

Supplementary Information to the following manuscript:

Complementation of an *Escherichia coli* K-12 mutant strain deficient in KDO synthesis by forming D-arabinose 5-phosphate from glycolaldehyde with fructose 6-phosphate aldolase (FSA)

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Table S1. PCR primers.

Name	Sequence (5'→ 3')
kdsD-del5'	ATGTCGCACGTAGAGTTACAACCGGGTTTTGACTTTCAGCA <u>ATTGTGTAGGCTGGAGCTGCTTCG</u>
kdsD-del3'	CGCAGTAAATCATGCATATGTAACACACCGAGTAAATGGT <u>CATATGAATATCCTCCTTAGTTC</u>
A _r -rbsK-5' (EcoRI)	TTTT <u>GAAATTC</u> AATGCAGAACCTGTTGACCGCTCATC
A _r -rbsK-3' (BstBI)	TTTTT <u>TTCGAATA</u> AAAATGCGCCACCGTGTTAGGGTG
C _r -rbsK-5' (PstI)	TTTT <u>CTGCAGTCA</u> ATAAAGATCGCTTCGTCAGTG

CrbsK-3' (HindIII)	TTTT <u>AAGCTT</u> AATAAGATCGCTGTCGCCATCGAAC
IrPtac- <i>fsaA</i> ^{A129S} -5' (BstBI)	TTTTT <u>TCGAAT</u> TGGTATGGCTGTGCAGGTCGTAAATC
IrPtac- <i>fsaA</i> ^{A129S} -3' (PstI)	TTTT <u>CTGCAGT</u> TTTATCAGACCGCTTCTGCGTTC
Universal pTarget	ACTAGTATTATACCTAGGACTGAGCTAGC
rbsK (CRISPR)	<u>GCTGCCATCACACTTTCGAGG</u> TTTTAGAGCTAGAAATAGC AAGTTAAAATAAGGCTAG
Test-gutQ-5'	TTCCAGCGTGAAACTATTCGTCAGG
Test-gutQ-3'	ATAAATTGTTTATGGGCACCGACGGC
Test-kdsD-5'	GCAATAGCGGGGGTTCGCAAAGG
Test-kdsD-3'	TGAGCGCACAAACGAATGCCATAACC

Table S2. Dose-response curve for various A5P concentrations, shake flask cultures, OD_{600 nm} measured. Details for growth curves and media used are given in Materials and Methods. Two biological replicates of BW25113 $\Delta gutQ \Delta kdsD::kan$ at A5P concentrations 0 μ M, 5 μ M, 10 μ M, 25 μ M, 40 μ M, 50 μ M, 60 μ M, 70 μ M, and 100 μ M.

	[A5P] (μ M)	Replicate	Time (h)				
			0	4	8	24	48
BW25113 $\Delta gutQ \Delta kdsD::kan$	0	1	0.046	0.079	0.054	0.082	0.031
		2	0.050	0.051	0.072	0.068	0.027
	5	1	0.042	0.041	0.043	0.288	0.835
		2	0.048	0.054	0.057	0.046	0.902
	10	1	0.045	0.043	0.040	0.436	1.206
		2	0.051	0.057	0.056	0.058	1.266
	25	1	0.047	0.070	0.059	0.673	1.808
		2	0.051	0.044	0.081	1.129	1.742
	40	1	0.045	0.045	0.047	0.058	2.916
		2	0.056	0.053	0.051	0.048	2.008
	50	1	0.045	0.055	0.063	0.715	2.382
		2	0.051	0.044	0.088	0.649	2.464
	60	1	0.045	0.048	0.045	0.046	3.113
		2	0.053	0.054	0.056	0.047	2.635
	70	1	0.045	0.050	0.052	0.054	3.232
		2	0.058	0.050	0.056	0.052	2.463
	100	1	0.049	0.050	0.063	0.088	2.532
		2	0.0531	0.05	0.066	0.119	2.949

Average OD_{600 nm} values from two biological replicates (see above):

	[A5P] (μM)	Time (h)				
		0	4	8	24	48
BW25113 <i>ΔgutQ ΔkdsD::kan</i>	0	0.048	0.065	0.063	0.075	0.029
	5	0.045	0.047	0.050	0.167	0.868
	10	0.048	0.050	0.048	0.247	1.236
	25	0.049	0.057	0.070	0.901	1.775
	40	0.051	0.049	0.049	0.053	2.462
	50	0.048	0.050	0.076	0.682	2.423
	60	0.049	0.051	0.050	0.047	2.874
	70	0.051	0.050	0.054	0.053	2.848
	100	0.051	0.050	0.065	0.104	2.741

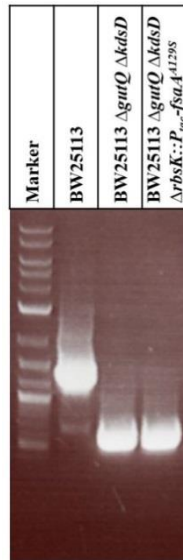
Table S3. Dose-response curve for various exogenous GoA concentrations, shake flask cultures, maximal OD_{600 nm} measured. Details for growth and media used are given in Materials and Methods. Two biological replicates.

GoA concentration (μM)	maximal OD _{600 nm}			
	BW25113 $\Delta gutQ \Delta kdsD::kan$ pJF119fsaA		BW25113 $\Delta gutQ \Delta kdsD::kan$ pJF119fsaA ^{A129S}	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	1.656	1.158	1.328	1.19
10	2.059	1.793	1.982	1.86
25	2.119	2.152	2.66	2.621
40	2.085	2.519	2.368	2.781
50	2.126	2.383	2.594	2.622
90	2.47	2.808	2.678	3.212
100	2.602	2.564	3.157	3.088
125	2.348	2.599	2.686	3.495

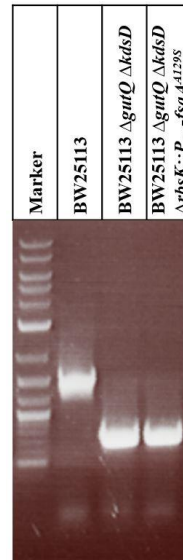
GoA concentration (μM)	maximal OD _{600 nm}			
	BW25113 Δ <i>gutQ</i> Δ <i>kdsD</i> pJF119 <i>fsaB</i>		BW25113 Δ <i>gutQ</i> Δ <i>kdsD</i> Δ <i>rbsK</i> ::P _{tac} - <i>fsaA</i> ^{A129S}	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
0	1.156	1.177	1.238	1.134
10	1.774	1.633	1.549	1.584
25	2.156	1.998	1.808	1.844
40	2.152	1.912	2.043	1.903
50	2.117	2.033	1.936	2.054
90	2.16	2.409	2.243	2.233
100	2.481	2.34	2.253	2.331
125	2.656	2.419	2.394	2.381
150	2.522	2.533	2.485	2.427
200	2.614	2.488	2.569	2.597
300	2.633	2.933	2.695	2.711
400	2.776	2.749	2.76	2.832

Figure S1. Agarose gel electrophoresis. Verification of gene deletions by colony PCR detection to study presence or deletion of the *gutQ*, *kdsD* or *rbsK* genes in the chromosome of BW25113, BW25113 $\Delta gutQ \Delta kdsD$ and BW25113 $\Delta gutQ \Delta kdsD \Delta rbsK::P_{tac-fsaA^{A129S}}$. For the confirmation of $\Delta rbsK::P_{tac-fsaA^{A129S}}$ a subsequent restriction with *EcoRI* was also necessary. BW25113 was used as a control for the deletions and BW25113 $\Delta gutQ \Delta kdsD$ for the integration of $P_{tac-fsaA^{A129S}}$ in the ribose operon.

$\Delta gutQ$



$\Delta kdsD$



$\Delta rbsK::P_{tac-fsaA^{A129S}}$

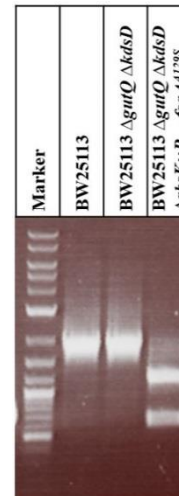
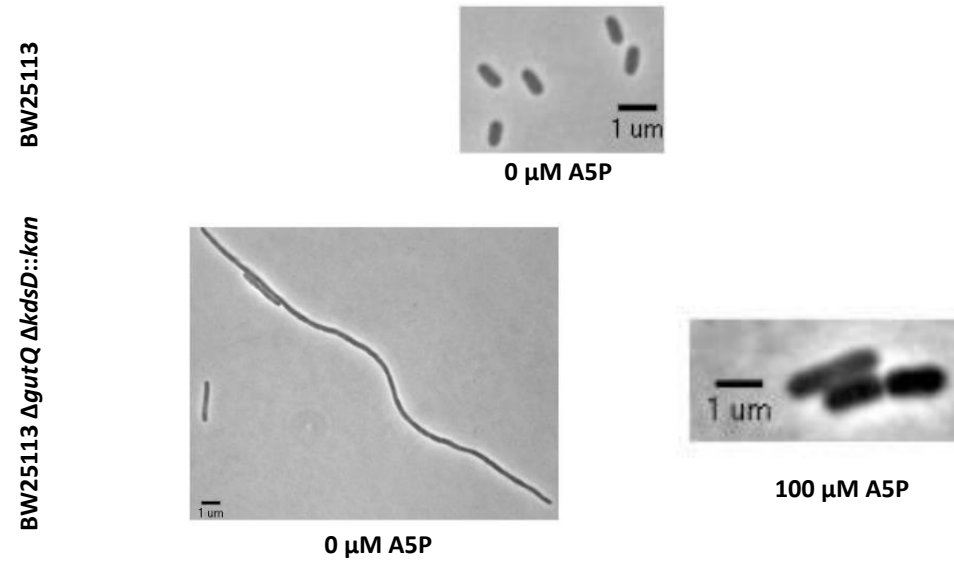
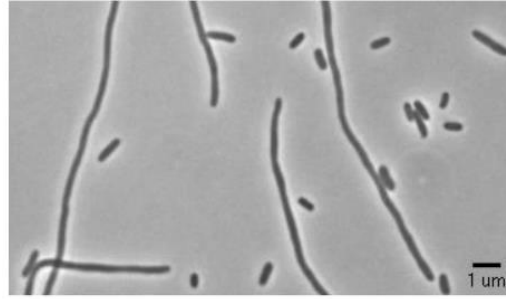


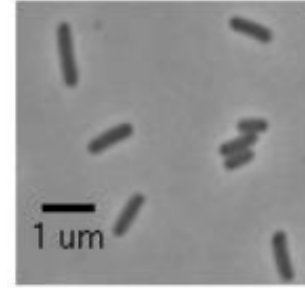
Figure S2. Light microscopy photos of cells of BW25113, BW25113 $\Delta gutQ \Delta kdsD::kan$ (left: in the absence of A5P, right: in the presence of 100 μM A5P), or BW25113 $\Delta gutQ \Delta kdsD \Delta rbsK::P_{tac-fsaA}^{A129S}$ (left: in the absence of A5P; right: in the presence of 100 μM GoA) after growth on MOPS-MM containing 28 mM glucose. BW25113 $\Delta gutQ \Delta kdsD \Delta rbsK::P_{tac-fsaA}^{A129S}$ was induced with 100 μM IPTG during growth.



BW25113 $\Delta gutQ \Delta kdsD \Delta rbsK::P_{tac}-fsaA^{A129S}$



0 μ M A5P



100 μ M GoA