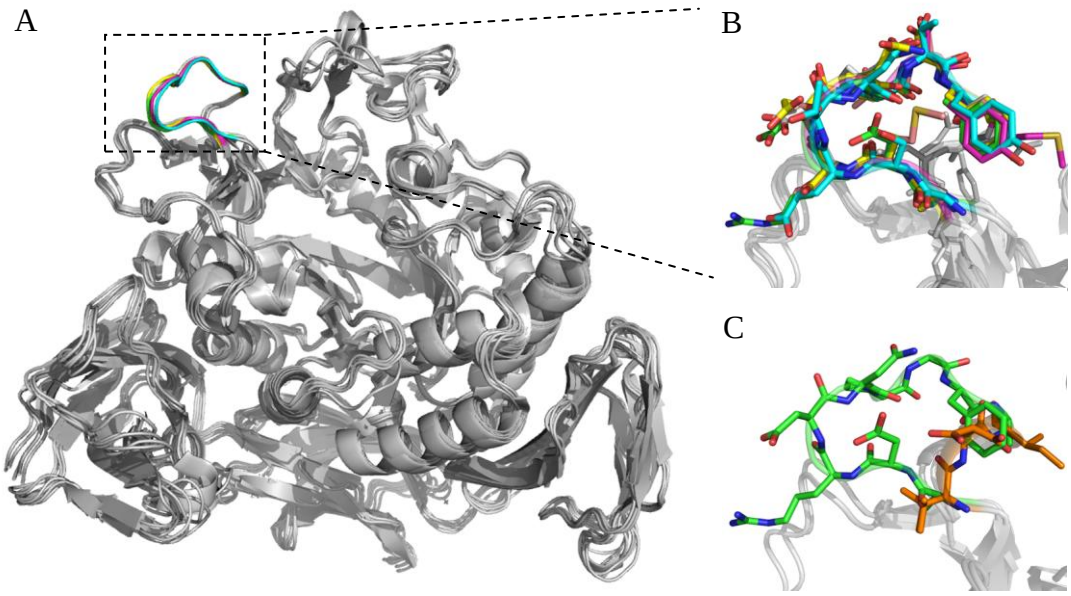

FIG S1 A, Structure alignment of parsed α-CGTases, α/β-CGTases and β-CGTase, the CCGTases are rendered in cartoon format and the loops constitute the subsite -7 are colorized: *Paenibacillus macerans* CGTase (PDB ID 4JCL), in green, *T. thermosulfurigenes* Em1 CGTase (PDB ID 1A47), in cygan, *B. stearothermophilus* N02 CGTase (PDB ID 1CYG), in magenta, *Bacillus circulans* 251 CGTase (PDB ID 1CDG), in yellow, *Bacillus circulans* 8 CGTase (PDB ID 1CGT), in salmon, *Bacillus sp.* 1011 CGTase (PDB ID 1PAM), in gray. The rest are coloured in gray. **B**, Partial view of the structures with the amino acid residues rendered in stick and coloured by element (N, blue; O, red; C, in accord with those in **A**). **C**, Comparison of *Bacillus clarkii* γ-CGTase (C, orange; N, blue; O, red; PDB ID 4JCM) with *Paenibacillus macerans* α-CGTase.

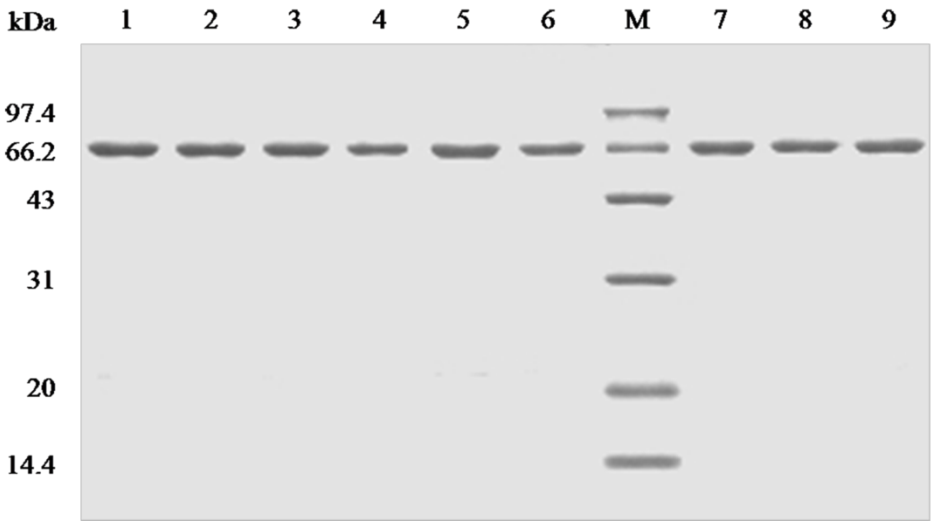

FIG S2 SDS-PAGE analysis of the purified wild-type and mutant proteins, Lanes: 1, wild-type CGTase; 2, mutant D145A; 3, mutant R146A; 4, mutant D147A; 5, mutant R146P; 6, mutant D147P; M, molecular mass marker; 7, mutant R146A/D147P; 8, mutant R146P/D147A and 7, mutant R146P/D147P.

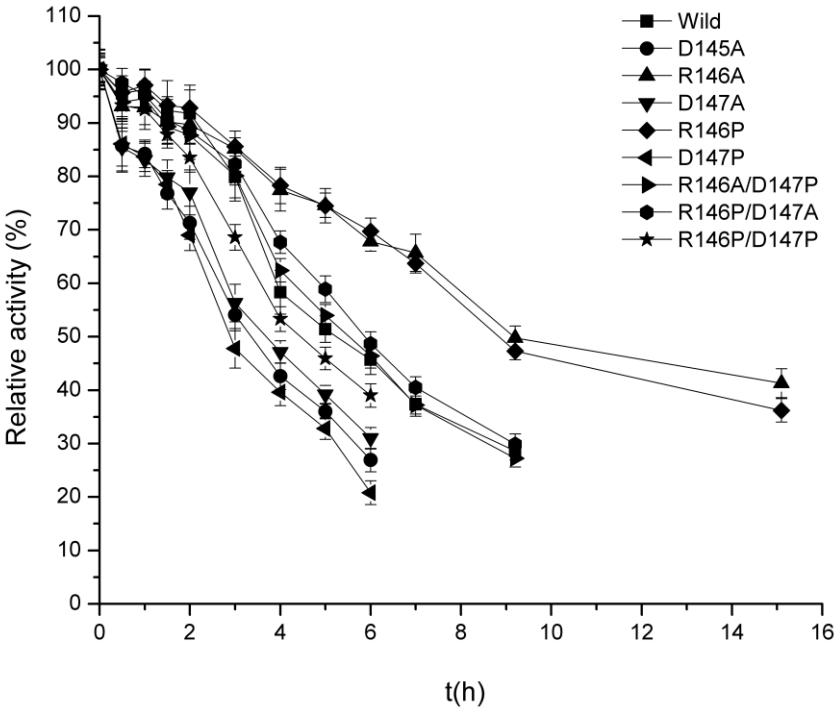

FIG S3 Stability of wild-type and mutant CGTases. Indicated enzymes were diluted to a concentration of 0.3 mg/ml with buffer at pH 5.5 and incubated at 45 ℃ for the indicated periods of time, then assayed for residual α-CGTase activity. Samples assayed prior to incubation at 45 ℃ were used as standards. Their activity was defined as 100%, and all subsequent activity measurements were scaled to these standards.

TABLE S1 Summary of isolation and purification procedures of CGTase

Enzyme	Purification step	Total activity[a] （U）	Total protein （mg）	Specific activity (U/mg)	Purification (fold)	Yield (%)
Wild-type	Crude enzyme	6951	343.80	20.22	1.00	100.0
	25% $(NH_4)_2SO_4$	5440	90.51	60.10	2.97	78.2
	DEAE-Sepharose	2320	19.40	119.60	5.91	33.4
D145A	Crude enzyme	6468	331.50	19.51	1.00	100.0
	25% $(NH_4)_2SO_4$	5392	97.32	55.40	2.84	83.4
	DEAE-Sepharose	2286	21.93	104.26	5.34	35.4
R146A	Crude enzyme	7970	352.20	22.63	1.00	100.0
	25% $(NH_4)_2SO_4$	6136	94.63	64.84	2.87	77.0
	DEAE-Sepharose	2518	19.09	131.90	5.83	31.6
D147A	Crude enzyme	6520	337.50	19.32	1.00	100.0
	25% $(NH_4)_2SO_4$	5591	98.67	56.67	2.93	85.7
	DEAE-Sepharose	2242	19.95	112.38	5.82	34.4
R146P	Crude enzyme	7622	358.70	21.25	1.00	100.0
	25% $(NH_4)_2SO_4$	5906	94.30	62.63	2.95	77.5
	DEAE-Sepharose	2406	18.47	130.30	6.13	31.6
D147P	Crude enzyme	7318	338.80	21.60	1.00	100.0
	25% $(NH_4)_2SO_4$	6006	92.78	64.74	3.00	82.1
	DEAE-Sepharose	2356	22.20	107.10	4.96	32.2
R146A/D147P	Crude enzyme	7158	350.40	20.43	1.00	100.0
	25% $(NH_4)_2SO_4$	5895	92.78	63.54	3.11	82.4
	DEAE-Sepharose	2375	20.80	114.20	5.59	33.2
R146P/D147A	Crude enzyme	7814	336.50	23.22	1.00	100.0
	25% $(NH_4)_2SO_4$	6329	88.50	71.51	3.08	81.0
	DEAE-Sepharose	2719	21.72	125.20	5.39	34.8
R146P/D147P	Crude enzyme	6603	325.30	20.30	1.00	100.0
	25% $(NH_4)_2SO_4$	5586	93.61	59.67	2.94	84.6
	DEAE-Sepharose	2238	19.77	113.20	5.58	33.9

[a] the activity was α-cyclodextrin forming activity