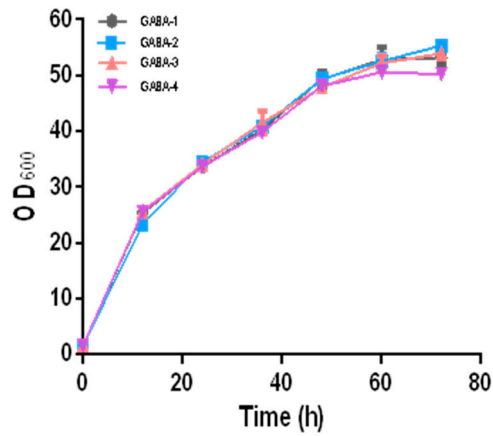
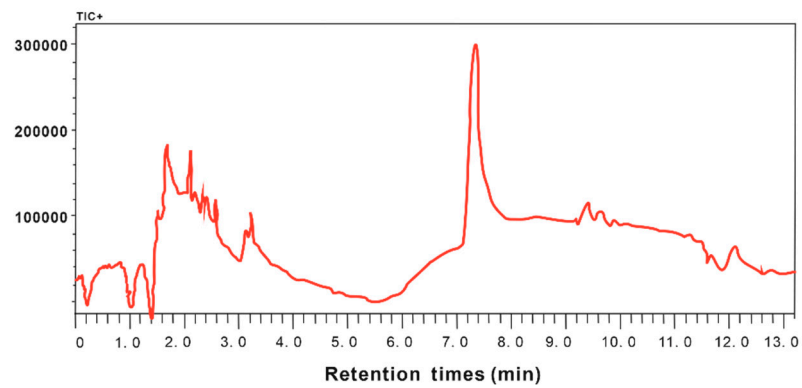


## Supplementary Materials



**Figure S1.** The growth profiles of engineered *C. glutamicum* strains harboring pXMJ19-*P<sub>tuf</sub>*-*guaB-gadM* in shake flask cultivations.



**Figure S2.** The analysis of butyrolactam in the shake flask cultivation using HPLC-MS. Time profiles of fermentation sample are shown.

**Table S1. Strains and plasmids used in this study.**

Strains	Characteristics	Source
<b><i>E. coli</i></b>		
EC135	TOP10 $\Delta dcm::FRTrecA^+\Delta dam::FRT$ , genotype of R-M systems:	(Zhang et. al., 2012)
BL21(DE3)	<i>mcrA</i> $\Delta(mrr-hsdRMS-mcrBC)\Delta dcm::FRT\Delta dam::FRT$	
<i>C. glutamicum</i>	F <sup>-</sup> <i>ompT gal dcm lon hsdS<sub>B</sub>(r<sub>B</sub><sup>-</sup> m<sub>B</sub><sup>-</sup>)</i> $\lambda$ (DE3)	Novagen
ATCC 13032	wild-type, biotin-auxotrophic	ATCC
GABA-1	13032 harboring pXMJ19- <i>P<sub>tuf</sub>-guaB-gadM</i>	This study
GABA-2	WT- <i>P<sub>tuf</sub>-acn</i> harboring pXMJ19- <i>P<sub>tuf</sub>-guaB-gadM</i>	This study
GABA-3	WT- <i>P<sub>tuf</sub>-acn-P<sub>tuf</sub>-icd</i> harboring pXMJ19- <i>P<sub>tuf</sub>-guaB-gadM</i>	This study
GABA-4	WT- <i>P<sub>tuf</sub>-acn-P<sub>tuf</sub>-icd</i> $\Delta$ <i>sucCD</i> harboring pXMJ19- <i>P<sub>tuf</sub>-guaB-gadM</i>	This study
GABA-5	WT- <i>P<sub>tuf</sub>-acn-P<sub>tuf</sub>-icd</i> $\Delta$ <i>sucCD</i> $\Delta$ <i>gabDT</i> harboring pXMJ19- <i>P<sub>tuf</sub>-guaB-gadM</i>	This study
GABA-6	WT- <i>P<sub>tuf</sub>-acn-P<sub>tuf</sub>-icd</i> $\Delta$ <i>sucCD</i> $\Delta$ <i>gabDT</i> $\Delta$ <i>gabP::potE</i> harboring pXMJ19- <i>P<sub>tuf</sub>-guaB-gadM</i>	This study
BLM-0	13032 harboring pXMJ19	This study
BLM-1	WT- <i>P<sub>tuf</sub>-acn-P<sub>tuf</sub>-icd</i> $\Delta$ <i>sucCD</i> harboring pXMJ19- <i>P<sub>tuf</sub>-guaB-gadM-act</i>	This study
BLM-2	WT- <i>P<sub>tuf</sub>-acn-P<sub>tuf</sub>-icd</i> $\Delta$ <i>sucCD</i> $\Delta$ <i>gabDT</i> harboring pXMJ19- <i>P<sub>tuf</sub>-guaB-gadM-act</i>	This study
BLM-3	WT- <i>P<sub>tuf</sub>-acn-P<sub>tuf</sub>-icd</i> $\Delta$ <i>sucCD</i> $\Delta$ <i>gabDT</i> $\Delta$ <i>gabP::potE</i> harboring pXMJ19- <i>P<sub>tuf</sub>-guaB-gadM-act</i>	This study
<b>Plasmids</b>		
pK18 <i>mobsacB</i>	Mobilizable vector, allows for selection of double crossover in <i>C. glutamicum</i> , <i>Kan<sup>r</sup></i>	(Schafer et al., 1994)
pK18 <i>mobsacB-P<sub>tuf</sub>-acn</i>	pK18 <i>mobsacB</i> derivate carrying <i>P<sub>tuf</sub>-acn</i> replacement	(Zhang et al., 2017)
pK18 <i>mobsacB-P<sub>tuf</sub>-icd</i>	pK18 <i>mobsacB</i> derivate carrying <i>P<sub>tuf</sub>-icd</i> replacement	This study
pK18 <i>mobsacB-<math>\Delta</math>sucCD</i>	pK18 <i>mobsacB</i> derivate carrying <i>sucCD</i> deletion	(Zhang et al., 2019)
pK18 <i>mobsacB-<math>\Delta</math>gabDT</i>	pK18 <i>mobsacB</i> derivate carrying <i>gabDT</i> deletion	This study
pK18 <i>mobsacB</i> $\Delta$ <i>gabP::potE</i>	pK18 <i>mobsacB</i> derivate carrying <i>gabP</i> deletion and <i>potE</i> integration	This study
pXMJ19	Shuttle vector (Cm <sup>r</sup> <i>P<sub>tac</sub> lacI<sup>q</sup> pBL1 oriV<sub>C. glutamicum</sub> pK18 oriV<sub>E. coli</sub></i> )	(Jakoby et al., 1999)
pXMJ19- <i>gadB</i>	pXMJ19 derivate carrying <i>P<sub>tac</sub>-gadB</i>	This study
pXMJ19- <i>gad</i>	pXMJ19 derivate carrying <i>P<sub>tac</sub>-gad</i>	This study
pXMJ19- <i>gadM</i>	pXMJ19 derivate carrying <i>P<sub>tac</sub>-gadM</i>	This study
pXMJ19- <i>P<sub>tuf</sub>-gadM</i>	pXMJ19 derivate carrying <i>P<sub>tuf</sub>-gadM</i>	This study
pXMJ19- <i>P<sub>tuf</sub>-tsf-gadM</i>	pXMJ19 derivate carrying <i>P<sub>tuf</sub>-tsf-gadM</i>	This study
pXMJ19- <i>P<sub>tuf</sub>-gsi-gadM</i>	pXMJ19 derivate carrying <i>P<sub>tuf</sub>-gsi-gadM</i>	This study
pXMJ19- <i>P<sub>tuf</sub>-guaB-gadM</i>	pXMJ19 derivate carrying <i>P<sub>tuf</sub>-guaB-gadM</i>	This study
pXMJ19- <i>P<sub>tuf</sub>-guaB-gadM-act</i>	pXMJ19 derivate carrying <i>P<sub>tuf</sub>-guaB-gadM-act</i>	This study
pET-28a	Kan <sup>r</sup> ; expression vector with an N-terminal hexahistidine affinity tag	Novagen
pET-28a- <i>act</i>	pET-28a derivate carrying <i>act</i> gene	This study

**Table S2. Primers used in this study.**

Primers	Sequence (5'-3')	Note
WZ4128	GCTTGCATGCCTGCAGGTCGACATGGAAGATGTTACATGCTGT ACGGT	<i>gadB</i> amplification to construct pXMJ19- <i>gadB</i>
WZ4129	GAGCTCGGTACCCGGGGATCCTCAGTGGGTGAAGCCGTAGGTTT TGT	
WZ4132	TAAGCTTGCATGCCTGCAGGTCGACATGGATAAGAAGCAAGTA ACGGATTTAAGGT	<i>gad</i> amplification to construct pXMJ19- <i>gad</i>
WZ4133	AATTCGAGCTCGGTACCCGGGGATCCTCAGGTATGTTTAAAGCT GTTCTGTTGGGCAAT	
WZ4134	TAAGCTTGCATGCCTGCAGGTCGACATGGATAAGAAGCAAGTA ACGGATTTAAGGT	<i>gadM</i> amplification to construct pXMJ19- <i>gadM</i>
WZ4135	AATTCGAGCTCGGTACCCGGGGATCCTCAGTGATCGCTGAGATA TTTCAGGGAAGCT	
WZ4136	AATTAAGCTTGCATGCCTGCAGGTCGACATGGATAAGAAGCAA GTAACGGAT	<i>gadM</i> amplification to construct
WZ4137	AATTAAGCTTGCATGCCTGCAGGTCGACATGGATAAGAAGCAA GTAACGGAT	pXMJ19- <i>P<sub>tuf</sub></i> - <i>gadM</i>
WZ4138	GTCGTATCCCACTACCGAGATTGGCCGTTACCCTGCGAATGT	<i>P<sub>tuf</sub></i> amplification to construct
WZ4139	GTCGACCTGCAGGCATGCAAGCTTAATTTGTATGTCCTCCTGGAC TTCGT	pXMJ19- <i>P<sub>tuf</sub></i> - <i>gadM</i>
WZ4141	TGCGGATGTTAAGAAGGAGGAATAAATGGATAAGAAGCAAGTA ACGGAT	<i>gadM</i> amplification to construct pXMJ19- <i>P<sub>tuf</sub></i> - <i>tsf-gadM</i>
WZ4137	AATTAAGCTTGCATGCCTGCAGGTCGACATGGATAAGAAGCAA GTAACGGAT	
WZ4142	AATTAAGCTTGCATGCCTGCAGGTCGACATGGCGAACTACACCG CTGCGG	<i>tsf</i> amplification
WZ4143	TTATTCCTCCTTCTTAACATCCGCAGCGGTGTAGTTCGCCAT	
WZ4144	AATAACAAAATAAGGAGGATTTACATATGAATGGATAAGAAGC AAGTAACGGAT	<i>gadM</i> amplification to construct pXMJ19- <i>P<sub>tuf</sub></i> - <i>gsi-gad</i>
WZ4137	AATTAAGCTTGCATGCCTGCAGGTCGACATGGATAAGAAGCAA GTAACGGAT	
WZ4145	AATTAAGCTTGCATGCCTGCAGGTCGACATGGCAGACAATAAC AAA	<i>gsi</i> amplification
WZ4146	TTCATATGTAAATCCTCCTTATTTTGTTATTGTCTGCCAT	
WZ4147	ATCGCCCAGTAAAGGAGGAATAAATGGATAAGAAGCAAGTAA CGGAT	<i>gadM</i> amplification to construct pXMJ19- <i>P<sub>tuf</sub></i> - <i>guaB-gadM</i>
WZ4137	AATTAAGCTTGCATGCCTGCAGGTCGACATGGATAAGAAGCAA GTAACGGAT	
WZ4148	AAGGAGGATCGCCCCGTAATGAGCCTTCAGACAAATCATC	<i>guaB</i> amplification
WZ4149	TTATTCCTCCTTTACTGGGCGATGATTTGTCTGAAGGCTCAT	
WZ4150	CCGGAATTCAAATCTGATTCTTTGCA	

Primers	Sequence (5'-3')	Note
WZ4151	TTCGCAGGGTAACGGCCACTTCATTATCCTAACAGTAC	Replacement of <i>acn</i> promoter with <i>P<sub>tuf</sub></i>
WZ4152	GTACTGTTAGGATAATGAAGTGGCCGTTACCCTGCGA	
WZ4153	AGTCACAGTGAGCTCCATTTCTATCCTCCTTTTGTATGTCCTCCTG	
WZ4154	ATACAAAAGGAGGATAGAAATGGAGCTCACTGCCT	
WZ4155	CCCAAGCTTTGGTGGTGTGGGAGTCG	
WZ4158	ATGTGCTGCAAGGCGATTAA	
WZ4159	TATGCTTCCGGCTCGTATGT	Replacement of <i>icd</i> promoter with <i>P<sub>tuf</sub></i>
WZ4160	TTGCATGCCTGCAGGTCGACGCGGTGTGGGAACCTCTTAA	
WZ4161	GAGCCCATCAACCAAGGAGACTCATGGCTAAGATCATCTGGAC	
WZ4162	GAGTCTCCTTGGTTGATGGGCTCTGTATGTCCTCCTGGACTTC	
WZ4163	ACTGTATTCTAGGTAGCTGAACAAAATGGCCGTTACCCTGCGAA T	
WZ4164	TTTTGTTTACGCTACCTAGAATACAGTGTTCTACTAATTGCTGGCG CCTA	
WZ4165	ATTCGAGCTCGGTACCCGGGGATCCATGAAACCGCAGCACCCG CAAT	<i>sucCD</i> knockout
WZ4166	TGCATCCATGGTTGCAACGTT	
WZ4167	AACCGCAGCACCCGCAATCGCGCGCATCCTCGAA	
WZ4176	ACAGCTATGACATGATTACGAATTCTGTAAAGACGCAGAAGG CTCT	
WZ4177	TCAGTAATAATCACGCACAGTGTGTCCTCATCAATACCAGTGAG	
WZ4178	CACTGTGCGTGATTATTACTGA	
WZ4179	CCTGCAGGTCGACTCTAGAGGATCCAGTGCCTTCTGAACCTGTC AC	<i>gadDT</i> knockout
WZ4180	GTCCTGCGCACAGATGAATACTCT	
WZ4181	GCTGAGCACCACGGATCCAAT	
WZ4182	TTGTAAAACGACGGCCAGTGCCACGATGGCTACTGCACCACCC AAAT	
WZ4183	AGCTCATGTGTGGTTCCTCCTGTGAGGTGAGATACA	
WZ4184	GGAGGAACCACACATGAGCTGTCCGGTGAATAACCCGAAGGAA	
WZ4185	CGAGCTCGGTACCCGGGGATCCAATGGGTGGAACACGATCAGG T	<i>potE</i> integration
WZ4186	TTGTAAAACGACGGCCAGTGCCACGATGGCTACTGCACCACCC AA	
WZ4187	GGTATTTATGTCAACACCGCCAGTTTTTTCCTTCGGGTATTACCC GGA	
WZ4188	TGGCGGTGTTGACATAAATACCACT	
WZ4189	TTAACCGTGTTTATTTTTCAGTTCAAAGCGT	
WZ4190	ACGCTTTGAACTGAAAAATAAACACGGTTAATCACTTCCTGTTG TGGCTGCCT	
WZ4191	AATTCGAGCTCGGTACCCGGGGATCCCTCTTCTAGCGCTTCACC ACCAG	

Primers	Sequence (5'-3')	Note
WZ4192	ATGCCTGCAGGTCGACTCTAGAAAAGGAGGAACTTATGAAAC GTCCGCTGGAAGGTA	<i>act</i> amplification to construct pXMJ19- <i>P<sub>tuf</sub></i> - <i>guaB</i> - <i>gadM</i> - <i>act</i>
WZ4193	TCCGCCAAAACAGCCAAGCTGAATTCGAGCTCTTAGATAACGTT TTTCTCTTCCA	
WZ867	CGTACTGCTGAAGGCTCTT	RT-PCR for <i>rpoB</i> gene
WZ868	TTTGCTACACCATCGGACT	
WZ981	ACCTACACCGACGACGCTGTTTCCG	RT-PCR for <i>acn</i> gene
WZ982	GTTGTCAGCTTCGACGCCGCCTTCA	
WZ983	TTCGTATGATCGGTTCCGCACAGGC	RT-PCR for <i>gltA</i> gene
WZ984	GTCGCCACCGTGGTTGCTCTTGATG	
WZ985	ACCGTTATCGAAGACTGCCGCAAGA	RT-PCR for <i>icd</i> gene
WZ986	TGAACCACACCGTCTGCTTCGATGC	
WZ987	CCAAAGCCAACCCAGGCAGAGCAGA	RT-PCR for <i>kgd</i> gene
WZ988	GCGGAGTCCATCAGTGGGATAAGTG	
WZ989	TATCCTGCTGGTCGCATTGGTTCTG	RT-PCR for <i>sucCD</i> gene
WZ990	AAGGAGTTGAAGCCGCCAACGAGGT	
WZ991	CAGGCTTCCACTCCCTCAACTACGG	RT-PCR for <i>aceA</i> gene
WZ992	AGCTGCCTTGAACCTACGGTTCTGC	
WZ993	CGCAACATTCTCACCATTCCAACCG	RT-PCR for <i>aceB</i> gene
WZ994	CACCGTGCTCAACCCAGCGCACAAC	

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