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ORIGINAL PAPER

Symbiotic *Streptomyces* sp. TN119 GH 11 xylanase: a new pH-stable, protease- and SDS-resistant xylanase

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Abstract A pH-stable and protease-resistant xylanase (XynB119) was identified from *Streptomyces* sp. TN119, a strain isolated from the gut luminal contents of longhorned beetle (*Batocera horsfieldi*) larvae. Using the GC TAIL-PCR method, the 1,026-bp coding gene (*xynB119*) with 67.3% GC content was successfully cloned and expressed in *Escherichia coli*. It encodes a 341-residue polypeptide with a calculated molecular mass of 35.9 kDa, including a putative 41-residue signal peptide, a catalytic domain of glycosyl hydrolase (GH) family 11, a short Gly/Pro-rich linker, and a family 2 cellulose-binding domain (CBM 2). The deduced amino acid sequence is most similar to (61.9% identity) an endo-1,4-β-xylanase from *Streptomyces thermoviolaceus* OPC-520. Purified recombinant XynB119 exhibited peak activity at 50°C and pH 7.0, remained stable over a broad pH range (retaining >70% activity after incubation at pH 1.0–11.0 for 1 h at 37°C without substrate), had strong protease resistance (retaining >90% activity after proteolytic treatment at 37°C for 1 h) and SDS resistance (at 100 mM). These properties make XynB119 promising for application in the feed industry and valuable for basic research. Compared to r-XynB119, the r-XynB119 derivative without CBM 2 and linker region (r-XynB119d) exhibited a decreased pH stability of >25% at extreme pHs (pH 1.0–3.0 and pH 11.0–12.0).

Keywords Longhorned beetles · pH stability · Protease resistance · *Streptomyces* sp. TN119 · Xylanase

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Introduction

Xylan is the most common hemicellulose, representing up to one-third of the total dry weight [1]. Endoxylanases (EC 3.2.1.8) are glycosidases and catalyze the hydrolysis of β-1,4-D-xylosidic linkages in xylan. Based on amino acid sequence similarities, the majority of endoxylanases fall into families 10 and 11 of the glycosyl hydrolase (GH) and are produced by various microorganisms, including bacteria, fungi and yeasts [2].

Endoxylanases have widespread potential for biotechnological applications in food processing, as a feed additive for monogastric and ruminant animals, in biobleaching of kraft pulps, and in the textile and others industries [3]. Xylanases are frequently utilized alone, but are more commonly used in conjunction with other enzymes, such as proteases [3].

Wood-feeding insects, such as larvae of longhorned beetles (Cerambycidae) that can develop deep within sapwood [4], are thought to be a potential source harboring diverse and novel symbiotic microorganisms [5]. Symbiotic microorganisms can produce various glycosyl hydrolases and play important roles in their host's ability to absorb nutrients [6–8]. From the symbiotic *Sphingobacterium* sp. TN19 harbored in the gut of *Batocera horsfieldi* larvae, we have previously isolated a GH 10 xylanase with high activity at low temperature and potential in the aquaculture and food

industries [9]. We also obtained a GH 10 xylanase from symbiotic *Streptomyces* sp. TN119 of the same source [10]. However, to date, reported enzymes from symbiotic microorganisms in the gut of longhorned beetles still remain extremely limited [11–13]. In this study, we obtained one more xylanase-encoding gene from *Streptomyces* sp. TN119. The gene was expressed in *Escherichia coli*, and the purified recombinant enzyme showed broad pH stability and very strong resistance to proteases and SDS.

Materials and methods

Strains, vectors and reagents

Streptomyces sp. TN119 (ACCC 03947) is a symbiotic bacterium that was isolated from the gut luminal contents of *B. horsfieldi* larvae [10]. The pGEM-T Easy vector (Promega, Madison, WI), pET-22b(+) vector (Novagen, San Diego, CA), *E. coli* Trans1-T1 (TransGen, Beijing, China) and *E. coli* BL21 (TransGen) were used for gene cloning and expression, respectively. The His-tagged protein was purified by Ni^{2+}-NTA Agarose (Qiagen, Valencia, CA). Genomic DNA and plasmid isolation and purification kits were purchased from TIANGEN (Beijing, China). Restriction endonucleases, T4 DNA ligase, DNA polymerase (*TaKaRa rTaq*™ and *Pyrobest*™), dNTPs, and GC buffer I were purchased from TaKaRa (Otsu, Japan). Substrates oat spelt xylan (X-0627), barley β-glucan, lichenan, and carboxymethyl cellulose sodium (CMC), and proteases including pepsin, trypsin, α-chymotrypsin, collagenase, and proteinase K were purchased from Sigma (St. Louis, MO). Isopropyl-β-D-1-thiogalactopyranoside (IPTG) was from Calbiochem (Darmstadt, Germany). All other chemicals were of analytic grade.

Gene cloning

A degenerate primer set (AXyn11F and AXyn11R; Table 1) was designed based on the two conserved motifs containing the catalytic glutamate residues (shown in bold): P-L-[I/V/A/M]-**E**-Y-Y and **E**–G-Y-[Q/K]-S–S-G—of GH 11 xylanases (Fig. 1), and was used to amplify the partial xylanase gene. Using the genomic DNA isolation kit, genomic DNA of strain TN119 was extracted and used as the PCR template. A touchdown PCR with the degenerate primer set was performed as follows: 94°C for 5 min, 10 touchdown cycles of 94°C for 30 s, 60°C for 30 s (decreasing 0.5°C each cycle), and 72°C for 30 s, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and a final extension step at 72°C for 7 min. The PCR product was gel purified, ligated to pGEM-T Easy vector, transformed into *E. coli* Trans1-T1, and sequenced by Biomed (Beijing, China).

A thermal asymmetric interlaced-PCR specific for GC-rich genes (GC TAIL-PCR) [10] was employed with a little modification (Fig. 2) to obtain the full-length xylanase gene

Table 1 Primers used in this study

Primer[a]	Sequence (5′ → 3′)[b]	Tm (°C)[c]
AXyn11F	TAYMTGDSNSTBTAYGGBTGG	60–55
AXyn11R	TRCCVCTVCTYTKRTAVCCYTC	
XynB119uSP1	**CGTTGGTGCGGGTCGCCTTGTA**	65
XynB119uSP2	**TGCTCGGGTTGTAGTTGCCGTAGTTC**	
XynB119dSP1	**TCGAGGGCACCAAGACCTTCACC**	65
XynB119dSP2	**CGGCACCATCACCAAGGCGAACA**	
rXynB119BF	CGGGATCCGGCCACGGTCATCACGTCCAACA	65
rXynB119HR	CCCAAGCTTGCCGCTGCTGCAGCTGACC	
rXynB119HRD	CCCAAGCTTCACCGTGATGTTGGAGCTGCCG	
GCAD1	NYCGASCKTSGWGCT	45.5–61.2
GCAD2	GTSGRCWGRSMCGSAT	41.2–69.9
GCAD3	TGYGSAGYASCRSMGA	35.0–73.9
GCAD4	TGCGNSGWMSCRSAG	43.9–70.4
GCAD5	AGWGISGSMNCSWGG	40.9–59.9
GCAD6	CAWCGSCNGWSRSGT	41.4–66.5
GCAD7	TCSGICGNACISKSGA	38.7–65.4
GCAD8	GTTSIKCSWGCWNSGC	43.1–56.4
GCAD9	TCRGSYGWCIGSNSTG	37.0–64.7
GCAD10	TCTYICGSRCSWNGGA	45.9–64.4
GCAD11	TGSWGNGCIRSWCG	44.0–60.0
GCAD12	GASYGWCSRGWGNSTC	31.2–57.6

[a] The specific primers (SP primers) are in bold, and GCAD primers were obtained from Zhou et al. [10]

[b] IUPAC/IUB symbols are used, and restriction sites are underlined

[c] *Tm* annealing temperature

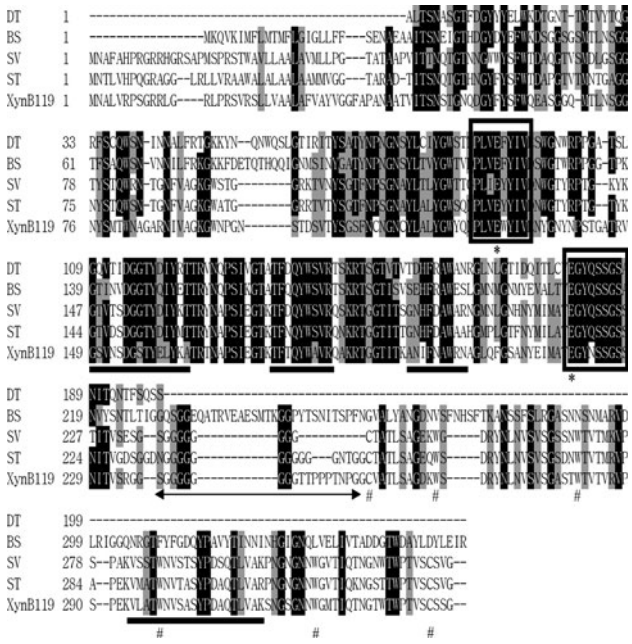

Fig. 1 Amino acid sequence alignment of XynB119 and homologous GH 11 xylanases, including DT from *Dictyoglomus thermophilum* RT46B.1 (AAC46361; PDB code: 1F5J), BS from *Bacillus* sp. 41 M-1 (AAS31755; PDB code: 2DCJ), SV from *Streptomyces viridosporus* T7A (AAF09501), and ST from *Streptomyces thermoviolaceus* OPC-520 (BAD02383). Identical residues are shaded in *black*. Conserved residues are shaded in *gray*. Blocks used for degenerate primer design are framed. The internal peptide sequences identified by MALDI-TOF/MS are *underlined* with *black bars*. *Asterisks* and *ash* symbols indicate the putative catalytic residues and conserved residues involved in cellulose binding, respectively. The putative linker is shown with the *arrow*

(*xynB119*). Briefly, we designed two nested insertion-specific primers (Table 1) for each round of TAIL-PCR. The undiluted primary PCR products (obtained by using SP1 and GCAD primers) were used as templates for both the secondary (using SP1 and GCAD primers) and tertiary (using SP2 and GCAD primers) PCR steps. Annealing temperature (*Tm*) of 65°C was used for high stringency. The PCR products with appropriate size were gel purified and directly sequenced. All primers were designed and analyzed using Primer Premier 5.0 (PREMIER Biosoft International, Palo Alto, CA) and Oligo 6.0 (Molecular Biology Insights, Cascade, CO) softwares and synthesized by Sangon (Beijing, China) or Invitrogen (Beijing, China).

Sequences and structure analysis

Sequence assembly was carried out using the Vector NTI 10.3 software (InforMax, Gaithersburg, MD). The signal peptide in the deduced amino acid sequence was predicted using SignalP [14]. Alignments of DNA and protein sequences were carried out with blastn and blastp [15], respectively. The conserved domains and classification of protein were identified with the website tool of Pfam [16].

Multiple sequence alignments were performed with ClustalW2 [17]. Homology model of protein structure was built with Automated Mode tool at SWISS-MODEL [18] and evaluated by Verify3D profile scores [19].

Recombinant expression of *xynB119* in *E. coli*

The coding sequence of the mature peptide was amplified by PCR using primers rXynB119BF and rXynB119HR (Table 1). The PCR product was gel purified, digested with *Bam*HI and *Hind*III, and cloned into the corresponding site of the pET-22b(+) vector. The recombinant plasmid (pET-*xynB119*) was transformed into *E. coli* BL21 (DE3) competent cells, which could putatively express the recombinant xylanase (r-XynB119) with pelB peptide at N-terminal for secretion to periplasm and His-tag sequence at the C-terminal for purification. The positive transformants were identified by PCR analysis and confirmed by DNA sequence.

Purification and identification of the recombinant xylanase

A positive transformant harboring pET-*xynB119* was picked from a single colony and inoculated into 2-l LB medium containing 100 μg ml^{-1} ampicillin and grown overnight at 37°C. The seed culture was transferred (1:100 dilution) into fresh LB supplemented with ampicillin and grown aerobically at 37°C to an A_{600} of 0.8. IPTG was then added to a final concentration of 0.7 mM for induction at 20°C for 20 h.

The recombinant His_6-tagged xylanase (r-XynB119) in the culture supernatant (~1.6 l in volume) was collected by centrifugation and concentrated using the Hollow Fiber Membrane Module (6 kDa MWCO; Motian Membrane Engineering and Technology, Tianjin, China) and an ultrafiltration membrane (PES5000; Sartorius Stedim Biotech, Goettingen, Germany). The concentrated supernatant was applied to a Ni^{2+}-NTA agarose gel column for purification with a linear imidazole gradient of 20–300 mM in Tris-HCl buffer (20 mM Tris-HCl, 500 mM NaCl, 10% glycerol, pH 7.6). The eluate was loaded onto an ultrafiltration membrane, further concentrated with solid polyethylene glycol (PEG8000) powder outside the ultrafiltration membrane, and finally dialyzed three times against 3 l deionized double-distilled H_2O (ddH_2O) to obtain 15-ml purified r-XynB119.

The homogeneity of the purified r-XynB119 was checked using Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with 12% running gel. To confirm the identity of the purified enzyme, the protein was digested in gel with trypsin and analyzed by the State Key Laboratory of Biology of Biomembrane and Membrane Technology (Institute of Zoology, Chinese Academy of Science) using the peptide fingerprinting method with

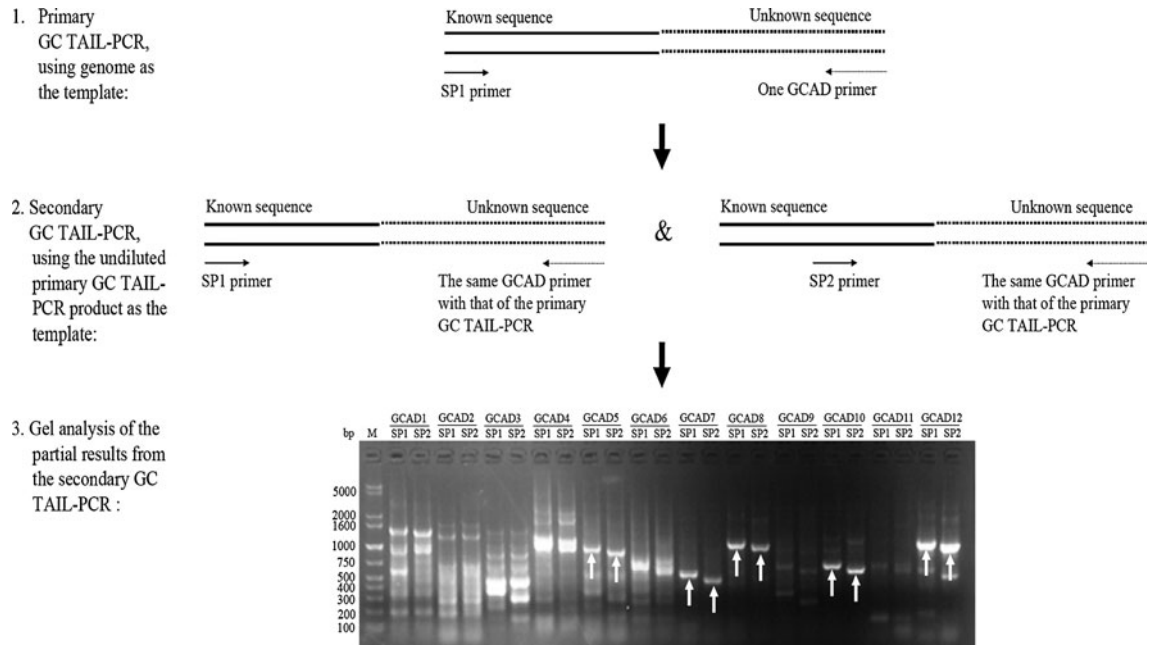

Fig. 2 The schematic representation of the modified GC TAIL-PCR strategy. The *white arrows* indicate the PCR products showing the expected differential shift. *Lanes M* 1 kb Plus DNA ladder; *SP1* PCR products from the secondary reaction using GCAD and SP1 primers; *SP2* PCR products from the secondary reaction using corresponding GCAD primers and SP2 primers

matrix-assisted laser desorption/ionization time-of-flight/time-of-flight mass spectrometry (MALDI-TOF/MS). The protein concentration was determined by the Bradford method [20] using bovine serum albumin as a standard.

Enzyme assay

Xylanase activity was determined by measuring the release of reducing sugar from oat spelt xylan using the 3,5-dinitrosalicylic acid (DNS) reagent [21]. The standard reaction was carried out at 37°C for 30 min with 0.1 ml of appropriately diluted enzyme and 0.9 ml of McIlvaine buffer (pH 7.0) containing 1% (w/v) oat spelt xylan. The reaction was stopped with 1.5 ml DNS reagent, boiled for 5 min and cooled down to room temperature. The absorption at 540 nm was measured. One unit (U) of xylanase activity was defined as the amount of enzyme that released 1 μmol of reducing sugar equivalent to xylose per minute. Under the standard conditions, cellulase and glucanase activity was also determined by the DNS method but using CMC and barley β-glucan (and lichenan) as the substrates, respectively.

Biochemical characterization

The optimal pH of the purified recombinant enzyme was determined at 37°C in buffers with pH ranging from 3.0 to 10.0. The optimal temperature of r-XynB119 was determined over the range of 20–70°C in McIlvaine buffer (pH 7.0). The pH stability was estimated by measuring the residual enzyme activity after pre-incubating the enzyme in different buffers of pH 1.0–12.0 at 37°C for 1 h; the thermostability of the enzyme was determined by pre-incubating the enzyme in McIlvaine buffer (pH 7.0) at 37, 50 or 60°C without substrate for various periods and measuring the residual enzyme activity under the standard conditions. The buffers used were: 0.1 M KCl-HCl for pH 1.0–2.0, McIlvaine buffer for pH 2.0–8.0, 0.1 M Tris-HCl for pH 8.0–9.0, and 0.1 M glycine-NaOH for pH 9.0–12.0.

According to the Lineweaver-Burk method [22], the K_m, V_{max} and k_{cat} values for the purified r-XynB119 were determined in McIlvaine buffer (pH 7.0) at 50°C using 1–10 mg ml^{-1} oat spelt xylan as the substrate.

The effects of different metal ions and chemical reagents on the purified recombinant enzyme activity were evaluated by individually adding 10 mM (final concentration) of NaCl, KCl, $CaCl_2$, LiCl, $CoCl_2$, $CrCl_3$, $NiSO_4$, $CuSO_4$, $MgSO_4$, $FeSO_4$, $MnSO_4$, $ZnSO_4$, $Pb(CH_3COO)_2$, $AgNO_3$, $HgCl_2$, EDTA, SDS, or β-mercaptoethanol to the reaction solution. The effect of adding 100 mM of SDS was also evaluated. To examine resistance to different proteases, the purified r-XynB119 (100 μg ml^{-1}) was incubated at 37°C for 1 h with 10 μg ml^{-1} pepsin (pH 2.0), trypsin (pH 7.0), α-chymotrypsin (pH 7.0), collagenase (pH 7.5), or proteinase K (pH 7.5), and the residual enzyme activity was measured under the standard conditions.

Construction and characterization of the r-XynB119 truncated derivative

To study the effect of CBM 2 on the pH stability of r-XynB119, the coding sequence of the truncated derivative of r-XynB119 without CBM 2 and linker region (*xynB119d*; from Gly51 to Val232) was amplified by PCR using primers rXynB119BF and rXynB119HRD (Table 1). The construction and expression of the recombinant plasmid (pET-*xynB119d*), and purification and pH stability assay of the recombinant xylanase (r-XynB119d) were performed like that of r-XynB119.

Results

Gene cloning and sequence analysis

A gene fragment of 334 bp was amplified by PCR using the degenerate primers AXyn11F and AXyn11R. Based on the partial sequence, four sequence-specific primers (XynB119uSP1, XynB119uSP2, XynB119dSP1, and XynB119dSP2; Table 1) were designed for amplification of the 5′ and 3′ flanking regions. The PCR products showing the expected differential shift were sequenced and then aligned with the known gene fragment. The resulting DNA sequence contains one open reading frame of 1,026 bp with 67.3% GC content, starting with ATG and ending with TGA codon (*xynB119*; accession number: GU984753).

The gene *xynB119* encodes a 341-residue protein (XynB119), including a 41-residue putative signal peptide (cleavage site between Ala41 and Ala42), a catalytic domain of GH 11 from Gly51 to Val232, a CBM 2 from Cys256 to Cys338, and a Gly/Pro-rich linker from Ser237 to Gly255 (SGGGGGGGGTTPPPTNPGG) between the two domains. A search of the NCBI database with blastp showed that XynB119 shared the highest identities of 61.9% with an endo-1,4-β-xylanase from *Streptomyces thermoviolaceus* OPC-520 (BAD02383), followed by the endo-1,4-β-xylanase from *Streptomyces viridosporus* T7A (61.6%; AAF09501). The amino acid sequence alignment of XynB119 with four other xylanases is shown in Fig. 1. Two conserved cysteines (Cys256 and Cys338) and four conserved tryptophans (Trp266, Trp282, Trp298, and Trp321) involved in cellulose binding (http://pfam.sanger.ac.uk/family/PF00553) were identified in the cellulose binding domain of XynB119.

Homology modeling of XynB119 was performed using a two-domain (GH 11 catalytic domain and CBM 6) xylanase XynJ from *Bacillus* sp. 41 M-1 (Fig. 1; PDB code: 2DCJ chain A; 38.4% identity) as the template. The Verify3D profile scores were all above zero throughout the entire model, suggesting the satisfactory fitness. Residues Glu129 and Glu220 were predicted as the catalytic sites of XynB119 based on amino acid alignment (Fig. 1) and structure comparison with *Dictyoglomus thermophilum* RT46B.1 xylanase (PDB code: 1F5J) [23].

Enzyme expression and purification

The structural gene (without the signal peptide coding sequence) coding for XynB119 was expressed in *E. coli* BL21 (DE3) and induced with 0.7 mM IPTG at 20°C for 20 h. The recombinant His_6-tagged xylanase (r-XynB119) was secreted into the culture supernatant and showed xylanase activity of 3.7 U mg^{-1} (0.8 U ml^{-1}). The same xylanase activity (0.8 U ml^{-1}) was also detected in the supernatant of sonication-disrupted cells, but no xylanase activity was detected in the cultures of uninduced transformants or induced transformants harboring the empty plasmid pET-22b(+).

Only the xylanase in the culture supernatant was concentrated and further purified to electrophoretic homogeneity by Ni^{2+}-NTA metal chelating affinity chromatography. SDS-PAGE showed a single band with a molecular mass of ~34 kDa (Fig. 3), which is identical to the calculated value of r-XynB119. Four internal peptides—VGSVNSDGSTYELYK, TFTQYWAVR, ANIFNAWR, and VLATWNVSASYPDAQTLVAK—of this protein (Fig. 1) matched their theoretical peptide mass fingerprints, confirming that the purified enzyme was indeed r-XynB119. When assayed at 37°C and pH 7.0, purified r-XynB119 showed the specific activity of 91.0 U mg^{-1} towards oat

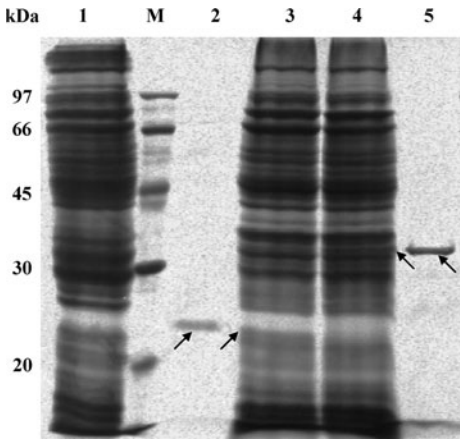

Fig. 3 SDS-PAGE analyses of r-XynB119 and r-XynB119d purification. *Lane M*, low-molecular weight markers; *lane 1* culture supernatant from an induced transformant harboring the empty plasmid pET-22b(+), *lane 2* r-XynB119d purified by Ni^{2+}-NTA chelating affinity chromatography, *lane 3* culture supernatant of an induced transformant harboring pET-*xynB119d*, *lane 4* culture supernatant of an induced transformant harboring pET-*xynB119*, *lane 5* r-XynB119 purified by Ni^{2+}-NTA chelating affinity chromatography. *Arrows* indicate the bands of interest

spelt xylan, 11.1 U mg^{-1} to barley β-glucan, 7.5 U mg^{-1} to lichenan, and no cellulase activity.

Biochemical properties of purified r-XynB119

When assayed at 37°C, purified r-XynB119 exhibited apparent optimal activity at pH 7.0 and retained >50% of the maximum activity between pH 5.0 and 9.0 (Fig. 4a). Following incubation at 37°C for 1 h without substrate, the enzyme retained more than 70% activity at pH 1.0–11.0 (Fig. 4b) and showed the maximum stability at pH 5.0. Enzyme activity was apparently optimal at 50°C when assayed at pH 7.0 and remained around 40% of the maximum activity when assayed at 37°C (Fig. 4c). r-XynB119 was stable at 37°C for ≥60 min, whereas at temperatures ≥50°C the enzyme activity decreased rapidly after 10 min pre-incubation (Fig. 4d). Based on a Lineweaver-Burk plot, the K_m, V_{max} and k_{cat} values were 15.1 mg ml^{-1}, 441.4 μmol min^{-1} mg^{-1}, and 251.0 s^{-1}, respectively, using oat spelt xylan as the substrate.

We tested the effects of various cations and compounds (10 mM final concentration) on the xylanase activity of r-XynB119. SDS, β-mercaptoethanol, and Ni^{2+} increased the activity. Ag^{+}, Hg^{2+}, and Cu^{2+} strongly inhibited the enzyme activity (>50%), whereas partial inhibition (<40%) was observed in the presence of Pb^{2+} and Mn^{2+}. The addition of other reagents at 10 mM and SDS at 100 mM final concentration had little or no effect on the enzyme activity (Table 2).

Table 2 Effect of 10 mM metal ions and chemical reagents on the xylanase activity of purified r-XynB119

Chemicals	Relative activity (%)[a]	Chemicals	Relative activity (%)
None	100.0 ± 2.6	EDTA	97.9 ± 1.8
β-Mercaptoethanol	126.5 ± 2.0	Co^{2+}	97.3 ± 2.8
Ni^{2+}	122.3 ± 2.8	Na^{+}	94.4 ± 9.8
SDS	110.8 ± 7.2	Zn^{2+}	93.0 ± 1.3
Cr^{3+}	103.3 ± 8.1	PbAc	77.1 ± 0.4
K^{+}	101.4 ± 2.6	Mn^{2+}	65.6 ± 0.9
Li^{+}	101.3 ± 3.6	Cu^{2+}	46.4 ± 0.3
Fe^{2+}	101.0 ± 1.6	Ag^{+}	25.0 ± 0.2
Mg^{2+}	99.0 ± 0.8	Hg^{2+}	0
Ca^{2+}	98.3 ± 5.1	SDS (100 mM)	103.6 ± 8.0

[a] Values represent the means ± standard deviation (SD, $n = 3$) relative to the untreated control samples

r-XynB119 was strongly resistant to protease digestion. After treatment at 37°C for 1 h with pepsin, trypsin, collagenase, α-chymotrypsin, and proteinase K, the enzyme retained 91.7, 105.8, 109.4, 121.6, and 129.0% of the maximum xylanase activity, respectively.

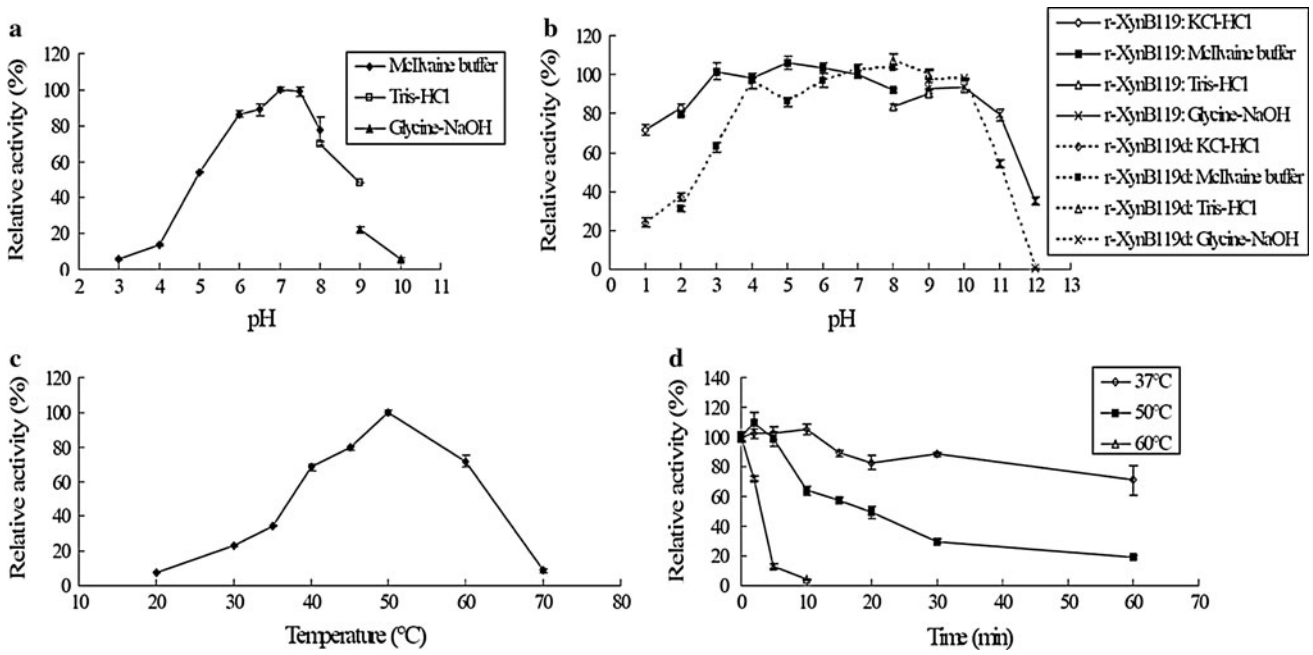

Fig. 4 Characterization of purified r-XynB119 and r-XynB119d enzymatic activity. **a** Effect of pH on the xylanase activity of r-XynB119. The enzyme activity was determined at 37°C from pH 3.0 to 10.0. **b** pH stability assay. After pre-incubation at pH 1.0–12.0 at 37°C for 60 min, the enzyme activity was determined in McIlvaine buffer (pH 7.0) at 37°C. **c** Effect of temperature on r-XynB119 activity measured in McIlvaine buffer (pH 7.0) at 20–70°C. **d** Thermostability assay. Purified r-XynB119 was pre-incubated in McIlvaine buffer (pH 7.0) at 37°C, 50°C, or 60°C. Aliquots were removed at specific time points for the measurement of residual activity at 37°C. The *error bars* represent the mean ± SD ($n = 3$)

Properties of r-XynB119d

The recombinant truncated xylanase r-XynB119d was expressed and purified to electrophoretic homogeneity after ultrafiltration and affinity chromatography (Fig. 3). The molecular mass was ~23 kDa, which is identical to the calculated value of r-XynB119d. Following incubation at 37°C for 1 h without substrate, the purified r-XynB119d retained more than 60% xylanase activity at pH 3.0–10.0 (Fig. 4b). Compared to r-XynB119, r-XynB119d exhibited a decreased pH stability of >25% at pH 1.0–3.0 and pH 11.0–12.0.

Discussion

The broad pH stability (maintaining more than 70% of its maximal activity at pH 1.0–11.0) and optimal activity at physiological pH (exhibiting 70–100% xylanase activity at pH 6.0–8.0) make r-XynB119 a good potential candidate for application in the feed industry to improve nutrient utilization. A few xylanases showed good pH stability over a broad acid and alkaline pH range, but not lower than pH 2.0 [24–30]. For example, the GH 10 xylanases XynA4 from *Alicyclobacillus* sp. A4 [24] and XynAS27 from *Streptomyces* sp. S27 [28] were stable at pH 2.6–12.0 and pH 2.2–12.0, respectively, very close to the pH stability profile of r-XynB119. But when incubated at pH 2.0, XynA4 and XynAS27 lost at least 40% xylanase activity, much more than that (20%) of r-XynB119. Furthermore, both xylanases were completely inhibited by 10 mM SDS, and their protease resistance has not been reported.

The excellent pH stability of r-XynB119 was partially ascribed to its two-domain structure. Compared to r-XynB119, r-XynB119d exhibited a decreased stability at extreme pHs (pH 1.0–3.0 and pH 11.0–12.0). The CBM 13 of xylanase XynAS27 from *Streptomyces* sp. S27 has been proved to contribute to the enzyme's pH stability [28]. Furthermore, the CBM 2 of XynB119 showed 91.9% similarity to its counterpart from *S. thermoviolaceus* OPC-520 (Fig. 1; BAD02383), a xylanase exhibiting binding ability to insoluble birchwood and oat spelt xylan, and thus probably increased the catalytic activity of XynB119 [31].

The strong resistance to acid and neutral proteases in vitro suggests XynB119 as a good candidate for animal feed supplement. To our knowledge, xylanases with proteolytic resistance are seldom reported. The xylanase SfXyn10 from *S. fradiae* var. k11 showed strong resistance to some neutral and alkaline proteases, but lost almost all of the activity after incubation with pepsin [29]. The *Trichoderma longibrachiatum* xylanase was resistant to pepsin at pH 3.0, but the xylanase activity decreased at a rate of 0.45% min^{-1} in the presence of pancreatin [32]. The recombinant xylanase XynA119 from *Streptomyces* sp. TN119 could retain about 60% of its activity after treatment of trypsin, but was sensitive to proteinase K [10]. XYL11B from *Bispora* sp. MEY-1 was the only reported enzyme that exhibited strong resistance to both pepsin and trypsin; however, XYL11B lost functions in the animal intestine due to inactivation at pH higher than 5.5 [33]. Furthermore, most xylanases are sensitive to SDS—an anionic detergent and a strong denaturant of proteins—when the concentration above ~7 mM in water [34]. XynAS9 is the only exception that retained 91.0% of the maximum xylanase activity in the presence of 10 mM SDS. In this study, r-XynB119 showed significant resistance to SDS, exhibiting ~100% of the relative activity in the presence of even 100 mM SDS. It has been proposed that SDS resistance is a common property of kinetically stable proteins, which have limited unfolded conformations susceptible to proteolytic attack and own strong proteolytic resistance [34, 35]. Accordingly, we infer that r-XynB119 is a kinetically stable protein with limited unfolded conformation. Further crystallization analysis of r-XynB119 will confirm our assumption.

In conclusion, a new xylanase-encoding gene, *xynB119*, was cloned from the symbiotic *Streptomyces* sp. TN119 harbored in the gut of *B. horsfieldi* larvae. Biochemical characterization of the recombinant xylanase showed some superior properties, such as broad pH stability, optimal activity at physiological pH, and strong resistance to proteases and SDS. These properties make XynB119 very promising for basic research and industrial applications, especially in the feed industry.

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