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Supplementary Materials

The PT/S-Box of Modular Cellulase AcCel12B Plays a Key Role in the Hydrolysis of Insoluble Cellulose

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Table S1 Oligonucleotides used as primers in PCR.

Enzyme	Number	Sequence	Direction
AcCel12B-wt	1	GCAATTCATATGTCAACGTGTTCACCTACCG	Forward
	2	TGAGACCTCGAGGCAGGTGAGTGTGGGTGGGGTGTTA	Reverse
AcCel12B-PT0	3	ACCACCAGACGACGATGTGCTGGACGTGCCGCTCGTCAC	Forward
	4	ACGAGCGGCACGTCCAGCACATCGTCGTCTGGTGGTG	Reverse
AcCel12B-PT1	5	GCGCCCAGCCCGTCCCCGAGCCCGAGCCCAACGCCCACGTCCAGCCCGACATCGTCGTCTGGTGGTGT	Forward
	6	ACATCGTCGTCTGGTGGTG	Reverse
AcCel12B-PT3	7	CCGACACCGACACCGTCTCCAAGCCCATCCCCGAGCCCCGCGACATCGTCGTCTGGTGGT	Forward
	8	GCTGGACGTGCCGCTCGT	Reverse

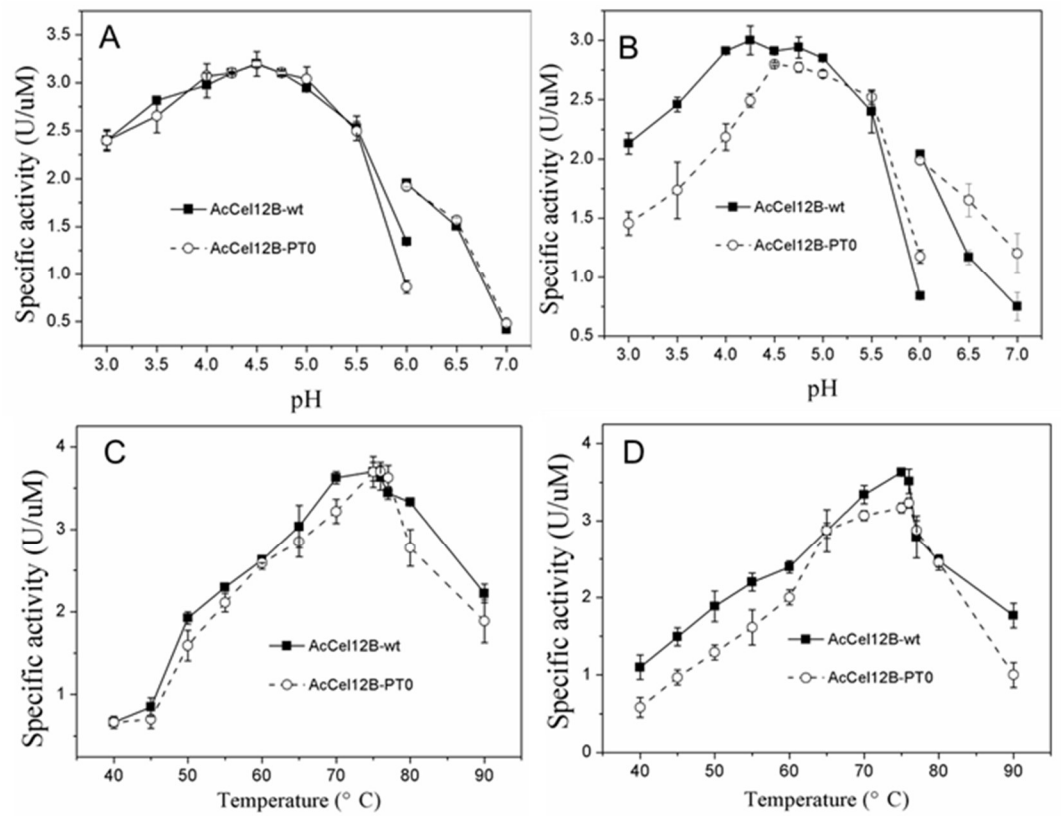

Figure S1. Effects of pH and temperature on activity of AcCel12B-wt (■) and mutant AcCel12B-PT0 (○) toward substrate CMC and substrate RAC. (A) (B): The optimum pH of AcCel12B-wt (■) and mutant AcCel12B-PT0 (○) toward CMC (A) and RAC (B) under conditions of 50 mM sodium acetate buffer (pH 3.0-6.0) and 50 mM phosphate buffer (pH 6.0-7.0) at 70 ºC. The optimum temperature of AcCel12B-wt (■) and mutant AcCel12B-PT0 (○) toward CMC (C) and RAC (D) under conditions of 50 mM sodium acetate buffer (pH 4.5). AcCel12B toward 1% (w / v) CMC or 0.5% (w / v) RAC were reacted in 5 or 15 min.

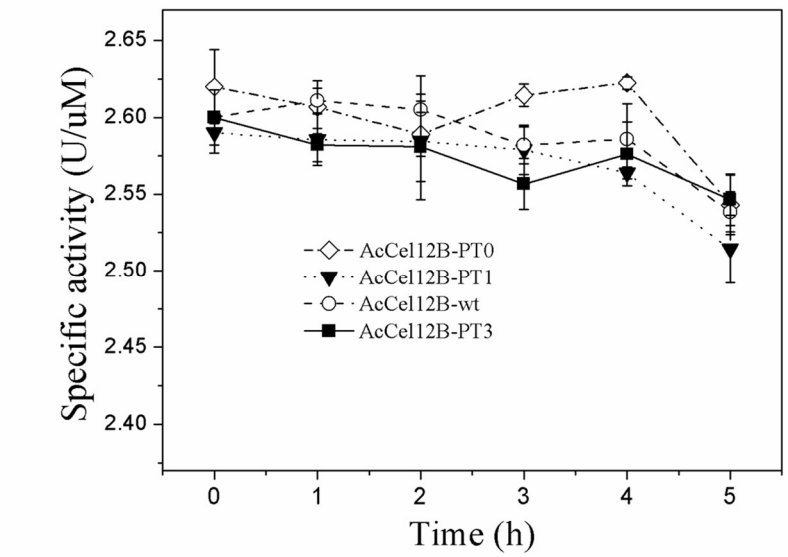

Figure S2. Thermal stability of AcCel12B and its mutants in at 60°C. The enzyme was incubated at 60 °C for various times under conditions of 50 mM sodium acetate buffer (pH 4.5). The residual activity of cellulases toward CMC was determined. All were described as AcCel12B-PT3 (solid line and ■), AcCel12B-wt (dash line and ○), AcCel12B-PT1 (dot line and ▲) and AcCel12B-PT0 (dast dot line and ◇).

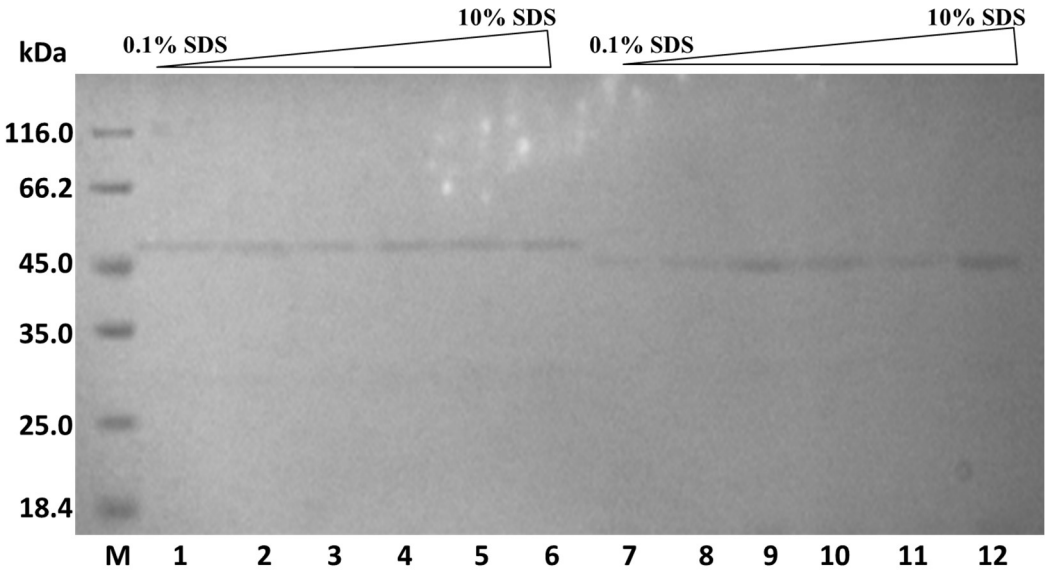

Figure S3. SDS-Polyacrylamide gel electrophoresis of AcCel12B-PT3 and AcCel12B-wt desorbing from RAC. Lane M: Protein Marker. Lane 1-6: AcCel12B-PT3 desorbing from Avicel under gradually increasing concentration SDS condition. Lane 7-12: AcCel12B-wt desorbing from Avicel under gradually increasing concentration SDS condition.

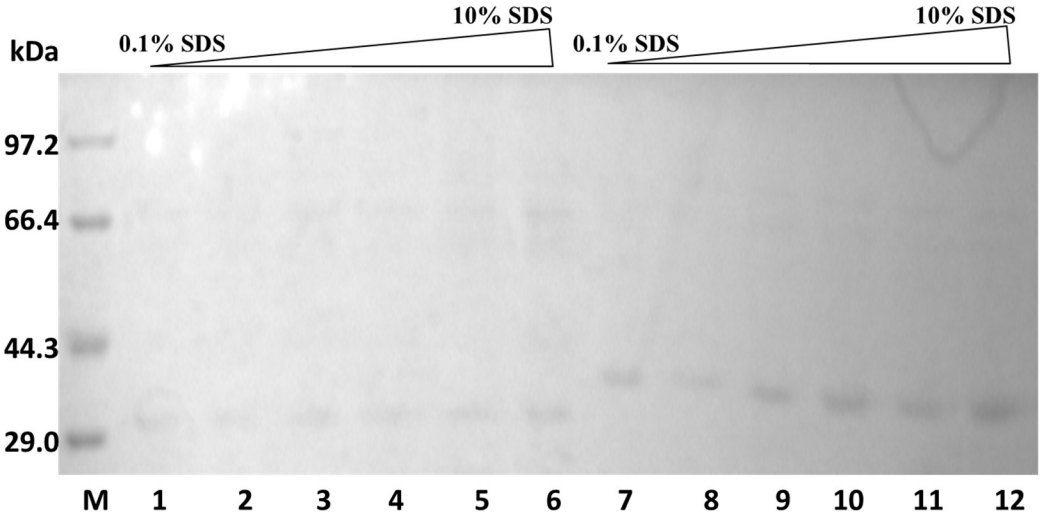

Figure S4. SDS-Polyacrylamide gel electrophoresis of AcCel12B-PT0 and AcCel12B-PT1 desorbing from Avicel. Lane M: Protein Marker. Lane 1-6: AcCel12B-PT0 desorbing from Avicel under gradually increasing concentration SDS condition. Lane 7-12: AcCel12B-PT1 desorbing from Avicel under gradually increasing concentration SDS condition.