Supporting Information

Structural and Functional Studies of *A. oryzae* Cutinase: Enhanced Thermostability and Hydrolytic Activity of Synthetic Ester and Polyester Degradation

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Supporting Method:

Gene Synthesis. The Protein Data Bank (PDB) and National Center for Biotechnology Information (NCBI) databases were used to obtain amino acid sequences of different cutinases. The gene sequences encoding cutinases were optimized by using Gene designer (DNA 2.0, Inc.). The codons were reassembled for *Pichia pastoris* in order to increase the expressed proteins. The cutinase genes were synthesized by DNA 2.0 (CA, USA), and cloned into the pPICZαA (Invitrogen, USA) vector. Recombinant plasmids were amplified in *E. coli*, and then extracted and linearized using Sac I for transformation. Competent cells and transformation were completed using Pichia Easycomp^{TM} kit (Invitrogen, USA). The transformants were spread onto the YPDS medium composed of yeast extract (1%, w/v), peptone (2%, w/v), dextrose (D-glucose, 2%, w/v), D-sorbitol (1 M) and Zeocin (100 μg/mL). The plates were incubated at 30 °C for 3-4 days.

Supporting Table:

Table S1. *Data Collection and Refinement Statistics*

Space group	$P3_221$
Unit-cell parameter (Å)	a= 45.299, b = 45.299, c =157.111
Highest resolution (Å)	1.75
Observed reflections	91845
Unique reflections	19394
Completeness (%)	98.1
I/σ (I)	8.9(5.3)
Mosaicity (º)	0.3
Redundancy	5(4.7)
R_{merge} (%)	0.042 (0.077)
V_M (A^3 Da^{-1})	1.94
Refinement	
Protein atoms	1370
Protein residues	187 (26-212)
Other atoms	275 water molecules
R_{work} (%)	19.4
R_{free} (%)	19.9
Mean temperature factor (excluding solvent) ($Å^2$)	11.3
R.m.s.d. bond lengths (Å)	0.004860
R.m.s.d. bond angles (º)	1.24894
Ramachandran most favored (%)	90.2

Supporting Figures:

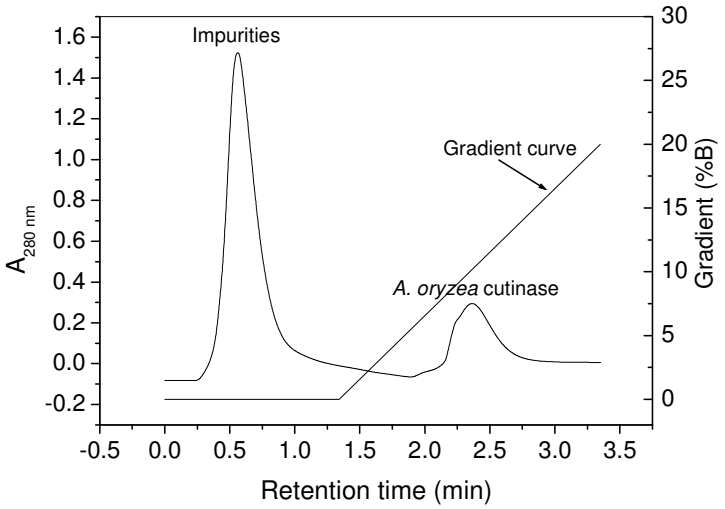

Figure S1. The FPLC trace for *A. oryzea* cutinase purification.

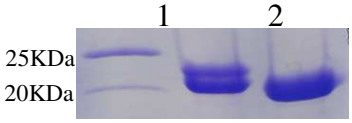

Figure S2. Purified cutinases on SDS-PAGE. 1, Protein Ladder; 2, *F. solani* cutinase (estimated MW, 21.4 KDa); and 3, *A. oryzea* cutinase (estimated MW, 20.6 KDa).

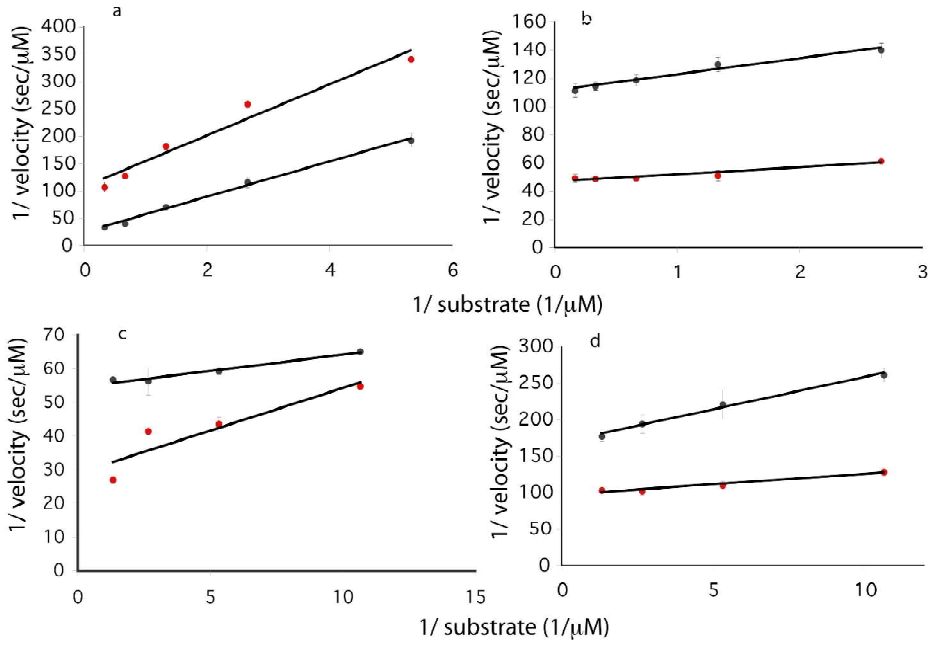

Figure S3. Lineweaver-Burke plots of AoC (red) and FsC (grey) for (a) pNPA, (b) pNPB, (c) pNPV and (d) pNPH. Plots represent an average of at least 3 trials where error bars signify the standard deviation.

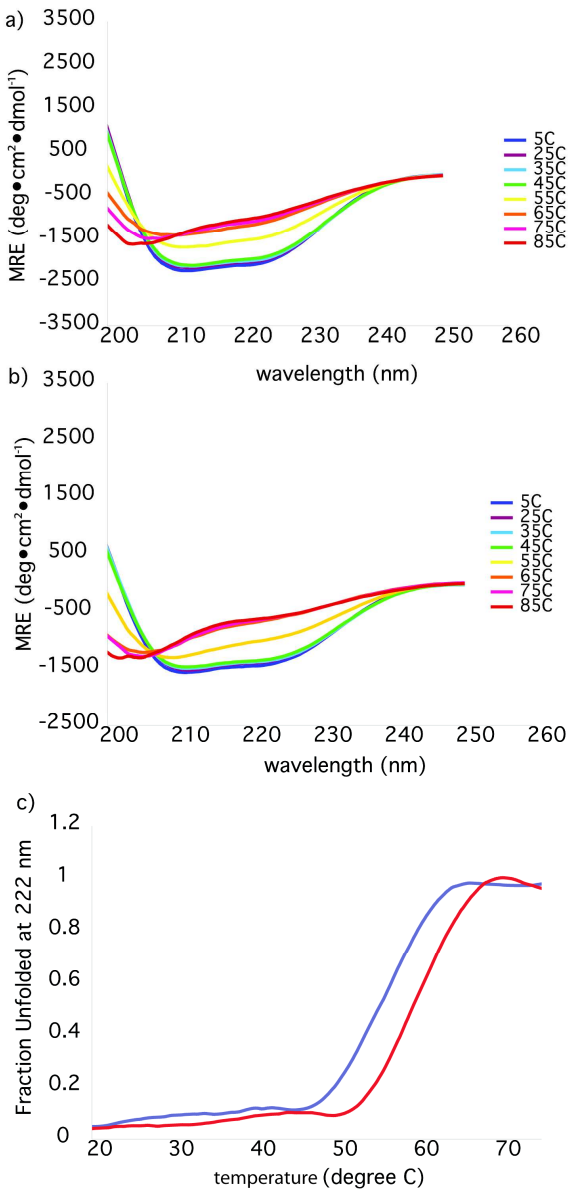

Figure S4. Wavelength scans of the a) FsC and b) AoC as a function of temperature. Comparative melts at 222 nm of c) FsC (blue) and AoC (red).

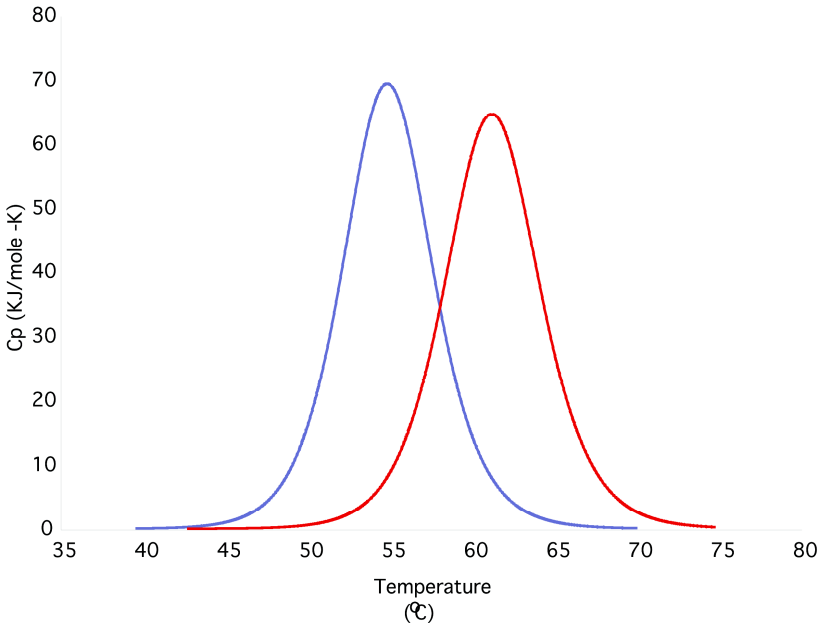

Figure S5. Overlay of AoC (red) and FsC (blue) DSC traces of heat capacity as a function of temperature. Traces represent and average of at least two trials.

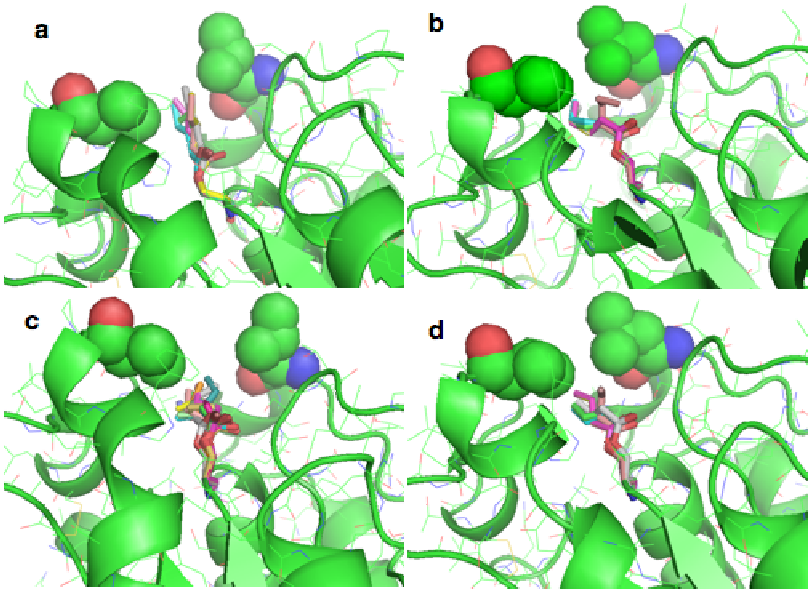

Figure S6. Predicted low energy conformations of serine valerate (a, b) and butyrate (c, d) esters for (a, c) *A. oryzae* and (b, d) *F. solani* cutinases. Ester heavy atoms are shown as sticks for all conformations within 5 kcal•mol^{-1} of the lowest energy conformation after systematic screening of torsions and minimization. The gatekeeper residues, Leu 87 and Val 190 in *A. oryzae* and Leu 81 and Val 184 in *F. solani* are shown as spheres.