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MINI-REVIEW

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Microbial xylanases and their industrial applications: a review

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Abstract Despite an increased knowledge of microbial xylanolytic systems in the past few years, further studies are required to achieve a complete understanding of the mechanism of xylan degradation by microorganisms and their enzymes. The enzyme system used by microbes for the metabolism of xylan is the most important tool for investigating the use of the second most abundant polysaccharide (xylan) in nature. Recent studies on microbial xylanolytic systems have generally focussed on induction of enzyme production under different conditions, purification, characterization, molecular cloning and expression, and use of enzyme predominantly for pulp bleaching. Rationale approaches to achieve these goals require a detailed knowledge of the regulatory mechanism governing enzyme production. This review will focus on complex xylan structure and the microbial enzyme complex involved in its complete breakdown, studies on xylanase regulation and production and their potential industrial applications, with special reference to biobleaching.

Introduction

Enzymes are the catalytic cornerstone of metabolism, and as such are the focus of intense worldwide research, not only in the biological community, but also with process designers/engineers, chemical engineers, and researchers working in other scientific fields. Since ancient times, enzymes have played a central role in many manufacturing processes, such as in the production of wine, cheese, bread, modification of starch etc. The latter half of the twentieth century saw an unprecedented expansion in our knowledge of the use of microorganisms, their metabolic products, and enzymes in a broad area of basic research and their potential industrial applications. Only in the past 2 decades, however, have microbial enzymes been used commercially in the pulp and paper industry.

The use of xylanases in this industry has increased significantly with the discovery of Viikarri et al. (1986). Since then researchers worldwide have focussed their attention toward newer microbial isolates, the xylanases from which can be used in the pulp and paper industries. The scientific interest in this field is reflected by the number of research papers published during recent years describing numerous xylanases from newer sources, as well as bleaching experiments reported using various hemicellulases, pulps, and bleaching sequence. The xylanases have been reported mainly from bacteria (Gilbert and Hazlewood 1993; Sunna and Antranikian 1997), fungi (Sunna and Antranikian 1997), actinomycetes (Ball and McCarthy 1989; Beg et al. 2000a), and yeast (Hrmova et al. 1984; Liu et al. 1998, 1999).

Over the years the number of possible applications of xylanases in the pulp and paper industry has increased steadily, and several have become, or are approaching, commercial use. Currently, the most effective application of xylanase is in prebleaching of kraft pulp to minimize use of harsh chemicals in the subsequent treatment stages of kraft pulp. While many applications of enzymes in paper industries are still in the research and developmental stage, several applications have found their way into the mills in an unprecedented short period of time in the last decade (Bajpai 1999).

Apart from its use in the pulp and paper industry, xylanases are also used as food additives to poultry (Bedford and Classen 1992), in wheat flour for improving dough handling and quality of baked products (Maat et al. 1992), for the extraction of coffee, plant oils, and starch (Wong and Saddler 1992), in the improvement of nutritional properties of agricultural silage and grain feed (Kuhad and Singh 1993), and in combination with pectinase and cellulase for clarification of fruit juices (Biely 1985) and degumming of plant fiber sources such as

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flax, hemp, jute, and ramie (Kapoor et al. 2001; Puchart et al. 1999; Sharma 1987). To really appreciate the rationale behind current studies on microbial xylanases, it is necessary to take a broad view that takes into account the undoubted commercial potential. In this present review, some industrial applications of microbial xylanolytic enzymes are discussed, with the main emphasis on biobleaching.

Xylan: occurrence and structure

Schulze (1891) first introduced the term 'hemicellulose' for the fractions isolated or extracted from plant materials with dilute alkali. Hemicelluloses include xylan, mannan, galactan, and arabinan as the main heteropolymers. The classification of these hemicellulose fractions depends on the types of sugar moieties present. The principal monomers present in most of the hemicelluloses are D-xylose, D-mannose, D-galactose, and L-arabinose. Xylans are heteropolymers consisting principally of D-xylose as its monomeric unit and traces of L-arabinose (Bastawde 1992). In plants, xylans or the hemicelluloses are situated between the lignin and the collection of cellulose fibers underneath. Consistent with their structural chemistry and side-group substitutions, the xylans seem to be interspersed, interwined, and covalently linked at various points with the overlying 'sheath' of lignin, while producing a coat around underlying strands of cellulose (Biely 1985) via hydrogen bonding (Joseleau et al. 1992). The xylan layer with its covalent linkage to lignin and its non-covalent interaction with cellulose may be important in maintaining the integrity of the cellulose in situ and in helping protect the fibers against degradation to cellulases (Uffen 1997).

Xylan is a complex polysaccharide comprising a backbone of xylose residues linked by β-1,4-glycosidic bonds (Fig. 1). The main chain of xylan is composed of β-xylopyranose residues (Whistler and Richards 1970). Xylan is the most common hemicellulosic polysaccharide in cell walls of land plants, representing up to 30%–35% of the total dry weight (Joseleau et al. 1992). Xylan is the major hemicellulose in hardwood from angiosperms, but is less abundant in softwood from gymnosperms; it accounts for approximately 15%–30% and 7%–12% of the total dry weight, respectively (Whistler and Richards 1970; Wong et al. 1988). The xylan from hardwood is 0-acetyl-4–0-methylglucuronoxylan. This polysaccharide consists of at least 70 β-xylopyranose residues [average degree of polymerization (DP) between 150 and 200], linked by β-1,4-glycosidic bonds. Every tenth xylose residue carries a 4-0-methylglucuronic acid attached to the 2 position of xylose. Hardwood xylans are highly acetylated (e.g., birchwood xylan contains more than 1 mol of acetic acid per 2 mol of xylose). Acetylation is more frequent at the C-3 than at the C-2 position. The presence of these acetyl groups is responsible for the partial solubility of xylan in water. These acetyl groups are readily removed when xylan is subjected to alkali extraction (Sunna and Antranikian 1997). Xylans from softwood are composed of arabino-4-0-methylglucuroxylans. They have a higher 4-0-methylglucuronic acid content than do hardwood xylans. The 4-0-methylglucuronic acid residues are attached to the C-2 position. Softwood xylans are not acetylated, and instead of an acetyl group they have α-L-arabinofuranose units linked by α-1,3-glycosidic bonds at the C-3 position of the xylose (Puls and Schuseil 1993). The arabinosyl substituents occur on almost 12% of the xylosyl residues (Wong et al. 1988). The ratio of β-D-xylopyranose, 4-0-

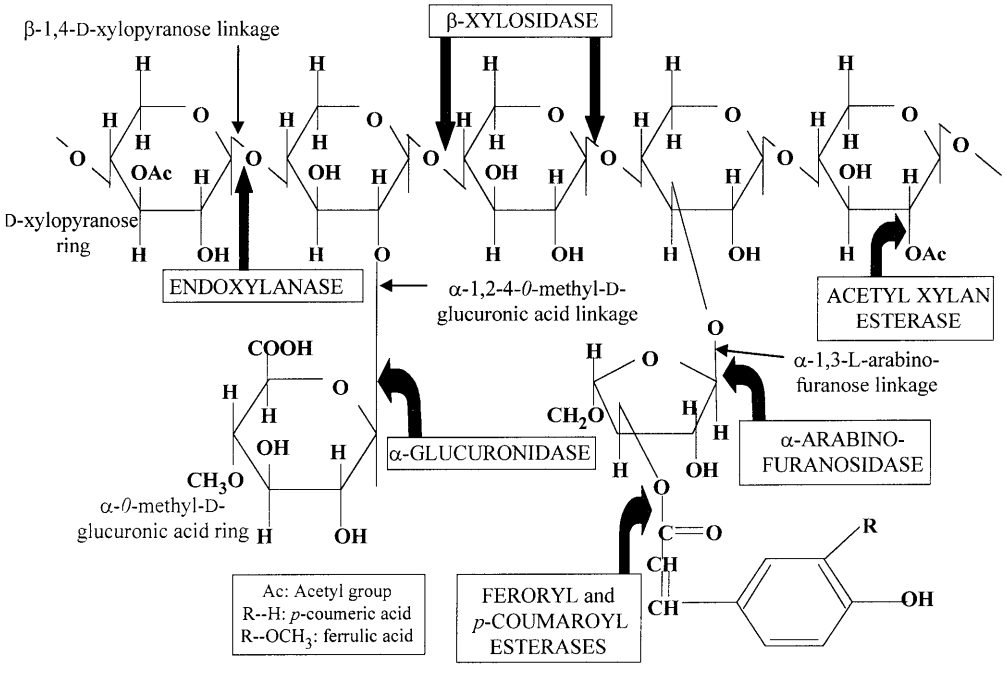

Fig. 1 A hypothetical plant xylan structure showing different substituent groups with sites of attack by microbial xylanases

methyl-α-D-glucuronic acid and L-arabinofuranose is 100:20:13 (Puls and Schuseil 1993). Softwood xylans are shorter than hardwood xylans, with a DP between 70 and 130. They are also less branched (Sunna and Antranikian 1997).

Most xylans occur as heteropolysaccharides, containing different substituent groups in the backbone chain and in the side chain (Biely 1985). The common substituents found on the backbone of xylan are acetyl, arabinosyl, and glucuronysyl residues (Whistler and Richards 1970). Homoxylans, on the other hand, consist exclusively of xylosyl residues. This type of xylan is not widespread in nature and has been isolated from esparto grass (Chanda et al. 1950), tobacco stalks (Eda et al. 1976), and guar seed husk (Montgomery et al. 1956). Xylans with β-1,3-linked backbone have been reported in marine algae (Dekker and Richards 1976). The mixed link of β-1,3- and β-1,4-xylans are found in seaweed such as *Palmeria palmata* (Barry and Dillon 1940).

Microbial xylanolytic system

Enzymatic hydrolysis of xylan

The main component of xylan is D-xylose, a five-carbon sugar that can be converted to single cell protein and chemical fuels by the cheapest 'chemical factories', microbial cells (Biely 1985). Due to the heterogeneity and complex chemical nature of plant xylan, its complete breakdown requires the action of a complex of several hydrolytic enzymes with diverse specificity and modes of action. Thus it is not surprising for xylan-degrading cells to produce an arsenal of polymer-degrading proteins. The xylanolytic enzyme system carrying out the xylan hydrolysis is usually composed of a repertoire of hydrolytic enzymes: β-1,4-endoxylanase, β-xylosidase, α-L-arabinofuranosidase, α-glucuronidase, acetyl xylan esterase, and phenolic acid (ferulic and *p*-coumaric acid) esterase (Fig. 1). All these enzymes act cooperatively to convert xylan into its constituent sugars. The presence of such a multifunctional xylanolytic enzyme system is quite widespread among fungi (Belancic et al. 1995; Biely et al. 1985), actinomycetes (Elegir et al. 1995), and bacteria (Dey et al. 1992). Table 1 summarizes the biochemical properties of some acidic, alkaline, and thermostable xylanases reported in literature.

Regulation of xylanase biosynthesis

Xylanases are usually inducible enzymes secreted in media containing pure xylan or xylan-rich residues (Balakrishnan et al. 1997). However, constitutive production of xylanase has also been reported (Khanna and Gauri 1993; Khasin et al. 1993; Lindner et al. 1994; Segura et al. 1998). Induction is mostly by xylan in *Trametes trogii* (Levin and Forschiassin 1998), *Aspergillus awamori* (Siedenberg et al. 1998), and *Streptomyces* sp. QG-11-3 (Beg et al. 2000a). However, in *Cellulomonas flavigena*, xylan is a poor inducer (Avalos et al. 1996). Induction of xylanase by several other compounds, such as L-sorbose, various xylooligosaccharides, xylose, and lignocellulosic residues, has been reported. L-Sorbose in medium induces the xylanase production in *Sclerotium rolfsii* (Sachslehner et al. 1998) and *Trichoderma reesei* PC-3–7 (Xu et al. 1998). In *Bacillus* sp. BP-7 (Lopez et al. 1998) and *Trichosporon cutaneum* SL409 (Liu et al. 1998), xylanase is induced by xylose, but is repressed in the presence of glucose. Several reports have shown xylanase induction by lignocelluloses such as wheat bran, rice straw, corncobs, and sugarcane bagasse (Beg et al. 2000a; Gupta et al., 2001; Kesker 1992; Kuhad et al. 1998; Puchart et al. 1999). In some cases, readily metabolizable sugars, such as glucose and/or xylose, are suppressors of xylanase synthesis (Bataillon et al. 1998; Beg et al. 2000a; Fernandez-Epsinar et al. 1992; Ishihara et al. 1997; Liu et al. 1999).

The enhancement of xylanase production in the presence of amino acids has also been shown in *Bacillus* sp. No. C-125 (Ikura and Horikoshi 1987), *Bacillus* sp. (NCL-87–6-10) (Balakrishnan et al. 1997), *Trametes trogii* (Levin and Forschiassin 1998), *Staphylococcus* sp. SG-13 (Gupta et al. 1999), and *Streptomyces* sp. QG-11-3 (Beg et al. 2000b). Synthetic calcium-containing zeoloite (CaA) at a concentration of 0.5% has also been reported to enhance xylanase production up to twofold in *Bacillus* sp. NCL 87–6-10 (Balakrishnan et al. 2000). Gupta et al. (2001) reported an improved xylanase production by *Staphylococcus* sp. SG-13 in a biphasic medium containing a solid lower layer of agar containing wheat bran and an upper liquid layer. The regulation of xylanase secretion by microorganisms is still not completely understood. Since xylan is unable to enter the microbial cell, the induction of xylanase is stimulated by low molecular weight xylan fragments, which are produced in the medium by a small amount of constitutively produced enzyme (Bastawde 1992; Kulkarni et al. 1999). Some positional isomers can also induce xylanase synthesis in yeast *Trichosporon cutaneum* (Hrmova et al. 1984).

Xylanase immobilization

For practical applications, immobilization of microorganism or enzymes on solid materials offer several advantages, including repeated usage of enzyme, ease of product separation, and improvement of enzyme stability. In immobilization studies, either the whole cell immobilization of organism to a solid support is performed, or sometimes the enzyme itself is immobilized on some reversible soluble-insoluble polymer. Gwande and Kamat (1998a) immobilized *Aspergillus* sp. strain 5 and *Aspergillus* sp. strain 44 on 400-mesh nylon bolting cloth in shake flask culture. They reported a 1.68-fold higher xylanase yield in immobilized *Aspergillus* sp. strain 5 than that freely suspended cells. The xylanase from the same organism was non-covalently immobilized on

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

Microorganism	Molecular weight (kDa)	Optimum		Stability		pI	K_m (mg/ml)	V_{max} (μM/mine per mg)	References
		pH	Temperature (°C)	pH	Temperature (°C)				
Bacteria									
Acidobacterium capsulatum	41	5	65	3–8	20–50	7.3	3.5	403	Inagaki et al. 1998
Bacillus sp. W–1	21.5	6	65	4–10	40	8.5	4.5	–	Okazaki et al. 1985
Bacillus circulans WL–12	15	5.5–7	–	–	–	9.1	4	–	Esteban et al. 1982
Bacillus stearothermophilus T–6	43	6.5	55	6.5–10	70	7, 9	1.63	288	Khasin et al. 1993
Bacillus sp. strain BP-23	32	5.5	50	9.5–11	55	9.3	–	–	Blanco et al. 1995
Bacillus sp. strain BP-7	22–120	6	55	8–9	65	7–9	–	–	Lopez et al. 1998
Bacillus polymyxa CECT 153	61	6.5	50	–	–	4.7	17.1	112	Morales et al. 1995
Bacillus sp. strain K-1	23	5.5	60	5–12	50–60	–	–	–	Ratannaka-nokchai et al. 1999
Bacillus sp. NG-27	–	7, 8.4	70	6–11	40–90	–	–	–	Gupta et al. 1992
Bacillus sp. SPS-0	–	6	75	6–9	85	–	–	–	Bataillon et al. 1998
Bacillus sp. strain AR-009	23, 48	9–10	60–75	8–9	60–65	–	–	–	Gessesse 1998
Bacillus sp. NCIM 59	15.8, 35	6	50–60	7	50	4, 8	1.58, 3.50	0.017, 0.742	Dey et al. 1992
Cellulomonas fimi	14–150	5–6.5	40–45	–	–	4.5–8.5	1.25–1.72	–	Khanna and Gauri 1993
Cellulomonas sp. N.C.I.M. 2353	22, 33, 53	6.5	55	–	–	8	1.7, 1.5	380, 690	Chaudhary and Deobagkar 1997
Micrococcus sp. AR-135	56	7.5–9	55	6.5–10	40	–	–	–	Gessesse and Mamo 1998
Staphylococcus sp. SG-13	60	7.5, 9.2	50	7.5–9.5	50	–	4	90	Gupta et al. 2000
Thermoanaerobacterium sp. JW/SL–YS 485	24–180	6.2	80	–	–	4.37	3	–	Shao et al. 1995
Thermotoga maritima MSB8	40, 120	5.4, 6.2	92–105	–	–	5.6	1.1, 0.29	374, 4760	Winterhalter and Liebel 1995
Fungi									
Acrophialophora nainiana	17	6	50	5	50	–	0.731, 0.343	–	Ximenes et al. 1999
Aspergillus niger	13.5–14.0	5.5	45	5–6	60	9	–	–	Frederick et al. 1985
Aspergillus kawachii IFO 4308	26–35	2–5.5	50–60	1–10	30–60	3.5–6.7	–	–	Ito et al. 1992
Aspergillus nidulans	22–34	5.4	55	5.4	24–40	–	–	–	Fernandez-Epsinar et al. 1992
Aspergillus fischeri Fxn1	31	6	60	5–9.5	55	–	4.88	5.88	Raj and Chandra 1996
Aspergillus sojae	32.7, 35.5	5, 5.5	60, 50	5–8, 5–9	50, 35	3.5, 3.75	–	–	Kimura et al. 1995

Table 1 (continued)

Microorganism	Molecular weight (kDa)	Optimum		Stability		pI	K_m (mg/ml)	V_{max} (μM/mine per mg)	References
		pH	Temperature (°C)	pH	Temperature (°C)				
Aspergillus sydowii MG 49	30	5.5	60	–	–	–	–	–	Ghosh and Nanda 1994
Cephalosporium sp.	30, 70	8	40	8–10	–	–	0.15	–	Bansod et al. 1993
Fusarium oxysporum	20.8, 23.5	6	60, 55	7–10	30	–	9.5; 8.45, 8.7	0.41, 0.37	Christakopolous et al. 1996
Geotrichum candidum	60–67	4	50	3–4.5	45	3.4	–	–	Radionova et al. 2000
Paecilomyces varioti	20	4	50	–	–	5.2	49.5	–	Kelly et al. 1989
Penicillium purpurogenum	33, 23	7, 3.5	60, 50	6–7.5, 4.5–7.5	40	8.6, 5.9	–	–	Belancic et al. 1995
Thermomyces lanuginosus DSM 5826	25.5	7	60–70	5–9	60	4.1	7.3	–	Cesar and Mrsa 1996
Thermomyces lanuginosus–SSBP	23.6	6.5	70–75	5–12	60	3.8	3.26	6300	Lin et al. 1999
Trichoderma harzianum	20	5	50	–	40	–	0.58	0.106	Tan et al. 1985
Trichoderma reesei	20, 19	5–5.5, 4–4.5	45, 40	3–8.5, 2.5–8.5	–	9, 5.5	3–6.8, 14.8–22.3	–	Tenkanen et al. 1992
Yeast									
Aureobasidium pullulans Y-2311–1	25	4.4	54	4.5	55	9.4	7.6	2650	Li et al. 1993
Cryptococcus albidus	48	5	25	–	–	–	5.7, 5.3	–	Morosoli et al. 1986
Trichosporon cutaneum SL409	–	6.5	50	4.5–8.5	50	–	–	–	Liu et al. 1998
Actinomycete									
Streptomyces sp. EC 10	32	7–8	60	–	–	6.8	3	–	Lumba and Pennickx 1992
Streptomyces sp. B–12–2	23.8–40.5	6–7	55–60	–	–	4.8–8.3	0.8–5.8	162–470	Elegir et al. 1994
Streptomyces T7	20	4.5–5.5	60	5	37–50	7.8	10	7610	Kesker 1992
Streptomyces thermoviolaceus OPC–520	33, 54	7	60–70	–	–	4.2, 8	–	–	Tsujibo et al. 1992
Streptomyces chattanoogensis CECT 3336	48	6	50	5–8	40–60	9	4, 0.3	78.2, 19.1	Lopez-Fernandez et al. 1998
Streptomyces viridisporus T7A	59	7–8	65–70	5–9	70	10.2–10.5	–	–	Magnuson and Crawford 1997
Streptomyces sp. QG-11-3	–	8.6	60	5.4–9.2	50–75	–	1.2	158.85	Beg et al. 2000a
Thermomonospora curvata	15–36	6.8–7.8	75	–	–	4.2–8.4	1.4–2.5	–	Stutzenberger and Bodine 1992

Eudragit S-100 for saccharification, which enabled its recovery and reuse for a longer period (Gwande and Kamat 1998b). Tokuda et al. (1997) also reported that maximum xylanase yield can be obtained by immobilizing *Aspergillus niger* on silk, rayon, and polyester fibers, which have several advantages over free enzyme. In a recent study by our group, the effectiveness of polyurethane foam (PUF) and three non-woven fabrics, namely cotton, silk, and polyester, as support materials for *Streptomyces* sp. QG-11-3 mycelia immobilization was investigated (Beg et al. 2000c). The xylanase yields were enhanced by 2.5-fold, 1.91-fold, 1.54-fold, and 1.47-fold using PUF, polyester, silk, and cotton, respectively, compared with the xylanase yield in liquid-batch fermentation. These results also indicated that the type of fiber material has a significant role in providing a favorable environment for enzyme production, thus having an influence on xylan hydrolysis activity of the immobilized mycelia of *Streptomyces* sp. QG-11-3. Mycelia grew inside the pores of fabric material and PUF particles. Unlike other techniques involving active immobilization, the use of PUF particles does not require the growth of cells prior to immobilization. The inert particles are simply placed in the fermentor before sterilization and the fermenter is inoculated in the normal way. Mycelia/cells become immobilized within the PUF pores as a natural consequence of growth, during an initial growth period. This technique has also been applied successfully to a wide variety of microbial cell systems for immobilization.

Xylanase production in solid-state fermentation

Solid-state fermentation (SSF) is the growth of microorganisms on moist substrates in the absence of free-flowing water. The advantage of SSF processes over liquid-batch fermentation include smaller volumes of liquid required for product recovery, cheap substrate, low cultivation cost for fermentation, and lower risk of contamination. The use of abundantly available and cost-effective agricultural residues, such as wheat bran, corn cobs, rice bran, rice husk, and other similar substrates, to achieve higher xylanase yields using SSF allows reduction of the overall manufacturing cost of biobleached paper. This has facilitated the use of this environment friendly technology in the paper industry. Several workers have also showed a high yield of xylanase at various moisture levels in SSF studies. In SSF using wheat bran and eucalyptus kraft pulp as the primary solid substrates, *Streptomyces* sp. QG-11-3 (Beg et al. 2000c) produces maximum xylanase yield at substrate-to-moisture ratio of 1:2.5 and 1:3, respectively. However, on increasing or decreasing the moisture level, the xylanase yield marginally decreased. In contrast, a lower solid substrate-to-moisture level of 1:1 has been reported for maximum xylanase production by *Bacillus* sp. A-009 (Gessesse and Mamo 1999). An improvement in xylanase production by fungal mixed culture (*Trichoderma reesei* LM-UC4 E 1, *Aspergillus niger* ATCC 10864, and *A. phoenicis* QM 329) using SSF has also been reported (Gutierrez-Correa and Tengerdy 1998). A higher xylanase yield using SSF compared with submerged fermentation using wheat straw and sugarcane bagasse has been reported from thermophilic *Melanocarpus albomyces* IIS-68 (Jain 1995).

The ‘xylanosome’

Xylanosomes are discrete, multifunctional, multienzyme complexes found on the surface of several microorganisms (Sunna and Antranikian 1997). These complexes play an important role in the degradation of hemicelluloses. The extracellular xylanosome complex B (CB) from *Butyvibrio fibrisolvens* H17c (Lin and Thomson 1991) exists as a multisubunit protein aggregate. The complex has a molecular weight >669 kilodaltons (kDa) and is composed of 11 protein bands with xylanase activity and 3 bands showing endoglucanase activity. *Clostridium papyrosolvens* C 7 possesses a multicomplex cellulase-xylanase system, which is responsible for hydrolysis of cellulose and xylan (Pohlschroder et al. 1994). This multiplex system consists of seven protein complexes whose molecular weight ranges from 500 to 660 kDa.

Synergistic action between multiple forms of xylanase

The hydrolysis of xylan requires the action of multiple xylanases with overlapping but different specificities (Wong et al. 1988). Multiplicity of xylanolytic enzymes has been reported in several microorganisms such as *Streptomyces* sp. (Godden et al. 1989), *Penicillium purpurogenum* (Belancic et al. 1995), *Melanocarpus albomyces* IIS 68 (Saraswat and Bisaria 1997), *Cellulomonas* sp. N.C.I.M 2353 (Chaudhary and Deobagkar 1997), and *Aeromonas caviae* W-61 (Okai et al. 1998). The production of a multienzyme system of xylanases, in which each enzyme has a special function, is one strategy for a microorganism to achieve effective hydrolysis of xylan. During xylan hydrolysis, synergism has been observed between enzymes acting on the 1,4-β-D-xylan backbone (β-1,4-endoxylanase) and side chain-cleaving enzymes (α-L-arabinofuranosidase, acetyl xylan esterase, and β-glucuronidase). The synergistic action between acetyl xylan esterase and endoxylanases results in the efficient degradation of acetylated xylan (Biely et al. 1986). The release of acetic acid by acetyl xylan esterase increases the accessibility of the xylan backbone for endoxylanase attack. The endoxylanase creates shorter acetylated polymers, which are preferred substrates for esterase activity (Biely et al. 1985, 1986). The thermophilic actinomycete *Thermomonospora fusca* possesses a multienzyme system of endoxylanase, β-xylosidase, α-L-arabinofuranosidase, and acetyl esterase activities (Bachmann and McCarthy 1991). β-Xylosidase enhances the

hydrolysis of xylan by endoxylanase by relieving the end-product inhibition of endoxylanases. Similarly, the addition of α-arabinofuranosidase to endoxylanase enhances the saccharification of arabinoxylan.

Molecular cloning of the xylanase gene

Recent advances in molecular biology and genetic engineering in the last 2 decades have opened up the areas of application of gene cloning and recombinant DNA technology. Recombinant DNA techniques offer new opportunities for construction of genetically modified microbial strains with selected enzyme machinery. To ensure the commercial utilization of hemicellulosic residues in the pulp and paper industries, the production of higher xylanase yields at low capital cost is required. In this respect, isolation and cloning of the xylanase gene represents an essential step in the engineering of the most efficient microorganism (Kulkarni et al. 1999). Several attempts have been made to clone and express xylanase from bacteria such as *Bacillus subtilis* (Bernier et al. 1983), *Bacillus* sp. (Jeong et al. 1998), and *Acidobacterium capsulatum* (Inagaki et al. 1998) into a non-cellulase producing strain of *Escherichia coli.* Similarly other reports (Honda et al. 1985a, b, c; 1986a, b) describe the production, purification, partial characterization, molecular cloning, sequencing, and expression of the alkaline xylanase gene from alkalophilic *Bacillus* sp. strain C-125 in *E. coli* carrying a plasmid pCX311. Such studies are essential to produce a more-efficient xylanase producer, which will allow improvement of paper quality (Kulkarni et al. 1999). Furthermore, biochemical studies on xylanase-secreting and non-secreting microorganisms could lead to better understanding of the xylanase secretory process and the development of cloning strategies that would guarantee secretion of desired products. Various molecular and biotechnological aspects of xylanase-producing microorganisms, such as the regulation of xylanase biosynthesis at the molecular level, and newer strategies, such as use of gene cloning, protein engineering, and site-directed mutagenesis for obtaining xylanase with novel properties, have been described in detail by Kulkarni et al. (1999).

Applications of xylanolytic enzymes

Xylanolytic enzymes from microorganism have attracted a great deal of attention in the last decade, particularly because of their biotechnological potential in various industrial processes (Bajpai 1999; Kuhad and Singh 1993; Niehaus et al. 1999; Wong and Saddler 1992), such as food, feed, and pulp and paper industries. Xylanases have shown an immense potential for increasing the production of several useful products in a most economical way. The main possibilities are the production of SCPs, enzymes, liquid or gaseous fuels, and solvents and sugar syrups, which can be used as such or as feed stock for other microbiological processes (Ball and McCarthy 1988; Kuhad and Singh 1993).

1. Currently, the most promising application of xylanases is in the prebleaching of kraft pulps (Bajpai 1999). Enzyme application improves pulp fibrillation and water retention, reduction of beating times in virgin pulps, restoration of bonding and increased freeness in recycled fibers, and selective removal of xylans from dissolving pulps. Xylanases are also useful in yielding cellulose from dissolving pulps for rayon production and biobleaching of wood pulps (Bajpai et al. 1994; Srinivasan and Rele 1999; Viikari et al. 1994a).
2. Depression in weight gain and feed conversion efficiency in rye-fed broiler chicks has been associated with intestinal viscosity. Incorporation of xylanase into a rye-based diet of broiler chickens results in reduced intestinal viscosity, thus improving both the weight gain of chicks and their feed conversion efficiency (Bedford and Classen 1992; vanParidon et al. 1992).
3. The efficiency of xylanases in improving the quality of bread has been seen with an increase in specific bread volume. This is further enhanced when amylase is used in combination with xylanase (Maat et al. 1992).
4. Xylan is present in large amounts in wastes from agricultural and food industries. Hence, xylanases are used for conversion of xylan into xylose in waste water. The development of an efficient process of enzymatic hydrolysis offers new prospects for treating hemicellulosic wastes (Biely 1985; Rani and Nand 1996).
5. Xylanase treatment of plant cells can induce glycosylation and fatty acylation of phytosterols. Treatment of tobacco suspension cells (*Nicotiana tabacum* CV. KY 14) with a purified endoxylanase from *Trichoderma viride* caused a 13-fold increase in the levels of acylated sterol glycosides and elicited the synthesis of phytoalexins (Moreau et al. 1994).
6. Xylanase are used concurrently with cellulase and pectinase for clarifying must and juices, and for liquefying fruits and vegetables (Biely 1985), and in the pretreatment of forage crops to improve the digestibility of ruminant feeds and to facilitate composting (Gilbert and Hazlewood 1993).
7. Alkyl glycosides are one of the most promising candidates for new surfactants. Commercially, they are produced from monomeric sugars such as D-glucose and a fatty alcohol. But the direct glycosylation using polysaccharide is more feasible for their industrial production, because hydrolysis of polysaccharide and subsequent steps can be omitted. Thus, use of xylanase in this process provides a challenging opportunity. Recently, xylanase from *Aureobasidium pullulans* has been used for direct transglycosylation of xylan, 1-octanal and 2-ethyl hexanol into octyl-β-D-xylobioside, xyloside, and 2-ethylhexyl-β-D-xylobioside, respectively (Matsumara et al. 1999).

Table 2 Commercial xylanases and their industrial suppliers

Supplier	Product trade name	Application
Alko Rajamaki, Finland	Ecopulp	Pulp bleaching
Sandoz, Charlotte, N.C. and Basle, Switzerland	Cartazyme	Pulp bleaching
Clarient, UK	Cartazyme HS 10, Cartazyme HT, Cartazyme SR 10 Cartazyme PS10, Cartazyme 9407 E, Cartazyme NS 10, Cartazyme MP	Pulp bleaching
Genercor, Finland; Ciba Giegy, Switzerland	Irgazyme 40–4X/Albazyme 40–4X, Irgazyme-10A, Albazyme-10A	Pulp bleaching
	Multifect xylanase	Baking, food
Voest Alpine, Austria	VAI Xylanase	Pulp bleaching
Novo Nordisk, Denmark	Pulpzyme HA, Pulpzyme HB, Pulpzyme HC	Pulp bleaching
	Biofeed Beta, Biofeed Plus	Feed
	Ceremix	Brewing
Bicon India, Bangalore	Bleachzyme F	Pulp bleaching
Rohn Enzyme 0Y; Primalco, Finland	Ecopulp X-100, Ecopulp X-200, Ecopulp X-200/4, Ecopulp TX-100, Ecopulp TX-200, Ecopulp XM	Pulp bleaching
Meito Sankyo, Nogaya Japan	Xylanase	Research
Rohm, Germany	Rholase 7118	Food
Solvay Interox, USA	Optipulp L-8000	Pulp bleaching
Thomas Swan, UK	Ecozyme	Pulp bleaching
Iogen, Canada	GS-35, HS70	Pulp bleaching
Sankyo, Japan	Sanzyme PX, Alpelase F	Feed
	Sanzyme X	Food
Enzyme Development, USA	Enzeko xylanase	Baking, food, feed

8. α-L-Arabinofuranosidase and β-D-glucopyranosidase have been employed in food processing for aromatizing musts, wines, and fruit juices (Spagna et al. 1998).
9. Some xylanases may be used to improve cell wall maceration for the production of plant protoplasts (Wong et al. 1988).
10. A recent application of a truncated bacterial xylanase gene from *Clostridium thermocellum* has been demonstrated in rhizosecretion in transgenic tobacco plants (Borisjuk et al. 1999).
11. Xylanase in synergism with several other enzymes, such as mannanase, ligninase, xylosidase, glucanase, glucosidase, etc., can be used for the generation of biological fuels, such as ethanol and xylitol, from lignocellulosic biomass (Dominguez 1998; Kuhad and Singh 1993; Olsson and Hahn-Hagerdal 1996). The biological process of ethanol fuel production requires delignification of lignocellulose to liberate cellulose and hemicellulose from their complex with lignin, followed by depolymerization of the carbohydrate polymers (cellulose and hemicellulose) to produce free sugars, and finally fermentation of mixed pentose and hexose sugars to produce ethanol (Lee 1997).
12. A potential application of the xylanolytic enzyme system in conjunction with the pectinolytic enzyme system is in the degumming of bast fibers such as flax, hemp, jute, and ramie (Puchart et al. 1999; Sharma 1987). A xylanase-pectinase combination is also used in the debarking process, which is the first step in wood processing (Bajpai 1999; Wong and Saddler 1992). The fiber liberation from plants is affected by retting, i.e., the removal of binding material present in plant tissues using enzymes produced in situ by microorganisms. Pectinases are believed to play a major role in this process, but xylanases may also be involved (Sharma 1987). Replacement of slow natural retting by treatment with artificial mixtures of enzymes could become a new fiber liberation technology in the near future (Bajpai 1999).

The strains reported for the commercial production of xylanases include *Trichoderma reesei* (Tenkanen et al. 1992), *Thermomyces lanuginosus* (Bajpai 1999; Gubitz et al. 1997), *Aureobasidium pullulans* (Christov et al. 1999a), *Bacillus subtilis* (Khanongnuch et al. 1999), and *Streptomyces lividans* (Ragauskas et al. 1994; Senior et al. 1992). Over the last decade, a number of microbial enzymes have been assessed for their potential applications in several industries. Several commercial products have been launched successfully worldwide in the past few years (Table 2).

Biobleaching process: past, present, and future

The most important application of xylanase enzymes is in the prebleaching of kraft pulp and xylanases are gaining importance as alternatives to toxic chlorine-containing chemicals (Bajpai and Bajpai 1992; Ragauskus et al. 1994; Vicuna et al. 1997; Viikari et al. 1994a). The main driving force has been the economic and environmental advantages the enzyme brings to the bleach plant. Such intense demand for the enzyme has pushed enzyme producers to develop an entirely new industry in a remarkably short time.

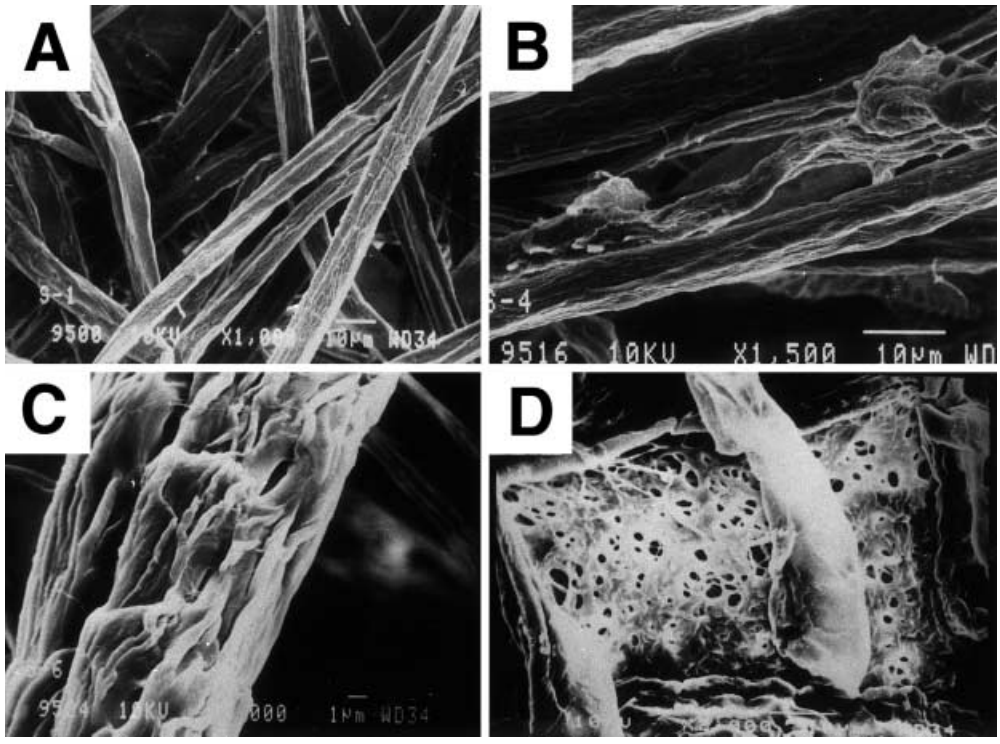

Fig. 2A–D Scanning electron micrographs of Eucalyptus kraft pulp. **A** Untreated eucalyptus kraft pulp showing smooth surfaces on kraft pulp. **B** Eucalyptus kraft pulp treated with xylanase from *Streptomyces* sp. QG-11-3 showing swelling and separation of pulp microfibrils. **C** Eucalyptus kraft pulp treated with xylanase from *Streptomyces* sp. QG-11-3 followed by chemical treatment with 4.5% Cl_2. **D** Growth of *Streptomyces* sp. QG-11-3 on eucalyptus kraft pulp fiber showing extent of penetration of organism mycelia in the eucalyptus kraft pulp

Biobleaching

The process of lignin removal from chemical pulps to produce bright or completely white finished pulp is called 'bleaching.' It is necessary for aesthetic reasons and for improvement of paper properties, because the left-over residual lignin after sulfite pulping imparts an undesirable brown color to the paper. Present-day bleaching of kraft pulp uses large amounts of chlorine-based chemicals and sodium hydrosulfite. These bleaching chemicals cause several effluent-based problems in the pulp and paper industries. Byproducts from using these chemicals are chlorinated organic substances, some of which are toxic, mutagenic, persistent, and bioaccumulate, and cause numerous harmful disturbances in biological systems (Onysko 1993). In response to government and environmental protection groups, paper industries are currently changing practices to minimize the use of chlorine-based chemicals. The available options are oxygen delignification, extended cooking, and substitution of chlorine dioxide for chlorine, hydrogen peroxide, and ozone. But most of these methods involve high capital investment for process change. Thus, an alternative and cost-effective method, i.e., use of enzymes, has provided a very simple and economic way to reduce the use of chlorine and other bleaching chemicals. Biobleaching involves using microorganisms and enzymes for bleaching pulp. It relies on the ability of some microorganisms to depolymerize lignin directly and on the use of microorganism or enzymes that attack hemicellulose and hence favor subsequent depolymerization (Jimenez et al. 1997).

To date, biological bleaching of pulp has been approached mainly by use of lignolytic (Bajpai and Bajpai 1992; Viikari et al. 1994b) and hemicellulolytic enzymes (Gubitz et al. 1997; Jimenez et al. 1997; Tenkanen et al. 1997). These enzymes are used commercially for pulp bleaching. Enzymes also offer a simple approach that allows for a higher brightness ceiling to be reached (Viikari et al. 1994a). The main enzyme needed to enhance the delignification of kraft pulp is endo-β-xylanase, but enrichment of other enzymes such as mannanase, lipase, and α-galactosidase has been shown to improve the effect of enzymatic treatment of kraft pulp (Elegir et al. 1995; Gubitz et al. 1997; Wong and Saddler 1992). Recently, Clarke et al. (2000) reported a comparative study of enzyme-aided bleaching of softwood pulp using a combination of xylanase, mannanase, and α-galactosidase.

The mechanism by which xylanases facilitate bleaching is not fully understood. The enzyme does not bleach pulp, but rather changes the pulp structure. One hypothesis is that they depolymerize hemicellulose precipitated on the surface of the fiber, thereby opening up the pulp structure to access by bleaching chemicals (Paice et al. 1992). However, it is also possible that xylanases release chromophores associated with carbohydrates (Patel et al. 1993). The cleavage of the carbohydrate portion of lignin-carbohydrate complex to produce smaller residual lignin molecules, which are easier to remove, is also a possible mechanism of xylanase prebleaching (Wong and Saddler 1992). Scanning electron-microscopic studies of *Streptomyces* sp. QG-11-3 xylanase-treated pulp (Fig. 2B) have shown that enzymatic prebleaching opens up the pulp structure compared with the smooth surfaces of untreated pulp (Fig. 2A), allowing access of chlorine and other chemicals used in later treatment stages (Fig. 2C) (Beg et al. 2000c). Therefore, enzymatic prebleaching of kraft pulp appears to be the most suit-

able step to facilitate bleach boosting of pulp. Xylanase enzyme can reduce the requirement for oxidizing chemicals by up to 20%–40% (Garg et al. 1998; Vicuna et al. 1997). When *Streptomyces* sp. QG-11-3 was grown on eucalyptus kraft pulp, the mycelia penetrated deep inside the fibers (Fig. 2D) and generated perforations at the site of attachment. These observations suggest that the additive effects of xylanase action and growth of microorganism rendered the pulp fibers more accessible to chemical bleaching agents.

Xylanase selection criteria for biobleaching

Several criteria are essential for choosing a microorganism to produce xylanases. To give the desired bleaching effect, the resulting enzyme preparation must be completely free of any cellulase activity (Srinivasan and Rele 1999; Subramanian and Prema 2000), since any cellulase activity will have serious economic implications in terms of cellulose loss, degraded pulp quality, and increased effluent treatment cost. Other major factors include pH, temperature, enzyme dosage and dispersion, consistency, and reaction time. The optimum pH for xylanase treatment varies among enzymes. Generally, xylanases of fungal origin are effective within the acidic pH range of 4–6 (Christov et al. 1999b; Maximo et al. 1998; Silva et al. 1994; Tenkanen et al. 1997), while those derived from actinomycetes (Garg et al. 1998; Beg et al. 2000c) and bacteria (Kulkarni and Rao 1996; Khanongnuch et al. 1999) are effective in over a broader pH range of 5–9. The optimum temperature for xylanase action ranges between 35 and 60°C. In the mills, xylanase pretreatment takes place in the brown stock high-density storage tanks, in which pulp is present at high temperature (approximately 60°C) and at alkaline pH. Therefore, xylanases that are active and stable at high temperature and alkaline pH are desirable. Thus, screening criteria for xylanases with better thermostability and possibly higher pH optima have received greater attention. To obtain the best results from enzyme use, enzyme dosage and pulp consistency must be optimized in each case to obtain effective dispersion of enzyme (Bajpai 1999). In general, the optimal dose lies within the range of 2–5 IU/g dry pulp, and 5%–10% pulp consistency is desirable. Most of the beneficial effects of xylanase prebleaching can be obtained after only 1–2 h of treatment (Beg et al. 2000c; Gupta et al. 2000).

New developments in xylanase technologies and future prospects

Appreciable quantities of xylan are present in materials released from wood during pulping and pulp processing. It is presently regarded as waste and often is deposited in streams and rivers where it is ecologically harmful. Considerable amounts are also present in agricultural residues. The conversion of xylan to useful products represents part of our efforts to strengthen the overall economics of the processing of the lignocellulose biomass, and also to develop new ways of energy production from renewable resources (Biely 1985; Kuhad and Singh 1993).

Several aspects of xylanases have stimulated research on the study of biochemical, regulatory, and molecular aspects of xylanolytic enzyme systems (Kulkarni et al. 1999). In order for xylanases to have significant impact on an industrial scale, they will need to be consistently effective under various operating conditions. Currently research is being directed towards the discovery of enzymes that are more robust with respect to their pH and temperature kinetics. Techniques used include protein engineering by identification of active site residues through chemical modification, X-ray crystallography and site-directed mutagenesis (Kulkarni et al. 1999). Realistic cost estimates and improvement in process economics are key factors in the commercial success of any technology. Programs involving microbiologists, biochemical and process engineers in collaboration with the paper industries should be established to view the overall perspective and to create effective and functional networks that are progressive and result oriented.

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