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Production of cellulase, exoglucanase and xylanase by different microorganisms cultivated on agricultural wastes.

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ABSTRACT

Out of 140 microbial isolates, 67 isolates were selected including 45 fungi, 12 actinomycetes and 10 bacteria based on the primary screening for cellulytic activity. These isolates were selected for secondary screening using alkali pretreated sugarcane bagasse (SCB) and Rice straw (RS) as agricultural wastes. The results showed high variation of FPase activity among fungal isolates using RS or SCB. The highest FPase activity (0.168 IU/ml) by *Aspergillus terreus*(F14) on SCB, which was about three times higher than those obtained on RS (0.053 IU/ml). On the other hand, the highest FPase activity (0.091 IU/ml) by *Scopulariopsishalophilica* (F61) using RS. The highest FPase activity of actinomycetes (0.051 IU/ml) was obtained by A6 isolate using both SCB and RS. The highest FPase activity (0.013 IU/ml) was obtained by the bacterial isolate B3 on SCB, while B4 isolate displayed highest FPase activity (0.013 IU/ml) on RS. The highest CMCase activity between the fungal isolates grown on SCB (0.213 IU/ml) was obtained by *Aspergillus terreus* (F14). While, *Penicilliumbrevicombactum* (F16) exhibited the highest CMCase activity (0.182 IU/ml) on RS. A3 isolate displayed the highest CMCase activity (0.156 IU/ml), on SCB. In addition, the highest CMCase activity (0.156 IU/ml) obtained by A3 on RS. While, the highest CMCase activity (0.028 IU/ml) displayed by B3 on SCB. On the other hand, B8 isolate displayed the highest CMCase activity (0.014±0.001 IU/ml) on RS. The highest xylanase activity (3.83 IU/ml) was recorded by *Aspergillus flavus* (F5) on SCB, while the highest xyalanase activity (4.16 IU/ml) was displayed by *Emericellanidulans* (F56) on RS. The highest xylanase activity among actinomycete isolates (4.02 IU/ml) was obtained by A6 and A15 on SCB. On the other hand, A15 isolate displayed the highest xylanase activity (4.20 U/ml) on RS. The highest xylanase activity (0.787 U/ml and 0.271) was recorded by B3 isolate on SCB and RS, respectively. The most cellulytic activity fungal isolates were identified microscopically to species level. Also, all actinomycetes and bacterial isolates were identified microscopically as morphological characteristics of bacterial colonies on nutrient agar. All bacterial isolates were gram positive bacilli .Morphological characteristics of actinomycetes were observed as filamentous bacteria that produce well-developed vegetative hyphae with branches. The genotypic characterization were done for the most active bacterial and actinomycetes isolates on the bases of 16S rRNA.

Keywords: cellulases, xylanase, agricultural wastes, bacteria, fungi, actinomycetes

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INTRODUCTION

In order to achieve a sustainable development, we must rely more on renewable resources instead of further depletion of environmental resources. To create sustainability, we must recognize that the waste is a resource that should be recycled and reused. If it is handled and treated correctly, waste can be a source of energy to complement other renewable energy sources. So, the use of fossil fuels can be reduced. In addition, the organic waste fractions contain high amounts of plant nutrients that ought to be recirculated back to agricultural fields [1]. Contrary to mineral fertilizers, the use of organic fertilizers (compost) supplies both nutrients and organic matter to agricultural land [2]. Additional benefits of composting as mechanism for waste management are production of valuable soil amendments, low operation costs, easy to be applied in most of developing countries, and encouragement of environmentally friendly practices such as reduction of the emission of greenhouse gases, promote the efficiency of fertilizer application [3].

To enable efficient degradation of plant polysaccharides, Microorganisms produce an extensive set of carbohydrate-active enzymes (CAZY). The variety of the enzyme set differs between Microorganisms and often corresponds to the requirements of its habitat. Carbohydrate-active enzymes can be organized in different families based on the amino acid sequence of the structurally related catalytic modules. Microbial enzymes involved in plant polysaccharide degradation are assigned to at least 35 glycoside hydrolase families, three carbohydrate esterase families and six polysaccharide lyase families [4].

Biotechnological conversion of cellulosic biomass is potentially sustainable approach to develop novel bioprocesses and products. Microbial cellulases have become the focal biocatalysts due to their complex nature and wide spread industrial applications [5].

The aim of this study was the Isolation of target microorganisms from different sources and Screening of the enzymatic activity of the isolated microorganisms to address the efficient microbial isolates that have high biodegradation activity.

MATERIAL AND METHODS

Samples collection

Different samples (soil, compost, bagasse and animal wastes) were collected from different localities of Egypt for isolation of different microorganisms (Table 1).

Table 1: Localities and sources of collected samples used for isolation of microorganisms

Location	Source	Location	Source
Qantara, Ismailia Governorate	Soil	Alexandria Governorate	Soil
Fayoum Governorate	Soil	Qaliubyia Governorate	Soil
Giza Governorate	Compost	Giza Governorate	Sugarcane bagasse
Sahl El-Hessenia, Sharkia Governorate	Soil	Giza Governorate	Animal wastes
South of Port Said Governorate	Soil	Giza Governorate	Soil
Port Said Governorate	Soil	Giza Governorate	Drainage canal

Isolation of Microorganisms

Microorganisms (Bacteria, fungi and actinomycetes) were isolated by transferring 20 g of samples (soil, compost, bagasse and animal wastes) to an autoclaved 250 ml Erlenmeyer flask containing 180 ml sterilized distilled water flasks were shaken at 120 rpm for 1 h at 28ºC.After shaking, 1 ml of sample suspension was transferred into 9 ml sterilized distilled water. Serial dilutions to 10^{-7} were conducted. 1 ml of 10^{-3} and 10^{-4} dilutions were used to isolate fungi using pour plate method [6]. Rose Bengal (65 ppm) was used to CZapek Dox Agar (CZ) as a bacteriostatic agent [7]. CZ agar with 10% NaCl was used for isolation of halophilic fungi .plates were incubated at 28ºC for five days to observe the growth of fungal mycelia. For isolation of Bacteria 100µl of 10^{-6} and 10^{-7} dilutions were spread on agar plates. Luria-Bertani Medium (L.B) [8] was used for isolation

of bacteria. Plates were incubated at 28ºC for one or two days to observe the growth of colonies. For isolation of actinomycetes, 100 µl of 10^{-3} and 10^{-4} dilutions were spread on agar plates of starch casein nitrate medium [9], plates were incubated at 28ºC for 1-2 weeks till colonies appeared. The isolated microorganisms were purified each on the appropriate medium till used.

Screening of cellulolytic microorganisms

Screening of cellulolytic fungal isolates

The isolated fungal cultures were screened for their ability to produce cellulases complex according to Teather and Wood [10]. Pores of 6 mm diameter were made in the solidified plates of fungal cellulase screening media and inoculated with 0.1 ml of spore suspension prepared from 7 day old slants. The plates were incubated at room temperature (28±2°C) for three days to allow fungal growth, then again incubated for 18 h at 50°C which is the optimum temperature for cellulases activity. After incubation, 10 ml of 1% Congo-Red staining solution was added to the plates that were shaken at 50 rpm for 15 min. The Congo-Red staining solution was then discarded, 10 ml of 1 N NaOH was added to the plates and shaken again at 50 rpm for 15 min. Finally 1 N NaOH was also discarded and the staining of the plates was examined by noticing the formation of yellow zones around the fungal spore inoculated wells.

Screening of cellulolytic Bacterial and Actinomycetes isolates

Confirmation of cellulose-degrading ability of bacterial and actinomycetes isolates was performed by streaking on the cellulose Congo-Red agar media. .The plates were incubated at 28±2°C (one or two days) in case of bacteria. Whereas actinomycetes require (3-5 days to observe the activity) then again for 18 h incubation at 50ºC, which is the optimum temperature for cellulases activity. After incubation 10 ml of 1 N NaOH was added to the plates and shaken again at 50 rpm for 15 min. Colonies showing discoloration of Congo-Red were taken as positive cellulose-degrading bacterial and actinomycetes colonies and only these were selected for further study.

Preparation of enzyme substrate

Pretreatment of Sugarcane bagasse (SCB) as a substrate

Sugarcane bagasse (collected from local market) was thoroughly washed, dried and milled to 1–2 mm particle size. The milled bagasse was mixed with 0.12 g NaOH/g dry weight and autoclaved at 121°C for 20 min. After autoclaving, the treated bagasse was washed with tap water, then distilled water till neutrality, and then dried at 80°C [11].

Pretreatment of Rice straw (RS) as a substrate

Chopped rice straw was pretreated by alkali, a total of 10 g chopped rice straw was suspended in 40 ml solution of 2% NaoH then autoclaved at 121ºC for 20 min. After autoclaving, the treated rice straw was washed with tap water, then distilled water till neutrality, and then dried at 80ºC [12].

Enzyme production

Enzymes production from fungi

Spores of the selected strains were washed from 5-day Potato Dextrose Agar (PDA) plates with 10 ml of 0.1% (v/v) Tween 80 solution, and this suspension (1×10^6 spores/ml) was used as inoculum. All inoculated flasks were incubated at 28ºC in a shaking incubator at 175 rpm for 7 days.

The experiments were conducted in two sets one SCB, the other with RS as substrates (sole carbon source). After incubation, the contents of the flasks were passed through Whatman filter paper No. 1 to separate mycelial mat from culture filtrate.The latter was then centrifuged in a

cooling centrifuge. Then the filtrate passed through syringe filter pore size 0.45 µm. The clear culture filtrate was taken to measure the final pH and determine protein content, reducing sugars and enzyme assays.

Enzymes production from bacteria and actinomycetes

The selected cellulolytic bacteria and actinomycetes isolates were cultured at 30ºCon shaking incubator at 200 rpm for five days and ten days, respectively. Enzyme production media were used for both bacteria [13] and actinomyctes[9].

The experiments were conducted in two sets, one with SCB and the other with RS. After five days of incubation, the contents of the flasks were passed through Whatman filter paper No. 1 to separate microbial cells and waste residues from culture filtrate.The latter was then centrifuged in a cooling centrifuge. Then the filtrate passed through syringe filter pore size 0.45 µm. The clear culture filtrate was taken to measure the final pH and determine protein content, reducing sugars and enzyme assays.

Cellulase assay

Enzyme activity was determined in culture filtrate by measuring the released sugars from substrates. One unit of enzyme activities was defined as the amount of enzymes required to release 1 µ mol of glucose or xylose/min under the assay conditions [14].

FPase Assay (Exoglucanase)

FPA is the most common total cellulase activity assay recommended by the International Union of Pure and Applied Chemistry (IUPAC) [15]. This assay is based on a fixed degree of conversion of substrate, i.e. a fixed amount (2 mg) of glucose (based on reducing sugars measured by the 3,5-dinitrosalicylic acid (DNS) assay released from 50 mg of filter paper within a fixed time (i.e., 60 min).This was done according to the method of[16]. One ml of culture filtrate from (fungi, bacteria and actinomycetes) as sources of enzyme was added to 1 ml of 0.05 M citrate-phosphate buffer with pH 4.8 containing 50 mg of filter paper (1.0×6.0cm) strips (Whatmen No. 1). The reaction was incubated at 50ºC for 60 min, then terminated by adding 3 ml of 3,5-dinitrosalicylic acid (DNS) reagent to the reaction mixture, then boiling all tubes for 15 min in water bath followed by transferring all test tubes into ice box for cooling. In these tests, reducing sugars were estimated spectrophotometrically at 540 nm with DNS [14] using glucose as standards.

Enzymatic activity calculations:

$$\text{FPase (IU/ml)} = \frac{\text{mg glucose released} \times 1000}{\text{volume of enzyme (culture filterate)} \times M.WT \text{ of glucose} \times Time}$$

Where

M.WT: molecular weight of glucose=180.16, Time: is the incubation time of reaction= 60 min.

Carboxymethylcellulase (CMCase) (Endoglucanase)

The reducing sugars are measured by the DNS method according to [17]. One ml of culture filtrate of fungi, bacteria and actinomycetes as sources of enzyme was added to 1 ml of 1% carboxymethylcellulose (CMC) in0.05 M citrate buffer (pH 4.8). Incubation ofthe reaction mixture was performed for 30 min at 50ºC.The reaction was terminated by adding 3ml of 3,5-dinitrosalicylic acid (DNS) to the reaction mixture, boiling all tubes for 15 min in water bath, then transferring all test tubes into ice box for cooling. In these tests, reducing sugars were estimated spectrophotometrically at 540 nm with 3,5-dinitrosalicylic acid [14] using glucose as standards.

Enzymatic activity calculations:

$$\text{CMCase (IU/ml)} = \frac{\text{mg glucose released} \times 1000}{\text{volume of enzyme (culture filterate)} \times \text{M.WT of glucose} \times \text{Time}}$$

Where

M.WT: molecular weight of glucose =180.16, Time: is the incubation time of reaction= 30 min.

Xylanase assay

This method is based on the determination of reducing sugar released from xylan by xylanase action [18]. Xylanase activity was determined by mixing 1 ml of 1% (w/v) birch wood xylan (prepared in 50 mM Acetate buffer, pH 5.3) with 1ml of culture filterate as a source of enzyme. The mixture was incubated at 50ºC for 5 min [19]. The reaction was stopped by the addition of 3 ml of 3,5-dinitrosalicylic acid (DNS) and the contents was boiled for 5 min. After cooling, the colour development was read at 540 nm. The amount of reducing sugar liberated was quantified using xylose as a standard. One unit of xylanase activity was defined as the amount of enzyme that liberates 1 µmol of xylose per min under the assay conditions.

Enzymatic activity calculations:

$$\text{Xylanase (IU/ml)} = \frac{\text{mg xylose released} \times 1000}{\text{volume of enzyme (culture filterate)} \times \text{M.WT of xylose} \times \text{Time}}$$

Where

M.WT: molecular weight of xylose =150.16, Time: is the incubation time of reaction= 5 min

Protein determination

The protein content of the enzyme preparation was determined by the method of Lowry et al. [20] as follows:1 ml of sample was added to 5 ml of Lowry C and left to stand for 10 min at room temperature. Then 0.5 ml of diluted FolinCiocalteus reagent was added and mixed immediately. The mixture was left for 20 min and the developed blue colour was measured spectrophotometrically at 750 nm. A standard curve was done with different concentrations of bovine albumin.

Genotypic characterization of the most efficient cellulolytic actinomycete and bacterial isolates

DNA Extraction

Genomic DNA of actinomycetes(A8 and A15) and bacterial (B3 and B8) isolates was isolated using Wizard® Genomic DNA purification Kit,Promega® Corporation, USA

PCR amplification of 16S rRNA gene

PCR amplification of 16SrRNA gene (1500 bp) was done using published primers 27F (5'-AGAGTTTGATCCTGG CTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') [21]. The PCR products were further cleaned up and purified using QIAquick Gel extraction kit, Qiagen® according to the manufacturer instructions.

Sequencing of 16S rRNA gene

Using the 16S rRNA specific primers [22], nearly full-length 16S rDNA gene regions were successfully amplified and sequenced.

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RESULTS

Isolation of microorganisms from different sources

Isolates of 100 fungi, 20 actinomycetes and 20 bacteria were isolated from different sources (soil, animal wastes, sugar cane bagasse and compost). All the above mentioned microbial isolates were subjected to the primary screening to test their ability for production of cellulolytic enzymes as qualitative screening.

Primary screening of different microbial isolates for their cellulolytic activity

The primary screening of 100 fungal,20 actinomycete and 20 bacterial isolates for their cellulolytic activity was estimated. The results showed that there was high variation in cellulolytic activity among all microorganisms (Tables 2, 3 and 4). After incubation period on carboxymethylcellulose–congo red media, the diameter of the yellow halo zone were observed. Production of cellulase is directly proportional to the diameter of yellow halo zone formed.

Table 2: Qualitative screening of fungal isolates for their cellulolytic activity

Isolate code	Cellulolytic activity	Isolate code	Cellulolytic activity	Isolate code	Cellulolytic activity	Isolate code	Cellulolytic activity
F1	+	F26	-	F51	+	F76	+
F2	+	F27	++	F52	+	F77	-
F3	-	F28	+	F53	++	F78	-
F4	++	F29	+	F54	-	F79	-
F5	++	F30	-	F55	+	F80	-
F6	-	F31	+	F56	+	F81	-
F7	-	F32	+++	F57	++	F82	-
F8	-	F33	-	F58	+	F83	-
F9	-	F34	++	F59	+	F84	-
F10	+	F35	-	F60	+	F85	-
F11	-	F36	-	F61	+++	F86	-
F12	++	F37	-	F62	+	F87	-
F13	++	F38	-	F63	-	F88	+
F14	+++	F39	++	F64	-	F89	-
F15	++	F40	-	F65	-	F90	-
F16	+	F41	++	F66	+	F91	+
F17	-	F42	-	F67	-	F92	-
F18	-	F43	-	F68	+	F93	-
F19	-	F44	+++	F69	+	F94	-
F20	-	F45	++	F70	+	F95	-
F21	+	F46	-	F71	+	F96	-
F22	-	F47	++	F72	+	F97	-
F23	-	F48	+	F73	+	F98	-
F24	-	F49	-	F74	+	F99	-
F25	-	F50	-	F75	-	F100	-

(+):weak activity,(++): moderate activity,(+++): high activeity,(-): no activity.

Table 3: Qualitative screening of the isolated actinomycetes isolates for their cellulolytic activity

Isolate code	Cellulolytic activity	Isolate code	Cellulolytic activity	Isolate code	Cellulolytic activity	Isolate code	Cellulolytic activity
A1	-	A6	+	A11	+	A16	+
A2	+	A7	-	A12	+	A17	-
A3	+	A8	++	A13	+++	A18	-
A4	+	A9	-	A14	+	A19	-
A5	-	A10	+	A15	+++	A20	-

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Table 4: Qualitative screening of the isolated bacterial isolates for their cellulolytic activity

Isolate code	Cellulolytic activity	Isolate code	Cellulolytic activity	Isolate code	Cellulolytic activity	Isolate code	Cellulolytic activity
B1	+	**B6**	++	**B11**	-	**B16**	-
B2	+	**B7**	+	**B12**	-	**B17**	-
B3	++	**B8**	++	**B13**	-	**B18**	-
B4	+	**B9**	+	**B14**	-	**B19**	-
B5	+	**B10**	+	**B15**	-	**B20**	-

(+):weak activity,(++): moderate activity,(+++): high activity,(-): no activity.

Identification of the most active cellulolytic microbial isolates

Depending on the results obtained from the primary screening of the isolated microorganisms for their cellulolytic activity as shown in (Tables 2, 3 and 4). Out of 140 microbial isolates, 67 isolates were selected to include 45fungi, 12 actinomycetes and 10 bacteria. These isolates were able to degrade carboxymethyl cellulose as a sole carbon source. The morphological characteristics of bacterial colonies on nutrient agar were round to irregular [2-4 mm in diameter]. All bacterial isolates were gram positive bacilli. Morphological characteristics of actinomycetes were observed as filamentous bacteria that produce well-developed vegetative hyphae with branches. The genotypic characterization were done for the most active bacterial and actinomycetes isolates which will be selected for the last degradation test (solid state fermentation).

In addition, The 45 cellulolytic fungal isolates were identified microscopically to species level by Arab society of Fungal Conservation (ASFC), Botany Department, faculty of science, Suez Canal University.The fungal isolates were belonged to the genus (*Aspergillus, Fusarium, Emericella, Trichoderma, penecillium, scopulariopsis, Eurotium, Stachybotry*and *Alternaria*).Identification using morphological characteristics of fungal isolates down to the species level were conducted according to standard identification keys, source and location of isolation are presented in Table (5).

Table 5: Localities, sources and identification to species level of cellulolytic fungal isolates

Isolate	location	source	Microscopic Identity
F1	Qantara, Ismailia Governorate	Soil	***Aspergillus niger***
F2	Qantara, Ismailia Governorate	Soil	***Aspergillus terreus***
F4	Qantara, Ismailia Governorate	Soil	***Aspergillus fumigatus***
F5	Qantara, Ismailia Governorate	Soil	***Aspergillus flavus***
F10	Qantara, Ismailia Governorate	Soil	***Penicilliumbrevicombactum***
F12	Fayoum Governorate	Soil	***Trichoderma harizianum***
F13	Fayoum Governorate	Soil	***Trichoderma viride***
F14	Fayoum Governorate	Soil	***Aspergillus terreus***
F15	Fayoum Governorate	Soil	***Aspergillus ochraceus***
F16	Fayoum Governorate	Soil	***Penicilliumbrevicombactum***
F21	Giza Governorate	Compost	***Aspergillus galucus***
F27	Sahl El-Hessenia, Sharkia Governorate	Soil	***Bisifusariumdimerum***
F28	Sahl El-Hessenia, Sharkia Governorate	Soil	***Sarocladiumstrictum***
F29	Sahl El-Hessenia, Sharkia Governorate	Soil	***Penicilliumislandicum***
F31	Sahl El-Hessenia, Sharkia Governorate	Soil	***Stachybotryschartarum***
F32	Sahl El-Hessenia, Sharkia Governorate	Soil	***Fusarium oxysporum***
F34	Sahl El-Hessenia, Sharkia Governorate	Soil	***Aspergillus ochraceus***

F39	South of Port Said Governorate	Soil	***Alternariaalternata***
F41	South of Port Said Governorate	Soil	***Eurotiumamstelodami***
F44	South of Port Said Governorate	Soil	***Fusarium oxysporum***
F45	Port Said Governorate	Soil	***Penicilliumislandicum***
F47	Port Said Governorate	Soil	***Aspergillus versicolor***
F48	Giza Governorate	Compost	***Emericellanidulans***
F51	Alexandria Governorate	Soil	***Trichoderma viride***
F52	Alexandria Governorate	Soil	***Trichoderma atroviridi***
F53	Qaliubyia Governorate	Soil	***Penicilliumchrysogenum***
F55	Qaliubyia Governorate	Soil	***Fusarium oxysporum***
F56	Qaliubyia Governorate	Soil	***Emericellanidulans***
F57	Giza Governorate	Sugarcane bagasse	***Fusarium oxysporum***
F58	Qaliubyia Governorate	Soil	***Aspergillus flavus***
F59	Qaliubyia Governorate	Soil	***Rhizopusstolonifer***
F60	Giza Governorate	Animal wastes	***Trichoderma harizianum***
F61	Giza Governorate	Animal wastes	***Scopulariopsishalophilica***
F62	Giza Governorate	Animal wastes	***Eurotiumchevalieri***
F66	Giza Governorate	Soil	***Aspergillus niger***
F68	Giza Governorate	Soil	***Aspergillus ochraceus***
F69	Giza Governorate	Soil	***Emericellanidulans***
F70	Giza Governorate	Soil	***Fusarium oxysporum***
F71	Giza Governorate	Drainage canal	***Aspergillus niger***
F72	Giza Governorate	Drainage canal	***Penicilliumpurpureogenum***
F73	Giza Governorate	Soil	***Emericellanidulans***
F74	Giza Governorate	Soil	***Emericellanidulans***
F76	Giza Governorate	Drainage canal	***Aspergillus versicolor***
F88	Giza Governorate	Sugarcane bagasse	***Trichoderma harizianum***
F91	Giza Governorate	Sugarcane bagasse	***Trichoderma harizianum***

Secondary screening for cellulolytic activity of microbial isolates

Based on the results of the primary screening of the microbial isolates, 45 fungal, 12 actinomycetes and10 bacterial isolates were selected for secondary screening to determine their ability of cellulolytic enzyme production under shaking flask techniques. Two agricultural wastes as a sole carbon source (both alkali pretreated sugar cane bagasse (SCB) and Rice straw (RS)) were used for different incubation conditions (7 days for fungal isolates at 28ºC, 5 days for bacterial isolates at 30ºC and 10 days for actinomycetes at 30ºC).

Effect of using different agricultural wastes on the cellulolytic enzymes production by microbial isolates

During the secondary screening for microbial isolates, natural lignocellulosic substrates can be used as the sole carbon source (alkali pretreated rice straw (RS) and alkali pretreated sugarcane bagasse (SCB) instead of synthetic (carboxymethylcellulose (CMC). The results indicated that using natural sources of carbon produces a well-balanced amount of core and accessory lignocellulosic enzymes required to degrade these substrates. These sources were chosen for solid state fermentation. To have efficient break down cellulose and hemicellulose into its monomers, the cellulase system applied should consist of three components, i.e. total cellulase (FPase), Endoglucanase (CMCase), and (Hemicellulase) xylanase that act synergistically to degrade both cellulose and hemicellulose as well.The culture filtrates were assayed for the cellulases, which include FPase (filter paper activity), CMCase (carboxymethylcellulase) and xylanase as well as extracellular protein formed in cultural filtrate of microbial isolates.

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Quantitative determination of Fpase activity (Exoglucanase)

FPase is a major component of cellulase enzymes complex, lack of whichcauses inefficient cellulose hydrolysis.The results in Table (6) showed high variation of FPase activity among fungal isolates using RS or SCB as sole carbon sources.Regarding to the FPase activity of fungal isolates Table (5) *Aspergillus terreus***(F14)**displayed the highest FPase activity by (0.168 U/ml) when grown on SCB as a sole carbon source which was about three times higher than those obtained on RS media (0.053 U/ml). On the other hand, variations were obtained when total activity compared to specific activity of each isolate due to the variation of protein concentration between each isolate on different substrates, in case of *Aspergillus fumigatus* **(F4)** which displayed highest FPase specific activity by (0.254 U/mg protein) whereas its total activity was (0.164 U/ml).Also, the data obtained in Table (6) showed that using RS as a sole carbon source resulted in FPase total activity ranged between (0.002 U/ml) obtained by *Aspergillus fumigatus* (**F4)** to (0.091 U/ml) by *Scopulariopsishalophilica* (**F61)**.

Table 6: FPase (exoglucanase) production by cellulolytic fungal isolates using alkali pretreated sugar cane bagasse and rice straw as a sole carbon source

Isolate	Sugarcane bagasse (SCB)		Rice straw (RS)	
	Total activity (U/ml)	Specific activity (U/mg protein)	Total activity (U/ml)	Specific activity (U/mg protein)
F1	0.049±0.001	0.094±0.001	0.030±0.001	0.035±0.001
F2	0.120±0.001	0.175±0.010	0.036±0.002	0.048±0.002
F4	0.164±0.005	0.254±0.004	0.002±0.000	0.002±0.000
F5	0.094±0.006	0.052±0.005	0.031±0.001	0.015±0.001
F10	0.094±0.003	0.142±0.005	0.053±0.002	0.037±0.002
F12	0.099±0.005	0.122±0.009	0.011±0.001	0.009±0.000
F13	0.118±0.001	0.224±0.011	0.052±0.004	0.041±0.003
F14	0.168±0.019	0.241±0.036	0.053±0.001	0.065±0.002
F15	0.087±0.006	0.096±0.005	0.022±0.001	0.037±0.002
F16	0.100±0.006	0.154±0.014	0.038±0.001	0.025±0.000
F21	0.104±0.001	0.143±0.009	0.059±0.002	0.044±0.003
F27	0.000±0.000	0.000±0.000	0.047±0.001	0.055±0.003
F28	0.052±0.004	0.104±0.009	0.034±0.002	0.053±0.003
F29	0.049±0.001	0.101±0.002	0.029±0.001	0.025±0.001
F31	0.000±0.000	0.000±0.000	0.049±0.002	0.037±0.001
F32	0.000±0.000	0.000±0.000	0.043±0.001	0.042±0.000
F34	0.114±0.002	0.177±0.004	0.026±0.000	0.032±0.001
F39	0.000±0.000	0.000±0.000	0.006±0.001	0.017±0.004
F41	0.073±0.002	0.111±0.000	0.046±0.002	0.042±0.003
F45	0.035±0.004	0.037±0.004	0.033±0.001	0.018±0.001
F47	0.093±0.008	0.132±0.014	0.009±0.001	0.012±0.000
F48	0.058±0.003	0.113±0.006	0.012±0.000	0.024±0.000
F51	0.008±0.001	0.006±0.006	0.013±0.001	0.024±0.001
F52	0.036±0.014	0.069±0.027	0.017±0.000	0.018±0.000
F53	0.005±0.000	0.015±0.004	0.026±0.002	0.072±0.007
F55	0.007±0.003	0.017±0.009	0.064±0.004	0.062±0.002
F56	0.032±0.010	0.026±0.008	0.031±0.004	0.026±0.003
F57	0.003±0.002	0.011±0.006	0.040±0.002	0.069±0.003
F58	0.061±0.013	0.105±0.024	0.013±0.001	0.013±0.000
F59	0.016±0.002	0.048±0.011	0.017±0.002	0.018±0.002
F60	0.069±0.008	0.130±0.009	0.032±0.001	0.041±0.002
F61	0.095±0.002	0.146±0.002	0.091±0.009	0.079±0.007
F62	0.000±0.000	0.000±0.000	0.005±0.003	0.006±0.004
F44	0.075±0.003	0.129±0.024	0.023±0.002	0.064±0.011
F66	0.024±0.008	0.034±0.012	0.022±0.001	0.031±0.003
F68	0.019±0.002	0.034±0.005	0.027±0.009	0.026±0.009

F69	0.000±0.000	0.000±0.000	0.018±0.009	0.024±0.005
F70	0.000±0.000	0.000±0.000	0.040±0.002	0.046±0.002
F71	0.003±0.002	0.003±0.002	0.027±0.006	0.028±0.006
F72	0.001±0.000	0.001±0.001	0.017±0.001	0.031±0.006
F73	0.022±0.007	0.026±0.023	0.010±0.002	0.012±0.002
F74	0.060±0.014	0.063±0.012	0.034±0.009	0.033±0.009
F76	0.024±0.003	0.027±0.003	0.043±0.008	0.039±0.006
F88	0.021±0.006	0.026±0.001	0.018±0.001	0.016±0.001
F91	0.046±0.005	0.070±0.013	0.008±0.001	0.007±0.001

Values are means ± SEM(standard error of means) of the three observations.

While, results in Table (7) showed that FPase total activity of actinomycetes ranged from 0.004-0.051 U/ml by **A10** and **A6** isolates, respectively using SCB as a sole carbon source. In the same context, in case of RS, **A6** displayed the highest FPase total activity by (0.062U/ml) as compared to **A10** which gave the lowest activity by (0.005 U/ml). Regarding specific activity on RS as a sole carbon source, actinomycetes isolates displayed FPase specific activity ranged from0.008-0.068 U/mg proteinby **A11**and**A8**, respectively.

Table 7: FPase (exoglucanase) production by cellulolytic actinomycete isolates using alkali pretreated sugarcane bagasse and rice straw as a sole carbon source

Isolate	Sugarcane bagasse(SCB)		Rice straw(RS)	
	Total activity (U/ml)	Specific activity (U/mg protein)	Total activity (U/ml)	Specific activity (U/mg protein)
A2	0.032±0.001	0.038±0.001	0.042±0.001	0.038±0.001
A3	0.036±0.001	0.035±0.000	0.038±0.003	0.035±0.003
A4	0.018±0.001	0.028±0.003	0.022±0.000	0.026±0.000
A6	0.051±0.002	0.046±0.001	0.062±0.003	0.038±0.005
A8	0.015±0.000	0.058±0.002	0.015±0.000	0.068±0.002
A10	0.004±0.001	0.028±0.000	0.005±0.002	0.017±0.000
A11	0.023±0.000	0.008±0.001	0.030±0.001	0.008±0.003
A12	0.039±0.001	0.020±0.001	0.055±0.001	0.028±0.001
A13	0.022±0.001	0.050±0.000	0.025±0.005	0.052±0.001
A14	0.026±0.000	0.029±0.003	0.043±0.004	0.024±0.005
A15	0.031±0.001	0.056±0.001	0.045±0.002	0.052±0.008
A16	0.014±0.000	0.021±0.001	0.028±0.001	0.029±0.001

Values are means ± SEM(standard error of means) of the three observations.

With respect to FPase total activity of bacterial isolates the obtained data presented inTable (8), showed that using SCB as a sole carbon source displayed activity ranged from (0.003 U/ml) by **B2** to (0.013 U/ml) by **B3** isolates, while using RS as a sole carbon source **B4** isolate displayed highest FPase activity by (0.013 U/ml) compared to **B5** isolate which did not exhibit any FPase activity. From the results of Table (8), FPase activity wasn't affected by using different carbon sources.

Table 8: FPase (exoglucanase) production by cellulolytic bacterial isolates using alkali pretreated sugarcane bagasse and rice straw as a sole carbon source

Isolate	Sugarcane bagasse (SCB)		Rice straw (RS)	
	Total activity (U/ml)	Specific activity (U/mg protein)	Total activity (U/ml)	Specific activity (U/mg protein)
B1	0.003±0.001	0.003±0.001	0.006±0.001	0.007±0.000
B2	0.003±0.000	0.003±0.000	0.004±0.000	0.007±0.000
B3	0.013±0.001	0.013±0.001	0.012±0.001	0.013±0.001
B4	0.011±0.000	0.011±0.000	0.013±0.001	0.015±0.001

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B5	0.003±0.000	0.003±0.000	0.000±0.000	0.000±0.000
B7	0.004±0.001	0.004±0.001	0.004±0.001	0.005±0.001
B8	0.012±0.001	0.012±0.001	0.010±0.001	0.012±0.001
B9	0.004±0.000	0.004±0.000	0.004±0.001	0.004±0.001
B10	0.005±0.001	0.005±0.001	0.003±0.001	0.004±0.001
B11	0.005±0.001	0.005±0.001	0.003±0.000	0.003±0.001

Values are means ± SEM(standard error of means) of the three observations.

Quantitative determination of CMCase activity (Endoglucanase)

As mentioned previously, effective cellulose hydrolysis requires more than one enzyme, CMCase endoglucanases (1,4-β-D-glucan-4-glucanohydrolase, EC 3.2.1.4) are more active against the amorphous regions ofcellulose and they can also hydrolyze substituted celluloses, such as carboxymethyl cellulose (CMC) and hydroxyethylcellulose (HEC) internally.

The CMCase activity among fungal isolates and the effect of using two different carbon sources alkali pretreated sugar cane bagasse and rice straw are shown in Table (9). The fungal isolates grown on SCB displayed CMCase total activity between (0.008 U/ml) by *Penicilliumpurpureogenum***(F72)** to (0.213U/ml) by *Aspergillus terreus* (**F14)**. While, the CMCase specific activity of fungal isolates on SCB was ranged from (0.012 U/mg protein) by *Penicilliumpurpureogenum* **(F72)** to (0.511 U/mg protein) by *Penicilliumchrysogenum*(**F53)**.Also, fungal isolates showed variation in its CMCase total activity on RS as a sole carbon source. *Penicilliumbrevicombactum*(**F16)** exhibited the highest CMCase total activity by (0.182U/ml)compared to *Eurotiumchevalieri***(F62)** which did not show any CMCase total activity. The results revealed that the CMCase activity of fungal activity were highly affected by type of agricultural wastes (carbon source) amended in production media *Aspergillus terreus*(**F14)** which displayed the highest CMCase total activity (0.213U/ml) using SCB , compared to less activity (0.164U/ml) on RS.

Table 9: CMCase (endoglucanase) production by cellulolytic fungal isolates using alkali pretreated sugarcane bagasse and rice straw as a sole carbon source

Isolate	Sugar cane bagasse (SCB)		Rice straw (RS)	
	Total activity (U/ml)	Specific activity (U/mg protein)	Total activity (U/ml)	Specific activity (U/mg protein)
F1	0.094±0.003	0.177±0.002	0.073±0.003	0.086±0.005
F2	0.201±0.004	0.293±0.013	0.127±0.001	0.170±0.000
F4	0.203±0.016	0.312±0.012	0.151±0.001	0.137±0.002
F5	0.119±0.008	0.065±0.003	0.100±0.001	0.050±0.001
F10	0.200±0.008	0.302±0.013	0.155±0.001	0.108±0.001
F12	0.188±0.002	0.230±0.005	0.073±0.001	0.063±0.001
F13	0.189±0.007	0.356±0.001	0.154±0.001	0.122±0.003
F14	0.213±0.002	0.304±0.009	0.164±0.001	0.201±0.003
F15	0.166±0.001	0.185±0.003	0.122±0.001	0.213±0.014
F16	0.178±0.001	0.274±0.009	0.182±0.002	0.117±0.001
F21	0.173±0.001	0.239±0.018	0.173±0.003	0.130±0.003
F27	0.051±0.001	0.267±0.001	0.139±0.001	0.164±0.003
F28	0.135±0.002	0.266±0.001	0.165±0.001	0.258±0.031
F29	0.099±0.003	0.202±0.008	0.092±0.001	0.078±0.002
F31	0.075±0.004	0.116±0.014	0.167±0.001	0.128±0.001
F32	0.087±0.005	0.424±0.037	0.158±0.003	0.158±0.004
F34	0.152±0.005	0.235±0.006	0.123±0.001	0.149±0.000
F39	0.074±0.003	0.335±0.012	0.036±0.000	0.108±0.007
F41	0.153±0.004	0.230±0.000	0.170±0.004	0.151±0.002
F45	0.085±0.006	0.090±0.030	0.089±0.002	0.050±0.000
F47	0.177±0.013	0.251±0.008	0.029±0.000	0.039±0.002
F48	0.074±0.004	0.147±0.014	0.053±0.000	0.102±0.003

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F51	0.068±0.001	0.126±0.007	0.056±0.001	0.100±0.004
F52	0.106±0.008	0.202±0.003	0.135±0.003	0.143±0.005
F53	0.158±0.001	0.511±0.015	0.091±0.000	0.170±0.018
F55	0.075±0.000	0.176±0.124	0.133±0.001	0.129±0.003
F56	0.164±0.003	0.131±0.014	0.087±0.002	0.074±0.001
F57	0.118±0.004	0.422±0.003	0.118±0.001	0.206±0.002
F58	0.097±0.005	0.166±0.044	0.081±0.002	0.080±0.001
F59	0.044±0.003	0.131±0.010	0.079±0.003	0.087±0.006
F60	0.114±0.005	0.219±0.020	0.166±0.001	0.211±0.012
F61	0.201±0.020	0.307±0.018	0.175±0.001	0.152±0.002
F62	0.018±0.001	0.029±0.021	0.000±0.000	0.000±0.000
F44	0.158±0.007	0.269±0.003	0.102±0.000	0.291±0.047
F66	0.077±0.001	0.107±0.001	0.082±0.001	0.118±0.001
F68	0.131±0.002	0.230±0.003	0.123±0.001	0.118±0.001
F69	0.066±0.001	0.194±0.005	0.134±0.001	0.187±0.005
F70	0.058±0.001	0.077±0.002	0.131±0.001	0.151±0.013
F71	0.061±0.001	0.054±0.001	0.110±0.001	0.111±0.000
F72	0.008±0.001	0.012±0.001	0.083±0.001	0.152±0.016
F73	0.052±0.002	0.119±0.005	0.117±0.000	0.148±0.005
F74	0.145±0.010	0.159±0.019	0.125±0.001	0.122±0.001
F76	0.078±0.002	0.089±0.003	0.135±0.002	0.124±0.001
F88	0.164±0.006	0.157±0.011	0.126±0.003	0.108±0.003

Values are means ± SEM (standard error of means) of the three observations.

Concerning to results in Table (10) showed the CMCase activity of actinomycete isolates,**A3** isolate displayed the highest CMCase total activity (0.156U/ml), while **A10** isolate exhibited the lowest activity (0.005U/ml) using SCB as a sole carbon source. The CMCase specific activity of**A15** isolate showed the highest activity (0.222U/mg protein), compared to the lowest specific activity (0.012U/mg protein) obtained by **A11** isolate using SCB as a sole carbon source. In addition, data in Table (10) showed that using RS as a sole carbon source resulted in highest CMCase total activity by **A3** (0.156U/ml)compared to the lowest activity (0.006U/ml) which recorded by **A10** isolate.

Table 10: CMCase (endoglucanase) production by cellulolytic actinomycete isolates using alkali pretreated sugarcane bagasse and rice straw as a sole carbon

	Sugarcane bagasse(SCB)		Rice straw (RS)	
Isolate	**Total activity (U/ml)**	**Specific activity (U/mg protein)**	**Total activity (U/ml)**	**Specific activity (U/mg protein)**
A2	0.129±0.002	0.157±0.002	0.134±0.001	0.121±0.001
A3	0.156±0.005	0.149±0.006	0.156±0.005	0.145±0.004
A4	0.074±0.001	0.115±0.004	0.100±0.000	0.118±0.001
A6	0.135±0.003	0.171±0.010	0.124±0.001	0.112±0.020
A8	0.037±0.000	0.156±0.003	0.044±0.001	0.135±0.001
A10	0.005±0.000	0.068±0.001	0.006±0.001	0.049±0.001
A11	0.127±0.000	0.012±0.000	0.148±0.000	0.011±0.002
A12	0.139±0.002	0.114±0.003	0.137±0.001	0.139±0.001
A13	0.100±0.000	0.175±0.003	0.128±0.005	0.131±0.003
A14	0.102±0.001	0.138±0.004	0.131±0.001	0.128±0.003
A15	0.115±0.001	0.222±0.002	0.130±0.000	0.160±0.010
A16	0.069±0.001	0.102±0.003	0.127±0.001	0.131±0.005

Values are means ± SEM (standard error of means) of the three observations.

With respect to bacterial isolates, Table (11) showed the highest CMCase total activity (0.028U/ml) displayed by **B3** isolate**,** while the lowest activity which obtained by **B11** isolate (0.001U/ml) when grown on SCB.On the other hand, using RS as a sole carbon source **B8** isolate displayed the highest CMCase total activity (0.014U/ml), while **B5** isolate didn't show any activity.

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Table 11: CMCase (endoglucanase) production by cellulolytic Bacterial isolates using alkali pretreated sugarcane bagasse and rice straw as a sole carbon source

Isolate	Sugarcane bagasse(SCB)		Rice straw(RS)	
	Total activity (U/ml)	Specific activity (U/mg protein)	Total activity (U/ml)	Specific activity (U/mg protein)
B1	0.003±0.000	0.003±0.000	0.006±0.001	0.006±0.001
B2	0.002±0.000	0.003±0.000	0.002±0.000	0.002±0.000
B3	0.028±0.001	0.029±0.002	0.009±0.001	0.009±0.001
B4	0.006±0.000	0.006±0.001	0.004±0.001	0.004±0.001
B5	0.003±0.001	0.002±0.000	0.000±0.000	0.000±0.000
B7	0.003±0.002	0.003±0.002	0.002±0.001	0.002±0.001
B8	0.026±0.001	0.026±0.000	0.014±0.001	0.014±0.001
B9	0.002±0.000	0.003±0.000	0.003±0.000	0.003±0.000
B10	0.002±0.001	0.002±0.001	0.002±0.001	0.002±0.001
B11	0.001±0.000	0.002±0.000	0.001±0.000	0.001±0.000

Values are means ± SEM (standard error of means) of the three observations.

Quantitative determination of Xylanase activity

Xylan (Hemicellulose) are the second most important natural polysaccharide material after cellulose. These compounds are generally found in the cell wall between lignin and cellulose i.e. at the middle lamella of plant cells.So its degradation is an important factor in recycling of agricultural wastes.Xylanase, a hemicellulolytic enzymes is generally required for the hydrolyisis of β 1, 4-xylanswhich is presented in lignocellulosic biomass.

With respect to results in Table (12) showed that the highest xylanase total activity of fungal isolates (3.83U/ml) recorded by*Aspergillus flavus* **(F5),** using SCB as a sole carbon source, while using RS as sole carbon source displayed highest xyalanase activity (4.16U/ml) by *Emericellanidulans* **(F56)** compared to *Eurotiumchevalieri* (**F62)** isolate which did not record any activity. Regarding to the effect of the carbon source on xylanase activity of fungal isolates, using RS as a sole carbon source enhanced xylanase activity of *Stachybotryschartarum***(F31) to** (3.83U/ml), while it didn't show any activity in case of SCB as a sole carbon source.

Table 12: Xylanase (Hemicellulase) production by cellulolytic fungal isolates using alkali pretreated sugarcane bagasse and rice straw as a sole carbon source

Isolate	Sugarcane bagasse(SCB)		Rice straw(RS)	
	Total activity (U/ml)	Specific activity (U/mg protein)	Total activity (U/ml)	Specific activity (U/mg protein)
F1	3.14±0.006	5.97±0.273	2.63±0.245	5.97±0.273
F2	3.51±0.032	5.13±0.292	3.73±0.012	5.13±0.292
F4	3.51±0.055	5.45±0.321	3.92±0.029	5.45±0.321
F5	3.83±0.087	2.10±0.113	3.97±0.107	2.10±0.113
F10	3.14±0.021	4.74±0.000	3.71±0.007	4.74±0.000
F12	3.44±0.015	3.24±1.100	3.69±0.012	3.24±1.100
F13	3.24±0.052	6.12±0.124	3.82±0.038	6.12±0.124
F14	3.40±0.041	4.86±0.234	3.98±0.017	4.86±0.234
F15	3.12±0.182	4.86±0.231	3.71±0.018	4.86±0.231
F16	3.14±0.092	3.50±0.280	3.56±0.012	3.50±0.280
F21	3.39±0.165	4.85±0.318	4.12±0.006	4.85±0.318
F27	0.02±0.006	4.65±0.104	3.45±0.069	4.65±0.104
F28	2.73±0.058	5.40±0.015	4.12±0.081	0.14±0.015
F29	2.82±0.032	5.75±0.017	3.46±0.049	5.75±0.017
F31	0.00±0.000	0.00±0.000	3.82±0.029	2.93±0.009

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F32	3.11±0.083	13.27±3.617	3.04±0.029	13.27±3.617
F34	3.53±0.035	5.47±0.006	3.76±0.009	5.47±0.006
F39	2.38±0.055	10.74±0.344	1.11±0.229	10.74±0.344
F41	3.13±0.101	4.76±0.012	3.82±0.041	4.76±0.012
F45	2.82±0.162	3.01±0.225	3.38±0.116	3.01±0.225
F47	2.90±0.046	4.13±0.115	3.87±0.061	4.13±0.115
F48	3.48±0.007	6.88±0.046	3.81±0.044	6.88±0.046
F51	1.62± 0.124	3.06±0.280	1.70± 0.012	3.06± 0.280
F52	2.81± 0.015	5.39±0.012	3.49± 0.188	5.39± 0.012
F53	1.72± 0.081	5.46±1.102	0.32± 0.014	0.53± 1.102
F55	0.65± 0.027	1.57±0.176	3.76± 0.018	3.63± 0.176
F56	2.93± 0.029	2.34±0.020	4.16± 0.020	3.53± 0.020
F57	0.09± 0.003	0.36±0.061	3.11± 0.041	5.42± 0.061
F58	3.61± 0.015	6.24±0.038	3.80± 0.058	3.80± 0.038
F59	1.34± 0.058	3.97±0.519	1.88± 0.003	2.06± 0.519
F60	2.93± 0.113	5.68±0.465	3.92± 0.006	4.95± 0.465
F61	2.72± 0.225	4.23±0.488	4.03± 0.035	3.52± 0.488
F62	2.32± 0.038	3.85±0.217	0.00± 0.000	000± 0 00
F44	3.56± 0.010	6.10±0.385	4.04± 0.018	11.04 ± 0.385
F66	2.77± 0.043	3.86±0.147	3.58± 0.006	5.14± 0.147
F68	3.11± 0.012	5.45±0.196	3.64± 0.023	3.51± 0.196
F69	0.47± 0.061	1.38±0.199	3.48± 0.061	4.83± 0.199
F70	0.53± 0.041	0.71±0.040	2.77± 0.023	3.16± 0.040
F71	2.74± 0.012	2.48±0.061	4.03± 0.012	4.08± 0.061
F72	0.00± 0.000	0.00±0.000	3.55± 0.029	6.34± 0.000
F73	3.33± 0.009	7.70±0.069	3.95± 0.072	4.99± 0.069
F74	3.64± 0.012	3.96±0.225	3.97± 0.032	3.87± 0.225
F76	3.33± 0.029	3.83±0.00	3.93± 0.038	3.61± 0.000
F88	3.07± 0.150	2.96±0.254	3.58± 0.026	3.08± 0.254
F91	3.21± 0.012	4.85±0.388	3.38± 0.009	2.86± 0.388

Values are means ± SEM (standard error of means) of the three observations.

The data presented in Table (13) xylanase activity of actinomycete isolates. Isolates **A6** and **A15** exhibited the highest xylanase activity (4.02U/ml), while **A10** isolate didn't show any activity upon SCB used as a sole carbon source.Regarding to the effect of carbon source on xylanase activity, **A14** isolate displayed the highest xylanase specific activity (8.66 U/mg protein) in case of SCB. This activity decreased (3.98 U/mg protein) when RS was used as a sole carbon source.Also, results in Table (13) showed that **A15** isolate displayed the highest xylanase activity (4.20 U/ml) in case of RS, while **A10** isolate didn't show any activity when grown on RS.AS a general observation data in Table (13) showed that **A10**isolate didn't record any activity when grown on SCB or RS.

Table 13: Xylanase production by cellulolytic actinomycete isolates using alkali pretreated sugarcane bagasse and rice straw as a sole carbon source

Isolate	Sugarcane bagasse(SCB)		Rice straw(RS)	
	Total activity (U/ml)	Specific activity (U/mg protein)	Total activity (U/ml)	Specific activity (U/mg protein)
A2	3.36±0.046	4.09±0.040	3.25±0.049	2.90±0.041
A3	3.95±0.009	3.78±0.052	3.88±0.020	3.60±0.049
A4	3.40±0.029	5.30±0.194	3.68±0.035	4.32±0.038
A6	4.02±0.012	4.58±0.009	3.90±0.055	3.61±0.617
A8	3.95±0.006	6.24±0.245	3.94±0.015	4.29±0.095
A10	0.00±0.000	0.00±0.000	0.00±0.000	0.00±0.000
A11	3.73±0.012	3.35±0.095	3.69±0.023	4.42±0.081
A12	3.73±0.009	5.01±0.020	4.01±0.003	3.48±0.055
A13	3.96±0.012	5.24±0.167	3.97±0.003	3.83±0.093
A14	3.96±0.015	8.66±0.052	4.02±0.038	3.98±0.038

A15	4.02±0.012	5.97±0.289	4.20±0.046	4.87±0.220
A16	3.38±0.049	5.02±0.179	3.73±0.046	3.88±0.173

Values are means ± SEM(standard error of means) of the three observations.

Bacterial isolates displayed xylanase activity ranged from (0.011 U/ml) by **B7** isolate to (0.787 U/ml) by **B3** isolate when SCB used as a sole carbon source Table (14) .Using RS as a sole carbon source resulted in xylanase activity ranged from (0.024 U/ml) by **B11** to (0.271 U/ml) by **B3** isolate.

Table 14: Xylanase production by cellulolytic bacterial isolates using alkali pretreated sugarcane bagasse and rice straw as a sole carbon source

Isolate	Sugarcane bagasse(SCB)		Rice straw(RS)	
	Total activity (U/ml)	Specific activity (U/mg protein)	Total activity (U/ml)	Specific activity (U/mg protein)
B1	0.035±0.001	0.033±0.001	0.098±0.004	0.113±0.005
B2	0.029±0.000	0.044±0.010	0.037±0.001	0.046±0.000
B3	0.787±0.001	0.801±0.007	0.271±0.005	0.301±0.002
B4	0.029±0.003	0.030±0.004	0.210±0.001	0.246±0.001
B5	0.032±0.000	0.038±0.001	0.030±0.000	0.041±0.001
B7	0.011±0.000	0.010±0.001	0.031±0.001	0.042±0.001
B8	0.136±0.001	0.137±0.001	0.151±0.003	0.171±0.009
B9	0.047±0.001	0.053±0.001	0.042±0.001	0.052±0.001
B10	0.051±0.005	0.068±0.003	0.039±0.001	0.048±0.001
B11	0.046±0.002	0.060±0.002	0.024±0.001	0.033±0.001

Values are means ± SEM (standard error of means) of the three observations.

Genotypic characterization of the most efficient cellulolytic actinomycete and bacterial isolates

The sequences of the isolated rDNA were submitted to the GenBank (http://www.ncbi.nlm.nih.gov) and subjected to a homology search using BLAST. Results in Table (15) summarize the information and the identification of local strains based on the sequence analysis, together with the sequence of some standard strains from the databank.

Table 15: Location, source of isolation and 16S rRNA accession numbers for the most efficient cellulolytic actinomycetes and bacterial local strains

Strain	Source	Sequence length (bp)	Accession no.	Homology of 16S rRNA
A8	Soil	1341	LC121588	*Streptomyces* sp.
A15	Soil	1350	LC121587	*Streptomyces* sp
B3	Soil	1394	LC121586	*Bacillus* sp.
B8	Soil	1394	LC121585	*Bacillus subtilis*

DISCUSSION

Microorganisms play an important role in degradation of plant residues in soil through excretion of different types of enzymes being hydrolases are the most abundant. It is well established that hydrolytic efficiency is a result of the concerted and synergistic actions of a multicomponent enzymatic system consisting of at least three major groups of enzymes to be endo -β-glucanases, exo -β-glucanases and β -glucosidases. Various enzymatic compositions secreted and produced by filamentous fungi and other microorganisms, have significant effects on biomass depolymerization .

Total of 140 isolated microorganisms (100 fungi, 20 actinomycetesand 20 bacteria) were screened for their ability to degrade cellulose on carboxymethyl cellulose-congo red media. Approximately, 48% of isolates were selected for secondary screening. In the present study, secondary screening of cellulolytic activity was performed using two types of agricultural wastes, namely sugarcane bagasse and rice straw. The ability of

microorganisms for producing cellulases and xylanases enhanced by using agricultural wastes as a sole carbonsource compared to using synthetic sources for production of enzymes.

Respect to the FPase (Exoglucanase) production by microorganisms, the obtained results showed that, high values of FPase were produced by fungal isolates compared to actinomycete and bacterialisolates. The FPase activity affected by type of agricultural wastes used. *Aspergillus terreus* **(F14)** recorded the highest value of total FPase activity (0.168U/ml) when SCB was used as a sole carbon source compared to the lowest FPase activity (0.053U/ml) in case of RS. These results are in agreement with those obtained by Kumar and Parikh [23] who reported that the cellulase activities, namely FPase produced by *Aspergillus terreus***(D34)** were significantly higher in the bagasse-grown culture extract than rice straw.

Also,the present study showed that actinomycetes produce considerable amounts of FPase enzymes, isolate A6 recorded the highest FPase total activity (0.051 U/ml) when SCB used as a sole carbon source which increased to (0.068 U/ml) in case of RS. In the same trend, strain A8 recorded the highest FPase specific activity by (0.068 U/mg protein) when RS used as a sole carbon source, compared to 0.058U/mg protein when SCB used as sole carbon source, These results are in harmony with the findings of Han [24] who reported that actinomycete isolatescultured in various carbon sources led to an obvious induction of the cellulolytic activity. Potential carbon sources for actinomycetes are rice husk powder and CMC. Wastes from rice (rice husk and rice straw) could be used as an optimal carbon source for actinomycetes strains.

With regard to the exoglucanase produced by bacterial isolates, our study showed that B3(*Bacillus* sp.)and B8 (*Bacillus subtilis*) displayed FPase activity by 0.013 and 0.012 U/ml, respectively when SCB used as a sole carbon source, this little amount of FPase produced by these bacterial isolates in agreement with Ladeira*et al.*[25] and Robson and Chambliss [26]who stated that *Bacillus* sp. hardly degrade crystalline forms of cellulose.Also, the obtained results are in harmony with those obtained by Kim *et al*. [27] who reported that *Bacillus* genus lack complete cellulolytic system.

Regarding to the CMCase (endoglucanase) activity recorded by microorganisms used in our study, fungal isolates gave the highest CMCase activity. *Aspergillus terreus* **(F14)** displayed the highest CMCase activity (0.213 U/ml) when SCB used as sole carbon source which compared to (0.164U/ml) in case of RS used as a sole carbon source.These observations indicated that the enzyme production affected by substrate type. These findings are in harmony with those obtained by Kumar and Parikh [23] who reported that CMCase produced by *Aspergillus terreus***(D34)** were significantly higher in the bagasse-grown culture extract than rice straw.

Respect to the CMCase specific activity, the obtained results revealed that *Penicilliumchrysogenum***(F53)** displayed high CMCase activity (0.511 U/mg protein) when SCB used as a sole carbon source which declined to (0.170 U/mg protein) in case of RS. These variations of CMCase obtained from different sources are in agreement with the findings of Picart*et al.* [28] who reported that *Penicillium***CR-313** and *Penicillium***CR-316**had higher yield of CMCase activity using media supplemented with rice straw than those supplemented with CMC as a carbon source.

Regarding to the CMCase activity produced during growth of actinomycete isolates on alkali pretreated sugar cane bagasse and rice straw, the results showed that strain **A15** displayed the highest CMCase specific activity (0.222U/mg protein) when SCB used as a sole carbon source, compared to(0.156U/mg protein) which displayed by isolate A3 when RS used as a sole carbon source. These results are in harmony with those obtained by Ibrahim and El-Diwany[29] and Prasad *et al*. [30]who reported that actinomycetecellulases are inducible extracellular enzymes produced during their growth on cellulosic materials but gave low activity when carboxymethyl cellulose used as a sole carbon source.

Concerning to the CMCase production by bacterial strains, B3 and B8 displayed high CMCase activity (0.028U/ml) and (0.026U/ml), respectively, when SCB used as a sole carbon source. These results are in agreement with Padilha*et al*. [31] who reported that CMCase activity of bacterial isolates increased when sugarcane bagasse used as sole carbon source compared to other sources.

Regarding to Xylanase activity of microbial isolates, filamentous fungi which produce xylanases are attracting greater attention than bacteria and yeast due to the fact they secrete much higher xylanolytic enzymes into the medium [32, 33].

Among the filamentous fungi employed to produce xylanase, *Aspergillus*is one of the most explored [34-37]. In the present study fungal isolates show considerable amounts of xyalanase by local strain **(F5)***Aspergillus flavus*which showed xylanase activity (3.83U/ml) and (3.97U/ml) when SCB and RS used as sole carbon sources, respectively. These results are in agreement with those obtained by Guimaraes*et al*. [38]who reported that *Aspergillus flavus*gave considerable xylanase activity when rice bran, sugarcane bagasse and rice straw were used as a sole carbon sources.

Xylanase activity was also detected in local isolate **(F56)** *Emericellanidulans* by (4.16U/ml) when rice straw used as a sole carbon source;these results are higher than those reported by Parihar and Rai [39] who assayed xylanase activity to be 0.11 U/ml,0.20U/ml produced by *Emericellanidulans* **NFCCI 2537** and *Emericellanidulans***NFCCI 2538**,respectively using wheat bran as a sole carbon source.

Respect to xylanase production by actinomycete isolates, our resultsshowed that **A6** isolate displayed the highest xylanase total activity (4.02U/ml), while **A14** isolate gave the highest xylanase specific activity (8.66U/mg protein) when SCB used as a sole carbon source compared to (3.98 U/mg protein) when RS was used.These results are in harmony with those obtained by Kumar *et al*. [40] who reported that agro industrial wastes used as a sole carbon source enhanced xylanase production by actinomycete isolates, compared to other carbon sources which considered as cost effective for commercial production of xylanase.

According to Esteban *et al*. [41]xylanases have been displayed in *Bacillus, Streptomyces* and other bacterial genera. In the same context, the obtained results revealed that local strain **B3** (*Bacillus* sp.)displayedthe highest xylanase specific activity by (0.801 U/mg protein) using SCB as a sole carbon source, these results are in agreement with those obtained by Stutzenberger[42]who reported that extracellular enzymes (amylase and xylanase) on sugarcane bagasse as a sole carbon source produced by *Thermomonosporacurvata*. On the other hand, Dhillon *et al*. [43] found that agro-industrial wastes (bagasse, rice straw, oat husk, rice husk and wheat straw) are the best inducers for the xylanase production by *Bacillus circulans* (AB 16) in submerged fermentation. Secondary screening was used to address the most active microorganisms to degrade agricultural wastes (sugar cane bagasse and rice straw) during solid state fermentation which can be used as a symbol for composting process.

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