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Review Article

Microbial xylanases and their biomedical applications: a review

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ABSTRACT

Xylanases have a great potential, mainly known for industrial applications. They can hydrolyze the xylose (Hemicellulose of plant cell wall) and can be used for bio-bleaching the kraft pulp. As it reduces the requirement of harsh chemicals in the process, it can be used further to a number of bio-products with a great aggregate value. Microbial-origin xylanases can also be used in improving the nutritional quality of animal feed (e.g. food additives to poultry, piggery or fishery) and indirectly affect the humans. Additionally they can be used directly in human food in bakery, clarification of juices and in xenobiotics like tobacco processing. The great value of xylanase as a bio-bleaching agent has now a new dimension of fiber digesting agent having relevance to food, drugs and cosmetics act. This review presents some important applications of Xylanases extended up to biomedical sciences.

Keywords: Xylan, Xylanase, Industry, Medical, Veterinary, Agricultural

INTRODUCTION

Enzymes are the central attraction point in metabolic and biochemical processes. In yonder days, people were also using the enzymes in various processes of distillery and bakery. As a result of that, they are widely studied not only by the biological community, but also by the process designers, chemical engineers, production experts and other scientific peoples. Though the concept of thermostable xylanase came in early 80's (Yoshioka et al[99], 1981; Uchino et al, 1981[86]), the application of xylanase in paper and pulp industry was first reported by Viikari *et al* in 1986.[92] In their study, they have claimed that endoxylanases decrease chemicals needed for bleaching kraft pulp. Many researchers (Paice *et al* 1988[68], Clark *et al* 1990[23]) have confirmed and extended this observation.

Kraft pulp is what we get after we place a chip of wood in a pressurized vessel in the presence of hot caustic soda and sodium sulfide. The cooking process attacks and eventually dissolves the phenolic material called lignin that glues the fibers to each other in the wood. The word "kraft" means "strong" in the language of its origin. German manufacturers of pulp discovered that addition of the sulfur to a "soda cook" improved the selectivity of the process - dissolving the lignin with less damage to the cellulose (Mini-Encyclopedia of Papermaking).[58] Xylanase treatment decreases ClO_2 demand, decreasing net bleaching costs by 3.4% and has several effects on the strength of the unrefined pulp without negatively affecting customer requirements (Tolan & Collins, 2004).[83]

The scope of revised version of Food and Drugs acts, 1940 has been recently grown far more extensive – cosmeceuticals and nutraceuticals now also fall under its purview. Food additives like preservatives, emulsifiers, coloring agents, flavoring agents, jellying agents, sweeteners etc are also under this observation. Xylanase is used in fodder industry for poultry (Momtazan et al[59], 2011, Barekatain, 2012[9]), piggery (Malagutti et al, 2010[57]) and fishery (Huichang C, 2006[37]; Yildirim 2010[98]) – thus indirectly effecting humans (Verhoeven et al, 2005).[88] Application of xylanase in juice, alcohol, tobacco and bakery industry has direct impact on human health. Thus the effect of xylanase is also medically and pharmacologically much more relevant heretofore.

BIOTECHNICAL BACKGROUND OF XYLANS

Plant cell walls have three major polymeric constituents: cellulose (Insoluble fibers of β-1,4-glucan), hemicellulose (non-cellulosic polysaccharides including glucans, mannans and xylans) and legnin (A complex poly phenolic structure). Xylan is the major hemicellulaose in wood from angiosperms but is less abundant in wood from gymnosperms like ferns (Akhtar et al, 1997).[3]

Lignocelluloses are mainly secondary plant cell-wall materials which consist of lignin, cellulose and hemicelluloses. D-xylan is the major hemi-cellulose found in woods and accounts for 20 - 35% of the total dry weight of hardwood and perennial plants. The basic structure of xylan is a β-D-(1, 4)-linked xylopyranosyl residue with a few branch points. The major backbone carries relatively short side chains of variable lengths. Due to the abundance and the structural heterogeneity of xylans, xylan-degrading enzymes are diverse (Lee et al., 2003).[51]

BIOTECHNICAL BACKGROUND OF XYLANASES

Typical xylan-degrading enzymes are endo-β-xylanases (EC 3. 2. 1. 8) which attack the main chain of xylans, and β-xylosidases (EC 3. 2. 1. 37) which hydrolyze xylooligo-saccharides into D-xylose. These two enzymes, also required for complete hydrolysis of native cellulose and biomass conversion, are produced by many bacteria and fungi. Potential applications of xylanase in biotechnology include bio-pulping of wood, pulp bleaching, treating animal feed to increase digestibility, processing food to increase clarification, and converting lignocellulosic substances into feed stocks and fuels (Kim et al., 2000).[46]

Filamentous fungi are attracting greater attention than bacteria as potential sources of plant cell wall hydrolyzing enzymes such as xylanases because they secrete high levels of the enzymes into the culture medium. In search for microorganisms capable of efficiently degrading lignocelluloses, some cellulolytic microfungi including a wild strain of Aspergillus niger (ANL301) were isolated from decomposing wood-wastes in Lagos, Nigeria (Nwodo-Chinedu et al., 2005).[68] This microfungus grows effectively in mineral salt medium supplemented with sawdust or sugarcane as sole carbon sources (Nwodo-Chinedu et al., 2007).[64]

Xylanase can be used as a bio-bleach for pre-bleaching the Kraft pulp to reduce the use of harsh chemicals in paper and pulp Industry. Xylanses are not only used in pulp and paper industry or degumming of plant fiber sciences like flex, hemp, jute and ramie (Karaduman et al, 2012)[43] but also in many food supplement, veterinary, and agricultural processing.

About 300 different chlorinated organic compounds have been identified in bleaching pulp mill effluents (Verma et al, 2012).[90] About 200 of these have chlorinated resin acids, chlorinated phenolics and dioxin. These compounds have been classified as acidic, phenolic and neutral and are partly responsible for oxygen demand (OD), effluent color, toxicity mutagenicity and carcinogenicity - untreated pulp and paper mill effluents can be extremely toxic to aquatic life too. This chemical pollution can be minimized by extensive use of xylanases (Zhang et al[100], 2008; Yadav et al[97], 2012).

MICROBIAL SOURCES OF XYLANASES

The multifunctional xylanolytic enzyme system is wide spread among Fungi (Belancic *et al* 1995)[12], *Actinomycetes* (Suneetha V et al, 2011)[81] and bacteria (Dey et al 1992).[25] Table 1 summarizes the biochemical properties of some acidic, alkaline, and thermostable xylanases reported in literature.

RECENT ARRIVALS

For obtaining industrially important xylanases alkaliphilic organism and thermophilic organism have been preferred. Alkaline xylanases are important due to their applications in pulp and kraft bleaching. Horikoshi and Atsukawa reported alkaliphilic bacteria for xylanase production for the first time in 1973.

The enzymes from Bacillus sp. TAR-1, C-125 (Nakamura et al 1994)[63] and alkaliphilic Bacillus sp (NCL-86-6-10) (Balakrishna *et al*., 1992)[6] were optimally active at pH 9-10. The Xylanase from *Cephalosporium* was the only one reported from an alkaliphilic fungus having activity at broad pH range of 6.5-9 (Bansod *et al* 1993).[7] Alkali/thermo-stable xylanase gene from *Bacillus* sp. StrainNG-27 in tobacco plants active up to 42^{o}C have been shown by the turn of century (Leelavathi et al, 2003).[52]

Soon halo-alkali-thermo-tolerant varieties were known - retaining more than 80% of activity at 30% NaCl and 76% of activity at pH 10.5 (Giridhar et al, 2010).[30] In some strains, optimal xylanase activity was observed at pH 6.4 and temperature 55°C and xylanase is active up to pH 9 (40.33 IU/ml) and temperature 85°C (48.81 IU/ml) (Agnihotri et al, 2010)[1] and A 27-fold higher production was achieved by cloning and expression (Verma et al, 2011).[89]

Recently thermostable xylanase retaining activity at 100^{o} C for 20 minutes have been found (Hokanson et al, 2011).[35] *Bacillus haloduras* is a wild (non-genetically manipulated) organism yielding thermo-alkali stable xylanase that acts in submerged fermentation at pH 10.0 (Kumar et al, 2012).[48] To the contrary, thermo-acid stable variants act at pH 2.0 and temperature 80°C (Apel et al, 2008).[4]

Table 1: Characteristics of xylanases from different microorganisms (kDa kilodaltons).

Microorganism	pl = Isoelectric Point	Molecular weight (kDa)	Optimum		References
			pH	Temperature (^{0}C)	
Bacteria					
Acidobacterium capsulatum	7.3	41	5	65	Inagaki et al. 1998[39]
Bacillus circulans WL-12	9.1	15	5.5-7	-	Joshi et al. 2008[41]
Bacillus stearothermophilus T-6	7,9	43	6.5	55	Khasin et al. 1993[45]
Bacillus polymyxa CECT 153	4.7	61	6.5	50	Morales et al. 1995[60]
Bacillus sp. strain K-1	-	23	5.5	60	Ratannaka- nokchai et al. 1999[72]
Bacillus sp. NG-27	-	-	7, 8.4	70	Gupta et al. 1992[31]
Cellulomonas fimi	4.5-8.5	14-150	5-6.5	40-45	Khanna 1993[44]
Cellulomonas sp. N.C.I.M. 2353	8	22,33,53	6.5	55	Chaudhary and Deobagkar 1997[17]
Staphylococcus sp. SG-13	-	60	7.5, 9.2	50	Gupta et al. 2000[32]
Thermoanaerobacterium sp. JW/SL-YS485	4.37	24-180	6.2	80	Shao et al. 1995[75]
Thermotoga maritima MSB8	5.6	40, 120	5.4, 6.2	92-105	Winterhalter and Liebel 1995[94]
Fungi					
Aspergillus niger ANL-301	9	13.5-14.0	5.5	45	Okafor et al 2010[66]
Aspergillus kawachii IFO 4308	3.5-6.7	26-35	2-5.5	50-60	Ito et al. 1992[40]
Aspergillus sojae	3.5,3.75	32.7, 35.5	5, 5.5	60,50	Kimura et al. 1995[47]
Aspergillus sydowii MG 49	-	30	5.5	60	Ghosh and Nanda 1994[29]
Cephalosporium sp.	-	30,70	8	40	Bansod et al. 1993[7]
Fusarium oxysporum	-	20.8,23.5	6	60,55	Christako-polous et al.1996[21]
Geotrichum candidum	3.4	60-67	4	50	Radionova et al. 2000[72]
Penicillium purpurogenum	8.6, 5.9	33,23	7,3.5	60,50	Belancic et al. 1995[12]
Thermomyces lanuginosus DSM 5826	4.1	25.5	7	60-70	Cesar and Mrsa 1996[16]
Trichoderma harzianum	-	20	5	50	Ahmed et al 2011[2]
Trichoderma reesei	9,5.5	20,19	5-5.5, 4-4.5	45,40	Tenkanen et al. 1992[82]
Yeast		-	-		
Aureobasidium pullulans Y-2311-1	9.4	25	4.4	54	Li et al. 1993[53]
Cryptococcus albidus	-	48	5	25	Morosoli et al. 1986[62]
Trichosporon cutaneum SL409	-	-	6.5	50	Liu et al. 1998[55]

Streptomyces sp B-12-2	4.8-8.3	23.8,40.5	6-7	55-60	Elegir et al. 1995[26]
Streptomyces thermoviolaceus OPC-520	4.2,8	33,54	7	60-70	Tsujibo et al. 1992[85]
Streptomyces viridisporus T7A	10.2-10.5	59	7-8	65-70	Magnuson and Crawford 1997[56]
Streptomyces sp. QG-11-3	-	-	8.6	60	Beg et al. 2000[10]
Thermomonospora curvata	4.2-8.4	15-36	6.8-7.8	75	Stutzenberger and Bodine 2008[80]

A cellulase-free, thermo-alkali-stable, salt- and solvent-tolerant xylanase (Bhxyl) showed 60% activity even at pH 12. It is highly tolerant (> 85%) to organic solvents (50% v/v) and surfactants (1%) (Woldesenbet et al, 2012).[95]

INDUSTRIAL PRODUCTION OF XYLANASE

Xylanase production depends on Media composition and inducing substrate. Filamentous fungi produce more Xylanase than the Yeast and bacteria but in the very beginning, fungal Xylanases was found generally associated with cellulase (Velkova et al, 2007).[87]

Trichoderma & Aspergillus species produces xylanase by using pure Xylan as substrate for enzyme production. These strains produces both cellulase and xylanase on using cellulose as a substrate, which may be due to the presence of traces of hemi cellulose in the cellulosic substrates (Biely 1993)[14], The process that controls the extra cellular enzyme-production according to the carbon sources of medium are influenced by the availability of precursors for protein synthesis.

Lower nitrogen/carbon ratio in the medium may be one of the strategies for cellulase free xylanase production (Biely 1991).[13] Cellulosic substrates in the medium were also found to be essential for the maximum xylanase production (Stutzenberger and Bodine 2008).[80] Agro waste substrates like corncob, rice straw, wheat straw, wheat bran, corn stalk and bagasse can be used as a substrate for xylanase production by certain microorganisms like *Aspergillus awanian* and *Penicilluim purpurogenum* (Ravanal et al, 2012).[74]

Cellulase free Xylanase producer has been reported in Bacillus sp and fungi (Dey *et al.*, 1992).[25] Xylanase activity is found to be higher in fungal system (with maximum activity of 3350 IU/ml In *Trichoderma reesei*) than Bacterial systems (Haapala *et al* 1994).[33] Maximum activity (22,700 IU/g) in solid-state formation was achieved from the fungus *Schizophyllum commune* (Haltrich *et al* 1995).[34]

Fungi generally require acidic pH but *Actinomycetes* and bacteria require neutral or alkaline pH optima for xylanase production. *Trichoderma reesei* (Tenkanen et *al* 1992)[82] *Thermomyces* (Bajpai 1999)[5], *Aureobasidium pullulans* (Christov *et al.*, 1999)[22]. *B. subtilis* (Wilson et al, 1999) are some of the strain for xylanase production at commercial level.[93]

Due to their Industrial potential, microbial xylanolytic enzymes have drawn a great attention in the last decade. The most promising and wide spread use of Xylanase is in the prebleaching of kraft pulps (Bajpai 1999).[5] On the laboratory scale, Xylanases come from Streptomyces roseiscleraticus (Patel *et al.*, 1993)[71] and Actinomycetes (Davis *et al.*, 1992)[24]. T. harzianum (Ahmed et al, 2011)[2] and Humeeala Sp. (Silva *et al.*, 1994)[77] have been used for enzymatic pulp treatment to check their bleach boosting abilities.

Xylanase enzyme from *Thermatoga maritima* was compared with commercial pulpzyme Hc and was found to be efficient in releasing lignin from kraft pulp (Chen *et al.*, 1997)[20]. The cloned xylanase expressed in *Bacillus cereus* (Tremblay and Archibold 1993)[84] and in E. coli (Chen et al, 2012)[20] have also been reported to improve the delignification of unbleached kraft pulps. Xylanases produced by many alkali tolerant strains having pH optima around 9 have been used for bio bleaching.

Thermostable Xylanase produced by *Dictyoglomus sp* has been evaluated for its suitability in pulp bleaching (Ratto *et al.*, 1994).[73] Xylanase from *Bacillus stereothermophilus* T-6 at 65^{o}C and pH 9 bleached the pulp effectively and has been industrially used in successful Metl trial (Lapidot 1996).[49] Novo Nordisk A/S under the brand name of "Pulpzyme HA' marketed first commercial xylanase produced by *T. ressei.* Later on, new enzymes from bacterial source were also sold under the same brand name.

Sandoz chemicals has also marketed it as 'Cartazyme HS". Ecopulp (from Alko-ICI), cartazyme NS-10 (from clariant) and pulpzyme (from Novo Nordisk) were tested with Eucalyptus kraft pulps and the significant decrease in ClO_2 & H_2O_2 consumption was observed (Vicuna *et al.*, 1997).[91] Some important commercial xylanase and their suppliers are given in Table 2. In February 2007 an application has been submitted by DANISCO Animal Nutrition (UK) for approval of DANISCO xylanase G and DANISCO xylanase L as a feed additive.

Table 2: Commercial Xylanase and their suppliers.

S. No.	Enzyme	Commercial Supplier
1.	Ecopulp	Alko Rajamaki, Finland
2.	Cartazyme	Sandoz,Charlotte,N.C. and Basel,Switzerland
3.	Cartazyme HS 10, Cartazyme SR 10 Cartazyme PS10, Cartazyme 9407, Cartazyme NS10	Clarient, UK
4.	Irgazyme 40-4X/Albazyme 40-4X, Irgazyme-10A,Albazyme-10A	Genercor, Finland; Ciba Giegy, Switzerland
5.	VAI Xylanase	Voest Alpine, Austria
6.	Pulpzyme HA, HB and HC	Novo Nordisk, Denmark
7.	Ecopulp X-100,200, 200/4,TX-100,TX200 and Ecopulp XM	Rohn Enzyme 0Y;Primalco, Finland
8.	Xylanase	Meito Sankyo, Nogaya Japan
9.	Ecozyme	Thomas Swan, UK
10.	GS-35, HS70	Iogen, Canada
11.	Sanzyme X,PX and Alpelase F	Sankyo, Japan
12.	Enzeko xylanase	Enzyme Development, USA

SCOPE OF XYLANASE USE IN INDUSTRY

1. Agro waste treatment: Hemicelluloses (Xylan) rich agro waste can be treated by Xylanase to convert xylan into xylose by enzymatic hydrolysis. Development of an efficient enzymatic hydrolysis process offers new prospects for treating hemicellulosic wastes and application in biogas production unit (Stalin et al, 2012).[79] As a pleasant surprise, xylanase can itself be generated from agro waste. The production of a low molecular weight xylanase by *Aspergillus carneus* M34 was investigated in solid-state fermentation using agricultural waste as the substrate (Fang et al, 2010).[27]

2. Degumming: Xylanase system with Pectinolytic enzyme system can be used for the degumming of bast fibers (Fu et al, 2008)[28] such as flax, hamp, jute and ramie. Xylanase pectinase combination can also be used in the debarking process, the first step in wood processing (Wong & Saddler 1997).[96] Pectinases are believed to play a major role in removal of binding materials from plant tissues, but xylanase may also be involved in this process.

3. Fermentation: A fungal B- glucanase from *A. niger* is used in the fermentation of beer to avoid the difficulties encountered in filtration and haze by B- glucans. Xylanase is also one of the components of 'Ultraflo L' produced by Humicola insolens, a heat stable multi active B- glucanase can be used in the mashing process in beer brewing to secure an efficient break down of B- glucans (Ostergaard et al, 2010[67], Simpson et al, 2012[78]). The activity of xylanase in composition with α-amylase and glucoamylase has been selected to achieve a higher ethanol yield in the distillate, by decreasing the concentrations of methanol, propanol, isobutanol and isoamyl & amyl alcohols' concentration (Juodeikiene et al, 2011).[42]

4. Biofuels: Xylanase in synergism with mannanase xylosidase, glucanase, ligninase , glucosidase etc, may be used for the generation of biological fuels, such as ethanol and xylital from lignocelluloic biomass (Stalin et al, 2012).[79] The bio process of ethanol fuel production requires de-lignification of lignocelluloses to liberate cellulose and Hemicellulose from their complex with lignin, followed by cellulose and hemicelluloses de-polymerization, to produce free sugars and finally fermentation of mixed pentose & hexose to produce ethanol (Lee 1987).[50]

USE OF XYLANASES IN BIOSCIENCES

Apart from the major application of Xylanases in pulp bleaching (Beg et al 2001)[11], some other applications of Xylanse are as follow.

1. Bread Quality Improvement: Xylanase improves the bread quality with an increase in specific bread volume. This can be further enhanced by combining amylase with Xylanse (Chen *et al.*, 2010).[19]

2. Non-bakery Food Industry: Xylanase with cellulase and pectinase are used for clarifying must and juices (Pal et al, 2011[69]; Sharma et al, 2012[76]), for liquefying fruits and vegetables (Biely 1991).[13] Xylanase is used in combination with peelinase and cellulase for clarification of fruit juices (Biely 1991).[13] α-L- arabinofuranosidase and β- D- gluco pyranosidase have been additionally employed for aromatizing musts wines and fruit juices (Li et al, 2011).[54]

3. Diet of pets: Xylanase incorporation to a rye-based diet of broiler chickens results in reduced intestinal viscosity, this improves both the weight gain of chicks and their feed conversion efficiency and given with proteases increases food conversion ratio (FCR). The same way, xylanase has applications in poultry (Momtazan et al, 2011[59], Barekatain, 2012[9]), piggery (Malagutti et al, 2010[57]) and fishery (Huichang C, 2006[37]; Yildirim 2010[98]).

4. Plant product processing: Xylanase can induce glycosylation and fatty acylation of phytosterols in plant cells Treatment of tobacco cell suspension (*Nicotiana tobaccum* CV. KY 14) with a purified endo-xylanase from *T.viride* caused a 13 – fold increase in the levels of acylated sterol glycosides and elicited the syntheses of phytoalexins (Moreau *et al.,* 1994).[61] Transgenic expression of endoglucanase and xylanase genes increases tobacco digestibility and biomass conversion (Pappan et al, 2009).[70] Few xylanases can be used for improving cell wall maceration for the production of plant protoplast. Truncated bacterial xylanase gene from *Clostridium thermocellum* has been demonstrated in rhizosecretion in transgenic tobacco plants (Borisjuk *et al.,* 1999).[15]

5. Seed germination: Xylanases from the germinating plant seed convert reserve food to the assimilable end product. It is proposed that xylanase play a role in cell elongation; fruit softening (Bapat et al, 2010).[8]

6. Miscellaneous: Xylanases are also used in wheat flour for improving dough handling and quality of baked products (Chen *et al* 2010)[19], for the extraction of coffee, plant oils, and starch (Wong & Saddller 1992)[96], in the improvement of nutritional properties of agricultural silage and grain feed (Iji et al, 2011).[38]

CONCLUSION

Seeing multiple sources of origin of xylanases and accordingly varying structural, chemical and physical properties vis a vis multiple uses of any given xylanase leading to varying accumulation into biomass directly or indirectly, finally leads to a much complex effect on human population. Human exposure to xylanase as a savior from chemical pollutants might have a sinister side of unexpected and untoward effect too which needs to be evaluated thoroughly.

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