Appl Biochem Biotechnol (2015) 177:1424–1436
DOI 10.1007/s12010-015-1823-8

Characterization of a Novel Xylanase Gene from Rumen Content of Hu Sheep

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Received: 22 June 2015 / Accepted: 18 August 2015 /
Published online: 10 September 2015

Abstract A novel xylanase gene, *xyn-lxy*, was cloned from a metagenomic fosmid library, which was previously constructed from the rumen contents of Hu sheep and was functionally characterized in *Escherichia coli*. The open reading frame was composed of 1923 bp and encoded for 640 amino acids, including a catalytic domain of glycosyl hydrolase family 10 and carbohydrate-binding module 9. The gene showed 97 % identity with uncultured bacterium Contig1552 but low similarity with xylanases from known cellulolytic-degrading microorganisms in the rumen. The recombinant XYN-LXY showed a specific activity of 664.7 U mg^{-1}. The optimal temperature and pH of the enzyme were 50 °C and 6.0, respectively. Specifically, XYN-LXY was exclusively activated by Mn^{2+} among all of the cations and reducing agents tested in this study. An enzymatic hydrolysis assay revealed that XYN-LXY degraded birchwood xylan into xylooligosaccharide with a low degree of polymerization. After incubation for 4 h, the concentration of the dominant product, xylobiose, was 2.297± 0.175 mg ml^{-1} (74.07 % of total product) followed by xylose with a concentration of 0.656 ±0.010 mg ml^{-1} (21.14 % of total product). The XYN-LXY exhibited deep degradation effects

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Electronic supplementary material The online version of this article (doi:10.1007/s12010-015-1823-8) contains supplementary material, which is available to authorized users.

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on the xylan substrate, which were rarely observed with endo-xylanase, making it a promising candidate for industrial application, especially in biofuel production.

Keywords Rumen · Xylanase · Enzyme properties · Hydrolysis · Xylooligosaccharide

Introduction

Xylan, as a major component of the plant cell wall, is the second most abundant renewable resource [1]. Xylanases (EC 3.2.1.8) are representative glycoside hydrolases (GH) that break long sugar chains of xylan into xylooligosaccharides (XOs); the synergic effects of both endo-xylanase and exo-xylosidase (EC 3.2.1.37) catalyze xylan into D-xylose [2]. According to their primary structure, xylanases are substantially classified into six GH families (GH 5, 8, 10, 11, 30, and 43) (http://www.cazy.org/Glycoside-Hydrolases.html). Most xylanases come from GH 10 and 11 and have been well studied [3].

Xylanases have been of tremendous interest for application in the feed, food, paper pulp, and bioenergy industries over the past several decades [4–6]. To suffice for industrial application, xylanases must remain stable and highly active at high temperatures and over a wide pH range. However, natural xylanases, derived from such an environment as horticultural soil, could not be adopted directly due to their faint activities or properties, and thus, protein engineering strategies are employed to modify the enzymes to handle industrial tasks [7–9].

In recent years, bioethanol production has been increasingly used as a complement to fossil fuel due to its low NOx emission [10]. Hexoses, such as glucose, have been widely used as traditional substrates for ethanol production through *Saccharomyces cerevisiae* fermentation. It is commonly known that the glucose in biofuel production is mostly isolated from maize, a main cereal for human and animals. In light of this, pentoses from lignocellulose biomass, such as xylose and arabinose, are promising candidates for biofuel production [11]. More recently, great efforts have been made to improve the efficiency of biofuel production from a xylose substrate [10, 12–16].

Although the hydrolysis pattern of xylanase showed certain variety, high concentrations of XOs with degrees of polymerization (DP) from two to six, with trace amounts of monosaccharide xylose, were frequently observed in hydrolysis products [17–24]. Metagenomic analysis is a powerful tool to reveal new genes from samples collected from extreme environments such as the gastrointestinal tract and volcanos [25]. We previously established a fosmid library from the rumen content of Hu sheep and screened 18 xylanase-positive clones from the library [26, 27]. In this study, we isolated a novel xylanase gene *xyn-lxy* from an uncultured strain in the rumen fluid. The effects of temperature, pH, metal cations, and reducing agents on the catalytic activities of the enzyme were characterized. The hydrolysis pattern toward birchwood xylan was also investigated.

Materials and Methods

Strains, Plasmids, and Reagents

A fosmid library of 393 MB, obtained from Hu sheep rumen fluid, was constructed in our previous study [26]. The Plasmid Midi isolation kit C25 and Ni-NTA 6× His-tag agarose were

purchased from Qiagen (Shanghai, China). The *Escherichia coli* BL21 (DE3) competent cells, T4 DNA ligase, and pGEM-T Easy vector were purchased from Promega (Madison, Wisconsin, USA). The restriction enzymes were purchased from Takara (Dalian, China). The pET30a(+) plasmid was obtained from Novagen (Madison, Wisconsin, USA). The birchwood xylan and standard XOs were purchased from Sigma (Saint Louis, MO, USA) and Megazyme (Wicklow, Ireland), respectively. All other chemicals were purchased from Sangon (Shanghai, China).

Screening and Sequence Analysis of Novel Xylanase Gene

The fosmid library constructed previously [26] was employed to screen for novel xylanase genes. Lysogeny broth (LB) agar plates (0.5 % yeast extract, 1 % tryptone, 1 % NaCl, and 2 % agar) containing 1 % birchwood xylan were used for Congo red staining [28]. Then, xylanolytic positive clones were picked and subjected to further comparison of xylanase activity by estimating the diameter of the halo generated in the plate. The clone L3 showing the highest catalytic activity was sequenced by Sangon (Shanghai, China). The open reading frame (ORF) of the potential gene and its function were predicted using the online software Softberry (http://linux1.softberry.com/berry.phtml?topic=fgenesb&group=programs&subgroup=gfindb) and PFAM (http://pfam.xfam.org/search), respectively. Sequence alignment was performed using a BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Cloning of a Novel Xylanase Gene *xyn-lxy*

A novel xylanase gene *xyn-lxy* was obtained according to the sequencing result of the L3 clone. To clone *xyn-lxy*, approximately 50 ng plasmid DNA was used as the template for amplification of the *xyn-lxy* gene by using a pair of primers Xyn-lxy-F: 5′ CCGGAATTCATGAAGAAGAAACTGACGAG3′(*Eco*RI) and Xyn-lxy-R: 5′ CCGCTCGAGTTATACCAGCTTGGCGTTACCAA3′(*Xho*I). PCR reaction was performed for 35 cycles consisting of 94 °C for 45 s, 55 °C for 45 s, and 72 °C for 2 min. The band of ~2 kb was purified, ligated with pGEM-T, transformed into DH5α competent cells, and streaked onto a 100 μg ml^{-1} ampicillin LB agar plate supplemented with 100 mM isopropyl-thio-β-D-galactopyranoside (IPTG) and 20 mg ml^{1} 5-bromo-4-chloro-indolyl-D-galactoside (X-gal). Then, the positive clones were picked and sequenced by Sangon (Shanghai, China). The resulting plasmid was designated as pGEM-T/*xyn-lxy*.

The pGEM-T/*xyn-lxy* plasmid was digested with *Eco*RI and *Xho*I and subjected to ligation with pET30a(+) (the vector map could have been acquired from http://www.synthesisgene.com/vector/pET-30a.pdf), which was previously digested with the same enzymes. Then, the ligation product was transformed into *E. coli* BL21 (DE3) and streaked onto an LB agar plate containing 100 μg ml^{-1} kanamycin. Plasmids of the transformants were isolated and used for screening by PCR (Supplemental Fig. S1). The resulting strain was designated as BL21/pET30a(+)/*xyn-lxy*.

Expression and Purification of XYN-LXY

The BL21/pET30a(+)/*xyn-lxy* was inoculated into 5-ml LB medium containing 100 μg ml^{-1} kanamycin and incubated at 37 °C overnight. On the next day, the culture was inoculated into a

250-ml flask containing 100-ml LB medium, followed by shaking at 200 rpm at 37 °C until $OD_{600}=0.5^{-1}$. After the addition of IPTG to a final concentration of 1 mM, the flask was further incubated at 150 rpm at 25 °C for 8 h. The culture was chilled on ice for 30 min and then centrifuged at 12,000 rpm at 4 °C for 15 min. The cell pellets were collected and resuspended in 30 ml PBS buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4). The pellets were sonicated on ice for 15 min, followed by centrifugation at 12,000 rpm at 4 °C for 15 min. Subsequently, the supernatant was assayed for xylanase activity and used for protein purification.

To purify xylanase, 6× His-tagged Ni-NTA agarose was mixed with total lysis and agitated gently on ice for 1 h in the presence of 20 mM imidazole. The beads were washed three times, and the target proteins were eluted with 250 mM imidazole. The purified xylanase was used for the enzymatic assays.

SDS-PAGE and Western Blotting Analysis

Both the crude lysis and purified protein were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, 12 % running gel and 4 % stacking gel) [29]. After gel electrophoresis, western blotting was carried out to detect the target protein using a mouse anti-His monoclonal antibody, according to the protocol described previously [9].

Enzyme Assay

The xylanase activity assay was carried out using 1 % birchwood xylan substrate, according to the method described by Bailey et al. [30], which quantified liberated reducing sugars released from the main chain of xylan with dinitrosalicylic acid (DNS). Briefly, 50-μl protein solution was mixed with 50 μl 1 % birchwood xylan and incubated at 50 °C for 10 min. To stop the reaction, 100 μl DNS was added into the solution, and the mixture was immediately boiled for 5 min. Then, the absorbance was determined spectrophotometrically at 540 nm. D-Xylose was employed as the standard. One unit (U) of xylanase activity was defined as the amount of the enzyme that produced reducing sugars at the rate of 1 μmol min^{-1}. The protein concentration was determined using the Bradford method [31], and bovine serum albumin (BSA) was used as the standard.

Enzymatic Characterization of XYN-LXY

In all the enzymatic assays of this section, approximately 10 μg purified XYN-LXY protein was used unless otherwise mentioned. The optimal pH of XYN-LXY was determined by incubating the enzyme in various pH buffers (pH 3.0–8.0, citrate/phosphate buffer, pH 8.0–9.0, 0.2 M Tris-HCl buffer, and pH 9.0–10.0, glycine-NaOH buffer) for 10 min. To estimate the pH stability of XYN-LXY, the enzyme was preincubated in different buffers at 50 °C for 30 min, and the residual activity was determined. The initial activity before preincubation was taken as 100 %.

To estimate the optimal temperature of XYN-LXY, the enzyme was incubated at 30–90 °C, pH 6.0, for 10 min. A thermostability assay was performed by evaluating the residual activity after preincubation in citrate/phosphate buffer (pH 6.0) at 30–60 °C for 60 min. Portions were sampled at different time intervals (2, 5, 10, 30, and 60 min). The initial activity before preincubation was taken as 100 %.

To investigate the effects of metal ions, EDTA, and SDS on the catalytic activity of XYN-LXY, the purified enzyme was incubated with various chemicals at 50 °C for 10 min. Then, the xylanase activity was assayed under the optimum conditions (pH 6.0, 50 °C).

The kinetic constants of XYN-LXY were determined under standard conditions (pH 6.0, 50 °C) for 10 min. The concentration of the birchwood xylan substrate ranged from 0.4 to 15 mg/ml. The data were fitted with linear regression for a Lineweaver-Burk plot using Microsoft Excel 2010.

Analysis of the Hydrolysis Products of Birchwood Xylan

To evaluate the hydrolysis profile of XYN-LXY, approximately 80 U pure enzyme was incubated with 1 % birchwood xylan at 50 °C. The portions were collected at different time intervals (5 min, 30 min, 1 h, 2 h, 4 h, and 24 h) and immediately boiled for 10 min. Then, all samples were subjected to a Waters Alliance HPLC system (separations module e2695, Waters, Milford, MA, USA) equipped with a Sugar-Pak TM 1 column (300 mm×6.5 mm) and refractive index detector (Waters 2414). Distilled water was applied as the mobile phase with a flow rate of 0.5 ml min^{-1}. Finally, the released sugars from the birchwood xylan were quantified by comparing the relative areas to standard xylose, xylobiose (X2), xylotriose (X3), xylotetroase (X4), and xylopentose (X5).

Results and Discussion

Identification of a Novel Xylanolytic Gene from a Fosmid Library

In our previous study, 18 clones out of 12,704 that produced halo zones on LB plates supplemented with 1 % xylan were obtained from a Hu sheep rumen fluid fosmid library [26]. The clone L3, showing the highest xylanolyic activity, was sequenced by shotgun pyrosequencing, and 44 potential ORFs were functionally predicted by the online softwares Softberry and PFAM. Among them, a xylanase-encoding ORF of 1923 bp, designated as *xyn-lxy*, was cloned. The gene coded for 640 amino acids (AA), with a GH 10 catalytic domain locating at its N-terminus (50–443 AA) and a carbohydrate-binding module (CBM) 9 locating at its C-terminus (456–640 AA) (http://pfam.xfam.org/search) and a linker sequence between them. The online software prediction indicated that the theoretical molecular weight and isoelectric point of XYN-LXY were 71.3 kDa and 4.95 (http://web.expasy.org/compute_pi/), respectively, with a signal peptide of 22 AA locating at the N-terminus (http://www.cbs.dtu.dk/services/SignalP/).

The data derived from BLAST revealed that XYN-LXY showed low similarity to xylanases from known cellulolytic-degrading microorganisms in the rumen, such as *Lachnospiraceae* bacterium (WP_035658042, 61 %), *Eubacterium ruminantium* (BAA09971, 59 %), *Roseburia intestinalis* (WP_022112477, 55 %), and *Butyrivibrio proteoclasticus* (WP_013279709, 52 %). However, it shared 97 % identity with the uncultured bacterium Contig1552 (AHF25111), which was recently uncovered in a metagenomic study of the rumen digesta of Jersey cows [32]. It was suggested that XYN-LXY was a novel xylanase gene of interest for use as a feed additive due to the high digestibility of the rumen toward cellulosic stuffs. The close relationship between XYN-LXY and xylanases from other rumen microbes was also confirmed via phylogenetic tree analysis (Fig. 1) and AA alignment (Fig. 2).

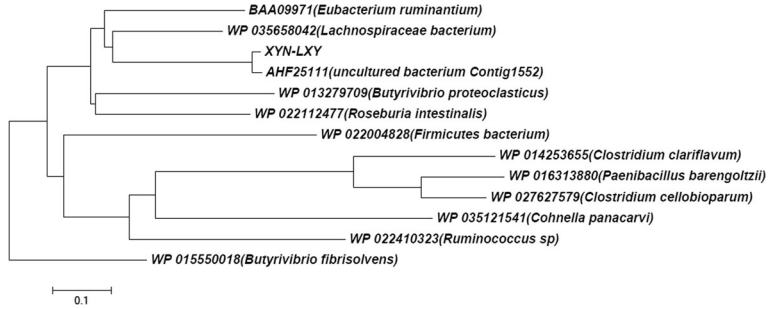

Fig. 1 Phylogenetic dendrogram of XYN-LXY and xylanases from related microorganisms

Expression and Purification of Recombinant XYN-LXY

The *E. coli* transformant carrying xylanase gene *xyn-lxy* was induced with 1 mM IPTG for 8 h, and the cell pellets were collected. After centrifugation and sonication, the soluble fractions were subjected to 6× His-tag purification. Both the crude supernatant and the purified protein were then analyzed by SDS-PAGE, and a distinguishable band with an estimated molecular mass of 80 kDa was observed (Fig. 3), which was in agreement with the calculated molecular mass. After purification, the purified XYN-LXY showed a specific activity of 664.7 U mg^{-1} (Table 1). The K_m and V_{max} of the recombinant XYN-LXY were 4.39 mg ml^{-1} and 3.20 μmol min^{-1}, respectively (Supplemental Fig. S2). The turnover number (k_{cat}) of the enzyme was 125.9 s^{-1}, suggesting that it was a promising candidate for hemicellulose hydrolysis.

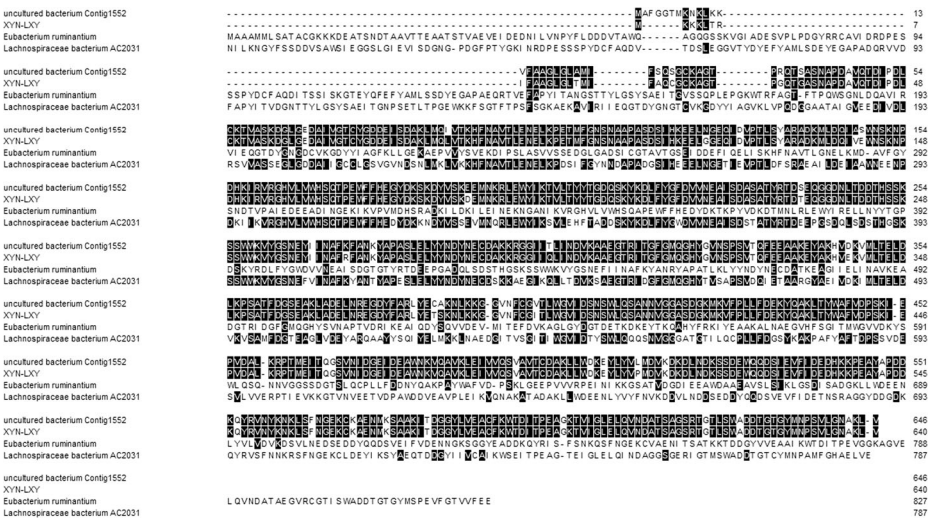

Fig. 2 Amino acid alignment of XYN-LXY and three closely related xylanases. The alignment includes xylanases from uncultured bacterium Contig1552 (AHF25111), *Lachnospiraceae* bacterium (WP_027439728), and *Eubacterium ruminantium* (BAA09971). Consensus amino acids were shaded in *black*. The figure was generated using DNASTAR Lasergene 10

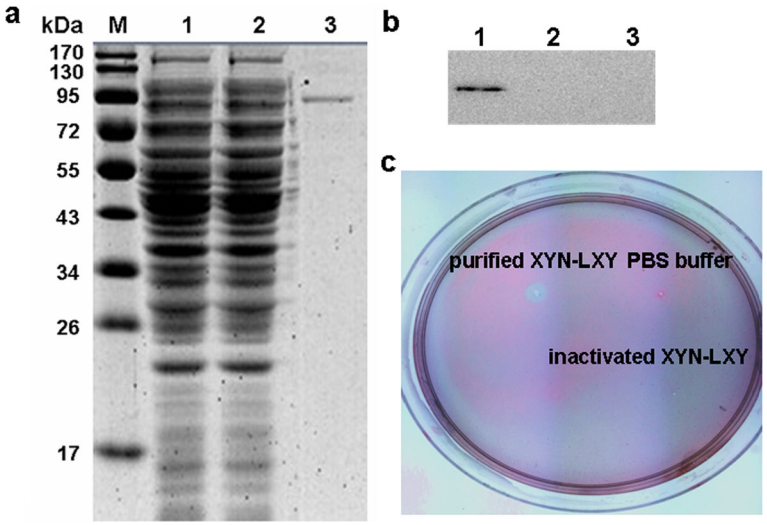

Fig. 3 Expression, purification, and enzymatic test of XYN-LXY. **a** SDS-PAGE analysis of crude and purified XYN-LXY; *M*: standard protein marker; *1*: crude supernatant from *E. coli* induced with 1 mM IPTG for 8 h; *2*: crude supernatant from *E. coli* without IPTG induction; *3*: purified XYN-LXY. **b** Western blot analysis of XYN-LXY; *1*: crude supernatant with IPTG induction; *2*: crude supernatant without IPTG induction; *3*: crude supernatant of *E. coli* harboring empty pET30a(+). **c** Enzymatic test using LB plate supplemented with 1 % xylan. The inactivated XYN-LXY was prepared by boiling the enzyme for 10 min

Optimum Temperature and Thermostability of XYN-LXY

The activity of the recombinant XYN-LXY increased with the rise of temperature and reached a maximum at 50 °C (Fig. 4a). The heat challenge assay suggested that XYN-LXY could maintain high xylanase activity below 40 °C, which was probably an adaptation to the rumen environment (38–41 °C). However, the enzyme lost its majority activity when exposed to temperatures above 50 °C for 10 min (Fig. 4c), suggesting that XYN-LXY was a mesophilic xylanase. XYN-LXY subtly started to lose activity at a temperature lower than the optimum temperature. However, similar phenomena were also observed in other studies [21, 24, 33]. To satisfy the requirement for application, a protein engineering approach, such as site mutagenesis and DNA shuffling, could be further carried out to improve the thermostability of XYN-LXY [7–9].

Optimum pH and Stability of XYN-LXY

The recombinant XYN-LXY was found to be active in a pH range from 5.0 to 7.0, with the highest activity at pH 6.0 (Fig. 4b). After preincubation in various pH buffers for 30 min,

Table 1 Purification of XYN-LXY expressed in *E. coli*

Purification steps	Volume (ml)	Total activity (U)	Total protein (mg)	Specific activity (U mg^{-1})	Yield (%)	Purification fold
Crude enzyme	20	1104	59.1	18.7	100	1
Purified enzyme	1	285.8	0.43	664.7	25.9	35.5

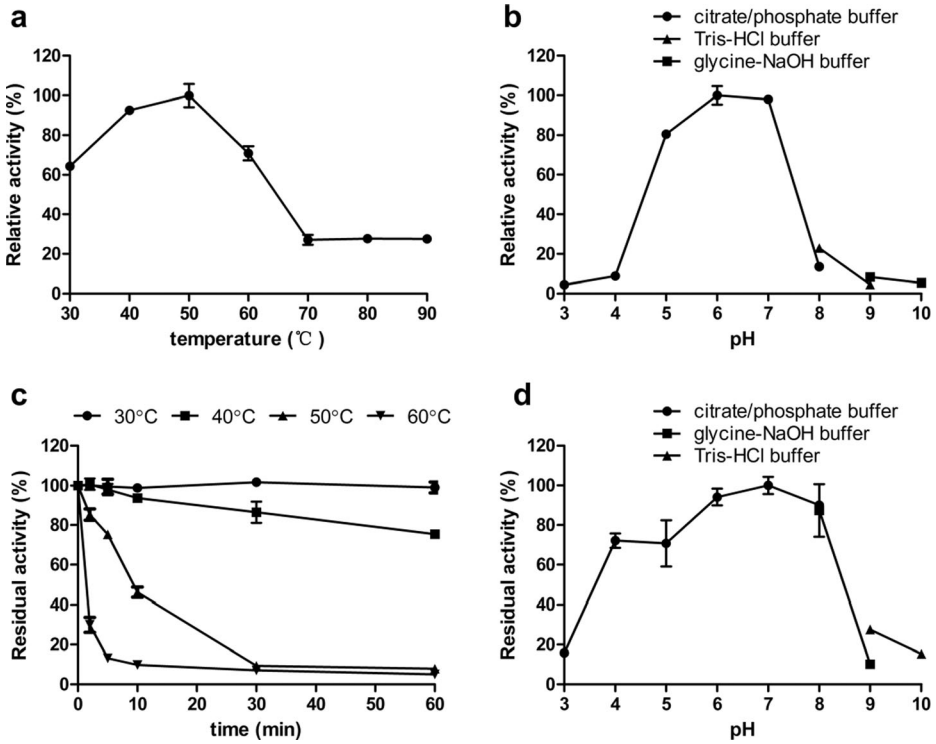

Fig. 4 Effects of temperature and pH on catalytic activity of XYN-LXY. **a** The optimal temperature of XYN-LXY. **b** The optimal pH of XYN-LXY. **c** Thermostability of XYN-LXY from 30 to 60 °C. **d** The pH stability of XYN-LXY. Assays were performed as described in the "Materials and Methods" section using 1 % birchwood xylan as the substrate. At the optimal pH and temperature, the highest xylanase activity was taken as 100 %. The xylanase activity under optimal conditions (50 °C, pH 6.0) was taken as 100 % in the assay to determine pH stability. For thermostability, the xylanase activity of the enzyme without a heat challenge was taken as 100 %. All of the assays were carried out in triplicate

XYN-LXY retained more than 90 % of its initial activity in the range from pH 6.0 to 8.0. However, the hydrolytic activity of the XYN-LXY dramatically declined when assays were conducted at pHs <6.0 or pHs >8.0 (Fig. 4d). A cold-active xylanase from XynGR40, also derived from rumen content, had an optimum pH at 6.5 and exhibited high activity from pH 5.5 to 7.5 [34]. Both XYN-LXY and XynGR40 showed high catalytic activity and good stability at neutral pHs, which was probably an adaptation to the normal environmental condition (pH 5.5–7.5) of the rumen for feed digestion.

Effects of Cations and Reducing Agents on XYN-LXY

The stimulatory or inhibitory effects of XYN-LXY by 10 mM of various metal cations, 10 mM EDTA, and 1 % SDS (*w*/*v*) were extensively investigated in this study. As seen in Table 2, EDTA, SDS, and most metal cations (Cu^{2+}, Fe^{3+}, Zn^{2+}, K^{+}, and Ag^{+}) tested in this study were found to dramatically inhibit XYN-LXY ($P<0.05$). However, the inhibitory effects of Co^{2+}, Ca^{2+}, Na^{+}, and Ni^{2+} were only marginal. Interestingly, Mn^{2+} was the only activator of the cations tested that significantly enhanced the hydrolytic activity of XYN-LXY ($P<0.05$). The promotive effect of Mn^{2+} was observed by two other studies [33, 35], although most studies

Table 2 Effects of various cations on activity of XYN-LXY

Reagents (10 mM)	Relative activity (%)
Control	100
Co^{2+}	91.0±4.3
Ca^{2+}	90.2±3.5
Cu^{2+}	80.5±4.7[a]
Na^{+}	87.6±14.6
Fe^{3+}	85.7±8.9[a]
Ni^{2+}	89.1±9.6
Zn^{2+}	84.1±11.0[a]
Mn^{2+}	117.2±11.5[a]
Mg^{2+}	79.9±7.8[a]
K^{+}	84.9±9.4[a]
Ag^{+}	16.0±1.8[a]
EDTA	72.1±8.5[a]
SDS[b]	0

[a] Xylanase activity was significantly increased or decreased compared to control ($P<0.05$)

[b] The concentration of SDS was 1 % (*w*/*v*)

revealed that Mn^{2+} showed inhibitory activity on xylanase activity [36–39]. Usually, cations such as Ca^{2+}, Co^{2+}, Cu^{2+}, and Zn^{2+} were believed to function as activators of xylanase activity [23, 35, 37]. However, it was also reported that Ca^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, and Fe^{3+} inhibited enzymatic activities, especially at high concentrations (5 to 20 mM) [39–42]. Taken together, the effects of cations on the catalytic activity of xylanase seem to be tricky, and further studies regarding the dosage-dependent effect and conformational change of enzymes need to be carried out to gain insight into the relationship of cations and xylanolytic activity.

Analysis of Hydrolysis Products of Birchwood Xylan

In the current study, the hydrolysis products of birchwood xylan by endo-xylanase were analyzed by HPLC (Fig. 5). Approximately 80 U purified XYN-LXY was employed to degrade the xylan substrate. After incubation at 50 °C for 5 min, the XOs with low DP

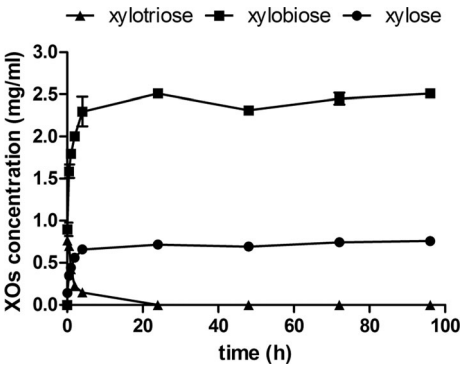

Fig. 5 Hydrolysis profile of birchwood xylan degradation by XYN-LXY. One percent birchwood xylan substrate was incubated with approximately 80 U XYN-LXY at 50 °C. Aliquots obtained at different time intervals (5 min, 30 min, 1 h, 2 h, 4 h, 24 h, 48 h, 72 h, and 96 h) were boiled for 10 min and subjected to HPLC analysis

(xylose, X2, and X3) accumulated rapidly. The concentrations of X2 and X3 were 0.897± 0.079 mg ml^{-1} (49.67 % of total product, the same below) and 0.764±0.024 mg ml^{-1} (42.35 %), respectively. However, xylose showed only a slight increase, with a concentration of 0.144±0.018 mg ml^{-1} (7.98 %). After hydrolysis for 5 min, the concentrations of xylose and X2 ascended as the reaction continued and reached a stationary phase at 4 h, with concentrations of 0.656±0.010 mg ml^{-1} (21.14 %) and 2.297±0.175 mg ml^{-1} (74.07 %), respectively. However, X3 started to reduce to a trace amount after hydrolysis for 4 h.

It is known that two key xylanases, endo-xylanase and xylosidase, are required for the hydrolytic degradation of the main chain of xylan. Endo-xylanase is believed to catalyze xylan into XOs, and partial products can be further converted into a monosaccharide xylose by xylosidase. Consequently, XOs, mainly X2 or/and X3, and a small amount of xylose were obtained after enzymatic hydrolysis of birchwood xylan by either GH 10 [20, 22, 24] or GH 11 [17–19, 21, 23] endo-xylanase. As for XOs with higher DP, such as X4 and X5, results varied among these studies. After adequate digestion (at least 12 h), birchwood xylan was able to release X4 only [24] or X5 only [22] or both X4 and X5 [18] directly or indirectly (via the transglycosylation reaction). Noticeably, negligible xylose was detected in all these studies. However, Ali et al. [43] reported that a Xyn10A from *Clostridium acetobutylicum* ATCC 824 exclusively released xylose from xylan or XOs, except X2, while X2 and X3 were found to be the main products when the substrates were catalyzed by xylanases from the same strain [44, 45]. Taken together, the various hydrolysis patterns above suggested that endo-xylanase probably functioned randomly on the main chain of xylan.

In the current study, a novel xylanase, XYN-LXY, derived from Hu sheep rumen content was employed to catalyze birchwood xylan substrate. Interestingly, X2 (74.07 %) was the prenominal product, followed by xylose (21.14 %), after hydrolysis for 4 h (Fig. 5). To our knowledge, comparable results were only found in two studies from the same group. The *Streptomyces* sp. S9 xylanase [46], isolated from the hottest place in China, Flaming Mountain in the Turpan basin of Xinjiang, with an air temperature of 47 °C during the daytime, and a cold-active xylanase, XynGR40 [34], derived from goat rumen content, were both reported to be capable of releasing high amounts of X2 and xylose from xylan in the absence of xylosidase. We speculated that the reason for the two enzymes and XYN-LXY in this study to exhibit an abnormal hydrolysis pattern was the extreme environmental conditions from which they were derived.

Conclusions

In this study, a novel xylanase gene, *xyn-lxy*, was cloned from an uncultured strain in the rumen fluid. The enzyme was optimally active at 50 °C and pH 6.0. The thermostability assay revealed that XYN-LXY was a mesophilic xylanase. Interestingly, it was demonstrated that birchwood xylan could be completely degraded into monosaccharide by XYN-LXY, indicating that the enzyme might be a potential candidate for application in the feed and biofuel industries.

Acknowledgments The authors acknowledge the financial support from the Innovation Team Program of Zhejiang province (2011R50025) and the Importation and Development of High-Caliber Talents Project of Beijing Municipal Institutions (CIT&CD20130324).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests.

Authors' Contribution Qian Wang drafted the manuscript. Yang Luo and Bo He carried out the studies and contributed to the drafting of the manuscript. Jia-Kun Wang, Jian-Xin Liu, and Lin-Shu Jiang participated in the project design and manuscript preparation. All authors read and approved the final manuscript.

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