**Additional file**

**Degradation patterns of GH11 xylanases for efficiently hydrolyzing xylan in *Aspergillus niger* An76**

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**Fig. S1 Growth condition of *A. niger* An76 on different carbon sources.** The growth curves by using 1.0% glycerol, xylose and XOS as carbon sources.

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**Fig. S2 Relative transcript levels of *xynE* in *A. niger* An76 induced by 1% glycerol, xylose and XOS at 0, 6, 12, and 24 h.**

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**Fig. S3 Sequence alignment of XynA, XynB, XynC and XynD with related GH11 and GH10 xylanases.** (**a**), Alignment of XynA, XynB, XynD from *Aspergillus. niger* An 76 and XylA from *Aspergillus. niger* CBS 513.88, XynC from *Talaromyces funiculosus* IMI-134756 and Xyn2 from *Trichoderma reesei* RUT-C30. (**b**) GH10 xylanases alignment includes XynC from *Aspergillus. niger* An 76 and XynA from *Aspergillus. niger* CBS 513.88 and Xyn2 from *Penicillium canescens* VKPM F178. Strictly conserved residues are highlighted by a red background, and conservatively substituted residues are boxed. Catalytic amino acids are shown as black dots.

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**Fig. S4 SDS-PAGE analysis of three GH11 xylanases from *A. niger* An76 expressed in *E. coli* strain BL21 (DE3).** Lane M: protein molecular weight marker; lane 1: XynA; lane 2: XynB; lane 3: XynD.

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**Fig. S5 The specific activities of the three xylanases on various substrates.**

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**Fig. S6 FACE analysis of the products after hydrolysis of various xylans by XynA, XynB and XynD.** Four substrates (beechwood xylan, wheat arabinoxylan, wheat bran, and corn cob) were hydrolyzed by XynA, XynB and XynD for 12 h under optimal conditions. Mixture of xylose (X1), xylobiose (X2), xylotriose (X3), xylotetraose (X4), xylopentaose (X5), xylohexaose (X6) was used as standards makers.

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**Fig. S7 FACE analysis of products following hydrolysis of xylohexaose (a) and xylotetraose (b) by XynD. a** The products of xylohexaose hydrolyzed by XynD. **b** The products of xylotetraose hydrolyzed by XynD. Xylose (X1), xylobiose (X2), xylotriose (X3), xylotetraose (X4), xylopentaose (X5), xylohexaose (X6).

**Table S1 Primers used in this study.**

|  |  |  |
| --- | --- | --- |
| **Primer** | **Primer purpose** | **Sequence (from 5′ to 3′)** |
| xynA-f | Expression primers | CATG*CCATGG*CGGCTCCTGCCCCGGGACCTGTTCTG |
| xynA-r | CCG*CTCGAG*TTAAGAAGAGATCGTGACACTGGCGCT |
| xynB-f | CATG*CCATGG*CGGTTCCCCACGACTCTGTCGTCGAG |
| xynB-r | CCG*CTCGAG*TTACTGAACAGTGATGGAGGAAGATCC |
| xynD-f | CATG*CCATGG*CGCTCCCCAACGGTAAGGCCCTGCTG |
| xynD-r | CCG*CTCGAG*TTAGCAGCTCTCCTCAGTGCTGTCAGA |
| gapdh-qf | Quantitative-PCR primers | ATTTTGGTGTTGCTCAGGG |
| gapdh-qr | CGGCGGTTCTTCTTGCTAT |
| xynA-qf | AAACGAACCGTCCATCACA |
| xynA-qr | GCAACAGTCACCGTTCCAG |
| xynB-qf | ACGGCTTCTACTACTCCTTCTG |
| xynB-qr | AGCCCTTTCCACCAACAA |
| xynC-qf | TCGGGATCGGATTACCTG |
| xynC-qr | TTTGTCCGTGATGGCTTG |
| xynD-qf | ACAGCGGATCTTGGGAAAC |
| xynD-qr | CAGAGGAGGGGTCGTAGTCA |

The italic sequences indicated the recognition sequence of restriction endonuclease *Nco*I and *Xho*I,, respectively.

**Table S2 Endo-β-1,4-xylanases of *Aspergillus niger* An76**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Gene ID** | **Gene name** | **cDNA\*/bp** | **CAZy family** | **EC** | **Signal peptide** | **Number of XlnR binding sitesa** |
| g9709.t1 | *xynA* | 585 | GH11 | 3.2.1.8 | + | 4 |
| g10033.t1 | *xynB* | 621 | GH11 | 3.2.1.8 | + | 2 |
| g1233.t1 | *xynC* | 906 | GH10 | 3.2.1.8 | + | 0 |
| g1345.t1 | *xynD* | 636 | GH11 | 3.2.1.8 | + | 0 |
| g3744.t1 | *xynE* | 687 | GH11 | 3.2.1.8 | + | 0 |

**a** Consensus region of XlnR binding sites: 5`-GGCTAATAA or 5`-GGCTAR (R: A or G).