Table S1. Primers used to generate full-length and truncated forms of SaGH74A and SaGH74B.

| Primers | Restriction sites in primers | Gene | region | Recombinant protein |
|---|---|---|---|---|
| F1, 5′-CATATGCGAAGAACCCGCATCTTCACGGCC-3′<br>R1, 5′-AAGCTTTCAGGCGACCGTGCAGTTCTGACC-3′ | NdeI/HindIII | *sav_1856* | 1-2649 | SaGH74A |
| F1, 5′-CATATGCGAAGAACCCGCATCTTCACGGCC-3′<br>R2, 5′-AAGCTTTCAGCCCGCGGTGTCGCCGTAGACGAT-3′ | NdeI/HindIII | *sav_1856* | 1-2292 | the catalytic domain of SaGH74A |
| F2, 5′-CCATGGGCGCCTGCACGGTGACATACAGGATCA-3′<br>R3,<br>5′-AAGCTTGGCGACCGTGCAGTTCTGACCGCCGAGTTTG-3′ | NcoI/HindIII | *sav_1856* | 2328-2649 | CBM2 (C-terminal His-tag fusion) |
| F2, 5′-CCATGGGCGCCTGCACGGTGACATACAGGATCA-3′<br>R4,<br>5′-AAGCTTTCAGGCGACCGTGCAGTTCTGACCGCCGAG-3′ | NcoI/HindIII | *sav_1856* | 2328-2649 | CBM2 |
| BF1, 5′-CATATGCGCACGCCCCGCCCGAGCAGACGA-3′<br>BR1, 5′-AAGCTTTCAGACCGGCTCCCCGTACTGGAT-3′ | NdeI/HindIII | *sav_2574* | 1-2220 | SaGH74B |

Table S2. The homologues of the catalytic domains and the CBM of *Streptomyces avermitilis* xyloglucanases.

| | Organism | Enzyme | GenBank accession number | Identity (%) and similarity (%) | 3D-Structure (PDB code) | GH family | CBM family | References |
|---|---|---|---|---|---|---|---|---|
| Catalytic domain | | | | | | | | |
| SaGH74 A | *Stretpomyces avermitilis* | Xyloglucanase (SaGH74A) | BAC69567 | | | GH74 | CBM2 | this study |
| | *Streptomyces coelicolor* A3(2) | Xyloglucanase (Sco6545) | CAA20642 | 83 and 91 | | GH74 | CBM2 | 1 |
| | *Thermobifida fusca* YX | Xyloglucanase (Xeg74) | AAZ55647 | 61 and 76 | 1WWO | GH74 | | 2 |
| | *Clostridium thermocellum* | Xyloglucanase (Xgh74A) | CAE51306 | 53 and 67 | 2CN2 | GH74 | | 3, 4 |
| | *Phanerochaete chrysosporium* K-3 | Xyloglucanase (Xgh74B) | BAF95189 | 49 and 64 | | GH74 | CBM1 | 5 |
| SaGH74 B | *Stretpomyces avermitilis* | Xyloglucanase (SaGH74B) | BAC70285 | 42 and 55 | | GH74 | CBM2 | this study |
| CBM | | | | | | | | |
| | *Streptomyces avermitilis* | Xyloglucanase (SaGH74A) | BAC69567 | | | GH74 | CBM2 | this study |
| | *Cellulomonas fimi* | Endo-β-1,4-xylanase | AEA30147 | 49 and 65 | 1EXG | GH10 | CBM2 | 6 |
| | *Clostridium cellulovorans* | Endo-β-1,4-glucanase/Endo-β-1,4-xylanase | AAA23233 | 40 and 60 | 3NDY | GH5 | CBM2 | 7 |

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Fig. S1

Schematic of the molecular architecture of SaGH74A and SaGH74B.
The modules in the enzymes are as follows: GH74, glycoside hydrolase family 74 catalytic module; CBM2, carbohydrate-binding module family 2. The primer sets used for the cloning are shown in Table S1.

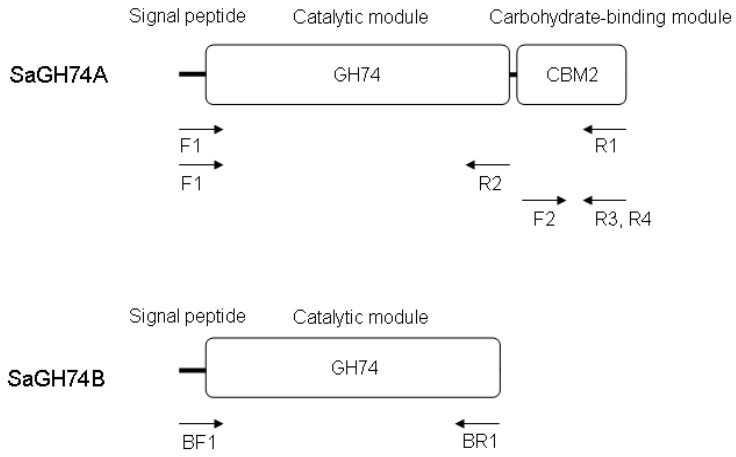

Fig. S2

SDS-PAGE analysis of purified recombinant enzymes.
Lane 1, molecular mass marker; lane 2, purified SaGH74A; lane 3, purified SaGH74B.
Approximately 1 μg of each sample was separated on 10% wt/vol polyacrylamide gel.

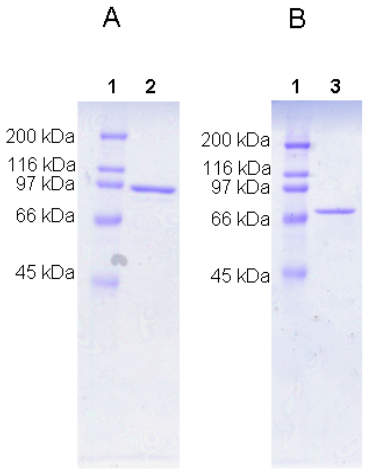

Fig. S3

HPAEC-PAD analysis of xyloglucan-derived oligosaccharides used in this study.

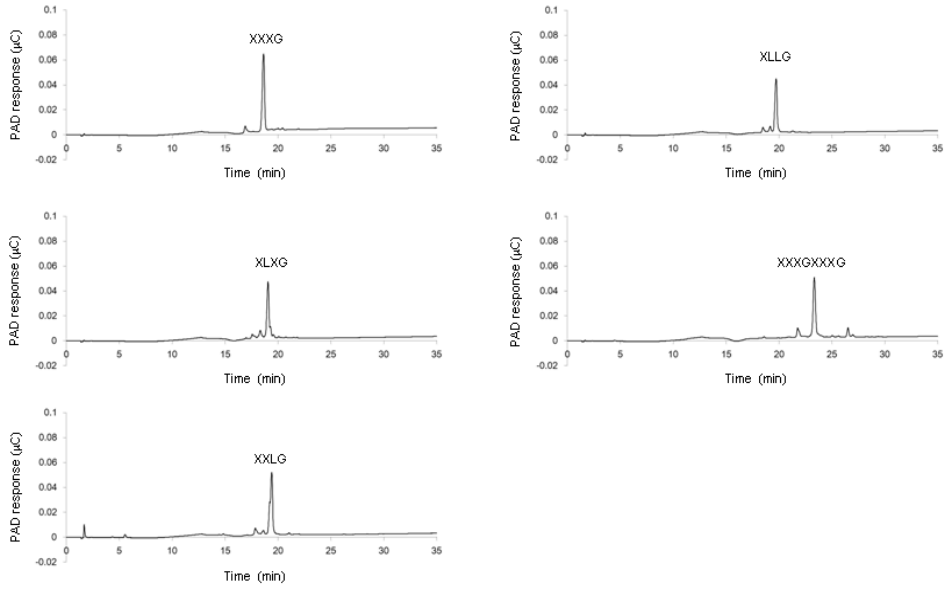

Fig. S4

Binding of SaGH74A.
The protein was incubated with insoluble polysaccharides, including avicel (crystalline cellulose) (A), insoluble oat spelt xylan (β-1,4-xylan) (B), insoluble birchwod xylan (β-1,4-xylan) (C), chitin (D), lichenan (β-1,3-β-1,4-glucan) (E), pachyman (β-1,3-glucan) (F), and ivory nut mannan (β-1,4-mannan) (G). After centrifugation, the proteins in supernatant (lane 2) and precipitate (lane 3) were analyzed by SDS-PAGE. Lane 1, molecular mass marker.