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DISTRIBUTION OF HYDROLYTIC ENZYMES AMONG NATIVE FUNGI: *ASPERGILLUS* THE PRE-DOMINANT GENUS OF HYDROLASE PRODUCER

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Abstract

One hundred and twenty eight fungi isolated from the native environments, including soil, plant, deteriorating distempered-wall and spoiled orange-juice samples and 32 isolates obtained from the culture collections were screened for hydrolytic enzyme production. A large proportion (71.25%) of fungal strains belonged to the genus *Aspergillus* followed by genera *Alternaria, Fusarium, Penicillium, Paecilomyces, Basidiomyces, Cladosporium, Curvularia*, *Rhizocotonia, Trichoderma, Macrophomina, Botryodiplodia, Monilia, Gliocladium* and *Rhizopus*. These strains were sorted on the basis of their hydrolytic potential of starch, cellulose, protein, xylan and pectin. Some selected strains that produce high levels of enzymes, grown in shake-flask and amylase, cellulase, protease, xylanase and pectinase were quantitatively evaluated. Members of the genus *Aspergillus* appeared to exhibit the greatest diversity in terms of high levels of all the investigated hydrolases.

Introduction

Many microbial enzymes are commercially exploited and successfully used on industrial scale to catalyze several chemical processes. These enzymes proved to be better, cheaper and environment friendly compared to the use of chemicals. Recently, enzymes have also been exploited in bioremediation of complex waste substances (Whiteley & Lee, 2006). Therefore, enzyme production now became a multi-billion dollar business (Bhat, 2000).

Plant biomass is one of those natural complex materials, containing lignin, cellulose and hemicelluloses, found in abundance and regarded as promising chemical feedstock (Kang *et al.,* 2004). Microbial enzymes, involved in the degradation and transformation of plant cell-wall polysaccharides, have found many biotechnological applications (De Vries & Visser, 2001). Ligninases, hemicellulases, cellulases, pectinases and amylases are the enzymes which are required to degrade not only the plant biomass to its completion but also have found applications in different industries and pharmaceutical preparations (Rao *et al.,* 1998).

Lignin is acted upon by lignin peroxidase, an enzyme that is not widely distributed among microorganisms. Hemicellulose (xylan and its derivatives), a heterogeneous group of hexoses, pentoses and some other sugars can be converted into fermentable sugars by xylanases (Gawande & Kamat, 1999). Cellulose is the most abundant biopolymer present on this planet and can be degraded to glucose when different types of cellulases act in synergy (Yang *et al.,* 2003). Pectin, the constituent of middle lamella in some plants

comprises of galacturonic acid and some other compounds that are frequently degraded by microbial pectinases (Fawole & Odunfa, 1992). Starch is a reserve source of glucose in plants and readily hydrolysed by amylases produced by almost all living organisms. However, microbial amylases are of great industrial importance (Aiyer, 2005). Beside these carbohydrolases, proteases are another important group of industrial enzymes that is widely used in detergent, baking and some other industries.

Fungi are ecologically involved in the degradation of a variety of complex materials, a property that is attributed to a battery of enzymes produced by these microorganisms. Fungal enzymes have been used in enzyme-technology industries for decades (Dalboge, 1997) and hence there is an ever-increasing demand for the isolation and screening of new fungal isolates. Though recombinant DNA technology has provided some new methods to obtain and develop microorganisms of industrial importance nonetheless, classical techniques are still in use (Kuznetsova *et al.,* 2005). These enzymes can degrade *in situ* plant cell-mass completely, however, the ecological interactions among these microbial agents and organic matter disintegration are poorly understood. Although, many consortia of microorganisms have been developed in laboratories for the degradation of complex plant materials, still there is and will remain a demand for new microorganisms which can secrete large amount of hydrolytic enzymes to decompose plant biomass. Keeping in view the importance of fungal enzymes, present study was initiated to isolate and screen the indigenous fungal strains and to explore their hydrolytic potential for their possible future applications.

Materials and Methods

Fungal isolates: Fungi were isolated from soil by pour-plate technique, either directly or after enriching it with different substrates like plant materials, cotton and filter paper. Some of the fungi were also isolated from infected wheat-plant and spoiled orange-juice samples. Other locally isolated strains were obtained from the culture collections available in the department of Microbiology and in the department of Botany, University of Karachi. All strains were maintained on Sabouraud's dextrose agar (SDA) slants. Fungi were identified on the basis of routine cultural and morphological characterization (Barnett & Hunter, 1998).

Screening for enzymes: Fungal strains were screened for amylase, endoglucanase, β-glucosidase, xylanase, pectinase and protease as follows:

Amylase: Cultures were spot inoculated on mineral salt medium (Mandel & Weber, 1969) that contained (w/v) 0.2% KH_2PO_4, 0.14% $(NH_4)_2SO_4$, 0.03% Urea, 0.03% $MgSO_4.7H_2O$, 0.01% $CaCl_2$, 0.1% Peptone, 0.0005% $FeSO_4.7H_2O$, 0.0016% $MnSO_4.H_2O$, 0.0029% $CoCl_2.6H_20$ and 0.0014% $ZnSO_4.7H_2O$, 1.5% agar-agar supplemented with 1% starch as a sole source of carbon. Clear zones of hydrolysis around the colonies after 5-days of incubation at 30^{o}C were noted either visually or after staining with Iodine vapour.

Pectinase: Pectinase activity was detected by growing fungi in a Petri plate on mineral salt medium containing 1 % (w/v) citrus pectin as sole carbon source. After incubation at

30°C for 5 days, stained with ruthenium-red and purple halos around the colonies were noted.

Xylanase: Birchwood xylan (0.5% w/v) was used as sole carbon source in mineral salt medium. Halos around fungal colonies after 5 days at 30°C indicated the xylanase production.

Protease: Protease activity was detected in the same manner as mentioned for xylanase activity with a difference that xylan was replaced with 0.5% (w/v) casein in mineral salt medium, a clear halo around the colonies was regarded as protease activity.

Enzyme assays: Cell-free culture filtrate was used as crude enzyme preparation to assay pectinase, xylanase, endoglucanase and β-glucosidase activities. Crude enzyme preparation (500 µL) was incubated in the presence of 500 µL (1% w/v) substrate; citrus pectin for pectinase, birhcwood xylan for xylanase, carboxymethyl cellulose for endoglucanase and salicin for β-glucosidase, in 50 mM Sodium citrate buffer, pH 4.8, at 50°C for 30 minutes. Amount of reducing-sugar was determined by DNS method after Miller (1969) against standard curves of glucose, xylose and galacturonic acid. One International Unit (IU) of enzyme was defined as the amount of enzyme that releases one micromole of reducing-sugar per minute under standard assay conditions.

Protease activity was measured according to Marco & Carlos (2002). One unit of protease activity was defined as amount of enzyme that causes an increase of 1.0 unit OD_{280} within 20 min.

Results and Discussion

Fungal isolates: Fungi constitute a group of microorganisms that are widely distributed in environment especially in soil (Boer *et al.*, 2005). Since they produce wide variety of hydrolytic enzymes and hence exist in nature in saprophytic mode (Ng, 2004). The action of enzymes is so important, for instance, wood-rotting fungi are classified on the basis of enzymatic deterioration of wood; white-rot fungi can degrade lignin and brown-rot fungi can degrade cellulose (Tortella *et al.*, 2005). Fungal hydrolytic enzymes share 40% of global enzyme market and have many industrial applications (Archer & Peberdy, 1997). Therefore, there is a need for screening these enzymes for improved characteristics. Keeping in view this objective, a total of 160 local fungal isolates either were isolated from environmental sources or obtained from departmental culture collections, were subjected to screening for the ability to elaborated extracellular hydrolases. The results indicate that a majority (79) of the strains were isolated from soil samples either directly or after enriching the soil with different substances including leaves, cotton and stem-bark, 2 fungal strains from deteriorating distempered-wall, 17 fungal strains from spoiled orange-juice samples and 2 strains were isolated from an infected wheat plant and 32 strains from departmental collections. The fungal strains and their genera/ species wise distribution are summarized in Table 1. Earlier, Duarte & Costa-Ferreira (1994) reported that *Aspergillus* is the most abundant genus of soil fungi, which supports the observation that we made during the present study.

Table 1. Fungal isolates and their sources.

Culture	Source						Total
	Soil	Spoiled orange juice	Enriched soil	Infected wheat plant	Deteriorating distempered wall	Culture collections	
Aspergillus niger	42	14	13	1	0	9	**79**
A. fumigatus	2	0	0	0	0	0	**2**
Aspergillus sp.	3	0	1	0	1	0	**5**
A. wentii	2	1	1	0	0	0	**4**
A. flavus	9	2	5	0	1	1	**18**
A. tereus	3	0	1	0	0	0	**4**
A. nidulans	1	0	1	0	0	0	**2**
Fusarium sp.	4	0	0	0	0	0	**4**
F. moniliforme	0	0	0	0	0	2	**2**
Curvularia sp.	2	0	0	0	0	2	**4**
Alternaria sp.	7	0	1	0	0	2	**10**
Penicillium sp.	2	0	0	1	0	2	**5**
Paecilomyces varioti	1	0	1	0	0	1	**3**
Basidiomycete sp.	1	0	0	0	0	0	**1**
Cunnighamella sp.	0	0	1	0	0	0	**1**
Rhizopus sp.	0	0	3	0	0	0	**3**
Cladosporium sp.	0	0	0	0	0	2	**2**
Trichoderma harzianum	0	0	0	0	0	1	**1**
T. longibrachiatum	0	0	0	0	0	1	**1**
Trichoderma sp.	0	0	0	0	0	3	**3**
Rhizoctonia solani	0	0	0	0	0	2	**2**
Monilia sp.	0	0	0	0	0	1	**1**
Botryodoplodia theobromae	0	0	0	0	0	1	**1**
Gliocladium virens	0	0	0	0	0	1	**1**
Macrophomina phaseolina	0	0	0	0	0	1	**1**
Total	**79**	**17**	**28**	**2**	**2**	**32**	**160**

Hydrolytic enzyme diversity: The present study was carried out on hydrolytic enzymes, which find their application in various fields from process industries to diagnostic laboratories. For instances cellulases are used in the production of biofuels and various other biotechnological processes (Bhat, 2000), pectinases in juice industry (Favela-Torres *et al.*, 2006), proteases in detergent industry (Rao *et al.*, 1998), amylases in starch

processing (Aiyer, 2005), xylanases in paper and pulp industry (Christov *et al.*, 1999). It was noted that a large number of isolates produced one or more hydrolytic enzymes investigated in this study; only three strains (1 strain of *Cladosporium* and 2 strains of *Fusarium moniliforme*) were non-producers of any of the hydrolytic enzymes studied. Eleven isolates produced all the investigated enzymes; among these enzyme-producing strains 9 were from soil (Table 2), one from spoiled orange-juice and one strain was obtained from departmental culture collection. Nine of these hydrolase-producers were identified as *Aspergillus niger*, one as *A. flavus* and one *Alternaria* sp. Production of as many as 6 different industrially important hydrolases by *Penicillium* and *Trichoderma* species has also been reported earlier (Berlin *et al.,* 2005). Fungal strains isolated from infected plant and deteriorating distempered-wall produced high levels of β-glucosidase, which can be used to derepress endoglucanase production by the other strains (Lynd *et al.,* 2002).

Seven isolates (*A. niger* MS78 and MS81, *Aspergillus* sp. MS20, *Penicillium* sp. MS66, *Trichoderma* sp. MS99, *T. harzianum* MS134 and *Botryodiplodia* MS100) produced only one of the studied hydrolytic enzymes and may have advantage in biotechnological applications because of ease in down-stream processing (Dalboge, 1997).

The present study on the diversity of hydrolases reveals that protease is the most widely distributed enzyme in fungi followed by xylanases and amylase. A large proportion (81.3%) of isolated fungal strains were proteolytic in nature. Further screening revealed that an appreciable number of isolates were capable to produce both, neutral and alkaline proteases (data not shown). Highly active proteolytic strains (produced up to 0.34 U/ml of protease) belonged to *A. niger, A. flavus, Alternaria* sp., *Curvularia* sp., and *Trichoderma* sp. (Table 3). Earlier reports also substantiates that the soil-borne strain of *A. fumigatus* (Wang *et al.,* 2005), *A. terreus* (Wu *et al.,* 2006), *A. oryzae* (Gomi *et al.,* 1993), and *Mucor* (Alves *et al.,* 2002) produce high levels of proteases.

Microbial xylanases have gained importance as they are used in paper and pulp industry for more than three decades (Beg *et al.,* 2001). These enzymes are reportedly produced by almost all groups of microorganisms including bacteria, actinomycetes, protozoa, algae, fungi and yeasts (Sunna & Antranikian, 1997). In the present case, only 30 fungal strains were high xylanase producers. When some over-producing species of *Aspergillus* (as determined by plate screening method) were grown in mineral salt medium containing 1% (w/v) birchwood xylan as a sole carbon source and assayed (see materials and methods), only *Aspergillus niger* MS80 and *Aspergillus flavus* MS172 were found to secrete high levels of xylanases (Table 4). Polizeli *et al*., (2005) reported that a number of species of *Aspergillus* possess xylanolytic potential.

Pectinases are important plant middle-lamella degrading enzymes as they are produced in large amounts and can alone macerate the plant tissues (Annis & Goodwin, 1997). During this study >50% isolates were found pectinolytic, among them 10 strains were over-producers. *Aspergillus* species are considered as the main pectinases producers (Godfrey, 1996). The present study on some selected over-producing strains of pectinases revealed that *Aspergillus flavus* MS50, *Penicillium* sp. MS119, *Aspergillus niger* MS41 and *Aspergillus niger* MS01 were over-producers (Table 5).

Ninety-seven strains were amylolytic and 11 were the good-producers as determined on the basis of size of halo around the colonies in plates containing media supplemented with 1% (w/v) starch, as sole carbon source. Many of the amylolytic strains belonged to the genus *Aspergillus,* as they are dominant amylolytic genera in nature (Norouzian *et al.,* 2006; Sohail *et al*., 2005).

Table 2. Hydrolytic potential of fungal isolates.

Source	Isolates	Xylanase	Protease	Pectinase	Amylase	Endoglucanase	β-glucosidase
Soil	*A. flavus* MS121	-	+	-	+	++	+++
	A. flavus MS122	-	+++	++	++	++	+
	A. flavus MS123	-	+++	-	+++	++	+
	A. flavus MS32	-	+	-	+	+++	+
	A. flavus MS64	+	++	-	++	-	-
	A. flavus MS67	+	-	-	+	-	+
	A. flavus MS71	+	++	+++	+	+	++
	A. flavus MS72	-	+	-	++	+	-
	A. flavus MS79	+	++	++	-	+	+
	A. fumigatus MS4	-	+++	-	+	+	+
	*A. fumigatus*MS16	+++	++	-	+++	+++	+++
	A. nidulans MS41	-	++	+++	-	-	-
	A. niger MS11	-	+	++	-	+	-
	A. niger MS12	++	+++	-	+	+	++
	A. niger MS124	-	+++	++	++	++	+++
	A. niger MS125	+	+++	-	++	+	+
	A. niger MS13	+	+++	-	+	-	+
	A. niger MS15	+	+++	+++	-	-	+
	A. niger MS177	++	-	-	+	+	+
	A. niger MS18	++	+++	++	++	-	-
	A. niger MS19	+	+	-	-	+++	+++
	A. niger MS21	-	+++	++	+	-	+
	A. niger MS22	-	++	++	++	-	+
	A. niger MS23	++	+++	++	+	+	++

Table 2. (Cont'd.).

Source	Isolates	Xylanase	Protease	Pectinase	Amylase	Endoglucanase	β-glucosidase
	A. niger MS24	+	+	+	++	+	-
	A. niger MS26	-	+++	+	++	-	+
	A. niger MS3	++	+++	+	-	+	+
	A. niger MS30	++	++	++	+	-	-
	A. niger MS31	++	++	+	++	+	-
	A. niger MS34	+++	+	++	-	-	-
	A. niger MS39	+	+	++	+++	-	-
	A. niger MS42	-	++	-	+++	-	-
	A. niger MS43	+	+	++	+	+	+
	A. niger MS45	+	++	+	++	-	-
	A. niger MS49	+	+++	+	++	+	++
	A. niger MS50	++	++	+++	-	-	-
	A. niger MS51	+	+	++	+	+++	++
	A. niger MS54	+	+++	-	++	-	-
	A. niger MS57	++	+++	-	+	+	+
	A. niger MS59	+	+++	++	+++	-	-
	A. niger MS6	++	++	++	-	+	-
	A. niger MS62	+	++++	++	+	+	+++
	A. niger MS65	+	+++	-	-	-	-
	A. niger MS69	-	+++	-	+	+	+
	A. niger MS7	+	++	+	+	+	-
	A. niger MS73	-	+	++	-	+	-
	A. niger MS75	-	+	-	+	+	-
	A. niger MS77	+	++	-	++	+	+

Table 2. (Cont'd.).

Source	Isolates	Xylanase	Protease	Pectinase	Amylase	Endoglucanase	β-glucosidase
	A. niger MS78	-	-	-	-	+	-
	A. niger MS80	+++	++	+	+	++	+++
	A. niger MS83	+	+	-	+	+	+
	A. niger MS88	-	+++	++	+	+	+
	A. niger MS9	-	+++	++	+	+	+
	A. tereus MS17	++	+++	-	+	+	+
	A. tereus MS53	-	+	+	+	-	-
	A. tereus MS60	-	-	-	-	+	-
	A. wentii MS38	+	++	-	-	-	-
	A. wentii MS8	+	+++	-	++	+	-
	A. niger MS1	+	+++	+++	+	-	+
	Alternaria sp. MS28	+	++	-	-	-	+
	Alternaria sp. MS33	++	++	+++	-	-	-
	Alternaria sp. MS37	+	+	++	++	-	+
	Alternaria sp. MS44	-	-	++	-	++	++
	Alternaria sp. MS48	+	+	++	-	++	-
	Alternaria sp. MS55	-	+++	-	-	-	+
	Alternaria sp. MS56	++	+	-	-	+	+
	Aspergillus sp. MS20	-	-	-	-	-	+
	Aspergillus sp. MS5	-	+++	++	++	+	+
	Aspergillus sp. MS176	-	+	-	-	+	-
	Basidiomycete sp. MS120	+	+	++	-	-	+
	Curvularia sp. MS10	-	+++	++	-	-	-
	Curvularia sp. MS27	+	++	-	-	-	-

Table 2. (Cont'd.).

Source	Isolates	Xylanase	Protease	Pectinase	Amylase	Endoglucanase	β-glucosidase
	Fusarium sp. MS14	+	+++	+	++	-	+
	Fusarium sp. MS25	+	++	+++	+	-	+
	Fusarium sp. MS29	+	++	-	-	-	-
	Fusarium sp. MS47	+	+++	+	+	-	-
	Paecilomyces varioti MS109	-	++	-	-	+++	+
	Penicillium sp. MS35	-	++	-	+	-	-
	Penicillium sp. MS58	-	+	+	-	-	++
Soil enriched with stem bark	*A. flavus* MS145	-	+	+	-	+	+
	A. nidulans MS114	++	+	-	+	+	+
	A. niger MS115	-	++	-	+	-	+
	A. niger MS117	+	+++	+	+	+	++
	A. niger MS146	-	++	+	-	+	+
	A. niger MS92	++	++++	++	-	-	+
	Alternaria sp. MS91	+	+	-	-	+	+
	Cunninghamella sp. MS116	+	++	-	-	+	+
	Rhizopus sp. MS144	+	+++	+	-	-	+
Soil enriched with leaves	*A. flavus* MS108	-	++	++	-	+	+
	A. niger MS110	-	++	++	+	+	+
	A. niger MS112	-	+++	-	-	+	+
	A. niger MS113	+	+	-	+	+	+
	Paecilomyces varioti MS111	-	+	+	-	+	+
Soil enriched with leaves & stem bark	*A. niger* MS150	++	++	-	+	+	+
	A. spp. MS149	-	++	-	-	+	+
	Rhizopus MS148	+	-	-	+	-	++

Table 2. (Cont'd.).

Source	Isolates	Xylanase	Protease	Pectinase	Amylase	Endoglucanase	β-glucosidase
Soil enriched with stem-bark & cotton	*A. niger* MS153	-	++	++	+++	+	+
Soil enriched with cotton	*A. flavus* MS103	-	++	++	+	-	-
	A. niger MS101	+	-	++	+++	++	-
	A. niger MS102	-	-		+	-	+
	A. tereus MS105	+++	+	-	-	+++	+++
Soil enriched with Whatman #1 Filter paper	*A. flavus* MS129	++++	++	-	+	+	+
	A. flavus MS131	-	++	++	+	-	-
	A. niger MS128	++	++	++	+	+	+
	A. niger MS130	++	+++	-	+	-	-
	A. wentii MS126	-	++++	-	-	+	+
	Rhizopus sp. MS127	++	++	+++	+++	-	+
Spoiled orange-juice	*A. flavus* MS167	-	++++	-	-	+	+
	A. flavus MS172	++++	-	-	+	-	-
	A. niger MS156	++	+	-	+	+++	+++
	A. niger MS158	+	++	++	+++	++	-
	A. niger MS159	+	++	++	+	++	-
	A. niger MS161	+	++	-	+++	+	-
	A. niger MS163	+	+++	-	++	++	-
	A. niger MS165	+	++	-	+	++	-
	A. niger MS166	+	++	++	-	++	-
	A. niger MS168	-	-	-	-	+	+
	A. niger MS169	-	+++	++	+++	+	+
	A. niger MS170	+	++	-	+	+	-

Table 2. (Cont'd.).

Source	Isolates	Xylanase	Protease	Pectinase	Amylase	Endoglucanase	β-glucosidase
	A. niger MS171	-	+++	+	++	+	+
	A. niger MS173	++	++	+++	+++	+	-
	A. niger MS174	+	+++	-	+	+	-
	A. niger MS175	+	+++	+	+	+	+
	A. wentii MS164	-	+++	+++	-	+++	+
Infected wheat plant	*A. niger* MS118	+	++	-	-	-	+
	Penicillium sp. MS119	-	++	+++	-	+	+
Deteriorating distempered-wall	*A. flavus* MS142	-	++	-	-	-	++++
	Aspergillus sp. MS143	-	-	-	+	-	+
Culture collection	*A. flavus* MS140	+	+++	-	-	-	-
	A. niger MS141	-	-	-	+	++	+
	A. niger MS2	++	+++	+	++	-	-
	A. niger MS81	-	-	-	-	+	-
	A. niger MS82	++	+	-	-	+++	+++
	A. niger MS86	+	+	-	+	+	++++
	A. niger MS87	++	++	++	++	++	-
	A. niger MS93	+	-	-	-	+	+++
	A. niger MS94	-	+	-	+	-	-
	A. niger MS98	-	+	-	-	+	-
	Alternaria sp. MS136	+	++++	++	+	++	++++
	Alternaria sp. MS85	+	+++	++	+	-	-
	Aspergillu sp. MS68	-	++	+	-	-	-
	Botryodiplodia MS100	-	-	-	-	+	-

Table 2. (Cont'd.).

Source	Isolates	Xylanase	Protease	Pectinase	Amylase	Endoglucanase	β-glucosidase
	Cladiosporium sp.MS61	-	++	-	-	+++	++
	*Cladiosporium*s p. MS52	-	-	-	-	-	-
	Curvularia sp. MS137	+	+++	-	-	+	+++
	Curvularia sp. MS89	-	+++	+	-	-	-
	F. moniliforme MS138	-	-	-	-	-	-
	F. moniliforme MS70				-	-	-
	Gliocladium virens MS132	-	+	++	-	+	+
	Macrophomina sp. MS139	-	++	+	-	+	-
	Monilia sp. MS95	+	+++	++	-	+++	-
	Paecilomyces varioti MS96	+	++	-	-	-	-
	Penicillium sp. MS107	-	+	++	-	-	-
	Penicillium Sp. MS66	-	+	-	-	-	-
	Rhizoctonia solani MS76	-	+++	++	-	++	-
	T. harzianum MS134	-	+	-	-	-	-
	T. longibrachiatum MS135	-	+	++	-	+	-
	Trichoderma sp. MS133	+	++	-	-	-	-
	Trichoderma sp. MS63	+	+++	+++	-	-	-
	Trichoderma sp. MS99	-	++++	-	-	-	-

Key: - = no enzyme activity; for Xylanase, protease, pectinase and amylase, + = zone size <5mm, ++ = 5.1-8mm, +++ = 8.1-10mm, ++++ = >10mm; for endoglucanase and β-glucosidase activity was determined on the basis of enzyme assays, + = 0.05-0.15 IU/ml, ++ = 0.16-0.3 IU/ml, +++ = > 0.3 IU/ml)

Table 3. Protease production by some fungal isolates in shake-flasks containing mineral salt medium supplemented with 1% (w/v) casein.

Organisms	Protease activity (U/ml)
A. niger MS26	0.336
A. niger MS49	0.22
A. flavus MS122	0.34
Alternaria sp. MS55	0.02
Alternaria sp. MS136	0.24
Trichoderma sp. MS99	0.036
Curvularia sp.137	0.312

Table 4. Pectinase production by some fungal isolates in shake-flasks containing mineral salt medium supplemented with 1% (w/v) citrus pectin.

Organisms	Pectinase activity (IU/ml)
A. niger MS01	0.2
A. niger MS15	0.153
A. niger MS41	0.279
A. flavus MS50	0.314
A. wentii MS164	0.048
Fusarium sp. MS25	0.119
Alternaria sp. MS33	0.127
Penicillium sp. MS119	0.241
Rhizopus sp. MS127	0.127
Trichoderma sp. MS63	0.147

Table 5. Xylanase production by some fungal isolates in shake-flasks containing mineral salt medium supplemented with 1% (w/v) birchwood xylan.

Organism	Xylanase activity (IU/ml)
A. flavus MS129	0.756
A. tereus MS105	0.317
A. niger MS80	1.243
A. fumigatus MS16	0.391
A. niger MS34	0.532
A. flavus MS172	1.243

Table 6. Endoglucanase and β-glucosidase production by some fungal isolates in shake-flasks containing mineral salt medium supplemented with 1% (w/v) Carboxymethyl cellulose.

Organism	Endoglucanase activity (IU/ml)	β-Glucosidase activity (IU/ml)
A. fumigatus MS16	0.311	0.389
A. tereus MS105	0.478	1.32
A. niger MS82	1.56	1.543
A. niger MS156	0.74	1.08
A. niger MS19	0.532	0.391

Though many bacteria and few animals also produce cellulases, nevertheless fungi are well known for their cellulytic potential (Jahangeer, 2005; Lynd *et al.,* 2002) and are frequently used in an array of processes (Bayer *et al.,* 2007; Howard *et al.,* 2003). There were 20 fungal strains producing higher levels of either endoglucanase or β-glucosidase; amongst them only 5 strains, all belonged to *Aspergillus*, were high-producers of both the enzymes (Table 6). Studies conducted by Das *et al*., (1997) indicate that the genus *Aspergillus* is the most abundant paper-degrading mycoflora that can produce endoglucanase. Because of its abundance and diversity, cellulase production from this organism is a topic of research in many laboratories around the world. Recently, it was reported that the cellulase production by an indigenous strain of *A. niger* MS82 was dependent on pH and temperature (Sohail *et al.,* 2009). Interestingly, it is imperative to note that almost all the cellulase over-producing Aspergilli were of soil origin; this explains the diversity of degradative processes that undergo in a particular reservoir of plant biomass. In addition to *A. niger*, other over-producing isolates from environments include *A. flavus, A. wenti, Alternaria* sp., *Curvularia* sp., *Monilia* sp., *Paecilomyces varioti, Cladosporium* sp., and *Rhizoctonia solani*.

The data presented here indicate that *Aspergillus* is a highly diverse group of naturally occurring fungi. Members of this genus are well known for their potential to degrade plant biomass and industrial applications since ancient time (Adeniran & Abiose, 2009; De Vries & Visser, 2001). Since aspergilli were also tested as the host of some heterologous proteins (Dalboge, 1997) there is a possibility to subject some of these isolates to genetic manipulation for developing a strain capable of complete degradation of plant-based biomass.

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