

XYLANASE WITH IMPROVED ENZYME ACTIVITY AND PREPARATION METHOD THEREFOR

Cross Reference to Related Applications

5 This application claims priority to Chinese Patent Application No. CN 202311613123.8, filed on November 28, 2023, the entire contents of which are incorporated herein by reference.

Technical Field

10 The present disclosure relates to xylanases and polynucleotides encoding the xylanases. The present disclosure further relates to recombinant vectors and host cells comprising these polynucleotides, as well as methods for preparing these xylanases and host cells.

Background Art

15 As a class of enzymes that degrade xylan, xylanases play an important role in the feed industry. By depolymerizing xylan—an anti-nutritional factor in feed that is indigestible and non-utilizable by animals—into oligosaccharides, xylose, and arabinose, the anti-nutritional factors are eliminated, thereby further improving
20 feed resource utilization efficiency. Additionally, this effectively reduces environmental pollution caused by animal excretion, playing a pivotal role in enhancing breeding economic benefits and developing environmentally friendly animal husbandry. There are numerous types of xylanases, but those applied in the feed industry require high thermal stability to meet the process demands of high-
25 temperature feed pelleting. Among them, the GH11 family xylanase derived from rumen fungus (*Neocallimastix patriciarum*) exhibits high enzyme activity and excellent heat resistance. Chen et al. obtained a recombinant xylanase Xyn-CDBFV with higher specific activity and better alkaline performance through random mutagenesis of a xylanase derived from *Neocallimastix patriciarum*
30 (Directed evolution to produce an alkalophilic variant from a *Neocallimastix patriciarum* xylanase, 2001 Dec; 47(12):1088-94. doi:10.1139/w01-118).

Subsequently, researchers conducted extensive modifications on Xyn-CDBFV to enhance specific activity and heat resistance. For example, Zhang et al. effectively increased the proportion of reducing sugars hydrolysed by the xylanase

through fusion expression of the carbohydrate-binding module CBM with CDBFV in *Pichia pastoris*. Chinese Patent CN 114807093 A significantly improved the thermal stability of xylanase Xyn-CDBFV by adding a fusion peptide segment at the C-terminus. Cui et al. achieved high-specification production of recombinant lysozyme and xylanase by fusing the N-terminus of lysozyme with the C-terminus of xylanase Xyn-CDBFV in *Pichia pastoris*, providing a new approach for the industrial application of compound feed.

Currently, numerous researchers have conducted studies on recombinant xylanase in *Pichia pastoris* by adding peptide segments to xylanase Xyn-CDBFV or through fusion expression. However, yeast exhibits issues such as excessive modification and protein folding when expressing heterologous proteins, thereby affecting the expression level and stability of heterologous proteins. Additionally, since *Pichia pastoris* typically requires methanol induction for heterologous protein production, there are certain hazards in actual industrial production. Therefore, developing a new platform to obtain novel xylanase products with high enzyme activity and excellent thermal stability holds significant importance, as it can effectively reduce production costs.

Summary of the Invention

The present disclosure provides a xylanase, which comprises a xylanase Xyn-CDBFV sequence and a fusion peptide segment linked to the C-terminus thereof, the fusion peptide segment comprising an amino acid sequence as set forth in SEQ ID NO: 1.

In some embodiments, the fusion peptide segment further comprises an amino acid sequence as set forth in SEQ ID NO: 2.

In some embodiments, the fusion peptide segment consists of amino acid sequences as set forth in SEQ ID NO: 1 and SEQ ID NO: 2.

In some embodiments, the xylanase Xyn-CDBFV sequence is as set forth in SEQ ID NO: 3.

In some embodiments, the xylanase comprises an amino acid sequence as set forth in SEQ ID NO: 4. In some embodiments, the xylanase comprises an amino acid sequence as set forth in SEQ ID NO: 5. In some embodiments, the xylanase consists of an amino acid sequence set forth in SEQ ID NO: 4. In some

embodiments, the xylanase consists of an amino acid sequence set forth in SEQ ID NO: 5.

The present disclosure further provides a polynucleotide encoding the xylanase.

5 In some embodiments, the polynucleotide comprises a nucleotide sequence as set forth in SEQ ID NO: 6. In some embodiments, the polynucleotide comprises a nucleotide sequence as set forth in SEQ ID NO: 7. In some embodiments, the polynucleotide consists of a nucleotide sequence as set forth in SEQ ID NO: 6. In some embodiments, the polynucleotide consists of a nucleotide sequence as set
10 forth in SEQ ID NO: 7.

The present disclosure further provides a recombinant vector comprising the polynucleotide, and a host cell comprising the polynucleotide or the recombinant vector.

15 In some embodiments, the host cell is *Aspergillus niger* or *Pichia pastoris*. In some embodiments, the host cell is *Aspergillus niger*.

The present disclosure further provides a method for preparing xylanase, comprising linking a fusion peptide segment to the C-terminus of the xylanase Xyn-CDBFV sequence, wherein the fusion peptide segment comprises an amino acid sequence as set forth in SEQ ID NO: 1.

20 In some embodiments, the fusion peptide segment further comprises an amino acid sequence as set forth in SEQ ID NO: 2.

In some embodiments, the fusion peptide segment consists of amino acid sequences as set forth in SEQ ID NO: 1 and SEQ ID NO: 2.

25 In some embodiments, the xylanase Xyn-CDBFV sequence is as set forth in SEQ ID NO: 3.

The present disclosure further provides a method for preparing an *Aspergillus niger* strain producing xylanase, comprising introducing the recombinant vector into the *Aspergillus niger* strain.

30 In some embodiments, the recombinant vector comprises an *Aspergillus niger* expression cassette sequence, and the *Aspergillus niger* expression cassette sequence comprises a gla promoter sequence of *Aspergillus niger* glucoamylase gene, a signal peptide nucleotide sequence of *Aspergillus niger* amylase, a xylanase nucleotide sequence as set forth in SEQ ID NO: 6 or 7, and a gla terminator sequence of *Aspergillus niger* glucoamylase gene.

In some embodiments, the method comprises screening the *Aspergillus niger* strain using an amdS selection marker plate after introducing the recombinant vector.

The present disclosure further provides use of the xylanase in feed.

5 The beneficial effects of the present disclosure are as follows: By adding specific fusion peptide segments to the C-terminus of the GH11 family xylanase Xyn-CDBFV, the problem of no enzyme activity when Xyn-CDBFV is expressed in the *Aspergillus niger* platform can be resolved. Moreover, compared to the expression of xylanases Xyn-307 and Xyn-309 in *Pichia pastoris*, the enzyme
10 activity levels of the xylanases Xyn-307 and Xyn-309 provided by the present disclosure after expression in the *Aspergillus niger* platform are significantly improved.

Brief Description of the Drawings

15 FIG. 1 compares the enzyme activity performance of recombinant xylanases Xyn-307 and Xyn-309 on the *Aspergillus niger* platform at the 50 L fermenter level.

FIG. 2 compares the enzyme activity performance of recombinant xylanases Xyn-307 and Xyn-309 on the yeast platform at the 50 L fermenter level.

20

Detailed Description of Embodiments

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

25 All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. Furthermore, the materials, methods, and examples described herein are illustrative only and not intended to be limiting.

30 The term "about", when used with a numerical variable, generally means that the value of the variable and all values of the variable are within measurement or experimental error (e.g., the 95% confidence interval of the mean) or within a broader range of the specified value (e.g., $\pm 5\%$ or $\pm 10\%$).

The term "comprise" or variations thereof such as "contain," "have," or "include" refers to the inclusion of the stated steps or elements but does not preclude the presence or addition of any other steps or elements. "consisting of" means excluding any steps or elements not listed. The term "comprise" a specified
5 step or element and variations thereof also includes situations "consisting of the specified step or element" and "consisting essentially of the specified step or element".

When referring to a numerical range, it shall be deemed to specifically disclose each specific value between the upper and lower limits, including all
10 intermediate ranges encompassed therein, such as intermediate ranges between either the upper or lower limit and any intermediate value, or between any two intermediate values. Furthermore, any intermediate ranges, sub-ranges, and all individual values within the described numerical range may be excluded from the stated numerical range.

15 The term "and/or" should be understood as referring to any one of the multiple elements modified by this term, or any combination of several of these elements.

The term "xylanase" refers to an enzyme, polypeptide, or protein capable of degrading xylan, for example, capable of catalysing the degradation of β -1,4
20 glycosidic bonds in xylan.

The term "recombinant", when used in reference to a nucleic acid, protein, or vector, indicates that the nucleic acid, protein, or vector has been modified by the introduction of a heterologous nucleic acid or protein or by alteration of the native nucleic acid or protein.

25 The term "expression", in the context of the present disclosure, encompasses any step involved in the production of the xylanase of the invention, including but not limited to transcription, post-transcriptional modification, translation, post-translational modification, and secretion.

The term "vector" may be an autonomously replicating vector, i.e., a vector
30 that replicates independently of the chromosome, such as a plasmid or an artificial chromosome, or may be a vector that integrates into the genome and replicates with the chromosome upon introduction into a host cell. The expression vector may comprise one or more selectable markers that allow for convenient selection of transformed cells, transfected cells, or transduced cells, such as markers

providing resistance to antibiotics, heavy metals, etc. The expression vector may further comprise an origin of replication to enable autonomous replication of the expression vector in host cells. The selection of these elements is routine technology in the art, and methods for constructing the recombinant expression
5 vectors of the present disclosure using these elements are well-known in the art, as can be seen, for example, in J. Sambrook, Molecular Cloning: A Laboratory Manual, Third Edition.

The term "host cell" refers to any cell type susceptible to transformation, transfection, transduction, etc., which may comprise the polynucleotide or
10 recombinant vector of the present disclosure. The term "host cell" encompasses any progeny of a parental cell that differs from the parental cell due to mutations occurring during replication.

The term "thermostable" means that the ability an enzyme to resist irreversible inactivation (typically caused by denaturation) at relatively high
15 temperatures, indicating that after exposure to a specified temperature for a given time period, the enzyme retains a specific amount of enzyme activity.

Unless otherwise indicated, nucleic acids are written from left to right in the 5' to 3' direction, and amino acid sequences are written from left to right in the amino- to carboxy-terminal (N- to C-terminal) direction.

The present disclosure provides xylanases comprising the xylanase Xyn-CDBFV sequence and a fusion peptide segment linked to its C-terminus, wherein the fusion peptide segment comprises an amino acid sequence as set forth in SEQ ID NO: 1 and/or SEQ ID NO: 2. The amino acid sequence of xylanase Xyn-CDBFV is as set forth in SEQ ID NO: 3. In specific embodiments, the amino acid
25 sequence of the fused xylanase Xyn-307 is as set forth in SEQ ID NO: 4 (wherein the underlined portion is the fusion peptide segment SEQ ID NO: 1). In specific embodiments, the amino acid sequence of the fused xylanase Xyn-309 is as set forth in SEQ ID NO: 5 (wherein the underlined portion is the fusion peptide segment sequence SEQ ID NO: 1, and the bold italicized portion is the fusion
30 peptide segment sequence SEQ ID NO: 2).

Those skilled in the art will appreciate that the xylanase may also be linked to other peptide segments (including but not limited to tag sequences, such as histidine tag sequences) without affecting the functional properties of the xylanase (including but not limited to enzyme activity, thermal stability, and other

properties described herein), and such xylanases are also encompassed within the scope of the xylanases described herein.

The present disclosure further provides a polynucleotide encoding the xylanase as described above. In specific embodiments, the polynucleotide encodes xylanase Xyn-307, and has a sequence as set forth in SEQ ID NO: 6. In specific
5 embodiments, the polynucleotide encodes xylanase Xyn-309, and has a nucleotide sequence as set forth in SEQ ID NO: 7.

The present disclosure further provides a recombinant vector comprising the polynucleotide as described above. In specific embodiments, the recombinant
10 vector comprises a polynucleotide encoding xylanase Xyn-307. In specific embodiments, the recombinant vector comprises a polynucleotide encoding xylanase Xyn-309.

The present disclosure further provides a host cell comprising the polynucleotide or recombinant vector as described above, wherein the host cell can
15 be *Aspergillus niger* or *Pichia pastoris*. In specific embodiments, the host cell is *Aspergillus niger*.

The present disclosure also provides a method for preparing a genetically engineered *Aspergillus niger* strain producing xylanase, comprising the following steps:

20 (1) Synthesizing a DNA fragment containing the gla promoter of *Aspergillus niger* glucoamylase gene (the nucleotide sequence of which is as set forth in SEQ ID NO: 8) and terminator (the nucleotide sequence of which is as set forth in SEQ ID NO: 9) by gene synthesis;

25 (2) Linking the signal peptide sequence of *Aspergillus niger* amylase (the nucleotide sequence of which is as set forth in SEQ ID NO: 10) and the nucleotide sequence of xylanase Xyn-307 or Xyn-309 in series between the promoter and the terminator to construct an *Aspergillus niger* expression cassette sequence;

 (3) Cloning the *Aspergillus niger* expression cassette sequence into a plasmid to obtain a recombinant expression plasmid;

30 (4) Cloning the recombinant expression plasmid into *Escherichia coli* competent cells DH5 α , picking positive transformants for colony PCR and DNA sequencing, and culturing the transformants with correct sequencing and extracting the recombinant expression plasmid on a large scale;

(5) Linearizing the extracted recombinant expression plasmid and then transforming into *Aspergillus niger* protoplasts, followed by screening using a selection marker amdS (the nucleotide sequence of which is as set forth in SEQ ID NO: 11) plate (0.8 M sucrose + 2 mL acetamide + 200 mL trace elements);

5 (6) Activating the grown transformants with TB3 low-sugar plates, inoculating the activated transformants into enzyme production medium and culturing for 144 h, collecting the supernatant by centrifugation, and performing protein electrophoresis identification and enzyme activity detection of the fermentation supernatant, selecting several transformants with high xylanase
10 activity therefrom, and conducting secondary screening for confirmation, thereby obtaining the genetically engineered *Aspergillus niger* strain for producing xylanase Xyn-307 or Xyn-309.

The present disclosure further provides a shake-flask cultivation method for recombinant strains of *Aspergillus niger* expressing xylanase Xyn-307 or Xyn-309,
15 comprising inoculating the screened positive transformants of Xyn-307 or Xyn-309 into shake flasks containing *Aspergillus niger* screening medium, culturing with shaking in a shaker, centrifuging to collect the supernatant after fermentation, and measuring the enzyme activity of xylanase.

In some embodiments, the culture temperature of the shaker is 30-35°C. In
20 preferred embodiments, the culture temperature of the shaker is 34°C.

In some embodiments, the shaker speed is 180-250 rpm. In preferred embodiments, the shaker speed is 220 rpm.

In some embodiments, the shaking culture period is 5-7 days. In preferred embodiments, the shaking culture period is 6 days.

25 The present disclosure is further described below in conjunction with specific examples:

Experimental materials and reagents

(1) Strains and Vectors

Escherichia coli DH5α was purchased from Invitrogen, *Aspergillus niger*
30 CICC2462 strain was purchased from China Centre of Industrial Culture Collection (CICC), and plasmid pUC57 was purchased from GenScript Biotech Corporation.

(2) Enzymes and related molecular biology reagents

The Agarose Gel DNA Purification Kit and Fragment Purification Kit Ver.2.0 were purchased from TaKaRa. T4 DNA ligase was purchased from Fermentas. Other biochemical reagents were purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd.

5 (3) Culture medium

LB Medium: 1% tryptone, 0.5% yeast extract, and 1% sodium chloride; Adjust the pH to 7.0 with NaOH; Add 1.5% (w/v) agar powder (solid medium).

TZ liquid medium: 0.8% beef extract powder, 0.2% yeast extract, 0.5% peptone, 0.2% NaCl, and 3% sucrose, pH 5.8.

10 STC solution: 1 M D-Sorbitol, 50 mM CaCl₂, 10 mM Tris, pH 7.5.

Acetamide medium: 3% sucrose, 0.05% KCl, 0.1% K₂HPO₄·3H₂O, 0.001% FeSO₄, 0.0244% MgSO₄, 0.06% acetamide, and 0.34% CsCl.

Enzyme production medium for *Aspergillus niger*: Glucose: 12%; Corn steep liquor powder: 1.4%; Bean cake powder: 3%; Ammonium sulphate: 1%;
15 Potassium dihydrogen phosphate: 1%; Magnesium sulphate: 0.02%; Calcium carbonate: 0.6%; Citric acid: 0.3%; Zinc sulphate: 0.005%; Ferrous sulphate: 0.005%.

Determination of xylanase activity

All determinations of xylanase activity in this document were performed
20 using the standard method specified in the National Standard "GB/T23874-2009 Determination of Xylanase Activity in Feed Additives by Spectrophotometry." Specifically, the colorimetric reaction between the reducing oligosaccharides and monosaccharides produced by the catalytic degradation of xylan by xylanase and the 3,5-dinitrosalicylic acid (DNS) reagent under boiling water bath conditions was
25 utilized and the enzyme activity of the tested xylanase was calculated based on the difference in absorbance at 540 nm between the enzyme reaction sample and the blank control sample.

The specific calculation formula for enzyme activity is as follows:

The activity of xylanase in the diluted enzyme solution for enzymatic
30 hydrolysis is calculated according to Formula (1) and Formula (2):

$$X_D = \frac{[(A_E - A_B) \times K + C_o]}{M \times t} \times 1000 \quad (1)$$

in the formula:

X_D — Activity of xylanase in the diluted enzyme solution, U/mL;

A_E — Absorbance of enzyme reaction solution;

A_B — Absorbance of enzyme blank sample;

K — Slope of the standard curve;

5 C_o — Intercept of the standard curve;

M — Molar mass of xylose, $M(C_5H_{10}O_5) = 150.2$ g/mol

t — Enzymatic reaction time, min;

1000 — Conversion factor, 1 mmol = 1000 μ mol

The X_D value should be between 0.04 U/mL and 0.1 U/mL. If it falls outside
10 this range, adjust the dilution factor of the enzyme solution and repeat the analysis.

$$X = X_D \times D_f \quad (2)$$

in the formula:

X — Activity of xylanase in the sample, U/g or U/mL;

D_f — Dilution factor of the sample.

15 **Example 1: Construction of expression plasmids for Xylanases Xyn-CDBFV, Xyn-307, and Xyn-309 in *Aspergillus niger* platform**

(1) The pUC57 plasmid was linearized using vector-F and vector-R primers;

(2) DNA fragments containing the gla promoter and terminator of *Aspergillus niger* glucoamylase gene and the selection marker amdS were synthesized by
20 GenScript;

(3) First, the primers amdS-F and amdS-R, as well as gla-F and gla-R, were used to PCR-amplify the amdS gene with recombination arms and the DNA fragment containing the gla promoter and terminator, respectively. The linearized pUC57 plasmid, the amdS gene, and the DNA fragments of the gla promoter and
25 terminator were then recombined using the Gibson Master Mix Kit (E2611, New England Biolabs) to obtain the pGla-amdS plasmid, and sequencing confirmed the correct sequence. This plasmid could be linearized at the AflIII site for the insertion of the xylanase gene sequences Xyn-CDBFV, Xyn-307, and Xyn-309.

(4) The construction of the expression plasmids for the xylanases Xyn-CDBFV, Xyn-307, and Xyn-309 was performed as follows: 1. The Xyn-CDBFV
30 gene with recombination arms was amplified by PCR using primers Xyn-F and Xyn-R1, and then the Xyn-CDBFV gene was recombined with the linearized pGla-amdS plasmid using the Gibson Master Mix Kit (E2611, New England Biolabs) to obtain the Xyn-CDBFV-amdS plasmid, whose sequence was confirmed by

sequencing. This plasmid could be linearized at the Hind III site for protoplast transformation. 2. The Xyn-307 gene with recombination arms was amplified by PCR using primers Xyn-F and Xyn-R2, and then the Xyn-307 gene was recombined with the linearized pGla-amdS plasmid using the Gibson Master Mix Kit (E2611, New England Biolabs) to obtain the Xyn-307-amdS plasmid, whose sequence was confirmed by sequencing. This plasmid could be linearized at the Hind III site for protoplast transformation. 3. The Xyn-309 gene with recombination arms was amplified by PCR using primers Xyn-F and Xyn-R3, and then the Xyn-309 gene was recombined with the linearized pGla-amdS plasmid using the Gibson Master Mix Kit (E2611, New England Biolabs) to obtain the Xyn-309-amdS plasmid, whose sequence was confirmed by sequencing. This plasmid could be linearized at the Hind III site for protoplast transformation.

The relevant primer sequences are as follows:

Table 1 Primers in the present disclosure

Primer name	Sequence (5'-3')
vector-F	CTTGCGTAATCATGGTCATAGC (SEQ ID NO. 12)
vector-R	CGGACCCCTCCGCCAATGGCCTTGCATGCAGGCCTCTGCA (SEQ ID NO. 13)
amdS-F	CTAGATCTACGCCAGGACCG (SEQ ID NO. 14)
amdS-R	ATGACCATGATTACGCCAAGCTTCTGGAAACGCAACCCTG (SEQ ID NO. 15)
gla-F	GCCATTGGCGGAGGGGTCCG (SEQ ID NO. 16)
gla-R	CGGTCCTGGCGTAGATCTAGATGCATTGAATGACAGTGAT (SEQ ID NO.17)
Xyn-F	CAGTCCTTCTGCTCCTCCGCCTCCC (SEQ ID NO. 18)
Xyn-R1	GTCACCGATGTAGACCTTGGCGTAGGG (SEQ ID NO. 19)
Xyn-R2	TTACTCGGAACCGCGGCGCAGGTGGG (SEQ ID NO. 20)
Xyn-R3	TTAGGAGGTGGAGGAGGAACCACCCTG (SEQ ID NO. 21)

15

Example 2: Transformation and Integration of Xylanase Xyn-CDBFV, Xyn-307, and Xyn-309 Expression Plasmids in *Aspergillus niger* Platform

The linearized xylanase Xyn-CDBFV, Xyn-307, and Xyn-309 expression plasmids were introduced into *Aspergillus niger* CICC2462 strain via protoplast transformation method. The detailed operational steps are described below:

20

(1) Preparation of protoplasts: The mycelia of *Aspergillus niger* were inoculated in nutrient-rich TZ liquid medium, cultured for 48 h, then filtered and

collected using Mira-cloth (Calbiochem), and washed with 0.7 M NaCl (pH 5.8); After draining the mycelia, they were transferred to an enzymatic solution (pH 5.8) containing 1% cellulase (Sigma), 1% snailase (Sigma), and 0.2% lywallzyme (Sigma), and enzymatically digested at 30°C and 65 rpm for 3 h; The enzymatic
5 digestion solution containing protoplasts was then placed on ice and filtered through four layers of lens paper. The filtrate was gently centrifuged at 3000 rpm and 4°C for 10 min, the supernatant was discarded, and the protoplasts adhering to the tube wall were washed once with STC solution (1 M D-sorbitol, 50 mM CaCl₂, 10 mM Tris, pH 7.5). Finally, the protoplasts were resuspended in an appropriate
10 amount of STC solution.

(2) Protoplast transformation: 10 µL of the xylanase expression plasmid linearized with HindIII (1000 ng/µL) was added to 100 µL of protoplast suspension, mixed evenly, and placed at room temperature for 25 min. Subsequently, 900 µL of PEG solution was added in three aliquots, mixed evenly, and placed at room
15 temperature for 25 min. The mixture was then centrifuged at 3000 rpm for 10 min at room temperature. After discarding the supernatant, the protoplasts adhering to the tube wall was resuspended in 1 mL of STC solution, mixed with acetamide medium pre-cooled to approximately 45°C, and poured onto plates. After the plates solidified, they were incubated in an incubator at 34°C for 4–5 days. The
20 transformants were then picked and transferred to fresh acetamide medium plates and incubated in the incubator at 34°C for an additional 4–5 days. The resulting transformants were designated as positive transformants.

Using the above protoplast transformation method, the linearized xylanase expression plasmids Xyn-CDBFV, Xyn-307, and Xyn-309 were transformed into
25 *Aspergillus niger* strains to obtain xylanase-positive transformants.

Example 3: Shake-flask cultivation of recombinant *Aspergillus niger* strains expressing xylanases Xyn-CDBFV, Xyn-307, and Xyn-309

The positive transformants of xylanases Xyn-CDBFV, Xyn-307, and Xyn-309 were inoculated into shake flasks containing 50 mL of *Aspergillus niger* enzyme
30 production medium and cultured at 34°C on a shaker at 220 rpm for 6 days. The fermentation supernatant was collected by centrifugation, and xylanase activity was determined. After 144 h of fermentation, the enzyme activity in the fermentation supernatant is shown in Table 2.

Table 2 Enzyme activity of xylanases Xyn-CDBFV, Xyn-307, and Xyn-309 in the fermentation supernatant in *Aspergillus niger* platform after 144 h of fermentation

Name	Enzyme activity in 144 h fermentation supernatant (u/mL)
Xyn-CDBFV	0
Xyn-307	40000-47000
Xyn-309	62000-69000

Example 4: Fermentation culture of recombinant *Aspergillus niger* strains expressing Xylanases Xyn-307 and Xyn-309 in a 50 L fermenter

The above xylanase Xyn-307 and Xyn-309 positive transformant of genetically engineered *Aspergillus niger* strains were subjected to fermentation experiments in a 50 L fermenter, with an actual working volume of 30 L for the enzyme production medium. Fermentation conditions: 34°C, stirring speed: 800 rpm, aeration rate: 25 LPM, fermentation duration approximately 186 h, with enzyme activity measured every 4 h during the fermentation process. The enzyme activity test data are shown in Table 3, and the enzyme activity change curve is shown in FIG. 1. The maximum whole-cell enzyme activity of Xyn-309 fermentation reached 256,000 u/g, while that of Xyn-307 fermentation reached 168,000 u/g.

Table 3 Whole-cell enzyme activity records of xylanases Xyn-307 and Xyn-309 in *Aspergillus niger* platform during fermentation

Fermentation duration (h)	Xyn-307 enzyme activity (u/g)	Xyn-309 enzyme activity (u/g)
19	4872	11752
23	5869	16182
27	7038	18691
31	9696	22064
35	12069	24477
39	15908	28692
43	17659	32442
47	27151	38407
51	30782	40083

55	35090	47633
59	39020	49323
63	42589	54185
67	47930	67501
71	58114	78723
75	63721	94377
79	74797	104846
83	81275	126238
87	82530	134678
91	95289	147123
95	104187	136142
99	110657	144557
103	113398	161172
107	123614	161190
111	118291	165066
115	125392	192894
119	150263	207134
123	152313	216790
127	156449	190800
131	155213	194522
135	155730	190137
139	154278	212091
143	158654	234893
147	161531	242564
151	157694	242089
155	164857	250810
159	164173	256931
163	168211	255511
166	168368	231464
170	165000	253033
174	145572	214525
178	142000	229011
182	145089	245104

186	139800	224225
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Example 5: High-efficiency expression of Xylanases Xyn-307 and Xyn-309 in *Pichia pastoris*

(1) Strains and Vectors

5 *Pichia pastoris* X-33 was obtained from GenScript Biotech Corporation, and the vector pPicZaA was purchased from Invitrogen.

(2) Enzymes and related molecular biology reagents

Restriction enzymes and ligases were purchased from TaKaRa, the Agarose Gel DNA Purification Kit and Fragment Purification Kit Ver.2.0 were purchased
10 from TaKaRa. T4 DNA ligase was purchased from Fermentas. Other biochemical reagents were purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd.

(3) Culture medium

LB Medium: 1% tryptone, 0.5% yeast extract, and 1% sodium chloride;
15 Adjust the pH to 7.0 with NaOH; Add 1.5% (w/v) agar powder (solid medium).

Yeast MD screening medium: 2% glucose, 1.5% agar powder, 0.00004% biotin, and 1.34% YNB; MM Medium: 0.5% methanol (volume fraction), 1.5% agar powder, 0.00004% biotin, and 1.34% YNB; RDB Medium: 1M sorbitol, 2% glucose, 0.00004% biotin, 1.34% YNB, and 0.005% amino acids.

20 Yeast BMGY induction medium: 1% yeast extract, 2% peptone, 1.34% YNB, 0.00004% biotin, and 1% glycerol (volume fraction).

Yeast BMMY induction medium: 1% yeast extract, 2% peptone, 1.34% YNB, 0.00004% biotin, and 0.5% methanol (volume fraction).

(4) Construction of recombinant plasmid and expression in *Pichia pastoris*

25 Using the synthesized xylanase Xyn-307 and Xyn-309 genes as templates, they were ligated into the expression vector pPicZaA, enabling the insertion of Xyn-307 and Xyn-309 genes downstream of the signal peptide sequence in the aforementioned expression vector, forming the correct reading frame with the α -MF signal peptide, thereby constructing the yeast expression vectors pPicZaA-Xyn-307 and pPicZaA-Xyn-309, which were then transformed into *Escherichia coli* competent cells DH5 α . Positive transformants were subjected to DNA sequencing, and those with correct sequences were used for large-scale preparation
30 of recombinant plasmids. Approximately 5 μ g of the recombinant expression

plasmid linearized with the restriction enzyme BglII was electroporated into yeast X-33 competent cells, spread onto Zeocin-resistant plates, and incubated at 30°C for 2-3 days. Transformants growing on the plates were picked for further expression experiments.

5 (5) Screening of *Pichia pastoris* transformants with high xylanase activity

Single colonies were picked from the plate containing transformants using sterilized pipette tips and spotted onto Zeocin plates according to numbering. The plates with spotted transformants were placed in a 30°C incubator and cultured for 1-2 days until colonies grew. Colony PCR verification and sequencing verification
10 of the expression cassettes carrying Xyn-307 and Xyn-309 were performed. After confirming the correct sequencing, transformants were picked from the plate according to the numbering and inoculated into shake flasks containing BMGY medium, followed by culture in a shaker at 30°C and 250 rpm for approximately 48 h. After 48 h of culture in the shake flasks, the culture broth was centrifuged at
15 $3,000 \times g$ for 15 min, the supernatant was discarded, and BMMY medium containing 0.5% (volume fraction) methanol was used for induction culture at 30°C and 260 rpm; After 48 h of induction culture, the culture broth was sampled, centrifuged at $3,000 \times g$ for 5 min, and the supernatant was collected for enzyme activity assay to screen transformants with high xylanase activity.

20 (6) Enzyme activity expression levels of Xyn-307 and Xyn-309 in genetically engineered *Pichia pastoris* strains in a 50 L fermenter

Fermenter: The above xylanase Xyn-307 and Xyn-309 positive transformant of genetically engineered yeast strains were subjected to fermentation experiments in a 50 L fermenter, with an actual working volume of 30 L for the enzyme
25 production medium. Fermentation conditions: 30°C, stirring speed: 800 rpm, aeration rate: 25 LPM, fermentation duration approximately 210 h, with enzyme activity monitored throughout the process. The enzyme activity test data are shown in Table 4, and the enzyme activity change curve is shown in FIG. 2. The maximum whole-cell enzyme activity of Xyn-309 fermentation reached 72,000 u/g,
30 while that of Xyn-307 fermentation reached 65,000 u/g.

Table 4 Whole-cell enzyme activity records of xylanases Xyn-307 and Xyn-309 in yeast platform during fermentation

Fermentation duration (h)	Xyn-307 enzyme activity	Xyn-309 enzyme activity
---------------------------	-------------------------	-------------------------

	(u/g)	(u/g)
22	885	1144
26	1480	1767
30	2313	3850
34	4338	5623
38	5138	8043
42	7270	11826
46	8059	12104
50	10686	16163
54	11887	17846
58	13018	19557
62	14309	20895
66	17899	21563
70	19169	24386
74	20352	25121
78	21887	28436
82	25762	29068
86	27256	28583
90	31045	34415
94	32410	36120
98	33595	37376
102	35290	38946
106	38099	43700
110	41255	44545
114	43944	47420
118	44693	46577
122	49129	50278
126	49907	49573
130	51409	45788
134	48708	46708
138	49796	53938
142	50024	56522
146	51259	57449

150	53196	58669
154	52858	60151
158	53256	60659
162	54832	61772
166	59611	65983
170	60590	66233
178	59500	68756
186	65000	71311
190	63451	72359
194	63876	70698
198	63910	71821
202	63948	72072
206	64779	72071
210	61716	69694

Comparison between Example 4 and Example 5 demonstrates that the xylanases Xyn-307 and Xyn-309 in the *Aspergillus niger* platform exhibit significantly enhanced enzyme activity compared to the xylanases Xyn-307 and Xyn-309 in the yeast platform. Specifically, the activity of Xyn-307 increased from 65,000 u/g to 168,000 u/g, representing a 158.5% improvement; the activity of Xyn-309 increased from 72,000 u/g to 256,000 u/g, representing a 255.6% improvement. Secondly, in the *Aspergillus niger* platform, the enzyme activity expression level of Xyn-309 was significantly improved compared to Xyn-307, reaching a maximum of 256,000 u/g, representing an increase of over 50% in enzyme activity relative to Xyn-307.

Example 6

To evaluate the practical performance of xylanases expressed in the *Aspergillus niger* platform for feed applications, we conducted water bath heat resistance tests on Xyn-307 and Xyn-309 in the *Aspergillus niger* platform and Xyn-307 in the yeast platform. Subsequently, pelletized products were prepared, and stability during feed pelleting as well as in vitro enzymatic hydrolysis effects were tested. The results are shown in Tables 5, 6, and 7, respectively.

Table 5 Water bath heat resistance test

Sample name	Enzyme activity retention rate, %		
	80°C	85°C	90°C
Xyn-307 (yeast)	69.96%	70.95%	64.46%
Xyn-307 (<i>Aspergillus niger</i>)	77.97%	73.71%	70.95%
Xyn-309 (<i>Aspergillus niger</i>)	75.4%	72.53%	74.14%

Table 6 Stability test during feed pelleting

Sample name	Enzyme activity retention rate, % (conditioning temperature 85°C)
Xyn-307 (yeast)	91%
Xyn-307 (<i>Aspergillus niger</i>)	95%
Xyn-309 (<i>Aspergillus niger</i>)	93%

Table 7 In vitro enzymatic digestion results

Sample name	Bran		Formulated diet	
	Average reducing sugar release, mg/g	Increase in reducing sugar, mg/g	Average reducing sugar release, mg/g	Increase in reducing sugar, mg/g
Blank	70.5074	0	81.9583	0
Xyn-307 (yeast)	87.8294	17.32	83.6052	1.65
Xyn-307 (<i>Aspergillus niger</i>)	93.3958	24.89	86.3422	4.38
Xyn-309 (<i>Aspergillus niger</i>)	96.6056	26.10	83.6799	1.72

5

The sequences involved in the present disclosure are as follows:

Amino acid sequence 1 SEQ ID NO: 1 of fusion peptide segment:

SRTKTHLRRGSE

Amino acid sequence 2 SEQ ID NO: 2 of fusion peptide segment:

10 GYESSGSSSITVSTQTTTLTSTKPTSTSTTTRTSTSTTSTQGGSSSTS

Amino acid sequence SEQ ID NO: 3 of xylanase Xyn-CDBFV:

QSFCSASHSGQSVKVTGNKVGITGGVGYELWADSGNNSATFYSDGSFSCTFQNA
GDYLCRSGLSFDSTKTPSQIGRMKADFKLVKQSSNVGYSYVGVGWTRSPLEYYIV
DNWLSPPFGDWVGNKKHGSFTIDGAQYTVYENTRTGPSIDGDTTFNQYFSIRQQARD
CGTIDISAHFDQWEKLGMTMGKLHEAKVLGEAGNVNGGASGTADFPYAKVYIGD

Amino acid sequence SEQ ID NO: 4 of xylanase Xyn-307:

QSFCSASHSGQSVKVTGNKVGITGGVGYELWADSGNNSATFYSDGSFSCFQNA
GDYLCRSGLSFDSTKTPSQIGRMKADFKLVKQNSSNVGYSYVGWYVWTRSPLEYYIV
DNWLSPPFPDWDVGNKKHGSFTIDGAQYTVYENTRTGPSIDGDTTFNQYFSIRQQARD
CGTIDISAHFDQWEKLGMTMGKLHEAKVLGEAGNVNGGASGTADFPYAKVYIGDSRT
KTHLRRGSE

Amino acid sequence SEQ ID NO: 5 of xylanase Xyn-309:

QSFCSASHSGQSVKVTGNKVGITGGVGYELWADSGNNSATFYSDGSFSCFQNA
GDYLCRSGLSFDSTKTPSQIGRMKADFKLVKQNSSNVGYSYVGWYVWTRSPLEYYIV
DNWLSPPFPDWDVGNKKHGSFTIDGAQYTVYENTRTGPSIDGDTTFNQYFSIRQQARD
CGTIDISAHFDQWEKLGMTMGKLHEAKVLGEAGNVNGGASGTADFPYAKVYIGDSRT
KTHLRRGSEGYESSGSSITVSTQTTTLTSTKPTSTSTTTTSTSTTTSTQGGSSSTS

5 Nucleotide sequence SEQ ID NO: 6 of xylanase Xyn-307:

CAGTCCTTCTGCTCCTCCGCCTCCCACTCCGGTCAGTCCGTCAAGGTCACCGGT
AACAAGGTCGGTACCATCGGTGGTGTCTCGGTTACGAGCTGTGGGCCGACTCCGGTAA
CAACTCCGCCACCTTCTACTCCGACGGTTCCTTCTCCTGCACCTTCCAGAACGCCGG
TGAATACCTGTGCCGCTCCGGTCTGTCTTTCGACTCCACCAAGACCCCTCCAGAT
CGGTTCGATGAAGGCCGACTTCAAGCTGGTCAAGCAGAACTCCTCCAACGTCGGTT
ACTCCTACGTCGGTGTCTACGGTTGGACCCGCTCCCCCTGGTCGAGTACTACATCG
TCGACAACCTGGCTGTCCCCCTTCCCCCCCCGGTACTGGGTTCGGTAACAAGAAGCAC
GGTTCCTTACCATCGACGGTGCCAGTACACCGTCTACGAGAACACCCGCACCGG
TCCCTCCATCGACGGTGACACCACCTTCAACCAGTACTTCTCCATCCGCCAGCAGGC
CCGCGACTGCGGTACCATCGACATCTCCGCCCACTTCGACCAGTGGGAGAAGCTGG
GTATGACCATGGGTAAAGCTGCACGAGGCCAAGGTCCTGGGTGAGGCCGGTAACGTC
AACGGTGGTGCCTCCGGTACCGCCGACTTCCCCTACGCCAAGGTCTACATCGGTGA
CTCCCGCACCAAGACCCACCTGCGCCGCGGTTCGAGTAA

Nucleotide sequence SEQ ID NO: 7 of xylanase Xyn-309:

CAGTCCTTCTGCTCCTCCGCCTCCCACTCCGGTCAGTCCGTCAAGGTCACCGGT
AACAAGGTCGGTACCATCGGTGGTGTCTCGGTTACGAGCTGTGGGCCGACTCCGGTAA
CAACTCCGCCACCTTCTACTCCGACGGTTCCTTCTCCTGCACCTTCCAGAACGCCGG
TGAATACCTGTGCCGCTCCGGTCTGTCTTTCGACTCCACCAAGACCCCTCCAGAT
CGGTTCGATGAAGGCCGACTTCAAGCTGGTCAAGCAGAACTCCTCCAACGTCGGTT
ACTCCTACGTCGGTGTCTACGGTTGGACCCGCTCCCCCTGGTCGAGTACTACATCG
TCGACAACCTGGCTGTCCCCCTTCCCCCCCCGGTACTGGGTTCGGTAACAAGAAGCAC
GGTTCCTTACCATCGACGGTGCCAGTACACCGTCTACGAGAACACCCGCACCGG
TCCCTCCATCGACGGTGACACCACCTTCAACCAGTACTTCTCCATCCGCCAGCAGGC
CCGCGACTGCGGTACCATCGACATCTCCGCCCACTTCGACCAGTGGGAGAAGCTGG
GTATGACCATGGGTAAAGCTGCACGAGGCCAAGGTCCTGGGTGAGGCCGGTAACGTC
AACGGTGGTGCCTCCGGTACCGCCGACTTCCCCTACGCCAAGGTCTACATCGGTGA
CTCCCGCACCAAGACCCACCTGCGCCGCGGTTCGAGGGTTACGAGTCTCCTCGGTT
CCTCCTCCATCACCCTCTCCACCCAGACCACCACCCTGACCACCTCCACCAAGCCC
ACCTCCACCTCCACCACCCGCACCTCCACCTCCACCACCTCCACCCAGGGTGG
TTCCTCCTCCACCTCCTAA

Nucleotide sequence SEQ ID NO: 8 of the gla promoter of *Aspergillus niger*

10 glucoamylase gene:

TGCCATTGGCGGAGGGGTCCGGACGGTCAGGAACTTAGCCTTATGAGATGAAT
GATGGACGTGTCTGGCCTCGGAAAAGGATATATGGGGATCATGATAGTAGCCAT
ATTAATGAAGGGCATATACCACGCGTTGGACCTGCGTTATAGCTTCCCGTTAGTTATA
GTACCATCGTTATACCAGCCAATCAAGTCACCACGCACGACCGGGGACGGCGAATC
CCCGGGAATTGAAAGAAATTGCATCCAGGCCAGTGAGGCCAGCGATTGGCCACCT
CTCCAAGGCACAGGGCCATTCTGCAGCGCTGGTGGATTTCATCGCAATTTCCCCCGG
CCCGGCCCGACACCGCTATAGGCTGGTTCTCCACACCATCGGAGATTCTGTCGCCTA
ATGTCTCGTCCGTTACAAGCTGAAGAGCTTGAAGTGCGAGATGTCTCTGCAGGA
ATTCAAGCTAGATGCTAAGCGATATTGCATGGCAATATGTGTTGATGCATGTGCTTCT
TCCTTCAGCTTCCCCTCGTGCAGATGAGGTTTGGCTATAAATTGAAGTGGTTGGTCG
GGGTTCCGTGAGGGGCTGAAGTGCTTCCTCCCTTTTAGACGCAACTGAGAGCCTGA
GCTTCATCCCCAGCATCATTACACCTCAGCA

Nucleotide sequence SEQ ID NO: 9 of the gla terminator of *Aspergillus niger*
glucoamylase gene:

ACAATCAATCCATTTTCGCTATAGTTAAAGGATGGGGATGAGGGCAATTGGTTATA
TGATCATGTATGTAGTGGGTGTGCATAATAGTAGTGAAATGGAAGCCAAGTCATGTG
ATTGTAATCGACCGACGGAATTGAGGATATCCGGAAATACAGACACCGTGAAAGCC
ATGGTCTTTTCCTTCGTGTAGAAGACCAGACAGACAGTCCCTGATTTACCCTTGCACA
AAGCACTAGAAAATTAGCATTCCATCCTTCTCTGCTTGCTCTGCTGATATCACTGTCA
TTCAATGCATAGCCATGAGCTCATCTTAGATCCAAGCACGTAATTCCATAGCCGAGG
TCCACAGTGGAGCAGCAACATTCCTCCATCATTGCTTTCCCCAGGGGCCTCCCAACG
ACTAAATCAAGAGTATATCTCTACCGTCCAATAGATCGTCTTCGCTTCAAATCTTTG
ACAATTCCAAGAGGGTCCCCATCCATCAAACCCAGTTCAATAATAGCCGAGATGCAT
5 GGTGGAGTCAATTAGGCAGTATTGCTGGAATGTCGGGGCCAGTTGGC

Nucleotide sequence SEQ ID NO: 10 of the signal peptide of *Aspergillus niger* amylase:

ATGGTCGCCTGGTGGTCCCTCTTCCTCTACGGTCTCCAGGTCGCCGCCCCCGCC
CTCGCCGCCACCCCCGCCGACTGGCGCTCC

Nucleotide sequence SEQ ID NO: 11 of the selection marker amdS:

CTGGAAACGCAACCCTGAAGGGATTCTTCCTTTGAGAGATGGAAGCGTGTCAT
ATCTCTTCGGTTCTACGGCAGGTTTTTTTTCTGCTCTTTCGTAGCATGGCATGGTCACT
TCAGCGCTTATTTACAGTTGCTGGTATTGATTTCTTGTGCAAATTGCTATCTGACACTT
ATTAGCTATGGAGTCAACACATTTCCCAGCAACTTCCCCACTTCCTCTGCAATCGCC
AACGTCCTCTCTTCACTGAGTCTCCGTCCGATAACCTGCACTGCAACCGGTGCCCA
TGGTACGCCTCCGGATCATACTCTTCCTGCACGAGGGCATCAAGCTCACTAACC GCC
TTGAAACTCTCATTCTTCTTATCGATGTTCTTATCCGCAAAGGTAACCGGAACAACC
ACGCTCGTGAAATCCAGCAGGTTGATCACAGAGGCATACCCATAGTACCGGAAC TG
GTCATGCCGTACCGCAGCGGTAGGCGTAATCGGCGCGATGATGGCGTCCAGTTCCTT
CCCCGCCTTTTCTTCAGCCTCCCGCCATTTCTCAAGGTACTCCATCTGGTAATTCCAC
TTCTGGAGATGCGTGTCCCAGAGCTCGTTCATGTTAACAGCTTTGATGTTGCGGTTC
AGTAGGTCTTTGATATTTGGAATCGCCGGCTCGCCGGATGCACTGATATCGCGCATTA
CGTCGGCGCTGCCGTACGCCGCTAGATATGGGAGATGAGATCGTGGCCGAAATCG
TGCTTGATGGCGTCCACGGGGTCACGGTGTGACCGGCTTTGGCGAGTGGCGGCAC
GGTGGTTTCCACGCCGCGCAGGATAGGAGGGTGTGGAAGGACATTGCCGTGCAAG
TTGTAGTAGCCGATATTGAGCCCGCCGTTCTTGATCTTGGAGGCAATAATGTCCGAC
TCGGACTGGCGCCAGGGCATGGGGATGACCTTGGAGTCGTATTTCCATGGCTCCTG
ACCGAGGACGGATTTGGTGAAGAGGCGGAGGTCTAACATACTTCATCAGTGACTGC
CGGTCTCGTATATAGTATAAAAAGCAAGAAAGGAGGACAGTGAGAGCCTGGTATAG
AGCAGGAAAAGAAGGAAGAGGCGAAGGACTCACCTCAACAGAGTGCGTAATCG
GCCCCACAACGCTGTGCACCGTCTCCTGACCCTCCATGCTGTTGCCATCTTTGCAT
ACGGCAGCCGCCCATGACTCGGCCTTAGACCGTACAGGAAGTTGAACGCGGCCCG
CACTCGAATCGAGCCACCGATATCCGTTCTACACCGATGACGCCACCACGAATCCC
AACGATCGCACCCCTCACACCAGA ACTGCCGCCGCACGACCAGTTCTTGTTGCGTG
GGTTGACGGTGCGCCCGATGATGTTGTTGACTGTCTCGCAGACCATCAGGGTCTGC
GGGACAGAGGTCTTGACGTAGAAGACGGCACCGGCTTTGCGGAGCATGGTTGTCA
GAACCGAGTCCCCTTCGTCGTA CTGTTTAGCCATGAGATGTAGCCATTGATGTTTC
GTAGCCCTGGTGGCATATGTTAGCTGACAAAAGGGACATCTAACGACTTAGGGGC
AACGGTGTACCTTGACTCGAAGCTGGTCTTTGAGAGAGATGGGGAGGCCATGGAGT
GGACCAACGGGTCTCTTGTGCTTTGCGTAGTATTCATCGAGTTCCCTTGCCCTGCGCG
AGAGCGGCGTCAGGGAAGAACTCGTGGGCGCAGTTTGTCTGCACAGAAGCCAGCG
TCAGCTTGATAGTCCCATAAGGTGGCGTTGTTACATCTCCCTGAGAGGTAGAGGGGA
CCCTACTAACTGCTGGGCGATTGCTGCCCGTTTACAGAATGCTAGCGTAACTTCCAC
CGAGGTCAACTCTCCGGCCGCCAGCTTGGACACAAGATCTGCAGCGGAGGCCTCT
GTGATCTTCAGTTCGGCCTCTGAAAGGATCCCCGATTTCTTTGGGAAATCAATAACG
CTGTCTTCCGCAGGCAGCGTCTGGACTTTCCATTATCAGGGATGGTTTTTTCGAGG
CGGGCGCGCTTATCAGCGGCCAGTTCTTCCCAGGATTGAGGCATTCTGTGTTAGCTT
ATAGTCAGGATGTTGGCTCGACGAGTGTA AACTGGGAGTTGGCATGAGGGTTATGT
AGGCTTCTTTAGCCCCGCATCCCCCTCATTCTCCTCATTGATCCCGGGGGAGCGGAT
GGTGTTGATAAGAGACTAATTATAGGGTTTAGCTGGTGCCTAGCTGGTGATTGGCTG
GCTTCGCCGAATTTACGGGCCAAGGAAAGCTGCAGAACCGCGGCACTGGTAAAC
GGTAATTAAGCTATCAGCCCCATGCTAACGAGTTTAAATTACGTGTATTGCTGATAAA
CACCAACAGAGCTTTACTGAAAGATGGGAGTCACGGTGTGGCTTCCCCACTGCGAT
TATTGCACAAGCAGCGAGGGCGAACTTGACTGTGTCGCTGAGCAGCCTGCAGTCA
AACATACATATATATCAACCGCGAAGACGTCTGGCCTTGAGAACACGACGCTCCCT
AGCAACACCTGCCGTGTCAGCCTCTACGGTTGTTACTTGCAATCAGGATGCTCTCCA
GCGGGCGAGCTATTCAA AATATTCAAAGCAGGTATCTCGTATTGCCAGGATTCAGCT
GAAGCAACAGGTGCCAAGGAAATCTGCGTCGGTTCTCATCTGGGCTTGCTCGGTCC
TGGCGTAGATCTAG

While the foregoing describes particularly preferred embodiments, it is to be understood that the present disclosure is not limited thereby. Those skilled in the art will envision various modifications to the disclosed embodiments, and such modifications are intended to fall within the scope of the present disclosure.

- 5 All publications, patent applications, and patents cited in this specification are incorporated herein by reference in their entirety.

CLAIMS

1. A xylanase, characterized in that the xylanase comprises a xylanase Xyn-CDBFV sequence and a fusion peptide segment linked to the C-terminus of the xylanase Xyn-CDBFV sequence, wherein the fusion peptide segment comprises an amino acid sequence as set forth in SEQ ID NO: 1.

2. The xylanase according to claim 1, characterized in that the fusion peptide segment further comprises an amino acid sequence as set forth in SEQ ID NO: 2.

3. The xylanase according to claim 1 or 2, characterized in that the fusion peptide segment consists of amino acid sequences as set forth in SEQ ID NO: 1 and SEQ ID NO: 2.

4. The xylanase according to claim 1, characterized in that the xylanase Xyn-CDBFV sequence is as set forth in SEQ ID NO: 3.

5. The xylanase according to claim 1, characterized in that the xylanase comprises an amino acid sequence as set forth in SEQ ID NO: 4.

6. The xylanase according to claim 1 or 2, characterized in that the xylanase comprises an amino acid sequence as set forth in SEQ ID NO: 5.

7. The xylanase according to claim 1, characterized in that the xylanase consists of an amino acid sequence as set forth in SEQ ID NO: 4.

8. The xylanase according to claim 1 or 2, characterized in that the xylanase consists of an amino acid sequence as set forth in SEQ ID NO: 5.

9. A polynucleotide, characterized in that the polynucleotide encodes the xylanase according to claim 1.

10. The polynucleotide according to claim 9, characterized in that the polynucleotide comprises a nucleotide sequence as set forth in SEQ ID NO: 6.

5 11. The polynucleotide according to claim 9, characterized in that the polynucleotide comprises a nucleotide sequence as set forth in SEQ ID NO: 7.

12. The polynucleotide according to claim 9, characterized in that the polynucleotide consists of a nucleotide sequence as set forth in SEQ ID NO: 6.

10 13. The polynucleotide according to claim 9, characterized in that the polynucleotide consists of a nucleotide sequence as set forth in SEQ ID NO: 7.

14. A recombinant vector, characterized in that the recombinant vector comprises the polynucleotide according to any one of claims 9-13.

15

15. A host cell, characterized in that the host cell comprises the polynucleotide according to any one of claims 9-13 or the recombinant vector according to claim 14.

20 16. The host cell according to claim 15, characterized in that the host cell is *Aspergillus niger* or *Pichia pastoris*.

17. The host cell according to claim 16, characterized in that the host cell is *Aspergillus niger*.

25

18. A method for preparing xylanase, characterized in that the method comprises linking a fusion peptide segment to the C-terminus of the xylanase Xyn-CDBFV sequence, wherein the fusion peptide segment comprises an amino acid sequence as set forth in SEQ ID NO: 1.

30

19. The method according to claim 18, characterized in that the fusion peptide segment further comprises an amino acid sequence as set forth in SEQ ID NO: 2.

20. The method according to claim 18 or 19, characterized in that the fusion peptide segment consists of amino acid sequences as set forth in SEQ ID NO: 1 and SEQ ID NO: 2.

5 21. The method according to claim 18, characterized in that the xylanase Xyn-CDBFV sequence is as set forth in SEQ ID NO: 3.

22. A method for preparing an *Aspergillus niger* strain producing xylanase, characterized in that the method comprises introducing the recombinant vector
10 according to claim 14 into the *Aspergillus niger* strain.

23. The method according to claim 22, characterized in that the recombinant vector comprises an *Aspergillus niger* expression cassette sequence, and the *Aspergillus niger* expression cassette sequence comprises a gla promoter
15 sequence of *Aspergillus niger* glucoamylase gene, a signal peptide nucleotide sequence of *Aspergillus niger* amylase, a xylanase nucleotide sequence as set forth in SEQ ID NO: 6 or 7, and a gla terminator sequence of *Aspergillus niger* glucoamylase gene.

20 24. The method according to claim 22 or 23, characterized in that the method comprises screening the *Aspergillus niger* strain using an amdS selection marker plate after introducing the recombinant vector.

25 25. The xylanase according to any one of claims 1-8 for use in feed.