Supporting information

Novel α-1,3/α-1,4-glucosidase from *Aspergillus niger* exhibits unique transglucosylation to generate high levels of nigerose and kojibiose

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Table S1. Oligonucleotides used in this study.

primer	sequence (5'→ 3' direction)[a]
EXT-Ⅰ	GGGGTGATCCCAGAGCCAACAT
EXT-Ⅱ	ATTAGCAGTAAGGGCACTATGT
AgdB-sig/EcoR1	TTTGAATTCATGTTGGGGTCTTTGCTT
AgdB-his/Xba1	GGGTCTAGAAACTTCAGCTTAAAGTTC
N170D-For	ACATACGACTACACGCGGACCCTTTGG
N170D-Rev	CGTGTAGTCGTATGTTGGCAAGCGCAT
N185D-For	ACTCCAGACAACACCAACTTGTACGGT
N185D-Rev	GGTGTTGTCTGGAGTGCCATACGCGTC
N221D-For	AAGATCGACCAAACGACAGATGGAAAG
N221D-Rev	CGTTTGGTCGATCTTGATGTCCATACC
N292D-For	GTCTACGACTACAGCCAGGCAAAGATT
N292D-Rev	GCTGTAGTCGTAGACCACCTCGGCAAG
N354D-For	GTAAGCGACAACACGGCATATATCAGC
N354D-Rev	CGTGTTGTCGCTTACGCTCACAGCCGG
N372D-For	AATCAGGACGGTAGCCTATACGAGGGT
N372D-Rev	GCTACCGTCCTGATTGTGAAGGAAAAC

[a]Codons producing the mutations are underlined.

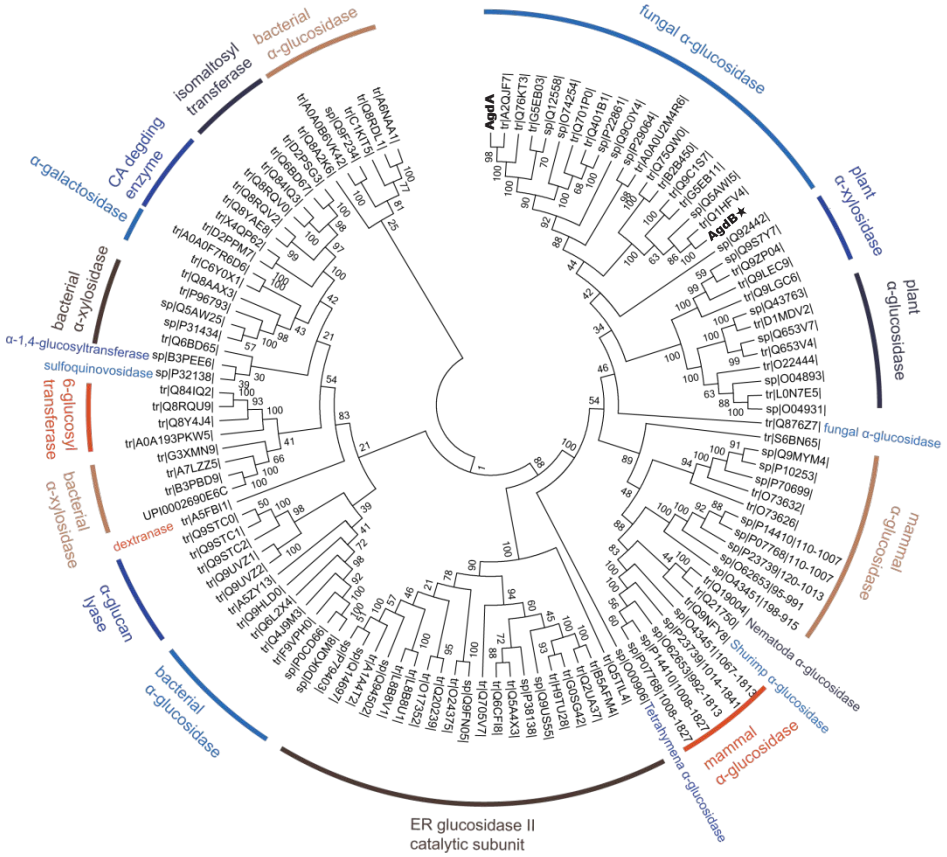

Figure S1. Evolutionary relationships of characterized GH31 proteins. The evolutionary history was inferred using the Neighbor-Joining method.[1] The bootstrap consensus tree inferred from 500 replicates[2] is taken to represent the evolutionary history of the GH31 proteins analyzed.[2] The evolutionary distances were computed using the Poisson correction method[3] and are in the units of the number of amino acid substitutions per site. The analysis involved 117 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 332 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.[4]

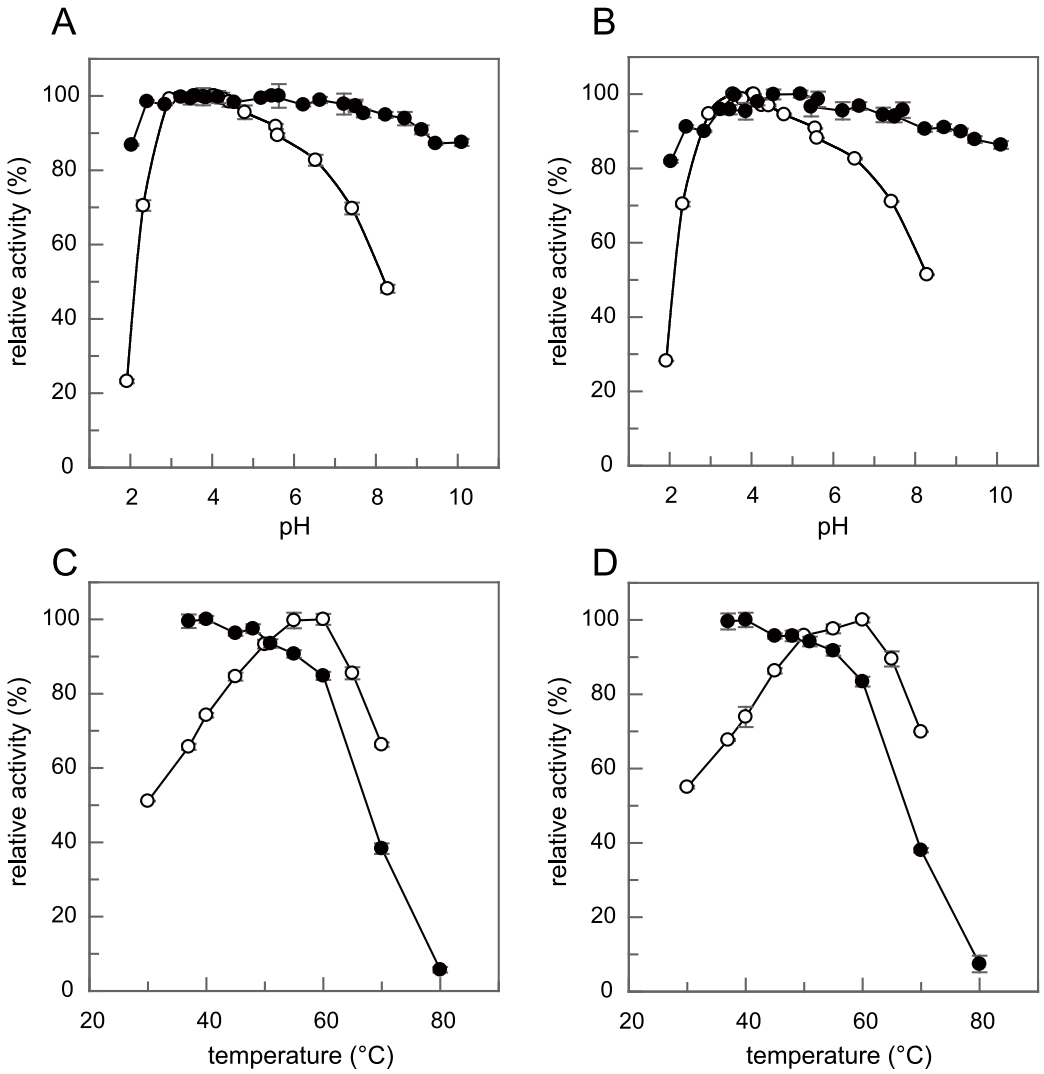

Figure S2. pH-activity (open circle) and –stability (closed circle) of (A) rAgdB and (B) N354D, and heat-activity (open circle) and –stability (closed circle) of (C) rAgdB and (D) N354D. The effect of pH on the activity to maltose was determined using the following buffers: 40 mM glycine-HCl (pH 1.9–4.0), 40 mM sodium acetate (pH 3.8–5.6), and citrate-phosphate (pH 5.6–8.3). All buffers contained 0.02% Triton X-100. The enzyme concentrations used were 2.38 nM (rAgdB) and 2.46 nM (N354D). The stability of rAgdB (238 nM) and N354D (246 nM) in various pH was evaluated the residual enzyme activity after an incubation in 20 mM glycine-HCl (pH 2.0–3.6), 20 mM sodium acetate (pH 3.5–5.6), 20 mM citrate-phosphate (pH 5.5–7.7), or 20 mM

glycine-NaOH (pH 7.5–10.1), every buffer contained 0.1% Triton X-100, at 4 °C for 24 h. The effect of temperature on the enzyme activity was determined by the maltose-hydrolytic activity at various temperatures (30–70 °C). The enzyme concentrations used were 1.36 nM (rAgdB) and 1.23 nM (N354D). To estimate the thermal stability, rAgdB (9.51 nM) and N354D (9.19 nM) in 20 mM sodium acetate buffer (pH 5.0) containing 0.1% Triton X-100 were kept at from 37 °C to 80 °C for 15 min, followed by measurement of their residual activities. Experiments were repeated three times and means are plotted (error bar, SD).

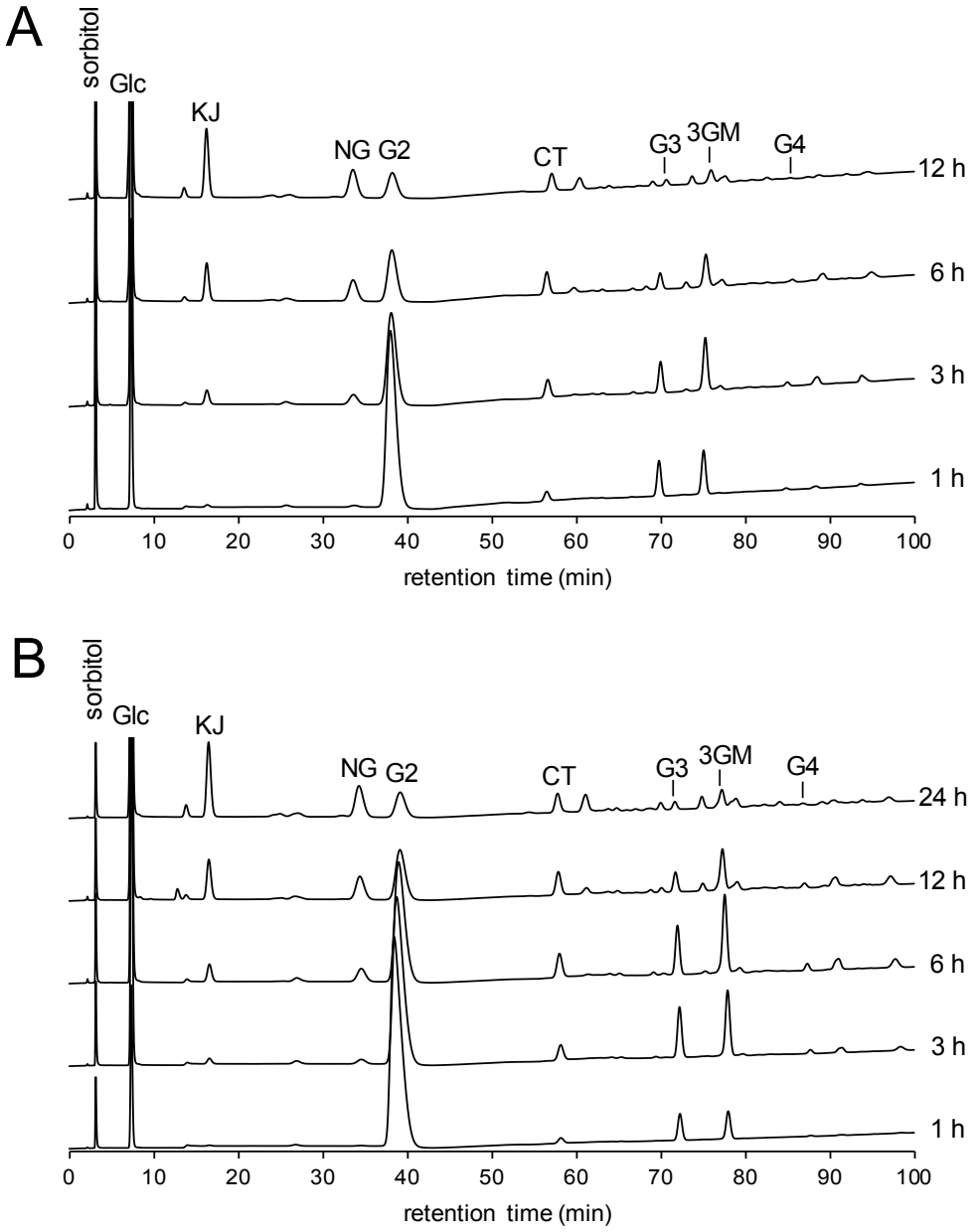

Figure S3. N354D-catalyzed transglycosylation of 100 mM or 500 mM G2. (A) HPAEC-PAD profiles of 1, 3, 6, and 12-h reactions of 100 mM G2 and (B) HPAEC-PAD profiles of 1, 3, 6, 12 and 24-h reactions of 500 mM G2. 3GM (3^{II}-*O*-α-glucosyl-maltose); CT (2^{I}-*O*-α-glucosyl-maltose).

References

(1) Saitou, N.; Nei, M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol*. **1987**, *4*, 406–425.

(2) Felsenstein, J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **1985**, *39*, 783–791.

(3) Zuckerkandl, E.; Pauling, L. Evolutionary divergence and convergence in proteins. In *Evolving Genes and Proteins*; Bryson, V., Vogel, H. J., Eds.; Academic Press: New York, NY, 1965; pp. 97–166.

(4) Kumar, S.; Stecher, G.; Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol. Biol. Evol*. **2016**, *33*, 1870–1874.