

Structural and functional characterization of a bifunctional GH30-7 xylanase B from the filamentous fungus *Talaromyces cellulolyticus*

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Supplementary Information

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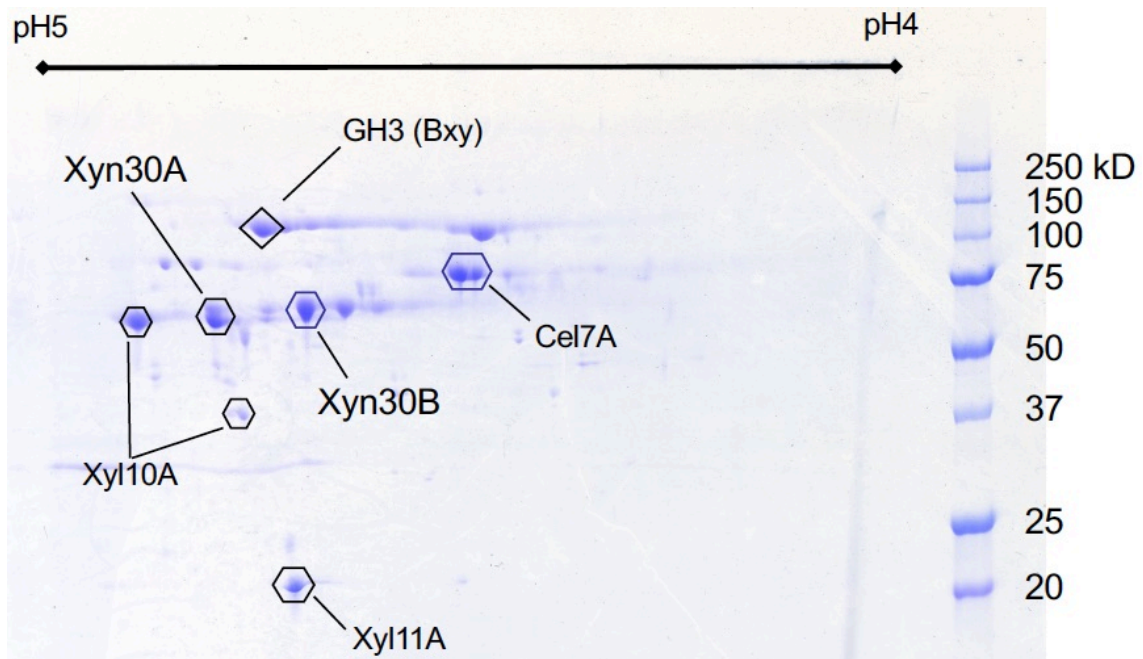


Figure S1. 2D gel electrophoresis analysis (pH4-5) of *T. cellulolyticus* CF-2612 culture grown on birchwood xylan.

CF-2612 was grown on a medium (pH 4.0) containing, per liter: 50 g birchwood xylan, 24 g KH_2PO_4 , 1 g Tween 80, 5 g $(\text{NH}_4)_2\text{SO}_4$, 1.2 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g $\text{MnSO}_4 \cdot 6\text{H}_2\text{O}$, 0.01 g $\text{CuSO}_4 \cdot 7\text{H}_2\text{O}$, and 4.0 g urea, at 30 °C, 200 rpm, for 72 h in an Erlenmeyer flask. The major protein spots on the gel were treated by endoproteinase Lys-C and analyzed by HPLC peptide mapping. Proteins from the resulting peptide sequences were assigned using a draft genome sequence of *T. cellulolyticus* Y-94. Xyn30B was identified from two peptide sequences (SAEDYLS, FYAESGVPVTHL), and Xyn30A was from two peptide sequences (ATVPPRAVQVF and DFLEIL). Bxy, GH3 β -xylosidase; Cel7A, GH7 cellobiohydrolase I; Xyl10A, GH10 xylanase A; Xyl11A, GH11 xylanase A.

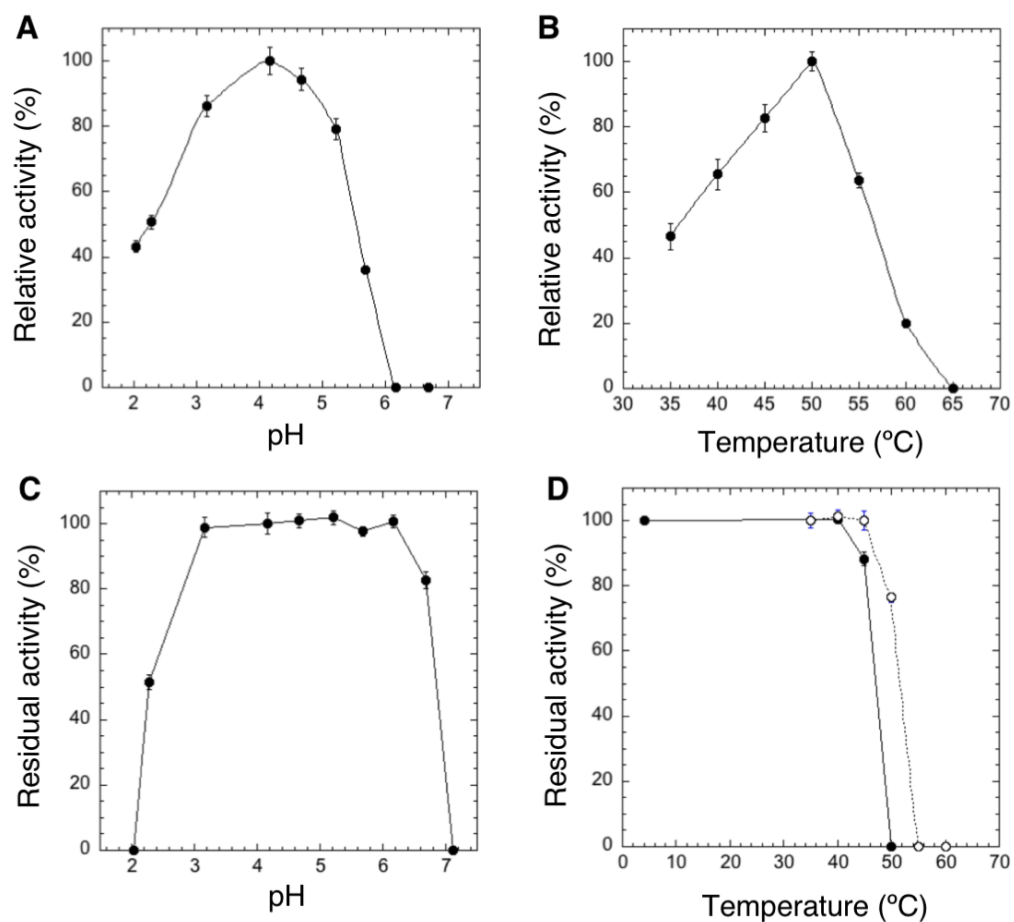


Figure S2. Characterization of purified Xyn30B.

Xylanase activity was measured in a reaction mixture containing purified Xyn30B and 10 mg mL⁻¹ beechwood xylan. (A) Optimal pH. Maximum activity is relatively taken as 100%. The reaction mixture was incubated at 40°C for 15 min in a pH range of 2.0–6.5. (B) Optimal temperature. Maximum activity is relatively taken as 100%. The reaction mixture was incubated at 35–60°C for 15 min in 50 mM sodium acetate (pH 4.0). (C) pH stability. The enzyme was preincubated in a pH range of 2.0–7.0 at 40°C for 30 min, and the residual activity was subsequently measured at 40°C for 15 min in 50 mM sodium acetate (pH 4.0). (D) Thermal stability. The enzyme was preincubated in 50 mM sodium acetate (pH 4.0) at 4–60°C for 30 min (open circle) or 24 hours (filled circle), and residual activity was measured at 40°C for 15 min.

Chain A

| | |
|--------|---|
| Asn60 | Man β 1 — 4GlcNAc β 1 — 4GlcNAc β |
| Asn88 | $\begin{array}{c} \text{Man } \alpha 1 \searrow 3 \\ \text{Man } \alpha 1 \swarrow 6 \end{array} \text{Man } \beta 1 \text{ — 4GlcNAc } \beta 1 \text{ — 4GlcNAc } \beta$ |
| Asn215 | GlcNAc β |
| Asn334 | Man β 1 — 4GlcNAc β 1 — 4GlcNAc β |
| Asn346 | GlcNAc β 1 |
| Asn412 | GlcNAc β 1 — 4GlcNAc β |

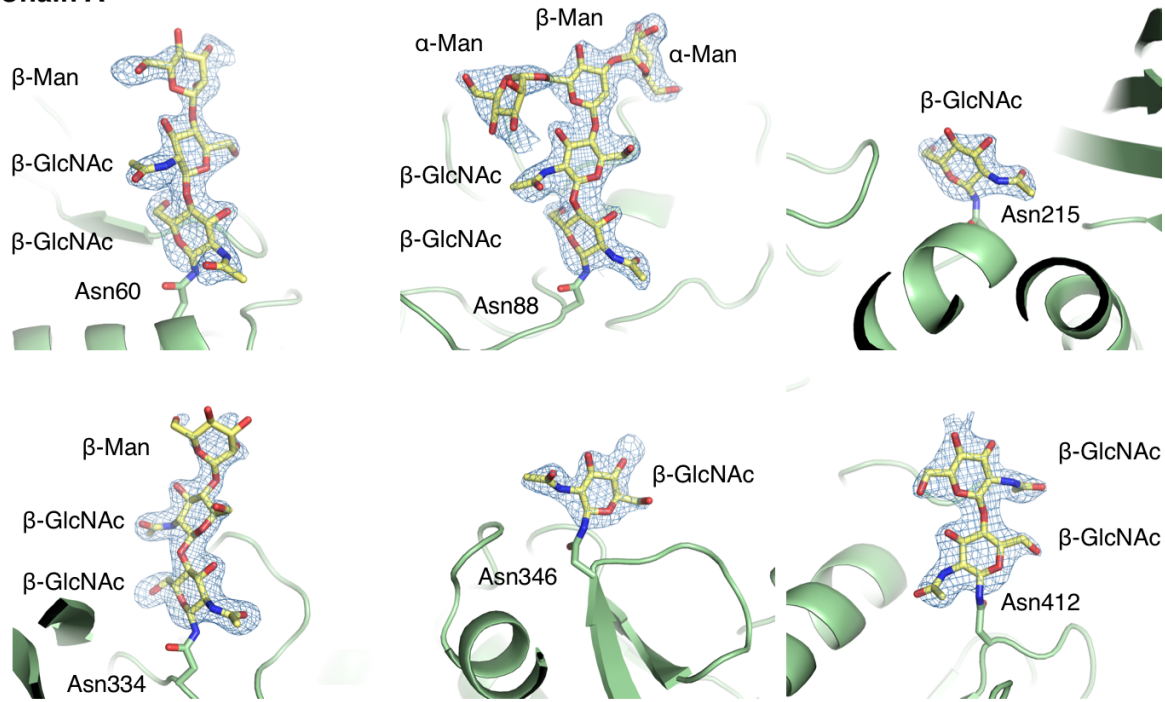
Chain B

| | |
|--------|---|
| Asn60 | Man α 1 — 6Man β 1 — 4GlcNAc β 1 — 4GlcNAc β |
| Asn88 | $\begin{array}{c} \text{Man } \alpha 1 \searrow 3 \\ \text{Man } \alpha 1 \swarrow 6 \end{array} \text{Man } \beta 1 \text{ — 4GlcNAc } \beta 1 \text{ — 4GlcNAc } \beta$ |
| Asn215 | GlcNAc β |
| Asn334 | Man β 1 — 4GlcNAc β 1 — 4GlcNAc β |
| Asn346 | GlcNAc β 1 — 4GlcNAc β |
| Asn412 | GlcNAc β 1 — 4GlcNAc β |

Figure S3. N-linked carbohydrate moieties and structures in Xyn30B assigned by electron density map shown in Fig. S4.

Two molecules of Xyn30B in an asymmetric unit in the crystal are named as chain A and B.

Chain A



Chain B

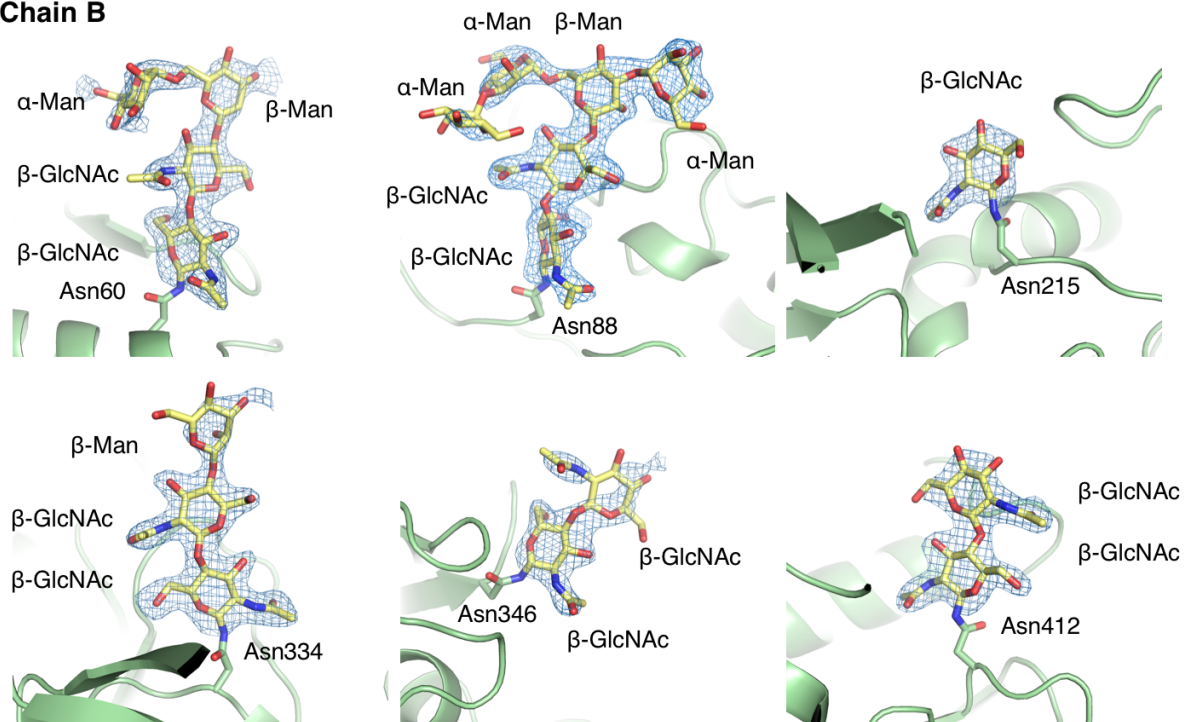


Figure S4. *F_o-F_c* omit map (blue) for *N*-linked carbohydrate moieties, which is calculated without sugar residues, is contoured at 3.0 σ .

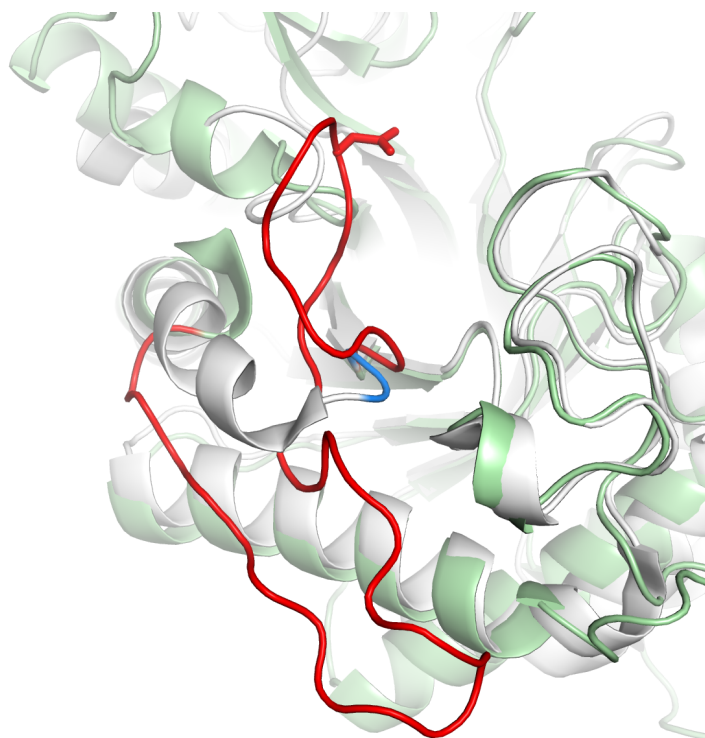


Figure S5. Structural differences in the $\beta 2$ - $\alpha 2$ loops of Xyn30B and *EcXynA*.

The $\beta 2$ - $\alpha 2$ loops of Xyn30B and *EcXynA* are shown by red and blue, respectively. Asn-93 of Xyn30B is shown as a stick. Atoms are colored as follows: green, C of Xyn30B; white, C of *EcXyn30A*.

| | 29 | | 46 | 51 | | | | | | | | | | | | | | | | | | | |
|--------------------------------------|----|---|----|----|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Xyn30B | Q | A | R | Y | Q | S | V | D | G | F | G | C | S | Q | A | F | Q | R | A | E | D | I | F |
| <i>T.reesei</i> XYN VI | G | S | K | F | Q | Q | I | D | G | F | G | F | S | Q | A | F | G | R | A | R | E | F | Q |
| Xyn30A | L | Q | R | Y | Q | E | M | I | G | G | G | C | S | G | A | F | G | W | A | C | Q | Q | F |
| <i>F.fujikuroi</i> CCT73001 | N | K | K | L | Q | V | I | D | G | F | G | V | S | E | A | Y | G | H | A | K | Q | F | Q |
| <i>T.reesei</i> XYN IV | R | Q | T | Y | Q | T | M | I | G | G | G | C | S | G | A | F | G | I | A | C | Q | Q | F |
| <i>T.terrestris</i> THITE 2123443 | G | V | R | Y | Q | T | M | M | G | G | G | C | S | G | A | F | G | V | A | C | D | Q | T |
| <i>A.oligospora</i> EGX45083 | G | T | T | Y | Q | T | I | E | G | F | G | F | S | Q | A | F | G | R | A | V | E | F | K |
| Bispore sp. XYLD | N | E | E | K | Q | I | V | D | G | F | G | F | S | E | A | F | G | R | A | E | N | V | F |
| <i>F.fujikuroi</i> CCT73045 | H | V | R | Y | Q | E | L | D | G | F | G | A | S | Q | A | F | Q | R | A | E | D | I | L |
| <i>L.maculans</i> CBX92400 | V | K | T | Y | Q | T | M | D | G | F | G | M | S | E | T | F | Q | R | A | N | Q | M | K |
| <i>L.maculans</i> CBX93583 | T | K | G | Y | Q | T | I | D | G | F | G | V | S | A | A | F | Q | R | A | N | L | I | V |
| <i>L.maculans</i> xyl38 | V | K | T | Y | Q | T | M | D | G | F | G | M | S | E | T | F | Q | R | A | N | Q | M | K |
| <i>P.comata</i> VBB81111 | S | R | T | F | Q | T | M | D | G | F | G | A | S | E | A | F | Q | R | A | V | T | M | K |
| <i>P.comata</i> VBB86450 | T | R | T | F | Q | K | M | D | G | F | G | F | S | L | A | F | Q | R | A | N | L | I | T |
| <i>S.lignohabitans</i> | A | Q | T | Y | Q | E | I | D | G | F | G | F | S | E | A | F | Q | R | A | N | D | L | Y |
| <i>T.purpureogenus</i> AKH40280 | K | V | Q | F | Q | E | V | D | G | F | G | A | S | Q | A | F | Q | R | A | E | D | I | F |
| <i>T.terrestris</i> THITE 30409 | G | T | K | Y | Q | R | I | D | G | F | G | F | S | Q | A | F | G | R | A | A | E | F | Q |
| <i>T.thermophila</i> AE055025 | S | T | T | Y | Q | R | I | D | G | F | G | T | S | E | A | F | Q | R | A | V | Q | M | S |
| <i>T.thermophila</i> AE057693 | S | Q | T | Y | Q | R | M | D | G | F | G | F | S | L | A | F | Q | R | A | N | L | I | T |
| <i>A.albispora</i> AXB42337 | A | R | R | H | Q | P | I | D | G | F | G | F | S | E | A | F | G | R | A | E | I | M | R |
| <i>A.derwentensis</i> SDT08346 | - | M | S | C | S | P | I | D | G | F | G | F | S | E | H | F | G | R | A | T | I | M | H |
| <i>A.orientalis</i> CAA11771 | R | H | S | L | Q | P | I | D | G | F | G | F | S | Q | A | F | Q | R | A | A | R | I | R |
| <i>N.gerenzanensis</i> SB093718 | R | V | R | H | Q | E | I | D | G | F | G | I | S | Q | A | F | R | R | N | E | L | L | E |
| <i>N.gerenzanensis</i> SB096059 | G | V | R | H | Q | T | I | D | G | F | G | I | S | T | A | F | R | R | G | E | L | L | K |
| <i>Nonomuraea</i> sp. AQZ61832 | S | Q | T | H | Q | T | I | D | G | F | G | Y | S | T | A | F | Q | R | A | T | L | V | H |
| <i>Nonomuraea</i> sp. AQZ61834 | Q | V | R | H | Q | E | I | D | G | F | G | I | S | T | A | F | R | R | N | E | L | L | K |
| <i>Nonomuraea</i> sp. AQZ65470 | R | V | R | H | Q | E | I | D | G | F | G | I | S | Q | A | F | R | R | N | E | L | L | K |
| <i>Plantactinospora</i> sp. ASW57759 | A | Q | T | H | Q | P | I | D | G | F | G | Y | S | I | A | F | Q | R | A | S | L | V | H |
| <i>S.bingchengensis</i> ADI10522 | G | Q | R | Q | Q | P | I | D | G | F | G | F | S | Q | A | F | Q | R | A | D | I | M | H |
| <i>S.hygroscopicus</i> AQW50539 | G | Q | H | L | Q | P | I | D | G | F | G | F | S | Q | A | F | Q | R | A | D | V | M | H |
| <i>S.iranensis</i> CDR10147 | G | R | R | L | Q | P | I | D | G | F | G | F | S | Q | A | F | Q | R | A | D | L | M | H |
| <i>S.lincolnensis</i> ANS64779 | S | T | T | H | Q | P | I | D | G | F | G | F | S | E | H | F | G | R | A | D | I | M | R |
| <i>S.lincolnensis</i> AXG57013 | S | T | T | H | Q | P | I | D | G | F | G | F | S | E | H | F | G | R | A | D | I | M | R |
| <i>S.rapamycinicus</i> AGP58059 | G | R | H | L | Q | P | I | D | G | F | G | F | S | Q | A | F | Q | R | A | D | L | M | H |
| <i>S.venezuelae</i> ALO06247 | R | A | A | L | Q | P | I | D | G | F | G | F | S | M | A | F | Q | R | A | D | L | L | H |
| <i>S.venezuelae</i> CCA60025 | R | A | E | L | Q | P | I | D | G | F | G | F | S | M | A | F | Q | R | A | D | L | L | H |
| <i>S.venezuelae</i> CUM43504 | R | A | A | L | Q | P | I | D | G | F | G | F | S | M | A | F | Q | R | A | D | L | L | H |
| <i>S.vietnamensis</i> AJF69043 | R | A | D | L | Q | P | I | D | G | F | G | F | S | M | A | F | Q | R | A | D | L | L | H |
| <i>Streptomyces</i> sp. ASQ94399 | G | Q | H | L | Q | P | I | D | G | F | G | F | S | Q | A | F | Q | R | A | D | V | M | H |
| <i>Streptomyces</i> sp. AXE85427 | S | T | R | H | Q | P | I | D | G | F | G | I | S | E | H | F | G | R | A | E | I | M | R |
| <i>A.derwentensis</i> SDT07978 | T | T | R | Y | Q | R | I | D | G | F | G | I | S | E | A | F | G | T | A | N | Q | L | R |
| BsXynC | S | A | E | K | Q | V | I | R | G | F | G | G | M | N | H | P | A | W | A | G | D | L | T |
| EcXynA | N | V | N | Y | Q | I | I | Q | G | F | G | G | M | S | G | V | G | W | I | N | D | L | T |

Figure S6. Amino acid sequence alignment of GH30-7 enzymes.

Amino acid sequence alignment of the region around Arg46 of Xyn30B was conducted using program MEGA7 (1). Amino acid sequences used for the alignment were from the following fungal GH30-7 enzymes: *T. cellulolyticus* Xyn30B (NCBI protein ID: GAM36763), *T.reesei* XYN VI (EGR45006), *T. cellulolyticus* Xyn30A (GAM43270), *T. reesei* XYN IV (AAP64786), *Arthrobotrys oligospora* ATCC24927 AOL_s00173g184

(EGX45083), *Bispora* sp. MEY-1 XylD (ADG62369), *Fusarium fujikuroi* IMI58289 FFUJ_12899 (CCT73001), *F. fujikuroi* IMI58289 FFUJ_12945 (CCT73045), *Leptosphaeria maculans* Xyl38 (AAO49459), *L. maculans* JN3 LEMA_P044840.1 (CBX93583), *L. maculans* JN3 LEMA_P051060.1 (CBX92400), *Podospora comate* PODCO_503770 (VBB81111), *P. comate* PODCO_702000 (VBB86450), *Sugiyamaella lignohabitans* AWJ20_568 (ANB12318), *Talaromyces purpureogenus* XynC (AKH40280), *Thermothelomyces thermophila* ATCC42464 Gxhl (AEO55025), *T. thermophila* ATCC42464 THITE_30409 (AEO57693) and bacterial GH30-7 enzymes: *Actinoplanes derwentensis* DSM43941 SAMN04489716_2447 (SDT07978), *A. derwentensis* DSM43941 SAMN04489716_2458 (SDT08346), *Amycolatopsis albispota* WP1 A4R43_07175 (AXB42337), *Amycolatopsis orientalis* PCZA361.14 (CAA11771), *Nonomuraea gerenzanensis* ATCC39727 BN4615_P5575 (SBO96059), *N. gerenzanensis* ATCC39727 BN4615_P3232 (SBO93718), *Nonomuraea* sp. ATCC55076 BKM31_32010 (AQZ65470), *Nonomuraea* sp. ATCC55076 BKM31_10440 (AQZ61834), *Nonomuraea* sp. ATCC55076 BKM31_10430 (AQZ61832), *Plantactinospora* sp. KBS50 CIK06_16110 (ASW57759), *Streptomyces bingchenggensis* BCW-1 SBI_07402 (ADI10522), *Streptomyces hygrosopicus* XM201 SHXM_04002 (AQW50539), *Streptomyces iranensis* SIRAN6727 (CDR10147), *Streptomyces lincolnensis* LC-G SLCG_5858 (AXG57013), *S. lincolnensis* NRRL2936 SLINC_2555 (ANS64779), *Streptomyces rapamycinicus* NRRL5491 M271_33220 (AGP58059), *Streptomyces* sp. 11-1-2 CGL27_16155 (ASQ94399), *Streptomyces* sp. Go-475 XynC (AXE85427), *Streptomyces venezuelae* ATCC10712 SVEN_6739 (CCA60025), *S. venezuelae* ATCC15439 BN2537_15973 (CUM43504), *S. venezuelae* ATCC15439 AQF52_0650 (ALO06247), *Streptomyces vietnamensis* GIM4.0001 SVTN_36865 (AJF69043) and bacterial GH30-8 enzymes: *Bacillus subtilis* subsp. *subtilis* str. 168 *Bs*XynC (CAA97612) and *Dickeya chrysanthemi* D1 *Ec*XynA (AAB53151). The amino acid numbering is according to the Xyn30B sequence. The Arg residues and the other residues corresponding to Arg-46 of Xyn30B are highlighted by green and blue, respectively. Highly conserved residues are highlighted by yellow.

References

1. Kumar, S., Stecher, G., and Tamura, K. (2016) MEGA7: molecular evolutionary genetics analysis version 7.0. *molecular biology and evolution. Mol. Biol. Evol.* **33**, 1870–1874

Table S1. Xylooligosaccharides generated by Xyn30B reaction

| Reaction time (h) | Products (mM) | | | | | |
|-------------------|---------------|------------------|------------------|------------------|------------------|------------------|
| | Xyl | Xyl ₂ | Xyl ₃ | Xyl ₄ | Xyl ₅ | Xyl ₆ |
| 1 | ND | 0.35 | 0.013 | 0.031 | ND | 0.025 |
| 6 | 0.0079 | 1.1 | 0.033 | 0.22 | 0.018 | 0.075 |
| 12 | 0.02 | 1.6 | 0.061 | 0.37 | 0.034 | 0.13 |

ND, not detected

Table S1. The release of linear xylooligosaccharides from 10 mg mL⁻¹ beechwood xylan was performed using 100 µg mL⁻¹ Xyn30B in 50 mM sodium acetate pH 4.0 at 40°C for 1, 6, or 12 hours. The reactions were stopped by incubation at 99°C for 5 min. Each resultant sample was analyzed by a HPAEC-PAD method.