Supplementary Methods | HPLC gradient program

Quantification of pyridoxal 5′-phosphate (PLP) by HPLC

PLP was quantified using an Agilent 1260 HPLC system. Samples were prepared by centrifuging fermentation broth at 9000 rpm, 4 °C for 10 min, and the supernatant was filtered through a 0.22 μm membrane prior to injection.

Chromatographic separation was performed on a C18 column (250 × 4.6 mm, 5 μm) at 30 °C with a flow rate of 1.0 mL/min and an injection volume of 10 μL. PLP detection was carried out at 388 nm.

Mobile phases

Mobile phases were prepared as follows: A, methanol; B, ultrapure water; C, acetonitrile/water (30:70, v/v) with pH adjusted to 6.0 using KH_2PO_4.

Column conditioning and elution program

Before analysis, the column was equilibrated with methanol/water (30:70, v/v) for 20 min, then switched to the above mobile-phase system and equilibrated for 30 min.

Supplementary Table S1 Strains & plasmids

Table S1. Strains and plasmids used in this study.

(A) Strains

Strain	**Description**
WT	*Corynebacterium glutamicum ssp. lactofermentum* CGMCC 1.542; parental strain used as the production host
S1	WT harboring pEC-Ptrc-*ecepd* (overexpression of *epd* from *E. coli*)
S2	WT harboring pEC-Ptrc-*ecepd-ecpdxB* (overexpression of *epd*/*pdxB* from *E. coli*)
S3	WT harboring pEC-Ptrc-*ecepd-ecpdxB-smpdxA-smpdxJ* (additional *pdxA*/*pdxJ* from *S. meliloti*)
E. coli DH5α	Used for plasmid construction

(B) Plasmids

Plasmid	**Key elements**	**Marker**
pEC-Ptrc-*ecepd*	Ptrc promoter; RBS AAGGAGGATATACAT; rrnB T1 + T7Te terminators; gene from *E. coli*	Kan
pEC-Ptrc-*ecepd-ecpdxB*	As above + *pdxB* from *E. coli*	Kan

Plasmid	Key elements	Marker
pEC-Ptrc-*ecepd-ecpdxB-smpdxA-smpdxJ*	As above + *pdxA/pdxJ* from *S. meliloti*	Kan

Supplementary Table S2 Primers

Table S2. Primers used in this study (5′→3′).

Primer	Sequence (5′→3′)
EPD-F	AAGCAGAAGCTTCGCGAAATTAATACGACTCACTATAGGG
EPD-R	TATGTATATCCTCCTTGTAAGTTTTTATTTTGCAGCCCAAA
PDXB-F	TCAAAAATGCTTGGTTATGTATATCCTCCTTGTAACTTCAA
PDXB-R	CCGCCTCCACCTTATATTAATCGATTCATTAAAAAGGGTGT
PDXA-F	TGACCAAGACGCGTCGGTTATGTATATCCTCCTTGTAACTTC
PDXA-R	AGGGCGTAGCCACCTTTATATTAATCGATTCATTAAAAAGGT
PDXJ-F	AACCAGATGTCGTGCCGGTTATGTATATCCTCCTTGTAACTTC
PDXJ-R	CGAGCTCGAATTCTGATTATATTAATCGATTCATTAAAAAGGT
pEC-F	AGTTACAAGGAGGATATACATAACCAAGCATTTTGATCGTAA
pEC-R	ATGAATCGATTAATATAAGGTGGAGGCGGTTATTGCTAGTTAT

Supplementary Table S3 Media compositions

Table S3. Media compositions used in this study (Article A).

Medium	Component	Amount (per L)	Typical use
LB	Yeast extract	5 g/L	Routine culture (if applicable)
	Tryptone	10 g/L	Routine culture (if applicable)
	NaCl	5 g/L	Routine culture (if applicable)
EPO	Yeast extract	5 g/L	Preparation of competent cells (if applicable)
	Tryptone	10 g/L	Preparation of competent cells (if applicable)
	NaCl	10 g/L	Preparation of competent cells (if applicable)
	Glycine	30 g/L	Preparation of competent cells (if

			applicable)
	Tween 80	10 g/L	Preparation of competent cells (if applicable)
LBHIS	Yeast extract	2.5 g/L	Recovery/routine cultivation
	Tryptone	5 g/L	Recovery/routine cultivation
	NaCl	5 g/L	Recovery/routine cultivation
	Brain heart infusion (BHI)	18.5 g/L	Recovery/routine cultivation
	Sorbitol	91 g/L	Recovery/routine cultivation
LBG	Yeast extract	5 g/L	Seed culture
	Tryptone	10 g/L	Seed culture
	NaCl	10 g/L	Seed culture
	Glucose	20 g/L	Seed culture
Fermentation medium	Glucose	65 g/L	Shake-flask fermentation
	Urea	5 g/L	Shake-flask fermentation
	Corn steep liquor powder	16 g/L	Shake-flask fermentation
	K2HPO4	1 g/L	Shake-flask fermentation
	KH2PO4	1.23 g/L	Shake-flask fermentation
	MgSO4·7H2O	29.4 mg/L	Shake-flask fermentation
	CaCl2·2H2O	0.4 mg/L	Shake-flask fermentation
	Biotin	0.4 mg/L	Shake-flask fermentation
	Vitamin B1 (thiamine)	0.4 mg/L	Shake-flask fermentation
	Trace element solution (1000×)	0.2% (v/v)	Shake-flask fermentation

Notes: Solid media were prepared by adding 1.5–2% (w/v) agar to the corresponding liquid media.