

Table S1. Main strains, plasmids, and primers used in this study.

Strain/plas- mid/primer	Description	Source
Strain		
MG1655	A substrain of <i>E. coli</i> K-12 strain, with a relevant GenBank ac- cession No: NC_000913	Lab stock
<i>E. coli</i> DH5 α	Gene cloning	Lab stock
<i>C. glutamicum</i> A21799	Provide gene <i>ddh</i>	Lab stock
<i>C. glutamicum</i> A13032	Provide gene <i>lysG</i>	Lab stock
S1	MG1655(pSB4K- <i>lysG</i> -P _{lysE} - <i>tetA</i>)	This study
MB1	MG1655(pSB4K5- <i>lysG</i> [*] -P _{lysE} - <i>tetA</i>)	This study
P1	MG1655 with <i>lysC</i> ^{T352I, S369F}	This study
P2	P1 with <i>dapA</i> ^{H118Y, A81V}	This study
P3	P1 (pTrc99a- <i>ddh</i>)	This study
P4	P2 (pTrc99a- <i>ddh</i>)	This study
P5	MG1655 with pTrc99a	This study
S3	P1 (pSB4K5- <i>lysG</i> [*] -P _{lysE} - <i>tetA</i>)	This study
S4	P4 (pSB4K5- <i>lysG</i> [*] -P _{lysE} - <i>tetA</i>)	This study
S5	P5 (pSB4K5- <i>lysG</i> [*] -P _{lysE} - <i>tetA</i>)	This study
Plasmid		
pCAGO	Ap ^R , containing Cas9 and gRNA	Lab stock
pTrc99A	Ap ^R , GenBank accession no.: U13872	Lab stock
pDM1	Provides tetracycline resistance gene <i>tetA</i>	Lab stock
pTrc99a- <i>lysC</i>	pTrc99A derivative, using promoter P _{trc} to overexpress <i>lysC</i>	This study
pTrc99a- <i>lysC</i> [*]	pTrc99A derivative, using promoter P _{trc} to overexpress <i>lysC</i> ^{T352I, S369F}	This study
pTrc99a- <i>dapA</i>	pTrc99A derivative, using promoter P _{trc} to overexpress <i>dapA</i>	This study
pTrc99a- <i>dapA</i> [*]	pTrc99A derivative, using promoter P _{trc} to overexpress <i>dapA</i> ^{H118Y, A81V}	This study
pTrc99a- <i>ddh</i>	pTrc99A derivative, using promoter P _{trc} to overexpress <i>ddh</i>	This study
pSB4K5	Km ^R , pSB4K5-I52002 (GenBank accession no.: EU496099) backbone	Lab stock
pSB4K5- <i>lysG</i> - <i>tetA</i>	pSB4K5 derivative, using promoter P _{trc} to overexpress <i>lysG</i> , promoter P _{lysE} and gene <i>tetA</i>	This study
pSB4K5- <i>lysG</i> [*] - <i>tetA</i>	pSB4K5 derivative, using promoter P _{trc} to overexpress <i>lysG</i> [*] , promoter P _{lysE} and gene <i>tetA</i>	This study
Primer		
psb-F	cgggccacctcgacctgaaacctctcacctcggc	
psb-R	tcgagggtattgcggccttaggcctgggggtgcctaata	
<i>lysG</i> -F	ctaaggccgcaatccctcga	
<i>lysG</i> -R	atgtatatctcttcttaaagtcactatagg	
<i>tetA</i> -F	agatgactttaagaaggagatatacatgaaatctaacaatgcgct	
<i>tetA</i> -R	tcaggtcgaggtggcccg	
99a-F	atagtaattgtttgagtaatcgctcaaggcgactcc	
99a-R	tggagacaacaatttcagacatcacgaattatgcagtgttacgacc	
<i>lysC</i> -F1	atgtctgaaattgtgtctccaa	
<i>lysC</i> -R1	ttactcaacaataactatgcagttt	
<i>lysC</i> -F2	gtggcattaatccttgataccacc	
<i>lysC</i> -R2	gggtgatcaaggattaatgccac	
<i>lysC</i> -F3	tgacgcaatttctgctgatg	
<i>lysC</i> -R3	catcagcagaaattgcgtca	
99a1-F	taagcatgccggtttgctgtaatcgctcaaggcgactcc	
99a1-R	gacaatacttcccgtgaacatcacgaattatgcagtgttacgacc	

<i>dapA</i> -F1	atgttcacgggaagtattgtc
<i>dapA</i> -R1	ttacagcaaaccggcatg
<i>dapA</i> -F2	ttgtatcagtatttcaaagccatcg
<i>dapA</i> -R2	cgatggctttgaaatactgatacaa
<i>dapA</i> -F3	cgctaacgttactcgacc
<i>dapA</i> -R3	ggctgcagtaacgttagcg
99a2-F	gatcgacgcgacgtctaactgctcaaggcgactcc
99a2-R	cctggtcgagcttaccgagacagaattatgcagtgatttacgacc
<i>ddh</i> -F	tctcggttaagctcgaccagg
<i>ddh</i> -R	ttagacgtcgcgtcgatc
UP-A-F	ggcaagctaaatttcgggtgc
UP-A-R	attaattaatgggccatcctctgtgcaaacaa
<i>dapA</i> -cat-F	gaggatggccatttaataatctcagtgtagac
<i>dapA</i> -cat-R	cttcggttcgatggactattacccccccctgcca
<i>dapA</i> -F	tagtccatcgaaccgaagtaagggaactgacacactgtttgcacagag- gatggcccatgttcacgggaagtattgtc
<i>dapA</i> -R	cagttccttacatgccatt
UP-C-F	gtgatgcggttttggagtaac
UP-C-R	attaattaataactacctcgtgtcaggggac
<i>lysC</i> -cat-F	cggagtagttattaataatctcagtgtagac
<i>lysC</i> -cat-R	cttcggttcgatggactattacccccccctgcca
<i>lysC</i> -F	tagtccatcgaaccgaagtaagggtgccaaggctgaaaatggatcccctgacacgagtag- ttatgtctgaaattgtgtctcca
<i>lysC</i> -R	gaacgggtccagtacgcc
EG-F	tcggcgaattgcctcaccacaaaccttcgccgacgg
EG-R	atcccgttaaccatgccatcaacgcagattcgctatccacat
EGV-F	ctcattaggcaccacagcctaaggccgaatccctcgattgctg
EGV-R	tgattgggtcttaacatggttaatatagcttcgaacccattcaactggacactttgct

Abbreviations: Ap^R, ampicillin resistance; Km^R, kanamycin resistance.

Table S2. The binding energy of LysG wild-type and lysine.

Name	Binding energy
lys-1	-6.00
lys-2	-5.80
lys-3	-5.66
lys-4	-5.57
lys-5	-4.84
lys-6	-4.62
lys-7	-4.57
lys-8	-4.35
lys-9	-3.78
lys-10	-3.33

Table S3. The binding energy of LysG^M and lysine.

Name	Binding energy
lys-1	-5.73
lys-2	-5.64
lys-3	-5.58
lys-4	-5.55
lys-5	-5.18
lys-6	-4.94
lys-7	-4.60
lys-8	-4.10
lys-9	-3.34
lys-10	-2.45

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