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Supporting information for article:

Expression and crystallization of a bacterial glycoside hydrolase family 116 β-glucosidase from *Thermoanaerobacterium xylanolyticum*

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S1. Supplementary methods

S1.1. Assay of activity

The activity of TxGH116 toward *p*NP-glycosides was assayed in 50 mM phosphate buffer, pH 6.0, with at the final substrate concentration of 5 mM and 0.1-1 μg of TxGH116, in the final volume of 140 μL. All the reactions were performed at 310 K for 30 min and the reactions were stopped by adding 70 μL of 0.4 M sodium carbonate (Na_2CO_3). The *p*-nitrophenol (*p*NP) released was monitored by measuring the absorbance at 405 nm with a microtiter plate reader (Thermo Labsystems, Finland).

Hydrolysis of cellobiose and cellotriose was reacted tested with 1 mM cellobiose or cellotriose and 1 μg of TxGH116 in 50 mM phosphate buffer, pH 6.0, at 333 K for 30 min. The reactions were stopped by boiling for 5 min, and kept on ice before TLC analysis. Reactions without enzymes were used as controls and the TLC plates were developed with 3:3 (v/v) acetronitrile : H_2O. The products were detected by staining with 10% sulfuric acid in ethanol followed by charring for 5 min at 373 K.

S1.2. Determination of pH and Temperature Optima

The optimum pH of TxGH116 for hydrolysis of *p*NPGlc was determined by reactions in which 0.4 μg of enzyme was incubated with 5 mM *p*NPGlc in 100 mM universal buffer (McIlvaine phosphate-citrate buffer) at pH ranging 2.0-12.0 at 0.5 pH unit intervals, at 310 K for 30 min. The temperature optimum was determined via the hydrolysis of 5 mM *p*NPGlc with 0.1 μg enzyme in 50 mM phosphate buffer pH 6.0, at temperatures of 288-363 K at 5 K intervals, for 10 min. The reaction volumes, stop and measurement of released *p*NP were as described in section S1.1.

S2. Results of Functional Assays

As shown in Table S1, TxGH116 had highest activity against pNPGlc (4.75 μmol min^{-1}/mg protein), with 35% as much activity toward *p*NP-β-D-galactoside and 6% toward *p*NP-*N*-acetyl β-D-glucosaminoside. It could not hydrolyze other *p*NP glycosides tested. TxGH116 also showed activity against cellobiose and cellotriose, as judged by TLC of the reactions. As seen in Figure S2, the pH optimum was 6.0, and Figure S2 shows that the temperature optimum in the 10 min assay was 358 K.

Table S1 Hydrolysis activity of TxGH116 purified from recombinant *E. coli*.

The activity was assayed at pH 6.0 and 310 K for 10 min *p*-nitrophenyl glycosides and 333 K for 30 min for oligosaccharides (cellobiose and cellotriose).

Substrate	Specific activity (μmol/min/mg protein)	Relative activity (percent)
*p*NP-β-D-glucoside	4.75	100
*p*NP-β-D-galactoside	1.64	35
*p*NP-β-D-N-acetyl-glucosaminoside	0.30	6.3
*p*NP-β-D-mannoside	N.H.	N.H.
*p*NP-β-D-xyloside	N.H.	N.H.
*p*NP-α-D-glucoside	N.H.	N.H.
*p*NP-α-D-galactoside	N.H.	N.H.
*p*NP-α-L-arabinoside	N.H.	N.H.
*p*NP-α-L-fucoside	N.H.	N.H.
cellobioside	+	+
cellotrioside	+	+

N.H. means no hydrolysis was detected. The + sign means hydrolysis detected by TLC.

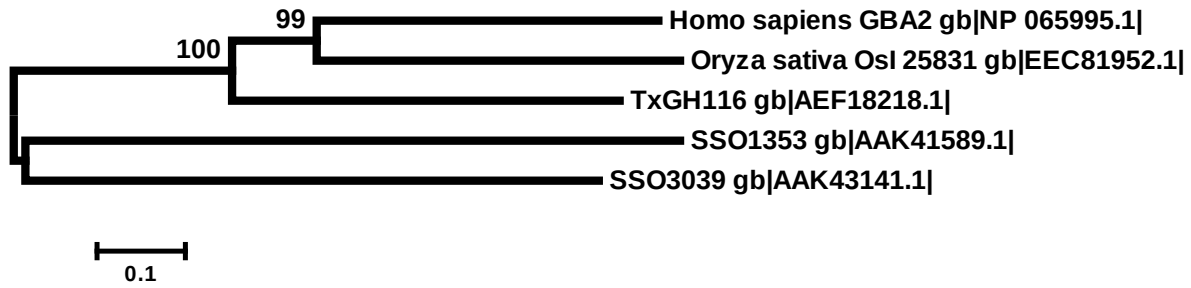

Figure S1 Protein sequence-based phylogenetic tree of GH116 proteins. The characterized proteins from Human (*Homo sapiens* GBA2), *S. solfataricus* (SSO1353 and SSO3039) and *T. xylanolyticum* TxGH116, are shown with a plant representative sequence from rice (*Oryza sativa*). The bacterial enzyme from *T. xylanolyticum* groups with the eukaryotic proteins from human and rice, while the enzymes from the Archaea *S. solfataricus* are more distantly related. The corresponding Genbank accession number is given after each sequence name. The percent bootstrap values supporting the clusters are given on the internal branches. The protein sequences were aligned with the MUSCLE algorithm and the tree drawn by the neighbor-joining algorithm within MEGA 5.

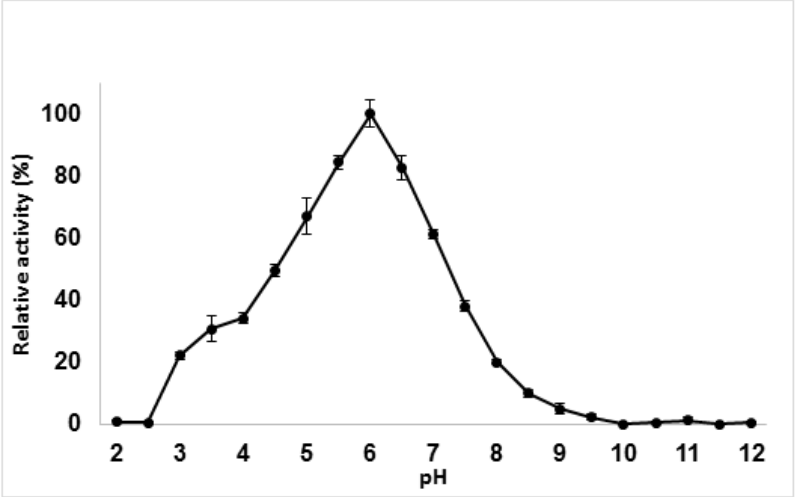

Figure S2 Activity vs. pH profile of TxGH116 expressed in recombinant *E. coli*. The activity was measured in universal citrate-phosphate buffers of the indicated pH. Values shown are means of three determinations with error bars indicating standard deviations. The curve indicates a sharp optimum at pH 6.0.

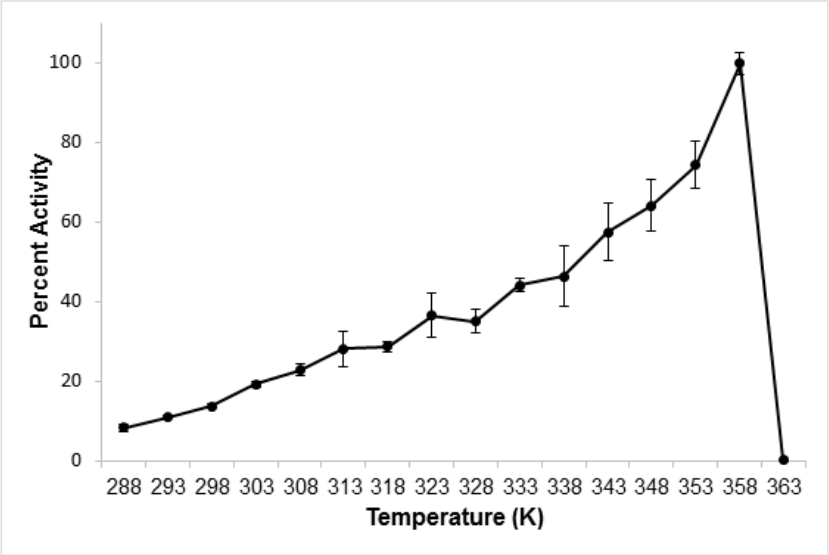

Figure S3 Activity vs. temperature assay of TxGH116 expressed in *E. coli*. The reactions were assayed for 10 min at the indicated temperatures, stopped and cooled before measuring the absorbance of the *p*-nitrophenol released from *p*NPGlc. Values shown are means of three determinations with error bars indicating standard deviations.

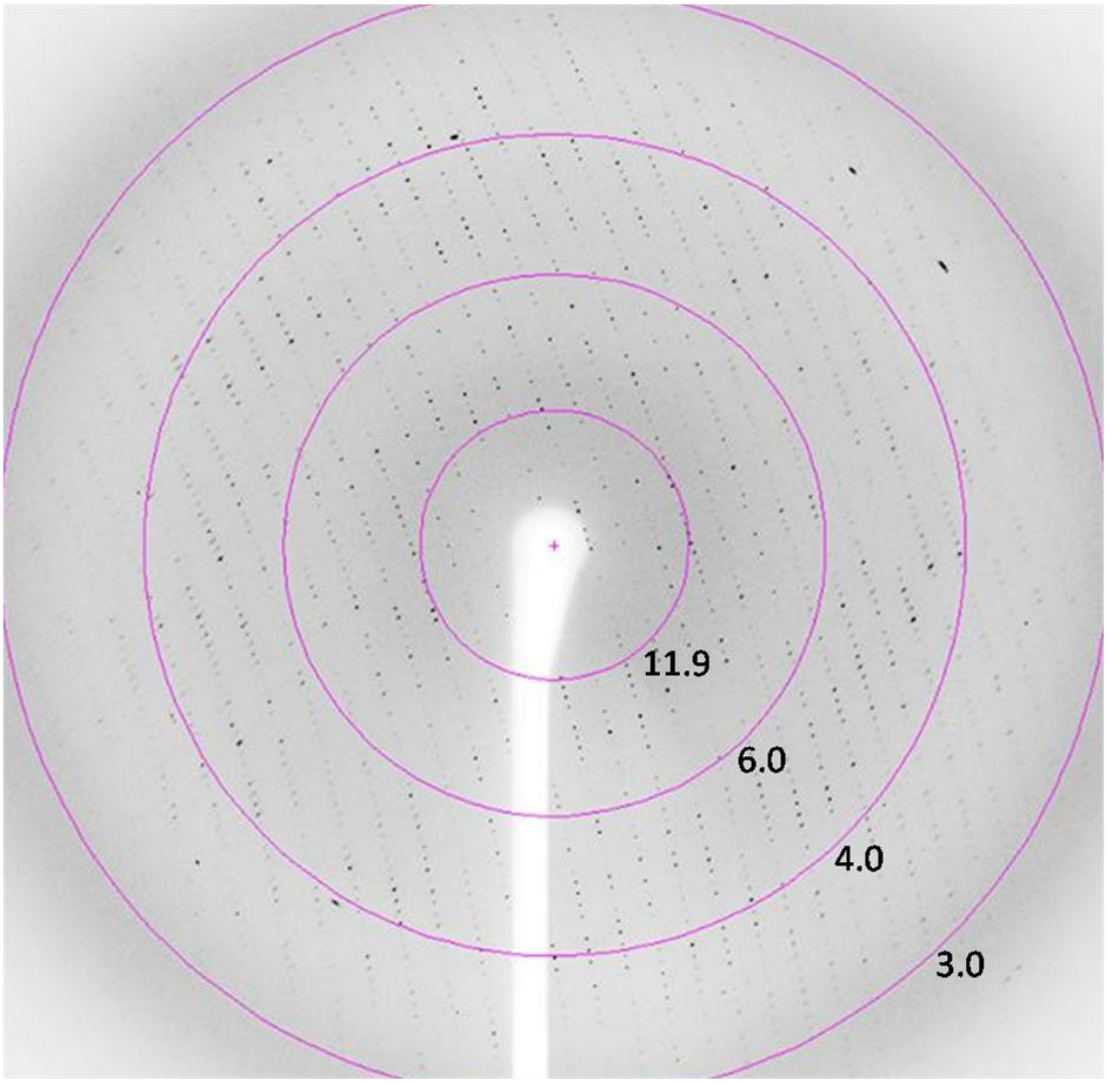

Figure S4 Example of a diffraction image from the TxGH116 protein crystal. The rings indicate the limits of diffraction in Å.