IDENTIFICATION AND CHARACTERIZATION OF DIVERSE XYLANASES FROM THERMOPHILIC AND THERMOTOLERANT FUNGI

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Thirteen fungal isolates included in this study expressed multiple xylanase isoforms as observed by xylan zymograms of polyacrylamide gel electrophoresis (PAGE) and isoelectrofocussing (IEF) fractionated proteins. Eighty-three xylanases produced by these thermophilic and thermotolerant strains were detected using the IEF profiling technique. Xylanases identified on the basis of their isoelectric points (pI) were functionally diverse and exhibited differential catalytic activities against various xylan types (birch wood xylan, larch wood xylan, oat spelt xylan, rye arabino xylan and wheat arabino xylan) as well as debranched arabinan. Thermophilic isolates, *Chaetomium thermophilum*, *Humicola insolens*, *Melanocarpus* sp., *Malbranchea* sp. and *Thermoascus aurantiacus*, were found to produce alkaline active xylanases that showed a bleach boosting effect on Decker pulp resulting in increased brightness (1.60-2.04 ISO units).

Keywords: Thermophilic and Thermotolerant fungi, Multiple xylanases, PAGE and IEF fractionated proteins, Specificities and catalytic activities, Alkaline active xylanases.

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INTRODUCTION

Xylan, the major hemicellulosic constituent of hardwood and softwood, is a branched heteropolysaccharide constituting a backbone of β-1,4 linked xylopyranosyl units substituted with arabinosyl, glucuronyl and acetyl residues (Shallom and Shoham 2003). The structure of xylan components from different sources depends upon extraction procedures as well as the frequency, number and type of substitutions (Viikari et al. 1994; Saha 2003). The hydrolysis of the xylan backbone is accomplished by endoxylanases (EC 3.2.1.8) and β-xylosidases (EC 3.2.1.37) along with a variety of debranching enzymes, i.e. α-L-arabinofuranosidases, α-glucuronidases and acetyl esterases (Collins et al. 2005). Many of the xylanase-producing microorganisms express multiple isoforms (Wong et al. 1988) that have been ascribed to a variety of reasons, i.e., heterogeneity and complexity of xylan structure, genetic redundancy and post-translational modifications, etc. (Kormelink and Voragnen 1993; Li et al. 2000).

Thermophilic fungi, a unique group of microorganisms, that thrive at high temperatures are often associated with piles of agricultural and forestry products and other composting materials (Maheshwari et al. 2000). The distribution and colonization of thermophilic fungal population in compost is closely related to their ability to produce a variety of cell wall degrading enzymes (Sharma 1989). Since these fungal strains function in amelioration of xylan substrate present in lignocellulosic waste, each xylanase

produced may be biotechnologically important and show specialized function. There is need to isolate and identify such novel xylanases from diverse indigenous strains. Some of the thermophilic fungi, *Chaetomium thermophile, Humicola insolens* (syn. *Scytalidium thermophilum*), *Thermomyces lanuginosus* and *Thermoascus aurantiacus* have been reported to produce biotechnologically-important, thermostable xylanases. These xylanases are used in a variety of applications, i.e., clarification of juice and wine, starch separation and production of functional food ingredients, improving the quality of bakery products and in animal feed biotechnology (Saha 2003). Xylanases showing transglycosylation activities can also be used for tailoring drugs and in the preparation of neoglycoproteins (Eneyaskaya et al. 2003). Alkaline-active xylanases of thermophilic fungi find application in bleaching of pulp in paper industry obviating the need for chlorine (to some extent) in ecofriendly process (Subramanium and Prema 2002).

Due to their huge potential, xylanases with novel properties must be isolated and identified. This study highlights the identification and characterization of multiple and catalytically diverse xylanases produced by thermophilic and thermotolerant fungi isolated from composting soils, and their application in pulp bleaching.

MATERIALS AND METHODS

Microorganisms and Culture Conditions

Diverse thermophilic and thermotolerant fungi, namely, *Absidia corymbifera* MTCC 4620, *Acrophialophora nainiana Edward* MTCC 6662, *Aspergillus caespitosus* MTCC 6326, *Aspergillus terreus* MTCC 6335, *Chaetomium thermophilum* MTCC 4981, *Chrysosporium lucknowense* MTCC 3921, *Emericella nidulans* var. *lata* MTCC 6327, *Humicola insolens* MTCC 4520, *Humicola fuscoatra* MTCC 6329, *Melanocarpus* sp. MTCC 3922, *Malbranchea* sp. MTCC 4887, *Penicillium lagena* MTCC 6334 and *Thermoascus aurantiacus* MTCC 4890 were isolated from composting soils and grown and maintained on yeast-starch agar (YpSs, pH 7.0) of following composition (% w/v): starch 1.5, yeast extract 0.4, K_2HPO_4 0.23, KH_2PO_4 0.2, $MgSO_4.7H_2O$ 0.05, citric acid 0.057 and agar 2.0 (Cooney and Emerson 1964). The fungi were cultured at 45-50 ^oC for 7-10 days and stored at 4 ^oC. These indigenous fungal strains were identified as described by Cooney and Emerson (1964) and have been deposited with Microbial Type Culture Collection (IMTECH, Chandigarh, India).

Enzyme Production

The cultures were grown in 250 ml Erlenmeyer flasks that contained 50 ml of production medium of the following composition (% w/v): corn cobs 2.0; oat spelt xylan 0.1, yeast extract 1.0, KH_2PO_4 0.3, $CaCl_2$, 0.05, $MgSO_4$ 0.05 and 1% v/v of trace element solution that contained (% w/v): $(NH_4)_2SO_4$ 0.2, KCl 0.5, $CaCl_2$ 0.1, $MgSO_4$ 0.5, $ZnSO_4$ 0.01 and $CuSO_4$ 0.005. The pH of the medium was adjusted to 6.0 prior to sterilization. The flasks were inoculated with 2 agar discs (2 mm in diameter) of 7-10 days old culture from YpSs agar plates and were incubated under shaking conditions (120 rpm) at 45/50 ^oC up to10 days (experiments performed in triplicates). The crude enzymes were filtered and centrifuged (11000 x g) for 10 min. The xylanase activity was estimated in these filtrates.

The xylanase assay of crude filtrate was determined using birch wood xylan as the substrate. The assay mixture which contained 500µl of 1 % birch wood xylan (Sigma, X0502) prepared in 50 mM Na-citrate buffer with pH 6.5 and 500µl suitably diluted enzyme was incubated at 50 ^{o}C for 5 minutes. The reaction was stopped by the addition of 3 ml dinitrosalicylic acid (DNS) reagent and the contents were boiled for 15 minutes. The developed color was read at 540 nm using Novaspec II spectrophotometer (Pharmacia). The amount of reducing sugar liberated was quantified using the xylose standard. Xylanase activity is expressed in terms of units per ml of xylanase solution. A unit of activity is defined as the micromoles of xylose released per minute under assay conditions (Gomez de Segura et al. 1998; Sunna and Berguist 2003).

EGTA-EDTA mix (each @ 1 mM) and PMSF (2 mM) were added to the filtrate 0.02% sodium azide in order to avoid contamination and inhibit protease activity. Further, the clear supernatants were desalted using a PD-10 column (Pharmacia) and lyophilized (HETO, Drywinner 3) and the concentrated samples were used for fractionation on PAGE and IEF.

Electrophoresis and Iso-Electric Focusing

The samples (protein-100µg) were fractionated by native-polyacrylamide gel electrophoresis (PAGE) that was performed in 7.5% gel with 4% stacking gel using the Mini-Protean II system of Biorad by the method of Laemmli (1970). Similarly, isoelectric-focusing (IEF) using 5% acrylamide gel containing 2.4% broad pH range (3.5-10.0) ampholine carrier ampholyte (Amersham Biosciences) was performed (Mini-Protean II system, BIORAD). Ethanolamine (0.4% v/v) and sulphuric acid (0.2% v/v) were used as cathodic and anodic electrolyte solutions, respectively (Bhat and Wood 1989). Isoelectrofocusing was carried out for 1 h each at a constant voltage of 100 V and 200 V followed by 500 V for 30 min.

Activity Staining

Xylanase activity in PAGE and IEF gels was detected by activity staining (Zymogram technique) with 1% agarose replica containing covalently dyed RBB-Xylan prepared from oat spelt xylan (SIGMA). Upon completion of electrophoresis, the gels were incubated in sodium acetate buffer (50 mM, pH 6.0) for 30 min and then overlaid on RBB-Xylan containing gel for 30-60 min at 50 ^{o}C. In order to avoid band diffusion, these gels were dried at 60 ^{o}C.

Quantification of Xylanases

After fractionating the proteins on IEF, the gel in each lane was sliced (1.25 mm thickness). Each slice was incubated in 500 µl sodium citrate buffer (50 mM, pH 6.0) for 72 h at 4 ^{o}C. The eluted protein in each fraction was assayed for xylanase activity against 1% birch wood xylan.

Substrate Specificity and Activity of Xylanase at Alkaline pH

The xylanase active fractions identified against birch wood xylan were also assayed for their catalytic action against different xylan types (oat spelt xylan, larch wood xylan, rye arabino xylan, wheat arabino xylan) and debranched arabinan. In order to

identify alkaline active xylanases, the assay under alkaline conditions using Tris-HCl buffer (50 mM, pH 9.0) was performed against birch wood xylan.

Bleaching of Pulp

Decker pulp (10 % w/w), provided by ABC paper mills (Hoshiarpur, India), was subjected to alkaline extraction (NaOH, 6.3 % w/v) for 30 min. at 50 ^{o}C, then was thoroughly washed with water and dried. A slurry of alkali treated pulp (6 % w/v pulp consistency) prepared in phosphate buffer (0.1 M, pH 7.0) was treated with 10 units xylanase/g (dry weight) at 65 ^{o}C for 120 min under mild shaking (60 rpm). No enzyme was added in the control. A post-alkaline extraction (NaOH, 2 % w/v; 6 % w/v pulp consistency) was performed at 55 ^{o}C under mild shaking (60 rpm).

The enzyme-mediated release of chromophoric material from pulp was measured spectrophotometrically at 237, 254, 280 and 465 nm in the enzyme filtrates after a 2 h incubation period using UV-VIS spectrophotometer (Shimadzu, UV mini 1240). Reducing sugars released from pulp were measured over the same incubation period according to the dinitrosalicylic acid method. The hand-sheets of bleached pulp were prepared according to standard TAPPI method (T 205 om-88) and their brightness was determined with an Elrepho 070 (Lorentzen and Wettre, Sweden).

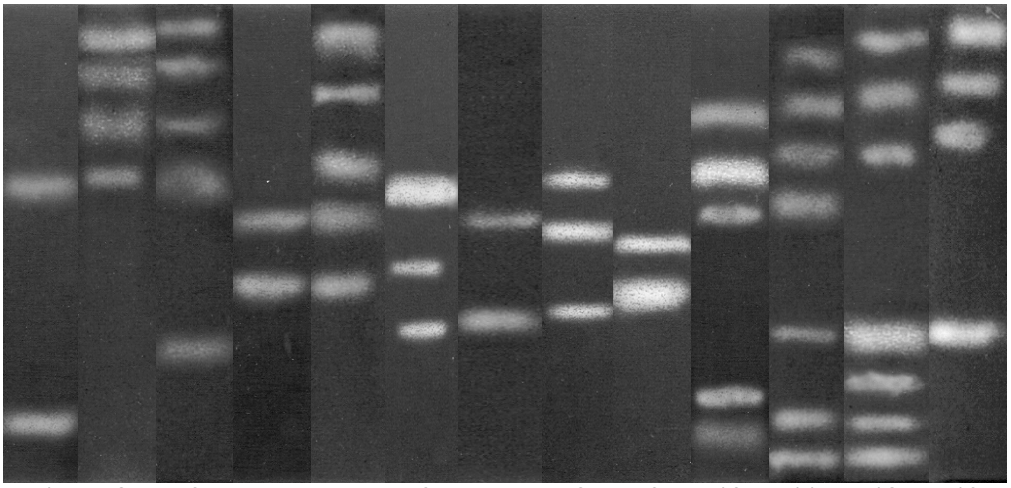
RESULTS AND DISCUSSION

Production and Multiplicity of Xylanases

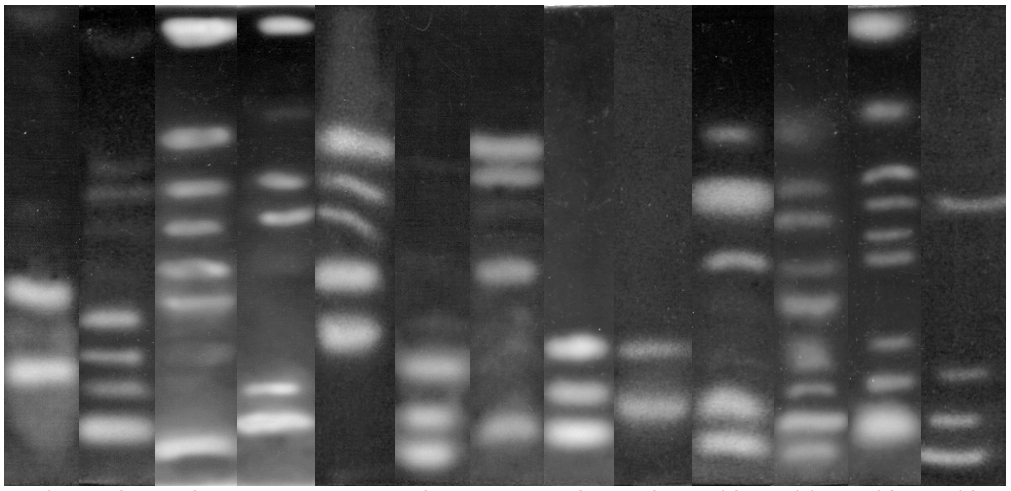
The fungal strains included in this study produced xylanases to a varied extent, where thermophilic fungal strains expressed high levels of xylanases as compared to thermotolerant fungi (Table 1). High xylanase titres produced by thermophilic fungi that are prevalent in composting soils possibly accelerate the process of natural decomposition. *Melanocarpus* sp. produced 264.2 units ml^{-1} of xylanases followed by *Che. thermophilum* and *Malbranchea* sp. (162.2 and 141.7 units ml^{-1}), respectively. Among thermotolerant fungal strains, maximum xylanase production was observed with *A. terreus* (38.0 units ml^{-1}) followed by *P. lagena* (32.4 units ml^{-1}) and *E. nidulans* var. *lata* (31.2 units ml^{-1}).

The analysis of crude enzymes produced by these fungal cultures revealed the presence of multiple xylanases. Even though multiplicity of xylanases is widespread in microorganisms, it has been demonstrated in few fungi including *Trichoderma* sp., *Sclerotium rolfsii* and *Aspergillus* sp. (Wong et al. 1988; Sachslehner et al. 1998; de Vries and Visser 2001), the thermophilic fungi, *Melanocarpus albomyces* IIS 68 and *Myceliophthora s*p. (Saraswat and Bisaria 2000; Badhan et al. 2004), and in an anaerobic fungal strain, *Neocallimastix frontalis* (Gomez de Segura et al. 1998). Studies revealing functionally diverse multiple isoforms of xylanases from thermophilic fungi isolated from the same ecosystem have not been reported earlier. Thirteen fungal cultures isolated (in this study) from composting soils produced 51 diverse xylanases identified based on their electrophoretic mobilities using PAGE zymograms (Fig. 1-A). Both *A. terreus* and *E. nidulans* var. *lata* produced seven xylanase isoforms with very high to low electrophoretic mobilities. Whereas, *Ac. nainiana*, *Che. thermophilum*, *H. fuscoatra* and *T. aurantiacus* produced two xylanases each with distinct banding pattern. Furthermore,

the resolution of filtrates on IEF gels showed 65 xylanases with distinct pI. *E. nidulans* var. *lata*, *H. fuscoatra* and *H. insolens* produced xylanases of highly basic to acidic pI~9.7-3.4 with a high degree of multiplicity (Fig. 1-B). The production of a wide variety of xylanases by thermophilic and thermotolerant fungi isolated from composting soils suggests that these xylanases possibly exhibit overlapping yet dissimilar specificities to achieve superior xylan hydrolysis (Wong et al. 1988). Studies on xylanases from *Myceliophthora* sp. from our laboratory have clearly shown that the multiplicity was not a result of proteolytic post-translational modification (Badhan et al. 2006). Multiple xylanases may be the product of different alleles of the same gene (allozymes) or distinct gene products produced by a fungus to enhance the utilization of xylan (Wong et al. 1988; Uffen 1997).



(A)



(B)

Fig. 1. Zymogram of PAGE gel (A) and IEF gel (B) representing xylanases produced by thermophilic fungal strains: (1) *Che. thermophilum*, (2) *Chy. lucknowense*, (3) *H. insolens*, (4) *H. fuscoatra*, (5) *Melanocarpus* sp., (6) *Malbranchea* sp. (7) *T. aurantiacus*, and thermotolerant fungal strains: (8) *Ab. corymbifera*, (9) *Ac. nainiana*, (10) *A. caespitosus*, (11) *A. terreus*, (12) *E. nidulans* var. *lata* and (13) *P. lagena*.

Table 1. Xylanase Production by Different Thermophilic and Thermotolerant Fungal Cultures Grown on Corn Cobs and Oat Spelt Xylan under Submerged Conditions.

Organisms	***Xylanase Activity (units ml^{-1})***
Thermophilic fungi	
Chaetomium thermophilum	162.2 ± 8.3
Chrysosporium lucknowense	96.6 ± 8.2
Humicola fuscoatra	12.9 ± 1.2
Humicola insolens	17.2 ± 2.5
Malbranchea sp.	141.7 ± 9
Melanocarpus sp.	264.2 ± 11
Thermoascus aurantiacus	18.3 ± 2.5
Thermotolerant fungi	
Absidia corymbifera	16.5 ± 1.5
Acrophialophora nainiana	9.7 ± 1.0
Aspergillus caespitosus	25.7 ± 2.1
Aspergillus terreus	38.0 ± 4.2
Emericella nidulans var. *lata*	31.2 ± 2.3
Penicillium lagena	32.4 ± 4.2

* Culture conditions: 250 ml Erlenmeyer flasks contained 50 ml of production medium; Incubation temperature - 45/50 ^{o}C; Shaking at120 rpm; Incubation time upto10 days.

The differential expression of multiple xylanases by each strain was quantified using IEF profiling by slicing of gels and eluting proteins. By this procedure the major and minor xylanases were identified based on their relative proportions (Fig. 2.A-M). Most of the fungi produced major xylanases with neutral to acidic pI. However, *H. fuscoatra*, *H. insolens* and *E. nidulans* var. *lata* produced major xylanases of highly basic pI ~ 9.7, 9.5 and 9.7 with relative proportion of 29%, 33% and 30%, respectively (Fig. 2.L). Whereas, in *Che. thermophilum*, *Chy. lucknowense* and *Malbranchea* sp., xylanases of acidic pI ~ 4.5, 5.5 and 3.4, and 3.5 with high level of relative proportion i.e., 56%, 31%, 31% and 45% were expressed, respectively (Fig. 2.A, B and E). The observed levels of minor xylanases were 2-10%. Though minor xylanases are not produced in large quantities, they may be responsible for some specialized functions (induction, transglycosylation, etc.) or may work in tandem with the major xylanases to attain superior xylan hydrolysis (Wong et al. 1988; Thomson 1993).

Substrate Specificity of Diverse Xylanases

In order to demarcate the observed xylanases as catalytically distinct and novel, various xylan types were used to analyze their substrate preferences. Most of the xylanases identified highly substituted wheat arabino-xylan as the preferred substrate for hydrolysis (Table 2 A and B, see Appendix). Xylanases from *H. fuscoatra* (HFX-VI), *Ac. nainiana* (ANX-VI) and *E. nidulans* var. *lata* (ENX-IV) showed 9.57, 9.71 and 8.26 fold higher activity, respectively, against wheat arabino xylan as compared to birch wood xylan (the usual substrate to assay xylanase activity). The activities of xylanases against

rye arabino xylan, another highly substituted form of xylan, were comparable to wheat arabino xylan. Xylanases identified from *H. insolens* (HIX-VI), *H. fuscoatra* (HFX-VI), *Ac. nainiana* (ANX-VI) and *E. nidulans* var. *lata* (ENX-IV) were highly active against rye arabino xylan and showed 7.76, 7.51, 8.38 and 6.26 fold higher activity, respectively, as compared to birch wood xylan. However, xylanases from *A. caespitosus* (ACSX-II and ACSX-VII), *A. terreus* (ATX-I and ATX-IX) and *E. nidulans* var. *lata* (ENX-I) showed exceptionally low activity against rye arabino xylan. Few of the xylanases of *H. fuscoatra* (HFX-I), *Ab. corymbifera* (ACX-V and ACX-VI), *A. caespitosus* (ASCX-II) and *E. nidulans* var. *lata* (ENX-I) identified oat spelt xylan as the most preferred substrate.

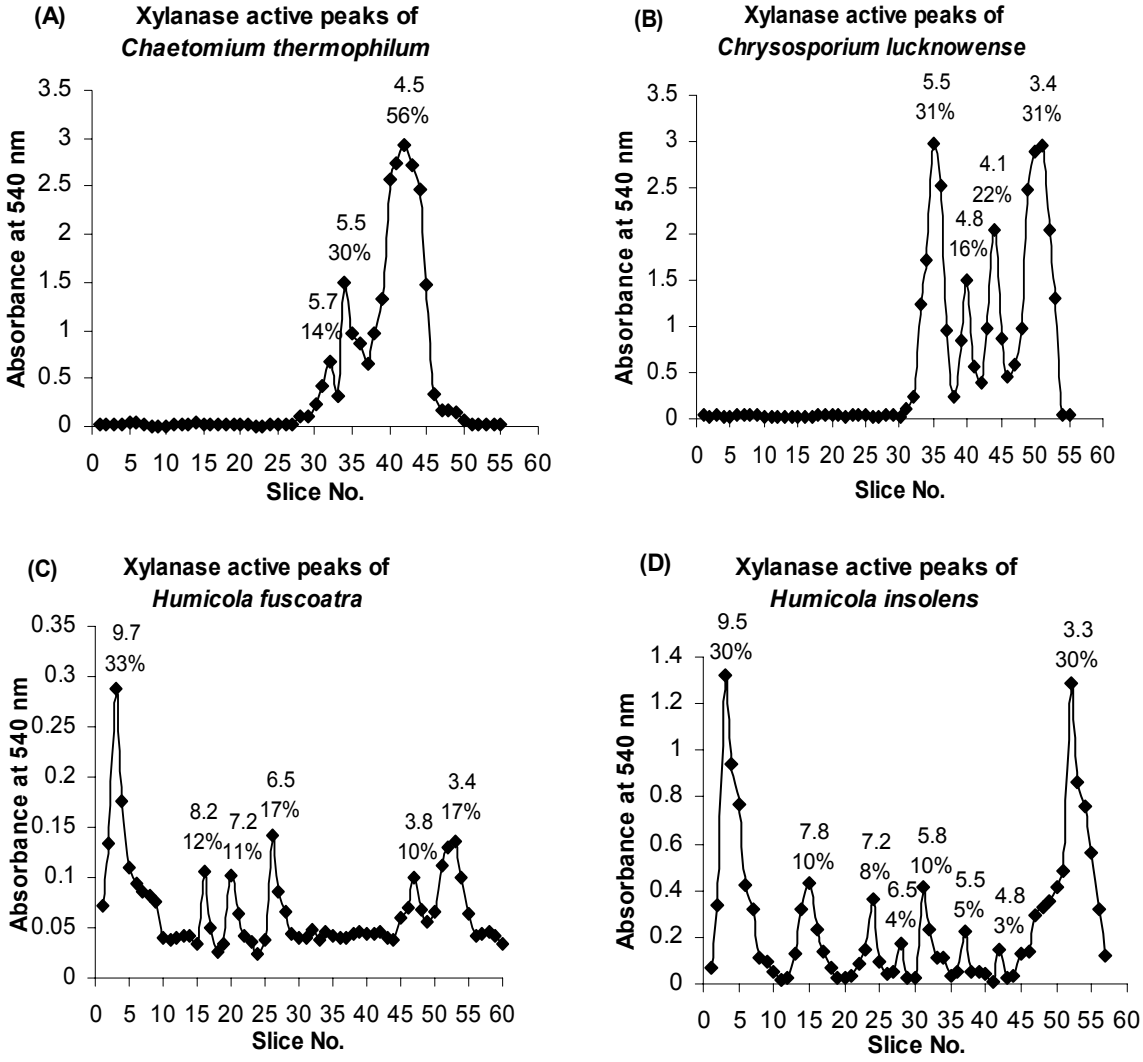
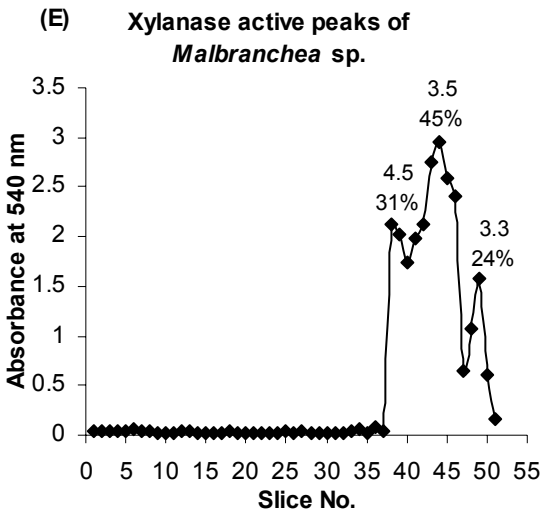
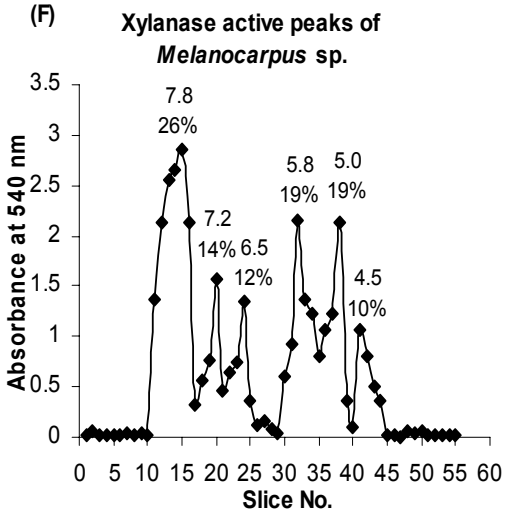


Fig. 2.A-D. Fractionation of thermophilic and thermotolerant fungi showing xylanase isoforms (pI and relative percent activity given at top of the each peak) using isoelectric focusing in polyacrylamide gel.

The activities against larch wood xylan were lower as compared to arabino-xylans, however, xylanases belonging to *H. fuscoatra* (HFX-II), *T. aurantiacus* (TAX-II), *A. terreus* (ATX-II) and *P. lagena* (PLX-I) used it as substrate of choice. The observed

differences in the activity of xylanases on structurally and chemically distinct xylan types (Table 2 A and B) suggest that they require the presence of a particular type of substituent in the vicinity to enhance their catalytic action (Silva et al. 2000). Furthermore, endoxylanases differ in their specificity towards the xylan polymer as some enzymes cut randomly between unsubstituted xylose residues, whereas the activity of other xylanases strongly depends on the substituents (arabinosyl, arabinofuranosyl, acetyl and glucuronyl residues) linked to the xylose residues neighboring the attacked residues (de Vries and Visser 2001). Minor xylanases produced by fungal strains showed appreciably higher activities (1.5-8.2 folds) against arabino-xylans, the main constituents in the straws (rice, oat, wheat, etc.), present in the composting soils and thus may be playing a significant role in its decomposition. Few of the xylanases belonging to *Ac. nainiana*, *A. terreus*, *P. lagena*, *Chy. lucknowense* and *H. fuscoatra* were catalytically versatile and also recognized debranched arabinan as substrate. Xylanases have cellulose binding domains with a few having the affinity to recognize xylan and cellulose (Subramanium and Prema 2002). These domains fold and function in an independent manner and may be responsible for such multi-functionality.





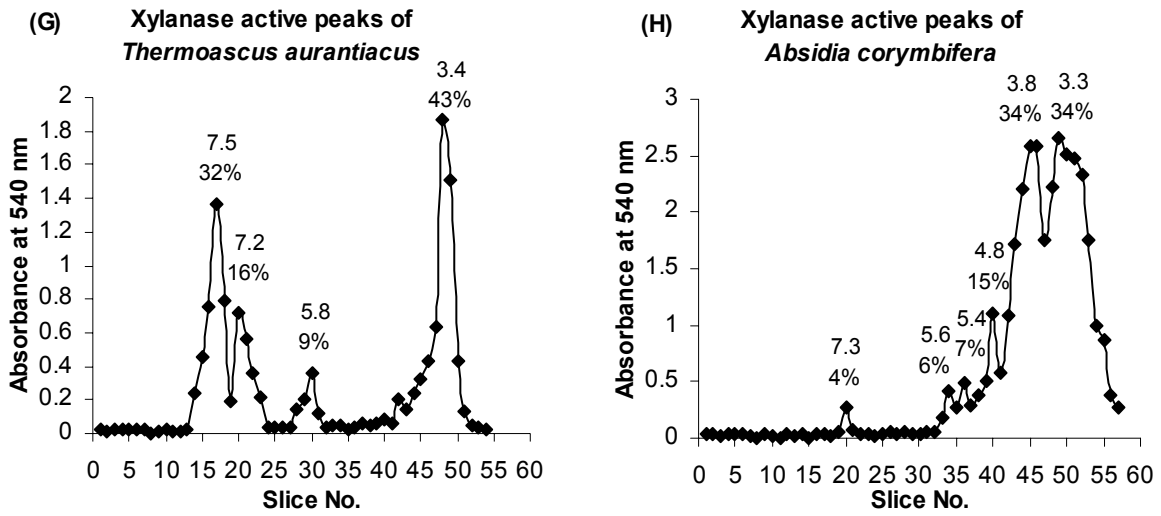
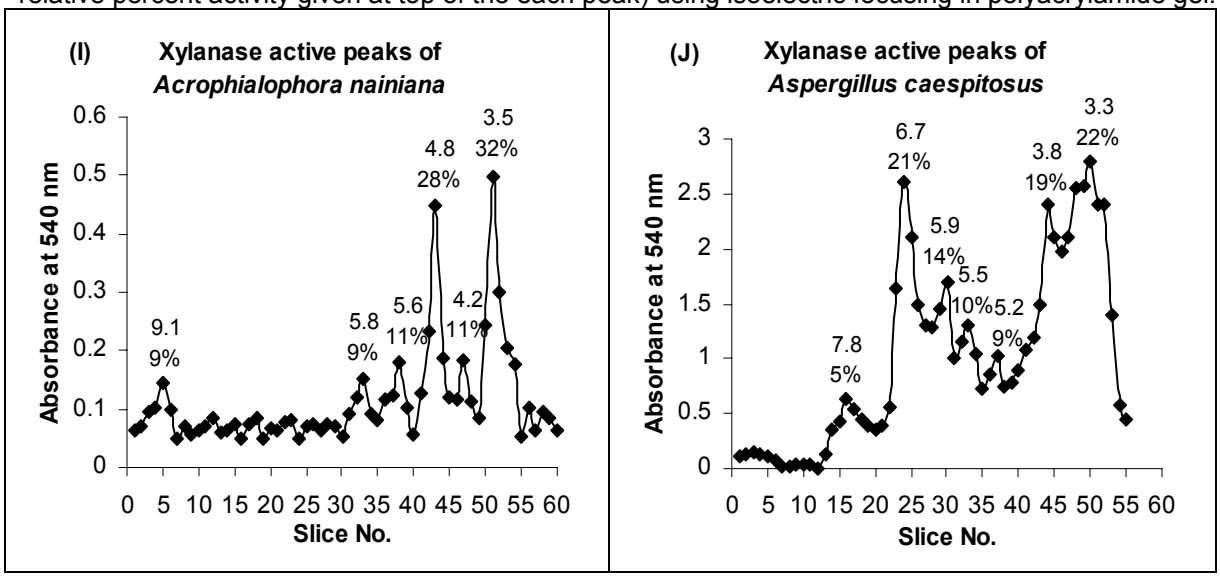


Fig. 2.E-H. Fractionation of thermophilic and thermotolerant fungi showing xylanase isoforms (pI and relative percent activity given at top of the each peak) using isoelectric focusing in polyacrylamide gel.



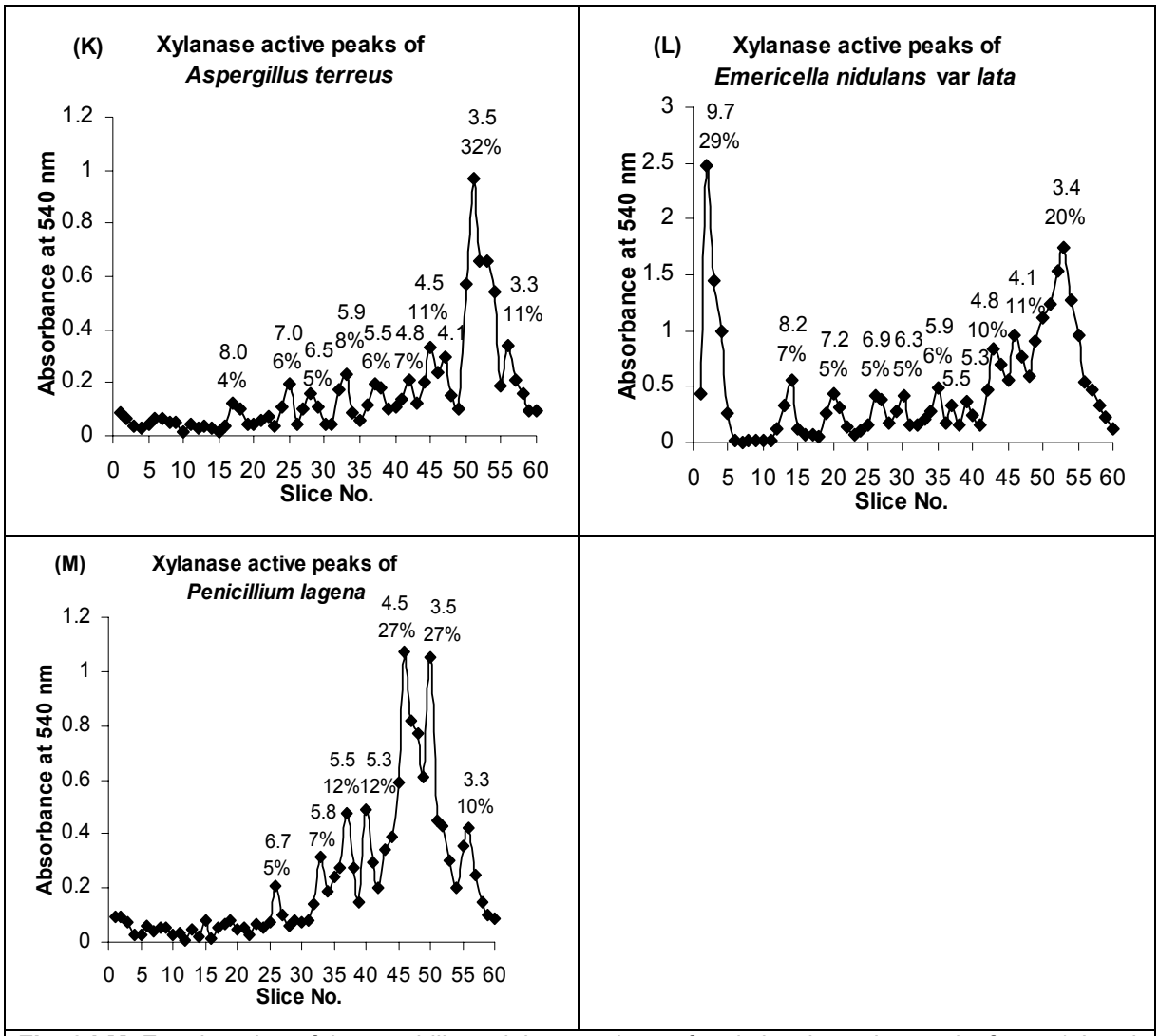


Fig. 2.I-M. Fractionation of thermophilic and thermotolerant fungi showing xylanase isoforms (pI and relative percent activity at top of the each peak) using isoelectric focusing in polyacrylamide gel.

Alkaline Active Xylanases

The alkaline active xylanases were identified on the basis of relatively higher xylanase activity against birch wood xylan at pH 9.0 as compared to at pH 6.0 (Table 2). Interestingly, only the xylanases of thermophilic fungal strains were found to be active under alkaline conditions. All the xylanases of *Che. thermophilum*, but just five of *H. insolens* and two each of *T. aurantiacus* and *Malbranchea* sp. were active under alkaline conditions. Of the six isoforms produced by *Melanocarpus* sp., only one xylanase isoform (MAX-I) was active at alkaline pH. There are evidences to suggest that geographically distinct strains produce xylanases of different physicochemical characters as *Che. cellulolyticum* isolated in Pushchino, Russia, produced three xylanases with pI, 8.9, 8.4 and 5.0 that were active at neutral pH (Baraznenok et al. 1999), while we observed the expression of three alkaline active xylanases of acidic pI, 5.7, 5.5 and 4.5 by *Che. thermophilum*.

Table 3. Effect of Xylanase (Xylanase of Diverse Thermophilic and Thermotolerant Fungi) Pretreatment on the Release of Chromophoric Material and Reducing Sugars from Pulp, and Brightness of Pulp.

Organisms	*Chromophore release*				*Reducing Sugar*	*Brightness*
	237nm	*254nm*	*280nm*	*465 nm*	*(mg g^{-1} Pulp)*	*(% ISO)*
Thermophilic fungi						
Control	0	0	0	0	0	32.40 ± 0.20
Che. thermophilum	0.604	0.497	0.408	0.083	0.379	34.01 ± 0.10
Chy. lucknowense	0.835	0.587	0.543	0.163	0.339	34.03 ± 0.10
H. fuscoatra	0.534	0.510	0.545	0.099	0.171	34.00 ± 0.10
H. insolens	0.546	0.536	0.524	0.173	0.209	34.04 ± 0.10
Malbranchea sp.	1.827	1.701	0.857	0.120	0.399	34.44 ± 0.15
Melanocarpus sp.	0.906	0.197	0.136	0.043	0.389	34.21 ± 0.10
T. aurantiacus	0.745	0.359	0.366	0.093	0.018	34.02 ± 0.10
Thermotolerant fungi						
Ab. corymbifera	0.123	0.095	0.065	0.046	0.098	32.48 ± 0.12
Ac. nainiana	0.211	0.102	0.098	0.035	0.121	33.81 ± 0.10
A. caespitosus	0.809	0.717	0.521	0.110	0.298	34.10 ± 0.10
A. terreus	0.289	0.156	0.110	0.065	0.235	33.07 ± 0.10
E. nidulans var. *lata*	0.221	0.142	0.118	0.061	0.169	33.95 ± 0.10
P. lagena	0.245	0.152	0.121	0.055	0.175	33.78 ± 0.10

Enzymatic Treatment of Pulp

Xylanase produced by thermophilic and thermotolerant fungal strains was used for the bleaching of Decker pulp. Interestingly, xylanases from all the thermophilic fungi showed a bleach boosting effect on the pulp, however, among thermotolerant fungi, only *A. caespitosus* xylanase was found to be effective. These biobleaching studies revealed that maximal chromophore release, reducing sugar release and brightness of pulp, was observed with alkaline active xylanases from novel strain of *Malbranchea* sp., with a gain of 2.04 ISO units (Table 3), and was much better as compared to other sources of alkaline active xylanases (1.63-1.81 ISO units) identified in this study. The observed increase in the pulp brightness by 2.04 units may result in decreased chlorine consumption by 20-25% as observed previously with commercial xylanases Novozyme 473 and VAI-Xylanase (Bajpai et al. 1994).

CONCLUSIONS

This paper highlights the use of simple techniques like PAGE and IEF for high through-put screening of diverse xylanases from different fungal cultures. Furthermore, the techniques can also be used for identification of xylanases with novel properties.

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Table 2A. The Relative Percent Activities of Xylanase Isoforms (Peak Fractions) of Different Thermophilic Fungi against Different Types of Xylan Substrates (Substituted and Unsubstituted) and Debranched Arabinan.

***Substrate*[a] → *Isoforms*↓**	***BWX*[b]**	***LWX*[b]**	***OSX*[b]**	***RAX*[b]**	***WAX*[b]**	***DA*[b]**	***BWX*[c]**
Thermophilic fungi							
Chaetomium thermophilum							
CTX-I (5.7)[d]	100	103	213	165	232	-*	122**
CTX-II (5.5)	100	127	143	162	209	-	114**
CTX-III (4.5)	100	159	171	165	204	-	108**
Chrysosporium lucknowense							
CLX-I (5.5)	100	128	228	217	231	50	41
CLX-II (4.8)	100	146	169	197	237	52	74
CLX-III (4.1)	100	107	153	138	183	50	80
CLX-IV (3.4)	100	192	263	233	307	43	58
Humicola fuscoatra							
HFX-I (9.7)	100	145	280	101	109	-	43
HFX-II (8.2)	100	140	121	115	115	-	0
HFX-III (7.2)	100	165	189	282	331	-	53
HFX-IV (6.5)	100	316	264	487	638	-	50
HFX-V (3.8)	100	250	142	415	669	12	63
HFX-VI (3.4)	100	228	321	751	957	-	56
Humicola insolens							
HIX-I (9.5)	100	121	218	292	322	-	189**
HIX-II (7.8)	100	182	175	195	256	-	120**
HIX-III (7.2)	100	192	180	126	259	-	115**
HIX-IV (6.5)	100	151	170	215	286	-	97
HIX-V (5.8)	100	192	212	253	276	-	82
HIX-VI (5.5)	100	471	247	776	423	-	95
HIX-VII (4.8)	100	125	119	211	381	-	184**
HIX-VIII (3.3)	100	149	224	197	259	-	149**
***Malbranchea* sp.**							
MFX-I (4.5)	100	142	171	146	204	-	116**
MFX-II (3.5)	100	134	144	124	174	-	107**
MFX-III (3.3)	100	112	149	147	167	-	80
***Melanocarpus* sp.**							
MAX-I (7.8)	100	153	155	137	182	-	107**
MAX-II (7.2)	100	123	155	188	185	-	71
MAX-III (6.5)	100	93	156	150	177	-	42
MAX-IV (5.8)	100	97	140	143	167	-	65
MAX-V (5.0)	100	87	137	137	159	-	87
MAX-VI (4.5)	100	100	96	168	200	-	46
Thermoascus aurantiacus							
TAX-I (7.5)	100	136	122	134	174	-	28
TAX-II (7.2)	100	139	127	116	129	-	74
TAX-III (5.8)	100	192	136	204	352	-	292**
TAX-IV (3.4)	100	140	157	253	377	-	209**

[a] Composition of each substrate:
BWX, Birch wood Xylan (> 90 % xylose);
LWX, Larch Wood Xylan (86 % xylose / 8.8 % arabinose / 1.2 % glucose / 3.2 % uronic acid);
OSX, Oat Spelt Xylan (75 % xylose / 10% arabinose / 15 % glucose);
RAX, Rye Arabino Xylan (49 % arabinose / 51 % xylose);
WAX, Wheat Arabino Xylan (41 % arabinose / 59 % xylose);
DA, Debranched Arabinan (arabinose).
Notes [b] through [d], *, ** See end of Table 2B

Table 2B. The Relative Percent Activities of Xylanase Isoforms (Peak Fractions) of Different Thermotolerant Fungi against Different Types of Xylan Substrates (Substituted and Unsubstituted) and Debranched Arabinan.

***Substrate*[a] → *Isoforms*↓**	***BWX*[b]**	***LWX*[b]**	***OSX*[b]**	***RAX*[b]**	***WAX*[b]**	***DA*[b]**	***BWX*[c]**
			Thermotolerant fungi				
Absidia corymbifera							
ACX-I (7.3)	100	185	110	352	428	-	83
ACX-II (5.6)	100	216	110	462	529	-	52
ACX-III (5.4)	100	123	103	247	303	-	88
ACX-IV (4.8)	100	108	171	165	281	-	79
ACX-V (3.8)	100	101	175	147	156	-	84
ACX-VI (3.3)	100	105	180	112	137	-	89
Acrophialophora nainiana							
ANX-I (9.1)	100	200	171	557	666	66	7
ANX-II (5.8)	100	152	184	456	604	72	14
ANX-III (5.6)	100	215	162	587	575	34	62
ANX-IV (4.8)	100	278	232	450	685	60	55
ANX-V (4.2)	100	208	174	448	756	32	46
ANX-VI (3.5)	100	322	466	838	971	66	41
Aspergillus caespitosus							
ASCX-I (7.8)	100	107	109	108	158	-	25
ASCX-II (6.7)	100	94	142	67	100	-	90**
ASCX-III (5.9)	100	75	70	101	135	-	32
ASCX-IV (5.5)	100	128	148	175	244	-	38
ASCX-V (5.2)	100	131	93	216	232	-	71
ASCX-VI (3.8)	100	104	120	162	214	-	98**
ASCX-VII (3.3)	100	94	102	99	131	-	96**
Aspergillus terreus							
ATX-I (8.0)	100	278	87	70	274	34	22
ATX-II (7.0)	100	403	150	146	300	53	7
ATX-III (6.5)	100	246	135	203	417	61	25
ATX-IV (5.9)	100	294	102	205	405	47	2
ATX-V (5.5)	100	212	201	398	306	30	17
ATX-VI (4.8)	100	277	120	283	368	18	21
ATX-VII (4.5)	100	185	99	179	273	-	17
ATX-VIII (4.1)	100	88	89	150	260	-	20
ATX-IX (3.5)	100	75	130	76	252	-	7
ATX-X (3.3)	100	226	96	234	321	-	13
Emericella nidulans* var. *lata							
ENX-I (9.7)	100	94	130	47	79	-	3
ENX-II (8.2)	100	134	123	125	156	-	36
ENX-III (7.2)	100	175	118	330	307	-	66
ENX-IV (6.9)	100	393	120	626	826	-	76
ENX-V (6.3)	100	226	140	463	200	-	66
ENX-VI (5.9)	100	163	103	343	350	-	70
ENX-VII (5.5)	100	179	63	401	384	-	68
ENX-VIII (5.3)	100	125	-	241	410	-	71
ENX-IX (4.8)	100	104	150	187	194	-	80
ENX-X (4.1)	100	134	153	219	293	-	58
ENX-XI (3.4)	100	96	145	173	211	-	44
Penicillium lagena							
PLX-I (6.7)	100	227	177	211	188	-	18
PLX-II (5.8)	100	307	115	456	433	28	26

PLX-III (5.5)	100	165	130	146	176	7	24
PLX-IV (5.3)	100	126	146	260	288	4	22
PLX-V (4.5)	100	130	208	169	234	3	69
PLX-VI (3.5)	100	115	173	162	229	3	62
PLX-VII (3.3)	100	264	191	355	400	9	52

[a] Composition of each substrate:
BWX, Birch wood Xylan (> 90 % xylose);
LWX, Larch Wood Xylan (86 % xylose / 8.8 % arabinose / 1.2 % glucose / 3.2 % uronic acid);
OSX, Oat Spelt Xylan (75 % xylose / 10% arabinose / 15 % glucose);
RAX, Rye Arabino Xylan (49 % arabinose / 51 % xylose);
WAX, Wheat Arabino Xylan (41 % arabinose / 59 % xylose);
DA, Debranched Arabinan (arabinose).

[b] Relative activity against different xylan substrates at pH 6.0.
[c] Relative activity against birch wood xylan at pH 9.0.
[d] pI of xylanase isoforms expressed in respective fungal strain.
* Activity not detected.
** Active under alkaline conditions.