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Bioinformatics Insights into Microbial Xylanase Protein Sequences

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Microbial xylanases represents an industrially important group of enzymes associated with hydrolysis of xylan, a major hemicellulosic component of plant cell walls. A total of 122 protein sequences comprising of 58 fungal, 25 bacterial, 19actinomycetes and 20 yeasts xylanaseswere retrieved from NCBI, GenBank databases. These sequences were *in-silico* characterized for homology,sequence alignment, phylogenetic tree construction, motif assessment and physio-chemical attributes. The amino acid residues ranged from 188 to 362, molecular weights were in the range of 20.3 to 39.7 kDa and pI ranged from 3.93 to 9.69. The aliphatic index revealed comparatively less thermostability and negative GRAVY indicated that xylanases are hydrophilic irrespective of the source organisms.Several conserved amino acid residues associated with catalytic domain of the enzyme were observed while different microbial sources also revealed few conserved amino acid residues. The comprehensive phylogenetic tree indicatedsevenorganisms specific,distinct major clusters,designated as A, B, C, D, E, F and G. The MEME based analysis of 10 motifs indicated predominance of motifs specific to GH11 family and one of the motif designated as motif 3 with sequence GTVTSDGGTYDIYTTTRTNAP was found to be present in most of the xylanases irrespective of the sources.Sequence analysis of microbial xylanases provides an opportunity to develop strategies for molecular cloning and expression of xylanase genes and also for identifying sites for genetic manipulation for developing novel xylanases with desired features as per industrial needs.

Keywords: Xylanases, Bioinformatics, Multiple sequence alignment, Phylogenetic tree, Source organisms.

Plant cell wall comprises of three major constituent namely cellulose, hemicelluloses and lignin.Xylan is the major hemicellulosic component and is the second most abundant polysaccharides in nature.Hemicellulose is a branched heteropolymer consisting of pentose and hexose sugars with xylose being most abundant[1] (Kumar *et al*., 2008). A repertoire of enzymes including endo-xylanase (endo-1,4-â-xylanase; E.C.3.2.1.8), â-xylosidase (xylan-1,4-â-xylosidase; E.C.3.2.1.37), á-glucosiduronase(E.C.3.2.1.139),á-arabinofuranosidase(E.C.3.2.1.55) and acetylxylan esterase,(E.C.3.1.1.72)are associated with complete hydrolysis of hemicellulose[2](Juturu and Wu, 2012).

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The endo-xylanase and â-xylosidase are key enzymes associated with hydrolysis of xylan and are collectively referred as xylanases.

Xylanasesbelongs to enzyme class of glycoside hydrolases (GH), which are classified into several families based on amino acid sequences. Xylanases is predominantly represented in two families GH10 and GH11[3-5] (Paes *et al*., 2012;Lafond *et al*., 2014; Chakdar *et al*., 2016).Xylanases have been reported from diverse microbial sources namely fungi, bacteria,actinomycetes and yeast and have been reviewed extensively over the years[2,6-10] (Beg *et al*., 2001; Collins *et al*., 2005; Nair *et al*., 2008; Juturu and Wu, 2012; Juturu and Wu, 2014; Walia *et al*., 2017).Xylanasesrepresents industrially important group of enzymes with diverse applications like pulp and paper bleaching, fruit juice clarification, bioethanol production, bioconversion etc.[2,11-14]. (Shatalov *et al*., 2008; Valls *et al*., 2010; Juturu and Wu, 2012; Singh *et al*., 2013; Walia *et al*., 2015).

Several bioinformatics studies have been done on various xylanases. In-silico analysis of structural attributes ofcommercially important xylanases from diverse sources structural has beenreported[15](Arora *et al*., 2009).Attempts have been made to study the structural dynamics changes of the *Trichodermalongibrachiatum*xylanase upon binding with xylohexaose and xylan ligands[16] (Uzuner *et al*., 2010). *In-silico* structural prediction of *Bacillus brevis*xylanase and its comparative assessment with few bacterial and fungal xylanases has been reported recently[17](Mathur *et al*., 2015). Efforts have been made to analyze several plant cell wall degrading enzymes (PCWDEs) including xylanses and polygalacturonases of*Fusariumvirguliforme*using bioinformatics tools to develop fungal resistant soyabean[18](Chang *et al*., 2016). Homology modeling of xylanase from *Aspergillusfumigatus* R1 isolate to get an insight into three dimensional structurehas been attempted[19] (Deshmukh *et al*., 2016).

This manuscript reports *in-silico* characterization of xylanase protein sequences retrieved from NCBI representing diverse microbial sources namely fungi, bacteria, actinomycetes and yeast. Bioinformatics assessment of these sequences for homology,sequence alignment, physio-chemical attributes, motif assessment and phylogenetic tree construction isreported.The bioinformatics driven characterization of available sequences of microbial xylanases could be utilized for developing appropriate strategies for molecular cloning and expression of xylanasegenes.Further, the sequence-structure-function relationship could be established from *in-silico* studies and novel xylanases could be derived using state-of-the art technologies either metagenomics or directed evolution approaches.

MATERIALS AND METHODS

Xylanase protein sequences representing different microbial sources were retrieved from GenBank, NCBI (http//www.ncbi.nlm.nih.gov/). The sequences retrieved were saved in FASTA format and truncated proteins were discarded. The major groups as source organisms represents fungi, bacteria, actinomycetes and yeast and the all the sequences of xylanases belongs to GH11 family.

Physio-chemical attributes

The physio-chemical attributes namely molecular weight, theoretical pI, aliphatic index, instability index, Grand Average of Hydropathicity (GRAVY) were analyzed by ProtParam tool (http://web.expasy.org/protparam/).[20](Gasteiger *et al*.,2005).

Multiple sequence alignment and Phylogenetic analysis

The protein alignment of full length amino acid sequences of xylanase were performed by CLUSTAL X version 2.1[21](Larkin *et al*., 2007). Phylogenetic tree was constructed by NJ method using the MEGA 7.0 program[22](Kumar *et al*., 2016) based on protein sequences.

Identification of conserved motifs

The protein sequences of xylanase were analyzed by Multiple EM for Motif Elicitation(MEME)program version 4.12.0 (http://meme.nbcr.net/meme/)[23](Timothy *et al*.,2009).The maximum number of motifswereset as 10.The minimum width of 6 and maximum width of 50 amino acids was s*et al*ong with other factors as default values.

RESULTS AND DISCUSSION

Physio-chemical characterization of xylanases

A total of the 122 xylanase protein

Table 1. List of xylanase protein sequences from different microbial sources with in-silico physio-chemical attributes revealed by Protparam.

S.No.	Source Organism	Accession No.	Amino acid	Mol. wt.	pI	Instability index	Aliphatic index	GRAVY
FUNGI								
1	*Aspergillus nomius*	XP_015411959	229	24.4	5.73	26.21	59.61	-0.33
2	*Aspergillus nomius*	XP_015411268	221	23.8	4.55	22.18	60.9	-0.427
3	*Aspergillus niger*	AAS46914	225	24.1	5.23	22.11	59.78	-0.396
4	*Aspergillus niger*	AAS46913	211	22.5	4	19.86	59.62	-0.178
5	*Aspergillus niger*	AAA99065	211	22.7	4.55	26.49	59.15	-0.18
6	*Aspergillus niger*	AFK10491	225	24	5.2	21.06	58.53	-0.38
7	*Aspergillus niger*	CAA03654	225	24	5.45	21.06	58.53	-0.384
8	*Aspergillus niger*	ACN89393	225	24	5.2	21.06	58.53	-0.38
9	*Aspergillus niger*	AAM95167	225	24.1	5.23	22.11	59.78	-0.396
10	*Aspergillus niger*	ABA00146	225	24.1	5.23	22.11	59.78	-0.396
11	*Aspergillus niger*	AGH29125	225	24.1	5.23	22.11	59.78	-0.396
12	*Aspergillus flavus*	KOC12560	232	24.6	5.49	21.53	58.84	-0.291
13	*Aspergillus fumigatus*	XP_751100	221	23.8	5.22	24.15	56.02	-0.422
14	*Aspergillus fumigatus*	XP_748354	228	24.4	6.27	23.44	53.9	-0.361
15	*Aspergillus fumigatus*	XP_748367	313	33	6.03	26.03	69.71	-0.093
16	*Aspergillus nidulans*	CAA90074	221	23.5	4.54	32.04	54.71	-0.378
17	*Aspergillus niger*	XP_001388522	225	24	5.2	21.06	58.53	-0.38
18	*Aspergillus niger*	ACJ26382	225	24	5.2	21.06	58.53	-0.384
19	*Aspergillus niger*	ACA24724	225	24	5.44	20.72	59.82	-0.346
20	*Aspergillus niger*	AAM08362	225	24.1	5.23	22.11	59.78	-0.396
21	*Aspergillus niger*	XP_001389848	231	24.8	3.94	26.61	61.69	-0.364
22	*Aspergillus niger*	GAQ35804	231	24.8	3.93	25.84	61.26	-0.373
23	*Aspergillus niger*	GAQ46944	211	22.5	4.07	17.72	60.52	-0.138
24	*Aspergillus niger*	EHA24718	236	25.7	4.4	21.78	65.64	-0.408
25	*Aspergillus niger*	ALN49265	256	28	4.7	34.64	70.04	-0.312
26	*Aspergillus niger*	AFK10490	211	22.5	4.31	23.47	58.72	-0.146
27	*Aspergillus niger*	ADO66655	211	22.6	4.31	22.55	58.72	-0.145
28	*Aspergillus niger*	XP_001401361	211	22.6	4.31	23.06	58.72	-0.156
29	*Aspergillus niger*	ACN82438	211	22.5	4.39	23.33	57.35	-0.17
30	*Aspergillus niger*	GAQ40597	256	28	4.76	34.57	70.78	-0.303
31	*Aspergillus oryzae*	XP_001823798	232	24.4	5.48	21.33	59.52	-0.276
32	*Aspergillus oryzae*	XP_001818666	221	23.7	4.67	19.63	61.76	-0.424
33	*Aspergillus clavatus*	XP_001273882	192	20.4	9.63	32.86	57.92	-0.357
34	*Aspergillus luchuensis*	GAT31123	225	24.1	5.74	22.92	60.22	-0.432
35	*Aspergillus luchuensis*	GAT25039	211	22.6	4.07	20.58	59.15	-0.129
36	*Aspergillus luchuensis*	OJZ80582	211	22.6	4.02	21.52	57.77	-0.153
37	*Aspergillus luchuensis*	GAT30893	256	28	4.76	34.57	69.26	-0.304
38	*Aspergillus luchuensis*	OJZ85846	256	28	4.84	35.16	68.87	-0.308
39	*Aspergillus versicolor*	ABM55503	206	21.9	4.24	16.32	62.96	-0.065
40	*Fusarium avenaceum*	KIL90649	287	29.8	4.53	29.56	42.93	-0.625
41	*Fusarium verticillioides*	XP_018753195	314	32.6	4.64	24.61	37.96	-0.832
42	*Fusarium verticillioides*	XP_018753196	296	30.6	4.87	21.85	39.93	-0.749
43	*Fusarium verticillioides*	XP_018755440	233	25.2	8.98	30.08	58.93	-0.436
44	*Fusarium verticillioides*	XP_018761356	231	25.7	6.41	34.6	51.95	-0.694
45	*Fusarium mangiferae*	CVK98696.1	231	25.6	6.41	34.56	51.52	-0.685
46	*Fusarium proliferatum*	CVL11933	231	25.6	6.05	36.73	53.2	-0.646
47	*Fusarium fujikuroi*	CCT69575	231	25.7	6.41	36.04	53.2	-0.662
48	*Fusarium*	XP_009253878	231	25.7	6.5	32.22	52.79	-0.666

	pseudograminearum							
49	*Fusarium langsethiae*	KPA38457.1	228	24.5	9.17	26.82	58.64	-0.472
50	*Fusarium oxysporum*	EWZ00952	277	29.2	4.91	23.97	41.59	-0.736
51	*Fusarium oxysporum*	EWZ46984	289	30.3	4.83	24.9	41.87	-0.754
52	*Fusarium oxysporum f. sp. vasinfectum*	EXM22239	271	28.3	5.19	22.81	43.21	-0.676
53	*Fusarium oxysporum f. sp. vasinfectum*	EXM22238	289	30.3	4.83	25.75	40.87	-0.77
54	*Fusarium oxysporum f. sp. lycopersici*	XP_018238679	277	28.8	5.06	23.67	42.64	-0.686
55	*Fusarium oxysporum f. sp. lycopersici*	XP_018238678	286	29.7	4.98	24.79	40.63	-0.737
56	*Fusarium oxysporum f. sp. lycopersici*	XP_018246999	232	25.1	8.96	25.04	57.46	-0.479
57	*Fusarium oxysporum f. sp. lycopersici*	XP_018256151	231	25.6	6.18	32.52	52.77	-0.655
58	*Fusarium oxysporum f. sp. cubense*	EMT73821	231	25.6	6.41	34.17	54.46	-0.638
BACTERIA								
59	*Bacillus cereus*	AAZ17391	213	23.3	9.44	15.87	54.46	-0.425
60	*Dictyoglomus thermophilum*	WP_012547705	360	39.7	8.68	25.12	72.28	-0.314
61	*Dictyoglomus thermophilum*	AAC46361	360	39.7	8.68	19.75	71.19	-0.333
62	*Dictyoglomus turgidum*	WP_012582654	356	39.4	8.45	22.94	71.99	-0.285
63	*Fibrobacter succinogenes*	WP_014546846	327	36.1	5.07	19.20	65.32	-0.350
64	*Paenibacillus jilunlii*	WP_062524300	212	23.2	9.10	24.53	56.08	-0.342
65	*Paenibacillus polymyxa*	ADK47978	211	22.7	9.55	17.82	61.47	-0.303
66	*Paenibacillus polymyxa*	KOS03251	212	23.2	9.40	21.41	51.93	-0.407
67	*Paenibacillus polymyxa*	WP_025720875	212	23.1	9.40	21.41	51.93	-0.419
68	*Paenibacillus polymyxa*	WP_061831741	212	23.1	9.15	21.05	51.93	-0.417
69	*Paenibacillus polymyxa*	WP_013308993	212	23.2	9.15	20.83	51.93	-0.432
70	*Paenibacillus polymyxa*	WP_016820426	212	23.1	9.30	22.35	55.14	-0.409
71	*Paenibacillus polymyxa*	WP_017425612	212	23.2	9.30	22.05	53.30	-0.417
72	*Paenibacillus polymyxa*	WP_023987219	212	23.1	8.94	20.56	51.93	-0.414
73	*Paenibacillus polymyxa*	WP_031462284	212	23.1	9.30	21.95	55.14	-0.407
74	*Paenibacillus polymyxa*	WP_013373220	212	23.2	9.40	21.91	53.77	-0.407
75	*Paenibacillus polymyxa*	WP_058831015	212	23.1	9.40	19.84	55.61	-0.394
76	*Paenibacillus polymyxa*	WP_039272535	212	23.1	9.18	23.35	52.41	-0.404
77	*Paenibacillus polymyxa*	WP_064797296	212	23.1	9.40	16.99	53.77	-0.445
78	*Paenibacillus polymyxa*	WP_023987332	362	39.5	7.69	26.27	59.78	-0.524
79	*Paenibacillus polymyxa*	WP_068938485	362	39.5	7.69	25.82	59.50	-0.525
80	*Paenibacillus polymyxa*	WP_071639791	362	39.5	7.69	19.53	62.18	-0.504
81	*Paenibacillus riograndensis*	WP_020430448	212	23.1	9.25	20.64	56.56	-0.316
82	*Paenibacillus riograndensis*	WP_060864761	212	23.2	9.25	20.73	56.08	-0.320
83	*Paenibacillus terrae*	WP_014280040	212	23.1	9.30	21.81	52.41	-0.405
ACTINOMYCETES								
84	*Actinobacteria*	WP_054228265	330	35.0	9.17	28.72	51.42	-0.415
85	*Hamadaea tsunoensis*	WP_027341164	327	33.8	9.16	26.33	56.70	-0.309
86	*Herbidospora cretacea*	WP_061296570	321	34.0	9.49	29.13	50.16	-0.513
87	*Herbidospora mongoliensis*	WP_066360436	322	33.8	9.37	25.86	48.79	-0.493
88	*Microbispora sp.*	WP_055478269	335	35.5	9.69	32.99	49.55	-0.569
89	*Micromonospora coxensis*	SCG34253	330	34.5	9.42	33.72	53.82	-0.366
90	*Micromonospora nigra*	SCL32394	329	34.5	9.61	32.39	54.83	-0.395
91	*Nocardiopsis dassonvillei*	WP_061080181	332	35.2	8.74	35.03	51.99	-0.497
92	*Nonomuraea jiangxiensis*	SDH13383	321	33.9	9.32	36.29	56.20	-0.397

93	*Planomonospora sphaerica*	WP_068897915	335	35.1	9.60	32.76	48.39	-0.503
94	*Saccharothrix syringae*	WP_033431747	329	34.8	9.67	30.96	53.98	-0.495
95	*Streptomonospora alba*	WP_040275156	339	35.8	5.17	35.81	44.31	-0.605
96	*Streptomyces hirsutus*	WP_055594006	337	35.9	9.30	26.42	52.37	-0.406
97	*Streptomyces reticuli*	WP_059255807	337	35.8	9.53	29.68	54.69	-0.413
98	*Streptomyces viridosporus*	AAF09501	329	35.1	9.55	26.02	50.09	-0.518
99	*Streptomyces davawensis*	WP_015659056	320	33.8	9.23	23.06	56.38	-0.351
100	*Streptomyces aureus*	WP_051901343	313	32.6	8.95	20.17	52.36	-0.394
101	*Thermobifida fusca*	WP_011291660	338	36.4	9.47	34.31	52.28	-0.495
102	*Thermobifida fusca*	WP_016188539	338	36.4	9.37	34.20	52.28	-0.504
YEAST								
103	*Aureobasidium melanogenum*	KEQ63689	218	23.3	4.73	30.22	70.78	-0.204
104	*Aureobasidium melanogenum*	KEQ63789	217	23.4	8.27	20.93	52.63	-0.369
105	*Aureobasidium melanogenum*	KEQ64351	221	23.4	4.86	17.74	60.90	-0.108
106	*Aureobasidium melanogenum*	BAB69655	221	23.3	4.86	17.74	61.36	-0.096
107	*Aureobasidium namibiae*	XP_013425857	225	24.1	9.25	24.83	60.71	-0.432
108	*Aureobasidium namibiae*	XP_013422490	218	23.1	6.40	25.16	72.11	-0.204
109	*Aureobasidium namibiae*	XP_013429521	221	23.2	5.71	18.17	62.26	-0.138
110	*Aureobasidium pullulans*	KEQ83780	229	25.0	8.84	22.97	48.52	-0.514
111	*Aureobasidium pullulans*	KEQ90048	224	24.1	9.03	24.80	58.39	-0.376
112	*Aureobasidium pullulans*	KEQ80629	218	23.2	5.54	35.83	75.23	-0.176
113	*Aureobasidium pullulans*	AAD51950	221	23.5	5.29	16.84	58.73	-0.181
114	*Aureobasidium subglaciale*	XP_013347844	224	24.1	8.84	29.17	58.35	-0.420
115	*Aureobasidium subglaciale*	XP_013339677	230	25.0	7.77	34.07	52.57	-0.464
116	*Baudoinia panamericana*	XP_007672582	188	20.3	6.54	23.04	61.22	-0.298
117	*Bispora sp.*	ADZ99365	205	21.8	4.21	14.67	59.41	-0.281
118	*Cryptococcus sp.*	BAA09699	209	22.7	5.46	19.73	50.81	-0.515
119	*Cryptococcus sp.*	BAA09698	209	22.7	5.46	19.73	50.81	-0.515
120	*Pseudozyma hubeiensis*	XP_012187186	261	27.9	9.15	24.47	50.84	-0.466
121	*Saitozyma flava*	AOS95422	209	22.7	6.25	18.09	51.29	-0.499
122	*Saitozyma flava*	ABY50453	209	22.7	6.25	21.81	52.20	-0.506

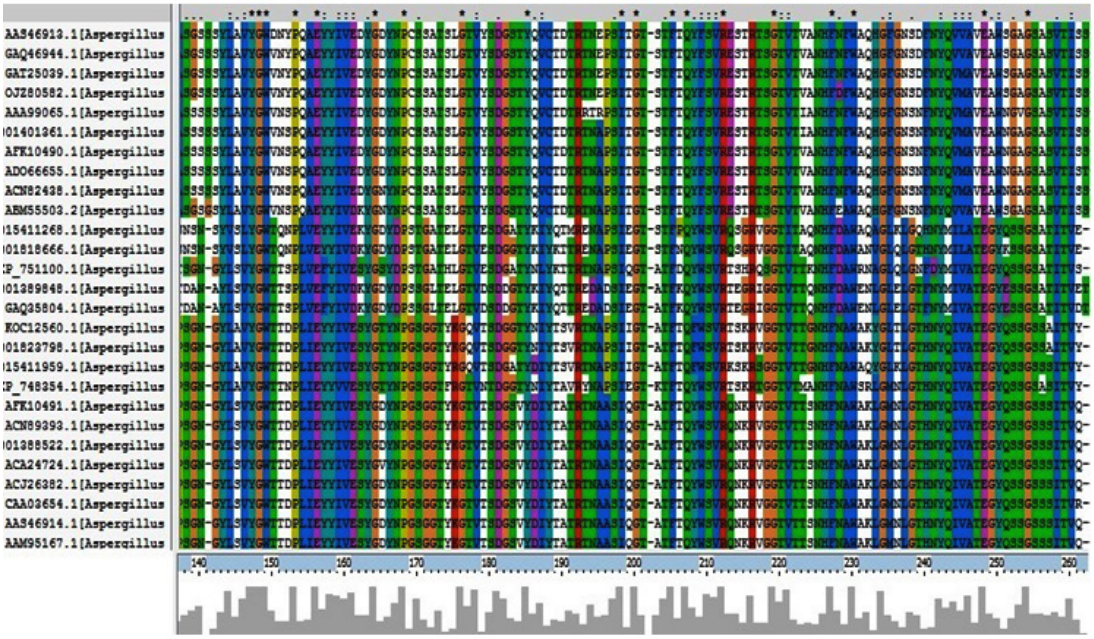
sequences belonging to GH11 family representing 58 fungal, 25 bacterial, 19 actinomycetes and 20 yeastxylanaseswere retrieved from NCBI databases (Table-1).It has been reported that bacterial xylanases generally represent GH10 family though fungal xylanases predominantly belongs to GH11 family[24](Liu *et al*., 2011). Theirphysio-chemical properties namely molecular weight, pI, instability index, aliphatic index, GRAVYwere analyzed using ProtParam tool (Table-1). Theamino acid residues ranged from 188-362 residueswhilemolecularweightwas in the range of 20.3-39.7 KDa. The Isoelectric point(pI) was in the range of 3.93-9.69.The molecular weight in the range of 8.5 to 85kDa and pI in the range of 4-10.3 has been reported for bacterial[5](Chakdhar *et al*.,2015) and fungal xylanases[25](Polizeli *et al*. 2005).

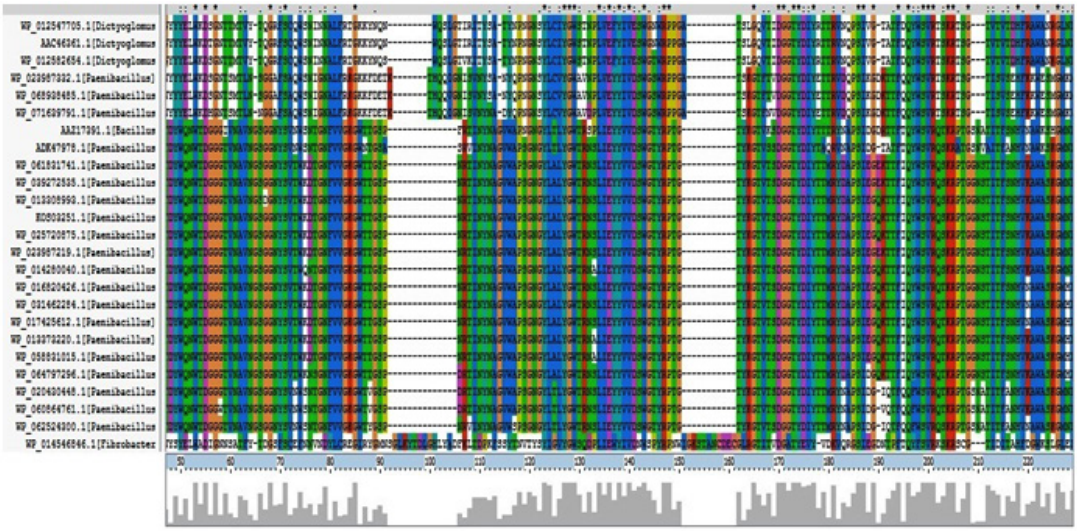
The stability –instability index method[26](Guruprasad *et al*. 1990) estimates the stability of the protein in a test tube. The instability index less than 40 predicts a stable protein whereas values higher than 40 denotes potentially unstable protein. The value of instability index of xylanases ranged from 14.67-36.73, which is less than 40 and hence represents stable protein.The stability -aliphatic index method[20](Gasteiger *et al*., 2005) reflects regional stability based on the relative volume occupied by aliphatic side chain and is a positive indicator of globular protein theromostability. The aliphatic index of xylanaseis the range of

37.96-75.23 as reported in literature[27](Walia *et al*., 2015) and high aliphatic index indicates stability of xylanases for wide temperature range. The aliphatic index of xylanases protein sequences from *Aspergillusniger* (ALN49265), *Dictyoglomusthermophilum*(WP_012582654, AAC46361), *Aureobasidiummelanogenum* (KEQ63689), *Aureobasidiumnamibiae* (XP_013422490) and *Aureobasidiumpullulans*(KEQ80629) was above 70 (Table-1).Another important physio-chemical attributeanalyzed by ProtParam is GRAVY value derived by calculating the sum of hydropathy values[28](Kyte and Doolittle, 1982) of all the amino acids, divided by the number of residues in the sequence[20](Gasteiger *et al*. 2005). Increasing positive score indicates a greater hydrophobicity. The microbial xylanase protein sequences revealed negative GRAVY value ranging from -0.832 to -0.093 indicating hydrophilic nature.

Multiple Sequence Alignment Analysis

Multiple sequence alignment





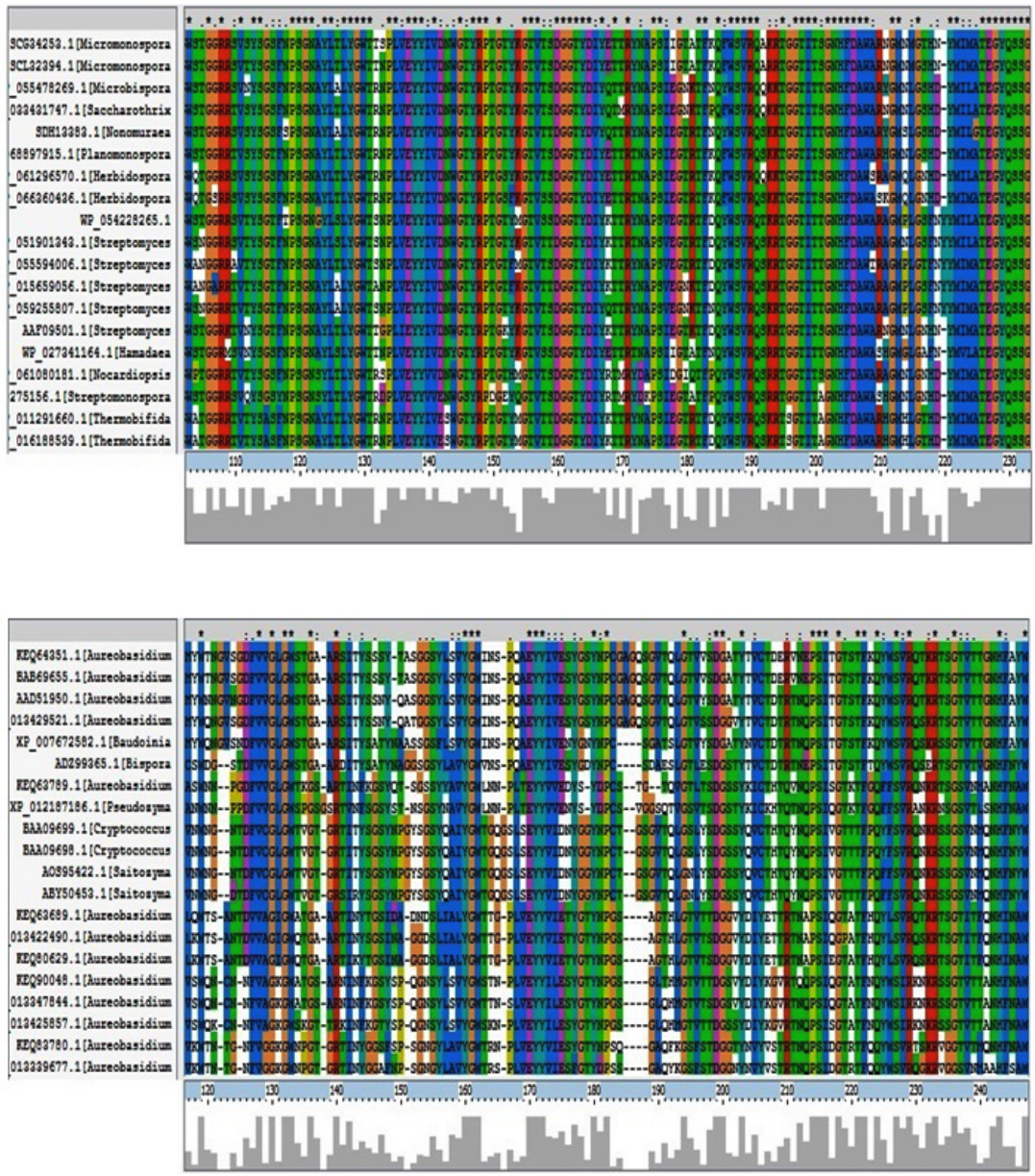


Fig. 1. Multiple sequence alignment of xylanase protein sequences from (A) Fungal (B) Bacterial (C) Actinomycetes and (D) Yeast sources. Strongly conserved amino acid residues are indicated by asterisk* above the alignment

ofretrievedxylanasesequenceswasperformed by CLUSTAL X version 2.1andis shown in Figure 1(A,B,C& D). Several conserved amino residues are observed for different source organisms while comprehensive multiple sequence alignment of all122xylanase sequences revealedtwohighly conserved residues namely YGW and EYYI (Figure-1E). The presence of these conserved amino acid residues has been reported for xylanases especially from fungal and bacterial sources[29-31](Ellouze *et al*.,2011, Sapag *et al*., 2002,Torronen *et al*.,1992). Similar conserved amino acid residues have been observed for xylanaseof*T. longibrachiatum*. Another conserved amino acid residues with

sequence RVNEPSIQGTATFNQYhas been reported, which plays significant role in stabilizing during substrate binding[16](Uzuner *et al*.,2010).

It has also been reported that glutamate amino acid residue responsible for catalysis is conserved in genera of ascomycetes and basidiomycetes representing GH11 and GH10 family of xylanases[32](Cervantes *et al*., 2016). Xylanase proteins representing actinomycetes revealed several conserved amino acid residues

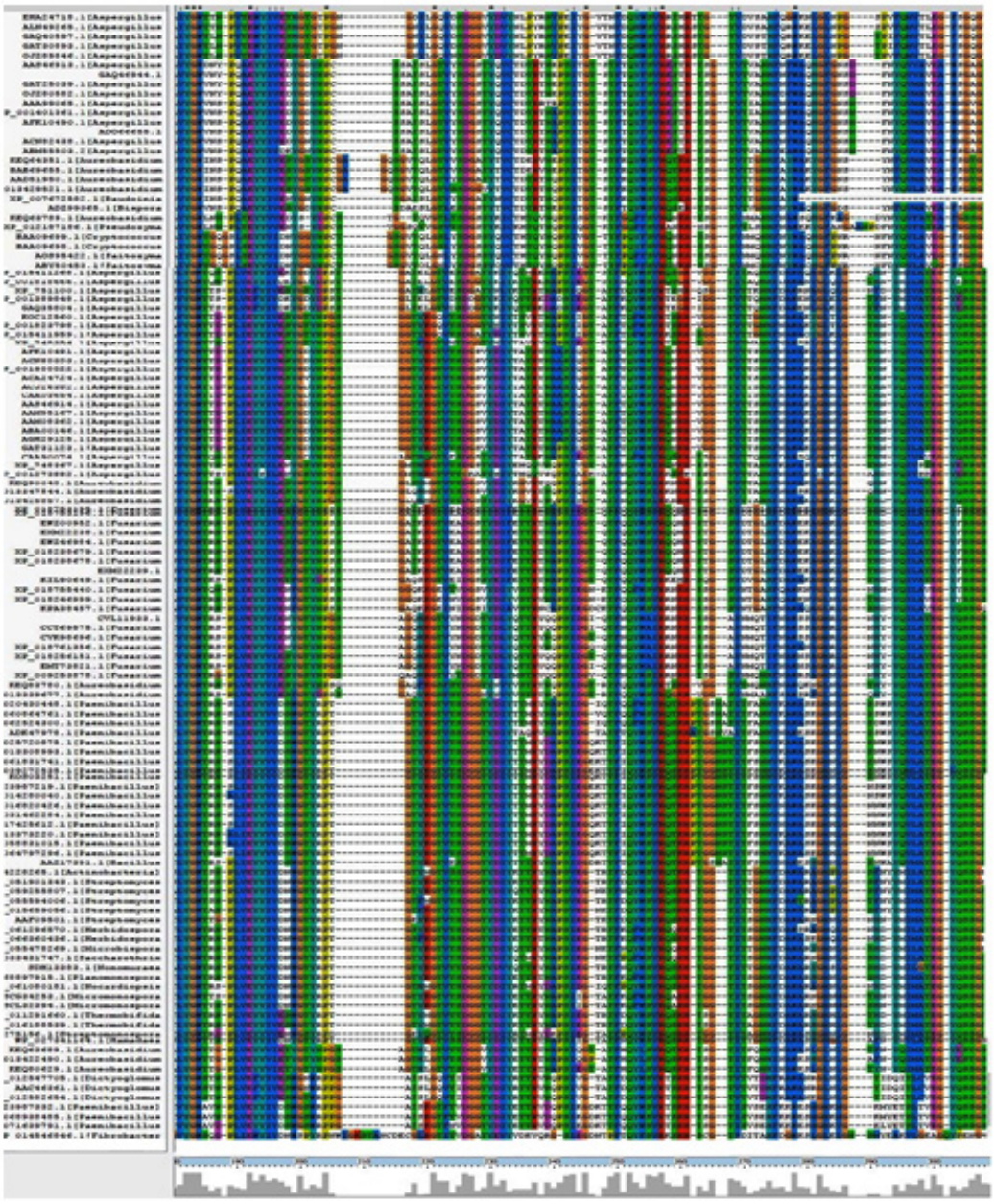


Fig. 1(E). Combined sequence alignment of a total 122 xylanases protein sequences from different microbial sources.Strongly conserved amino acid residues are indicated by asterisk* above the alignment

at positions 119-131,155-169,185-191,197-208 and 221-233 along with YGW and EYY residues (Figure-1C). Similarly alignment of 20 xylanase protein sequences of GH11 family from source organism yeast revealed several conserved amino acid residues at position 214-216 (Figure-1D). The presence of conserved amino acid residues provides an insight into the catalytic activity of the enzyme based on the fact that there exits sequence-structure-function relationship. The multiple

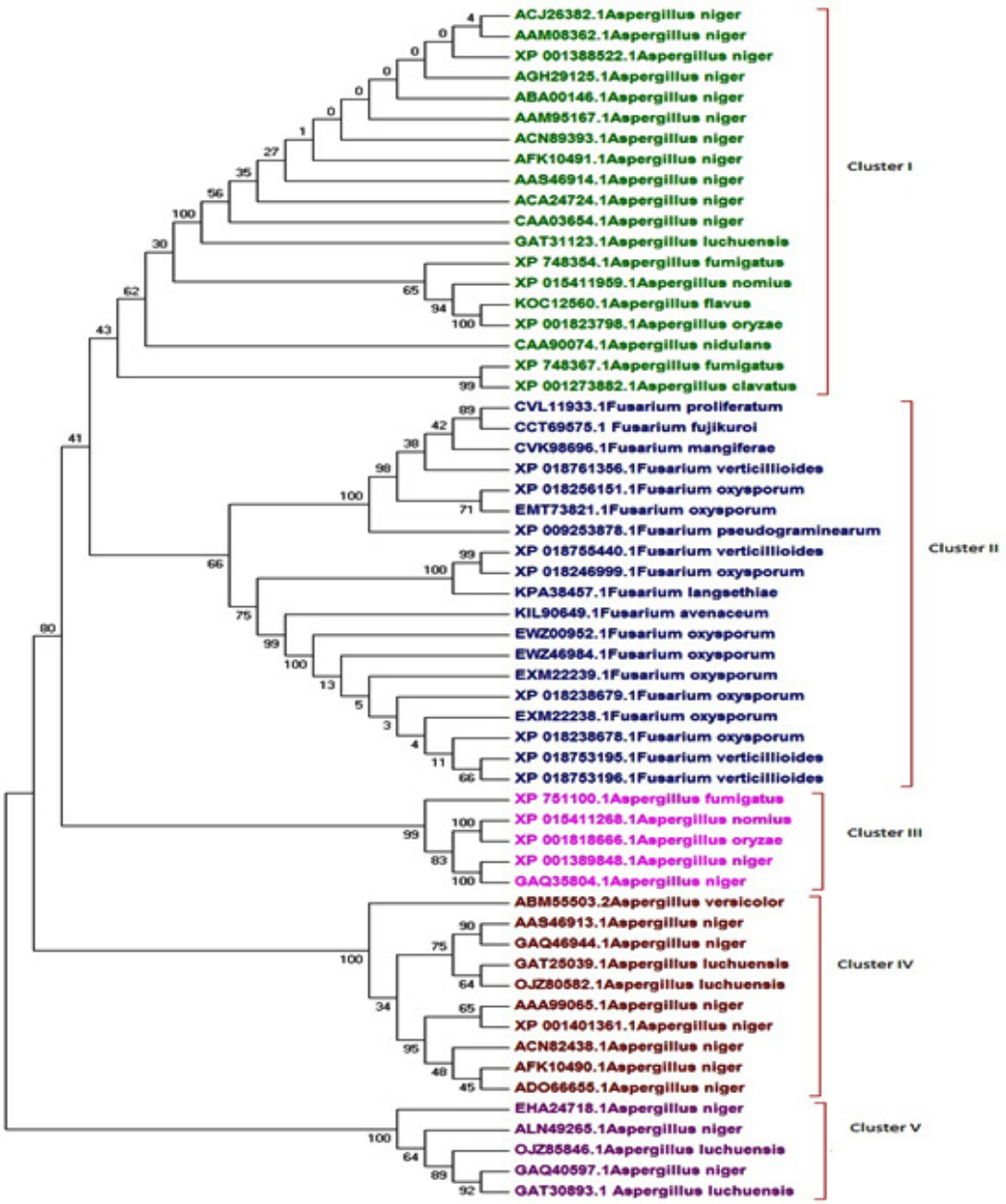


Fig. 2(A). Phylogenetic tree constructed using protein sequences of 58 fungal xylanase. The distinct major clusters designated as I, II, III, IV and V comprising of 19, 19, 5, 10 and 5 members respectively are highlighted

sequence alignment also provides an opportunity to design appropriate degenerate primers for amplification of xylanase genes from different microbial sources.

Phylogenetic analysis

The phylogenetic tree based on microbial xylanase protein sequenceswere constructed by NJ method (Figure-2 A, B, C, D). The phylogenetic tree representing fungal xylanase protein sequences revealed 5 distinct major clusters designated as I,II,III,IV and V group (Figure-2A).

Genera specific clusters for different species of *Aspergillus* and *Fusarium* were observed. This indicates sequence level similarity among xylanases representing specific genera and could be utilized to decipher specific sequence features for designing genera specific probe or primers exclusively for xylanase genes. Further distinct sub-clusters representing multiple strains of predominately *Aspergillusniger* and *Fusariumoxysporum* were also observed (Figure-2A). In case of bacterial xylanases two distinct clusters designated as I and II comprising exclusively for*Paenibacillus* and*Dictyoglomus* species were observed (Figure-2B).

Xylanase from *Fibrobacter succinogenes* occupied distinct place in the phylogenetic tree. The major clusters I and II represented predominantly multiple strains of *Paenibacillus polymyxa* and *Dictyoglomusthermophilum* indicating strain specific sequence similarity. Similarly, the phylogenetic tree for xylanases fromactinomycetes revealed two major clusters I and II with 15 and 4 members respectively. The major cluster I was further divided into three subclusters i.e. A, B, C (Figure-2C).

In case of xylanases from yeast sources, two major clusters I and II with 12 and 8 sequences were observed, which were further divided into two sub-clusters A and B respectively (Figure-

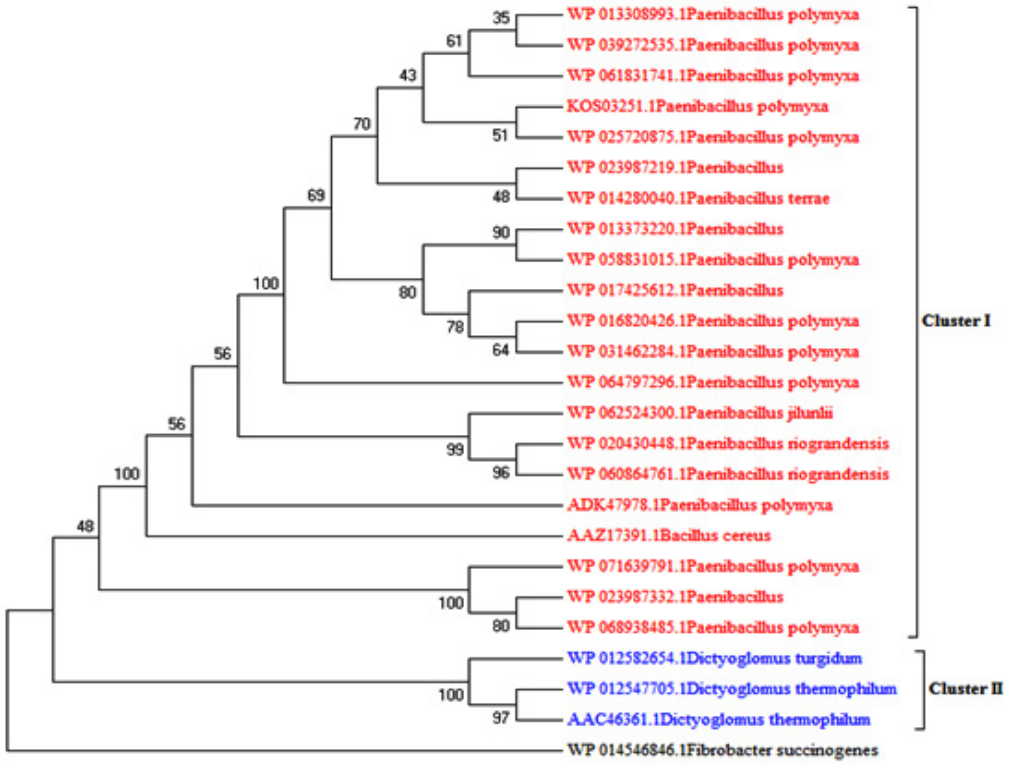


Fig. 2(B). Phylogenetic tree constructed using 25 protein sequences of xylanases from bacterial sources. The distinct major clusters designated as I and II comprising of 21 and 3 members respectively are highlighted

2D).The phylogenetic tree comprising of all the 121 sequences representing different microbial sources revealed seven distinct major clusters designated as A, B, C, D, E, F and G. These major clusters represented specific source organisms (Figure-2D). The major cluster A comprising of 19 sequences represents actinomycetes source organism exclusively while B represented bacterial sources. The major cluster C with 12 sequences represents both bacterial and fungal sources while D comprises of 22 sequences exclusively from *Aspergillus* genera.

The major cluster E included 3 sequences of yeast genera, F included 21 sequences

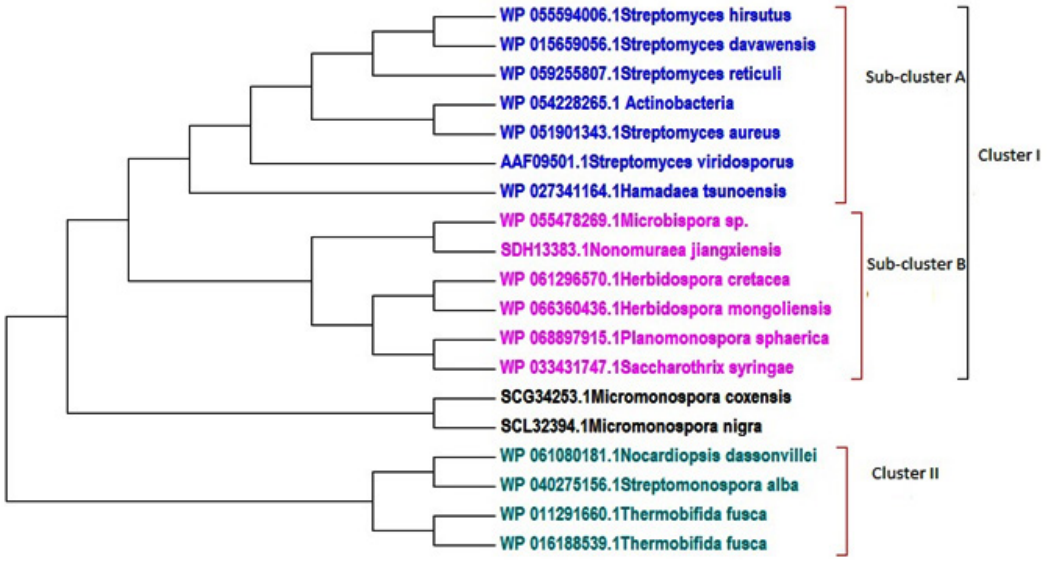


Fig. 2(C). Phylogenetic tree constructed using 19 xylanase protein sequences of actinomycetes. The distinct major clusters designated as I and II comprising of 15 and 4 members respectively are highlighted

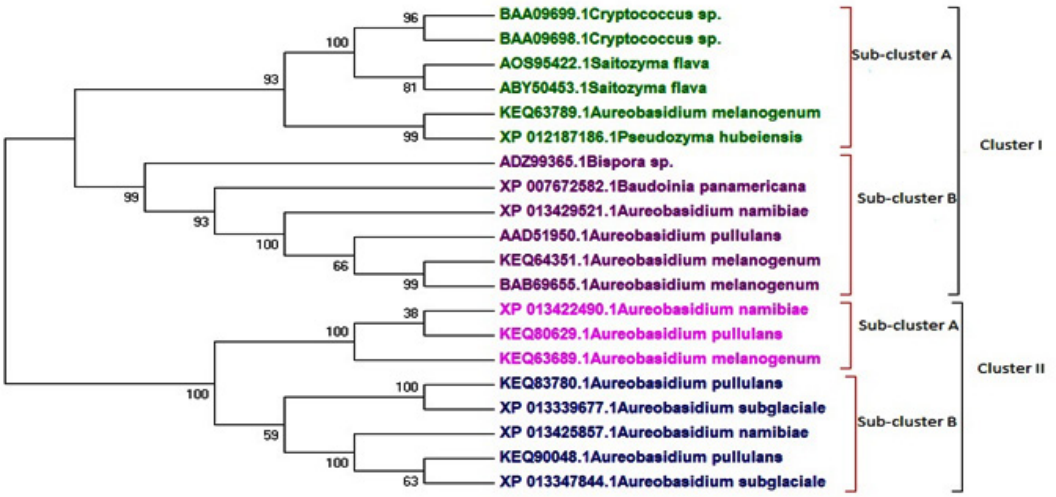


Fig. 2(D). Phylogenetic tree constructed using 20 xylanase protein sequences of yeast. The distinct major clusters designated as I and II comprising of 12 and 8 members respectively are highlighted

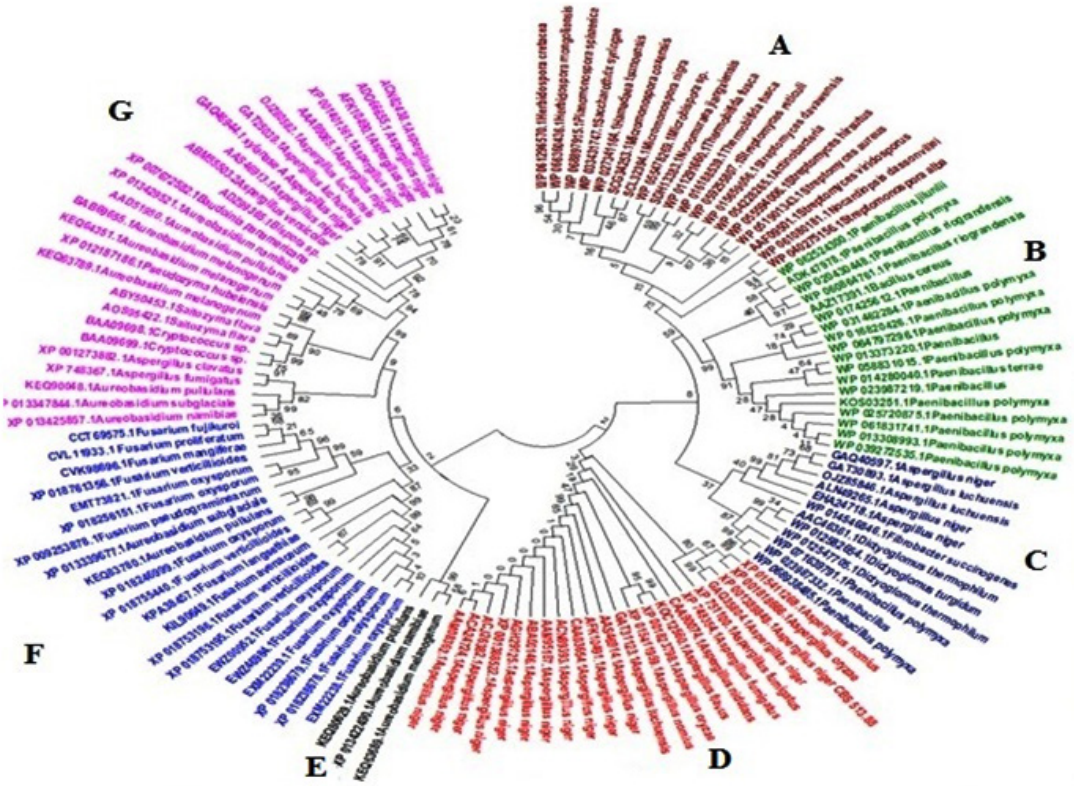


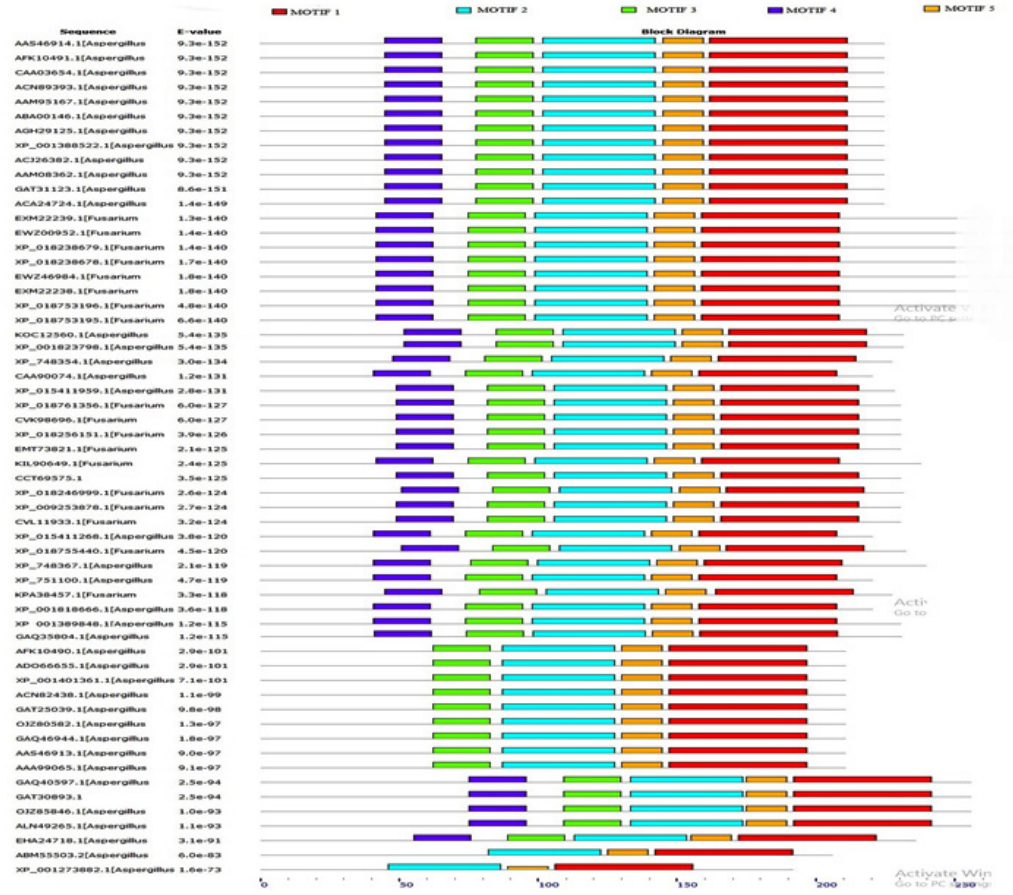
Fig. 2(E). Phylogenetic tree constructed using 122 protein sequences of xylanase representing different microbial sources. The major clusters designated as A,B,C,D,E,F and G is highlighted

predominantly from *Fusarium*genera along with some sequences from yeast and the major cluster G with 27 sequences comprises of both fungal and yeast source organisms (Figure-2D). Phylogenetic tree revealing xylanases representing GH10 and GH11 family and also basidiomycetes and ascomycetes specific fungal groups have been reported[32,29](Cervantes*et al*.,2016; Ellouz*et al*.,2011). Distinct clades representing GH10, GH11 and GH30 family revealing evolutionary relatedness based on 22 protein sequences of xylanaseswere also deciphered[33](Liao *et al*., 2015).

The conserved motifs deduced by MEME are generally analyzed for biological function using protein BLAST and domains are characterized by Interproscanto reveal the best possible match based on highest similarity score.The distribution of five motifs among microbial xylanase protein sequencesis shown in Figure 3A, B, C and D.

The distribution of five motifs among 58 fungal xylanase protein sequences was analyzed (Figure3A) and motifs with width and best possible match amino acid sequences is shown in Table-2A. The predominance of motifs with conserved domain representing unique feature of GH11 family was observed. The motif 1 with amino acid sequence IDGTATFTQYWSVRQNKR S S G T V T T S N H F N A W A K L G M N L G T H N Y Q I V A T E and motif 2 with sequence P S G N G Y L S V Y G W TTNPLVEYYIVESYGTYNPGSGGTYKGTV was uniformly distributed among fungal xylanases. Similarly the motif assessment for bacterial (Figure-3B, Table-2B), actinomycetes (Figure-3C, Table-2C) and yeast (Figure-3D, Table-2D) source organisms revealed predominance of conserved domains specific to GH11 family.

The comprehensive analysis of all the xylanases sequences, irrespective of source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E-value
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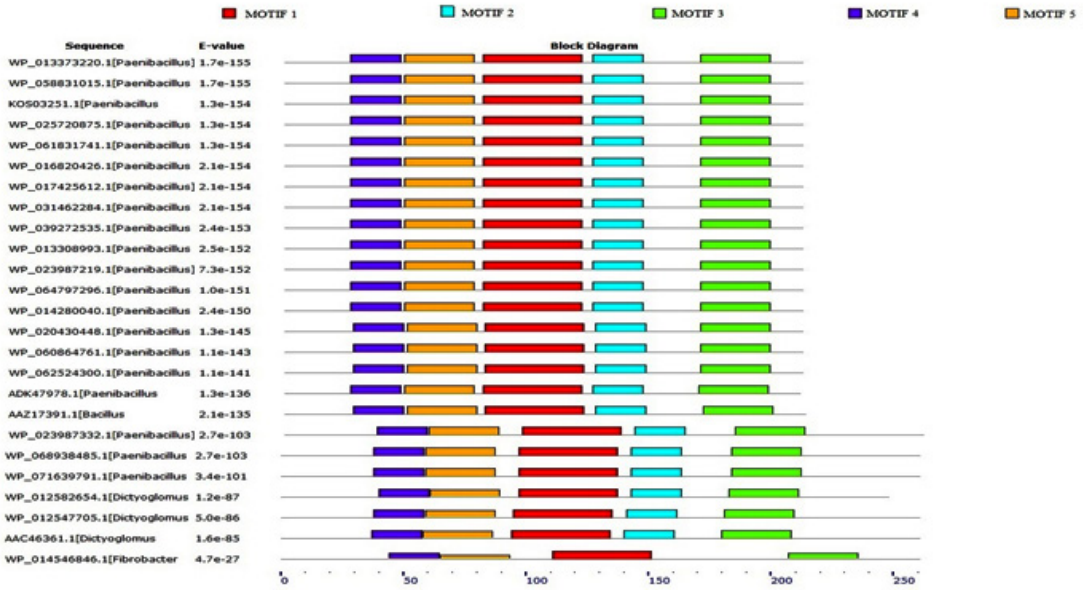
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MOTIF 5
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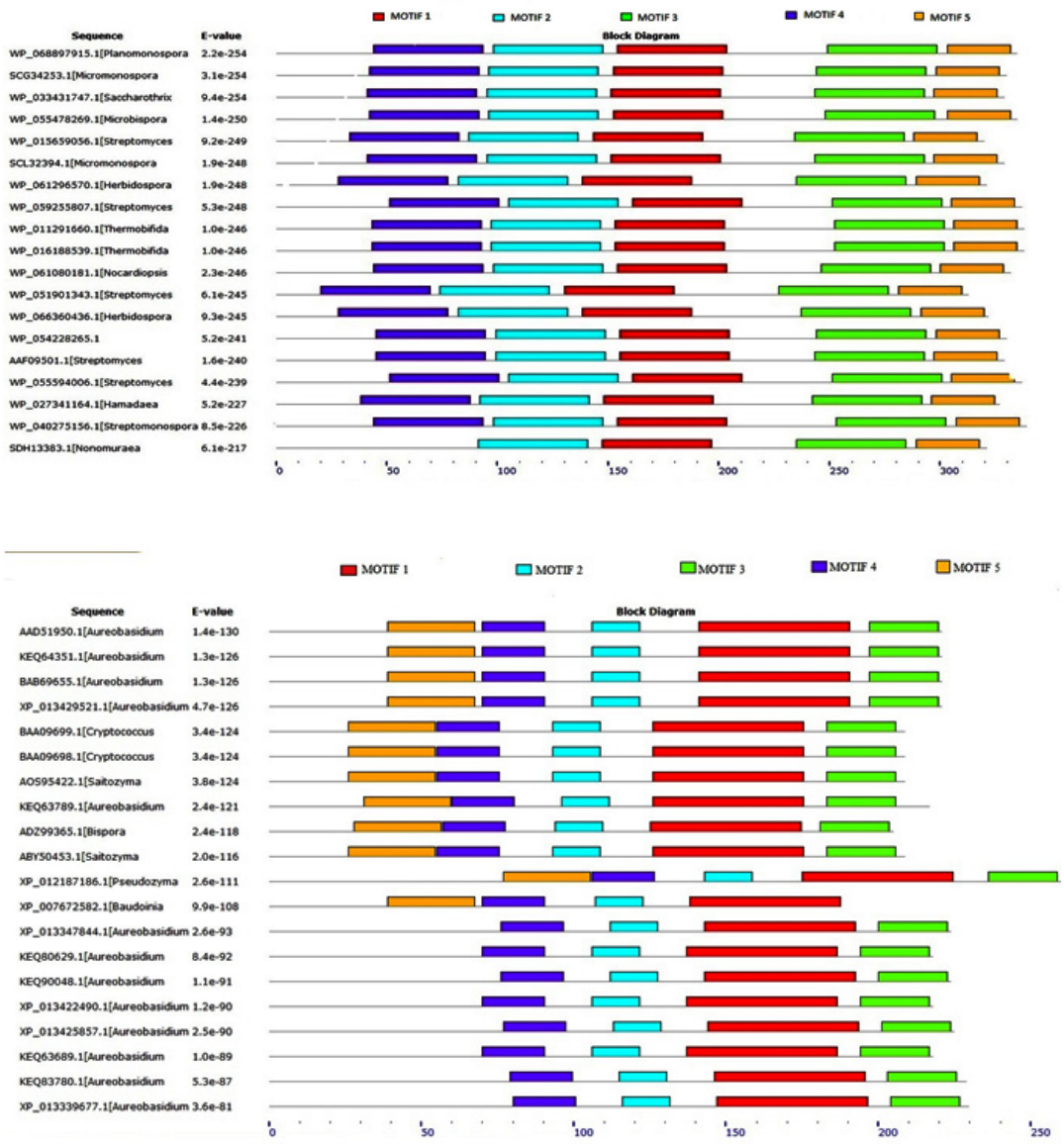
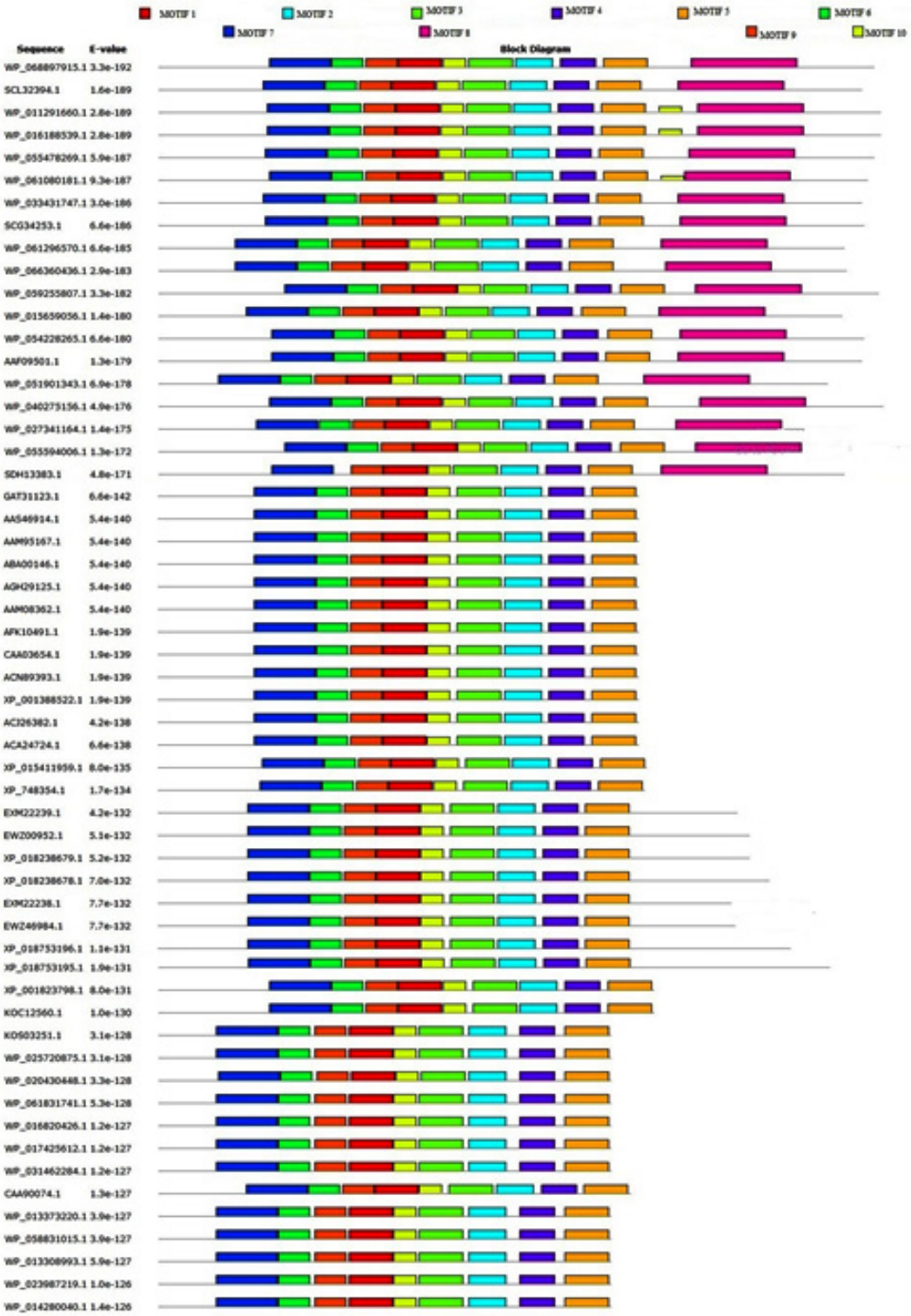


Fig. 3. Distribution of 5 commonly observed motifs among xylanases representing different microbial sources (A) Fungal (B) Bacterial (C) Actinomycetes and (D) Yeast sources

organisms for motif distribution and domain characterization is shown in Figure-3E and Table-2B respectively. A total of 10 motifs among the 122 xylanase protein sequences revealed 6 motifs with domains specific to GH11 family. The Motif 3 with sequence GTVTSDGGTYDIYTTTRTNAP was found to be highly conserved and was found uniformly among most of the microbial xylanase sequences analyzed. The sequence motifs could be considered as signature sequence revealing the functional identity of the proteins or enzymes and could be targeted for enzyme engineering. The motif assessment also provides an insight into the structural and functional diversity of the enzymesas reported[34] (Mohammed *et al*., 2011). Motif assessment for Endo-1,4-â-xylanase of GH11 family from source organism *Paecilomycesvariotii*, *Schizophyllum commune* and

Table 2(A). The best possible match amino acid sequences of five motif with respective domains observed for xylanasesin*Fungal, Bacterial, Actinomycetes and Yeast*

Motif no.	Sequence length	Sequence	Occurrence at different site	Conserved Domain
		(A) *FUNGAL*		
1	50	IDGTATFTQYWSVRQNKRSSGTVTTSNHFNAWAKLGMNLGTHNYQIVATE	58	GH11family
2	41	PSGNGYLSVYGWTTNPLVEYYIVESYGTYNPGSGGTYKGTV	58	GH11family
3	21	GNFVGGKGWNPGSARTITYSG	56	GH11family
4	21	NNGFYYSFWTDGGGDVTYTNG	47	GH11family
5	15	DGSTYDIYTTTRTNA	57	No information
		(B) *BACTERIAL*		
1	41	AGVWAPSGNGYLALYGWTRNSLIEYYVVDSWGTYRPTGTYK	25	GH11family
2	21	SDGGTYDIYTTMRYBAPSIEG	24	GH11family
3	29	ITFSNHVKAWASKGMNLGSNWSYQVLATE	21	GH11family
4	21	AATDYWQNWTDGGGTVNAVNG	21	No information
5	29	GGNYSVTWKBTGNFVVGKGWTTGSPNRTI	21	GH11family
		(C) *ACTINOMYCETES*		
1	50	YDIYKTTRYNAPSIEGTRTFDQYWSVRQSKRTGGTITSGNHFDAWARAGM	19	GH11family
2	50	RRSVTYSGSFNPSGNAYLTLYGWTRNPLVEYYIVDNWGTYRPTGTYKGTV	19	GH11family
3	50	CTATLSAGQQWSDRYNLNVSVSGSSNWTVTMNVPSPAKVJSTWNVSASYP	19	No information
4	50	VTTNQTGTNNGYFYSFWTDSQGTVSMELGSGGNYSTSWRNTGNFVAGKGW	18	GH11family
5	29	LTARPNGNGNNWGVTIQHNGNWTWPTVSC	19	No information
		(D) *YEAST*		
1	50	SDGSTYDVCTDTRTNQPSITGTSTFKQYWSVRQNKRTSGTVTTQNHFNYW	20	GH11family
2	16	WTNSPLVEYYVIESYG	20	GH11family
3	23	GSYNYQVMATEGFSGSGSASVTV	19	GH11family
4	21	NTDFVVGLGWSTGAARTITYS	20	GH11family
5	29	INYVQNYNGNVANFTYNZNAGTYSMNWNN	12	No information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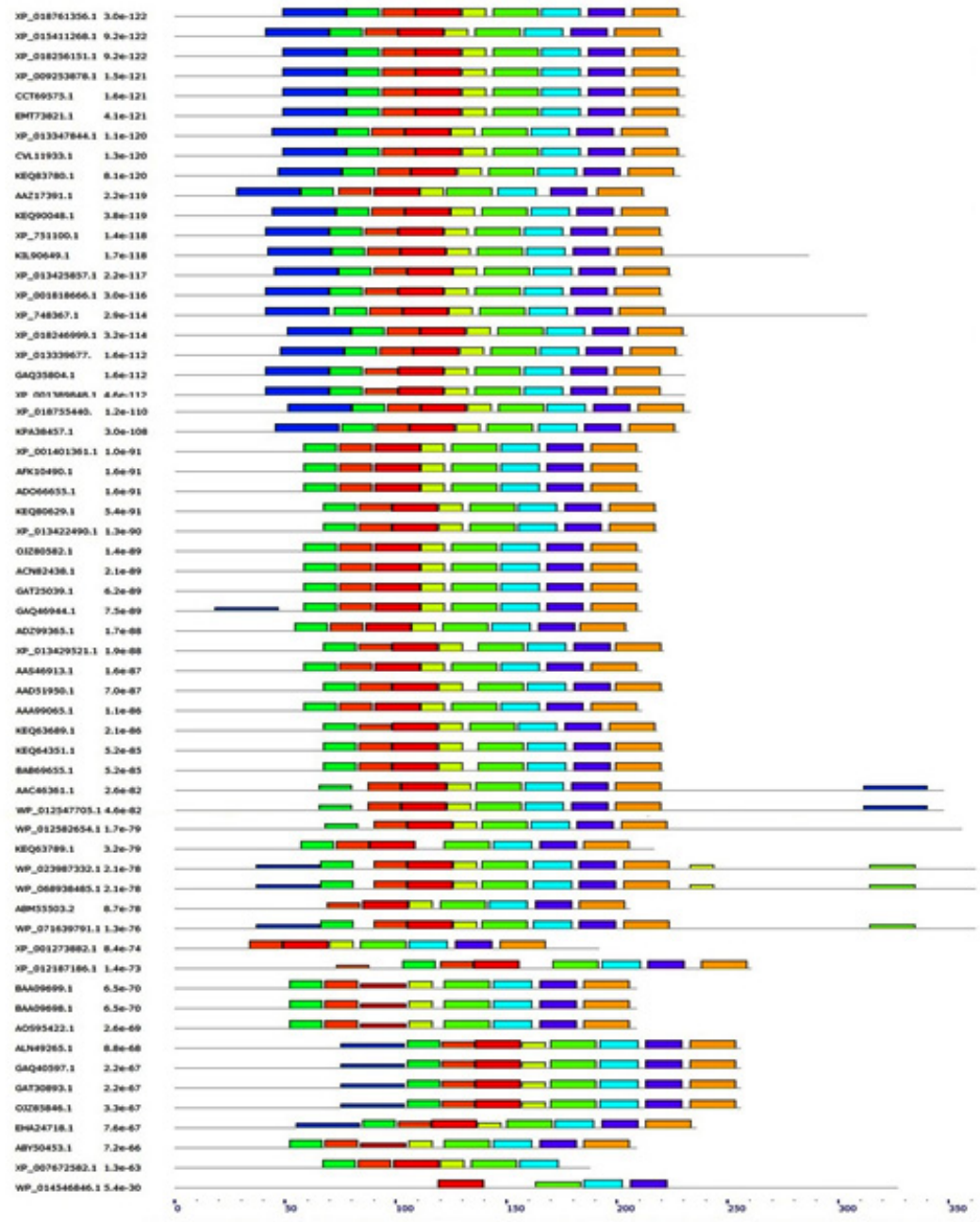

Fig. 3(E). Distribution of 10 commonly observed motifs among 122 xylanase protein sequences

*Trichodermaharzianum*has been reported[15](Arora *et al*.,2009).

The relevance of bioinformaticsin enzyme engineering has been witnessed in recent years and several *in-silico* tools mainly focusing on prediction of three dimensional structure of enzyme based on the availability of the protein sequences is now being routinely used[35,36](Damborsky and Brezovsky, 2014; Suplatov *et al*., 2015). The *in-silico* analysis of the sequences of genes/proteins of several industrially important enzymes mainly focusing on homology search, multiple sequence

Table 2(B). The best possible match amino acid sequences of 10 motifs with respective conserved domain observed among 122 protein sequences of xylanases from different microbial sources

Motif no.	Sequence length	Sequence	Occurrence at different site	Conserved Domain
1	21	NSYLAVYGWTRNPLVEYYIVE	118	GH 11Family
2	18	IDGTATFTQYWSVRQSKR	118	GH 11Family
3	21	GTVTSDGGTYDIYTTTRTNAP	121	GH11Family
4	17	TVTTGNHFBAWASLGMN	120	GH11Family
5	21	HBYQILATEGYQSSGSSSITV	120	GH11Family
6	15	WSNTGNFVGGKGWNT	115	No information
7	29	NNGYYYSFWTDGGGTVTYTNGSGGNYSVE	84	GH11Family
8	50	CTATLSAGQQWSDRYNLNVSVSGSSNWTVT MNVPSPAKVJSTWNVSASYP	19	No information
9	15	SARTITYSGSFNPSG	120	No information
10	11	SYGTYNPGSGY	119	No information

alignment, phylogenetic tree construction and motif assessment has been reported.[37-48](Yadav*et al*., 2009; Dubey*et al*., 2010; Yadav*et al*., 2010; Malviya*et al*., 2011; Dubey *et al*., 2012; Morya*et al*., 2012; Yadav *et al*., 2012; Kumar *et al*., 2012; Dwivedi and Mishra, 2014; Mathew *et al*., 2014; Morya*et al*., 2016;; Yadav*et al*., 2017).

Molecular cloning of relevant genes coding for enzymes and its expression needs bioinformatics interventiontargeting forsubstantial improvement in enzyme for desired features. Recently,functional diversity of multiple xylanases from *Penicilliumoxalicum* GZ-2, revealing functional redundancy using bioinformatics approach has been reported.[33](Liao *et al*., 2015)

CONCLUSIONS

Using bioinformatics approach, an attempt has been made to characterize microbial xylanase sequences for several important attributes, which could be targeted for enzyme engineering to develop novel xylanases. The knowledge about the sequences is being applied for deciphering the three dimensional structure using appropriate *in-silico*toolsprior to wet-lab experimentation. The tools of bioinformatics are also relevant in the era of genomics, where several microbial genome sequences have been deciphered. This provides an opportunity to perform genome-wide identification and characterization of multigene families of industrially important enzymes and analyze the functional redundancy.There has been substantial improvement in advanced enzyme technologies including metagenomics and directed evolution based on recent bioinformatics driven approaches.

ACKNOWLEDGMENTS

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