

Mutational Analysis of Phenolic Acid Decarboxylase from *Bacillus subtilis* (BsPAD), which Converts Bio-derived Phenolic Acids to Styrene Derivatives

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Supporting Information

Variant	Forward Primer	Reverse Primer
WT	CCAGGGACCAGCAAGGAAAACCTTTA TCGGAAGCCACATGATTTTATACGT	GAGGAGAAGGCGCGTTATTATAATC TTCCCGCGCGAATATCGTCTGTTCAT
Y11F	CGGAAGCCACATGATTTTACGTAT GAAAACGGATG	CATCCGTTTTTCATACGTAAAAATCA TGTGGCTTCCG
Y13F	GAAGCCACATGATTTTATACGTTTGA AAACGGATGGGAATACG	CGTATTCCCATCCGTTTTCAAACGTA TAAATCATGTGGCTTC
Y19A	CGTATGAAAACGGATGGGAAGCGG AGATTTATATTA AAAACGAC	GTCGTTTTTAATATAAATCTCCGCTT CCCATCCGTTTTTCATACG
R41A	CATAGCGGAATGGTTGCCGGAGCGT GGGTTTCGAGATCAGGAAGTG	CACTTCCTGATCTCGAACCCACGCT CCGGCAACCATTCCGCTATG
E64A	TATAAAGTGTCTTGGACAGCGCCGA CTGGCACGGATGTTT	AAACATCCGTGCCAGTCGGCGCTGT CCAAGACACTTTATA
T68V	GGACAGAGCCGACTGGCGTGGATGT TTCATTAAAC	GTTTAATGAAACATCCACGCCAGTC GGCTCTGTCC
T98A	GCATGAACATCCTGAAATTGCGGTT TGCTACCAA AATG	CATTTTGGTAGCAAACCGCAATTTT AGGATGTTCATGC
Y11F/ Y13F *	CTTTATCGGAAGCCACATGATTTTTA CGTTTGAAAACGGATGGGAATAC	TATTCCCATCCGTTTTCAAACGTAAA AATCATGTGGCTTCCGATAAAG

Table S1. Primers used for PCR in amplification of gene encoding *BsPAD* from genomic DNA of *B. subtilis* and subsequent site-directed mutagenesis experiments using the plasmid encoding the WT as a template. *Primers containing two mutagenic codons were used in this instance.

<i>BsPAD</i> complexed with coumaric acid	
Beamline	Diamond io4 , 23rd October 2010
Wavelength (Å)	0.9763 Å
Resolution (Å)	53.60-3.03 (3.10-3.03)
Space Group	$P3_221$
Unit cell (Å)	a = b = 107.21; c = 92.80
Unique reflections	11708 (778)
Completeness (%)	99.2 (98.2)
R_{merge} (%)	0.11 (0.86)
$R_{\text{p.i.m.}}$	0.051 (0.39)
Multiplicity	10.8 (11.0)
$\langle I/\sigma(I) \rangle$	21.4 (3.3)
Protein atoms	3852
$R_{\text{cryst}}/R_{\text{free}}$ (%)	20.1/ 26.3
r.m.s.d 1-2 bonds (Å)	0.011
r.m.s.d 1-3 angles (°)	1.44
Avg B (Å ²)	52

Table S2. Data Collection and Refinement Statistics for Tyr19Ala mutant of *BsPAD* complexed with coumaric acid

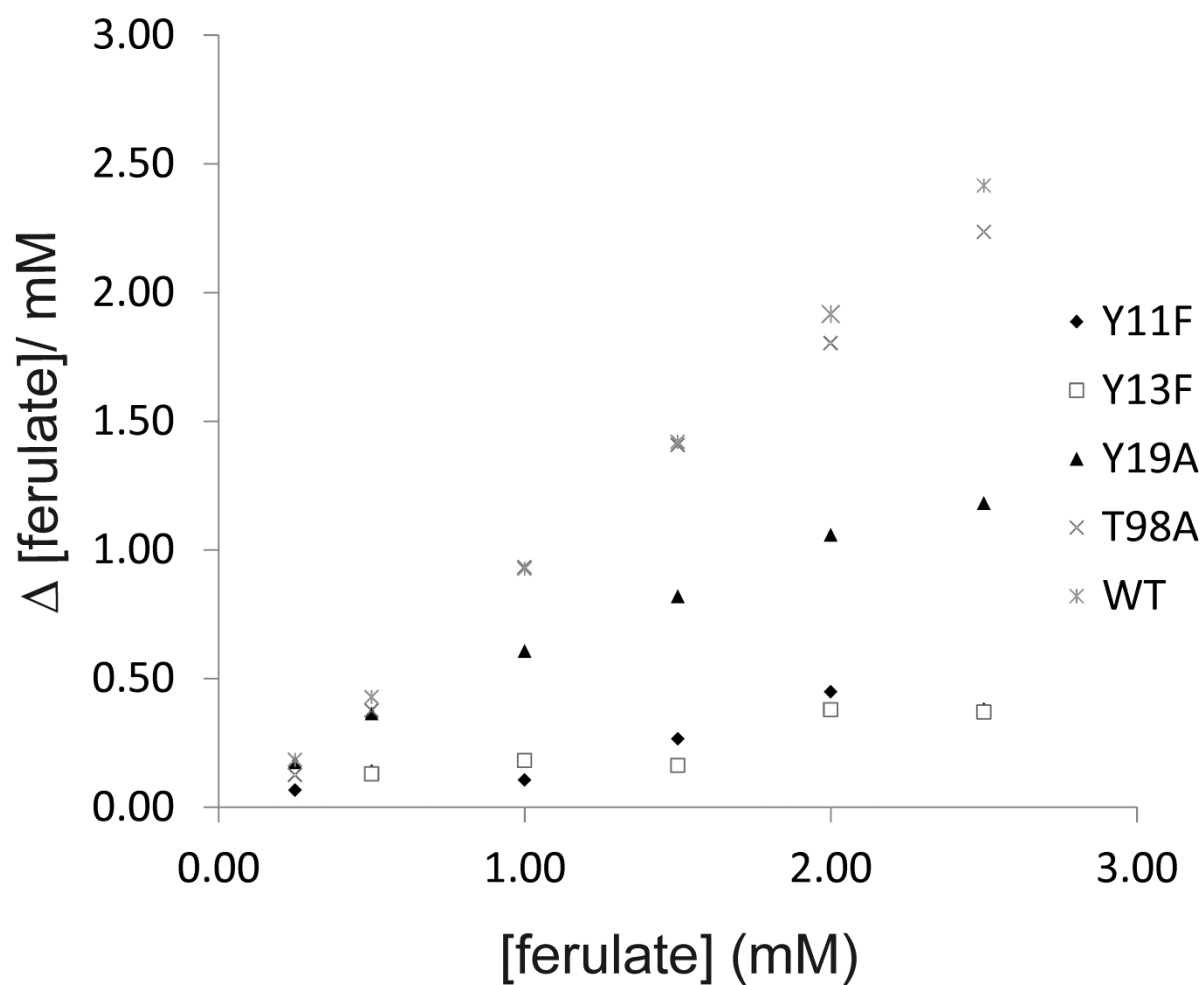


Figure S1. Graph illustrating results of an HPLC-based kinetic study of wild-type *BsPAD* using ferulic acid as substrate. Experiments were carried out as detailed in the Experimental Section.