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REVIEW ARTICLE

Microbial Xylosidases: Production and Biochemical Characterization

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ABSTRACT

Degradation of complex mixture of biomass is a prerequisite for the production of easily accessible sugar residues in industrial processes. A concerted action of multiple enzyme systems is implicated for the complete hydrolysis of hemicelluloses into monosaccharides. β-Xylosidase being an important enzyme of this system has been purified and biochemically characterized from many potential bacterial genera such as *Bacillus, Clostridium, Streptomyces, Thermoanaerobacterium, Thermomyces,* and fungal genera like *Trichoderma, Aspergillus, Penicillum, Fusarium.* Recent breakthroughs in biotechnology have overcome the tedious purification steps of enzyme production by fermentation. Large scale expression of these enzymes in both homologous and heterologous expression hosts is facilitated with the advent of genetic engineering. However, screening of new organisms for production of native and superior version of current enzymes, conventional and molecular mutagenesis and improvement of enzymes by protein engineering still needs meticulous attention. This review encompasses sources, structure based classification and comparative properties of fungal and bacterial β-xylosidases along with potential solutions of current problems especially with reference to industrial enzymes.

INTRODUCTION

Plant cell wall is made up of a complex mixture of polysaccharides (cellulose, hemicellulose and pectin), proteins, aromatic compounds including lignins and other polymers that are tightly interconnected (Malherbe and Cloete, 2003). Cellulose, in spite of being the most abundant component of plant cell wall polysaccharide consisting of exclusively 1,4-linked β-D-glucose units is a least complex polymer (Ahmed et al., 2009a). In contrast to cellulose, hemicelluloses occur in a large variety of structural types and are the second largest component of total plant biomass on the planet after cellulose (Korner, 2003). Hemicelluloses are classified into xylans, xyloglucans, mannans and mixed linkage β-glucans (Ebringerova et al., 2005). Lignin being the less prominently present polymer in most plant biomass is bound to xylans by an ester linkage (Subramaniyan and Prema, 2002). Xylans are the most abundant hemicelluloses that comprise about three quarter of the entire hemicelluloses pool in hardwood species and grasses. It consists of β-1,4-linked xylose residues which are linked together with different sugars and sugar acid residues (Silva et al., 2012).

Release of easily accessible sugar residues from the plant cell wall polysaccharides is necessary for biotechnological and industrial processes (Brink and Vries, 2011). Degradation of plant cell wall components is a result of coordinated series of biochemical processes. Concerted action of a battery of hydrolytic enzymes is implicated for complete hydrolysis of hemicelluloses into monosaccharides (Coughlan and Hazlewood, 1993; Cosgrove, 1997; Kulkarni et al., 1999). One of the major hemicellulases is xylanase to improve the yield of short xylooligomers by hydrolysis of xylan backbone that is further hydrolyzed into single xylose units by β-xylosidase (Puls and Schuseil 1993; Smith and Harris, 1999; Saha 2003; Willfor et al. 2005). Thus production of xylose as a major industrial raw material is accomplished cooperatively by endo-1,4-β-xylanase and β-xylosidase. Non-reducing ends of xylooligosaccharides produced from the activity of endo-1,4-β-xylanase are further hydrolyzed by β-xylosidases to release xylose (Fig 1).

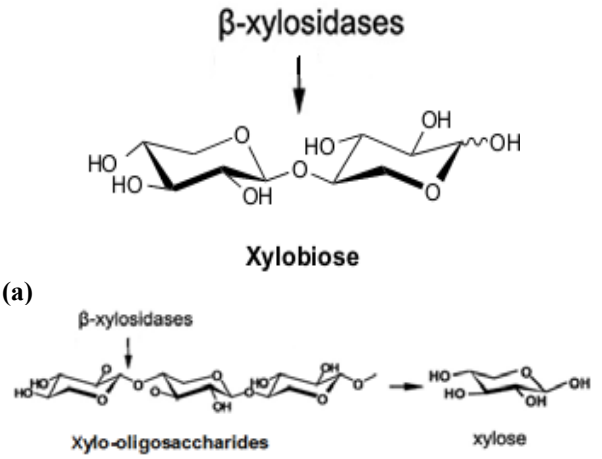

Fig 1: Schematic outline showing hemicellulose degradation by β-xylosidases which release xylose from xylobiose (a) and xylo-oligosaccharides (b)

For production of ethanol fuels, xylose being abundant plant biomass is a significant economic resource.

Xylosidase is a group of enzymes in xylanolytic system that hydrolyzes complex α or β linkages and heterogenous hemicellulose. The enzymes of this group are endo-1,4-β-xylanases hydrolysing 1,4-β-D-xylosidic linkages, endo-1,3-β-D-xylanase hydrolyzing 1,3-β-D-xylosidic linkages. Xylan 1,4-β-D-xylosidase or simply the β-xylosisdase being the most considerable enzyme of this review catalyzes the exo-hydrolysis of xylan at its non-reducing ends whereas 1,3-β-D-linkages are hydrolyzed by xylan 1,3-β-xylosidase at the non-reducing ends of xylan. Efficient enzymatic and microbial hydrolysis of xylanolytic substrates from waste plant material has generated demand of xylan degrading enzymes with different modes of action, novel activity and improved stability from some attractive source (Risna and Suhirman, 2002). Diverse and widespread world of microorganisms has been recognized as a source for screening the production of enzymes with novel and desirable characteristics specifically dedicated to degrade plant polysaccharides. However level of production and biochemical characteristics of a particular enzyme varies markedly between species and even between strains of the same species.

Although a number of β-xylosidases have been produced from a variety of sources including plant, animal and microbial sources, filamentous fungi have mostly been used for high productivity and stability of the enzyme for industrial applications. In this respect large scale commercial production of biocatalysts and their wide applications in biomass conversion, pulping processes, food and textile industries is a considerable interest of applied research (Banerjee et al., 2010).

Considering xylosidase as an enzyme of commercial and metabolic importance, literature regarding biochemical and molecular characterization of the enzyme has not been detailed. This attempt addresses the updated information on characterization of the enzyme. Major emphasis of the review is on the comparative properties of different xylosidase sources.

Xylan; structure and xylanolytic enzymes

Xylan is a heteropolysaccharide containing β-1,4 linked xylopyranose units at its backbone along with substituent groups of acetyl, 4-*O*-methyl-D-glucopyranosyl and α-arabinofuranosyl. Xylan, specific for its linear backbone of D-xylose monomers is found in secondary cell wall and is a constituent of several types of cells and tissues of all terrestrial plants. It accounts for 15-30% of hardwoods and 7-10% of softwoods having *O*-acetyl-4-*O*-methyl-D-glucuronic acid and arabino- 4-*O*-methyl-D-glucuronic acid residues respectively.

Heterogeneity and complexity of xylan requires a battery of cooperatively acting enzymes for complete hydrolysis of main-chain and side-chains. Two key enzymes of this system include endoxylanase (endo-1,4-β-xylanase, E.C.3.2.1.8) which randomly hydrolyzes xylan backbone by its depolymerisation and β-xylosidase (xylan-1,4-β-xylosidase, E.C.3.2.1.37) that converts xylooligomers produced by endoxylanase into xylose. α-glucuronidase (α-glucosiduronase, E.C.3.2.1.139), α-arabinofuranosidase (α-L-arabinofuranosidase, E.C.3.2.1.55) and acetylxylan esterase (E.C.3.1.1.72) are the other hydrolyzing enzymes that liberate side groups present in xylan including α-D-xylosidases.

Linked xylose residues from xyloglucan are released by α-D-xylosidases. Very few α-xylosidases have been purified characterized from fungi such as *Aspergillus* and are specific for xylose residues which are D-linked. *Aspergillus flavus* gives constitutive expression of α-D-xylosidase-I whereas xylose induces α-D-xylosidase-II expression from this fungus (Matsushita et al., 1985, 1987; Yoshikawa et al., 1993). Thus α-D-xylosidases and β-D-xylosidases are the most prominent enzymes of the xylanolytic enzyme system to convert xylooligosaccharides into xylose units which is further metabolized by microorganisms as a carbon source.

Sources of xylosidase

Due to diversity of microorganisms in nature, bulk production of xylosidases from bacterial and fungal sources is a prerequisite for industrially important polysaccharide degrading enzymes. Microorganisms have been selected as a source of enzymes with novel and useful characteristics. Bacterial and fungal species are able to produce xylanases and xylosidases by use of

xylan and recycling of carbon source. Thus a number of xylanolytic enzymes have been studied to understand their physical and biochemical characteristics (Sunna and Antranikian, 1997).

Degradation of hemicelluloses imposes several intrinsic problems for microorganisms. Since the polymer is insoluble or associated with cellulose and lignin, it is a high molecular weight molecule with variable structure, so a concerted action of enzymes is required for its degradation. Aerobic microorganisms such as fungi give a mass production of hemicellulases to degrade polymer into mono or disaccharides which are further used by surrounding microorganisms (Mushtaq and Jamil, 2012).

Aerobic bacteria secrete polysaccharide backbone degrading enzymes to convert complex polymer into oligosaccharides which are sensed by anaerobic bacteria to integrate cellulolyic and hemicellulolytic enzymes for the complete hydrolysis of oligosaccharides. So, microorganisms depending upon their substrate specificity make complex material to be more accessible for hydrolysis (de Vries and Visser, 2001; Shallom and Shoham, 2003).

Xylan degradation by synthesis of 1,4-β-D-endoxylanases and β-xylosidases has been reported to be quite widespread among filamentous fungi, bacteria and yeast (Ahmed et al., 2012; Collins et al., 2005). However there are reports regarding xylosidase production from plants (Subramaniyan and Prema, 2002). Cell wall polysaccharide degrading enzymes well studied from prokaryotic microorganisms include *Bacillus*, *Streptomyces*, *Clostridium* and other bacterial genera that play an extensive role in cell wall polysaccharide degradation.

Fungal plant pathogens produce an extensive set of cell wall degrading enzymes. Upon their penetration partial degradation of the cell wall occurs (Subramaniyan, 2000). Purification and biochemical characterization of cell wall polysaccharide degrading enzymes have been reported from fungi such as *Humicola* sp., *Neomallimatrix* sp., *Talaromyces* sp. (Saha, 2003) and filamentous fungi including *Aspergillus awamori* (Kurakake et al., 2005), *Aspergillus japonicus strain* MU-2 (Hayashi et al., 1992), *Aspergillus nidulans* (Perez-Gonzalez et al., 1998), *Aspergillus niger* (van Peij et al., 1997), *Aspergillus oryzae* (Kitamoto et al., 1999), *Talaromyces emersonii* (Reen et al., 2003), *Trichoderma harzianum* (Ahmed et al., 2009b), *Trichoderma reesei* (Margolles-Clark et al., 1996) and *Penicillium herquei* (Ito et al., 2003). Thermophilic fungi including *Humicola grisea* (de Almeida et al., 1995), *Scylatidium thermophilum* (Zanoelo et al., 2004) and *Sporotrichum thermophile* (Katapodis et al., 2006) have also been studied for the purification of β-xylosidases. Furthermore, production of β-xylosidases has also been studied in yeast (Rajoka and Riaz, 2005; Yanai and Sato, 2001) and plants (Martinez et al., 2004; Chinen et al., 1982; Ronen et al., 1990).

With respect to the localization of β-xylosidases, filamentous fungi have been reported to retain the enzyme within the mycelia, and are detected or liberated in cell extract and growth medium (extracellular). β-xylosidases have been purified from the cell extract and the culture media of *Humicola grisea* var. thermoidea (de Almeida et al., 1995) and *Aspergillus phoenicis* (Rizzatti et al., 2001) respectively. But β-xylosidases produced from bacteria and yeasts however, are mostly cell-associated (Polizeli et al., 2005). Therefore, filamentous fungi are the preferred choice for industrial production of enzymes due to ease of downstream processing. Filamentous fungi including *Aspergillius* sp. and *Penicillum* sp. are particularly promising producers of xylanolytic enzymes for industrial use because they are secreted into the medium. Higher levels of the enzymes have been found from fungal cultures as compared to those from yeasts or bacteria (Wakiyama et al., 2006).

Classification of xylosidases

Based on amino acid sequence similarities, β-xylosidases are classified into seven glycoside hydrolase (GH) families: 3, 30, 39, 43, 51, 52 and 54 (Shallom and Shoham, 2003). Majority of β-xylosidases from bacteria such as *Bacillus* sp. and *Closyridium* sp. are found in families 39 and 43 (Wagschal et al., 2007; Suryani et al., 2003) but fungal enzymes have been described so far only for families 3, 43, and 54 (http://www.cazy.org/). These enzymes are either extracellular or intracellular and contribute to the hydrolysis of xylan (Henrissat, 1991).

GH family 3 consists of β-glucosidase (EC 3.2.1.21), xylan 1,4-β-xylosidase (EC 3.2.1.37), β-N-acetyl hexosaminidase (EC 3.2.1.52), glucan 1,3-β-glucosidase (EC 3.2.1.58), glucan 1,4-β-glucosidase (EC 3.2.1.74), exo-1,3-1,4-glucanase (EC 3.2.1.-), and α-L-arabinofuranosidase (EC.3.2.1.55). Family-3 β-xylosidases from archea, bacteria, and eukaryota have been reported. Many fungal β-xylosidases generally fall into this family.

GH family 43 consists of β-xylosidase, β-1,3-xylosidase (EC 3.2.1.-), α-L-arabinofuranosidase, arabinanase (EC 3.2.1.99), xylanase, and galactan 1,3-β-galactosidase (EC 3.2.1.145). Most enzymes in family 43 are produced by bacteria. Family-43 β-xylosidases from fungi have been known only for *Cochliobolus carbonurn* and *Penicillium herquei* (Wegener et al., 1999; Ito et al., 2003). Family-43 β-xylosidases involve bifunctional β-xylosidases/α-L-arabinofuranosidase activities, and some β-xylosidases have additional xylanase activity, defined as the ability to release free xylose from xylan. GH43 β-xylosidases from bacteria including *Bacillus subtilis*, *Bacillus*

halodurans, *Clostridium acetobutylicum* and *Geobacillus stearothermophilus* T-6 have been recently studied (Barker et al., 2010).

Family-54 enzymes include only β-xylosidases and α-L-arabinofuranosidases. The majority of family-54 enzymes involve α-L-arabinofuranosidase activities. Family-54 β-xylosidase from fungi has been reported for *Trichoderma koningii* (Li et al., 2000).

Structure of β-xylosidase

β-xylosidase being the member of glycosidase hydrolase family, hydrolyses the glycosidic bond by either retention or inversion of the anomeric configuration of the substrate (Bravman et al., 2001). A common structural feature of GH43 enzymes is a 5-bladed β-propeller domain that comprises the catalytic acid and the catalytic base (Jordan and Braker, 2007). Each XynB3 monomeric subunit is organized in two domains: an N-terminal five-bladed β-propeller catalytic domain and a β-sandwich domain (Fig 2). The active site possesses a pocket topology which is mainly constructed from the β-propeller domain residues and is closed on one side by a loop that originates from the β-sandwich domain. This loop restricts the length of xylose units that can enter the active site, consistent with the exo mode of action of the enzyme (Barker et al., 2010). GH43 β-xylosidases crystal structures from *Bacillus pumilus* (Xu et al., 1991), *Bacillus halodurans* (Liang et al., 2009), *Butyrivibrio fibrisolvens* (Utt et al., 1991), *Clostridium acetobutylicum* (Lee and Forsberg, 1987) and *Geobacillus stearothermophilus* T-6 have also been determined (Jordan and Braker, 2007).

Double displacement mechanisms used by retaining glycosidases whereby one catalytic residue acts as a nucleophile and the other as a general acid-base, and single displacement mechanism is used by inverting glycosidases in which one carboxylic acid acts as a general acid for the glycon as a better leaving group. The second carboxylate acts as a general base by activating a water molecule (Fig 3) promoting a nucleophilic attack of the anomeric carbon from the opposite side of the glycosidic bond, thus resulting in inversion of the configuration (Brux et al., 2006).

Glycosidase hydrolases in family-39 were found to be "retaining" enzymes when studied in *Thermoanaerobacterium saccharolyticum* (Shao et al., 2011), *Caldocellum saccharolyticum* (Hudson et al., 1991) and *Geobacillus stearothermophilus* (Czjzek et al., 2005). The first crystal structure of a GH39 β-xylosidase revealed a multi-domain organization with the catalytic domain having the canonical (β/α) 8 barrel fold. Enzymes from this family are currently found in bacteria and eukaryotes, although one gene sequence encoding a putative family GH39 enzyme from archaea has been reported (Li et al., 2000).

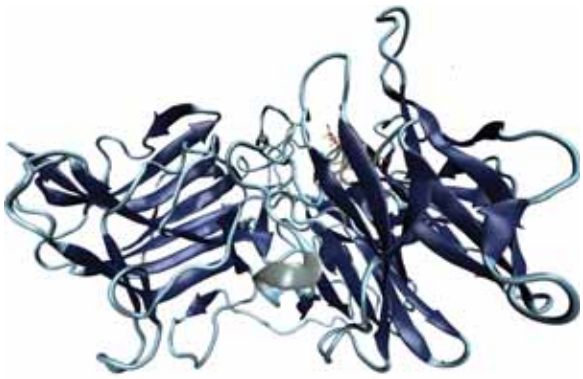

Fig 2: Structure of XynB3 showing its β-sandwich domain (left) and N-terminal five-bladed β-propeller domain (right) that form a pocket-shaped active site (Figure adopted from Barker et al. 2010)

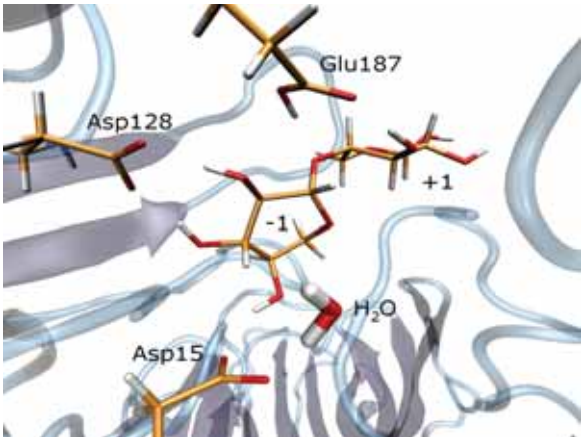

Fig 3: A view of the XynB3 active site with xylobiose as substrate, general base Asp15 coordinating the catalytic water molecule and general acid Glu187 (Figure adopted from Barker et al. 2010)

Multiple forms of xylosidase

There are reports regarding monomeric forms of fungal β-xylosidases but two or three subunits have also been reported (Sunna and Antranikian, 1997). The purified proteins have been found with molecular mass between 26,000-36,000 Da. Ito et al. (2003) produced two β-D-xylosidases S1 and S2 that were purified from the culture filtrate of *Penicillium herquei* having molecular masses of 103,700 and 37,460 Da respectively. Characterization of both enzymes showed different properties regarding optimum pH and temperature. Substrate specificities of the xylosidases were examined by using various substrates. S2 did not show activity on all substrates except p-nitrophenyl-β-D-xylopyanoside whereas S1 acted preferentially on this substrate and had little activity on p-nitrophenyl-β-D-glucopyanoside

and p-nitrophenyl-β-D-glalactopyanoside. The amino acid sequence of S2 showed similarity to the members of family 43 glycoside hydrolases (Ito et al., 2003).

A β-D-xylosidase was produced from the concentrated culture filtrate of *Penicillium wortmanni* strain IFO 7237 and was designated as xylosidase 1, 2, 3 and 4 (Matsuo et al., 1987). Molecular masses of xylosidase-1, -2, -3, and -4 were estimated to be 110,000, 195,000, 210,000, and 180,000 Da with their isoelectric points to be 3.7, 4.28, 4.6, and 4.8 respectively. The pH optima of β-xylosidase activities were in a range from 3.0 to 4.5. The optimum temperature for enzyme activities was found to be from 55 °C to 65 °C. On the enzymatic hydrolysis of phenyl β-D-xyloside, the reaction product of each enzyme was found to be β-D-xylose with retention of configuration. With respect to the hydrolysis patterns xylosidase-2 was a distinct enzyme from other three. Xylosidase-1 was a monomeric protein and was placed in a separate group although xylosidase-3 and -4 as dimeric proteins showed closely related action patterns as a different group (Matsuo et al., 1987).

Characterization of β-xylosidases

β-xylosidases have mostly been isolated and purified by different chromatographic techniques such as ion-exchange and size exclusion chromatography. Studies have been conducted related to characterization of β-xylosidases from many fungi (Table 1) and bacteria (Table 2) concerning their mode of action and biochemical properties. β-xylosidases isolated and purified from fungi have gained much attention as compared to bacteria and the characterized fungal β-xylosidases are mostly extracellular (Susan et al., 1989). A broad range of pH optima have been found, that lie mostly between 2.0-9.5. The optimum temperature varies from 30 °C to 80 °C, but most β-xylosidases give best assay results at 60 °C. Their thermostability is highly variable and depends on the organism. *Aspergillus phoenicis* produced β-xylosidase with retaining 100% of its activity after 4 h at 60 °C or 21 days at room temperature (Rizzatti et al., 2001).

A thermostable β-xylosidase from *Penicilium janczewskii* showed half-life of 144 min at 50 °C however, it was less thermostable than the enzyme from *Penicilium scleroticum* (Knob and Carmona, 2009). This enzyme is active at 50 °C for 4 h and reduced its activity up to 3.8 h at 55 °C and was stable in acidic pH. These characteristics are the basis of the enzymes for their use in biotechnological processes and specifically in animal feed industry. Purified β-xylosidase from thermophilic fungus *Paecilomyces thermophila* (Yan et al., 2008) is more thermostable than *Fusarium proliferatum* (Saha, 2003).

Trichoderma viride β-xylosidase was found to be similar in some properties to that of *P. wortmanni* with respect to pH, temperature optima, isoelectric points and monomeric structure (Matsuo and Yasui, 1984). Due to such properties the enzymes were considered to be promising biocatalysts in the enzymatic bleaching of pulps. A different set of β-xylosidases was purified from *P. wortmanii* having four enzymes. Temperature and pH effects on the activity and stability disclosed that four β-xylosidases from *P. wortmanni* were different enzymes. All the four enzymes had favorable actions on a series of β-xylosides, showing no activities of other enzymes (Matsuo et al., 1987).

Most of the fungal β-xylosidases exhibit molecular mass of more than 100 kDa (Rizzatti et al., 2001; Herrmann et al., 1997; Kumar and Ramon, 1996; Hayashi et al., 1992) although some exhibits lower molecular mass (de Almeida et al., 1995). In addition to high molecular mass enzymes with two identical subunits, monomeric forms of β-xylosidases have also been purified from some fungi (de Almeida et al., 1995; Herrmann et al., 1997; Rizzatti et al., 2001; Katapodis et al., 2006). Bacterial β-xylosidases however were found to have molecular mass less than 100 kDa in most cases.

Recombinant enzymes purified from different fungi and bacteria show variation from the native enzymes. A recombinant β-xylosidase from *Aspergillus japonicus* (Table 1) had an apparent mass of 118.4 kDa, which was slightly larger than that of the native enzyme (113.2 kDa) originally secreted by the fungus (Hayashi et al., 1992). The difference in molecular mass between the native and recombinant enzymes might be because of possible post-translational modifications. Many recombinant β-xylosidases have been reported to have characteristics suitable for industrial use. For example the enzyme produced by *A. niger* exhibited significant activity at high temperatures, in acidic growth media and in the presence of reducing agents, thus having a good potential to be used in animal feed, enzymatic synthesis and the fruit-juice industry (Amaro-Reyes et al., 2011).

Immobilized enzymes show better thermostability than soluble enzymes in most of the reports. β-xylosidase from *A. niger* and *A. fumigates* were immobilized by adsorption on a polyamide membrane with 30% of enzyme being immobilized. The immobilized enzyme kept only 6.8% of the maximal activity of the free enzyme but showed a better thermal stability than the free enzyme. However, further investigations are needed in this area so as to achieve maximum potential of this technique for β-xylosidases at commercial scale.

Km of enzymes gives clue about affinity of the enzyme with substrate, hence their suitability for commercial utilization. Km of β-xylosidases, both from fungal and bacterial sources, was found in lower range in most of the cases demonstrating efficient conclusion of the substrate into products. Mixed trend was observed for maximal initial velocity (Vmax) of the β-xylosidases.

Table 1: Properties of fungal β-xylosidases

Microorganism	Mol mass (kDa)	Purification fold	Yield (%)	Optimum pH & Temperature		Stabilities at[a]		pI	K_m (mM)	V_{max} (umol/ min/mg)	Specific Activity (U/mg)	References
				pH	Temp (°C)	pH	Temp (h)					
Aspergillus awamori K4	117	13.9	28.7	4	70	4-6	60 (1)	-	-	-	19.58	Kurakake et al., 1997
Aspergillus fumigatus fresenius no. 4-45-IF Soluble Immobilized				4.5 4	65 65	4.5-5.0 4-5	50-55 (1) 60 (3days)		8.7 7.4			Yongpanichkul, 1994
Aspergillus japonicas	113.2 118.4[rec]	59.4	12.3	4	70	2-7	60	-	0.31 4	114	112	Wakiyama et al., 2008
Aspergillus nidulans	180			5	50				1.1	25.6		Kumar and Ramon, 1996
Aspergillus ochraceus	137	-	-	3-5.5	70	3-6	70 (1)	-	0.66	39		Michelin et al., 2012a
Aspergillus phoenicis	132	17.7	9.8	4-4.5	75	-	60 (4)	3.7	2.36	-		Rizzatti et al., 2001
Fusarium proliferatum	912	331	3.8	4.5	60	3-6	50	7.8	0.77	75	53	Saha, 2003
Paecilomyces thermophila	53.5	31.9	2.27	6.5	55	6-9	55 (30 min)	-	4.3	-	43.4	Yan et al., 2008
Penicillium scleroticum	-	-	-	2.5	60	2-4	50 ($4^{1/2}$)-55 ($3.8^{1/2}$)	-	0.75	0.48	8.96[b]	Knob and Carmona, 2009
Penicilium herquei S1	100	34.1	0.7	4	50	5-6.5	40	-	-	-	353.6	Ito et al., 2003
S2	40	21.7	6.6	6.5	30	7.5-9.5	30	-	-	-	224.6	
Penicilium janczewskii	67-94			4	75	1.6-5.5	50 ($2.4^{1/2}$)				171.8	Terrasan et al., 2010
Sporotricum thermophile	45			7	50	6-8		4.2	1.1	114		Katapodis et al., 2006
Trichoderma harzianum	60			4-4.5	70				0.05 3		3.4	Ximenes et al., 1996
Trichoderma reesei	100	-	-	4	60	3-6	55 (1)	4.7	0.42, 20.0	-		Herrmann et al., 1997
Trichoderma viride	102	-	25.5	4.5	55	3-4	55	4.4 5	5.8	-	10.8	Matsu and Yasui, 1984
Talaromyces emersonii	975	166	62.7	2.5	60	-	-	8.9	0.13	1.7	149.4	Tuohy et al., 1993
Xylaria regalis	44.9	23	24.2	5.5	50	5-9					3.996	Shenq-Chyi et al., 2005

a: The stability in hours is given in bracket. Numbers preceding $^{1/2}$ represent half-life; b: Specific activity against oat spelt xylan; rec: Recombinant

Table 2: Properties of bacterial β-xylosidases

Microorganism	Mol. Mass (kDa)	Purification fold	Yield (%)	Optimum pH and Temperature		Stabilities at[e]		pI	K_m (mM)	V_{max} (umol/ min/mg)	Specific activity (U/mg)	References
				pH	Temp	pH	Temp (hrs)					
Aeromonas caviae[rec]	75	--	-	6	50	5-8	40	-	0.34	33		Suzuki et al., 2001
Bacillus halodurans	61	1.0	70	7.0	45	4.5-9.0	35-45		1.9	0.65	172.9	Liang et al., 2009
Bacillus stearothermophilus	150	72.5	16.1	6.0	70	6-8	60	4.2	1.2		34.2	Nanmori et al., 1990
Bacillus thermentarcitcus	150	160	13.75	6.0	70		60 ($1^{1/2}$)	4.2	0.5	-	160	Lama et al., 2004
Caldocellum saccharolyticum[rec]		550	4.3	5.7	65		65 ($4.85^{1/2}$), 70($0.67^{1/2}$)	4.3	10	64	49	Hudson et al., 1991
Clostridium acetobutylicum	85[a], 63[b]	159	23	6.0-6.5	45	6.0-8.0	40	5.85	3.7	19.6	17.50	Lee and Forsberg, 1987
Clostridium cellulolyticum	43	169	4.4	7.5	35	-	-	-	0.40	-	15.11I	Saxena et al., 1995
Streptomyces Sp.[d]	42	10	15.07	7.5	45	5-8	25 (48)	-	13.5	-		Belfaquih et al., 2002
Thermoanaerobacter Ethanolicus	85	72	14	5.9	93	-	-	4.6	-	-	66	Shao and Weigel, 1992
Thermomonospora Fusca[c]	168, 56		95	5-9	40-60	6-8	65 ($8^{1/2}$)			0.89	8.0	Susan et al., 1989
Thermoanaerobacterium Saccharolyticum	78	46	12	6.0	65	7.0	67 ($1^{1/2}$)	4.45	28	276	45.8	Shao et al., 2011

a: dimer b: monomer; c: Trimer. Each subunit of 56 kDa; rec: recombinant; d: bifunctional xylosidase-isomerase enzymes. e: The stability in hours is given in bracket. Number preceding $^{1/2}$ represent half life.

Overall, the kinetics of the reported β-xylosidases was found to be in reasonable range for efficient utilization of the enzymes.

Concluding remarks and future outlook

Degradation of a complex mixture of biomass is necessary for the production of easily accessible sugar

residues in industrial processes. Enhanced production of industrial biocatalysts with novel and desirable characteristics has better prospects in terms of both increased economic pressure and industrial developments. Since living systems are responsible for enzyme production thus thermodynamically they are not stable, so they are intrinsically expensive. Physical and chemical recalcitrance of complex biomass mixture requires high enzyme loading to obtain a reasonable degradation rate. Thus ultimate cost is the major hindrance in the development of biofuel industry (Banerjee et al., 2010).

Thermophilic organisms are considered to be better source of β-xylosidase production however, limitations and constrains are there regarding practical applications of the secreted enzyme. Researchers have been attracted to explore natural sources for the production of the enzymes with outstanding properties, e.g., maintenance of activity during processing at high temperatures, high specific activity, elevated thermal tolerance, synergistic action with other enzymes, ability to be efficiently translated, resistance to proteases and efficient secretion (Turner et al., 2007). However, extreme conditions of fermentation make it difficult to propagate such microorganisms at industrial scale, and the procedure is expensive as well (Banerjee et al., 2010; Ahmed et al., 2009a). Furthermore, manipulation of the enzymes from less stable to a more stable form requires a compromise of stability over functional state of proteins. Strain improvement, media development and optimization of fermentation conditions are also being considered to get high yields of enzymes for use in biofuel industries (Bhalla et al., 2013).

Overproduction of β-xylosidase could not receive much attention due to limited information regarding crystallographic structure of the enzyme; however recombinant enzymes with pH and temperature stability are a good alternative to the native enzymes for their effective utilization. Search for new sources to incorporate recyclable agricultural wastes with enhanced production of potential enzymes requires mining of new genes from nature to meet industrial needs. Screening of new organisms including fungi for the production of native and superior version of the current enzymes, mutagenesis at conventional and molecular level and improvement of enzymes by protein engineering needs meticulous attention (Meraj et al., 2012).

The ways to improve β-xylosidase production would include: i. Use of genetic approach to introduce microbial strains with hyperproduction of the enzymes, ii. Exploitation of new strategies to design β-xylosidase with extreme pH and temperature, iii. Improvement of the enzymes with respect to their industrial applications, and iv. Modification of genetic makeup of organisms by gene cloning followed by their expression in high expression hosts.

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