Cellulase and Xylanase Production by Sugarcane Bagasse Mycobiota

[1]Ahmad M. Moharram, [1]Abdel-Naser A. Zohri and [2]Dalia A. Hussein

[1]Department of Botany and Microbiology, Faculty of Science, Assiut University,
[2]Moubasher Centre for Mycology, Assiut University, Egypt
Corresponding author: A. M. Moharram, E-mail: ***ahmed.marzouk@science.aun.edu.eg***

Abstract

Lignocelluloses of sugarcane represent a precious and cheap source for enzyme production by several fungal species. In the present study 25 samples of sugarcane bagasse were analyzed for their fungal content. The growing fungal strains were then screened for their abilities to produce cellulolytic and xylanolytic enzymes in solid and broth cultures. A total of 62 fungal species belonging to 31 genera were identified from the tested samples. The most prevalent genera were *Aspergillus* and *Fusarium* being isolated from 96% of the tested samples. *Mucor*, *Penicillium* and *Talaromyces* colonized 45% - 52% of bagasse samples.The most prevalent species were *A. flavus*, *A. niger* and *F. oxysporum*. Internal trans. Spacer (ITS) sequences of rDNA confirmed the identification of two new records (*Ceratocystis adiposa*, *Phaeoacremonium viticola*) in addition to *Sarocladium kiliense*, *Stachybotrys chartarum* and *S. elegans*. Cellulolytic activities were detected in solid cultures of 73.7% of fungal strains with the most active being *Aspergillus tamarii*, *Exserohilum rostratum* and *S. chartarum.* Xylanolytic activities were exhibited by59.8% of fungal strains and the active xylanase producers were A. *niger*, *A. tamarii*, *A. tubingensis*, *C. adiposa* and *E. rostratum.* Spectrophotometric measurements using dinitrosalicylic acid (DNS) reagents howed high cellulase concentration in broth cultures of *S. chartarum* followed by *A. niger*, *C. adiposa* and *S. elegans* (10.2- 52.0 IU/ml). The relative activity of fungal cellulase ranged from 0.2-1.7 IU/ml/min whereas the specific activity fluctuated between 2.471-14.590 IU/mg protein. Xylanase concentration, relative and specific activities were markedly high especially in cultures of *A.*

niger (360.6 IU/ml, 32.0IU/ml/min. and 64.611 IU/mg protein, respectively).

Keywords: Sugarcane bagasse, fungi, cellulases, xylanases,enzymes

Introduction

Agricultural crop wastes such as bagasseofsugarcane (*Saccharum officinarum* L.), consist mainly of cellulose and hemicellulose while, the rest comprises lignin, nitrogenous compounds and ash (**Abdullah and Zafar, 1999**). These agro-industrial wastes have been recognized as important renewable sources of biofuels and other value-added products (**Moretti *et al.*, 2012**). Sugarcane bagasse contains approximately 35-45% cellulose, 25% hemicellulose and 20-25% lignin. Due to its high availability, it could serve as a substrate formicrobial production of value-added products, such as protein-rich animal feed, enzymes, amino acids, organic acids and compounds of pharmaceuticalimportance (**Parameswaran 2009**), and also source of carbon for the growth of filamentous fungi (**Martins *et al*. 2011**).

There has been increasing interest in obtaining new, stable and more specific enzymes using low-cost carbon sources, such as sugarcane bagasse, and in searching for new and suitable microbial strains for large-scale cultivation for biotechnological processes (**Parameswaran 2009**).**Dos Santos *et al*. (2015)** isolated nineteen molds and seven yeast strains from sugarcane bagasse piles located at Brazilian Cerrado. Four species of them were identified as *Kluyveromyces marxianus*, *Aspergillus niger*, *A. sydowii* and *A. fumigatus.* The isolates were screened for the production of key enzymes in the saccharification of lignocellulosic material. Among them, three strains were selected as good producers of hemicellulolytic enzymes: *A. niger (*SBCM3), *A. sydowii* (SBCM7) and *A. fumigatus* (SBC4). The best xylosidase producer was *A.niger*SBCM3 strain. This crude enzyme presented optimal activity at pH 3.5 and 55 °C (141 U/g). For glucosidase and xylanase, the best producer was *A. fumigatus* SBC4 strain, whose enzymes presented maximum activities at pH 3.5 and 60 °C (54

U/g) and 4.0 (573 U/g), respectively. All these crude enzymes presented stability around pH 3.0-8.0 and up to 60 °C, which can be very useful in industrial processes. These characteristics among these fungal crude enzymes suggest that they can be used synergistically in cocktails in future studies of biomass conversion with potential application in several biotechnological sectors.

A recent report by **Mohammad *et al*. (2019)**confirmed that sugarcane bagasse can be used as substrate for cellulase enzyme production as it is cheap and readily available.*A. fumigatus* and *A. niger* can be used forcellulase enzyme production because they are easily isolated from the environment as well as from agricultural byproducts in addition to the both species were recorded by several researchers as highly producers of cellulases (**Moretti *et al.*, 2012 and Souza *et al.*, 2012**). **Bansal *et al.* (2012)** analyzed various agricultural and kitchen wastes to support the production of cellulases by a strain of *A. niger* isolated from decaying residues and obtained appreciable levels of different cellulases. **Rocha *et al.* (2013)** showed the importance of ethanol production using crude enzymatic complexes produced by *A. niger* and agro-industrial biomass**. Ferreira *et al.* (2018)** assessed the enzymatic activity of six strains of filamentous fungi grown in liquid media containing 1%sugarcane bagasse as the sole carbon source. They reported that all fungal strains were able to use this agro-industrial residue producing various types of enzymes, such as cellulases, xylanases, amylases, pectinases, and laccases. However, *Aspergillus japonicus* was the most efficient producer, showing the highest enzymatic activity for endo-β-1,4-xylanase(3.55 U mL^{-1}) and β-xylosidase (9.74 U mL^{-1}) at fourteen and twenty-one days in culture, respectively. Furthermore, the endo-β-1,4-xylanases and β-xylosidases of *A. japonicas* showed maximum activity at 50°C, and pH 5.5 and pH 3.5-4.5respectively.**Pinotti *et al.* (2020)** investigated the production of cellulases by submerged fermentation using *Trichoderma koningii, Penicillium* spp and *Rhizomucor* spp. They found that the best fungus was *T. koningii* (3130.4 IU/L) using 2.7% natural sugarcane bagasse at 28°C followed by *Penicillium* spp. (111.8 IU/L at 33°C using 1.6%

w/v acid-alkaline pretreated bagasse) and *Rhizomucor* spp. (63.5 IU/L at 28°C using 1.6% w/v natural bagasse).

In Egypt, sugarcane production in 2019/20 has been expected to reach 13.8 million metric tons (MMT) showing an increase of 1.30 MMT above last year's estimate (**Abdi and Omar 2019).**The current study was designed to explore the mycobiota associated with sugarcane bagasse and to determine their efficiency for production of cellualases and xylanase enzymes which are of great value in environmental and industrial biotechnology.

Material's and Methods.

1- Collection of samples

A total of 25 samples (500 g each) of sugarcane bagasse were randomly collected from different places in Assiut Governorate, Egypt. The samples were brought in clean plastic bags to the laboratory and kept individually in the refrigerator (3-5 ºC) till of fungal analysis.

2- Media used for isolation and identification of fungi

a- Cellulose-Czapek's agar (Eggins& Pugh 1962): This medium was used for isolation of cellulose decomposing fungi. It has a composition of (g/L): cellulose powder 10, $NaNO_3$ 3.0, K_2HPO_4 1.0, $MgSO_4.7H_2O$ 0.5, KCl 0.5,$FeSO_4.7H_2O$ 0.01, agar agar 20, chloramphenicol 0.25 and Rose Bengal (1/15000). The medium was then autoclaved at 121ºC for 20 min.

b- Czapek's agar without carbon source: This medium was used for isolation of fungi able to consume bagasse as sole carbon source. The medium contains (g/L): $NaNO_3$ 3.0, K_2HPO_4 1.0, $MgSO_4.7H_2O$ 0.5, KCl 0.5, $FeSO_4.7H_2O$ 0.01, agar agar 20, chloramphenicol, 0.25and rose Bengal (1/15000). The medium was then autoclaved at 121ºC for 20 min.

c- Czapek's Yeast Extract Agar (CYA): This medium was used for identification of *Aspergillus, Pencillium* and species. It contained the following ingredients (g/l): sucrose 30, yeast extract 5, K_2HSO_4 1, Na NO_3 3, K Cl 0.5, Mg $SO_4.7$ H_2O 0.5, Fe $SO_4 7H_2O$ 0.01, $ZnSO_4.7H_2O$ 0.01, $CuSo_4 5H_2O$ 0.005and agar agar 20. The medium was autoclaved at 121°C for 20 min.

d- Potato Dextrose agar (PDA): It is preferred for identification of *Fusarium* species. It composed of (g/l): potato extract of 200, dextrose 20, agar agar 15, and 1000 distilled water then autoclaved at 121 °C for 15 min.

e- Malt extract agar (MEA): The medium is preferred for identification of Dematiaceous Hyphomycetes. It contained (g/l): malt extract powder 20, glucose 20, peptone 1, agar 20, and 1000 distilled water then autoclaved at 121 °C for 15 min.

3- Methods used for isolation of fungi

a- Direct-plating technique (Modified from Johnson *et al.* 1960): Four pieces of 1 cm of bagasse were put onto the agar surface in Petri plates containing either cellulose-Czapek's agar or Czapek's agar without carbon source. Five plates were prepared for each medium which were then incubated at 25°C for 7-10 days. The developing colonies were counted, isolated and identified. Fungal counts were calculated in each individual sample as colony forming units (CFUs) per 20 bagasse pieces.

b- Dilution technique (Modified from Johnson *et al.* 1972): A known weight of bagasse (5 g) was put in 195 ml of sterilized distilled water and shaken at 150 rpm on an orbital shaker (Stuart Model SSL1) for 30 min, then 8 ml of the washing water were transferred to 92 ml of sterilized distilled water to give a final dilution of 1/500.Aliquots of one ml of the suspension was put in each of 5 Petri dishes. The cellulose-Czapek's agar medium was poured into the Petri dishes (5 plates for each sample). Plates were rotated clock- and anti-clockwise to distribute the CFUs homogenously. The cultures were incubated at 25°C for 7-10 days. The developing colonies were isolated and identified. Counts of the growing fungal colonies were calculated in each individual sample as CFUs per g bagasse.

4- Identification of fungi

a- Phenotypic identification of fungi: Fungal genera and species were identified basically on their macroscopic and microscopic features using the following references: These keys included **Raper and Fennell (1965), Simmons (2007), Ellis (1976), Pitt**

(1979), Sivanesan (1987), Moubasher (1993), Bensch *et al.* (2012), Domsch*et al.* (2007), and Ismail *et al.* (2015),

Molecular identification of fungi:

To confirm the identification of somefungal isolates they were firstly grown in plates containing Czapek's yeast extract agar (CYA) and incubated at 25° C for 7 days. A small amount of the fungal growth was scraped and suspended in 200 µl of distilled water and boiled at 100° C for 15 minutes (**Moubasher *et al.*, 2016**) and sent to SolGent Company, Daejeon, South Korea, for PCR and rDNA sequencing. Fungal DNA was extracted and isolated using SolGent purification bead in SolGent Company. ITS sequences of nuclear ribosomal DNA were amplified using the universal primers ITS 1 (5'-TCC GTA GGT GAA CCT GCG G-3'), and ITS 4 (5'-TCC TCC GCT TAT TGA TAT GC-3'). Amplification was performed using the polymerase chain reaction (PCR) (ABI, 9700). The PCR reaction mixtures were prepared using Solgent EF-Taq as follows: 10X EF-Taq buffer 2.5 µl, 10 mM dNTPs (T) 0.5 µl, primer (F-10p) 1.0 µl, primer (R-10p) 1.0 µl, EF-Taq (2.5U) 0.25µl, template1.0 µl, distilled water to 25 µl. Then the amplification was carried out using the following PCR reaction conditions: one round of amplification consisting of denaturation at 95 °C for 15 min followed by 30 cycles of denaturation at 95 °C for 20 sec, annealing at 50 °C for 40 sec and extension at 72 °C for 1 min, with a final extension step at 72 °C for 5 min. The PCR products were then purified with the SolGent PCR Purification Kit-Ultra (SolGent, Daejeon, South Korea) prior to sequencing. Then the purified PCR products were reconfirmed (using size marker) by electrophoreses of the

b- PCR products on 1% agarose gel. Then these bands were eluted and sequenced. Each sample was sequenced in the sense and antisense direction. Contigs were created from the sequence data using CLCBio Main Workbench program. The sequence obtained from each isolate was further analyzed using BLAST from the National Center for Biotechnology Information (NCBI) website. Sequences obtained with those retrieved from GenBank database were subjected to Clustal W analysis using Meg Align (DNA Star)

software version 5.05 for the phylogenetic analysis. Sequence data were deposited in GenBank and accession numbers were given for them.

5- Screening for enzyme production by the common fungal species

a- Cellulase production: The tested fungal strains were cultured on a medium containing (g/L): $(NH_4)_2SO_4$ 0.5, L-asparagine 0.5, KH_2PO_4 1.0, KCl0.5, $MgSO_4.7H_2O$ 0.2, $CaCl_2$ 0.2, Yeast extract 0.5, carboxymethyl cellulose (CMC) 10, and agar 20 **(Eggins& Pugh,1962).**Cultures were incubated at 25°Cfor 7 days. Erlenmeyer conical flasks containing liquid medium of the previous composition (the pH was adjusted to 5.4 using acetate buffer) were inoculated with the tested fungi, and incubated at 25ºC for 7 days. Using a sterile cork borer, wells (10 mm diameter) were made in agar medium of the same composition. Each well was filled with 50 µl of the filtrated crud enzyme and the plates were incubated at 25ºC for 24 hour. The plates were then flooded with 1 % iodine solution (KI 15 g, I_2 3 g /L distilled water). Clear zones around wells indicate hydrolysis of cellulose by the resulting cellulase enzyme (endo-1, 4-β-glucanase).

b- Xylanse production: The fungal isolates were subjected for screening their xylanase activity using the plate screening method on minimal agar medium with 0.5% corn copxylan as the only carbon source **(Sridevi and Charya, 2013).** The medium contained the following constituents (g/L): $MgSO_4 \cdot 7H2O$ 0.05, $CaCl_2$0.005, $NaNO_3$0.005, $FeSO_4.7H2O$ 0.009, ZnSO40.002, $MnSO_4$0.012, KCl 0.23, $KH2PO_4$0.23, peptone 2, agar 19 **(Adesina and Onilude, 2013).** The Petri dishes containing the medium were inoculated with the microorganism in the center. The cultures were incubated at 28±2ºC for 10 days, and analyzed at every 24 hours for the occurrence and evaluation of the halo diameter. The cultures were flooded with 0.4% Congo red dye and after 10 minutes washed with 1M NaCl. Clear zones around wells indicate hydrolysis of xylan by the resulting xylanase enzyme.

6- Quantitative determination of enzyme concentration and activity

a- Cellulase (CMCase) activity was determined by mixing 0.5 of CMC in 50 mM acetate buffer pH 5 with 0.5 ml of suitably diluted enzyme and incubating at 50°C for 20 min **(Ghose, 1987).** The reaction was terminated by addition of 2 ml of 3,5-dinitrosalicylic acid (DNS) and the contents was boiled for 10 min. The colour developed was read at 540 nm (UV spectrophotometer model T80+). The amount of reducing sugar liberated was quantified using glucose as standard. One unit of cellulase is defined as the amount of enzyme that liberates 1 µ mole of glucose equivalents per minute under the assay conditions.A blank was also prepared that contains 1 ml of distilled water instead of enzyme.

b- Xylanase activity was determined by mixing 0.5 g of corncob xylan (Sisco research laboratories SRL-30565) in 50 mM acetate buffer, pH 5 with 0.5 ml of diluted enzyme and the mixture was incubated at 50°C for 20 min **(Bailey *et al*., 1992).** The reaction was terminated by addition of 2 ml of 3,5-dinitrosalicylic acid (DNS) and the contents was boiled for 10 min **(Miller, 1959).** After cooling, the colour developed was read at 540 nm (UV spectrophotometer model T80+). The amount of reducing sugar liberated was quantified using xylose as standard. One unit of xylanase is defined as the amount of enzyme that liberates 1 µ mol of xylose equivalents per minute under the assay conditions. A blank was also prepared that contains 1 ml of distilled water instead of enzyme.

c- Determination of protein: The concentration of soluble protein was estimated using bovine serum albumin as the standard **(Lowry, 1951).**

Results and Delusion

Mycobiota isolated from bagasse samples:

A total of 62 fungal species attributed to 31 genera were isolated. Plating technique produced relatively higher number of fungi either on carbon free Czapek's agar (41 species and 19 genera) or on cellulose-Capek's agar (41 and 20). Using the

dilution method and Cellulose-Czapek's agar the total number of fungi was 34 species belonging to 17 genera as shown in table (1). The most common genera were *Aspergillus* and *Fusarium* which were represented in 18-24 and 14-24 samples analyzed on Czapek's without carbon source and cellulose-Czapek's agar respectively. *Mucor* occurred in high incidence on the first (13 samples) and second medium (12 samples) using plating technique but it was rare (3 samples) when the dilution plate technique was employed. Other genera such as *Cladosporium*, *Myrothecium*, *Penicillum* and *Talaromyces* were appeared in moderate frequency of occurrence on sugarcane bagasse analyzed by direct plating or dilution plate methods and their frequencies were fluctuated between 7-13 samples out of the 25 samples under study (Table1).Reports from Thailand (**Boonyuen *et al.*, 2014**) indicated the prevalence of *Aspergillus* and *Penicillium* in sugar cane bagasse. In China the common genera on sugarcane were identified as *Bipolaris, Chaetomium, Colletotrichum, Curvularia, Phoma, Fusarium, Nigrospora* and *Trichoderma* **(Raza et al., 2019)**

In the current study, the most common species were *Aspergillus flavus*, *A. niger* and *Fusarium oxysporum* which colonized 14-24 samples out of 25. Each of *Cladosporium cladosporioides*, *Fusarium semitectum, F. subglutinans, F. verticillioides, Mucor racemosus* and *Penicillium aurantiogriseum* were mostly occurred in moderate incidence on sugarcane bagasse. The remaining fungal genera and species were less common (Table 1). Some unidentified yeasts appeared in the isolation plates in moderate incidences (8-10) in case of plating technique and in high incidence (20) in case of dilution method. In Thailand, **Boonyuen *et al.* (2014**) performed mycological analysis of sugarcane bagasse and were able to identify 13 isolates including *A. flavus*, *A. niger*, *A. fumigatus*, *P. citrinum*, *Penicillium piceum* and *P. verruculosum*. In Brazil, **Dos Santos *et al.* (2015)** identified three filamentous species namely *A. niger, A. sydowii, A. fumigatus* in addition to the yeast fungus *Kluyveromyces marxianus* from sugarcane bagasse. In Iraq, **Abdullah and Saleh (2010)** described 10 teleomorphic ascomycetous fungi from leaves and stems of sugarcane. Among these fungi *Chaetomium globosum* was

considered a new record from sugarcane in Iraq. This species appeared in the current study in rare incidence and has been considered by many investigators as one of active cellulolytic fungi **(Domsch *et al.*, 2007).**Reports from Nigeria (**Olaniran and Adebayo, 2014**) showed that 46 fungal species belonging to 30 genera were isolated from 60 samples of *Saccharrum officinarum* collected from Sabo Sugarcane market, Ogbomoso, Nigeria. The common genera included *Aspergillus*, *Trichoderma* and *Mucor* whereas the most prevalent species were represented by *A. niger*, *A. flavus*, *A. ustus*, *A. terreus* and *A. wentii*, *A. nidulans* and *Trichoderma viride*. In Egypt, **Abdel-sater *et al.* (2015)** collected Twenty five samples of Egyptian cane molasses were collected from different five sugar factories (5 for each) in Egypt named Abo Qurqas, Nag Hammady, Dishna, Qus and Kom Ombo. They isolated15 fungal species belonging to the genera *Aspergillus, Curvularia, Fusarium, Microascus, Paecilomyces* and *Penicillium.*More recently *Bipolaris* and *Chaetomium* were isolated as mycopathogens involved in leaf spot diseases of sugarcane in Cameron **(Bechem and Mbella, 2019).**

Using the dilution plate technique it was possible to collect 133300 fungal colonies/25g (=5332 CFU/g) including the unidentified yeasts which shared with 72000colonies. The highest number of colony forming units (CFU) was given by *Aspergillus* species (26800 CFU/g matching 20.11% of total fungal population). This high count was mainly due to the high densities of *A. flavus*, *A. tubingensis* and *A. niger* (5600, 5100 and 14800 CFU/ g, respectively) as shown in Table (1).

Fusarium and *Penicillium* counts (11700 and 8900 CFU/g) contributed 8.78% and 6.68% of total fungal count respectively. Among *Fusarium* species, the highest number of colonies was shown by *F. proliferantum*(3300 CFU/g) followed by *F. semitectum*, *F. solani*, *F. oxysporum*, *F. subglutinans s*and *F. circinatum* (900-1600 CFU/g). In case of *Penicillium* the highest count was observed with *P. aurantiogriseum* (5900 CFU/g) matching 4.48% of total fungi followed by *P. chrysogenum* and *P. cittrinum* (2800 and 200 CFU/g respectively). Fungal species such as *Purpureocillium lilacinum, Stachybotrys elegans, Talaromyces*

duclauxii shared with 1100-2100 CFU/g (0.83-0.90 % of total fungal count). The following six fungal species appeared only when samples of sugarcane bagasse were analyzed using the dilution plate technique. These were generally of rare incidence and called *Acremonium implicatum*, *Aspergillus nidulans*, *Exophiala jeanselmi*, *E. spinifera*, *Purpureocillium lilacinum* and *Phoma glomerata* as shown in table (1). Most of these fungi were reported among the mycobiota of sugarcane (**Abdullah and Saleh 2010, Boonyuen *et al.*, 2014, Abdel-Sater *et al.*, 2015, Dos Santos *et al.*, 2015 and Olaniran and Adebayo, 2014).**

Fungi identified by molecular techniques

Five different fungal cultures were subjected to molecular characterization to confirm their identity. Sequencing results revealed the identification of two new fungal records to Egypt namely *Ceratocystis adiposa* (AUMC14646) and *Phaeoacremonium viticola* (AUMC 14713).The remaining 3 strains were identified as *Sarocladium kiliense*(AUMC14712), *Stachybotrys chartarum* (AUMC14705) and *S. elegans* (AUMC14706) as shown in Figure (1). ITS sequences of these strains showed 99.63 -100% similarities with several closely related strains accessed from the GenBank except in case of *S. elegans* which exhibited 97.45% identity with related strains.

In Mexico, five fungal strains were isolated from sugarcane bagasse **(Cort´es-Espinosa *et al.*, 2006)**. These strains were identified on molecular basis as *A. fumigatus*, *A. niger*, *A. terreus*, *Penicillium glabrum* and *Cladosporium cladosporioides*. With the exception of *A. terreus* and *P. glabrum*, these species were found in sugarcane bagasse analyzed in the current study.Both *A. fumigatus* (10 strains) and *A. niger* (2 strains) were also isolated from sugarcane bagasse in Brazil **(Dos Santos *et al.* (2015).** *A.fumigtus*, *Bipolaris* sp. and *Exophiala spinifera* have been reported from sugarcane in USA **(Shrestha *et al.*, 2015).**

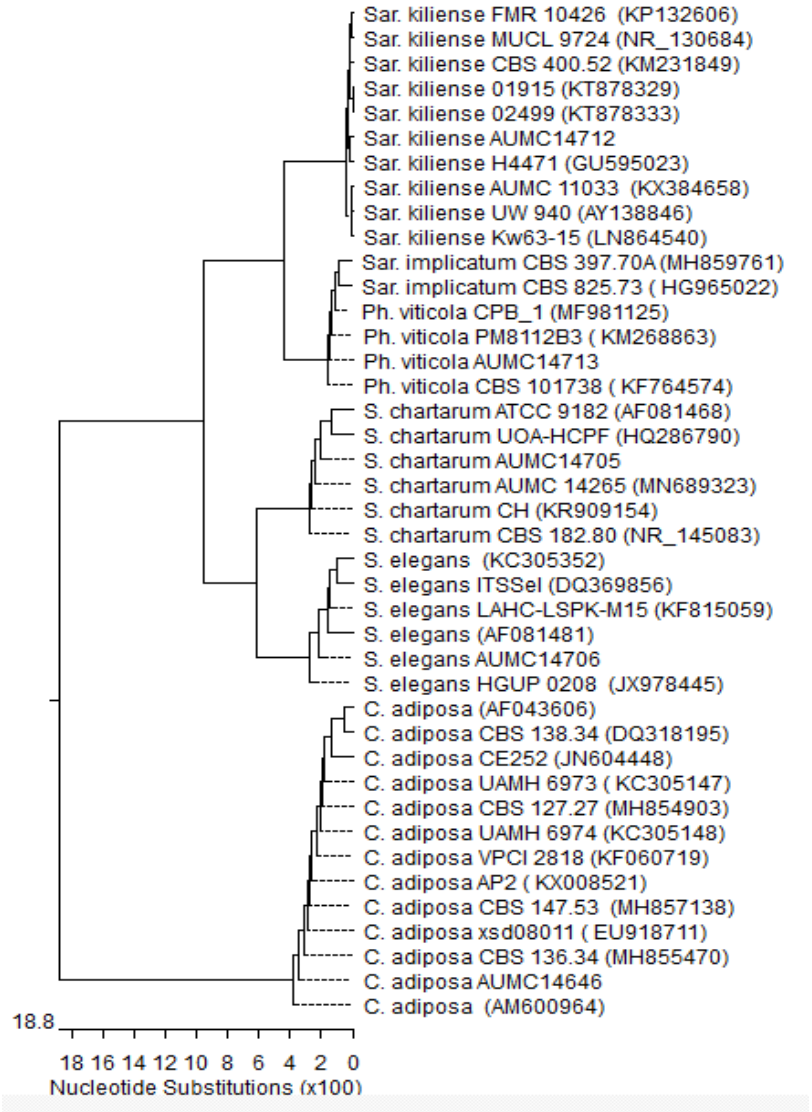

Figure (1): Phylogenetic tree based on ITS sequences of r DNA gene of the fungal samples isolated in the present study (arrowed)) aligned with closely related strai
s accessed from the GenBank. These samples showed 97.45%-100% identity with several related strains

Table (1): Counts (CFU), frequency (F) and occurrence remarks (OR) of fungi isolated from sugar cane bagasse.

Medium / Fungal taxa	Plating method						Dilution method		
	On Capek's (*) Without carbon source			On Capek's cellulose			On Capek's cellulose		
	CFU	%CFU	F &OR	CFU	%CFU	F &OR	CFU	%CFU	F &OR
Acremonium	0	0	0	2	0.14	1 R	800	0.60	3 R
A. blochii (Matr.)W.Gams	0	0	0	0	0	0	200	0.15	1 R
A. hyalinulum (Sacc. W.Gams	0	0	0	2	0.14	1 R	0	0	0
A. implicatum (J.C. Gilman & E.V. Abbott) W. Gams	0	0	0	0	0	0	600	0.45	2 R
Albifimbria verrucaria (Albertini & Schweinitz) Ditmar	1	0.06	1 R	0	0	0	0	0	0
Alternaria	17	0.97	4 L	8	0.56	4 L	800	0.60	5 L
A. alternata (Fries) Keissler	13	0.74	3 R	6	0.42	3 R	800	0.60	5 L
A. tenuissima (Kunze) Wiltshire	4	0.23	2 R	2	0.14	1 R	0	0	0
Arthrinium sacchari (Speg.) M.B. Ellis	0	0	0	7	0.49	1 R	0	0	0
Aspergillus	826	47.34	24H	721	50.42	23 H	26800	20.11	18 H
*A. brasiliensis*Varga, Frisvad & Samson	7	0.40	2 R	2	0.14	1 R	100	0.08	1 R

Medium / Fungal taxa	Plating method						Dilution method		
	On Capek's (*) Without carbon source			On Capek's cellulose			On Capek's cellulose		
	CFU	%CFU	F &OR	CFU	%CFU	F &OR	CFU	%CFU	F &OR
***A. flavus*Link**	448	25.67	24 H	356	24.90	22 H	5600	4.20	11 M
***A.fumigatus* Fresenius**	5	0.29	3 R	16	1.12	6 L	800	0.60	7 M
***A. latus* (Thom & Raper) A.J. Chen, Frisvad & Samson**	2	0.11	2 R	0	0	0	0	0	0
***A. nidulans* (Eidam) G. Winter**	0	0	0	0	0	0	100	0.08	1 R
***A. niger* VanTiegh**	344	19.71	20 H	316	22.10	17 H	14800	11.10	13 H
***A. ochraceus* G.Wilh**	0	0	0	4	0.28	1 R	0	0	0
***A. parasiticus* Speare**	20	1.15	4 L	2	0.14	2 R	300	0.23	3 R
***A. tubingensis* Mosseray**	0	0	0	25	1.75	1 R	5100	3.83	2 R
***Bipolaris spicifera* (Bainier) Subramanian**	3	0.17	3 R	0	0	0	200	0.15	1 R
***Cephaliophora tropica* Thaxt**	0	0	0	2	0.14	1 R	0	0	0
***Ceratocystisadiposa* (E.J. Butler) C. Moreau**	1	0.06	1 R	5	0.35	1 R	0	0	0

Medium / Fungal taxa	Plating method						Dilution method		
	On Capek's (*) Without carbon source			On Capek's cellulose			On Capek's cellulose		
	CFU	%CFU	F &OR	CFU	%CFU	F &OR	CFU	%CFU	F &OR
Chaetomium globosum Kunze	0	0	0	4	0.28	2 R	0	0	0
Cladosporium	3	0.17	3 R	0	0	0	2900	2.18	7 M
C. cladosporioides (Fresenius) G.A. de Vries	1	0.06	1 R	0	0	0	2900	2.18	7 M
C. sphaerospermum Penz	2	0.11	2 R	0	0	0	0	0	0
Clonostachys rosea (Link) Schroers, Samuels, K.A. Seifert & W. Gams	2	0.11	2 R	0	0	0	0	0	0
Cochliobolus australiensis (Tsuda & Ueyama) Alcorn	0	0	0	1	0.07	1 R	0	0	0
Cunninghamella echinulata (Thaxt.)	1	0.06	1 R	5	0.35	3 R	0	0	0
Didymella glomerata (Corda) Qian Chen & L. Cai	0	0	0	0	0	0	2500	1.88	2 R
Epicoccum nigrum Link	0	0	0	1	0.07	1 R	0	0	0
Exophiala	0	0	0	0	0	0	900	0.68	2 R

Medium / Fungal taxa	Plating method						Dilution method		
	On Capek's (*) Without carbon source			On Capek's cellulose			On Capek's cellulose		
	CFU	%CFU	F &OR	CFU	%CFU	F &OR	CFU	%CFU	F &OR
(Langeron) *E. jeanselmei* McGinnis & A.A. Padhye	0	0	0	0	0	0	400	0.30	1 R
E. spinifera (H.S. Nielsen & Conant) McGinnis	0	0	0	0	0	0	500	0.38	1 R
Exserohilum rostratum (Drechsler) K.J. Leonard & Suggs	6	0.34	2 R	0	0	0	0	0	0
Fusarium	471	26.99	24 H	429	30.00	23 H	11700	8.78	14 H
F. anthophilium (A. Braun) Wollenw.	0	0	0	8	0.56	1 R	0	0	0
F. arthrosporioides Sherb.	22	1.26	3 R	0	0	0	0	0	0
F. circinatum Nirenberg & O'Donnell	45	2.58	4 L	12	0.84	2 R	900	0.68	2 R
F. incarnatum (Desm.) Sacc.	83	4.76	7 M	41	2.87	4 L	1600	1.20	3 R
F. oxysporum Schltdl	125	7.16	14 H	175	12.24	14 H	1500	1.13	4 L
F. proliferatum (Matsush.) Nirenberg	29	1.66	3 R	6	0.42	1 R	3300	2.48	4 L

Medium / Fungal taxa	Plating method						Dilution method		
	On Capek's (*) Without carbon source			On Capek's cellulose			On Capek's cellulose		
	CFU	%CFU	F &OR	CFU	%CFU	F &OR	CFU	%CFU	F &OR
***F. pseudonygamai* O'Donnell & Nirenberg**	1	0.06	1 R	1	0.07	1 R	0	0	0
***F. solani* (Mart.) Sacc.**	8	0.46	1 R	57	3.99	4 L	1600	1.20	2 R
***F. subglutinans* (Wollenw. & Reinking) P.E. Nelson, Toussoun & Marasas**	59	3.38	9 M	93	6.50	8 M	1500	1.13	3 R
***F. verticillioides* (Sacc.) Nirenberg,**	99	5.67	9 M	36	2.52	9 M	1300	0.98	4 L
***Graphium penicillioides* Corda**	0	0	0	0	0	0	100	0.08	1 R
***Lichtheimia corymbifera* (Cohn) Vuill**	1	0.06	1 R	0	0	0	0	0	0
***Microascus brevicaulis*S.P. Abbott**	1	0.06	1 R	0	0	0	0	0	0
Mucor	41	2.35	13 H	43	3.01	12 H	700	0.53	3 R
***M. circinelloides* Tiegh.**	6	0.34	2 R	16	1.12	5 L	0	0	0
***M. hiemalis* Wehmer,**	13	0.74	5 L	7	0.49	4 L	600	0.45	2 R

Medium / Fungal taxa	Plating method						Dilution method		
	On Capek's (*) Without carbon source			On Capek's cellulose			On Capek's cellulose		
	CFU	%CFU	F &OR	CFU	%CFU	F &OR	CFU	%CFU	F &OR
***M. racemosus* Fresen.**	22	1.26	7 M	20	1.40	6 L	100	0.08	1 R
***Nigrospora oryzae* (Berk. & Broome) Petch**	1	0.06	1 R	1	0.07	1 R	0	0	0
Penicillium	33	1.89	11 M	55	3.85	11M	8900	6.68	11 M
***P. aurantiogriseum* Dierckx**	23	1.32	8 M	19	1.33	5 L	5900	4.43	9 M
***P. chrysogenum* Thom**	10	0.57	4 L	36	2.52	6 L	2800	2.10	5 L
***P. citrinium* Thom**	0	0	0	0	0	0	200	0.15	1 R
***Phaeoacremonium viticola* J. Dupont**	3	0.17	2 R	3	0.21	1 R	300	0.23	3 R
***Purpureocillium lilacinum* (Thom) Samson**	0	0	0	0	0	0	2100	1.58	2 R
***Rhizopus oryzae* Went & H.C. Prinsen Geerligs,**	0	0	0	3	0.21	1 R	0	0	0
***S. elegans* Pidopl. ex W. Gams**	9	0.52	3 R	0	0	0	1200	0.90	2 R

Medium / Fungal taxa	Plating method						Dilution method		
	On Capek's (*) Without carbon source			On Capek's cellulose			On Capek's cellulose		
	CFU	%CFU	F &OR	CFU	%CFU	F &OR	CFU	%CFU	F &OR
Talaromyces	18	1.04	12 M	9	0.70	4 L	1100	0.83	5 L
T.duclauxii (Delacr.) Samson, N. Yilmaz, Frisvad & Seifert	12	0.69	5 L	8	0.56	3 R	1100	0.83	5 L
T. funiculosus (Thom) Samson, N. Yilmaz, Frisvad &	3	0.17	2 R	1	0.07	1 R	300	0.17	2 R
T. pinophilus (Hedgc.) Samson, N. Yilmaz, Frisvad & Seifert	3	0.18	3R	1	0.07	1 R	100	0.08	1 R
Trichoderma	2	0.12	2 R	2	0.14	2 R	100	0.08	1 R
T. harzianum Rifai	1	0.06	1 R	1	0.07	1 R	100	0.08	1 R
T. koningii Oudem	1	0.06	1 R	1	0.07	1 R	0	0	0
T. aureoviride Rifai	1	0.06	1 R	0	0	0	0	0	0
Yeast sp.	306	17.54	8 M	126	8.81	10 M	72000	54.01	20 H

Medium / Fungal taxa	Plating method						Dilution method		
	On Capek's (*) Without carbon source			On Capek's cellulose			On Capek's cellulose		
	CFU	%CFU	F &OR	CFU	%CFU	F &OR	CFU	%CFU	F &OR
Total CFUs	1745			1430			133300		
Total No. of genera	23			21			17		
Total No. of species	44			42			37		

(*)= Capek's agar without carbon source, CFU= Colony forming units. F= Frequency, OR= Occurrence remarks

Cellulases and xylanases detected in fungal cultures

All tested strains (51); Cellulase 39 of 51, xylanase 31 of 51 exhibited cellulolytic and/or xylanolytic activities in solid media containing either cellulose or xylan as a carbon source (Table 2). 19 strains produced both cellulose and xylanase and these were *Alternaria alternata* (AUMC 14566), *Ceratocystis adiposa* (AUMC 14644) and *Fusarium semitectum* (AUMC 14670). Among the 20 fungal strains which exhibited only cellulolytic activity, *Aspergillus tamarii* was the best producer where the calculated enzyme index was 2.4. Each of *Bipolaris spicifera* (AUMC 14659), *Alternaria alternata* (AUMC 14564), *Aspergillus flavus* (AUMC14587), *Stachybotrys elegans* (AUMC 14706) and *Fusarium incarnatum* (AUMC 14669) showed moderate ability of cellulose production. The remaining positive strains were of low cellulolytic ability (Table, 2).

When fungal strains were tested for xylanolytic ability, it was found that 31out of 51 produced detectable amounts of the enzyme. Based on enzyme index, 17 fungal strains mainly belong to *A.niger* secreted relatively high levels of xylanase with enzyme indices ranging from 2.1 to 2.9. Moderate levels of xylanase were secreted by 9 strains (enzyme indices between 1.6 – 1.9). The remaining 5 fungal strains exhibited low levels of the enzyme as shown in table (2).

Concentrations and activities of fungal enzymes

In the current study 16 active fungal strains were selected for quantitative determination of enzymes concentration as well as relative and specific activities of cellulases and xylanases.

When CMC was used as a substrate the highest cellulase concentration was recorded in broth culture of *Stachybotrys chartarum* AUMC 14705 (7.9 IU/ml and 1579.6 IU/gds).The produced cellulase had a relative activity of 1.7 IU/ml/min and specific activity of 14.590 IU/mg protein. Two strains of *A. niger* (AUMC 14614 and AUMC 14625) produced also high quantities of cellulase at concentrations of 7.8 and 7.1 IU/ml matching 1551.5 and 1424.5 IU/gds. Relative and specific activities of the enzyme

produced by these two strains were recorded as 1.7 and 1.6 IU/ml/min and 11.909 and 12.130 IU/mg protein respectively.

The lowest enzyme concentration as well as relative and specific activities of cellulase was detected in the culture of *Ceratocystis adiposa*. The remaining 7 fungal strains showed intermediate values of enzyme concentrations and activities as shown in table (3).

Cellulases are industrially important enzymes, which are widely used to bioethanol production from lignocellulosic biomass (**Zhang and Sang 2012, Behera and Ray 2016).** They are a cocktail of three types of complex enzymes namely, cellobiohydrolases (EC3.2.1.91), endoglucanases or CMCases (EC 3.2.1.4) and β-glucosidases (EC 3.2.1.21), acting synergistically to convert the cellulose into glucose (**Satyamurthy *et al*., 2016** and **Prasanna *et al*., 2016**). Early enzymatic exploration of several fungi that could be cultured in sugarcane bagasse **(Prayuwidayati *et al*., 2008)** revealed that *Trichoderma viride*, *A. niger, A. oryzae, Rhizopus oryzae* has cellulase activity of 0.034, 0.007, 0.007, 0.004 units/ml respectively. **Mohammad *et al*. (2019)** tested cellulase production by Wild Strain of *A. niger* using sugar cane bagasse as substrate. They reported that the highest cellulase enzyme activity was observed after the 5th day of incubation (0.243U/mL). **Liu *et al.* (2012)** compared the endoglucanase activity of 4 species of fungi (*Trichoderma koningii*, *T. reesei*, *T. viride* and *A. niger*) and found that *T. koningii* was the best cellulase producer (31,300 IU/L), followed by *T. viride* (22,000 IU/L), in a medium containing 20 g/L of microcrystalline cellulase.

Boonyuen *et al.* (2014) screened thirty-six fungal isolates for the presence of polyphenol oxidase, endoglucanase and xylanase activities. They found that five isolates belonging to *Aspergillus flavus*, *Aspergillus niger* and *Penicillium citrinum* were the best enzyme producers. Additional 15 moderately to highly xylolytic, cellulolytic and ligninolytic isolates were added to construct fungal mixes for enzyme production**. Lokhande and Pethe (2016)** recorded peak cellulase activities at day 5 of incubation with *Aspergillus niger* while **Gautam *et al.* (2010)**

reported the maximum time of cellulase production was 4 days of fermentation period for *Aspergillus niger* by using municipal solid waste residue. **Ogel *et al.* (2001)** reported that time course required to reach maximum levels of activity may be affected by several factors like the presence of different ratios of amorphous to crystalline cellulose. In process economics, longer periods of fermentation are quite uneconomical and such processes are prone to contamination **(Gautam *et al.*, 2010).**

As shown in table (4) xylanase concentration was ranging from 15.0-360.6 IU/ml and 2996.7 – 72110.0 IU/gds. The highest quantities were observed cultures of *A. niger* (AUMC 14611) which yielded xylanase with relative activity of 23.0 IU/ml/min and specific activity of 61.611 IU/mg protein. The lowest values of xylanase concentrations and activities were detected in cultures of *Exserohilum rostratum* AUMC 14656. Fungal strains of *A. niger* (AUMC 14611 and AUMC 14613), A. *tamarii* (AUMC 14632) and *Ceratocystis adiposa* (AUMC 14644) produced relatively high quantities of xylanase where the calculated enzyme concentrations ranged from 293.8-360.6 IU/ml and from 58757.7-72110.0 IU/gds. Relative and specific activities of xylanase produced by these fungi were considerably high. The remaining ten strains yielded lower quantities of xylanase as recorded in table (4).

According to **Polizeli *et al.* (2005)** two hemicellulases have a significant role for the depolymerization of hemicellulose: xylanases and the xylosidases. The former are applied for xylan hydrolysis, the main polysaccharide of hemicellulose and xylosidases act after the hydrolysis of xylan by xylanases, cleaving oligomers of D-xylopyranosyl and xylobiose in free xylose. A strain of *A niger* was reported by **Dos Santos *et al*. (2015)** to be a potential xylosidase producer showing high activity (141U/g). Lower values of -xylosidase were obtained by other species (**Dogaris *et al.*, 2009 and de Oliveira *et al.*, 2019**). In a similar study carried out with an *A. niger* strain isolated from copra paste, the xylosidase could not be detected in the crude extract (**Díaz-Malváez *et al.*, 2013**). **Tallapragada and Venkatesh (2011)**

successfully isolated, identified and studied the optimization conditions for xylanase enzyme produced by *Aspergillus niger* under submerged fermentation.

In conclusion, the current study introduces new fungal candidates for active production of cellulolytic and xylanolytic enzymes from cheap agro-industrial wastes. More work is needed for optimization of cultural and environmental conditions to achieve higher enzymatic yield and to obtain fermentable sugars required for various biotechnological applications.

Table (2): Cellulolytic and xylanolytic activities of fungi isolated from sugarcane bagasse.

Fungal species	AUMC No.	Cellulase			Xylanase		
		Colony Diam. (mm)	Clear zone diam. (mm)	Enzyme index	Colony Diam. (mm	Clear zone diam. (mm))	Enzyme index
Alternaria alternata	14563	20	26	1.3 L	-	-	-
Alternaria alternata	14566	10	15	1.5 M	20	25	1.2 L
Alternaria alternata	14565	15	20	1.3 L	0	0	0
Alternaria alternata	14564	20	35	1.75 M	0	0	0
Alternaria alternata	14568	0	0	0	15	28	1.8 M
Aspergillus flavus	14585	23	33	1.4 L	0	0	0
Aspergillus flavus	14587	23	40	1.7 M	0	0	0
Aspergillus flavus	14586	28	40	1.4 L	0	0	0
Aspergillus fumigatus	14594	29	36	1.2 L	0	0	0
Aspergillus latus	14595	28	34	1.2 L	0	0	0
Aspergillus niger	14598	23	24	1.4 L	0	0	0
Aspergillus niger	14597	29	36	1.2 L	0	0	0
Aspergillus niger	14611	24	35	1.9 M	28	76	2.7 H
Aspergillus niger	14607	10	15	1.5 M	30	58	1.9 M
Aspergillus niger	14603	0	0	0	30	35	1.6 M
Aspergillus niger	14601	20	25	1.25 L	26	70	2.6 H
Aspergillus niger	14609	22	23	1.04 L	24	64	2.6 H
Aspergillus niger	14600	24	35	1.9 M	32	78	2.4 H
Aspergillus niger	14625	10	17	1.7 M	23	58	2.5 H
Aspergillus niger	14622	10	17	1.7 M	30	82	2.7 H

Aspergillus niger	14608	0	0	0	36	70	1.9 M
Aspergillus niger	14599	0	0	0	30	88	2.9 H
Aspergillus niger	14605	0	0	0	34	82	2.4 H
Aspergillus niger	14602	0	0	0	24	82	3.4 H
Aspergillus niger	14606	0	0	0	30	78	2.6 H
Fungal species	AUMC No.	Cellulase			Xylanase		
		Colony Diam. (mm)	Clear zone diam. (mm)	Enzyme index	Colony Diam. (mm	Clear zone diam. (mm))	Enzyme index
Aspergillus niger	14612	0	0	0	32	68	2.1 H
Aspergillus niger	14604	0	0	0	28	70	2.5 H
Aspergillus niger	14610	0	0	0	28	64	2.2 H
Aspergillus niger	14614	24	35	1.9 M	38	74	1.9 M
Aspergillus niger	14615	20	25	1.25 L	28	66	2.3 H
Aspergillus niger	14613	0	0	0	30	76	2.5 H
Aspergillus ochraceus	14626	22	23	1.04 L	NT	NT	NT
Aspergillus tamarii	14632	9	22	2.4 H	32	78	2.4 H
Aspergillus tubingensis	14640	0	0	0	28	64	2.2 H
Ceratocystis adiposa	14644	20	25	1.25 L	45	49	1.08 L
Ceratocystis adiposa	14645	20	25	1.25 L	45	49	1.08 L
Ceratocystis adiposa	14646	22	23	1.04 L	25	30	1.2 L
Cladosporium cladosporioides	14648	10	12	1.2 L	0	0	0
Exserohilum rostratum	14655	10	17	1.7 M	30	35	1.6 M
Exserohilum rostratum	14656	10	25	2.5 H	38	74	1.9 M

Bipolaris spicifera	**14660**	**20**	**25**	**1.25 L**	**0**	**0**	**0**
Bipolaris spicifera	**14659**	**10**	**20**	**2 M**	**0**	**0**	**0**
Fusarium incarnatum	**14669**	**20**	**30**	**1.5 M**	**0**	**0**	**0**
Fusarium incarnatum	**14670**	**25**	**33**	**1.3 L**	**7**	**9**	**1.2 L**
Fusarium oxysporum	**14674**	**25**	**30**	**1.2 L**	**0**	**0**	**0**
Fusarium proliferatum	**14675**	**29**	**37**	**1.2 L**	**0**	**0**	**0**
Fusarium proliferatum	**14677**	**35**	**40**	**1.1 L**	**0**	**0**	**0**
Fusarium verticillioids	**14682**	**27**	**33**	**1.2 L**	**0**	**0**	**0**
Fusarium verticillioids	**14683**	**29**	**36**	**1.2 L**	**0**	**0**	**0**
Stachybotrys chartraum	**14705**	**5**	**25**	**5.0 H**	**30**	**35**	**1.6 M**
Stachybotrys elegans	**14706**	**10**	**17**	**1.7 M**	**15**	**28**	**1.8 M**

Enzyme index: High =More than 2, Moderate 1.5-2, low=Less than 1.5.

Table (3): Concentration, relative and specific activities of fungal cellulose

Fungal species	AUMC	Abs.	Abs. (×3)	Conc. Of Glucose (g/L)	Conc. Of Enzyme (IU/ml)	Conc. Of Enzyme (IU/gds)	Total Protein (mg/ml)	Relative activity (IU/ml/min)	Specific activity (IU/mg protein)
Aspergillus niger	14614	1.381	4.143	3.956	7.8	1551.5	0.142	1.7	11.909
Aspergillus niger	14609	0.347	1.041	0.994	1.9	389.8	0.106	0.4	4.008
Aspergillus niger	14615	0.8	2.4	2.292	4.5	898.8	0.15	1.0	6.531
Aspergillus niger	14625	1.268	3.804	3.633	7.1	1424.5	0.128	1.6	12.130
Aspergillus tamarii	14632	0.224	0.672	0.642	1.3	251.7	0.111	0.3	2.471
Ceratocystis adiposa	14646	0.163	0.489	0.467	0.9	183.1	0.097	0.2	2.058
C. adiposa	14644	0.919	2.757	2.633	5.2	1032.4	0.163	1.1	6.904
Exserohilum rostratum	14655	0.498	1.494	1.427	2.8	559.5	0.106	0.6	5.753
Exserohilum rostratum	14656	0.726	2.178	2.080	4.1	815.6	0.104	0.9	8.548
Stachybotrys chartarum	14705	1.406	4.218	4.028	7.9	1579.6	0.118	1.7	14.590
Stachybotrys elegans	14706	0.764	2.292	2.189	4.3	858.3	0.112	0.9	8.353

Table (4): Concentration, relative and specific activities of fungal Xylanase.

Fungal species	AUMC No.	Abs.	Abs. (×30)	Conc. Of Xylose (g/L)	Conc. Of Enzyme (IU/ml)	Conc. Of Enzyme (IU/gds)	total Protein (mg/ml)	Relative activity (IU/ml/min)	Specific activity (IU/mg protein)
Aspergillus niger	14614	0.087	2.61	2.475	16.5	3300.1	0.2	1.1	5.273
Aspergillus niger	14609	0.414	12.42	11.778	78.5	15704.1	0.253	5.0	19.835
Aspergillus niger	14615	0.475	14.25	13.514	90.1	18018.0	0.284	5.8	20.273
Aspergillus niger	14625	0.409	12.27	11.636	77.6	15514.5	0.25	5.0	19.830
Aspergillus niger	14613	1.841	55.23	52.376	349.2	69834.0	0.368	22.3	60.639
Aspergillus niger	14611	1.901	57.03	54.083	360.6	72110.0	0.374	23.0	61.611
Aspergillus niger	14622	1.709	51.27	48.620	324.1	64826.9	0.341	20.7	60.748
Aspergillus niger	14600	1.839	55.17	52.319	348.8	69758.2	0.345	22.3	64.611
Aspergillus niger	14601	1.549	46.47	44.068	293.8	58757.7	0.315	18.8	59.606
Aspergillus tamarii	14632	0.331	9.93	9.417	62.8	12555.7	0.214	4.0	18.748
Ceratocystis adiposa	14646	0.108	3.24	3.073	20.5	4096.7	0.297	1.3	4.408
Ceratocystis adiposa	14644	0.294	8.82	8.364	55.8	11152.2	0.315	3.6	11.313
Exserohilum rostratum	14655	0.18	5.4	5.121	34.1	6827.9	0.188	2.2	11.605
Exserohilum rostratum	14656	0.079	2.37	2.248	15.0	2996.7	0.14	1.0	6.840
Stachybotrys chartarum	14705	0.26	7.8	7.397	49.3	9862.5	0.275	3.2	11.460
Stachybotrys elegans	14706	0.249	7.47	7.084	47.2	9445.2	0.234	3.0	12.898

References

Abdel-Sater M. A., Zohri A. A., Ragab W. S. M. and Nor El-Din S. M. (2015): c Filamentous Fungi and their Enzymatic Activities Associated with Sugar Cane Molasses. Egyptian Sugar Journal, 8: 53-68.

Abdi, A. and Omar, S. R.(2019): Increasing sugar supply on expanded beet production. Egypt Sugar Annual, GAIN Report Number: EG-19006 (9pages).

Abdullah S. K. and **Saleh** Y. A. (2010): Mycobiota Associated with Sugarcane (*Saccharumofficinarum* L.) cultivars in Iraq. Jordan Journal of Biological Sciences 3(4):193-202.

Abdullah, N. and Zafar, S.I. (1999): Ligno-cellulose biodegradation by white rot basidiomycetes: Overview. Int. J. Mush. Sci. 2: 59-78.

Adesina F.C., and Onilude A.A., (2013): Isolation, identification and screening of xylanase and glucanase- producing microfungi from degrading wood in Nigeria. African Journal of Agricultural Research 8(34): 4414-4421**.**

Bailey M.J., Biely P., and Poutanen K., (1992): Interlaboratory testing of methods for assay of xylanase activity. Journal of Biotechnology 23(3): 257-270.

Bansal N., Tewari R., Soni R.*et al.* (2012): Production of cellulases from Aspergillus niger NS-2 in solid state fermentation on agricultural and kitchen waste residues. Waste Manage. 32:1341-1346.

Bensch K., Braun, U., Schubert K. Groenewald J. Z. and Crous P. (2012): The genus *Cladosporium* **Studies in Mycology 72: 1-401.**

Bechem E. E. T. and Mbella C. E. J.(2019): Survey of Symptoms of Fungal Disease in Sugarcane (*Saccharumofficinarum* L.) in Buea, in the South West Region of Cameroon Annual Research & Review in Biology 31(3): 1-12.

Behera S.S. and Ray R.C. (2016): Solid state fermentation for production of microbial cellulases: recent advances and improvement strategies. Int. J. Biol. Macromol. 86, 656–669.

Boonyuen, N. ; Manoch, L. ; Luangsa-ard , J. J. ; Piasai , O. ; Chamswarng , C. ; Chuaseeharonnachai , C. ; Ueapattanakit c J.;, Arnthong , J. and Sri-indrasutdhi V. (2014): Decomposition of sugarcane bagasse with lignocellulose-derived thermotolerant and thermoresistant Penicillia and Aspergilli.International Biodeterioration & Biodegradation.92. 86-100.

Cort´es-Espinosa,D. V. ; Fern ´andez-Perrino,F. J. ; Arana-Cuenca,A. ; Esparza-Garc´ıa,F. ;Loera,O. andRodr´ıguez-V ´azquez R. (2006): Selection and identification of fungi isolated from sugarcane bagasse and their application for phenanthrene removal from soil. Journal of Environmental Science and Health 41:475–486.

de Oliveira Simões L.C., da Silva R.R., de Oliveira Nascimento C.E., Boscolo M., Gomes E. and da Silva R. (2019): Purification and physicochemical characterization of a novel thermostable xylanase secreted by the fungus Myceliophthora heterothallica F.2.1.4. Appl Biochem Biotechnol**.**

Díaz-Malváez F.I., García-Almendárez B.E., Hernández-Arana A., Amaro-Reyes A. and Regalado-González C. (2013): Isolation and properties of β-xylosidase from *Aspergillus niger* GS1 using corn pericarp upon solid state fermentation,Process Biochemistry, 48(7): 1018-1024, ISSN 1359-5113.

Dogaris I., Vakontios G., Kalogeris E., Mamma D. and Kekos D. (2009): Induction of cellulases and hemicellulases from Neurospora crassa under solidstate cultivation for bioconversion of sorghum bagasse ethanol. Ind. Crops Products., 29: 404-411.

Domsch K.H., Gams W. and Anderson T. (2007): Compendium of soil fungi. (2nd ed.), IHW-Verlag, pp. 672.

Dos Santos B. S. L., Gomes A. F. S., Franciscon E. G., de Oliveira J. M., Baffi M. A. (2015): Thermotolerant and mesophylic fungi from sugarcane bagasse and their prospection for biomass-degrading enzyme production. Brazilian Journal of Microbiology 46 (3): 903-910.

Eggins H. O. W. and Pugh G. J. F. (1962): Isolation of cellulose decomposing fungi from soil. Nature London 193: 94-95.

Ellis MB (1976): More Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England. Pp 481.

Ferreira F. L., Dall'Antonia C. B., Shiga E. A., Alvim L. J. and Bom Pessoni R. A. (2018): Sugarcane bagasse as a source of carbon for enzyme production by filamentous fungi. Hoehnea 45(1): 134-142.

Gautam S.P., Bundela P.S., Pandey A.K., Jamaluddin M.K.and Sarsaiya S. (2010): Fusarium sp. from municipal solid waste compost: ability to produce extracellular cellulases enzyme. International Journal of Biotechnology and Bioengineering Research, 2(2): 239– 245**.**

Ghose, T.K. and Bisaria, V.S. (1987): Measurement of hemicellulase activities part 1: xylanases. Pure and Applied Chemistry 59(12): 1739–1752.

Ismail M.A., Abdel-Hafez S.II, Hussein N.A. and Abdel-Hameed N.A. (2015): Contributions to the genus Fusarium in Egypt with dichotomous keys for identification of species. Tmkarpiński Publisher, Suchy Las, Poland, Pp. 175

Johnson L. E., Curl E. A., Bond J. H. and Fribourg H. A. (1960): Methods for studing soil microflora-plant disease relationship. Burgess publishing company, Minneapolis, USA.178pp.

Johnson L. E., Eiroy A. and Curl (1972): Methods for research on the ecology of soil-borne pathogens. Minneapolis, MN 55415: Burgress publishing Company, pp. 426.

Liu HQ, Feng Y., Zhao DQ, Jiang J. X. (2012): Evaluation of cellulases produced from four fungi cultured on furfural residues and microcrystalline cellulose. Biodegradation 23:465-472.

Lokhande S. and Pethe A. S. (2016): Isolation, screening and partial purification of cellulase from cellulase producing fungi. European Journal of Pharmaceutical and Medical Research. 3(9), 301-306.

Lowry, O.H., Rosebrough N.J., Farr A.L. and Randall R.J., (1951): Protein measurement with the folin phenol reagent. Journal of General Microbiology 131: 3017-3027.

Martins, D.A.B., Prado, H.F.A., Leite, R.S.R., Ferreira, H., Moretti, M.M.S., Silva, R. & Gomes, E. (2011). Agroindustrial wastes as substrates for microbial enzymes production and source of sugar for bioethanol production. In: S. Kumar (ed.). Integrated Waste Management - Volume II, 319-360.

Miller, G.L. (1959): Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry 31(3): 426-428**.**

Moretti MMS, Martins DAB, Da Silva R et al. (2012): Selection of thermophilic and thermotolerant fungi for the production of cellulases and xylanases under solid-state fermentation. Braz. J. Microbiol 43:1062-1071.

Moubasher A.H. (1993): Soil fungi in Qatar and other Arab Countries. Centre for Scientific and Applied Research, University of Qatar, Doha, Qatar. Pp 566

Moubasher AH, Abdel-Sater MA and Soliman Z (2016): Biodiversity and molecular characterization of yeast and filamentous fungi in the

air of citrus and grapevine plantations in Assiut area, Egypt. Mycosphere 7(3): 236–261.

Mohammad, I., Abdullahi, I. O., and Tijjani, M. B. (2019): Optimization of culture conditions for cellulase production by wild strain of Aspergillus niger using sugarcane bagasse as substrate. Journal of Sciecnce Technology and Education 7(3), 94-100.

Ogel, Z. B., Yarangumeli, K., Du, H.,and Ifrij, J. (2001). Submerged cultivation of Scytalidium thermophilum on complex lignocellulosic biomass. Enzyme Micobiol Technol, 28, 689-695.

Oja O. A., Olaniran O. A. and Adebayo T. A. (2014): Incidence of Mycotoxigenic fungi Associated with Decayed *Saccharrumofficinarum* and the Effect of Chlopyriphos insecticide on their mycelia Growth and Glutamic-oxaloacetic acid Activity. Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) 8(8): 37-44.

Parameswaran, B. (2009): Sugarcane bagasse. In: P.S.N.Nigam, P., A. Pandey (ed.). Biotechnology for agroindustrial residues utilisation - Utilisation of agroresidues. Springer, pp. 239-252.

Pinotti L. M., Paulino L. B., Agnezi J. C., Santos P. A., da Silva H. N. L., Zavarise J. P., Salomão G. S. B. and Tardioli P. W. (2020): Evaluation of different fungi and bacteria strains for production of cellulases by submerged fermentation using sugarcane bagasse as carbon source: Effect of substrate concentration and cultivation temperature. African Journal of Biotechnology 19(9):625-635**.**

Pitt JI (1979): The genus Penicillium and its teleomorphic states Eupenicillium and Talaromyces. Academic Press Inc. Ltd, London, Pp. 635**.**

Polizeli M., Rizzatti A.C.S., Monti R. et al. (2005): Xylanases from fungi: properties and industrial applications. Appl Biochem Microb 67:577-591

.**Prasanna, H.N., Ramanjaneyulu, G. and Reddy, B.R., (2016).** Optimization of cellulase production by *Penicillium* sp. 3 Biotech 162, 1–11.

Prayuwidayati, M., Irawan B., and Muhtarudin. (2008). Enzymatic decomposition ability test of fungi on sugarcane bagasse. Research Report. Research Institute, University of Lampung. (in Indonesian).

Raper K.B. and Fennell D.I. (1965): The genus *Aspergillus*. The Williams and Wilkins Company, Baltimore, USA, Pp. 686.

Raza M., Zhang Z-F., Kevin D., Diao H. Y-Z. and Cai L. (2019): Culturable plant pathogenic fungi associated with sugarcane in southern China. Fungal Diversity 99:1–104.

Satyamurthy, P., Jain, P., Karande, V.S., Nadanathangam, V., (2016): Nanocellulose induces cellulase production in *Trichoderma reesei*. Process Biochem. 51, 1452–1457.

Shrestha P., Ibáñez A. B., Bauer S., Glassman S. I., Szaro T. M. , Bruns T. D. and Taylor J. W. (2015): Fungi isolated from miscanthus and sugarcane: biomass conversion, fungal enzymes, and hydrolysis of plant cell wall polymers. Biotechnology

Simmons E.G., (2007): *Alternaria*: An Identification Manual. Utrecht, Netherlands: CBS Fungal Biodiversity Centre.

Sivanesan A. (1987): *Graminicolous* species of *Bipolaris, Curvularia, Drechslera, Exserohilum* and their teleomorphs. Mycological Papers 158: 1-261.

Souza D.T., Bispo A.S., Bon E.P. et al. (2012): Production of thermophilic endo-beta-1,4-xylanases by *Aspergillus fumigates* FBSPE-05 using agro-industrial by-products. Appl Biochem Biotech 166:1575-1515.

Sridevi B., and Charya M.A.S., (2013): Isolation, identification and screening of potential cellulase-free xylanase producing fungi. African Journal of Biotechnology 10(22): 4624-4630.

Tallapragada, P.and Venkatesh, K. (2011). Isolation, identification and optimization of xylanase enzyme produced by Aspergillus niger under submerged fermentation. Journal of Microbiology and Biotechnology Research 1: 137-147.

Zhang, H.,and Sang, Q., (2012). Statistical optimization of cellulases production by *Penicillium chrysogenum* QML-2 under solid-state fermentation and primary application to chitosan hydrolysis. World J. Microbiol. Biotechnol. 28, 1163 – 1174**.**

الملخص العربي

إنتاج إنزيمات السيليوليز والزيلانيز من الفطريات المعزولة من مصاصة قصب السكر

أحمد محمد محرم[1]، عبد الناصر أحمد زهرى[1] و داليا عبدالله محمد حسين[2]

[1] قسم النبات والميكروبيولوجي – كلية العلوم

[2] مركز أ. د/ عبد العال مباشر لعلوم الفطريات – جامعة أسيوط

يمثل الليجنوسليلوز فى قصب السكر مصدرًا ثمينًا ورخيصًا لإنتاج الإنزيمات بواسطة العديد من الأنواع الفطرية. وقد تم في هذه الدراسة إجراء التحليل الميكولوجى لخمسة وعشرين عينة من مصاصة قصب السكر ، ثم اختبار مقدرة السلالات الفطرية المعزولةعلى إنتاج الإنزيمات المحللة للسيلولوز والزيلان في البيئات الغذائية الصلبة والسائلة. أمكن تعريف 62 نوعًا من الفطريات تنتمي إلى 31 جنسًا من العينات المختبرة ، وكانت الأجناس الأكثر انتشارًا هي *Aspergillus* و *Fusarium* حيث تم عزلهما من 96٪ من عينات مصاصة قصب السكر يليهما *Mucor* و *Penicillium* و *Talaromyces* بنسب تراوحت بين 45% و 52 % من العينات ، وكانت الأنواع الأكثر انتشارًا هي *Aspergillus flavus* ، *A. niger* و *Fusarium oxysporum*. وبدراسة تتابع النيوكليوتيدات فى منطقة ITS لـلحمض النووى rDNA فى خمسة عزلات فطرية أمكن تعريف نوعين جديدين على البيئة المصرية وهما *Ceratocystis adiposa* و *Phaeoacremonium viticola* بالإضافة إلى تأكيد تعريف ثلاثة أنواع أخرى هى *Sarocladium kiliense* و *Stachybotrys chartarum* و *Stachybotrys elegans*. وكذلك تم الكشف عن بعض الأنشطة الإنزيمية للفطريات النامية على المزارع الصلبة وأثبتت النتائج مقدرة 73.7٪ من السلالات الفطرية على إنتاج الإنزيمات المحللة للسليلوز وكان أكثرها نشاطاً هي *Aspergillus tamarii* و *Exserohilum rostratum* و *S. chartarum*. كما لوحظ أن 59.8٪ من السلالات الفطرية استطاعت إنتاج الإنزيات المحللة لمركب الزيلان وكانت أفضل الفطريات إنتاجا للزيلانيز هى *A. niger* و *A. tamarii* و *A. tubingensis* و *C. adiposa* و *E.*

rostratum. كما أوضحت القياسات الطيفية باستخدام حمض الساليسيليك ثنائى النترات (DNS) إنتاج السليولين بتركيز عالٍ في المزارع السائلة لفطرة ***S. chartarum*** تليها ***A. niger*** و ***C. adiposa*** و ***S. elegans*** (10.2- 52.0 IU / ml). كما تراوح النشاط النسبي لانزيم السليوليز بين 0.2- و1.7 وحدة دولية / مل / دقيقة ، بينما تراوح النشاط النوعي بين 2.471- 14.590 وحدة دولية / ملجم بروتين. وكان تركيز الزيلانين وكل من النشاطا النسبي والنوعي له عالٍ بشكل ملحوظ خاصة في مزارع ***A. niger*** (360.6 وحدة دولية / مل ، 32.0 وحدة دولية / مل / دقيقة و 64.611 وحدة دولية / مجم بروتين على التوالي).