AcademicPres

Available online: www.notulaebotanicae.ro

Print ISSN 0255-965X; Electronic 1842-4309

Not Bot Horti Agrobo, 2019, 47(3):xxx-xxx. DOI:10.15835/nbha47311500

Original Article

Notulae Botanicae Horti Agrobotanici Cluj-Napoca

Optimization and Characterization of Extracellular Cellulase Produced by Native Egyptian Fungal Strain

Gamal El-BAROTY[1], Faten ABOU-ELELLA[1], Hassan MOAWAD[2], Talaat N. El-SEBAI[2], Fatma ABDULAZIZ[1]*, A. A. KHATTAB[3]

[1]*Cairo University, Faculty of Agriculture, Biochemistry Department, Giza, Egypt, Giza Governorate 12613, Egypt; elbarotys@hotmail.com; faten2010bio@yahoo.com; fatma.ali@agr.cu.edu.eg (*corresponding author)*

[2]*National Research Centre, Agricultural Microbiology Department, Cairo, 33 El Buhouth St, Cairo Governorate 12622, Egypt; hassanmoawad@hotmail.com; talaatnelsebai@gmail.com*

[3]*National Research Centre, Genetic and Cytology Dept., Cairo, 33 El Buhouth St, Dokki, Cairo Governorate 12622, Egypt; khattababdelnasser69@yahoo.com*

Abstract

Since accumulation of agricultural wastes represents a huge problem, it was important to explore the available methods to help eliminate agricultural wastes safely, and simultaneously produce functional enzyme like cellulase. Six native Egyptian fungal strains were isolated, morphologically identified and screened for cellulose biodegradation potential, which was determined as endoglucanase or as carboximethylcellulase (CMCase). The most promising isolate (*Aspergillus terreus*) was selected for molecular characterizations based on sequencing of internal transcribed spacer (ITS). Further optimization experiments were accomplished on the selected strain. The strain with cellulolytic activity, 2.26 IU mL^{-1} was identified using ITS nucleotides (genes) sequences and the result confirmed that the strain is 99.8% homology with *A. terreus.* Then, it was submitted to GeneBank and given an accession number. Further optimization experiments revealed that 35°C is the optimum temperature for cellulase production and raised the enzyme activity (EA) up to 3.19 IU mL^{-1}. Out of two organic nitrogen sources; peptone at concentration 6 gL^{-1} was found to be the optimum nitrogen source for cellulase production with the highest activity 4 IU mL^{-1}. Whereas, the different four carbon sources: microcellulose, corn stalks, wheat straw and rice straw showed significant differences in EA with values 11.07, 9.68, 7.87 and 3.71 IU mL^{-1}, respectively at pH 3. The maximum EA was recorded to be within 5-7 days of incubation, dependent on type of carbon sources. The optimization of different incubation conditions raised cellulolytic activity from 2.26 IU mL^{-1} up to 11.18 IU mL^{-1}.

Keywords: *Aspergillus terreus*; corn stalk; endoglucanase;rice straw; wheat straw

Abbreviations: agricultural wastes (agro-wastes); Basic Local Alignment Search Tool (BLAST); carboximethylcellulase (CMCase); enzyme activity (EA); greenhouse gases (GHGs); million tons (MT); internal transcribed spacer region (ITS); National Center Biotechnology Information (NCBI)

Introduction

Agricultural waste biomass represents the most abundant renewable carbon source on earth, which could be used for production of bioenergy (bioethanol, biogas and biodiesel), chemicals, enzymes and other valuable products. During the procedure of the plant food processing, large quantities of by products are generated, that creates several environmental problems like water contamination, odor and greenhouse gases (GHGs) emission due to anaerobic mineralization process of the massive volumes of organic matters (McKendry, 2002).

In Egypt, according to the Egyptian Ministry of State for Environmental Affairs (EEAA, 2016), the amount of annual Egyptian agricultural wastes (agro-wastes) is about 31 million tons (MT). Nearly 4 MT of corn wastes, 6 MT of wheat wastes and 3 MT of rice straw are produced annually (FAO, 2001), a massive amount of these agricultural wastes are burned every year which cause a black smog and health problems such as respiratory diseases and allergies (Abdulrahman and Huisingh, 2018).

Cellulose is the most important component of agriculture and food wastes which represents most of the accumulated organic plant matter (about 60 % of agro-wastes) (Clarke, 1996), and it is a branched polymer, consists of glucose units linked by a β (1→4) D- glycosidic

Received: 00 Mar 2018. Received in revised form: 00 May 2018. Accepted: 00 May 2019. Published online: 00 Sep 2019.

bond (Gielkens *et al*., 1999). The degradation of cellulosic biomass demonstrates a critical part of the carbon cycle within the biosphere (Béguin and Aubert, 1994).

Cellulose is degraded by cellulases which cleave the β-1,4 bond (Béguin and Aubert, 1994). Recently, cellulase has shown potential application in pulp and paper industrial, biofuel production, food, feed and agriculture industrial. Furthermore, there has been an increasing interest in the biodegradation of cellulose to glucose using cellulases, which produced by a diversity of microorganisms including both fungi and bacteria during their growth on cellulosic materials (Kulkarni *et al*., 2018; Islam and Roy 2018; Srivastava *et al*., 2018).

Fungi are the most important soil microorganisms which produce cellulases (Lynd *et al*., 2002). Fungal cellulases are complex consist of at least 3 groups of enzymes: endo-1,4-β-glucanase (EC, 3.2.1.4), exoglucanase reducing (E.C. 3.2.1.176) and non-reducing ends (E.C. 3.2.1.91) and β- glucosidase (E.C. 3.2.1.21). These enzymes are belong to the glycoside hydrolase (GH) family and catalyze the hydrolysis of glyosidic linkages, that depolymerizing cellulose synergistically to fermentable sugars (glucose) (Shahriarinour and Wahab, 2011).

However, the mechanism of cellulose degradation by aerobic fungi is similar to that of aerobic bacteria. The exoglucanase (Exg) acts on the ends of the cellulose chain to produce ß-cellobiose unites as the end product, endoglucanase (EG) randomLy attacks the internal O-glycosidic bonds, resulting in glucan chains of different lengths and the ß-glycosidases act specifically on the ß-cellobiose disaccharides and produce glucose (Bayer *et al*., 1991).

Recently, several researchers investigated the ability of fungi to use agricultural wastes as carbon sources rich in cellulosic material, of which *A. terreus* species found to be a promising source of cellulose (Olanbiwoninu and Odunfa 2016; Lee *et al*., 2017; Ranjith *et al*., 2018). An isolated species of *A. terreus* from soil showed high levels of cellulases (Sohail *et al*., 2016). The efficiency of the isolated *A. terreus* cellulase was increased through fermentation with delignified rice straw (Narra *et al*., 2014), however it still important to study the stimulation and optimization of cellulase production, using fermentation of *A. terreus* with different agro wastes as carbon sources. Besides, the application of cellulases enzymes to hydrolyze agro waste cellulose under mild conditions, is an attractive process as the enzymatic depolymerization of agro waste is environmentally friendly (Kuhad *et al*., 2011).

Therefore, in order to avoid agro wastes problems in Egypt, this study were aimed to isolate some native Egyptian fungi strains and study there ability to produce cellulases, and identify the superior cellulase producer isolate by sequencing of the ribosomal ITS region. Different cultivation media contained agricultural wastes and physical conditions were compared in terms of cellulase activity.

Materials and Methods

Substrates, chemicals and media

Three agro waste as carbon sources corn stalks, wheat straw and rice straw were collected from local farm in Giza, Egypt. They were air dried, milled with electric mill (model, CO.) and sieved into 2mm sieve. All reagents and chemicals were obtained from commercial sources and were of analytical grade.

Isolation of fungi from soil

Isolation was made from soil by using enrichment technique. The soil samples were supplemented with saw dust and synthetic modified medium (Mandels *et al*., 1976) containing Whatman # 1 filter paper strip of 6x1cm, peptone (0.1%), urea (0.03%), $MnSO_4.H_2O$ (0.0016%), $ZnSO_4.7H_2O$ (0.0014%), $(NH_4)2SO_4$ (0.14%), $MgSO_4.7H_2O$ (0.03%), $FeSO_4.7H_2O$ (0.05%), $CaCl_2$ (0.01%), $CoCl_2.6H_20$ (0.0029%), KH_2PO_4 (0.2%). This mixture was kept for about one month at room temperature followed by dilution of soil sample sand followed by 1-2 weeks incubation at room temperature. Fungal growth appearing was isolated and transferred to Sabouraud dextrose agar (SDA) slants.

Morphological identification of isolates

Six fungal strains were isolated from soil sample collected from the National Research Centre NRC farm, Dokki, Cairo, Egypt. These strains were identified in the Fungal Identification units in the Regional Center of Mycology and Biotechnology (RCMB, Al-Azhar University, Cairo, Egypt).

Molecular identification

The internal transcribed spacer region (ITS) was used to identify the superior cellulase producer isolate based on molecular genetic analysis. Partial sequences of the isolates 18S rDNA were obtained. The universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG -3') and (ITS4) (5'-TCC TCC GCT TAT TGA TAT GC -3') (Operon Technologies Company, Netherlands) were used. For the amplification of the ITS region, 12 ng of the used primer and 40 ng of the purified DNA sample were added to each polymerase chain reaction (PCR) vial. The total volume of the amplification reaction was completed to 25 µL using sterile distilled water. Then, the amplification was carried out under the following thermal conditions: 95 °C for 5 min, 35 cycles of (95 °C for 1 min, 50 °C for 1 min and 72 °C for 2 min). A final extension for 5min at 72 °C followed by an infinite hold at 4 °C finished the run. The amplified DNA products from ISSR analysis was electrophoresed against plus DNA ladder and photographed using Gel Documentation System with UV Trans eliminator after staining with red safe dye. The PCR product was cleaned up using GeneJET™ PCR purification Kit (Thermo K0701). The DNA sequencing of the purified PCR products was directly sequenced by using ABI 3730xl DNA sequencer (GATC Company, Germany) and forward primer. The DNA sequences were compared with the sequences available by the Basic Local Alignment Search Tool (BLAST) in the program of National Center Biotechnology Information (NCBI), GenBank database (http://www.ncbi.nlm.nih.gov). The sequences were aligned together with those of reference taxa retrieved from public databases. The evolutionary distances were generated based on parameter model (Jukes and Cantor, 1969) and

phylogenetic tree was constructed by using the neighbor-joining method (Saitou and Nei, 1987). Alignment of ITS DNA sequences was done using Clustal_X program (Thompson *et al*., 1997).

Inoculums preparation

Fungal spores of each tested isolate fungus were collected from Potato Dextrose agar (PDA) slants which had been inoculated and incubated for 5 days at 37 ºC. Spores were collected by adding 0.01% (v/v) tween 80 with the aid of wire loop. Each of fermentation flasks was inoculated with 5 mL containing almost (7 ×106 spores mL^{-1}) (Shahriarinour and Wahab, 2011).

Enzyme production

Each fungal isolate was inoculated in mineral salt medium containing microcellulose (30 gL^{-1}), Peptone (3 gL^{-1}), Yeast extract (1 gL^{-1}), Tween 80 (1 mL), 5 mL Trace element solution (1.6 gL^{-1} $MnSO_4.H_2O$, 1.4 gL^{-1} $ZnSO_4.7H_2O$, 2 gL^{-1} $CoCl_2$), Urea (1.5 gL^{-1}), NH_4Cl (5 gL^{-1}), Na_2HPO_4 (10 gL^{-1}), $CaCl_2$. $2H_2O$ (0.4 gL^{-1}), $MgSO_4.7H_2O$ (0.3 gL^{-1}) and $FeSO_4.7H_2O$ (0.005 gL^{-1}) with pH value 5.3 adjusted with 1 M KOH and incubated at 30 °C in a shaking water bath at 115 rpm for 6 days (Haapala *et al*., 1995). In all fermentations, 40 mL of the medium was dispensed into a 100 mL Erlenmeyer flask, sterilized at 121 °C for 20 min and then inoculated with different fungal strains.

Collection and storage of samples

After incubation, the cell-free culture supernatant was centrifuged at 1050 xg for 2 min and then the aliquots of supernatant were dispensed in sterile Eppendorf tubes and stored at -20 °C until used as a source of enzyme in further studies.

Determination of enzyme activity

Carboxymethyl cellulose (CMC, 1% W/V in citrate buffer, pH 4.6) was used as substrate to determine Endoglucanase or carboxymethylcellulase (CMCase) EA of tested fungal strains isolates. The reducing sugars released from CMC were spectrophotometrically measured as glucose at 540 nm using 3,5-Dinitrosalicylic acid (DNS) method. One unit of EA was defined as the amount of enzyme required to release 1 µmol of reducing sugar per min under assay conditions. The following equation: 1 IU = 1 µmol min^{-1} of glucose equivalent released = 0.18 mg min^{-1} of glucose was used to calculate EA of the tested fungi (Wood and Bhat, 1988).

Optimization of culture conditions

The most promising cellulolytic isolate fungus strain *A. terreus* was selected for further studies to optimize the culture conditions for increasing cellulase activity.

Nitrogen sources

Only two types of nitrogen source [peptone and yeast extract] at different concentrations (6, 9, and 12 gL^{-1}) were used separately in the medium formulation as described by Shahriarinour and Wahab (2011).

Carbon source

Three agro wastes; corn stalk, wheat straw and rice straw as well as microcellulose at concentration 30 gL^{-1} were used as a carbon source.

Initial culture pH

The optimum pH was determined by incubation of *A. terreus* with the different carbon sources at pH values ranging from two to seven. The culture initial pH was adjusted using 4M HCl or KOH.

Temperature

Optimum temperature of the enzyme was determined by conducting incubation at different temperatures degrees (20, 25, 30, 35, 40 and 45 °C).

Incubation time

To detected the optimum incubation time, the inoculated flasks were incubated at 35 ± 2 °C temperature and the EA was measured daily for 11 days.

Statistical analysis

All measurements were carried out in triplicate. Statistical analyses were performed using one way analysis of variance (ANOVA), and the equality of variance between means was determined by Bartlett's test, CoStat Software Computer Program. Differences at $p < 0.05$ or less were considered statistically significant. The results were presented as mean values ± SD, standard deviations (CoStat, 2004).

Results

Morphological identification

Based on identification of the six isolated native fungi from Egyptian soil by the RCMB, the investigation data revealed that five of them belonged to genera *Aspergillus*: *A. parasiticus, A. fumigatus var. ellipticus, A. japonicus, A. terreus and A. fumigatus.* The sixth isolated fungus was *T. harzianum*. Fig. 1 demonstrates microscopic examination of the most cellulolytic promising strain *A. terreus*. Colonies of *A. terreus* on Czapek agar at 25 ºC attaining a diameter of 3.0-3.5 cm within 7 days gives buff to yellow brown color.

Screening of CMCase activity

According to the results of CMCase EA determined by DNS method out of six fungal isolates produces the enzyme by various degrees, the most promising CMCase producer was *A. terreus* with EA 2.26 ± 0.04 IU mL^{-1}. The rest of the five fungi strains could not be considered as extremely cellulolytic active strains.

Molecular identification of fungal strain associated with the highest cellulase production

Fungal DNA isolation and amplification by ITS primers

The fungal DNA was isolated and determined using spectrophotometer, the ITS1 and ITS4 primers were used to amplify the region of the rDNA repeat unit that includes ITS1, 5.8S, ITS2 and 28S from the genomic DNA of the fungal strain. A band of approximately 500 to 550 bp was obtained after performing PCR (Fig. 2).

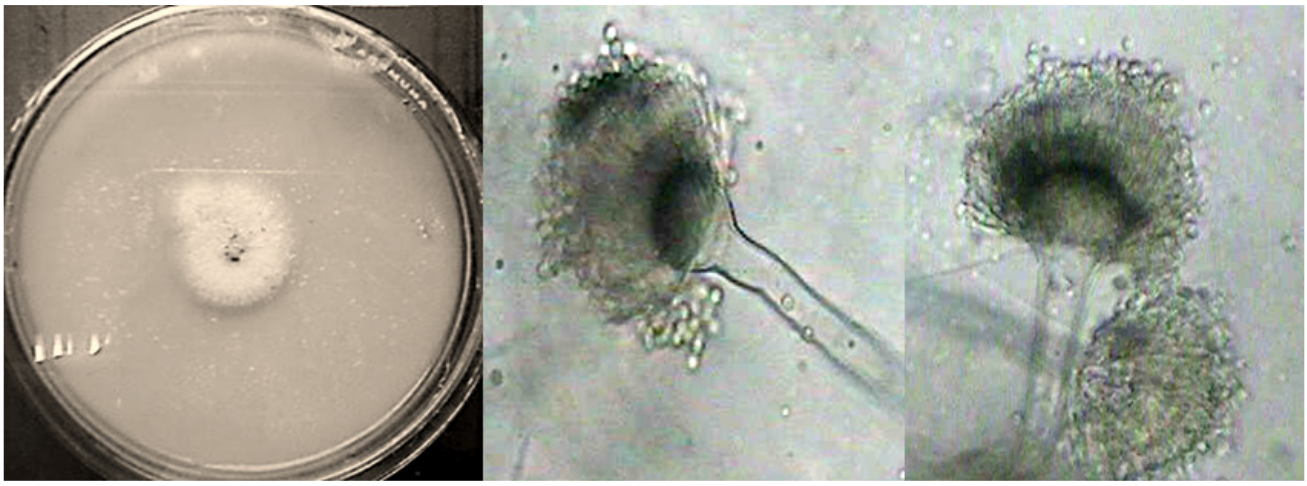

Fig. 1. Characterization and microscopic examination of *A. terreus*

Table 1. CMCase activity of six fungal isolates

Isolate	CMCase (IU/ mL)
A. terreus	2.26±0.04[a**]
A. fumigatus	0.82±0.08[b]
T. harzianum	0.23±0.003[c]
A. japonicus	0.02±0.004[d]
A. parasiticus	0.02±0.002[d]
A. fumigatus var. ellipticus	0.02±0.002[d]

Note: Values are means of triplicate ± standard deviation. Different superscripts mean significantly difference (P < 0.05)."

DNA sequencing of the purified PCR products was done using ABI 3730xl DNA sequencer (GATC Company, Germany) by the use of forward primer, the obtained DNA sequence with the identified fungal strain was applied and conserved in the GenBank under the following accession number (MH997666.1). DNA sequencing of the ITS1, 5.8S, ITS2 and 28S regions was conducted for the differentiation of fungal strain in comparison with the reference strains from GeneBank. The amplified sequences showed that this strain had the highest homology with *Aspergillus terreus* strain. Furthermore, the phylogenetic tree of the fungal strain which isolated from soil was recorded in Fig. 3.

Optimization

Effect of temperature on Endoglucanase enzyme activity

CMCase activity of *A. terreus* was tested during incubation at different temperatures (Fig. 4). The data revealed that the highest CMCase activity (3.19 ± 0.093 IU mL^{-1}) was recorded at range 35 - 40 °C, after 7 days of incubation. Also it was observed that the EA decreased to almost 84% at 30 °C and 70.5% at 25 °C while the EA significantly decreased to 35.7 and 33.5% at 20 and 45 °C.

Effect of nitrogen source

Various studies has investigated the effect of organic and inorganic nitrogen sources on cellulase production during fungal cultivation and demonstrated that using of organic nitrogen sources causes high efficiency (Shahriarinour and Wahab 2011; Narra *et al.*, 2014; Yadav *et al.*, 2016; Lee *et*

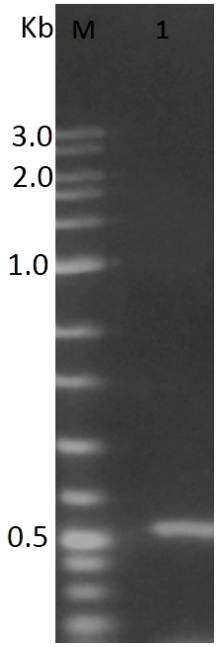

Fig. 2. Photograph of ITS-DNA amplified band for fungal strain (*A. terreus*) isolated from soil (lane 1) using ITS1 and ITS4 primers against 100 bp ladder DNA marker (lane M)

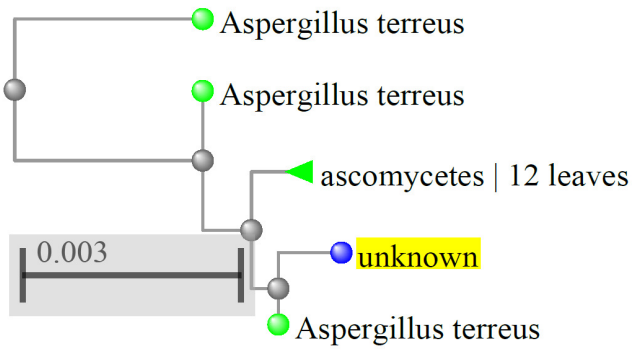

Fig. 3. Phylogenetic tree of sequences of the strain *A. terreus* and the fungi most closely related to it

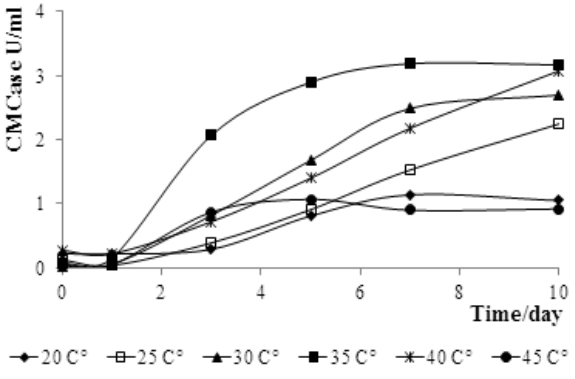

Fig. 4. CMCase activity at different incubation temperatures

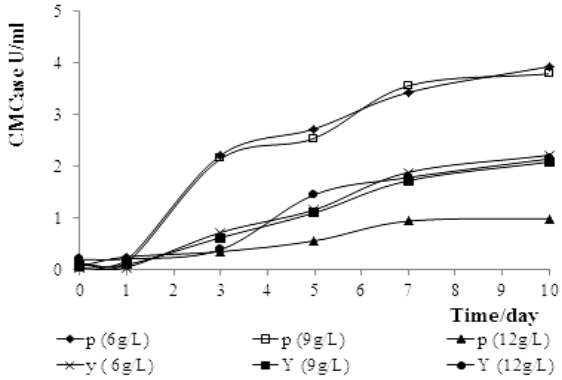

Fig. 5. CMCase activity at different nitrogen sources and concentrations

al., 2017). Based on these studies the effect of two organic nitrogen sources peptone and yeast extract was studied with three different concentrations (6, 9, 12 gL^{-1}). The results highlighted that peptone concentrations 6 and 9 gL^{-1} had the greatest effect on cellulase production and increased the activity from 3 IU mL^{-1} to 4 IU mL^{-1}. While the EA decreased by 74.8% at peptone concentration 12 gL^{-1}. No significant difference was found among the different concentrations of yeast extract and the activity was significantly decreased compared to peptone at 6 gL^{-1} (Fig. 5). These results are analogous to the previous studies (Gautam *et al*., 2011; Narra *et al*., 2014; Yoon *et al*., 2014).

Effect of initial culture pH and different carbon sources

A range of pH (2-7) was used with corn stalk and wheat straw, and a range of pH (2-5) was used with rice straw, in case of rice straw there was no activity detected at pH 6 and 7. The production of CMCase using the three carbon sources was compared by microcellulose as carbon source during incubation. It was observed that optimum pH for *A. terreus* growing on all the four carbon sources was pH 3 and the highest EA with microcellulose, corn stalk, wheat straw and rice straw was 11.07 ± 0.322, 9.68 ± 0.388, 7.87 ± 0.017 and 3.71 ± 0.016 IU mL^{-1} respectively. The enzyme was active in the pH range of (3-6) during incubation with

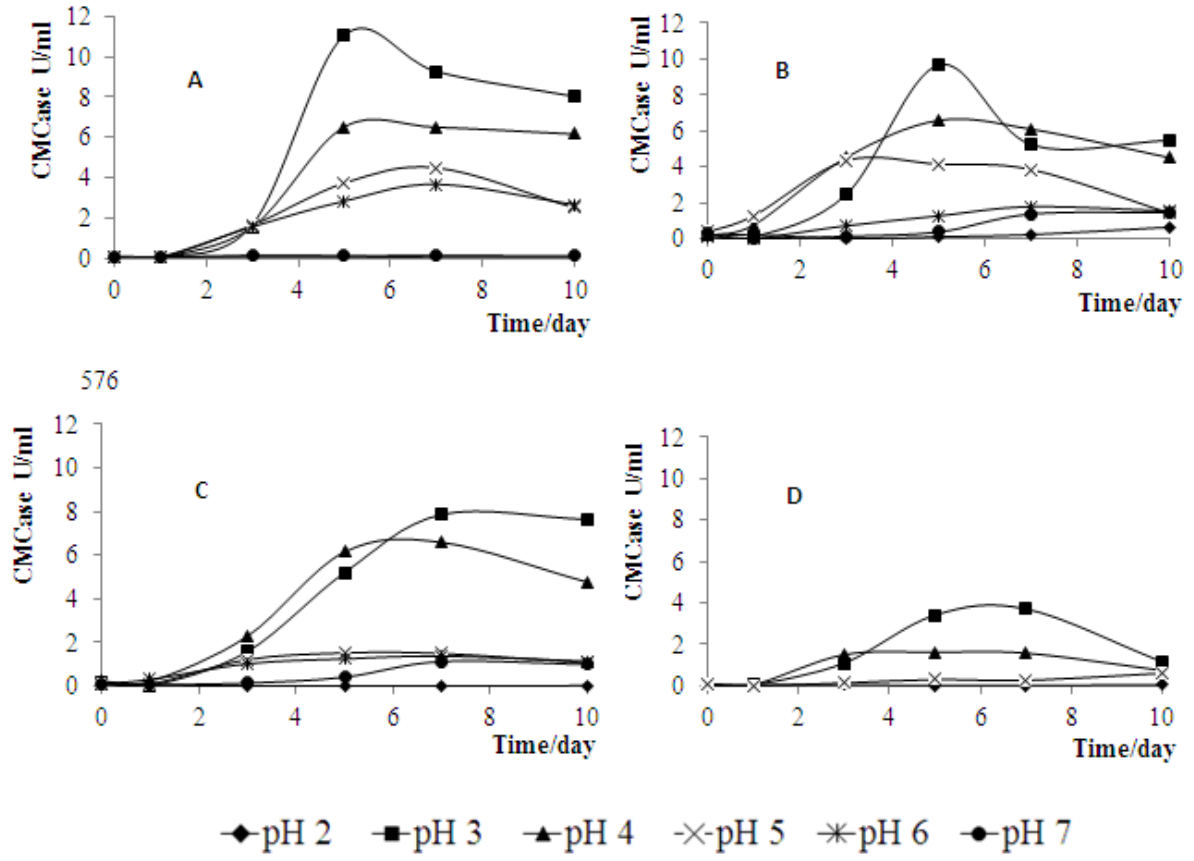

Fig. 6. Effect of different carbon sources (A: Microcellulose, B: Corn stalks, C: Wheat straw, D: Rice straw) on CMCase activity at different pH during incubation

xxx

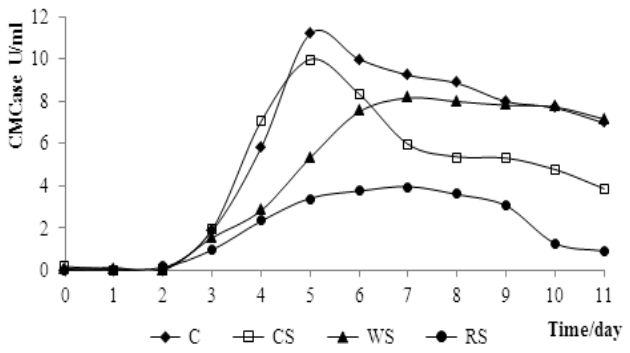

Fig. 7. CMCase activity during incubation with (C: microcellulose, CS: corn stalks, WS: wheat straw and RS: rice straw) as carbon sources

microcellulose and corn stalk, while the pH range was narrower (3-5) during incubation with wheat straw and even narrowest (3, 4) with rice straw. Fig. 6 illustrates the effect of the different carbon sources on EA during incubation at different pH rang.

Optimization of incubation time

On the way to detect the optimum incubation time for the highest EA for each one of the carbon sources, fermentation was carried out up to 11 days. The greatest CMCase activity was found to be 11.18 ± 0.625 IU mL^{-1} at day 5, in case of microcellulose as carbon source; corn stalk also gave the highest activity 9.97 ± 0.163 IU mL^{-1} after 5 days (Fig. 7) .Otherwise wheat straw and rice straw gave the maximum EA after 8 days of incubation with values of 8.15 ± 0.13, 3.96 ± 0.072 IU mL^{-1}, respectively.

Discussion

Screening of CMCase activity

Several Aspergilli species were studied for cellulolytic activity, such as *A. nidulans, A. niger, A. fumigatus and A. terreus,* the last one was recorded with a high cellulolytic enzyme capacity and this is similar to the results of this study (Jahangeer *et al*., 2005; Gao *et al*., 2008; Liu *et al*., 2011; Shahriarinour and Wahab, 2011; Narra *et al*., 2014; Benoit *et al*., 2015). Cellulolytic microorganisms can exist in all biota where cellulosic waste assembles. They usually arise in mixed populations consist of cellulolytic and non-cellulolytic species, which often cooperate synergistically. These cooperation lead to the complete utilization of cellulose, and eventually transform it into carbon dioxide and water (Béguin and Aubert, 1994).

Effect of temperature on Endoglucanase enzyme activity

Enzymes activity is proportional to the temperature because of the increasing of the collisions frequent between the substrate and the enzyme. After a specific temperature degree the thermal agitation affect the tertiary conformation of the enzyme, causing denaturation and an intense derease in solubility (Palmer and Bonner, 2007). The optimum growth at 35 °C may be attributed to the optimal growth rate of *A. terreus* as it is a thermotolerant fungi (Mueller *et al*., 2011). The decline of EA may be because of the effect of temperature on enzymatic Km (Michaelis constant) and this directly affects the enzyme velocity and carbon utilization and consequently affect the fungal growth and enzyme production (Copeland, 2004).

Effect of nitrogen source

Peptone is a result of incomplete hydrolysis of protein which contains peptides and amino acids while yeast extract is the soluble part of digested yeast cell (Sumbali and Mehrotra, 2009). The peptone composition which is a source for additional carbon minerals and vitamins may be responsible for its enhancing effect, because these nutrients are important for fungal growth (Karim *et al*., 2015). This theory had been confirmed by Ilmén *et al*. (1997) who reported that enhancing effect of peptone is not attributed to cellulase gene induction but to intensive fungal growth. On the other hand, the decline of the enzyme production in case of yeast extract can be related to nitrogen availability and chemical component interaction (Panagiotou *et al*., 2003; Sarria-Alfonso *et al*., 2013).

Effect of initial culture pH and different carbon sources

The main structure of enzymes is proteins which make them very sensitive to pH, every enzyme has a specific pH range at which its activity can be detected and this range differs from fungi to another according to species. Cellulases activity is based on acid hydrolysis mechanism in which the enzyme break down the β-1,4- glucosidic bonds via a proton donor, so it was expected to see a different EA during incubation at different pH (Lynd *et al*., 2002). Optimal pH results consistent with previous studies at which suitable pH range for cellulolytic fungi is between 3-5 (Milala *et al*., 2005; Kadarmoidheen *et al*., 2012; Sivaramanan 2014). pH range may effect on the affinity between the enzyme and the substrate and the substrate ionization, and this might be a good reason for the different enzyme activity between pH ranges at each one of the carbon sources (Copeland, 2004; Sivaramanan, 2014).

Optimization of incubation time

EA at different incubation time was found to be similar to previous studies which showed the best incubation period to produce the highest CMCase activity was ranged between 3-7 incubation days (Ahmed *et al*., 2009; Narra *et al*., 2014; Sohail *et al*., 2016). Incubation period may be affected by different factors such as the microorganism species, heterogeneity of the substrate, changing in pH during fermentation, byproduct production and diminishment of nutrients in the medium (Narasimha *et al*., 2006; Karim *et al*., 2015). This difference in CMCase activity and fermentation period may be due to the difference in the carbon bioavailability of the four sources; it was found that corn stalk contains high cellulose content followed by wheat straw, followed by rice straw (Bakker *et al*., 2013; Daud *et al*., 2014). Lignin content of the substrates may be attributed, since lignin can affect the attachment between cellulose and cellulase via lignin-cellulase interaction (Saini *et al*., 2016), consequently more studies on pretreatment of agricultural wastes like delignification are required.

Conclusions

Results of this study demonstrate that a native Egyptian fungal strain (*A. terreus*) can be cultured using agricultural wastes as carbon sources to produce highly active cellulolytic enzymes. Production of cellulase was optimized and enhanced to reach 11.18, 9.97 IU mL-1 in 5 days. This highly active cellulolytic fungus can be used in agricultural processes like composting piles to reduce the required time for agricultural wastes decomposing and enhancing saccharification of agricultural wastes to produce biofuels. Also it may be beneficial in reducing the enzyme production cost, thus it could be used in many industrial applications like textile, paper and pulp, and laundry and detergent industry. Further researches are required to focus on the other enzymes that *A. terreus* can produce like exoglucanase and β- glucosidase and determine cellulase characteristics like molecular weight.

Conflicts of interest

The authors declare that there are no conflicts of interest related to this article.

Acknowledgements

This work was supported by Agricultural Microbiology Dept., National Research Centre who provided help that greatly assisted the research, although they may not agree with all of the conclusions of this paper.

References

Abdulrahman AO, Huisingh D (2018). The role of biomass as a cleaner energy source in Egypt's energy mix. Journal of Cleaner Production 172:3918-3930.

Ahmed S, Bashir A, Saleem H, Mubshara S, Jamil A (2009). Production and purification of cellulose - degrading enzymes from a filamentous fungus. Pakistan Journal of Botany 41(3):1411-1419.

Bakker RRC, Elbersen HW, Poppens RP, Lesschen JP (2013). Rice straw and wheat straw. Potential feedstocks for the biobased economy. NL Agency. Report No. 448025. Retrieved from https://library.wur.nl/WebQuery/wurpubs/448025.

Bayer EA, Morag E, Lamed R (1991). The cellulosome - a treasure- trove for biotechnology. Journal of Biological Chemistry 266(253):9241-9250.

Béguin P, Aubert J-P (1994). The biological degradation of cellulose. Federation of European Microbiological Societies Microbiology Reviews 13(1):25-58.

Benoit I, Culleton H, Zhou M, Difalco M, Osorio GA, Battaglia E, … Vries R P (2015). Closely related fungi employ diverse enzymatic strategies to degrade plant biomass. Biotechnology for Biofuels 8(1):107.

Clarke A (1996). Biodegradation of cellulose: enzymology and biotechnology. CRC Press (1st ed), Lancaster.

Copeland RA (2004). Enzymes: A practical introduction to structure, mechanism, and data analysis (2nd ed). New York.

CoStat (2004). Monterey, CA, USA: CoHort Software. www.cohort.com.

Daud Z, Hatta MZM, Kassim ASM, Awang H, Aripin AM (2014). Exploring of agro waste (pineapple leaf, corn stalk, and napier grass) by chemical composition and morphological study. BioResources 9(1):872-880.

EEAA (Egyptian Environmental Affair Agency) (2016). Annual report for solid waste management in Egypt. Retrieved 2018 Nov 11 from http://www.eeaa.gov.eg/enus/mediacenter/reports/annualreports.aspx.

FAO (2001). FAO - Food and Agricultural Organization of the United Nations. Production Yearbook. Retrieved 2018 Nov 11 from http://www.fao.org/docrep/017/i3138e/i3138e.pdf.

Gao J, Weng H, Zhu D, Yuan M, Guan F, Xi Y (2008). Production and characterization of cellulolytic enzymes from the thermoacidophilic fungal *Aspergillus terreus* M11 under solid-state cultivation of corn stover. Bioresource Technology 99(16):7623-7629.

Gautam SP, Bundela PS, Pandey AK, Khan J, Awasthi MK, Sarsaiya S (2011). Optimization for the production of cellulase enzyme from municipal solid waste residue by two novel cellulolytic fungi. Biotechnology Research International 2011:1-8.

Gielkens MMC, Dekkers E, Visser J, De Graaff LH (1999). Two cellobiohydrolase-encoding genes from *Aspergillus niger* require D - xylose and the xylanolytic transcriptional activator XlnR for Their expression. Applied and Environmental Microbiology 65(10):4340-4345.

Haapala R, Parkkinen E, Suominen P, Linko S (1995). Production of extracellular enzymes by immobilized *Trichoderma reesei* in shake flask cultures. Applied Microbiology and Biotechnology 43(5):815-821.

Ilmén M, Saloheimo A, Onnela ML, Penttilä ME (1997). Regulation of cellulase gene expression in the filamentous fungus *Trichoderma reesei*. Applied and Environmental Microbiology 63(4):1298-1306.

Islam F, Roy N (2018). Screening, purification and characterization of cellulase from cellulase producing bacteria in molasses. BMC Research Notes 11(1):1-6.

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

Jukes T H, Cantor C R (1969) Evolution of protein molecules. (Munro H N, eel.) Mammalian protein mnetabolism, *III*. New York: Academic Press, pp 21-132.

Kadarmoidheen M, Saranraj P, Stella D (2012). Effect of cellulolytic fungi on the degradation of cellulosic agricultural wastes. International Journal of Applied Microbiology Science 1(2):13-23.

Karim A, Nawaz MA, Aman A, Ul Qader SA (2015). Hyper production of cellulose degrading endo (1,4) β-d-glucanase from *Bacillus licheniformis* KIBGE-IB2. Journal of Radiation Research and Applied Sciences 8(2):160-165.

Kuhad RC, Gupta R, Singh A (2011). Microbial cellulases and their industrial applications. Enzyme Research 2011:280696.

Kulkarni N, Vaidya T, Rathi G (2018). Production of cellulase by *Aspergillus* sp. under solid state fermentation. Journal of Pharmaceutical Innovation 7(1):193-196.

Lee H, Lee YM, Heo YM, Lee J, Kim JS, Kang KY, Kim J-J (2017). Utilization of agricultural residues for enhancement of cellulolytic enzyme production and enzymatic saccharification by *Trichoderma harzianum* KUC1716. Industrial Crops and Products 109:185-191.

Liu D, Zhang R, Yang X, Wu H, Xu D, Tang Z, Shen Q (2011).

Thermostable cellulase production of *Aspergillus fumigatus* Z5 under solid-state fermentation and its application in degradation of agricultural wastes. International Biodeterioration and Biodegradation 65(5):717-725.

Lynd RL, Weimer JP, van Zyl HW, Pretorius SI (2002). Microbial cellulose utilization: fundamentals and biotechnology. Microbiology and Molecular Biology Reviews 66(3):506-577.

Mandels M, Andreotti R, Roche C (1976). Measurement of saccharifying cellulase. Biotechnology and Bioengineering Symposium 6:21-33.

McKendry P (2002). Energy production from biomass (part 1): Overview of biomass. Bioresource Technology 83(1):37-46.

Milala M, Shugaba A, Gidado A, Ene A, Wafar J (2005). Studies on the use of agricultural wastes for cellulase enzyme production by *Aspegillus niger*. Research Journal of Agriculture and Biological Sciences 1(4):325-328.

Mueller GM, Bills GF, Foster MS (2011). Biodiversity of fungi: inventory and monitoring methods. Elsevier Academic Press (2nd ed), San Diego.

Narasimha G, Sridevi A, Buddolla V, Subhosh, Chandra M. Rajasekhar RB (2006). Nutrient effects on production of cellulolytic enzymes by *Aspergillus niger*. African Journal of Biotechnology 5(5):472-476.

Narra M, Dixit G, Divecha J, Kumar K, Madamwar D, Shah AR (2014). Production, purification and characterization of a novel GH 12 family endoglucanase from *Aspergillus terreus* and its application in enzymatic degradation of delignified rice straw. International Biodeterioration and Biodegradation 88:150-161.

Olanbiwoninu AA, Odunfa SA (2016). Production of cellulase and xylanase by *Aspergillus terreus* KJ829487 using cassava peels as substrates. Advances in Microbiology 06(07):502-511.

Palmer T, Bonner PL (2007). Ezymes: biochemistry, biotechnology and clinical chemistry. Elsevier Academic Press (2nd ed), Hawthorne, CA, USA.

Panagiotou G, Kekos D, Macris BJ, Christakopoulos P (2003). Production of cellulolytic and xylanolytic enzymes by *Fusarium oxysporum* grown on corn stover in solid state fermentation. Industrial Crops and Products 18(1):37-45.

Ranjith S, Kanchana D, Pratheep S (2018). Cellulase production from *Aspergillus niger* using paddy straw as a substrate and immobilization. International Journal of Pure and Applied Bioscience 6(2):1081-1084.

Saini JK, Patel AK, Adsul M, Singhania RR (2016). Cellulase adsorption on lignin: A roadblock for economic hydrolysis of biomass. Renewable Energy 98:29-42.

Saitou N, Nei M (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4(4):406-425.

Sarria-Alfonso V, Sánchez-Sierra J, Aguirre-Morales M, Gutiérrez-Rojas I, Moreno-Sarmiento N, Poutou-Piñales RA (2013). Culture media statistical optimization for biomass production of a ligninolytic fungus for future rice straw degradation. Indian Journal of Microbiology 53(2):199-207.

Shahriarinour M, Wahab M (2011). Effect of medium composition and cultural condition on cellulase production by *Aspergillus terreus*. African Journal of Biotechnology 10(38):7459-7467.

Sivaramanan S (2014). Biodegradation of saw in sulching. Research Journal of Agriculture and Forestry Sciences 2(2):13-19.

Sohail M, Ahmad A, Khan SA (2016). Production of cellulase from *Aspergillus terreus* MS105 on crude and commercially purified substrates. 3 Biotech 6(1):1-8.

Srivastava N, Srivastava M, Manikanta A, Ramteke PW, Singh RL, Mishra PK, Upadhyay SN (2018). Fungal cellulases production for biodegradation of agriculture waste. Microorganisms for Green Revolution pp 75-89.

Sumbali G, Mehrotra RS (2009). Principles of Microbiology. Tata McGraw-Hill, New Delhi.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997). The CLUSTAL X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25(24):4876-4882.

Wood TM, Bhat KM (1988). Biomass part A: Cellulose and hemicellulose. Methods in Enzymology 160:87-112.

Yadav PR, Chauhan PB, Gahlout M (2016). Isolation, screening and optimization of process parameters for enhanced production of cellulase by solid state fermentation. International Journal of Advanced Research in Biological Sciences 3(5):21-27.

Yoon LW, Ang TN, Ngoh GC, Chua ASM (2014). Fungal solid-state fermentation and various methods of enhancement in cellulase production. Biomass and Bioenergy 67:319-338.