



The International Journal of Biotechnology

journal homepage: <http://aessweb.com/journal-detail.php?id=5022>



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, Penicillium, Aureobasidium have been identified. As tolerance to higher pH and temperature are desirable properties of xylanase for effective use in pulp treatment, thermophilic 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 β -1, 4-glycosidic bonds in xylan. First reported in 1955 (1)- 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- β -xylanase, but commonly used synonymous terms include xylanase, endoxylanase, β -1, 4-D-xylan-xylanohydrolase, endo-1, 4- β -D-xylanase, β -1, 4-xylanase and β -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 β -1, 4-endoxylanase, β -xylosidase, α -L-arabinofuranosidase, α -glucuronidase, acetyl xylan esterase and phenolic acid (ferulic and p-coumaric acid) esterase (2). 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 (3-5). 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 (6-10). 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 (11). Therefore, screening of thermophilic organisms is of special interest as a source of novel thermostable xylanases (12-15) for their major application in paper and pulp industry for pre-bleaching of Kraft pulp (16)

Xylanase: Xylan hydrolyzing enzymes

The complex structure of xylan needs different enzymes for its complete hydrolysis. Endo-1, 4- β -xylanases (1, 4- β -D-xylanxylanohydrolase, E.C.3.2.1.8) depolymerise xylan by the random hydrolysis of xylan backbone and 1, 4- β -D-xylosidases (1,4- β -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 (6, 17). However, there are certain reports regarding their origin from plants (18) and some members of higher animals also (19). There are lots of reports on microbial xylanases starting from 1960, but with an angle of plant pathology related problems (20, 21). Only during 1980's, the great impact of xylanases has been recognized in the area of biobleaching.

Exo-1, 4- β -D-xylosidase (EC 3.2.1.37) catalyses the hydrolysis of 1, 4- β -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 β -xylosidases. There are reports about *Bacillus* sp. (22) and different fungi (23) producing intracellular β -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 (24). Two different polypeptides with α -arabinofuranosidase activity from *Bacillus polymyxa* were characterized at the gene level (25). 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 (26). 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 (27).

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 (28). One family has the designation of Family 10 or (F) and the other is Family 11 or (G). (29) 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 (30). 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 (31).

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 xylanase activity. (32, 33) after extensive study on the differences in catalytic properties among the xylanase families concluded that endoxylanases of family 10 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- β -D-xylosidase activity. After conducting an extensive factor analysis study, (30) 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 (30).

Diverse forms of xylanases

Streptomyces sp. B-12-2 produces five endoxylanases when grown on oat spelt xylan (34). The culture filtrate of *Aspergillus niger* was composed of 15, and *Trichoderma viridae* of 13 xylanases (31). 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 (35). There are several reports regarding fungi and bacteria producing multiple forms of xylanases (29, 35). The filamentous fungus *Trichoderma viridae* and its derivative *T. reesii* produce three cellulase free β -1, 4- endoxylanases (32). 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 (29). The fact that protein modification (e.g. post

translational cleavage) leads to the genesis of multienzymes has been confirmed by various reports (36, 37). 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 (32). Multiple xylanases can also be the product from different alleles of the same gene (29). However, some of the multiple xylanases are the result of independent genes (2).

Xylanase producing microorganisms

Several microorganisms including bacteria, yeasts, filamentous fungi and actinomycetes, have been reported to be readily hydrolyzing xylan by synthesizing 1, 4- β -D endoxylanases (E.C. 3.2.18) and β -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 β -xylanases together with cellulose degrading enzymes play a major role during primary invasion of the host tissues (38). There are reports regarding the induction of the biosynthesis of ethylene (39) and two classes of pathogenesis-related proteins in tobacco plants by the microbial xylanases (40). 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 (41, 42). Most of the fungal plant pathogens produce plant cell wall polysaccharide degrading enzymes (20, 21). 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 (38). Since the introduction of xylanases in paper and pulp and food industries (32, 43) there have been many reports on xylanases from both bacterial and fungal microflora (17).

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 (34, 41-47). 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* (48) has thus far proven noncompliant to cloning with family 10 and 11 consensus primers (47) 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. (49), *Caldicellulosiruptor* sp. (50), *Rhodothermus marinus*



(51), *Bacillus stearothermophilus* (52), *Thermoascus aurantiacus* (53) and *C. thermocellum*(54). 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(55). While less frequently studied, family 11 thermophilic xylanases have also been isolated from *Thermomyces lanuginosus* (44, 56), *Paecilomyces varioti*(57), *Caldicellulosiruptor* sp Rt69B.1. (58), *Dictyoglomus thermophilum* (59), *Chaetomium thermophilum* (60) and *Nonomuraea flexuosa* (60). However, *Bacillus* strain D3(45, 61) 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*(62) , *Pyrococcus furiosus*(62), *Sulfolobus solfataricus*(46), *Pyrodictium abyssi* (63) and a number of *Thermophilum* strains(63).

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 (60, 61), an improved internal packing(60), an increased number of charged surface residues (64), the presence of tandem repeats of thermostabilising domains(49, 50, 65) and/or the introduction of disulphide bridges, particularly at the N- or C- termini or in the α -helix regions (57, 66, 67). Recently, the thermostabilising role of calcium on a modular family 10 xylanase was demonstrated(52), 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 (45, 68). 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 (69). This bacterium produces a xylanase activity of 506 IU/ml in the optimized medium (21). Earlier (70) 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 (53, 71, 72). *Streptomyces cuspidosporus* produced 40-49 U/ml in xylan

medium and was associated with cellulases (CMCase, 0.29 U/ml) (73). *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 (74). Another *Bacillus* sp., *Bacillus circulans* AB-16 produces 19.28 U/ml of xylanase when grown on rice straw medium (75). *Streptomyces* sp. QG-11-3 was found to be producing both xylanase (96 U/ml) and polygalacturonase (46 U/ml) (76). *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 (77, 78). 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) (79). The strict thermophilic anaerobe *Caldocellum saccharolyticum* possesses xylanases with optimum activity at pH values 5.5-6.0 and at temperature 70°C (80). (81) 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						
<i>Aeromonas caviae</i> ME1	20	7	50	3.0-4.0	6.5-8	(82)
<i>Bacillus amyloliquefaciens</i>	18.5-19.6	6.8-7.0	80	9	50	(83)
<i>Bacillus circulans</i> WL-12	85	5.5-7	-	-	-	(69)
<i>Bacillus</i> sp. strain SPS-0	99	6.0	75	-	70 (4)	(84)
<i>Bacillus</i> sp.strain 1(36)	41-36	9	50	-	-	(85)
<i>Bacillus</i> sp.strain TAR-1	40	6	75	-	-	(86)
<i>Bacillus</i> sp. strain K-1	23	5.5	60	12	50	(87)
<i>Bacillus stearothermophilus</i> T-6	43	6.5	75	-	70 (14 ^{1/2})	(53)
<i>Streptomyces</i> T-7	20.643	4.5-5.5	60	5.0 (144)	37 (264)	(88)
<i>Thermotoga maritima</i>		6.5	85		95 (12 ^{1/2})	(89)
<i>Thermotoga thermarum</i>	266	6	80	-	-	(90)
<i>Bacillus pumilus</i>	-	8.5	55	-	-	(91)



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 (53).

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						
<i>Acrophialophora nainiana</i>	22	7.0	55	-	60 (1)	(92)
<i>Asperqillus awamori</i>	39	5.5-6	55	-	-	(93)
<i>Asperqillus nidulans</i>	34	6	56	4.0-6.7	56	(94)
<i>Asperqillus sojae</i>	32.7	5.0	60	5-8 (24)	50 (10)	(95)
<i>Aureobasidium pullulans</i> Y-	25	4.8	54	4.5	50	(96)
<i>Aureobasidium pullulans</i>	21	3-4.5	35	-	-	(97)
<i>Cephalosporium</i> sp.strain RYM-202	35	7.5-8.0	50	-	-	(96)
<i>Humicola insolens</i>	6.0	6-6.65	55-60	-	-	(97)
<i>Penicillium purpurogenum</i>	33	7.0	60	6.0-7.5	40 (3)	(98)
<i>Trichoderma</i>	37.7	5-6	45	5	-	(99)
<i>Trichoderma viridae</i>	22	5	53	-	-	(100)
<i>Thermomyces lanuginosus</i> -	-	6.0	55	5-9	55	(101)

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. (102) 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 (103). 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 (104). (105) 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 (106). Reports on fungal xylanases with negligible cellulolytic activity are very rare like the *Thermomyces lanuginosus* xylanase with traces of cellulase activity (107). 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 (108). 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 (70, 79, 102, 104, 107-111), there is considerable overlapping in the molecular biology and biochemistry of prokaryotic and fungal xylanases (112).

Xylanases: Industrial aspects

Bacterial xylanases having broad range of temperature and pH stability are preferred in industry (17). Similarly, xylanases extracted from actinomycetes are also operational over a broad range of reaction parameters (113) whereas, fungal xylanases are stable under acidic pH conditions, varying from pH 4 to 6 only (37).

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 (112). 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 (72). 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 (108). 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 (71). 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 (114).

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 (115, 116). 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 (29). 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 (112). 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 thermophilic alkaline form of xylanases needs meticulous attention of microbiologists and molecular biologists for their commercial exploitation.

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