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Review

# Thermostable xylanases from thermophilic fungi and bacteria: Current perspective

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ABSTRACT

Thermostable xylanases from thermophilic fungi and bacteria have a wide commercial acceptability in feed, food, paper and pulp and bioconversion of lignocellulosics with an estimated annual market of USD 500 Million. The genome wide analysis of thermophilic fungi clearly show the presence of elaborate genetic information coding for multiple xylanases primarily coding for GH10, GH11 in addition to GH7 and GH30 xylanases. The transcriptomics and proteome profiling has given insight into the differential expression of these xylanases in some of the thermophilic fungi. Bioprospecting has resulted in identification of novel thermophilic xylanases that have been endorsed by the industrial houses for heterologous over- expression and formulations. The future use of xylanases is expected to increase exponentially for their role in biorefineries. The discovery of new and improvement of existing xylanases using molecular tools such as directed evolution is expected to be the mainstay to meet increasing demand of thermostable xylanases.

## 1. Introduction

Xylanase represents a key component of hemicellulolytic enzymes that catalyzes the breakdown of β 1–4 linkage present in the xylan backbone of hemicelluloses for subsequent conversion to xylose monomers. Xylanases have found beneficial role in feed and food industry liberating essential nutrients through hydrolysis/cleavage of non-digestible hemicellulose fibers (Leisola et al., 2002). Commercially, xylanase as an important ingredient has attained a global market proportion for paper and pulp industry (Kumar et al., 2016). Besides, some of the potential applications of microbial xylanases include, as a food additive ingredient for poultry, in baked products, fruit extraction, agriculture silage, as well as functional food. In baking industry, xylanases are being employed for enhancing the quality through such as stability, flexibility and extensibility by acting on soluble as well as non- soluble pentosans in flour (Butt et al., 2008; Dornez et al., 2014). The xylanase market is expected to boost during the forecast period due to increase requirement in animal feed industry. The estimated market share of this enzyme is between 200 and 300 million dollars and slated to reach 500 million dollars by 2023. In animal feed, nutritional additives are a prime need for poultry industry as they are involved in improving digestibility of broiler chickens by lowering the viscosity level in their intestines thus leading to the improvement in the weight gain and feed conversion efficiency (Collins et al., 2005; Nagar et al., 2012). In comparison to other operational costs, the expenses of animal feed in production of poultry and livestock are very high. Thus, to improve the feed digestivity of livestock and cut down the expenses with profit gain, enzymes are the best and safe option to be employed in feed industry. In addition to animal feed industry, the paper and pulp industry also aids in the growth of global xylanase market. The eco-friendly use of xylanase enzyme over the harsh chemical usage further fuels the global xylanase market. On the basis of grade, global xylanase market is segmented into: feed grade and food grade whereas on the basis of their application, the global xylanase market is dissected according to their use in different sectors such as feed and livestock, bleaching of pulp, as an additive (in poultry), bakery, and bioconversion of agro-wastes. The global xylanase market is geographically divided in to five key regions including North America, Latin America, Europe, Asia-Pacific and Middle East Africa. Among these, Asia Pacific, especially China, Indonesia holds maximum share of xylanase market due to major poultry production, followed by Asia Pacific is North America, Europe, Latin America and MEA. In Europe, the presence of poultry companies such as PHW-Gruppe Lohmann & CO. AGxylanase, Plukon Food Group, LDC Group momentous the growth of xylanase market.

Xylanases are produced by diverse variety of micro-organisms including extremophilic fungi, bacteria, yeast etc. which are classified in

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several glycoside hydrolase (GH) families (5, 7, 8, 10, 11, 26, 30 and 43). Xylanases from these sources have been characterized and possess different characteristics such as pH and temperatures to retain their functionality. The industrial application of xylanase demands that the enzymes must be able to withstand the harsh conditions such as acid/alkaline environment and elevated temperature, where alkaline xylanase is beneficial for pulp and kraft bleaching while the preferred xylanases in poultry feed must be active under acidic to neutral pH, the prevalent conditions in the gut. Therefore, bioprospecting for thermostable xylanases keeping in mind the pH and temperature optima has been considered as two major criteria for their application besides being resistant to proteases, metal ion, etc. This review keeping in view the immense potential of xylanases from thermophilic fungi and bacteria primarily focuses on the research and development that has taken place in last 5–8 years and explore the methodologies being used for discovery of novel xylanases.

### 1.1. Xylanase coding genes in thermophilic fungi

It is well recognized that thermophilic fungi produce multiple xylanases (Hinz et al., 2009). The current advances in genome sequencing, annotation and analysis of thermophilic fungi (www. fungal genomics.ca) shows the presence of multiple genes coding for xylanases (Table 1). The majority of the xylanases from thermophilic fungi harbor either GH10 or GH11 xylanase except for *Myceliophthora sepedonium, Pseudocercosporella, Thermomyces stellatus, Scytalidium thermophilum* that also code for functionally distinct GH 30 xylanases (Table 1). GH30 xylanases specifically active against methyl-glucouronoxylans have been studied in thermophilic *Clostridium thermocellum* (st John et al., 2016) but have not been functionally purified from thermophilic fu... Of the different thermophilic fungi genome sequenced, *Myceliop... sepedonium,* codes for maximum xylanase genes (seventeen) inclu... eight that belongs to GH10 and six categorized to GH11. The genome... other strains *Pseudocerco sporella* and *Myceliophthora ...phila* al... coded for 14 and 13 xylanase genes, respectively ... *Thermo... myces lanuginosus*, considered as one of the most ...ific xy...ase producer and *Thermoascus aurantiacus* coded for s... GH1... xylanase genes (Singh et al., 2003; Winger... al., 2... thermophi... bacterial genome, however, do not conta... uch elabo... iversity and multiplicity of xylanases possibly owi... aller genom... Most of thermophilic bacterial genome has... netic ...rmation for GH 10/GH 11 xylanases (Chakdar et al., 2...6).

### 1.2. Mode of action of th...ophilic ...nases

The mode of action of t... ...nases h... ...en elucidated in excellent reviews previou... ...lins et ...2005; ...odd and Cann, 2009). Based on recent understanding on the mode of action and structure of the thermophilic xylanases belonging to different families (Table 2), the GH10 family xylanase from *Thermoascus aurantiacus* revealed the presence of $(\alpha/\beta)_8$ Tim barrel fold comprising of eight major parallel β-strands and eight major α helices. In addition to this, six short helices are present along the polypeptide chains. Side view of the molecule resembles a "Salad bowl" as the upper face of the molecule on the β-barrel side possess a greater radius due to the extended loops of β-α loops. The bottom face has a narrow radius with α-β turns (Pollet et al., 2010). The catalytic site of enzyme comprises of two glutamate residues where one acts as an acid/base catalyst and the other behaves as a nucleophile. The catalytic domain comprisin... of 250 to 450 amino acids are highly conserved in xylanases ... ...inity differences between these sub-sites significantly a... their mo... of action, substrate preference and product prefere... Bar et al., 2... In order to decipher the mechanism underl...g th... eat stabil... the crystal structure of many thermostal... xylanases ... be... resolved. The stability at high temperatu... thoug... to res... n intermolecular and intra-molecular inte...ons (li...hydrogen a...d disulfide bonds), interactions with carb...hyd...ing unit...nd stabilized C- and N-terminal ends. Th...gh these ...ractio... ...e N-terminal region increases the conf...tional stabil... revents the destabilization of proteins at hi...r t...eratures (Che... et al., 2014). Since the terminal regions of the protei... generally more flexible than other parts of the str... an increase... minal flexibility may enhance the process of d...turation and promo... irreversible aggregation. Thus, the form...n of a disulfide bond increases the surface pKa and hydrogen b...s for stabil... the N-terminal random structure, which are the ke... terminant... r the catalytic activity under conditions of high temp...re a...l (Boonyapakron et al., 2017).

The s...al elucidation of GH11 xylanase exhibits the presence of ...ytic domain that displays a β-jelly-roll architecture comprising of ...arallel β sheets and one single major α-helix forming fingers and palm. The presence of loops in between the structure depicts thumb and cord. The structure is compact and closely packed and mimics partially closed right hand. However, a unique structure for a GH11 xylanase (XynCDBFV) has been reported in ruminant fungus *Neocallimastix patriciarum*. It harbors an extended N-terminal region comprising 11 amino acids that adheres to several β strands and spanned the convex side of the palm β-sheet. The N-terminal region is attached to the catalytic core by hydrogen bonds with stacking forces along a disulfide bond between Cys-4 and Cys- 172. Through these interactions, the N-terminal region clumps the catalytic core into a more restrained structure, which confers to its stabilization at elevated temperatures (Cheng et al., 2014). The active site of thermophilic GH11 xylanase is a deep (~9 Å), narrow (~4 Å) and long (~25 Å) cleft with the catalytic dyad located in the middle, which supports the endo mechanism of GH11 xylanases. The active site residues are two molecules of glutamic acid acting as a nucleophile and an acid/base catalyst that actively participates in a double-displacement catalytic mechanism and the existence of a covalent glycosyl-enzyme intermediate complex. Xylanases from the GH11 family have low molecular weight and high pI values (Alvarez-cervantes et al., 2016).

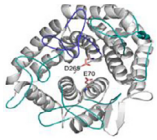
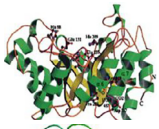
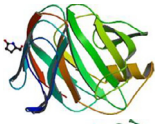
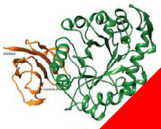
Endoxylanases, belonging to GH family30 (GH30), are classified into 8 different subfamilies of which subfamily 7 (GH30-7) is derived from fungi and subfamily 8 (GH30-8) belongs to bacterial strains and are single -domain enzymes that act on xylans that are decorated with 4-O-methylglucuronic acid (MeGlcA) moieties to yield glucouronic acid (GlcA) containing xylooligosaccharides or aldouronates (Valenzuela et al., 2012; Padhila et al., 2014). However, unlike well characterized GH30 glucurono-xylanases from *Bacillus subtilis* and *Erwinia chrysanthemi* (st John et al., 2016), xyn30D reported from the secretome of *Paenibacillus barcinonensis* is a modular enzyme comprising of carbohydrate binding module belonging to family CBM35 (Sainz-polo et al., 2014). Moreover it has been reported that recent GH30-8 xylanase from thermophilic anaerobic bacterium *Clostridium thermocellum* (CtXyn30A)

**Table 1**
Genome wi... ...ly... ...c fungi coding for diverse xylanases.

| Fungal strains | Number of genes | GH 7 | GH 10 | GH 11 | GH 30 |
|---|---|---|---|---|---|
| *Chaetomium thermophi...* | 8 | | 3 | 4 | 1 |
| *Myceliophothora sepedoni...* | 17 | | | | |
| *Myceliophothora fergussi* | 9 | | 4 | 5 | 0 |
| *Malbranchea cinnamomea* | 4 | | 3 | 1 | |
| *Rasamsonia byssochlamys* | 6 | 1 | 2 | 1 | 1 |
| *Pseudocercosporella* | 14 | | 6 | 6 | 2 |
| *Rhizomucus pusilus* | 7 | | | | |
| *Scytalidium thermophilium* | 10 | | 7 | 3 | 1 |
| *Thermoascus aurantiacus* | 1 | | 1 | | |
| *Thermomyces lanuginosus* | 1 | | | 1 | |
| *Thermomyces stellatus* | 10 | | 4 | 4 | 2 |
| *Thielavia australiensis* | 7 | | 3 | 3 | 1 |
| *Thielavia terrrestaris* | 9 | | 5 | 4 | |
| *Myceliophthora themophilum* | 13 | | 5 | 8 | |

www.fungalgenomics.ca (Prof Adrian Tsang, Principal Invest.

**Table 2**
Structure and folding topologies of xylanases of different GH families.

| GH families | Organism | Folding topology | Structure | Mechanism | References |
|---|---|---|---|---|---|
| 8 | *Paenibacillus barcinonensis* | $(\alpha/\alpha)_6$ |  | Inverting | Valenzuela et al.,2016 |
| 10 | *Thermoascus auranticus* | $(\beta/\alpha)_8$Tim barrel |  | Retaining | Pollet et al., 2010 |
| 11 | *Neocallimastix patriciarum* | β Jelly roll |  | Retaining | Ch al., 2014 |
| 30 | *Clostridium thermocellum* | $(\beta/\alpha)_8$Tim barrel |  | Retaini | eire et al., 2016 |
| 43 | *Butyrivibrio proteoclasticus* | β-propeller |  | Inver | Tillet al., 2014 |



show preference for GlcA containing substrates as well as pos 2014).
significantly high activity on wheat arabinoxylan (WAX) and se
other biomass derived polysaccharides that do not contain GlcA (Ve
et al., 2016). The three dimensional structure of endo 1–4 xylana
(CtXyn30A) showed $(\beta/\alpha)_8$ TIM domain which is spec cteristi
of clan A. This domain comprises of Val11–Pro295 ues, n a core
of eight β strands and is surrounded by eight ces. W reas, the
strongly attached side β-domain contains His3 to 0
Val386 residues. The structure analysis h vealed Glu 136 and
Glu 225 are absolutely conserved withi e subfamily re the key
constituents of the catalytic residue tance betwe ese car-
boxyl residues is less than 5Å thus suppor ntion mechanism for
hydrolysis. CtXyn30A is activ r a broad ran pH and displays an
optimum temperature of 7 The thermostabilit the enzyme may
be attributed to the pr ce of er number of salt bridges and
strong hydrogen bondin ne side chain residues (Freire et al., 2016).

GH family S th *ena* -β nases also contain cellulases, chitosanases menas The col pted xylanase act on xylan to get converted primari xylotriose and xylotetraose and was found to be most active gosaccharides. Modeling of the three-dimensional ( tructure of Rex8A from *Paenibacillus barcinonensis* BP-23 shows an ( barrel fold topology where the loops connecting the α-helices contou e active site. Moreover Rex8A enzyme has been reported for the first time that acts on branched *xylo*-oligosaccharides (Valenzuela et al., 2016). It has been proposed that like GH11 family xylanases, GH 8 xylanases contain the catalytic site in the middle alongwith the large substrate binding cleft comprising of at least six xylose binding residues. But they differ from GH10 and GH11 by their inverting single displacement reaction mechanism (Pollet et al., 2010). According to CAZy database, GH 43 family includes an array of enzymes such as β-xylosidase, α-L-arabinofuranosidase, arabinase, xylanase and galactan 1,3-β-galactosidase (Lombard et al., 2013). The structural elucidation of GH 43 family shows that proteins of this family contain five bladed β-propeller domains and possess inverting mechanism where three residues are involved in catalysis (Till et al.,

## cteristics, properties and applications of xylanases from hermophilic fungi

### 2.1. Myceliophthora strains

*M. thermophila,* a thermophilic fungus (optimum growth temperature of 45 °C) previously identified as *Chrysosporium lucknowense* has been found to be an efficient source of hemicellulolytic enzymes including xylanases (Hinzet al., 2009). The genome of *C. lucknowense* strain was annotated to contain 11 xylanases genes for GH10 and seven for GH11 (Hinz et al., 2009). Whereas, *Myceliophthora thermophila* genome was later found to contain 12 xylanase genes (Karnaouriet al., 2014). A thermophilic strain identified as *Myceliophthora* sp (IMI, 38079) by IMI Kew, UK and as *C. lucknowense* (MTCC) by Microbial Type Culture Collection, Chandigarh, India, had also been reported previously to produce functionally distinct multiple xylanases (Badhanet al., 2004). Ten different functionally diverse xylanases from *Myceliophthora* sp. were resolved electrophoretically using PAGE/IEF and were found to show characteristically different activity against unsubstituted xylans, arabinoxylans and methyl-glucouroxylan (Badhan et al., 2004). Ustinov and co-workers (2008) purified six xylanases from *C. lucknowense* (3 each of GH10 and GH11 xylanases) and found that GH11 in comparison to GH10 xylanases displayed high substrate specificity when subjected to catalysis against different xylans (Table 3). Structurally two of the GH10 xylanases (Xyl 10B and Xyl 10C) were found to be distinct with modular configuration and were tethered by CBM1 at N and C terminal, respectively. Further they found these xylanases to be catalytically versatile and exhibited high thermostability. *Myceliophothora thermophila* is also known to produce low molecular weight xylanase (14 kDa) that is active under extreme alkaline condition (Boonrung et al., 2018). *Myceliophthora* is now known to contain four species namely *M. theromophila, M. heterothallica, M. Hinulea and M. fergusis* (van de Brink et al., 2013), where *M. thermophila* is better producer of xylanases when compared to *M. heterothallica*. The

**Table 3**
Production and properties of purified xylanases from thermophilic and thermotolerant fungi.

| Fungal strain, (xylanase activity) | MW (kDa) /pI | Optimum pH/Temp | Km (mg/ml) /Vmax (U/mg/min) | GH Family | Reference |
|---|---|---|---|---|---|
| *Humicola insolens* (16.4 units/ml) | Xyn W (44.0)[w]/NR | 6.0/70 °C | 2.8 / 311.8 | GH10 | Du et al.,2013 |
| | Xyn A (41.5)[a]/NR | 6.0/80 °C | 1.6 / 974.4 | GH10 | |
| | Xyn B (42.0)[a]/NR | 7.0/70 °C | 1.1 / 306.8 | GH10 | |
| | Xyn C (44.0)[a]/NR | 6.0/70 °C | 2.1 / 287.7 | GH10 | |
| *T. lanuginosus* DSM 10,635 (1180 units/ml) | (25.5)/N.R | 6.5/70 °C | 3.85/ N.R | NR | Xiong et al.,2004Le and Wang, 2014 |
| *T. lanuginosus*[a] (36 units/ml) | (44.0)[f]/N.R | 6.0/65 °C | N.R/ N.R | GH 11 | |
| *Thermoascus aurianticus* (575.9 units/ml) | (33.0)/N.R | 4.0–4.5/70–75 °C | N.R/ N.R | GH10 | Kalogeris et al., 2001 |
| *Chaetomium thermophile* (61.1 units/ml) | Xyl I (26.0)/N.R | 5.4–6.0/ | 0.55/58.8 | NR | Ganju et al., 1989Katapodis et al., |
| | Xyl II (7.0)/N.R | 70 °C4.8–6.4/ 60 °C | 0.1/5.26 | | 2007 |
| *Melanocarpus albomyces* (415 units/ml) | Xyl I (38.0)/N.R | 6.6/65 °C | 0.30/311 | NR | Bisw[illegible] |
| | Xyl II (24.0)/N.R | 5.5/65 °C | 1.69/500 | | |
| *Myceliophthora* sp. IMI 387,099 (16.2 units/ml) | Xyl Ia (53.0)/5.2 | 6.0/75 °C | 1.63/55.5 | GH10 | [illegible]ha et al., 2004 |
| | Xyl IIa (53.0)/4.8 | 6.0/75 °C | 1.69/33.3 | GH10 | |
| *Malbranchea flava* MTCC 4889 (164 units/ml) | MFX I (25.2)/4.5 | 9.0/70 °C | 1.25/1666 | GH11 | Shar[illegible]al., 2010 |
| | MFX II (30.0)/3.7 | 9.0/70 °C | 3.7/1923 | GH[illegible] | |
| *Chrysosporiium lucknowenese* (96.6 units/ml) | Xyn 10A (47.8)/4.7 | 5.5–7.0/65–70 °C | N.D/65[d] | [illegible] | [illegible]tinov et |
| | Xyn 10a (31.0)/8.9 | 5.5–7.0/65–70 °C | N.D/83[d] | [illegible]10 | |
| | Xyn 10B (57.0)/4.4 | 5.5–7.0/80–85 °C | N.D/39[d] | [illegible] | |
| | Xyn 10b (46.0)/4.3 | 5.5–7.0/80–85 °C | N.D/85[d] | GH[illegible] | |
| | Xyn 10C(40.0)/4.8 | 5.0/80 °C | N.D/32[d] | GH1[illegible] | |
| | Xyn 11A(24.0)/7.7 | 5.5–7.0/70 °C | N.D/395[d] | GH11 | |
| | Xyn 11B (23.0)/8.4 | 5.5–7.0/65–70 °C | N.D/169[d] | GH11 | |
| | Xyn 11C (22.0)/6.7 | 4.5/65 °C | N.D/300[d] | GH11 | |
| Sporotrichum thermophile | StXyn I (24.0)/8.7 | 5.0/60 °C | NR | [illegible] | Vafiadi et al., 2010 |
| | StXyn II (48.0)/8.0 | 5.0/60 °C | | G[illegible]0 | |
| Remersonia thermophila | rtXylI (42.0)/N.R | 6.5/60 °C | 2[illegible]/5.79 | GH 10 | McPhillips et al., 2014 |

[a] recombinant xylanases;
[f] fusion protein xylanase;
[w] wild;
[d] (units/mg).



transcriptome profiling of *M. thermophilia* showed that the expressio[illegible] GH 1 xylanases was up-regulated when cultured in pres[illegible] monoc[illegible] cereals, whereas, the expression of GH10 showed [illegible] up-reg[illegible] ulation in presence of either monocots/dicots as [illegible]trate a[illegible] its level remained unchanged (Kolbusz et al., 2014). *M[illegible]rmo[illegible]* strain from Brazil (Pereiera et al., 2015) w[illegible] foun[illegible] oduce [illegible] (units/g) of xylanase on sugarcane ba[illegible], whereas [illegible]*celiophthora* sp., isolated from composting soils [illegible] was reporte[illegible] produce 2366 (units/g) of xylanase when ct[illegible]ed o[illegible] straw emp[illegible]ying solid state fermentation (Badhan et [illegible] 2007). Rec[illegible] two GH11 xylanase genes MYCTH_49824 and M[illegible]1H_56237 from[illegible] *thermophila* have been cloned and expres[illegible]n *Pich[illegible] pastoris*. The multiple template alignment for modellin[illegible]ther r[illegible]uled their catalytic domains (Basit et al., 2018).

### 2.2. Therm[illegible]es lan[illegible]osus

*Thermon[illegible] l[illegible]gu[illegible]* optimum growth temperature of 50°C is considered [illegible]e of the most prolific producer of alkaline active virtually cellulas[illegible]e thermostable xylanases that exhibited 3500 (units/ml) and 48,0[illegible] (units/g substrate) of activity under shake flask and solid substrate fermentation, respectively (Sonia et al., 2005; Kumar et al., 2009; Winger et al., 2014). However, *T. lanuginosus* strains isolated from different geographical regions vary in their xylanase production capacity. A *T. lanuginosus* strain VAPS isolated from decaying wood sample produced as low as (61 units/ml) that was improved to 132.5 (units/ml) using combination of optimization approaches such as Genetic Algorithm-Response Surface Methodology (GA-RSM), Artificial Neural Network (ANN), Genetic Algorithm-Artificial Neural Network (GAANN) (Kumar et al., 2016). Heterologous production of cloned *T. lanuginosus* gene using *E. coli* and *Pichia pastoris* as host has also been reported (Mchunu et al., 2009). Upon comparison, it was found that expression of xylanase by alkaline stable variant [illegible]38 *P. pastoris* (261. 7 ± 0.61Uml) was 545-folds higher than observed in *E. coli* (Mchunu et al., 2009). Engineering of an N-terminal disulfide bridge resulted in improved thermal performance of recombinant T. lanuginosus GH11 xylanase as evidenced by apparent upward temperature optima shift upwards at pH 6.5 by about 10 °C to 75 °C (Wang et al., 2012). *T. lanuginosus* produces low molecular weight (25.0 kDa) and highly thermostable xylanase that is active between pH 5.0 and 9.0 and has been classified as GH 11 family member. The secretome based analysis of *T. lanuginosus* grown on corn-cob based medium showed high proportion of protein as xylanase (22–30%) (Winger et al. 2014) and may be the reason for its purification using simple two-step process involving weak anion exchanger and gel filtration (Bennet et al., 1998). Recent studies have shown that the xylanase purification was also possible by partitioning using PEG1000/NaCit based two phase aqueous system. The separation based on partitioning is best because of high Kp value of 17.7 ± 0.3 thus making it applicable for industrial purification of xylanase (Loureiro et al., 2017). Xylanase from *T. lanuginosus* is commercially produced as recombinant enzyme using *Fusarium venentatum* as host organism and is marketed as Novozyme 899 as processing aid in baking industry to improve dough stability and crumb structure. Similarly, Pentopan Mono BG, a recombinant xylanase from *T. Lanuginosus* expressed in *A. oryzae,* is known to improve bread loaf volume by 41% (Butt et al., 2008). Ronozyme WX CT, an endoxylanase derived from *T. Lanuginosus* employing submerged fermentation by Novozymes A/S (Denmark), and marketed by DSM Nutritional Products (Switzerland) is authorized in the EU (Commission Regulations (EC) No 1332/2004 and No 2036/2005) as feed enzyme. The xylanase product was found to positively influence the feed conversion efficiency in non-ruminants (Nielsen et al., 2007). The treatment of Kraft pulp during milling with *T. lanuginosus* xylanase was found to partially hydrolyze xylan associated with cellulose fibres thus resulting in subtle changes in the pulp structure and enhanced susceptibility of the pulp to refining (refining energy was

significantly reduced) and improved the static strength properties of paper (Buzala et al., 2016).

### 2.3. Malbranchea strains as source of xylanase

*Malbranchea* strains produce multiple forms of thermostable alkaline active xylanases (Sharma et al., 2010). The culture produces high levels of xylanases (27193 units/g substrate) at 45°C on solidified culture medium containing rice straw as carbon source (Mahajan et al., 2014). Employing transcriptomics profiling it was observed that the expression of *M. cinnamomea* GH11 xylanase gene was up-regulated by 301.8 times when cultured on xylan and 22.79 times on wheat bran as compared to glucose (Huttner et al., 2017). The xylanases MEX-I(GH11) and MEX-II(GH10) from a thermophilic isolate *Malbranchea flava* were purified and characterized and found to be optimally active at pH 9.0 and 70°C (Sharma et al., 2010). The secretome profiling of the *M. Cinnamomea* indicated it to be a source of thermostable metal dependent glycosyl hydrolases (Mahajan et al., 2016) including xylanases that are involved in boosting the hydrolytic potential of commercial cellulase Cellic Ctec 2 during saccharification of rice straw, carrot grass and corn stover (Sharma et al.,. 2016). The cloning of 50 kDa thermostable GH10 xylanase MpXyn10A and its expression in *Aspergillus nidulans* was carried out and the expressed enzyme was optimally active at pH 5.8 and 80 °C and found to be 16% glycosylated and thermostable, preserving 85% activity after 24 h at 65 °C. Circular dichroism showed high alpha-helical content consistent with the canonical GH10 family $(\beta/\alpha)_8$ barrel fold observed in molecular modeling. The xylanase resulted in effective hydrolysis of native and pretreated sugarcane bagasse (Rebeiro et al., 2014). In a recent study, two xylanase genes (GH10 and GH11) designated as XYN10A_MALCI and XYN11A_MALCI from *Malbranchea cinnamomea*, respectively, were cloned and exp in *P. pastoris* X33. The resultant clones of GH11 and GH10 prod 573.3 and 24.3 (units/ml) of xylanase, respectively, when fed v methanol under shake flask cultures (Basotra et al., 2018) were purifi and the recombinant rXYN11A MALCI was found to at 70 °C and catalytically active against a variety of substit (ara xylans) as well as unsubstituted xylans. The recombina anases found to act synergistically with commercial cellu ases and 1.58 folds improved hydrolysis of aci d alkali ed rice straw (Basotra et al., 2018).

### 2.4. Thermoascus aurantiacus as source of xyl

*T.aurantiacus* (with op l growth temperat at 50°C), a well known source of GH10 ase, h en reported to produce xylanase both under solid state fe ta where heat bran supported 6543 (units/g substrate) (Jainet a 5) and e flask culture (130 units/ml) using corn bstrate t al., 2010). *T.aurantiacus* has also been f to ex ss xyla onstitutively using xylose as inducer wh Schue et al. (2017) showed that feeding xylose continuously in b ited in production of 80 (units/ml) of xylanase. The al structure of *T. aurantiacus* GH10 xylanases had been shown to co with xylobiose substitute with arabinofuranosyl feraloyl ester side ch and the enzyme. The enzyme showed fourfold higher substrate specificity for xylotriose which is linked to arabinose at non reducing xylan moiety compared to xylose alone (Vardakou et al., 2005). *Thermoascus aurantiacus* M-2, producing novel acidophilic and thermostable xylanase 39.07 (U/mL) was purified. The purified xylanase (31.0 kDa) was characterized to be active at 75 °C and pH 5.0 and maintained stability at 80 °C (Ping et al., 2018).

### 2.5. Scytalidium thermophilium and other thermophilic fungal strains as source of xylanases

Xylanases produced from *Humicola insolens* (syn. *S. thermophilum* and now *Mycothermus thermophilus*) being GRAS organism has been approved by FDA and is used commercially for application in food (brewery, bread, starch extraction from wheat) and feed industry. The xylanases from *H. Insolens* are capable of improving the starch extraction from wheat and is sold commercially as Shearzyme by Novozymes. The optimum growth temperature of *H. insolens* is 45 °C (Basotra et al., 2016). Four GH10 xylanases from *H. insolens* have been purified and characterized to be optimally active between 70 and 80 °C and at pH 6.0–7.0 (Du et al., 2013). Later Shi et al., (2015) cloned and expressed multi-modular GH11 xylanases form *H. insolens*Y1that was found to be alkaline tolerant.

Besides the strains cited above, different thermophilic fungus like *Thielavia terrestaris* (Berka et al., 2011; Garcia-Haunte et al., 2017), *Corynascus thermophilus* (van de Brink et a izomucor pusillus* (Huttner et al., 2018) have also been re ed as so f xylanase. *T. terrestris* was found to produce hyper mophilic act xylanase (Tt Xyn A) with molecular weight of kDa as found be optimally active at pH 5.5 and at 85 he xyla exhi d half life of 23.1 days at 65 °C. The ther tability as fou associated with gain in secondary struct t high mperature (Garcia-Haunte et al., 2017). *C. thermophilu* prev own as *ceriophthora fergusii,* reported to produce levels lanase an de Brink et al., 2013), was taken up f pression of anase for heterologous over expression i *pa* To obtain p ct expression, the 870 bp gene sequence wa codon imized and synthesized. The recombinant Xyn11A found to b timally active at pH 7.4 and 70 °C. The enz was resistant to me ions and protease such as pepsin which m s it suitable for biotechnological applications (Yang and Zhang, 2 ). *Paecilomy thermophile*, a thermotolerant fungus that had been re d to prod high levels of thermostable xylanases was cloned and ced i *pastoris*. The recombinant xylanase produced 8.1 (g/L) of pr and 2940 (U/ml) of activity in 5 L fermenter fed se- tially with methanol. The recombinant enzyme was active at 80 °C n (Fanet al., 2012). A highly catalytically efficient thermophilic xylanase belonging to family GH10 from *Achaetomium* Sp X2-8 was stable at 75 °C and at broad range of pH 4.0 to 10.0 with Kcat/ Km of 3710/m/s/mg (Zhao et al., 2013). The xylanase was comparable to Ultraflo, commercially produced from *H. insolens* by Novozyme and was used for filtration of brewing mash and was found to perform much better in combination with β-glucanase when compared to Ultraflo. Surprisingly, the genome of thermophilic fungus *Rhizomucor pusillus* which do not harbor any gene for xylanase (Huttner et al., 2018) was reported to produce 6 (Units/ml) of xylanase when cultured on beechwood xylan (Huttner et al., 2018). *R. pusillus,* isolate from maize silage, has also previously been shown to produce 824 (U/g) of xylanase which was found to be stable at 75 °C (Robeldo et al., 2016). The observed xylanase activity in the secretome can be ascribed to the broad specificity of some of other glycosyl hydrolases like GH5 and even auxiliary activity proteins in the secretome against the xylan as substrate (Fromhaggen et al., 2016). *Talaromyces emersonii* now renamed as *Rasmasonia emersonii* is an important source of xylanases that is commercially produced by Novozymes DSM and is in the list of food enzymes approved for use in mashing process in breweries as well as biorefineries. A thermotolerant strain *T. Leycettanus,* reported as a novel source of the thermostable GH10 xylanase, was cloned and expressed in *P. pastoris.* The purified recombinant TlXyn10A was acidic and hyperthermophilic and retained stability over the pH range of 2.0–6.0 and at 90 °C (Wang et al., 2017a,b). Later Wang and co-workers (2017) prepared three mutants of GH10 xylanases by swapping gene of a xylanase of another fungal strain *Bispora* sp., MEY-1 reported to be source of thermostable xylanases and showed site directed mutation of E229I, F232E and G145D resulted in weakened substrate specificity and improved catalytic efficiency. *Melanocarpus albomyces*, a thermophilic and non-sporulating fungus that is considered as proficient strain for production of alkaline active xylanase with application in paper and pulp industry was subjected to strain development. The mutant *M. albomyces* IITD3A produced 415 (IU/mL) xylanase on alkaline lignocellulosic

extract in a 14 L bioreactor with volumetric productivity of 11,530 (IU/L/h),which was 8-fold higher than that of the wild-type strain (Biswas et al., 2010, Gupta et al., 2013). The cyclic maintenance of pH of fermentation medium between 7.8 and 8.2 increased the productivity to 16, 670 (IU/L/h), it was found that change in the fungal morphology to a pellet form resulted in very high xylanase productivity of 22,000 IU/L/h (Biswas et al., 2010a,b).

## 3. Thermostable xylanases from thermophilic bacteria

### 3.1. Geobacillus strains as source of thermostable xylanase

*Geobacillus* perhaps is the most widely reported thermophilic bacteria for its ability to produce thermostable xylanase. *Geobacillus* sp. WSUCEF1 (grows optimally at 60°C) isolated from soil (Bhalla et al., 2015) and strain DUSELR 13 isolated from deep gold mines of South Dakota (Bibra et al., 2018) have been reported for producing highly thermostable enzyme whereas xylanase from the former strain showed half life of 18 and 12 days at 60 °C and 70 °C, respectively, while DUSELR 13 produced 31 (U/ml) after optimization and exhibited t ½ of 13 days at 60 °C and 70 °C. The enzymes also showed better hydrolysis of beechwood xylan when compared to commercial xylanases Cellic HTEC 2 and Accelarase XY. A GH10 xylanase from *Geobacillus* sp WSUCFI cloned and expressed in *E. coli* exhibited 461 (units/mg) of specific activity and exhibited t ½ at 60 °C after 60 h (Bhalla et al., 2015). *G. stearothermophilus* KIBGE-IB29, a soil isolate was found to produce xylanase optimally at 60 °C and at pH 6.0 after 24 h of incubation period (Bibi et al.,2014).The GH10 xylanase from *G. stearothermophilus* and *Rhodothermus marinus* (Mathew et al., 2018) produced *xylo*-oligosaccharides (XOS) and *arabino*-xylooligosaccharides (AXOS) such as A3X, A2XX and A2 + 3XX. The strain produced a 45 kDa xylanase; however unlike *G. Stearothermophilus*, xylose was not an inducer of the resultant xylanase (Gerasimova & Kuisiene, 20). The xylanase expression in *G. stearothermophilus* was found to be controlled by multiple regulatory mechanisms including XylR as master negative regulator in addition to carbon catabolite repression. The genes for xylanase expression were positively regulated in presence of xylose as inducer which negates the effect of XylR. Quorum sensing played an important role as evidenced from 3 fold increase in expression of xylanase during exponential growth phase (Talami et al., 2014). *G. galactosidasius* BS61 isolated from geothermal source in Turkey producing (15 U/ml) xylanase was purified and applied for clarification of juices of orange and pomegranate evident by reduced turbidity (Sari et al., 2018). *Geobacillus thermodenitrificans* JK1 is another well studied strain for the production of xylanase that was shown to produce two xylanases (XynA and XynA2) isoforms that synergistically act with β-xylosidase and arabinofuranosidase from efficient hydrolysis of birchwood xylan (Irfan et al., 2017). The xylanase gene GtxynA1 of *G. thermodenitrificans* was found to be associated with HUS (hemicellulose utilization) locus. This GH10 family protein was optimally active at pH of 3.0–9.0 and its stability was shown to be improved by applying protein engineering where mutants of *G. thermodenitrificans* with enhanced temperature by 11 °C substituting histidine, tryptophan and aspartic acid with glutamic acid and proline (H82E/ W185P/ D186E) was reported (Irfan et al., 2018).

### 3.2. Caldicellulosiruptor as source of thermostable xylanase

*C. bescii*. *C. lactoaceticus* and *C. owensensis* have been reported for the secretion of thermostable xylanases in recent past (An et al., 2015, Jia et al., 2014; Liu et al., 2017). The GH10 xylanase from *C. bescii* was over-expressed in *E. coli* and found to exhibit high catalytic activity with half life of 7.7 h at 60 °C and the enzyme was optimally active at 70 °C at pH 7.0 with 85% activity at pH 4.0 and 12.0 (Anet al., 2014). The N-terminal domain of GH10 xylanase from *C. bescii* was found to be multidomain cbxyn10C/cel48B capable of degrading crystalline cellulose besides xylan and barley β-glucan (Xue et al., 2015), thus making it useful candidate for bioconversion. *C. lactoaceticus* is also known to express GH10 xylanase (47 kDa) that exhibited good stability at 80 °C at pH4.5. The enzyme was shown to liberate branched XOS with methyl-glucouronic acid sub-chains (Jia et al., 2014). Recently, *C. owensensis* has been shown to produce highly thermostable xylanase which is optimally active at 90 °C. The recombinant CoXynA was shown to exhibit half life of 1 h at 80 °C. The authors further solved crystal structure of the recombinant xylanase. The relative high thermostability of the enzyme was proposed to be due to increased overall protein rigidity resulting from reduced length and fluctuation of loop 7 (Liu et al., 2017). The hemicellulase from *C. owensensis* was capable of degrading hemicellulose of native corn stover efficiently (Peng et al., 2015). *Herbivorax saccincola* AZ isolated through enrichment culture using untreated corn stover as carbon source was found to grow optimally at 55 °C and at alkaline pH (9). The draft genome of the culture revealed the presence of GH11 xylanase and essential genes for xylose metabolism, xylose isomerase, xylose transporter etc. (Aikawa et al., 2018). A high xylanase producing *Caldicellulosiruptor algeriensis* isolated from hydrothermal spring of Guelma produced 250 (U/ml) of xylanase in a medium comprising birchwood and oat spelt xylan at 70 °C in 24 h (Amel et al., 2016). Modular trifunctional xylanolytic Axy43A has been reported from *Paenibacillus curdlanolyticus* strain B-6. This GH43 hydrolase with family 6 carbohydrate binding module exhibits endoxylanase, xylosidase and arabinoxylan hydrolyzing activities (Veeravivattanaki et al., 2016). Modular family 8 xylanase from marine bacteria *Glaciecola mesophila* was found to have new type of CBM with eight strands (Chen et al., 2018). Improved expression of *Thermoanaerobacterium aotearoense* SCUT27 GH10 xylanase harbouring CBM three surface layer homology domains (Huang et al., 2015) expressed in *B. subtilis* was optimally active at 80 °C and at pH . The hydrolysis of beechwood xylan resulted in xylobiose and methyl-glucurono-xylotriose as the main products.

### 3.3. Thermopolyspora flexuosa as source of industrial xylanase

A GH10 xylanase from *Thermopolyspora flexuosa* (*Nonomuraea flexuosa*) DSM43186 which is an actinomycete known to produce thermophilic xylanase exhibited temperature optima of 65–70 °C against insoluble xylan and 75–80 °C in presence of 3% xylan was found to be stabilized in presence of ionic liquid [EMIM]OAc at inactivating temperatures (80–90 °C). The increased stability of TfXYN10A indicated to the binding of IL molecules that stabilized the protein structure. Furthermore, the xylanase exhibited low $K_m$ (~1 mg/mL) demonstrated higher tolerance to ILs than xylanases with higher $K_m$ (Anbarasan et al., 2017). The GH10 xylanase from *T. flexuosa*was was taken up for commercial production. The gene was cloned and expressed in *Trichoderma reesei* by AB enzymes. The enzyme produced in GRAS organism has been cleared by Canadian government for application in baking, brewery, potable alcohol and grain processing (GRAS Notice No. GRN000628). The recombinant xylanase produced by *T. Reesei* at industrial scale was subjected to ultrafiltration and spray drying and is marketed in powdered form that has an activity of 2,28,200 (units/g).

In addition, some of the novel thermophilic bacterial strains capable of producing xylanase including, *Caldoprocter, Herbinix*, *Herbivorax saccinola* from bovine manure as well as biogas digester beside *Rhodothermus marinus* from marine environment have also been reported (Amel et al., 2016; Mechelke et al., 2017; Aikawa et al., 2018; Mathew et al., 2018).

## 4. Metagenomic DNA as source of thermostable xylanases

Owing to the search for novel enzymes for industrial exploitation, metagenomic approaches can overcome the limitations of culture-dependent methods and can facilitate the discovery of enzymes from

uncharacterized microorganisms. The screening of metagenomic libraries coding for xylanases has resulted in few novel ORF's coding for thermostable xylanases. The environmental DNA isolated from different niches such as insect gut, manure, waste water, hot environmental samples, chicken cecum, crater of Avachinsky volcano have been reported as sources of xylanases (Mientus et al., 2013). The xylanases from the chicken cecum metagenome was found to require high concentration of salt and organic solvents for their activity (Darkazali et al., 2017). Furthermore it was proposed that poultry feed is primarily composed of high ratio of non-starch polysaccharides that include xylans and arabinoxylans so the microorganisms capable of hydrolyzing these polysaccharides should be abundant in chicken intestine. A novel alkali-stable and thermostable GH11 endoxylanase encoding gene (Mxyl) was retrieved by functional screening of a compost soil metagenome. The recombinant xylanase (1077 bp) exhibited activity at 80 °C and pH 9.0 (Verma et al., 2013). The thermostability of this enzyme was subsequently engineered through the enrichment of surface β-sheets with arginine residues by substituting serine/threonine by site directed mutagenesis (Verma and Satyanarayana, 2013) whereas xylanases from volcano metagenome was found to be maximally active at 95 °C and exhibited half life of 22 h at 85 °C (Mientus et al., 2013). Similarly, the expression of xylanase gene xyl7 derived from termite gut, in *E. coli* showed higher thermostability with 10 °C increase in optimal temperature and was active under broad pH range of 5.5–10.0 (Qian et al., 2015). Ming-Zhe et al. (2015) cloned xylanase gene from metagenomic DNA of cow dung and expressed in *B. Megaterium*. The recombinant xylanase was found to be optimally active at pH7 and 75 °C.

## 5. Conclusion

Bioprospecting of new genes for novel xylanases from diverse [e]teria and metagenomics can be exploited for future applicati[ons]. Furthermore, through taking advantage of rapid development genome editing and synthetic biological techniques [in] the en[zy]me producing microorganisms are compelling st[ud]ies to [ex]plore the growing importance of thermophilic xylanase[s] in vario[us] industrial applications. The development of technolog[y] base[d] is foreseen as they will be able to cater [to] the grow[ing] needs of the industry. Therefore this area of rese[arch] with expect[ed] increase in market share by three folds in next [few] y[ears] will be hotly pursued.

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## Referenc[es]

Ahmed, Z., Butt, M[.S.], hmed, A., Riaz, M., Sabir, S.M., Rehman, F.U., 2014. Effect of *Aspergillus niger* [xy]e on dough characteristics and bread quality attributes. J. Food Sci. Technol. [51], 45–2453.

Aikawa, S., Baramee, S., S[it]nsathanaswadi, J., Thianheng, P., Tachaapaikoon, C., Shikata, A., Waeonukul, R., Pason, P., Ratanakhanokchai, K., Kosugi, A., 2018. Characterization and high-quality draft genome sequence of *Herbivorax saccincola* A7, an anaerobic, alkaliphilic, thermophilic, cellulolytic, and xylanolytic bacterium. Sys. Appl. Microbiol. 41, 261–269.

Álvarez-Cervantes, J., Díaz-Godínez, G., Mercado-Flores, Y., Gupta, V.K., Anducho-Reyes, M.A., 2016. Phylogenetic analysis of β-xylanase SRXL1 of *Sporisorium reilianum* and its relationship with families (GH10 and GH11) of Ascomycetes and Basidiomycetes. Sci. Rep. 6, 24010.

Amel, B.D., Nawel, B., Khelifa, B., Mohammed, G., Manon, J., Salima, K.G., Farida, N., Hocine, H., Bernard, O., Jean-Luc, C., Marie-Laure, F., 2016. Characterization of a purified thermostable xylanase from *Caldicoprobacter algeriensis* sp. nov. strain TH7C1T. Carbohydr. Res. 419, 60–68.

An, J., Xie, Y., Zhang, Y., Tian, D., Wang, S., Yang, G., Feng, Y., 2015. Characterization of a thermostable, specific GH10 xylanase from *Caldicellulosiruptor bescii* with high catalytic activity. J. Mol. Catal B: Enzym. 117, 13–20.

Anbarasan, S., Wahlström, R., Hummel, M., Ojamo, H., Sixta, H., Turunen, O., 2017. High stability and low competitive inhibition of thermophilic *Thermopolyspora flexuosa* GH10 xylanase in biomass-dissolving ionic liquids. Appl. Microbiol. Biotechnol. 101, 1487–1498.

Badhan, A.K., Chadha, B.S., Kaur, J., Saini, H.S., Bhat, M.K., 2007. Production of multiple xylanolytic and cellulolytic enzymes by thermophilic fungus *Myceliophthora* sp. IMI 387099. Bioresour. Technol. 98, 504–510.

Badhan, A.K., Chadha, B.S., Sonia, K.G., Saini, H.S., Bhat, M.K., 2004. Functionally diverse multiple xylanases of thermophilic fungus *Myceliophthora sp.* IMI 387099. Enzy. Microb. Technol. 35, 460–466.

Bar, M., Golan, G., Nechama, M., 2004. A new crystal form of XT6 enables a significant improvement of its diffraction quality and resolution. Acta. Crystallogr. D.60, 545–549.

Basit, A., Liu, J., Miao, T., Zheng, F., Rahim, K., Lou, H., Jiang, W., 2018. Characterization of two Endo-β-1, 4-Xylanases from *Myceliophthora thermophila* and their saccharification efficiencies, Synergistic with Commercial C[ellulase] ront. Microbiol. 9, 233.

Basotra, N., Joshi, S., Satyanarayana, T., Pati, P.K. [...] a, B.S., 2018. Expression of catalytically efficient xylanases [...] thermophi[lic] gus *Malbranchea cinnamomea* for synergistically enhancing [...] ysis of lignocel[lu]ics. Int. J. Biol. Macromol. 108, 185–192.

Basotra, N., Kaur, B., Falco, M. Di., Tsa[ng] Chad[ha] 2016. M[y]thermus thermophilus (Syn. Scytalidium therm[o]um): Reper[t]of a di[verse] array of efficient cellulases and hemicellulases i[n] secretom[e] revea[l] ur. Technol. 222, 413–421.

Bennett, N.A., Ryan, J., Biely, P[.], nska, [...] Kremnicky, L., Macris, B.J., Kekos, D., Christakopoulos, P., K[o]podi[s], [...]ssens, M. [...]nckx, W., 1998. Biochemical and catalytic prope[rties] of an en[do]nase pur[ified] from the culture filtrate of *Thermomyces lan[u]sus* ATCC 468[...]bo[l]. Res. 306, 445–455.

Bhalla, A., Bischo[fs], ani, R.K., 2015. [...] thermostable xylanase production from a the[rmo]ilic [Geo]cillus *sp.* strain [WC]UCF1 utilizing lignocellulosic biomass. Front. Bioen[g]. Biotech[nol]. 84.

Berka, R.M., Grigoriev, I.V., O[...]R., Salamov, A., Grimwood, J., Reid, I., Ishmael, N., Jo[hn]rmond, C., Moi[...]C., Henrissat, B., 2011. Comparative genomic [an]ysis of the thermophilic bi[omass]-degrading fungi *Myceliophthora thermophila* and *[C.] lelavia terrestris*. Nat. Biotechnol. 29, 922–927.

Bi[...], Ansari, A., [...], R.R., Aman, A., Qader, S.A.U., 2014. Production of xylan [de]grading endo-[1,4]-xylanase from thermophilic *Geobacillus stearothermophilus* [KF]-IB29. J. R[oyal] [...]t. Res. Appl. Sci. 7, 478–485.

Bibra[...] [...]areddy, Sani, R., 2018. Thermostable Xylanase Production by *Geobacillus sp.* S[train] [WC]R13, and Its Application in Ethanol Production with Lignocellulosic Biomass. Microorganisms 6, 93.

[...], Sahai, V., Mishra, S., Bisaria, V.S., 2010a. Bioprocess strategies for enhanced [pro]ion of xylanase by *Melanocarpus albomyces* IITD3A on agro-residual extract. J. Biosci. Bioeng. 110, 702–708.

Biswas, R., Sahai, V., Mishra, S., Bisaria, V.S., 2010b. Development of mutants of *Melanocarpus albomyces* for hyperproduction of xylanase. Biotechnol. Bioproc. Eng. 15, 800–809.

Boonrung, S., Katekaew, S., Mongkolthanaruk, W., Aimi, T., Boonlue, S., 2018. Purification and characterization of low molecular weight extreme alkaline xylanase from the thermophilic fungus *Myceliophthora thermophila* BF1-7. Mycosci. 57, 408–416.

Boonyapakron, K., Jaruwat, A., Liwnaree, B., 2017. Structure-based protein engineering for thermostable and alkaliphilic enhancement of endo-b-1,4-xylanase for applications in pulp bleaching. J Biotech. 259, 95–102.

Butt, M.S., Tahir-Nadeem, M., Ahmad, Z., Sultan, M.T., 2008. Xylanases and their applications in baking industry. Food Technol. Biotechnol. 46.

Buzała, K.P., Przybysz, P., Kalinowska, H., Derkowska, M., 2016. Effect of cellulases and xylanases on refining process and kraft pulp properties. PloS one 11, 0161575.

Chadha, B.S., Badhan, A., Mello, F., Bhat, M.K., 2004. Two endoxylanases active and stable at alkaline pH from newly isolated thermophilic fungus, *Myceliophthora* sp.IMI 387099. J. Biotechnol. 109, 227–237.

Chakdar, H., Kumar, M., Pandiyan, K., Singh, A., Nanjappan, K., Kashyap, P.L., Srivastava, A.K., 2016. Bacterial xylanases:biology to biotechnology. 3. Biotech. 6, 150.

Chen, X.L., Zhao, F., Yue, Y.S., Zhang, Y.Z., Li, P.Y., 2018. A new group of modular xylanases in glycoside hydrolase family * from marine bacteria. Appl. Environ. Microbiol. 84, 01785–1818.

Cheng, Y.S., Chen, C.C., Huang, C.H., 2014. Structural analysis of a glycoside hydrolase family 11 xylanase from *Neocallimastix patriciarum*: insights into the molecular basis of a thermophilic enzyme. J Biol Chem. 289, 11020–11028.

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

Darkazali, H.A.L., Meevootisom, V., Isarangkul, D., Wiyakrutta, S., 2017. Gene Expression and Molecular Characterization of a Xylanase from Chicken Cecum Metagenome. Int. J. Microbiol. https://doi.org/10.1155/2017/4018398.

Dodd, D., Cann, I.O., 2009. Enzymatic deconstruction of xylan for biofuel production. GCB Bioenergy 1, 2–17.

Du, Y., Shi, P., Huang, H., Zhang, X., Luo, H., Wang, Y., Yao, B., 2013. Characterization of three novel thermophilic xylanases from *Humicola insolens* Y1 with application potentials in the brewing industry. Bioresour. Technol. 130, 161–167.

Fan, G., Katrolia, P., Jia, H., Yang, S., Yan, Q., Jiang, Z., 2012. High-level expression of a xylanase gene from the thermophilic fungus *Paecilomyces thermophila* in *Pichia pastoris*. Biotechnol. Lett. 34, 2043–2048.

Freire, F., verma, A., Bule, P., Alves, V.D., Fontes, C.M.G., Goyal, A., Najmudin, S., 2016. Conservation in the mechanism of glucuronoxylan hydrolysis revealed by the structure of glucuronoxylan xylanohydrolase (CtXyn30A) from *Clostridium thermocellum*.

Str. Biol. D72, 1162–1173.
Frommhagen, M., Koetsier, M.J., Westphal, A.H., Visser, J., Hinz, S.W., Vincken, J.P., Berkel, W.J., Kabel, M.A., Gruppen, H., 2016. Lytic polysaccharide monooxygenases from *Myceliophthora thermophila* C1 differ in substrate preference and reducing agent specificity. Biotechnol. Biofuels. 9, 186.
Ganju, R.K., Vithayathil, P.J., Murthy, S.K., 1989. Purification and characterization of two xylanases from *Chaetomium thermophile* var. coprophile. Can. J. Microbiol. 35, 836–842.
Garcia- Haunte, Y., Cayetano- Cruz, M., Hernandez, A.S., Ramirez, C.C., Moreno, R.M., Campos, J.E., Osorio, G.A., Cardoza, C.G.B., Estrada, S.T., Lara, M.E.H., 2017. The thermophilic biomass-degrading fungus *Thielavia terrestris* Co3Bag! Produces a hyperthermophilc and thermostable β-1,4-xylanase with exo- and endo-activity. Extremophiles 21, 175–186.
Gerasimova, J., Kuisiene, N., 2012. Characterization of the novel xylanase from the thermophilic *Geobacillus thermodenitrificans* JK1. Microbiol. 81, 418–424.
Gupta, G., Sahai, V., Gupta, R.K., 2013. Optimization of xylanase production from *Melanocarpus albomyces* using wheat straw extract and its scale up in stirred tank bioreactor. Ind. J. Chem. Technol. 20, 282–289.
Hinz, S.W., Pouvreau, L., Joosten, R., Bartels, J., Jonathan, M.C., Wery, J., Schols, H.A., 2009. Hemicellulase production in *Chrysosporium lucknowense* C1. J. Cereal Sci 50, 318–323.
Huang, D., Liu, J., Qi, Y., Yang, K., Xu, Y., Feng, L., 2017. Synergistic hydrolysis of xylan using novel xylanases, β-xylosidases, and an α-l-arabinofuranosidase from *Geobacillus thermodenitrificans* NG80-2. Appl. Microbiol. Biotechnol. 101, 6023–6037.
Huang, X., Li, Z., Du, C., Wang, J., Li, S., 2015. Improved expression and characterization of a multidomain xylanase from *Thermoanaerobacterium aotearoense* SCUT27 in *Bacillus subtilis*. J. Agricul. Food Chem. 63, 6430–6439.
Hüttner, S., Granchi, Z., Nguyen, T.T., van Pelt, S., Larsbrink, J., Thanh, V.N., Olsson, L., 2018. Genome sequence of *Rhizomucor pusillus* FCH 5.7, a thermophilic zygomycete involved in plant biomass degradation harbouring putative GH9 endoglucanases. Biotechnol. Rep. 20, 00279.
Hüttner, S., Nguyen, T.T., Granchi, Z., Chin-A-Woeng, T., Ahrén, D., Larsbrink, J., Thanh, V.N., Olsson, L., 2017. Combined genome and transcriptome sequencing to investigate the plant cell wall degrading enzyme system in the thermophilic fungus *Malbranchea cinnamomea*. Biotechnol. Biofuels 10, 265.
Irfan, M., Gonzalez, C.F., Raza, S., Rafiq, M., Hasan, F., Khan, S., Shah, A.A., 2018. Improvement in thermostability of xylanase from *Geobacillus thermodenitrificans* C5 by site directed mutagenesis. Enz. Microb. Technol. 111, 38–47.
Jain, K.K., Dey, T.B., Kumar, S., Kuhad, R.C., 2015. Production of thermostable hydrolases (cellulases and xylanase) from *Thermoascus aurantiacus* RCKK: a potenti fungus. Bioprocess Biosys. Eng. 38, 787–796.
Jia, X., Mi, S., Wang, J., Qiao, W., Peng, X., Han, Y., 2014. Insight into glycoside drolases for debranched xylan degradation from extremely thermophilic bacter *Caldicellulosiruptor lactoaceticus*. PloS one 9, e106482.
Kalogeris, E., Christakopoulos, P., Vršanská, M., Kekos, D., Biely, P B.J., 200 Catalytic properties of the endoxylanase I from *Thermoascus* Mol. Catal. B: Enz. 11, 491–501.
Karnaouri, A., Topakas, E., Antonopoulou, I., Christakopoul 2014. G ic insights into the fungal lignocellulolytic system of *Myceliophthor* Microbiol. 5, 281.
Katapodis, P., Christakopoulou, V., Kekos, D., Chri oulos, P., Optimization of xylanase production by Chaetomium therm m in wheat str g response surface methodology. Biochem. Eng. J. 3
Kolbusz, M.A., Di Falco, M., Ishmael, N., M teau, isan, M.C., da Silva Baptista, C., Powlowski, J., Tsang, A., 2014. Transcriptome a proteome analysis of utilization of plant-derived biomas *yceliophthora th ila*. Fungal Genet. Biol. 72, 10–20.
Kumar, K.S., Manimaran, A., P u, K., Si S., 2009. Production of β-xylanase by a *Thermomyces lanuginosu* 34 mut n corn cobs and its application in biobleaching of bagasse pulp sc eng. 107 94–498.
Kumar, V., Marin-Navarro, J., Sh 2016. Th table microbial xylanases for pulp and paper : trend licatio d further perspectives. W.J. Microbiol. Bi 34.
Le, Y., Wang, 14. Hi vel soluble ssion of a thermostable xylanase from thermo fungus *T omyces lanugi osus* in *Escherichia coli* via fusion with OsmY protein. Ex
Leisola, M., Jok stinen, O., nen, O., Schoemaker, H., 2002. Industrial use of enzymes. In: E pedia of Life Support Systems (EOLSS), EOLSS Publishers Co. Oxford UK.
Liu, X., Liu, T., Zhang, Y F., Mi, S., Wen, B., Gu, T., Shi, X., Wang, F., Sun, L., 2017. Structural insights into the thermophilic adaption mechanism of endo-1, 4-β-xylanase from *Caldicellulosiruptor owensensis*. J. Agric. food Chem. 66, 187–193.
Lombard, V., Golaconda Ramulu, H., Drula, E., Coutinho, P.M., Henrissat, B., 2013. The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res. 42, D490–D495.
Loureiro, D.B., 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. Purify. 129, 25–30.
Mahajan, C., Basotra, N., Singh, S., Di Falco, M., Tsang, A., Chadha, B.S., 2016. *Malbranchea cinnamomea*: a thermophilic fungal source of catalytically efficient lignocellulolytic glycosyl hydrolases and metal dependent enzymes. Bioresour. Technol 200, 55–63.
Mahajan, C., Chadha, B.S., Nain, L., Kaur, A., 2014. Evaluation of glycosyl hydrolases from thermophilic fungi for their potential in bioconversion of alkali and biologically treated *Parthenium hysterophorus* weed and rice straw into ethanol. Bioresour. Technol. 163, 300–307.
Mathew, S., Aronsson, A., Karlsson, E.N., Adlercreutz, P., 2018. Xylo-and arabinoxylooligosaccharides from wheat bran by endoxylanases, utilisation by probiotic bacteria, and structural studies of the enzymes. Appl. Microbiol Biotechnol. 102, 3105–3120.
Mchunu, N.P., Singh, S., Permaul, K., 2009. Expression of an alkalo-tolerant fungal xylanase enhanced by directed evolution in *Pichia pastoris* and *Escherichia coli*. J. Biotechnol. 141, 26–30.
McPhillips, K., Waters, D.M., Parlet, C., Walsh, D.J., Arendt, E.K., Murray, P.G., 2014. Purification and characterisation of a β-1, 4-xylanase from *Remersonia thermophila* CBS 540.69 and its application in bread making. Appl. Biochem Biotechnol. 172, 1747–1762.
Mechelke, M., Koeck, D.E., Broeker, J., Roessler, B., Krabichler, F., Schearz, W.H., Zverlov, V.V., Liebl, W., 2017. Characterization of the arabinoxylan-degrading machinery of the thermophilic bacterium *Herbinixhemicellulosilytica*-six new xylanase, three arabinofuranosidases and one xylosidase. J. Biotechnol 257, 122–130.
Mientus, M., Brady, S., Angelov, A., Zimmerman, P., W Schuldes, A., Daniel, R., Liebl, W., 2013. Thermostable Xylanase and ca ived from the Metagenome of the Avachinsky Crater in Ka ka (Russia) s://doi.org/10.2174/22115501029991312815025 7.
Ming-zhe, S., Hong-chen, Z., Ling-cai, M., Jun-she i, S., Yun-jua , Hai-sheng, P., Zheng, Y., Xiu-qing, Z., Jing-sheng, -han, ing, L., 2 Direct cloning, expression of a thermostable xyla gene from t gen DNA of cow dung compost and enzymatic produ of xylo gosacca corn cob. Biotechnol. Lett. 37, 1877–
Nagar, S Anuradha, Mittal, Dav Kum 012. Lalit Kumar, Vijay Kumar Gupta. Immobilization of xyla se o hyde acti d aluminum oxide pellets for increasing digestibi poultry Process em. 47, 1402–1410.
Nielsen, P.H., Oxenb M., Wenzel, H to-gate environmental assessment of enzyme pr duced industria mark by Novozymes A/S. Int. J. Life Cycle Ass. 2 2.
Oliveira, D.S., Mo erb-Din anco, C.M., Gomes, E., Da-Silva, R., 2010. Production of crude xylanase from *Ther s aurantiacus* CBMAI 756 aiming the baking process. J 75, 588–594.
Pad .Q., Valenzuela Mayorga, Grisi, T.C., Díaz Lucea, P., de Araújo, D.A., Pastor sco, F.I.J., 2014. A glucuronoxylan-specific xylanase from a new *Paenibacillus visporus* strain ted from tropical soil of Brazil. Int. Microbiol. 17, 175–184.
Pe Qiao, W., M Jia, X., Su, H., Han, Y., 2015. Characterization of hemicellulase cellulase fro e extremely thermophilic bacterium *Caldicellulosiruptor owensensis* and their ential application for bioconversion of lignocellulosic biomass with ment. Biotechnol. Biofuels 8, 131.
Pereira, J.C., Marques, N.P., Rodrigues, A., Oliveira, T.B., Boscolo, M., da Silva, R., s, E., Martins, B., 2015. Thermophilic fungi as new sources for production of and xylanase with potential use in sugarcane bagasse saccharification. J. Appl. Microbiol. 118, 928–939.
Ping, L., Wang, M., Yuan, X., Cui, F., Huang, D., Sun, W., Zou, B., Huo, S., Wang, H., 2018. Production and characterization of a novel acidophilic and thermostable xylanase from *Thermoascus aurantiacus*. Int. J. Biol Macromol. 109, 1270–1279.
Pollet, A., Delcour, J.A., Courtin, C.M., 2010. Structural determinants of the substrate specificities of xylanases from different glycoside hydrolase families. Crit. Rev. Biotechnol. 30, 176–191.
Qian, C., Liu, N., Yan, X., Wang, Q., Zhaou, Z., Wang, Q., 2015. Engineering a high performance metagenomic derived novel xylanase with improved soluble protein yield and thermostability 70, 35–41.
Ribeiro, L.F., De Lucas, R.C., Vitcosque, G.L., Ribeiro, L.F., Ward, R.J., Rubio, M.V., Damásio, A.R., Squina, F.M., Gregory, R.C., Walton, P.H., Jorge, J.A., 2014. A novel thermostable xylanase GH10 from *Malbranchea pulchella* expressed in *Aspergillus nidulans* with potential applications in bioTechnol. Biotechnol. Biofuels 7, 115.
Robledo, A., Aguilar, C.N., Belmares-Cerda, R.E., Flores-Gallegos, A.C., Contreras-Esquivel, J.C., Montañez, J.C., Mussatto, S.I., 2016. Production of thermostable xylanase by thermophilic fungal strains isolated from maize silage. CyTA-J. Food 14, 302–308.
Sainz-Polo, M.A., Valenzuela, S.V., González, B., Pastor, F.J., Sanz-Aparicio, J., 2014. Structural analysis of glucuronoxylan specific Xyn30D and its attached CBM35 domain give insights into the role of modularity in specificity. J. Biol. Chem. M114.
Sari, B., Faiz, O., Genc, B., Sisecioglu, M., Adiguzel, A., Adiguzel, G., 2018. New xylanolytic enzyme from *Geobacillus galactosidasius* BS61 from a geothermal resource in Turkey. Int. J Biol. Macromol. 119, 1017–1026.
Schuerg, T., Prahl, J.P., Gabriel, R., Harth, S., Tachea, F., Chen, C.S., Miller, M., Masson, F., He, Q., Brown, S., Mirshiaghi, M., 2017. Xylose induces cellulase production in *Thermoascus aurantiacus*. Biotechnol. Biofuels. 10, 271.
Sharma, M., Chadha, B.S., Saini, H.S., 2010. Purification and characterization of two thermostable xylanases from *Malbranchea flava* active under alkaline conditions. Bioresour. Technol. 101, 8834–8842.
Sharma, M., Mahajan, C., Bhatti, M.S., Chadha, B.S., 2016. Profiling and production of hemicellulases by thermophilic fungus Malbranchea flava and the role of xylanases in improved bioconversion of pretreated lignocellulosics to ethanol. 3 Biotech, 6, p. 30.
Shi, P., Du, Y., Yang, H., Huang, H., Zhang, X., Wang, Y., Yao, B., 2015. Molecular characterization of a new alkaline-tolerant xylanase from *Humicola insolens* Y1. BioMed. Res. Int.
Shulami, S., Shenker, O., Langut, Y., Lavid, N., Gat, O., Zaide, G., Zehavi, A., Sonenshein, A.L., Shoham, Y., 2014. Multiple regulatory mechanisms control the expression of the *Geobacillus stearothermophilus* gene for extracellular xylanase. J. Biol. Chem. M114.
Singh, S., Madlala, A.M., Prior, B.A., 2003. *Thermomyces lanuginosus*: properties of strains and their hemicellulases. FEMS Micobiol. Rev. 27, 3–16.
Sonia, K.G., Chadha, B.S., Saini, H.S., 2005. Sorghum straw for xylanase hyper-production by *Thermomyces lanuginosus* (D2W3) under solid-state fermentation. Bioresour.

Technol. 96, 1561–1569.
St John, F.J., Crooks, C., Dietrich, D., Hurlbert, J., 2016. Xylanase 30 A from *Clostridium thermocellum* functions as a glucuronoxylan xylanohydrolase. J. Mol. Catal. B: Enzy. 133, S445–S451.
Teeravivattanakit, T., Baramee, S., Phitsuwan, P., Waeonukul, R., Pason, P., Tachaapaikoon, C., Sakka, K., Ratanakhanokchai, K., 2016. A Novel Trifunctional Xylanolytic Enzyme Axy43A from *Paenibacillus curdlanolyticus* B-6 Exhibiting Endo-Xylanase, β-d-Xylosidase, and Arabinoxylan Arabinofuranohydrolase Activities. Appl. Environ. Microbiol. AEM-02256.
Till, M., Goldstone, D., Card, G., Attwood, G.T., Moon, C.D., Arcus, V.L., 2014. Structural analysis of the GH43 enzyme Xsa43E from *Butyrivibrio proteoclasticus*. Struc. Comm. F70, 1193–1198.
Ustinov, B.B., Gusakov, A.V., Antonov, A.I., Sinitsyn, A.P., 2008. Comparison of properties and mode of action of six secreted xylanases from *Chrysosporium lucknowense*. Enz. Microb. Technol 43, 56–65.
Vafiadi, C., Christakopoulos, P., Topakas, E., 2010. Purification, characterization and mass spectrometric identification of two thermophilic xylanases from *Sporotrichum thermophile*. Proc. Biochem. 45, 419–424.
Valenzuela, S.V., Diaz, P., Pastor, F.J., 2012. A modular glucuronoxylan-specific xylanase with a carbohydrate binding module of family CBM35. Appl. Environ. Microbiol. AEM-07932.
Valenzuela, S.V., Lopez, S., Biely, P., Sanz-Aparico, J., Pastor, F.I.J., 2016. The glycoside hydrolase family 8 reducing-end xylose-releasing exo-oligoxylanase Rex8A from *Paenibacillus barcinonensis* BP-23 is active on branched xylooligosaccharides. Appl. Environ. Micobiol. 82, 5116–5124.
Van den Brink, J., Van Muiswinkel, G.C.J., de Vries, R.P., 2013. Efficient plant biomass degradation by thermophilic fungus *Myceliophthora heterothalica*. Appl. Environ. Micrbiol. 79, 1316–1324.
Vardakou, M., Flint, J., Christakopoulos, P., Lewis, R.J., Gilbert, H.J., Murray, J.W., 2005. A family 10 *Thermoascus aurantiacus* xylanase utilizes arabinose decorations of xylan as significant substrate specificity determinants. J. Mol. Biol. 352, 060–1067.
Verma, A.K., Goyal, A., 2016. A novel member of family 30 glycoside hydrolase subfamily 8 glucuronoxylan endo-β-1, 4-xylanase (CtXynGH30) from *Clostridium thermocellum* orchestrates catalysis on arabinose decorated xylans. J. Molecul. Catal. B: Enzy. 129, 6–14.
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, 52459.
Verma, D., Satyanarayana, T., 2013. Improvement in thermostability of metagenomic GH11 endoxylanase (Mxyl) by site-directed mutagenesis and its applicability in paper pulp bleaching process. J. Ind. Microbiol. Biotechnol. 40, 1373–1381.
Wang, X., Ma, R., Xie, X., Liu, W., Tu, T., Zheng, F., You, S., Ge, J., Xie, H., Yao, B., Luo, H., 2017a. Thermostability improvement of a *Talaromyces leycettanus* xylanase by rational protein engineering. Sci. Rep. 7, 15287.
Wang, X., Zheng, F., Wang, Y., Tu, T., Ma, R., Su, X., You, S., Yao, B., Xie, X., Luo, H., 2017b. Improvement of the catalytic efficiency of a hyperthermophilic xylanase from *Bispora sp*. MEY-1. PloS one 12 e0189806.
Wang, Y., Fu, Z., Huang, H., Zhang, H., Yao, B., Xiong, H., Turunen, O., 2012. Improved thermal performance of *Thermomyces lanuginosus* G[illegible]se by engineering of an N-terminal disulfide bridge. Bioresour. Technol[illegible]
Winger, A.M., Heazlewood, J.L., Chan, L.J.G., Pe[illegible] C.J., Perm[illegible] Singh, S., 2014. Secretome analysis of the thermophilic xy[illegible] hyper-produce[illegible]*rmomyces lanuginosus* SSBP cultivated on corn cobs. J. Ind. [illegible]biol. Biotechn[illegible]1, 1687–1696.
Xiong, H., Nyyssölä, A., Jänis, J., Pastine[illegible] von W[illegible]n, N., Leis[illegible]M., Turunen, O., 2004. Characterization of the xyl[illegible] produced b[illegible]nerg[illegible]tivation of *Thermomyces lanuginosus* DSM [illegible]5. Enzy[illegible]Microb. [illegible], 93–99.
Xue, X., Wang, R., Tu, T., Shi, P[illegible], R., Lu[illegible]Yao, B., S[illegible] 2015. The N-terminal GH10 domain of a multim[illegible]prot[illegible]om *Caldicellulosiruptorbescii* is a versatile xylanase/β-glucanase [illegible]t ca[illegible]rystalline [illegible]lose. Appl. Environ Microbiol AEM-00432.
Yang, Z., Zhang, Z., [illegible] Codon-optimi[illegible]pr[illegible]n and characterization of a pH stable fungal [illegible]in Pichia pastor[illegible]Biochem. 53, 80–87.
Zhao, L., Meng [illegible]di, P[illegible]Y., Luo, H., Hu[illegible]H., Wang, Y., Yang, P., Yao, B., 2013. A novel thermo[illegible]philic xyl[illegible]from *Achaetomium sp*. Xz-8 with high catalytic efficiency and application potential[illegible]e brewing and other industries. Proc. Biochem. 48, 1[illegible]