Supporting Information

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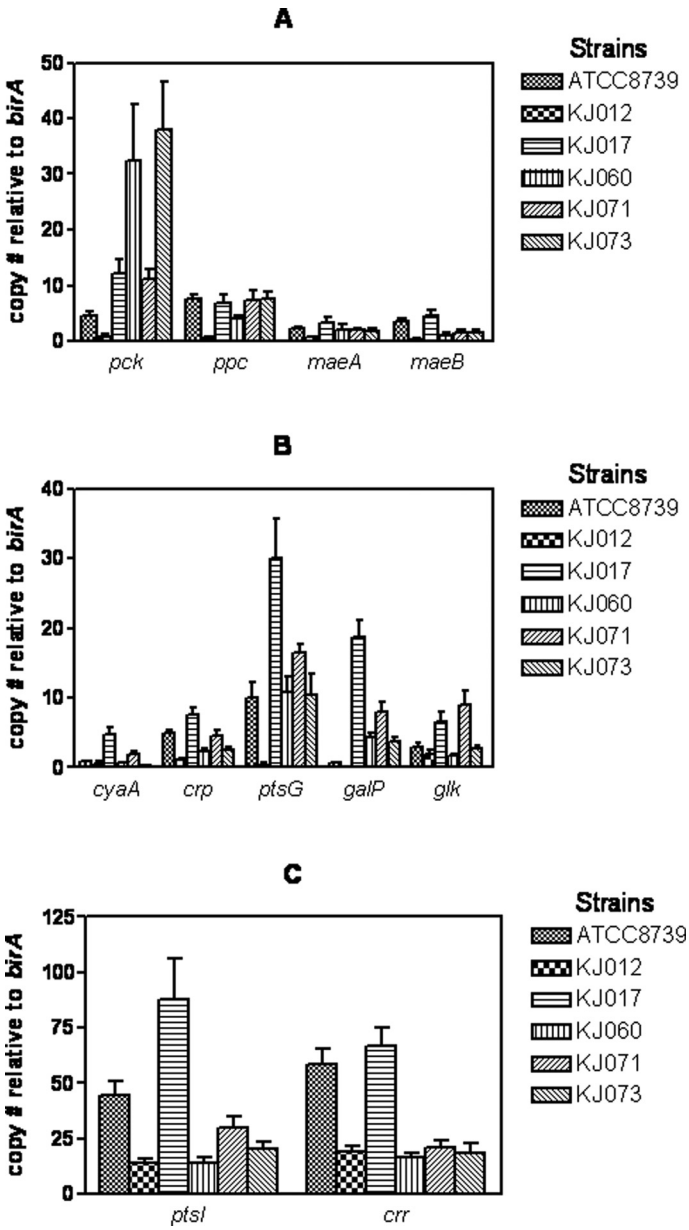

Fig. S1. Comparison of transcript abundance of the engineered succinate-producing strains. (*A*) carboxylation genes, including *pck*, *ppc*, *maeA* and *maeB*. (*B*) Glucose use genes, including *cyaA*, *crp*, *ptsG*, *galP* and *glk*; (*C*) *ptsI*, and *crr* gene.

Table S1. Sources and characteristics of *E. coli* strains, plasmids, and primers used in this study

	Relevant characteristics	Source or reference
Strains		
ATCC 8739	Wild type	Lab collection
KJ012	Δ *ackA,* Δ *ldhA,* Δ *adhE*	3
KJ017	Δ *ackA,* Δ *ldhA,* Δ *adhE*	3
KJ060	Δ *ackA,* Δ *ldhA,* Δ *adhE,* Δ *pflB*	3
KJ071	Δ *ackA,* Δ *ldhA,* Δ *adhE,* Δ *pflB,* Δ *mgsA*	3
KJ073	Δ *ackA,* Δ *ldhA,* Δ *adhE,* Δ *pflB,* Δ *mgsA,* Δ *poxB*	3
XZ320	KJ073, Δ *ppc*	This study
XZ332	KJ073, Δ *pck*	This study
XZ341	KJ073, Δ *maeA*	This study
XZ396	KJ073, Δ *maeB*	This study
XZ613	KJ017, Δ *ptsI*	This study
XZ615	KJ060, Δ *ptsI*	This study
XZ616	KJ060, restored *ptsI* to wild type	This study
XZ618	KJ017, *pck**	This study
XZ620	KJ071, *pck**	This study
XZ622	KJ060, restored *pck* to wild type	This study
XZ624	KJ073, restored *pck* to wild type	This study
XZ626	KJ017, Δ *cra*	This study
XZ627	KJ060, Δ *cra*	This study
XZ642	KJ012, Δ *crp*	This study
XZ643	KJ017, Δ *crp*	This study
Plasmids		
pCR2.1-TOPO	*bla kan*; TOPO TA cloning vector	Invitrogen
pLOI4162	*bla cat;* plasmid to provide *cat-sacB* cassette	19
pLOI4677	*bla kan*; *pck* (including ribosomal binding site, coding and terminator fragment) from *E.coli* ATCC8739 cloned into pCR2.1-TOPO vector	This study
maeA deletion		
pLOI4283	*bla kan*; *maeA* (PCR) from *E.coli* ATCC 8739 cloned into pCR2.1-TOPO vector	This study
pLOI4284	*cat-sacB* cassette (*Sma*I-*Sfo*I fragment of pLOI4162) cloned into *maeA* of pLOI4283	This study
pLOI4285	*Pac*I digestion of pLOI4284, and self-ligated	This study
ppc deletion		
pLOI4264	*bla kan*; *ppc* (PCR) from *E.coli* ATCC 8739 cloned into pCR2.1-TOPO vector	This study
pLOI4265	*cat-sacB* cassette (*Sma*I-*Sfo*I fragment of pLOI4162) cloned into *ppc* of pLOI4264	This study
pLOI4266	*Pac*I digestion of pLOI4265, and self-ligated	This study
pck deletion		
pLOI4641	*bla kan*; *pck* (PCR) from *E.coli* ATCC 8739 cloned into pCR2.1-TOPO vector	This study
pLOI4642	*cat-sacB* cassette (*Sma*I-*Sfo*I fragment of pLOI4162) cloned into *ppc* of pLOI4641	This study
pLOI4643	*Pac*I digestion of pLOI4642, and self-ligated	This study
maeB deletion		
pLOI4728	*bla kan*; *maeB* (PCR) from *E.coli* ATCC 8739 cloned into pCR2.1-TOPO vector	This study
pLOI4729	*cat-sacB* cassette (*Sma*I-*Sfo*I fragment of pLOI4162) cloned into *maeB* of pLOI4728	This study
pLOI4730	*Pac*I digestion of pLOI4729, and self-ligated	This study
ptsI mutation change		
pLOI4734	*bla kan*; *ptsI* (*ptsI*-D-up/D-down) from *E.coli* ATCC8739 cloned into pCR2.1-TOPO vector	This study
pLOI4735	*cat-sacB* cassette (*Sma*I-*Sfo*I fragment of pLOI4162) cloned into *ptsI* of pLOI4734	This study
pck mutation change		
pLOI4736	*bla kan*; *pck-P* (pck-Pro-up/Pro-down) from *E.coli* ATCC873 cloned into pCR2.1-TOPO vector	This study
pLOI4737	*cat-sacB* cassette (*Sma*I-*Sfo*I fragment of pLOI4162) cloned into *pck-P* of pLOI4736 (pck-Pro-1/Pro-2)	This study
Primers		
pck mutation change		
pck-Pro-up	CACGGTAGCAACAACATTGC	This study
pck-Pro-down	AGAAAGCGTCGACAACGAAC	
pck-Pro-1	ATGCGCGTTA ACAATGGTTT	
pck-Pro-2	ATGGATAACG TTGAACTTTC	
ptsI mutation change		
ptsI-D-up	CGCATTATGTTCCCGATGAT	This study
ptsI-D-down	GCCTTTCAGTTCAACGGTGT	
ptsI-D-1	CGGCCCAATTTACTGCTTAG	
ptsI-D-2	ATCCCCAGCAACAGAAGTGT	
pck sequencing		
pck-F	TTGGCTAAGGAGCAGTGAAATGCGCGTTA	This study

	Relevant characteristics	Source or reference
pck-R	CACGACAAAAGAAGGGTAAATAAAC	
pck-2	TTGTTAACGCGCATTTCACT	
pck-3	GCGATAGCGGCTACTGTCAT	
maeA deletion		
maeA-up	CTATGCTTGATCGGCAACCT	This study
maeA-down	ACGATCGCCTGGTTTTAATG	
maeA-1	TACCGCCGTACCTCCATCTA	
maeA-2	CGTAAGGGATATAAAGCGAACG	
ppc deletion		
ppc-up	TCAAACGATG CCCAACTGTA	This study
ppc-down	TTTAATCCGC TTCGGAAAGA	
ppc-1	GTCACTATTG CCGGGATTGC	
ppc-2	CAATGCGGAA TATTGTTCGT	
pck deletion		
pck-up	TCCGGGCAGTAGTATTTTGC	This study
pck-down	ATGGCTGGATCAAAGTCAGC	
pck-1	CCTGGCGAAACTGTTTATCG	
pck-2	TTGTTAACGCGCATTTCACT	
maeB deletion		
maeB-up	GCATCCTGGGGATGATAATG	This study
maeB-down	TTTCTTCGCCAGTTCCTCAC	
maeB-1	AACCCAACCGCTGTAATTTTT	
maeB-2	CTGGAACTGGAAATTCATGG	
Primers for sequencing*		
pck-F	TTGGCTAAGGAGCAGTGAAATGCGCGTTA	This study
pck-R	CACGACAAAAGAAGGGTAAATAAAC	
pck-2	TTGTTAACGCGCATTTCACT	
pck-3	GCGATAGCGGCTACTGTCAT	
cra-up	GCGGTAAGCTTGATGCATTT	This study
cra-down	CTTCCCCGGTTAACAGTCCT	
ptsHI-up1	TCATCGGGTGAGCGTTATTT	This study
ptsHI-down1	TGACCGTCCAGCGTAATAGC	
ptsHI-up2	CATCCTGGGCCTGAAGATTA	
ptsHI-down2	AGCAATACCATCACCAACGA	
crr-up	CCCGCGCATTAAGAAGATTA	This study
crr-down	CTCATCAGTGGCTTGCTGAA	
ptsG-up	GAAGAACTGGCGCAGGTAAC	This study
ptsG-down	AAGGAAACGCCGTTAATCCT	
cyaA-up	TCGCCATCAACTTGTCTTTG	This study
cyaA-down	AAAGGCGATGAGTGGATTTG	
crp-S-up	TGAGTTGCCGTCCATTAAAA	This study
crp-S-down	AATCGTAATTCGCCAAGCAT	
cpdA-S-up	GAAGTGTGTTCAAGCCAGCA	This study
cpdA-S-down	AGGACAATGGATTCCAGCAG	
ygiF-S-up	ATCAGTGTCGCTACGCAAAG	This study
ygiF-S-down	GCTGTCCTGCACAAAATCAC	
sxy-S-up	TTTACTTGCTGCGGATGAGA	This study
sxy-S-down	TATCTCAGCCCTCGGTGCTC	
csrA-up	CAGCGTTAGCCAGTGTGAAA	This study
csrA-down	ACGCCTCTTACGAGTGCTTC	
csrB-S-up	CTGTAGGAGATCGCCAGGAA	
csrB-S-down	TCTAACAAATCGTGCATTCG	
csrC-S-up	GCCATACGCTTTGTGAGACA	
csrC-S-down	AGTCACGCCCAATGGAATAG	
csrD-S-up1	ATGTGCATGATGGATTGGAA	
csrD-S-down1	CGGTATCCTGACCACTACGC	
csrD-S-up2	GTGATTTTGCTGCGCTGTTA	
csrD-S-down2	ACAAGGCGCAAAAATCATCT	
uvrY-s-up1	CCTCGTCATGTTGCAATGAA	This study
uvrY-s-down1	TATCATCGCGTAGCAAAACG	
barA-s-up1	TTTTGCTTCGCTGCTGTAAA	This study
barA-s-down1	TCAGGCACGTCGCTTTTAAT	
barA-s-up2	CGCGATCACCTGAATACGAT	
barA-s-down2	CTGGCTGGACGTTCGATAAC	
barA-s-up3	TGGCCTATGTCGAACCAAAC	

	Relevant characteristics	Source or reference
mlc-s-up1	CTGGCAAATAACCCGAATGT	This study
mlc-s-down1	CCAGGGCATCTTTATTACGC	
mlc-s-up2	TGAAACTGAAGCCTGGCACT	

*Primers for sequencing were used to amplify the upstream, coding, and terminator fragment of all genes.

Table S2. Effects of mutations in *pck*, *ptsI*, and *cra* on PEP carboxykinase activity

Strain	Genotype	PEP-carboxykinase¶ U·mg protein^{-1}
KJ017†	*pck*$^+$, *ptsI*$^+$, *cra*$^+$	0.7 ± 0.1
XZ618	KJ017, *pck**	6.4 ± 0.2
XZ613	KJ017, δ *ptsI*	0.6 ± 0
XZ626	KJ017, δ *cra*	0.5 ± 0
KJ060‡§	*pck**, *ptsI*^, *cra*$^+$	8.4 ± 0.7
XZ622‡	KJ060, - *pck*$^+$	1.1 ± 0.1
XZ615	KJ060, δ *ptsI*	10.3 ± 1.6
XZ627	KJ060, δ *cra*	7.2 ± 0.8
KJ071†§	*ptsI*^,–*pck*$^+$, *cra*$^+$	0.7 ± 0.1
XZ620	KJ071, *pck**	6.2 ± 0.4
KJ073‡§	*pck**, *ptsI*^, *cra*$^+$	7.3 ± 0.5
XZ624‡	KJ073,–*pck*$^+$	0.9 ± 0.1

†The *pck* in KJ017 and KJ071 was mutated (G to A) to *pck** in XZ618 and XZ620, respectively.

‡The *pck** mutation in KJ060 and KJ073 was restored to wild type (A to G, - *pck*$^+$) in XZ622 and XZ624.

§The abbreviation *ptsI*^ refers to a frame-shift mutation, a single base deletion in the carboxy-terminal region.

¶Crude extract was prepared from mid-log cells during fleaker fermentation.

Table S3. Deletion of *galP* dramatically reduced succinate production from glucose by derivatives of KJ073 (5% glucose NBS medium)

Strain	Time (h)*	Succinate (mM)
KJ073	72	339 ± 3
KJ073, Δ*ptsG*	72	405 ± 18
KJ073, Δ*galP*	>72	51 ± 1
KJ073, Δ*manX*	72	414 ± 3
KJ073, Δ*galP* Δ*manX*	>72	24 ± 3
KJ073, Δ*ptsG* Δ*manX*	72	391 ± 22
KJ073, Δ*ptsG* Δ*galP*	>72	58 ± 3

*Time required to complete fermentation.