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Supporting information for article:

Crystal structure determination of *Pseudomonas stutzeri* A1501 endoglucanase Cel5A: the search for a molecular basis for glycosynthesis in GH5_5 enzymes

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Table S1 Structure homologues identified using the Dali server

Endoglucanase	PDB ID (chain)	Dali Z-score	GH5 subfamily	Reference
RBcel1	4EE9 (A)	47.2	5	(Delsaute <i>et al.</i> , 2013)
Xac0030	5HHN (C)	42.5	5	Unpublished
Xac0029	5HOS (A)	41.8	5	Unpublished
Ta_Cel5A	1GZJ (A)	39.9	5	(Lo Leggio & Larsen, 2002)
An_Cel5A	5I78 (B)	39.9	5	(Yan <i>et al.</i> , 2016)
Tv_Cel5A	5I6S (A)	39.6	5	Unpublished
Hj_Cel5A	3QR3 (A)	34.7	5	(Lee <i>et al.</i> , 2011)
Gl_Cel5A	5D8W (A)	34.6	5	(Liu <i>et al.</i> , 2016)
Fn_Cel5A	3NCO (A)	28.6	25	Unpublished
Tm_Cel5A	3AMD (B)	28.4	25	(Wu <i>et al.</i> , 2011)

Abbreviations: *Xanthomonas axonopodis* (Xac), *Thermoascus aurantiacus* (Ta), *Aspergillus niger* (An), *Talaromyces verruculosus* (Tv), *Hypocrea jecorina* (Hj), *Ganoderma lucidum* (Gl), *Fervidobacterium nodosum* (Fn), *Thermotoga maritima* (Tm).

Table S2 Amino acid residues forming the active centre of Ps_Cel5A endoglucanase

Amino acids	Characteristic and functional role	References
Arg-56	Structurally and functionally important residue, providing an ideal geometric and electrostatic environment for the nucleophile glutamate.	(Domínguez <i>et al.</i> , 1996; Sakon <i>et al.</i> , 1996; Cutfield <i>et al.</i> , 1999; Lo Leggio & Larsen, 2002)
Asn-143	Probably involved in the stabilization of transition state through the formation of a critical hydrogen bond with the sugar 2-hydroxy group at the -1 subsite.	(Davies <i>et al.</i> , 1997)
Glu-144	Catalytic proton donor	(Ducros <i>et al.</i> , 1995; Sakon <i>et al.</i> , 1996; Larsson <i>et al.</i> , 2006; Lee <i>et al.</i> , 2011; Yan <i>et al.</i> , 2016)
His-206	Forms an unusually short hydrogen bond (2.51 Å in Ps_Cel5A) with the proton donor glutamate, contributing to its positioning and ionization.	(Ducros <i>et al.</i> , 1995; Sakon <i>et al.</i> , 1996; Sabini <i>et al.</i> , 2000; Larsson <i>et al.</i> , 2006; Delsaute <i>et al.</i> , 2013)
Tyr-208	Function remains to be elucidated. Some authors proposed that it stabilizes the oxocarbenium-like ion of the transition state. Other studies suggested that it orients and/or influences the charged state of the nucleophile.	(Ducros <i>et al.</i> , 1995; Gebler <i>et al.</i> , 1995)
Glu-252	Catalytic nucleophile	(Ducros <i>et al.</i> , 1995; Domínguez <i>et al.</i> , 1996; Sakon <i>et al.</i> , 1996; Lo Leggio & Larsen, 2002; Liu <i>et al.</i> , 2016)
Trp-283	Involved in a non-proline cis peptide bond (conserved in GH5 and many other (β/α) ₈ -barrel GH). It participates in stacking interactions with the nucleophilic glutamate and interacts with the sugar.	(Sakon <i>et al.</i> , 1996; Domínguez <i>et al.</i> , 1996)

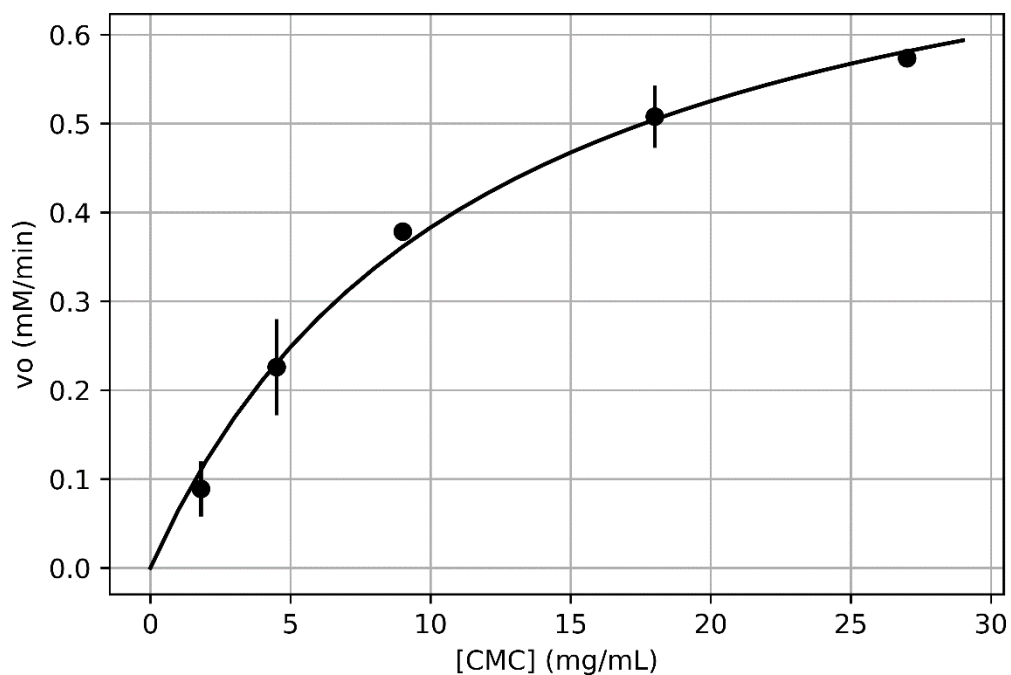


Figure S1 Michaelis-Menten plots deduced from the initial rates measured with increasing CMC initial concentrations. CMC hydrolysis in presence of Ps_Cel5A was determined by measuring the release of reducing sugars using dinitrosalicylic acid assay (DNS). Error bars represent standard deviations from two independent measurements.

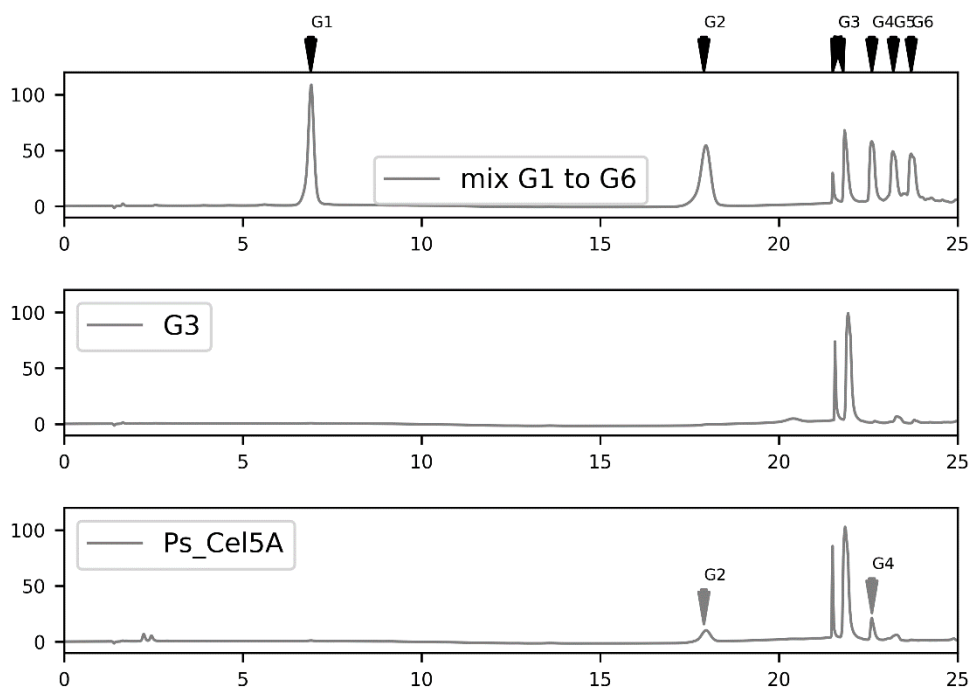


Figure S2 HPAEC-PAD elution profiles of products generated from cellotriose (G3) by Ps_Cel5A after 24 hours incubation at 37°C.

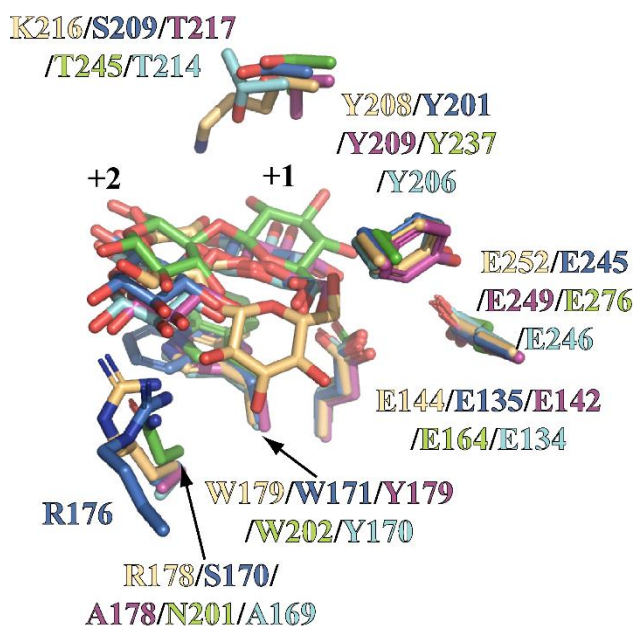


Figure S3 Position of cellobiose in the +1 and +2 subsites of Ps_Cel5A (PDB ID 6R2F, gold), RBcel1 (PDB ID 4M24, blue), Tv_Cel5A (PDB ID 5L9C, magenta), Gl_Cel5A (PDB ID 5D8Z, green), and Xac0030 (PDB ID 4W7V, cyan).

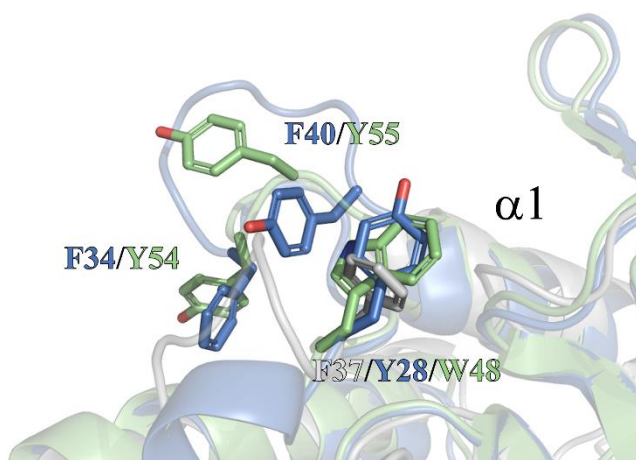


Figure S4 The -4 subsite adaption in Hj_Cel5A and Gl_Cel5A structures. The binding residue of the -4 subsite is Phe-37, Tyr-28, and Trp-48 for Ps_Cel5A (PDB ID 4LX4, light grey), Hj_Cel5A (PDB ID 3QR3, blue (Lee *et al.*, 2011)), and Gl_Cel5A (PDB ID 5D8W, green (Liu *et al.*, 2016)), respectively.

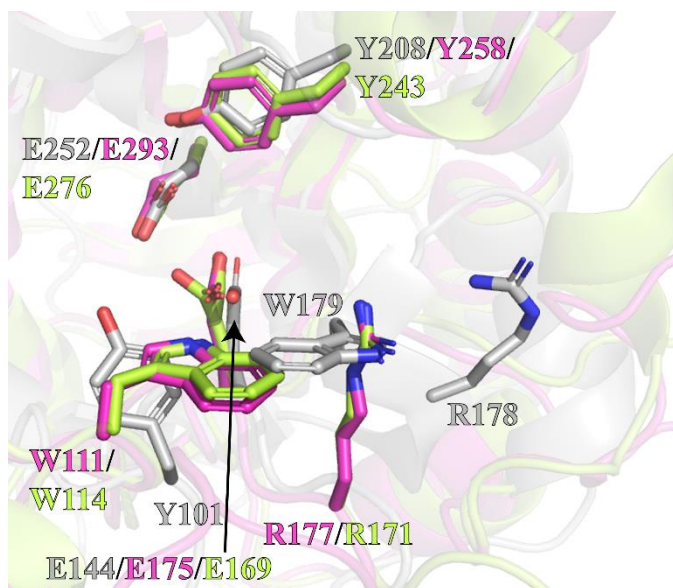


Figure S5 The adaptation of the +1 and +2 subsites in Ps_Cel5A and GH5_7 mannases. Ps_Cel5A structure (PDB ID 6R2F, light grey) was superimposed on *Rhizomucor miehei* Man5A (PDB ID 4QP0, magenta (Zhou *et al.*, 2014)) and *Trichoderma reesei* Man5A (PDB ID 1QNR, green, (Sabini *et al.*, 2000)).