Supplementary Material

Change of the product specificity of a cyclodextrin glucanotransferase by semi-rational mutagenesis to synthesize large-ring cyclodextrins

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Table S1. Accession numbers for the sequences used in the multiple protein alignment. Accession numbers are shown together with the bacterial origin and the main cyclodextrins (CD) product synthesized (CD6, CD7 and CD8, respectively).

Acc.Nr. NCBI/PDB	Bacterial orign	Main CD product
WP_003323850.1	*Alkalophilic Bacillus sp.* G-825-6	CD8
CAA01436.1	*Bacillus firmus/lentus* 290-3	CD7, CD8
BAH14968.1	*Bacillus clarkii* 7364	CD8
AEO89319.1	*Bacillus* sp. BPED101	-
P31746.1	*Bacillus* sp. 1-1	CD7
AGT45478	*Bacillus cereus*	-
O30565.1	*Brevibacillus brevis* CD162	CD7, CD8
AAV38118.2	*Bacillus sp.* G1	CD7
BAA02380.1	*Bacillus sp.* KC201	CD7
ADY17981.1	*Bacillus sp*. NR5 UPM	-
AGR66230.1	*Bacillus firmus*	CD7
AEL33336.1	*Bacillus* sp. 20RF	CD7
P27036.2	*Bacillus obhensis*	CD7
CAH61550.1	Anaerobranca gottschalkii	CD6
AAP31242.1	*Bacillus agaradhaerens* LS-3C	CD7
ABN14270.1	*Bacillus* sp. BL-31	CD7
P26827.2	Thermoanaerobacterium *thermosulfurigenes* Em1	CD7
WP_022587620.1	*Caldanaerobacter suberraneus*	-
AAD00555.1	*Geobacillus stearothermophilus* ET1	CD7
WP_007544393.1	*Haloferax larsenii*	-
AAC04359.1	*Paenibacillus macerans*	CD6
CAO05752.1	*Paenibacillus pabuli*	-
P42279.1	*Bacillus circulans* 251	CD7
P05618.1	*alkalophiler Bacillus sp*. 1011	CD7
	Paenibacillus sp. T16	CD7, CD8
AAV38117.1	*Bacillus sp.* TS1-1	CD7
ETT36448.1	*Paenibacillus* sp. FSL R5-808	-
P31747.1	*Bacillus* sp. 6.3.3	-
WP_021879762.1	*Paenibacillus sp*. P22	-
P14014.1	*Bacillus licheniformis*	CD6, CD7
M19880.1	*Bacillus sp. 38-2*	CD7

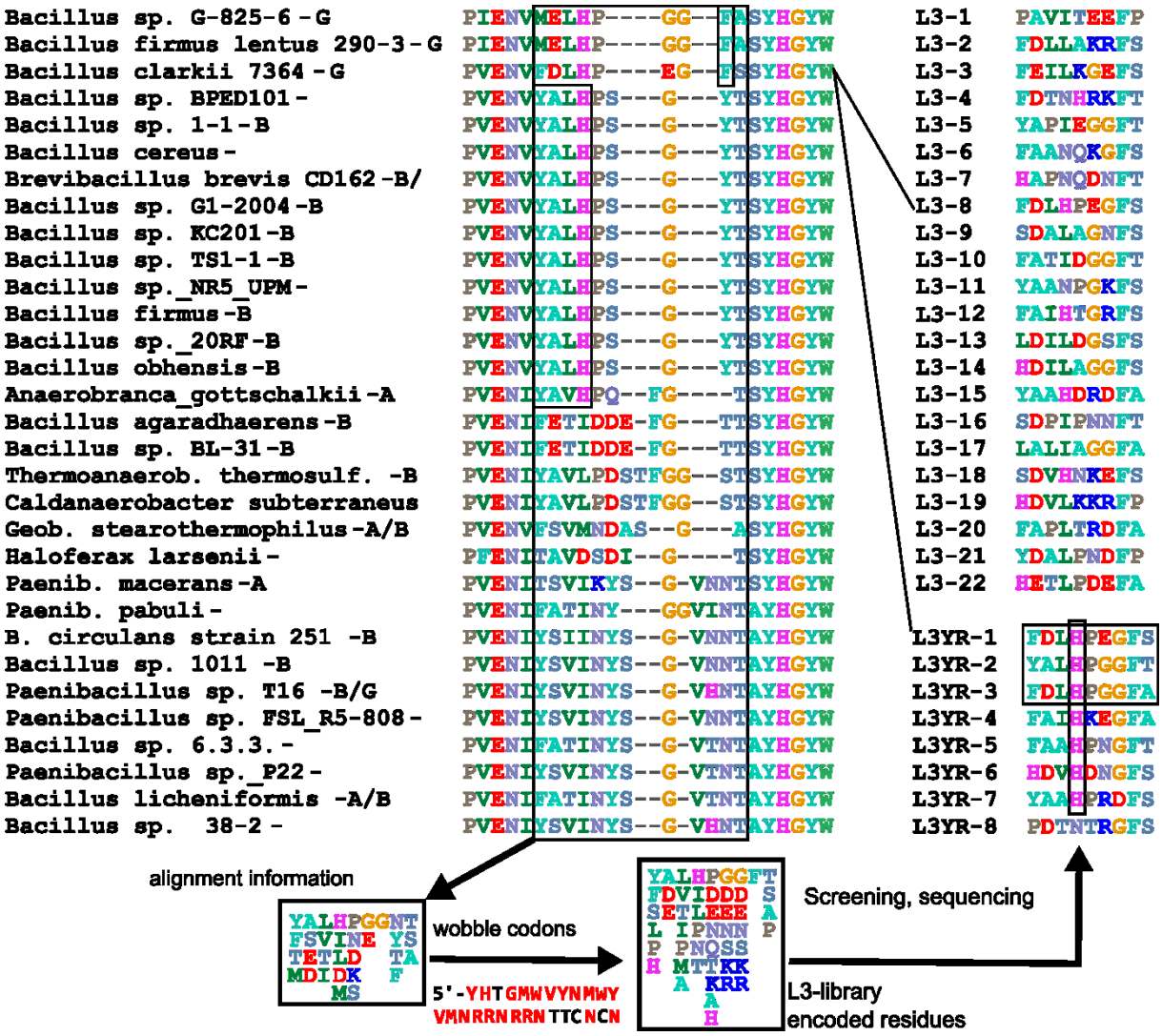

Figure S1. Multiple sequence alignment of cyclodextrin glucanotransferases (CGTases) and library design. Comparison of the variable loop 81-89 and flanking constant sequence elements of CGTase G825-6 with 30 other CGTase sequences. Based on the loop sequence diversity, a set of residues was selected to be encoded for the library design using a degenerative primer. Loop sequences of characterized variants from the experiments L3 and L3YR are shown.

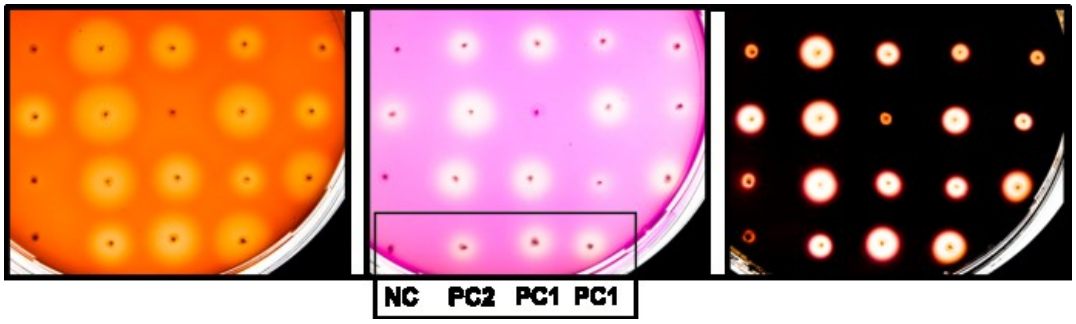

Figure S2. Agar plate screening of L3 clones for CD7 (left) and CD8 (middle) synthesis activity, and for starch-degrading activity (right). NC, negative control; PC,positive control.

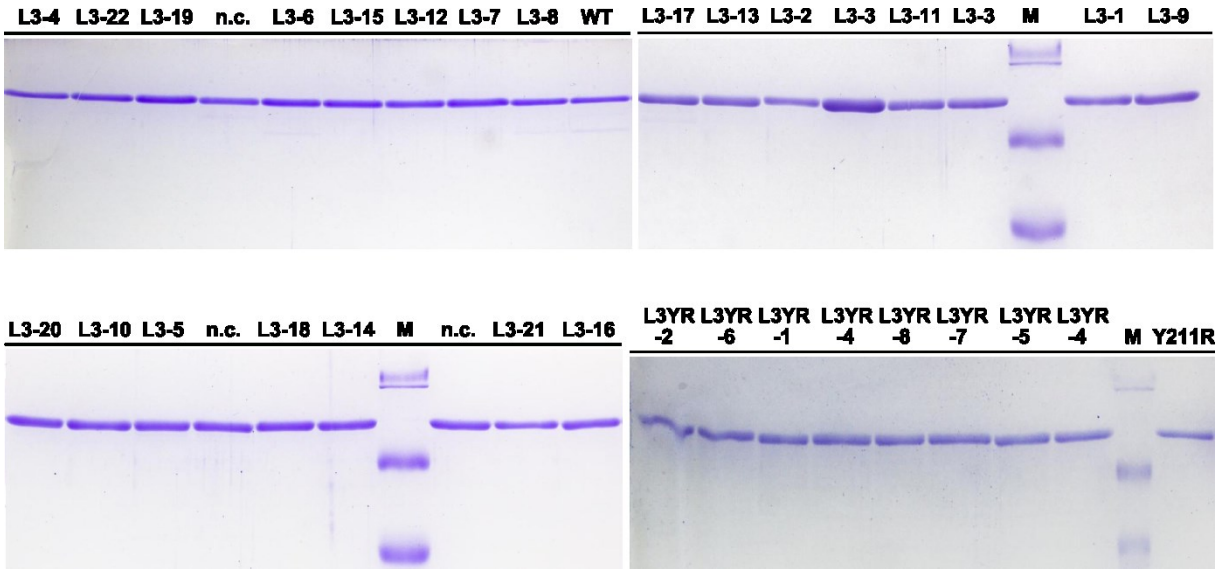

Figure S3. SDS-PAGE of purified L3 and L3YR variant CGTases.

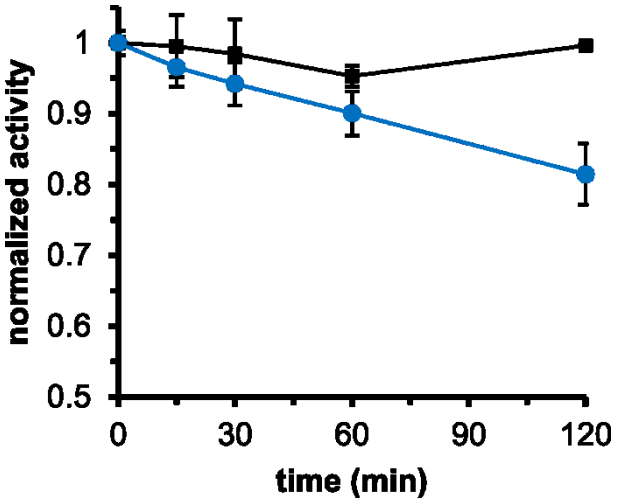

Figure S4. Thermal stability of the CGTase Y183R (black) and the variant L3YR-6 (blue). Mean values (n=3) ± S.D. are shown.

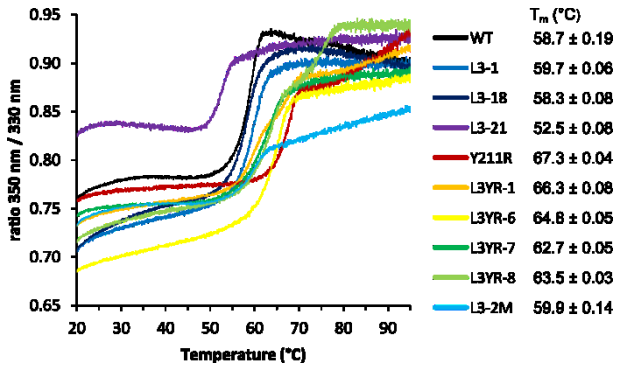

Figure S5. Melting curves of GTase variants. Averaged values for variants from both experiments determined by nano-differential scanning fluorimetry are shown. T_m values were calculated as inflection point of the first derivative. Mean values (n=3) are shown.

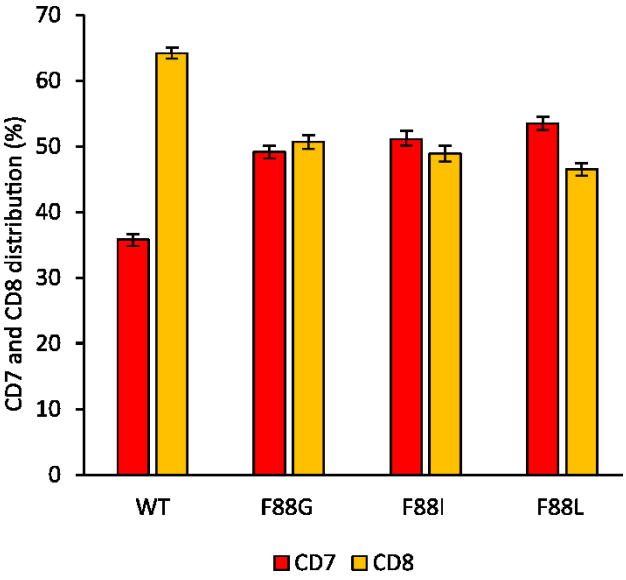

Figure S6. Influence of the amino acid residue 88 in the CGTase G825-6 (WT) on the synthesis of CD7 and CD8. The variants F88G, F88I and F88L produce a lower proportion of CD8. Mean values (n=3) ± S.D. are shown.

References

1. Sonnendecker, C.; Melzer, S.; Zimmermann, W. Engineered cyclodextrin glucanotransferases from Bacillus sp. G-825-6 produce large-ring cyclodextrins with high specificity. *Microbiologyopen* **2018**, e757, doi:10.1002/mbo3.757.