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Research Article

Characterization of Two Wheat-Derived Glycoside Hydrolase Family-10 Xylanases Resistant to Xylanase Inhibitors

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Xylanase inhibitors inhibit the activities of microbial xylanases and seriously compromise the efficacy of microbial xylanases added to modify cereals. Cereal endogenous xylanases are unaffected by these xylanase inhibitors, but little information is available regarding their effects in improving cereal quality, a neglected potential application. As a strategy for circumventing the negative effects of xylanase inhibitors, the objective of this study was to use genetic engineering to obtain sufficient amounts of active endo-1,4-β-D-xylanase from wheat to analyze the characteristics of its structure. The endo-1,4-β-D-xylanase from wheat was heterologously expressed. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), western blotting, MALDI-TOF/TOF (MS) analyses, and enzyme activity determination confirmed 2 active endo-1,4-β-D-xylanases (EXY3 and EXY4) were successfully obtained. The molecular weights (MW) and isoelectric point (pI) of EXY3 were 36.108 kDa and 5.491, while those of the EXY4 protein were 41.933 kDa and 5.726. They both contained the same catalytic domain of GH10 xylanases from G266 to V276 and have the same catalytic site, Glu273. They shared the same putative N-glycosylation sites (N62-T63-S64 and N280–V281–S282) and 3 putative O-glycosylation sites (Ser8, Ser9, and Thr21), but EXY4 had an additional O-glycosylation site (Thr358). EXY3 was smaller than EXY4 by 51 amino acids because of a nonsense mutation and premature termination. They both had the 8-fold beta/alpha-barrel (TIM-barrel) fold. The specific activities of EXY3 and EXY4 were 152.0891 and 67.2928 U/mg, respectively. This work demonstrates a promising way to obtain wheat xylanases by genetic engineering; the properties of the enzymes indicate their potential application in cereal-based industries.

1. Introduction

As an excellent raw material, wheat has been widely used in beer production. Wheat bran, the major by-product of flour milling, accounts for about 25% of the grain weight and is used as a major ingredient in animal feeds (typically up to 65%) [1]. Wheat milling annually produces up to 150 million tons of bran [2]. The cell wall of starchy wheat endosperm contains abundant arabinoxylans (AX); when dissolved, it has a relatively high viscosity and has a negative impact on beer production and in the animal intestine [3]. It is feasible to reduce the viscosity by degrading the AX, for example, with endo-1,4-β-D-xylanase (EC 3.2.1.8), which cleaves the xylan backbone and plays a key role in the depolymerization of AX [4]. The degradation of AX by xylanase has become a topic of interest and urgently needs to be better understood [5]. Recently, more than 300 xylanases have been identified from bacteria and fungi, some of which have been used industrially [6]. Many xylanase activities are reduced by specific conditions such as excessive temperature, nonoptimal pH, and a variety of endoxylanase inhibitors.

Wheat contains three xylanase inhibitors: TAXI (*Triticum aestivum* xylanase inhibitor) [7], XIP (xylanase inhibiting protein) [8], and TLXI (thaumatin-like xylanase inhibitor) [9]. The content of these inhibitors varies greatly in different wheat types [10]. Pelleting is a process of transforming feed mash into pellets, and the pelleting temperature increases during pelleting. The activities of xylanase inhibitors are only partly reduced (46%) during pelleting at 85 ˚C or higher temperatures, indicating their resistance to high temperatures and that pelleting cannot completely eliminate their inhibiting effects [11]. It is important to note that xylanase inhibitors can partially inhibit the activities of xylanases in fungi and bacteria but are unable to inhibit the endogenous xylanases from cereals. For example, XIP inhibits fungal GH10 and GH11 xylanase, and TAXI inhibits GH11 xylanase from bacteria and fungi [12]. These properties of xylanase inhibitors seriously compromise the efficiency of microbial xylanases added to wheat. In order to alleviate or overcome the negative effects of xylanase inhibitors, it would be of great significance to obtain active wheat-derived xylanases by genetic engineering; they would be resistant to inhibition.

In the process of grain germination, endo-1, 4-β-D-xylanases are synthesized to degrade the cell walls, and during wheat germination, the activities of endo-1,4-β-D-xylanases increase significantly. First, the 67 kDa precursor protein is synthesized, and then, the proposed intermediate forms and mature processed forms of 50 kDa, 43 kDa, and 34 kDa proteins are formed [13]. For barley, the precursor is a 61.5 kDa protein, while the proposed intermediate forms and mature processed forms are 41 kDa, 34 kDa, and 29 kDa proteins [14]. In 2007, Van Campenhout et al. were the first to clone and heterologously express the different forms of barley endo-1,4-β-D-xylanases in order to obtain sufficient amounts of active endo-1,4-β-D-xylanases from barley. By expressing the 34, 41, and 61.5 kDa forms of endo-1,4-β-D-xylanases in *Escherichia coli* (*E. coli*), they obtained the active precursor, while the endo-1,4-β-D-xylanases of 34 kDa and 41 kDa forms showed no activities [15].

The objective of this study was to clone and heterologously express the endo-1,4-β-D-xylanase from wheat in *E. coli*. The amino acid sequence and the spatial configuration of the wheat xylanase obtained are described. The potential applications of recombinantly derived wheat endo-1,4-β-D-xylanase in the cereal-based industry are discussed.

2. 2. Materials and Methods

2.1. Cloning and Constructing the Recombinant Expression Plasmids of Xylanases. The one-step method using a Trizol kit (Sangon, Shanghai, China) was adopted to extract genomic deoxyribonucleic acid (DNA) from germinated wheat seeds. Polymerase chain reaction (PCR) primers were designed based on the exon 3 sequence of endo-1,4-β-D-xylanase from "Chinese Spring" wheat (GenBank AF156977.2). Restriction enzyme digestion sites and the protective nucleotides were added to the primer sequences. The primers are as follows: a forward primer with the EcoR V restriction site (underlined) 5′-TCGATATCATGCAGCTGGACAACGCCTT-3′, and reverse primer EcoR I restriction site (underlined) 5′-TCGAATTCTGCGTCCGTCTTCCACTCCC-3′. The xylanase DNA fragment was amplified on a Bio-Rad S1000 PCR machine (Hercules, CA) by the following protocol: 95˚C for 3 minutes; 35 cycles of 95˚C for 30 seconds, 60˚C for 30 seconds, 72˚C for 1 minute, and finally 72˚C for 10 minutes. The PCR products of appropriate size were purified with a TaKaRa MiniBest DNA fragment purification kit ver. 4.0 (Dalian, China).

The purified products and pET 30a + vector were double digested with EcoR V and EcoR I at 37˚C for 8 hours. After that, the two digestion fragments were purified and ligated with T4 DNA ligase to build the recombinant plasmids. The recombinant plasmids were transformed into *E. coli* DH5α cells and selected on Luria–Bertani (LB) agar plates containing 50 μg/mL kanamycin. After verification by PCR, double restriction digestion, and DNA sequencing, two positive recombinant plasmids were confirmed. According to design, one has a complete coding sequence and was named pET-EXY4, and the other with the terminator mutant in the 3′-terminus was named pET-EXY3. The two recombinant plasmids, pET-EXY3 and pET-EXY4, were used in the subsequent experiments.

2.2. Expression of Recombinant pET-EXYs in E. coli BL21. Individual transformant clones of *E. coli* BL21 (DE3) transformed with pET-EXY3 and pET-EXY4 were expanded in 5 mL LB medium with 50 μg/mL kanamycin and grown at 37˚C with shaking at 220 rpm for 10 hours. After that, the earlier stated culture medium was inoculated into a new culture medium at 37˚C rotating at 220 rpm to achieve an $OD6_{00}$ of 0.5, and then, isopropyl-β-D-1-thiogalactopyranoside (IPTG) was added to a final concentration of 0.1 mM to induce the expression of pET-EXY recombinant proteins at 37 ˚C with shaking at 220 rpm for 12 hours. The expressed proteins were named EXY3 and EXY4, respectively. The bacterial cells were harvested by centrifugation at 8,000 g for 10 minutes at 4 ˚C.

2.3. Extraction and Purification. The harvested cells were resuspended in PBS and lysed by three cycles of freezing at −80 ˚C for 30 minutes and thawing. The cells were subjected to three 15-s bursts of sonication (Vibra CellTM, VC-505, Sonics and Materials, Newtown, CT) separated by 30 seconds of cooling in an ice bath. The bacterial lysate was clarified by centrifugation at 10,000 g for 30 minutes at 4 ˚C, and the supernatant was collected.

Then, the supernatant was loaded onto 5 mL HisTrap HP columns (GE Healthcare), washed, and eluted with the different concentrations of Tris-HCl buffer (pH 7.9) according to the instruction. The eluent was pooled and dialyzed into a storage buffer before the enzyme was stored. The protein concentration was determined with a Micro BCA Protein Assay Kit (Beyotime, Haimen, China).

2.4. SDS-PAGE and Western Blot Analyses. The expression of recombinant EXY3 and EXY4 was assessed by SDS-PAGE using 5% stacking and 12% separating gels. The isolated protein bands were visualized in the gel after staining with

Coomassie Blue R-250, and the MW was calculated by EditSeq 7.10 (DNAstar, Inc. Wisconsin, USA). For the western blot analysis, protein samples and prestained protein MW markers (Thermo Fisher, Waltham, MA) were run on SDS-PAGE and transferred to polyvinylidene difluoride (PVDF) membrane using a Bio-Rad Trans-Blot, SD Semi-Dry transfer cell. The membrane was blocked with 5% nonfat dry milk in phosphate-buffered saline and then incubated with HRP-conjugated anti-6×His tag mouse monoclonal antibody for 5 hours at room temperature. Immunoreactive protein was visualized with a chromogenic substrate and recorded by a Bio-Rad ChemiDoc XRS imaging system.

2.5. Mass Spectrometric Identification. For the MS identification, the Coomassie-stained recombinant protein bands were excised from the SDS-PAGE gel. The sample bands were washed three times with 100 mM NH4HCO3 and then destained twice with 50% acetonitrile (v/v) in 100 mM NH4HCO3. After drying in a vacuum centrifuge for 30 minutes, the sample bands were reduced with 10 mM DTT in 100 mM NH4HCO3 and alkylated with 40 mM iodoacetamide in 100 mM NH4HCO3. After dehydrating and drying again, proteins in the gel were digested overnight at 37 ˚C with 12.5 ng/μL trypsin (Madison, WI, USA) in 50 mM NH4HCO3. The peptides were extracted three times with 0.5% trifluoroacetic acid (TFA) in 50% acetonitrile and lyophilized.

For MALDI-TOF/TOF MS analysis [16,17], the tryptic digests were dissolved in 5 mg/mL *a*-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 0.1% TFA. The peptides were analyzed using MALDI-TOF/TOF MS to proteomics analysis (ABI 4800plus, Applied Biosystems, Foster City, CA). After peptide mass fingerprints (PMF) and the MS/MS spectra were obtained, the data were analyzed using the GPS Explorer software (Applied Biosystems) and the Mascot 2.0 software (Matrix Science, Mount Prospect, IL). Based on the National Center for Biotechnology Information nonredundant (NCBInr) search, peptide and protein identification were performed without the restriction of protein species, protein molecular mass (Mr), or pI. Proteins were accepted when identifications exceeded a 95% confidence level.

2.6. Determination of the Activities of Recombinant Xylanases. The activities of xylanases were determined by the 3,5-dinitrosalicylic acid (DNS) method, according to protocol GB/T 23874–2009 of the Standardization Administration of China (Beijing, China) [18]. The activities of xylanases were determined by measuring the reducing sugars released from 0.5% (w/v) beechwood xylan as the substrate and D-xylose as the standard. One unit of xylanase activity was the amount of enzyme releasing 1 μmol reducing sugar equivalents per minute from the substrate under the standard assay conditions. For each assay, the mean of triplicate measurements was calculated.

2.7. Analysis of Amino Acid and DNA Sequences. The InterPro (http://www.ebi.ac.uk/interpro/) was used to analyze the domain composition and important sites of EXY3 and EXY4. The NetNGlyc program 10 (http://www.cbs.dtu.dk/services/NetNGlyc/) and NetOGlyc 4.0 server (http://www.cbs.dtu.dk/services/NetOGlyc/) were applied to locate the putative N-glycosylation and O-glycosylation sites of EXY3 and EXY4. The three-dimensional (3-D) structures of EXY3 and EXY4 were modeled through the Swiss-Model protein-modeling server (http://swissmodel.expasy.org/), based on a crystal structure of the 1,4-beta-D-xylan-xylanohydrolase from the GH10.

3. Results

3.1. Construction of the pET-EXY Plasmids. Using wheat genomic DNA as a template, a single PCR amplicon of about 891 bp was obtained (Figure 1). Sequencing confirmed that the amplicon corresponded to the expected amplicon size of the xylanase fragment. By PCR screening, various transformant clones harboring recombinant pET-EXY plasmids revealed an amplicon of about 891 bp (Figure 1). DNA sequencing confirmed that the recombinant pET-EXY plasmids had 96.0% similarity with GenBank AF156977.2. Amino acid sequence alignment showed that pET-EXY3, pET-EXY4, and reference target had 96.0% similarity, but the pET-EXY3 had a nonsense mutation leading to a C-terminal truncation (Figure 2). Compared with pET-EXY4, pET-EXY3 had 51 residues lost from the C terminus.

3.2. Expression of the Recombinant EXY3 and EXY4 Proteins. The recombinant pET-EXY plasmids were successfully induced and expressed. The MW and pI of EXY3 were 36.108 kDa and 5.491, while those of the EXY4 protein were 41.933 kDa and 5.726. SDS-PAGE analysis revealed the presence of protein bands of EXY3 and EXY4 (Figure 3). The induced proteins were further confirmed by western blotting and successfully detecting the 6×His tags (Figure 4). MS analysis further revealed that the induced proteins were the (1,4)-beta-xylan endohydrolase from *Triticum aestivum* (Figure 5).

3.3. Primary Structure Analyses of the EXY3 and EXY4 Proteins. Through InterPro analysis, EXY3 and EXY4 both belong to the glycoside hydrolase superfamily and contain the GH10 domain found in G266 to V276 and the putative catalytic site Glu273 (Figure 2). Through NetNGlyc 1.0 prediction, EXY3 and EXY4 share two putative N-glycosylation sites (N62-T63-S64 and N280–V281–S282). Through NetOGlyc 4.0 prediction, EXY3 has three putative O-glycosylation sites (Ser8, Ser9, and Thr21), whereas EXY4 has four (the three just listed plus Thr358).

3.4. Enzyme Activity. The specific activity of EXY3 was 152.0891 U/mg, and those of EXY4 protein were 67.2928 U/mg.

3.5. Three-Dimensional Structure Analyses of the EXY3 and EXY4 Proteins. Based on the crystal structure of the 1,4-beta-d-xylan-xylanohydrolase from GH10, the 3-D structures of the EXY3 and EXY4 are shown (Figure 6). From one

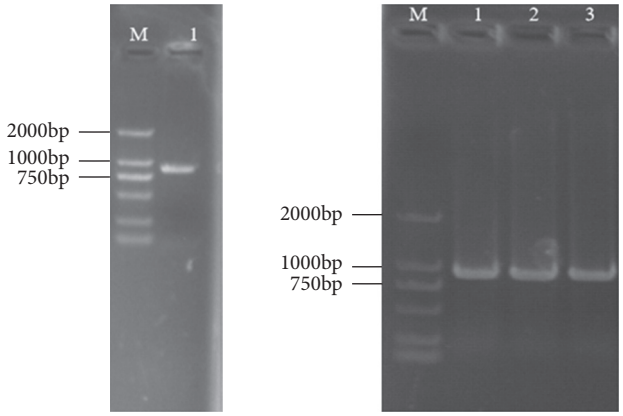


Figure 1: PCR amplification and PCR identification.

EXY4	MHHHHHH*SS*GLVPRGSGMKE*T*AAAKFERQHMDSPDLGTDDDDKAMADIM	49
EXY3	MHHHHHH*SS*GLVPROSOMKE*T*AAAKFERQHMDSPDLGTDDDDKAMADIM	49
EXY4	QLDNAFPFGTCINTSVIHNPVFLDFFTNHFDWAVFENELKWYHTEAQQGQLN	101
EXY3	QLDNAFPFOTCINTSVIHNPVFLDLFTSHFDWAVFENELKWYHTEAQEGQLN	101
EXY4	YADADALLAFCDRQGKHVRGHCVFWSVDGDVQQWVKDLNRDQLRSAMQ	149
EXY3	YADADALLALCDRQGKHVRGHCVFWSVDGDVQQWVKNLNRDQLRSAMQ	149
EXY4	SRLEOLVSRYAGRFKHYDVNNEMLHGRFFRDRLGDEDIPAYMFKEVARLGPE	201
EXY3	SRLEGLVSRYAGRFKHYDVNNEMLYGRFFRDRLGDEDIPAYMFKEVARLDPE	201
EXY4	PALFVNDYNVERGNDPNATPEKYAKQVAWLQGRGAVVGGIGLQGHVQNPV	251
EXY3	PSLFVNDYNVERGNDPNATPEKYAKQVAWLQGRGAVVGGIGLQGHVQNPV	251
EXY4	GEVIGAAIDRLAKTGVPIWFTELDVPEYNVSLRAKDLEVVLREAYAHPAVEGI	304
EXY3	GEVIGAAIDRLAKTGVPIWFTELDVPEYNVSLRAKDLEVVLREAYAHPAAEG	303
EXY4	VFWGFLQGTMWRENAWLVDADOTVNEAGQMFLNLQREWKTDAEFELRRQ	353
EXY3	IVIWGFLQGTMWRENA.	319
EXY4	ACGR*T*RAPPPPPLRSGC.	370

Figure 2: The deduced amino acid sequences of EXY3 and EXY4. The GH10 catalytic domains from G266 to V276 are underlined. The catalytic sites Glu273 are boldly underlined. The N-glycosylation sites are indicated in a gray box. The O-glycosylation sites are italic underlined.

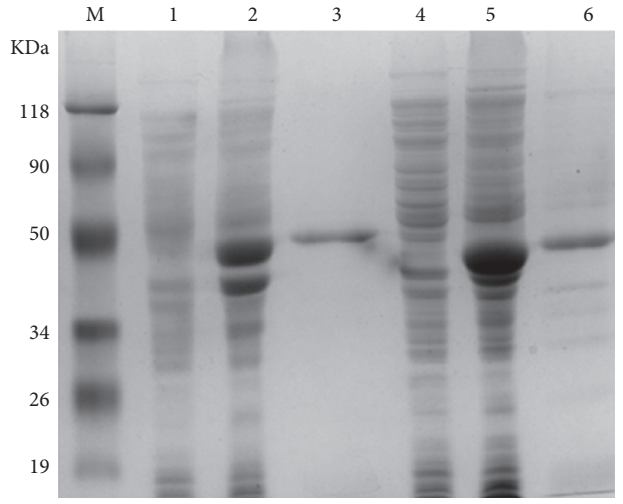


Figure 3: Analysis of recombinant fusion proteins through SDS-PAGE. SDS-PAGE gel of recombinant proteins: lane M-low MW protein markers; lane 1-crude supernatant of *E. coli* cells without IPTG induction; lane 2-crude EXY 4 supernatant of *E. coli* cells with IPTG induction; lane 3-purified EXY 4 proteins; lane 4-crude supernatant of *E. coli* cells without IPTG induction; lane 5-crude EXY4 supernatant of *E. coli* cells with IPTG induction; and lane 6-purified EXY 3 proteins.

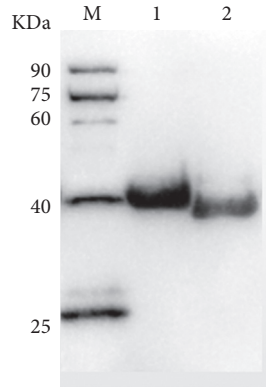
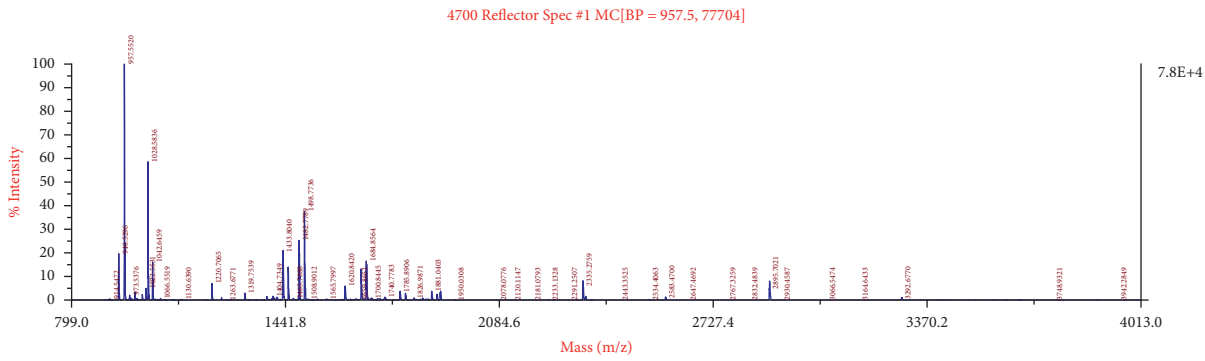
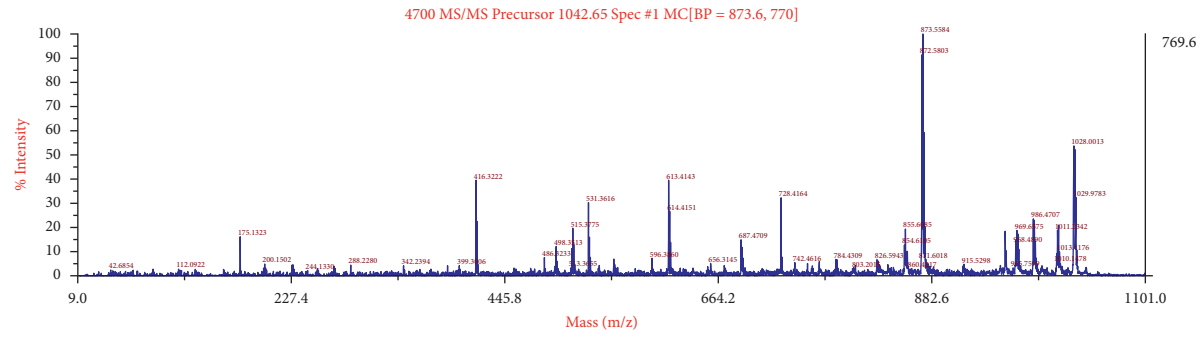


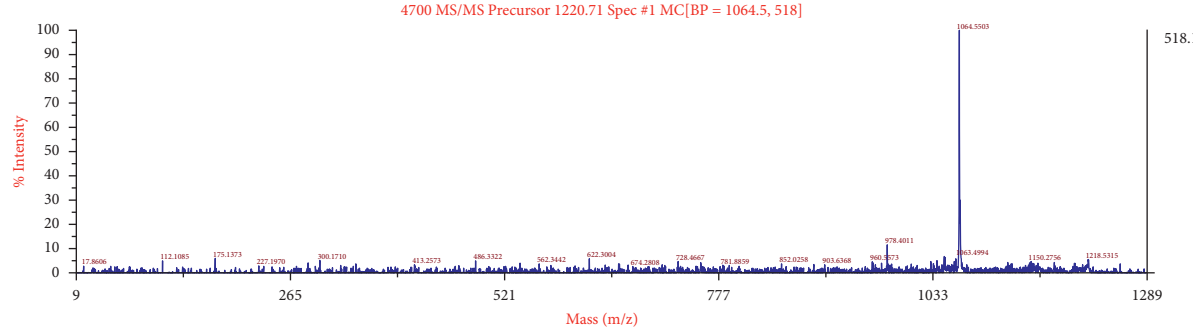
Figure 4: Western blot analysis of EXY3 and EXY4. Lane M-standard protein marker; lane 1-purified EXY 4 proteins; lane 2-purified EXY 3 proteins.



(a)



(b)



(c)

Figure 5: Continued.

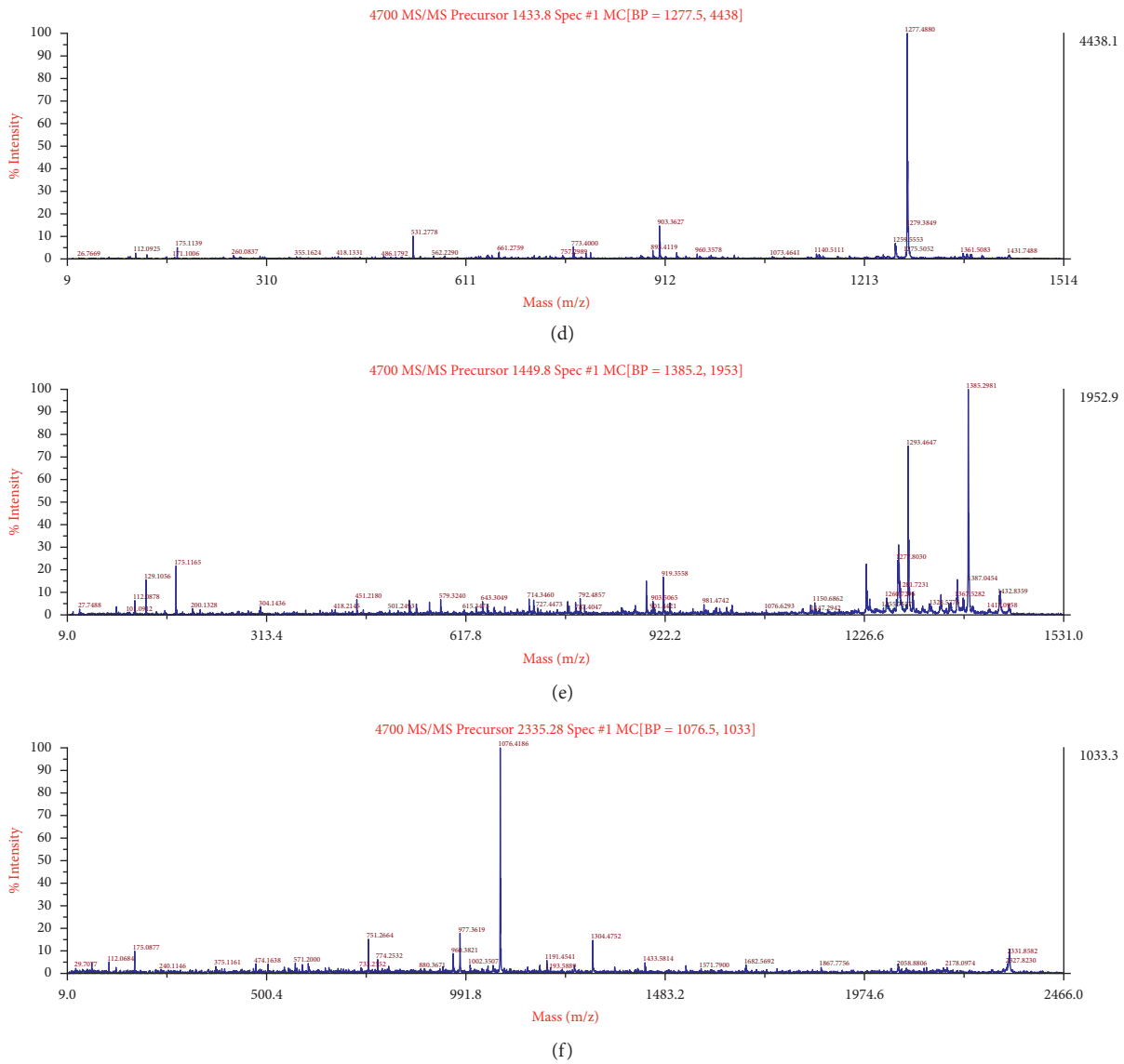


Figure 5: The peptide fingerprint of protein bands extracted from the gel. Peptide mass fingerprinting (a) and tandem mass spectra (b–f).

face of the molecule, the 3-D structure of EXY3 and EXY4 had a typical deep groove in the center and can be likened to a "salad bowl." From the opposite face of the molecule, the 3-D structure of EXY3 and EXY4 was mainly composed of the (α/β) 8 TIM-barrel fold. The catalytic residue (Glu273) of EXY3 and EXY4 was located in the β-bulge on the β7 strand. There were some differences in the structure between EXY3 and EXY4, whereas the main one is shown in the red box. Because of the C-terminal truncation, the structure of EXY3 was simpler than that of EXY4.

4. Discussion

Wheat cultivars contain xylanase inhibitors, the contents of TAXI, XIP, and TLXI are 17–200 μg/g, 156–560 μg/g, and 51–150 μg/g, respectively [19, 20]. The inhibition by xylanase inhibitors on microbial xylanase directly relates to their levels. Some inhibition exceeded 50%, and the maximum inhibition even reached 90% [21, 22]. Thus, xylanase inhibitors remarkably increased the quantity of xylanase needed to achieve optimal hydrolysis [7, 23]. It was shown that TAXI and XIP used a competitive exclusion mechanism to inhibit the activity of microbial xylanases [24]. Theoretically, adding more xylanase after the xylanase inhibitors are saturated with substrate should provide active xylanase, but that may not be the case. Based on the concentration and type of enzyme and inhibitor, the concentrations of XIP and TAXI were approximately 100–700 times greater than the amount of xylanase added to the feed [25]. In commercial practice, due to the low moisture content of the feed, xylanase inhibitors present in the feed might not be in contact with added exogenous xylanase, so the latter might not be completely inactivated [26]. The above studies suggested that the use of uninhibitable xylanases may be a promising way to circumvent problems related to endogenous xylanase inhibitors.

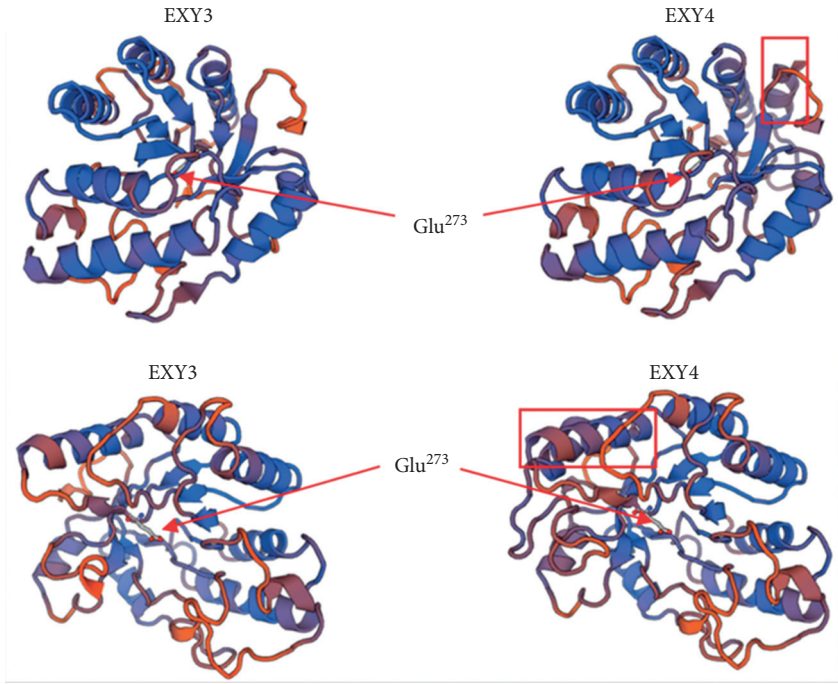


Figure 6: The three-dimensional structure models of the EXY3 and EXY4 proteins. The Glu273 is the putative catalytic site in both EXY3 and EXY4. Upper, one face, and lower, the opposite faces of EXY3 and EXY4, and the main difference between EXY3 and EXY4 are shown in the red box.

Plant endogenous xylanases are resistant to xylanase inhibitors, so wheat xylanases may be very suitable for industrial wheat applications [27]. Wheat cultivars contain small quantities (0.69–1.4 mU/g) of xylanases [22]. During wheat germination, the content of xylanase increased 10-fold [28]. In 2004, Scott et al. demonstrated that dry pregerminated grain significantly increased the feed intake and growth of broilers, but the two extra steps of pregerminating and subsequent drying of wheat would significantly increase costs [29]. Extraction and purification of an endo-1,4-β-xylanase from wheat have also been done, but the purification processes were time-consuming and laborious [5]. In the present work, wheat xylanase was successfully expressed using biotechnology, and two active xylanases of 36.108 kDa and 41.933 kDa were obtained. The wheat xylanases were highly similar to the barley xylanases. The present result was different from that of Van Campenhout et al. (2007), who expressed barley xylanases of 34, 41, and 61.5 kDa in *E. coli*, whereas only the 61.5 kDa form was expressed in the cytoplasmic soluble fraction and was active xylanase [15]. The 61.5 kDa form was the precursor, which could be activated to a 41 kDa intermediate and subsequent 34 kDa mature form [30]. The possible intermediate and mature forms were obtained here by genetic engineering. Compared with previously used pregermination of wheat, extraction, and purification, genetic engineering may be the most appropriate way to obtain amounts of wheat xylanase.

Most efforts at purifying and characterizing xylanases from cereals were aimed at studies on their functionality in cell wall degradation during seed germination. Little information is available on their use to improve cereal quality; this application has been disregarded until recently [31]. Fortunately, some researchers have paid attention to its advantage recently. In order to solve the problem of using cereal xylanases to control reasonable degradation of cereal AXs, Guo et al. extracted and purified an endo-1,4-β-xylanase from wheat malt. The MW was 27.8 kDa, and it was purified 12.08-fold to achieve a specific activity was 4.47 U/mg [5]. In the present study, the specific activities of EXY3 and EXY4 were 152.0891 and 67.2928 U/mg, respectively, much higher than that of Guo et al. The results further demonstrated that genetic engineering could obtain plenty of active wheat xylanases for industrial wheat applications.

The two endo-1,4-β-D-xylanases obtained here came from the same DNA, but because of a nonsense mutation, EXY3 was 51 amino acid residues shorter. This is similar to maize endo-1,4-β-D-xylanase where removal of 198 and 48 residues from the N- and C-termini of the precursor converts it into the 35 kDa form [32]. Like many other endo-1,4-β-xylanases, the proteins obtained here contained a single GH10 catalytic domain and the same catalytic site [33]. With the exception of one additional O-glycosylation site in EXY4, they shared two putative N-glycosylation sites and three putative O-glycosylation sites. The 3-D structures had the TIM-barrel fold; there were subtle differences.

The protein sequences of the interior/core region of the TIM-barrel fold were conserved, contrasting with the sequences around the terminal regions being less conserved [34]. Some N- and C-terminal regions were even in a disordered form, and these regions were not necessary for catalysis. After these sequences are truncated, the catalytic efficiency or other properties of the enzyme may be improved. As such, the thermostability and activity of GH10 xylanase from *A. niger* significantly increased when it was truncated by

five N-terminal and one C-terminal disordered residues [35]. In this regard, EXY3 was the same as EXY4 truncated by 51 C-terminal residues. The main structure and active site did not change, but the activity of EXY3 was higher than that of EXY4. The truncation of the C-terminal residues may be the main reason for the improvement of EXY3 activity.

Compared with GH11 xylanases, GH10 xylanases can degrade both linear xylans, decorated heteroxylans, and low-molecular-weight xylooligosaccharides, generating xylobiose and xylose as the main hydrolysis products [36]. Moreover, barley xylanase degrades different heteroxylans and xylooligosaccharides, generating xylobiose, and xylose as the major hydrolysis products [15]. Because of the high similarity, wheat xylanases should have similar actions to barley xylanase. The activity of wheat xylanase remains stable in the range of 40 ˚C to 50 ˚C and is rapidly inactivated above 55 ˚C [37]. The activities of rye xylanases were optimal between pH 3.8 and 5.3 [38]. If these grain-derived xylanases can be added to the feeds after the pelleting process, their activities must remain high at the temperature (approximately 40 ˚C) and pH (approximately pH 4.8) of the intestinal tract [39]. The activities of grain-derived xylanases were not affected in a simulated digestion process [11]. The present work, along with these previous studies, indicates that wheat xylanases, as obtained here by biotechnology, have potential applications for modifying cereal-based feeds.

5. Limitations

Several limitations exist in this study and deserve discussion. First, we cloned the main active domain region of xylanase in this study, but not the full mRNA sequence. However, we are not sure whether there is any regulatory element beyond the cloned sequence, which may affect the bio-active of xylanase. Second, this study was carried out at the laboratory level and did not attempt to express the recombinant xylanase by large-volume fermentation. Third, the biochemical properties of the recombinant xylanase were not analyzed, such as the optimum reaction temperature and pondus hydrogenii (PH). For use as a bio-additive, further experimental studies are needed.

6. Future Perspectives

In the present study, we expressed and purified two active xylanases isoforms: EXY3 and EXY4. They may be used in industry, especially in the production of feed, paper making, and other crop raw materials. First, as we know, the existence of xylan is not conducive to the absorption and utilization of nutritional factors in feed. Second, xylan can hinder the effect of lignin dissolution in pulp, which will affect the quality of the pulp. In order to make the pulp whiter and brighter, the amount of chlorine used in the bleaching process cannot be controlled. Third, the soluble xylan in the outer layer of fruit and vegetable will make the viscosity of the extract too large, resulting in subsequent concentration and drying work difficult. Adding xylanase as bio-additive to degrade the integrity of xylan and other antinutritional factors not only can reduce the viscosity of raw materials and promote the digestion and absorption of feed/food nutrients but also make it easier to get the high-quality pulp.

7. Conclusion

In the present study, two active xylanases from wheat were successfully obtained by genetic engineering. The two active xylanases are GH10 xylanases and have potential application in the cereal-based industry, especially for modifying cereal-based feeds.

Highlights.

(1) Expression and purification of two isoforms from wheat glycoside hydrolase family-10 xylanases

(2) The specific activities of EXY3 and EXY4 were 152.0891 and 67.2928 U/mg

(3) The bioinformatics analysis of EXY3 and EXY4

(4) The two xylanases might be the intermediate and mature forms of wheat xylanases

(5) The two active xylanases have potential application in cereal-based industries

Data Availability

All processed data used in this study can be obtained from the corresponding author on reasonable request.

Ethical Approval

The experiment was approved by the Institutional Animal Care and Use Committee of Guangxi University and the Experimental Ethics Committee of the College of Animal Science and Technology, Guangxi University.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors' Contributions

Fangfang Liang contributed to the methodology. Yi Mo supported the research. Suleman Shah and Ying Xie contributed to the figure preparation of the study. Arshad Mehmood drafted and revised the article. Hesheng Jiang designed and coordinated the research. Yafen Guo performed the analysis and modeling of the data.

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