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PLANT CELL-WALL HYDROLYZING ENZYMES FROM INDIGENOUSLY ISOLATED FUNGI GROWN ON CONVENTIONAL AND NOVEL NATURAL SUBSTRATES

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Abstract

Fungi elaborate a variety of plant-hydrolyzing enzymes including cellulases, xylanases, pectinases and amylases. Although these enzymes have potential biotechnological applications, their production at industrial level is limited because of higher costs of the purified substrates. Hence, the present study was aimed to explore the novel, natural and cheaper substrates for enzyme production. Indigenously isolated fungal strains of *Aspergillus* sp. were grown on banana-peels, grapefruit-peels, pomegranate-peels, sugarcane bagasse, *Eucalyptus camaldulensis*-leaves and shoots of two halophytic plants including *Halopyrum mucronatum* and *Desmostachya bipinnata* under solid-state fermentation (SSF) and submerged fermentation (Smf) conditions. The crude enzyme preparation was screened for cellulase (endoglucanase, β-glucosidase and filter-paperase), hemicellulase (xylanase), pectinase and amylase production. The results revealed that among all investigated enzymes, the xylanase titers were highest using *D. bipinnata*- shoots and *H. mucronatum*- shoots as substrates under solid state fermentation conditions, suggesting their exploitation at commercial scale.

Keywords: Plant-hydrolyzing enzymes, Natural substrates, Solid-state fermentation, Submerged fermentation.

Introduction

The plant cells are composed of about 90% polysaccharides including cellulose, hemicellulose and pectin which serve as source of nutrients and energy for microorganisms. The extensive research on these plant-hydrolyzing enzymes has indicated their potential for a wide variety of biotechnological applications including food, feed, pharmaceutical, paper, textile and leather industries (de Vries & Visser, 2001). However, the large-scale production of these enzymes is limited by the high cost of the purified substrates, and requires inexpensive natural substrates for enzyme production.

On the other hand, a large amount of natural wastes are generated from food industries like sugarcane bagasse from sugar industries, fruit-pulp and peels from fruit-juice processing industries (Abdel-Halim, 2014). The improper disposal of these and other similar wastes to the environment causes pollution, thereby affecting the public health (Sathiyavathi & Parvatham, 2013). Hence, the utilization of these natural wastes as substrate for enzyme production may not only reduce the environmental pollution but also provide the industrially-important enzymes at low cost (Seyis & Aksoz, 2005; Norazlina *et al.*, 2013).

Although the enzymes can be produced by a variety of microorganisms, the filamentous fungi are preferred because of their easy handling, low cost for growth in large bioreactors and higher enzyme productivity (Aro *et al*., 2005; Jun *et al.*, 2011). Among filamentous fungi, *A. niger* has widely been used for enzyme production due to its ability to secrete highly active proteins (Robl *et al.*, 2015).

In the present research, seven different types of natural substrates were screened for production of various plant hydrolyzing enzymes including cellulase, xylanase, pectinase and amylase by three different strains of *Aspergillus* sp. The novel substrates used in the study were the shoots of *Halopyrum mucronatum* and *Desmostachya bipinnata* which are halophytic plants. These salt tolerant grasses occupy the saline agricultural lands which have been affected due to the shortage of fresh water. To the best of our knowledge of our knowledge, it is for the first time that the halophytic plants are being used as substrate for enzyme production. These plants were explored as substrate for enzyme production, and compared with the conventional substrates including banana-peels, pomegranate-peels, grapefruit-peels, sugarcane bagasse and *Eucalyptus camaldulensis*-leaves.

Materials and Methods

Culture: Fungal strains, *Aspergillus niger* MS34, *Aspergillus niger* MS80 and *Aspergillus flavus* DK5 were procured from the culture collection of the Department of Microbiology, University of Karachi, and maintained on Sabouraud's dextrose agar slants at 4°C.

Collection and preparation of substrates: Banana-peels, grapefruit-peels, pomegranate-peels and sugarcane bagasse were collected from the local juice shop, and *Eucalyptus Camaldulensis*-leaves were obtained from a local tree. The shoots of *Halopyrum mucronatum* and *Desmostachya bipinnata* were kindly provided by the Institute of Sustainable Halophyte Utilization, University of Karachi. All the substrates were washed with distilled water followed by air-drying and oven drying at 60ºC. They were then grinded and passed through sieve of 100 mesh-size.

Solid-state fermentation (SSF): The substrates (1g) were supplemented with mineral-salt medium (0.00016g $MnSO_4$, 0.00014g $ZnSO_4$, 20g $(NH_4)_2SO_4$, 0.03g $MgSO_4$, 0.0005g $FeSO_4$, 0.03g $CaCl_2$, 0.0002g $CoCl_2$, 0.2g KH_2PO_4, 0.1g peptone and 100ml distilled H_2O) to obtain a relative moisture content of about 65%, and autoclaved at 121°C and 15 psi for 20 minutes. The fungal culture (1×10^5 spores, as counted by hemocytometer) was inoculated and incubated at 28ºC for 5 days. For enzyme extraction, 25ml of sodium-citrate buffer (pH 4.8) was added, followed by shaking at 28°C and 150 rpm for 2 hours. It was then filtered through a double layered

Whattman filter-paper no. 1, and centrifuged at x5000g for 30 minutes. The cell-free culture supernatant (CFCS) was then analyzed for enzyme titers.

Submerged fermentation (Smf): The substrates (0.1g) were suspended in 10ml of mineral-salt medium (composition was same as mentioned above), and autoclaved at 121°C and 15 psi for 20 minutes. The fungal culture (1×10^5 spores) was inoculated, and incubated at 28°C at 150 rpm for 4 days. CFCS was obtained by centrifugation at x 5000 g for 30 minutes and determined for enzyme titers.

Assays for endoglucanase, β-glucosidase, xylanase, pectinase and amylase enzymes: The enzyme assays were performed by DNS method (Miller, 1959). The reaction mixture containing CFCS (25µl) and sodium-citrate buffer (25µl; pH4.8) supplemented with 0.5% of respective substrates (carboxy-methyl cellulose, salicin, beechwood xylan, pectin and starch for endoglucanase, β-glucosidase, xylanase, pectinase and amylase assays respectively) was incubated at ambient temperatures for appropriate time periods (at 50°C for 30 minutes for endoglucanase, β-glucosidase, pectinase and amylase assays, and at 63°C for 15 minutes for xylanase assay). The reaction was stopped by adding 150µl of DNS, boiled for 10 minutes and chilled on ice. The volume was made up to 920µl with distilled water, A_{550} determined and results compared with standard curves of relevant reducing sugars (glucose for endoglucanase, β-glucosidase and amylase assays, xylose for xylanase assay and β-galactouronic acid for pectinase assay). One unit of enzyme activity was considered as the amount of reducing sugars produced by 1ml of enzyme in 1 minute.

Assay for filter-paperase enzyme: CFCS (250µl) was mixed with sodium citrate buffer (250µl; pH 4.8) containing filter-paper strips (3×0.5cm) and incubated at 50°C for 60 minutes. DNS (1.5ml) was added and reaction mixture boiled for 10 minutes followed by cooling on ice. The volume was made up to 9.2ml with distilled water, A_{550} noted and compared with standard curve for glucose. An international filter paper unit (IFPU) was considered as the amount of glucose produced by 1ml of filter-paperase enzyme in 1minute.

Statistical analysis: All the experiments were performed in triplicate and the results are reported as the mean and standard deviation of three values.

Results and Discussion

The experimental data suggested that among all the investigated enzymes, higher levels of xylanase were observed followed by pectinase and amylase. However, cellulase titers were very low. This difference in enzyme titers could be ascribed to the variation in the chemical composition of different substrates used in the study as the chemical makeup of plants vary not only from one another but also among the cells of the same plant (Have *et al.,* 2002).

Among all the substrates used in the study, *D. bipinnata*-shoots showed the highest xylanase production followed by *H. mucronatum*-shoots (Fig. 1). It could be linked to the presence of about 24 and 28% hemicellulosic content in the cell walls of *D. bipinnata* and *H. mucronatum* respectively (Abideen *et al.,* 2012). Since these plants grow on saline lands which have been formed due to the shortage of fresh water, their use as substrate for enzyme production may not only save the cost invested on purified substrates but also explore the potential utilization of these lands. Moreover, their ability to grow throughout the year without any need of high-quality water, fresh sowing and fertilizers make them attractive as substrate for enzyme production (Aziz *et al.,* 2005; Qasim *et al.,* 2014).

The findings of the present study did not show significant xylanase production in presence of banana-peels, sugarcane bagasse and *E. camaldulensis*-leaves. It contradicts some of the studies which report them as potential xylanase inducers (Rezende *et al.,* 2002; Fang *et al.,* 2010; Sathiyavathi & Parvatham, 2013; Roy *et al.,* 2013; Sanchez-Vazquez *et al.,* 2013; Guilherme *et al.,* 2015). Since the chemical constituents of the plant may vary with the growth and maturity level, it might be one of the reasons for low xylanase levels using the same substrate.

It was noted that *A. niger* MS34 and *A. niger* MS80 produced the maximum pectinase titers when grown on *E. camaldulensis*-leaves under Smf conditions (Fig. 2). Also, grapefruit-peels showed considerable pectinase levels under Smf conditions by all the three fungal strains; *A. niger* MS34 exhibited significant pectinase titers under SSF conditions also. It could be associated with their higher pectin content. Although, the grapefruit-peel waste generated by the grapefruit juice processing industries, is used as cattle-feed but it does not generate enough revenue to compensate the cost invested by the grapefruit juice processing industries. Hence, this waste if utilized as substrate for pectinase production may benefit the concerned industrial sectors (Mark *et al.,* 2007). Besides, significant pectinase titers were also notied when *A. niger* MS80 was grown on pomegranate-peels under Smf conditions.

Furthermore, significant levels of amylase were obtained by *A. flavus* DK5 when cultivated in the growth medium containing sugarcane bagasse under Smf conditions; the same strain also showed considerable amylase induction in presence of *E. camaldulensis*-leaves under both SSF and Smf conditions (Fig. 3). Moreover, appreciable levels of endoglucanase enzyme were obtained when *A. flavus* DK5 was grown on grapefruit peels under Smf conditions. Besides, banana peels also induced endoglucanase production (Fig. 4). This observation is in lines with the studies conducted earlier (Phiriyawirut & Maniaw, 2012). It was followed by β-glucosidase production from *A. flavus* DK5 in presence of *H. mucronatum*-shoots under Smf conditions; banana-peels and *D. bipinnata*-shoots also induced β-glucosidase production under SSF conditions by *A. flavus* DK5 and *A. niger* MS34 respectively (Fig. 5). It can be linked to the higher cellulosic content in *H. mucronatum* and *D. bipinnata* as they are reported to possess 37% and 26% cellulose (Abideen *et al.,* 2011; Abideen *et al.,* 2012). Nonetheless, the filter-paperase levels were negligible (Fig. 6).

The calculation of volumetric productivities revealed that xylanase from *A. niger* MS34 exhibited the highest volumetric productivity when cultivated in presence of *D. bipinnata*-shoots under SSF conditions. Overall, xylanases and *D. bipinnata*-shoots showed higher values amongst all studied enzymes and substrates respectively. Nonetheless, *H. mucronatum*-shoots and sugarcane bagasse also showed significant volumetric productivity by *A. niger* MS34 under SSF and Smf conditions respectively. Additionally, comparatively higher values were obtained under SSF conditions than Smf (Tables 1 & 2).

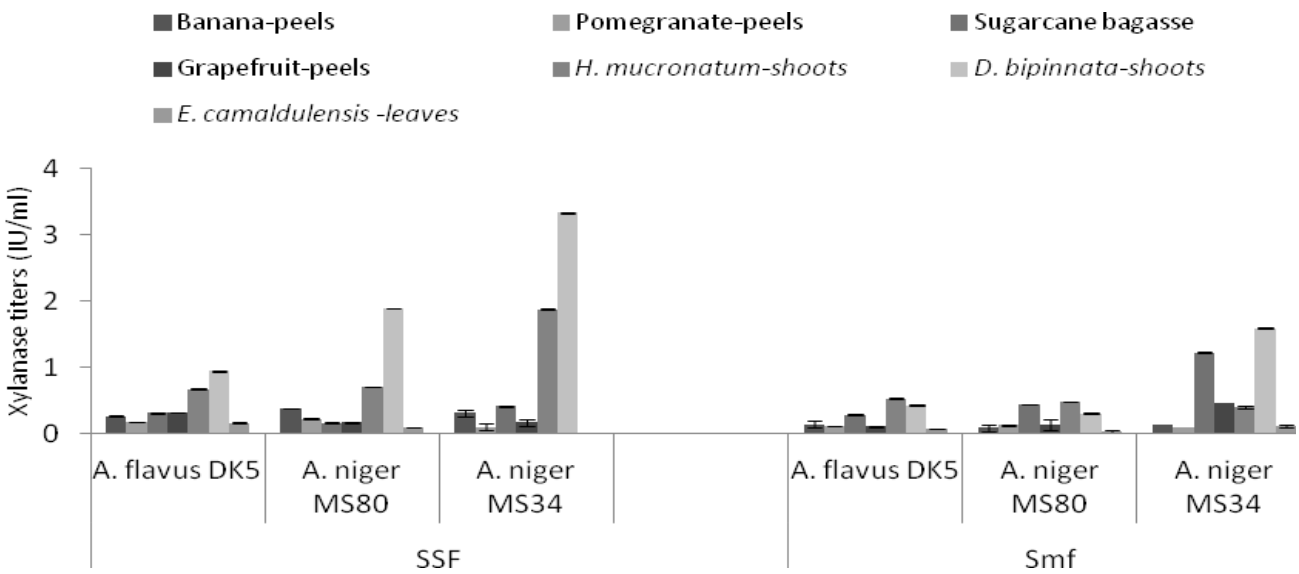

Fig. 1. Xylanase titers by fungal strains grown in presence of natural substrates under SSF & Smf conditions.

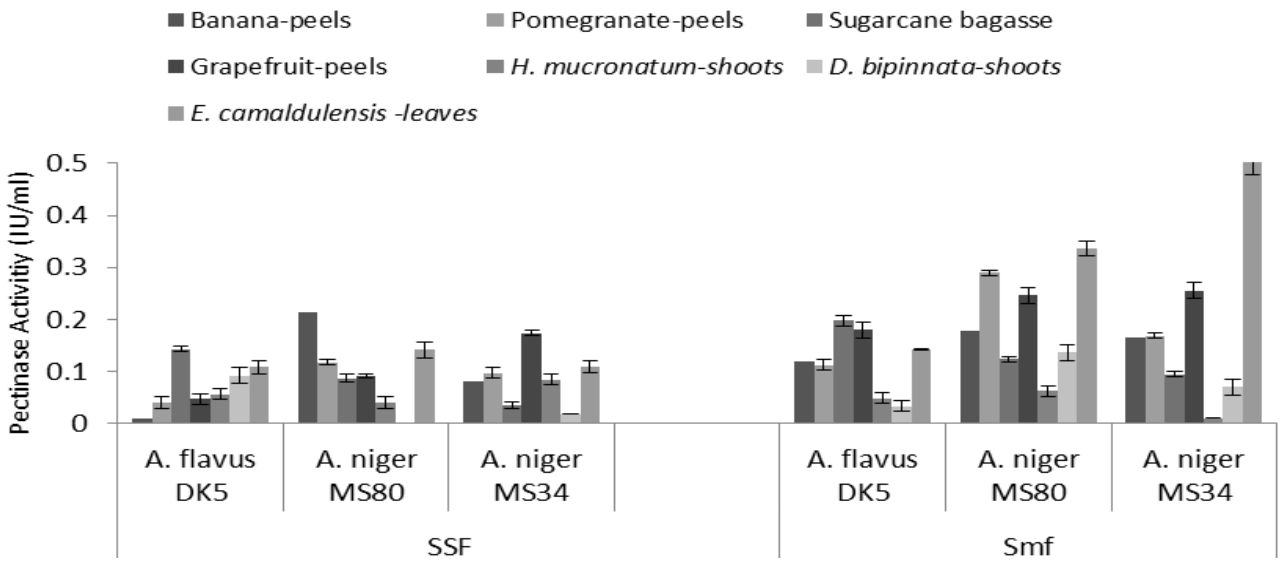

Fig. 2. Pectinase titers by fungal strains grown in presence of natural substrates under SSF & Smf conditions.

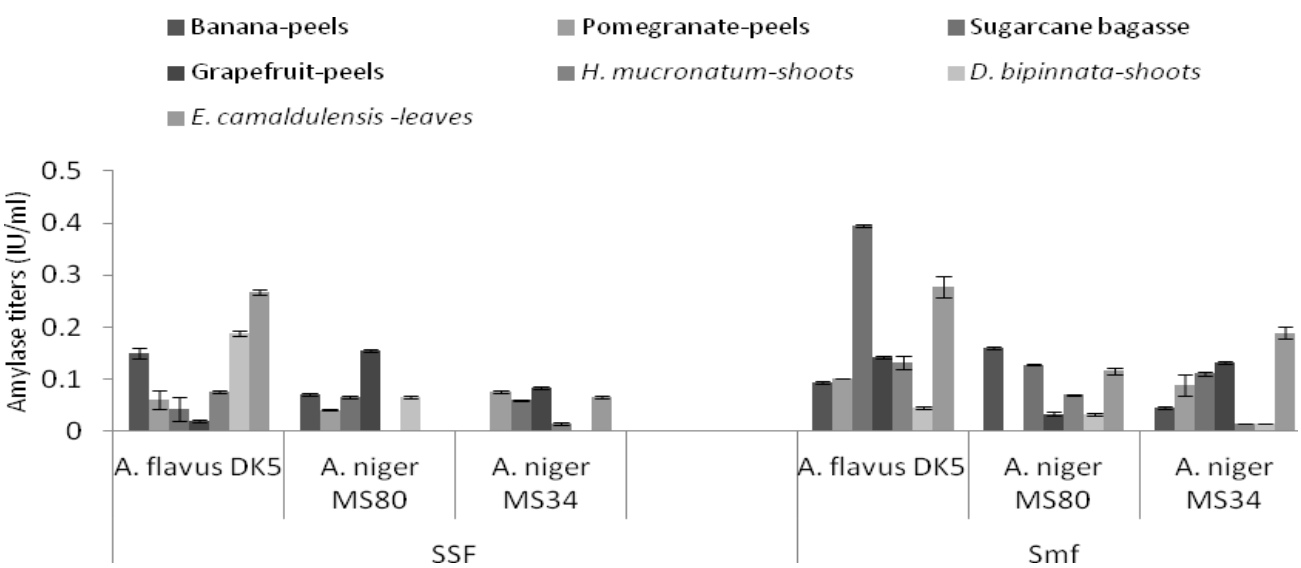

Fig. 3. Amylase titers by fungal strains grown in presence of natural substrates under SSF & Smf conditions.

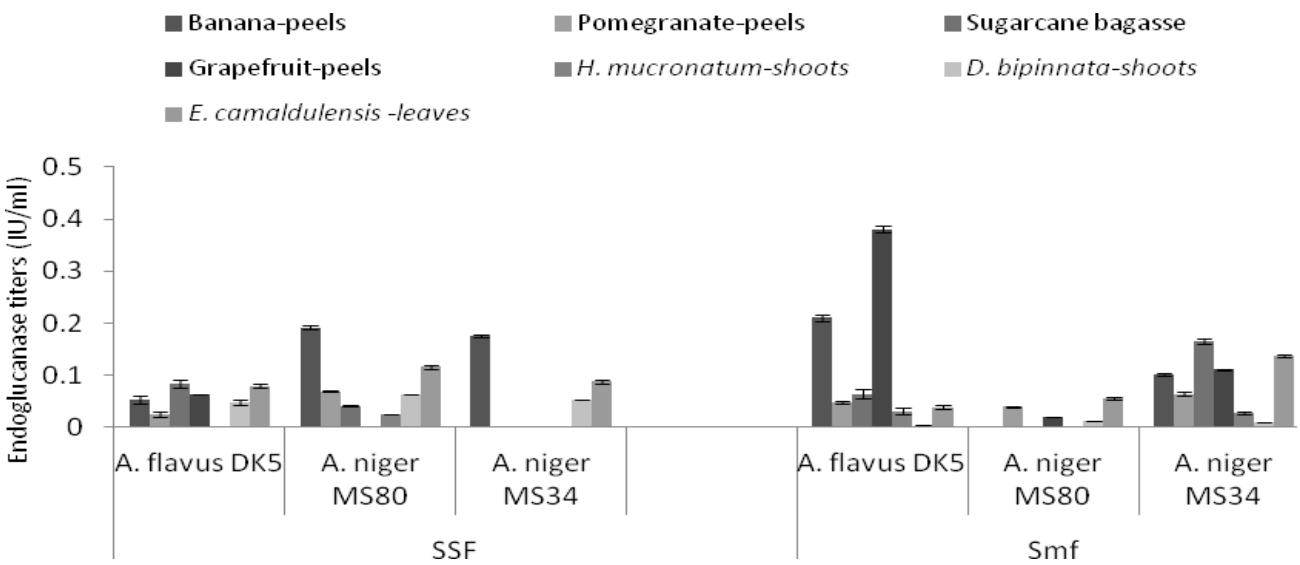

Fig. 4. Endoglucanase titers by fungal strains grown in presence of natural substrates under SSF & Smf conditions.

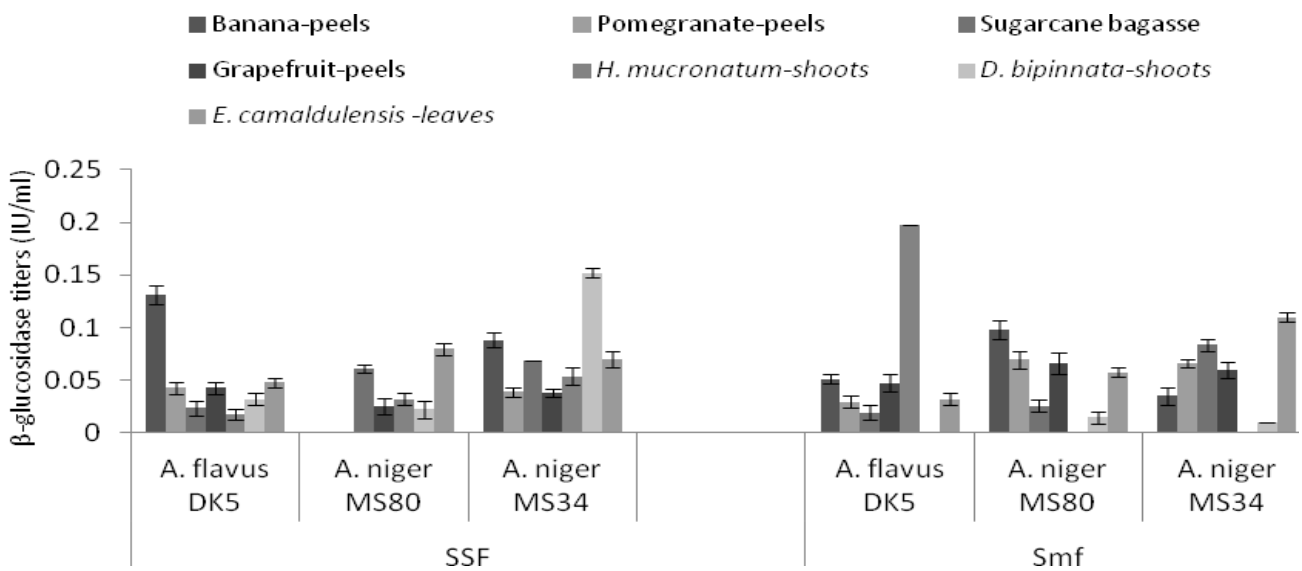

Fig. 5. β-glucosidase titers by fungal strains grown in presence of natural substrates under SSF & Smf conditions.

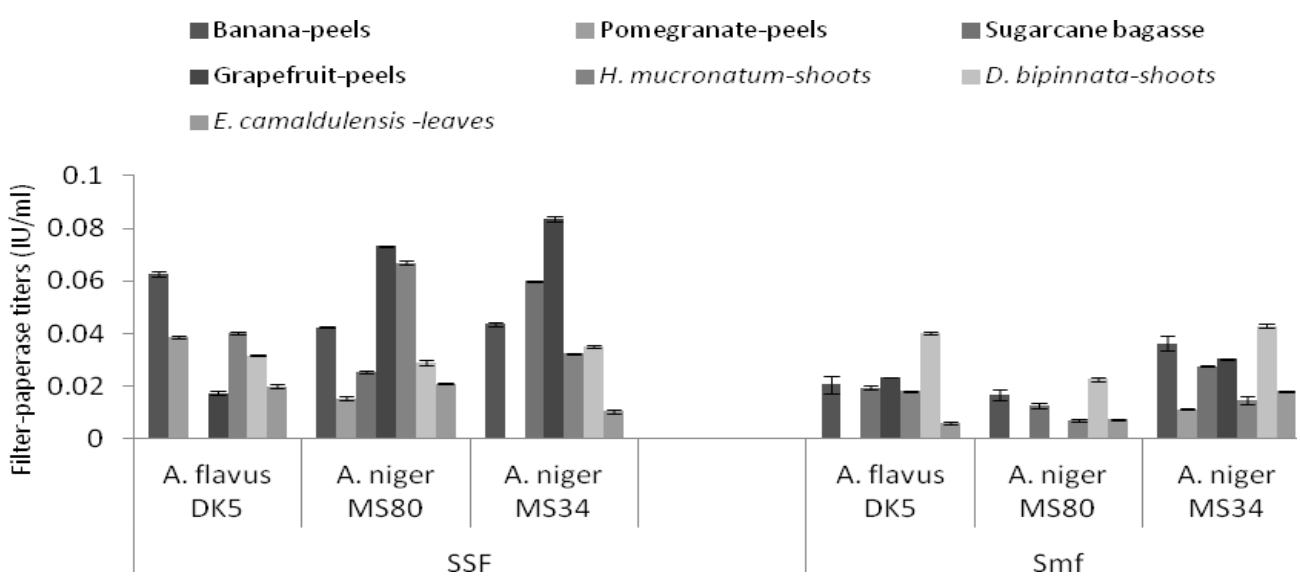

Fig. 6. Filter-paperase titers by fungal strains grown in presence of natural substrates under SSF & Smf conditions.

Table 1. Volumetric productivities of plant-hydrolyzing enzymes from fungal strains grown in presence of natural substrates under SSF conditions.

Substrates	*A. flavus* DK5					
	Xylanase	**Pectinase**	**Amylase**	**Endoglucanase**	**β-glucosidase**	**Filter-paperase**
Banana-peels	32160 ± 0.003	0	17960 ± 0.009	6360 ± 0.008	15720 ± 0.08	7520 ± 0.001
Pomegranate-peels	20880 ± 0.005	4880 ± 0.0121	7320 ± 0.017	3040 ± 0.005	5120 ± 0.005	4640 ± 0.004
Sugarcane bagasse	37440 ± 0.005	17160 ± 0.005	5120 ± 0.023	10040 ± 0.007	2840 ± 0.007	0
Grapefruit-peels	38520 ± 0.007	5640 ± 0.010	2480 ± 0.002	7680 ± 0.004	5120 ± 0.005	2080 ± 0.0007
H. mucronatum- shoots	80920 ± 0.005	6720 ± 0.010	9100 ± 0.003	0	2128 ± 0.004	4840 ± 0.0004
D. bipinnata-shoots	113648± 0.007	11040 ± 0.015	22560 ± 0.005	5840 ± 0.005	3848 ± 0.005	3800 ± 0.0002
E. camaldulensis-leaves	19640 ± 0.008	13000 ± 0.012	32080 ± 0.004	9560 ± 0.004	5760 ± 0.004	2400 ± 0.0009
Substrates	***A. niger* MS80**					
	Xylanase	**Pectinase**	**Amylase**	**Endoglucanase**	**β-glucosidase**	**Filter-paperase**
Banana-peels	45760 ± 0.008	25560 ± 0.008	8480 ± 0.002	23040 ± 0.003	0	5080 ± 0.00008
Pomegranate-peels	27280 ± 0.004	14160 ± 0.004	4960 ± 0.002	8320 ± 0.002	0	1840 ± 0.004
Sugarcane bagasse	19800 ± 0.005	10440 ± 0.008	7920 ± 0.003	4960 ± 0.002	7320 ± 0.004	3040 ± 0.005
Grapefruit-peels	20520 ± 0.005	10920 ± 0.004	18600 ± 0.003	0	3040 ± 0.007	8800 ± 0.005
H. mucronatum- shoots	85244 ± 0.006	4880 ± 0.012	0	2872 ± 0.002	3848 ± 0.005	8040 ± 0.006
D. bipinnata-shoots	228000 ± 0.005	0	7844 ± 0.002	7696 ± 0.003	2660 ± 0.008	3480 ± 0.008
E. camaldulensis-leaves	11680 ± 0.008	17000 ± 0.015	0	13920 ± 0.003	9560 ± 0.005	2520 ± 0.09
Substrates	***A. niger* MS34**					
	Xylanase	**Pectinase**	**Amylase**	**Endoglucanase**	**β-glucosidase**	**Filter-paperase**
Banana-peels	37440 ± 0.051	9840 ± 0.005	0	21040 ± 0.002	10600 ± 0.007	5240 ± 0.051
Pomegranate-peels	12000 ± 0.051	11640 ± 0.010	9040 ± 0.002	0	4680 ± 0.004	0 ± 0.051
Sugarcane bagasse	50400 ± 0.010	4200 ± 0.005	7200 ± 0.001	0	8160 ± 0.004	7200 ± 0.01
Grapefruit-peels	20520 ± 0.051	20880 ± 0.005	10040 ± 0.002	0	4560 ± 0.008	10040 ± 0.051
H. mucronatum- shoots	225840 ± 0.004	10080 ± 0.010	1768 ± 0.003	0	6436 ± 0.004	3880 ± 0.004
D. bipinnata-shoots	400660 ± 0.007	2160 ± 0.011	0	6212 ± 0.003	18232 ± 0.007	4200 ± 0.007
E. camaldulensis-leaves	0	13056 ± 0.012	7844 ± 0.002	10580 ± 0.004	8424 ± 0.008	1248 ± 0.008

Table 2. Volumetric productivities of plant-hydrolyzing enzymes from fungal strains grown in presence of natural substrates under Smf conditions.

Substrates	*A. flavus* DK5					
	Xylanase	**Pectinase**	**Amylase**	**Endoglucanase**	**β-glucosidase**	**Filter-paperase**
Banana-peels	14144 ± 0.046	11424 ± 0.015	9024 ± 0.003	20224 ± 0.002	4928 ± 0.015	1984 ± 0.001
Pomegranate-peels	11328 ± 0.010	10784 ± 0.012	9632 ± 0.013	4544 ± 0.003	2848 ± 0.005	0
Sugarcane bagasse	27552 ± 0.004	19008 ± 0.010	37856 ± 0.036	6144 ± 0.003	1856 ± 0.005	1872 ± 0.0004
Grapefruit-peels	10752 ± 0.004	17280 ± 0.010	13696 ± 0.002	36544 ± 0.004	4544 ± 0.007	2240 ± 0.0007
H. mucronatum- shoots	51427.2 ± 0.008	4704 ± 0.015	12704 ± 0.002	2960 ± 0.003	19001.6 ± 0.008	1728 ± 0.0004
D. bipinnata-shoots	41472 ± 0.002	3168 ± 0.015	4304 ± 0.002	288 ± 0.004	0	3872 ± 0.0002
E. camaldulensis-leaves	7072 ± 0.004	13600 ± 0.015	26560 ± 0.005	3712 ± 0.002	3040 ± 0.005	576 ± 0.0009
Substrates	***A. niger* MS80**					
	Xylanase	**Pectinase**	**Amylase**	**Endoglucanase**	**β-glucosidase**	**Filter-paperase**
Banana-peels	9024 ± 0.051	16992 ± 0.005	15456 ± 0.001	0	9408 ± 0.004	1600 ± 0.0003
Pomegranate-peels	12480 ± 0.005	27840 ± 0.010	0	3744 ± 0.001	6656 ± 0.005	0
Sugarcane bagasse	42592 ± 0.004	11904 ± 0.010	12256 ± 0.002	0	2432 ± 0.007	1216 ± 0.0008
Grapefruit-peels	13056 ± 0.005	23616 ± 0.015	3168 ± 0.003	1984 ± 0.003	6336 ± 0.008	0
H. mucronatum- shoots	46880 ± 0.003	5952 ± 0.010	6745.6 ± 0.001	0	0	672 ± 0.0004
D. bipinnata-shoots	29542.4 ± 0.006	13056 ± 0.010	3078.4 ± 0.002	1241.6 ± 0.002	1417.6 ± 0.005	2176 ± 0.0002
E. camaldulensis-leaves	4032 ± 0.005	32323.2 ± 0.001	11104 ± 0.02	5408 ± 0.029	5536 ± 0.006	704 ± 0.0001
Substrates	***A. niger* MS34**					
	Xylanase	**Pectinase**	**Amylase**	**Endoglucanase**	**β-glucosidase**	**Filter-paperase**
Banana-peels	13344 ± 0.051	15840 ± 0.02	4384 ± 0.002	9760 ± 0.006	3360 ± 0.008	3488 ± 0.0007
Pomegranate-peels	9024 ± 0.004	16128 ± 0.005	8480 ± 0.001	6144 ± 0.002	6336 ± 0.008	1088 ± 0.0002
Sugarcane bagasse	117888 ± 0.006	9024 ± 0.005	10656 ± 0.003	15808 ± 0.009	8032 ± 0.005	2656 ± 0.0008
Grapefruit-peels	43840 ± 0.075	24480 ± 0.015	12768 ± 0.001	10656 ± 0.006	5728 ± 0.01	2912 ± 0.0004
H. mucronatum- shoots	39168 ± 0.003	982.4 ± 0.010	1440 ± 0.003	2604.8 ± 0.002	0	1408 ± 0.0004
D. bipinnata-shoots	153408 ± 0.004	6720 ± 0.015	1417.6 ± 0.003	934.4 ± 0.002	934.4 ± 0.005	4128 ± 0.0006
E. camaldulensis-leaves	11904 ± 0.017	55584 ± 0.015	18112 ± 0.006	13139.2 ± 0.002	10560 ± 0.004	1728 ± 0.0009

Conclusion

The current research was conducted with an aim to search cheaper natural sources as substrate for plant-hydrolyzing enzymes. The data indicates that the halophytic plants including *Halopyrum mucronatum* and *Desmostachya bipinnata* have promising potential as substrate for xylanase production. Nevertheless, the xylanase production using these substrates needs to be optimized so that it could be exploited at the industrial level. Moreover, other halophytic plants should also be screened to explore their potential as substrate for enzyme production.

References

Abdel-Halim, E.S. 2014. Chemical modification of cellulose extracted from sugarcane bagasse: Preparation of hydroxyethyl cellulose. *Arab. J. Chem.,* 7: 362-371.

Abideen, Z., R. Ansari and M.A. Khan. 2011. Halophytes: Potential source of ligno-cellulosic biomass for ethanol production. *Biomass Bioenergy,* 35: 1818-1822.

Abideen, Z., R. Ansari, B. Gul and M.A. Khan. 2012. The place of halophytes in Pakistan's biofuel industry. *Biofuels,* 3: 211-220.

Aro, N., T. Pakula and M. Penttila. 2005. Transcriptional regulation of plant cell wall degradation by filamentous fungi. *FEMS Microbiol. Rev.,* 29: 719-739.

Aziz, I., S. Gulzar, M. Noor and M.A. Khan. 2005. Seasonal variation in water relations of *Halopyrum mucronatum* (L.) Stapf. growing near sandspit, Karachi. *Pak. J. Bot.,* 37: 141-148.

de Vries, R.P and J. Visser. 2001. *Aspergillus* Enzymes involved in degradation of plant cell wall polysaccharides. *Microbiol. Mol. Biol. Rev.,* 65: 497-522.

Dean, R.A. and W.E. Timberlake. 1989. Production of Cell wall-degrading enzymes by *Aspergillus nidulans*: A model system for fungal pathogenesis of plants. *Plant Cell,* 1: 265-273.

Fang, T.J., B-C. Liao and S-C. Lee. 2010. Enhanced production of xylanase by *Aspergillus carneus* M34 in solid-state fermentation with agricultural waste using statistical approach. *New Biotechnol.,* 27: 25-32.

Guilherme, A.A., P.V.F. Dantas, E.S. Santos, F.A.N. Fernandes and G.R. Macedo. 2015. Evaluation of composition, characterization and enzymatic hydrolysis of pretreated sugarcane bagasse. *Braz. J. Chem. Eng.,* 32: 22-33.

Have, A.T., K.B. Tenberge, J.A.E. Benen, P. Tudzynski, J. Visser and J.A.L. Van Kan. 2002. The contribution of cell wall degrading enzymes to pathogenesis of fungal plant pathogens. In: (Ed.): Kempken, F. *The Mycota*. Springer Verlag Berlin Heidelberg, Germany. pp. 341-360.

Jun, H., T. Kieselbach andABID L.J. Jönsson. 2011. Enzyme production by filamentous fungi: analysis of the secretome of *Trichoderma reesei* grown on unconventional carbon source. *Microbial Cell Factories,* 10: 68.

Mark, R.W., W.W. Widmer, K. Grohmann and R.G. Cmeron. 2007. Hydrolysis of grapefruit peels waste with cellulose and pectinase enzymes. *Bioresource Technol.,* 98: 1596-1601.

Miller, G.L., 1959. Use of dinitrosalicyclic acid reagent for determination of reducing sugar. *Anal. Chem.,* 31: 426-428.

Norazlina I., N. Meenalosani and Halim K.H.K. 2013. Production of xylanase by *Trichoderma* sp. *via* solid state culture using sugarcane bagasse. *Int. J. Energ. Sci.,* 3: 99-105.

Phiriyawirut, M. and P. Maniaw. 2012. Cellulose Microfibril from Banana Peels as a Nanoreinforcing Fillers for Zein Films. *Open J. Polymer Chem.,* 2: 56-62.

Qasim, M., Z. Abideen, M.Y. Adnan, R. Ansari, B. Gul and M.A. Khan. 2014. Traditional ethno-botanical uses of medicinal plants from coastal areas of Pakistan. *J. Coastal Life Med.,* 2: 22-30.

Rezende, M.I., A.M. Barbosa, A.F.D. VascoFAnelos and A.S. Endo. 2002. Xylanase production by *Trichoderma harzianum* Rifai by solid state fermentation on sugarcane bagasse. *Braz. J. Microbiol.,* 33: 67-72.

Robl, D., P.S. Delabona, P.S. Costa, D.J.S. Lima, S.C. Rabelo, I.C. Pimentel, F. Büchli, F.M. Squina, G. Padilla and J.G.C. Pradella. 2015. Xylanase production by endophytic *Aspergillus niger* using pentose-rich hydrothermal liquor from sugarcane bagasse. *Biocatalysis Biotransformation,* 33: 175-187.

Roy, S., T. Dutta, T.S. Sarkar and S. Ghosh. 2013. Novel xylanases from *Simplicillium obclavatum* MTCC 9604: comparative analysis of production, purification and characterization of enzyme from submerged and solid state fermentation. *Springer Plus,* 2: 382.

Sanchez-Vazquez, S.A., H.C. Hailes and J.R. Evans. 2013. Hydrophobic Polymers from Food Waste: Tesources and Synthesis. *Polymer Rev.,* 53: 627-694.

Sathiyavathi, M and R. Parvatham. 2013. Industrial Application of xylanase in the crude enzyme extract from *Trichoderma* sp. MS 2010. *Asian J. Pharm. Clin. Res.,* 6: 90-96.

Seyis, I. and N. Aksoz. 2005. Xylanase Production from *Trichoderma harzianum* 1073 D3 with Alternative Carbon and Nitrogen Sources. *Food Technol. Biotechnol.,* 43: 37-40.

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