A review on chimeric xylanases: methods and conditions

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Abstract Multi-functional enzymes are one of the nature’s solutions to facilitate metabolic pathways, thus several reactions are regulated and performed simultaneously on one polypeptide chain. Inspired by nature, artificial chimeric proteins have been designed to reduce the production costs and improve the performance. One of the interesting applications of this method is in the plant-based industries such as feed additive, waste treatment, biofuel production, and pulp and paper bleaching. In fact, the heterogeneous texture of plants needs using a combination of different enzymes to achieve an optimal quality in the manufacturing process. Given that xylans are the most abundant non-cellulosic polysaccharides in nature, xylanases are widely utilized in the mentioned industries. In this regard, several studies have been conducted to develop the relevant chimeric enzymes. Despite the successes that have been attained in this field, misfolding, functional or structural interference, and linker breakage have been reported in some cases. The present paper reviews the research to introduce the prerequisites to design an appropriate chimeric xylanase.

Keywords Domain insertion · Linker · Protein engineering · Tandem fusion

Introduction

Xylanases (EC 3.2.1.8) as hemicellulose-degrading enzymes are widely used in various plant-based industries including pulp and paper bleaching, feed additive, dough processing, juice clarification, textile manufacturing, protoplast preparation, degumming of bast fibers, extraction of coffee, oil, and starch, and production of bioethanol, xylose, xylitol, SCPs (single-cell proteins), solvents, and sugar syrups (Beg et al. 2001; Chen et al. 2015).

Due to the heterogeneous nature of plant cell walls, the presence of diverse enzymes is often necessary to complete degradation of them. Accordingly, many microorganisms mainly possess two approaches to facilitate plant decomposition. The first one includes using complexes such as xylanosomes or cellulosomes in which different enzymatic chains have been attached to a non-catalytic scaffold via a cohesin-dockerin interaction (Bayer et al. 2004). In another strategy some bi- or multi-functional enzymes hold several catalytic activities in a single polypeptide chain. Interestingly, the catalysis can be done in one or several domains, such as what has been reported for xylanase–glucanase complex in Paenibacillus sp. E18 and Ruminococcus flavefaciens 17, respectively (Flint et al. 1993; Shi et al. 2010). Sequence analysis suggests that the multi-functional enzymes have been appeared to improve the performance of enzymatic reactions during evolution. As a consequence, these enzymes expedite intermediate transfer and regulation mechanisms to increase the efficiency of the metabolic pathway (Elleuche 2015).

Inspired by nature, artificial chimeric proteins have been designed for the multi-billion dollar markets. For example, chimeric drugs had sales of 8.3 billion dollars in 2010 (Schmidt 2013). Another promising usage can be in xylanase-based industries in which a combination of different
Chimeric xylanases with domain insertion

Domain insertion exists in about 9% of natural multi-domain proteins, especially in \( \alpha/\beta \) classes (Yu et al. 2015). The most critical step in this method is selecting an appropriate cut-site to avoid disruption of the protein conformation. Hence, a domain has to be inserted in the places that are not directly involved in enzyme activity, such as surface loops and turns. However, domains should not interfere with each other to find the correct folding after insertion. Generally, computational methods such as SCHEMA algorithm can be used to take a closer investigation into the impact of a segment entrance on the protein conformation (Smith and Arnold 2014). Apart from the mentioned challenges, this method has a notable advantage as a result of using one domain instead of whole enzyme. In this way, a severe increase in the molecular weight, observed in tandem fusion, is not expected. This not only reduces metabolic burden on the host cells, but also is more appropriate for the enzyme penetration through the polymeric substrates like hemi-cellulose. On the other hand a lower activity is predicted because of the steric hindrance resulting from getting too close. Thus, it has been suggested using a domain with its surrounding areas for insertion.

One example of this kind of chimeric xylanases was produced by insertion of the whole sequence of xylanase (XynA) into a surface loop of thermophile laccase (CotA). Both enzymes were from Bacillus subtilis. The chimeric enzyme showed nearly two times greater laccase activity. In addition, the solubility and the resistance to proteolytic attacks were enhanced. Although the optimum pH and temperature of the chimeric enzyme was almost as the same as the parent enzymes, the xylanase thermostability had significantly improved. In fact, the N- and C-terminal regions of CotA had a considerable influence on the thermostability of the surrounded XynA (Ribeiro et al. 2011).

In addition to catalytic domains, other regions can also be transferred for domain study and/or physicochemical advancement. Modules of the family F/10 xylanases have drawn the most attention in this regard. A module embraces a compact cluster of 10–40 adjacent amino acid residues which are supposed to act as independent functional blocks (Ahsan et al. 2001). Endo-\( \beta \)-1,4-xylanase from Cellulomonas fimii (Cex) is one of the well-characterized xylanases that contains 22 modules on the basis of the crystal structure analysis. It has been demonstrated that the modules 4, 6, 7, 10, 15, 19 and 20 of Cex are responsible for substrate binding. On account of this, several articles have reported DNA shuffling between Cex and its homologues xylanases. For example, an exchange of M10 between Cex and FXYN (from Streptomyces olivaceoviridis E-86) boosted the catalytic activity of FXYN. Since the M10 is conserved among family 10 xylanases, its replacement leaves no adverse effects on the folding (Kaneko et al. 1999; Mesta et al. 2001). In another study, a tenfold increase in the catalytic activity was observed by substitution of M15–M22 in FXYN with the respective modules from Cex. Interestingly, an inactive enzyme was obtained by shuffling of M1–M14 (Kaneko et al. 2004). Besides the catalytic activity, a thermostable Cex was created by replacing the modules 4 and 5 with the corresponding modules of thermophile XylA from Thermomonospora alba (Wang and Xia 2007). The same result was achieved using self-priming overlapping PCR for the flexible modules 3, 4, 14 and 15 XylA and FXYN. In spite of the improvement in temperature and pH profiles of both FXYN-M3/4-XylA and FXYN-M14/15-XylA, the thermal stability only was noted for FXYN-M3/4-XylA. It suggests that the modules 4–14 of XylA may be responsible for inducing thermal stability (Ahsan et al. 2001).
A survey on the relationship between alkaline xylanase regions and pH-activity was done by overlapping PCR between XynA and XynB from Bacillus halodurans C-125 and Clostridium stercorarium F9, respectively. Both xylanases have a homology in four regions: N-terminal region (A), proton donor region (P), catalytic nucleophile region (N), and C-terminal region (C). Among these regions, A and P sections indicated a significant importance to activity in alkaline pH (Nishimoto et al. 2002).

A combination of alkaline and thermostable xylanases for pre-bleaching of paper pulp was generated by DNA shuffling between G53 and G41, two mutants of xynA from Thermomyces lanuginosus xylanase DSM 5826. G41 was a thermostable xylanase with 75% activity at 80 °C, and 40% activity at pH 10. On the contrary, G53 was an alkaline stable xylanase with 93% activity at pH 10, and 10% activity at 80 °C. The result of DNA shuffling, which inherited mutations from both parents, showed 85% stability at 80 °C and 60% stability at pH 10. Interestingly, the authors noted that the thermostable variants displayed higher arginine content and lower activity. It has been suggested that arginine can affect the stability of protein by making salt bridges on the protein surface (Stephens et al. 2014).

Although most of the researches were done on the family 10 xylanases, here is a report on the relevant regions to alkaline activity of BadX from family GH11. For this purpose a gene shuffling for five segments was performed between BadX from Bacillus agaradhaeren and XynB6 from Dictyoglomus thermophilum by degenerate oligonucleotide gene shuffling (DOGS) method. However, the results indicated no significant improvement in alkaline pH characteristics of the enzymes, thus the authors concluded that a few mutations is not adequate to alter the pH optimum of family 11 xylanases (Gibbs et al. 2010). Conversely, a thermostable xylanase was created in GH11 family by substituting the N-terminus of SoxB from Streptomyces olivaceovirdis with TfxA from Thermomonospora fusca. The half-life of the produced xylanase was about 493 min in 70 °C which was nearly 136-fold higher than wild SoxB (Zhang et al. 2010).

**End to end fusion; simple and prevalent method**

Tandem fusion is the most common method to construct chimeric proteins because it is less dependent on the knowledge of protein structure and using special equipment. In this approach two or more sequences are connected through end-to-end fusion with or without a linker. For this purpose, the first gene should be free of stop codon and the last gene should be without ribosome binding site and signal peptide sequence. Although a reduction in expression level is probable as a result of sequence length increasing, this restriction can be controlled by codon optimization, adjustment of the mRNA secondary structure, and putting a well-expressed protein at the N-terminal domain (Elleuche 2015). Finally, it is suggested to check the structure of the chimer with different software, such as I-TASSER, MOLMOL and PSIPRED.

The chimeric xylanases produced by end-to-end method along with the alternations which have taken place in their properties are summarized in Table 1. As it is clear, most of the products showed no significant variation in the optimum temperature and pH. However, presence of more alkaline enzyme at N-terminal region may decrease the optimum pH of chimeric enzyme.

It should be noted that the chimeras in Table 1 have been created from two enzymes, while some examples exist with more enzymes. For instance, XAR-XYN embraces xyllosidase-arabinosidase (XAR) and xylanase (XYN) activities (Wang et al. 2010; Xue et al. 2009). In addition to enzyme fusion, there is a report for xylanase–ELPs (Elastin-like polypeptides) coupling. This connection increased the optimum temperature, specific activity, and storage capability of enzyme (Li and Zhang 2014). In another example, an improvement in the optimum temperature and specific activity along with a decline in thermostability was created by linking OsmC-region (osmotically induced protein) to Xyn8 of Pseudoalteromonas arctica (Elleuche et al. 2011).

Although tandem fusion seems simple, several parameters have to be considered before getting started; such as sequence arrangement and style of connection. The position of enzymes is affected by the strength of the translation initiation, flexibility of the connecting domains, and conformation, structure and physicochemical property of each parent protein. According to the N-end rule, the N-terminal residues have a determining role in the half-life of a protein. For this reason, putting the more thermostable enzyme in the N-terminus of chimer is desirable to improve the thermal stability of the next enzyme (Zhang et al. 2010). Moreover, it has been illustrated that the N-terminal domain of XynA needs being free for making a significant effect on the thermostability of the adjacent enzyme (Diogo et al. 2015).

One of the critical points in making chimeric enzymes refers to protein linkage. The easiest way is end-to-end fusion in which the unstructured C- or N-terminal of the enzymes acts as a bridge and provides enough space between catalytic domains. However, a non-functional protein can be made if the terminals are short, rigid or essential for protein activity. These have contributed to steric hindrance, misfolding, reduction in protein dynamics, and prevention of interaction between protein subunits. In this situation an external sequence called linker can be
Table 1 Chimeric xylanases constructed by tandem fusion along with the physicochemical properties

| Character of N-terminal enzyme | Linker | Character of C-terminal enzyme | References |
|-------------------------------|--------|--------------------------------|------------|
| $k_{\text{cat}}$ | $K_m$ | Activity | Tp (°C) | pH | N-terminal Enzyme | C-Terminal enzyme | pH | Tp (°C) | Activity | $K_m$ | $k_{\text{cat}}$ |
| 50 ▼ | 6.5 | Endo-1,4-β-xylanase | ETTTGEVDDC EYEQAQQQAG PEVTYE | β-xylosidase | 6.5 | 35 ▲ | (Diogo et al. 2015) |
| 65 | 8 ▼ | β-D-xylanase | GGGGADQLAIGPMYNQVYQYPN | β-D-xylosidase | 6 | 60 | (Fan et al. 2009a) |
| 65 | 8 ▼ | β-D-xylanase | GGGGADQLAIGPMYNQVYQYPN | β-L-arabinofuranosidase | 6 | 70 | (Fan et al. 2009a, b) |
| 0.75 ▲ | 0.72 ▼ | 6 | 5 | Feruloyl esterase A | GSTYSSGSSSGSSSSS | XYNB | 5.5 ▲ | 50 ▼ | 386 ▲ | 6.6 ▲ | (Levasseur et al. 2005) |
| 173 ▼ | 7.87 ▼ | 3.73 ▼ | 50 | 6.5 | XynA | 4-glycine linker | bgLS (lichenase) | 6.5 | 50 | 3.65 ▲ | 3.39 ▼ | 197.2 ▲ | (Cota et al. 2013) |
| 7 ▲ | 21.12 | 27 ▼ | Xylanase (Xy111) | (GGGGS)$_4$ | Endo-1,4-β-mannanase | 6.4 | 40 | 1181.8 ▼ | 4.06 ▼ | 11,149 ▼ | (Qiao and Cao 2012) |
| 1363 ▲ | 62 ▼ | 6 ▼ | XynCDBFV | (GGGGS)$_2$ | Acetylxylan esterase AxelS20E | (Kim et al. 2015) |
| 1941.5 ▲ | 15.3 ▲ | 727.21 ▼ | 70 | 7 ▼ | Xyl10g | (GGGGS)$_2$ | Endoglucanase (Cel5B) | 6.4 | 40 | 1181.8 ▼ | 4.06 ▼ | 11,149 ▼ | (Qiao and Cao 2012) |
| 642 | 50 | 7 | β-1,4-endoglucanase (Endo5A) | (GGGGS)$_2$ | Xyn11D | 7 | 50 | 389 ▲ | (Adlakha et al. 2011) |
| 6565 ▲ | 4.2 ▼ | 40 | 9 | β-glucanase | Without linker | Xylanase | 9 | 60 ▼ | 4.95 ▲ | 6491 ▼ | (Lu and Feng 2008; Lu et al. 2006) |
| 49.2 ▲ | 12.3 ▼ | 47 ▲ | 3.8 | Xylanase | PEVLPLPK ESRISEGEAV VVG | Glucanase | 3.8 | 96 | 1.87 ▲ | 2.6 ▼ | (Liu et al. 2012) |
| 949 ▼ | 2.36 ▲ | 324 ▼ | 50 | 3.4 | XynA | AAAAA | β-1,3-1,4-glucanase (BglGlu) | 6.4 | 40 | 1181.8 ▼ | 4.06 ▼ | 11,149 ▼ | (Qiao and Cao 2012) |
| 7.41 | 75 ▲ | 80 ▼ | 7 ▼ | XylT | VDKTKYTAS | Cel5A | 6 | 90 ▼ | 1431 ▲ | 7.41 | (Rizk et al. 2015) |

No data have been reported for the blank cells.

Symbols ▲ and ▼, respectively, represent increase and decrease in the parameters after constructing chimeric enzyme. Those are asymptomatic were without change.
used. A database of various linkers has been presented by the Centre for Integrative Bioinformatics VU (IBIVU) at the Vrije University of Amsterdam (http://www.ibi.vu.nl/programs/linkerdwwww/). In addition to online databases, a genetic algorithm can be used to determine the appropriate connector (Zhang et al. 2009). Generally, linkers can be categorized based on the length, amino acid content, hydrophobicity, secondary structure, solvent accessibility and sensitivity to proteases. However, the following classification is more prevalent and comprehensive form (Yu et al. 2015).

1. Flexible linker Small amino acids such as Gly and Ala can make the flexible linkers. The presence of polar amino acids like Ser, Thr, Glu and Lys lead to stability of the linker in the aqueous solvents by making hydrogen bond (Chen et al. 2013). The most popular flexible linker is (GGGGS)ₙ (usually n ≤ 6) which forms a loop. However, this glycine-rich region can reduce the level of protein expression by creating a stable mRNA secondary structure. Furthermore, the GC-rich content (codon GGN for glycine) may be unacceptable for some microorganisms (Lu and Feng 2008). It is worth to note that the humdrum rhythm of Gly in (GGGGS)₂ reduces the functional activity of enzymes. Thus, using a combination of other small amino acids such as Ala and Ser can help to overcome this problem (Wang et al. 2010).

2. Rigid linker This is useful when a defined distance is necessary between enzymes. As a result of being tough and stable, they can inhibit interactions between proteins and support correct folding. The most common type is (EAAAK)ₙ (usually n ≤ 6), which forms helices and salt bridges between its Glu⁻–Lys⁺. Another type is proline-rich sequences (XP)ₙ, where X can be any amino acid, preferably Ala, Lys or Glu. This kind of linkers naturally has been discovered in proteases and pyruvate dehydrogenases. Since Pro has a circular and solid structure, it makes a firm and independent connection. Moreover, lack of hydrogen on the amide group of Pro prevents from interaction with other amino acids (Chen et al. 2013).

3. Cleavable linker These linkers apply to separation of chimeric proteins when exposed to solvents or proteases. MEROPS is a database to determine the sensitivity to peptidase.

In contrast to the flexible and rigid linkers, there is no report for the usage of the cleavable linkers in the chimeric xylanases (Table 1). Therefore, the first two categories will be discussed in the following.

A comparison between [GGGGS]ₙ and [EAAAK]ₙ linkers (n = 2–4) has illustrated that xylanase-mannase chimers which were created by [EAAAK]₃ had a better thermostability. However, the flexible linker with n = 3 causes more catalytic activity. In addition, K_m was greater with rigid linkers (Guo et al. 2013). Similar results were obtained by the mentioned linkers (n = 1–3) for xylanase-glucanase chimer (Lu and Feng 2008). Amino acid composition of linkers can also affect post-translational modifications and thus protein stability. A good example of this was reported by Levasseur et al. (2005) in which the proteolytic cleavage of linker was avoided using a hyperglycosylated linker. Furthermore, the authors believed that the glycosylation of linker can be useful for increasing the secretion and production yield of recombinant proteins (Levasseur et al. 2005).

In addition to amino acid content, length of linkers has been investigated. Researches have shown that the solvent accessibility and hydrophilicity are related to the length of linker. Accordingly, access to solvent for long (average length of 21.0 ± 7.6 residues), medium (9.1 ± 2.4) and short (4.5 ± 0.7) linkers are about 42.5, 26.3 and 14.4%, respectively (Chen et al. 2013; George and Heringa 2003). Although a linker should be long enough so that each enzyme acts independently, the possibility of breakage increases if the linker be too long (Qiao and Cao 2012). It has been demonstrated that a 31 amino acids linker is suitable not only to prevent collision of two enzymes, but also to make great flexibility to provide free access and binding to the substrate (Diogo et al. 2015). In another study, Fan and coworkers found that a five glycine residues linker cannot make enough distance between two enzymes; therefore, they used a 24 residues linker (Fan et al. 2009a). Although the mentioned results provide useful information for designing an appropriate linker, further investigation may be required depending on the aim of study and properties of the target protein.

Conclusion

The simple structure and diverse industrial usages of xylanases have led to perform several studies to create relevant chimeric enzymes to reduce the production costs and improve the characteristics of enzymes. The two main methods discussed here were domain insertion and tandem fusion. In both methods, maintenance of the protein conformation and inhibition of overall interference are the most critical criteria. In this regard, selection of cut-site is the important step in domain insertion and mostly takes place in loops or turns that does not have a direct effect on enzyme activity or folding. However, in domain replacement a high similarity and accordance between enzymes is necessary. Most of the reports in this area have been done on the family 10 xylanases because modules of Cex from this family have been identified and can be used to advance
other xylanases from the same family without making misfolding (Kaneko et al. 1999). However, this method requires knowledge about the structure of enzymes, and also is associated with high possibility of steric hindrance, folding interference, and inactivity, thus most of the researchers employ tandem fusion. The features of parent enzymes as well as the communication bridge have significant effects on the results. When a suitable terminal domain does not exist for direct connection, a linker is used. Linkers have various applications in biotechnology, such as the production of bifunctional enzymes and antibodies and as a means of FRET analysis (Chichili et al. 2013). These linkers often are derived from nature. Amino acid residues that are common to all natural linker are Pro, Thr, and Gln (Chen et al. 2013). Generally, a linker should be without a protease cleavage site and enough distances provided between the two independent activities of each enzyme fusion. According to the studies, an average length of 15 amino acid residues is suitable for connection. Selection of the type of linker also depends on the purpose of the research. It is proposed here to use rigid and flexible linkers for thermal stability and enhanced activity, respectively. Although there is no report for cleavable linkers in the creation of chimeric xylanases, they can at least be used to reduce the cost of production and purification of enzymes.

In spite of the selected method, terminal domains, N-terminal domains are especially important in protein stability. Therefore, it is suggested that the more stable enzyme is put in this position. In addition, using post-translational modification sites such as N-acetylation or glycosylation sites can improve the enzyme stability.

Compliance with ethical standards

Conflict of interest The author declares that there is no conflict of interest regarding the publication of this paper.

References

Adlakha N, Rajagopal R, Kumar S, Reddy VS, Yazdani SS (2011) Synthesis and characterization of chimeric proteins based on cellulase and xylanase from an insect gut bacterium. Appl Environ Microbiol 77:4859–4866

Ahsan MM, Kaneko S, Wang Q, Yura K, Go M, Hayash K (2001) Capacity of thermomonospora alba Xyla to impart thermotability in family F/10 chimeric xylanases. Enzyme Microb Technol 28:8–15

Bayer EA, Belaych J-P, Shoham Y, Lamed R (2004) The cellulases: multienzyme machines for degradation of plant cell wall polysaccharides. Annu Rev Microbiol 58:521–554

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

Chen X, Zaro J, Shen W-C (2013) Fusion Protein Linkers: property, design and functionality. Adv Drug Deliv Rev 65:1357–1369

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

Chichili VPR, Kumar V, Sivaramam J (2013) Linkers in the structural biology of protein–protein interactions. Protein Sci 22:153–167

Cota J, Oliveira LC, Damasio ARL, Citadini AP, Hoffmann ZB, Alvarez TM, Codima CA, Leite VBP, Pastore G, Oliveira-Neto Md et al (2013) Assembling a xylanase–lignenase chimera through all-atom molecular dynamics simulations. Biochim Biophys Acta 1834:1492–1500

Diogo JA, Hoffmann ZB, Zanphorlin LM, Cota J, Machado CB, Wolf LD, Quina F, Damasio ARL, Murakami MT, Ruller R (2015) Development of a chimeric hemicellulase to enhance the xyloseproduction and thermostolerance. Enzyme Microb Technol 69:31–37

Elleuche S (2015) Bringing functions together with fusion enzymes—from nature’s inventions to biotechnological applications. Appl Microbiol Biotechnol 99:1545–1556

Elleuche S, Piascheck H, Antranikian G (2011) Fusion of the OsmC domain from esterase EstO confers thermostability to the cold-active xylanase Xyn8 from Pseudalteromonas arctica. Extremophiles 15:311–317

Fan Z, Wagschal K, Lee CC, Kong Q, Shen KA, Maiti IB, Yuan L (2009a) The construction and characterization of two xylan-degrading chimeric enzymes. Biotechn Bioeng 102:684–692

Fan Z, Werkman JR, Yuan L (2009b) Engineering of a multifunctional hemicellulase. Biotechnol Lett 31:751–757

Flint HJ, Martin J, McPherson CA, Daniel AS, Zhang JX (1993) A bifunctional enzyme, with separate xylanase and beta(1,3-1,4)-glucanase domains, encoded by the xynD gene of Ruminococcus flavefaciens. J Bacteriol 175:2943–2951

Gibbs MD, Reeves RA, Choudhary PR, Bergquist PL (2010) Alteration of the pH optimum of a family 11 xylanase, XynB6 of Dictyoglomus thermophilum. N Biotechnol 27:803–809

George RA, Heringa J (2003) An analysis of protein domain linkers: their classification and role in protein folding. Protein Eng 15:871–879

Guo N, Zheng J, L-s Wu, Tian J, H-b Zhou (2013) Engineered bifunctional enzymes of endo-1,4-β-xylanase/endo-1,4-β-mannanase were constructed for synergistically hydrolyzing hemicellulose. J Mol Catal B Enzyme 97:311–318

Kaneko S, Kano A, Fujimoto Z, Shimizu D, Machida S, Sato Y, Yura K, Go M, Mizuno H, Taira K et al (1999) An investigation of the nature and function of module 10 in a family F/10 xylanase FXYN of Streptomyces olivaceoviridis E-86 by module shuffling with the Cex of Cellulomonas fimii and by site-directed mutagenesis. FEBS Lett 460:51–66

Kaneko S, Ichinose H, Fujimoto Z, Kuno A, Yura K, Go M, Mizuno H, Kusakabe I, Kobayashi H (2004) Structure and function of a family 10 β-xylanase chimera of Streptomyces olivaceoviridis E-86 FXYN and Cellulomonas fimii Cex. J Biol Chem 279:26619–26626

Khandeparker R, Numan MT (2008) Bifunctional xylanases and their potential use in biotechnology. J Ind Microbiol Biotechnol 35:635–644

Kim HM, Jung S, Lee KH, Song Y, Bae H-J (2015) Improving lignocellulose degradation using xylanase–cellulase fusion protein with a glycin-serine linker. Int J Biol Macromol 73:215–221

Levasseur A, Navarro D, Punt PJ, Belach J-P, Asther M, Record ER (2005) Construction of engineered bifunctional enzymes and their overproduction in Aspergillus niger for improved enzymatic tools to degrade agricultural by-products. Appl Environ Microbiol 71:8132–8140

Li C, Zhang G (2014) The fusions of elastin-like polypeptides and xylanase self-assembled into insoluble active xylanase particles. J Biotechnol 177:60–66
Liu L, Wang L, Zhang Z, Guo X, Li X, Chen H (2012) Domain-swapping of mesophilic xylanase with hyper-thermophilic glucanase. BMC Biotechnol. doi: 10.1186/1472-6750-12-28

Lu P, Feng M-G (2008) Bifunctional enhancement of a β-glucanase-xylanase fusion enzyme by optimization of peptide linkers. Appl Microbiol Biotechnol 79:579–587

Lu P, Feng M-G, Li W-F, Hu C-X (2006) Construction and characterization of a bifunctional fusion enzyme of Bacillus-sourced β-glucanase and xylanase expressed in Escherichia coli. FEMS Microbiol Lett 261:224–230

Mesta L, Rascal C, Durand R, Fevre M (2001) Construction of a chimeric xylanase using multidomain enzymes from Neocallimastix frontalis. Enzyme Microb Technol 29:456–463

Nishtmoto M, Kitaoka M, Hayashi K (2002) Employing chimeric xylanases to identify regions of an alkaline xylanase participating in enzyme activity at basic pH. J Biosci Bioeng 94:395–400

Pai C-K, Wang H-T, Guo R-T, Liu J-R (2012) The construction of bifunctional fusion xylanolytic enzymes and the prediction of optimum reaction conditions for the enzyme activity. Bioresources 7:5647–5665

Qiao J, Cao Y (2012) In-fusion expression and characterization of β-xylanase and β-1,3-1,4-glucanase in Pichia pastoris. Biologia 67:649–653

Ribeiro LF, Furtado GP, Lourenzoni MR, Costa-Filho AJ, Santos CR, Nogueira SCP, Betini JA, Polizeli MdLT, Murakami MT, Ward RJ (2011) Engineering bifunctional laccase-xylanase chimeras for improved catalytic performance. J Biol Chem 286:43026–43038

Rizk M, Elleuche S, Antranikian G (2015) Generating bifunctional fusion enzymes composed of heat-active endoglucanase (Cel5A) and endoxylanase (XylT). Biotechnol Lett 37:139–145

Schmidt SR (2013) Fusion protein technologies for biopharmaceuticals applications and challenges. John Wiley & Sons, Canada

Shi P, Tian J, Yuan T, Liu X, Huang H, Bai Y, Yang P, Chen X, Wu N, Yao B (2010) Paenibacillus sp strain E18 bifunctional xylanase-glucanase with a single catalytic domain. Appl Environ Microbiol 76:3620–3624

Smith MA, Arnold FH (2014) Designing libraries of chimeric proteins using SCHEMA recombination and RASPP. Methods Mol Biol 1179:335–343

Stephens DE, Khan FL, Singh P, Bisetty K, Singh S, Permaul K (2014) Creation of thermostable and alkaline stable xylanase variants by DNA shuffling. J Biotechnol 187:139–146

Wang Q, Xia T (2007) Enhancement of the thermostability and hydrolytic activity of GH10 xylanase by module shuffling between Cellulomonas fimi Cex and Thermomonospora alba XylA. World J Microbiol Biotechnol 23:1047–1055

Wang R, Xue Y, Wu X, Song X, Peng J (2010) Enhancement of engineered trifunctional enzyme by optimizing linker peptides for degradation of agricultural by-products. Enzyme Microb Technol 47:194–199

Xue Y, Peng J, Wang R, Song X (2009) Construction of the bifunctional enzyme associating the Thermoaerobacter ethanolicus xylosidase-arabinosidase with the Thermomyces lanuginosus xylanase for degradation of arabinoxylan. Enzyme Microb Technol 45:22–27

Yu K, Liu C, Kim B-G, Lee D-Y (2015) Synthetic fusion protein design and applications. Biotechnol Adv 33:155–164

Zhang J, Yun J, Shang Z, Zhang X, Pan B (2009) Design and optimization of a linker for fusion protein construction. Prog Nat Sci 19:1197–1200

Zhang S, Zhang K, Chen X, Chu X, Sun F, Dong Z (2010) Five mutations in N-terminus confer thermostability on mesophilic xylanase. Biochem Biophys Res Commun 395:200–206