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Unveiling the Effect of *NCgl0580* Gene Deletion on 5-Aminolevulinic Acid Biosynthesis in *Corynebacterium glutamicum*

Table S1. Strains and plasmids used in this study.

Strains/Plasmids	Description	Source
Strains		
E. coli DH5α	Host for plasmid construction	Lab stock
J0	C. glutamicum ATCC13032△*ldhA*△*pqo*△*cat*△*pta*△*ackA*△*pck* P*ppc*::P*sod*	Lab stock
JT	J0 harboring pXPT	This study
JS	J0 harboring pXPS	This study
JP	J0 harboring pXPP	This study
JG	J0 harboring pXPG	This study
JSC	JS harboring pEC-XK99E	This study
JSC1	JS harboring pEC-NCgl0580	This study
JSC2	JS harboring pEC-NCgl2065	This study
JSD1	JS△*NCgl0580*	This study
JSD1C	JS△*NCgl0580* harboring pEC-XK99E	This study
JSD1C1	JS△*NCgl0580* harboring pEC-NCgl0580	This study
JSD2	JS△*NCgl2065*	This study
JSD3	JS derivative with the insertion of three termination codon TAA before 10th, 18th and 27th amino acids of *NCgl0580*	This study
JSD4	JS△*NCgl0581*	This study
JSD5	JS△*NCgl0580*△*NCgl0581*	This study
JSC3	JS harboring pEC-NCgl0581	This study
JSCZ1	JS harboring pEC-NCgl1445	This study
JSCZ2	JS harboring pEC-iolT1	This study
JSCZ3	JS harboring pEC-pyk2	This study
JSCZ4	JS harboring pEC-aceA	This study
JSCZ5	JS harboring pEC-afuABC	This study
JSCZ6	JS harboring pEC-pstSCAB	This study
JSCZ7	JS harboring pEC-ugpQ	This study
JSDZ1	JS △*sdhCAB*	This study
JSDZ2	JS △*gdhA*	This study
DZ1C	JS△*sdhCAB* harboring pEC-XK99E	This study
DZ1C1	JS△*sdhCAB* harboring pEC-NCgl1445	This study
DZ1C2	JS△*sdhCAB* harboring pEC-iolT1	This study
DZ1C3	JS△*sdhCAB* harboring pEC-pyk2	This study
DZ1C4	JS△*sdhCAB* harboring pEC-aceA	This study
DZ1C5	JS△*sdhCAB* harboring pEC-afuABC	This study
DZ1C6	JS△*sdhCAB* harboring pEC-pstSCAB	This study
DZ1C7	JS△*sdhCAB* harboring pEC-ugpQ	This study
DZ2C	JS△*gdhA* harboring pEC-XK99E	This study
DZ2C1	JS△*gdhA* harboring pEC-NCgl1445	This study
DZ2C2	JS△*gdhA* harboring pEC-iolT1	This study
DZ2C3	JS△*gdhA* harboring pEC-pyk2	This study
DZ2C4	JS△*gdhA* harboring pEC-aceA	
DZ2C5	JS△*gdhA* harboring pEC-afuABC	This study
DZ2C6	JS△*gdhA* harboring pEC-pstSCAB	This study
DZ2C7	JS△*gdhA* harboring pEC-ugpQ	This study

Plasmids		
pXMJ19	Cm^r, *C. glutamicum*/*E. coli* shuttle vector	Lab stock
pXK	Derived from pXMJ19, deletion of *tac* promoter and lacIq	Lab stock
pXPS	Derived from pXK, carrying hemA from *Rhodobacter palustris* with amino acid sequence mutation C75A/R365K under the control of *sod(C131T)* promoter	This study
pXPT	Derived from pXK, carrying hemA from *Rhodobacter palustris* with amino acid sequence mutation C75A/R365K under the control of *tuf* promoter	This study
pXPP	Derived from pXK, carrying hemA from *Rhodobacter palustris* with amino acid sequence mutation C75A/R365K under the control of *pgk* promoter	This study
pXPG	Derived from pXK, carrying hemA from *Rhodobacter palustris* with amino acid sequence mutation C75A/R365K under the control of *glyA* promoter	This study
pEC-XK99E	Kan^r; *C. glutamicum*/*E. coli* shuttle vector	Lab stock
pEC-NCgl0580	Derived from pEC-XK99E, for overexpression of *NCgl0580*	This study
pEC-NCgl2065	Derived from pEC-XK99E, for overexpression of *NCgl2065*	This study
pEC-NCgl0581	Derived from pEC-XK99E, for overexpression of *NCgl0581*	This study
pEC-NCgl1445	Derived from pEC-XK99E, for overexpression of *NCgl1445*	This study
pEC-iolT1	Derived from pEC-XK99E, for overexpression of *iolT1*	This study
pEC-pyk2	Derived from pEC-XK99E, for overexpression of *pyk2*	This study
pEC-aceA	Derived from pEC-XK99E, for overexpression of *aceA*	This study
pEC-afuABC	Derived from pEC-XK99E, for overexpression of *afuABC*	This study
pEC-pstSCAB	Derived from pEC-XK99E, for overexpression of *pstSCAB*	This study
pEC-ugpQ	Derived from pEC-XK99E, for overexpression of u*gpQ*	This study
pD-SacB	Kan^r; vector for gene deletion	Lab stock
pD-NCgl0580	pD-SacB containing upstream and downstream of gene *NCgl0580*	This study
pD-NCgl0581	pD-SacB containing upstream and downstream of gene *NCgl0581*	This study
pD-NCgl0580TAA	pD-SacB containing upstream and downstream of gene *NCgl0580* and insertion of three termination codon TAA before 10th, 18th and 27th amino acids of *NCgl0580*	This study
pD-gdhA	pD-SacB containing upstream and downstream of gene *gdhA*	This study
pD-sdhCAB	pD-SacB containing upstream and downstream of gene *sdhCAB*	This study

Table S2. Primers used in the construction of plasmids.

Primers	DNA sequence (5′ - 3′)	Digest Sites
pXPP-1	cgtgaggtaccggttgtttcctggtacgacaacg	*Kpn*I
pXPP-2	cgtagttcatgccgtactccttggagatttgattg	
pXPP-3	caaggagtacggcatgaactacgaagcctacttccgtc	
pXPP-4	ctacagaattcttaggcggcttttgcgaggcccac	*Eco*RI
pXPT-1	gctccggtacctggccgttaccctgcgaatgtccac	*Kpn*I
pXPT-2	ggaagtaggcttcgtagttcattgtatgtcctcctggacttcgtggtg	
pXPT-3	ccacgaagtccaggaggacatacaatgaactacgaagcctacttcc	
pXPT-4	ctacagaattcttaggcggcttttgcgaggcccac	*Eco*RI
pXPG-1	cgtgaggtaccgaatccattgtgcaccttagctactc	*Kpn*I
pXPG-2	tcgtagttcatcaggtcagctaacctttcacaagac	
pXPG-3	gttagctgacctgatgaactacgaagcctacttccgtc	
pXPG-4	ctacagaattcttaggcggcttttgcgaggcccac	*Eco*RI
pXPS-1	cgtgaggtacctagctgccaattattccgggcttgtg	*Kpn*I
pXPS-2	gtaggcttcgtagttcatgggtaaaaaatcctttcgtag	
pXPS-3	cctacgaaaggatttttacccatgaactacgaagcctacttc	
pXPS-4	ctacagaattcttaggcggcttttgcgaggcccac	*Eco*RI

NCgl0580-F	gcacgtctagaaaaggaggacaaccatgaataaacagtccgctgcag-tgtt	*Xba*I
NCgl0580-R	cgcgtcctgcaggttaactaggtgtgtgtactcg	*Sda*I
NCgl2065-F	gcacgtctagaaaaggaggacaaccgtgaatgatgctggcttgaagac	*Xba*I
NCgl2065-R	cgcgtcctgcaggctaggaggggcgtcgcaaag	*Sda*I
NCgl0581-F	gcacggagctcaaaggaggacaaccgtgctcaatctcaaccgcttacac	*Sac*I
NCgl0581-R	ctgcaggatcctcactctactagacgagcctcca	*Bam*HI
NCgl1445-F	gcacgggtaccaaaggaggacaaccatggcggtcaccgcaagaac	*Kpn*I
NCgl1445-R	cgcgttctagattagtggctagctttcctacccaaag	*Xba*I
iolT1-F	gcacgggtaccaaaggaggacaaccatggctagtaccttcattcagg	*Kpn*I
iolT1-R	cgcgtcctgcaggttagtgcacctttccttttcggatg	*Sda*I
pyk2-F	gcacgtctagaaaaggaggacaaccatgcaggcccgcctcaag	*Xba*I
pyk2-R	cgcgtcctgcaggttactcttcccagctcttcaccttg	*Sda*I
aceA-F	gcacgtctagaaaaggaggacaaccatgtcaaacgttggaaagccac	*Xba*I
aceA-R	cgcgtcctgcaggctagttgtggaactggccttcttc	*Sda*I
afuABC-F	gcacgggtaccaaaggaggacaaccatgtcttcgaagcacccttttg	*Kpn*I
afuABC-R	gctagcccgggttaatccgcgcggtagaccatc	*Sma*I
pstSCAB-F	gcacgggatccaaaggaggacaaccatgaacctcactcttaagcgctc	*Bam*HI
pstSCAB-R	gcacgtctagattatccgaagcggccggagatg	*Xba*I
ugpQ-F	gcacggagctcaaaggaggacaaccatgaaagtcatcgcgcaccgag	*Sac*I
ugpQ-R	gcacgggtaccttagtcttctttgccatgcgca	*Kpn*I
pD-NCgl0580-1	gctagcccgggactctactagacgagcctccaaataag	*Sma*I
pD-NCgl0580-2	ggtaagcctgcacggcccttgattattgccaaagaaac	
pD-NCgl0580-3	caagggccgtgcaggcttacctttggaag	
pD-NCgl0580-4	ctgcaggatcctggcatattgacgcctacaagtttg	*Bam*HI
pD-NCgl2065-1	gctagcccgggtgtccaagctcagtggggtgag	*Sma*I
pD-NCgl2065-2	ggtgtgggaaaccgatcacgacaccttacttcagc	
pD-NCgl2065-3	gtgtcgtgatcggtttcccacaccgccagtc	
pD-NCgl2065-4	ctgcatctagaaaggtgtaggaacgagactcaatac	*Xba*I
pD-NCgl0580TAA-1	ctacaggatccgacggcggcggcgaatgccggttcgagg	*Bam*HI
pD-NCgl0580TAA-2	acagtccgctgcagtgttgatgtaagtg-taaatgtaaggttccgccctatccctgcaat	
pD-NCgl0580TAA-3	caaattgcagggatagggcggaaccttacatttacacttacatcaacac-tgcagcggac	
pD-NCgl0580TAA-4	cgtgtaagctttttaatggctggatgcccctggcgcccttccct	*Hin*dIII
pD-NCgl0581-1	gctagcccgggttaactaggtgtgtgtactcgc	*Sma*I
pD-NCgl0581-2	gtactgggcgatgatttgtctgaaggctcacaagaacgacacctcc	
pD-NCgl0581-3	gtaatactgaacaattttggaggtgtcgttcttgtgagccttcagac	
pD-NCgl0581-4	gcggtcctgcaggctactcccattcgatggttcctggtg	*Sda*I
pD-NCgl0580-NCgl0581-1	gctagcccgggctcttcaggagctgcacgcag	*Sma*I
pD-NCgl0580-NCgl0581-2	gcggtgctttttccagcagcgccatttgttg	
pD-NCgl0580-NCgl0581-3	cgctgctggaaaaagcaccgcggtcagggaagg	
pD-NCgl0580-NCgl0581-4	ctgcaggatcccacggtggaaccttctggag	*Bam*HI
pD-sdhCAB-1	ctgcatctagagcggcggtcaagggcatc	*Xba*I
pD-sdhCAB-2	cttggattaaagaggcacctccagtgtcgttg	
pD-sdhCAB-3	gaggtgcctctttaatccaagtaagtaccggttcagaca	
pD-sdhCAB-4	ctgcacctgcaggcttcaaagtagttcagggtgaggcac	*Sda*I
pD-gdhA-1	gctagcccgggtgcccgccgaaaactgagga	*Sma*I

pD-gdhA-2	cgcaggggtcgatttcctcgttcccatctcggctg	
pD-gdhA-3	cgaggaaatcgacccctgcgctttacttaaacc	
pD-gdhA-4	ctgcacctgcaggccggcaggcttgaaatcaactc	*Sda*I

Table S3. Codon-optimized coding sequence of gene *hemA* from *Rhodopseudomonas palustris* (*hemA*$_{RP}$).

Gene	DNA sequence (5′ - 3′)
hemA$_{RP}$	atgaactacgaagcctacttccgtcgccagctggatggtctgcatcgcgaaggccgctac- cgtgtgttcgccgatctggaacgtcatgccggttccttcccacgcgcaacccatcaccgtccagagggtgccggc gacgtcaccgtctggtgctccaacgattatctgggtatgggtcagcatccagcagtgctgac- cgccatgcacgaagcactggattctgccggcgccggtgccggtggcactcgtaacattgccggcaccaaccac taccacgtgctgctggagcaagaactcgcagcactgcacggcaaggaatccgcac- tcctcttcacctccggctatgtctccaactgggcctctctgtccactctggcatcccgcatgccgggctgcgtgatcc tctccgacgaactgaatcacgcctccatgatcgaaggcatccgtcac- tcccgctctgaaacccgcatctttgcccacaacgatcctcgcgatctggaacgtaagctcgcagatctggatcctc acgccccaaagctggtcgcattcgagtccgtgtactccatggacggcga- tatcgccccaatcgcagaaatctgcgacgtggccgatgcacacaacgccatgacctatctggatgaggtccacg gtgtcggcctctacggtccaaacggcggtggtatcgccgatcgcgaaggcatttcccac- cgtctgaccatcatcgaaggcactctggccaaggcattcggtgtggtcggtggctacattgccggctcttccgcc gtgtgcgacttcgtgcgctcctttgcctccggctttatcttctctacctctcctccac- cagccgtggcagccggcgcactggcctctatccgtcatctccgcgcatcctctgccgagcgcgaacgtcatcaa gatcgcgtcgcacgtctgcgcgcacgtctggatcaagccggcgtgg- cacacatgccaaacccatctcacatcgtgccagtgatggtcggtgacgcagccctctgcaaacagatctctgacg agctcatctcccgctatggcatctacgtgcagcctatcaactatccaaccgtgccaaaggg- caccgagcgtctccgtatcactccatccccacaacacaccgacgccgatatcgaacacctcgtgcaagcactgtc cgaaatctggactcgcgtgggcctcgcaaaagccgcctaa

Table S4. Primers used for RT-qPCR.

Primers	DNA sequence (5′ - 3′)
sdhA-U	gcgtcatcgaccacatgaacg
sdhA-D	cggtttgtccacgggtgtag
afuA-U	gggtcatgagctcgatgtcag
afuA-D	cgctacatcaacttcctgctgtc
hemB-U	cccacgcactaattcgtcgtc
hemB-D	gagatctcacgtgcctcagtg
hemE-U	cctgagtacaagaaggtccgtg
hemE-D	gcatccacatcatgacgacga
groES-U	ggcaaccgttatcgcagttg
groES-D	ccaccgaacttgatctcggtg
groL-U	ccaacgacggtgtcaccattg
groL-D	gcagagttgcagtcgtggtg
NCgl0581-U	cgaagtgttggcctcagaaac
NCgl0581-D	gaaggaggtgactttcagcgttc
gltA-U	gctgtctgagactggactgatca
gltA-D	gtggcattctcagccagatcag
urtD-U	cgtccacttcctcatcggtg
urtD-D	gcacaggggttcccagaatc
sucA-U	gcatgttcgggatcagctctg
sucA-D	gctaccctaacgctgaggaag
cydB-U	gcgttccagatatccaggctc
cydB-D	gcactgatcaaagaccgcgatg

atpG-U	gatcaccaaggctcaagagctca
atpG-D	cagcattgggtggtctagggag
pfkA-U	ccaccgtcgttggttatcaag
pfkA-D	ggccttaaacttgtccggatgg
NCgl1445-U	ccgatgtcggtgaacagga
NCgl1445-D	ctggcacattgttctcgctg
hemH-U	ctcgataacgcgcttgagctct
hemH-D	gagctgatggaagaagccgaga
acn-U	cctatgactacttcgccctctctg
acn-D	cctcaatgtgctcgttggtgatg
gluD-U	gccgtgttctcggtgatctt
gluD-D	cggaatggctcggaaagtct
gdhA-U	ctctgcacaggtcttgaagatg
gdhA-D	ggagatgcagcagaacgcttc
qcrB-U	gcacggatgtcttctggatcag
qcrB-D	gcccagttgatgaccatggtc
ctaC-U	ggagtcacggaagctgatgtac
ctaC-D	gctgtgcagaaatgtgcggtac
aceA-U	gcagaccaggtagctgatctg
aceA-D	gccaagcgcgttgatgtatc
sucD-U	gcatgccccatggtctttc
sucD-D	gtgagatcggtggagatgcag
pstA-U	catcagtggaagggaagcct
pstA-D	gcactgtccggtatcgtcac
glk2-U	gacagccctatcgagcagatcc
glk2-D	ccctctggtcccagaattacac
pyk2-U	ccttgatggcgtcgttgatg
pyk2-D	ccatctcgcgcagaaatcac
hrrA-U	ggatttcaccgtggcttctg
hrrA-D	cccacgtgaactggaagttc
hrrS-U	cctgcccatatgcagattccact
hrrS-D	gagccttcggaagtgtccagtac
sfcA-U	gcgacatgcgcgatctttc
sfcA-D	cgcgacggtgtttccaatg

Table S5. The raw data of significant differentially expressed genes between JSD1 and JS.

Details in Excel Table S5

Table S6. The relative transcriptional level of genes in metabolic pathways of *C. glutamicum* JS and JSD1.

Gene	Product	log_2FoldChange	
		RT-PCR	RNA-seq
pyk2	pyruvate kinase	1.80	2.42
pfkA	6-phosphofructokinase	2.53	1.62
glk2	glucokinase	1.16	1.23
NCgl1445	MFS transporter	2.12	4.18
gltA	citrate synthase	-0.38	-2.04
sucA	pyruvate and 2-oxoglutarate dehydrogenases, E1 component	-1.07	-0.82
acn	aconitate hydratase	-1.24	-2.57
sucD	succinate-CoA ligase subunit alpha	1.62	1.71

aceA	isocitrate lyase	0.79	1.94
sfcA	NADP-dependent malic enzyme	1.27	1.40
hemB	porphobilinogen synthase	-0.49	-0.41
hemE	uroporphyrinogen decarboxylase	-0.69	-2.14
hemH	ferrochelatase	0.52	-0.86
gdhA	NADP-specific glutamate dehydrogenase	-1.46	-2.21
hrrS	sensor histidine kinase	-2.30	-1.11
hrrA	response regulator transcription factor	0.41	1.95
groES	co-chaperone	2.27	2.59
groL	chaperonin	1.88	1.43
NCgl0581	LysR substrate-binding domain-containing protein	4.07	6.92
atpG	F0F1 ATP synthase subunit gamma	-0.58	-1.10
qcrB	cytochrome bc1 complex cytochrome b subunit	-2.26	-2.20
ctaC	cytochrome c oxidase subunit II	-1.41	-2.22
cydB	cytochrome d ubiquinol oxidase subunit II	-2.91	-4.84
sdhA	fumarate reductase/succinate dehydrogenase flavoprotein subunit	-3.32	-3.99
afuA	ABC transporter substrate-binding protein	2.35	4.48
pstA	phosphate ABC transporter permease	3.76	3.95
gluD	amino acid ABC transporter permease	-1.70	-1.12
urtD	urea ABC transporter ATP-binding protein	-5.17	-7.44

Table S7. The raw data of pathway enrichment analysis of the differentially expressed genes between JSD1 and JS.

Details in Excel Table S7

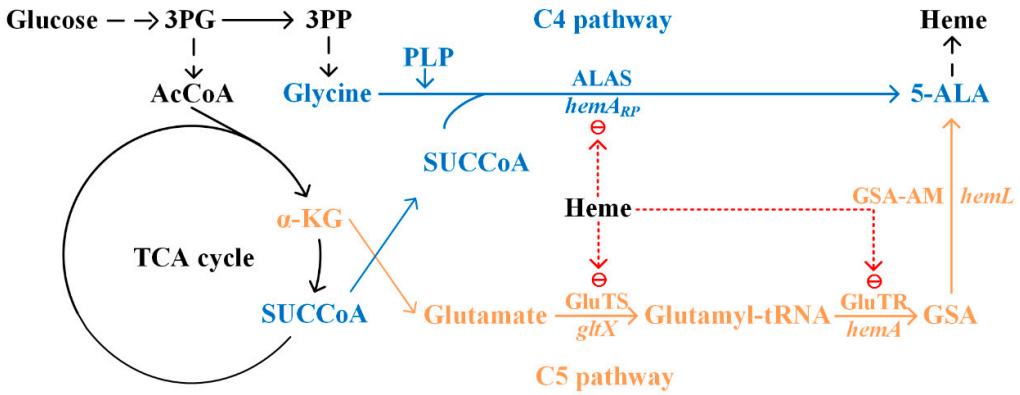

Figure S1. An overall metabolic pathway for 5-ALA biosynthesis. The blue font and lines represent the C4 pathway. The orange font and lines represent the C5 pathway. The dashed black line indicates several continuously enzymatic reactions, and the dashed red line indicates feedback inhibition. 3PG, 3-phosphoglycerate; α-KG, α-oxoglutarate; SUCCoA, Succinyl-CoA; PLP, Pyridoxal 5′-phosphate; GluTS, glutamyl–tRNA synthetase; GluTR, glutamyl-tRNA reductase; GSA, glutamate-1-semialdehyde; GSA-AM, glutamate-1-semialdehyde aminotransferase.

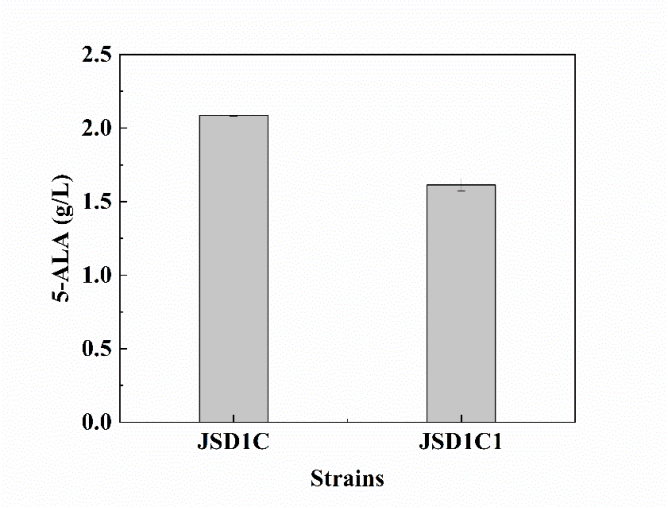

Figure S2. Effect of plasmid-based *NCgl0580* overexpression in JSD1 strain on 5-ALA biosynthesis. JSD1C1 is the strain overexpressing *NCgl0580* compared with the JSD1C strain (JSD1 harboring pEC-XK99E).

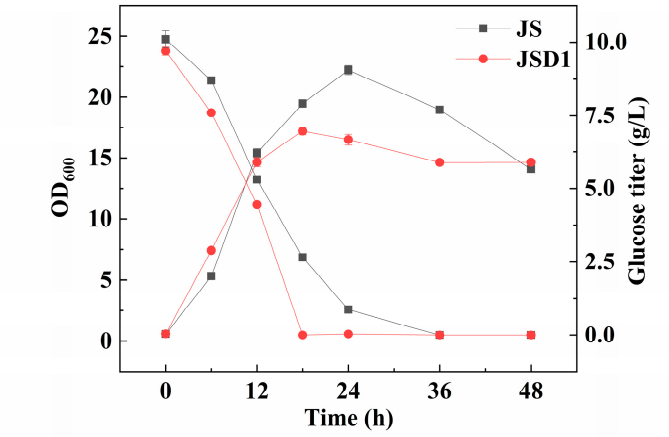

Figure S3. Determination of cell growth and sugar consumption of strains JS and JSD1.