*49b8r also produced soluble sugar (1.94 mg/ml) by DNS Method at 35°C in 11 days. The fungal isolates in the present investigation need to be further studied in depth for their cellulolytic potential of conversion of cellulosic waste material into useful products.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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