Acetylated Xylan Degradation by Glycoside Hydrolase Family 10 and 11 Xylanases from the White-rot Fungus *Phanerochaete chrysosporium*

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Abstract: Endo-type xylanases are key enzymes in microbial xylanolytic systems, and xylanases belonging to glycoside hydrolase (GH) families 10 or 11 are the major enzymes degrading xylan in nature. These enzymes have typically been characterized using xylan prepared by alkaline extraction, which removes acetyl sidechains from the substrate, and thus the effect of acetyl groups on xylan degradation remains unclear. Here, we compare the ability of GH10 and 11 xylanases, *Pc* Xyn10A and *Pc* Xyn11B, from the white-rot basidiomycete *Phanerochaete chrysosporium* to degrade acetylated and deacetylated xylan from various plants. Product quantification revealed that *Pc* Xyn10A effectively degraded both acetylated xylan extracted from *Arabidopsis thaliana* and the deacetylated xylan obtained by alkaline treatment, generating xylooligosaccharides. In contrast, *Pc* Xyn11B showed limited activity towards acetyl xylan, but showed significantly increased activity after deacetylation of the xylan. Polysaccharide analysis using carbohydrate gel electrophoresis showed that *Pc* Xyn11B generated a broad range of products from native acetylated xylans extracted from birch wood and rice straw, including large residual xylooligosaccharides, while non-acetylated xylan from Japanese cedar was readily degraded into xylooligosaccharides. These results suggest that the degradability of native xylan by GH11 xylanases is highly dependent on the extent of acetyl group substitution. Analysis of 31 fungal genomes in the Carbohydrate-Active enZymes database indicated that the presence of GH11 xylanases is correlated to that of carbohydrate esterase (CE) family 1 acetyl xylan esterases (AXEs), while this is not the case for GH10 xylanases. These findings may imply co-evolution of GH11 xylanases and CE1 AXEs.

Key words: xylanase, glycoside hydrolase, acetylated xylan, *Phanerochaete chrysosporium*, biomass utilization

INTRODUCTION

Plant cell walls are composed of three main components, i.e., cellulose, hemicellulose and lignin, and enzymatic saccharification is a mild and effective way to utilize cellulose and hemicellulose as a source of biofuel and bio-based chemicals that can replace fossil resources. Recently, it was shown that the degradation of hemicellulose is the bottleneck in enzymatic saccharification of plant biomass,¹ and thus degradation of hemicellulose is as important as that of cellulose. In plant cell walls, hemicelluloses interact strongly with other components. For instance, xylan, a common hemicellulose of terrestrial plants, can be on the surface of cellulose² and can also be chemically linked to lignin to form lignin-carbohydrate complexes.³⁴

The main chain of xylan consists of β-1,4-linked xylose residues, whereas the side chains, such as glucuronic acid (GlcA), arabinofuranose (Araf) and acetyl groups (Ac), differ among different plant species. Acetylation in hardwood secondary cell walls is largely on alternate xylose residues and can substitute O2 and/or O3 of xylose residues with a degree of acetylation over 0.5.⁵ In gymnosperm, softwoods,
xylan is not acetylated, while there are moderate levels of acetylation in grasses.\(^7\) In nature, xylan is degraded by a series of enzymes produced by fungi and bacteria.\(^8,9\) Main chain degradation is performed by endo-xylanases (Xyn, EC 3.2.1.8), which can degrade xylan polymer into xylooligosaccharides, and then β-xylosidase (EC 3.2.1.37) produces xylose monomer from the xylooligosaccharides, with an aid of accessory enzymes. GcLa, Araf, and Ac are removed by xylan α-1,2-glucuronosidase (EC 3.2.1.131), α-L-arabinofuranosidase (EC 3.2.1.55), and acetyl xylan esterase (AXE, EC 3.2.1.72), respectively.

Most of fungal xylanases, the key enzymes of the xylan degradation system, are classified into glycoside hydrolase (GH) families 10, 11, and 30 in the Carbohydrate-Active enZymes (CAZy) database (http://www.cazy.org/).\(^10\) GH10 and 11 xylanases, the major enzymes among wood-decay fungi,\(^11\) are reported to show no distinct substrate preferences, i.e., both enzymes are capable of degrading various xylooligosaccharides and non-acetylated xylan, based on their reaction properties and crystal structures.\(^12,13,14,15,16\) However, although those substrates lack acetyl groups, native xylans from plants other than gymnosperm are modified with acetyl groups, and the products of degradation of acetylated xylan by GH10 and 11 xylanases are quite diverse.\(^9\) Thus, the substrate specificity of xylanases towards acetylated xylan remains unclear. The aim of this work was to investigate the substrate preferences of GH10 and 11 xylanases from the basidiomycete Phanerochaete chrysosporium\(^7\) (PcXyn10A and PcXyn11B) by means of time course experiments and analysis of the final products generated from native acetylated xylan, deacetylated xylan obtained by alkaline treatment and non-acetylated xylan.

**MATERIALS AND METHODS**

**Cloning.** *P. chrysosporium* strain K-3 was grown on Kremer and Wood medium\(^9\) containing 2 % Avicel (Sigma-Aldrich, MA, USA) as the sole carbon source. Total RNA was extracted from approximately 100 mg of frozen mycelial powder using RNeasy (QIAGEN, Limburg, Netherlands), and mRNA was purified using Oligo dt Latex (Takara Bio Inc., Shiga, Japan), as per the instructions. cDNA from mRNA was synthesized using a GeneRacer kit (Invitrogen, Waltham, MA, USA). The primers for amplification from mRNA were designed based on the genomic sequences of *P. chrysosporium* as shown in Table S1 (see J. Appl. Glycosci. Web site). To determine and obtain the full-length sequences of *pcxyn10a*, the primers FW_PcXyn10A_5'UTR and RV_PcXyn10A_3'UTR were used. For *pcxyn11b*, FW_PcXyn11B_5'UTR and RV_PcXyn11B_3'UTR were used.

All PCR products were cloned using a Zero Blunt TOPO PCR Cloning kit for Sequencing (Thermo Fisher Science Inc., Waltham, MA, USA). The amino acid sequences of *PcXyn10A* and *PcXyn11B* were analyzed by SignalP 5.0\(^9\) and Pfam 31.0\(^9\) to identify signal peptides and domain structures, respectively. *pPICZα* plasmid with Ste13 cleavage sites removed and *pcxn110a* or *pcxyn11b* were fused using an In-Fusion HD cloning Kit (Takara Bio Inc.). Each sequence was located between Kex2 and Xba I sites in the *pPICZα* plasmid. After transformation of the methylotrophic *Pichia pastoris* strain KM71H by electroporation, transformants were selected using Zeocin according to the manual.

**Enzyme preparations.** Recombinant proteins were obtained by using a 5-L jar fermenter (TSC-MSL; Takasugi Seisakusho, Tokyo, Japan) with methanol feed according to the reported method.\(^12,13\) After protein production was finished, each medium was collected by centrifugation at 5,000 × G for 30 min.

The collected medium was passed through a 100 kDa filter and concentrated using a 10 kDa filter (Millipore, Corporation, Billerica, MA, USA). The concentrate was purified on a hydrophobic interaction column (TOYOPEARL Phenyl 650M, Tosoh Corporation, Tokyo, Japan). After equilibration with 50 mM sodium acetate buffer (pH 5.0) containing 1 M ammonium sulfate (FUJIFILM Wako Pure Chemical Co., Osaka, Japan), enzymes were eluted with 50 mM sodium acetate buffer (pH 5.0). An anion-exchange column (TOYOPEARL 650S, Tosoh Corporation) was used for further purification. After equilibration with 50 mM Tris-HCl buffer (pH 7.0), the enzymes were eluted with 50 mM Tris-HCl buffer (pH 7.0) containing 1 M NaCl. To confirm purity, 12 % SDS-PAGE was performed.

GH10 xylanase from *Cellvibrio japonicus* (CGGH10)\(^23\) was a kind gift from Professor Harry Gilbert (York University) and GH11 xylanase from *Neocallimastis patriciarum* (NpGH11)\(^5\) was purchased from Megazyme (Wicklow, Ireland).

Crude protein was quantified using the Bradford Protein Assay (Bio-Rad Laboratories, Inc., Hercules, CA, USA) with bovine serum albumin as a standard. Purified enzymes were quantified with a NanoDrop2000 (Thermo Fisher Scientific Inc.).

**Preparation of native xylans from various plants.** Acetylated xylan from *Arabidopsis thaliana* was extracted based on the method reported by Busse-Wicher et al.\(^8\) Approximately 100 mg of alcohol-insoluble residue from stems of *A. thaliana*, prepared as described previously,\(^24\) was depectinated in 0.5 (w/v) % ammonium oxalate at 85 °C for 2 h. The sample was then washed with water and delignified in 11 % peracetic acid at 85 °C for 30 min. Finally, acetylated xylan was extracted from holocellulose with dimethyl sulfoxide (DMSO), and the solvent was changed to distilled water (DW) using a PD-10 column (GE Healthcare, Chicago, IL, USA). For analysis of the sugar content, TFA hydrolysis of this acetylated xylan and high-pressure ion chromatography using Dionex (Dionex Corporation, Sunnyvale, CA, USA) were conducted.

Native xylans were also extracted from birch wood, Japanese cedar, and rice straw. Wood blocks and rice straw were dried, milled andmeshed as described previously.\(^9\) Samples were depectinated as described above and delignified according to Klaudiz.\(^25,26\) One gram of powder sample, 0.8 g of NaClO; and 150 mg of acetic acid (FUJIFILM Wako Pure Chemical Co.) were mixed with 11 mL of DW and incubated at 37 °C for 40 h. Acetylated xylans were extracted from birch wood and rice straw with DMSO, and non-acetylated xylan was extracted from Japanese cedar with 4 M KOH. After extraction, the solvents were replaced with DW as described above.

To estimate the ratio of xylan components, \(^1\)H-NMR spectra were acquired at 20 °C on a 500 MHz spectrometer.
Since xylose residues doubly substituted with GlcA and Ac are counted twice, the total is not 100%.

Table 1. Ratios of xylan components.

| Xylan components                        | Free Xyl (%) | GlcA-Xyl (%) | Ac-Xyl (%) | Araf-Xyl (%) |
|----------------------------------------|--------------|--------------|------------|--------------|
| Acetylated xylan from Arabidopsis thaliana | 34.7±34       | 12.1±30       | 53.2±20    | n.d.         |
| Acetylated xylan from birch wood         | 45.5 ± 1.2    | 6.6 ± 0.9     | 51.1 ± 0.8 | n.d.         |
| Acetylated xylan from rice straw         | 64.5 ± 0.7    | 0.8 ± 0.1     | 25.9 ± 0.8 | 8.7 ± 0.3    |
| Non-acetylated xylan from Japanese cedar | 74.8 ± 0.4    | 17.4 ± 0.2    | n.d.       | 7.8 ± 0.6    |

The ratio of components of acetylated xylan from A. thaliana was taken from the previous reports. Based on NMR spectra data for acetylated xylans from dicots, acetylated xylans from grasses, and non-acetylated xylan from softwood, the peaks were assigned and the ratio of xylan components was estimated from the peak areas. Free Xyl means free xylose residue. GlcA-Xyl, Ac-Xyl, and Araf-Xyl mean GlcA-substituted xylose residue, acetylated xylose residue, and Araf-substituted xylose residue. n.d. means not detected in this study. Since xylose residues doubly substituted with GlcA and Ac are counted twice, the total is not 100%.
RESULTS AND DISCUSSION

Cloning and preparation of PcXyn10A and PcXyn11B.

*P. chrysosporium* has 6 genes encoding GH10 xylanase and one gene encoding GH11 xylanase. Previous reports confirmed that *P. chrysosporium* secretes two GH10 xylanases, PcXyn10A and PcXyn10C, during culture using only cellulose or cellulose and xylan as carbon resources and that *PcXyn10A* is more constantly expressed than *PcXyn10C*. Therefore, *PcXyn10A* was selected as the representative GH10 xylanase for *P. chrysosporium* in this paper. Cloning of *pcxyn10a* and *pcxyn11b* was successful (Fig. S1; see J. Appl. Glycosci. Web site). *PcXyn10A* and *PcXyn11B* consisted of 389 and 271 amino acids, respectively, and lacked any signal peptide according to SignalP 5.0 (Fig. 1). The amino acid sequences were analyzed by HMMER, which indicated the presence of a CBM1 domain at the N-terminal and C-terminal ends of *PcXyn10A* and *PcXyn11B*, respectively.

Recombinant *PcXyn10A* and *PcXyn11B* were produced in a jar-fermenter and purified by ultrafiltration and column chromatography. Purity was examined by SDS-PAGE, as shown in Fig. S2 (see J. Appl. Glycosci. Web site), and the purest samples were used for activity testing. Since both proteins contain CBM1 and a linker, the molecular weights are higher than those of the catalytic domain alone. The theoretical molecular weight of *PcXyn10A* is 42,000 and that of *PcXyn11B* is 29,000, whereas the observed values were around 45,000 and 37,000, respectively, in SDS-PAGE. Larger molecular weight of *PcXyn11B* in appearance is consistent with previous results and the large difference between the two values for *PcXyn11B* is due to glycosylation.

Analysis of the average DP after digestion of acetylated xylan.

The results of digestion of acetylated xylan extracted from *A. thaliana* resemble more than half of the xylose residues are acetylated (Table 1), as shown in Fig. 2. To compare xylanases from *P. chrysosporium* and well-characterized xylanases, CjGH10 and NpGH11 without CBMs were also tested, because CjGH10 and NpGH11 were known as efficient enzymes in GH10 and GH11 xylanases, as additive enzyme to commercial enzymes cocktails in the previous report. *GH10* xylanases degrade even this densely acetylated xylan into oligosaccharides. Although the τ-values, the time constant, of *PcXyn10A* (0.21 ± 0.02 h) and CjGH10 (0.12 ± 0.03 h) were somewhat different, the S-values, the concentration of degradable regions of the substrate, were similar at 28.0 ± 0.9 and 24.6 ± 1.6 μM, respectively, indicating that both enzymes have similar ability to degrade acetylated xylan (Table 2). Based on the S-values, the average DP after reaction was estimated as 9.6 and 11 for *PcXyn10A* and CjGH10, respectively. Since the DP of xylan polymer is approximately 150, *GH10* xylanases cleaved one molecule of acetylated xylan approximately 14 times. After removal of acetyl groups by alkaline treatment, the S-values of *PcXyn10A* and CjGH10 were 47.3 ± 1.2 and 37.4 ± 1.2 μM, respectively, being 1.7 and 1.5 times larger than the values for acetylated xylan. Compared among GH10 xylanases, CjGH10 showed the smaller values of τ and S, suggesting that CjGH10 can bind substrates more selectively than *PcXyn10A*.

In contrast, GH11 xylanases attacked acetylated xylan from *A. thaliana* much less effectively than did GH10 xylanases, and the S-values were 5.2 ± 0.8 μM for *PcXyn11B* and 7.8 ± 0.2 μM for NpGH11. The average DPs after digestion were 45 and 32, respectively, indicating that only 3.3 and 4.7 cleavages occurred per acetylated xylan molecule. However, after deacetylation, the S-values of *PcXyn11B* and NpGH11 were 38.0 ± 2.2 and 32.6 ± 0.4 μM, respectively, being 7.3 and 4.2 times higher than those for acetylated xylan. Comparing all xylanases tested, NpGH11 showed the smallest value of τ for deacetylated xylan, suggesting that NpGH11 may have evolved to act specifically on deacetylated xylan.

It was reported that deacetylation contributes to the resistance of xylan to enzymatic degradation, and our results show that deacetylation has a greater negative effect on GH11 xylanases than on GH10 xylanases. As shown in Table 1, since half of xylose residues are acetylated in *A. thaliana* xylan, the probability of three connected free xylose groups will appear in every 8 residues. However, in the present results, GH11 xylanases, which require three free xylose residues, failed to saccharify in the previous report.

> **PcXyn10A**
> QSPVWGCGGIGWTGPTCTAGNVCQEYSAYYQCICPASQATSVTSVTSTAPNPPTSTHTSTSSAPSGASTSTAKLNTLAIAKGLKLYFTATDNLGDSYAITAIILDNTMFQGGITFPANSKMDATPEQQGQTSPSGDQ1IANLAKSNGMLLRHNCWYNQLPSWSNGKPTAAQLTSIIOQHCSLTLVTHYKQVYAWDVVNEPNDDGSWRTDVFYNTLTGSYSVQAILEAAARADPPDKLYINEYIYAGAKATSSLNLVKTLKAAVPLLDIQGFSQHVFQCVPTQLQSQLTFAAG
> GVEVAITELDRMTPPLSTPALLAQQKTDSNYIKACASVEACVGVTTWDWTKYSWPVNTPFSGQGAACPWDQN芙
> RKPAYDQIAIGFNG

> **PcXyn11B**
> FPFZEFNTHVFRQSTPACTGTTNNGFYFWSWTDGGGSVTVNNGNFAEVSYTWSNAفنNFVAGKWNPGSAQAISFTANTQPQNSLYSVYGTSTPNLVLVEYILEDFTYNPASVTLHKGTLTSDGATDYETRGRVNPESPSIQQTATFNQWISIRSSKRSSGTVTANFAHAAWQLGPGPFLTFFNQTVATTGEYQSSGSLTVNPAGVGTSPCTAPGSSSVSTFTS
> GSPSSSSPSVPGSCAYLHYCGGCGGTGPTCCSSGTCFKNSWYSQCL

Fig. 1. Amino acid sequences of the xylanases.

*PcXyn10A* consists of CBM1 (orange) and the GH10 catalytic domain (blue), whereas *PcXyn11B* consists of the GH11 catalytic domain (blue) and CBM1 (orange). The red residues are motifs for binding cellulose. CBM1 from *PcXyn10A* has a WYY motif while that of *PcXyn11B* has a YWY motif.
residues, showed less activity than expected, indicating the side chain of acetylated xylan from *A. thaliana* should be regular, which is unfavorable to GH11 activity. Moreover, assuming that the acetyl group modification is every other xylose residue, the degradation of GH10 xylanases did not complete, suggesting that the position of the acetyl group substitutions is important for GH10 to accommodate acetylated xylan. Further analysis of crystal structures with acetylated xylan is necessary to reveal the details.

**Table 2.** *S* and *r* values and final average DP from time course study.

| Enzyme | Substrate  | *S* (µM) | *r* (h) | Average DP |
|--------|------------|----------|---------|------------|
| *Pc*Xyn10A | Acetylated xylan | 28.0 ± 0.9 | 0.21 ± 0.02 | 9.6 |
| C/GH10  | Acetylated xylan | 24.6 ± 1.6 | 0.12 ± 0.03 | 11 |
|         | Deacetylated xylan | 37.4 ± 1.2 | 0.11 ± 0.01 | 7.3 |
| *Pc*Xyn11B | Acetylated xylan | 5.2 ± 0.8 | 0.09 ± 0.05 | 45 |
|         | Deacetylated xylan | 38.0 ± 2.2 | 0.34 ± 0.05 | 7.2 |
| *Np*GH11 | Acetylated xylan | 7.8 ± 0.2 | 0.06 ± 0.01 | 32 |
|         | Deacetylated xylan | 32.6 ± 0.4 | 0.05 ± 0.01 | 8.3 |

Values of *S* and *r* were determined based on fitting curves for first-order reaction, as shown in Fig. 2. The average DP after reaction was calculated from the *S* value.

**Fig. 2.** Time course study of digestion of acetylated xylan and deacetylated xylan.

Reducing-ends during the reaction were measured by the BCA method with few modifications. The reaction solution consisted of 4 nM purified enzyme, 1.67 µM acetylated xylan from *A. thaliana*, and 50 mM ammonium acetate buffer (pH 5.0). The same amount of DMSO-extracted xylan was treated with alkali for deacetylation using 4 M NaOH for 20 min and then neutralized by adding 1 M HCl. The ratio of xylan solution, 4 M NaOH and 1 M HCl solution was 200:5:20. Curve fitting was performed as described in Materials and Methods section.

**Analysis of final products by PACE.**

A time course study revealed that longer xylooligosaccharides remained undegraded even after prolonged incubation, especially with GH11 xylanases, and the distribution of final products was still unclear. Therefore, 10 % PACE gel was used to separate oligosaccharides with various DPs. As shown in Fig. 3, xylan and longer xylooligosaccharides appeared as a smear at the upper side of the gels, while several peaks appeared after hydrolysis. *Pc*Xyn10A degraded acetylated xylan mainly into xylooligosaccharides with DP < 10, which is consistent with the value estimated from reducing-sugar analysis. After deacetylation, as expected, *Pc*Xyn10A xylanases produced smaller oligosaccharides, again in agreement with the biochemical results.

In contrast to *Pc*Xyn10A, *Pc*Xyn11B degraded acetylated xylan mainly into large compounds which remained at the upper part of the gel, suggesting that the action of *Pc*Xyn11B is clearly blocked by acetyl group substitution. However, after deacetylation, *Pc*Xyn11B produced smaller oligosaccharides and the final products of deacetylated xylan had a DP of 6 or less (Fig. 3B). Although more data is needed to identify final products completely, it was confirmed that acetylated xylan from *A. thaliana* is not a favorable substrate for *Pc*Xyn11B, in contrast to *Pc*Xyn10A.

**Comparison of degradation of various xylans.**

To compare the degradation of various native xylans by
PcXyn10A and PcXyn11B, we focused on two acetylated xylans from birch wood and rice straw and one non-acetylated xylan from Japanese cedar. As summarized in Table 1, acetylated xylan from birch wood consisted of 45.5 ± 1.2 % free xylose residues, 51.1 ± 0.8 % acetylated xylose residues and 6.6 ± 0.9 % GlcA-substituted xylose residues. While the acetylation ratio is similar to that of acetylated xylan from A. thaliana, the ratio of GlcA/Xyl is slightly smaller. Acetylated xylan from rice straw consisted of 64.5 ± 0.7 % free xylose residues, 25.9 ± 0.8 % acetylated xylose residues, 0.8 ± 0.1 % GlcA-substituted xylose residues and 8.7 ± 0.3 % Araf-substituted xylose residues. It has been reported that acetylated xylans from grasses contain 60–80 % free xylose residues, while other xylose residues are substituted mostly with acetyl groups, as well as Araf and GlcA in that order. Non-acetylated xylan from Japanese cedar consisted of 74.8 ± 0.4 % free xylose residues, 17.4 ± 0.2 % GlcA-substituted xylose residues and 7.8 ± 0.6 % Araf-substituted xylose residues. The ratios of GlcA/Xyl and Araf/Xyl are approximately 1/6 and 1/13, respectively. Two major motifs of non-acetylated xylan from softwood were previously identified as α-L-arabinofuranosyl-α-D-glucuronyl xylohexaose and α-D-glucuronyl xylohexaose, and small amounts of other motifs are also present. These values are similar to those of non-acetylated xylan from softwoods.

The results of digestion of the three selected xylans by PcXyn10A and PcXyn11B are shown in Fig. 4. PcXyn10A degraded acetylated xylan from birch wood similarly to acetylated xylan from A. thaliana. The pattern of degradation products of acetylated xylan from rice straw was similar to that of acetylated xylan from birch wood (Fig. 4A), whereas their components are different. PcXyn10A produced smaller oligosaccharides from acetylated xylan of rice straw as compared with those from acetylated xylan of birch wood due to less acetylation of rice straw xylan. The peak intensities were quantified by Image J as shown in Fig. 4B. The final products of acetylated xylans from both plants were mainly oligosaccharides with DP < 15, as in the case of A. thaliana. The results for PcXyn11B are also shown in Fig. 4. PcXyn11B did not degrade acetylated xylans as well as PcXyn10A, as was the case for the acetyl xylan from A. thaliana. However, non-acetylated xylan from Japanese cedar was degraded well not only by PcXyn10A, but also by PcXyn11B. These results are consistent with the idea that acetyl group substitution can interfere with the action of GH11 enzymes, but not GH10 enzymes much.

The evolutionary relationship between fungal xylanases and AXEs.

Figure 5 shows the putative relationship between fungal evolution and the numbers of GH10, GH11 xylanases and CE1 enzymes which are classified as AXEs, based on previous research. To examine the relationships of these enzymes in fungal xylan degradation systems, the correlation coefficients between the numbers of enzymes were calculated (Table 3). The correlation between numbers of GH10 and GH11 enzymes is weak (correlation coefficient less than 0.5), while numbers of CE1 enzymes show high correlation coefficients of approximately 0.7 with the numbers of both GH10 and GH11 enzymes. These results suggest that fungi possessing a large number of xylanases also tend to possess a large number of AXEs.

Furthermore, the correlation coefficient between the existence of GH10 and CE1 enzymes is low, under 0.4,
while that between the existence of GH11 and CE1 enzymes is approximately 0.6. Nine of 10 fungi having GH11 xylanase genes have CE1 genes, though only 14 fungi carry CE1 genes among the 31 fungi considered here. These results suggest that coevolution of these families’ enzymes may have occurred. This seems plausible because GH11 enzymes would require deacetylation by CE1 AXEs in order to work well in hydrolyzing xylan.

It is worth noting that acetyl group(s) should be removed even after degradation by GH10 enzymes in order to facilitate the reaction of glycosidases. Thus, there may be so-far-unidentified AXEs associated with GH10. In evolutionary terms, it seems that GH10 xylanases appeared much earlier than GH11 xylanases, and fungi and molds may have acquired GH11 and CE1 concomitantly from the Carboniferous period to the Permian period, when gymnosperms and angiosperms appeared. Brown-rot fungi tend to have fewer GH11 and CE1 enzymes in their genome, which is reasonable because these fungi prefer softwood to hardwood. Thus, the evolution of plant species having acetyl side chains on

Table 3. The correlation coefficients between the numbers (left, bottom) and the existence (right upper) of GH10, GH11, and CE1 enzymes.

|          | GH10 |          |          |
|----------|------|----------|----------|
| GH10     | 0.41 | GH11     | 0.39     |
| GH11     | 0.47 | CE1      | 0.62     |
| CE1      | 0.69 |          | 0.72     |

Data on the numbers and the existence of GH10, GH11, and CE1 enzymes were taken from Floudas et al.35)
xylan may have led to the appearance of relevant enzymes in fungal xylan-degrading systems.

We believe the present findings will be helpful not only in understanding how plant biomass is degraded in nature, but also in improving the efficiency of human utilization of cellulosic biomass.

CONFLICTS OF INTEREST

The authors declare no conflict of interests.

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