

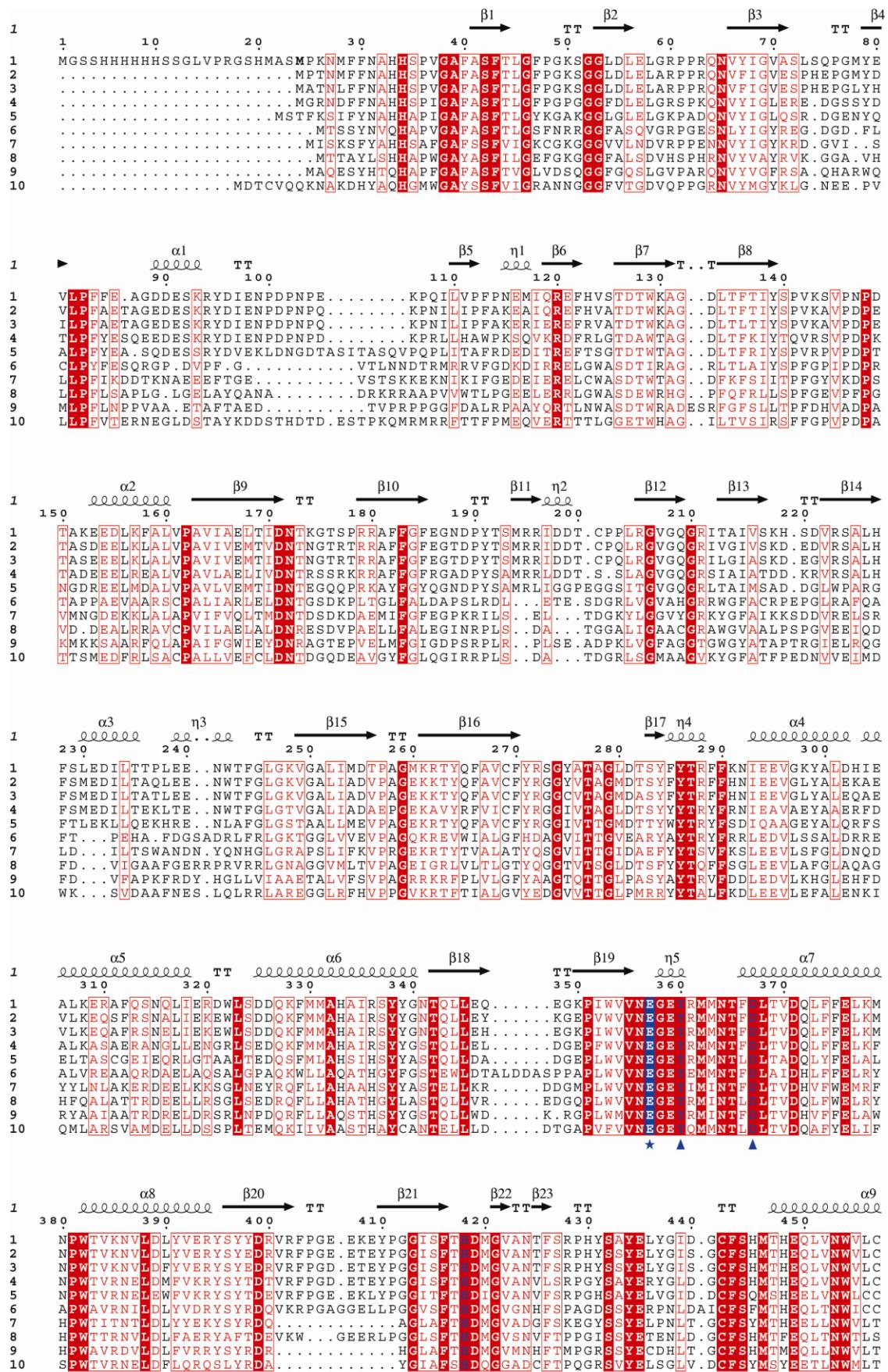
# Acta Crystallographica Section D

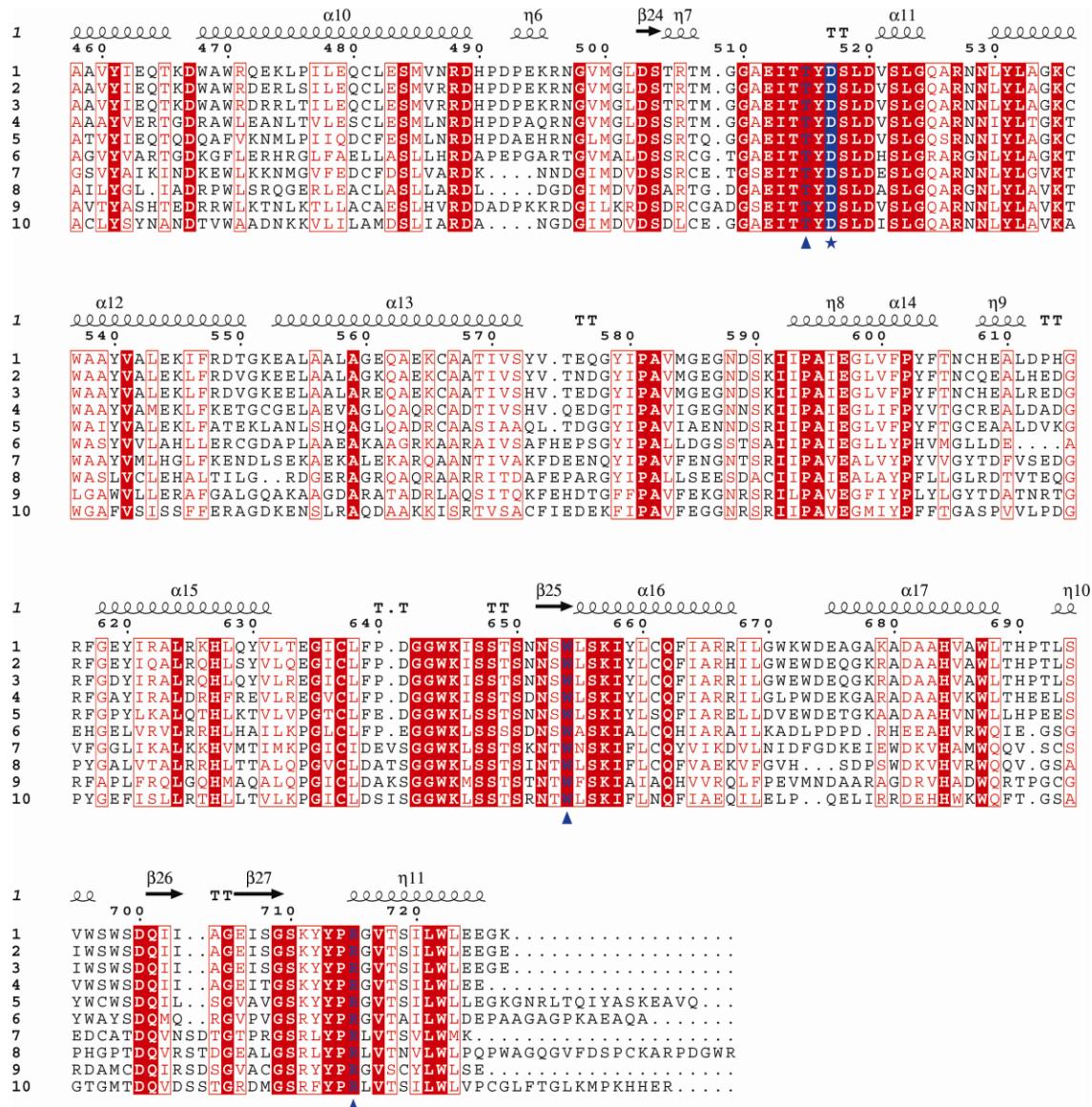
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Supporting information for article:

**A novel  $\beta$ -xylosidase structure from *Geobacillus thermoglucosidasius*: the first crystal structure of a glycoside hydrolase family GH52 enzyme reveals unpredicted similarity with other glycoside hydrolase folds**

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**Figure S1** Structure-based sequence alignment produced using Clustal Omega and displayed using ESPript 3.06 (Gouet *et al.*, 1999) of representative family 52  $\beta$ -xylosidases available in CAZY (Cantarelet *et al.*, 2009). Conserved residues are shown in red and strictly-conserved residues are shown in white on a red background. Aminoacids involved in the interaction with substrate are highlighted in blue (the catalytic residues are indicated by blue stars, and the others by blue triangles, beneath the alignment). Secondary structure of *Geobacillus thermoglucosidasius* TM242 $\beta$ -xylosidase is shown above the alignment with  $\beta$ -Strands as arrows and helices as coils. 1) *Geobacillus thermoglucosidasius* TM242; 2) *Geobacillus thermoglucosidasius* C56-YS93 (AEH48193.1, 87% identity); 3) *Geobacillus stearothermophilus* T-6 NCIMB 40222 (ABI49956.1, 86% identity); 4) *Paenibacillus* sp. DG-22 (ABV46494.1, 71% identity); 5) *Aeromonas caviae* ME-1 (BAA74507.1, 60% identity); 6) *Sorangium cellulosum* So0157-2 (AGP38099.1, 43% identity); 7) *Thermoanaerobacterium saccharolyticum* JW/SL-YS485 (AFK86458.1, 41% identity); 8) *Deinococcus geothermalis* DSM 11300 (ABF44123.1, 40% identity); 9) *Opitutus terrae* PB90-1 (AEC01950.1, 39% identity); 10) *Spirochaeta coccoides* DSM 17374 (AEC01950.1, 36% identity). It should be noted that the sequence of the *Geobacillus thermoglucosidasius* TM242  $\beta$ -xylosidase is of the recombinant His-tagged protein, as crystallised. The native protein would begin at residue 24 (in bold) in this alignment.

**Table S1** Glycosidase hydrolase families classified in CAZy that contain at least one sequence with xylosidase (EC 3.2.1.37) activity: their mechanism of action, structure (with CATH classification where known), and a summary of the xylosidase structures deposited in the PDB.

GH family	Mechanism	Domain structure		Organism	PDB codes
1	Retaining	( $\alpha/\beta$ ) <sub>8</sub> barrel 3.20.20.80			
3	Retaining	( $\alpha/\beta$ ) <sub>8</sub> barrel 3.20.20.300	Rossmann 3.40.50.1700		
30	Retaining	( $\alpha/\beta$ ) <sub>8</sub> barrel 3.20.20.80	$\beta$ -sandwich 2.60.40.1180		
39	Retaining	( $\alpha/\beta$ ) <sub>8</sub> barrel 3.20.20.80	$\beta$ -sandwich 2.60.40.1500	<i>Caulobacter crescentus</i> CB15	4EKJ
				<i>Geobacillus stearothermophilus</i> T-6 NCIMB 40222	1W91, 2BFG, 2BS9
				<i>Thermoanaerobacterium saccharolyticum</i> B6A-RI	1PX8, 1UHV
43	Inverting	$\beta$ -propellor 2.115.10.20	$\beta$ -sandwich 2.60.120.200	<i>Bacillus halodurans</i> C-125	1YRZ
				<i>Bacillus subtilis</i> 168	1YIF
				<i>Clostridium acetobutylicum</i> ATCC 824	1Y7B, 1YI7, 3CPN, 3K1U
				<i>Geobacillus stearothermophilus</i> T-6 NCIMB 40222	2EXH, 2EXI, 2EXJ, 2EXK
				<i>Selenomonas ruminantium</i> GA192	3C2U
52*	Retaining	$\alpha/\alpha_6$ barrel	$\beta$ -sandwich		4C1O, 4C1P
54	Retaining	$\beta$ -sandwich 2.60.120.200	$\beta$ -trefoil 2.80.10.50		
116	Retaining	unknown			
120*	Retaining	$\beta$ -helix	$\beta$ -sandwich	<i>Thermoanaerobacterium saccharolyticum</i> JW/SL-YS485	3VST, 3VSU, 3VSV

\* these families contain only EC 3.2.1.37 xylosidase sequences.

**Table S2** Structures identified as similar to *G.thermoglucosidasius* $\beta$ -xylosidase by DaliLite V.3 (Holm & Rosenström, 2010) including only those structures that shared similarity across both domains.

PDB code	Description	Organism	Family	% Id	Z score	RMSD Å	L ali	Nres
1UG9	Glucodextranase	<i>Arthrobacter globiformis</i>	GH 15	8	25.9	4.7	379	1019
1LF6	Glucoamylase	<i>Thermoanaerobacterium thermosaccharolyticum</i>	GH 15	8	24.5	5.4	491	674
3W7S	Uncharacterized Protein Ygjk	<i>Escherichia coli K-12</i>	GH 63	13	24.4	5.1	525	760
1V7V	Chitobiose Phosphorylase	<i>Vibrio proteolyticus</i>	GH 94	9	21.0	4.3	512	779
3CIH	Putative Alpha-Rhamnosidase	<i>Bacteroides thetaiotaomicron</i>	GH 78	10	20.9	3.6	338	714
4J5T	Mannosyl-Oligosaccharide Glucosidase	<i>Saccharomyces cerevisiae</i>	GH 63	8	20.5	8.2	492	788
2CQS	Cellobiose Phosphorylase	<i>Cellvibrio gilvus</i>	GH 94	10	19.1	4.2	533	822
3RRS	Cellobiose Phosphorylase	<i>Cellulomonas uda</i>	GH 94	9	19.6	4.3	530	822
3QDE	Cellobiose Phosphorylase	<i>Clostridium thermocellum</i>	GH 94	11	19.4	4.3	524	811
1H54	Maltose Phosphorylase	<i>Lactobacillus brevis</i>	GH 65	8	17.3	5.5	504	754
3W5M	Putative Rhamnosidase	<i>Streptomyces avermitilis</i>	-	9	17.5	3.4	326	1030
2RDY	Bh0842 Protein	<i>Bacillus halodurans</i>	-	7	12.9	4.7	494	787
2WW2	Alpha-1,2-Mannosidase	<i>Bacteroides thetaiotaomicron</i>	GH 92	6	11.8	5.7	385	737
2XSG	Ccman5	<i>Cellulosimicrobium cellulans</i>	GH 92	7	10.3	4.9	376	765
2EAB	Alpha-Fucosidase	<i>Bifidobacterium bifidum</i>	GH 95	10	11.6	4.8	495	888

Z = Z-score (statistical significance) of the best domain-domain alignment (distance, in standard deviations, between the observed alignment RMSD and the mean RMSD for random pairs of the same length, with the same or fewer gaps).

RMSD = root-mean-square deviation of C $\alpha$  atoms in rigid-body superimposition.

Lali = total number of structurally-equivalent residues.

%Id = percentage of sequence identity over equivalenced positions.