Supporting Information

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SI Text

Methods for Gene Deletion and Integration. The methods of seamless chromosomal deletion, gene replacement, and integration were described previously using Red recombinase technology (1). In general, primers "up" and "down" were used to amplify target genes and adjacent regions (200–400 bp upstream and downstream to ORF). Resulting PCR products were cloned into the pCR2.1 TOPO vector. Primers with the designation "1" and "2" ("10" and "20" in some cases) were used to amplify the backbone of the plasmid by inside-out PCR, omitting the coding region of target gene. The PCR fragments were ligated to *cat-sacB* cassette (amplified from pLOI4162) to create the template for integration (1). After removal of *cat-sacB*, the self-ligated plasmid contains only the adjacent regions of target region allowing a seamless deletion (1). Plasmids and primers used in strain constructions are listed in Table S1.

Constructions of Plasmids for *fucO-ucpA* Expression and Chromosomal Integration. ***pLOI5229 (pTrc* fucO-ucpA*).*** The DNA sequence of *fucO* (ribosome-binding site, coding region, and terminator) was cloned previously into pTrc99a (pLOI4319) (2). The whole plasmid of pLOI4319 (2) was amplified by PCR using primers pTrcFucO-UcpA left and pTrcFucO-UcpA right to open the plasmid precisely after *fucO* stop codon and to create the fragment containing the plasmid backbone and *fucO* ORF. The fragment containing intergenic sequence (AATTGAAGAAGGAATAAGGT) and *ucpA* ORF was assembled by PCR using *Escherichia coli* genomic DNA as template and primers pTrcFucO-UcpAORFup and pTrcFucO-UcpAORFdown. Both PCR fragments contain a more than 50-bp identical sequence at each end provided by primers. The two pieces of DNA were joined by CloneEZ PCR Cloning Kit from GenScript to produce pLOI5229. The protein level of FucO produced from pLOI5229 is equal to that from pLOI4319 (~0.7 U/mg protein) (Fig. S1) (2).

pLOI4857 (cloning wild-type* ackA *and its adjacent region into pACYC184). The fragment of *E. coli ackA* ORF and its adjacent region (200 bp upstream and downstream from coding region) was amplified by PCR using primers *ackA*up200 and *ackA*down200. Using primers pACYC-up and pACYC-down, the plasmid backbone of pACYC184 excluding *tet* ORF (1.2 kb) was also amplified. After phosphorylation, these two DNA fragments were ligated to form plasmid pLOI4857.

pLOI4859 (replacing* ackA *ORF with* fucO-ucpA *to create* ackA::fucO-ucpA *cassette). Primers *ackA* 1 and *ackA* 2 were used to amplify the sequence from pLOI4857 precisely excluding the *ackA* ORF by PCR. Primers *ackA*pAC up and *ackA*pAC down were used to amplify the *fucO-ucpA* fragment from pLOI5229. The two pieces of DNA were joined by CloneEZ PCR Cloning Kit, designated pLOI4859.

pLOI4869 (reducing the size of pLOI4859). Primers pACY PacI and pACY HindIII were used to amplify the backbone of pACYC184 omitting *tet* and downstream sequence (1.9 kb). PacI and HindIII sites in primers were added to the two ends of the PCR fragment. Primers HindIII *ackA fucO* and *ackA fucO* PacI were used to amplify the *fucO-ucpA* cassette with flanking *ackA′* regions using pLOI4859 as a template. These primers included PacI and HindIII sites at the ends. These two PCR products were ligated to create plasmid pLOI4869.

pLOI4870 (adding unique BamHI site and ribosomal-binding region). The full length of plasmid pLOI4869 was amplified by inside-out PCR using primers *fucO* RBS and *fucO* BamHI. After phosphorylation and self-ligation, the resulting plasmid was designated pLOI4870. This plasmid contained a promoter-probe cassette consisting of a unique BamH1 site for ligation of Sau3A1 fragments followed by an *adhE* ribosomal-binding site, *fucO* ORF, an intergenic sequence and *ucpA* ORF (Fig. S1). This cassette is bordered by sequence homologous to upstream (omitting part of *ackA* native promoter and ribosomal binding site) and downstream sequences to *ackA* ORF that can be used to guide chromosomal integration (Fig. S1).

Growth-Based Screen for Surrogate Promoters to Express the *fucO-ucpA* Cassette. *E. coli* genomic DNA was completely digested with Sau3AI and ligated into BamHI-treated pLOI4870 to create a plasmid library containing varied sequences between *ackA* upstream sequences (*ackA′*) and the ribosomal binding site of *fucO* (Fig. S1). More than 10,000 colonies were pooled and used to prepare a master library of plasmid DNA. The plasmid library of surrogate promoters was transformed into XW092 (LY180 Δ*yqhD*) with selection on AM1–xylose plates containing 12 mM furfural and 40 mg/L chloramphenicol. Plates were incubated under argon. Large colonies (176 clones) were isolated from more than 10,000 transformants. These were further screened using a BioScreen C growth curve analyzer (BioScreen). Control strains XW092(pACYC184) and XW092(pLOI4870) and clones with a large colony phenotype were inoculated in a 100-well honeycomb plate containing 400 μL of AM1 xylose medium with 40 mg/L chloramphenicol. Optical density was measured at 30-min intervals with 10-s shaking immediately before each reading. After incubation for 16 h, these seed cultures were diluted to an initial optical density of 0.1 and inoculated again in AM1 media containing 12 mM furfural and 40 mg/L chloramphenicol. Growth curves were monitored. The single clone with the highest furfural resistance was selected and designated pLOI5237 (Fig. S1 *B* and *C*). XW092(pLOI5237) also showed much stronger NADH-linked furfural reductase activities (~0.7 U/mg protein) (Fig. S1*D*) and the enhanced putative FucO and UcpA bands (Fig. S1*E*) compared with XW092(pLOI4870).

The promoter fragment in pLOI5237 (1.6 kb) was composed of 10 independent Sau3AI fragments (Fig. S1*B*), each from a different region of the *E. coli* genome. It does not have any known promoter and any complete gene. Approximately 1 kb of upstream sequence containing eight of these fragments was deleted by digestion with BamH1-AatII (self-ligation to create pLOI5259) (Fig. S1*B*), with no decline in furfural tolerance (Fig. S1*C*) or furfural reductase activity (Fig. S1*D*). Analysis of this sequence with the Web-based program Neural Network Promoter Prediction 2.2 (www.fruitfly.org/seq_tools/promoter.html) and BPROM (http://linux1.softberry.com/berry.phtml) both predicted a promoter in an internal segment of the y*adC* coding region near the center of this fragment (Fig. S1*B*).

Sequences of Promoter Fragments from pLOI5237 and pLOI5259 (Subclone). The predicted promoter region (BPROM and Neural Network Promoter Prediction) is underlined and bold. The sequences of *ackA′* upstream and partial *fucO* ORF (downstream) are italicized and underlined.

Promoter Fragment (1.6 kb) from pLOI5237. *TACTTGAGTCGTCAAATTCATATACATTATGCCATTGGCTGAAAATTACGCAAAATGGCATAGACTCAAGATATTTCTTCCATCATGCAAAAAAAATTTGCAGTGCATGATGTTAATCATAAATGTCGGTGTCATCATGCGCTACGCTCTATGGCTCCCTGACGTTTTTTTAGCCAGG*(*ackA′* upstream sequence)

ATCCACGTTTTGTATTAGCAAACGTCAAACTCTCAT-
CGCTGACAGAACTCACCGCAAAAGACCTTCTCGGTTA-
ATGACCAGGGGCAGTGATCGTCTCATGGCCTTGCCAT-
GGTGTTCTCTATGTTGCTGGCGGCGATTATCTGGAAC-
CTGGGTACCTGGTACTTTGGTTTACCTGCATCCAGCTC-
TCATACGCTGATTGGCGCGATCGCTTCTAAAGCAGCC-
CGCATGCGTTCCATCGTCGTTCCTGCGCCAGAAGCGCA-
AAATGATCGGCCAGCCCACCAGGAAGCCCAGCGAGTA-
AATTAAGCCGTCATAGCCGGAGGTAAACACCAGCGCG-
GAGATCTCATTTTTAACCGCGTCAATCAGCATTGAAG-
AGTCCTGGCTTAAGTCATAGCCCGGCGGATTAACCAC-
CTGCATTTCCAGTTCAATACCGAGGGTAAAAGGTTCA-
GAAACATGAAAATCGGGTAATGGCATAGGTTTCTCTT-
AAGTTGGCGTTTTCTATTCAGTATAGAAGTCGGAGCG-
GCTGGGCGAGATGCGGAAGTTCTGGAATGTTTCTTTT-
TTTGGTGATGGTGACTGAAGCAATTTGGCTACTTTTG-
CAATGTGACAAGTTATGGCACGGCTGGCTGGTGGCG-
AAGAATTTTGACGATTGAGGCATGCAGAAAAAAAAC-
GGGTTCAGCTTTCAGTTGATCCTCCCAGAACTTTGCT-
CTGGGGGGGATACGGTCCCCGCTGTTCCCCGTCGCTTA-
ATCTGCATTATGCCGCGTAACTATGGCGCGGCGTTTA-
AGTTTCCTTGCCGATAGCGGCGGCTGGCAGCGTTGGT-
TCTTTGCCGGTATTGCGATTGGTATTAGCGTGATCAA-
ATTCCGCTGGCGGTTATCTCTGGCCCAACGTTTGCGA-
AAGAACTGGCGGCAGGTTTACCTACAGCTATTTCGCT-
GGCCTCGACCGATCAGGAATGCCCAGTGTTGTATTCA-
GACGTCCACGTGACTTATTAAAGATCTTTACTGCGGC-
TATACTCCTGCGACGCTAATTGAGCAGCTTTTTGGTA-
AGATTGATCAAAAATGGAGAGAAACGGGGCCGAATG-
GCGATGCTACTGTCATATTCAGATATGCAACAAGTAC-
AAATAATTTAGTTTTCTACAAACCGACGCAGCTTGGA-
CCTACAGGTGTAAAATTACAGTGGAGTCAGTTAGATA-
CCGCTTCTGGTGGTGGTTTTCTTTATTGCAACAGAAG-
TGATAGCACAAGTGGTAGCGCAATGCGTA**TTGAAAAT-**
GCAATGGTTGACTCAGGTAAAATGTATGGCTCCCATAAAT-
TATTTAATACATCAGTTCCTGGTTTGTATTACACATTATTA-
ATTTCAAACATGTGGTCAGCTTACGGTACCGTAACTA-
ACGTTAGTTCACCTGGGATATACATTGGTGACTCTGC-
AGAACAATATTTTTCGTGGTATAATCCAAGCGAAGAC-
GTGTTATATTGGAGTTGCAATAATGCGAATAGCACCC-
GTAAATACTGGGCTGTAGGTGGTATTTATCAGACCCT-
TACAATTGAATTCTATACAGATACAAACTTTGATCCAT-
ATCAGGAGAGCATT*ATGATGGCTAACAGAATGATTCTG-*
AACG…(fucO downstream ORF)

Subcloned Promoter Fragment (0.6 kb) from pLOI5259. *GTCGTCA-*
AATTCATATACATTATGCCATTGGCTGAAAATTACGCAA-
AATGGCATAGACTCAAGATATTTCTTCCATCATGCAAAA-
AAAATTTGCAGTGCATGATGTTAATCATAAATGTCGGTG-
TCATCATGCGCTACGCTCTATGGCTCCCTGACGTTTTTT-
TAGCCAGG(ackA′ upstream sequence)

ACCACGTGACTTATTAAAGATCTTTACTGCGGCTAT-
ACTCCTGCGACGCTAATTGAGCAGCTTTTTGGTAAGA-
TTGATCAAAAATGGAGAGAAACGGGGCCGAATGGCG-
ATGCTACTGTCATATTCAGATATGCAACAAGTACAAA-
TAATTTAGTTTTCTACAAACCGACGCAGCTTGGACCTA-
CAGGTGTAAAATTACAGTGGAGTCAGTTAGATACCGC-
TTCTGGTGGTGGTTTTCTTTATTGCAACAGAAGTGAT-
AGCACAAGTGGTAGCGCAATGCGTA**TTGAAAATGCAA-**
TGGTTGACTCAGGTAAAATGTATGGCTCCCATAAATTATT-
TAATACATCAGTTCCTGGTTTGTATTACACATTATTAATT-
TCAAACATGTGGTCAGCTTACGGTACCGTAACTAACG-
TTAGTTCACCTGGGATATACATTGGTGACTCTGCAGAA-
CAATATTTTTCGTGGTATAATCCAAGCGAAGACGTGT-
TATATTGGAGTTGCAATAATGCGAATAGCACCCGTAA-
ATACTGGGCTGTAGGTGGTATTTATCAGACCCTTACA-
ATTGAATTCTATACAGATACAAACTTTGATCCATATCA-
GGAGAGCATT*ATGATGGCTAACAGAATGATTCTGAACG…*
(fucO ORF)

1. Jantama K, et al. (2008) Eliminating side products and increasing succinate yields in engineered strains of *Escherichia coli* C. *Biotechnol Bioeng* 101(5):881–893.

2. Wang X, et al. (2011) Increased furfural tolerance due to overexpression of NADH-dependent oxidoreductase FucO in *Escherichia coli* strains engineered for the production of ethanol and lactate. *Appl Environ Microbiol* 77(15):5132–5140.

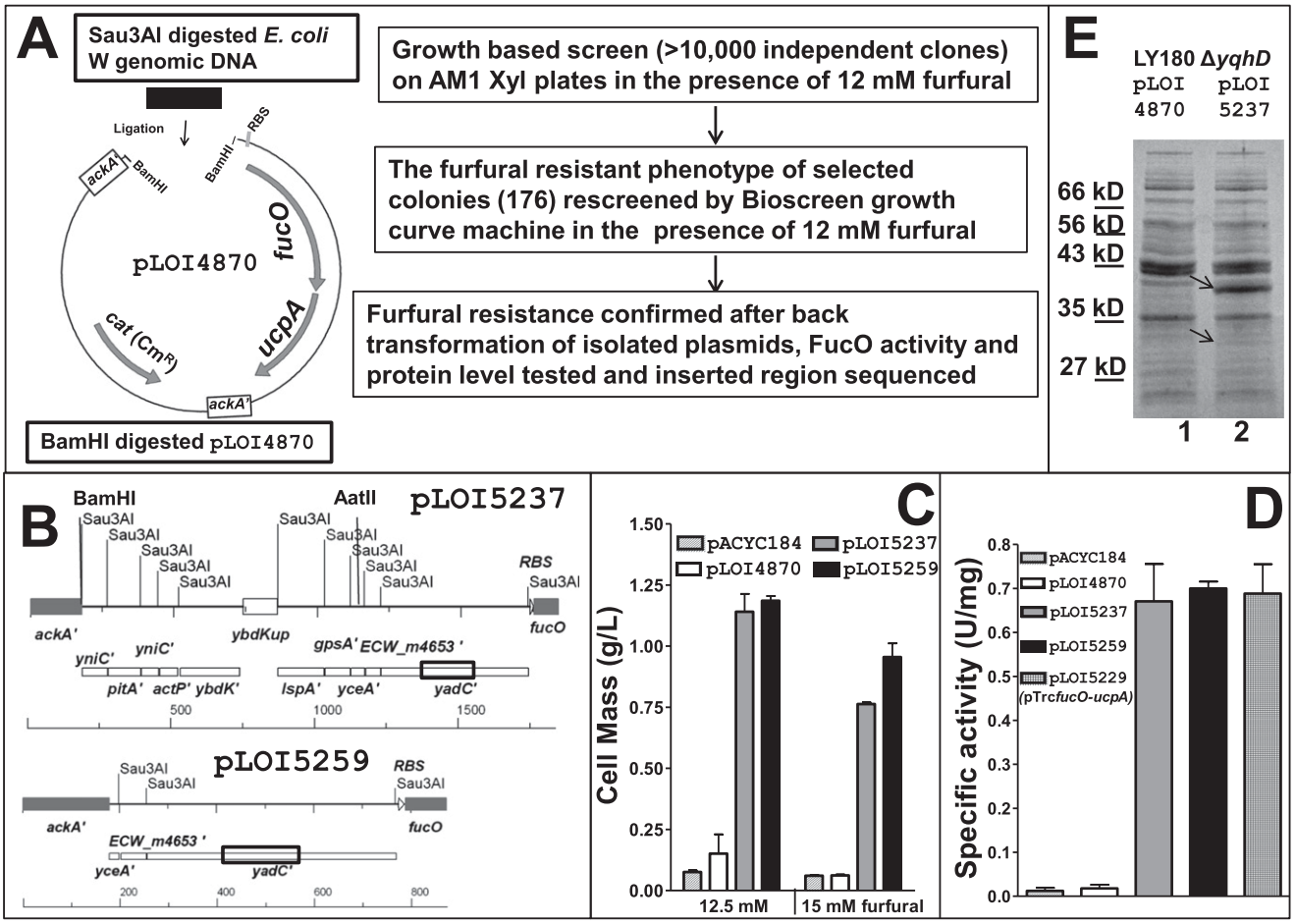

Fig. S1. Isolation and characterization of the surrogate promoter for chromosomal expression of *fucO-ucpA* cassette. (*A*) Promoter–probe plasmid pLOI4870 was used to isolate Sau3A1 fragments that serve as surrogate promoters for expression of *fucO-ucpA*. Two rounds of the growth-based screen were used in AM1 medium containing furfural. (*B*) Isolation and identification of promoter fragment by sequencing pLOI5237 and pLOI5259. A putative promoter (boxed region) was predicted within this fragment using BPROM and Neural Network Promoter Prediction. (*C*) Growth of strains containing furfural-resistance plasmids expressing the *fucO-ucpA* cassette. Tube cultures ($n = 3$) were grown for 48 h in AM1 medium containing 50 g/L xylose, 20 mg/L chloramphenicol, and 12.5 or 15 mM furfural as described previously (2). (*D*) The NADH-linked furfural reductase activity in plasmid strains containing *fucO-ucpA* cassettes. (*E*) SDS/PAGE of cytoplasmic extracts from strains harboring *fucO-ucpA* cassettes. Arrows indicate the predicted size of FucO (molecular mass, 40.5 kDa; thick band) and UcpA (molecular mass, 27.8 kDa; not easily seen).

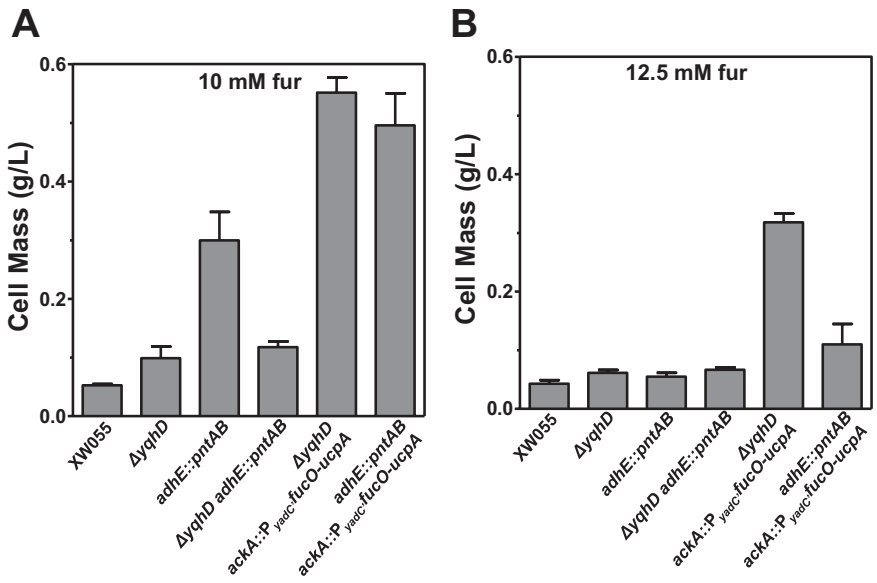

Fig. S2. Effects of furfural resistance traits in succinate-producing strains. Cell mass was measured from tube cultures (n = 3) grown for 48 h in AM1 minimal media containing 50 g/L xylose with 10 mM (*A*) or 12.5 mM (*B*) furfural, 100 mM 3(N-morpholino)propanesulfonic acid (MOPS), and 50 mM $KHCO_3$. Data represent averages of at least three experiments with SDs.

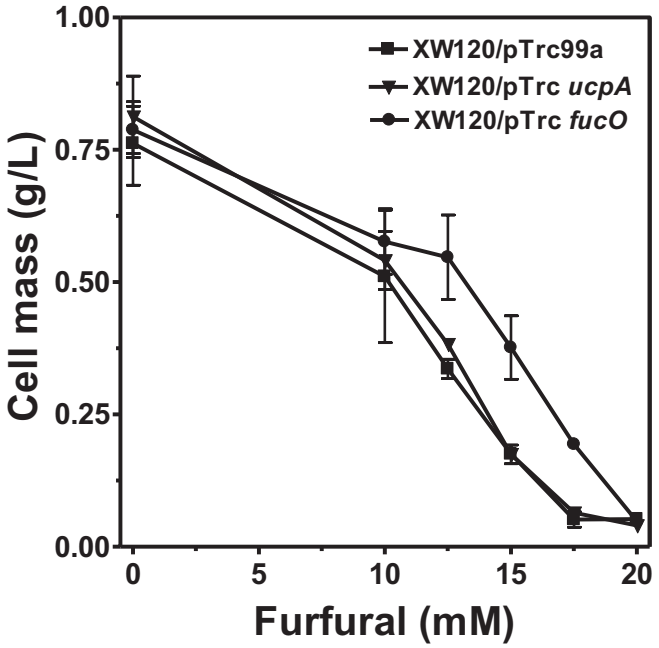

Fig. S3. Effect of plasmid-expressed *fucO* and *ucpA* on furfural tolerance of XW120 (XW055, Δ*yqhD ackA*::P_{yadC}-*fucO-ucpA*) during succinate production from xylose. Tube cultures (n = 3) were grown for 48 h in AM1 medium containing 50 g/L xylose, 100 mM MOPS, 50 mM $KHCO_3$, 0.1 mM isopropyl β-D-1-thio-galactopyranoside, and 12.5 mg/L ampicillin with varying concentrations of furfural. Only plasmid pTrc *fucO* improved the furfural tolerance of strain XW120.

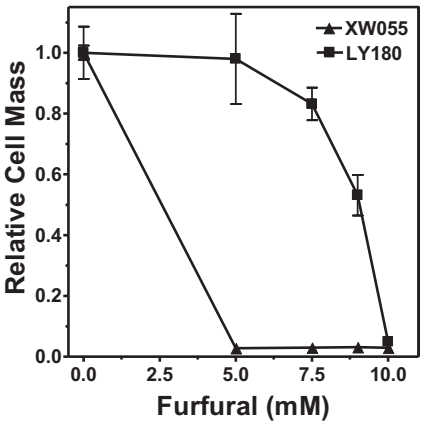

Fig. S4. Comparison of furfural resistance between strains XW055 and LY180. Cell mass was measured from tube cultures (n = 3) grown for 48 h in AM1 minimal media containing 50 g/L xylose with varied concentrations of furfural (additional 100 mM MOPS and 50 mM $KHCO_3$ included for XW055). Data represent averages of at least three experiments with SDs. Cultures were inoculated to an initial density of 22 mg dry cell weight (dcw) per liter.

Table S1. Strains, plasmids, and primers

Strains, plasmids and primers	Relevant characteristics	Source
Strains		
LY180	Δ*frdBC::(ZmfrgcelY$_{Ec}$)*, *ldhA::(ZmfrgcasAB$_{Ko}$)*,*adhE::(ZmfrgestZ$_{Pp}$*FRT*)*, Δ*ackA::*FRT, *rrlE::(pdc adhA adhB* FRT*)*, Δ*mgsA::*FRT	Ref. 1
XW092	LY180 Δ*yqhD*	This study
XW103	LY180 *adhE::pntAB*	This study
XW109	LY180 Δ*yqhD adhE::pntAB*	This study
XW115	LY180 Δ*yqhD ackA::fucO-ucpA*	This study
XW116	LY180 *adhE::pntAB ackA::fucO-ucpA*	This study
XW129	LY180 Δ*yqhD ackA::*P$_{yadC}$*:fucO-ucpA*	This study
XW131	LY180 *adhE::pntAB ackA::*P$_{yadC}$*:fucO-ucpA*	This study
KJ122	Δ*adhE* Δ*ldhA* Δ*focA-pflB* Δ*tdcDE* Δ*mgsA* Δ*citF* Δ*poxB* Δ*aspC* Δ*sfcA* Δ*ackA*	Ref. 2
XW055	KJ122 after serial transfer with xylose; succinate production strain	This study
XW056	XW055 Δ*yqhD*	This study
XW058	XW055 *adhE::pntAB*	This study
XW082	XW055 Δ*yqhD adhE::pntAB*	This study
XW120	XW055 Δ*yqhD ackA::*P$_{yadC}$*:fucO-ucpA*	This study
XW135	XW055 *adhE::pntAB ackA::*P$_{yadC}$*:fucO-ucpA*	This study
XW136	XW055 Δ*yqhD ackA::*P$_{yadC}$*:fucO-ucpA adhE::fucO*	This study
Plasmids		
Characterization of epistatic interactions among furfural resistance traits		
pCR2.1-TOPO	*Bla*, *kan*	Invitrogen
pTrc99a	pTrc *bla oriR rrnB lacI*^{q}	Laboratory collections
pTrc *fucO* (pLOI4319)	*fucO* in pTrc99a	Ref. 3
pTrc *ucpA* (pLOI4856)	*ucpA* in pTrc99a	Ref. 4
pTrc *fucO-ucpA* (pLOI5229)	The intergenic region AATTGAAGAAGGAATAAGGT and *E. coli ucpA* ORF cloned after *fucO* ORF in pLOI4319	This study
Promoter engineering and integration into *ackA* site		
pACYC184	*cat tet* p15A	NEB
pLOI4162	*bla*, *cat-sac* cassette	Ref. 2
pLOI4810	PCR product of *ackA* region (*ackA*::FRT and its adjacent regions) of LY180 cloned into the pCR2.1-TOPO vector (primers used: *ackA* up and *ackA* down)	This study
pLOI4823	*cat-sacB* cassette cloned into *ackA* region of pLOI4810 (primer used: *ackA* 10 and *ackA* 20)	This study
pLOI4857	*E. coli ackA* ORF and its adjacent regions (200 bp upstream and downstream from coding region) (PCR) cloned into pACYC184 by blunt ligation (primers used: *ackA* up 200/*ackA* down 200; pACYC-up/pACYC-down)	This study
pLOI4859	*ackA* ORF in PLOI4857 was replaced by *fucO-ucpA* ORF (from pLOI5229) by CloneEZ PCR Cloning Kit (primers used: *ackA* 1/*ackA* 2; *ackA*pAC up/*ackA*pAC down)	This study
pLOI4869	*fucO-ucpA* ORF and *ackA* adjacent regions from pLOI4859 was cloned into pACYC184. The *tet* ORF and its downstream sequences (total 1.9 kb) were removed to reduce the size of the plasmid smaller. (primers used: pACYC PacI/pACYC HindIII: HindIII *ackA fucO*/*ackA fucO* PacI)	
pLOI4870	BamHI site and *adhE* RBS integrated before *fucO-ucpA* ORF in pLOI4869 to provide ligation site to Sau3AI digested fragments (primers used: *fucO* RBS and *fucO* BamHI)	This study
pLOI5237	Furfural resistant plasmid isolated by promoter screen	This study
pLOI5259	pLOI5237 digested by BamHI and AatII and self-ligated; it contains *ackA*::P$_{yadC}$*:fucO-ucpA* for chromosomal integration	This study
Plasmids used for strain constructions		
Deletion of *yqhD*		
pLOI5203	*E. coli yqhD* and its adjacent regions (PCR) cloned into the pCR2.1-TOPO vector	This study
pLOI5204	*cat-sacB* cassette cloned into *yqhD* of pLOI5203	This study
pLOI5205	PacI digestion of pLOI5204 and self-ligated to delete *yqhD* ORF	This study
Integration of *adhE::pntAB*		
pLOI5167	*E. coli adhE* and its adjacent regions (PCR) from *E. coli* cloned into the pCR2.1-TOPO vector	Ref. 3
pLOI5168	*cat-sacB* cassette cloned into *adhE* of pLOI5167	Ref. 3
pLOI5169	PacI digestion of pLOI5168 and self-ligated to delete *adhE* ORF	Ref. 3

Table S1. Cont.

Strains, plasmids and primers	Relevant characteristics	Source
pLOI5210	Backbone of pACYC184 (PCR) bluntly ligated to *adhE* adjacent regions (from pLOI5169) (primers used: pACYC-up/pACYC-down; *adhE* up/*adhE* down)	This study
pLOI5214	*E. coli pntAB* cloned into *adhE* adjacent regions in pLOI5210 to accurately replace *adhE* ORF by CloneEZ PCR Cloning Kit (primers used: *adhE-pntAB* ORF up/*adhE-pntAB* ORF down; *adhE-pntAB* 1/*adhE-pntAB* 2)	This study
Integration of *adhE::fucO*		
pLOI5209	*E. coli fucO* ORF cloned into pLOI5167 to replace *adhE* ORF by CloneEZ PCR Cloning Kit (primers used: *adhE-fucO* ORF up/*adhE-fucO* ORF down; *adhE-fucO* 1/*adhE-fucO* 2)	This study
Primers		
Deletion of *yqhD*		This study
yqhD up	TATGATGCCAGGCTCGTACA	This study
yqhD Down	GATCATGCCTTTCCATGCTT	This study
yqhD 1	GCTTTTTACGCCTCAAACTTTCGT	This study
yqhD 2	TACTTGCTCCCTTTGCTGG	This study
Integration of *adhE::pntAB*		
adhE up	CAATACGCCTTTTGACAGCA	Ref. 3
adhE down	GCCATCAATGGCAAAAAGTT	Ref. 3
adhE-pntAB ORF up	TACTAAAAAAGTTTAACATTATCAGGAGAGCATTATGCGAATTGGCATACCAAGAG	This study
adhE-pntAB ORF down	TGCCAGACAGCGCTACTGATTACAGAGCTTTCAGGATTGCA	This study
adhE-pntAB 1	TGCAATCCTGAAAGCTCTGTAATCAGTAGCGCTGTCTGGCA	This study
adhE-pntAB 2	CTCTTGGTATGCCAATTCGCATAATGCTCTCCTGATAATGTTAAACTTTTTTAGTA	This study
pTrc *fucO-ucpA* construction		
pTrcFucO-UcpA left	CTTGCCCGTGAGTTTACCCATACCTTATTCCTTCTTCAATTTTACCAGGCGGTATGGTAAAGCT	This study
pTrcFucO-UcpA right	CGGTTAGCGTCGGTATCTGAATGCGCTGATGTGATAATGCCGGAT	This study
pTrcFucO-UcpA ORFup	AATTGAAGAAGGAATAAGGTATGGGTAAACTCACGGGCAAG	This study
pTrcFucO-UcpA ORF down	ATCCGGCATTATCACATCAGCGCATTCAGATACCGACGCTAACCG	This study
Integration of *ackA::fucO-ucpA*		
ackA 10	GACTCTTCCGGCATAGTCTG	This study
ackA 20	GCATGAGCGTTGACGCAATC	This study
ackA up	CTGGTTCTGAACTGCGGTAG	This study
ackA down	CGCGATAACCAGTTCTTCGT	This study
*ackA*up 200	TTAGCAGCCTGAAGGCCTAA	This study
*ackA*down 200	ACGACTTCAGCGTCTTTGGT	This study
pACYC-up	CACCTCGCTAACGGATTCAC	This study
pACYC-down	GGATGACGATGAGCGCATTG	This study
ackA 1	TTTCACACCGCCAGCTCAGC	This study
ackA 2	GGAAGTACCTATAATTGATACGTGGCTAAAAAAACGT	This study
*ackA*pAC up	GTATCAATTATAGGTACTTCCATGATGGCTAACAGAATGATTCTG	This study
*ackA*pAC down	GCTGAGCTGGCGGTGTGAAATCAGATACCGACGCTAACCGTCTCC	This study
pACYC PacI	GCATTTAATTAAcctgtggaacacctacatct	This study
pACYC HindIII	AACCAAGCCTATGCCTACAG	This study
HindIII *ackA fucO*	GCATAAGCTTTTAGCAGCCTGAAGGCCTAAGTAGTACATATTCAT	This study
ackA fucO PacI	GCATTTAATTAAACGACTTCAGCGTCTTTGGTGTTAGCGTG	This study
fucO RBS	TATCAGGAGAGCATTATGATGGCTAACAGAATGATTCTGAACGAAACG	This study
fucO BamHI	GGATCCTGGCTAAAAAAACGTCAGGGAGCCATAGAGCGTAGCGCATGATGA	This study
Integration of *adhE::fucO*		
adhE-fucO ORF up	TACTAAAAAAGTTTAACATTATCAGGAGAGCATTATGATGGCTAACAGAATGATTCTGAAC	This study
adhE-fucO ORF down	TGCCAGACAGCGCTACTGATTACCAGGCGGTATGGTAAAG	This study
adhE-fucO 1	CTTTACCATACCGCCTGGTAATCAGTAGCGCTGTCTGGCA	This study
adhE-fucO 2	GTTCAGAATCATTCTGTTAGCCATCATAATGCTCTCCTGATAATGTTAAACTTTTTTAGTA	This study
Sequencing of pLOI4870		
fucO ORF left	ACCAGCGTTTTATCGGTGAC	This study
ackA up 200	TTAGCAGCCTGAAGGCCTAA	This study

NEB, New England Biolabs.

1. Miller EN, et al. (2009) Silencing of NADPH-dependent oxidoreductase genes (*yqhD* and *dkgA*) in furfural-resistant ethanologenic *Escherichia coli*. *Appl Environ Microbiol* 75(13): 4315–4323.
2. Jantama K, et al. (2008) Eliminating side products and increasing succinate yields in engineered strains of *Escherichia coli* C. *Biotechnol Bioeng* 101(5):881–893.
3. Wang X, et al. (2011) Increased furfural tolerance due to overexpression of NADH-dependent oxidoreductase FucO in *Escherichia coli* strains engineered for the production of ethanol and lactate. *Appl Environ Microbiol* 77(15):5132–5140.
4. Wang X, Miller EN, Yomano LP, Shanmugam KT, Ingram LO (2012) Increased furan tolerance in *Escherichia coli* due to a cryptic *ucpA* gene. *Appl Environ Microbiol* 78(7):2452–2455.