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THERMOSTABLE XYLANASES OF MICROBIAL ORIGIN: RECENT INSIGHTS AND BIOTECHNOLOGICAL POTENTIAL

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ABSTRACT

Xylanases are hydrolases which depolymerise the plant cell wall component-xylan, the second most abundant polysaccharide. They are mainly produced by microorganisms but can also be found in plants, marine algae, protozoans, crustaceans, insects, and snails. Because of their ability to break down xylan, these enzymes especially of microbial origin, have attracted more attention due to their potential role in pulping and bleaching processes, in food and feed industry, textile processes and organic waste treatment. Xylanases are more suitable in paper and pulp industry than lignin degrading enzymes. Owing to the increasing biotechnological importance of thermostable xylanases, many potential thermophilic and hyperthermophilic bacterial genera like Bacillus, Thermotoga, Streptomyces, Thermomyces, Pyrococcus and Sulfolobus and some fungal genera like Trichoderma, Aspergillus, Penicillum, Aureobasidium have been identified. As tolerance to higher pH and temperature are desirable properties of xylanase for effective use in pulp treatment, thermophillic organisms are of special interest as a source of novel thermostable xylanases. But for large scale production of xylanases, reduction of cost is still very challenging. This review encompasses the sources, classification, industrial and future prospects of xylanases with special reference to thermostable ones.

Key Words: Thermophilic Xylanases, Bacterial Xylanases, Fungal Xylanases , Enzyme, Diversity, Applications

INTRODUCTION

Xylanases are glycosidases (O-glycoside hydrolases) which catalyze the endohydrolysis of ß-1, 4-glycosidic bonds in xylan. First reported in 1955 (Whistler R. and Masek E., 1955)- they were originally termed pentosanases, and were recognized by the International Union of Biochemistry and Molecular Biology (IUBMB) in 1961 when they were assigned the enzyme code EC 3.2.1.8. Their official name is endo-1, 4-ß-xylanase, but commonly used synonymous terms include xylanase, endoxylanase, ß-1, 4-D-xylan-xylanohydrolase, endo-1, 4-ß-D-

xylanase, ß-1, 4-xylanase and ß-xylanase. Biodegradation of xylan is a complex process that requires the synergistic action of several enzymes. A typical xylan degrading enzyme system is composed of ß-1, 4-endoxylanase, ß-xylosidase, α-L-arabinofuranosidase, α-glucuronidase, acetyl xylan esterase and phenolic acid (ferulic and p-coumaric acid) esterase (Coughlan M.P. and Hazlewood G.P., 1993). Production of multiple xylanases is a strategy that a microorganism uses for complete hydrolysis of xylan. A variety of microorganisms, including bacteria, actinomycetes, yeasts and filamentous fungi have been reported to produce xylanases (Nascimento R.P. *et al*., 2002; Poorma C.A. and Prema P., 2007; Bakri Y. *et al*., 2008). Recently the interest in xylanases has markedly increased due to the potential application in pulping and bleaching processes using cellulase free preparations, in food and feed industry, textile processes, the enzymatic saccharification of lignocellulosic materials and organic waste treatment (Van der Broeck H.C. *et al*., 1990; Gilbert M. *et al*., 1992; GodfreyT. and West S., 1996; Mechaly A. *et al*., 1997; Wong K.K.Y. *et al*., 1998). Such enzymes provide eco-friendly alternative for effective bleaching of paper pulp without the use of toxic chlorine compounds and without adversely affecting the quality of the paper pulp. Most of these processes are carried out at high temperature, so thermostable enzymes would be of more advantageous (Sonnleitner B. and Fiechter A., 1983). Therefore, screening of thermophillic organisms is of special interest as a source of novel thermostable xylanases (Becker P. *et al*., 1997; Lee D.W. *et al*., 1999; Beg Q.K. *et al*., 2000; Touzel J.P. *et al*., 2000) for their major application in paper and pulp industry for pre-bleaching of Kraft pulp (Christov L.P. *et al*., 1999)

Xylanase: Xylan hydrolyzing enzymes

The complex structure of xylan needs different enzymes for its complete hydrolysis. Endo-1, 4-ß-xylanases (1, 4-ß-D-xylanxylanohydrolase, E.C.3.2.1.8) depolymerise xylan by the random hydrolysis of xylan backbone and 1, 4-ß-D-xylosidases (1,4-ß-D-xylan xylohydrolase E.C.3.2.1.37) split off small oligosaccharides. The side groups present in xylan are liberated by α -L-arabinofuranosidase, α –D-glucuronidase, galactosidase and acetyl xylan esterase.

Endo-xylanases are reported to be produced mainly by microorganisms like bacteria and fungi (Wong K.K.Y. *et al*., 1998; Kulkarni N. *et al*., 1999). However, there are certain reports regarding their origin from plants (Cleemput G. *et al*., 1997) and some members of higher animals also (Yamura I. *et al*., 1997). There are lots of reports on microbial xylanases starting from 1960, but with an angle of plant pathology related problems (Subramaniyan S., 2000; Lebeda A. *et al*., 2001). Only during 1980's, the great impact of xylanases has been recognized in the area of biobleaching. Exo-1, 4-ß-D-xylosidase (EC 3.2.1.37) catalyses the hydrolysis of 1, 4-ß-D-xylo-oligosaccharides by removing successive D-xylose residues from the non-reducing end. The endoxylanases reported to release xylose during hydrolysis of xylan but no activity against xylobiose which

could be easily hydrolysed by ß-xylosidases. There are reports about *Bacillus* sp. (La-Grange D.C. *et al*., 2000) and different fungi (Poutanen K., 1998) producing intracellular ß-xylosidases.

α -Arabinofuranosidases (EC 3.2.1.55) hydrolyse the terminal, non-reducing α -L-arabinofuranosyl groups of arabinans, arabinoxylans, and arabinogalactans. A number of microorganisms including fungi, actinomycetes and some bacteria have been reported to produce α -arabinosidases. The extreme thermophile *Rhodothermus marinus* is reported to produce α -L-arabinofuranosidase with a maximum yield of 6.6 IU/ml (Gomes J. *et al*., 2000). Two different polypeptides with α -arabinofuranosidase activity from *Bacillus polymyxa* were characterized at the gene level (Morales P. *et al*., 1995). α -D-glucuronidases (EC 3.2.1.1) are required for the hydrolysis of α -1, 2-glycosidic linkages between xylose and D-glucuronic acid or its 4-O-methyl ether linkage. The hydrolysis of the far stable α - (1, 2) - glycosidic linkage is the bottleneck in the enzymatic hydrolysis of xylan and the reported α - glucuronidases have different substrate requirements. Similar to lignin carbohydrate linkage, 4-O-methylglucuronic acid linkage forms a barrier in wood degradation. There are number of microorganisms reported to be producing α – glucuronidases (Hazlewood G. P. and Gilbert H. J., 1993). The complete hydrolysis of natural glucuronoxylans requires esterases to remove the bound acetic and phenolic acids. Esterases break the bonds of xylose to acetic acid [acetyl xylan esterase (EC 3.1.1.6)], arabinose side chain residues to ferulic acid (feruloyl esterase) and arabinose side chain residue to p-coumaric acid (p-coumaroyl esterase). Cleavage of acetyl, feruloyl and p-coumaroyl groups from the xylan is helpful in the removal of lignin. It may contribute to lignin solubilisation by cleaving the ester linkages between lignin and hemicelluloses. If used along with xylanases and other xylan degrading enzymes in biobleaching of pulps, the esterases could partially disrupt and loosen the cell wall structure (Puls J., 1997).

Xylanases: Families and catalytic sites

Different types of xylanases come under the category of glycosyl hydrolases and these can be further classified in to two families (Collins T. *et al*., 2005). One family has the designation of Family 10 or (F) and the other is Family 11 or (G). (Wong K.K.Y. *et al*., 1988) classified microbial xylanases into two groups on the basis of their physicochemical properties such as molecular mass and isoelectric point (pI), rather than on their different catalytic properties. While one group consists of high molecular mass enzymes with low pI values, the other group comprises of low molecular mass enzymes with high pI values, but exceptions are there. The above observation was later found to be in tune with the classification of glycanases on the basis of hydrophobic cluster analysis and sequence similarities (Sapag A. *et al*., 2002). The high molecular weight endoxylanases with low pI values belong to "glycanase family 10" formerly known as family 'F' while the low molecular mass endoxylanases with high pI values are classified as "glycanase family 11," formerly family G (Kuno A. *et al*., 2000).

Recently there has been the addition of 123 proteins in Family 11, out of which, 113 are xylanases/ORFs for xylanases, 1 unnamed protein and 9 sequences from US patent collection. But, 150 members are present in family 10, of which 112 are having xlyanase activity. (Biely P. *et al*., 1985; Biely P. *et al*., 1997) after extensive study on the differences in catalytic properties among the xylanase families concluded that endoxylanases of family10 in contrast to the members of family 11, are capable of attacking the glycosidic linkages next to the branch points and towards the non-reducing end. While endoxylanases of family 10 require two unsubstituted xylopyranosyl residues between the branches, endoxylanases of family 11 require three unsubstituted consecutive xylopyranosyl residues. According to them, endoxylanases of family 10 possess several catalytic activities, which are compatible with β-xylosidases. The endoxylanases of family 10 liberate terminal xylopyranosyl residues attached to a substituted xylopyranosyl residue, but they also exhibit aryl-ß-D-xylosidase activity. After conducting an extensive factor analysis study, (Sapag A. *et al*., 2002) applied a new method without referring to previous sequence analysis for classifying Family 11 xylanases, which could be subdivided in to six main groups. Groups I, II and III contain mainly fungal enzymes. The enzymes in groups I and II are generally 20 kDa from *Ascomyceta* and *Basidiomyceta*. The group I enzymes have basic pI values while those of group II exhibit acidic pI. Enzymes of group III are mainly produced by anaerobic fungi. Meanwhile, the bacterial xylanases are divided in to three groups (A, B and C). Group A contains mainly enzymes produced by members of the *Actinomycetaceae* and the *Bacillaceae* families, strictly aerobic gram-positive ones. Groups B and C are more closely related and contain mainly enzymes from anaerobic gram-positive bacteria, which usually live in the rumen. Xylanases from aerobic gram-negative bacteria are found in subgroup Ic as they closely resemble to the fungal enzymes of group I. Unlike previous classifications, they also reported a fourth group of fungal xylanases consisting of only two enzymes (Sapag A. *et al*., 2002).

Diverse forms of xylanases

Streptomyces sp. B-12-2 produces five endoxylanases when grown on oat spelt xylan (Vieille and Zeikus G.J., 2001). The culture filtrate of *Aspergillus niger* was composed of 15, and *Trichoderma viridae* of 13 xylanases (Kuno A. *et al*., 2000). The most outstanding case regarding multiple forms of xylanases was production of more than 30 different protein bands separated by analytical electrofocusing from *Phanerochaete chrysosporium* grown in avicel (Tsujibo H. *et al*., 1997). There are several reports regarding fungi and bacteria producing multiple forms of xylanases (Wong K.K.Y. *et al*., 1988; Tsujibo H. *et al*., 1997). The filamentous fungus *Trichoderma viridae* and its derivative *T. reesii* produce three cellulase free β-1, 4- endoxylanases (Biely P. *et al*., 1985). Due to the complex structure of heteroxylans, all of the xylosidic linkages in the substrates are not equally accessible to xylan degrading enzymes. Therefore, hydrolysis of xylan requires the action of multiple xylanases with overlapping but different specificities (Wong K.K.Y. *et al*., 1988). The

fact that protein modification (e.g. post translational cleavage) leads to the genesis of multienzymes has been confirmed by various reports (Leathers T.D., 1988; Li X.L. and Ljungdahl L.G., 1994). However, several other factors could be responsible for the multiplicity of xylanases. These include differential mRNA processing, post-secretional modification by proteolytic digestion, and post translational modification such as glycosylation and autoaggregation (Biely P. *et al*., 1985). Multiple xylanases can also be the product from different alleles of the same gene (Wong K.K.Y. *et al*., 1988) . However, some of the multiple xylanases are the result of independent genes (Coughlan M.P. and Hazlewood G.P., 1993).

Xylanase producing microorganisms

Several microorganisms including bacteria, yeasts, filamentous fungi and actinomycetes, have been reported to be readily hydrolyzing xylan by synthesizing 1, 4-ß-D endoxylanases (E.C. 3.2.18) and ß-xylosidases (EC.3.2.1.37). According to many of the early reports, pathogenicity of xylanase producers to plants was a unifying character and it was thought that ß -xylanases together with cellulose degrading enzymes play a major role during primary invasion of the host tissues (Esteban R. *et al*., 1982). There are reports regarding the induction of the biosynthesis of ethylene (Fuchs Y. *et al*., 1989) band two classes of pathogenesis-related proteins in tobacco plants by the microbial xylanases (Lotan T. and Fluhr R., 1990). Thus these points reveal that certain xylanases can elicit defense mechanisms in plants. These actions may be mediated by specific signal oligosaccharides, collectively known as oligosaccharins or it may be due to the functioning of enzymes themselves or their fragments as the elicitors (Dean J.F.D. and Anderson J.D., 1991; Dean J.F.D. *et al*., 1991). Most of the fungal plant pathogens produce plant cell wall polysaccharide degrading enzymes (Subramaniyan S., 2000; Lebeda A. *et al*., 2001). These enzymes result in the softening of the region of penetration by partial degradation of cell wall structures. Xylanases have been reported in *Bacillus*, *Streptomyces* and other bacterial genera that do not have any role related to plant pathogenicity (Esteban R. *et al*., 1982). Since the introduction of xylanases in paper and pulp and food industries (Biely P. *et al*., 1985; Viikari V. *et al*., 1994) there have been many reports on xylanases from both bacterial and fungal microflora (Kulkarni N. *et al*., 1999).

Thermophilic xylanases

A number of thermophilic (optimal growth at 50–80 °C) and hyperthermophilic (optimal growth at >80 °C) xylanase producing microorganisms have been isolated from a variety of sources, including terrestrial and marine solfataric fields, thermal springs, hot pools and self-heating decaying organic debris (Dean J.F.D. and Anderson J.D., 1991; Dean J.F.D. *et al*., 1991; Viikari V. *et al*., 1994; Harris G.W. *et al*., 1997; Vieille and Zeikus G.J., 2001; Singh S. *et al*., 2003; Sunna A. and Bergquist P.L., 2003; Cannio R. *et al*., 2004). The majority of the xylanases produced have

been found to belong to families 10 and 11, with as yet, no reported studies of thermophilic xylanases belonging to any of the other glycoside hydrolase families. Interestingly, the gene for the thermostable xylanase (half life of 8 minutes at 100 °C) from the extreme thermophilic archaeon *Thermococcus zilligii* (Sunna A. *et al*., 1997) has thus far proven noncompliant to cloning with family 10 and 11 consensus primers (Sunna A. and Bergquist P.L., 2003) suggesting that this enzyme may belong to one of the other less well studied glycoside hydrolase families i.e.,families 5, 7, 8 or 43 or indeed to another as yet unknown xylanase family.

Family 10 xylanases have been isolated from various thermophilic and hyperthermophilic organisms, including *Thermotoga* sp. (Winterhalter C. *et al*., 1995), *Caldicellulosiruptor* sp.(Zverlov V. *et al*., 1996), *Rhodothermus marinus* (Luthi E. *et al*., 1990), *Bacillus stearothermophilus* (Abou-Hachem M. *et al*., 2002), *Thermoascus aurantiacus* (Khasin A. *et al*., 1993) and *C. thermocellum(Lo Leggio L. et al., 1999)*. Indeed, a family 10 xylanase, XynA from *Thermotoga* sp. strain FjSS3-B.1 is one of the most thermostable xylanases reported to date with an apparent optimum temperature for activity of 105 °C, and a half life of 90 minutes at 95 °C(Simpson H.D. *et al*., 1991). While less frequently studied, family 11 thermophilic xylanases have also been isolated from *Thermomyces lanuginosus* (Schlacher A. *et al*., 1996; Singh S. *et al*., 2003), *Paecilomyces varioti(Kumar P.R. et al., 2000)*, *Caldicellulosiruptor sp* Rt69B.1. (Morris D.D. *et al*., 1999), *Dictyoglomus thermophilum* (McCarthy A.A. *et al*., 2000), *Chaetomium thermophilum* (Hakulinen N. *et al*., 2003) and *Nonomuraea flexuosa* (Hakulinen N. *et al*., 2003). However, *Bacillus* strain D3(Harris G.W. *et al*., 1997; Gruber K. *et al*., 1998) was the most thoroughly investigated. Xylanases from *Nonomuraea flexuosa* and *Dictyoglomus thermophilum* are among the most stable, with apparent temperature optima of 80 and 85 °C, respectively. In addition to the above mentioned xylanase producing bacteria, a number of xylanase producing hyperthermophilic archaea have also been recently reported e.g. *Thermococcus zilligii(Cady S.G. et al., 2001)* , *Pyrococcus furiosus(Cady S.G. et al., 2001)*, *Sulfolobus solfataricus(Cannio R. et al., 2004)*, *Pyrodictium abyssi (Andrade C.M.M.C. et al., 1999)* and a number of *Thermofilum strains(Andrade C.M.M.C. et al., 1999)*.

Crystal structure analyses, sequence alignments and mutagenesis studies have indicated that mesophilic and thermophilic xylanases are very similar. Enhanced stability of thermophilic xylanases is probably due to an array of minor modifications which include: an increase in the number of salt bridges and hydrogen bonds (Gruber K. *et al*., 1998; Hakulinen N. *et al*., 2003), an improved internal packing(Hakulinen N. *et al*., 2003), an increased number of charged surface residues (Turunen O. *et al*., 2002), the presence of tandem repeats of thermostabilising domains(Fontes C.M. *et al*., 1995; Winterhalter C. *et al*., 1995; Zverlov V. *et al*., 1996) and/or the introduction of disulphide bridges, particularly at the N- or C- termini or in the α-helix regions (Wakarchuk W.W. *et al*., 1994; Kumar P.R. *et al*., 2000; Turunen O. *et al*., 2001). Recently, the thermostabilising role of calcium on a modular family 10 xylanase was demonstrated(Abou-

Hachem M. *et al*., 2002), while the *Bacillus* D3 xylanase was also shown to use a very unique adaptation strategy. Here a series of surface aromatic residues form clusters or "sticky patches" between pairs of molecules and these intermolecular hydrophobic interactions are believed to contribute to the thermostability of this enzyme (Harris G.W. *et al*., 1997; Connerton I. *et al*., 1999). Collectively, or singly, all the above mentioned modifications may improve the network of interactions within the protein, thereby leading to a more rigid and stable enzyme.

Bacterial Xylanases

Bacteria, just like in case of many other industrial enzymes, fascinated the researchers for alkaline thermostable xylanase producing trait (Table 1). Noteworthy bacteria producing high levels of xylanase activity at alkaline pH and high temperature are the members of the genus *Bacillus*. *Bacillus* SSP-34 produces higher levels of cellulose deficient xylanase activity under optimum nitrogen condition (Subramaniyan S. *et al*., 2001). This bacterium produces a xylanase activity of 506 IU/ml in the optimized medium (Subramaniyan S., 2000). Earlier (Ratto M. *et al*., 1992) reported xylanase with an activity of 400 IU/ml from *Bacillus circulans*. It had optimum activity at pH 7 and 40% of its activity was retained at pH 9.2. *Bacillus stearothermophilus* strain T6, reported to be producing cellulase free xylanases, actually had some cellulolytic activity (Shoham Y. *et al*., 1992; Khasin A. *et al*., 1993; Lundgren K.R. *et al*., 1994). *Streptomyces cuspidosporus* produced 40-49 U/ml in xylan medium and was associated with cellulases (CMCase, 0.29 U/ml) (Maheswari M. U. and Chandra T. S., 2000). *Bacillus* sp. strain NCL 87-6- 10 produced 93 U/ml of xylanase in the zeolite induced medium which was more effective than Tween 80 medium (Balakrishnan H. *et al*., 2000). Another *Bacillus* sp., *Bacillus circulans* AB- 16 produces 19.28 U/ml of xylanase when grown on rice straw medium (Dhillon A. *et al*., 2000). *Streptomyces* sp. QG-11-3 was found to be producing both xylanase (96 U/ml) and polygalacturonase (46 U/ml) (Beg Q.K. *et al*., 2000). *Rhodothermus marinus* was found to be producing thermostable xylanases of approximately 1.8-4.03 IU/ml but a detectable amount of thermostable cellulolytic activity was also seen (Dahlberg L. *et al*., 1993; Hreggvidsson G.O. *et al*., 1996). Most of the other bacteria which degrade hemicellulosic materials are reported to be potent cellulase producers which include *Streptomyces roseiscleroticus* NRRL-B-11019 (xylanase 16.2 IU/ml and cellulase 0.21 IU/ml) *(Grabski A.C. and Jeffries T.W., 1991)*. The strict thermophilic anaerobe *Caldocellum saccharolyticum* possesses xylanases with optimum activity at pH values 5.5-6.0 and at temperature 70°C (Luthi E. *et al*., 1990) . (Mathrani I.M. and Ahring B.K., 1992) reported xylanases from *Dictyoglomus* sp. having optimum activities at pH 5.5 and 90°C, but with significant pH stability at pH values 5.5-9.0.

Table-1. Profile of thermostable bacterial xylanases

Microorganism	Mol. Wt. (KDa)	Optimum pH and Temperature		Stabilities at		Reference
		pH	Temperature	pH (hrs)	Temp. (hrs)	
BACTERIA						
Aeromonas caviae ME1	20	7	50	3.0-4.0	6.5-8	(Kubata *et al.*, 1992)
Bacillus amyloliquefaciens	18.5-19.6	6.8-7.0	80	9	50	(Breccia *et al.*, 1998)
Bacillus circulans WL-12	85	5.5-7	-	-	-	(Subramaniyan S. *et al.*, 2001)
Bacillus sp. strain SPS-0	99	6.0	75	-	70 (4)	*(Bataillon et al., 2000)*
*Bacillus sp.*strain 41-1(36)	36	9	50	-	-	(Nakamura *et al.*, 1993)
*Bacillus sp.*strain TAR-1	40	6	75	-	-	(Nakamura *et al.*, 1994)
Bacillus sp. strain K-1	23	5.5	60	12	50	(Ratanakhanokchai *et al.*, 1999)
Bacillus stearothermophilus T-6	43	6.5	75	-	70 (14 1/2)	(Khasin A. *et al.*, 1993)
Streptomyces T-7	20.643	4.5-5.5	60	5.0 (144)	37 (264)	(Keskar *et al.*, 1989)
Thermotoga maritima		6.5	85		95 ($12^{1/2}$)	(Bergquist *et al.*, 2001)
Thermotoga thermarum	266	6	80	-	-	(Bergquist *et al.*, 2001)
Bacillus pumilus	-	8.5	55	-	-	(Mahilrajan *et al.*, 2012)

Fungal xylanases and associated problems

The optimum pH for xylan hydrolysis is around 5 for most of the fungal xylanases although they are normally stable at pH 3 - 8 (Table 2). Most of the fungi produce xylanases, which tolerate temperatures below 50 °C. In general, with rare exceptions, fungi reported to be producing xylanases having an initial cultivation pH lower than 7. Nevertheless it is different in the case of bacteria (Table 1). The pH optima of bacterial xylanases are in general slightly higher than the pH optima of fungal xylanases (Khasin A. *et al.*, 1993).

Table-2. Profile of thermostable fungal xylanases

Microorganisms	Mol. Wt. (KDa)	Optimum pH and Temperature		Stabilities at		Reference
		pH	Temperature	PH (hrs)	Temp. (hrs)	
FUNGI						
Acrophialophora nainiana	22	7.0	55	-	60 (1)	(Salles *et al.*, 2000)
Aspergillus awamori	39	5.5-6	55	-	-	(Kormelink *et al.*, 1993)
Aspergillus nidulans	34	6	56	4.0-6.7	56	(Fernadez *et al.*, 1994)
Aspergillus sojae	32.7	5.0	60	5-8 (24)	50 (10 minutes)	(Kimura *et al.*, 1995)
Aureobasidium pullulans Y-2311-1	25	4.8	54	4.5	50	(Kang *et al.*, 1996)
Aureobasidium pullulans ATCC 42023	21	3-4.5	35	-	-	(Vadi *et al.*, 1996)
*Cephalosporium sp.*strain RYM-202	35	7.5-8.0	50	-	-	(Kang *et al.*, 1996)
Humicola insolens	6.0	6-6.65	55-60	-	-	(Vadi *et al.*, 1996)
Penicillium purpurogenum	33	7.0	60	6.0-7.5 (24)	40 (3)	(Belancic *et al.*, 1995)
Trichoderma longibrachiatum	37.7	5-6	45	5	-	(Chen *et al.*, 1997)
Trichoderma viridae	22	5	53	-	-	(Ujiie *et al.*, 1991)
Thermomyces lanuginosus C_{la}	-	6.0	55	5-9	55	(Mendoza *et al.*, 2006)

In most of the industrial applications, especially paper and pulp industries, the low pH required for the optimal growth and activity of xylanase necessitates additional steps in the subsequent stages which make fungal xylanases less suitable. Although high xylanase activity was reported from several fungi, the presence of considerable amount of cellulase activity and lower pH optima make the enzyme less suitable for pulp and paper industries. (Gomes J. *et al.*, 1992) reported xylanase activity of 188.1 U/ml at optimum pH 5.2 and FPase activity of 0.55 U/ml at optimum pH 4.5 from *Trichoderma viridae*. Similar to *T.viridae*, *T. reesei* was also known to produce higher xylanase activity, approximately 960 IU/ml and cellulase activity - 9.6 IU/ml (Bailey M.J. *et al.*, 1993). Like *Trichoderma spp.*, *Schizophillum commune* is also one of the high xylanase producers

with a xylanase activity of 1244 U/ml, CMCase activity of 65.3 U/ml and FPase activity of 5.0 U/ml . Among white rot fungi, a potent plant cell wall degrading fungus - *Phanerochaete chrysosporium* produces a xylanase activity of 15-20 U/ml in the culture medium, but it also produces high amounts of cellulase activity measuring about 12% of maximum xylanase activity (Copa-Patino J.L. *et al*., 1993). (Singh S. *et al*., 2000) reported a xylanase activity of 3576 U/ml from *Thermomyces lanuginosus* strain. *Aspergillus niger sp.* produces only 76.60 U/ml of xylanase activity after 5.5 days of fermentation (Bi R. *et al*., 2000). Reports on fungal xylanases with negligible cellulolytic activity are very rare like the *Thermomyces lanuginosus* xylanase with traces of cellulase activity (Gomes J. *et al*., 1993). Another major problem associated with fungi is, the reduced xylanase yield in fermenter studies. Agitation is normally used to maintain the medium homogeneity, but the shearing forces in fermenter can disrupt the fragile fungal biomass leading to low productivity (Subramaniyan S. and Prema P., 2002). Higher rate of agitation speed leads to hyphal disruption which may reduce xylanase activity. Even though there are differences in the growth conditions including pH, agitation, aeration and optimum conditions for xylanase activity (Steiner W. *et al*., 1987; Grabski A.C. and Jeffries T.W., 1991; Gomes J. *et al*., 1992; Ratto M. *et al*., 1992; Copa-Patino J.L. *et al*., 1993; Gomes J. *et al*., 1993; Subramaniyan S. *et al*., 1997; Subramaniyan S. and Prema P., 1998; Subramaniyan S. and Prema P., 2002), there is considerable overlapping in the molecular biology and biochemistry of prokaryotic and fungal xylanases (Gilbert H.J. and Hazelwood G.P., 1993).

Xylanases: Industrial aspects

Bacterial xylanases having broad range of temperature and pH stability are preferred in industry *(Kulkarni N. et al., 1999)*. Similarly, xylanases extracted from actinomycetes are also operational over a broad range of reaction parameters (Beg Q. K. *et al*., 2001) whereas, fungal xylanases are stable under acidic pH conditions, varying from pH 4 to 6 only(Li X.L. and Ljungdahl L.G., 1994) .

Biobleaching of Pulp

Xylan doesn't form tightly packed structures hence is more accessible to hydrolytic enzymes. Consequently, the specific activity of xylanase is 2-3 times greater than the hydrolases of other polymers like crystalline cellulose (Gilbert H.J. and Hazelwood G.P., 1993). In the pulping process, the resultant pulp has a characteristic brown colour owing to the presence of residual lignin and its derivatives. In order to obtain white and bright pulp suitable for manufacturing good quality paper, it is necessary to bleach the pulp to remove the constituents such as lignin and its degradation products (Lundgren K.R. *et al*., 1994). Biobleaching of pulp is reported to be more effective with xylanases than with lignin degrading enzymes. This is because the lignin is cross-linked mostly to the hemicelluloses and the hemicellulose is more readily depolymerised

than lignin (Subramaniyan S. and Prema P., 2002). Removal of even a small portion of the hemicellulose can be sufficient to open up the polymer and facilitate removal of the residual lignin by mild oxidants. The principal objective of the application of biotechnological methods is the achievement of selective hemicellulose removal without degrading cellulose. Degradation of cellulose is the major problem associated with conventional pulping process, which invariably affects the cellulose fibre, and thus the quality of paper (Shoham Y. *et al*., 1992). Removal of xylan from the cell wall leads to a decrease in energy demand during bleaching. Therefore enzymatic treatments of pulp using xylanases have better prospects in terms of lower cost and improved fiber quality.

Bio- processing of Fabrics

Enzymatic treatment can significantly increase the water absorbing properties of fiber by removing complex impurities situated in the primary cell wall. The advantage associated with enzymatic treatment is the highly specific action of the enzyme. Xylanases specifically act on the hemicellulosic impurities and cause their removal. Enzymatic treatment does not cause any strength loss of the fiber (Dhiman S. S. *et al*., 2008).

Other applications of xylanolytic enzymes

In cereals like barley, arabinoxylans form the major non-starch polysaccharide. The arabinoxylanases are partly water soluble and result in a highly viscous aqueous solution. This high viscosity of cereal grain water extract might be involved in brewing problems (decreased rate of filtration or haze formation in beer) and is a negative parameter for the use of cereal grains in animal feed (Dervilly G. *et al*., 2001; Dervilly G. *et al*., 2002) . A better solution for this problem could be derived from the application of xylanases for pretreating the arabinoxylan containing substrates.

Sugars like xylose, xylobiose and xylooligomers can be prepared by the enzymatic hydrolysis of xylan (Wong K.K.Y. *et al*., 1988). Bioconversion of lignocelluloses to fermentable sugars has the possibility to become a small economic prospect. For example, xylitol- a five- carbon sugar is used as a natural food sweetener and its recovery from the xylan fraction is about 50-60% or 8-15% based on the raw material employed. A product of hemicellulosic hydrolysate, 2, 3- butanediol, is a valuable chemical feedstock because of its application as a solvent, liquid fuel, and as a precursor of many synthetic polymers and resins. Dehydration of 2, 3- butanediol yields the industrial solvent methyl ethyl ketone, which is more suited as a fuel. Another value added product obtained from hemicellulose hydrolysate is lactic acid, which is used in food, pharmaceutical and cosmetic industries.

Pretreatment of agricultural silage and grain feed by xylanases has been reported to improve its nutritional value (Gilbert H.J. and Hazelwood G.P., 1993). Most of low quality feed stuff contains large amounts of incompletely digestible nutrients and energy values. Pretreatment of these low-quality feed stuffs with xylanases improve their digestibility as it reduces viscosity and increases absorption by breaking down the non- starch polysaccharides in high fiber rye and barley based feeds.

Future prospects

A principal hurdle in the commercialization of enzymatic processes is the bulk production of enzymes at a cost effective rate. In order to meet this goal, such strategies should be explored by which cost-efficient bulk production can be achieved. Therefore, coming years will see advancement in production methods to exploit such microbial species that can easily metabolize the available waste material by using the simplest techniques at affordable prices. In future, deep knowledge of molecular aspect of xylanase and cloning in suitable expression vectors will be the major target. This is so because new industrial uses of xylanases have been explored, and such kinds of xylanases are required that are stable and active over a broad range of pH and temperature. Therefore, cloning of genes encoding for the thermophillic alkaline form of xylanases needs meticulous attention of microbiologists and molecular biologists for their commercial exploitation.

REFERENCES

Abou-Hachem M., Olsson F. and Nordberg Karlsson E., 2002. Probing the stability of the modular family 10 xylanase from rhodothermus marinus. Extremophiles 7: 483–491.

Andrade C.M.M.C., Pereira N. and Antranikian G., 1999. Extremely thermophilic microorganisms & their polymer- hydrolytic enzymes. Revista de Microbiologia, 30: 287–298.

Bailey M.J., Buchert J and Viikari., 1993. Applications of enzymes in pulp & paper industry. Biotechnol Progress 5: 147-157.

Bakri Y., Manal A. and Ghassan A., 2008. Xylanase production by a newly isolated aspergillus niger ss7 in submerged culture. Polish Journal of Microbiology, 57: 248-251.

Balakrishnan H., Srinivasan M.C., Rele M.V., Chaundhari K. and Ch&wadkar A.J., 2000. Effect of synthetic zeolites on xylanase production from an alkalophilic bacillus sp. Curr Sci 79: 95-97.

Bataillon, M., C. Nunes, A. P., N. Castillon and F. Duchiron, 2000. Purification and characterization of a moderately thermostable xylanase from bacillus sp. Strain sps-0. Enzyme Microb Technol 26: 187-192.

Becker P., Abu-Reesh I. and Markossian S., 1997. Determination of the kinetic parameters during continuous cultivation of the lipase-producing thermophile bacillus sp. Ihi-91 on olive oil. Appl Microbiol Biotechnol 48: 184-190.

Beg Q. K., Kapoor M., Mahajan L. and Hoondal G.S., 2001. Microbial xylanases & their industrial applications. App Microbiol Biotechnol 56: 326-338.

Beg Q.K., Bhushan B., Kapoor M. and Hoondal G.S., 2000. Production & characterization of thermostable xylanase & pactinase from streptomyces sp. Qg-11-3. J Ind Microbiol Biotechnol 24: 396-402.

Beg Q.K., Bhushan B., Kapoor M. and Hoondal G.S., 2000. Production & characterization of thermostable xylanase & pactinase from streptomyces sp. Qg-11-3. J Ind Microbiol Biotechnol 24: 396-402.

Belancic, A., J. Scarpa, A. Peirano, R. Diaz, J. Steiner and J. Eyzaguirre, 1995. Pencillium purpurogenum produces several xylanases: Purification and properties of two of the enzymes. J Biotechnol 41: 71-79.

Bergquist, P., M.D. Gibbs, D.D. Morris, D.R. Thompson, A.M. Uhl and R.M. Daniel, 2001. Hyperthermophilic xylanases. In Methods Enzymol 330: 301-319.

Bergquist, P., M.D. Gibbs, D.D. Morris, D.R. Thompson, A.M. Uhl and R.M. Daniel, 2001. Hyperthermophilic xylanases. In Methods Enzymol 330: 310-329.

Bi R., Sun X. and Ren S., 2000. The study on xylanase fermentation by aspergillus niger sp. Ind Microbiol 30: 53-59.

Biely P., Markovik O. and Mislovicova D., 1985. Sensitive detection of endo-1, 4 - glucanases & endo-1,4-xylanases in gels. Anal Biochem 144: 147-151.

Biely P., Vrsanska M., Tenkanen M. and Kluepfel D., 1997. Endo-xylanases families: Differences in catalytic properties. J Biotechnol 57: 151-166.

Breccia, J.D., F. Sineriz, M.D. Baigori, G.R. Castro and K. Hatti, R. , 1998. Purification and characterization of a thermostable xylanase from bacillus amyloliquefaciens enzyme Microb Technol 22: 42-49.

Cady S.G., Bauer M.W., Callen W., Snead M.A., Mathur E.J., Short J.M. and Kelly R.M., 2001. Beta-endoglucanase from pyrococcus furiosus. Methods Enzymol 330: 346–354

Cannio R., Di Prizito N., Rossi M. and Morana A., 2004. A xylan-degrading strain of sulfolobus solfataricus: Isolation & characterization of the xylanase activity. Extremophiles 8: 117–124.

Chen, C., J.L. Chen and T.Y. Lin, 1997. Purification and characterization of a xylanase from trichoderma longibrachiatam for xylooligosaccharide production. Enzyme Microb Technol 21: 91-96.

Christov L.P., Zakacs G. and Balakis H., 1999. Production, partial characterization & use of fungal cellulase free xylanases in pulp bleaching. Process Biochem 34: 511-517.

Cleemput G., Hessing M., van Oort M., Deconynck M. and Delcour J. A., 1997. Purification & characterization of ß -d-xylosidase & an endo-xylanase from wheat flour. Plant Physiol 113: 377-386.

Collins T., Gerday C. and Feller G., 2005. Xylanases, xylanase families & extremophilic xylanases. Microbiol Rev 29 3-23.

Connerton I., Cummings N., Harris G.W., Debeire P. and Breton C., 1999. A single domain thermophilic xylanase can bind insoluble xylan: Evidence for surface aromatic clusters. Biochimica et Biophysica ACTA 1433: 110–121.

Copa-Patino J.L., Kim Y.G. and Broda P., 1993. Production & initial characterization of the xylan-degrading system of phanerochaete chrysosporium. App Microbiol Biotechnol 40: 69-76.

Coughlan M.P. and Hazlewood G.P., 1993. ß-1, 4-d-xylan–degrading enzyme systems: Biochemistry, molecular biology & applications. Biotechnol App Biochem 17: 259-289.

Dahlberg L., Holst O. and Kristjansson J.K., 1993. Thermostable xylanolytic enzymes from rhodothermus marinus grown on xylan. App Microbiol Biotechnol 40: 63-68.

Dean J.F.D. and Anderson J.D., 1991. Ethylene biosynthesis inducing xylanase ii: Purification & physical characterization of the enzyme produced by trichoderma viridae. Plant Physiol, 95: 316-323.

Dean J.F.D., Gross K.C. and Anderson J.D., 1991. Ethylene biosynthesis inducing xylanase iii: Product characterization. Plant Physiol 96: 571-576.

Dervilly G., Leclercq C., Zimmerman D., Roue C., Thibault J.F. and Saulnier L., 2002. Isolation & characterization of high molecular mass water-soluble arabinoxylans from barley malt. Carbohydrate Res 47: 143-149.

Dervilly G., Thibault J.F. and Saulnier L., 2001. Experimental evidence for a semi-flexibile conformation for arabinoxylans. Carbohydr Res 330: 365-372.

Dhillon A., Gupta J.K., Jauhari B.M. and Khanna S., 2000. A cellulase-poor, thermostable, alkali tolerant xylanase produced by bacillus circulans ab -16 grown on rice straw & its application in biobleaching of eucalyptus pulp. Bioresour Technol 73: 273-277.

Dhiman S. S., Sharma J. and Battan B., 2008. Pretreatment processing of fabrics by alkalothermophilic xylanase from bacillus stearothermophilus sdx. Enzyme Microb Technol 43: 262-269.

Esteban R., Villanueva I.R. and Villa T.G., 1982. D-xylanases of bacillus circulans wl-12. Can J Microbiol 28: 733-739.

Fernadez, E., M.T., F. Pinaga, L. deGraaff, J. Visser, D. Ramon and S. Valles, 1994. Purification, characterization and regulation of the synthesis of an aspergillus nidulans acidic xylanase. App Microbiol Biotechnol 42: 555-564.

Fontes C.M., Hall J., Hirst B.H., Hazlewood G.P. and Gilbert H.J., 1995. The resistance of cellulases & xylanases to proteolytic inactivation. App Microbiol Biotechnol 43: 52–57.

Fuchs Y., Saxena A., Gamble H.R. and Anderson J.D., 1989. Ethylene biosynthesis inducing protein from cellulysin in an endoxylanase. Plant Physiol 89: 138-143.

Gilbert H.J. and Hazelwood G.P., 1993. Bacterial cellulases & xylanases. J Gen Microbiol 139: 187-194.

Gilbert M., Breu C., Aguchi M. and Saddler J.N., 1992. Purification & characterization of xylanase from the thermophillic ascomycete thielavia terrestris 255b. App Biochem Biotechnol 34: 247-259.

GodfreyT. and West S., 1996. Industrial enzymology. 2nd ed Edn., New York: New York,Stockton Press.

Gomes J., Gomes I., Steiner W. and Esterbauer H., 1992. Production of cellulase & xylanase by a wild strain of trichoderma viridae. App Microbiol Biotechnol 36: 701-707.

Gomes J., Gomes I., Terler K., Gubala N., Ditzelmuller G. and Steiner W., 2000. Optimization of culture medium & conditions for α - l- arabinofuranosidase production by the extreme thermophilic eubacterium rhodothermus marinus. Enzyme Microb Technol 27: 414-422

Gomes J., Purkarthofer H., Hayn M., Kapplmuller J., Sinner M. and Steiner W., 1993. Production of a high level of cellulase- free xylanase by the thermophilic fungus thermomyces lanuginosus in laboratory & pilot scales using lignocellulosic materials. App Microbiol Biotechnol 39: 700-707.

Grabski A.C. and Jeffries T.W., 1991. Production, purification & characterization of β -(1-4)- endoxylanase of streptomyces roseiscleroticus. Appl Environ Microbiol 57: 987-992.

Gruber K., Klintschar G., Hayn M., Schlacher A., Steiner W. and Kratky C., 1998. Thermophilic xylanase from thermomyces lanuginosus: High-resolution x-ray structure & modeling studies. Biochem 37: .13475–13485. .

Hakulinen N., Turunen O., Janis J., Leisola M. and Rouvinen J., 2003. Three-dimensional structures of thermophilic beta-1,4-xylanases from chaetomium thermophilum & nonomuraea flexuosa" comparison of twelve xylanases in relation to their thermal stability. Euro J Biochem 270: 1399–1412.

Harris G.W., Pickersgill R.W., Connerton I., Debeire P., Touzel J.P., Breton C. and Perez S., 1997. Structural basis of the properties of an industrially relevant thermophilic xylanase. Proteins, 29: 77–86.

Hazlewood G. P. and Gilbert H. J., 1993. Molecular biology of hemicellulases. London: Hemicelluloses & Hemicellulases, M. P. Coughlan, & G. P. Hazlewood, Eds, Portl and Press.

Hreggvidsson G.O., Kaiste E., Holst O., Eggertsson G., Palsdottir A. and Kristjansson J.K., 1996. An extremely thermostable cellulase from the thermophilic eubacterium rhodothermus marinus. Appl Environ Microbiol 62: 3047-3049.

Kang, M.K., P.J. Maeng and Y.H. Rhee, 1996. Purification and characterization of two xylanases from alkalophilic cephalosporium sp. Strain rym-202. Appl Environ Microbiol 62: 3480-3482.

Keskar, S.S., M.C. Srinivasan and V.V. Deshpande, 1989. Chemical modification of a xylanase from thermotolerant streptomyces: Evidence for essential tryptophan and cysteine residues at the active site. Biochem J 261: 49-55.

Khasin A., Alchanati I. and Shoham Y., 1993. Purification & characterization of a thermostable xylanase from bacillus stearothermophilus t-6. Appl Environ Microbiol 59: 1725-1730.

Kimura, I., H. Sasahara and S. Tajima, 1995. Purification and characterization of two xylanases and an arabinofuranosidase. Aspergillus sojae. J. Fermen. Bioeng 80: 334-339.

Kormelink, F.J.M., M.G.F.S.L. Leeuwen, T.M. Wood and A.G.J. Voragen, 1993. Purification and characterization of three endo (1,4)- xylanases and one xylosidase. Aspergillus awamori. J Biotechnol 27: 249.

Kubata, K.B., H. Horitsu, K. Kawai, K. Takamizawa and T. Suzuki, 1992. Xylanase i of aeromonas caviae me-i isolated from the intestine of a herbivorous insect (samia cynthia pryeri) Biosci Biotechnol Biochem 56: 1463-1146.

Kulkarni N., Shendye A. and Rao M., 1999. Molecular & biotechnological aspects of xylanases. FEMS Microbiol Rev 23: 411-456.

Kumar P.R., Eswaramoorthy S., Vithayathil P.J. and Viswamitra M.A., 2000. The tertiary structure at 1.59 a° resolution & the proposed amino acid sequence of a family-11 xylanase from the thermophilic fungus paecilomyces varioti bainier. J Mol Bio, 295: 581–593.

Kuno A., Kaneko S., Ohtsuki H., Ito S., Fujimoto Z., Mizuno H., Hasegawa T., Taira K., Kusakabe I. and Hayashi K., 2000. Novel sugar-binding specificity of the type xiii xylan-binding domain of a family f/10 xylanase from streptomyces olivaceoviridis e-86. FEBS Lett 482: 231-236.

La-Grange D.C., Claeyssens M., Pretorius I.S. and van-Zyl W.H., 2000. Co-expression of the bacillus pumilus beta-xylosidase (xynb) gene with the trichoderma reesei beta-xylanase-2 (xyn2) gene in the yeast saccharomyces cerevisiae. Appl Microbiol Biotechnol 54: 195-200.

Leathers T.D., 1988. Amino acid composition & partial sequence of xylanase from aureobasidium. Biotechnol Lett 10: 775-780.

Lebeda A., Luhová L., Sedlá M. and Jan D., 2001. The role of enzymes in plant-fungal pathogens interactions. Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz, 08: 89-111.

Lee D.W., Koh Y.S., Kim K.J., Kim B.C., Choi H.J., Kim D.S., Suhartono M.T. and Pyun Y.R., 1999. Isolation & characterization of a thermophillic lipase from bacillus thermoleovorans id-1. FEMS Microbiol Lett 179: 393-400.

Li X.L. and Ljungdahl L.G., 1994. Cloning, sequencing & regulation of a xylanase gene from the fungus aureobasidium pullulans y-2311-1. Appl Environ Microbiol 60: 3160-3166.

Lo Leggio L., Kalogiannis S., Bhat M.K. and Pickersgill R.W., 1999. High resolution structure & sequence of t. Aurantiacus xylanase i: Implications for the evolution of thermostability in family 10 xylanases & enzymes with (beta)alpha-barrel architecture. Proteins, 36: 295–306.

Lotan T. and Fluhr R., 1990. Xylanase, a novel elicitor of pathogenesis related proteins in tobacco, uses a non-ethylene pathway for induction. Plant Physiol 193: 811-817.

Lundgren K.R., Bergkvist L., Hogman S., Joves H., Eriksson G., Bartfai T., Laan J. V.D., Rosenberg E. and Shoham Y., 1994. Tcf mill trial on softwood pulp with korsas thermostable & alkaline stable xylanase t6. FEMS Microbiol Rev 13: 365-368.

Luthi E., Jasmat N.B. and Bergquist P.L., 1990. Xylanase from the extremely thermophilic bacterium caldocellum saccharolyticum: Overexpression of the gene in escherichia coli & characterisation of the gene product. Appl Environ Microbiol 36: 2677-2683.

Luthi E., Jasmat NB. and Bergquist P.L., 1990. Xylanase from the extremely thermophilic bacterium caldocellum saccharolyticum: Overexpression of the gene in escherichia coli & characterisation of the gene product. Appl Environ Microbiol 36: 2677-2683.

Maheswari M. U. and Chandra T. S., 2000. Production & potential applications of a xylanase from a new strain of streptomyces cuspidosporus. World J Microbiol Biotechnol 16: 257-263.

Mahilrajan, S., S. Balakumar and V. Arasaratnam, 2012. Screening and identification of a thermophillic and alkalophillic bacterium producing xylanase. Adv App Sci Res 3: 242-250.

Mathrani I.M. and Ahring B.K., 1992. Thermophilic & alkalophilic xylanases from several dictyoglomus isolates. Appl Microbiol Biotechnol 38: 23-27.

McCarthy A.A., Morris D.D., Bergquist P.L. and Baker E.N., 2000. Structure of xynb, a highly thermostable beta-1,4-xylanase from dictyoglomus thermophilum rt46b.1, at 1.8a°resolution. Acta Crystallogr. D: Biol. Crystallogr 56: 1367–1375

Mechaly A., Belakhov V., Shoham Y. and Bassov T., 1997. An efficient chemical-enzymatic synthesis of 4-nitrophenyl ß- xylobiosede: A chromogenic substrate for xylanase. Carbohydr Res 304: 111-115.

Mendoza, N.S., T.E.M. Caliwara and N.M. Unciano, 2006. A local isolate of non-cellulolytic, xylanolytic, and pectinolytic thermophilic fungus thermomyces lanuginosus cla Philipp J Sci 135: 113-119.

Morales P., Sendra J.M. and Perez-Gonzalez J.A., 1995. Purification & characterization of an arabinofuranosidase from bacillus polymyxa expressed in bacillus subtilis. Appl Microbiol Biotechnol 44: 112-117.

Morris D.D., Gibbs M.D., Ford M., Thomas J. and Bergquist P.L., 1999. Morris, d.D., gibbs, m.D., ford, m., thomas, j. & bergquist, p.L. Extremophiles 3: 103-111.

Nakamura, S., R. Nakai, K. Wakabayashix, Y. Ishiguro, R. Aono and K. Horikoshi, 1994. Thermophilic alkaline xylanase from newly isolated alkalophilic and thermophilic bacillus sp. Strain tar-1 Biosci Biotechnol Biochem 58: 78-81.

Nakamura, S., K. Wakabayashix, R. Nakai, R. Aono and K. Horikoshi, 1993. Purification and some properties of an alkaline xylanase from alkalophilic bacillus sp. Strain 41m-1 Appl Environ Microbiol 59: 2311-2316.

Nascimento R.P., Coelho R.R., Marques Alver S.L., Girio E.P.S. and Amaral-Collago M.T., 2002. A novel strain of streptomyces malaysiensisi from brazilian soil produces high endo-1, 4 xylanase titres. Enzyme Microb Technol 31: 549-555

Poorma C.A. and Prema P., 2007. Production of cellulose free endoxylanase from from novel alkalophilic thermotolerant bacillus pumilus by solid state fermentation & its application in waste paper recycling. Bioresour Technol 98: 485-490.

Poutanen K., 1998. Characterization of xylanolytic enzymes for potential applications. Espoo: Technical Research Centre of Finl&, Publications, Espoo.

Puls J., 1997. Chemistry & biochemistry of hemicelluloses: Relationship between hemicellulose structure & enzymes required for hydrolysis. Macromol Symp 120: 183-196.

Ratanakhanokchai, K., K.L. Kyu and M. Tanticharoen, 1999. Purification and properties of a xylan-binding endoxylanase from alkalophilic bacillus sp.Strain k-1 Appl Environ Microbiol 65: 694-697.

Ratto M., Poutanen K. and Viikari L., 1992. Production of xylanolytic enzymes by an alkali tolerant bacillus circulans strain. Appl Microbiol Biotechnol 37: 470-473

Salles, B.C., R.B. Cunha, W. Fontes, M.V. Sousa and E.X.F. Filho, 2000. Purification and characterization of a new xylanase Acrophialophora nainiana. J Biotechnol 81: 199-204.

Sapag A., Wouters J., Lambert C., P. Ioannes, Eyzaguirre J. and Depiereux E., 2002. The endoxylanases from family 11: Computer analysis of protein sequences reveals important structural & phylogenetic relationships. J Biotechnol 95: 109–131.

Schlacher A., Holzmann K., Hayn M., Steiner W. and Schwab H., 1996. Cloning & characterization of the gene for the thermostable xylanase xyna from thermomyces lanuginosus. J Biotechnol 49: 211–218.

Shoham Y., Schwartz Z., Khasin A., Gat O., Zosim Z. and Rosenberg E., 1992. Delignification of wood pulp by a thermostable xylanase from bacillus stearothermophilus strain t-6. Biodegradation, 3: 207-218.

Simpson H.D., Haufler U.R. and Daniel R.M., 1991. An extremely thermostable xylanase from the thermophilic eubacterium thermotoga. Biochem J, 277: 413–417.

Singh S., Madlala A.M. and Prior B.A, 2003. Thermomyces lanuginosus: Properties of strains & their hemicellulases. FEMS Microbiol Rev 27: 3–16.

Singh S., Pillay B., Dilsook V. and Prior B. A., 2000. Production & properties of hemicellulases by a thermomyces lanuginosus strain. J Appl Microbiol, 88: 975-982.

Sonnleitner B. and Fiechter A., 1983. Advantages of using thermophiles in biotechnological process: Expectations & reality. Trends Biotechnol 1: 74-80.

Steiner W., Lafferty R. M., G. I. and Esterbauer H., 1987. Studies on a wild strain of schizophyllum commune: Cellulase & xylanase production & formation of the extracellular polysaccharide schizophyllan. Biotechnol. Bioeng 30: 169-178.

Subramaniyan S., 2000. Studies on the production of bacterial xylanases. Ph.D. Thesis, Cochin University of Science & Technology, Kerala, India.

Subramaniyan S. and Prema P., 1998. Optimization of cultural parameters for the synthesis of endo-xylanases from bacillus ssp-34. J Sci Ind Res 57: 611-616.

Subramaniyan S. and Prema P., 2002. Biotechnology of microbial xylanases: Enzymology, molecular biology, & application. Crit Rev Biotechnol 22: 33-64.

Subramaniyan S., Prema P. and Ramakrishna S.V., 1997. Isolation & screening for alkaline thermostable xylanases. Isolation & screening for alkaline thermostable xylanases, 37: 431-437.

Subramaniyan S., Sandhia G.S. and Prema P., 2001. Control of xylanase production without protease activity in bacillus sp. By the selected nitrogen source. Biotechnol Lett 23: 369-371.

Sunna A., Moracci M., Rossi M. and Antranikian G., 1997. Glycosyl hydrolases from hyperthermophiles. Extremophiles 1: 2–13.

Sunna A. and Bergquist P.L., 2003. A gene encoding a novel extremely thermostable1,4-beta-xylanase isolated directly from an environmental DNA sample. Extremophiles 17: 63–70.

Touzel J.P., O'DonohueM., Debeire P., Samain E. and Breton C., 2000. Thermobacillus xylanilyticus gen. Nov., sp. Nov., a new aerobic thermophillic xylan-degrading bacterium isolated from farm soil. Int. J. Syst. Evol. Microbiol 50: 315-320.

Tsujibo H., Ohtsuki T., Iio T., Yamazaki I., Miyamoto K., Sugiyama M. and Inamori Y., 1997. Cloning & sequence analysis of genes encoding xylanases & acetyl xylan esterases from streptomyces opc-520. Appl Environ Microbiol 63: 661-664.

Turunen O., F.F. Etuaho K., Vehmaanpera J., Wu X., Rouvinen J. and Leisola M., 2001. A combination of weakly stabilizing mutations with a disulfide bridge in the alpha-helix region of trichoderma reesei endo-1,4-beta-xylanase ii increases the thermal stability through synergism. J Biotechnol 88: 37–46.

Turunen O., Vuorio M., Fenel F. and Leisola M., 2002. Engineering of multiple arginines into the ser/thr surface of trichoderma reesei endo-1,4-beta-xylanase ii increases the thermotolerance & shifts the ph optimum towards alkaline ph. Prot Eng, 15: 141–145.

Ujiie, M., C. Roy and M. Yaguchi, 1991. Low molecular weight xylanase from trichoderma viridae. Appl Environ Microbiol 57: 1860-1862.

Vadi, R.M., B.R.H. Strohfus and T.P. West, 1996. Characterization of a xylanase from aureobasidium pullulans. Microbios 85: 179-188.

Van der Broeck H.C., De Graaff L.L., Hille J.D.R., Van Ooyen A.J.J. and Harder A., 1990. Cloning & expression of fungal xylanase genes & use of the xylanase in bread making & preparation of feed & paper products. Eurp Pat Appl 90: 202-220.

Vieille, C. and Zeikus G.J., 2001. Hyperthermophilic enzymes: Sources, uses & molecular mechanisms for thermostability. Microbiol Mol Bio Rev 65: 1–43.

Viikari V., Kantelinen J. and Linko M., 1994. Xylanases in bleaching: From an idea to the industry. FEMS Microbiol Rev 13: 335-350.

Wakarchuk W.W., Sung W.L., Campbell R.L., Cunningham A., Watson D.C. and Yaguchi M., 1994. Thermostabilization of the bacillus circulans xylanase by the introduction of disulfide bonds. Prot Eng 7: 1379–1386.

Whistler R. and Masek E., 1955. Enzymatic hydrolysis of xylan. J Am Chem Soc 77: 1241–1243.

Winterhalter C., Heinrich P., Candussio A., Wich G. and Liebl W., 1995. Identification of a novel cellulose-binding domain within the multidomain 120 kda xylanase xyna of the hyperthermophilic bacterium thermotoga maritime. Mol Microbiol 15: 431–444.

Wong K.K.Y., Tan L.U. and Saddler J.N., 1988. Multiplicity of ß-1,4- xylanases in microorganism: Functions & applications. Microbiol Rev 52: 305-317.

Wong K.K.Y., Tan L.U. and Saddler J.N., 1998. Multiplicity of ß-1,4- xylanases in microorganism: Functions & applications. Microbiol Rev, 52: 305-317.

Yamura I., Koga T., Matsumoto T. and Kato T., 1997. Purification & some properties of endo-1,4-d-xylanse from a fresh water mollusc, pomacea insularus (de ordigny). Biosc Biotech Biochem, 61: 615-620

Zverlov V., Piotukh K., Dakhova O., Velikodvorskaya G. and Borriss R., 1996. The multidomain xylanase a of the hyper- thermophilic bacterium thermotoga neapolitana is extremely thermoresistant. Appl Microbiol Biotechnol 45: 245–247.