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Fungi are Unique Cellulase-and Zylanase-Producing Microbiota

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ABSTRACT

The objective of this study is to screen cellulose- and zylan-hydrolytic fungi in samples collected from different rotten agricultural wastes (strawberry, grapes, fig and artichoke), decomposed wood and compost. One hundred and seven isolates were secured on cellulose or zylan containing medium. They were tested for their ability to analyze both of cellulose and zylan. Sixty-nine isolates were positive for cellulases and zylanase activities. Fifteen isolates were found to be the most effective producers of cellulolytic enzymes. Seven isolates were identified as *Fusarium oxysporum-I*, *Aspergillus versicolor-1 and 2, Aspergillus oryzae, Penicillium citrinum, Penicillium brevicompactum* and *Mucor indicus*. These isolates were compared with some other fungi known to produce cellulases and zylanase. The latter are *Phanerochaete chrysosporium, Pleurotus ostreatus, Fusarium oxysporum, and Rhizoctonia solani*. *Phanerochaete chrysosporium*, *Pleurotus ostreatus* and *Fusarium oxysporum* were the superior producers of cellulolytic enzymes. To study the cellulases and zylanase activities**,** corncobs (a recycled agricultural waste) and carboxymethylcellulose (CMC) (an intermediate cellulolytic product) were selected and added to Mandel's medium, as carbon sources. The activity of filter paper cellulose activity (FP-ase) of all strains, on the medium containing corn cobs, was higher than its activity on the medium containing CMC. On corn cobs containing medium, *P. brevicompactum* and *A. versicolor-2* gave the highest FP-ase activities (7.44 and 7.29 U/ml, respectively). While on CMC containing medium, *A. versicolor-1* was the most efficient as, it gave 15.7 U/ml. The activity of CMC-ase was the highest when produced by *A. versicolor-2 and A. versicolor-1* on the medium containing corn cobs, which gave 32 and 27.55 IU/ml, respectively. While on the medium containing CMC, *A. versicolor-1* gave 33.4 IU/ml. In respect to β-glucosidase, *Pleurotus ostreatus* was the superior on the medium containing CMC, it gave 570.64 IU/ml. followed by *F. oxysporum-I, F. oxysporum, A. oryzae*, (353.57, 349.45 and 343.96 IU/ml. respectively). The isolate *Mucor indicus* gave 325.45 IU/ml on the medium containing corn cobs. Regarding zylanase, its activity tested the isolates was higher than its activity among the known strains on both medium containing corn cobs and CMC. *A. versicolor-2* was the superior in its production; it gave 35.24 IU/ml.

Keywords: Cellulase; zylanase, *Phanerochaete* sp., *Pleurotus* sp., *Fusarium* sp., *Aspergillus* sp., *Penicillium* sp., *Mucor indicus.*

Introduction

Agricultural and industrial wastes are among the main causes of environmental pollution. Their conversion into useful products may reduce the intensity of such problems environmental. Economically, the most important industrial materials other than food stuffs affected by microorganisms are cellulose and wood products (Debing Jing *et al.*, 2007). Proper utilization of these wastes in the environment will eliminate pollution and convert them into useful byproducts (Milala *et al.*, 2005).

Lignocellulosic biomass, such as bioenergy crops and trees (*e.g.*, switch grass, aspen wood) or agricultural and forestry wastes (*e.g.*, wood residues, corn stover, straw) are potential low cost sources of fermentable sugars. Lignocellulosic material consists of mainly three different types of polymers namely cellulose, hemicellulose and lignin which are associated which each other (Mohan *et al.,* 2012). Cellulose is the most abundant renewable natural product in the biosphere. Much of the cellulose in nature exists as waste paper. The potential of cellulose as an alternative energy source has stimulated research into bioconversion process which hydrolyzes cellulose to soluble sugars for feedstock, in alcoholic fermentations and other industrial processes (Bakare *et al.*, 2005).

Since cellulose is very difficult to degrade as a component of plant cell walls, only a few microorganisms specialized for plant cell wall degradation can hydrolyze cellulose. The ability to utilize lignocellulosic material is well established among anaerobic and aerobic genera of domain fungi from chytridiomycetes to basidiomycetes. The basidiomycetes (aerobic fungi) produce extracellular enzymes allowing lignocelluloses degradation *e.g.* lacasses, hemicellulases, and cellulases, some ascomycetes are able to degrade cellulosic compounds as well (Rosa and Jorge, 2013).

Fungi have generally been considered the main organisms responsible for decomposition of cellulose. The researchers are trying to discover cellulolytic fungi and are developing mutant strains to enhance the production

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of cellulases (Khalid *et al.*, 2006). Nowadays, more than 14,000 fungi, which are active against cellulose, are known (Gautam *et al.,* 2012; Wilson, 2011). One of the most studied fungi, due to its industrial relevance, is *Phanerochaete chrysosporium*.

Cellulase is a complex enzyme having chiefly endo and exo 1-4 glucanase and β-glucosidase activities. A synergistic action of these enzymes is required for the complete hydrolysis of cellulose (Pothiraj *et al.*, 2006). Cellulases are inducible enzymes by cellulosic substrates, which are synthesized by a large diversity of microorganisms including fungi during their growth on cellulosic materials. Sun and Cheng (2002) mentioned that the genus *Aspergillus* is the most extensively studied cellulases producer.

The objective of this study is to screen cellulose- and zylan-hydrolytic fungi that satisfy industrial requirement (high cellulases and zylanase activities) as well as to provide useful ethanol fermentation from cellulosic substrate, which is all vital to reduce the processing cost of bio-ethanol.

Materials and Methods

Isolation and identification of Fungi:

Some fungal isolates were obtained from different rotten agricultural wastes (strawberry, grapes, fig and artichoke), decomposed wood and compost, using a tenfold serial dilution-plating technique on potato dextrose agar (PDA) containing chloramphenicol. The culture was daily observed and was successfully sub cultured onto fresh plates of PDA until pure isolates were obtained. One hundred and seven isolates were secured. The pure cultures were then transferred to PDA slants and maintained at 4 ^{0}C.

The isolated fungi were identified after growth on PDA medium by observing their macroscopic (color, texture, appearance, and diameter of colonies) and microscopic (microstructures) characteristics according to Domsch *et al.* (1980).

Screening of cellulolytic and zylanolytic fungi:

Mandel's medium (Patel *et al*., 2007) was supplemented with carboxy-methyl cellulose (CMC) as a sole carbon source. It was prepared by adding the following reagents (g/l): urea 0.3, (CMC) 10, $MgSO_4.7H_2O$ 0.3, KH_2PO_4 2, $CaCl_2.2H_2O$ 0.3, $(NH_4)_2 SO_4$ 1.4, Bactopeptone 1, Tween 80 0.1, trace elements: $FeSO_4.7H_2O$ 5mg, $MnSO_4.H_2O$ 16 mg, $ZnCl_2.2H_2O$ 17 mg, $CoCl_2.6H_2O$ 2 mg, agar 15. The plates were strained by congo red to record visually the cellulolytic activity of fungal isolates after incubation at 32^o C for 5 days (Lo *et al*., 2009). The cellulase-producing fungi showed the zone of clearance on agar.

Similar procedure was followed for the identification of zylan-hydrolytic activity of the fungal isolates. Strains were grown on Mandel's medium (Patel *et al*., 2007) amended with zylan as a sole carbon source and strained by congo red (Lo *et al*., 2009). The cellulase- and zylanase- producing fungi showed the zone of clearance on agar.

A qualitative measurement of cellulase and zylanase production was obtained by calculating the ratio of clear zone size to colony diameter (Hankin and Anagnotakis, 1977).

Produced cellulase activity (absolute units):

$$= \frac{clear\ zone\ size\ (mm^3)}{colony\ diameter\ (mm)}$$

$$= \frac{(Y/_2)^2 \times 3.14 \times agar\ thickness\ (mm)}{x}$$

Y= clear zone diameter
X= colony diameter

The best fungal isolates were enriched by inoculation in Mandel's medium at 32^{0}C, shaking at 125 rpm for 5 days.

Basal medium for enzyme production:

The basal medium used for enzyme production was Mandel's medium (Patel *et al*., 2007) The carbon source, carboxymethyl cellulose (CMC) (Sigma Chemical Co.) was added at 1% concentration for measuring cellulases and zylanase activities and then sterilized at 121°C for 15 min. CMC was supplemented as an intermediate cellulolytic product. While corn cobs were added as another carbon source for the same reason. Corn cob was added as a recycled agricultural waste. It was converted into fine powder by hammer milling and

sieving. Pre-treatment was then carried out by refluxing the powder with 0.2M NaOH for 2 hours then neutralized with HCl. The pre-treated sample was dried in an oven at 65^0C (Roy *et al*., 1993).

The enriched cultures of the best fungal strains were re-inoculated (5% v/v) into both tested medium. the pH was adjusted at 5. After inoculation, it was incubated at room temperature for 5 days on a shaker at 125 rpm. The experimental flasks were allocated in a complete randomized design with three replicates. After five days, the mycelium was separated by filtration through Whatman filter paper No. 1. The filtered broth was collected for determination of enzymatic activities.

The chemical analysis of corn cobs is shown in Table (1) according to Ansah *et al.* (2012).

Table 1: Chemical analysis of corn cobs.

Composition	Percentage (%)
Crude protein	3.5
Phosphorus	0.08
Calcium	0.35
Cellulose	45
Hemicellulose	35
Lignin	6.7-13.90

Enzyme assay:

The cellulase activity was estimated in terms of filter paper cellulase activity (FP-ase) using Whatman No.1 filter paper according to Ghose (1987), carboxy-methyl-cellulose (CMC-ase activity) using CMC according to Mandel *et al*. (1976) and β-glucosidase activity, using silicine as the substrate, according to Chahal (1985). While zylanase activity was determined in terms of zylan, according to Ilieva *et al*. (1995). The glucose for FPA, CMC-ase, β-glucosidase and zylose for zylanase, produced after hydrolysis, were measured with 3, 5-dinitrosalicilic acid according to Miller (1959). One international unit (IU) of enzyme activity was defined as the amount of enzyme that releases 1 µmol product per min (glucose equivalents for FPA, CMC-ase, β-glucosidase and zylose equivalents for zylan).

The cellulolytic activities of chosen fungi were investigated under the some environmental conditions, such as pH 5, temperature of 32^oC and incubation period of 5 days.

Data were statistically analyzed for least significant differences (LSD) adopting the procedure mentioned by Gomez and Gomez (1984).

Results and Discussion

The use of microbial enzymes in lignocellulosic waste treatment has been shown to be an alternative that is efficient and cost-effective (Mohamed, 2013). Chemical hydrolysis of lignocellulose is accompanied with the formation of toxic components that are toxic to the environment (Kulkarni *et al.* 1999). Hence, the need to explore the uses of microorganisms and their enzymes, which have high specificity, mild reaction conditions and negligible substrate loss (Ninawe and Kuhad 2005).

Cellulase, a group of enzymes which hydrolysis cellulosic biomass is thought to be critical for the successful utilization of cellulosic materials (Nwodo-Chinedu *et al*., 2007). Cellulases are produced by the microorganisms grown over cellulosic matters. They can be produced by fungi, bacteria or actinomycetes, but the most common producers are fungi (Ariffin *et al*., 2006). Bakri *et al*. (2003) reported that filamentous fungi are preferred for commercially important enzymes production, because the levels of the enzymes produced by these cultures are higher than those obtained from bacteria.

Zylans are the main carbohydrate in the hemicellulosic fraction of vegetable tissues and form an interface between lignin and the other polysaccharides. Hydrolysis of zylan and cellulose are essential steps towards the efficient utilization of lignocellulosic materials in nature (Mohamed, 2013).

Isolation and screening of cellulolytic and zylanolytic fungi:

In this study, one hundred and seven isolates of fungi were obtained from different rotten agricultural wastes, decomposed wood and compost. All were tested for their ability to analyze both of cellulose and zylan. Sixty nine isolates showed positive results for cellulases and zylanase activities (Table 2). Fifteen isolates were the most effective producers of cellulolytic enzymes (Fig 1). Seven isolates were identified, they were belonging to *Fusarium oxysporum-I, Aspergillus versicolor-1 and 2, Aspergillus oryzae, Penicillium citrinum, Penicillium brevicompactum* and *Mucor indicus* (Fig 2). They were chosen as best producers of cellulolytic enzymes that gave diameters of clear zones between 5 and 6 mm to describe the relative cellulolytic activity between 12.62 and 14.88 and the relative zylanytic activity between 12.18 and 13.95.

Table 2. Measurements of cellulases and zylanase produced by some cellulolytic and zylanolytic isolates of fungi.

No. of Fungal isolates	Cellulases activities [(a)]		Zylanase activity [(b)]	
	Diameter of clear zone (mm)	[*] Relative cellulolytic activity	Diameter of clear zone (mm)	[*]Relative zylanytic activity
1	Nil	Nil	0.7	0.18
2	2.7	2.73	5.3	10.53
3	5.5	11.34	5.2	10.14
4	0.6	0.14	2.2	1.82
5	2.4	2.16	5.5	11.34
6	6.2	14.42	5.8	12.62
7	Nil	Nil	5.4	10.94
8	5.6	11.76	Nil	Nil
9	1.2	0.54	0.9	0.30
10	5.6	11.76	5.5	11.34
11	5.5	11.34	0.6	0.14
12	1.4	0.74	5.3	10.53
13	Nil	Nil	0.7	0.18
14	5.5	11.34	5.6	11.76
15	0.6	0.14	0.9	0.30
16	4.9	9	5.4	10.94
17	0.7	0.18	1	0.38
18	6.3	14.88	5.8	12.62
19	5.4	10.94	Nil	Nil
20	0.8	0.24	1.3	0.63
21	0.6	0.14	Nil	Nil
22	0.3	0.03	Nil	Nil
23	6.1	11.76	Nil	Nil
24	5.8	12.62	5.7	12.18
25	Nil	Nil	5.5	11.34
26	5.5	11.34	1.1	0.45
27	Nil	Nil	2.4	2.16
28	5.6	11.76	2.9	3.15
29	5.3	10.53	Nil	Nil
30	1	0.38	2.3	1.98
31	Nil	Nil	5.2	10.14
32	1.3	0.63	0.5	0.1
33	2.3	1.98	Nil	Nil
34	0.8	0.24	Nil	Nil
35	Nil	Nil	0.6	0.14
36	Nil	Nil	5.3	10.53
37	5.8	12.62	5.7	12.18
38	2	1.5	Nil	Nil
39	5.3	10.53	0.8	0.24
40	6.1	13.95	5.9	13.1
41	Nil	Nil	1.2	0.54
42	Nil	Nil	1.4	0.74
43	Nil	Nil	5.4	10.94
44	Nil	Nil	2.5	2.34
45	5.1	9.75	Nil	Nil
46	0.7	0.18	0.7	0.18
47	1.2	0.54	0.6	0.14
48	1.1	0.45	0.7	0.18
49	5.4	10.94	1.4	0.74
50	2.5	2.34	4.9	9
51	5.5	11.34	5.5	11.34
52	2.2	1.82	Nil	Nil
53	5.6	11.76	5.4	10.94
54	5.3	10.53	5.6	11.76
55	1.9	1.35	5.5	11.34
56	0.9	0.30	5.1	9.75
57	6.2	14.42	5.7	12.18
58	5.3	10.53	4.9	9
59	0.6	0.14	Nil	Nil
60	Nil	Nil	2.2	1.82
61	0.5	0.1	5.2	10.14
62	Nil	Nil	5.6	11.76
63	Nil	Nil	5.2	10.14
64	0.9	0.30	Nil	Nil
65	Nil	Nil	5.4	10.94
66	Nil	Nil	5.3	10.53
67	Nil	Nil	1.8	1.22
68	5.4	10.94	2.8	2.94
69	6	13.5	6.1	13.95

[(*)] the ratio between clear zone size and colony diameter
[(a)] Mandel's medium supplemented with CMC as a sole carbon source
[(b)] Mandel's medium supplemented with corn cobs as a sole carbon source

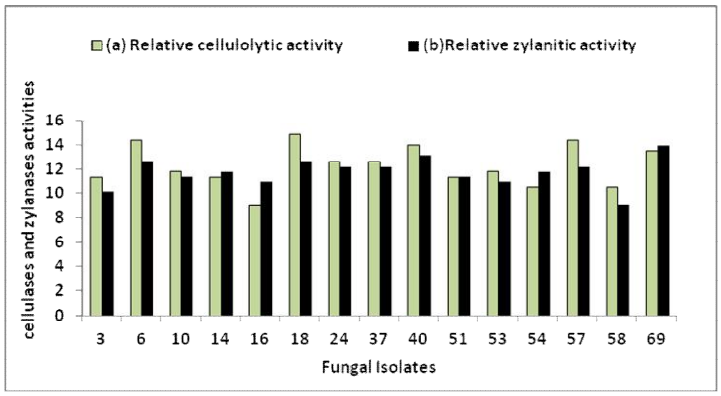

Fig. 1: Cellulases and zylanase produced by the effective producers of the cellulolytic and zylanolytic isolates of fungi. **(a)** Mandel's medium supplemented with CMC as a sole carbon source **(b)** Mandel's medium supplemented with corn cobs as a sole carbon source.

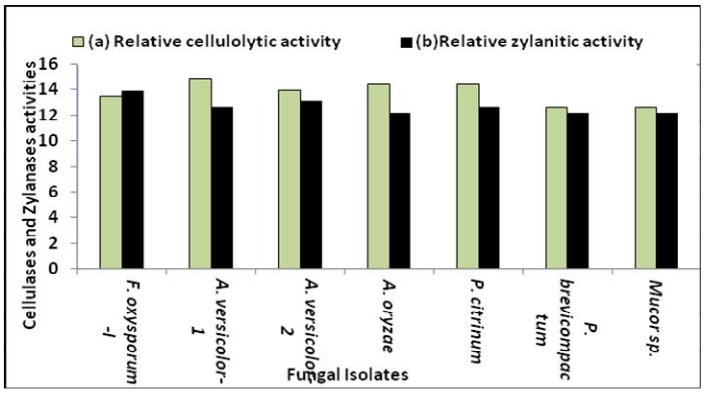

Fig 2: The superior producers of cellulases and zylanase enzymes (a) Mandel's medium supplemented with CMC as a sole carbon source (b) Mandel's medium supplemented with Corn Cobs as a sole carbon source

These isolates were compared with other fungi known with their ability to produce cellulases and zylanase enzymes. Those were *Phanerochaete chrysosporium, Pleurotus ostreatus, Fusarium oxysporum* and *Rhizoctonia solani* (Table 3).

Table 3: Measurements of cellulases and zylanase enzymes produced by some cellulolytic and zylanolytic fungi.

Fungi	Cellulases activities [(a)]		Zylanases activities [(b)]	
	Diameter of clear zone (mm)	[(*)]Relative cellulolytic activity	Diameter of clear zone (mm)	[(*)]Relative zylanitic activity
Phanerochaete chrysosporium	6.1	13.95	5.9	13.05
Pleurotus ostreatus	5.8	12.62	5.6	11.76
Fusarium oxysporum	5.7	12.18	5.7	12.18
Rhizoctonia solani	Nil	Nil	0.7	0.18

[(*)]The ratio between clear zone size and colony diameter
[(a)] Mandel's medium supplemented with CMC as sole carbon source
[(b)] Mandel's medium supplemented with Corn Cobs as sole carbon source

Enzymolysis of native cellulose is carried out by three components of cellulase as: (1) exo- β-1-4, glucanase, it acts on the non-reducing end of the cellulose chain and successively removes single glucose units, (2) endo-β-1-4, glucanase, it randomly attacks the internal β-1-4, linkages and (3) β-glucosidases or cellobiases. The cellulose system also contains cellobiase, which eventually breaks down cellobiose, the building unit of cellulose to glucose.

The breakdown of cellulose into sugar can be achieved by acid hydrolysis as well as by enzymatic hydrolysis. But, Mandel *et al*. (1976) reported that the enzymatic hydrolysis is mostly preferred because it produces fewer by-products and proceeds under milder condition.

In the present study, carboxy-methyl-cellulose (CMC) (an intermediate cellulolytic product) and corn cobs (a recycled agricultural waste) were added to Mandel's medium, as a sole carbon sources, for measuring cellulases and zylanase activities (Tables 4 and 5). Corn cobs were pretreated before use following the method described by Roy *et al*. (1993). The pretreatment facilitated the degradation of hemi-cellulosic components and increased the reducing sugar concentration either in the form of glucose from cellulose or zylose from hemicelluloses (Mohan *et al*., 2012). Many factors like lignin content, crystallinity of cellulose and particle size limit the digestibility of hemicellulose and cellulose present in the lignocellulosic biomass. Pretreatment is a goal to improve the digestibility of the lignocellulosic biomass (Hendriks and Zeeman, 2009). For the enzymatic conversion of lignocellulose biomass to ethanol and other chemical products, a pretreatment stage is required to break the lignin structure and to partially solubilize the polysaccharides (Keller *et al*., 2003).

Three fungal strains (*Phanerochaete chrysosporium*, *Pleurotus ostreatus* and *Fusarium oxysporum*), known by their ability to produce cellulases and zylanase, and seven fungal isolates (*Fusarium oxysporum –I, A. versicolor -1* and *-2, A. oryzae, Penicillium citrinum, Penicillium brevicompactum* and *Mucor indicus)* were selected to grow on CMC and corn cobs containing media.

All of the cellulases enzymes (Fp-ase, CMC-ase, β-glucosidase and zylanase) activities of the ten cultures were observed on the 5th day of growth. Mohan *et al*. (2012) mentioned that the fungal growth almost stopped after 5 days of cultivation and the biomass maintained at a relatively stable value. After this period, although small increases, in the enzymatic activity, on 6th day, were observed, there was a consequent decrease in productivity. They also reported that the enzymes were produced both during growth and stationary phases, indicating that the process of these enzymes production and secretion is a growth-associated one. Osman *et al*. (2008) mentioned that the cellulolytic activity was related to fungal growth.

Table 4: Cellulases and zylanase enzymes released from the best fungal isolates and the identified fungi grown on Mandel's medium supplemented with *(CMC) as a sole carbon source.

Fungal strains	Fp-ase activity (FPU ml^{-1})	CMC-ase activity (IUml^{-1})	β-glucosidase activity (IUml^{-1})	zylanase activity (IUml^{-1})
Known Fungi				
Phanerochaete chrysosporium	2.91	4.24	262.00	12.80
Pleurotus ostreatus	2.91	4.80	570.64	14.32
Fusarium oxysporum	3.26	4.03	349.45	10.07
Fungal Isolates				
Fusarium oxysporum -I	4.03	7.29	353.57	20.16
A. versicolor -1	15.7	33.4	168.72	20.85
A. versicolor-2	6.94	9.09	314.81	20.13
A. oryzae	4.46	6.00	343.96	11.96
Penicillium citrinum	4.54	8.01	257.54	19.93
Penicillium brevicompactum	5.32	5.49	246.91	10.56
Mucor indicus	6.09	6.00	216.74	12.45
LSD (0.05)	0.08	0.07	0.79	0.03

*Carboxymethyl cellulose

Table 5: Cellulases and zylanase enzymes released from the best fungal isolates and the identified fungi grown on Mandel's medium supplemented with corn cobs as a sole carbon source

Fungal strains	Fp-ase activity (FPU ml^{-1})	CMC-ase activity (IUml^{-1})	β-glucosidase activity (IUml^{-1})	zylanase activity (IUml^{-1})
Known Fungi				
Phanerochaete chrysosporium	4.89	5.06	214.68	9.911
Pleurotus ostreatus	5.66	6.52	244.17	13.47
Fusarium oxysporum	5.32	4.97	223.25	20.25
Fungal Isolates				
Fusarium oxysporum -I	6.94	8.74	158.44	19.47
A. Versicolor -1	7.12	27.55	183.12	22.44
A. Versicolor-2	7.29	32.8	234.91	35.24
A. oryzae	6.94	6.94	173.87	19.98
Penicillium citrinum	6.60	8.23	202.67	19.22
Penicillium brevicompactum	7.44	10.8	231.48	19.71
Mucor indicus	5.40	5.66	325.45	8.75
LSD (0.05)	0.1	0.09	0.03	0.03

In general, the enzymatic activities of all the isolated fungal strains exceeded those of the known correspondings.

FP-ase activity:

Results in Tables (4) and(5), show that corn cobs cultures had greater FP-ase activity (about 5 and 7 U mL^{-1}) than CMC cultures (about 3 and 5 U mL^{-1}) for both identified and isolated strains, respectively. This indicated that the milling of corn cobs did reduce the crystallinity of lignocellulose and exposed the available cellulose

surface area for enzyme activity. These results are in accordance with Rajoka (2004). He reported that in the medium containing CMC as a sole carbon source, the organism synthesis low enzyme for filter paper activity, while a high level of enzymes was synthesized when it grown on other cellulosic substrates. While, Narasimha *et al*. (2006) reported that CMC was the best carbon for the growth on Czapek-Dox medium and cellulase production by *Aspergillus* sp. The presence of cellulose not only induces cellulases, but also induces the production of zylanase (Aro *et al*., 2001).

On the CMC containing medium (Table 4), it was found that there was a significant difference between all the tested strains in their FP-ase activities. While, there were no significant differences between *A. oryzae* and *P. citrinum* and between *Phanerochaete chrysosporium* and *Pleurotus ostreatus*. However, the FPA activity of *A. Versicolor -1* was the highest for all the tested strains (15.7 UmL^{-1}). *A. niger and Fusarium oxysporum* were employed by Azzaz *et al*. (2012) for cellulase production. Jecu (2000) and Sharada *et al*. (2012) reported that *Aspergillus* sp. is a known good producer of cellulases. In addition, Ojumu *et al*. (2003) indicated that the filter paper activity of *A. flavus* on saw dust gave the highest cellulase activity of 0.0743 U/ml.

Significant differences among all the tested strains in their FP-ase activities when grown on corn cobs containing medium were scored (Table 5). *P. brevicompactum* and *A. Versicolor -2* scored the highest activities *e.g.* 7.44 and 7.29 U mL^{-1}, respectively. No significant differences between *A. Versicolor -1* and *F. oxysporum*-I and between *A. Versicolor -1* and *A. Versicolor -2*, were recorded.

CMC-ase activity:

Results of the present work indicated that the CMC-ase activity was higher with all the tested strains when grown on corn cobs medium than on CMC medium. Significant differences were found between all the isolates and identified strains grown on CMC containing medium, but between *A. oryzae* and *Mucor indicus,* there was no significant differences. While, the isolate *A.versicolor*-1 was the best in its CMC-ase activities (33.4 $IUml^{-1}$) as presented in Table 4.

However, results in Table 5 revealed that the isolates *A.versicolor-2 and 1,* attained the maximum CMC-ase activities of 32.8 and 27.55 $IUml^{-1}$, respectively, when grown on corn cobs medium, followed by *P. brevicompactum* (10.8 $IUml^{-1}$). Sharada *et al.* (2013) mentioned that some species of *Penicillium* produced significant quantities of cellulase, when grown under different conditions. There were significant differences between all the tested strains except *Phanerochaete chrysosporium* and *F. oxysporum*.

β-glucosidase activity:

The successful β-glucosidase activity was on the CMC medium (Table 4). Significant differences were scored between all the tested strains. For example, *Pleurotus ostreatus* gave 570.64 $IUml^{-1}$ followed by the isolated *F. oxysporum*-I and the identified one, then, the isolate of *A. oryzae*. They gave, respectively, 353.57; 349.45 and 343.96 $IUml^{-1}$. *Aspergillus* strains are known with their ability to produce β-glucosidase with significantly high yields according to Tamas *et al*. (2003). Also, Wong *et al*. (2008) reported that *Aspergillus* species exhibited high β -glucosidase activity. While Sharada *et al.* (2013) reported that *A. oryzae* is one of the three highest producers of cellulases enzymes. It gave the maximum production when grown on orange peels and decomposed it.

Also, significant differences were reported between all the tested strains when grown on corn cobs containing medium (Table 5). The greatest activity of β–glucosidase was obtained by *Mucor indicus* which gave 325.45 $IUml^{-1}$ followed by *Pleurotus ostreatus* (244.17 $IUml^{-1}$).

One of the problems related to the economic viability of the enzymatic hydrolysis of cellulose is due to low β - glucosidase levels (Umikalsom *et al*., 1997).

Zylanase activity:

Considering zylanase, its activity from *A. versicolor-2* was the highest with corn cobs (35.24 $IUml^{-1}$), followed by *Aspergillus, Penicillium* and *Fusarium* sp. which gave 19 - 23 $IUml^{-1}$. It was also obvious that the enzyme activities from these isolates were similar to those on the CMC containing medium but with little variation. Comparing the isolates with the identified strains grown on CMC containing medium, it was found that the zylanase activities were higher from the isolates than from the identified ones. Ghosh *et al*. (1993) reported that zylose, the ultimate breakdown product of zylan, serves as a good inducer of this enzyme. It has been well documented that the carbon source is an important variable for the production of zylanases, with lignocellulose materials perhaps being better substrates than zylan for the production of zylanolytic enzymes (Damaso *et al*., 2000; Papinutti and Forchiassin, 2007). In addition, Haltrich *et al*. (1996) suggested that low molecular mass degradation products of zylan and cellulose hydrolysis penetrate into the cells and induce the production of hydrolytic enzymes.

Conclusion:

It could be concluded that corn cobs can be used as a sole carbon source for cellulases and zylanase production. It is recommended that the isolates *Aspergillus versicolor-1 and 2* can be used for FP-ase, CMC-ase and zylanase production and both of the isolate *Mucor indicus* and the strain *Pleurotus ostreatus* can be used for β-glucosidase production.

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