

Supplementary materials

Semi-rational design of *Proteus mirabilis* L-amino acid deaminase for expanding its substrate specificity in α -keto acid synthesis from L-amino acids

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Table S1. Primers used in this study.

Primers	Sequence
F96A_f	CTCGAGCGTATGGTCAAGCTATTAGCTATAAAATGCCAGATGAAACCT
F96A_r	AGCTTGACCATA <u>CG</u> CTGAGATGATTGTTGCCGGCAATAT
F96C_f	CTCGATGCTATGGTCAAGCTATTAGCTATAAAATGCCAGATGAAACCT
F96C_r	AGCTTGACCATA <u>AC</u> GTGAGATGATTGTTGCCGGCAATAT
F96D_f	CTCGAG <u>A</u> CTATGGTCAAGCTATTAGCTATAAAATGCCAGATGAAACCT
F96D_r	AGCTTGACCATA <u>CT</u> GTGAGATGATTGTTGCCGGCAATAT
F96E_f	CTCGAG <u>AA</u> ATGGTCAAGCTATTAGCTATAAAATGCCAGATGAAACCT
F96E_r	AGCTTGACCATA <u>TT</u> TCGAGATGATTGTTGCCGGCAATAT
F96G_f	CTCGAG <u>GG</u> TATGGTCAAGCTATTAGCTATAAAATGCCAGATGAAACCT
F96G_r	AGCTTGACCATA <u>AC</u> CGAGATGATTGTTGCCGGCAATAT
F96H_f	CTCGAC <u>AC</u> ATGGTCAAGCTATTAGCTATAAAATGCCAGATGAAACCT
F96H_r	AGCTTGACCATA <u>AG</u> TCGAGATGATTGTTGCCGGCAATAT
F96I_f	CTCGA <u>AT</u> CTATGGTCAAGCTATTAGCTATAAAATGCCAGATGAAACCT
F96I_r	AGCTTGACCATA <u>AG</u> TCGAGATGATTGTTGCCGGCAATAT
F96K_f	CTCG <u>AA</u> ATGGTCAAGCTATTAGCTATAAAATGCCAGATGAAACCT
F96K_r	AGCTTGACCATA <u>TT</u> TCGAGATGATTGTTGCCGGCAATAT
F96L_f	CTCG <u>AC</u> TGATGGTCAAGCTATTAGCTATAAAATGCCAGATGAAACCT
F96L_r	AGCTTGACCATA <u>AG</u> TCGAGATGATTGTTGCCGGCAATAT
F96M_f	CTCGA <u>AT</u> GTATGGTCAAGCTATTAGCTATAAAATGCCAGATGAAACCT
F96M_r	AGCTTGACCATA <u>AC</u> TCGAGATGATTGTTGCCGGCAATAT
F96N_f	CTCG <u>AA</u> CTATGGTCAAGCTATTAGCTATAAAATGCCAGATGAAACCT
F96N_r	AGCTTGACCATA <u>TT</u> TCGAGATGATTGTTGCCGGCAATAT
F96P_f	CTCG <u>AC</u> GTATGGTCAAGCTATTAGCTATAAAATGCCAGATGAAACCT
F96P_r	AGCTTGACCATA <u>AG</u> GTGAGATGATTGTTGCCGGCAATAT
F96Q_f	CTCG <u>AC</u> GTATGGTCAAGCTATTAGCTATAAAATGCCAGATGAAACCT
F96Q_r	AGCTTGACCATA <u>AG</u> TCGAGATGATTGTTGCCGGCAATAT
F96R_f	CTCG <u>AC</u> GTATGGTCAAGCTATTAGCTATAAAATGCCAGATGAAACCT
F96R_r	AGCTTGACCATA <u>AG</u> TCGAGATGATTGTTGCCGGCAATAT
F96S_f	CTCG <u>AT</u> CTATGGTCAAGCTATTAGCTATAAAATGCCAGATGAAACCT
F96S_r	AGCTTGACCATA <u>AG</u> TCGAGATGATTGTTGCCGGCAATAT
F96T_f	CTCG <u>AC</u> CTATGGTCAAGCTATTAGCTATAAAATGCCAGATGAAACCT
F96T_r	AGCTTGACCATA <u>GG</u> TCGAGATGATTGTTGCCGGCAATAT
F96V_f	CTCG <u>AG</u> TTATGGTCAAGCTATTAGCTATAAAATGCCAGATGAAACCT
F96V_r	AGCTTGACCATA <u>AC</u> TCGAGATGATTGTTGCCGGCAATAT
F96W_f	CTCG <u>AT</u> GGTATGGTCAAGCTATTAGCTATAAAATGCCAGATGAAACCT
F96W_r	AGCTTGACCATA <u>AC</u> TCGAGATGATTGTTGCCGGCAATAT
F96Y_f	CTCG <u>AT</u> CTATGGTCAAGCTATTAGCTATAAAATGCCAGATGAAACCT
F96Y_r	AGCTTGACCATA <u>AT</u> TCGAGATGATTGTTGCCGGCAATAT
Q278A_f	GCTTAT <u>GC</u> GTACAGCAATTAAATTAGCGCAGCACCAATGCGC
Q278A_r	AATTGCTGTGAC <u>GC</u> CATAAGCAGGTAATGTTGGTACATCAACAT

Q278C_f	GCTTATT <u>GCTCACAGCAATTAAATTAGCGCAGCACCAAATGCGC</u>
Q278C_r	AATTGCTGTGA <u>ACGATAAGCAGGTAAATGTTGGTACATCAACAT</u>
Q278D_f	GCTTAT <u>GACTCACAGCAATTAAATTAGCGCAGCACCAAATGCGC</u>
Q278D_r	AATTGCTGT <u>GACTGATAAGCAGGTAAATGTTGGTACATCAACAT</u>
Q278E_f	GCTTAT <u>GAATCACAGCAATTAAATTAGCGCAGCACCAAATGCGC</u>
Q278E_r	AATTGCTGT <u>GACTTATAAGCAGGTAAATGTTGGTACATCAACAT</u>
Q278F_f	GCTTATT <u>TCACAGCAATTAAATTAGCGCAGCACCAAATGCGC</u>
Q278F_r	AATTGCTGT <u>GAAAGATAAGCAGGTAAATGTTGGTACATCAACAT</u>
Q278G_f	GCTTAT <u>GGTCACAGCAATTAAATTAGCGCAGCACCAAATGCGC</u>
Q278G_r	AATTGCTGT <u>GACCAATAAGCAGGTAAATGTTGGTACATCAACAT</u>
Q278H_f	GCTTAT <u>CACTCACAGCAATTAAATTAGCGCAGCACCAAATGCGC</u>
Q278H_r	AATTGCTGT <u>GAGTGATAAGCAGGTAAATGTTGGTACATCAACAT</u>
Q278I_f	GCTTAT <u>ATCTCACAGCAATTAAATTAGCGCAGCACCAAATGCGC</u>
Q278I_r	AATTGCTGT <u>GATAAGATAAGCAGGTAAATGTTGGTACATCAACAT</u>
Q278K_f	GCTTATA <u>AAATCACAGCAATTAAATTAGCGCAGCACCAAATGCGC</u>
Q278K_r	AATTGCTGT <u>GATTATAAGCAGGTAAATGTTGGTACATCAACAT</u>
Q278L_f	GCTTAT <u>CTGCACAGCAATTAAATTAGCGCAGCACCAAATGCGC</u>
Q278L_r	AATTGCTGT <u>GAGACATAAGCAGGTAAATGTTGGTACATCAACAT</u>
Q278M_f	GCTTAT <u>ATGTCACAGCAATTAAATTAGCGCAGCACCAAATGCGC</u>
Q278M_r	AATTGCTGT <u>GATAACATAAGCAGGTAAATGTTGGTACATCAACAT</u>
Q278N_f	GCTTATA <u>ACTCACAGCAATTAAATTAGCGCAGCACCAAATGCGC</u>
Q278N_r	AATTGCTGT <u>GATTGATAAGCAGGTAAATGTTGGTACATCAACAT</u>
Q278P_f	GCTTAT <u>CCGTACAGCAATTAAATTAGCGCAGCACCAAATGCGC</u>
Q278P_r	AATTGCTGT <u>GAGGCATAAGCAGGTAAATGTTGGTACATCAACAT</u>
Q278R_f	GCTTAT <u>CGTCACAGCAATTAAATTAGCGCAGCACCAAATGCGC</u>
Q278R_r	AATTGCTGT <u>GAGCAATAAGCAGGTAAATGTTGGTACATCAACAT</u>
Q278S_f	GCTTATT <u>CTCACAGCAATTAAATTAGCGCAGCACCAAATGCGC</u>
Q278S_r	AATTGCTGT <u>GAAGAATAAGCAGGTAAATGTTGGTACATCAACAT</u>
Q278T_f	GCTTAT <u>ACCTCACAGCAATTAAATTAGCGCAGCACCAAATGCGC</u>
Q278T_r	AATTGCTGT <u>GATGGATAAGCAGGTAAATGTTGGTACATCAACAT</u>
Q278V_f	GCTTAT <u>GTTCACAGCAATTAAATTAGCGCAGCACCAAATGCGC</u>
Q278V_r	AATTGCTGT <u>GACAATAAGCAGGTAAATGTTGGTACATCAACAT</u>
Q278W_f	GCTTAT <u>GGTCACAGCAATTAAATTAGCGCAGCACCAAATGCGC</u>
Q278W_r	AATTGCTGT <u>GAACCATAAGCAGGTAAATGTTGGTACATCAACAT</u>
Q278Y_f	GCTTATT <u>ACTCACAGCAATTAAATTAGCGCAGCACCAAATGCGC</u>
Q278Y_r	AATTGCTGT <u>GAATGATAAGCAGGTAAATGTTGGTACATCAACAT</u>
E417A_f	CAGAT <u>CGAACCCAATTATCTCTGATGTTAAAGAGTATCCAGGTCT</u>
E417A_r	GATAATT <u>GGGTCGCATCTGGTCAATGCCATCGCAC</u>
E417C_f	CAGATT <u>GCAACCCAATTATCTCTGATGTTAAAGAGTATCCAGGTCT</u>
E417C_r	GATAATT <u>GGGTAACGATCTGGTCAATGCCATCGCAC</u>
E417D_f	CAGAT <u>GACAACCCAATTATCTCTGATGTTAAAGAGTATCCAGGTCT</u>
E417D_r	GATAATT <u>GGGTCGATCTGGTCAATGCCATCGCAC</u>

E417F_f	CAGATTCAACCCATTATCTGATGTTAAAGAGTATCCAGGTCT
E417F_r	GATAATTGGGTT <u>AAG</u> ATCTGGTCAATGCCATCGCAC
E417G_f	CAGAT <u>GG</u> TAACCCATTATCTGATGTTAAAGAGTATCCAGGTCT
E417G_r	GATAATTGGGTT <u>CCA</u> ATCTGGTCAATGCCATCGCAC
E417H_f	CAGAT <u>CACA</u> ACCCATTATCTGATGTTAAAGAGTATCCAGGTCT
E417H_r	GATAATTGGGTT <u>GT</u> GATCTGGTCAATGCCATCGCAC
E417I_f	CAGAT <u>ATCA</u> ACCCATTATCTGATGTTAAAGAGTATCCAGGTCT
E417I_r	GATAATTGGGTT <u>AGA</u> TCTGGTCAATGCCATCGCAC
E417K_f	CAGAT <u>AAA</u> ACCCATTATCTGATGTTAAAGAGTATCCAGGTCT
E417K_r	GATAATTGGGTT <u>TT</u> TATCTGGTCAATGCCATCGCAC
E417L_f	CAGAT <u>CTGA</u> ACCCATTATCTGATGTTAAAGAGTATCCAGGTCT
E417L_r	GATAATTGGGTT <u>GAC</u> ATCTGGTCAATGCCATCGCAC
E417M_f	CAGAT <u>ATGA</u> ACCCATTATCTGATGTTAAAGAGTATCCAGGTCT
E417M_r	GATAATTGGGTT <u>AC</u> ATCTGGTCAATGCCATCGCAC
E417N_f	CAGAT <u>ACA</u> ACCCATTATCTGATGTTAAAGAGTATCCAGGTCT
E417N_r	GATAATTGGGTT <u>TTG</u> ATCTGGTCAATGCCATCGCAC
E417P_f	CAGAT <u>CCG</u> AACCCATTATCTGATGTTAAAGAGTATCCAGGTCT
E417P_r	GATAATTGGGTT <u>GGC</u> ATCTGGTCAATGCCATCGCAC
E417Q_f	CAGAT <u>CAGA</u> ACCCATTATCTGATGTTAAAGAGTATCCAGGTCT
E417Q_r	GATAATTGGGTT <u>GTC</u> ATCTGGTCAATGCCATCGCAC
E417R_f	CAGAT <u>CGT</u> AACCCATTATCTGATGTTAAAGAGTATCCAGGTCT
E417R_r	GATAATTGGGTT <u>GCA</u> ATCTGGTCAATGCCATCGCAC
E417S_f	CAGAT <u>CTA</u> ACCCATTATCTGATGTTAAAGAGTATCCAGGTCT
E417S_r	GATAATTGGGTT <u>AGA</u> ATCTGGTCAATGCCATCGCAC
E417T_f	CAGAT <u>ACCA</u> ACCCATTATCTGATGTTAAAGAGTATCCAGGTCT
E417T_r	GATAATTGGGTT <u>GG</u> GATCTGGTCAATGCCATCGCAC
E417V_f	CAGAT <u>GTT</u> ACCCATTATCTGATGTTAAAGAGTATCCAGGTCT
E417V_r	GATAATTGGGTT <u>CAA</u> ATCTGGTCAATGCCATCGCAC
E417W_f	CAGAT <u>GG</u> AACCCATTATCTGATGTTAAAGAGTATCCAGGTCT
E417W_r	GATAATTGGGTT <u>ACC</u> ATCTGGTCAATGCCATCGCAC
E417Y_f	CAGAT <u>TAC</u> AACCCATTATCTGATGTTAAAGAGTATCCAGGTCT
E417Y_r	GATAATTGGGTT <u>ATG</u> ATCTGGTCAATGCCATCGCAC

Table S2. The rank of docking results of each ligand.

Ligand input	Rank	Docked energy
L-Ala	Alanine1_1	-3.29
	Alanine1_2	-3.26
	Alanine1_3	-3.03
	Alanine1_4	-2.93
	Alanine1_5	-2.91
	Alanine1_6	-2.80
	Alanine1_7	-2.78
	Alanine1_8	-2.68
	Alanine1_9	-2.67
	Alanine1_10	-2.48
L-Val	Valine1_1	-4.82
	Valine1_2	-4.80
	Valine1_3	-4.80
	Valine1_4	-4.75
	Valine1_5	-4.74
	Valine1_6	-4.71
	Valine1_7	-4.69
	Valine1_8	-4.66
	Valine1_9	-4.54
	Valine1_10	-4.38
L-Leu	Leucine1_1	-4.70
	Leucine1_2	-4.58
	Leucine1_3	-4.54
	Leucine1_4	-4.32
	Leucine1_5	-4.00
	Leucine1_6	-3.93
	Leucine1_7	-3.91
	Leucine1_8	-3.83
	Leucine1_9	-3.72
	Leucine1_10	-3.71
L-Phe	Phenylalanine1_1	-6.26
	Phenylalanine1_2	-5.83
	Phenylalanine1_3	-5.70
	Phenylalanine1_4	-5.63
	Phenylalanine1_5	-5.52
	Phenylalanine1_6	-5.50
	Phenylalanine1_7	-5.07
	Phenylalanine1_8	-4.92
	Phenylalanine1_9	-4.90

	Phenylalanine1_10	-4.78
L-Trp	Tryptophan1_1	-7.04
	Tryptophan1_2	-6.51
	Tryptophan1_3	-6.39
	Tryptophan1_4	-6.26
	Tryptophan1_5	-6.25
	Tryptophan1_6	-6.15
	Tryptophan1_7	-6.13
	Tryptophan1_8	-5.93
	Tryptophan1_9	-5.73
	Tryptophan1_10	-5.52
L-Ile	Isoleucine1_1	-4.53
	Isoleucine1_2	-4.51
	Isoleucine1_3	-4.48
	Isoleucine1_4	-4.33
	Isoleucine1_5	-4.06
	Isoleucine1_6	-4.02
	Isoleucine1_7	-3.96
	Isoleucine1_8	-3.96
	Isoleucine1_9	-3.80
	Isoleucine1_10	-3.60
L-Asp	Aspartic_acid_1_1	-5.12
	Aspartic_acid_1_2	-5.09
	Aspartic_acid_1_3	-4.96
	Aspartic_acid_1_4	-4.74
	Aspartic_acid_1_5	-4.42
	Aspartic_acid_1_6	-4.33
	Aspartic_acid_1_7	-4.31
	Aspartic_acid_1_8	-4.27
	Aspartic_acid_1_9	-4.12
	Aspartic_acid_1_10	-3.95

Table S3. The retention times of high-performance liquid chromatography (HPLC).

Corresponding Substrate	Keto acids	Retention time of keto acids (min)
L-Ala	Pyruvic acid	9.627
L-Asp	Oxalacetic acid	8.067
L-Leu	4-Methyl-2-oxovaleric acid	13.167
L-Ile	3-Methyl-2-oxovaleric acid	11.360
L-Phe	Phenylpyruvic acid	18.233
L-Trp	Indole-3-pyruvic acid	17.326
L-Val	3-Methyl-2-oxobutanoic acid	10.287

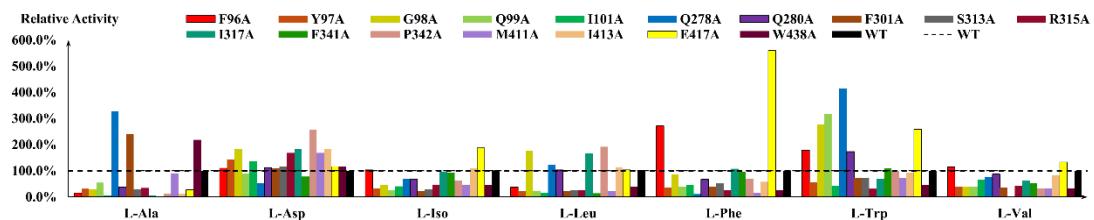


Figure S1. The results of alanine scanning. The black dotted line indicates the relative activity (100%) of wild-type PmiLAAD.

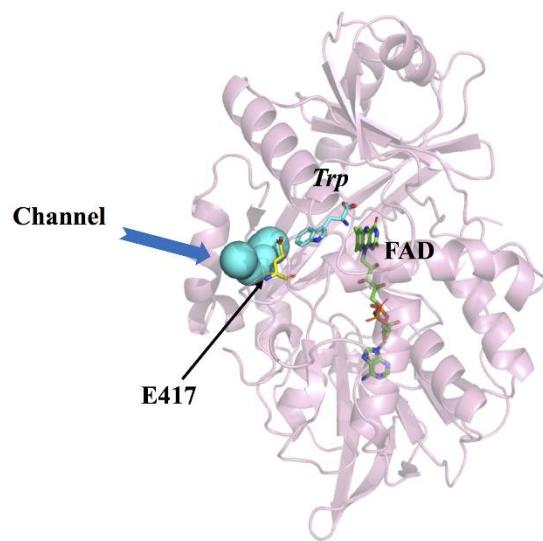


Figure S2. Structure of PmiLAAD. The substrate channel is shown in translucent sky blue. The residue E417 located in the substrate channel is showed as yellow sticks.

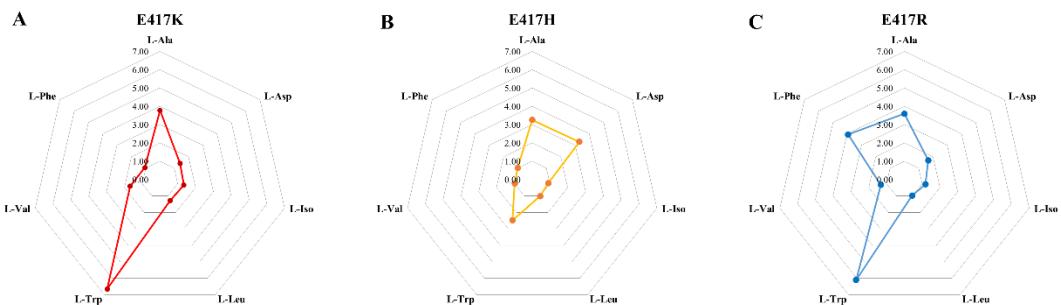


Figure S3. Relative activity of PmLAAD^{E417K} (A), PmLAAD^{E417H} (B), and PmLAAD^{E417R} (C) toward seven kinds of L-amino acids.

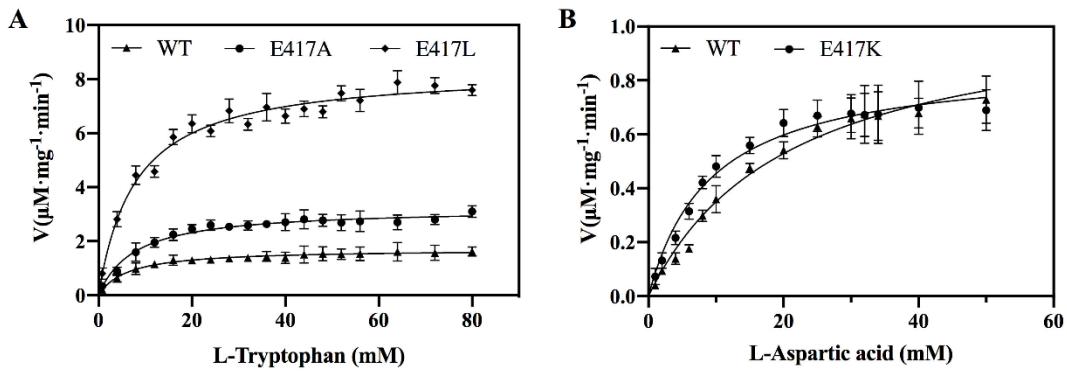


Figure S4. Plots for the apparent kinetic parameter determination of PmiLAAD variants and wild-type. (A) Plots for kinetic parameter determination of PmiLAAD^{E417A}, PmiLAAD^{E417L} and PmiLAAD wild-type. The K_m and V_{max} of PmiLAAD^{E417A} toward L-Trp were 7.92 ± 1.59 mM and 3.25 ± 0.14 $\mu\text{M}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$, respectively. The K_m and V_{max} of PmiLAAD^{E417L} toward L-Trp were 7.33 ± 1.76 mM and 8.53 ± 0.36 $\mu\text{M}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$, respectively. The K_m and V_{max} of PmiLAAD wild-type toward L-Trp were 6.010 ± 0.92 mM and 1.76 ± 0.05 $\mu\text{M}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$, respectively. (B) Plots for kinetic parameter determination of PmiLAAD^{E417K} and PmiLAAD wild-type. The K_m and V_{max} of PmiLAAD^{E417K} toward L-Asp were 11.61 ± 2.11 mM and 0.94 ± 0.06 $\mu\text{M}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$, respectively. The K_m and V_{max} of PmiLAAD wild-type toward L-Asp were 21.88 ± 5.20 mM and 1.17 ± 0.133 $\mu\text{M}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$, respectively. Error bars represent the standard deviation of three independent experiments.