

Title: New insights into the biosynthesis of succinic acid by *Actinobacillus succinogenes* with the help of its engineered strains

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Table S1 The strains and plasmids used in this study.

Table S2 The primers used in this study.

Table S3 The PAM sequences used in this study.

Table S1 The strains and plasmids used in this study

Strains and plasmids	Description	Reference or source
Strains		
JM109	<i>E. coli</i> , cloning strain	Our laboratory
<i>A. succinogenes</i> M 2012036	<i>A. succinogenes</i> , wild type	Our laboratory
Δ ackA	<i>A. succinogenes</i> derivative, contains <i>ackA</i> knockout	Reference 30
Δ ackA:: <i>ackA</i>	Δ ackA derivative, contains <i>ackA</i> from <i>A. succinogenes</i>	This study
Δ ackA:: <i>ppk</i>	Δ ackA derivative, contains <i>ppk</i> from <i>Pseudomonas aeruginosa</i>	This study
Δ ackA:: <i>pyc</i>	Δ ackA derivative, contains <i>pyc</i> from <i>Corynebacterium acetoacetophilus</i>	This study
Δ ackA:: <i>pepc</i>	Δ ackA derivative, contains <i>pepc</i> from <i>Corynebacterium acetoacetophilus</i>	This study
Δ Asuc_0914	<i>A. succinogenes</i> derivative, contains <i>Asuc_0914</i> knockout	This study
Δ Asuc_0023	<i>A. succinogenes</i> derivative, contains <i>Asuc_0023</i> knockout	This study
Δ Asuc_0715	<i>A. succinogenes</i> derivative, contains <i>Asuc_0715</i> knockout	This study
Δ Asuc_0716	<i>A. succinogenes</i> derivative, contains <i>Asuc_0716</i> knockout	This study
Δ Asuc_0750	<i>A. succinogenes</i> derivative, contains <i>Asuc_0750</i> knockout	This study
Δ Asuc_2056	<i>A. succinogenes</i> derivative, contains <i>Asuc_2056</i> knockout	This study
Δ Asuc_0715-0716	<i>A. succinogenes</i> derivative, contains <i>Asuc_0715</i> and <i>Asuc_0716</i> knockout	This study
Plasmids		
Td-CBE	the cytosine base editor	Data unpublished
1034-Td-CBE	Td-CBE derivative, contains gRNA expression cassette targeting <i>Asuc_1034</i>	This study
0914-Td-CBE	Td-CBE derivative, contains gRNA expression cassette targeting <i>Asuc_0914</i>	This study
0301-Td-CBE	Td-CBE derivative, contains gRNA expression cassette targeting <i>Asuc_0301</i>	This study
0302-Td-CBE	Td-CBE derivative, contains gRNA expression cassette targeting <i>Asuc_0302</i>	This study
0715-Td-CBE	Td-CBE derivative, contains gRNA expression cassette targeting <i>Asuc_0715</i>	This study
0716-Td-CBE	Td-CBE derivative, contains gRNA expression	This study

	cassette targeting <i>Asuc_0716</i>	
0023-Td-CBE	Td-CBE derivative, contains gRNA expression cassette targeting <i>Asuc_0023</i>	This study
0750-Td-CBE	Td-CBE derivative, contains gRNA expression cassette targeting <i>Asuc_0750</i>	This study
2056-Td-CBE	Td-CBE derivative, contains gRNA expression cassette targeting <i>Asuc_2056</i>	This study

Table S2 The primers used in this study

Primers	Sequence (5' to 3')
Pyc-F	ttatcaatgaggtgatctagaGTGTCGACTCACACATC TTCAACG
Pyc-R	acggccagtgaattcgagctcTTAGGAAACGACGACG ATCAAGT
Pepc-F	ttatcaatgaggtgatctagaatgactgatttttacgga
Pepc-R	acggccagtgaattcgagctcctagccggagtgcgcagc
Ppk-F	ttatcaatgaggtgatctagaatgttcgaatccgcggaagttggcca c
Ppk-R	acggccagtgaattcgagctccagcggcgcggtgcggct
ackA-F	ttatcaatgaggtgatctaga atgtccaaattagtttta
ackA-R	acggccagtgaattcgagctc ttagaacaagacgggcg
CBfdh-F	ttatcaatgaggtgatctagaATGAAAATTGTGCTGGT GCTGTA
CBfdh-R	acggccagtgaattcgagctcTTATTTCTTATCATGTTT GCCATAGGC
DVfdh-F	ttatcaatgaggtgatctagaATGACCGTGACCCGTCG TCA
DVfdh-R	acggccagtgaattcgagctcTTACGCACGCGGACGG AATA
0914-td-sg-F	cgacaggttattgataaactGTTTTAGAGCTAGAAATAG CAAG
0914-td-sg-R	agtttatcaataacctgtcggatattttctccaataaaaagt
1034-td-sg-F	ggatccgcaaacggcgattcGTTTTAGAGCTAGAAATA GCAAG
1034-td-sg-R	gaatcgccgtttgcggatccgatattttctccaataaaaagt
0023-td-sg-F	tgctcagtcctaaacttaaccGTTTTAGAGCTAGAAATAG CAAG
0023-td-sg-R	ggttaagtttgactgagcagatattttctccaataaaaagt
0715-td-sg-F	attgtgcaaatcaattcttaGTTTTAGAGCTAGAAATAGC AAG
0715-td-sg-R	taagaattgattgcacaatgatattttctccaataaaaagt
0716-td-sg-F	catgcagttgcagcacgggcGTTTTAGAGCTAGAAATA GCAAG
0716-td-sg-R	gcccgtgctgcaactgcatggatattttctccaataaaaagt
0301-td-sg-F	ctaccgcaagcggcatctatGTTTTAGAGCTAGAAATAG CAAG
0301-td-sg-R	atagatgccgcttgcggtaggatattttctccaataaaaagt
2056-td-sg-F	tatccaatctcaccatacgtGTTTTAGAGCTAGAAATAG CAAG
2056-td-sg-R	acgtatggtgagattggatagatattttctccaataaaaagt

0750-td-sg-F	ctggcaagtcggcgtattggGTTTTAGAGCTAGAAATAG CAAG
0750-td-sg-R	ccaatacgccgacttgccagatatttttccaataaaaagt
0302-td-sg-F	attacaagcagtgaaaaaacGTTTTAGAGCTAGAAATAG CAAG
0302-td-sg-R	gttttttactgcttgtaatgatattttccaataaaaagt
0914-cexu-F	atggatttcccgaattg
0914-cexu-R	aggcgcatggcgaaagta
1575-cexu-F	atgacaacgggcagtttac
1575-cexu-R	actacggccgataaataga
0023-cexu-F	atgtccgatattgcagtaa
0023-cexu-R	caaatcccgaaggatacg
0715-cecu-F	gcctgcatgggtattgtact
0715-cecu-R	gacgatagaataacagacga
0716-cexu-F	ggcttgaatatgcaggtga
0716-cexu-R	agtacaatacccatgcagg
0292-cexu-F	tacatgaaatgccggatgt
0292-cexu-R	ccgaattatcgctttcttc
0301-2-cexu-F	accgcaccgatggcgggcaa
0301-2-cexu-R	acatggtttcaagctggtt
0750-cexu-F	atgccaaataagtaaataag
0750-cexu-R	gactttcgccgtcgcccggt
2056-cexu-F	tagcccgatgtccgcttate
2056-cexu-R	ccgacagacaaacggccgtc

Table S3 The PAM sequences used in this study

序列 (5'→3')	PAM	目的
cgacaggttattgataaact	CGG	Targeting <i>Asuc_0914</i>
ccagcttgaaccatgtcaa	TGG	Targeting <i>Asuc_0301</i>
attacaagcagtgaaaaaac	AGG	Targeting <i>Asuc_0302</i>
ttacggagaacaatgatgac	GGG	Targeting <i>Asuc_0716</i>
tgctcagtccaaacttaacc	AGG	Targeting <i>Asuc_0023</i>
attgtgcaaatcaattctta	CGG	Targeting <i>Asuc_0715</i>