3 Biotech (2018) 8:78
https://doi.org/10.1007/s13205-018-1091-8

REVIEW ARTICLE

Molecular structure and catalytic mechanism of fungal family G acidophilic xylanases

Protyusha Dey[1] · Amit Roy[1]

Received: 19 August 2017 / Accepted: 4 January 2018 / Published online: 15 January 2018
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

Industrial applications of xylanases have made this enzyme an important subject of applied research work. Function of this particular enzyme is to degrade or hydrolyze the plentiful polysaccharide xylan, an important component of hemicellulose. It mainly cleaves the backbone of xylan that is made up of a number of xylose residues connected with β-1,4-glycosidic linkages. Fungi with mycelia are regarded as the best producer of xylanases. These varied xylanases not only differ in their sizes and shapes but also differ in their physicochemical properties. Depending on the optimum pH in which they work best, they have been classified into (1) acidophilic xylanases active at low pH or acidic pH range, (2) alkaliphilic xylanases that are active at high or alkaline pH range and (3) neutral xylanases having pH optima in the neutral range between pH 5 and 7. Other researchers have classified the xylanases also on the basis of their structural properties, kinetic parameters, etc. This review discusses the molecular structures of some acidophilic xylanases and the molecular basis of low pH optima observed for their activities. It also discusses their unique catalytic mechanism and actual role of the catalytic residues found in them. Apart from these, the review also discusses different applications of these acidophilic xylanases in different industries. The article concludes with brief suggestions about how these acidophilic xylanases can be created employing the techniques of genetic engineering and concepts of synthetic evolution, using the traits of the known acidophilic xylanases discussed in the review.

Keywords Fungal family G/11 acidophilic xylanase · Molecular structure · Low pH optima · Catalytic mechanism · Biobleaching

Introduction

Xylanases are the active biological molecules that chew up the heteropolysaccharide, xylan. Xylan is one of the important constituents of the lignocellulose biomass. It is the second most abundant natural polysaccharide (Collins et al. 2005). In the last few decades, the xylanolytic products obtained from the enzymatic breakdown of xylan have gained a lot of industrial attentions such as in paper making, biobleaching, animal feed production and in biofuel production. As a result, a lot of research attentions are aimed at recombinant xylanolytic enzymes production. Microorganisms are the key producer of xylanolytic enzymes (Wong et al. 1988; Kuhad and Singh 1993; Kuhad et al. 1997; Beg et al. 2001). Evolutionary pressure for survival has resulted in many microorganisms developing cellular mechanisms to derive energy from the plant biomass by secreting various carbohydrate-degrading enzymes. Such enzymatic degradation of biomass by these microorganisms is performed by complex mixtures of several enzymes such as cellulases (Bayer et al. 1998), hemicellulases (Ljungdahl 2008) and ligninases (Sanchez 2008). The hemicellulases include various types of xylanases that degrade xylan from the biomass, to liberate pentose sugar xylose. Since xylan constitutes approximately 30% of the biomass, it forms a vast source of cheap xylose which can be fermented to ethanol. Presently bio-ethanol is a hot area of renewable biofuel research (Weng et al. 2008).

As mentioned above, various bacteria and fungi are known to produce different kinds of xylanases. Fungi, in particular, are potent sources of biomass-degrading enzymes and serve as an important resource for the production of heterologous recombinant proteins. These eukaryotic cells have a short generation time, small haploid genomes and protein production is "Generally Regarded As Safe

✉ Amit Roy
amit.roy@visva-bharati.ac.in

[1] Department of Biotechnology, Visva-Bharati University, Santiniketan 731235, West Bengal, India

(GRAS)" (Moore 2009). Also, xylanase yield from fungal culture is much higher than yeast or bacteria (Motta et al. 2013). So they have gained much more industrial research interest. Generally, fungi require moist and damp medium with slightly acidic pH for their growth. Thus, they mainly grow on decaying wood, plant deteriorate and also in the rumen of ruminants. They derive their energy by degrading complex polysaccharides present in the medium with the help of polysaccharide splitting enzymes such as xylanases. As xylan is very complex and heterogeneous in nature, a variety of xylanases are found in nature having different substrate specificity and mode of actions. Amongst them, the class endo-1,4-β-xylanase is regarded as the most important one. Furthermore, the members of this family can be subdivided into different groups based on different properties.

On the basis of physicochemical properties such as pH, the endo-1,4-β-xylanase can be categorized into three types (Fushinobu et al. 2011); the first category is for acidophilic xylanases, i.e. they are stable and active in very low pH range. The second category reserves the xylanases that are stable and active near the neutral pH range and known as neutrophilic xylanases and the third category consists of the alkaliphilic xylanases which have pH optimum at alkaline range. In most of the cases, the acidophilic xylanases are found to be produced by fungi (Enshasy et al. 2016). These acid-stable xylanases have many industrial values (Tenkanen et al. 1997; Pokhrel et al. 2013; Chen et al. 2016). It has been found that the acidic media accelerates the breakdown of xylan present in lignocellulosic biomass. So from that point of view, acidophilic xylanases are very important as the acidic reaction conditions not only help the enzymes to work efficiently but also make the substrate more sensitive towards the treatment. Literature suggests that the acidophilic xylanases are also desirable in biobleaching (Tenkanen et al. 1997), baking, and fruit juice clarification or xylooligosaccharides production (Sharma et al. 2016). Because of the so many benefits of acidophilic xylanases, research is being carried out to shift the pH optima of neutral/alkaliphilic xylanases towards acidic side (Pokhrel et al. 2013). This review presents a concise idea about the industrial usefulness of acidophilic xylanases (Table 1) that are available in Protein Data Bank in addition to their structure–function relationship.

Structure and distribution of Xylan

In 1891, Schulze coined the term 'Hemicellulose' (Schulze 1891). It is the fraction of plant material that can be extracted with the treatment of dilute alkali. It is composed of xylan which is the major constituent, then galactan, mannan and arabinan (Whistler and Richards 1970; Viikari et al. 1994; Uffen 1997; Ebringerova and Heinze 2000). Xylan is the polymer of the pentose sugar, xylose. The free hydroxyl groups of xylose residues present in the backbone are often found to be substituted with diversified chemical groups resulting in the formation of heterogeneous xylan (Singh et al. 2003). Depending on the substituent, xylan can be categorized into five basic types, namely homoxylan, arabinoxylan, glucuronoxylan, glucuronoarabinoxylan and galactoglucuronoarabinoxylan (Voragen et al. 1992). Homoxylan (Fig. 1a) is composed of solitary xylopyranosyl residues linked with β-1,4 and β-1,3-glycosidic linkages forming the skeleton of the polysaccharide. The homoxylan is found in nature rarely. They have been found to be present in esparto grass, tobacco stalks and guar seed husks (Sunna and Antranikian 1997). In addition to that, arabinose residues are very often found to be present within the xylose residues making it arabinoxylan (Fig. 1b). This can be observed in the cell wall structure of rice, barley, oat, rye, etc.; in glucuronoxylan (Fig. 1c), the free hydroxyl group of C-2 is replaced with glucuronic acid. They are found in the secondary cell wall structure of plants neighboring cellulose and lignin thereby contributing to the integrity of the cells; in glucuronoarabinoxylan (Fig. 1d), the substituent is glucuronic acid and arabinose. They are mainly present in soft wood and can be readily hydrolyzed by acid; galactoglucuronoarabinoxylan contains galactose moieties, glucuronic acid moieties as well as arabinose. Apart from these mentioned substituent acetyl group, feruloyl group and *p*-coumaroyl group can also be seen in the structure of xylan. All these substituents are the branches of the heteropolysaccharide that correspond to the solubility and reactivity of the particular variety of xylan.

Enzymatic hydrolysis of xylan

The presence of diverse substituents (arabinose, glucuronic acid, galactose, acetyl group, feruloyl group and p-coumaroyl group, etc.) that are found to be linked with the free hydroxyl groups of xylose residues is accountable for the heterogeneity and complexity of xylan. So simplification of the particular biomass requires simultaneous action of several hydrolytic enzymes with different modes of action. Thus, microorganisms capable of degrading xylan possess a xylanolytic system sometimes called xylanosome that constitutes different enzymes including endo-1,4-β-xylanases (E.C.3.2.1.8), β-xylosidase (E.C.3.2.1.37), α-arabinofuranosidase (E.C.3.2.1.55), acetylxylan esterase (E.C.3.1.1.72), glucuronidase (E.C.3.2.1.139), *p*-coumaric acid esterase, ferulic acid esterase, etc. (Polizeli et al. 2005; Juturu and Wu 2011; Motta et al. 2013). Among these, the most important one is the endo-1,4-β-xylanases also known as endoxylanases (E.C. 3.2.1.8) as it cleaves the primary chain, i.e. the β-1,4-glycosidic linkage of the structure resulting in the production of lower molecular weight fragments of xylan as well as xylooligosaccharides. Studies revealed that xylanases may occur in

Table 1 Some acidophilic xylanases

S. no.	Name of the organism	Name of the enzyme	PDB code	Size (no. of amino acids)	Optimum pH	Isoelectric point (pI)	References
1.	*Trichoderma reesei*	XYNI	1XYN	178	3.5	5.2	Törrönen and Rouvinen (1995)
2.	*Aspergillus niger*	Xylanase I	1UKR	184	3	Not available	Krengel and Dijkstra (1996)
3.	*Aspergillus kawachii*	XynC	1BK1	184	2	3.5	Fushinobu et al. (1998)
4.	*Termitomyces clypeatus*	Endoxylanase	Not available	Not available	3.5	4.0	Mukherjee and Sengupta (1985)
5.	*Penicillium* sp. 40	Xyn A	Not available	190	2	Not available	Kimura et al. (2000)
6.	*Cryptococcus* sp. S-2	Xylanase CS	Not available	184	2	7.4	Iefuji et al. (1996)
7.	*Aureobasidium pullulans* var. *melanigenum*	*xynI*	Not available	187	2	6.7	Ohta et al. (2001)
8.	*Penicillium sclerotiorum*	Xylanase I	Not available	Not available	2.5	Not available	Knob and Carmona (2010)
9.	*Scytalidium acidophilum*	XYL1	3M4F	181	3.2	Not available	Michaux et al. (2010)
10.	*Penicillium occitanis* Pol6	PoXyn3	Not available	197	3	Not available	Driss et al. (2011)
11.	*Penicillium glabrum*	Xylanase	Not available	Not available	3	Not available	Knob et al. (2013)
12.	*Penicillium oxalicum GZ-2*	xyn11A	Not available	316	4	Not available	Liao et al. (2015)
13.	*Aspergillus awamori* ATCC 11358	EXLA	Not available	181	3.5	3.7	Hessing et al. (1994), Berrin et al. (2000)
14.	*Aspergillus niger* IBT90	Xyn6	Not available	167	3.5	Not Available	Korona et al. 2006
15.	*Aspergillus niger* SCTCC400264	XYNA1	Not available	205	3.0	Not available	Yi et al. (2010)
16.	*Aspergillus sulphureus*	XynA	Not available	204	2.4–3.4	Not available	Cao et al. (2007)
17.	*Bispora* sp. MEY-1	XYL11 B	Not available	228	2.6	Not available	Luo et al. (2009)
18.	*Cryptococcus flavus* I-11	Cfxyn 1 p	Not available	193	3.0	Not available	Parachin et al. (2009)
19.	*Penicillium purpurogenum*	Xyn B	Not available	176	3.5	5.9	Belancic et al. (1995)

multiple isoforms. It has been observed that existence of these isoforms are the resultant of various pre or post-translational modifications such as differential mRNA processing, partial proteolysis or differences in the degree of amidation and glycosylation (Biely 1985). β-xylosidase is the enzyme that acts upon the product of endoxylanases thereby producing the monomer, xylose. Beside these two enzymes, there are accessory enzymes such as α-arabinofuranosidase, acetylxylan esterase and glucuronidase that cleave the substituents according to their specificity (Coughlan and Hazlewood 1993). A schematic diagram (Fig. 2) of xylan hydrolysis is given below.

Types of Endo-1, 4-β-xylanase

Xylanases have been subdivided in many ways. In 1988, Wong et al. classified xylanases based on their molecular weight and pI (Wong et al. 1988). One class contained xylanases with molecular weight < 30 kDa and pI of alkaline range and another class contained the xylanases with molecular weight > 30 kDa and low pI. But near about 1/3 of xylanases especially fungal xylanases did not fit into these two categories. Later, many classification systems have come up based on different properties of the enzyme such as pH, kinetic parameter and substrate specificity, and

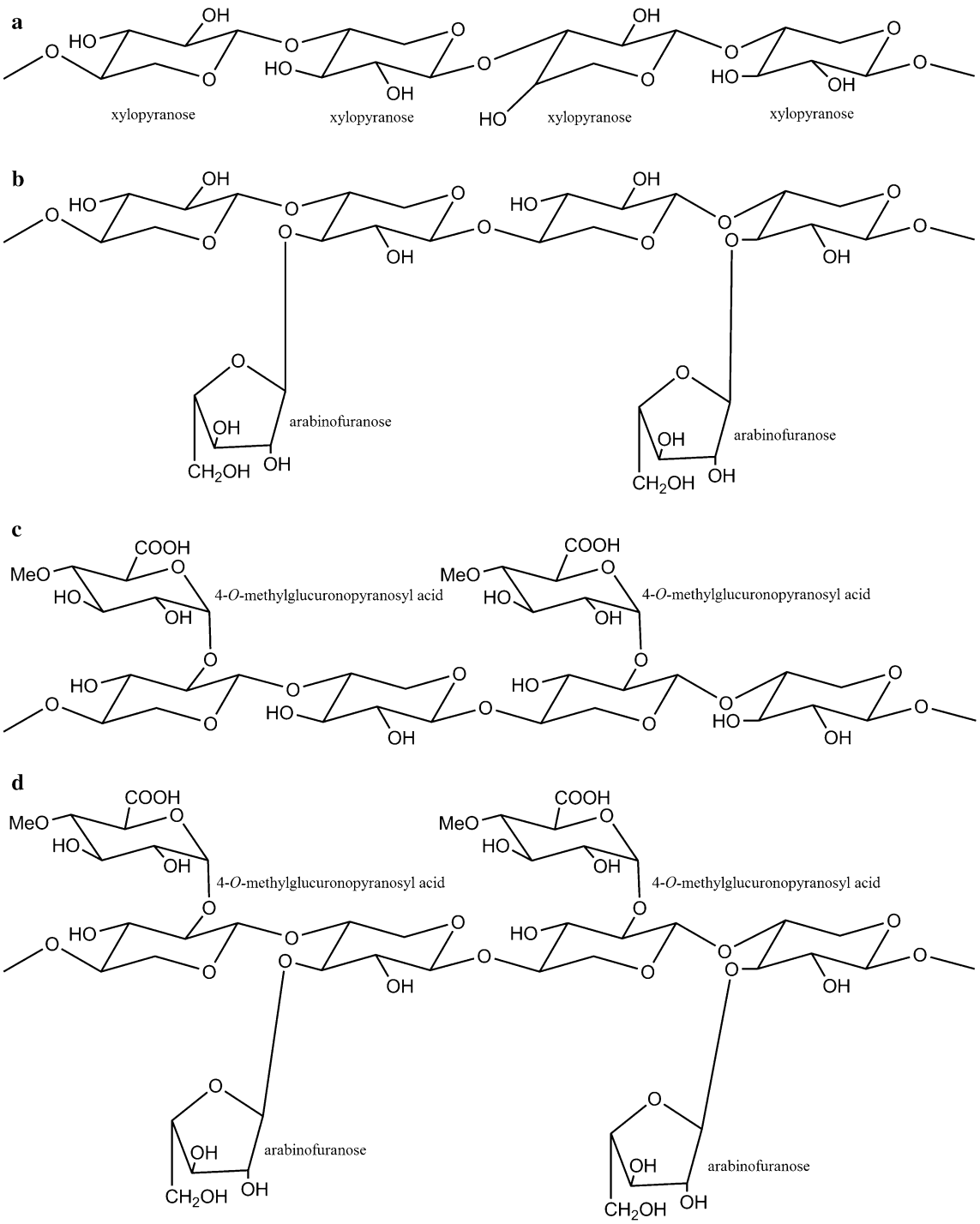

Fig. 1 **a** Structure of homoxylan. **b** Structure of arabinoxylan. **c** Structure of glucuronoxylan. **d** Structure of glucuronoarabinoxylan

crystal structure (Fushinobu et al. 2011; Motta et al. 2013). Literature reveals three categories of xylanases based on the optimum pH of the xylanases (Fushinobu et al. 2011) which are mainly determined by the presence of Asp residue or Asn residue. Acidophilic xylanases have got an Asp residue around the acid/base catalyst Glu, whereas neutrophilic and alkaliphilic xylanases carry an Asn residue instead of Asp. But there are some exceptions. For example, neutrophilic XynC of *Fibrobacter succinogenes* (Zhu et al. 1994) and Xyl-11 from *A. versicolor* (Carmona et al. 1998) having a pH optima of 6.0 carries an Asp at the position. Xylanases differing in their optimum pH have got specific industrial importance in various fields which are already discussed in the review and because of the usefulness of the acidophilic

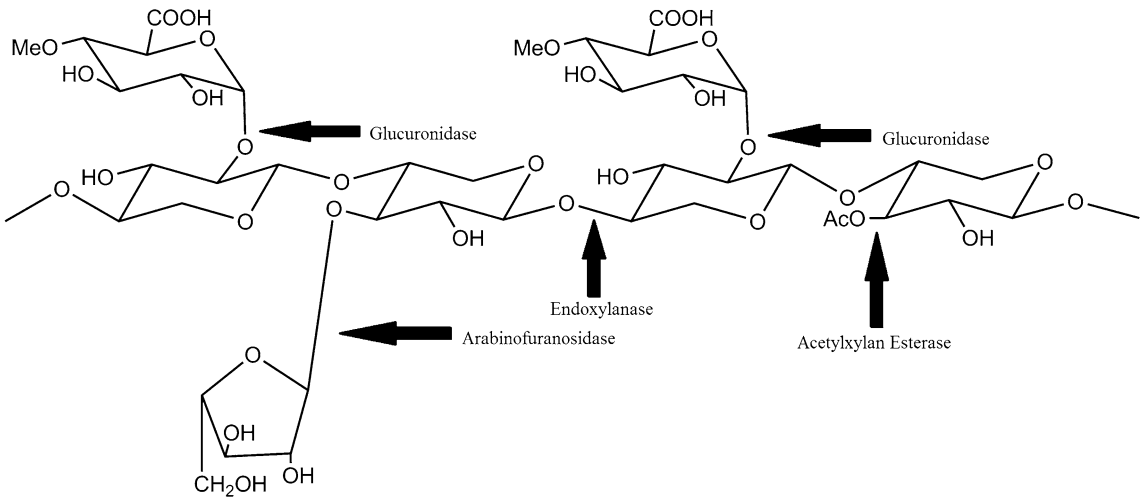

Fig. 2 Schematic diagram of hydrolysis of xylan with different xylanases (endoxylanase, arabinofuranosidase, glucuronidase, acetyl esterase, etc.)

enzymes; several attempts are being made to shift the pH optima of the xylanases in the acidic range (Pokhrel et al. 2013).

Afterward, another classification system was introduced that in general classifies glycosidases (E.C.3.2.1-) into different category based on their primary structure comparisons of the catalytic domain (which forms a database named CAZy) (Henrissat et al. 1989; Henrissat and Coutinho 2001; Collins et al. 2005). Initially, cellulases and xylanases were classified into six families and the numbers of the families are growing as new xylanases are being identified. As of now there are 146 different glycosyl hydrolase (GH) families enlisted in the CAZy database. According to CAZy database, xylanases fit into 14 different GH families (GH family 5, 7, 8, 9, 10, 11, 12, 16, 26, 30, 43, 44, 51 and 62). But sequence analyses have shown that only 6 out of 14 families (5, 7, 8, 10, 11 and 43) contain truly distinct catalytic domain for xylanase activity. It has been recently found that the GH5 family members are specialized for arabinoxylan digestion (Biely et al. 2016) and are α-1,3-linked arabinofuranoside appendage-dependent (Correia et al. 2011). Paes et al. (2012) have discussed the structure/function relationships of GH11 xylanases in some detail in a review earlier. Family 16, 51 and 62 contain xylanases that are bi-functional in nature and the rest of the families contain members (9, 12, 26, 30 and 44) that have got residual or secondary xylanase activity (Collins et al. 2005). For example, the GH30 xylanases are found to be appendage-dependent meaning that they require free 4-*O*-methyl-D-glucuronosyl residues for their activities (Paes et al. 2012).

The true endo-1,4-β-xylanases have finally been subdivided into two glycosyl hydrolase (GH) family that are GH 10 and GH 11 on the basis of the amino acid sequence and the hydrophobic cluster analysis of the catalytic domain (Henrissat and Bairoch 1993; Davies and Henrissat 1995; Jeffries 1996; Sapag et al. 2002; Verma and Satyanarayana 2012). Most of the members of these two families have been studied extensively. Comprehensive studies on the members of these two families disclose that they not only differ in their shape and size but also differ in their action upon substrates (4-*O*-methyl-D-glucurono-D-xylan and rhodymenan). Members of the family GH 10 yield aldotetrauronic acid and isomeric xylotriose whereas members of GH 11 family produce aldopentauronic acid and isomeric xylotetraose from 4-*O*-methyl-D-glucurono-D-xylan and rhodymenan upon action, respectively (Biely et al. 1997).It has been found that these two families share dissimilarities in substrate-binding sites too. Family GH 10 has got smaller substrate-binding site than members of GH 11 family (Biely et al. 1997). It has been observed that endoxylanases of fungal origin have a broad range of temperature optima (40–80 °C) as well as pH optima (2.0–6.0) like some of them have optimum pH at 2.0 (in case of Xyn C of *Aspergillus kawachii*), whereas others have their pH optima at pH 3.5-6.0 (XynI and XynII of *Trichoderma reesei*). In this review, we have focused on the details of the members of GH 11 xylanases only that are acidophilic in nature and belong to fungal origin.

Molecular structure of acid-stable xylanases

The endoxylanases that are active in low pH have got a shape that resembles the shape of a right hand (Törrönen et al. 1994) (Fig. 3) and possess much more acidic residues (Asp and Glu) than basic (Arg, Lys, His) to give the overall structure a net negative charge. They are mainly comprised of a single domain containing β-sheets and assume β-jelly roll fold architecture and contain a number of subsites for substrate binding. This is a characteristic feature for GH family

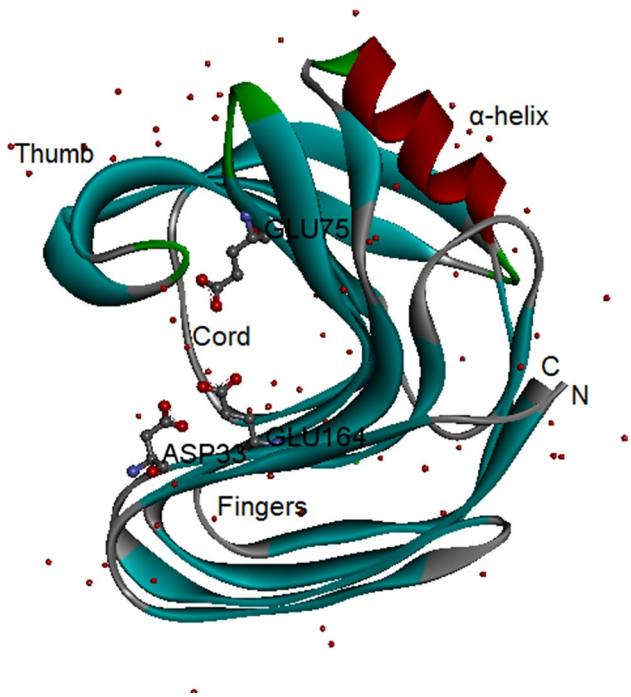

Fig. 3 A representative structure of fungal family G, acidophilic xylanase. The overall fold of XYNI in *Trichoderma reesei* is depicted with a cartoon model (PDB ID, 1XYN) showing different parts of the right-handed enzyme structure

G/11 xylanases. Molecular structures of some acidophilic xylanases (that are available in Protein Data Bank) (Berman et al. 2000) are discussed below.

XYNI of *T. reesei*

XYNI is composed of 178 amino acid residues. The catalytic residues for the enzymes are Glu 75 and Glu 164. The former one acts as "nucleophile" and the later one acts as "acid–base catalyst". The enzyme is composed of two β-sheets and one α-helix. The two β-sheets antiparallely form the "Fingers" of the right hand structure. One pair of β-strands and the α-helix are found to be engaged to form the "Palm". A long loop connecting the β-strands B7 and B8 shapes the "thumb" region. A five amino acid residues long loop that connects the β-strands B6a and B9 looks like a "Cord" that reaches the cleft. It has got three subsites in the active site of the molecule that were marked as − 2, − 1 and + 1. Among the subsites, two are present in the non-reducing end, and one is in the reducing site suggesting the direction of ligand binding which is directed towards the non-reducing end of the molecule. It has been found that the surroundings of the catalytic glutamic acid residue Glu 75 are highly conserved among family 11 xylanases and these are Tyr 66, Trp 68, Tyr 77, Gln 132, and Tyr 158. According to Wakarchuk et al., the Tyr 66 and Tyr 158 are involved in ligand binding (Wakarchuk et al. 1994). It has been predicted that all these conserved residues may act to fine-tune the catalytic property of active glutamic acid residues (Törrönen and Rouvinen 1995). On the contrary, the surroundings of the other catalytic glutamic acid residue Glu 164 is found to be much less conserved. An aspartic acid residue at position 33 plays a critical role that confers the enzyme to possess low pH optima. Torronen and Rouvinen have found that the Oδ1 of Asp 33 and Oε1 of Glu 164 are very close to each other (2.9 Å) and they proposed the existence of a proton in between the two acidic amino acid residues that probably decreases the pKa of Glu 164 (Törrönen and Rouvinen 1995).

Xylanase I of *Aspergillus niger*

The Xylanase I of *A. niger* is 184 amino acid long. Krengel and Dijkstra (1996) performed the X-ray crystallography with the enzyme to have a look on its molecular structure. They too have the similar observations for the catalytic residues. Here, Glu 79 and Glu 170 have been observed to be the catalytic residues where Glu 79 is the nucleophile and Glu 170 is the acid-base catalyst. According to them, two β-sheets comprised of 13 β-strands are twisted antiparallely, thereby forming a deep cleft that contains the substrate-binding sites. The cleft is lined with many aromatic amino acid residues mostly Tyr. Among these, Tyr 70 and Tyr 81 play important role which is to place the two catalytic glutamic acid residues (Glu 79 and Glu 170) perfectly in position by creating hydrogen bonds. It contains only one α-helix that is found to be embedded on the rear side of the cleft and electrostatically interacts with highly conserved amino acids.

Xyn C of *A. kawachii*

The Xyn C of *A. kawachii* is found to be the most interesting acidophilic xylanases because it has got a pH optima of 2.0 and can remain active at pH 1.0. Fushinobu et al. (1998) determined the crystal structure of the enzyme. Like the two other acidophilic xylanases mentioned here, it is made up of two antiparallel β-sheets and one α-helix that confers the structure resembling a right hand. They found a high degree of structural similarity with the XYNI of *T. reesei*. A few dissimilarities were there which includes three regions that correspond to the "tip" and base of the little finger and the "tip" of the ring finger. These three regions comprised of two short 3_{10} helices in the segment Asn 11-Gly 15, Asp 20-Gly 24 and a short turn in Glu 31-Gly 33, respectively. The fourth region (Ala 153 to Asp 161) is adjacent to the helix and the fifth one lies in the "Cord" region (Gly 87-Ala 95) with a disulfide linkage between Cys 92 and Cys 111 on the β-strand B8. The crystal structure of Xylanase I from *A. niger* was published during the structural study

of Xyn C from *A. kawachii* and they have found that these two enzymes only differs in three amino acid residues. The positions are 64, 154 and 167. In the "Cord" region, there is a conserved proline residue which is thought to be a determinant of the "Cord" conformation. The active site of this enzyme is made up of aromatic amino acid residues stretching the inner part of the cleft. Among them, Tyr 10, Phe 131 and Trp 172 are important. These three amino acids were found to be conserved in acidophilic xylanases, whereas in alkaliphilic xylanases, these amino acids are Trp, Trp and Tyr, respectively. The convex flat face of β-sheet A contains a large number of negatively charged residues that are Asp 16, Asp 20, Glu 21, Glu 31, Asp 32 and Glu 57. In other xylanases of family G/11, this region is occupied by Ser and Thr residues (Fushinobu et al. 1998; Törrönen et al. 1994) which is called Ser/Thr surface in family G/11 xylanases.

XYL1 of *Scytalidium acidophilum*

The XYL1 of *S. acidophilum* has been first reported by Al Balaa et al. (2006). This enzyme is composed of 181 amino acids and showed its optimum activity at pH 3.2. Its low pH optimum led the scientists to have a look on its crystal structure to establish its basis for acidic pH optima and they have found a quite similar structure to those of other acidophilic xylanases. They have also got 13 β-strands packed in two antiparallel β-sheets and assuming a shape of right hand. One α-helix is present at the rear side of the molecule. At the active site (Cord region), a buffer CAPS (*N*-cyclohexyl-3-aminopropanesulfonic acid) moiety was found to be present. The sulfate group of the CAPS molecule interacts with Arg 138 and a water molecule present in the structure. A disulfide linkage (Cys 115 and Cys 134) is also found in the central cord region just like the other members acidophilic xylanase class (Michaux et al. 2010).

Catalytic mechanisms of acid-stable xylanases

Acidophilic xylanases along with some alkaliphilic xylanases have been shown to cleave the β-1,4-glycosidic bonds of xylan with the retention of anomeric configuration of the sugar moiety (Coutinho and Henrissat 1999) via two successive nucleophilic displacement (S_N2) mechanisms (McCarter and Withers 1994; Rye and Withers 2000; Zechel and Withers 2000). This particular reaction mechanism is analogous to those catalyzed by α-amylase and lysozyme (VršanskáIlona et al. 1982). The mechanism of the reaction is found to be little tricky but can be understood from the Fig. 4. Upon substrate binding, the reaction starts with the active participation of the nucleophile which is generally a glutamic acid residue present in the B6 β-strand. It attacks the C-1 of the xylan molecule to displace the first xylose residue from the reducing end and forms a bond to stabilize the intermediate. Then the acid–base catalyst, another glutamic acid residue, comes to play its role. It acts as an acid at that moment and protonates the departing xylose residue. In the next step, a water molecule comes and attacks the C-1 to displace the nucleophile, thereby releasing the second product. The water molecule also gives a proton to the acid–base catalyst, glutamate that makes the residue ready for its next round of reaction. However, the frequencies of the glycosidic bonds cleavage of xylopentaose, xylotetraose and xylotriose highly depend on the initial concentration of the substrate molecule (Biely et al. 1981; VršanskáIlona et al. 1982).

Molecular basis for the low pH optima of acid xylanases

The catalytic mechanism of an enzyme is determined by the nature of the amino acid residues that are responsible for catalysis. The local environment is the key player which forces an amino acid to act as an acid or base. Generally, the pKa of the catalytic residues decide the pH profile of enzymes. A number of studies regarding the catalytic mechanism of acid-stable endoxylanase reveal the existence of an aspartic acid or glutamic acid residue playing the pivotal role (Fig. 5).

XYNI of *T. reesei*

The XYNI of *T. reesei* exhibits a pH range of 3.0–6.0 and maximally active at pH 3.5. Its isoelectric point is nearly 5.2. This enzyme undergoes substrate hydrolysis via double-displacement mechanism same as proposed for hen egg-white lysozyme where two carboxyl groups are participating in catalysis. One of them is acting as a nucleophile that stabilizes the reaction intermediate and the other one is the acid–base catalyst. The Glu 75 and Glu 164 act as catalytic residue for the enzyme. Glu 75 is the nucleophile that attacks the C-1of the substrate to release the first product and Glu 164 works as an acid that donates a proton to the departing xylose or xylo-oligosaccharide. In 1995, Torronen and Rouvinen reported the presence of an Asp residue at position 33 that goes close to the Glu, that is, at position 164 due to protein folding and makes a hydrogen bond which is of 2.9 Å long. They proposed that this Asp residue probably lowers the pKa of Glu 164 creating a negatively charged environment, thereby lowering the pH optimum of the enzyme (Törrönen and Rouvinen 1995).

Xylanase I of *A. niger*

This enzyme has a lot of industrial applications as it is most effective in low pH (pH 3.0) and thus it is produced commercially by Gist Brocades Company in Delft, The Netherlands (van den Broeck et al. 1992). In case of xylanase I of

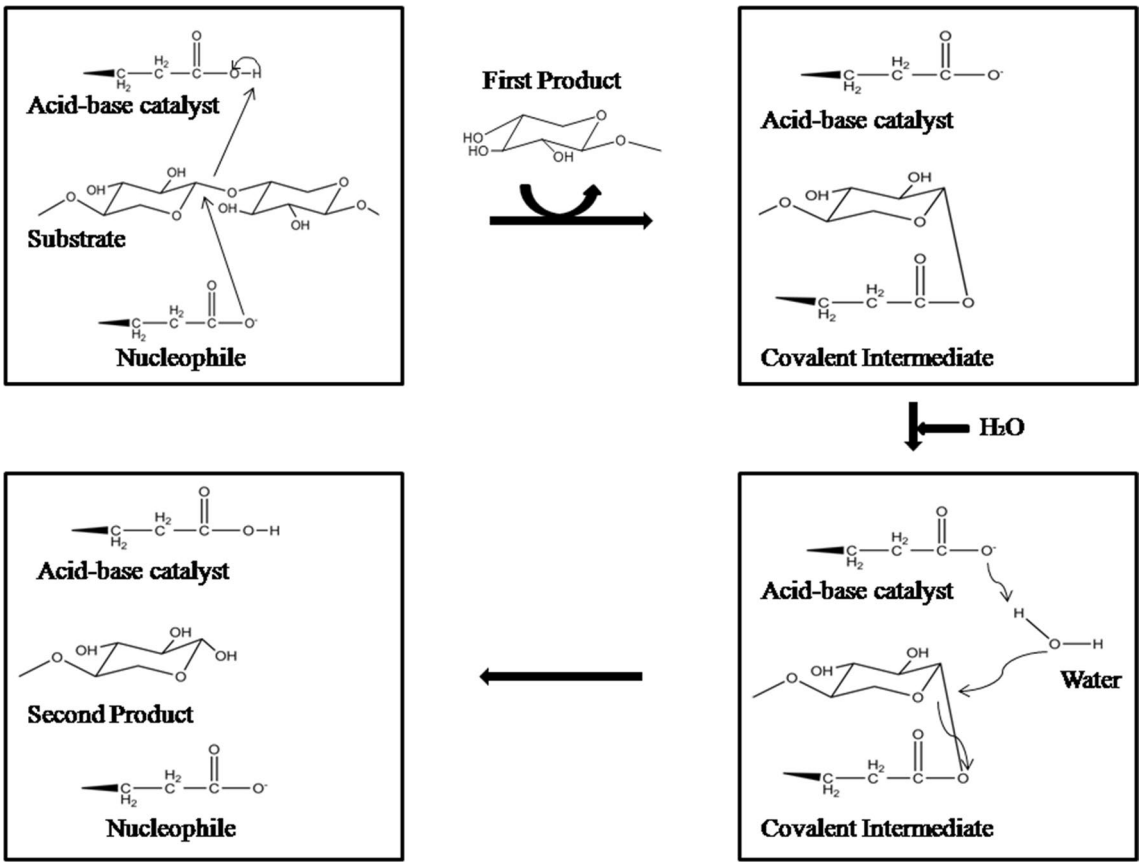

Fig. 4 Catalytic mechanism of acidophilic xylanases. The reaction takes place via two successive nucleophilic displacement or S_N2 pathway

A. niger, Glu79 is predicted to be the nucleophile and Glu 170 is the acid–base catalyst. The Asp 37 residue was proposed to be the basis for its low pH optima. The carboxylic atom of the Asp 37 forms a hydrogen bond of near about 2.8 Å with the acid–base catalyst, Glu170 and it was thought that the bonding distance is responsible for low pH optima. It was observed that the other endoxylanases that carries Asn instead of Asp at the said position exhibit a higher pH optima and the bonding distance with the acid–base catalyst is also larger than that of Asp-containing xylanases. But the exact role of this particular residue is still not clear. Further studies such as site-directed mutagenesis of this residue are needed to establish its specific role (Krengel and Dijkstra 1996).

Xylanase C of *A. kawachii*

The xylanase C from *A. kawachii* exhibits pH optima of 2.0. Glu 79 and Glu 170 were found to be the catalytic residues acting as nucleophile and acid–base catalyst, respectively. It is found that the Asp 37 is highly responsible for the low pH optima as the replacement of Asp with Asn shifts the pH optima to the higher limit. X-ray crystallographic study reveals that the short distance (2.8 Å) between the Asp 37 and Glu 170 influences the pH profile. In case of xylanases with alkaline pH optima, the Asp was found to be replaced with Asn and has a bonding distance of 3.1-3.5 Å with the acid base catalyst. Structural comparison with other xylanase having higher pH optima shows a remarkable feature that the xylanase C has got a number of acidic amino acid residues at the edge of the catalytic cleft and these are Asp16, Asp 20, Glu 21, Glu 31, Asp 32, Glu 57. All these acidic amino acids create a strong negative-charged environment that corresponds for the low pI of the enzyme (Fushinobu et al. 1998).

XYL1 of *S. acidophilum*

The XYL1 possesses two glutamic acid residues (Glu 104 and Glu 192) as its catalytic machinery too. Along with that it contains the Asp (Asp 60) residue near the acid–base catalyst Glu 192 which is the main key factor for maintaining the low pH optima. The hydrogen bonding distance between these two residues was found to be 2.45 Å. This hydrogen bonding distance is the lowest among the observed acidophilic xylanases. An experiment by mutating the Asp 60 to

```
XynA          QTITSSQTGTNNGYYYSFWTNGGGTVQYTNGAAGEYSVTWENC--GDFTSGKGWSTGSAR
XYNI          ----------ASINYDQNYQT-GGQVSY-SPSNTGFSVNWNTQ--DDFVVGVGWTTGSSA
XylanaseI     ---------SAGINYVQNYNGNLGDFTY-DESAGTFSMYWEDGVSSDFVVGLGWTTGSSK
XYNC          ---------SAGINYVQNYNGNLADFTY-DESAGTFSMYWEDGVSSDFVVGLGWTTGSSN
XylanaseCS    -----------TGNYVQNYNGNVANFEY-SQYDGTFSVNWNGN--TDFVCGLGWTVGTGR
                            * . :    . . * .     :*: *:     **. * **:.*:.

XynA          DITFEGTFNPSG-NAYLAVYGWTTSP-LVEYYILEDYGDYNPGNS--MTYKGTVTSDGSV
XYNI          PINFGGSFSVNSGTGLLSVYGWSTNP-LVEYYIMEDNHNYPAQ----GTVKGTVTSDGAT
XylanaseI     AITYSAEYSASGSSSYLAVYGWVNYP-QAEYYIVEDYGDYNPCSS--ATSLGTVYSDGST
XYNC          AISYSAEYSASGSSSYLAVYGWVNYP-QAEYYIVEDYGDYNPCSS--ATSLGTVYSDGST
XylanaseCS    TITYSGSYNPGYSGSYQAIYGWTGQGSLSEYYVIDNYGGYNPCTGSGVTQLGSLYSDGSS
               *.: . :. .   .  ::***       ***::::  .*        *  *:: ***:

XynA          YDIYEHQQVNQPSISGTATFNQYWSIRQNTRSSGTVTTANHFNAWAKLGMNLGSFNYQIV
XYNI          YTIWENTRVNEPSIQGTATFNQYISVRNSPRTSGTVTVQNHFNAWASLGLHLGQMNYQVV
XylanaseI     YQVCTDTRTNEPSITGTSTFTQYFSVRESTRTSGTVTVANHFNFWAQHGFGNSDFNYQVM
XYNC          YQVCTDTRTNEPSITGTSTFTQYFSVRESTRTSGTVTVANHFNFWAQHGFGNSDFNYQVM
XylanaseCS    YQVCTHTQYNQPSIVGTTTFPQYFSVRQNKRSSGSVNMQNHFNYWAQHGFPNRNFNYQVL
              * :  . : *:*** **:** ** *:*:. *:**:*.  **** **. *:   .:***::

XynA          STEGYESSGSSTITVS--
XYNI          AVEGWGGSGSASQSVSN-
XylanaseI     AVEAWSGAGSASVTISS-
XYNC          AVEAWSGAGSASVTISS-
XylanaseCS    AVEGFSGSGNANMKLISG
              :.*.: .:*.:. .:
```

Fig. 5 Sequence Alignment (Goujon et al. 2010; Sievers et al. 2011; McWilliam et al. 2013) of acidophilic xylanases from *A. niger* (van den Broeck et al. 1992), *A. kawachii* (Fushinobu et al. 1998), *Cryptococcus* sp. S-2 (Iefuji et al. 1996), *T. reesei* (*T. reesei* I; Törrönen and Rouvinen 1995), and *Penicillium* sp. 40 (Kimura et al. 2000) showing the three important amino acid residues (noncatalytic aspartic acid, nucleophile glutamic acid and the acid–base catalyst glutamic acid)

Asn 60 of XYL1 was performed to check its importance in maintaining the acidic pH optima which resulted in a shift of pH optima by 0.9 units (Michaux et al. 2010).

Applications of acid-stable xylanases

The application of xylanases for industrial purposes has increased many folds in current years (Wong et al. 1988; Golugiri et al. 2012; Beg et al. 2001). Xylanases has occupied 20% of the global market for industrially important enzymes along with cellulases and pectinases (Polizeli et al. 2005) replacing the use of hazardous chemicals. As we are looking forward to a better environment, ecofriendly tools are gaining the popularity, thereby making enzyme biotechnology a potent domain of research work. Xylanases are being applied in industries for paper and pulp production, food and feed production, pharmaceuticals production, textile and most importantly in biofuel production. The special characteristic exhibited by the acidophilic xylanases which is to remain active at low pH seems advantageous for many applications discussed below.

Biobleaching in paper-pulp industry

The importance of using xylanase in paper-pulp industries lies in the hydrolysis of the xylan that corresponds to the lignin removal. Elimination of lignin whitens the paper thereby enhancing its quality. In the past years, chlorine, the chemical bleach, was being used to remove lignin but it has got a number of drawbacks that include generation of products hazardous to human health. These by-products are sometimes found to be toxic, mutagenic, persistent and highly resistant to biodegradation indicating a high degree of environment pollution. The application of xylanases in pulp to remove lignin is known as biobleaching. Several reports suggest that the use of acid-tolerant xylanases for pulp-bleaching has increased as it is advantageous in a number of aspects (Tenkanen et al. 1997; Schell et al. 2003). A study carried out by Tenkanen et al. (1997) proved that

the use of xylanase (XynC) from *A. kawachii* for bleaching of pulp is highly efficient and due to its low optimum pH, metal ions associated with the pulp can easily be removed. Thus, the application of acidophilic xylanase omits the step that involves the addition of chelating agent such as EDTA thereby decreasing the cost associated with bleaching. Another advantage is associated with the pretreatment of kraft pulp by thermo-chemical techniques. This step lowers the pH of the medium (pH 0.8–1.2) as it involves the use of dilute sulfuric acid (0.5–1.5%) (Schell et al. 2003). At this point, the use of highly acid-stable xylanases such as XynC of *A. kawachii,* Xyn A of *Penicillium* sp. 40, Xylanase CS of *Cryptococcus* sp whose optimum pH are 2.0 and retain 75% of its activity at pH 1.0 (Fushinobu et al. 1998; Kimura et al. 2000; Iefuji et al. 1996, respectively) not only minimizes the steps but also lowers the cost associated with biomass residue neutralization (Goldman and Kole 2014).

Food and feed industry

Acidophilic xylanases are very important for food and feed industries. They are highly desirable in clarifying juices, bread making, xylooligosaccharide production, etc. (Sharma et al. 2016). For the purpose of bread making in food industries, acidophilic xylanases are applied because the pH of the dough lies in acidic range (Sharma et al. 2016). The Xylanase I of *A. niger* is commercially produced by the company Gist-Brocades in Delft, The Netherlands (van den Broeck et al. 1992) to use it widely in the food and feed industry. These acidophilic enzymes are also preferable in animal food as supplement. Their presence in the food induces effective feeding by increasing the flora of lactic acid (lactobacilli and bifido) bacteria and decreasing the number of *E. coli* present in the intestine. According to Kittelmann and Janssen (2011), this healthy balance of intestinal micro flora is highly important to keep an animal healthy. Presence of them in animal food also reduces excretion as well as its low pH helps to release important nutrients from the diet in the alimentary tract. Apart from that, it has also been proved that use of acid-stable xylanase in animal food leads to better digestibility because the pH of the intestine tends to be acidic (Chen et al. 2016). In acidic environment, only enzymes having low pH optima will act better than those having alkaline pH optima. Another advantage of using these acidophilic enzymes in animal food is they exert an improving effect on average daily gain (ADG) of animals (Chen et al. 2016).

Biofuel production

The over consumption of fossil fuels in last few decades not only lead to rapid exhaustion of the stock but also exert deleterious impacts on the environment. Such negative effects of using fossil fuel gives rise to thinking of alternatives which should be ecofriendly as well as sustainable in nature. Nowadays, renewable energy in the form of biofuel from lignocellulosic substrate has become one of the important modes of sustainable energy productions. The lignocellulosic biomass is highly abundant as the production of this biomass is estimated to be 10–50 billion dry tons worldwide (Claassen et al. 1999; Galbe and Zacchi 2002). The production of ethanol from this biomass does not compete with the food source and it is cost effective too. But this biomass is recalcitrant in nature and seeks pretreatment which includes the introduction of an acidic environment that makes the structure more fragile and accelerates the penetration of active molecules that can cleave the bonds within it (Himmel et al. 2007) thereby increasing the ethanol yield (Galbe and Zacchi 2007; Tomás-Pejó et al. 2008). This pretreatment process involves the addition of dilute sulfuric acid which increases the accessible surface area of the biomass and effectively solubilizes the hemicellulosic part present in the biomass (Alvira et al. 2010). So the pretreatment process involving sulfuric acid makes the medium acidic and at this point the use of acidophilic xylanases would be beneficial as no pH adjustment step for downstream process is needed.

Conclusion

The molecular basis of the acidophilic xylanases discussed here increases the understanding about their structure–function relationship which is of immense use for those engaged in protein engineering for their commercial uses. The major commercial applications of xylanases lie in biofuel making industry, paper-pulp industry and food-feed industry. For biofuel making industry, it is cost effective to use acidophilic xylanases. In kraft-pulp biobleaching, application of these acidophilic enzymes prior to ozone or chlorine dioxide treatment are found to be beneficial and environment friendly too. Alkaliphilic and neutral xylanases possess elevated ratio of positive to negatively charged amino acid residues but acidophilic xylanases are just opposite of them. The Ser/Thr surface of neutral and alkaliphilic xylanases are found to be replaced with acidic residues (Asp16, Asp 20, Glu 21, Glu 31, Asp 32, Glu 57) in the acidophilic enzyme of *A. kawachii*. Construction of acidic xylanases (shifting the optimum pH to the lower end) can be done by performing site-directed mutagenesis in these sites of alkaliphilic or neutral xylanases. The dictator of the acidophilic property of these xylanases is observed to be an Asp residue that forms a short-distance hydrogen bond with the acid–base catalyst. In neutral and alkaliphilic xylanases, this Asp residue has been replaced with an Asn residue that forms a weak hydrogen bond with the acid–base catalyst Glu thereby elevating the pI of these enzymes. This particular Asp residue can be

engineered to shift the optimum pH of these enzymes to the acidic side. In *A. kawachii*, the active cleft of the XynC is found to be surrounded by aromatic amino acids like all other family G xylanases. But the orientations of the aromatic amino acids are different. In *A. kawachii*, these are Tyr 10, Phe 131 and Trp 172 and in alkaliphilic xylanases these are replaced with Trp, Trp and Tyr, respectively. All the three sites may serve as potent locations for creating mutant enzyme with low pH optimum using recombinant DNA methodologies. Nowadays directed evolution also has become an important strategy for creating superior extremophilic industrial enzymes (Ruller et al. 2014). This tool, which mimics the process of natural selection, can also be used to screen for desirable acid-stable xylanase enzyme for industrial uses. A number of attempts have already been initiated to produce more thermo-stable acidophilic xylanase, such as in the case of a GH11 enzyme by Yang et al. (2017) using this concept. As very few acidic xylanases are reported to be available in nature till now, the guidance of this article, which is about the structure or the amino acid composition of this specific class of acidophilic enzyme, will assist in the creation of more genetically engineered acid-stable xylanases by researchers.

Acknowledgements PD is thankful to the University Grant Commission, India and Visva-Bharati University for financial assistance.

Compliance with ethical standards

Conflict of interest There is no conflict of interest between any of the author.

Ethical statement This article does not contain any studies with human participants or animals performed by any of the authors.

References

Al Balaa B, Wouters J, Dogne S, Rossini C, Schaus JM, Depiereux E, Vandenhaute J, Housen I (2006) Identification, cloning, and expression of the Scytalidium acidophilum XYL1 gene encoding for an acidophilic xylanase. Biosci Biotechnol Biochem 70:269–272

Alvira P, Tomás-Pejó E, Ballesteros M, Negro MJ (2010) Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. Bioresour Technol 101:4851–4861

Bayer EA, Chanzy H, Lamed R, Shoham Y (1998) Cellulose, cellulases and cellulosomes. Curr Opin Struct Biol 8:548–557

Beg QK, Kapoor M, Mahajan L, Hoondal GS (2001) Microbial xylanases and their industrial applications: a review. Appl Microbiol Biotechnol 56:326–338

Belancic A, Scarpa J, Peirano A, Diaz R, Steiner J, Eyzaguirre J (1995) *Penicillium purpurogenum* produces several xylanases: purification and properties of two of the enzymes. J Biotechnol 41:71–79

Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig Shindyalov HIN, Bourne PE (2000) The protein data bank. Nucl Acids Res 28:235–242

Berrin JG, Williamson G, Puigserver A, Chaix JC, McLauchlan WR, Juge N (2000) High-level production of recombinant fungal endo-β-1,4-xylanase in the methylotrophic yeast *Pichia pastoris*. Protein Expr Purif 19:179–187

Biely P (1985) Microbial xylanolytic systems. Trends Biotechnol 3:286–290

Biely P, Vrsanská M, Krátký Z (1981) Mechanisms of substrate digestion by endo-1,4-beta-xylanase of *Cryptococcus albidus*. Lysozyme-type pattern of action. Eur J Biochem 119:565–571

Biely P, Vrsanská M, Tenkanen M, Kluepfel D (1997) Endo-beta-1,4-xylanase families: differences in catalytic properties. J Biotechnol 57:151–166

Biely P, Singh S, Puchart V (2016) Towards enzymatic breakdown of complex plant xylan structures: state of the art. Biotechnol Adv 34:1260–1274

Cao YH, Qiao JY, Li YH, Lu WQ (2007) De novo synthesis, constitutive expression of *Aspergillus sulphureus* β-xylanase gene in *Pichia pastoris* and partial enzymic characterization. Appl Microbiol Biotechnol 76:579–585

Carmona EC, Brochetto-Braga MR, Pizzirani-Kleiner AA, Jorge JA (1998) Purification and biochemical characterization of an endoxylanase from *Aspergillus versicolor*. FEMS Microbiol Lett 166:311–315

Chen Q, Li M, Wang X (2016) Enzymology properties of two different xylanases and their impacts on growth performance and intestinal microflora of weaned piglets. Anim Nutr 2:18–23

Claassen PAM, van Lier JB, Contreras AML, van Niel EWJ, Sijtsma L, Stams AJM, de Vries SS, Weusthuis RA (1999) Utilisation of biomass for the supply of energy carriers. Appl Microbiol Biotechnol 52:741–755

Collins T, Gerday C, Feller G (2005) Xylanases, xylanase families and extremophilic xylanases. FEMS Microbiol Rev 29:3–23

Correia MAS, Mazumder K, Brás JLA, Firbank SJ, Zhu Y, Lewis RJ, York WS, Fontes CMGA, Gilbert HJ (2011) Structure and function of an arabinoxylan-specific xylanase. J Biol Chem 286:22510–22520

Coughlan GP, Hazlewood MP (1993) β-1,4-D-xylan-degrading enzyme system: biochemistry, molecular biology, and applications. Biotechnol Appl Biochem 17:259–289

Coutinho PM, Henrissat B (1999) Carbohydrate-active enzyme server (CAZY). http://afmb.cnrs-mrs.fr/~cazy/CAZY/

Davies G, Henrissat B (1995) Structures and mechanisms of glycosyl hydrolases. Structure 3:853–859

Driss D, Bhiri B, Elleuch L, Bouly N, Stals I, Miled N, Blibech M, Ghorbel R, Chaaboun SE (2011) Purification and properties of an extracellular acidophilic endo-1,4-β-xylanase, naturally deleted in the "thumb", from *Penicillium occitanis* Pol6. Process Biochem 46:1299–1306

Ebringerova A, Heinze T (2000) Xylan and xylan derivatives— biopolymers with valuable properties. 1. Naturally occurring xylans structures, isolation procedures and properties. Macromol Rapid Commun 21:542–556

Enshasy HAE, Kandiyli SK, Malek R, Othman NZ (2016) Microbial xylanases: sources, types and their application. In: Gupta VK (EDS) Microbial enzymes in bioconversions of biomass, Chap 7. Springer, Switzerland, pp 151–214

Fushinobu S, Ito K, Konno M, Wakagi T, Matsujawa H (1998) Crystalographic and mutational analyses of an extremely acidophilic and acid-stable xylanase: biased distribution of acidic residues and importance of Asp37 for catalysis at low pH. Protein Eng 11:1121–1128

Fushinobu S, Uno T, Kitaoka M, Hayashi K, Matsuzawa H, Wakagi T (2011) Mutational analysis of fungal family 11 xylanases on pH optimum determination. J Appl Glycos 58:107–114

Galbe M, Zacchi G (2002) A review of the production of ethanol from softwood. Appl Microbiol Biotechnol 59:618–628

Galbe M, Zacchi G (2007) Pretreatment of lignocellulosic materials for efficient bioethanol production. Adv Biochem Eng Biotechnol 108:41–65

Goldman SL, Kole C (2014) Compendium of bioenergy plants: Corn. CRC press Taylor and Francis group

Golugiri BR, Thulluri C, Cherupally M, Nidadavolu N, Achuthananda D, Mangamuri LN, Addepally U (2012) Potential of thermo and alkali stable xylanases from *Thielaviopsis basicola* (MTCC-1467) in biobleaching of wood Kraft pulp. Appl Biochem Biotechnol 167:2369–2380

Goujon M, McWilliam H, Li W, Valentin F, Squizzato S, Paern J, Lopez R (2010) A new bioinformatics analysis tools framework at EMBL-EBI. Nucl Acids Res 38(Suppl):W695–W699. https://doi.org/10.1093/nar/gkq313

Henrissat B, Bairoch A (1993) New families in the classification of glycosyl hydrolases based on amino acid sequence similarities. Biochem J. 293:781–788

Henrissat B, Coutinho PM (2001) Classification of glycoside hydrolases and glycosyltransferases from hyperthermophiles. Methods Enzymol 330:183–201

Henrissat B, Claeyssens M, Tomme P, Lemesle L, Mornon JP (1989) Cellulase families revealed by hydrophobic cluster analysis. Gene 81:83–95

Hessing JGM, van Rotterdam C, Verbakel JMA, Roza M, Maat J, van Gorcom RFM, van den Hondel CAMJJ (1994) Isolation and characterization of a 1,4-β-endoxylanase gene of *A. awamori*. Curr Genet 26:228–232

Himmel ME, Ding SY, Johnson DK, Adney WS, Nimlos MR, Brady JW, Foust TD (2007) Biomass recalcitrance: engineering plants and enzymes for biofuels production. Science 315:804–807

Iefuji H, Chino M, Kato M, Iimura Y (1996) Acid xylanase from yeast *Cryptococcus* sp. S-2: purification, characterization, cloning, and sequencing. Biosci Biotech Biochem 60:1331–1338

Jeffries TW (1996) Biochemistry and genetics of microbial xylanases. Curr Opin Biotechnol 7:337–342

Juturu V, Wu JC (2011) Microbial xylanases: engineering, production and industrial applications. Biotechnol Adv. https://doi.org/10.1016/j.biotechadv.2011.11.006

Kimura T, Ito J, Kawano A, Makino T, Kondo H, Karita S, Sakka K, Ohmiya K (2000) Purification, characterization, and molecular cloning of acidophilic xylanase from *Penicillium* sp. 40. Biosci Biotechnol Biochem 64:1230–1237

Kittelmann S, Janssen PH (2011) Characterization of rumen ciliate community composition in domestic sheep, deer, and cattle, feeding on varying diets, by means of PCR-DGGE and clone libraries. FEMS Microbiol Ecol 75:468–481

Knob A, Carmona EC (2010) Purification and characterization of two extracellular xylanases from *Penicillium sclerotiorum*: a novel acidophilic xylanase. Appl Biochem Biotechnol 162:429–443

Knob A, Beite SM, Fortkamp D, Terrasan CRF, Almeida AFD (2013) Production, purification and characterization of a major *Penicillium glabrum* xylanase using brewer's spent grain as substrate. Biomed Res Int 2013:728–735

Korona B, Korona D, Bielecki S (2006) Efficient expression and secretion of two co-produced xylanases from *Aspergillus niger* in *Pichia pastoris* directed by their native signal peptides and the *Saccharomyces cerevisiae* alpha-mating factor. Enzyme Microb Technol 39:683–689

Krengel U, Dijkstra BW (1996) Three-dimensional structure of endo-1,4-b-xylanase I from *Aspergillus niger*: molecular basis for its low pH optimum. J Mol Biol 263:70–78

Kuhad RC, Singh A (1993) Lignocellulose biotechnology: current and future prospects. Crit Rev Biotechnol 13:151–172

Kuhad RC, Singh A, Eriksson KE (1997) Microorganisms and enzymes involved in the degradation of plant fiber cell walls. Adv Biochem Eng Biotechnol 57:45–125

Liao H, Zheng H, Li S, Wei Z, Mei X, Ma H, Shen Q, Xu Y (2015) Functional diversity and properties of multiple xylanases from *Penicillium oxalicum* GZ-2. Sci Rep 5:12631

Ljungdahl LG (2008) The cellulase/hemicellulase system of the anaerobic fungus *Orpinomyces* PC-2 and aspects of its applied use. Ann NY Acad Sci 1125:308–321

Luo HY, Wang Y, Li J, Wang H, Yang J, Yang YH, Huang H, Fan Y, Yao B (2009) Cloning, expression and characterization of a novel acidic xylanase, XYL11B, from the acidophilic fungus *Bispora* sp. MEY-1. Enzyme Microb Technol 45:126–133

McCarter JD, Withers SG (1994) Mechanisms of enzymatic glycoside hydrolysis. Curr Opin Struct Biol 4:885–892

McWilliam H, Li W, Uludag M, Squizzato S, Park YM, Buso N, Cowley AP, Lopez R (2013) Analysis tool web services from the EMBL-EBI. Nucl Acids Res 41:W597–W600

Michaux C, Pouyez J, Mayard A, Vandurm P, Housen I, Wouters J (2010) Structural insights into the acidophilic pH adaptation of a novel endo-1,4-beta-xylanase from *Scytalidium acidophilum*. Biochimie 92:1407–1415

Motta FL, Andrade CCP, Santana MHA (2013) Review of xylanase production by the fermentation of xylan: classification, characterization and applications. In: Chandel AK, Silva SSd (eds) Sustainable degradation of lignocellulosic biomass—techniques, applications and commercialization. InTech http://dx.doi.org/10.5772/53544

Moore MM (2009) Genetic engineering of fungal cells. In: Doelle HW, Rokem S (eds) Biotechnology, Fundamentals in Biotechnology, Encyclopedia of life support systems (EoLSS) vol. 3, pp.36–66

Mukherjee M, Sengupta S (1985) An inducible xylanase of the mushroom *Termitomyces clypeatus* differing from the xylanase/amylase produced in dextrin medium. J Gen Microbiol 131:1881–1885

Ohta K, Moriyama S, Tanaka H, Shige T, Akimoto H (2001) Purification and characterization of an acidophilic xylanase from *Aureobasidium pullulans* var. melanigenum and sequence analysis of the encoding gene. J Biosci Bioeng 92:262–270

Paes G, Berrin JG, Beaugrand J (2012) GH11 xylanases: structure/function/properties relationships and applications. Biotechnol Adv 30:564–592

Parachin NS, Siqueira S, deFaria FP, Torres FAG, deMoraes LMP (2009) Xylanases from *Cryptococcus flavus* isolate I-11: enzymatic profile, isolation and heterologous expression of CfXYN1 in *Saccharomyces cerevisiae*. J Mol Catal B Enzym 59:52–57

Pokhrel S, Joo JC, Yoo YJ (2013) Shifting the Optimum pH of *Bacillus circulans* xylanase towards acidic side by introducing arginine. Biotechnol Bioproc E 18:35–42

Polizeli LTM, Rizzatti ACS, Monti R, Terenzi HF, Jorge JA, Amorim DS (2005) Xylanases from fungi: properties and industrial applications. Appl Microbiol Biotechnol 67:577–591

Ruller R, Alponti J, Deliberto LA, Ward RJ (2014) Concomitant adaptation of GH11 xylanase by directed evolution to create an alkali tolerant/thermophilic enzyme. Protein Eng Des Sel 27:255–262

Rye CS, Withers SG (2000) Glycosidase mechanisms. Curr Opin Chem Biol 4:573–580

Sanchez C (2008) Lignocellulosic residues: biodegradation and bioconversion by fungi. Biotechnol Adv 27:185–194

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

Schell DJ, Farmer J, Newman M, McMillan JD (2003) Dilute-sulfuric acid pretreatment of corn stover in pilot-scale reactor: investigation of yields, kinetics, and enzymatic digestibilities of solids. Appl Biochem Biotechnol 105:69–85

Schulze E (1891) Information regarding chemical composition of plant cell membrane. Ber Dtsch Chem Ges 24:2277–2287

Sharma A, Parashar D, Satyanarayana T (2016) Acidophilic microbes: biology and applications. In: Rampelotto PH (ed) Biotechnology of extremophiles: advances and challenges, Chap 7. Switzerland: Springer, 215–242

Sievers F, Wilm A, Dineen DG, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J, Thompson JD, Higgins D (2011) Fast scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Sys Biol 7:539

Singh S, Madlala AM, Prior BA (2003) *Thermomyces lanuginosus*: properties of strains and their hemicellulases. FEMS Microbiol Rev 27:3–16

Sunna A, Antranikian G (1997) Xylanolytic enzymes from fungi and bacteria. Crit Rev Biotechnol 17:39–67

Tenkanen M, Viikari L, Buchert J (1997) Use of acid-tolerant xylanase for bleaching of kraft pulps. Biotechnol Tech 11:935–993

Tomás-Pejó E, Oliva JM, Ballesteros M (2008) Realistic approach for full-scale bioethanol production from lignocellulose: a review. J Sci Ind Res 67:874–884

Törrönen A, Rouvinen J (1995) Structural comparison of two major endo-1,4-xylanases from *Trichoderma reesei*. Biochemistry 34:847–856

Törrönen A, Harkki A, Rouvinen J (1994) Three-dimensional structure of endo-1,4-β-xylanase II from *Trichoderma reesei*: two conformational states in the active site. EMBO J 13:2493–2501

Uffen RL (1997) Xylan degradation: a glimpse at microbial diversity. J Ind Microbiol Biotechnol 19:1–6

van den Broeck HC, de Graaff LH, Hille JDR, van Ooyen AJ, Visser J, Harder A (1992) Cloning and expression of xylanase genes from fungal origin. European Patent Application EPA 0463706A1, Gist-Brocades, Delft

Verma D, Satyanarayana T (2012) Molecular approaches for ameliorating microbial xylanases. Bioresour Technol 17:360–367

Viikari L, Kantelinen A, Sundquist J, Linko M (1994) Xylanases in bleaching, from an idea to the industry. FEMS Microbiol Rev 13:335–350

Voragen AGJ, Gruppen H, Verbruggen MA, Vietor RJ (1992) Characterization of cereals arabinoxylans. In: Visser J et al. (eds) Xylan and xylanases, vol 7. Elsevier Amsterdam, pp 51–67

VršanskáIlona M, Gorbacheva IV, Krátký Z, Biely P (1982) Reaction pathways of substrate degradation by an acidic endo-1,4-β-xylanase of *Aspergillus niger*. Biochim Biophys Acta Protein Struct Mol Enzymol 704:114–122

Wakarchuk WW, Campbell RL, Sung WL, Davodi J, Yaguchi M (1994) Mutational and crystallographic analyses of the active site residues of the *Bacillus circulans* xylanase. Protein Sci 3:467–475

Weng JK, Li X, Bonawitz ND, Chapple C (2008) Emerging strategies of lignin engineering and degradation for cellulosic biofuel production. Curr Opin Biotechnol 19:166–172

Whistler RL, Richards EL (1970) Hemicelluloses. In: Pigman W, Horton D (eds) The carbohydrates. Academic, pp 447–469

Wong KK, Tan LU, Saddler JN (1988) Multiplicity of β-1,4-xylanase in microorganisms: functions and applications. Microbiol Rev 52:305–317

Yang W, Yang Y, Zhang L, Xu H, Guo X, Yang X, Dong B, Cao Y (2017) Improved thermostabilty of an acidic xylanase from Aspergillus sulphureus by combined disulphide bridge introduction and proline residue substitution. Sci Rep 7:1587

Yi XL, Shi Y, Xu H, Li W, Xie J, Yu RQ, Zhu J, Cao Y, Qiao D (2010) Hyperexpression of two *Aspergillus niger* xylanase genes in *Escherichia coli* and characterization of the gene products. Braz J Microbiol 41:778–786

Zechel DL, Withers SG (2000) Glycosidase mechanisms: anatomy of a finely tuned catalyst. Acc Chem Res 33:11–18

Zhu H, Paradis FW, Krell PJ, Phillips JP, Forsberg CW (1994) Enzymatic specificities and modes of action of the two catalytic domains of the XynC xylanase from *Fibrobacter succinogenes* S85. J Bacteriol 176:3885–3894