Green Chemistry

Supplementary information

Supplementary method

Calculation of the amount of wastewater

The titer of L-alanine produced by the fermentation method is about 120.8 g/L (Applied Biochemical Biotechnology, 178:324-337), and the wastewater for producing 1 ton of L-alanine is 1/0.12=8.3 tons.

Whole-cell catalysis was carried out in a reactor containing 2.5 M maleic acid and recombinant cells with OD_{600}=20. The OD_{600} of cells cultivated by high-density fermentation was about 100, and cells were harvested by centrifuge and used for 5 batches of biocatalysis. During the catalysis-extraction circulation process, one liter of the reaction solution was re-utilized, and 5 batches of fresh cells were supplemented. After 5 batches of biocatalysis, the final reaction was precipitated with methanol (about 600 mL) on ice. The total yield of L-alanine was 890 g, and the total amount of wastewater was approximate 2.5 L (1 liter was from the fermentation broth, and 1.5 liters was from the reaction and precipitation solution). Thus, the wastewater for 1 ton L-alanine is 2.5/0.89=2.8 tons.

Supplementary Tables

Table S1. Primers constructed for the triple-enzyme cascade

Plasmids	Primes	Sequences (5'-3')
pAM	EcoRI-ASD-F	gcgccgaattcgatgagcaaggattatcagag
	NotI-ASD-R	tttgcggccgctcagcgcttgttcc
	NdeI-MaiA-F	gcgcatatgatgagcaaccactac
	XhoI-MaiA-R	tttctcgagtcaataagcgccgg
pMA	BamHI-MaiA-F	tttggatccatgagcaaccactaccgc
	HindIII-MaiA-R	gttaagctttcaataagcgccggacag
	NdeI-ASD-F	catatgatgagcaaggattatcagagtctggcgaac
	XhoI-ASD-R	tttctcgagtcagcgcttgttcc

Note: restriction sites are underlined.

28 **Table S2. Conversion rates of the whole cell catalysis**

Host strain	Biomass (OD_{600})	Concentration of substrate (M)	Time (h)	Molar conversion (%)
E. coli BL21	20	2.0	6	22.2
			10	71.0
			22	86.8
E. coli BL21 -△*fumA/fumC*	20	2.0	6	68.9
			10	81.2
			22	89.0
E. coli BL21 -△*fumA/fumC*-T_7/AspA	10	2.0	6	71.3
			10	93.6
	20	2.0	4	78.1
			6	94.9
	20	2.5	6	69.6
			10	95.6
	20	3.0	6	31.5
			10	69.5
			15	95.6

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Supplementary Figures

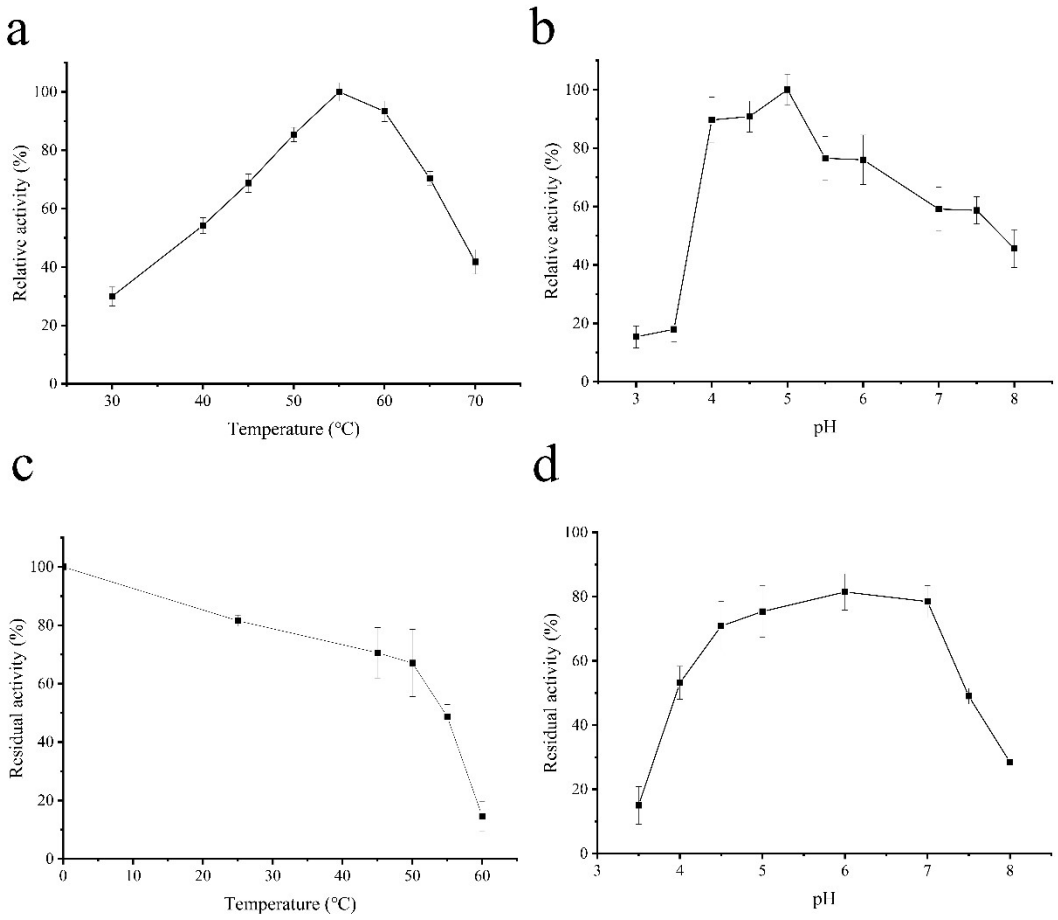

Fig S1. Enzymatic properties of purified *At*ASD. (a) The optimum temperature. The activities of *At*ASD were determined in phosphate buffer (pH 5.0) at various temperatures, and the highest activity was plotted as 100%. (b) The optimum pH. The activities of *At*ASD were determined at various pH values (phosphate buffer) and 55℃, and the highest activity was plotted as 100%. (c) The thermostability. *At*ASD was incubated at various temperatures for 30 min, and the activity of *At*ASD without treatment was plotted as 100%. (d) The pH stability. *At*ASD was incubated in phosphate buffer (pH 3.0-8.0) on ice overnight, and the activity of *At*ASD without treatment was plotted as 100%. All reactions contained 0.5 mM PLP and 1 mM α–ketoglutaric acid.

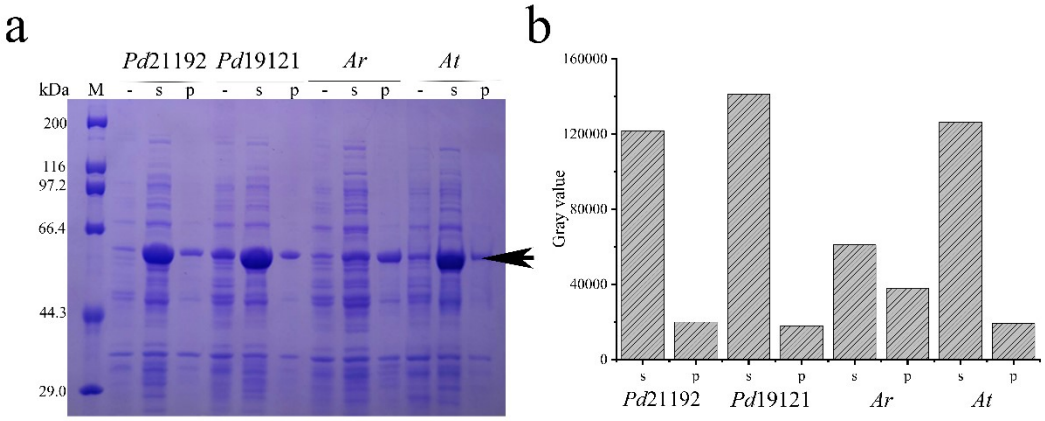

Fig S2. Expression level analysis of recombinant ASDs. (a) SDS-PAGE analysis of recombinant ASDs. -: noninduced cells; S: supernatants of cell lysates; P: precipitation of cell lysates. Recombinant ASDs were indicated by an arrow. (b) Semiquantitative analysis by ImageJ software. The gray value of the target protein was plotted as Y-axis.

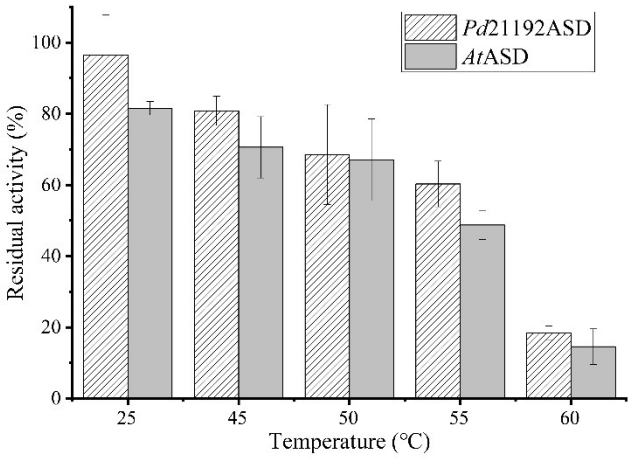

Fig S3. Comparison of thermostability of purified *At*ASD and *Pd*21192ASD. *At*ASD and *Pd*21192ASD were incubated at various temperatures for 30 min, and the activities of purified *At*ASD and *Pd*21192ASD without treatment were plotted as 100%. The activity of *At*ASD was determined at pH 5.0 and 55℃, and the activity of *Pd*21192ASD was determined at pH 5.0 and 45℃. All reactions contained 0.5 mM PLP and 1 mM α-ketoglutaric acid.

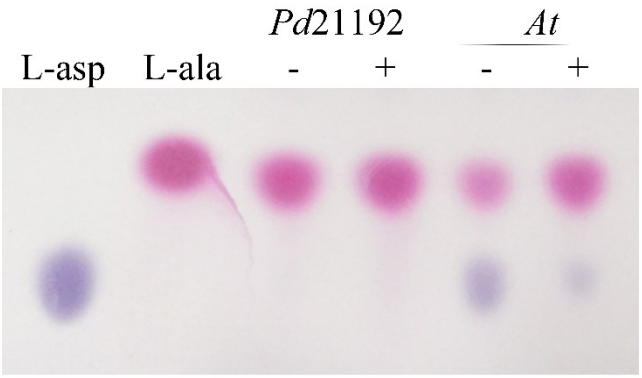

Fig S4. Effects of PLP on the catalytic abilities of recombinant strains expressing *At*ASD and *Pd*21192ASD. The amounts of L-alanine and L-aspartic acid synthesized from fumaric acid were detected by thin plate chromatography. The reaction was carried out at pH 5.0 and 37℃ for 4 h. The biomass was OD_{600}=10, and the substrate of fumaric acid was 0.2 M. The standards of 0.1 M L-aspartic acid (L-asp) and L-alanine (L-ala) were dotted in the left columns. -: reactions without the addition of PLP; +: reactions containing 0.1 mM PLP.

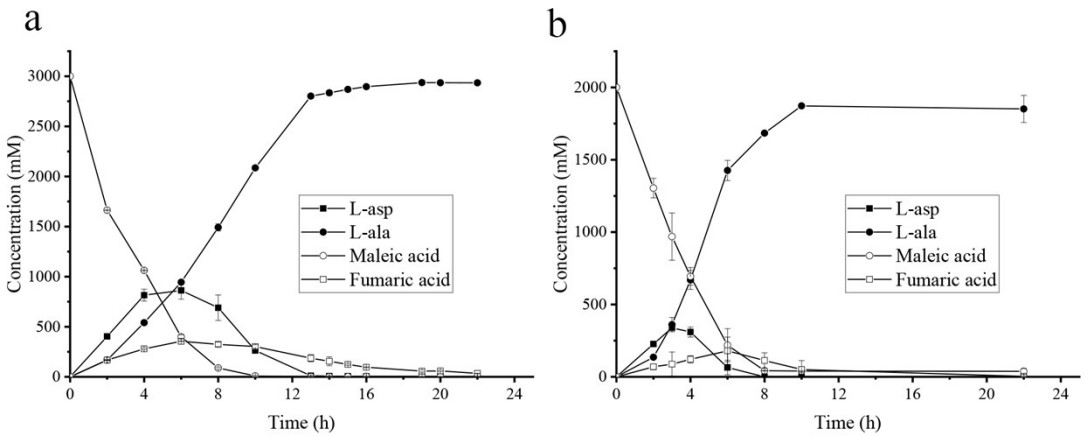

Fig S5. Whole-cell catalysis using the recombinant strain *E. coli* BL21-△*fumA*/*fumC*-T_7/AspA harboring pAM. (a) The biomass was OD_{600}=20 and the substrate of maleic acid was 3.0 M. (b) The biomass was OD_{600}=10 and the substrate of maleic acid was 2.0 M. The reaction was carried out at pH 7.5 and 37°C. The concentrations of L-alanine (L-ala, solid circle), L-aspartic acid (L-asp, solid square), fumaric acid (open square), and maleic acid (open circle) were measured by HPLC.