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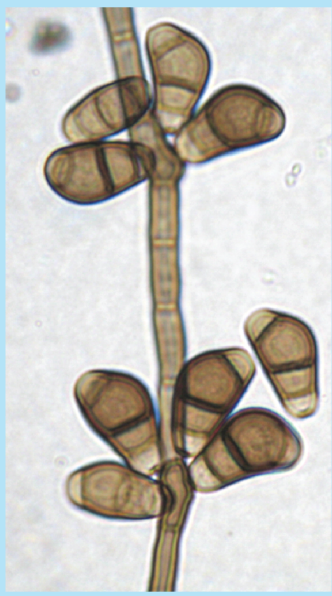
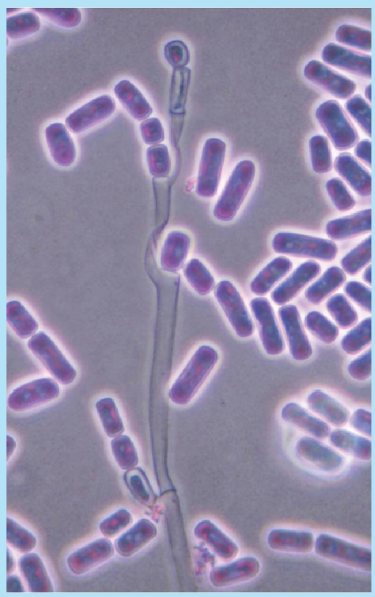

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Worthwhile enzyme production and eco-friendly bioconversion of three agricultural residues by *Aspergillus curvatus* and *Aspergillus gaarensis*, promising enzyme-producers isolated from extreme environment

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Abstract: Xylan polymer was extracted from oat spelt, wheat bran, corn cobs and corn stalks, and confirmed by High Performance Liquid Chromatography (HPLC). Enzymatic ability of two newly-discovered *Aspergillus curvatus* and *A. gaarensis* in the submerged fermentations (SmF) with starch, pectin as well as xylan isolated from oat spelt, wheat bran, corn cobs and corn stalks, was pursued. Both strains were able to produce various enzymes at different concentrations per gram of dry substrate (gds) using SmF, reaching 1720 IU/gds for amylase, 2664 IU/gds for endoglucanase, 4630 IU/gds for exoglucanase, 8392 IU/gds for pectinase, and 4274 IU/gds for xylanase. The two *Aspergillus* species have converted lignocellulosic biomass of date palm leaves, sugarcane bagasse and rice straw into useful enzymes, namely amylase, endoglucanase, exoglucanase, laccases, pectinase and xylanase, in solid-state fermentation (SSF). Augmented production of all enzymes has been shown by both strains reaching 3859 IU/gds for amylase, 24112 IU/gds for exoglucanase, and 14182 IU/gds for xylanase by *A. curvatus*, and 8411 IU/gds for endoglucanase, 854.3 IU/gds for laccase, 12824 IU/gds for pectinase by *A. gaarensis*. As a result, both strains used in this research are thought to be promising producers of a wide range of enzymes from low-cost materials such as plant residues.

Kew words: *Aspergillus*, bioconversion, enzymes, residues, submerged, solid-state, fermentation.

Introduction

Globally five billion metric tons of lignocellulosic biomass have been produced yearly from agriculture including rice bran, rice straw, sugarcane bagasse, fruits and vegetable wastes, wheat bran, cotton leaf scraps. It is therefore very important to turn these wastes efficiently and effectively into valuable products of industrial and commercial value and also to reduce the negative environmental effects of these wastes (Wang *et al.* 2016). About half of the plant matter consists of lignocellulose, which is the most abundant green organic matter in the soil (Singh *et al.* 2012). It consists of cellulose (35–50%), hemicellulose (20–35%) and lignin (15–25%) which are closely bound together by a combination of non-covalent and covalent bonds (Limayem and Ricke 2012, Ismail *et al.* 2018).

Cellulases are used in the biofuel, textile, pulp, paper, detergent, animal feed and food industries as well as in ligand binding tests (Sukumaran *et al.* 2005, Gupta *et al.* 2012, Bharathiraja *et al.* 2017). Xylanases are being used for clarity of fruit juices, for de-icing of paper waste, for biobleaching and for improving the consistency of milk, feed and fiber as well as for the saccharification of hemicelluloses to xylose sugars (Soni and Kango 2013). Lignin-degrading enzymes are widely used for the pretreatment of recalcitrant lignocellulosic biomass for biofuel production and also applied in textile, food, paper, cosmetic, pharmaceutical industries, organic synthesis, wastewater treatment, and bioremediation (Abdel-Hamid *et al.* 2013, Bharathiraja *et al.* 2017).

Because of its diverse applications including dye decolourization, wastewater detoxification and biological remediation, laccase is currently the main priority. A vast array of substrates, such as ortho and paradiphenols, methoxy-substituted phenols,

aromatics, phenolic acids and many other compounds, can be oxidized by laccase (Atalla *et al.* 2013, Abd El Monssef *et al.* 2016).

In Egypt, they remain largely unexplored and create significant pollution issues and health risks when burning in the fields. Fortunately, such residues are typically rich in sugars, minerals and proteins and thus should not be considered 'waste' but 'natural resources' for other manufacturing processes (Mussatto *et al.* 2012, Ismail *et al.* 2018) and may be used as a good source of enzyme production. Thus, their biodegradation by microorganisms involves the action of more than one enzyme. Fungi may use these residues to generate useful materials and chemicals (Kango *et al.* 2003). Therefore, in the synthesis of several enzymes in submerged fermentation, two newly-described *Aspergillus* strains were used and their enzyme production potential is utilized to bioconvert several plant residues to useful enzymes by using a solid-state fermentation (SSF) method.

Materials and methods

1. Extraction and preparation of xylans from different sources

Alkaline xylan extraction was carried out from oat spelts, wheat bran, corn cobs and corn stalks. The extraction procedure was carried out using the (Puls *et al.* 2005) method with some modification. A fine-milled 100 g of each substrate was soaked at a tenth volume of 5% NaOH. The mixture was steamed at 105°C for 120 min. The mixture was centrifuged at 5,000 ×g for 30 min and the supernatant was pooled. Double volume of methanol was applied to the supernatant to precipitate the xylan. The residual lignin had to be degraded by addition of 0.4% hydrogen peroxide and the eventual washing by methanol. After centrifugation, the xylan could dry in freeze dryer (VirTis, USA), and the prepared xylan was used for enzyme production.

2. High Performance Liquid Chromatography (HPLC) assay of obtained xylans

Characterization of the previously prepared xylan using High Performance Liquid Chromatography (HPLC) with fluorescent detection was performed at the Analytical Chemistry Unit at the Faculty of Science, Assiut University. A wave length of 295 nm was used as an excitation and 345 nm as an emission one. 0.5 g of each xylan type was dissolved separately in 5 ml of Milli-Q water, and then 10, 25 and 50 ng/µl of this solution was injected at a retention time of 3.528 min (Figure 1).

3. Fungal strains

Aspergillus curvatus AUMC 11038 (Al-Bedak *et al.* 2020a) and *A. gaarensis* AUMC 11046 (Al-Bedak and Moubasher 2020) were involved in this research.

4. Fermentation medium

Sucrose-free Czapek's mineral medium was used as fermentation medium. The medium has the following composition ($g.L^{-1}$): Na_2NO_3, 2.0; K_2HPO_4, 1.0; KCl, 0.5; $MgSO_4.7H_2O$, 0.5; $FeSO_4$, 0.01; $ZnSO_4$, 0.01; $CuSO_4$, 0.005.

5. Quantitative assessment of enzymatic capacity in SmF

Fungal strains were separately grown in a 250 ml Erlenmeyer conical flasks each containing 50 ml of sucrose free-Czapek's broth medium, complemented by 1% soluble starch as the sole carbon source for amylase production, or 1% xylan (for endoglucanase, exoglucanase and xylanase production), or 1% citrus peel pectin (for pectinase production). Every flask was inoculated with 1 ml of spore suspension (1.5 x 10^8 spores/ml) from 7-day-old cultures.

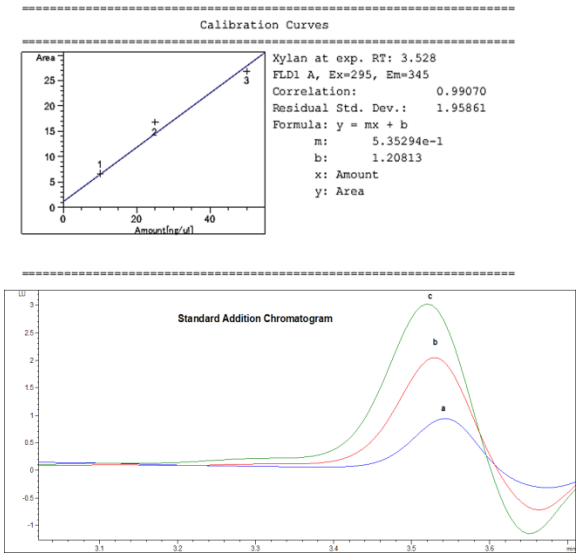

Figure 1: HPLC calibration curve of standard Birchwood xylan

5.1. Extraction of enzymes

After incubation, the medium was filtered into Whatman No. 1 filter paper and the cell-free supernatant was collected through centrifugation (10,000 rpm at 4°C for 10 min) and used as an enzyme supply.

5.2. Enzymes assay and protein determination

Amylase, endoglucanase, exoglucanase, pectinase and xylanase production were determined by mixing 0.5 ml filtered crude enzyme with 0.5 ml of 1% of each starch, carboxymethyl cellulose (CMC), microcrystalline cellulose (MCC), pectin and oat spelts xylan respectively (prepared in 50 mM Na-citrate buffer, pH 5.0). The reaction mixture was incubated at 50°C for 15 min and the process was stopped by applying 2 ml of 3, 5-dinitrosalicylic acid (DNS) and boiling in a water bath for 10 min (Miller 1959). After cooling, the color absorbance was measured at 540 nm using UV-Visible spectrophotometer (T80+, UK). The amount of reducing sugar liberated was quantified using standard curves of glucose (for amylase, endoglucanase, exoglucanase and pectinase), or xylose (for xylanase). One unit of the enzyme is defined as the amount of enzyme that liberates 1 µmol of the reducing sugar (glucose or xylose) equivalent per minute under the standard assay conditions (Ghose and Bisaria 1987). Total protein content was measured by the method suggested by Lowry *et al.* (1951) using bovine serum albumin (BSA) as the standard.

5.3. Calculations of enzyme production and activity

One unit of amylase, endoglucanase, exoglucanase or pectinase activity is defined as the amount of enzyme that liberate 1 µmole of glucose equivalent per minute under the standard assay conditions, and one unit of xylanase activity is defined as the amount of enzyme that liberate 1 µmole of xylose equivalent per minute under the standard assay conditions (Ghose and Bisaria 1987). Enzyme production and activity of amylase, endoglucanase, exoglucanase or pectinase can be calculated as the following equations **(Al-Bedak *et al.* 2020b)**

$$\textbf{Glucose or xylose} = \frac{\text{Absorbance}}{\text{slope}} \quad \text{mg/ml (= g/L)}$$

$$\textbf{Enzyme concentration} = \frac{\text{Sugar concentration (g/L)}}{(0.00018) \text{ or } (0.00015)} \quad \text{IU/L}$$

$$\textbf{Activity} = \text{Abs.} \times \text{DF} \times \left(\frac{1}{x}\right)\left(\frac{1}{y}\right)\left(\frac{1}{t}\right)\left(\frac{1}{\text{slope}}\right) \quad \text{IU/ml/min}$$

$$\textbf{Specific activity} = \frac{\text{Activity}}{\text{Total protein (mg/mL)}} \quad \text{IU/mg protein}$$

Where: DF = the dilution factor for enzyme; x = the volume of enzyme used; y = the volume of hydrolysate used for assay of reducing sugars; t = the time of hydrolysis; slope was determined by the standard curves of glucose or xylose; 0.00018 was employed for amylase, endoglucanase, exoglucanase and pectinase, and 0.00015 was used for xylanase.

For calculation of laccase production and activity, a calibration curve of gallic acid (Figure 2) was used. One unit of laccase enzyme was defined as the amount of laccase that liberates one µmol gallic acid

per ml per minute under the standard assay conditions.

$$\textbf{Gallic acid concentration} = \frac{\text{Absorbance}}{\text{slope } (=0.0011)} \quad \text{mg//L}$$

$$\textbf{Laccase concentration} = \frac{\text{Gallic acid (g/L)}}{0.00017} \quad \text{IU/L}$$

Where: DF = the dilution factor for enzyme; x = the volume of enzyme used; y = the volume of hydrolysate used for assay of reducing sugars; t = reaction time; slope was determined by the standard curves of gallic acid.

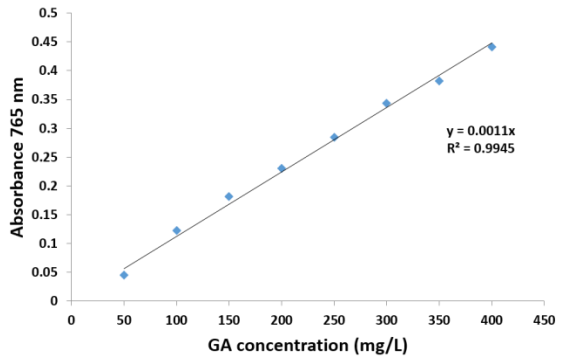

Figure 2: Standard curve of gallic acid

6. Bioconversion of agro-industrial residues into enzymes

6.1. Substrate pretreatment

Three separate agro-industrial residues, namely date palm leaves (DPL), rice straw (RS) and sugarcane bagasse (SB), were selected for enzyme processing in solid state fermentation (SSF). All these substrates were collected from public markets in Assiut and New Valley, Egypt. They were cleaned with purified water, oven dried at 50°C to a constant weight and ground with fine particles to move through 2 mm sieve.

6.2. Enzymes production in solid-state fermentation (SSF)

Two sets of Erlenmeyer flasks (500 ml), each containing 10 g of each of agro-industrial residues tested, were prepared. The first set was moistened with 20 ml of the fermentation medium amended with 0.1% sucrose and the second with 20 ml of the fermentation medium supplemented with 0.1% oat spelt xylan. Distilled water was then applied until the moisture content reached 80%. The flasks were then autoclaved for 20 min at 121°C. After cooling, every flask was inoculated with 1 ml spore suspension containing $1x10^8$ (spore/ml) from the 7–day–old culture of *A. curvatus* AUMC 11038 and *A. gaarensis* AUMC 11046. The inoculated flasks were incubated in a stagnant state at 30°C for 15 days.

6.3. Extraction and assay of cocktail enzyme

The flask content was collected and harvested in 150 ml of 50 mM sodium citrate buffer (pH 5.0) and the fermented slurry were filtered through double cheese cloth. The filtrates were centrifuged at 10,000 rpm for 20 min at 4ºC. The cell-free supernatants have been used for enzymes assay (amylase, endoglucanase, exoglucanase, pectinase and xylanase) as described above.

Results

1. Extraction and preparation of xylans from different sources

Calibration curve was built using a standard xylan solution (Birchwood xylan, Sigma-Aldrich) to measure the concentration of xylan. A strong linearity was achieved with $R^2 = 0.9907$. Out of 100 g extractable substrates after alkaline extraction, 27.4 g xylan can be obtained from oat spelts, 20.1 g from wheat bran, 13.7 g from corn stalks, and 12.5 g from corn cobs. The HPLC study showed the resemblance of the peaks for extracted xylans to the standard at the same retention time (Figures 3-6).

2. Quantitative assessment of enzymatic capacity in SmF

The results from this study indicated that both *Aspergillus* strains used in this research were able to produce multiple enzymes using the SmF technique

in differing amounts (Table 1). *A. curvatus* can produce a higher amylase enzyme level (1720 IU/gds) than that generated by *A. gaarensis* (1390 IU/gds). The amylase enzyme of *A. curvatus* displayed a higher relative activity of 1.65 IU/ml/min and a specific activity of 1.65 IU/ml/min compared to that of *A. gaarensis* (1.336 IU/ml/min and 5.26 IU/mg respectively).

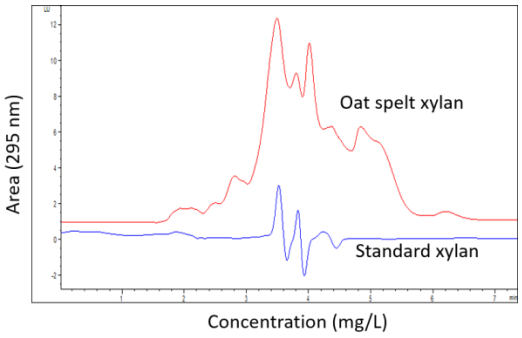

Figure 3: Addition (1:1) of 50 ppm of standard xylan in blue color and extracted oat spelts xylan in red color

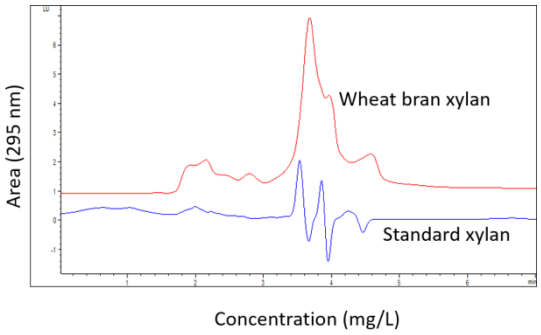

Figure 4: Addition (1:1) of 50 ppm of standard xylan in blue color and extracted wheat bran xylan in red color

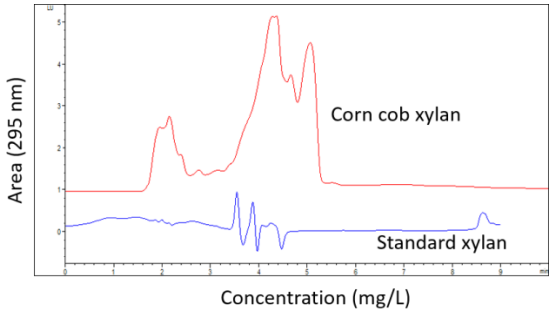

Figure 5: Addition (1:1) of 50 ppm of standard xylan in blue color and extracted corn cob xylan in red color

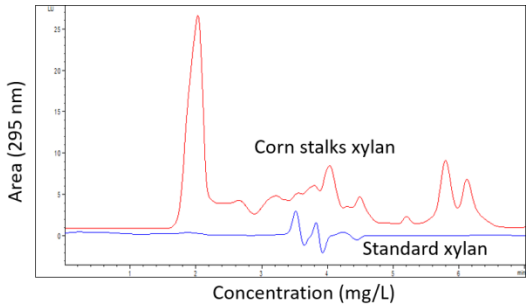

Figure 6: Addition (1:1) of 50 ppm of standard xylan in blue color and extracted corn stalks xylan in red color.

On the other hand, *A. gaarensis* was superior with 8392 IU/gds and 8.068 IU/ml/min in pectinase development over *A. curvatus* (6882 IU/gds and 6.614 IU/ml/min respectively); but *A. curvatus* had a pectinase-specific activity of 16.54 IU/mg greater than *A. gaarensis* (15.0 IU/mg). Using various types of extracted xylans in the SmF process carried out by *A. curvatus* and *A. gaarensis* in this study demonstrated the ability of both strains to degrade xylans and produce endoglucanase, exoglucanase and xylanase enzymes (Table 1). The oat-spelt xylan was the desirable carbon source for *A. curvatus* producing 4262 IU/gds xylanase enzyme with 3.43 IU/ml/min relative activity and 17.2 IU/mg real activity. The other three forms of xylan (wheat bran xylan, corn cob xylan and corn stalk xylan) could also be fermented but would yield 3580 IU/gds, 633 IU/gds and 70 IU/gds xylanase productivity, 2.88 IU/ml/min, 0.51 IU/ml/min and 0.056 IU/ml/min relative activities and 16.0 IU/mg, 3.2 IU/mg and 0.2 IU/mg specific activities respectively. On the other hand, xylan isolated from wheat bran was the ideal substrate for *A. gaarensis* to generate the enzyme xylanase. It could yield 4052 IU/gds with a relative activity of 3.27 IU/ml/min and a specific activity of 16.34 IU/mg. However, xylanase enzyme produced by *A. gaarensis* from corn cob xylan has hit the third level of productivity and relative activity and has attained the maximum specific activity of 20.61

IU/mg (Table 1). Endoglucanase enzyme was produced with considerably high level by both fungi when corn cob xylan was used in the fermentation process yielding 2281 IU/gds and 2664 IU/gds with 2.193 IU/ml/min and 2.55 IU/ml/min relative activity and 13.7 IU/mg and 17.466 IU/mg specific activity for *A. curvatus* and *A. gaarensis* respectively (Table 1). In the other hand, the oat spelt xylan was the most undesirable substrate in endoglucanase production for both fungi with the lowest production levels of 250 IU/gds and 17.0 IU/gds and the least relative activity (0.024 IU/ml/min and 0.016 IU/ml/min) and specific activity (1.2 IU/mg and 0.0816 IU/mg) for *A. curvatus* and *A. gaarensis*, respectively (Table 1). Xylan isolated from the wheat bran was the optimal medium for the synthesis of the exoglucanase enzyme by *A. curvatus* containing 3763 IU/gds of exoglucanase with 3.62 IU/ml/min of relative activity and 20.11 IU/mg of specific activity.

Corn cob xylan came next registering 3513 IU/gds, 3.37 IU/ml/min and 21.1 IU/mg followed by corn stalks xylan (3413 IU/gds, 3.28 IU/ml/min and 12.0 IU/mg) and oat spelt xylan (3386 IU/gds, 3.25 IU/ml/min and 16.27 IU/mg). In contrast *A. gaarensis* could ferment the corn stalks xylan perfectly to produce 4630 IU/gds of the exoglucanase enzyme with 4.45 IU/l/min relative activity and 14.134 IU/mg specific activity. Wheat bran xylan was the runner of corn stalks xylan followed by oat spelt xylan and corn cobs xylan producing 4274 IU/gds, 3996 IU/gds and 2064 IU/gds of exoglucanase with 4.11 IU/ml/min, 3.84 IU/ml/min and 1.984 IU/ml/min relative activity and 18.67 IU/mg, 19.2 IU/mg and 13.588 IU/mg specific activity respectively (Table 1).

3. Bioconversion of agro-industrial residues into valuable enzymes in SSF

Three agro-industrial residues have been fermented by *A. curvatus* and *A. gaarensis* in the present work using SSF. Exploitation of plant residues in amylase, endoglucanase, exoglucanase, laccase, pectinase, and xylanase in incremental amounts has been demonstrated by current results.

Amylase enzyme has been produced from all substrates fermented by both strains at different amounts. RS+S was the best fermented residue by *A. curvatus* yielding 3859 IU/gds amylase enzyme with 3.4 IU/mg of specific activity followed by RS+X registering 2891 IU/gds of amylase enzyme with 2.536 IU/mg of specific activity. On the other side, *A. gaarensis* was able to record its highest amylase production (2975 IU/gds) from SB+X followed by SB+S which produced 2879 IU/gds of amylase (Table 2), although the latter substrate reported higher specific activity (2.67 IU/mg) than the first (1.653 IU/mg).

Cellulases (endoglucanase and exoglucanase) enzymes were also generated by both *Aspergillus* strains when grown on the plant residues. *Aspergillus curvatus* yielded its highest endoglucanase production (8364 IU/gds) with 7.5 IU/mg specific activity on SB+S and the maximum exoglucanase (24112 IU/gds) with 21.22 IU/mg specific activity on RS+S. While *A. gaarensis* achieved its highest output of endoglucanase (8411 IU/gds) with 7.566 IU/mg specific activity on DPL+X and maximum exoglucanase (10347 IU/gds) with 9.3 IU/mg specific activity on DPL+X (Table 2).

Laccase enzyme was produced by *A. gaarensis* at the maximum level (854.3 IU/gds) with the highest specific activity of 426.4 IU/mg on RS+X followed by RS+S (271.4 IU/gds) with 146.5 IU/mg specific activity. While *A. curvatus* was runner of *A. gaarensis* in its maximum laccase production on DPL+S (241.7 IU/gds) with 125.6 IU/mg specific activity followed by RS+S (206.3 IU/gds) with 107.2 IU/mg specific activity (Table 2).

Table 1: Enzyme production, Relative activity, total protein and specific activity of amylase, endoglucanase, exoglucanase, pectinase and xylanase enzymes produced by *A. curvatus* AUMC 11038 and *A. gaarensis* AUMC 11046 in SmF at 30 °C.

Substrates	Enzymes	*A. curvatus* AUMC 11038				*A. gaarensis* AUMC 11046			
		Enzyme production IU/gds	Relative activity IU/ml/min	Total protein mg/ml	Specific activity IU/mg	Enzyme production IU/gds	Relative activity IU/ml/min	Total protein mg/ml	Specific activity IU/mg
Soluble starch	Amylase	1720	1.65	0.15	11.0	1390	1.336	0.254	5.26
Pectin	Pectinase	6882	6.614	0.4	16.54	8392	8.068	0.537	15.0
Oat spelts xylan	Xylanase	4262	3.43	0.2	17.2	3885	3.134	0.2	15.67
	Endoglucanase	250	0.24		1.2	17	0.016		0.0816
	Exoglucanase	3386	3.25		16.27	3996	3.84		19.2
Wheat bran xylan	Xylanase	3580	2.88	0.18	16.0	4052	3.27	0.22	16.34
	Endoglucanase	1250	1.2		6.67	266	0.255		1.275
	Exoglucanase	3763	3.62		20.11	4274	4.11		18.67
Corn cob xylan	Xylanase	633	0.51	0.16	3.2	3730	3.01	0.146	20.61
	Endoglucanase	2281	2.193		13.7	2664	2.55		17.466
	Exoglucanase	3513	3.37		21.1	2064	1.984		13.588
Corn stalks xylan	Xylanase	70	0.056	0.273	0.2	3552	2.866	0.315	9.1
	Endoglucanase	1720	1.65		6.0	2414	2.32		7.37
	Exoglucanase	3413	3.28		12.0	4630	4.45		14.134

Table 2: Enzyme production and specific activity of amylase, endoglucanase, exoglucanase, laccase, pectinase and xylanase enzymes released by *A. curvatus* AUMC 11038 and *A. gaarensis* AUMC 11046 from some agro-industrial plant residues in SSF at 30 °C.

Fungal strains	AUMC	Substrate	Amylase		Endoglucanase		Exoglucanase		Laccase		Pectinase		Xylanase		Total Protein mg/ml
			IU/gds	IU/mg	IU/gds	IU/mg	IU/gds	IU/mg	IU/gds	IU/mg	IU/gds	IU/mg	IU/gds	IU/mg	
A. curvatus	11038	DPL + S	2748	2.2	5711	4.586	14888	11.95	241.7	125.6	10275	8.25	11163	7.52	0.788
		RS + S	3859	3.4	7599	6.69	24112	21.22	206.3	107.2	9654	8.5	11846	8.75	0.7188
		SB + S	1374	1.23	8364	7.5	10610	9.52	43.3	20.85	10514	9.43	8173	6.15	0.705
		DPL + X	2067	2.0	7360	7.2	11279	11.0	58.6	33.85	9391	9.18	13014	10.68	0.647
		RS + X	2891	2.536	4970	4.36	8937	7.84	65.0	33.68	8961	7.86	14182	10.43	0.7215
		SB + X	2831	2.64	3823	3.57	9104	8.5	112.4	61.94	9511	8.87	7945	6.22	0.678
A. gaarensis	11046	DPL + S	1314	1.116	5902	5.013	5233	4.44	93.14	46.7	10323	8.77	8344	5.94	0.745
		RS + S	1254	1.15	8292	7.582	5655	5.17	271.4	146.5	8650	7.91	11220	8.61	0.692
		SB + S	2879	2.67	5496	5.1	4827	4.48	55.4	30.34	10777	9.996	9654	7.513	0.6822
		DPL + X	2521	2.27	8411	7.566	10347	9.3	89.93	47.77	12824	11.53	13270	10.0	0.7034
		RS + X	2258	1.91	8292	7.0	10036	8.48	854.3	426.4	9439	7.98	12131	8.6	0.7486
		SB + X	2975	1.653	6476	3.6	7097	3.94	91.5	30.0	9128	5.1	9198	4.29	1.139

DPL+S: date palm leaves+0.1% sucrose-Czapek's; RS+S: rice straw+0.1% sucrose-Czapek's; SB+S: sugarcane bagasse+0.1% sucrose-Czapek's; DPL+X: date palm leaves+sucrose free-Czapek's with 0.1% xylan; RS+X: rice straw+sucrose free-Czapek's with 0.1% xylan; SB+X: sugarcane bagasse+ sucrose free-Czapek's with 0.1 % xylan; gds: gram dry substrate.

The pectinase enzyme was distributed to all substrates in increased concentrations by both strains. Maximum productivity of *A. gaarensis* (12824 IU/gds) with the maximum specific activity of 11.53 IU/mg was reported from DPL+X followed by SB+S (10777 IU/gds) with a specific activity of 9.996 IU/mg. Whereas *A. curvatus* achieved its highest production (10514 IU/gds) with a maximum specific activity of 9.43 IU/mg from SB+S followed by DPL+S (10275 IU/gds and 8.25 IU/mg specific activity) (Table 2).

In both strains the xylanase enzyme could be produced on all substrates in high productivity. The highest production (14182 IU/gds) with a 10.43 IU/mg specific activity was attained by *A. curvatus* from RS+X accompanied by DPL+X, RS+S and DPL+S harboring 13014 IU/gds, 11846 IU/gds and 11163 IU/gds of xylanase production with 10.68 IU/mg, 8.75 IU/mg and 7.52 IU/mg specific activity, respectively. The DPL+X followed by RS+X and RS+S reported high level of xylanase production of 13270 IU/gds, 12131 IU/gds and 11220 IU/gds by *A. gaarensis* with specific activity of 10.0 IU/mg, 8.6 IU/mg and 8.61 IU/mg respectively (Table 2).

Discussion

With the global annual production of large quantities of agricultural residues, alternative approaches for biomass disposal are now required in an environmentally sustainable way. It is a valuable practice with important economic advantages that agricultural wastes are used as substrates to manufacture industrial essential products such as enzymes, polysaccharides, organic acids, aromas and flavor compounds. Low-value agricultural residues that can effectively be converted to fermentable sugars and enzymes. Efficient strains of microorganisms that can consume cellulose directly without the need for enzymatic or acid hydrolysis should be studied. These powerful strains make the process economically and eco-friendly. Improvements to the strain would help to maximize the yield of sugars and enzymes, allowing large-scale production more competitive and feasible.

In Egypt, date palm leaves, rice straw and sugarcane bagasse are desirable opportunities for the commercial production of valuable enzymes. In present study, three agricultural residues have been converted to certain useful enzymes by two *Aspergillus* strains, previously isolated and described as new fungi namely *Aspergillus curvatus* (Al-Bedak *et al.* 2020a) and *A. gaarensis* (Al-Bedak and Moubasher 2020) (Figure 7). Both strains have shown outstanding ability to break down all residues used and produce high yields of six valuable enzymes namely amylase, endoglucanase, exoglucanase, laccase, pectinase and xylanase. The first species was recovered from the alkaline waters of Lake Khadra and the second was recovered from a soil sample near Lake El-Gaar in Wadi-El-Natron, a typical extreme environment in Egypt. Extremophilic fungi are known to be a promising category of microorganisms for the industrial processing of complex waste products, since they exhibit special characteristics and are most adaptable to harsh environmental conditions (Ismail *et al*. 2017).

Industrial bioprocesses for the manufacturing of industrial products are planned to be developed in the near future (Bhatia and Paliwal 2011). As far as we know, no researches have been published on the production of enzyme from lignocellulosic date palm residues in Egypt, irrespective of the substantial lignocellulosic biomass. In the present work, the ground date palm leaf residues obtained from the New Valley Governorate have been transformed into highly-valued enzymes using SSF by two newly-identified *Aspergillus* species. The date palm (*Phoenix dactylifera* L.) is a major fruit crop in the governorate of the New Valley, Egypt. It has historically been tied to the preservation of human life and the ancestral heritage of the inhabitants of

the New Valley as a major agricultural crop. Residues of date palm leaves (DPL) are a pruning waste containing mainly of hemicellulose (16–18%), cellulose (27–41%), and lignin (10–19%) (Bahman *et al*. 1997, Pascual *et al*. 2000, Arhab *et al*. 2009, Rad *et al.* 2015). The average naturally dried leaf (including leaflets and rachis) has a weight of 2-3 kg (Rezende *et al*. 2011). Egypt generates approximately 18% of the global production and 23% of the Arab production of dates (Rageh *et al.* 2020). There are around 2.5 million date palm trees in the New Valley Government. It is therefore calculated that each date palm yields approximately 50 kg of leaf residue per year (Rad *et al.* 2015). This means that the annual yield from date palm leaf residues is over 125,000 tons, but unfortunately, a large part of this waste remains largely underutilized, and causes potential waste disposal problems.

The current findings have shown that *A. curvatus* and *A. gaarensis* perfectly uses rice straw as an enzyme production substrate in SSF, in which the six studied enzymes can be produced in high quantities. Rice straw is one of the most widespread lignocellulosic waste products in the world. It is produced annually in large quantities of approximately 731 million tons distributed in Africa (20,9 million tons), Asia (667,6 million tons), Europe (3,9 million tons) and America (37.2 million tons) (Karimi *et al*. 2006). Egypt is the main producer of rice in the Near East with over 1,428,600 feddan rice cultivated area with an estimated farm yield of 6.0 tons/feddan (Hammoud *et al*. 2020) and an approximate straw production of 2.4 tons/feddan (Sabaa and Sharaf 2000). As a result, 3,428,640 million tons of unused lignocellulosic biomass from rice straw residues are produced annually.

Farm outdoor public burning is actually the primary practice of eliminating such large quantities of post-harvest rice residues. While field burning leads to the successful removal of weed seeds and pathogenic microbial spores, the black smoke emitted is a threat to public health. In Egypt, the farming of rice and the construction sectors has resulted in the production of significant amounts of agricultural waste rich in cellulose, hemicellulose and lignin, which are available free of charge throughout the year (Sherief *et al.* 2010, Ragab *et al.* 2014).

In the current study, sugarcane bagasse was fermented by *A. curvatus* and *A. gaarensis* and can produce high levels of amylase, endoglucanase, exoglucanase, laccase, pectinase and xylanase enzymes in SSF. Around the world, 54 million tons of dry bagasse are produced annually (Moubarik and Grimi 2015), of which about 4.7 million tons are produced in Egypt annually (Mohamed *et al*. 2015). The quest for an acceptable application for the use of this waste is therefore a research subject that leads to the protection of the ecosystem. Sugarcane bagasse, which is considered to be one of the lignocellulosic waste materials, has gained significant interest in its ability as a bioadsorbent for the treatment of wastewater (Peñafiel et al., 2021; Tony, 2021), a promising substrate for the production of ethanol (Cardona et al., 2010; Bhatia and Paliwal, 2011; Faisal and Saeed, 2021; Ntimbani et al., 2021) as well as a safe source for the production of enzymes using microorganisms.

Conclusion

The use of lignocellulosic materials to manufacture value-added products, such as enzymes, fermentable sugars and organic acids, is met by improved energy requirements and reduced energy supplies. One of the key barriers to bioconversion processes is the morphological heterogeneity and crystallinity of lignocellulosic biomass. The manufacture of enzymes has become economical with the processing of agricultural commodities.

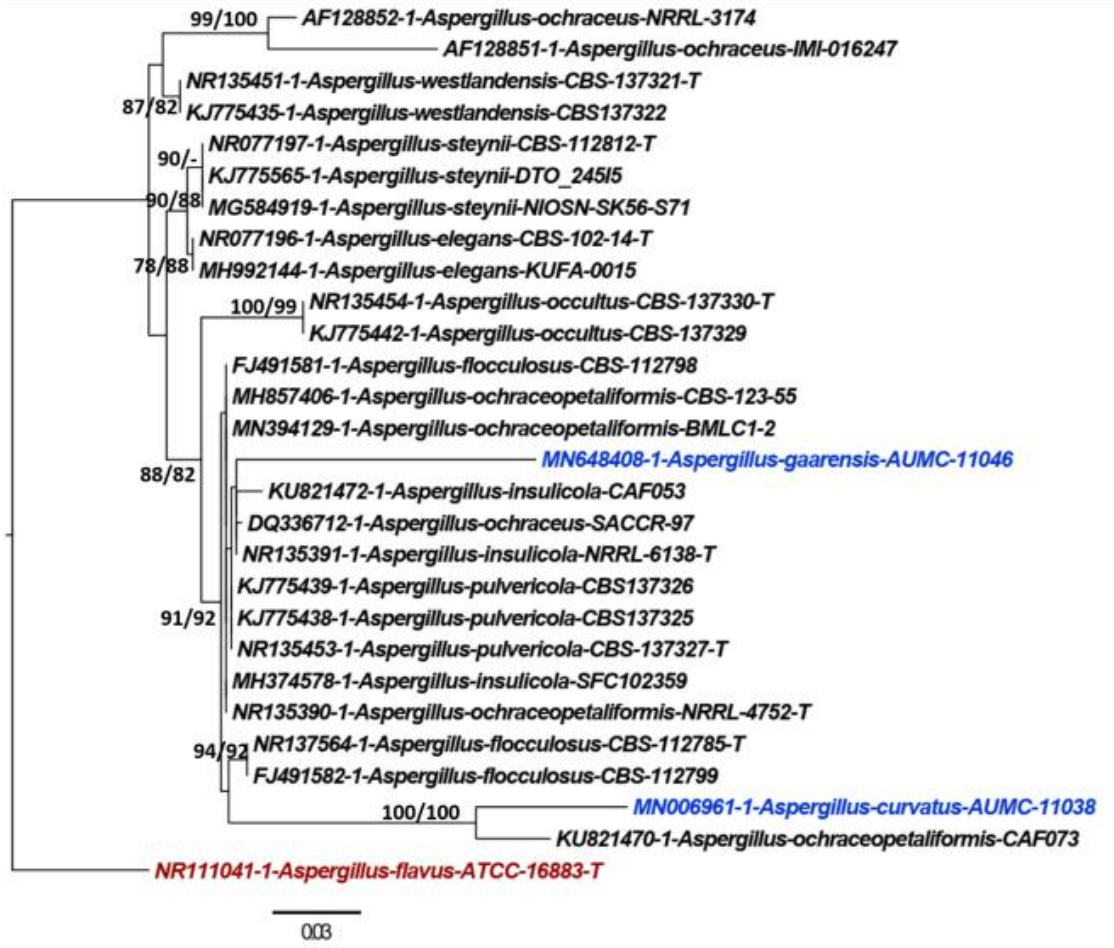

Figure 7: Maximum Likelihood (ML/MP combination) phylogenetic tree of *A. curvatus* AUMC 11038 and *A. gaarensis* AUMC 11046 aligned with other related *Aspergillus* taxa in section *Circumdati* based on the ITS gene sequences. Sequence of the new species are in blue color. The numbers near the branches are the bootstrap values (100 replications). Values <70 % are not shown. The tree is rooted to *A. flavus* ATCC 16883. T = type strain.

This creates the opportunity to start recycling such waste in the production of products with tremendous financial benefits for the national economy which will generate thousands of new jobs under the 2030 vision.

Compliance with ethical standards

The manuscript is original. No part of the manuscript has been published before nor is any part of it under consideration for publication at another journal.

Declaration of competing interest

The authors declare that there are no potential conflicts of interest regarding the publication of this paper.

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