REVIEW　　　　　　　　　　　　　　　　　　　　**Open Access**

A detailed overview of xylanases: an emerging biomolecule for current and future prospective

Nisha Bhardwaj[†], Bikash Kumar[†] and Pradeep Verma[*]

Abstract

Xylan is the second most abundant naturally occurring renewable polysaccharide available on earth. It is a complex heteropolysaccharide consisting of different monosaccharides such as L-arabinose, D-galactose, D-mannoses and organic acids such as acetic acid, ferulic acid, glucuronic acid interwoven together with help of glycosidic and ester bonds. The breakdown of xylan is restricted due to its heterogeneous nature and it can be overcome by xylanases which are capable of cleaving the heterogeneous β-1,4-glycoside linkage. Xylanases are abundantly present in nature (e.g., molluscs, insects and microorganisms) and several microorganisms such as bacteria, fungi, yeast, and algae are used extensively for its production. Microbial xylanases show varying substrate specificities and biochemical properties which makes it suitable for various applications in industrial and biotechnological sectors. The suitability of xylanases for its application in food and feed, paper and pulp, textile, pharmaceuticals, and lignocellulosic biorefinery has led to an increase in demand of xylanases globally. The present review gives an insight of using microbial xylanases as an "Emerging Green Tool" along with its current status and future prospective.

Keywords: Xylan, Xylanase, Glycoside hydrolase, Green tool

Introduction

The major constituent of the plant cell wall is "lignocelluloses", as the name suggests it consists of lignin (15–20%), hemicellulose (25–30%) and cellulose (40–50%) (Gray et al. 2006; Singla et al. 2012). These components together form a three-dimensional complex network with the help of covalent and non-covalent interactions (Sánchez 2009). Hemicelluloses consist of xylan, a heteropolysaccharide substituted with monosaccharides such as L-arabinose, D-galactose, D-mannoses and organic acids such as acetic acid, ferulic acid, glucuronic acid interwoven together with help of glycosidic and ester bonds (Collins et al. 2005; Ahmed et al. 2007; Motta et al. 2013; Sharma 2017). Xylan is readily available in nature, followed by cellulose the second most abundant polysaccharide which

covers 33% of total lignocellulosic biomass found on the globe (Collins et al. 2005; Polizeli et al. 2005; Chávez et al. 2006; Walia et al. 2017). It accounts for 15–30% in hardwoods and 7–10% in softwood (Walia et al. 2017). There is a need for depolymerization of this complex polymer for its efficient utilization in different industrial application. Xylanase is a group of enzymes consisting of *endo*-1,4-β-D-xylanases (EC 3.2.1.8), β-D-xylosidases (E.C. 3.2.1.37), α-glucuronidase (EC 3.2.1.139) acetylxylan esterase (EC 3.1.1.72), α-L-arabinofuranosidases (E.C. 3.2.1.55), *p*-coumaric esterase (3.1.1.B10) and ferulic acid esterase (EC 3.1.1.73) involved in the depolymerization of xylan into simple monosaccharide and xylooligosaccharides (Gomez et al. 2008; Juturu and Wu 2014; Walia et al. 2017; Romero-Fernández et al. 2018).

Xylanases are produced by different living organisms such as microorganisms, protozoans, and molluscs, and also found in the rumen of higher animals (Beg et al. 2001). The xylanases are mainly produced by microorganisms, e.g., bacteria, fungi, and actinomycetes at

*Correspondence: vermaprad@yahoo.com; pradeepverma@curaj.ac.in
[†]Nisha Bhardwaj and Bikash Kumar contributed equally to this work
Bioprocess and Bioenergy Laboratory, Department of Microbiology, Central University of Rajasthan, NH-8, Bandarsindri, Kishangarh, Ajmer 305817, India

industrial scale (Motta et al. 2013). The utilization of lignocellulosic biomass (LCB) for production of different biochemicals such as bioethanol, enzymes, and value-added compounds has tremendously improved in recent years. It results in providing opportunities for scientists to explore the hydrolytic potential of xylanase for efficient saccharification of LCB for ethanol and xylooligosaccharides generation. Xylanase also finds application in several industries like pulp and paper bleaching, food, feed, and pharmaceuticals.

Xylanase is required in huge amount for industrial level application with characteristic properties to survive the harsh industrial level processing's (Qiu et al. 2010). Therefore, there is a need to select potent microorganisms for xylanase production, followed by optimization of media components for enhanced production. The understanding of the genetic constituents of the microbe will help in deducing the mode of action of the enzyme. This will help in regulating the enzyme action for employment in desired industrial application. The microorganisms also produce other protein and metabolites with desired xylanase enzyme. Therefore, purification of the crude enzyme is a prerequisite to obtain purified enzymes. The characterization of purified xylanase will help in elucidating its stability and specificity toward different substrates. This will help in selecting the suitable industrial process in which it can be utilized. With the advent of advanced biotechnological techniques such as recombinant DNA technology, several attempts have been made to identify, isolate and clone the gene encoding for xylanase in a suitable system. This approach helps in the engineering of efficient microorganisms for enhanced xylanase production with desired properties. This review gives a comprehensive insight into xylanase classification, its mode of action, different xylanase sources with available production methods and its optimization strategies for enhanced production. The review also gives a brief idea about different strategies employed for xylanase purification and characterization, biotechnological approach for enhanced xylanase production with desired properties which are further used for different industrial applications.

Structure of xylan and role of xylanolytic enzymes in its breakdown

Xylan consists of D-xylose backbone linked with β-1,4-glycosidic bonds and L-arabinose traces forming into a complex heteropolymeric structure. Xylan is present in various biomasses that have several forms such as in hardwoods as *O*-acetyl-4-*O*-methylglucuronoxylan, in softwoods as arabino-4-*O*-methylglucuronoxylan and in grasses and annual plants as arabinoxylans. These residues can be substituted with acetyl, feruloyl,
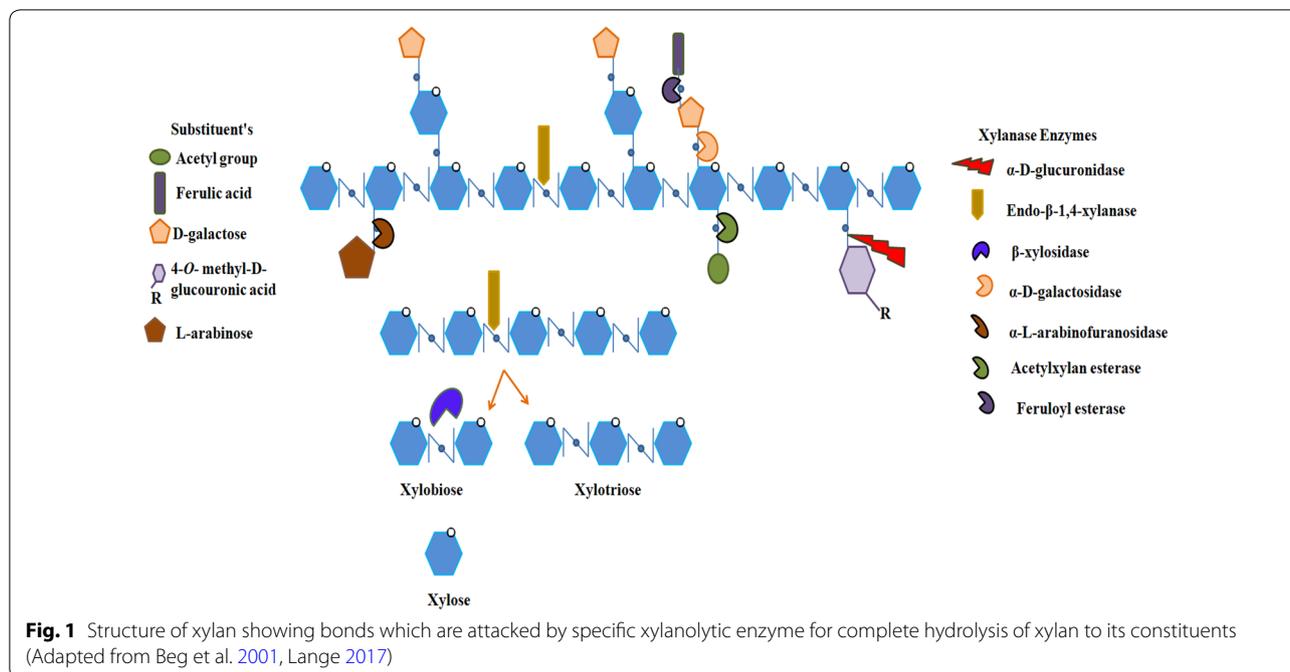
glucopyranosyl, 4-*O*-methyl-D-glucuronopyranosyl, *p*-coumaroyl or α-L-arabinofuranosyl side-chain groups with varying degrees. Xylanolytic enzymes play a key role in the breakdown of the complex structure of xylan. Hence, for complete and efficient hydrolysis of xylan into its constituent sugars requires synergistic action of various enzymes with specifically targeting appropriate bonds of xylan.

The multifunction xylanolytic system exists in bacteria (Zhang et al. 2016a, 2016b), fungi (Driss et al. 2011; Bhardwaj et al. 2018) and actinomycetes (Hunt et al. 2016) where xylan backbone is randomly cleaved by the action of *endo*-1,4-β-D-xylanases; xylose polymer is broken down to its monomeric form by action of β-D-xylosidases. Acetyl and phenolic side branches were removed by the action of α-glucuronidase and acetylxylan esterase. α-L-Arabinofuranosidases catalyze the removal of the side groups. The ester bonds present on the xylan are cleaved by the action of *p*-coumaric esterase and ferulic acid esterase (Beg et al. 2001; Collins et al. 2005; Chakdar et al. 2016; Walia et al. 2017). The schematic structure of xylan showing bonds which are attacked by a specific xylanolytic enzyme for complete hydrolysis of xylan to its constituent monomeric units is represented in Fig. 1.

Classification of xylanase

Xylanase can be broadly classified into three types on the basis of (a) molecular mass and isoelectric point, (b) crystal structure and (c) catalytic/kinetic property (Wong et al. 1988; Jeffries 1996; Biely et al. 1997; Liu and Kokare 2017). On basis of molecular mass and isoelectric point, the xylanase was classified into two groups, i.e., (a) high-molecular weight with low isoelectric (acidic) point (HMWLI) and (b) low-molecular weight with high isoelectric (basic) point (LMWHI). However, several exceptions to this classification have been observed where not all xylanases fall in the category of HMWLI (above 30 kDa) or LMWHI (below 30 kDa) (Collins et al. 2002, 2005). Therefore, a more appropriate system including primary structure (crystal), comparison of catalytic domain with mechanistic features such as kinetic, catalytic property, substrate specificity, and product description was introduced (Henrissat and Coutinho 2001; Collins et al. 2005). The genomic, structural (3D crystal structure) and functional information of xylanase is available under glycoside hydrolase (GH) families available on carbohydrate-active enzyme (CAZy) database.

The CAZy is knowledge-based, highly curated database on enzymes that play a key role in breakdown, modification, and assembly of glycosidic bonds in carbohydrates and glycoconjugates. It consists of genomic, sequence annotation, family classifications, structural (3D crystal)

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Fig. 1 Structure of xylan showing bonds which are attacked by specific xylanolytic enzyme for complete hydrolysis of xylan to its constituents (Adapted from Beg et al. 2001, Lange 2017)

and functional (biochemical) information on carbohydrate-active enzyme from publicly available resources such as National Center for Biotechnology Information, NCBI (Lombard et al. 2014).

The major GH families associated with xylanase are 5, 7, 8, 9, 10, 11, 12, 16, 26, 30, 43, 44, 51, and 62. The GH families 5, 7, 8, 10, 11, and 43 have a single distinct catalytic domain, whereas enzymes grouped under GH families 16, 51, and 62 have two catalytic domains with bi-functional property (Collins et al. 2005). The enzyme grouped under GH families 9, 12, 26, 30, and 44 has secondary xylanase activity. Based on the hydrophobic cluster analysis of the catalytic domains along with similarities studies of amino acid sequences, xylanases have been primarily classified as GH 10 and GH 11 (Verma and Satyanarayana 2012a). The catalytic properties of GH 10 and GH 11 have been studied extensively, whereas the information on GH families 5, 7, 8 and 43 is very limited (Taibi et al. 2012). Different structural and functional properties of different GH families are tabulated in Table 1.

Mode of action of xylanases grouped under various GH families

There is the difference in structure, physicochemical properties, substrate specificities and mode of action of members of GH families 5, 7, 8, 10, 11 and 43 (Collins et al. 2005). The hydrolysis of xylan by xylanase may occur by two different mechanisms, i.e., retention or

inversion (Subramaniyan and Prema 2002; Lombard et al. 2014).

Retention

This process is represented by double displacement mechanism with α-glycosyl and *oxo*-carbonium intermediate formation followed by its subsequent hydrolysis. Glutamate residues play a vital role in the catalytic mechanism. First, two carboxylic acid residues present in the active site result in α-glycosyl enzyme intermediate formation. The intermediate formation occurs via protonation of the substrate by a carboxylic acid residue acting as an acid catalyst and departure of the leaving group due to nucleophilic attack caused by another carboxylic acid. This collectively results in β to α inversion due to the α-glycosyl enzyme intermediate formation. Second, the first carboxylate group abstracts a proton from a nucleophilic water molecule and attacks the anomeric carbon resulting in second substitution, where the anomeric carbon gives rise to product with the β configuration (α to β inversion) via a transition state of oxo-carbonium ions (Fig. 2) (Collins et al. 2005; Lombard et al. 2014). Enzymes of families 5, 7, 10, and 11 mostly work on the principle of retention.
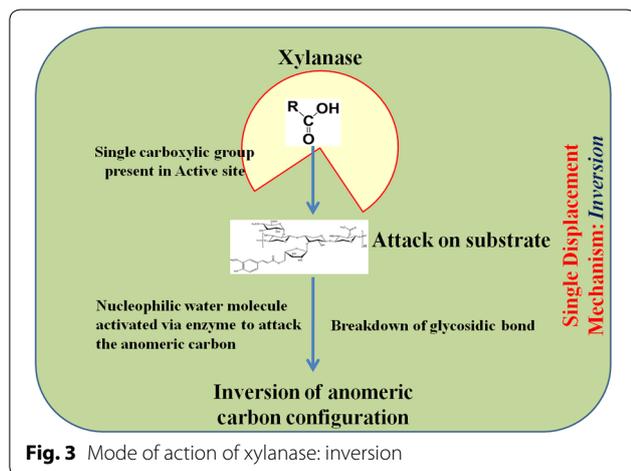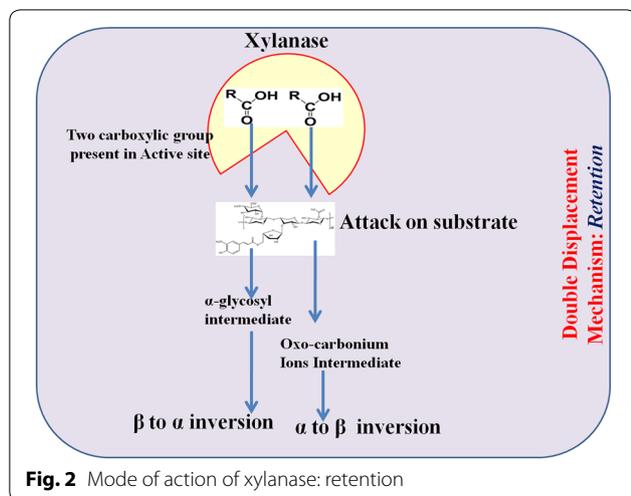
Inversion

The enzymes of families 8 and 43 act via inversion of the anomeric center with glutamate and aspartate as the major catalytic residue. This is a single displacement mechanism, in which only one carboxylate ion offers

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Table 1 Classification of glycoside hydrolases (GH) family consisting of xylanase

GH family	Clan	Activities in family	Properties		Mode of action	References
			Structure	Functional		
GH 5	GH-A	*endo*-β-1,4-Xylanase; β-glucosidase cellulase	Consist of seven amino acid (β/α)₈ barrel fold Highly conserved	Largest GH family Substitute on main xylan chain	CNB-Glu CPD-Glu Retaining	Collins et al. (2005), Lombard et al. (2014)
GH 7	GH-B	*endo*-β-1,4-Glucanase	High-molecular weight and low pI β-Jelly roll small substrate-binding site (4 subsite and 1 catalytic site)	Common characteristics with family 10 and 11	CNB-Glu CPD-Glu Retaining	Collins et al. (2005), Lombard et al. (2014)
GH 8	GH-M	Cellulases; chitosanases; lichenases; *endo*-1,4-β-xylanases	(α/α)₆ fold Large substrate-binding cleft	Cold-adapted Break xylan into X3 and X4 Highly active on long-chain XOS	CNB-Asp CPD-Glu Inversion of the anomeric	Collins et al. (2002, 2005), Lombard et al. (2014)
GH 10 Family G	GH-A	*endo*-1,4-β-Xylanases; *endo*-1,3-β-xylanases	Low-molecular mass high pI (α/β)₈ barrel fold Small yet numerous (4–5) substrate-binding sites	Attack on aryl β-glycosides and aglyconic bond of X2 and X3, respectively Highly active on short XOS	CNB-Glu CPD-Glu Retaining	Ahmed et al. (2009), Collins et al. (2005), Lombard et al. (2014), Motta et al. (2013)
GH11	GH-C	xylanases "true xylanases" (active on xylose substrate)	High MW and lower pI values β-Jelly roll Small size Large substrate-binding clefts (7 subsites)	Attack on aryl β-glycosides and aglyconic bond of X2 and X3, respectively Inactive on aryl cellobiosides High substrate selectivity and catalytic efficiency Wide pH and temperature stability Cold-adapted Highly active on long-chain XOS	CNB-Glu CPD-Glu Retaining	Ahmed et al. (2009), Collins et al. (2005), Lombard et al. (2014), Motta et al. (2013)
GH 43	GH-F	β-Xylosidase α-L-Arabinofuranosidase; xylanase	β-Propeller (5 blade) fold Catalytic residue glutamate and aspartate in the center	Debranching and degradation of hemicellulose polymer	CNB-Asp CPD-Glu Inverting single displacement mechanism	Collins et al. (2005), Lombard et al. (2014), Mewis et al. (2016)

X2, xylobiose; X3, xylotriose; X4, xylotetraose; XOS, xylooligosaccharides; CNB, catalytic nucleophile/base; CPD, catalytic proton donor; Asp, aspartic acid; Glu, glutamic acid

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Fig. 2 Mode of action of xylanase: retention

Fig. 3 Mode of action of xylanase: inversion

for overall acid catalyzed group departure (Fig. 3). This enzyme also acts as the base for activating a nucleophilic water molecule to attack the anomeric carbon (depending on the distance between two molecules) for breaking the glycosidic bonds and causing inversion of anomeric carbon configuration (Collins et al. 2005; Motta et al. 2013; Lombard et al. 2014).

Several attempts have been made to understand the mode of action of xylanase obtained for different organisms. An unusual mode of action of GH8 xylanase (β-xylosidase, an α-arabinofuranosidase, and an acetylesterase activity) was observed in *Pseudoalteromonas atlantica*, which showed the presence of a long tail of unsubstituted xylose residue on the reducing end of oligosaccharides produced (Ray et al. 2019). Thermophilic xylanase obtained from *Bacillus licheniformis* DMS has novel hydrolysis properties similar to GH30. It breaks the linear β-(1-4) linkage of beech wood and birchwood xylan along with glucuronoxylan and arabinoxylan. *B. licheniformis* DMS xylanase had both the properties of endoxylanase and appendage dependent xylanase activity. It showed equal production of both xylobiose and xylotriose by hydrolysis of the commercial substrate and agro-waste such as corn cob (Ghosh et al. 2019).

Thermothelomyces thermophila (*Tt*Xyn30A) that hydrolyzes xylan into xylose and two acidic xylooligosaccharides, namely xylotriose ($\text{MeGlcA}^2\text{Xyl3}$) and xylobiose, i.e., $\text{MeGlcA}^2\text{Xyl}_2$, was studied. TtXyn30A catalyzed the release of the disaccharide xylobiose from the non-reducing end of xylooligosaccharides, thus exhibiting an exo-acting catalytic behavior. TtXyn30A also showed the capability to cleave linear parts of xylan and uronic xylo-oligosaccharides as well as resulting in the formation of aldotriuronic and aldotetrauronic acid (Katsimpouras et al. 2019). Puchart et al. (2018) have reported the mode of action of hydrolysis of eucalyptus plant using endoxylanase belonging to GH10, GH11, and GH30 family. All the endoxylanse resulted in the formation of acetylated XOS. The GH10 endoxylanase results in short xylo-oligosaccharides, whereas GH30 endoxylanase results in longer xylooligosaccharides. An acetyl esterase (AcXEs) played a key role in understanding the plant decay or depolymerization mechanism and also showed efficiency in plant biomass bioconversion (Rytioja et al. 2014).

A novel modular endoxylanase with transglycosylation activity was reported from *Cellulosimicrobium* sp. HY-13 belonging to GH6 family (Ham et al. 2012). A GH30 family xylanase XynA was reported from *Erwinia chrysanthemi* belonging to subfamily 8 with the special property of hydrolyzing 4-*O*-methyl-glucuronoxylan (Urbániková et al. 2011). Xyn11B from thermophilic fungus *Humicola insolens* Y1 encoding multi-cellular xylanase belonged to GH11 reported by Shi et al. (2015). *Bacteroides intestinalis* DSM17393, a xylan degrading human gut bacterium, reported the presence of two putative GH8 xylanases which hydrolyze both xylopentose and xylohexose (Hong et al. 2014). *Endo*xylanase XynB from marine bacterium *Glaciecola mesophila* KMM241 with xylan binding ability and GH8 catalytic domain was reported by Guo et al. (2013).

Mechanism for glycosidic hydrolase family 10 (GH10)

Among all the above-mentioned GH families, GH 10 consists of endoxylanase, e.g., *endo*-1,4-β-xylanases, *endo*-1,3-β-xylanases and cellobiohydrolases (Collins et al. 2005). *Endo*-1,4-β-xylanases or xylanase mainly comes under this GH10 family. It usually consists of high-molecular weight xylanase with low isoelectric points and displays an $(\alpha/\beta)_8$-barrel fold. This structure mimics the shape of a 'Salad Bowl', because of an enlarged loop architecture, one face of the molecule is having

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~ 45 Å large radius and the other face is having ~ 30 Å radius because of simple (α/β) turns (Zhang et al. 2016a, 2016b). However, these two categories are relatively the same because along with sharing similar fold also shares some common residues and has similar catalytic mechanisms. The xylanase belonging to GH 10 family has low substrate specificity and, however, exhibits high catalytic versatility than that of GH 11 family. Xylanase belonging to GH10 family exhibits greater catalytic versatility and lower substrate specificity as compared to those belonging to GH11 (Biely et al. 1997; Faulds et al. 2006; Motta et al. 2013). GH10 xylanase attacks the xylose linkages which are closer to the side-chain residues (Dodd and Cann 2009). This could be explained by fact that the xylose residues bind at subsites (Fig. 4) on xylanase that causes cleavage of the bond between the monomeric residues at the non-reducing (-1) and the reducing end ($+1$) of the polysaccharide substrate (Davies et al. 1997).

Maslen et al. (2007) demonstrated that when arabinoxylan was hydrolyzed by GH10 and GH11 xylanase, the products generated have arabinose residues substituted on xylose at the $+1$ subsite and $+2$ subsites, respectively. Therefore, xylanases from family 11 and 10 preferentially cleave the unsubstituted regions of the arabinoxylan backbone and the unhampered substituted regions along the xylan backbone (Biely et al. 1997; Motta et al. 2013). The degree of side-chain decorations of xylan influences the specificity of the enzyme toward substrates and, thus, has an important implication on the hydrolytic product formation by xylan deconstruction (Dodd and Cann 2009). Yang and Han (2018) demonstrated the positional binding and substrate interaction of GH10 xylanase of *Thermotoga maritime* using molecular docking approach.

Researchers have reported in their previous studies that GH10 endoxylanase had better performance than GH11 in synergy with cellulase enzyme for pretreated lignocellulosic biomass hydrolysis. The reason behind this may be because GH11 endoxylanase has the lower accessibility toward acetylated xylan backbone (Faulds et al.

2006). Hu et al. (2011) proposed a model holocellulosic substrate, i.e., mixture of pure cellulose and 10% predeacetylated commercial birchwood xylan to understand the synergism between two family xylanase and cellulase (during the xylan extraction process). This study showed that substrate deacetylation has increased the hydrolytic performance of GH11 as the acetyl group restricted the accessibility of xylan more for GH11 than GH10. Thermostability is the second factor for better performance of GH10 endoxylanase over GH11 because lignocellulosic biomass hydrolysis occurs better at high temperature (50 °C) and 2–3 days long-time duration.

Source for xylanase production

The xylanase is ubiquitous in nature and its presence is observed diversely in a wide range of living organisms, such as marine, terrestrial and rumen bacteria (Chakdar et al. 2016), thermophilic and mesophilic fungi (Chadha et al. 2019; Singh et al. 2019), protozoa (Devillard et al. 1999; Béra-Maillet et al. 2005), crustaceans (Izumi et al. 1997), snails (Suzuki et al. 1991), insects (Brennan et al. 2004), algae (Jensen et al. 2018), plants and seeds (immature cucumber seeds and germinating barley) (Bae et al. 2008; Sizova et al. 2011). Bacteria and fungus are widely used for industrial production of xylanase. Several microbial sources of xylanase are classified in Table 2.

Bacterial sources of xylanase

Among bacteria, *Bacillus* species have been reported widely as the most potent xylanolytic enzyme producers such as *Bacillus* sp., *B. halodurans* (Gupta et al. 2015), *B. pumilus* (Thomas et al. 2014), *B. subtilis* (Banka et al. 2014), *B. amyloliquefaciens*, *B. circulans*, and *B. stearothermophilus* (Chakdar et al. 2016). Xylanase with high temperature stability, acid/alkali stability, and cold adaptability have been isolated and purified from a wide range of bacteria found in extreme environment. Thermotolerant xylanase active at a very high temperature of 60–70 °C has been reported from *Bacillus* spp. (Thomas
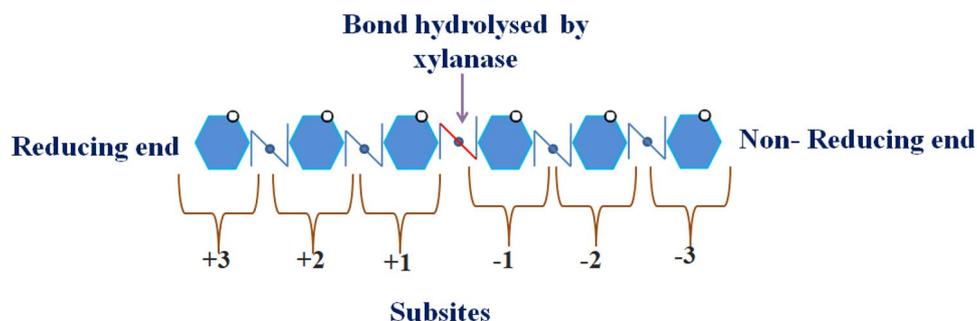

Fig. 4 Schematic representation of site for attack of GH10 xylanase on xylan

Table 2 Microbial sources of xylanase

Xylanase producing microorganisms

Major group	Genus	Species	References
Bacteria	Arthrobacter	Arthrobacter sp.	Murugan et al. (2011)
	Geobacillus	Geobacillus thermoleovorans, Geobacillus stearothermophilus Geobacillus thermodenitrificans	Gerasimova and Kuisiene (2012), Verma and Satyanarayana (2012a, b), Bibi et al. (2014)
	Pediococcus	Pediococcus acidilactici	Adiguzel et al. (2019)
	Bacillus	Bacillus firmus, Bacillus arseniciselenatis, Bacillus licheniformis, Bacillus amylolique-faciens, Bacillus subtilis	John et al. (2006), Bajaj and Manhas (2012), Kamble and Jadhav (2012), Amore et al.(2015)
	Clostridium	Clostridium thermocellum, Clostridium papyrosolvens	Pohlschroder et al. (1994), Heinze et al. (2017)
	Dictyoglomus	Dictyoglomus thermophilum	Zhang et al. (2010b)
	Paenibacillus	Paenibacillus sp., Paenibacillus xylanilyticus, Paenibacillus terrae, Paenibacillus barcinonensis, Paenibacillus macquariensis	Shi et al. (2010), Valenzuela et al. (2010), Sharma et al. (2013), Song et al. (2014)
	Rhodothermus	Rhodothermus marinus	Abou Hachem et al. (2000)
	Staphylococcus	Staphylococcus aureus, Staphylococcus sp.	Gupta et al. (2000), Iloduba et al. (2016)
	Pseudomonas	Pseudomonas sp., Pseudomonas aeruginosa, Pseudomonas fluorescens subsp. cellulose, Pseudomonas boreopolis, Pseudomonas stutzeri	Raghothama et al. (2000), Xu et al. (2005), Iloduba et al. (2016), Lin et al. (2017), Purkan et al. (2017), Lee et al. (2018)
	Thermoactinomyces	Thermoactinomyces thalophilus, Thermoactinomyces vulgaris	Kohli et al. (2001), Selim (2016)
	Thermotoga	Thermotoga maritima Thermotoga thermarum Thermotoga neapolitana	Velikodvorskaya et al. (1997), Shi et al. (2013), Yoon et al. (2014)
Actinomycetes	Streptomyces	Streptomyces sp., Streptomyces lividans	Shibuya et al. (2000), Verma and Satyanarayana (2012a, b)
	Kitasatospora	Kitasatospora sp.	Rahmani et al. (2018)
	Nonomuraea	Nonomuraea flexuosa, Nonomuraea jabiensis sp.	Paloheimo et al. (2007), Camas et al. (2013)
	Actinomadura	Actinomadura geliboluensis, Actinomadura sp.	Verma and Satyanarayana (2012a, b), Adigüzel and Tunçer (2017)
	Cellulomonas	Cellulomonas fimi, Cellulomonas flavigena, Cellulomonas uda	Notenboom et al. (1998), Murugan and Jampala (2015), Lisov et al. (2017)
	Microbacterium	Microbacterium xylanilyticum sp., Microbacterium soli sp.	Kim et al. (2005), Srinivasan et al. (2010)
	Micrococcus	Micrococcus luteus, Micrococcus sp.	Gessesse and Mamo (1998), Mmango-Kaseke et al. (2016)
	Thermomonospora	Thermomonospora fusca, Thermomonospora curvata	Irwin et al. (1994), Stutzenberger (1994)

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Table 2 (continued)

Xylanase producing microorganisms

Major group	Genus	Species	References
Fungus	Talaromyces	*Talaromyces byssochlamydoides, Talaromyces emersonii*	Yoshioka et al. (1981), Waters et al. (2011)
	Thermomyces	*Thermomyces lanuginosus*	Alam et al. (1994)
	Thermoascus	*Thermoascus aurantiacus, Thermoascus aurantiacus var. levisporus*	Vardakou et al. (2005), Chanwicha et al. (2015)
	Melanocarpus	*Melanocarpus albomyces*	Gupta et al. (2013)
	Chaetomium	*Chaetomium thermophilum, Chaetomium globosum*	Gandhi et al. (1994), Latif et al. (2006)
	Fusarium	*Fusarium sp., Fusarium solani, Fusarium proliferatum, Fusarium oxysporum, Fusarium heterosporum, Fusarium roseum*	Saha (2002), Imad et al. (2011), Paulo et al. (2014), Adesina et al. (2017), Gascoigne and Gascoigne (2019)
	Humicola	*Humicola sp. Humicola insolens, Humicola lanuginosa, Humicola grisea*	da Silva et al. (1994), Monti et al. (2003), Kamra and Satyanarayana (2004), Shi et al. (2015)
	Paecylomyces	*Paecylomyces varioti, Paecilomyces themophila*	Krishnamurthy and Vithayathil (1989), Zhang et al. (2010a)
	Scytalidium	*Scytalidium thermophilum, Scytalidium acidophilum*	Joshi and Khare (2012) Al Balaa et al. (2006)
	Thielavia	*Thielavia sp., Thielavia terrestaris*	Garcia-Huante et al. (2017), Thanh et al. (2019) Berka et al. (2011)
	Corynascus	*Corynascus sepedonium*	Sharma et al. (2008)
	Myceliophthora	*Myceliophthora heterothallica, Myceliophthora fergusii*	van den Brink et al. (2013)
	Sporotrichum	*Sporotrichum thermophile*	El-Naghy et al. (1991), Sadaf and Khare (2014), Topakas et al. (2003)
	Rhizomucor	*Rhizomucor pusillus*	Hüttner et al. (2018)
	Trichoderma	*Trichoderma inhamatum, Trichoderma piluliferum, Trichoderma viride, Trichoderma longibrachiatum, Trichoderma asperellum, Trichoderma stromaticum, Trichoderma harzianum, Trichoderma reesei*	Azin et al. (2007), da Silva et al. (2015), Carvalho et al. (2017), Tang et al. (2017), Syuan et al. (2018), da Costa et al. (2019), Ezeilo et al. (2019)
	Aspergillus	*Aspergillus niger, Aspergillus flavus, Aspergillus niveus, Aspergillus ochraceus, Aspergillus foetidus, Aspergillus fumigates, Aspergillus terreus, Aspergillus tamari*	Gawande and Kamat (1999), Betini et al. (2009), Ang et al. (2013), Dhulappa and Lingappa (2013), de Guimaraes et al. (2013), De Queiroz Brito Cunha et al. 2018a, b

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et al. 2014), *Bacillus Halodurans* TSEV1 (Kumar and Satyanarayana 2014), *Clostridium thermocellum* (Fernandes et al. 2015), *Rhodothermus marinus* (Karlsson et al. 2004), *Streptomyces* sp. (Sukhumsirichart et al. 2014), *Stenotrophomonas maltophila* (Raj et al. 2013), *Thermotoga thermarum* (Shi et al. 2013). Psychrophilic xylanases are not very common but found to be isolated from several bacteria such as *Clostridium* sp. PXLY1 (Akila and Chandra 2003), *Flavobacterium* sp. MSY-2 and *Flavobacterium frigidarium* (Humphry et al. 2001; Dornez et al. 2011) *Pseudoalteromonas haloplanktis* TAH3A (Van Petegem et al. 2002).

Several alkali stable xylanases have been isolated from firmicutes such as *B. pumilus* (Thomas et al. 2014), *B. halodurans* TSEV1 (Kumar and Satyanarayana 2014) and *Geobacillus thermoleovorans* (Verma and Satyanarayana 2012b) and actinomycetes such as *Actinomadura* sp. Cpt20 (Taibi et al. 2012) and *Streptomyces althioticus* LMZM (Luo et al. 2016).

Fungal sources of xylanases

The mesophilic fungi of genera *Aspergillus* and *Trichoderma* are well known to be potent xylanase producer and most widely used for commercial production. *Thielavia terrestris,* (Garcia-Huante et al. 2017), *Talaromyces thermophilus* (Maalej et al. 2009), *Paecilomyces thermophile* (Fan et al. 2012), *Achaetomium* sp. X2-8 (Chadha et al. 2019), *Rhizomucor pusillus* (Hüttner et al. 2018), *Rasamsonia emersonii,* (Martínez et al. 2016) *T. Leycettanus* (Wang et al. 2017), *Melanocarpus albomyces* (Gupta et al. 2013) and *Aspergillus oryzae* LC1 (Bhardwaj et al. 2019) were found to be producer of hyper-thermophilic active xylanase. Several alkali stable xylanases were obtained from different fungal strains such as *Paenibacillus barcinonensis* (Valenzuela et al. 2010), *Aspergillus fumigatus* MA28 (Bajaj and Abbass 2011), *Cladosporium oxysporum* (Guan et al. 2016) and *Aspergillus oryzae* LC1 (Bhardwaj et al. 2019).

Strategies employed for xylanase production from different microbial sources

The production of xylanase from microorganisms is affected by the fermentation process employed, choice of substrate and different media components. These components are often regulated by different process optimization for enhanced production of the enzyme for its application at large scale.

Different fermentation process employed for xylanase production: submerged and solid-state fermentation

Xylanases are produced by a different fermentation process using various microorganisms. The better understanding of the physiology and different metabolic processes of the microbial system has led to an improvement in the fermentation process. However, there is still an opportunity to improve the yield of enzymes. The optimization of the xylanase production will be discussed in a later section.

The xylanase production has been carried out under submerged fermentation (SmF) and solid-state fermentation (SSF) (Motta et al. 2013). The choice of the fermentation process usually depends on the type of microorganisms used (Table 3). Bacteria require a high amount of water during growth; therefore, SmF is preferred whereas fungi due to its mycelia nature require less moisture and can be grown under SSF (Walia et al. 2017). Several reports suggest that submerged fermentation using bacteria and fungi is the most preferred method for xylanase production. Statistically speaking approximately 90% of total xylanase is produced globally through SmF. During SmF, the synergistic effect of different xylan degrading enzymes can be observed and even result in better biomass utilization for enhanced xylanase production (Polizeli et al. 2005; Bajpai 2014). Xylanase production utilizes soybean residues and rice straw as a substrate under SmF by *Aspergillus oryzae* LC1 and *Aspergillus foetidus* (Bhardwaj et al. 2017; De Queiroz Brito Cunha et al. 2018a, b). Similarly, Irfan et al. (2016) suggested the production of xylanase under SmF by *B. subtilis* BS04 and *B. megaterium* BM07. Different advantages of the SmF are homogenous condition throughout medium; method is well characterized and can be easily scaled up (Guleria et al. 2013). There are some disadvantages to SmF as well which limit its industrial application, i.e., high maintenance cost, energy intensive and complex downstream (Virupakshi et al. 2005; Walia et al. 2017).

Recent trends suggest that xylanase production by SSF is also gaining popularity (Walia et al. 2014). *Bacillus* sp. was used for the production of thermo-alkalophillic extracellular xylanase under SSF using wheat bran as substrate (Kamble and Jadhav 2012). Similarly, SSF of *Trichoderma koeningi* using corn cob supplemented with pineapple peel powder showed enhanced production of xylanase (Bandikari et al. 2014). It has several advantages such as low cultivation, operation and capital cost, a lower rate of contamination, easy enzyme recovery, and high productivity per reactor volume. The disadvantages associated with SSF are not suitable for all microorganisms (preferred for the fungal system) and require proper aeration and humidity control and up-scaling is a tedious process (Mienda et al. 2011).

Selection of suitable substrate for xylanase production

Quantity and quality of the fermentation product vary with different substrates. There are various commercially

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Table 3 Production system, characteristic properties and application of xylanase from different microorganisms

Microbes	Substrate	Fermentation	MW (kDa)	Optimum		Stability		Metal activator	Application	References
				Temperature (°C)	pH	Temperature (°C)	pH			
Bacteria										
Bacillus altitudedinus	Sorghum straw	SmF	98	50	8	35–70	3–10	Ca, Mn, K, Na, Fe	Deinking of waste hand-written paper pulp	Adhyaru et al. (2017)
Paenibacillus barengoltzii	Birch wood xylan, beechwood xylan, oat-spelt xylan	SmF	116	60	6.5	20–65	4–11	Mg, Ca, Ba, Co	XOS production	Liu et al. (2018)
Burkholderia sp. DMAX	Wheat Bran	SmF	–	50	8.6	30–50	6–9	Co, Na, Ba, Mg, K	Hydrolysis of ligno-cellulosic biomass	Mohana et al. (2008)
Bacillus licheniformis	Wheat Bran	SmF	46	60	9	30–100	4–11	Fe, Mn Mg, Zn Ca, Ni	Kraft pulp bio-bleaching	Raj et al. (2018)
Anoxybacillus kamchatkensis NASTPD13	Beech wood xylan	SmF	37	65	9	30–65	6–9	–	Thermo-alkaline stable xylanase suitable for industrial application	Yadav et al. (2018)
Pediococcus acidilactici GC25	Oatmeal	SmF	48.15	40	7	20–50	2–9	K, Ba, Cd, Co, Sr, Mg, Ca, Al, Zn, Ni	Clarification of fruit juice	Adiguzel et al. (2019)
Pseudomonas boreopolis LUQ1	Wheat Bran	SmF	20	65	6	55–75	5–9	–	Thermo-alkali stable xylanase for industrial application	Lin et al. (2017)
Geobacillus sp. Strain DUSELR13	Corncob, beech-wood	SmF	45	75	7	50–85	5–9	Cu, Zn, K, Fe, Ca, Zn, Mg, Na	Ethanol Generation	Bibra et al. (2018)
Actinomycetes										
Streptomyces olivaceus (MSU3)	Birch wood Xylan	SmF	23	40	7	20–70	5–11	Na, Cu, Co, Fe, Zn, Mg, Mn	Pretreated biomass hydrolysis for bioethanol production	Sanjivkumar et al. (2017)
Streptomyces thermovulgaris TISTR1948	Beech wood xylan	SmF	46.2	65	6.5	50–70	4–11.5	Ca, Co, Mn	XOS production	Boonchuay et al. (2016)
Kitasatospora sp	Sugarcane bagasse	SmF	49.3	50	5	30–80	4–7	Zn, EDTA	XOS production	Rahmani et al. (2019)
Actinomadura sp. strain Cpt20			20.11	80	10	60–90	5–10	Mn, Ca, Cu	Paper and Pulp Industry	Taibi et al. (2012)
Fungus										
Trichoderma longibrachiatum KT693225	Rice straw	SSF	–	60	5	30–60	3–10	–	Textile processing efficient desizing and bioscouring,	El et al. (2018)

Table 3 (continued)

Microbes	Substrate	Fermentation	MW (kDa)	Optimum			Stability		Metal activator	Application	References
				Temperature (°C)	pH		Temperature (°C)	pH			
Trichoderma viride VKF3	Rice bran, sugarcane bagasse, coconut oil cake, ground-nut oil cake, neem oil cake	SSF	14	50	7		25–50	3–9	Zn, Fe, Mg, Mn, Ca	Bio-bleaching of newspaper	Nathan et al. (2017)
Trichoderma pilu-liferum	Wheat bran	SSF	–	50	4.5		30–60	3–10	–	Additives for bovine feeding	da Costa et al. (2019)
Humicola insolens Y1	wheat bran	SmF	44	70–80	6–7		40–80	5–10		Brewing Industries and XOS genera-tion	Du et al. (2013)
Pichia stipitis	corn cob wheat bran	SSF	31.6	50	6		40–60	3.0–11.0	Cu, K	XOS generation	Ding et al. (2018)
Fusarium sp	Wood shaving	SSF	–	–	–		25–55	4–9	–	Paper and pulp bleaching	Adesina et al. (2017)
Thermomyces lanuginosus	wheat bran	SmF	–	47.9	7.3		30–65	3.0–10.0	Mg, Na, K	Fermentable sugar production, pulp bleaching	Kumar and Shukla (2018)
Thermoascus aurantiacus	Wheat Bran	SmF	31	75	5		30–80	2–10	Mn, Ag	–	Ping et al. (2018)
Schizophyllum com-mune ARC-11	Rice straw	SSF	–	55	5		30–65	4–7	Na, K, Ca	Kraft pre-bleaching	Gautam et al. (2018)
Aspergillus oryzae LC1	Rice straw	SmF	35	25	5		25–60	3–10	Fe, Ag, Mg, Mn, Co	Hydrolysis of Agro-residues and XOS Production	Bhardwaj et al. (2017, 2019)

SmF, submerged fermentation; SSF, solid-state fermentation; XOS, xylooligosaccahrides

available substrates, i.e., xylan, carboxymethyl cellulose (CMC), pectin, and starch for, i.e., xylanase, cellulase, pectinase, and amylase, respectively (Barman et al. 2015; Bhardwaj et al. 2017; Kumar et al. 2018a). Due to the high cost of commercial substrates and considering the economic feasibility of the process, scientists are working from past several years to find alternative substrates for the production of these enzymes.

Agrowastes and other organic wastes (domestic and industrial) are used as a carbon source for the production of xylanase with the focus on sustainability and best utilization of these wastes (Table 3). Some of the most commonly used agro-residues for xylanase production are wheat bran, wheat husk (Kumar et al. 2018a, b, c, d), rice straw (Bhardwaj et al. 2017), rice husk, sugarcane bagasse (Suleman and Aujla 2016), coconut coir, coconut oil cake (Rosmine et al. 2019), groundnut shell (Namasivayam et al. 2015), wood pulp (Kalpana and Rajeswari 2015), sawdust, chilli post-harvest (Sindhu et al. 2017), corncobs, molasses, sugar beet pulp fruit, and vegetable waste (Bandikari et al. 2014). Recent studies also showed that wastewater from pulp industry was reused as media for xylanase production (de Queiroz-Fernandes et al. 2017).

Role of important media components used for xylanase production

Naturally, xylanolytic enzymes are induced by the different intermediate products generated by their own action. Xylan is found to be best xylanase inducer (Taibi et al. 2012; Guleria et al. 2013; Walia et al. 2013, 2014). However, xylan being a high-molecular weight polymer cannot stimulate xylanase as it cannot enter the microbial cells. Therefore, a small amount of constitutive enzyme produced in the media results in the generation of low-molecular weight fragments, i.e., xylobiose, xylotriose, xylotetraose, xylose from the breakdown of xylan and further induces the xylanolytic enzymes for enhanced enzyme production (Walia et al. 2017). Cellulose, synthetic alkyl, aryl β-ᴅ xylosides, and methyl β-ᴅ-xyloside also act as an inducer for xylanolytic enzyme production (Thomas et al. 2013). Busk and Lange (2013) observed that poor quality paper can efficiently induce the xylanase production in *Thermoascus aurantiacus* even in the absence of xylan and xylooligosaccharides.

Nitrogen is an important structural element required for the metabolic processes in the microbial system. Therefore, the choice of nitrogen source is important for the growth of microorganisms that subsequently affect the overall enzyme yield. Peptone, tryptone, soymeal, yeast extract, etc. have found to be suitable nitrogen source. The requirement of these nitrogen sources varies for different microorganisms; therefore, optimizing the type and level of nitrogen source in the media is an important parameter (Seyis and Aksoz 2005; Naveen et al. 2014; Irfan et al. 2016). Trace elements, amino acids, and vitamins are also important parameters for the growth of different microorganisms (Simair et al. 2010; Bibra et al. 2018). Therefore, regulating their levels in the media is important for regulating the production of xylanase. Also, the addition of biosurfactant such as Tween 80 affected the level of xylanase production (Liu et al. 2006; Kumar et al. 2013).
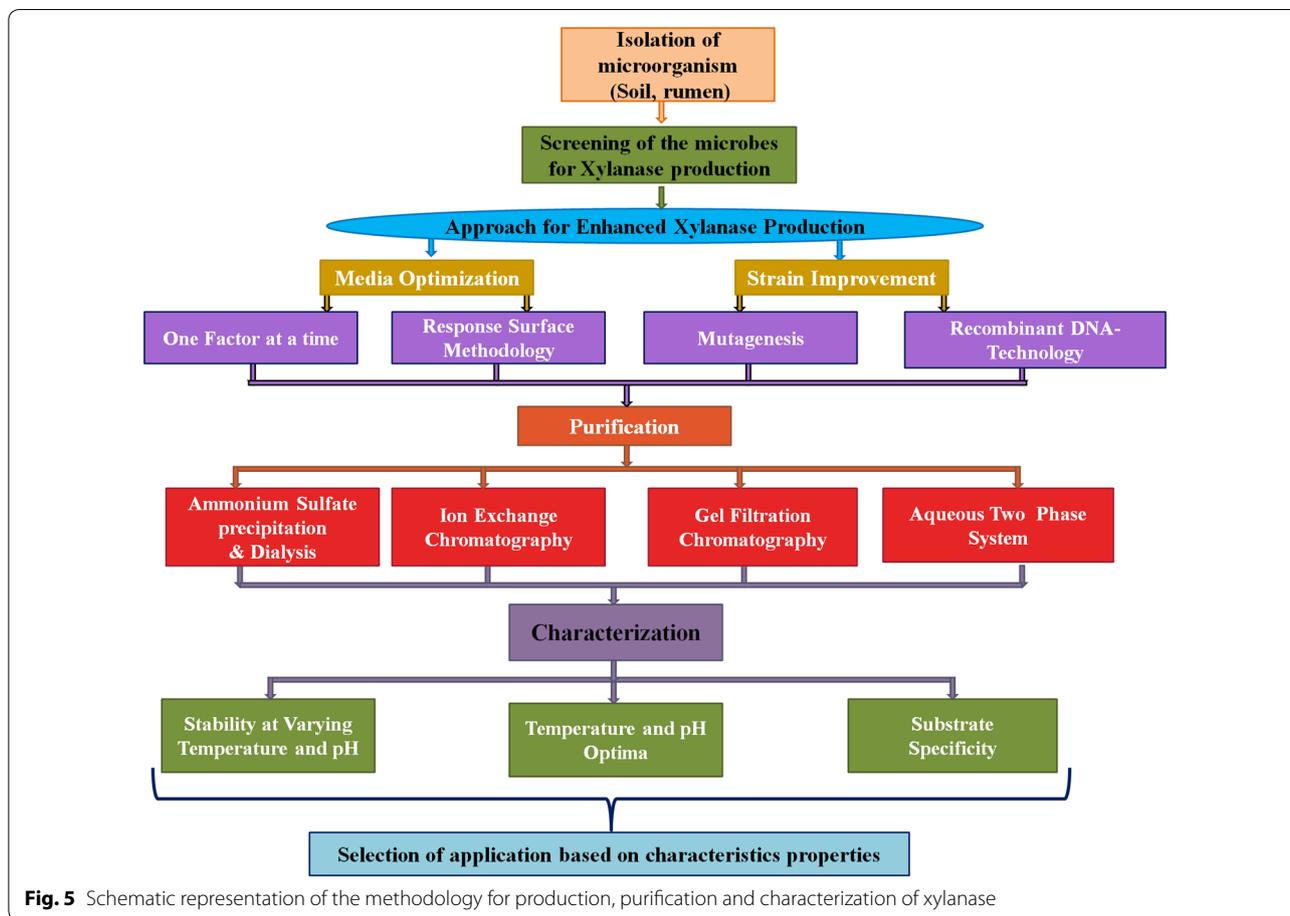
Strategies employed for the selection of the method of xylanase production and its optimization

Intially a common minimal media providing essential nutrients to the growth of microorganisms are used. This will allow to check the strains are capable of producing required enzymes/metabolite of desired interest. Then, the process is further optimized for higher production of enzymes from the strain (Walia et al. 2017). For the production of desired product, different strategies are used for improving yield such as optimization of media components, regulating physical growth parameters, and improving the strain by use of the different biotechnological tool (Sharma 2017). The schematic representation of the methodology adapted for production, purification and characterization of xylanase are shown in Fig. 5. In this section, the focus will be on the optimization of media and growth parameters and biotechnological tool approach will be discussed in a later section. During SmF for enzyme production, different components which need to be optimized are selection of substrate and microorganisms, regulation of nutrients concentration in media, i.e., carbon, nitrogen, trace elements, vitamins and amino acids, and physical parameters, i.e., temperature, pH, agitation, aeration, inoculum sizes, and incubation period (Motta et al. 2013; Walia et al. 2015a, 2017).

During optimization of the SSF, there is requirement of regulating particle size, pretreatment, humidity, water content and water activity (a_w) of substrate, type and size of the inoculums, removal of extra heat generated during microbial metabolism and most importantly maintaining the uniform environment (temperature) and evolution of CO_2 and consumption of O_2, i.e., gaseous system (Murugan et al. 2011; Behera and Ray 2016; Behnam et al. 2016; Leite et al. 2016; Walia et al. 2017).

Approach for enhanced xylanase production: one factor at a time (OFAT)

To proceed for the optimization of the xylanase production, one factor at a time (OFAT) approach is used for the selection of important factors affecting the xylanase

Fig. 5 Schematic representation of the methodology for production, purification and characterization of xylanase

yield. In the OFAT approach, one factor is kept variable keeping other factors at constant (Bhardwaj et al. 2018). The factor may be important physical or nutritional parameters regulating the growth of microorganisms and its enzyme yield. Ramanjaneyulu et al. (2017) have evaluated several operating parameters for nutritional (different substrates and their concentrations, additional carbon and nitrogen sources) and physical factors (incubation temperature, pH, agitation) along with inoculum size of *Fusarium* sp. BVKT R2 in a shake flask culture (SmF) by OFAT approach. The high xylanase yield of 4200 U/mL was obtained with birch wood xylan in mineral salt medium with 1.5% sorbitol (additional carbon source), 1.5% yeast extract (nitrogen source) at temperature of 30 °C, pH of 5.0, agitation of 200 rpm and inoculum of agar plugs (6) for only 5 days incubation. Under unoptimized condition, xylanase yield was only 1290 U/mL after 7 days of incubation, thus improving by 3.2-fold. Bhardwaj et al. (2018) also optimized xylanase production using *Aspergillus oryzae* LC1 using OFAT approach. The physical parameters (liquid to solid ratio, pH, inoculums size, incubation time and temperature) and nutrient

parameters (substrate concentration) were optimized using OFAT approach for enhanced production of xylanase by *T. viride*-IR05 under SSF (Irfan et al. 2014).

Statistical approach for enhanced xylanase production

The OFAT approach is tedious and requires a large set of experiments for optimization. The recent trend suggested the application of the statistical approach to design experiments considering different factors as variable and performing the interaction studies among several physical and nutritional parameters. The statistical-based approach has shown satisfactory results for optimization of xylanase production using fungal and bacterial strains with the minimum number of experimental sets (Guleria et al. 2015, 2016a; Walia et al. 2015b; Bhardwaj et al. 2017).

Response surface methodology (RSM) was employed to optimize the fermentation medium constituents and the physical factors affecting xylanase production using *Bacillus tequilensis* strain ARMATI under SmF (Khusro et al. 2016). The experimental design consists of central composite design (CCD) with four (4) independent

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variable (carbon and nitrogen source, temperature and time) resulting in 30 experimental runs. The central composite design gave an optimum parameter for studied variable (1.5% w/v birchwood xylan, 1% w/v yeast extract, temperature 40 °C, time 24 h) showing 3.7-fold enhanced xylanase production as compared to OFAT. High coefficient of determination (R^2) of 0.9978 with $p < 0.05$ as obtained by analysis of variance (ANOVA) analysis suggested the accuracy of the overall process at a significant level. The R^2 value of 0.9978 represents that sample variation of 99.78% and only 0.21% of the total variation in the response cannot be explained by the model. The xylanase obtained has shown high thermal (60 °C) and alkali stability (pH 9). Bhardwaj et al. (2017) optimized nutritional components (rice straw, $MgSO_4$, and $CaCl_2$ concentration) and physical parameters (temperature and pH) for enhanced xylanase production with an *Aspergillus oryzae* LC1 under submerged fermentation using CCD-RSM. The statistical design suggested optimum condition of 1% rice straw (w/v), 1.0 g/L $CaCl_2$, and 0.3 g/L $MgSO_4$, with pH 5 and 25 °C. It resulted in maximum xylanase activity of 935 ± 2.3 IU/mL which is 3.8-fold higher than the un-optimized Mendel's Stenberg Basal Salt medium (245 ± 1.9 IU/mL). The enzyme showed thermal (25–60 °C) and pH (3–10) stability. The xylanase also showed potential for efficient enzymatic hydrolysis of different lignocellulosic agro-residues.

Similarly, Tai et al. (2019) reported the optimization of five physical and two nutritional parameters using the RSM approach for enhanced xylanase production. Indigenous fungus *Aspergillus niger* DWA8 was grown under SSF on an oil palm frond. One physical (moisture content 75%) and one nutritional parameters (substrate concentration 2.5 g) have significant effect on xylanase production. Under optimum condition, an increase in xylanase yield by 78.5% was observed as compared to an unoptimized condition. The xylanase was efficiently used for saccharification of biomass. The statistical optimization method for enhanced xylanase production has been applied and widely accepted for SSF and SmF that helped in overcoming several limitations of classical empirical (OFAT) methods.

Biotechnological approach for enhanced xylanase production

There is a need of high yield of the enzyme with specific properties such as high stability over a wide range of temperature and pH, high substrate specificity and strong resistance to metal cations and chemicals for the industrial application (Garg et al. 2010; Qiu et al. 2010). The native enzyme is usually produced in low quantity and also lacks all the characteristics to meet the industrial needs (Ahmed et al. 2009). Therefore, different biotechnological approaches are used for improving the yield and imparting characteristic properties to the desired enzyme. These approaches involve genetic manipulation involving mutation and recombinant DNA technology.

Mutagenesis of microorganisms for enhanced xylanase production

Several researchers suggested that the application of physical mutagens such as UV radiation (Rahim et al. 2009; Abdel-Aziz et al. 2011) and chemical mutagens such as *N*-methyl *N*-nitro *N*-nitroso guanidine (MNNG) (Haq et al. 2004, 2008) resulted in enhanced xylanase production. Burlacu et al. (2017) demonstrated the improvement of xylanase production in fungal strains, i.e., *Aspergillus brasiliensis* and *Penicillium digitatum* by physical mutagenesis (5–50 min, exposure to UV light) and chemical mutagenesis (150 µg/mL of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine or ethyl methane sulfonate). The exposure to physical and chemical mutagens has resulted in significant changes in the mutant strain as compared to the wild type. Han et al. (2017) demonstrated the site-directed mutagenesis of XynCDBFV gene of ruminal fungus *Neocallimastix patriciarum* for improving the thermostability of XynCDBFV, a glycoside hydrolase (GH) family 11 xylanase. Similar work has also been carried out in different bacterial strains, a rifampin-resistant mutant of *Cellulomonas biazotea*, designated 7Rf, resulting in elevated levels of xylanases production as compared to the parental strain. After mutation, maximum xylanase and β-xylosidase production of 493 IU/L/h and 30.7 IU/L/h of β-xylosidase were obtained respectively. This increase in xylanase and β-xylosidase yield were 1.21- and 2.29-fold higher respectively as compared to the parental strain (Rajoka et al. 1997). *Bacillus mojavensis* PTCC 1723 when subjected to UV light exposure (280 nm, 30 s) resulted in the xylanase yield 330.6 IU/mL which is 3.45 times higher as compared to 95.7 IU/mL for wild strain (Ghazi et al. 2014). Lu et al. (2016) demonstrated mutation of XynHB, alkaline stable xylanase from *Bacillus pumilus* HBP8 at N188A. The mutant XynHBN188A is expressed in *E. coli* and *Pichia pastoris* with improved xylanase yield by 1.5- and 7.5-fold, respectively. The codon-based optimization and high-density fermentation using *Pichia pastoris* system were utilized for improving the xylanase yield.

Gene cloning and expression of xylanase genes using recombinant DNA

The recombinant xylanases are designed to have equivalent or better properties than the wild-type enzymes with high yield in the expression system which can be employed in the fermentation industry. The highly

thermo-alkalophilic xylanase producing strains can be directly employed during simultaneous saccharification and fermentation for ethanol generation using lignocellulosic biomass. The inherited stability will enable the xylanase to work efficiently even at high temperature and varying pH range of the fermentation system.

Several reports suggests that desired xylanase gene was cloned into the suitable vector followed by its expression in the suitable microbial systems such as bacteria, yeasts, and fungus (Belancic et al. 1995; Goswami et al. 2014; Jhamb and Sahoo 2012; Juturu and Wu 2012; Motta et al. 2013; Nevalainen and Peterson 2014; Verma et al. 2013).

Expression in bacteria

Goswami et al. (2014) demonstrated the expression of a xylanase gene from *Bacillus brevis* in *E. coli* BL21. The recombinant strain predominantly secreted xylanase in the culture medium with 30 IU/mL xylanase activity. The culture filtrate is free from cellulase activity and found to be active in a wide range of pH and temperature. A thermo-alkali stable xylanase encoding gene (Mxyl) was retrieved from compost-soil metagenome library construct and cloned into pET28a vector expressed in *E. coli* BL21(DE3). The recombinant xylanase has shown half-life of 2 h and 15 min at 80 °C and 90 °C, respectively. The recombinant xylanase has pH and temperature optima of 9.0 and 90 °C, respectively (Verma et al. 2013).

Escherichia coli is preferred and most widely used expression host due to its inexpensive growth conditions, easy manipulation, simple transformation techniques requirement, high level of product accumulation in the cell cytoplasm (Jhamb and Sahoo 2012). However, efficient and functional expression of many xylanase genes is not possible with *E. coli* which may be due to repetitive appearance of rare codons and the requirement for specific translational modifications (disulfide-bond formation and glycosylation) (Belancic et al. 1995; Jhamb and Sahoo 2012; Juturu and Wu 2012; Motta et al. 2013). One of the other important concerns associated with *E. coli* is the presence of endotoxins (lipopolysaccharide) which makes the protein purification process very tedious. *Lactobacillus* and *Bacillus* species are used for heterologous expression of xylanase than in *E. coli*. It is capable of performing N-glycosylation, generally regarded as safe (GRAS) due to the absence of endotoxins and their secretory production is beneficial in industries (Bron et al. 1998; Subramaniyan and Prema 2002; Upreti et al. 2003; Juturu and Wu 2012). Zhang et al. (2010a, b) demonstrated the expression and characterization of the xylanase gene (xynB) from *Dictyoglomus thermophilum* Rt46B.1 in *Bacillus subtilis* system. The pH and temperature optima for the purified recombinant enzyme were 6.5 and 85 °C, respectively. The xylanase was stable up to 95 °C and retained its activity in surfactants such as EDTA, DTT, Tween-20 and Triton X-100.

Expression in yeast

The heterologous protein expression in yeast system is highly attractive due to its ability to perform eukaryotic post-translational modifications and can grow to very higher cell densities with the ability to secrete enzyme into the fermentation system. Most of the yeasts are considered as GRAS organisms and do not produce toxins (Juturu and Wu 2012). *Saccharomyces cerevisiae* already established as an industrial microorganism, thus, can be conveniently used for xylanase production (Ahmed et al. 2009).

The application of *Pichia pastoris* as expression system has gained impetuous because it can promote the expression of the protein on their own using alcohol oxidase as promoter using methanol utilization pathway (Ahmed et al. 2009; Juturu and Wu 2012; Motta et al. 2013). *Pichia pastoris* as expression system is preferred as it can grow to very high cell densities, inherit strong and tightly regulated promoters, and produce high titer of recombinant protein (g/L) both intracellularly and in the secretory manner (Ahmad et al. 2014). Basit et al. (2018) demonstrated the cloning of two GH11 xylanase genes, *MYCTH_56237* and *MYCTH_49824*, from thermophilic fungus *Myceliophthora thermophila* and its expression in *Pichia pastoris*. The specific activities of purified recombinant xylanase were observed at 1533.7 U/mg and 1412.5 U/mg for *MYCTH_56237* and *MYCTH_49824*, respectively. The recombinant xylanase showed stability under harsh condition (high pH and temperature) and high efficiency for biomass saccharification. However, the application of *Pichia pastoris* at large scale is limited due to health and fire hazards of methanol (Ahmed et al. 2009). In the case of *P. pastoris* as expression system, lower protein yield was obtained while expressing membrane-attached protein or proteolytic degradation prone protein and complex protein such as hetero-oligomers (Ahmad et al. 2014).

Expression in filamentous fungi

Filamentous fungi can be efficiently used for heterologous and homologous gene expression resulting in high yield of recombinant gene products (Su et al. 2012; Motta et al. 2013; Nevalainen and Peterson 2014; Nevalainen et al. 2018). Similar to yeast, it can regulate expression yields with their own promoters and can provide eukaryotic style post-translational modification of proteins such as N-glycosylation, proteolytic processing, or formation of multiple disulfide bonds (Ahmed et al. 2009; Fleissner and Dersch 2010; Landowski et al. 2015).

The application of fungi as an expression system also has advantages associated with cost-effectiveness of the overall process due to low-cost substrate and downstream processing. Further, fungi have already been subjected to many strain improvement procedures for enhanced production of xylanase. Therefore, the native xylanase expressing machinery can be efficiently used for functional expression of a foreign xylanase gene from other sources. Xyn2 xylanase gene was expressed in *T. reesei* by homologous expression resulting in the 1.61 g/L of xylanase 2 on glucose-containing medium (Li et al. 2012). Godlewski et al. (2009) demonstrated xylanase B(XynB) gene expression in *T. reesei.* Similarly, the expression of xylanase 2 (XYN2) and xylanase gene from the thermophilic fungus *Humicola grisea var. thermoidea* and *P. griseofulvum* was expressed in *Trichoderma reesei* and *Aspergillus oryzae*, respectively (De Faria et al. 2002; Motta et al. 2013). Nevalainen and Peterson (2014) presented a comprehensive review on application of filamentous fungus as expression system and suggested that research is now focused on understanding the cellular mechanisms for better internal protein quality control and secretion stress. The better utilization of "omics" tools can help in improving the regulation of xylanase production using filamentous fungus as an expression system.

Strategies for enhanced purification and characterization of xylanase for industrial application

The microbial system produces a wide range of biochemical's during different growth and development of the microorganisms. These biochemical's are enzymes, secondary metabolites, etc. which are of great importance to human applications. Similarly, enzymes are produced by microorganisms along with other enzymes or metabolites. Therefore, purification is prerequisites for obtaining pure enzyme with minimum or no impurities (Zhang et al. 2012). The characterization of the purified enzyme such as evaluation of temperature and pH optimum, thermal and acid/alkali stability, role of metal ions and inhibitors in regulation of enzyme activity, and substrates specificity was performed for selecting the suitable industrial process (Bhardwaj et al. 2019). There are different enzyme purification strategies for the xylanase such as ammonium sulfate precipitation (salting in) followed by dialysis (salting out), gel permeation chromatography, ion exchange chromatography, recently developed techniques aqueous phase chromatography and ultrafiltration (Walia et al. 2014; Guleria et al. 2016b; Bhardwaj et al. 2019).

Ammonium sulfate precipitation followed by dialysis

The crude xylanase preparation is subjected to different ranges of ammonium sulfate concentration (30–90%) for selection of suitable salt concentration for precipitation of the enzyme. The precipitated enzyme is then subjected to dialysis for removal of the salt. The crude xylanase obtained from *Streptomyces* P12-137 was subjected to ammonium sulfate precipitation (40–90%) followed by dialysis. The purification fold of 4.18 was observed with two different endoxylanase observed as F5 (65%) and F6 (80%) with the specific activity of 45.4 U/mg and 36.5 U/mg, respectively. This was also confirmed by HPLC analysis. The purified enzyme was further characterized by incubating at different temperature and pH followed by analyzing the enzyme for xylanase activity. The optimum pH and temperature of pH 7.0, 60 °C and 6.5, 60 °C, for F5 and F6 xylanase, respectively, were obtained (Coman et al. 2013).

Bhardwaj et al. (2017) performed partial purification of the crude xylanase obtained from *Aspergillus oryzae* LC1 using ammonium sulfate (60%) precipitation followed by dialysis against 50 mM acetate buffer (pH 5.0). The partially purified enzyme was further characterized which showed stability over a wide pH range of 3 to 10 and thermal stability over the temperature range of 25 to 60 °C. Similarly, Kumar et al. (2018d) have demonstrated the purification and characterization of xylanase obtained from sea sediment bacteria using a combination of ammonium sulfate precipitation and dialysis. The improvement in specific activity and characteristic properties of xylanase was observed. The major limitations of the precipitation are needed to remove salt from protein sample so further processing in the form of dialysis or chromatography is required. Further, for dialysis, there is a need to have a better understanding of the protein solubility. It is also stated that ammonium precipitation concentrates the protein rather than purifying it. Thus, contaminant present in the crude sample may also be present along with the protein sample even after precipitation and dialysis (Biosciences 2019). The xylanase is also concentrated or precipitated using trichloroacetic acid (TCA) and acetone. However, the TCA may denature the protein; therefore, it is not advisable to use TCA when the protein is required in the folded state (for activity assay) and the toxicity of TCA also limits its applications (Koontz 2014).

Chromatography techniques for enhanced xylanase yield employed for purification

Usually, it has been observed that xylanase purification was performed by the multi-step process where

the concentration of protein using ammonium acetate/TCA/acetone precipitation or ultracentrifugation was followed by a single step or series of chromatography techniques. Yadav et al. (2018) demonstrated the purification and characterization of extracellular xylanase obtained from *A. kamchatkensis* NASTPD13 cultures. The crude xylanase was subjected to ammonium sulfate (80%) precipitation followed by dialysis. The dialyzed sample was further subjected to Sephadex G100 column chromatography. The fractions collected showing maximum xylanase activity were concentrated and analyzed by SDS-PAGE (MW obtained was 37 kDa). The two-step purification has led to increased xylanase activity by 11-fold with a 33 U/mg specific activity. The characterization of purified protein showed pH and temperature optimum of 9.0 and 65 °C, respectively, and also retained more than 50% of its activity over a wide range of 6–9 pH and 30–65 °C temperature. An insight into several purification strategies employed for xylanase from different microorganisms along with the process efficiency in terms of recovery potential and kinetics property is tabulated in Table 4.

Purification of endoxylanase obtained from *Bacillus pumilus* B20 was performed in three steps (Geetha and Gunasekaran 2017). The first step was ammonium sulfate precipitation (60–80%) followed by FPLC using DEAE Sepharose column as the second step and further subjecting the eluted sample onto a Sephacryl S-200 column as the third purification step. At each step, the specific activity was improved as compared to the crude enzyme by 5 to 14.8-fold with maximum 755.8 U/mg specific activity at the end of all the three purification steps. After the purification, the fractions showing maximum xylanase activity were subjected to xylanase assay and other characterization studies such as SDS-PAGE, zymography, temperature and pH stability. The SDS-PAGE and zymography analysis showed the purified enzyme of ~ 85 kDa, i.e., endoxylanase (XylB). The purified enzyme was stable in a pH range of pH 6.5 to 7.5 and the temperature range of 20 to 50 °C. The purified enzyme was highly specific to different commercial and natural xylan substrate and has the potential to generate xylooligosaccharides.

Aqueous two-phase system employed for purification of xylanase

The conventional multistep purification techniques are time consuming, which increases the cost of the overall process and also results in loss of protein at each step (Iqbal et al. 2016; Ramakrishnan et al. 2016). The 60–70% of total processing cost in enzyme downstream process comes from the purification step (Loureiro et al. 2017; Bhardwaj et al. 2019). Therefore, several scientists suggested a single step liquid–liquid fractionation technique,

i.e., aqueous two-phase system (Naganagouda and Mulimani 2008; Yasinok et al. 2010; Glyk et al. 2015).

Garai and Kumar (2013) purified alkaline xylanase from *Aspergillus candidus* using aqueous two-phase system (ATPS) composed of PEG 4000/NaH_2PO_4 system. The critical factors of ATPS such as PEG molecular weight, PEG and phosphate salt concentration using Box–Behnken design approach were used for the optimization of enhanced xylanase purification. The optimum condition was PEG 4000 at 8.66% w/w with a high salt concentration of 22.4 w/w that resulted in 8.41% purification fold. The enzyme was stable at alkaline pH and activity is enhanced with Mn^{2+} ions. Ng et al. (2018) demonstrated the recovery of xylanase from *Bacillus subtilis* fermentation broth with an alcohol/salt ATPS. The ATPS system consists of 26% (w/w) 1-propanol and 18% (w/w) ammonium sulfate resulting in 5.74 ± 0.33 purification fold and yield of $71.88\% \pm 0.15$.

Gómez-garcía et al. (2018) demonstrated purification of xylanase by *Trichoderma harzianum* using ATPS with PEG/salt system. The PEG molecular weight, PEG, phosphate salt concentration, and salt conditions were optimized. The best enzyme recovery and purification fold of 62.5% and 10% respectively was obtained using 20.2% PEG 8000, 14.8% K_2HPO_4, and tie to a length of 45% w/w. Bhardwaj et al. (2019) subjected crude xylanase from *Aspergillus oryzae* LC1 to four different single-step purification by ammonium sulfate precipitation, ion exchange, gel filtration chromatography and ATPS PEG/Salt system. The xylanase purification using single-step ATPS system resulted in highest purification yield (PY) of 86.8% and 13-fold purification fold (PF) which was much higher than other purification strategy, i.e., ammonium precipitation (PY-21%, PF-4.1), anion exchange (PY-31.9%, PF-3) and gel filtration (PY-78.7%, PF-6.6).

Therefore, ATPS exhibits several advantages over traditional purification techniques, i.e., it requires low-cost materials, low energy consumption with high yield and better resolution (Naganagouda and Mulimani 2008; Yasinok et al. 2010; Glyk et al. 2015). The ATPS method is independent of protein concentration and does not affect the native property of protein (Iqbal et al. 2016; Ramakrishnan et al. 2016).

Structural properties of xylanase responsible for thermal and pH stability required for industrial application

The high stability of xylanase was due to the presence of intrinsic structural properties. The presence of extra disulfide and salt bridges, hydrophobic side chains, and N-terminal proline residues helps in reduction of conformational freedom of the protein structure. Thus, it help in providing more stability to protein at the higher

Table 4 Purification strategy employed for xylanase from different microorganisms

Source organism	Purification methodology	Specific activity (IU/mg)	Fold purification	Recovery (%)	K_m (mg/mL)	V_{max} (µmol/mg/min)	Application of purified enzyme	References
Combined purification strategy								
Bacillus sp. SV-34S	Ammonium sulfate precipitation (80%)	2803.1	10.62	88.0	3.7	133.33 IU/mL	Paper and Pulp industry	Mittal et al. (2013)
	Carboxymethyl Sephadex C-50 cation exchange chromatography	3417.2	12.94	13.44				
Bacillus sp. GRE7	Ammonium sulfate (40–80%)	191.1	3.9	71	2.23	296.8 IU/mg	Eucalyptus kraft pulp bio-bleaching	Kiddinamoorthy et al. (2008)
	DEAE-Cellulose	582.9	11.9	48				
	Sephadex G-75	1392.6	28.5	27				
Arthrobacter sp.	Ammonium sulfate	162	2	74	0.9	3571	–	Khandeparkar and Bhosle (2006)
	Sephadex G-200	282.6	3.5	62				
	DEAE-Sepharose FF	444.2	5.5	49				
	CM-Sepharose FF	1697.7	21	14				
Thermotoga thermarum	Ni-affinity chromatography	192	6.9	82.3	1.8	769	XOS generation	Shi et al. (2014)
Glaeocola mesophila KMM 241	Ammonium sulfate Dialysis Ni^{+2}-NTA Agarose resins	77	9.1	17.4	1.22	98.31	XOS generation, Sea and saline food processing, bakery industries	Guo et al. (2009)
Streptomyces olivaceus (MSU3)	Ammonium sulfate fractionation	37.35±1.33	1.045±0.002	60.69±2.38	8.16	250.01	High sugar yield and bioethanol generation ability using lignocellulosic biomass	Sanjivkumar et al. (2017)
	Dialysis	82.40±2.50	2.298±0.021	38.29±1.53				
	DEAE–cellulose column	116.98±2.28	3.260±0.024	28.15±1.87				
	Sephadex-G-75 column	153.11±2.11	4.270±0.026	15.57±0.85				
Penicillium glabrum	Ammonium sulfate fractionation	291.78	3.25	84.19	1.2–5.3	212.10–393.17	Purified enzyme suitable for animal feed additives, clarification, and maceration of juices and wines	Knob et al. (2013)
	Molecular exclusion chromatography (Sephadex G-75 column)	457.89	5.10	76.92				
Trichoderma inhamatum	Dialysis followed by ion exchange chromatography (DEAE Sephadex A-50 column)	XylI-1257.7 XylII-1216.4	XylI, II-1.9	XylI-62.7 XylII-3.7	XylI-1.6–14.5 XylII-4.0–10.7	XylI-462.2–2680.2 U/mg XylII-1972.7–4553.7 U/mg	XOS generation	da Silva et al. (2015)

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Table 4 (continued)

Source organism	Purification methodology	Specific activity (IU/mg)	Fold purification	Recovery (%)	K_m (mg/mL)	V_{max} (µmol/mg/min)	Application of purified enzyme	References
Chrysoporthe cubensis	Q Sepharose anion exchange column followed by Sephacryl S-200 gel filtration column	–	P2-1.2 P3-1.4	P2-13.4 P3-1.3	P2-1.81 P3-1.18	–	Efficient saccharification of sugarcane bagasse	de Sousa et al. (2017)
Myceliophthora heterothallica F.2.1.4	Ni Sepharose® resin containing His-Trap HP column	102.2	44	7	13.4 ± 1.5	274.5 ± 1.0	Pulp bleaching	de Amo et al. (2019)
Aspergillus flavus	Ammonium sulfate fractionation (40–60%)	621.4	2.7	46.7	–	–	Efficiently hydrolyzed pretreated corncobs for generation of XOS	Chen et al. (2019)
	Gel filtration (HiPrep 16/60 Sephacryl S–100 HR column) chromatography	838.2	3.7	34.8				
Penicillium chrysogenum	Ion exchange chromatography (CM Sephadex C-50 column)	438.3	9.8	49.3	2.3 mM	731.8 U/mg	Beverage, bakery, and feed industry. For production of xylooligosaccharides and bioethanol	Terrone et al. (2018)
	Size exclusion chromatography (Sephadex G-100 column)	834.2	18.7	31.1				
Single step purification strategy								
Streptomyces P12-137	Ammonium sulfate precipitation followed by dialysis	F5-45.44 F6-36.48	4.18	F5-60.63 F6-73.02	F5-0.2012 F6-0.0388	F5-0.4742 s⁻¹ F6-0.6314 s⁻¹	Efficient XOS generation	Coman et al. (2013)
Sorangium cellulosum So9733-1	Ni-affinity followed by dialysis and concentration	4.11	4.03	43.84	38.13	10.69	Hydrolysis of xylan rich substrates and food industries	Wang et al. (2012)
Aspergillus tamarii Kita	Single step purification carboxymethyl-cellulose (CM-cellulose) chromatography	1215.89	7.43	36.72	7.59–8.13	1178.56–1330.20	XOS generation	Heinen et al. (2018)

Table 4 (continued)

Source organism	Purification methodology	Specific activity (IU/mg)	Fold purification	Recovery (%)	K_m (mg/mL)	V_{max} (μmol/mg/min)	Application of purified enzyme	References
Scytalidium thermophilum ATCC No. 16454	Ammonium sulfate precipitation (50%)	125.9	2.5	52	2.4	168.6 IU/mL	Agricultural biomass hydrolysis for application in bioethanol refinery	Kocabacs et al. (2015)
	ATPS (7% Triton X-114 top phase)	79.8	2.7	79				
	10 kDa molecular weight cut-off (MWCO)	841.1	4.3	25				
Aspergillus oryzae LC1	Ammonium sulfate Precipitation followed by dialysis	631	4.1	21	0.2	172.2	XOS generation	Bhardwaj et al. (2019)
	Anion exchange Chromatography (DEAE-Sephadex A-50)	469	3	31.9				
	Gel Filtration Chromatography (Sepharose G-100)	101.5	6.6	78.7				
	ATPS with Triton X-114 (5% v/v)	508.9	3.3	69.4				
	ATPS with PEG (22.5% MgSO$_4$ Salt 11.3% PEG 8000)	2004.3	13	86.8				

temperature (Turunen et al. 2001; Chen et al. 2015). Different structural modifications such as high Thr/Ser ratio and high charged residues, i.e., Arg, cause enhanced polar interaction and improved stabilization of the alpha-helix region and secondary structures (Hakulinen et al. 2003). The xylanase protein has a large number of ion pairs/aromatic residues on the surface of protein resulting in enhanced interactions (Polizeli et al. 2005; Chen et al. 2015). The low average protein rigidity i.e. low B factor, low flexibility results in high rigidity at extreme physical conditions (Xie et al. 2014). The presence of divalent metal ions and removal of N or C terminal disordered residues protect xylanase from heat and protease inactivation (Andrews et al. 2004; Chen et al. 2015). The presence of carbohydrate-binding modules (CBM22 and CBM9) at N or C terminal often imparts heat stability to xylanase. The pH stability of the xylanases is often affected by the presence of several amino acids near the catalytic residues (Singh et al. 2019).

Cost estimation of the xylanase production

Polizeli et al. (2005) suggested that 20% of the total global enzyme production is from biomass hydrolysis enzymes, i.e., xylanase, cellulase and pectinases. An extensive study on cost involved in each step of xylanase production at industrial scale is unavailable on public domain. Klein-Marcuschamer et al. (2012) performed a study on the cost analysis of application of enzymes during the lignocellulosic biomass based biofuel production and suggested breakdown of the operating cost (annual) in their enzyme production facility. They suggested percentage of cost involved for each component, i.e., raw materials (28%), labor (7%), transportation (1%), consumables (4%), utilities (10%), facility dependent (48%), and waste treatment (2%). This clearly shows that maximum contribution of 48% comes from the capital investment followed by cost of substrate (28%). Klein-Marcuschamer et al. (2012) also suggested the baseline production cost of hydrolysis enzyme as $10.14/kg.

Da Gama Ferreira et al. (2018) performed techno-economic analysis of the β-glucosidase enzyme production from *E. coli* on industrial scale. They showed major cost during industrial production are facility dependent (45%) followed by raw materials (25%) and consumables (23%), that are similar to observations made by Klein-Marcuschamer et al. (2012). Capital investment/facility-dependent cost is required for development of infrastructure (i.e., equipments), insurance, maintenance and depreciation. This upstream and downstream process during enzyme production involves the cost on part of capital investment along with the cost of consumables and utilities. da Gama Ferreira et al. (2018) performed sensitivity analyses of process scale, inoculation volume with

respect to volumetric productivity, which suggested that remarkable reduction in cost of enzyme production may be observed under optimized conditions. Thus, based on the above studies, we can suggest that xylanase production can be based on the cost of substrate and consumable, along with the cost of each step involved in upstream and downstream processing. Therefore, utilizing cheap raw materials, less number of steps during upstream and downstream process (such as single step purification instead of multistep process) can help in keeping the enzyme production cost as low as possible.

Xylanase employed as a greener tool in different industries

Xylanase with such unique characteristics of thermo-alkali tolerant nature has a diverse range of application in different industries such as paper and pulp, deinking, biomass utilization and food feed industries (Fig. 6).

Xylanase employed in the food and feed industry
Bakery

The xylanase finds application in food industries such as bakery. The bread is made up of wheat consisting of hemicelluloses such as arabinoxylan. The xylanase can solubilize the water unextractable arabinoxylan into water-extractable arabinoxylan (Courtin and Delcour 2002). This help in uniform water distribution and improvement in gluten network formation throughout the dough. The addition of xylanase improves the rheological properties of dough such as softness, extensibility, and elasticity along with bread-specific volume and crumb firmness (Harbak and Thygesen 2002; Camacho and Aguilar 2003; Butt et al. 2008). The breakdown products of arabinoxylan, i.e., arabino-xylooligosaccharides in bread have its health benefits (Polizeli et al. 2005; Bajpai 2014).

Butt et al. (2008) demonstrated the role of GH11 endoxylanases from *B. subtilis* in solubilizing the arabinoxylan. This increases the viscosity and volume of dough and decreases gluten agglomeration and dough firmness resulting in the development of uniform and fine crumbs. GH11 xylanase (0.12 U/g flour) from *Penicillium occitanis* Pol6 resulted in improvement of bread-making process such as the decrease in water absorption (8%) and an increase in dough rising (36.8%), volume (17.8%), specific volume (34.9%) and cohesiveness. The bread has improved rheological and sensory properties (texture, taste, flavor, softness, and overall acceptability). Low springiness and gumminess were observed in the bread prepared using xylanase (Driss et al. 2013). Partially purified microbial xylanase was used by Ghoshal et al. (2013) to produce whole-wheat bread with better sensory properties (brighter color). The addition of xylanase
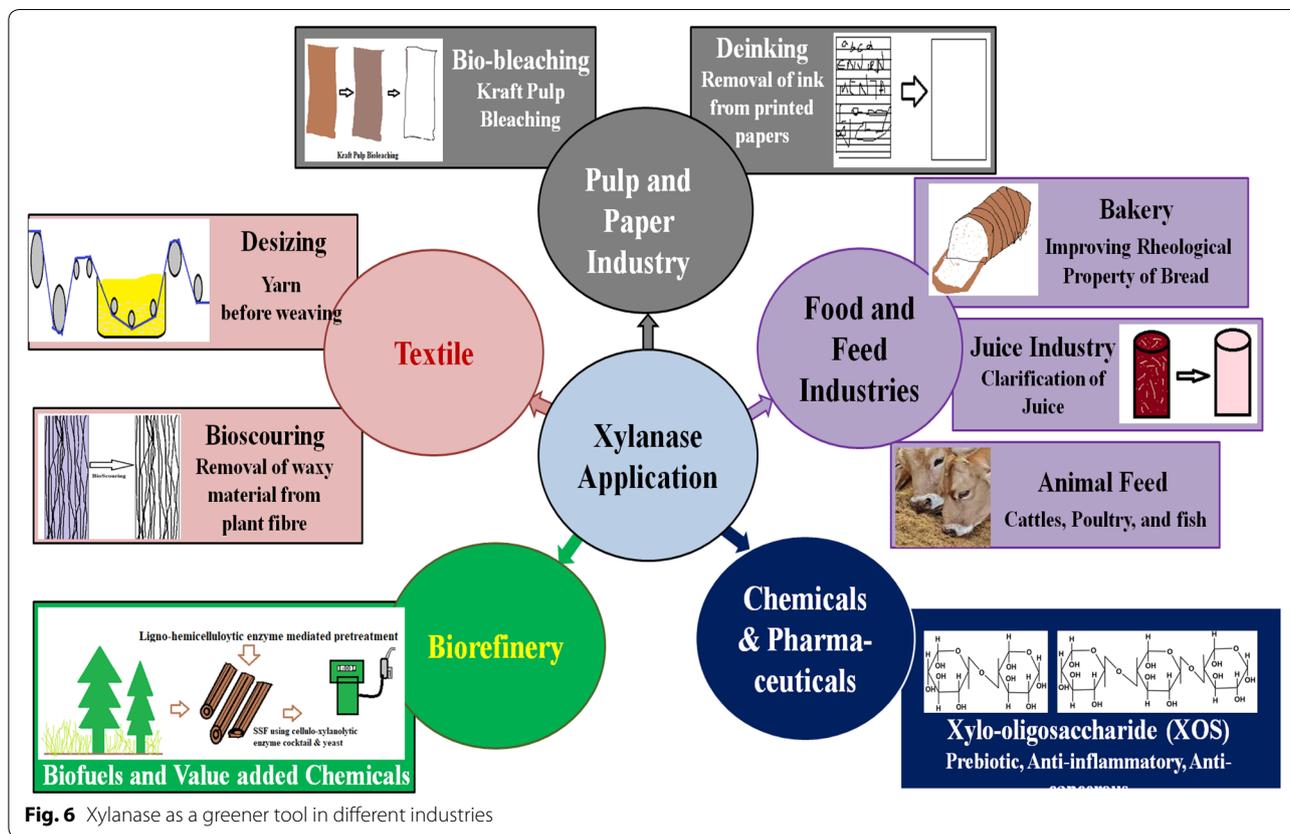
Fig. 6 Xylanase as a greener tool in different industries

also resulted in increased specific volume, and shelf life, with lower firmness and reduced staling during storage. Panzea, new generation xylanase obtained from *Bacillus Licheniformis*, can help in improving dough properties at low enzyme dosage. It helps in achieving the desired texture, appearance, loaf volume and crumb structure (Bajpai 2014). Similarly, recombinant xylanase (r-XynBS27) obtained from *Pichia pastoris (*xynBS27 gene from *Streptomyces* sp. S27) used as an additive during bread-making process. The recombinant xylanase resulted in improvement in a specific volume and reducing sugar content with a decrease in firmness, consistency, and stiffness (De Queiroz Brito Cunha et al. 2018a, b).

Fruit juice clarification

The enzymatic process in fruit juice extraction and clarification is widely used. Raw juices of fruit contain polysaccharides such as cellulose, hemicellulose, starch pectin and surface-bound lignin and decrease the quality of the juice, e.g., hazy color and high viscosity (Danalache et al. 2018). The use of enzymes decreases the viscosity and avoids the formation of clusters, by removing the suspended and undissolved solid using centrifugation and filtration methods. This increases the clarity, aroma, and color of the juice (Danalache et al. 2018). Xylanase from

Streptomyces sp was used for the clarification of orange, mousambi, and pineapple with 20.9%, 23.6% and 27.9% clarity, respectively (Rosmine et al. 2017). Immobilized xylanase obtained from *Bacillus pumilus* VLK-1 was used for orange (29%) and grape juice (26%) enrichment (Kumar et al. 2014). Xylanase immobilized on 1,3,5-tri-azine-functionalized silica-encapsulated magnetic nanoparticles was reported to clarify the three different types of fruit juices after five hours of incubation at 50 °C (Shahrestani et al. 2016). Partially purified xylanase from *Streptomyces* sp AOA40 was used in fruit juice industry for increased clarity of juices from apple (17.8%), orange (18.4%) and grape (17.9%) (Adigüzel and Tunçer 2016). Glutaraldehyde-activated immobilized xylanase was used for the clarification of tomato juice. Xylanase from *P. acidilactici* GC25 was used to treat the kiwi, apple, peach, orange, apricot, grape, and pomegranate in which increase in the amount of reducing sugar was observed along with the decrease in turbidity of the juice (Adiguzel et al. 2019).

Animal feed

Xylanases plays an important role in animal feed by breaking the feed ingredient arabinoxylan and reduces the raw material viscosity. *Aspergillus japonicus* C03 with

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good endoxylanase and cellulase production ability with high stability in the presence of goat ruminal environment showed ruminant feed applications (Facchini et al. 2011). A number of studies reported the availability of distillers dried grains with soluble (DDGS) to be utilized in animal feeds and use of exogenous xylanase in poultry diets to treat the higher fiber content (Pirgozliev et al. 2016; Whiting et al. 2019). The exogenous enzymes effectively improved the nutritional value of co-products of bioethanol as reported previously with DDGS obtained from corn (Liu et al. 2011). Xylanases have been involved in animal feed over decades, as it reduces the viscosity of digesta in poultry. Xylanase addition showed the weight gain improvement and enhanced feed conversion ratio because of the improvement in the arabinoxylan digestibility in monogastric animal diets (Paloheimo et al. 2010; Van Dorn et al. 2018). Xylanase utilized as a dietary supplement for the nutrients digestibility, digesta viscosity growing pigs fed corn intestinal morphology diet based on soybean meal was reported by Passos et al. (2015). ECONASE XT a well-known commercial *endo*-1,4-β-xylanase which has been used as feed additives for chicken fattening, weaned piglets and fattening for pigs (Rychen et al. 2018).

Xylanase in paper and pulp industries
Bio-bleaching
The process of removal of lignin from wood pulp to produce bright and completely white finished paper is known as bleaching (Beg et al. 2001). Traditionally, chemical bleaching agents (such as chlorine) were used for bleaching (Subramaniyan and Prema 2002). The use of ligno-hemicellulolytic enzymes for bleaching has gained impetuous all over the world. Xylanases are capable of hydrolyzing xylan which is linked to the cellulose and lignin of the pulp fiber. Thus, xylan disruption will eventually lead to the separation between these components, enhance swelling in the fiber wall, and improve lignin extraction from the pulp (Thomas et al. 2015). Thus, xylanase in combination with lignin-degrading enzyme help in increasing the brightness of pulp (Viikari et al. 1994; Sunna and Antranikian 1997; Pérez et al. 2002; Motta et al. 2013). The exposures of the cellulose fiber to enzymatic pulping enhance the bonding forces of paper and improve paper strength via degradation of xylan and removal of lignin during enzymatic treatment (Lin et al. 2018). The enzymatic system has been highly selective, non-toxic, environmentally friendly approach for bio-bleaching (Bajpai 2012).

Paper and biomass pulp processing takes place at varying pH and temperature. Therefore, thermo-alkali stable xylanases are required for the bio-bleaching. An alkaliphilic *Bacillus* strain produced thermoactive cellulase-free xylanase using agro-residues active at high temperature 60 °C and pH 6–10 and was utilized for bio-bleaching of kraft pulp (Azeri et al. 2010). *Paenibacillus campinasensis* BL11 xylanase pretreatments showed the increased brightness and viscosity of hardwood kraft pulp (Ko et al. 2010). *S. thermophilum* xylanase active at high temperature (50–70 °C) was used for the bleaching of bagasse pulp (Joshi and Khare 2011). *T. lanuginosus* VAPS24 xylanase was stable at wide range pH that can be useful in both alkaline and acidic bioprocesses (Kumar and Shukla 2018). An alkaliphilic *Bacillus liceniformis* Alk-1 xylanase was utilized in a purified form for the enzymatic pretreatment on eucalyptus kraft pulp bleaching (Raj et al. 2018). The xylanase preparation obtained from white-rot fungi, *S. commune* ARC-11 was capable of ethanol soda pulp pre-bleaching from *Eulaliopsisbinata* (Gautam et al. 2018). The paper manufacturing units of various countries, i.e., Japan. South America, North America and Europe are slowly replacing chemical pulp bleaching by xylanase mediated pulp bleaching. Canada is known to be the leading producer of pulp and they are bleaching more than 10% its pulp via xylanase (Dhiman et al. 2008a). In addition to that, several reports also suggest that the xylanase enzyme-mediated pretreatment can help in generation of cellulosic nanofibres (CNF) with improved crystallinity from unbleached bagasse and eucalyptus pulp (Nie et al. 2018; Zhang et al. 2018; Tao et al. 2019). Zhang et al. (2018) suggested that application of commercial Novozyme X2753 can simplify the CNF's production and purification process. Tao et al. (2019) demonstrated that xylanase can directly act on the unbleached pulp, where it acts on the covalent bond between hemicellulose molecule and hydrogen bond between hemicelluloses and cellulose. The presence of a small amount of hemicelluloses in cellulose nanofibrils increases light blockage efficiency and subsequently the energy storage capacity of solar cells. Thus, xylanase-mediated bio-bleached pulp acts as a potential substrate for flexible solar cells.

Deinking of waste paper
The dislodgement of ink from the waste used paper is required for its recycling and reuse. Chemical-based methods involving chlorine or chlorine-based derivatives, ClO^-, $NaOH$, $NaCO_3$, H_2O_2, Na_2SiO_2, have been used for removing ink from the paper. This resulted in generation of hazardous effluents and required tedious treatment before disposal to the environment (Maity et al. 2012). The enzyme-based methods utilizing xylanase and laccase have been suggested for the removal of ink from paper and pulp industries effluents (Chandra and Singh 2012; Dhiman et al. 2014).

Virk et al. (2013) explored the deinking efficiencies of bacterial alkalophilic laccase and xylanase along with physical deinking methods such as microwave and ultrasound for recycling of waste paper. The combination of xylanase and laccase enzymes showed an increase in brightness of different waste pulp old newsprint pulp (21.6%), inkjet print pulp (4.1%), laser print pulp (3.1%), magazine pulp (8.3%), and xerox paper pulp (1.9%) only. Gupta et al. (2015) reported that synergistic action of xylanase and laccase enzyme (co-cultivation of *Bacillus* sp. and *B. halodurans* FNP135) resulted in improvement of physical properties like freeness, breaking length, burst factor and tear factor by 17.8%, 34.8%, 2.77%, and 2.4%, respectively, of old newspaper. The appearance of the newspaper was also improved with an increase in 11.8% brightness and 39% whiteness. The effective dose of commercial cellulase and xylanase from *Bacillus halodurans* TSEV1 for removal of ink was determined at 1.2. U/mg (each enzyme) by Kumar and Satyanarayana (2014). The cellulase and xylanase complex obtained from *Escherichia coli* SD5 facilitated the reduction in hexenuronic acid (Hex A) and kappa number, increase in brightness (10%) and tear strength of recycled paper (Kumar et al. 2018c).

Xylanase employed in textile industries

The textile processing can be broadly divided into desizing, scouring and bleaching. Desizing involves removal of adhesive material from plant fibers and scouring to remove the inhibitory material from desized fibers (Hartzell et al. 1998; Dhiman et al. 2008b). The conventional method used for desizing and scouring involves the application of high temperature under the influence of oxidizing agents in the alkaline system. This method is not only chemical intensive but also non-specific that causes hamper to the useful cellulosic fractions compromising the overall strength of the textile fibers. Therefore, application of highly thermo-alkali stable cellulase-free xylanolytic enzyme can efficiently be used for desizing and scouring (Csiszár et al. 2001; Losonczi et al. 2005; Dhiman et al. 2008b; Bajpai 2014).

Dhiman et al. (2008b) demonstrated the application of alkalo-thermophilic xylanase from *Bacillus stearothermophilus* SDX for processing of cotton and microply fabrics. The desizing and bioscouring treatments were performed using 5 g/L of xylanase at 70 °C, pH 9.5, for 90 min. This resulted in weight loss for 0.91% in microply and 0.83% in cotton with overall whiteness index of 11.81% for cotton and 52.15% for micropoly. The processed fabric has increased tensile strength (1.1–1.2%) and tearness value (1.6–2.4%) as compared to control.

Garg et al. (2013) demonstrated the application of alkalo-thermostable xylanase from *Bacillus pumilus* ASH in bioscouring of jute fabric. The oven-dried jute fabric when incubated with a small dose of 5 IU/g xylanase at 55 °C for 2 h resulted in an increase in 4.3% whiteness and 10.7% brightness of fabric. Further, it also helped in decreasing in yellowness of fabric by 5.57%. Similarly, xylanase from *Bacillus pumilus* was studied for enzymatic desizing of cotton and micropoly fabrics (Battan et al. 2012). The enzymatic desizing with enzyme load of 5 IU/g at pH 7.0, temperature 60 °C for 90 min resulted in improved whiteness of 0.9% with respect to the chemical process. The addition of surfactant such as EDTA improved the desizing and bioscouring efficiency (Losonczi et al. 2005; Battan et al. 2012; Garg et al. 2013).

The synergistic action of xylanase and pectinase enzyme was used for scouring of cotton fabrics. The bioscouring was performed with 5.0 IU xylanase and 4.0 IU pectinase from *Bacillus pumilus* strain AJK (MTCC 10414) along with surfactants such as 1.0 mM EDTA and 1% Tween-80 at high pH 8.5 for 1 h at 50 °C. They observed improvement in whiteness, brightness, and reduction in yellowness by 1.2%, 3.2%, and 4.2% respectively that is better in comparison to chemical-based alkaline scouring method (Singh et al. 2018). El et al. (2018) reported improvement in desizing, bioscouring and bio-finishing efficiency using xylanase obtained from *T. longibrachiatum* KT693225 without any requirement of additives.

Xylanase employed in chemical and pharmaceutical industries

The non-digestible sugar molecules together form oligomers known as xylooligosaccharides, which are made up of xylose monomers (Vazquez et al. 2000). XOS has various applications in biotechnology, pharmaceutical, food and feed industries (Chang et al. 2017). XOS plays a vital role as prebiotic as it is not hydrolyzed or absorbed in the gastrointestinal tract. Thus, XOS selectively stimulates the growth of important gastrointestinal microorganisms regulating the human digestive health (Roberfroid 1997; Collins and Gibson 1999; Vazquez et al. 2000). The potential of XOS as an efficient feed alternative is established by the fact that it help in cholesterol reduction, inhibit starch retro-gradation, improve the bioavailability of calcium thus improving the nutritional and sensory properties of food (Voragen 1998; Motta et al. 2013). XOS has shown the application in pharmaceutical sectors due to its immunomodulatory (Chen et al. 2012), anti-cancerous (Gupta et al. 2018), anti-microbial, antioxidant (Kallel et al. 2015b), anti-allergy, anti-inflammatory (Aachary and Prapulla 2011),

and anti-hyperlipidemic activity (Li et al. 2010). XOS have also shown phyto-pharmaceutical and feed applications such as growth regulatory activity in aquaculture and poultry. These properties may be due to the presence of uronic substituents in acidic oligosaccharides.

The process of XOS synthesis involves the physical (autohydrolysis), chemical (hydronium ions generated by water autoionization and in situ organic acids) or enzymatic hydrolysis (xylanase or β-xylosidase) of hemicellulose-rich agricultural wastes (Aachary and Prapulla 2011). Several reports suggested that XOS can be enzymatically produced from different agro-residues such as hardwoods (Huang et al. 2016), straws (Gullón et al. 2008; Kallel et al. 2015a; Moniz et al. 2016) corn cobs (Chapla et al. 2013; Gowdhaman and Ponnusami 2015), bran (Otieno and Ahring 2012), sugarcane bagasse (Jayapal et al. 2013) and bamboo (Xiao et al. 2013) using microbial xylanases.

Alkaline xylanase from *Bacillus mojavensis* A21 utilized corncob xylan for the release of xylotriose and xylobiose (Haddar et al. 2012). *Bacillus aerophilus* KGJ2 xylanase showed efficiency toward XOS synthesis, e.g., xylobiose, xylotriose, and xylose after hydrolysis of xylan (Gowdhaman et al. 2014). A cellulase free xylanase (EX624) from *Streptomyces* sp. CS624 produced xylose, xylobiose and xylotriose with commercial beech wood xylan and wheat bran (Mander et al. 2014). Using deoiled *Jatropha curcas* seed cake as substrate, *Sporotrichum thermophile* xylanase was produced which showed the efficiency to produce XOS by the hydrolysis of oat spelt xylan, with 73% xylotetraose, 15.4% xylotriose and 10% xylobiose (Sadaf and Khare 2014). Xylanase obtained from the mixed microbial culture of *Cellulomonas uda* NCIM 2523 and *Acetobacter xylinum* NCIM 2526 using *Prosopis juliflora* showed the potential to produce XOS with probiotic activity from beech wood xylan (Anthony et al. 2016). A xylanase gene PbXyn10A isolated from *Paenibacillus barengoltaii* cloned in *E. coli* showed 75% XOS yield from xylan extracted from raw corncobs (Liu et al. 2018). The hydrolysis of xylan using xylanase from *Pichia stipitis* produced 2% XOS consisting of xylotetraose 14%, xylotriose 49% and xylobiose 29% (Ding et al. 2018). Bhardwaj et al. (2019) demonstrated the partially purified xylanase obtained from *Aspergillus oryzae* LC1 resulted in the generation of xylobiose, xylotriose, and xylotetraose.

Xylanase employed in biorefinery

Efficient conversion of lignocellulosic biomass (LCB) into fuel-grade ethanol has become a world priority for producing environmentally friendly renewable energy at a reasonable price for the transportation sector. The process of bioconversion of lignocellulosic biomass requires hydrolysis of the pretreated biomass for the conversion of complex carbohydrate polymer of LCB to the simple monomers which will be further converted to ethanol by fermentation. The xylanolytic enzyme in combination with cellulolytic enzyme plays an important role in the hydrolysis process.

Several reports suggest that xylanase obtained from several microorganisms plays an important role in saccharification of LCB for lignocellulosic-based biorefinery (Hu et al. 2011; Choudhary et al. 2014; Ramanjaneyulu et al. 2017; Basit et al. 2018). Hydrolysis and fermentation are important steps in biomass to bioethanol generation. Initially, several groups demonstrated separate hydrolysis of biomass followed by fermentation (SHF). SHF is a time-consuming process and thus increases the overall cost of the process. Later on, different integrated process (combined hydrolysis and fermentation) have been developed such as simultaneous saccharification and co-fermentation (SSCF), simultaneous saccharification and fermentation (SSF), and consolidated bioprocessing (CB) (Malhotra and Chapadgaonkar 2018). These strategies resulted in an enhancement in reaction rates and ethanol yields (Eklund and Zacchi 1995; Sun and Cheng 2002). Bibra et al. (2018) showed thermostable xylanase production using *Geobacillus* sp. DUSELR13, which is applied further for ethanol generation from LCB. SSF *Geobacillus* sp. DUSELR13 and *Geobacillus thermoglucosidasius* are co-cultured for SSF of prairie cord grass (PCG), and corn stover (CS). The SSF resulted in 3.53 and 3.72 g/L ethanol from PCG and CS, respectively.

Hu et al. (2011) suggested that xylanase causes fiber swelling improving porosity that helps in improving the accessibility of cellulose. To ferment both cellulose-derived hexoses (C6) and xylan-derived pentoses (C5), simultaneous saccharification and co-fermentation (SSCF) was introduced which causes ethanol production using single microorganisms co-cultured with cellulase and xylanase producing strain. Yasuda et al. (2014) demonstrated bioethanol generation by SSCF of anhydrous ammonia-pretreated *Pennisetum purpureum* Schumach (Napier grass) using *Escherichia coli* KO11 and *Saccharomyces cerevisiae* cellulase, and xylanase. SSCF for 96 h was reported to have a maximum 74% ethanol yield as compared to theoretical yield calculated based on glucan and xylan yield of 397 mg/g and 214 mg/g, respectively.

Bondesson and Galbe (2016) designed experimental setup of SSCF for ethanol production from steam-pretreated, acetic acid-impregnated wheat straw using a pentose fermenting *S. cerevisiae* KE6-12b strain. The highest 37.5 g/L ethanol concentration with 0.32 g/g ethanol yield was obtained based on the glucose–xylose available in the pretreated wheat straw. Shariq and Sohail (2018) demonstrated that yeast strain *Candida tropicalis*

MK-160 can help in xylanase and endoglucanase production as well as ethanol production. Therefore, it can be potentially used for SSCF involves single microorganism.

The consolidated processing or simultaneous delignification, saccharification, and fermentation involve the cultivation of ligno-hemicellulolytic enzyme-producing strains along with ethanol-producing strain in a reactor. It may be monoculture or co-culture of different microorganisms. It will help to decrease the overall process cost required for bioreactor and operation of different enzyme production and ethanol generation (Chadha et al. 1995).

To design a monoculture-based consolidated processing, different engineered microorganisms are used having ligno-hemicellulolytic enzymes having the capability along with ethanol generating potential. Shen et al. (2012) engineered a thermostable self-splicing bacterial intein-modified xylanase for consolidated lignocellulosic biomass processing. Sun et al. (2012) demonstrated expression of recombinant *Saccharomyces cerevisiae* strain having an engineered minihemicellulosome. It has the capability of converting xylan directly to ethanol. Horisawa et al. (2019) suggested direct ethanol production from lignocellulosic materials by consolidated bioprocessing using the mixed culture of wood rot fungi, i.e., *Schizophyllum commune, Bjerkandera adusta*, and *Fomitopsis palustris*.
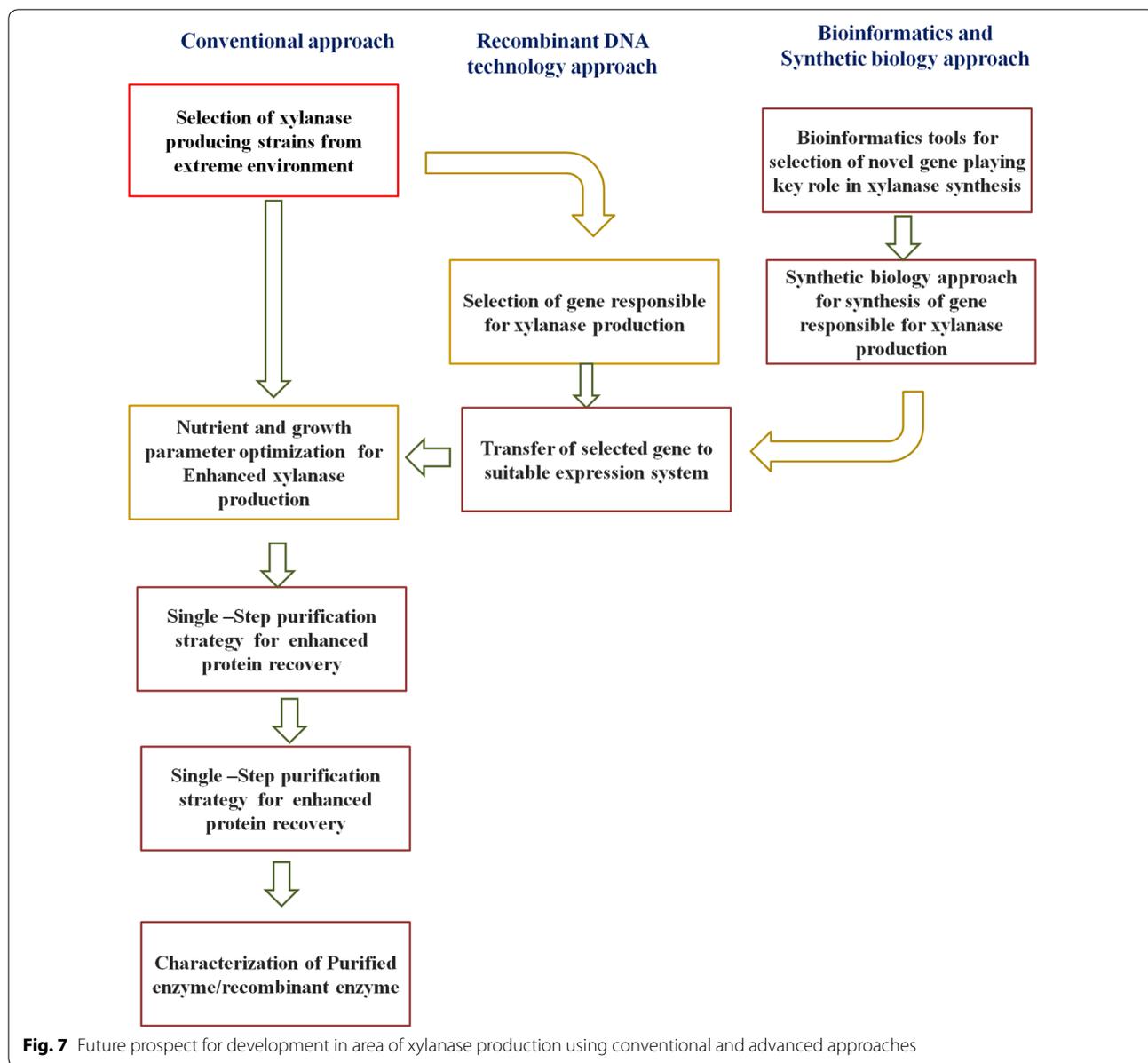
Fig. 7 Future prospect for development in area of xylanase production using conventional and advanced approaches

Commercial xylanase enzyme and their application

The xylanase enzyme is commercially employed in several industries such as pulp bleaching, food, feed, and brewing. The major application of the xylanase is in pulp bleaching and is produced by different companies around the world with various trade name such as Bleachzyme (Biocon, India), Cartzyme (Sandoz, US), Cartzyme MP (Clarient, UK), Ecozyme (Thomas Swan, UK), Irgazyme 10 A & Irgazyme 40–4× (Genercor, Finland), Ecopulp (Alko Rajamaki, Finland), VAI Xylanase (Voest Alpine, Austria), and Rholase 7118 (Rohm, Germany). Xylanase is commercially produced by several international industries for its application in food and feed industries. Sankyo from Japan, Ciba Giegy from Switzerland produces xylanase as trade name Sanzyme, and Albazyme-10A, respectively, and has been used commercially in food and baking industries. A Danish firm Novo Nordisk, produces three commercial xylanases namely Pulpzyme (HA, HB, HC), Biofeed (Beta, plus) and Ceremix and used in pulp bleaching, feed and brewing industries, respectively (Walia et al. 2017). An American firm Alltech, Inc., commercially produces xylanase with trade name Allzym PT and Fibrozyme and has been used for the upgrading the animal feed. A Japanese firm named Amano Pharmaceutical Co, Ltd. produced xylanase enzyme named Amano 90 and it has been used in food, feed and pharmaceutical industries. Most of the commercial xylanases are produced by fungal source due to its high production potential.

Challenges and future trends in commercial production, purification and application of xylanase

The search of super xylanase is still on, therefore, searching for new microbial source with the ability to produce highly active and stable xylanase is going on around the world. The strains isolated from different extreme habitat can be of potential applications as these strains already possesses the ability to withstand different stress such as high temperature and pH variations. The selection of such thermal and pH tolerant strains and subjecting them to different optimization strategies for enhanced xylanase production can be one alternative. The advancement in biotechnological tools and techniques (Recombinant DNA technology or genetic engineering) provides an opportunity to select the gene responsible for xylanase production that can be isolated and efficiently transferred to the expression system. These expression systems can be regulated for enhanced production of xylanase with desired property for specific industrial applications. The availability of a high amount of genomics, proteomics and metabolomics data can be used via different bioinformatics tools to develop different approaches for enhanced xylanase production. The combination of new technology such as synthetic biology (DNA oligo-synthesis) and conventional recombinant DNA technology can be used for attaining the objective of high xylanase production with desired industrial properties (Fig. 7). However, limitations associated with mimicking the natural system into synthetic system need to be taken care before full-scale applications. Also, the ethical, socio-economic and health concerns need to taken care before commercial exploitation of the developed strategies.

Conclusion

The enzymatic breakdown of the xylan into its constituent component requires the synergistic action of xylanases and other debranching enzymes. The key enzyme used for xylan hydrolysis is *endo*-1,4-β-xylanase that cleaves β-1,4-glycoside linkage of xylan. The xylanase can be grouped under different GH families with major xylanases from GH10 or GH11 families, followed by GH5, GH7, GH8 and GH43. The xylanase enzyme acts as a "Green" alternative to already existing industrial processes for processing of xylan to different industrially important product such as paper, textile, food, feed, pharmaceuticals, and biofuels. The application of xylanases in the production of the above-mentioned products can regulate the overall economics of the process. Therefore, already existing method can be further improved or new strategies may be developed for enhanced and cost-efficient production of xylanase with desired characteristics. Further, it is often observed that native enzyme cannot meet the industrial process requirement; thus, the combination of already existing technology with new technology such as synthetic biology (DNA oligo-synthesis), rational engineering and directed evolution can be used for attaining the objective of high xylanase production with desired industrial properties.

Abbreviations
LCB: lignocellulosic biomass; EC: enzyme commission; HMWLI: high-molecular weight with low isoelectric point; LMWHI: low-molecular weight with high isoelectric point; CAZy: carbohydrate-active enzyme database; NCBI: National Centre for Biotechnological Information; GH: glycoside hydrolase; XOS: xylo-oligosaccharides; X2: xylobiose; X3: xylotriose; X4: xylotetraose; CNB: catalystic nucleophile/base; CPD: catalystic proton donor; Asp: aspartic acid; Glu: glutamic acid; SmF: submerged fermentation; SSF: solid-state fermentation; CMC: carboxymethyl cellulose; OFAT: one factor at a time; CCD: central composite design; ANOVA: analysis of variance; RSM: response surface methodology; GRAS: generally regarded as safe; TCA: trichloroacetic acid; SDS–PAGE: sodium dodecyl sulfate-polyacrylamide gel electrophoresis; DEAE: diethylaminoethyl; FPLC: fast protein liquid chromatography; ATPS: aqueous two-phase system; PEG: polyethylene glycol; DDGS: distillers dried grains with soluble; SSCF: simultaneous saccharification and co-fermentation; SSF: simultaneous saccharification and fermentation; CB: consolidated bioprocessing; PCG: prairie cord grass; CS: corn stover.

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Availability of data and materials
Not applicable.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

References
Aachary AA, Prapulla SG (2011) Xylooligosaccharides (XOS) as an emerging prebiotic: microbial synthesis, utilization, structural characterization, bioactive properties, and applications. Compr Rev Food Sci Food Saf 10:2–16

Abdel-Aziz MS, Talkhan FN, Fadel M et al (2011) Improvement of xylanase production from *Streptomyces pseudogriseolus* via UV mutagenesis. Aust J Basic Appl Sci 5:1045–1050

Abou Hachem M, Nordberg Karlsson E, Bartonek-Roxâ E et al (2000) Carbohydrate-binding modules from a thermostable *Rhodothermus marinus* xylanase: cloning, expression and binding studies. Biochem J 345(Pt 1):53–60

Adesina FC, Onilude AA, Oluboyede OA et al (2017) Production and application of xylanase by *Fusarium* spp. using wood shavings as substrate. EC Microbiol 6:4–13

Adhyaru DN, Bhatt NS, Modi HA, Divecha J (2017) Cellulase-free-thermo-alkali-solvent-stable xylanase from *Bacillus altitudinis* DHN8: over-production through statistical approach, purification and bio-deinking/bio-bleaching potential. Biocatal Agric Biotechnol 12:220–227

Adigüzel AO, Tunçer M (2016) Production, characterization and application of a xylanase from *Streptomyces* sp. AOA40 in fruit juice and bakery industries. Food Biotechnol 30:189–218

Adigüzel AO, Tunçer M (2017) Production and characterization of partially purified thermostable endoxylanase and endoglucanase from novel *Actinomadura geliboluensis* and the biotechnological applications in the saccharification of lignocellulosic biomass. BioResources 12:2528–2547. https://doi.org/10.15376/biores.12.2.2528-2547

Adiguzel G, Faiz O, Sisecioglu M et al (2019) A novel endo-β-1,4-xylanase from *Pediococcus acidilactici* GC25; purification, characterization and application in clarification of fruit juices. Int J Biol Macromol 129:571–578

Ahmad M, Hirz M, Pichler H, Schwab H (2014) Protein expression in *Pichia pastoris*: recent achievements and perspectives for heterologous protein production. Appl Microbiol Biotechnol 98:5301–5317. https://doi.org/10.1007/s00253-014-5732-5

Ahmed S, Jabeen A, Jamil A (2007) Xylanase from *Trichoderma harzianum*: enzyme characterization and gene isolation. J Chem Soc Pak 29:176–182

Ahmed S, Riaz S, Jamil A (2009) Molecular cloning of fungal xylanases: an overview. Appl Microbiol Biotechnol 84:19–35

Akila G, Chandra TS (2003) A novel cold-tolerant *Clostridium* strain PXYL1 isolated from a psychrophilic cattle manure digester that secretes thermolabile xylanase and cellulase. FEMS Microbiol Lett 219:63–67

Al Balaa B, Wouters J, Dogne S et al (2006) Identification, cloning, and expression of the *Scytalidium acidophilum* XYL1 gene encoding for an acidophilic xylanase. Biosci Biotechnol Biochem 70:269–272. https://doi.org/10.1271/bbb.70.269

Alam M, Gomes I, Mohiuddin G, Hoq MM (1994) Production and characterization of thermostable xylanases by *Thermomyces lanuginosus* and *Thermoascus aurantiacus* grown on lignocelluloses. Enzyme Microb Technol 16:298–302. https://doi.org/10.1016/0141-0229(94)90170-8

Amore A, Parameswaran B, Kumar R et al (2015) Application of a new xylanase activity from *Bacillus amyloliquefaciens* XR44A in brewer's spent grain saccharification. J Chem Technol Biotechnol 90:573–581. https://doi.org/10.1002/jctb.4589

Andrews SR, Taylor EJ, Pell G et al (2004) The use of forced protein evolution to investigate and improve stability of family 10 xylanases the production of Ca^{2+}-independent stable xylanases. J Biol Chem 279:54369–54379

Ang SK, Ariyani LP et al (2013) Production of cellulases and xylanase by *Aspergillus fumigatus* SK1 using untreated oil palm trunk through solid state fermentation. Process Biochem 48:1293–1302. https://doi.org/10.1016/j.procbio.2013.06.019

Anthony P, Harish BS, Jampala P et al (2016) Statistical optimization, purification and applications of xylanase produced from mixed bacteria in a solid liquid fermentation using Prosopis juliflora. Biocatal Agric Biotechnol 8:213–220

Azeri C, Tamer UA, Oskay M (2010) Thermoactive cellulase-free xylanase production from alkaliphilic *Bacillus* strains using various agro-residues and their potential in biobleaching of kraft pulp. Afr J Biotechnol 9:63

Azin M, Moravej R, Zareh D (2007) Production of xylanase by *Trichoderma longibrachiatum* on a mixture of wheat bran and wheat straw: optimization of culture condition by Taguchi method. Enzyme Microb Technol 40:801–805. https://doi.org/10.1016/j.enzmictec.2006.06.013

Bae H-J, Kim HJ, Kim YS (2008) Production of a recombinant xylanase in plants and its potential for pulp biobleaching applications. Bioresour Technol 99:3513–3519

Bajaj BK, Abbass M (2011) Studies on an alkali-thermostable xylanase from *Aspergillus fumigatus* MA28. 3 Biotech 1:161–171. https://doi.org/10.1007/s13205-011-0020-x

Bajaj BK, Manhas K (2012) Production and characterization of xylanase from *Bacillus licheniformis* P11(C) with potential for fruit juice and bakery industry. Biocatal Agric Biotechnol 1:330–337. https://doi.org/10.1016/j.bcab.2012.07.003

Bajpai P (2012) Biobleaching. In Bajpai P (eds) Biotechnology for pulp and paper processing. Springer, Berlin, pp 93–138

Bajpai P (2014) Sources, production, and classification of xylanases. Xylanolytic Enzyme Academic Press (Imprint Elsevier), Tokyo, pp 43–52

Bandikari R, Poondla V, Obulam VSR (2014) Enhanced production of xylanase by solid state fermentation using *Trichoderma koeningi* isolate: effect of pretreated agro-residues. 3 Biotech 4:655–664. https://doi.org/10.1007/s13205-014-0239-4

Banka AL, Guralp SA, Gulari E (2014) Secretory expression and characterization of two hemicellulases, xylanase, and β-xylosidase, isolated from *Bacillus subtilis* M015. Appl Biochem Biotechnol 174:2702–2710

Barman S, Sit N, Badwaik LS, Deka SC (2015) Pectinase production by *Aspergillus niger* using banana (*Musa balbisiana*) peel as substrate and its effect on clarification of banana juice. J Food Sci Technol 52:3579–3589. https://doi.org/10.1007/s13197-014-1413-8

Basit A, Liu J, Miao T et al (2018) Characterization of two endo-β-1,4-xylanases from *Myceliophthora thermophila* and their saccharification efficiencies, synergistic with commercial cellulase. Front Microbiol 9:1–11. https://doi.org/10.3389/fmicb.2018.00233

Battan B, Dhiman SS, Ahlawat S et al (2012) Application of thermostable xylanase of *Bacillus pumilus* in textile processing. Indian J Microbiol 52:222–229

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

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Behera SS, Ray RC (2016) Solid state fermentation for production of microbial cellulases: recent advances and improvement strategies. Int J Biol Macromol 86:656–669

Behnam S, Karimi K, Khanahmadi M, Salimian Z (2016) Optimization of xylanase production by *Mucor indicus*, *Mucor hiemalis*, and *Rhizopus oryzae* through solid state fermentation. Biol J Microorg 4:1–11

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

Béra-Maillet C, Devillard E, Cezette M et al (2005) Xylanases and carboxymethylcellulases of the rumen protozoa *Polyplastron multivesiculatum*, *Eudiplodinium maggii* and *Entodinium* sp. FEMS Microbiol Lett 244:149–156. https://doi.org/10.1016/j.femsle.2005.01.035

Berka RM, Grigoriev IV, Otillar R et al (2011) Comparative genomic analysis of the thermophilic biomass-degrading fungi *Myceliophthora thermophila* and *Thielavia terrestris*. Nat Biotechnol 29:922

Betini JHA, Michelin M, Peixoto-Nogueira SC et al (2009) Xylanases from *Aspergillus niger*, *Aspergillus niveus*, and *Aspergillus ochraceus* produced under solid-state fermentation and their application in cellulose pulp bleaching. Bioprocess Biosyst Eng 32:819–824. https://doi.org/10.1007/s00449-009-0308-y

Bhardwaj N, Chanda K, Kumar B et al (2017) Statistical optimization of nutritional and physical parameters for xylanase production from newly isolated *Aspergillus oryzae* LC1 and its application in the hydrolysis of lignocellulosic agro-residues. BioResources 12:8519–8538

Bhardwaj N, Verma VK, Chaturvedi V, Verma P (2018) GH10 XynF1 and Xyn11A: the predominant xylanase identified in the profiling of extracellular proteome of *Aspergillus oryzae* LC1. Ann Microbiol 68:731–742

Bhardwaj N, Kumar B, Agarwal K et al (2019) Purification and characterization of a thermo-acid/alkali stable xylanases from *Aspergillus oryzae* LC1 and its application in xylo-oligosaccharides production from lignocellulosic agricultural wastes. Int J Biol Macromol 122:1191–1202. https://doi.org/10.1016/j.ijbiomac.2018.09.070

Bibi Z, Ansari A, Zohra RR et al (2014) Production of xylan degrading endo-1, 4-β-xylanase from thermophilic *Geobacillus stearothermophilus* KIBGE-IB29. J Radiat Res Appl Sci 7:478–485. https://doi.org/10.1016/j.jrras.2014.08.001

Bibra M, Kunreddy V, Sani R (2018) Thermostable xylanase production by *Geobacillus* sp. strain DUSELR13, and its application in ethanol production with lignocellulosic biomass. Microorganisms 6:93. https://doi.org/10.3390/microorganisms6030093

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

Biosciences G (2019) Ammonium sulfate protein precipitation-the key to salting-out. https://info.gbiosciences.com/blog/ammonium-sulfat

Bondesson PM, Galbe M (2016) Process design of SSCF for ethanol production from steam-pretreated, acetic-acid—impregnated wheat straw. Biotechnol Biofuels 222:1–12. https://doi.org/10.1186/s13068-016-0635-6

Boonchuay P, Takenaka S, Kuntiya A et al (2016) Journal of molecular catalysis b: enzymatic purification, characterization, and molecular cloning of the xylanase from *Streptomyces thermovulgaris* TISTR1948 and its application to xylooligosaccharide production. J Mol Catal B Enzyme 129:61–68. https://doi.org/10.1016/j.molcatb.2016.03.014

Brennan Y, Callen WN, Christoffersen L et al (2004) Unusual microbial xylanases from insect guts. Appl Environ Microbiol 70:3609–3617

Bron S, Bolhuis A, Tjalsma H et al (1998) Protein secretion and possible roles for multiple signal peptidases for precursor processing in bacilli. J Biotechnol 64:3–13

Burlacu A, Israel-Roming F, Cornea CP et al (2017) Fungal strains improvement for xylanase over production through physical and chemical mutagenesis. AgroLife Sci J 6:40–47

Busk PK, Lange L (2013) Cellulolytic potential of thermophilic species from four fungal orders. AMB Express 3:47

Butt MS, Tahir-Nadeem M, Ahmad Z, Sultan MT (2008) Xylanases and their applications in baking industry. Food Technol Biotechnol 46:22–31

Camacho NA, Aguilar OG (2003) Production, purification, and characterization of a low-molecular-mass xylanase from *Aspergillus* sp. and Its application in baking. Appl Biochem Biotechnol Part A Enzyme Eng Biotechnol 104:159–171. https://doi.org/10.1385/ABAB:104:3:159

Camas M, Sazak A, Spröer C et al (2013) *Nonomuraea jabiensis* sp. nov., isolated from arid soil. Int J Syst Evol Microbiol 63:212–218. https://doi.org/10.1099/ijs.0.039362-0

Carvalho EA, dos Santos Góes LM, Uetanabaro APT et al (2017) Thermoresistant xylanases from *Trichoderma stromaticum*: application in bread making and manufacturing xylo-oligosaccharides. Food Chem 221:1499–1506. https://doi.org/10.1016/j.foodchem.2016.10.144

Chadha BS, Kanwar SS, Garcha HS (1995) Simultaneous saccharification and fermentation of rice straw into ethanol. Acta Microbiol Immunol Hung 42:71–75

Chadha BS, Kaur B, Basotra N et al (2019) Thermostable xylanases from thermophilic fungi and bacteria: current perspective. Bioresour Technol 277:195–203

Chakdar H, Kumar M, Pandiyan K et al (2016) Bacterial xylanases: biology to biotechnology. 3 Biotech 6:1–15. https://doi.org/10.1007/s13205-016-0457-z

Chandra R, Singh R (2012) Decolourisation and detoxification of rayon grade pulp paper mill effluent by mixed bacterial culture isolated from pulp paper mill effluent polluted site. Biochem Eng J 61:49–58

Chang S, Chu J, Guo Y et al (2017) An efficient production of high-pure xylo-oligosaccharides from corncob with affinity adsorption-enzymatic reaction integrated approach. Bioresour Technol 241:1043–1049

Chanwicha N, Katekaew S, Aimi T, Boonlue S (2015) Purification and characterization of alkaline xylanase from *Thermoascus aurantiacus* var. levisporus KKU-PN-I2-1 cultivated by solid-state fermentation. Mycoscience 56:309–318. https://doi.org/10.1016/j.myc.2014.09.003

Chapla D, Dholakiya S, Madamwar D, Shah A (2013) Characterization of purified fungal endoxylanase and its application for production of value added food ingredient from agroresidues. Food Bioprod Process 91:682–692

Chávez R, Bull P, Eyzaguirre J (2006) The xylanolytic enzyme system from the genus Penicillium. J Biotechnol 123:413–433

Chen HH, Chen YK, Chang HC, Lin SY (2012) Immunomodulatory effects of xylooligosaccharides. Food Sci Technol Res 18:195–199

Chen C-C, Ko T-P, Huang J-W, Guo R-T (2015) Heat- and alkaline-stable xylanases: application, protein structure and engineering. ChemBioEng Rev 2:95–106. https://doi.org/10.1002/cben.201400035

Chen Z, Zaky AA, Liu Y et al (2019) Purification and characterization of a new xylanase with excellent stability from *Aspergillus flavus* and its application in hydrolyzing pretreated corncobs. Protein Expr Purif 154:91–97. https://doi.org/10.1016/j.pep.2018.10.006

Choudhary J, Saritha M, Nain L, Arora A (2014) Enhanced saccharification of steam-pretreated rice straw by commercial cellulases supplemented with xylanase. J Bioprocess Biotechnol 4:1

Collins MD, Gibson GR (1999) Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. Am J Clin Nutr 69:1052s–1057s

Collins T, Meuwis M-A, Stals I et al (2002) A novel family 8 xylanase, functional and physicochemical characterization. J Biol Chem 277:35133–35139

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

Coman G, Georgescu L, Bahrim G (2013) *Streptomyces* p12-137 endoxylanases characteristics evaluation in order to obtain xylo-oligosaccharides. Rom Biotechnol Lett 18:8086–8096

Courtin CM, Delcour JA (2002) Arabinoxylans and endoxylanases in wheat flour bread-making. J Cereal Sci 35:225–243. https://doi.org/10.1006/jcrs.2001.0433

Csiszár E, Urbánszki K, Szakács G (2001) Biotreatment of desized cotton fabric by commercial cellulase and xylanase enzymes. J Mol Catal B Enzyme 11:1065–1072. https://doi.org/10.1016/S1381-1177(00)00149-1

Cunha L, Martarello R, de Souza PM et al (2018) Optimization of Xylanase Production from *Aspergillus foetidus* in Soybean Residue. Enzyme Res 2018:1–7. https://doi.org/10.1155/2018/6597017

da Costa AC, Cavalheiro GF, de Queiroz Vieira ER et al (2019) Catalytic properties of xylanases produced by *Trichoderma piluliferum* and *Trichoderma viride* and their application as additives in bovine feeding. Biocatal Agric Biotechnol 19:101161. https://doi.org/10.1016/j.bcab.2019.101161

Da Gama Ferreira R, Azzoni AR, Freitas S (2018) Techno-economic analysis of the industrial production of a low-cost enzyme using *E. coli*: the case of recombinant β-glucosidase. Biotechnol Biofuels 11(81):1–13

Bhardwaj *et al. Bioresour. Bioprocess.* (2019) 6:40

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da Silva R, Yim DK, Park YK (1994) Application of thermostable xylanases from *Humicola* sp. for pulp improvement. J Ferment Bioeng 77:109–111. https://doi.org/10.1016/0922-338X(94)90221-6

da Silva LA, Terrasan CRF, Carmona EC (2015) Purification and characterization of xylanases from *Trichoderma inhamatum*. Electron J Biotechnol 18:307–313. https://doi.org/10.1016/j.ejbt.2015.06.001

Danalache F, Mata P, Alves VD, Moldão-martins M (2018) Enzyme-assisted extraction of fruit juices. Elsevier Inc., New York

Davies GJ, Wilson KS, Henrissat B (1997) Nomenclature for sugar-binding subsites in glycosyl hydrolases. Biochem J 321:557

de Amo GS, Bezerra-Bussoli C, da Silva RR et al (2019) Heterologous expression, purification and biochemical characterization of a new xylanase from *Myceliophthora heterothallica* F.2.1.4. Int J Biol Macromol 131:798–805. https://doi.org/10.1016/j.ijbiomac.2019.03.108

De Faria FP, Te'o VSJ, Bergquist PL et al (2002) Expression and processing of a major xylanase (XYN2) from the thermophilic fungus *Humicola grisea* var. thermoidea in Trichoderma reesei. Lett Appl Microbiol 34:119–123

de Guimaraes NCA, Sorgatto M, de Peixoto-Nogueira SC et al (2013) Bioprocess and biotechnology: effect of xylanase from *Aspergillus niger* and *Aspergillus flavus* on pulp biobleaching and enzyme production using agroindustrial residues as substract. Springerplus 2:1–7. https://doi.org/10.1186/2193-1801-2-380

De Queiroz Brito Cunha CC, Gama AR, Cintra LC et al (2018) Improvement of bread making quality by supplementation with a recombinant xylanase produced by *Pichia pastoris*. PLoS ONE 13:1–14. https://doi.org/10.1371/journal.pone.0192996

de Queiroz-Fernandes GM, Martins BL, Rustiguel CB (2017) Reuse of wastewater from pulp industry for the optimization of fungal xylanase production. Acta Sci Biol Sci 39:21–26. https://doi.org/10.4025/actascibiolsci.v39i1.34459

de Sousa Gomes K, Maitan-Alfenas GP, de Andrade LGA et al (2017) Purification and characterization of xylanases from the fungus *Chrysoporthe cubensis* for production of xylooligosaccharides and fermentable sugars. Appl Biochem Biotechnol 182:818–830. https://doi.org/10.1007/s12010-016-2364-5

Devillard E, Newbold CJ, Scott KP et al (1999) A xylanase produced by the rumen anaerobic protozoan *Polyplastron multivesiculatum* shows close sequence similarity to family 11 xylanases from Gram-positive bacteria. FEMS Microbiol Lett 181:145–152. https://doi.org/10.1016/S0378-1097(99)00525-X

Dhiman SS, Sharma J, Battan B (2008a) Industrial applications and future prospects of microbial xylanases: a Review. BioResources 3(4):1377–1402

Dhiman SS, Sharma J, Battan B (2008b) Pretreatment processing of fabrics by alkalothermophilic xylanase from *Bacillus stearothermophilus* SDX. Enzyme Microb Technol 43:262–269. https://doi.org/10.1016/j.enzmictec.2008.03.016

Dhiman SS, Garg G, Sharma J et al (2014) Reduction in acute ecotoxicity of paper mill effluent by sequential application of xylanase and laccase. PLoS ONE 9:e102581

Dhulappa A, Lingappa K (2013) Xylanase-screening and biosynthesis from *Aspergillus tamarii*. Int J Curr Microbiol Appl Sci 2:79–83

Ding C, Li M, Hu Y (2018) High-activity production of xylanase by *Pichia stipitis*: purification, characterization, kinetic evaluation and xylooligosaccharides production. Int J Biol Macromol 117:72–77

Dodd D, Cann IKO (2009) Enzymatic deconstruction of xylan for biofuel production. Gcb Bioenergy 1:2–17

Dornez E, Verjans P, Arnaut F et al (2011) Use of psychrophilic xylanases provides insight into the xylanase functionality in bread making. J Agric Food Chem 59:9553–9562

Driss D, Bhiri F, Elleuch L et al (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

Driss D, Bhiri F, Siela M et al (2013) Improvement of breadmaking quality by xylanase GH11 from *Penicillium occitanis* Pol6. J Texture Stud 44:75–84. https://doi.org/10.1111/j.1745-4603.2012.00367.x

Du Y, Shi P, Huang H et al (2013) Characterization of three novel thermophilic xylanases from *Humicola insolens* Y1 with application potentials in the brewing industry. Bioresour Technol 130:161–167. https://doi.org/10.1016/j.biortech.2012.12.067

Eklund R, Zacchi G (1995) Simultaneous saccharification and fermentation of steam-pretreated willow. Enzyme Microb Technol 17:255–259

El AAAA, Saleh SA, Eid EB et al (2018) Thermodynamics characterization and potential textile applications of *Trichoderma longibrachiatum* KT693225 xylanase. Biocatal Agric Biotechnol 1:129–137. https://doi.org/10.1016/j.ultramic.2015.06.008

El-Naghy MA, El-Ktatny MS, Attia AA (1991) Factors affecting activity of CMC-ase and xylanase produced by *Sporotrichum thermophile* and fractionation of the enzymes. Zentralbl Mikrobiol 146:379–385. https://doi.org/10.1016/S0232-4393(11)80172-1

Ezeilo UR, Lee CT, Huyop F et al (2019) Raw oil palm frond leaves as cost-effective substrate for cellulase and xylanase productions by *Trichoderma asperellum* UC1 under solid-state fermentation. J Environ Manage 243:206–217. https://doi.org/10.1016/j.jenvman.2019.04.113

Facchini FDA, Vici AC, Reis VRA et al (2011) Production of fibrolytic enzymes by *Aspergillus japonicus* C03 using agro-industrial residues with potential application as additives in animal feed. Bioprocess Biosyst Eng 34:347–355

Fan G, Katrolia P, Jia H et al (2012) High-level expression of a xylanase gene from the thermophilic fungus *Paecilomyces thermophila* in *Pichia pastoris*. Biotechnol Lett 34:2043–2048

Faulds CB, Mandalari G, Lo Curto RB et al (2006) Synergy between xylanases from glycoside hydrolase family 10 and family 11 and a feruloyl esterase in the release of phenolic acids from cereal arabinoxylan. Appl Microbiol Biotechnol 71:622–629

Fernandes AC, Fontes CMGA, Gilbert HJ et al (2015) Homologous xylanases from *Clostridium thermocellum*: evidence for bi-functional activity, synergism between xylanase catalytic modules and the presence of xylan-binding domains in enzyme complexes. Biochem J 342:105–110. https://doi.org/10.1042/bj3420105

Fleissner A, Dersch P (2010) Expression and export: recombinant protein production systems for *Aspergillus*. Appl Microbiol Biotechnol 87:1255–1270

Gandhi JP, Rao KK, Dave PJ (1994) Characterization of extracellular thermostable xylanase from *Chaetomium globosum*. J Chem Technol Biotechnol 60:55–60. https://doi.org/10.1002/jctb.280600109

Garai D, Kumar V (2013) Aqueous two phase extraction of alkaline fungal xylanase in PEG/phosphate system: optimization by Box–Behnken design approach. Biocatal Agric Biotechnol 2:125–131

Garcia-Huante Y, Cayetano-Cruz M, Santiago-Hernández A et al (2017) The thermophilic biomass-degrading fungus *Thielavia terrestris* Co3Bag1 produces a hyperthermophilic and thermostable β-1, 4-xylanase with exo-and endo-activity. Extremophiles 21:175–186

Garg N, Kumar A, Mahatman KK (2010) Xylanase: applications and biotechnological aspects: biotechnological aspects of xylanase. LAP LAMBERT Academic Publ, Lambert

Garg G, Dhiman SS, Gautam R et al (2013) Bioscouring of jute fabric by cellulase-free alkalo-thermostable xylanase from *Bacillus pumilus* ASH. J Mol Catal B Enzym 85–86:43–48. https://doi.org/10.1016/j.molcatb.2012.08.002

Gascoigne JA, Gascoigne MM (2019) The Xylanases of *Fusarium roseum*. J Gen Microbiol 1:242–248

Gautam A, Kumar A, Bharti AK, Dutt D (2018) Rice straw fermentation by *Schizophyllum commune* ARC-11 to produce high level of xylanase for its application in pre-bleaching. J Genet Eng Biotechnol 16:693–701

Gawande PV, Kamat MY (1999) Production of *Aspergillus* xylanase by lignocellulosic waste fermentation and its application. J Appl Microbiol 87:511–519. https://doi.org/10.1046/j.1365-2672.1999.00843.x

Geetha K, Gunasekaran P (2017) Purification of endoxylanase from *Bacillus pumilus* B20 for production of prebiotic xylooligosaccharide syrup; an in vitro study. Iran J Biotechnol 15:232-240

Gerasimova J, Kuisiene N (2012) Characterization of the novel xylanase from the thermophilic *Geobacillus thermodenitrificans* JK1. Microbiology 81:418–424. https://doi.org/10.1134/s0026261712040066

Gessesse A, Mamo G (1998) Purification and characterization of an alkaline xylanase from alkaliphilic *Micrococcus* sp AR-135. J Ind Microbiol Biotechnol 20:210–214. https://doi.org/10.1038/sj.jim.2900503

Ghazi S, Sepahy AA, Azin M et al (2014) Uv mutagenesis for the overproduction of xylanase from *Bacillus mojavensis* ptcc 1723 and optimization of the production condition. Iran J Basic Med Sci 17:844–853

Ghosh A, Sutradhar S, Baishya D (2019) Delineating thermophilic xylanase from *Bacillus licheniformis* DM5 towards its potential application in

Bhardwaj *et al. Bioresour. Bioprocess.* (2019) 6:40

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xylooligosaccharides production. World J Microbiol Biotechnol 35:1–18. https://doi.org/10.1007/s11274-019-2605-1

Ghoshal G, Shivhare US, Banerjee UC (2013) Effect of xylanase on quality attributes of whole-wheat bread. J Food Qual 36:172–180. https://doi.org/10.1111/jfq.12034

Glyk A, Scheper T, Beutel S (2015) PEG-salt aqueous two-phase systems: an attractive and versatile liquid–liquid extraction technology for the downstream processing of proteins and enzymes. Appl Microbiol Biotechnol 99:6599–6616

Godlewski M, Kautto L, Nevalainen H (2009) The elusive pulse in protein production in *Trichoderma reesei*. Poster Light Life Sci Conf Melbourne, Aust November, p 2

Gomez LD, Steele-King CG, McQueen-Mason SJ (2008) Sustainable liquid bio-fuels from biomass: the writing's on the walls. New Phytol 178:473–485

Gómez-garcía R, Medina-morales MA, Farruggia B et al (2018) Production of a xylanase by *Trichoderma harzianum* (*Hypocrea lixii*) in solid-state fermentation and its recovery by an aqueous two-phase system. Can J Biotechnol 2:108–115

Goswami GK, Krishnamohan M, Nain V et al (2014) Cloning and heterologous expression of cellulose free thermostable xylanase from *Bacillus brevis*. Springerplus 3:1–6. https://doi.org/10.1186/2193-1801-3-20

Gowdhaman D, Ponnusami V (2015) Production and optimization of xylo-oligosaccharides from corncob by *Bacillus aerophilus* KGJ2 xylanase and its antioxidant potential. Int J Biol Macromol 79:595–600

Gowdhaman D, Manaswini VS, Jayanthi V et al (2014) Xylanase production from *Bacillus aerophilus* KGJ2 and its application in xylooligosaccharides preparation. Int J Biol Macromol 64:90–98

Gray KA, Zhao L, Emptage M (2006) Current opinion in chemical biology. Bioethanol Curr Opin Chem Biol 10:141–146

Guan G-Q, Zhao P-X, Zhao J et al (2016) Production and partial characterization of an alkaline xylanase from a novel fungus *Cladosporium oxysporum*. Biomed Res Int 2016:1–7. https://doi.org/10.1155/2016/4575024

Guleria S, Walia A, Chauhan A, Shirkot CK (2013) Optimization of cultural conditions for cellulase-free xylanase production by mutant strain of alkalophilic *Cellulosimicrobium* sp. CKMX1 in submerged fermentation. Appl Biol Res 15:137–144

Guleria S, Walia A, Chauhan A et al (2015) Mutagenesis of alkalophilic *Cellulosimicrobium* sp. CKMX1 for hyper-production of cellulase-free xylanase in solid state fermentation of apple pomace. Proc Natl Acad Sci India Sect B Biol Sci 85:241–252

Guleria S, Walia A, Chauhan A, Shirkot CK (2016a) Optimization of milk-clotting enzyme production by *Bacillus amyloliquefaciens* SP1 isolated from apple rhizosphere. Bioresour Bioprocess 3:30

Guleria S, Walia A, Chauhan A, Shirkot CK (2016b) Purification and characterization of detergent stable alkaline protease from *Bacillus amyloliquefaciens* SP1 isolated from apple rhizosphere. J Basic Microbiol 56:138–152

Gullón P, Moura P, Esteves MP et al (2008) Assessment on the fermentability of xylooligosaccharides from rice husks by probiotic bacteria. J Agric Food Chem 56:7482–7487

Guo B, Chen XL, Sun CY et al (2009) Gene cloning, expression and characterization of a new cold-active and salt-tolerant endo-β-1,4-xylanase from marine *Glaciecola mesophila* KMM 241. Appl Microbiol Biotechnol 84:1107–1115. https://doi.org/10.1007/s00253-009-2056-y

Guo B, Li PY, Yue YS et al (2013) Gene cloning, expression and characterization of a novel xylanase from the marine bacterium, *Glaciecola mesophila* KMM241. Mar Drugs 11:1173–1187. https://doi.org/10.3390/md11041173

Gupta S, Bhushan B, Hoondal GS (2000) Isolation, purification and characterization of xylanase from *Staphylococcus* sp. SG-13 and its application in biobleaching of kraft pulp. J Appl Microbiol 88:325–334. https://doi.org/10.1046/j.1365-2672.2000.00974.x

Gupta G, Sahai V, Gupta RK (2013) Optimization of xylanase production from *Melanocarpus albomyces* using wheat straw extract and its scale up in stirred tank bioreactor. Indian J Chem Technol 20:282–289

Gupta V, Garg S, Capalash N et al (2015) Production of thermo-alkali-stable laccase and xylanase by co-culturing of *Bacillus* sp. and *B. halodurans* for biobleaching of kraft pulp and deinking of waste paper. Bioprocess Biosyst Eng 38:947–956

Gupta PK, Agrawal P, Hedge P, Akhtar MS (2018) Xylooligosaccharides and their anticancer potential: an update. Anticancer plants: natural products and biotechnological implements. Springer, New York, pp 255–271

Haddar A, Driss D, Frikha F et al (2012) Alkaline xylanases *Bacillus mojavensis* A21: production and generation of xylooligosaccharides. Int J Biol Macromol 51:647–656

Hakulinen N, Turunen O, Jänis J et al (2003) Three-dimensional structures of thermophilic β-1,4-xylanases from *Chaetomium thermophilum* and *Nonomuraea flexuosa*: comparison of twelve xylanases in relation to their thermal stability. Eur J Biochem 270:1399–1412

Ham S-J, Kim HJ, Kim J et al (2012) Novel modular endo-*β*-1,4-xylanase with transglycosylation activity from *Cellulosimicrobium* sp. strain HY-13 that is homologous to inverting GH family 6 enzymes. Bioresour Technol 107:25–32

Han N, Miao H, Ding J et al (2017) Improving the thermostability of a fungal GH11 xylanase via site-directed mutagenesis guided by sequence and structural analysis. Biotechnol Biofuels 10:133

Haq I, Tasneem M, Raana K et al (2004) Optimization of cultural conditions for the production of xylanase by chemically mutated strain of *Aspergillus niger* GCBCX-20. Int J Agric Biol 6:1115–1118

Haq I, Hussain R, Hameed U, Javad M (2008) Selection of *Aspergillus niger* mutant using antimetabolite 2-deoxy D-glucose after N-methyl N-nito N-nitroso guanidine (MNNG) treatment. Pak J Bot 40:2613–2623

Harbak L, Thygesen HV (2002) Safety evaluation of a xylanase expressed in *Bacillus subtilis*. Food Chem Toxicol 40:1–8. https://doi.org/10.1016/S0278-6915(01)00092-8

Hartzell MM, Hsieh Y (1998) Enzymatic scouring to improve cotton fabric wettability. Text Res J 68:233–241. https://doi.org/10.1177/004051759806800401

Heinen PR, Bauermeister A, Ribeiro LF et al (2018) GH11 xylanase from *Aspergillus tamarii* Kita: purification by one-step chromatography and xylooligosaccharides hydrolysis monitored in real-time by mass spectrometry. Int J Biol Macromol 108:291–299

Heinze S, Mechelke M, Kornberger P et al (2017) Identification of endoxylanase XynE from *Clostridium thermocellum* as the first xylanase of glycoside hydrolase family GH141. Sci Rep 7:1–10. https://doi.org/10.1038/s41598-017-11598-y

Henrissat B, Coutinho PM (2001) Classification of glycoside hydrolases and glycosyltransferases from hyperthermophiles. Methods in enzymology. Elsevier, New York, pp 183–201

Hong P-Y, Iakiviak M, Dodd D et al (2014) Two New xylanases with different substrate specificities from the human gut bacterium bacteroides intestinalis DSM 17393. Appl Environ Microbiol 80:2084–2093. https://doi.org/10.1128/aem.03176-13

Horisawa S, Inoue A, Yamanaka Y (2019) Direct ethanol production from lignocellulosic materials by mixed culture of wood rot fungi *Schizophyllum commune, Bjerkandera adusta*, and *Fomitopsis palustris*. Fermentation 5:21. https://doi.org/10.3390/fermentation5010021

Hu J, Arantes V, Saddler JN (2011) The enhancement of enzymatic hydrolysis of lignocellulosic substrates by the addition of accessory enzymes such as xylanase: is it an additive or synergistic effect? Biotechnol Biofuels 36:1–13. https://doi.org/10.1186/1754-6834-4-36

Huang C, Jeuck B, Du J et al (2016) Novel process for the coproduction of xylo-oligosaccharides, fermentable sugars, and lignosulfonates from hardwood. Bioresour Technol 219:600–607

Humphry DR, George A, Black GW, Cummings SP (2001) *Flavobacterium frigidarium* sp. nov., an aerobic, psychrophilic, xylanolytic and laminarinolytic bacterium from Antarctica. Int J Syst Evol Microbiol 51:1235–1243

Hunt CJ, Tanksale A, Haritos VS (2016) Biochemical characterization of a halotolerant feruloyl esterase from *Actinomyces* spp.: refolding and activity following thermal deactivation. Appl Microbiol Biotechnol 100:1777–1787

Hüttner S, Granchi Z, Nguyen TT et al (2018) Genome sequence of *Rhizomucor pusillus* FCH 5.7, a thermophilic zygomycete involved in plant biomass degradation harbouring putative GH9 endoglucanases. Biotechnol Rep 20:e00279. https://doi.org/10.1016/j.btre.2018.e00279

Iloduba MI, Milala MA, Ali A (2016) Isolation and partial characterization of crude cellulase-free xylanase from *Pseudomonas aeruginosa* and *Staphylococcus aureus* for possible use in paper industry. Int J Microbiol 3:8

Imad M, Arabi E, Bakri Y, Jawhar M (2011) Extracellular xylanase production by *Fusarium* species in solid state fermentation. Polish J Microbiol 60:209–212

Bhardwaj *et al. Bioresour. Bioprocess.* (2019) 6:40

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Iqbal M, Tao Y, Xie S et al (2016) Aqueous two-phase system (ATPS): an overview and advances in its applications. Biol Proced Online 18:18

Irfan M, Nadeem M, Syed Q (2014) One-factor-at-a-time (OFAT) optimization of xylanase production from *Trichoderma viride*-IR05 in solid-state fermentation. J Radiat Res Appl Sci 7:317–326. https://doi.org/10.1016/j.jrras.2014.04.004

Irfan M, Asghar U, Nadeem M et al (2016) Optimization of process parameters for xylanase production by *Bacillus* sp. in submerged fermentation. J Radiat Res Appl Sci 9:139–147. https://doi.org/10.1016/j.jrras.2015.10.008

Irwin D, Jung ED, Wilson DB (1994) Characterization and sequence of a *Thermomonospora fusca* xylanase. Appl Environ Microbiol 60:763–770

Izumi Y, Koga T, Matsumoto T, Kato T (1997) Purification and some properties of endo-1,4-beta-ᴅ-xylanase from a fresh-water mollusc, *Pomacea insularus* (de Ordigny). Biosci Biotechnol Biochem 61:615–620

Jayapal N, Samanta AK, Kolte AP et al (2013) Value addition to sugarcane bagasse: xylan extraction and its process optimization for xylooligosaccharides production. Ind Crops Prod 42:14–24

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

Jensen JK, Busse-Wicher M, Poulsen CP et al (2018) Identification of an algal xylan synthase indicates that there is functional orthology between algal and plant cell wall biosynthesis. New Phytol 218:1049–1060

Jhamb K, Sahoo DK (2012) Production of soluble recombinant proteins in *Escherichia coli*: effects of process conditions and chaperone co-expression on cell growth and production of xylanase. Bioresour Technol 123:135–143

Joshi C, Khare SK (2011) Utilization of deoiled *Jatropha curcas* seed cake for production of xylanase from thermophilic *Scytalidium thermophilum*. Bioresour Technol 102:1722–1726

Joshi C, Khare SK (2012) Induction of xylanase in thermophilic fungi *Scytalidium thermophilum* and *Sporotrichum thermophile*. Brazilian Arch Biol Technol 55:21–27. https://doi.org/10.1590/S1516-89132012000100003

Juturu V, Wu JC (2012) Microbial xylanases: engineering, production and industrial applications. Biotechnol Adv 30:1219–1227

Juturu V, Wu JC (2014) Microbial exo-xylanases: a mini review. Appl Biochem Biotechnol 174:81–92

Kallel F, Driss D, Bouaziz F et al (2015a) Production of xylooligosaccharides from garlic straw xylan by purified xylanase from *Bacillus mojavensis* UEB-FK and their in vitro evaluation as prebiotics. Food Bioprod Process 94:536–546. https://doi.org/10.1016/j.fbp.2014.07.012

Kallel F, Driss D, Chaabouni SE, Ghorbel R (2015b) Biological activities of xylooligosaccharides generated from garlic straw xylan by purified xylanase from *Bacillus mojavensis* UEB-FK. Appl Biochem Biotechnol 175:950–964

Kalpana VN, Rajeswari VD (2015) Production of xylanase from various lignocellulosic waste materials by *Streptomyces* sp. and its potential role in deinking of newsprint. Asian J Biochem 10:222–229

Kamble RD, Jadhav AR (2012) Isolation, purification, and characterization of xylanase produced by a new species of *Bacillus* in solid state fermentation. Int J Microbiol 2012:683193. https://doi.org/10.1155/2012/683193

Kamra P, Satyanarayana T (2004) Xylanase production by the thermophilic mold *Humicola lanuginosa* in solid-state fermentation. Appl Biochem Biotechnol Part A Enzym Eng Biotechnol 119:145–157. https://doi.org/10.1385/ABAB:119:2:145

Karlsson EN, Hachem MA, Ramchuran S et al (2004) The modular xylanase Xyn10A from *Rhodothermus marinus* is cell-attached, and its C-terminal domain has several putative homologues among cell-attached proteins within the phylum Bacteroidetes. FEMS Microbiol Lett 241:233–242. https://doi.org/10.1016/j.femsle.2004.10.026

Katsimpouras C, Dedes G, Thomaidis NS, Topakas E (2019) A novel fungal GH30 xylanase with xylobiohydrolase auxiliary activity. Biotechnol Biofuels 12:1–14. https://doi.org/10.1186/s13068-019-1455-2

Khandeparkar RDS, Bhosle NB (2006) Isolation, purification and characterization of the xylanase produced by *Arthrobacter* sp. MTCC 5214 when grown in solid-state fermentation. Enzyme Microb Technol 39:732–742. https://doi.org/10.1016/j.enzmictec.2005.12.008

Khusro A, Kaliyan BK, Al-Dhabi NA et al (2016) Statistical optimization of thermo-alkali stable xylanase production from *Bacillus tequilensis* strain ARMATI. Electron J Biotechnol 22:16–25. https://doi.org/10.1016/j.ejbt.2016.04.002

Kiddinamoorthy J, Anceno AJ, Haki GD, Rakshit SK (2008) Production, purification and characterization of *Bacillus* sp. GRE7 xylanase and its application in eucalyptus Kraft pulp biobleaching. World J Microbiol Biotechnol 24:605–612. https://doi.org/10.1007/s11274-007-9516-2

Kim KK, Park HY, Park W et al (2005) *Microbacterium xylanilyticum* sp. nov., a xylan-degrading bacterium isolated from a biofilm. Int J Syst Evol Microbiol 55:2075–2079. https://doi.org/10.1099/ijs.0.63706-0

Klein-Marcuschamer D, Oleskowicz-Popiel P, Simmons BA, Blanch HW (2012) The challenge of enzyme cost in the production of lignocellulosic biofuels. Biotechnol Bioeng 109(4):1083–1087

Knob A, Beitel SM, Fortkamp D et al (2013) Production, purification, and characterization of a major *Penicillium glabrum* xylanase using Brewer's spent grain as substrate. Biomed Res Int 2013:1–8. https://doi.org/10.1155/2013/728735

Ko C-H, Lin Z-P, Tu J et al (2010) Xylanase production by *Paenibacillus campinasensis* BL11 and its pretreatment of hardwood kraft pulp bleaching. Int Biodeterior Biodegrad 64:13–19

Kocabacs DS, Güder S, Özben N (2015) Purification strategies and properties of a low-molecular weight xylanase and its application in agricultural waste biomass hydrolysis. J Mol Catal B Enzyme 115:66–75

Kohli U, Nigam P, Singh D, Chaudhary K (2001) Thermostable, alkalophilic and cellulase free xylanase production by *Thermoactinomyces thalophilus* subgroup C. Enzyme Microb Technol 28:606–610. https://doi.org/10.1016/S0141-0229(01)00320-9

Koontz L (2014) TCA precipitation. Methods in enzymology. Elsevier, New York, pp 3–10

Krishnamurthy S, Vithayathil PJ (1989) Purification and characterization of endo-1,4-β-xylanase from *Paecilomyces variotii* Bainier. J Ferment Bioeng 67:77–82. https://doi.org/10.1016/0922-338X(89)90183-9

Kumar V, Satyanarayana T (2014) Production of endoxylanase with enhanced thermostability by a novel polyextremophilic *Bacillus halodurans* TSEV1 and its applicability in waste paper deinking. Process Biochem 49:386–394

Kumar V, Shukla P (2018) Extracellular xylanase production from *T. lanuginosus* VAPS24 at pilot scale and thermostability enhancement by immobilization. Process Biochem 71:53–60. https://doi.org/10.1016/j.procbio.2018.05.019

Kumar L, Nagar S, Kumar D et al (2013) Production of an alkali tolerant extracellular xylanase from *Bacillus pumilus* VLK-1 in solid state fermentation and its use in tomato juice clarification. Int J Cell Sci Biotechnol 2:1–10

Kumar L, Nagar S, Mittal A et al (2014) Immobilization of xylanase purified from *Bacillus pumilus* VLK-1 and its application in enrichment of orange and grape juices. J Food Sci Technol 51:1737–1749

Kumar B, Bhardwaj N, Alam A et al (2018a) Production, purification and characterization of an acid/alkali and thermo tolerant cellulase from Schizophyllum commune NAIMCC-F-03379 and its application in hydrolysis of lignocellulosic wastes. AMB Express 8:1–16. https://doi.org/10.1186/s13568-018-0696-y

Kumar BA, Amit K, Alok K, Dharm D (2018b) Wheat bran fermentation for the production of cellulase and xylanase by *Aspergillus niger* NFCCI 4113. Res J Biotechnol 13:5

Kumar NV, Rani ME, Gunaseeli R, Kannan ND (2018c) Paper pulp modification and deinking efficiency of cellulase-xylanase complex from *Escherichia coli* SD5. Int J Biol Macromol 111:289–295

Kumar PS, Yaashikaa PR, Saravanan A (2018d) Isolation, characterization and purification of xylanase producing bacteria from sea sediment. Biocatal Agric Biotechnol 13:299–303

Landowski CP, Huuskonen A, Wahl R et al (2015) Enabling low cost biopharmaceuticals: a systematic approach to delete proteases from a well-known protein production host *Trichoderma reesei*. PLoS ONE 10:e0134723

Lange L (2017) Fungal enzymes and yeasts for conversion of plant biomass to bioenergy and high-value products. Microbiol Spectr. 5:1. https://doi.org/10.1128/microbiolspec.FUNK-0007-2016.10.1128/microbiolspec.FUNK-0007-2016

Latif F, Asgher M, Saleem R et al (2006) Purification and characterization of a xylanase produced by *Chaetomium thermophile* NIBGE. World J Microbiol Biotechnol 22:45–50. https://doi.org/10.1007/s11274-005-5745-4

Lee JH, Kim YG, Lee J (2018) Thermostable xylanase inhibits and disassembles *Pseudomonas aeruginosa* biofilms. Biofouling 34:346–356. https://doi.org/10.1080/08927014.2018.1440551

Bhardwaj *et al. Bioresour. Bioprocess.* (2019) 6:40

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Leite P, Salgado JM, Venâncio A et al (2016) Ultrasounds pretreatment of olive pomace to improve xylanase and cellulase production by solid-state fermentation. Bioresour Technol 214:737–746

Li T, Li S, Du L et al (2010) Effects of haw pectic oligosaccharide on lipid metabolism and oxidative stress in experimental hyperlipidemia mice induced by high-fat diet. Food Chem 121:1010–1013

Li J, Wang J, Wang S et al (2012) Achieving efficient protein expression in *Trichoderma reesei* by using strong constitutive promoters. Microb Cell Fact 11:84

Lin C, Shen Z, Zhu T, Qin W (2017) Bacterial xylanase in *Pseudomonas boreopolis* LUQ1 is highly induced by xylose. Can J Biotechnol 1:73–79. https://doi.org/10.24870/cjb.2017-000112

Lin X, Wu Z, Zhang C, Liu S, Nie S (2018) Enzymatic pulping of lignocellulosic biomass. Ind crop prod 120:16–24

Lisov AV, Belova OV, Lisova ZA et al (2017) Xylanases of *Cellulomonas flavigena*: expression, biochemical characterization, and biotechnological potential. AMB Express. 5:7. https://doi.org/10.1186/s13568-016-0308-7

Liu X, Kokare C (2017) Chapter 11—microbial enzymes of use in industry. In: Brahmachari G (ed) Biotechnology of microbial enzymes. Academic Press, Cambridge, pp 267–298

Liu J, Yuan X, Zeng G et al (2006) Effect of biosurfactant on cellulase and xylanase production by *Trichoderma viride* in solid substrate fermentation. Process Biochem 41:2347–2351

Liu N, Ru YJ, Tang DF et al (2011) Effects of corn distillers dried grains with solubles and xylanase on growth performance and digestibility of diet components in broilers. Anim Feed Sci Technol 163:260–266

Liu X, Liu Y, Jiang Z et al (2018) Biochemical characterization of a novel xylanase from *Paenibacillus barengoltzii* and its application in xylooligosaccharides production from corncobs. Food Chem 264:310–318. https://doi.org/10.1016/j.foodchem.2018.05.023

Lombard V, Golaconda Ramulu H, Drula E et al (2014) The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res 42:490–495. https://doi.org/10.1093/nar/gkt1178

Losonczi A, Csiszár E, Szakács G, Bezúr L (2005) Role of the EDTA chelating agent in bioscouring of cotton. Text Res J 75:411–417. https://doi.org/10.1177/0040517505053812

Loureiro DB, Braia M, Romanini D, Tubio G (2017) Partitioning of xylanase from *Thermomyces lanuginosus* in PEG/NaCit aqueous two-phase systems: structural and functional approach. Protein Expr Purif 129:25–30

Lu Y, Fang C, Wang Q et al (2016) High-level expression of improved thermostable alkaline xylanase variant in *Pichia Pastoris* through codon optimization, multiple gene insertion and high-density fermentation. Sci Rep 6:1–10. https://doi.org/10.1038/srep37869

Luo L, Cai J, Wang C et al (2016) Purification and characterization of an alkaliphilic endo-xylanase from *Streptomyces althioticus* LMZM and utilization in the pulp paper industry. J Chem Technol Biotechnol 91:1093–1098

Maalej I, Belhaj I, Masmoudi NF, Belghith H (2009) Highly thermostable xylanase of the thermophilic fungus *Talaromyces thermophilus*: purification and characterization. Appl Biochem Biotechnol 158:200–212

Maity C, Ghosh K, Halder SK et al (2012) Xylanase isozymes from the newly isolated *Bacillus* sp. CKBx1D and optimization of its deinking potentiality. Appl Biochem Biotechnol 167:1208–1219

Malhotra G, Chapadgaonkar SS (2018) Production and applications of xylanases—an overview. BioTechnologia 99:59–72

Mander P, Choi YH, Pradeep GC et al (2014) Biochemical characterization of xylanase produced from *Streptomyces* sp. CS624 using an agro residue substrate. Process Biochem 49:451–456

Martínez PM, Appeldoorn MM, Gruppen H, Kabel MA (2016) The two *Rasamsonia emersonii* α-glucuronidases, ReGH67 and ReGH115, show a different mode-of-action towards glucuronoxylan and glucuronoxylo-oligosaccharides. Biotechnol Biofuels 9:1–10. https://doi.org/10.1186/s13068-016-0519-9

Maslen SL, Goubet F, Adam A et al (2007) Structure elucidation of arabinoxylan isomers by normal phase HPLC–MALDI-TOF/TOF-MS/MS. Carbohydr Res 342:724–735

Mewis K, Lenfant N, Lombard V, Henrissat B (2016) Dividing the large glycoside hydrolase family 43 into subfamilies: a motivation for detailed enzyme characterization. Appl Environ Microbiol 82:1686–1692

Mienda BS, Idi A, Umar A (2011) Microbiological features of solid state fermentation and its applications—an overview. Res Biotechnol 2:21–26

Mittal A, Nagar S, Gupta VK (2013) Production and purification of high levels of cellulase-free bacterial xylanase by *Bacillus* sp. SV-34S using agro-residue. Ann Microbiol 63:1157–1167. https://doi.org/10.1007/s13213-012-0574-9

Mmango-Kaseke Z, Okaiyeto K, Nwodo UU et al (2016) Optimization of cellulase and xylanase production by *Micrococcus* species under submerged fermentation. Sustain 8:1–15. https://doi.org/10.3390/su8111168

Mohana S, Shah A, Divecha J, Madamwar D (2008) Xylanase production by *Burkholderia* sp. DMAX strain under solid state fermentation using distillery spent wash. Bioresour Technol 99:7553–7564. https://doi.org/10.1016/j.biortech.2008.02.009

Moniz P, Ho AL, Duarte LC et al (2016) Assessment of the bifidogenic effect of substituted xylo-oligosaccharides obtained from corn straw. Carbohydr Polym 136:466–473

Monti R, Cardello L, Custódio MF et al (2003) Production and purification of an endo-1,4-β-xylanase from *Humicola grisea* var. thermoidea by electro-elution. Brazilian J Microbiol 34:124–128

Motta FL, Andrade CCP, Santana MHA (2013) A review of xylanase production by the fermentation of xylan: classification, characterization and applications. Sustainable degradation of lignocellulosic biomass-techniques, applications and commercialization. InTechOpen, New York, pp 251–275

Murugan P, Jampala P (2015) Production of xylanase from A mixed culture system of *Acetobacter xylinum* and *Cellulomonas uda* in submerged fermentation. Biosci Biotechnol Res Asia 12:1615–1622

Murugan S, Arnold D, Pongiya UD, Narayanan PM (2011) Production of xylanase from *Arthrobacter* sp. MTCC 6915 using saw dust as substrate under solid state fermentation. Enzyme Res 2011:696942

Naganagouda K, Mulimani VH (2008) Aqueous two-phase extraction (ATPE): an attractive and economically viable technology for downstream processing of *Aspergillus oryzae* α-galactosidase. Process Biochem 43:1293–1299

Namasivayam S, Babu M, Bharani RS (2015) Evaluation of lignocellulosic agro wastes for the enhanced production of extracellular cellulase and xylanase by *Trichoderma harzianum*. Nat Environ Pollut Technol 14:47–52

Nathan VK, Rani ME, Rathinasamy G, Dhiraviam KN (2017) Low molecular weight xylanase from *Trichoderma viride* VKF3 for bio-bleaching of newspaper pulp. BioResources 12:5264–5278. https://doi.org/10.15376/biores.12.3.5264-5278

Naveen M, Saroj Y, Pramod T et al (2014) Role of nitrogen source for the production of xylanase from *Aspergillus* sp. J Drug Deliv Ther 4:147–149

Nevalainen H, Peterson R (2014) Making recombinant proteins in filamentous fungi-are we expecting too much ? Front Microbiol 5:1–10. https://doi.org/10.3389/fmicb.2014.00075

Nevalainen H, Peterson R, Curach N (2018) Overview of gene expression using filamentous fungi. Curr Protoc Protein Sci 92:e55

Ng HS, Chai CXY, Chow YH et al (2018) Direct recovery of *Bacillus subtilis* xylanase from fermentation broth with an alcohol/salt aqueous biphasic system. J Biosci Bioeng. 125:585–589. https://doi.org/10.1016/j.jbiosc.2017.12.010

Nie S, Zhang K, Lin X, Zhang C, Yan D, Liang H, Wang S (2018) Enzymatic pretreatment for the improvement of dispersion and film properties of cellulose nanofibrils. Carbohyd Polym 181:1136–1142

Notenboom V, Birsan C, Warren RAJ et al (1998) Exploring the cellulose/xylan specificity of the β-1,4-glycanase Cex from *Cellulomonas fimi* through crystallography and mutation. Biochemistry 37:4751–4758. https://doi.org/10.1021/bi9729211

Otieno DO, Ahring BK (2012) A thermochemical pretreatment process to produce xylooligosaccharides (XOS), arabinooligosaccharides (AOS) and mannooligosaccharides (MOS) from lignocellulosic biomasses. Bioresour Technol 112:285–292

Paloheimo M, Mäntylä A, Kallio J et al (2007) Increased production of xylanase by expression of a truncated version of the xyn11A gene from *Nonomuraea flexuosa* in *Trichoderma reesei*. Appl Environ Microbiol 73:3215–3224. https://doi.org/10.1128/AEM.02967-06

Paloheimo M, Piironen J, Vehmaanperä J et al (2010) Xylanases and cellulases as feed additives. Enzymes in farm animal nutrition. Nutr Finnfeeds Malborough, Wiltshire, pp 12–53

Passos AA, Park I, Ferket P et al (2015) Effect of dietary supplementation of xylanase on apparent ileal digestibility of nutrients, viscosity of digesta,

Bhardwaj *et al. Bioresour. Bioprocess.* (2019) 6:40

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and intestinal morphology of growing pigs fed corn and soybean meal based diet. Anim Nutr 1:19–23

Paulo RH, Caroline H, Rosane MP et al (2014) Xylanase from *Fusarium heterosporum*: properties and influence of thiol compounds on xylanase activity. African J Biotechnol 13:1047–1055. https://doi.org/10.5897/AJB2013.13282

Pérez J, Munoz-Dorado J, la Rubia T, Martinez J (2002) Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview. Int Microbiol 5:53–63

Ping L, Wang M, Yuan X et al (2018) Production and characterization of a novel acidophilic and thermostable xylanase from *Thermoascus aurantiacu*. Int J Biol Macromol 109:1270–1279. https://doi.org/10.1016/j.ijbiomac.2017.11.130

Pirgozliev V, Whiting I, Rose SP et al (2016) Variability between wheat dry distillers grains with solubles samples influence the effectiveness of exogenous enzymes when fed to broiler chickens. Vet Med Anim Stud 6:61–69

Pohlschroder M, Leschine SB, Canale-Parola E (1994) Multicomplex cellulase–xylanase system of *Clostridium papyrosolvens* C7. J Bacteriol 176:70–76. https://doi.org/10.1128/jb.176.1.70-76.1994

Polizeli M, Rizzatti ACS, Monti R et al (2005) Xylanases from fungi: properties and industrial applications. Appl Microbiol Biotechnol 67:577–591

Puchart V, Fravnová L, Krogh KBRM et al (2018) Action of different types of endoxylanases on eucalyptus xylan in situ. Appl Microbiol Biotechnol 102:1725–1736

Purkan P, Huruniawati E, Sumarsih S (2017) Xylanase enzyme from a local strain of *Pseudomonas stutzeri*. J Chem Technol Metall 52:1079–1085

Qiu Z, Shi P, Luo H et al (2010) A xylanase with broad pH and temperature adaptability from *Streptomyces megasporus* DSM 41476, and its potential application in brewing industry. Enzyme Microb Technol 46:506–512

Raghothama S, Simpson PJ, Szabó L et al (2000) Solution structure of the CBM10 cellulose binding module from *Pseudomonas* xylanase A. Biochemistry 39:978–984. https://doi.org/10.1021/bi992163+

Rahim T, Ray AL, Beauty SP, Gomes DJ (2009) Induction of mutation in *Neurospora crassa* with ultraviolet radiation and evaluation of cellulase and xylanase activities. Bangladesh J Bot 38:201–203

Rahmani N, Kahar P, Lisdiyanti P et al (2018) Xylanase and feruloyl esterase from actinomycetes cultures could enhance sugarcane bagasse hydrolysis in the production of fermentable sugars. Biosci Biotechnol Biochem 82:904–915. https://doi.org/10.1080/09168451.2018.1438169

Rahmani N, Kahar P, Lisdiyanti P et al (2019) GH-10 and GH-11 Endo-1,4-β-xylanase enzymes from *Kitasatospora* sp. produce xylose and xylooligosaccharides from sugarcane bagasse with no xylose inhibition. Bioresour Technol 272:315–325. https://doi.org/10.1016/j.biortech.2018.10.007

Raj A, Kumar S, Singh SK (2013) A highly thermostable xylanase from *Stenotrophomonas maltophilia*: purification and partial characterization. Enzyme Res 2013:429305

Raj A, Kumar S, Singh SK, Prakash J (2018) Production and purification of xylanase from alkaliphilic *Bacillus licheniformis* and its pretreatment of eucalyptus kraft pulp. Biocatal Agric Biotechnol 15:199–209

Rajoka MI, Bashir A, Malik KA (1997) Mutagenesis of *Cellulomonas biazotea* for enhanced production of xylanases. Bioresour Technol 62:99–108

Ramakrishnan V, Goveas LC, Suralikerimath N et al (2016) Extraction and purification of lipase from *Enterococcus faecium* MTCC5695 by PEG/phosphate aqueous-two phase system (ATPS) and its biochemical characterization. Biocatal Agric Biotechnol 6:19–27

Ramanjaneyulu G, Sridevi A, Seshapani P et al (2017) Enhanced production of xylanase by *Fusarium* sp. BVKT R2 and evaluation of its biomass saccharification efficiency. 3 Biotech 7:1–17. https://doi.org/10.1007/s13205-017-0977-1

Ray S, Vigouroux J, Bouder A et al (2019) Functional exploration of *Pseudoalteromonas atlantica* as a source of hemicellulose-active enzymes: evidence for a GH8 xylanase with unusual mode of action. Enzyme Microb Technol. 127:6–16. https://doi.org/10.1016/j.enzmictec.2019.04.007

Roberfroid MB (1997) Health benefits of non-digestible oligosaccharides. Dietary fiber in health and disease. Springer, New York, pp 211–219

Romero-Fernández M, Moreno-Perez S, Orrego AH et al (2018) Designing continuous flow reaction of xylan hydrolysis for xylooligosaccharides production in packed-bed reactors using xylanase immobilized on methacrylic polymer-based supports. Bioresour Technol 266:249–258

Rosmine E, Sainjan NC, Silvester R et al (2017) Statistical optimisation of xylanase production by estuarine *Streptomyces* sp. and its application in clarification of fruit juice. J Genet Eng Biotechnol 15:393–401

Rosmine E, Edassery Sainjan NC, Silvester R, Varghese SA (2019) Utilisation of agrowaste xylan for the production of industrially important enzyme xylanase from aquatic *Streptomyces* sp. and potential role of xylanase in deinking of newsprint. Int J Curr Microbiol Appl Sci 8:2061–2076. https://doi.org/10.20546/ijcmas.2019.801.216

Rychen G, Aquilina G, Azimonti G et al (2018) Safety and efficacy of ECONASE®XT (endo-1, 4-$β$-xylanase) as a feed additive for pigs for fattening. EFSA J 16:e05217

Rytioja J, Hildén K, Yuzon J et al (2014) Plant-polysaccharide-degrading enzymes from basidiomycetes. Microbiol Mol Biol Rev 78:614–649. https://doi.org/10.1128/MMBR.00035-14

Sadaf A, Khare SK (2014) Production of Sporotrichum thermophile xylanase by solid state fermentation utilizing deoiled *Jatropha curcas* seed cake and its application in xylooligosaccharide synthesis. Bioresour Technol 153:126–130

Saha BC (2002) Production, purification and properties of xylanase from a newly isolated *Fusarium proliferatum*. Process Biochem 37:1279–1284. https://doi.org/10.1016/S0032-9592(02)00012-2

Sánchez C (2009) Lignocellulosic residues: biodegradation and bioconversion by fungi. Biotechnol Adv 27:185–194

Sanjivkumar M, Silambarasan T, Palavesam A, Immanuel G (2017) Biosynthesis, purification and characterization of β-1,4-xylanase from a novel mangrove associated actinobacterium *Streptomyces olivaceus* (MSU3) and its applications. Protein Expr Purif 130:1–12. https://doi.org/10.1016/j.pep.2016.09.017

Selim S (2016) Molecular characterization of thermostable xylanase producing *Thermoactinomyces vulgaris* HSB4. J Pure Appl Microbiol 10:1965–1969

Seyis I, Aksoz N (2005) Effect of carbon and nitrogen sources on xylanase production by *Trichoderma harzianum* 1073 D3. Int Biodeterior Biodegradation 55:115–119

Shahrestani H, Taheri-Kafrani A, Soozanipour A, Tavakoli O (2016) Enzymatic clarification of fruit juices using xylanase immobilized on 1, 3, 5-triazine-functionalized silica-encapsulated magnetic nanoparticles. Biochem Eng J 109:51–58

Shariq M, Sohail M (2018) Application of *Candida tropicalis* MK-160 for the production of xylanase and ethanol. J King Saud Univ. https://doi.org/10.1016/j.jksus.2018.04.009

Sharma M, Chadha BS, Kaur M et al (2008) Molecular characterization of multiple xylanase producing thermophilic/thermotolerant fungi isolated from composting materials. Lett Appl Microbiol 46:526–535. https://doi.org/10.1111/j.1472-765X.2008.02357.x

Sharma M, Mehta S, Kumar A (2013) Purification and characterization of alkaline xylanase secreted from *Paenibacillus macquariensis*. Adv Microbiol 03:32–41. https://doi.org/10.4236/aim.2013.31005

Sharma PK (2017) Xylanases current and future perspectives : a review Pawan Kumar Sharma. New Biol Rep 6:122

Shen B, Sun X, Zuo X et al (2012) Engineering a thermoregulated intein-modified xylanase into maize for consolidated lignocellulosic biomass processing. Nat Biotechnol 30:1131–1136

Shi P, Tian J, Yuan T et al (2010) *Paenibacillus* sp. Strain E18 bifunctional xylanase–glucanase with a single catalytic domain. Appl Environ Microbiol 76:3620–3624. https://doi.org/10.1128/AEM.00345-10

Shi H, Zhang Y, Li X et al (2013) A novel highly thermostable xylanase stimulated by Ca^{2+} from *Thermotoga thermarum*: cloning, expression and characterization. Biotechnol Biofuels 6:1–9. https://doi.org/10.1186/1754-6834-6-26

Shi H, Zhang Y, Zhong H et al (2014) Cloning, over-expression and characterization of a thermo-tolerant xylanase from *Thermotoga thermarum*. Biotechnol Lett 36:587–593. https://doi.org/10.1007/s10529-013-1392-2

Shi P, Du Y, Yang H et al (2015) Molecular characterization of a new alkaline-tolerant xylanase from *Humicola insolens* Y1. Biomed Res Int 2015:1–7. https://doi.org/10.1155/2015/149504

Shibuya H, Kaneko S, Hayashi K (2000) Enhancement of the thermostability and hydrolytic activity of xylanase by random gene shuffling. Biochem J 349:651–656

Bhardwaj *et al. Bioresour. Bioprocess.* (2019) 6:40

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Simair AA, Dahot MU, Mangrio GS et al (2010) Enhancing effect of amino acids and vitamins on xylanase production by *Pleurotus Eryngii* through submerged fermentation. Pak J Biotechnol 7:123–126

Sindhu R, Binod P, Mathew AK et al (2017) Development of a novel ultrasound-assisted alkali pretreatment strategy for the production of bioethanol and xylanases from chili post harvest residue. Bioresour Technol 242:146–151

Singh A, Kaur A, Patra AK, Mahajan R (2018) A sustainable and green process for scouring of cotton fabrics using xylano-pectinolytic synergism: switching from noxious chemicals to eco-friendly catalysts. 3 Biotech 8:184

Singh S, Sidhu GK, Kumar V et al (2019) Fungal xylanases: sources, types, and biotechnological applications. Recent advancement in white biotechnology through fungi. Springer, New York, pp 405–428

Singla A, Paroda S, Dhamija SS et al (2012) Bioethanol production from xylose: problems and possibilities. J Biofuels 3:1–17

Sizova MV, Izquierdo JA, Panikov NS, Lynd LR (2011) Cellulose-and xylan-degrading thermophilic anaerobic bacteria from biocompost. Appl Environ Microbiol 77:2282–2291

Song HY, Lim HK, Kim DR et al (2014) A new bi-modular endo-β-1,4-xylanase KRICT PX-3 from whole genome sequence of *Paenibacillus terrae* HPL-003. Enzyme Microb Technol 54:1–7. https://doi.org/10.1016/j.enzmictec.2013.09.002

Srinivasan S, Kim MK, Sathiyaraj G et al (2010) *Microbacterium soli* sp. nov., an α-glucosidase-producing bacterium isolated from soil of a ginseng field. Int J Syst Evol Microbiol 60:478–483. https://doi.org/10.1099/ijs.0.012526-0

St. John FJ, Rice JD, Preston JF (2006) Characterization of XynC from *Bacillus subtilis* subsp. subtilis strain 168 and analysis of its role in depolymerization of glucuronoxylan. J Bacteriol 188:8617–8626. https://doi.org/10.1128/JB.01283-06

Stutzenberger F (1994) Extracellular enzyme production by *Thermomonospora curvata* grown on bagasse. J Ind Microbiol 13:35–42. https://doi.org/10.1007/BF01569660

Su X, Schmitz G, Zhang M et al (2012) Heterologous gene expression in filamentous fungi. In: Gadd GM, Sariaslani S (eds) Advances in applied microbiology. Academic Press, Cambridge, pp 1–61

Subramaniyan S, Prema P (2002) Biotechnology of microbial xylanases: enzymology, molecular biology, and application. Crit Rev Biotechnol 22:33–64

Sukhumsirichart W, Deesukon W, Kawakami T et al (2014) Expression and characterization of recombinant GH11 xylanase from thermotolerant *Streptomyces* sp. SWU10. Appl Biochem Biotechnol 172:436–446

Suleman M, Aujla MI (2016) Production and characterization of xylanase from *Aspergillus niger* using wheat bran, corn cobs, and sugar cane bagasse as carbon sources with different concentrations. J Bioresourc Mang. 3:1-9. https://doi.org/10.35691/JBM.5102.0040

Sun Y, Cheng J (2002) Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresour Technol 83:1–11

Sun J, Wen F, Si T et al (2012) Direct conversion of xylan to ethanol by recombinant *Saccharomyces cerevisiae* strains displaying an engineered mini-hemicellulosome. Appl Environ Microbiol. 78:3837–3845. https://doi.org/10.1128/AEM.07679-11

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

Suzuki M, Yoshida K, Ashida K (1991) Purification and characterization of xylanase from the mid-gut gland of the apple snail (*Pomacea canaliculata*). Agric Biol Chem 55:693–700

Syuan KY, Ai LOG, Suan TK (2018) Evaluation of cellulase and xylanase production from *Trichoderma harzianum* using acid-treated rice straw as solid substrate. Mater Today Proc 5:22109–22117. https://doi.org/10.1016/j.matpr.2018.07.077

Tai WY, Tan JS, Lim V, Lee CK (2019) Comprehensive studies on optimization of cellulase and xylanase production by a local indigenous fungus strain via solid state fermentation using oil palm frond as substrate. Biotechnol Prog. 35:e2781. https://doi.org/10.1002/btpr.2781

Taibi Z, Saoudi B, Boudelaa M et al (2012) Purification and biochemical characterization of a highly thermostable xylanase from *Actinomadura* sp. strain Cpt20 isolated from poultry compost. Appl Biochem Biotechnol 166:663–679

Tang F, Chen D, Yu B et al (2017) Improving the thermostability of *Trichoderma reesei* xylanase 2 by introducing disulfide bonds. Electron J Biotechnol 26:52–59. https://doi.org/10.1016/j.ejbt.2017.01.001

Tao P, Wu Z, Xing C, Zhang Q, Wei Z, Nie S (2019) Effect of enzymatic treatment on the thermal stability of cellulose nanofibrils. Cellulose 26:7717–7725

Terrone CC, de Freitas C, Terrasan CRF et al (2018) Agroindustrial biomass for xylanase production by *Penicillium chrysogenum*: purification, biochemical properties and hydrolysis of hemicelluloses. Electron J Biotechnol 33:39–45. https://doi.org/10.1016/j.ejbt.2018.04.001

Thanh VN, Thuy NT, Huong HTT et al (2019) Surveying of acid-tolerant thermophilic lignocellulolytic fungi in Vietnam reveals surprisingly high genetic diversity. Sci Rep 9:1–12. https://doi.org/10.1038/s41598-019-40213-5

Thomas L, Joseph A, Arumugam M, Pandey A (2013) Production, purification, characterization and over-expression of xylanases from actinomycetes. Indian J Exp Biol 51:875–884

Thomas L, Ushasree MV, Pandey A (2014) An alkali-thermostable xylanase from Bacillus pumilus functionally expressed in *Kluyveromyces lactis* and evaluation of its deinking efficiency. Bioresour Technol 165:309–313

Thomas L, Sindhu R, Binod P, Pandey A (2015) Production of an alkaline xylanase from recombinant *Kluyveromyces lactis* (KY1) by submerged fermentation and its application in bio-bleaching. Biochem Eng J 102:24–30

Topakas E, Katapodis P, Kekos D et al (2003) Production and partial characterization of xylanase by *Sporotrichum thermophile* under solid-state fermentation. World J Microbiol Biotechnol 19:195–198. https://doi.org/10.1023/A:1023207429410

Turunen O, Etuaho K, Fenel F et al (2001) A combination of weakly stabilizing mutations with a disulfide bridge in the α-helix region of *Trichoderma reesei* endo-1,4-β-xylanase II increases the thermal stability through synergism. J Biotechnol 88:37–46

Upreti RK, Kumar M, Shankar V (2003) Bacterial glycoproteins: functions, biosynthesis and applications. Proteomics 3:363–379

Urbániková L, Vršanská M, Krogh KBRM et al (2011) Structural basis for substrate recognition by *Erwinia chrysanthemi* GH30 glucuronoxylanase. FEBS J 278:2105–2116

Valenzuela SV, Díaz P, Javier Pastor FI (2010) Recombinant expression of an alkali stable GH10 Xylanase from *Paenibacillus barcinonensis*. J Agric Food Chem 58:4814–4818. https://doi.org/10.1021/jf9045792

van den Brink J, van Muiswinkel GCJ, Theelen B et al (2013) Efficient plant biomass degradation by thermophilic fungus *Myceliophthora heterothallica*. Appl Environ Microbiol 79:1316–1324

Van Dorn R, Shanahan D, Ciofalo V (2018) Safety evaluation of xylanase 50316 enzyme preparation (also known as VR007), expressed in *Pseudomonas fluorescens*, intended for use in animal feed. Regul Toxicol Pharmacol 97:48–56

Van Petegem F, Collins T, Meuwis M-A et al (2002) Crystallization and preliminary X-ray analysis of a xylanase from the psychrophile *Pseudoalteromonas haloplanktis*. Acta Crystallogr Sect D 58:1494–1496. https://doi.org/10.1107/S0907444902011666

Vardakou M, Flint J, Christakopoulos P et al (2005) A family 10 *Thermoascus aurantiacus* xylanase utilizes arabinose decorations of xylan as significant substrate specificity determinants. J Mol Biol 352:1060–1067. https://doi.org/10.1016/j.jmb.2005.07.051

Vazquez MJ, Alonso JL, Dominguez H, Parajo JC (2000) Xylooligosaccharides: manufacture and applications. Trends Food Sci Technol 11:387–393

Velikodvorskaya TV, Iy Volkov, Vasilevko VT et al (1997) Purification and some properties of *Thermotoga neapolitana* thermostable xylanase B expressed in *E. coli* cells. Biochemistry (Mosc) 62:66–70

Verma D, Satyanarayana T (2012a) Molecular approaches for ameliorating microbial xylanases. Bioresour Technol 117:360–367

Verma D, Satyanarayana T (2012b) Cloning, expression and applicability of thermo-alkali-stable xylanase of *Geobacillus thermoleovorans* in generating xylooligosaccharides from agro-residues. Bioresour Technol 107:333–338

Verma D, Kawarabayasi Y, Miyazaki K, Satyanarayana T (2013) Cloning, expression and characteristics of a novel alkalistable and thermostable xylanase encoding gene (Mxyl) retrieved from compost-soil metagenome. PLoS ONE 8:e52459

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

Bhardwaj *et al. Bioresour. Bioprocess.* (2019) 6:40

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Virk AP, Puri M, Gupta V et al (2013) Combined enzymatic and physical deinking methodology for efficient eco-friendly recycling of old newsprint. PLoS ONE 8:e72346

Virupakshi S, Babu KG, Gaikwad SR, Naik GR (2005) Production of a xylanolytic enzyme by a thermoalkaliphilic *Bacillus* sp. JB-99 in solid state fermentation. Process Biochem 40:431–435

Voragen AGJ (1998) Technological aspects of functional food-related carbohydrates. Trends Food Sci Technol 9:328–335

Walia A, Mehta P, Chauhan A, Shirkot CK (2013) Optimization of cellulase-free xylanase production by alkalophilic *Cellulosimicrobium* sp. CKMX1 in solid-state fermentation of apple pomace using central composite design and response surface methodology. Ann Microbiol 63:187–198. https://doi.org/10.1007/s13213-012-0460-5

Walia A, Mehta P, Chauhan A et al (2014) Purification and characterization of cellulase-free low molecular weight endo β-1,4 xylanase from an alkalophilic *Cellulosimicrobium cellulans* CKMX1 isolated from mushroom compost. World J Microbiol Biotechnol 30:2597–2608

Walia A, Mehta P, Guleria S, Shirkot CK (2015a) Modification in the properties of paper by using cellulase-free xylanase produced from alkalophilic *Cellulosimicrobium cellulans* CKMX1 in biobleaching of wheat straw pulp. Can J Microbiol 61:671–681

Walia A, Mehta P, Guleria S, Shirkot CK (2015b) Improvement for enhanced xylanase production by *Cellulosimicrobium cellulans* CKMX1 using central composite design of response surface methodology. 3 Biotech 5:1053–1066

Walia A, Guleria S, Mehta P et al (2017) Microbial xylanases and their industrial application in pulp and paper biobleaching: a review. 3 Biotech 7:1–12. https://doi.org/10.1007/s13205-016-0584-6

Wang SY, Hu W, Lin XY et al (2012) A novel cold-active xylanase from the cellulolytic myxobacterium *Sorangium cellulosum* So9733-1: gene cloning, expression, and enzymatic characterization. Appl Microbiol Biotechnol 93:1503–1512. https://doi.org/10.1007/s00253-011-3480-3

Wang X, Ma R, Xie X et al (2017) Thermostability improvement of a *Talaromyces leycettanus* xylanase by rational protein engineering. Sci Rep 7:1–9. https://doi.org/10.1038/s41598-017-12659-y

Waters DM, Ryan LAM, Murray PG et al (2011) Characterisation of a *Talaromyces emersonii* thermostable enzyme cocktail with applications in wheat dough rheology. Enzyme Microb Technol 49:229–236. https://doi.org/10.1016/j.enzmictec.2011.04.006

Whiting IM, Rose SP, Mackenzie AM et al (2019) Effect of wheat distillers dried grains with solubles and exogenous xylanase on laying hen performance and egg quality. Poult Sci 98:3756–3762

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

Xiao X, Bian J, Peng X-P et al (2013) Autohydrolysis of bamboo (*Dendrocalamus giganteus* Munro) culm for the production of xylo-oligosaccharides. Bioresour Technol 138:63–70

Xie Y, An J, Yang G et al (2014) Enhanced enzyme kinetic stability by increasing rigidity within the active site. J Biol Chem 289:7994–8006. https://doi.org/10.1074/jbc.M113.536045

Xu ZH, Bai YL, Xu X et al (2005) Production of alkali-tolerant cellulase-free xylanase by *Pseudomonas* sp. WLUN024 with wheat bran as the main substrate. World J Microbiol Biotechnol 21:575–581. https://doi.org/10.1007/s11274-004-3491-7

Yadav P, Maharjan J, Korpole S et al (2018) Production, purification, and characterization of thermostable alkaline xylanase from *Anoxybacillus kamchatkensis* NASTPD13. Front Bioeng Biotechnol 6:65

Yang J, Han Z (2018) Understanding the positional binding and substrate interaction of a highly thermostable GH10 xylanase from *Thermotoga maritima* by molecular docking. Biomolecules 8:64. https://doi.org/10.3390/biom8030064

Yasinok AE, Biran S, Kocabas A, Bakir U (2010) Xylanase from a soil isolate, *Bacillus pumilus*: gene isolation, enzyme production, purification, characterization and one-step separation by aqueous-two-phase system. World J Microbiol Biotechnol 26:1641–1652

Yasuda M, Nagai H, Takeo K, et al (2014) Bio-ethanol production through simultaneous saccharification and co-fermentation (SSCF) of a low-moisture anhydrous ammonia (LMAA)-pretreated napiegrass (*Pennisetum purpureum* Schumach). SpringerPlus 3:333

Yoon H, Han NS, Kim CH (2014) *E. coli* and characterization of the recombinant enzyme. Microb Cell Fact 13:45

Yoshioka H, Chavanich S, Nilubol N, Hayashida S (1981) Production and characterization of thermostable xylanase from *Talaromyces byssochlamydoidesyh*-50. Agric Biol Chem 45:579–586. https://doi.org/10.1080/00021369.1981.10864586

Zhang M, Jiang Z, Yang S et al (2010a) Cloning and expression of a *Paecilomyces thermophila* xylanase gene in *E. coli* and characterization of the recombinant xylanase. Bioresour Technol 101:688–695. https://doi.org/10.1016/j.biortech.2009.08.055

Zhang W, Lou K, Li G (2010b) Expression and characterization of the *Dictyoglomus thermophilum* Rt46B.1 xylanase gene (xynB) in *Bacillus subtilis*. Appl Biochem Biotechnol 160:1484–1495. https://doi.org/10.1007/s12010-009-8634-8

Zhang F, Hu S-N, Chen J-J et al (2012) Purification and partial characterisation of a thermostable xylanase from salt-tolerant *Thermobifida halotolerans* YIM 90462T. Process Biochem 47:225–228

Zhang D, Wang Y, Zheng D et al (2016a) New combination of xylanolytic bacteria isolated from the lignocellulose degradation microbial consortium XDC-2 with enhanced xylanase activity. Bioresour Technol 221:686–690

Zhang Y, An J, Yang G et al (2016b) Structure features of GH10 xylanase from *Caldicellulosiruptor bescii*: implication for its thermophilic adaption and substrate binding preference. Acta Biochim Biophys Sin (Shanghai) 48:948–957. https://doi.org/10.1093/abbs/gmw086

Zhang K, Zhang Y, Yan D, Zhang C, Nie S (2018) Enzyme-assisted mechanical production of cellulose nanofibrils: thermal stability. Cellulose 25(9):5049–5061

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