Isolation and Characterization of Xylanose by Thermophilic Fungal Strain from Telangana Region

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ABSTRACT:- *Xylanase secretion by three strains of T. lanuginosus under study was specific with the carbon source present in the medium. Xylose was the best carbon source for the production of xylanase by GSLMBKU-10 and starch, glycerol and sucrose were next preferred carbon sources for the production of xylanase. Rest of the carbon sources induced varying amount of xylanase. D-Maltose followed by D-xylose was the best carbon source for xylanase production by GSLMBKU-12. On the other hand succinic acid, lactose and D-galactose were unfavorable for xylanase production. GSLMBKU-14 preferred D-xylose for the production of xylanase. Maltose, starch and mannose were the next preferred carbon sources for the production of xylanase, where as D-ribose was least preferred source. GSLMBKU-10 showed increasing trend with the progress of incubation period on succinic acid carbon source, where as other carbon sources were responsible for decrease in xylanase production after nine days of incubation period.PH and strains are reported in respective **Tables15-20.***

KEYWORDS - Xylanase, T. lanuginosusstrain, GSLMBKU-12.

INTRODUCTION

Xylan, is the second most-abundant polysaccharide in nature, accounting for approximately one-third of the renewable organic carbon on Earth. Schulze first introduced the term 'hemicellulose' to extracted from plant materials using a dilute alkali[1-4]. It constitutes the major component of hemicellulose, a complex of polymeric carbohydrates, including xylan, xyloglucan (heteropolymer of D-xylose and D-glucose), glucomannan (heteropolymer of D-glucose and D-mannose), galactoglucomannan (heteropolymer of D-galactose, D-glucose and D-mannose) and arabinogalactan (heteropolymer of D-galactose and arabinose).

Xylan is a branched hetero polysaccharide constituting a backbone of β-1, 4 linked xylopyranosyl units substituted with arabinosyl, glucuronyl and acetyl residues. The xylan structure, however, can differ greatly depending on its origin[5-7]. They were classified according to the nature of the linkages joining the xylose residues. β -1, 3 linked xylans were found only in marine algae, whereas the xylans containing a mixture of β -1,3 and β -1,4 linkages is present only in seaweeds and β-1, 4 linked xylans occur in softwoods, hardwoods and grasses.

The complete hydrolysis of xylan requires the combined action of various enzymes such as Endoxylanase, β-xylosidase, α-glucuronidase, α-arabinofuranosidase (α-L arabinofuranosidase and acetyl xylan esterase[8-10]. Among all xylanases, endoxylanases are the most important due to their direct involvement in cleaving the glycosidic bonds and in liberating short xylooligosaccharides. In general, the endoxylanases show peak activity between 40 and 80°C, and between pH 4.0 and 6.5, but optimal conditions have been found outside these ranges[11-13].

The degradation of xylan by microorganisms was first reported by Hoppe-Seyler. Since then number of xylanase producing microorganisms including bacteria, yeasts, Actinomycetes and filamentous fungi are identified and their xylanase production is studied. Individual fungi and bacteria can exhibit a multiplicity of endoxylanases; in some cases three or more enzyme activities have been separated from a single culture[14]. Even though various microorganisms are actively involved in the degradation of hemicelluloses, from the industrial point of view the filamentous fungi are more useful. This is because of many reasons like higher xylanase levels than those of bacteria and yeast and they can be cultivated very easily.

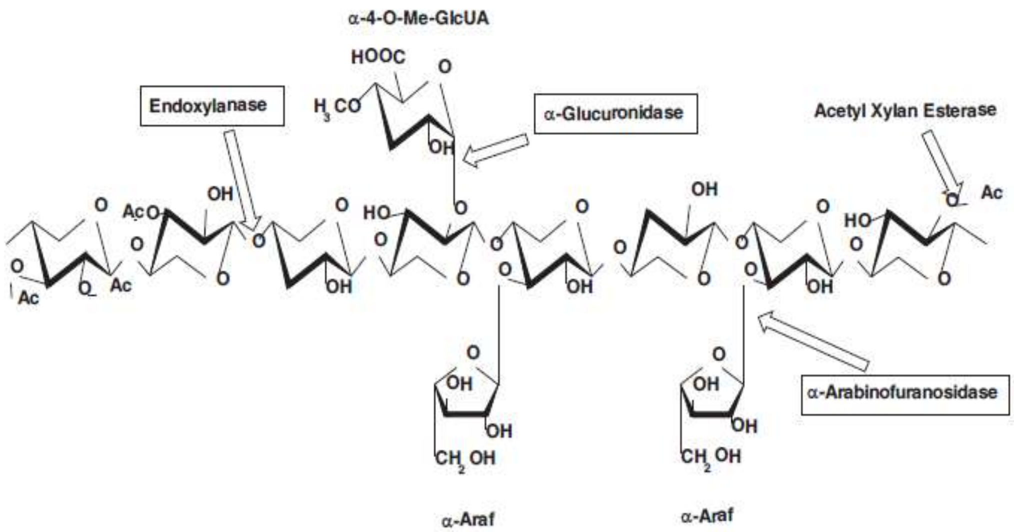

Fig1. Structure of xylan and the xylanolytic enzymes involved in its degradation. Ac: Acetyl group; α-Araf: α-arabinofuranose; α-4-O-Me -GlcA: α-4-O-methyl glucuronic acid .

Xylanases are commercially used in the pulp and paper, food, beverage, textile and animal feed industrie. In the paper and pulp industry xylanases are used for biobleaching and bioprocessing of pulp. In animal feed, they are used to improve the digestability of animal feed. Recently xylanases are also used in the production of biofuels.

MATERIALS AND METHOD

Total of thirty two fungal strains belonging to 22 thermophilic and 10 thermotolerants were screened for the xylanase activity and the results are presented in Table 15.

Xylanase production by three strains of *T. lanuginosus* on different synthetic media was studied. Simultaneously vegetative growth and pH changes were also recorded and the results are summarized in Table 16 .

A.flavus, A.nidulans, A.niger, A.terreus, Humicola grisea, *H.stellata, R. miehei, R. pusillus, Rhizopus arrhizus, R.microsporus, R.rhizopodiformis, M.pulchella*, *T.luteus,* all the strains of *T.lanuginosus* and *Torula thermophila* were exhibited clear zones around colonies on xylanase screening medium, suggesting their potential to secret exylanase, while remaining fungi were failed to produce xylanase.

Table 16 reveal that Medium E was more suitable for xylanase production by *GSLMBKU-14* whereas, medium F was poor substratum for xylanase production. In case of *GSLMBKU-10* and *GSLMBKU-12* medium C was responsible for maximum xylanase production and medium D with lowxylanase activity. Xylanase production by all the three strains of *T. lanuginosus* is significantly influenced by the addition of 0.1% xylan. Nine days incubation was optimum for the enzyme production. Medium C followed by medium B were best substrates for the growth of *GSLMBKU-10* and *GSLMBKU-12* while *GSLMBKU-14* opted medium E followed by C. Nine days incubation period was optimum for the vegetative growth in all the media tried. Minimum pH changes were recorded and the final pH remained near neutral.

Table 17 shows that *GSLMBKU-10* could grow in the pH range of 5.0 to 8.0. Maximum xylanase production observed at pH 8.0. *GSLMBKU-12* could grow in the pH range of 5.0 to 8.0. pH 7.5 followed by pH 7.0 was most suitable for xylanase production. *GSLMBKU-14* was able to grow in the pH range of 5.5 to 8.0. It failed to grow at pH 8.0. pH 7.5 followed by pH 7.0 was optimum for xylanase production. Nine days incubation period was optimum for all the pH range tried. Padmavathi and Kavya, 2011 observed pH 8.0 was most favorable for xylanase production by *A.niger.* pH 6.5 followed by pH 6.0 was most suitable for mycelial growth of *GSLMBKU-12* and *GSLMBKU-14.* But it was reverse in the case of *GSLMBKU-10* where pH 6.0 followed by pH 6.5 was most suitable for optimum vegetative growth.

RESULTS AND DISCUSSION

Table 15. Production of xylanase by different thermophilic fungi.

Name Of the Fungus

Absidia corymbifera

Acremonium thermophilum

Aspergillus fumigatus

A. nidulans

A. terreus

A. flavus

A.niger

Chaetomium thermophile. V caprophile

C. thermophile. V dissit um

Chrysosporium fergusii

Humicola grisea

H.fuscoatra

Humicola insolens

H.stellata

Rhizomucor miehei

R. pusillus

Rhizopus arrhizus

R.rhizopodiformis

Malbranchea pulchella

Myriococcum albomyces

Pencillium duponti

P.purpurogenum

Talaromyces luteus

Thermoascus aurantiacus

Thermomyces lanuginosus GSLMBKU-10

Thermomyces lanuginosus GSLMBKU-11

Thermomyces lanuginosus GSLMBKU-12

Thermomyces lanuginosus GSLMBKU-13

Thermomyces lanuginosus GSLMBKU-14

Torula thermophila

(+) appearance of zone (positive)

(-) No clearance zone (negative)

Table 16. Production of xylanase*, mycelial growth and pH changes on different synthetic media by three strains of T.lanuginosus

		GSLMBKU-10			*GSLMBKU-12*			*GSLMBKU-14*		
	Days of incubation	Dry wt (µg/ml)	pH	Xylanase (µg/ml)	Dry wt (µg/ml)	pH	Xylanase (µg/ml)	Dry wt (µg/ml)	pH	Xylanase (µg/ml)
Yeast extract Starch	6	146	5.8	106	135	6.3	92	158	6	85
medium[A]	9	197	6.9	146	154	6.7	126	175	6.5	97
	12	251	7.2	124	235	7.2	75	194	7	65
Medium A+	6	169	6.2	128	147	6.5	105	191	5.9	93
0.1% xylan[B]	9	221	6.5	163	160	6.9	143	214	6.7	122
	12	260	7.4	120	247	7.1	112	238	7.2	82
Medium A+	6	191	6.4	130	148	5.9	114	195	6.2	109
1% xylan[C]	9	246	6.9	176	202	6.4	161	232	6.8	128
	12	266	7.2	142	259	6.9	103	257	7.2	95
Yeast extract Glucose	6	124	6.2	95	157	6	81	126	6.4	76
medium[D]	9	139	7	127	175	6.7	93	135	5.9	134
	12	159	7.2	108	163	7.2	78	153	6.9	113
Medium D+	6	132	5.8	104	172	6.2	92	147	5.8	87
0.1% xylan[E]	9	147	6.5	132	189	6.6	116	168	6.3	148
	12	176	6.9	101	168	6.9	98	151	7.1	91
Modified Czepek	6	183	6	126	104	6.8	83	121	6.1	43
dox medium[F]	9	192	6.6	134	134	6.2	106	127	6.5	57
	12	212	7.1	93	146	6.9	60	134	7.2	32

*Expressed in terms of xylose units (xylose/0.1ml enzyme) during 30 min of incubation.

Table 17. Effect of pH on xylanase* production, mycelial growth and pH changes by three strains of *T.lanuginosus*

		GSLMBKU-10		GSLMBKU-12			GSLMBKU-14		
	Days								
pH	of	pH	Xylanase	Dry wt	pH	Xylanase	Dry wt	pH	Xylanase
	incubation		(in units)	(µg/ml)		(in units)	(µg/ml)		(in units)
5	6	5.1	24	85	5.3	32	--	--	--
	9	5.6	39	98	5.6	53	--	--	--
	12	6	26	116	6.1	4[illegible]	--	--	--
5.5	6	5.6	77	119	6	66	123	5.8	56
	9	5.9	89	125	6.2	74	141	5.5	63
	12	6.2	52	139	6.7	52	155	6.4	37
6	6	6.2	63	152	6.2	69	153	6.2	64
	9	6.7	105	179	6.8	80	172	6.6	92
	12	7.1	86	220	7.2	55	191	6.9	46
6.5	6	6.5	101	165	6.6	83	161	6.3	108
	9	6.8	135	183	6.2	122	197	6.7	116
	12	7.2	117	216	7.1	96	209	7	86
7	6	7.3	108	128	7.2	91	137	7.3	87
	9	6.9	146	142	7.6	136	149	7.4	121
	12	7.2	126	153	7.3	117	158	7.5	92
7.5	6	7.2	125	121	7.4	115	117	7.8	97
	9	7.5	171	138	7.6	164	128	7.4	143
	12	7.1	132	146	7.8	109	137	7.6	116
8	6	7.4	124	93	7.5	66	108	7.9	58
	9	7.6	186	115	7.8	78	121	8.2	82
	12	7.8	136	107	8	52	132	7.6	53

*Expressed in terms of xylose units (xylose/0.1ml enzyme) during 30min of incubation.

Table 18. Effect of Temperature on xylanase* production, mycelial growth and pH changes by three strains of *T.lanuginosus*

Temp -	Days	GSLMBKU-10		GSLMBKU -12			GSLMBKU -14	
arature	of	Dry wt	Xylanase	Dry wt	pH	Xylanase	Dry wt	pH
	incubation	(µg/ml)	(in units)	(µg/ml)		(in units)	(µg/ml)	
35	6	91	31	95	6.2	28	84	5.5
	9	115	45	125	5.4	35	98	6.7
	12	142	36	136	7	22	121	7.1
40	6	154	68	134	5.8	34	135	6.2
	9	187	122	161	6.8	83	153	7.2
	12	195	93	175	7.2	51	165	7.4

45	6	181	97	169	6.1	76	159	6.1
	9	227	146	183	7.2	132	174	7.2
	12	253	117	236	7.6	97	193	7.4
50	6	162	71	148	6.4	62	127	6.2
	9	189	112	164	7.3	90	139	7.2
	12	145	86	185	7.6	54	156	7.5
55	6	134	54	112	6.1	38	103	6.3
	9	148	62	123	7.3	41	116	7.1
	12	152	38	108	7.5	29	96	7.6

Table 19. Influence of different carbon sources on xylanase* production, mycelial growth and pH changes by three strains of *T.lanuginosus*

Carbon source	Days of incubation	*GSLMBKU-10*		*GSLMBKU-12*			*GSLMBKU-14*	
		Dry wt (µg/ml)	Xylanase (µg/ml)	Dry wt (µg/ml)	pH	Xylanase (µg/ml)	Dry wt (µg/ml)	pH
D-glucose	6	132	55	116	5.7	39	141	6.1
	9	145	82	134	6.4	56	164	6.7
	12	155	73	149	6.9	43	169	7.3
D-fructose	6	137	87	171	6.2	73	152	5.9
	9	148	116	215	6.5	94	202	6.4
	12	165	91	234	6.8	68	217	7
D-galactose	6	172	72	135	6.1	64	146	6.2
	9	179	128	146	6.7	107	153	6.9
	12	185	97	153	7.1	87	168	7.2
D-mannose	6	176	115	157	6.4	91	162	6.3
	9	187	145	176	7	126	173	6.6
	12	228	139	191	7.3	119	198	7.1
L-sorbose	6	179	62	163	6.3	47	155	6.5
	9	193	86	185	6.9	74	175	6.8
	12	181	51	194	7.5	39	188	7.2
D-ribose	6	127	57	153	5.9	41	121	6
	9	143	89	175	6.7	56	139	6.8
	12	156	47	184	7.2	39	145	7
D-xylose	6	142	134	136	6.1	98	121	5.8
	9	158	186	142	6.8	162	139	6.4
	12	178	129	152	7.4	119	159	7.1
Sucrose	6	147	138	134	6.4	83	136	6.2
	9	185	175	149	7.1	114	153	7
	12	194	122	162	6.9	96	175	7.4

Table 20. Influence of different nitrogen sources on xylanase* production, mycelial growth and pH changes by three strains of *T.lanuginosus*

Nitrogen source	Days of incubation	Dry wt (µg/ml)	Xylanase (in units)	*GSLMBKU-12* Dry wt (µg/ml)	pH	Xylanase (in units)	Dry wt (µg/ml)	pH
Ammonium chloride	6	163	63	132	6	51	155	6.8
	9	213	78	151	6.7	65	172	6.3
	12	203	55	163	7.1	45	189	7.2
Ammonium nitrate	6	165	58	121	6.2	64	125	6.3
	9	209	62	134	6.1	73	149	6.7
	12	182	51	152	6.9	57	167	7
Ammonium sulphate	6	178	112	158	5.8	97	182	5.9
	9	227	175	184	6	156	197	6.5
	12	205	141	192	6.6	124	207	6.9
L-arginine	6	182	84	119	6.1	71	132	5.8
	9	246	124	146	6.8	103	164	6.9
	12	229	114	162	7	94	172	7.2
L-aspargine	6	187	101	165	5.8	84	154	5.6
	9	239	132	181	6	106	224	6.3
	12	216	93	238	6.5	81	257	6.5
L-aspartic acid	6	143	39	132	5.1	32	118	4.7
	9	206	46	158	5.7	43	142	5.6
	12	174	31	173	6.7	29	159	6.4
L-glutamine	6	163	53	125	5.6	45	143	6.1
	9	211	74	146	6.4	68	172	6.6
	12	184	51	162	7.1	39	192	6.9
L-glutamic acid	6	186	46	131	5.9	41	132	5
	9	224	57	159	6.7	48	164	5.8
	12	195	41	169	6.9	42	185	6.7
L-Glycine	6	167	93	129	6.5	72	149	6.2
	9	193	108	145	6.9	95	172	6.6
	12	218	83	182	7.5	65	182	7.1

L-methionine	6	152	77	151	5.9	64	136	6.4
	9	174	105	169	6	86	150	6.9
	12	192	86	173	6.2	59	176	7.2
L-histidine	6	148	82	124	6	79	148	6.4
	9	186	97	132	6.2	95	173	6.8
	12	172	65	140	6.8	72	191	7.1
L-lysine	6	167	54	117	5.3	46	135	5.5
	9	189	63	132	6.1	51	180	6.3
	12	197	43	155	6.6	57	208	6.9
L-tryptophan	6	181	126	143	5.9	107	126	5
	9	215	152	169	6.3	163	141	5.8
	12	232	108	187	6.7	121	178	6.4
L-tyrosine	6	162	66	135	6.4	46	139	5.1
	9	211	91	159	6.8	75	187	6.2
	12	179	78	179	7.2	40	216	6.7
Yeast extract	6	151	102	152	6.6	69	182	5.9
	9	224	132	197	7.1	148	201	6.5
	12	244	112	214	7.3	114	211	7.2

*Expressed in terms of xylose units (xylose/0.1ml enzyme) during 30min of incubation.

CONCLUSIONS

Ammonium sulphate followed by tyrosine and L-asparagine supported maximum production of xylanase by *GSLMBKU-14* while aspartic acid, glysine and glutamic acid were poor nitrogen sources for the production of xylanase.

GSLMBKU-10 could achieve good mycelial growth during its growth on L-arginine, L-asparagine, tryptophan and yeast extract, while it was least in medium containing L-histidine and L-methionine. Yeast extract was more suitable nitrogen source for the vegetative growth of *GSLMBKU-12,* whereas histidine and ammonium nitrate was least preferred. L-asparagine and yeast extract were preferred nitrogen sources for maximum vegetative growth of *GSLMBKU-14* and L-aspartic acid and L-methionine were least preferred Rest of nitrogen sources supported intermediate amount of mycelial growth. Twelve days incubation period was optimum for the vegetative growth of all the three strains under investigation.

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