Table S1. The catalytic parameters of MaiA and AspA

Enzyme	Specific activity (U/mg)	Half-life[a] (d)	pH_{opt}	T_{opt} (℃)
AspA	169.8	28	8.5	37
Original MaiA	41.1	2.5	8.0	45

[a]The experiments were performed at room temperature.

Table S2. Primers used for the construction of MaiA variants

Primers	Sequence (5'→3')
A27M-F	CCGGCGATGCTGATGGCGCGCCAG
A27M-R	TCGGCGTCCATCGCCGCCAATT
I32F-F	TGGCGGCGCGCCAGCTGTTCCGCCCGGAGC
I32F-R	TCGGCGTCCATCGCCGCCAATT
N50Y-F	GCGCATGAAACACGTCTACAAAGAAGAAT
N50Y-R	TCGGCGTCCATCGCCGCCAATT
G77A-F	GGTCGACGTGCTCGCCTACGCCTG
G77A-R	CCATGATGGCCACCAGGCAG
Q96E-F	CACCGCGAATCGGAGGCCCGGCTGG
Q96E-R	GATGACCGGCGCGGCGGCC
A100M-F	GGCCCGGCTGATGCAGGTGACGAAAG
A100M-R	GATGACCGGCGCGGCGGCC
A109F-F	GACGAAAGACAATCAGGCCTTCGCGCCGGTCAT
A109F-R	CGCGCCGGCGCTGCTGATGAC
N121M-F	GGCGCGCTGGTCATGGGCCTGAAGGTG
N121M-R	TCCATCTCGGCGACGATCC
A212F-F	GACGGTGGAGTTCCAAACCGGCAAAC
A212F-R	CGGCGCCGGGAACGATCGGT
E234G-F	CTGACCGCGCTGGGCCTGGAACCGAT
E234G-R	CGGCGCCGGGAACGATCGGT

The mutation site were represented with an underline.

Table S3. Primers used for plasmid optimization

Primers	Sequence (5'→3')
$RBS^{weakened}$-F	CTTAGTATATTAGTTAAGTATAAGCAGGAGATATACATATTCATATGTC
$RBS^{weakened}$-R	GACATATGAATATGTATATCTCCTGCTTATACTTAACTAATATACTAAG
AspA/ΔT_7-F	GGATGCTGCTGGCTACCCTGTGGAACCCCATCTTAGTATATTAGTTAAG TATAAGAAGGAG
AspA/ΔT_7-R	TTCCACAGGGTAGCCAGCAGCATCCATTTCGATTATGCGGCCGTGTAC AA
MaiA/2T_7-F	CTAATACGACTCACTATAGGGGCGACCGCATCAGGCGCTCTATGCGAC TCCTGCATTAGGAAT
MaiA/2T_7-R	GAGCGCCTGATGCGGTCGCCCCTATAGTGAGTCGTATTAGAGGGAGAG CGTCGAGATCC
MaiA/3T_7-F	CAGGTCGTATAATACGACTCACTATAGGGGTCCACGAGTTGCGACTCC TGCATTAGGAAT
MaiA/3T_7-R	ACTCGTGGACCCCTATAGTGAGTCGTATTATACGACCTGTAGAGCGCCT GATGCGGTCG
MaiA/5T_7-F	GTCGTAACGTCATTCCAGTAATACGACTCACTATAGGCGACCGCATCA GGTCGTAACGTCATTCCAGTAATACGACT
MaiA/5T_7-R	CTGATGCGGTCGCCTATAGTGAGTCGTATTACTGGAATGACGTTACGA CCTGATGCGGTCGCCCC
2MaiA-F	CGCAGCCCGGACTCGGCCCCTGTAGAAATAATTTTG
2MaiA-R	CCAACGCGCAGCCCGGACACGATTACTTTCTGTTCGACT
2MaiA-vector-F	CCGAGTCCGGGCTGCGCGACTTAAGCATTATGCGGC
2MaiA-vector-R	GTCCGGGCTGCGCGTTGGAACAGAAAGTAATCGTATTGTACACG
3MaiA-F	GACGCACAATCCCACTATCCCATAATGCTTAAGTCGCGCAGC
3MaiA-R	GCGCACACCGTGGGGCCAGGCGGACACGATTACTTTCTGTTCGACTTA AGC
3MaiA-vector-F	CCTGGCCCCACGGTGTGCGCTGCGCGTTGGAACAGAAAGTAATC
3MaiA-vector-R	GGATAGTGGGATTGTGCGTCCGGACACGATTACTTTCTGTTCGACTTAA G
4MaiA-F	GGATCCCGCGAAATTAATACGAATGAACTCGAACATAGTCTTAAGGAG GT
4MaiA-R	TCGATGCTCTGGATCGCGAATTCAATAAGCGCCGGACAGCAG
4MaiA-vector-F	ATTCGCGATCCAGAGCATCGACTGCGCGTTGGAACAGAAAGTAATCGT ATTGTACAC
4MaiA-vector-R	ATTCGTATTAATTTCGCGGGATCCGCACACCGTGGGGCCAG

The mutation site were represented with an underline.

Table S4. Candidates of MaiA variants

Variants	Fold X (kcal/mol)	Rosetta (kcal/mol)	Conserved
A27M	-1.25	-16.81	N
Q30E	-0.57	-	N
I32F	-1.41	-25.80	N
N50Y	-1.36	-21.38	N
G77A	-1.06	-12.38	N
Q96E	-0.69	-	N
A100M	-1.66	-14.34	N
Q107G	0.05	-	N
A109F	-1.01	-14.36	N
A110L	-1.42	-7.33	N
S114T	-1.53	-	Y
V120I	-1.06	-2.97	N
N121M	-1.44	-19.88	N
G122S	-1.33	-7.21	N
V134I	-1.18	-8.39	N
Q150E	0.03	-	N
D169E	-0.81	-	N
G180Q	-1.04	-3.04	N
T209L	-1.18	-11.28	N
A212F	-1.17	-15.97	N
A228M	-2.02	-11.29	N
T231M	-1.52	-2.31	N
A232M	-1.26	-5.28	N
E234G	-0.62	-	N
I238M	-1.06	-9.15	N
A244F	-1.32	-3.07	N

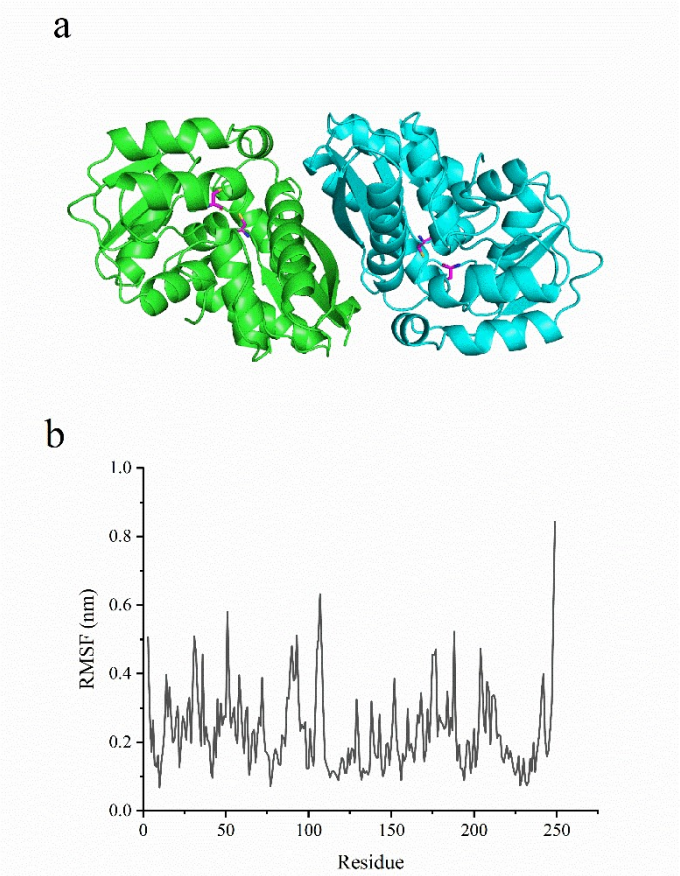

Fig. S1. Rational molecular redesign for the original MaiA. (a) The three-dimensional structure of the original MaiA that was generated using the SWISS-MODEL software. MaiA from *Pseudomonas putida* S16 (PDB: 4fq7) was used as a template, which showed 71.89% sequence identity. The active center was represented by two pink Cys residues involved in catalysis. (b) RMSF (root mean square fluctuation). Molecular dynamics simulations were performed on the original MaiA using NAMD 2.14 software at a temperature of 300 K.

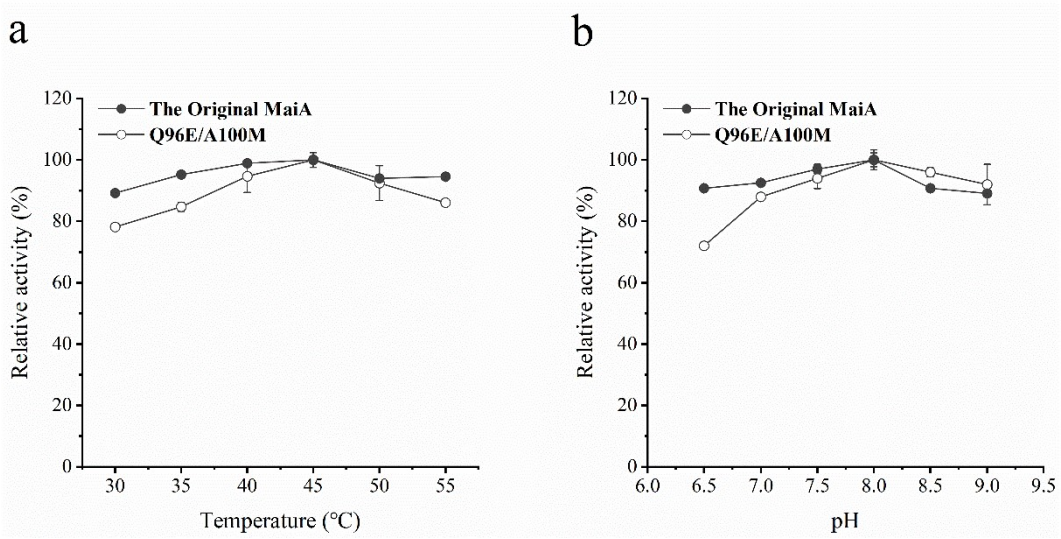

Fig. S2. The effect of temperature and pH on the activity of the Q96E/A100M variant. (a) The enzyme activity was assessed at various temperatures ranging from 30°C to 55°C to identify the optimal temperature. (b) The enzyme activity was evaluated at pH values of 6.5, 7.0, 7.5, 8.0, 8.5, and 9.0 to determine the optimal pH. These experiments were performed using pure enzymes, with the highest activity set as a reference of 100%. The reaction was carried out in Na_2HPO_4-KH_2PO_4 buffer (20 mmol/L, pH 8.0) at 40°C for 10 min, followed by heat inactivation at 100°C for 10 min. After centrifugation, the supernatant was collected, appropriately diluted, and subjected to HPLC to determine the concentration of fumaric acid.

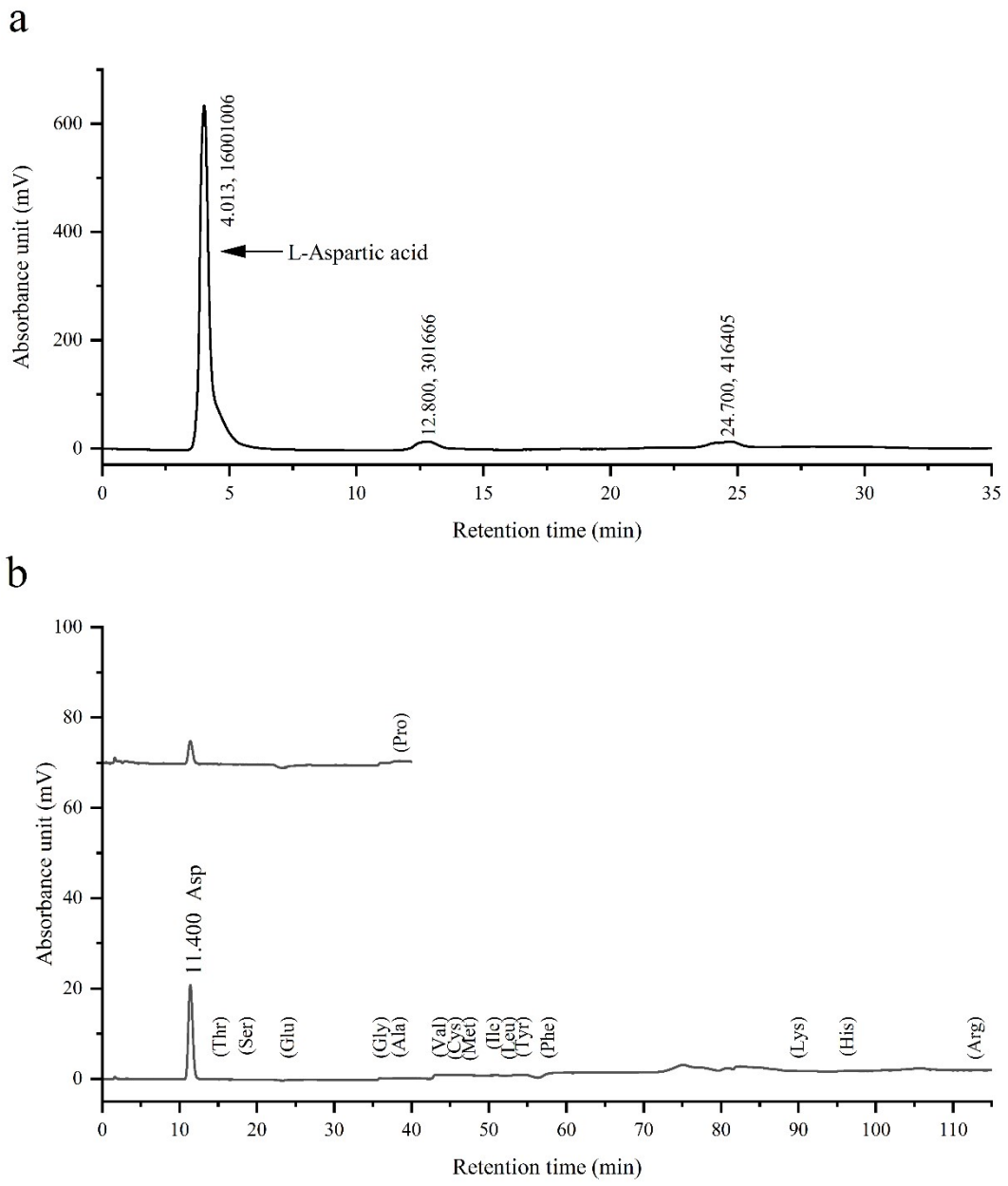

Fig. S3. Identification of the product purity. (a) The product of whole-call catalysis was analyzed by HPLC using a La Chrom C18 column (4.6 mm×250 mm, 5 μm) after PITC derivatization. (b) After precipitation and drying, the product is dissolved in hydrochloric acid and analyzed for purity using an amino acid analyzer (Hitachi L-8900, Japan).