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Optimization of cellulases production under fermintation conditions by halophilic *Aspergillus niger*

Assmaa Abd El-kader [a, *], Nabil Abu El-kassem Abu-Hamed [a], Mohamed Eweis [b], Tharwat E.E. Radwan [a]

[a] *Botany Department, Faculty of Science, Fayoum Universtity.*
[b] *Botany and Microbiology Department, Faculty of Science, Cairo Universtity.*

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ABSTRACT

A halophilic black *Aspergillus niger* isolate has showen high cellulolytic activity and was identified by both morphological and molecular identification, and the result was halophilic *Aspergillus niger* AUMC 15543. The later showed high stability after cultivated for about 12 generations. It was used for cellulases production under submerged fermentation (Smf) conditions. It has been found that optimum temperature and pH yielded maximum cellulases enzymes, dry weight, extracellular protein and soluble sugars production by *A. niger* were 30°C for culture temperature, and pH5, by yielding maximum dry weight, extracellular protein and soluble sugars of (1170.333 mg/50ml, 1065 mg/ml and 585.333 mg/ml) respectively and maximum titers of cellulases enzymes FPase, CMCase and ß- glucosidase of (1.704 IU/ml, 0.852667 IU/ml and 2.121333 IU/ml) respectively. Culture medium with lactose 1% addition yielded higher titers of FPase, CMCase and ß- glucosidase of (2.106333 IU/ml, 1.111667 IU/ml and 2.615333 IU/ml) respectively and best dry weight, extracellular protein and soluble sugars of (1489.333 mg/50ml, 1376 mg/ml and 886.6667 mg/ml) compared to control, glucose, maltose and sucrose respectively.

1. Introduction

The use of plant biomass with high starch continent is primarily for human consumption, raising food security concerns [1]. Plant biomass derived from agricultural waste, such as wheat straw, corn straw, husk, and so on, can also be used to produce second generation biofuels [2].They have advantages like being low-cost because their production is big enough and a significant amount of waste has been observed in the form of agricultural residues, sugarcane bagasse, Stover, and so on [3].This biomass is primarily composed of lignocellulose, which is made up of lignin, hemicellulose, and cellulose. Cellulose accounts for the majority of total biomass (30-50%). The hemicelluloses and lignin composition varies. The complex composition of lignocellulose and the crystalline nature of cellulose, which makes it difficult to hydrolyze, limit the use of this energy-rich source. Cellulose has a crystalline structure composed of chains held together by hydrogen bonding and β-1,4 linkages, preventing even water entry and making degradation tough [4]. Many fungi in nature produce enzymes that can hydrolysis cellulose. To complete cellulose hydrolysis, three key components of the cellulase enzyme system are required: Endoglucanases, Exoglucanases or cellobiohydrolases and ß-glucosidase. Endoglucanases attack the noncrystalline region of glucose, while cellobiohydrolases or exoglucanases attack the crystalline region [5]. These generate cellobiose units, which are then digested by ß-glucosidase.

Cellulases rank third in the global enzyme market. By 2015, the global market for industrial enzymes had grown to $4.4 billion. They work in a number of industries, including textiles, paper and pulp, food and feed, and detergents [6, 7]. Even after widespread use, their high cost remains an issue for the industry.

Although both bacteria and fungi have been found to produce a variety of cellulases, fungal cellulases have received more attention due to their greater adaptability. Fungal cellulases have all three cellulase enzyme system components, whereas the bacterial cellulase system lacks one of the cellulolytic enzymes, Filter Paperases (FPases) [8]. The present study is being undertaken to optimize physical and chemical factors (incubation temperature, pH and various carbon sources) for maximum cellulases enzymes synthesis by halophilic *A. niger* isolated from Qarun Lake employing submerged fermentation (Smf) conditions.

* Corresponding author.
E-mail address: aa699@fayoum.edu.eg (*A.Abd-Elkader*); Tel.: +2 01003151228
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2. Materials and methods

All the experiments of this study were conducted with only Czapek' glucose liquid medium because it gives higher yields of cellulase production by the isolated fungus than any other media. Czapek-glucose agar medium containing g/l dis.H_2O (20 glucose, 2NaNO3, 1KH_2PO_4, 0.5 KCl, 0.5 $MgSO_4.7H_2O$, 0.001 $FeSO_4.7H_2O$, 0.001 $MnCl_2$, 0.001 $ZnSO_4$,20 Agar-agar,15 NaCl and 1000 dis.H_2O [9].

2.1. Collection and isolation

Five soil samples were taken from the Qarun Lake Beach. Samples were placed in clean plastic bags and were transferred to the lab. 300 g of each soil sample was cleaned from gravels and organic matters kept in a refrigerator later for fungal flora isolation. To make a 100 ml soil suspension, 10 g of each sample was added to 90 ml of distilled water.Serial dilutions for soil suspension were made (0.1 mM, 0.01 mM, 0.001 mM, 0.0001 mM, 0.00001 mM and 0.000001 mM). 1ml of each dilution was taken and was spread over the surface of Czapek's glucose agar medium with addition of 15% NaCl and final pH 5 pre-autoclaved in an autoclave and then poured into one sterilized 10 cm Petri dish for 7 days, plates were incubated at 30 °C. The same for water samples collected from the lake. 10 ml of each sample centrifuged at 3000 rpm. Then 1 ml of each centrifuged sample was spread over the surface of Czapek's glucose agar medium with addition of 30 g NaCl /l and pH 5. Pre-autoclaved in an autoclave at 121°c, 1atm pressure for 20 minutes [10], then poured in sterilized one use 10 cm Petri plates. For 5 days, petri plates were incubated at 28 °C. The developing colonies after 5 days from both soil and water samples were inoculated in new Czapek's glucose agar medium plates of 15% salinity and pH5 For 20 minutes, the media were pre-autoclaved at 121°C and 1 atm pressure in an autoclave. The halophilic fungal isolates were stored at 4°C on Czapek's glucose agar slants and sub-cultured once a month. They later were tested for their ability of cellulase enzymes production.

2.2. Morphological identification of halophilic fungal isolate species

For morphological identification, Czapek Yeast agar, a medium regarded as a standard for *Aspergillus* characterization, was utilised [11]. The medium was enriched with 15% NaCl. For 8 days, infested plates were kept in the dark at 25+1 °C, and examined for colony features including as growth rate, sporulation extent, mycelial colour, cleistothecia production, and the backside colony colour Scanning. Electron Microscope (SEM) was used for microscopic structures examination. A 1 cm^2 piece of an 8-day-old fungal culture plate had been utilised as a specimen. The sample was submerged in 2.5% glutaraldehyde and stored at 4 °C overnight. The tissue was rinsed three times with 0.1 M phosphate buffer before being submerged in 0.5% Osmium Tetroxide solution and washed in 0.1 M phosphate buffer. To dehydrate the tissue, it was submerged in 30%, 50%, 70%, 80%, 90%, 95%, and 100% alcohol. The material was placed in a 1:2 solution of hexamethyldisilazane (HMDS): 100% ethanol (EtOH) and stored for 20 minutes. A new solution of 2:1 HMDS:EtOH was added, and the solution was then put in 100% HMDS. Each step was performed twice for a total of 20 minutes. After drying overnight, the sample was mounted on a stub and sputtered with gold-palladium before being mounted on a Carl Zeiss EVO18 Scanning Microscope. The specimen was examined under low vacuum for characteristics such as conidial head form, seriation, morphology, and ornamentation [11].

2.3. Molecular identification of fungal isolates

Czapek's agar (CZA) medium was used to cultivate the fungal isolates, which were incubated at 28°C for 5 days [12]. DNA extraction was performed at the Molecular Biology Research Unit, Assiut University using Patho-gene-spin DNA/RNA extraction kit (Intron Biotechnology Company, Korea). Polymerase chain reaction (PCR) and sequencing were done with the help of SolGent Company, Daejeon, South Korea. The ITS regions of rRNA gene for samples 3 and F8 were amplified using the universal primers ITS1 (forward) and ITS4 (reverse) which were incorporated in the reaction mixture. Primers have the following composition: ITS1 (5' - TCCGTAGGTGAA CCTGCGG - 3'), and ITS4 (5'- TCCTCCGCTTATTGATATGC-3'). The purified PCR product was sequenced with the same primers with the incorporation of ddNTPs in the reaction mixture [13].

2.4. Qualitative screening for cellulolytic activity of fungal isolates isolated from Qarun Lake

In liquid medium, the cellulolytic activity and the growth of the halophilic fungal isolate was visually examined in Czapek-Dox liquid medium with 1 % wheat bran, pH5 and 15% salinity. After transferring 50 mL of the latter medium to 100 mL Erlenmeyer flasks, it was autoclaved in an autoclave at 121°C for 20 minutes. After 7 days, 14 days, and 21 days, the flasks containing media were inoculated with 5 mm agar discs of the fungal isolate and incubated at 30 °C. Flasks containing media without inoculation by the fungal isolate were used as control. For each treatment (incubation period), three replicates were used. Degradation of wheat bran was observed using the following scale: (-) no degradation of wheat bran, (+) mild degradation of wheat bran, (++) moderate degradation of wheat bran, (+++) very high degradation of wheat bran, and (++++) full degradation of wheat bran.

2.5. Biomass determination

The dry weight of fungal biomass was evaluated by collecting mycelium from a 50 ml culture. (liquid culture media) by filtration with pre-weighing Whattman filter paper no 1. Mycelium was then rinsed with tap water and dried in a 70 °C oven before being weighed on a weighing scale. The weight of the Whattman filter paper no 1 was cut from the final weight.

2.6. Determination of extracellular protein secretions

Filtrate was collected in sterile flasks, while the developing culture of the fungal isolate in the flask produced from different tests was filtered using Whatman No 1 filter paper at suitable intervals. An aliquot of this culture filtrate, diluted appropriately, was used to calculate the soluble protein content [13]. As a protein standard, bovine serum albumin was used. 5 mL of an alkaline solution was added to aliquots of filter. After 10 minutes, 0.5

ml of adequately diluted Folin-Ciocalteau solution was applied, resulting in a blue color after 30 minutes. The color developed was measured using a Spectrophotometer at 700 nm. The following reagents are prepared. Na_2CO_3 2%, $CuSO_4.5H_2O$ 1%, NaOH 4%, Potassium sodium tartrate $C_4H_4O_6KNa•4H_2O$) 1% and Folin-Ciocalteau reagent [14]. A volume of 0.1% copper sulfate ($CuSO_4$) in Potassium sodium tartrate ($C_4H_4O_6KNa•4H_2O$) 1% was mixed just before use (reagent A). A test tube was filled with 1 ml of the tested culture filtrate.The mixture was then treated with 0.2 mL of NaOH and 0.5 mL of freshly produced reagent A. For 10 minutes, the mixture was left at room temperature. Later, 0.1 ml of Folin-Ciocalteau reagent was added. For half an hour, the test tubes were left at room temperature in a dark environment. The ended mixture's volume was completed to 5 ml. After incubation, the absorbance was measured at 700 nm. Extracellular protein secretions were calculated using a casein curve.

2.7. Determination of soluble sugars

Filtrate was collected in sterile flasks while the developing culture of the fungal isolate in the flask produced from different tests was filtered using Whatman No 1 filter paper at suitable intervals. An aliquot of this culture filtrate, with an appropriate dilution factor, was utilized to calculate total soluble sugar concentration using the method of Lorenz [15]. Glucose was used as a sugar standard.

2.8. Enzmes assay

Cellulase enzymes released in the culture medium of the investigated fungus were calculated using techniques provided by [16].

2.8.1. An assay for filter paper

It is a measure of total cellulolytic action induced by the combined action of different enzyme elements detected in culture medium filtrate. It was determined according to the method of Mary and Weber [17], taking a glucose calibration curve as a reference. To measure activity, a Whatman filter paper strip (1x6 cm) weighing 50 mg was submerged in one ml of 0.05 M sodium citrate buffer (pH 4.8) in a 50°C water bath. The prior combination was incubated at 50°C for 60 minutes with appropriate aliquots of enzyme source. The same as mentioned above, enzyme blanks (without enzyme) were made. After incubating for 60 minutes at 50°C, 3ml of 3,5-Dinitrosalicylic acid (DNS) had been added. All blanks and standards of glucose samples were severely boiled for 5 minutes in a boiling water bath [15]. All samples were cooled, and the color generated in tubes was measured in a Spectrophotometer at 540 nm. Cellulase enzyme activity was measured in filter paper units. the amount of enzyme necessary to liberate one mole of reducing sugar per minute from filter paper called One filter paper unit (FPU).

2.8.2. Carbxymethyle cellulose assay

0.2 M acetate buffer (pH 5.0) was added to 1.0 ml of 1% carboxymethyl cellulose. For 20 minutes, the resultant mixture was incubated in a 50°C water bath. The latter was incubated for one hour at 50°C in a water bath with 0.2 ml of the examined culture filtrate [18]. Simultaneously, a suitable control (without substrate or enzyme) was produced. The 3, 5-dinitrosalicylic acid technique was used to determine the reducing sugars generated in the mixture [15]. The combination was then treated with 3 mL of 3, 5-Dinitrosalicylic acid reagent, and the color produced was measured at absorbance of 540 nm by a Spectrophotometer with a glucose calibration curve as a reference. The amount of enzyme that yielded one mole of reducing sugar per minute was classified as endoglucanase activity.

2.8.3. An assay for ß-D-glucosidase

The activity of ß-D-glucosidase activity was measured in the culture filtrate under investigation by the method of [19]. In 0.05 M citrate buffer pH 4.8, 0.2 ml of 5 mM p-nitrophenyl ß-D glucopyranoside (PNPG) and 0.2 ml of diluted enzyme solution with controls (no enzyme or substrate) were dissolved. They were then incubated at 50°C for 30 minutes. The reaction was stopped by adding to it 4 ml of 0.05 M NaOH-glycine buffer (pH 10.6). At absorbance of 405 nm, the liberated yellow color of the p-nitro phenol was measured using a spectrophotometer. A glucose calibration curve was taken as a reference [20]. The amount of enzyme yielded one mole of p-nitrophenol per minute under standard test conditions was classified as ß-D-glucosidase activity.

2.9. Effect of temperature on halophilic fungal isolate growth and cellulase enzymes production

To find out how incubation temperature affects cellulase enzymes production, 50 ml of sterile Czapek-Dox liquid medium devoid of agar-agar with 1% (W/V) cellulose (replacing glucose) and 15% NaCl was autoclaved for 20 minutes at 121°C and inoculated with 5 mm agar disc of the tested fungus. The flasks were incubated for 14 days at different temperatures (20, 30, and 40°C) in a rotary shaker of 180 rpm to ensure that all enzymes were distributed evenly throughout the liquid media. After 14 days of growth on the medium, in the culture filtrate of the studied fungus, the biomass of the fungus, extracellular protein levels, total soluble sugars, and activity of particular enzyme parts of the cellulase enzymes were measured.

2.10. The effect of pH on fungal growth and cellulases production

Czapek-Dox liquid medium containing 1% cellulose and 15% NaCl was made with varied initial pH of (3, 5, and 7) by adding 1 N HCl or 1 N NaOH before being distributed into 250 ml Erlenmeyer flasks and autoclaved in an autoclave at 121°C for 20 minutes. To ensure that the enzymes were distributed evenly throughout the liquid medium, the flasks were inoculated with a 5 mm agar disc of the tested fungus and incubated for 14 days at 30 °C with a salinity of 15% in a rotary shaker of 180 rpm as stated above. After only 14 days of development on the medium, the biomass of the fungus, extracellular protein levels, total soluble sugars, and activity of particular enzyme parts of the cellulase enzymes were measured.

2.11. Effect of carbon source on fungal growth and cellulases production

Czapek-Dox liquid media containing 1% cellulose was made with 15% salinity, the PH was adjusted to 5, and 50 ml was distributed into 100ml Erlenmeyer flasks before autoclaving for 20 minutes at 121°C. External carbon sources (lactose, glucose, maltose, and sucrose) were sterilized separately and added separately to the fermentation media to a final concentration of 1% (W/V), after which they were infected with a 5mm agar disc of the tested fungus. To ensure that the enzymes were distributed evenly throughout the liquid medium, the flasks were incubated for 14 days at 30°C in a rotary shaker of 180 rpm as indicated above..

3. Results and discussion

Halophilic *A. niger* had showed the highest cellulolytic activity and stability was identified by both morphological and molecular identification, and the result was halophilic *A. niger* AUMC 15543. The later was taken to be tested for best incubation temperature, pH and carbon sources. Isolate 1 was *A. niger* AUMC 15543 (555 letters). As halophilic isolate number 1 *A. niger* AUMC 15543 (555 letters) (black fungus) gave high ability of wheat bran and cellulolytic activity, it was selected for further experimentation and studies.

With a synthesis rate of 1010 tons per year, cellulose is the most common polymer in the biosphere [3; 21; 22]. A wide range of microorganisms can produce cellulases; however, fungi are thought to be most effective against the most abundant natural polymer. In this context, many Trichoderma sp. strains have received the greatest attention. Several investigations indicated that *A. spergillus* sp. produces substantial quantities of endoglucanase and ß-glucosidase. However, its low exoglucanase levels combined with high protein levels make it a suitable organism for industrial use. In this study we isolated halophilic fungal isolates from Qarun Lake, by culturing samples from Qarun Lake over the surface of Czapek's glucose agar medium with the addition of 15% NaCl pre-autoclaved in an autoclave and then poured in sterilized one use 10 cm Petri plates. The later was incubated for 7 days at 30 °c, as demonstrated in the above method. After collection and isolation of *A. niger*, it was identified by microscopic examination at Assiut University Mycological center (AUMC). The later was tested for it is cellulolytic activity and had showed the high cellulolytic activity. *A. niger* was identified by molecular identification at Assiut University Mycological center (AUMC), and the result was halophilic *A. niger* AUMC 15543. The later was taken to be tested for best temperature, pH and carbon source.

The temperature of incubation is an important element in enzyme synthesis [23]. In studying the effect of temperature on halophilic *A. niger* dry weight, extracellular protein secretions, soluble sugars and cellulase enzymes production. It was found that the culture incubated at 30 °C gave the highest dry weight (1170.333 mg/50ml). The culture broth also contained higher extracellular protein content (1065 mg/ml) and (590.3333 mg/ml) total soluble sugars content. This culture filtrate also produced higher activity in terms of FPase with (1.709 IU/ml), CMCase with (0.851 IU/ml) and ß-glucosidase with (2.0363 IU/ml). Maximum activities were obtained with biomass and maximum production rates were observed at the exponential growth phase, suggesting that cellulases of the fungal species halophilic *A.ngier* were produced as primary metabolite. Poor growth of the culture at 20°C was reflected by recovery of the low dry weight of *A.niger* (571.66 mg/50ml). Under the same conditions, secretion of extracellular protein in smaller amounts (556 mg/ml) and total soluble sugar content (309.33mg/ml) were observed in the culture incubated at 20°C. Lower cellulases activities of FPase (0.955 IU/ml), CMCase (0.034667 IU/ml) and ß-glucosidase (0.813 IU/ml) were recorded with filtrate derived from the culture incubated at 20°C. This present study is similar to [24] shown in (Tables 1 and 2).

Through studying the effect of pH on dry weight, extracellular protein secretions, soluble sugars and cellulase enzymes production, it was found that *A. niger* produced maximum growth and extracellular protein secretions but less soluble sugars when cultured at pH 5.0 as it gave the highest yields of fungal dry weight of (1170.333 mg/50ml), extracellular protein (1065 mg/ml) and total soluble sugars (585.333 mg/ml). It gave also high activities of FPase 1.704 IU/ml), CMCase(0.852667 IU/ml) and (3-glucosidase (2.121333 IU/ml), as it reached the recorded at Table 4, similar to the study in Kassim and Ghazi [25]. Low activities of the later desired enzymes occurred in culture initial medium initial pH adjusted at pH 3.0 and pH 7.0, but the extracellular protein secretions were higher at pH 7.0 when compared to pH 3.0. The total soluble sugars content was more at pH 3.0 than pH 5.0 and pH 7.0. Similarly Uma [26] had made approve that acidic pH 5.5 was optimal for cellulase production. In the present study, pH measurement of the culture medium at the end of 14-day incubation indicated the occurrence of changes in the pH of the medium and the initial pH of 3.0, 5.0 and 7.0 dropped down to 1.5, 3.5 and 5.0 respectively within 14- day. Low production of biomass in the medium with an initial pH of 3.0 and in turn could secrete low amounts of extracellular protein including cellulolytic enzymes. Utilization of soluble sugars at lower rate by low biomass and releasing of soluble sugars in extremely acidic conditions by chemical means may be attributed to more accumulation of soluble sugars in the medium at pH 3.0, as during metabolic utilization of cellulose by *A.niger* acidic metabolites have been formed resulting in lowering of the pH of the broth and utilization of these acidic metabolites at subsequent interval resulted in recovery of pH of the broth. Controling of pH during the process of growth improved the cellulase production by *A. niger*, by addition of NaOH after 2 days of incubation under shaking conditions (Tables 3 and 4).

In studying the effect of different types of carbon sources on dry weight, extracellular protein secretions, soluble sugars and cellulase enzymes production produced by the halophilic *A.niger*, the later produced maximum growth, extracellular protein secretions and total soluble sugars with external carbon source lactose, as it gave highest yields of fungal dry weight of (1489.333 mg/50ml), extracellular protein (1376 mg/ml) and total soluble sugars (886.6667 mg/ml). It also gave high levels of FPase (2.106333 IU/ml), CMCase (1.111667 IU/ml) and ß-glucosidase (2.615333 IU/ml) recorded at Table 6. Followed by glucose then maltose compared with control. This is almost similar to [27, 28]. However Sucrose 1% made repression of dry weight, extracellular protein and soluble sugar, it also gave low activities of FPase, CMCase and ß-glucosidase recorded in Tables 5 and 6.

GTAACCAAAATCGGTGCTGCTTTCTGGTACGTATACAACTGCCGTTGGATTGGGGATGGAACATCGTC
TCTTAGGCTATCTCAGCTTGAGTTCAGATGTTGTCCATTAGGTACATGCTATCGGTCTAAGAACACGTC
TAACAATTCAACAGGCAGACCATCTCTGGCGAGCACGGCCTTGACGGCTCCGGTGTGTAAGTGCAACT
TTTTCACACCTCTCAATTGGTCAACAATGGGCAAAGGGTTGGGTCTTCTGACACGCAGGATAGTTACA
ATGGCACCTCCGACCTCCAGCTGGAGCGCATGAACGTCTACTTCAACGAGGTGAGATCCATCGGACCT
TGGCTTTTACACGACAATATCATCAATGTCCTAATCACTTCAGCAGGCTAGCGGTAACAAGTATGTTCC
TCGTGCCGTCCTCGTCGACCTCGAGCCCGGTACCATGGACGCCGTCCGTGCCGGTCCTTTCGGCCAGCT
CTTCCGCCCCGACAACTTCGTCTTCGGCCAGTCCGGTGCTGGTAACAACTGGGCCAAGGGTCACTACA
CTGAGGGT

Fig 1 : *Aspergillus niger* AUMC 15543

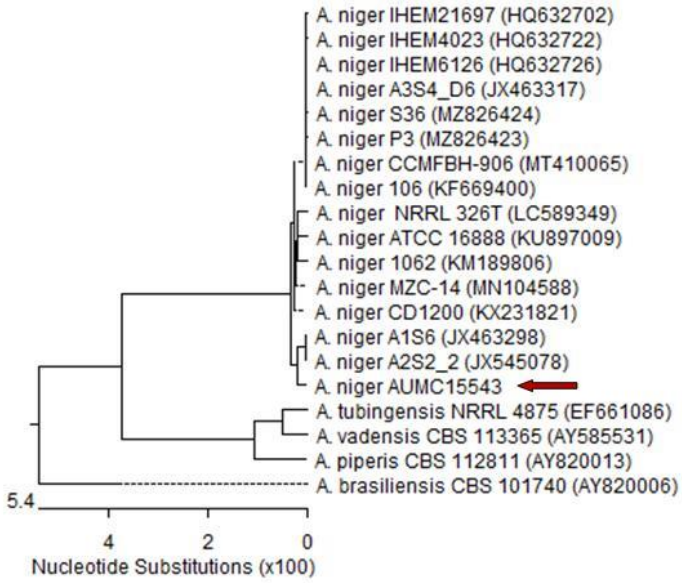

Fig 2: Phylogenetic tree based on Beta tubulin gene sequences of rDNA of the fungal sample isolated in the present study (*A. niger* AUMC15543, arrowed) aligned with closely related strains accessed from the GenBank including the type strain *A. niger* NRRL326T with GenBank accession no. LC589349. *A. niger* AUMC15543 showed 99.46% - 100% identity and 99% 100% coverage with several strains of the same species

Table 1. Effect of temperature on dry weight of halophilic *A.niger*, extracellular protein secretions and soluble sugars

Temp(°C)	Dry weight (mg/50 ml)	Extracellular proteins (mg/ml)	Soluble sugars (mg/ml)
20°C	571.67 ± 4.9	485.67 ± 2.33	309.33± 5.2
30°C	1170.33 ± 10.1	1065.00 ± 7.8	590.33 ± 9.1
40°C	745.00 ± 2.8	611.67 ± 4.4	464.00 ± 4.9

The values in the table are the averages of three separate studies.

Table 2. Effect of temperature on cellulases enzymes released by halophilic *A.niger.*

Temp(°C)	FPase	CMCase	B-glucosidase
20°C	0.9550 ± 0.0026	0.035 ± 0.003	0.813 ± 0.0036
30°C	1.7097 ± 0.0046	0.852 ± 0.021	2.036 ± 0.023
40°C	1.1267 ± 0.0066	0.074 ± 0.005	1.093 ± 0.009

The values in the table are the averages of three separate studies. FPase is measured in filter paper units. One unit is the amount of enzyme necessary to liberate one mole of reducing sugar per minute from filter paper. CMCase is measured in units. One unit is the amount of enzyme necessary to liberate one mole of reducing sugar per minute from carboxymethyl cellulose. ß-glucosidase activity is expressed in terms of units. One unit is the amount of enzyme necessary to liberate one mole of reducing sugar per minute from p-nitrophenol.

Table 3. Effect of pH on *A.niger* dry weight, extracellular protein secretions, soluble sugars.

PH	Dry weight (mg/flask)	Extracellular proteins (mg/ml)	Soluble sugars (mg/ml)
3	281.00 ± 4.9	190.00 ± 2.88	673.67 ± 4.0
5	1170.33 ± 10.1	585.33 ± 14.1	585.33 ± 14.1
7	588.33 ± 6	594.67 ± 1.2	301.67 ± 1.7

The values in the table are the averages of three separate studies

Table 4. Effect of pH on cellulase enzymes production.

PH	FPase (IU/ml)	CMCase (IU/ml)	B-glucosidase (IU/ml)
3	0.479 ± 0.0015	0.123 ± 0.0012	0.673 ± 0.0020
5	1.704 ± 0.0030	0.853 ± 0.0017	2.121 ± 0.0018
7	1.117 ± 0.0014	0.388 ± 0.0011	1.448 ± 0.0014

The values in the table are the averages of three separate studies. FPase is measured in filter paper units. One unit is the amount of enzyme necessary to liberate one mole of reducing sugar per minute from filter paper. CMCase is measured in units. One unit is the amount of enzyme necessary to liberate one mole of reducing sugar per minute from carboxymethyl cellulose. ß-glucosidase activity is expressed in terms of units. One unit is the amount of enzyme necessary to liberate one mole of reducing sugar per minute from p-nitrophenol.

Table 5. Effect of carbon source on *A. niger* dry weight, extracellular protein secretions and soluble sugars

Carbon source	Dry weight(mg/50ml)	Extracellular proteins mg/ml	Soluble sugars mg/ml
Control	1170.3 ± 10.1	1078.3 ± 7.8	585.3 ± 14.1
Lactose 1%	1489.3± 1.76	1376.0 ± 3.21	886.7 ± 1.76
Glucose 1%	1394.3 ± 2.3	1278.0 ± 1.52	795.0 ± 1.7
Maltose 1%	1375.7 ± 2.33	1242.0 ± 3.71	695.0 ± 2.30
Sucrose 1%	566.3 ± 3.2	474.3 ± 2.96	248.3 ± 1.7

The values in the table are the averages of three separate studies.

Table 6. Effect of carbon sources on cellulase enzymes producton by halophilic *A. niger*

Carbon source	FPase (IU/ml)	CMCase (IU/ml)	B-glucosidase (IU/ml)
Control	1.704 ± 0.040	0.853 ± 0.01	2.121 ± 0.04
Lactose 1%	2.106 ± 0.001	1.112± 0.002	2.615 ± 0.002
Glucose 1%	1.927 ± 0.03	1.007 ± 0.002	2.306 ±0.002
Maltose 1%	1.887 ± 0.003	0.991 ± 0.002	2.285 ± 0.002
Sucrose 1%	0.906 ± 0.002	0.395 ± 0.003	1.126 ± 0.002

The values in the table are the averages of three separate studies. FPase is measured in filter paper units. One unit is the amount of enzyme necessary to liberate one mole of reducing sugar per minute from filter paper. CMCase is measured in units. One unit is the amount of enzyme necessary to liberate one mole of reducing sugar per minute from carboxymethyl cellulose. ß-glucosidase activity is expressed in terms of units. One unit is the amount of enzyme necessary to liberate one mole of reducing sugar per minute from p-nitrophenol.

4. Conclusions

Halophilic *A. niger* isolated from Qarune Lake had showed the highest cellulolytic activity and stability was identified by both morphological and molecular identification, and the result was halophilic *A. niger* AUMC 15543. The later was tested for it is cellulolytic activity and had showed high cellulolytic activity. *A. niger* was cultured in Czapek-Dox liquid medium with 1% cellulose and 15% salinity and had been tested for best temperature ,pH and carbon sources and that was resulting in the following: Optimum temperature of the culture medium yielded maximum titers of cellulases enzyme, maximum dry weight, extracellular protein and soluble sugars production by *A. niger* was at 30°C. Optimum pH yielded maximum titers of cellulases enzymes, maximum dry weight, extracellular protein and soluble sugars production by *A. niger* was at pH5. Controlling of pH during the process of the growth improved the cellulases production by *A. niger*. Culture medium with lactose 1% addition yielded higher titers of cellulases enzyme, higher dry weight, extracellular protein and soluble sugars production by *A. niger* compared to control, glucose, maltose and sucrose. The general conclution from this study that halophilic *A. niger* AUMC 15543 isolated from Qarun Lake is highly recommended to be used in cellulases enymes production. Because of it is high growth, stability and productivity under hard conditions like high salinity and the low cost as it can be cultivated easily in labs, *A. niger* can be tested for other enzymes activities in future work as that can be used in many fields and industries with low cost.

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Author Contributions

All authors contributed to this work. A. Abd-Elkader prepared the samples and completed the experimental measurements. N. Abu El-kassem (late), proposed the aim and protocol of the current work. M. Eweis shared the frist author writing and followed the performance of the experiments, followed the revision and submission of the manuscript for publication, helped the first author complete the paper writing and analyzing the data. T.E.E. Radwan share the second author in proposing the aim and protocol of the current work. In addition, helping the frist auther in preparation of the samples, completing the experimental measurements, completing the paper writing, analyzing the data, and validation and followed the revision and submission of the manuscript for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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