

Electronic supplementary information

Energy-efficient butanol production by *Clostridium acetobutylicum* with histidine kinase knockouts to improve strain tolerance and process robustness

Guangqing Du,^{a,b} Chao Zhu,^{a,b} Mengmeng Xu,^c Lan Wang,^d Shang-Tian Yang^c and Chuang Xue^{*a}
,^b

^aEngineering Research Center of Application and Transformation for Synthetic Biology, School of Bioengineering, Dalian University of Technology, Dalian 116024, China

^bNingbo Institute of Dalian University of Technology, Ningbo 315016, China

^cDepartment of Chemical and Biomolecular Engineering, The Ohio State University, 151 West Woodruff Avenue, Columbus, OH 43210, USA

^dState Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 10090, China

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*Correspondence: Tel & Fax: 86-0411-8470 6625; Email: xue.1@dlut.edu.cn (C. Xue).

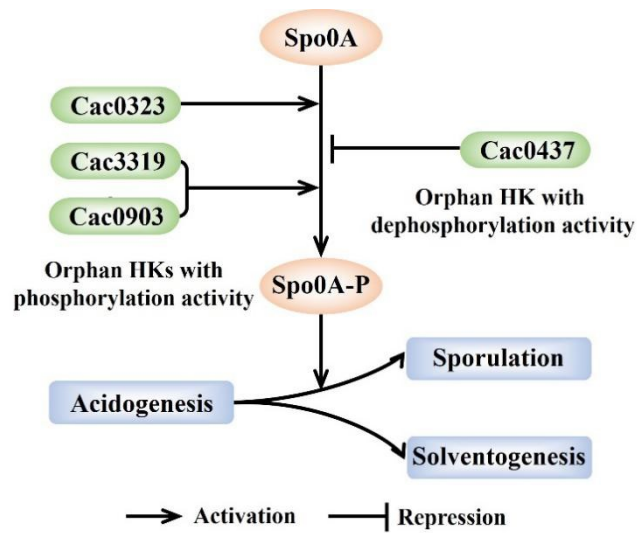


Fig. S1. Phosphorylation and dephosphorylation of Spo0A via orphan HKs in *Clostridium acetobutylicum*. Spo0A is phosphorylated via two parallel routes: the first one is via Cac3319 and Cac0903; the second one is via Cac0323. Dephosphorylation of Spo0A is via Cac0437. These HKs work in coordination to regulate the degree of Spo0A phosphorylation, which in turn regulates sporulation and solventogenesis in *C. acetobutylicum*.

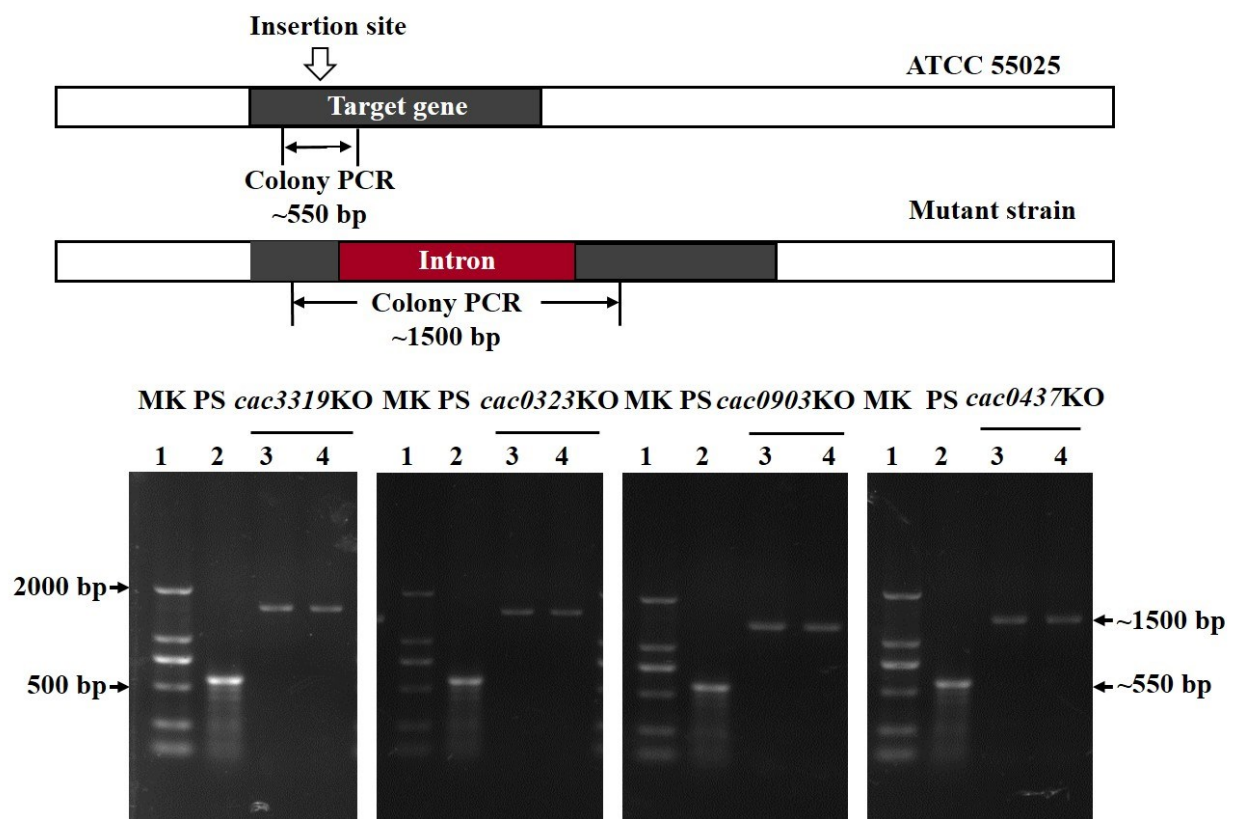


Fig. S2. Confirmation of the gene knockout in *C. acetobutylicum* by colony PCR. The target genes were disrupted by inserting an intron (~950 bp). MK, DNA Marker (DL2000); PS, parental strain.

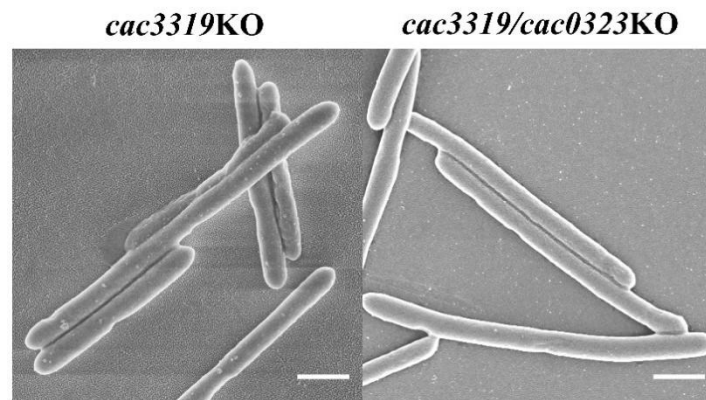


Fig. S3. Cell morphologies of *cac3319KO* and *cac3319/cac0323KO* in the decline phase of fermentation. Scale bar, 1 µm.

Table S1. Strains and plasmids used in this study.

Relevant characteristics		Source or reference
Bacterial strains		
ATCC 55025	Mutant of ATCC 4259	ATCC
<i>cac3319</i> KO	ATCC 55025 with <i>cac3319</i> ::intron	This study
<i>cac3319</i> DHpKO	<i>cac3319</i> ::intron (Intron located at DHp domain)	This study
<i>cac3319</i> H230R	<i>cac3319</i> KO, carries the pIMP1-P _{thl} - <i>cac3319</i> H230R plasmid	This study
<i>cac0323</i> KO	ATCC 55025 with <i>cac0323</i> ::intron	This study
<i>cac0903</i> KO	ATCC 55025 with <i>cac0903</i> ::intron	This study
<i>cac0437</i> KO	ATCC 55025 with <i>cac0437</i> ::intron	This study
<i>cac3319/cac0323</i> KO	ATCC 55025 with <i>cac3319</i> ::intron, <i>cac0323</i> ::intron	This study
<i>cac3319/cac0903</i> KO	ATCC 55025 with <i>cac3319</i> ::intron, <i>cac0903</i> ::intron	This study
<i>cac3319/cac0437</i> KO	ATCC 55025 with <i>cac3319</i> ::intron, <i>cac0437</i> ::intron	This study
<i>cac0323/cac0903</i> KO	ATCC 55025 with <i>cac0323</i> ::intron, <i>cac0903</i> ::intron	This study
<i>cac0323/cac0437</i> KO	ATCC 55025 with <i>cac0323</i> ::intron, <i>cac0437</i> ::intron	This study
<i>cac0903/cac0437</i> KO	ATCC 55025 with <i>cac0903</i> ::intron, <i>cac0437</i> ::intron	This study
<i>E. coli</i> Top10	General cloning host strain	Invitrogen
<i>E. coli</i> ER2275	<i>hsdR mcr recA1 endA1</i>	New England Biolabs
Plasmids		
pSY6	Group II intron, ltrA	Reference 1
pAN1	Cmr, Φ3T I gene, p15A origin	Reference 2
pIMP1-P _{thl}	Expression vector with pIM13 replicon and <i>thl</i> promoter, <i>Amp^r</i> , <i>MLS^r</i> ; repL, ColE1 origin	Reference 3
pSY6- <i>cac3319</i>	Vector for intron insertion in <i>cac3319</i> at 364/365 nt	This study
pSY6- <i>cac3319</i> DHp	Vector for intron insertion in <i>cac3319</i> at 816/817 nt	This study
pSY6- <i>cac0323</i>	Vector for intron insertion in <i>cac0323</i> at 420/421 nt	This study
pSY6- <i>cac0903</i>	Vector for intron insertion in <i>cac0903</i> at 495/496 nt	This study
pSY6- <i>cac0437</i>	Vector for intron insertion in <i>cac0437</i> at 414/415 nt	This study
pIMP1-P _{thl} - <i>cac3319</i> H230R	pIMP1-P _{thl} carrying <i>cac3319</i> ^{H230R} gene	This study

Reference

- 1 L. Shao, S. Hu, Y. Yang, Y. Gu, J. Chen, Y. Yang, W. Jiang and W. Yang, *Cell Res.*, 2007, **17**, 963.
- 2 L. D. Mermelstein and E. T. Papoutsakis, *Appl. Environ. Microbiol.*, 1993, **59**, 1077–1081.
- 3 H. Xiao, Y. Gu, Y. Ning, Y. Yang, W. J. Mitchell, W. Jiang and S. Yang, *Appl. Environ. Microbiol.*, 2011, **77**, 7886–7895.

Table S2. Primers used in this study.

Primer name	Sequence (5'→ 3')
<i>cac3319-364/365s</i> -IBS	CCGCTCGAGATAATTATCCTTATTGGTCCTAATTGTGCGCCCAGAT AGGGTG
<i>cac3319-364/365s</i> -EBS2	TGAACGCAAGTTTCTAATTTTCGGTTACCAATCGATAGAGGAAAGTG TCT
<i>cac3319-364/365s</i> -EBS1d	CAGATTGTACAAATGTGGTGATAACAGATAAGTCCTAATTGTTAAC TTACCTTTCTTTGT
<i>cac3319-816/817s</i> -IBS	CCGCTCGAGATAATTATCCTTACTAAACACTATGGTGCGCCCAGAT AGGGTG
<i>cac3319-816/817s</i> -EBS2	TGAACGCAAGTTTCTAATTTTCGGTTTTTAGTCGATAGAGGAAAGTG TCT
<i>cac3319-816/817s</i> -EBS1d	CAGATTGTACAAATGTGGTGATAACAGATAAGTCACTATGGTTAA CTTACCTTTCTTTGT
<i>cac0323-420/421s</i> -IBS	CCGCTCGAGATAATTATCCTTAAATAACGATAAGGTGCGCCCAGAT AGGGTG
<i>cac0323-420/421s</i> -EBS2	TGAACGCAAGTTTCTAATTTTCGGTTTTATTCCGATAGAGGAAAGTG TCT
<i>cac0323-420/421s</i> -EBS1d	CAGATTGTACAAATGTGGTGATAACAGATAAGTCGATAAGTATAA CTTACCTTTCTTTGT
<i>cac0903-495/496s</i> -IBS	CCGCTCGAGATAATTATCCTTAGATATCATTGCAGTGCGCCCAGAT AGGGTG
<i>cac0903-495/496s</i> -EBS2	TGAACGCAAGTTTCTAATTTTCGATTATATCTCGATAGAGGAAAGTG TCT
<i>cac0903-495/496s</i> -EBS1d	CAGATTGTACAAATGTGGTGATAACAGATAAGTCATTGCAATTAA CTTACCTTTCTTTGT
<i>cac0437-414/415s</i> -IBS	CCGCTCGAGATAATTATCCTTACCTTTCTTAGTAGTGCGCCCAGAT AGGGTG
<i>cac0437-414/415s</i> -EBS2	TGAACGCAAGTTTCTAATTTTCGGTTAAAGGTCGATAGAGGAAAGT GTCT
<i>cac0437-414/415s</i> -EBS1d	CAGATTGTACAAATGTGGTGATAACAGATAAGTCTTAGTAATTAAC TTACCTTTCTTTGT
EBS universal	CGAAATTAGAACTTGCGTTCAGTAAAC
<i>cac3319-s</i>	GGGATAGCTTTATTGGTACTG
<i>cac3319-a</i>	GCTTTGAATAGACTCCACAC
<i>cac3319DHP-s</i>	GCTTATGCTTCTTTTACCTGC
<i>cac3319DHP-a</i>	GGATTGCATTAGATATGAGG
<i>cac0323-s</i>	GGTCGTTACATTAAGGTATGG
<i>cac0323-a</i>	CCATAAGGGTACTACTTAAGGC
<i>cac0903-s</i>	CTTGATTTTAACTGCCGGA
<i>cac0903-a</i>	AATAGAATTTGGTTGCCGC
<i>cac0437-s</i>	GTGGTTGAGTGTATACTTC
<i>cac0437-a</i>	GAATAAAGATAGCCTCTGG
<i>cac3319-For</i>	CGGGATCCATGGAAAACCTTTTCAG
<i>cac3319-Rev</i>	CCGGAATTCCTTACATCATTAATTAAT
<i>cac3319H230R-For</i>	GCAGTTCGTGACCTAAAGAATCCATTATCAGTAAT
<i>cac3319H230R-Rev</i>	TAGGTCACGAAGTGCAGTTGCTAGAAGCTTTGAAGTT
<i>cac0323-For</i>	CGGGATCCATGGATTATTCTAAAATAG
<i>cac0323-Rev</i>	TCCCCCGGGTTACTCCAAATATATATC
<i>cac0903-For</i>	CGGGATCCGTGTTTAATAGATTGAAG
<i>cac0903-Rev</i>	CGGGGTACCCTATAGATCTGAAAACCTC
<i>cac0437-For</i>	CGGGATCCATGAGTTATTTATTG

<i>cac0437</i> -Rev	CGGGGTACCTTAAATCAAGAGATCCG
pIMP1-P _{thl} -S	TCTTACTCGAATTTGGGAAC
pIMP1-P _{thl} -a	TGCGGGCCTCTTCGCTATTA
<i>qcac1050</i> -For	ATGAAAGACAGAGAGAAGAACGC
<i>qcac1050</i> -Rev	ACAACAAGAGCAAGGGTGGA
<i>qcap0162</i> -For	AATCGACGCAAGGATAGAGCTA
<i>qcap0162</i> -Rev	TCCGTAGGGTTCATTTTCGTTT
<i>qcap0035</i> -For	CAAAGCAAACAGCATTTCCC
<i>qcap0035</i> -Rev	TTTATTTATTCCAGCGGCAC
<i>qcac0028</i> -For	ATGGTAGAGGTAGAAGGCAAGG
<i>qcac0028</i> -Rev	CTTTTACTTCATCGGATTCTGTG
<i>qcac2071</i> -For	AGTTGCATGGTCACGTGGA
<i>qcac2071</i> -Rev	AGTCTTAGCTTGTGCAATCATAG
<i>qcac0903</i> -For	GACGGGTATGCCTTGTGATT
<i>qcac0903</i> -Rev	AGGTTGTTGTGGCTGCTTCT
<i>qcac3315</i> -For	TGTAATAAGCCGTTTCGGTTGA
<i>qcac3315</i> -Rev	CCAGGTGTCTCTTTTTCGCAT
<i>qcac0570</i> -For	TGAGACTTGGACAGCCAGATT
<i>qcac0570</i> -Rev	AGCAACTCCAACCGCAA
<i>qcac0877</i> -For	TGACAGTTTAGAGCAGGCACAG
<i>qcac0877</i> -Rev	TAAGTTCTCCCCAACCGCA
<i>qcac2162</i> -For	AGGTTTACCTTTTGATAACACAGC
<i>qcac2162</i> -Rev	TATCGCTAAAGCAAGAACTCCA
<i>qcac2306</i> -For	TCAAGATGATGGGTCTCCTGTT
<i>qcac2306</i> -Rev	ATTTGCGACCTGAGTTTGAGTT

Table S3. Q-RT-PCR verification for selected genes.

Locus tag	Gene	Gene annotation	Fold change (RNA-Seq)				Fold change (Q-RT-PCR)			
			<i>cac3319</i> KO		<i>cac0437</i> KO		<i>cac3319</i> KO		<i>cac0437</i> KO	
			AG	SG	AG	SG	AG	SG	AG	SG
<i>cac0028</i>	<i>hydA</i>	Hydrogen dehydrogenase	-2.4	-4.2	2.0	3.2	-2.1	-3.7	1.5	3.1
<i>cac0570</i>	<i>glcG</i>	Glucose PTS IICBA	16.3	-3.7	4.9	6.4	13.5	-3.6	3.4	4.9
<i>cac0877</i>	<i>cfa</i>	Cyclopropane fatty acid synthase	3.6	7.1	2.3	7.3	3.7	5.0	1.3	5.9
<i>cac0903</i>		Sensory transduction histidine kinase	-13.0	-24.6	-4.4	-4.4	-8.1	-20.6	-2.9	-2.3
<i>cac2071</i>	<i>spo0A</i>	Transcriptional regulator	-2.2	-3.3	-2.7	-2.2	-1.7	-1.7	-3.9	-3.6
<i>cac2162</i>	<i>fliF</i>	Flagellar MS-ring protein	1.4	2.0	3.4	7.9	1.3	1.7	2.7	6.8
<i>cac2306</i>	<i>sigF</i>	Sporulation sigma factor	-13.7	-56.5	-9.9	-10.0	-11.1	-44.7	-7.6	-8.1
<i>cac3315</i>	<i>htpG</i>	Heat shock protein 90	2.5	3.1	1.2	2.2	2.8	4.0	1.6	2.4
<i>cap0035</i>	<i>adhE2</i>	Alcohol dehydrogenase	-2.2	-2.5	-1.1	-1.6	-1.9	-2.4	-1.5	-2.1
<i>cap0162</i>	<i>adhE1</i>	Alcohol dehydrogenase	-1.4	4.0	-41.6	-9.0	-1.4	3.8	-33.9	-8.3

Abbreviations: AG, acidogenesis; SG, solventogenesis.

Table S4. Expression levels of megaplasmid genes in ATCC 55025 and *cac3319/cac0903KO*.

Locus tag	FPKM			
	ATCC 55025		<i>cac3319/0903KO</i>	
	AG	SG	AG	SG
<i>cap0001</i>	8.83	18.42	0	0
<i>cap0002</i>	11.55	30.02	0	0
<i>cap0003</i>	3.97	12.2	0.1	0.21
<i>cap0004</i>	361.66	788.64	0	0
<i>cap0005</i>	21.1	6.94	0	0
<i>cap0006</i>	1131.73	1100.24	0	4.49
<i>cap0007</i>	5.19	0	0	0
<i>cap0008</i>	9.79	19.37	0	0
<i>cap0009</i>	179.19	221.33	0	0.6
<i>cap0010</i>	236.58	336.96	1.34	0
<i>cap0011</i>	154.36	178.73	0.26	0.26
<i>cap0012</i>	129.76	128.6	0	0.94
<i>cap0013</i>	199.44	171.84	0	0.36
<i>cap0014</i>	329.78	329.17	0	0.7
<i>cap0015</i>	25.23	75.79	0	0
<i>cap0016</i>	14.42	40.92	0	0
<i>cap0017</i>	27.99	107.89	0	0
<i>cap0018</i>	20.42	82.35	0	0
<i>cap0019</i>	4.9	3.79	0	0
<i>cap0020</i>	1.5	33.42	0	0
<i>cap0021</i>	3.64	13.85	0	0
<i>cap0022</i>	26.78	59.8	0	0
<i>cap0023</i>	1884.89	5169.64	0.32	0.17
<i>cap0024</i>	1325.78	3761.46	0	1.18
<i>cap0025</i>	124.52	212.93	0.14	0.3
<i>cap0026</i>	59.49	41.76	0.59	1.22
<i>cap0027</i>	3.37	5.09	0	0
<i>cap0028</i>	1.53	6.96	0	0
<i>cap0029</i>	30.33	23.33	0	0
<i>cap0030</i>	2.95	2.26	0	0
<i>cap0031</i>	12.95	19.83	0	0
<i>cap0032</i>	241.54	467.98	0	0
<i>cap0033</i>	452.86	1110.56	0.1	0.73
<i>cap0034</i>	67.78	113.29	0	0
<i>cap0035</i>	20.23	35.85	0	0
<i>cap0036</i>	19.96	20.47	0	1.76
<i>cap0037</i>	12.82	83.19	0	0
<i>cap0038</i>	214.34	302.05	0	0.21
<i>cap0039</i>	2.4	5.5	0	0
<i>cap0040</i>	1.57	11.96	0	0

<i>cap0041</i>	26.53	48.95	0	0
<i>cap0042</i>	18.88	45.97	0.29	0
<i>cap0043</i>	68.14	66.52	0	0
<i>cap0044</i>	4.55	13.84	0	0
<i>cap0045</i>	40.27	103.5	0	0
<i>cap0046</i>	21.87	38.4	0	0.51
<i>cap0047</i>	70.42	61.7	0	0
<i>cap0048</i>	94.78	63.91	0	0.38
<i>cap0049</i>	78.83	159.17	0	0
<i>cap0050</i>	14.02	85.56	0	0
<i>cap0051</i>	111.35	3260.08	0	0
<i>cap0052</i>	80.2	2219.81	0	0
<i>cap0053</i>	16.53	144.83	0	0.78
<i>cap0054</i>	428.64	199.03	0	0
<i>cap0055</i>	2580.73	818.93	0	0
<i>cap0056</i>	79795.05	46307.49	1.04	3.23
<i>cap0057</i>	224.79	270.09	0	0.49
<i>cap0058</i>	1.27	2.9	0	0.99
<i>cap0059</i>	0	62.98	0	0
<i>cap0060</i>	29.72	100.52	0	0
<i>cap0061</i>	60.27	1131.33	0	0
<i>cap0062</i>	15.78	260.75	0	0
<i>cap0063</i>	328.36	438.82	0	0
<i>cap0064</i>	731.45	2544.61	0	0.92
<i>cap0065</i>	690.28	2148.83	0	0.39
<i>cap0066</i>	680.86	2678.32	0	1.33
<i>cap0067</i>	33.15	162.08	0	0
<i>cap0068</i>	353.16	424.85	0.7	3.25
<i>cap0069</i>	8.2	6.2	0	0
<i>cap0070</i>	17030.95	9710.99	0	0
<i>cap0071</i>	348.25	119.57	0	0
<i>cap0072</i>	289.81	56.11	0	0
<i>cap0073</i>	74.23	20.89	0	0
<i>cap0074</i>	116.83	60.13	0	0.81
<i>cap0075</i>	190.47	113.03	0	0.29
<i>cap0076</i>	170.23	51.9	0	0
<i>cap0077</i>	981.13	165.86	0	0
<i>cap0078</i>	22.75	17.22	0	0
<i>cap0079</i>	19.09	32.39	0	0
<i>cap0080</i>	53.07	50.74	0	0
<i>cap0081</i>	156.97	264.5	0.4	0.82
<i>cap0082</i>	439.8	530.96	0	0
<i>cap0083</i>	95.2	127.09	0	0
<i>cap0084</i>	236.59	363.32	0.23	0.24
<i>cap0085</i>	899.24	1200.43	1.18	0.61
<i>cap0086</i>	1543.88	1996.07	0.67	2.32

<i>cap0087</i>	167.33	196.56	0	2.03
<i>cap0088</i>	57.46	99.61	0	0
<i>cap0089</i>	17.91	27.69	0	0
<i>cap0090</i>	72.78	122.83	0	0
<i>cap0091</i>	18.23	27.6	0	0
<i>cap0092</i>	59.15	112.32	0	0
<i>cap0093</i>	217.7	329.74	0	0.68
<i>cap0094</i>	14.64	121.58	0	0
<i>cap0095</i>	28.95	76.31	0	0
<i>cap0096</i>	295.01	693.28	0	0
<i>cap0097</i>	106.43	108.52	0	0
<i>cap0098</i>	218.16	1064.29	0.08	0.08
<i>cap0099</i>	35.36	79.35	0	0
<i>cap0100</i>	117.17	187.87	0	0
<i>cap0101</i>	113.93	174.06	0	1.94
<i>cap0102</i>	106.52	21.47	0	0.63
<i>cap0103</i>	48.18	32.33	0	0.82
<i>cap0104</i>	50.94	69.03	0	0.64
<i>cap0105</i>	37.47	67.24	0	0
<i>cap0106</i>	273.73	358.41	0	0
<i>cap0107</i>	345.94	443.81	0	0
<i>cap0108</i>	12052.59	14330.28	0	0
<i>cap0109</i>	71.17	48.6	0	1.82
<i>cap0110</i>	2.5	26.05	0	0
<i>cap0111</i>	1.75	21.59	0	0
<i>cap0112</i>	63.38	88.88	0.3	0
<i>cap0113</i>	24.47	46.48	0	0.45
<i>cap0114</i>	13.94	29.2	0	0.16
<i>cap0115</i>	9.93	21.13	0	0
<i>cap0116</i>	10.44	23.36	0	0
<i>cap0117</i>	12.7	7.75	0	0.34
<i>cap0118</i>	3.49	6.18	0	0
<i>cap0119</i>	47.4	20.06	0	0
<i>cap0120</i>	70.51	62.63	0	0.25
<i>cap0121</i>	141.79	94.05	0	0
<i>cap0122</i>	212.04	226.79	0	0.94
<i>cap0123</i>	18.3	23.83	0	0.51
<i>cap0124</i>	7.2	70.74	0	0
<i>cap0125</i>	13195.67	11734.13	0	0.43
<i>cap0126</i>	2.12	14.56	0	0
<i>cap0127</i>	55.55	70.76	0	0.58
<i>cap0128</i>	38.63	40.02	0	0
<i>cap0129</i>	505	649.08	0	0
<i>cap0130</i>	246.8	282.14	0	0
<i>cap0131</i>	186.98	255.94	0	0
<i>cap0132</i>	217.39	380.42	0	0

<i>cap0133</i>	174.08	307.77	0	0
<i>cap0134</i>	220.63	335.71	0	0
<i>cap0135</i>	82.23	46.82	0	0
<i>cap0136</i>	4.6	11.73	0	0
<i>cap0137</i>	33.9	924.38	0	0
<i>cap0138</i>	30.58	900.23	0	0
<i>cap0139</i>	12.02	366.69	0	0
<i>cap0140</i>	18.61	572.77	0	0
<i>cap0141</i>	7.64	471.07	0	0
<i>cap0142</i>	15.65	647.71	0.19	0.2
<i>cap0143</i>	0	10.38	0	0
<i>cap0144</i>	374.61	286.96	0	0
<i>cap0145</i>	780.35	430.78	0.64	1.54
<i>cap0146</i>	329.36	677.13	0	0
<i>cap0147</i>	459.05	584.08	0.12	0.96
<i>cap0148</i>	422.62	505.94	0	1.2
<i>cap0149</i>	13.35	20.46	0	0
<i>cap0150</i>	18.66	34.93	0	0.48
<i>cap0151</i>	21.29	342.01	0	0
<i>cap0152</i>	21.48	51.4	0	0
<i>cap0153</i>	2.29	16.48	0	0
<i>cap0154</i>	17.31	36.92	0	0.22
<i>cap0155</i>	5.45	12.47	0	0
<i>cap0156</i>	19.16	110.28	0	0
<i>cap0157</i>	107.92	77.81	0	0.31
<i>cap0158</i>	6662.32	26861.7	0.7	1.35
<i>cap0159</i>	4439.54	17623.08	0	1
<i>cap0160</i>	8410.91	33556.24	1.43	0.98
<i>cap0161</i>	6320.42	19489.24	0.42	0.87
<i>cap0162</i>	0	5.68	0	0
<i>cap0163</i>	7.77	37.81	0	0
<i>cap0164</i>	98.76	454.81	0	0
<i>cap0165</i>	14.42	7.44	0	0
<i>cap0166</i>	9.79	11.37	0	0
<i>cap0167</i>	0	14.88	0	0
<i>cap0168</i>	24.48	37.9	0	0
<i>cap0169</i>	109.23	140.53	0	0
<i>cap0170</i>	123.9	246.32	0	0
<i>cap0171</i>	110.67	200.52	0.29	1.18
<i>cap0172</i>	182.54	179.68	0	0
<i>cap0173</i>	191.58	72.64	0	0
<i>cap0174</i>	64.09	54.7	0	0

Abbreviations: AG, acidogenesis; SG, solventogenesis.

Table S5. RNA-Seq analysis of differential gene transcription in *cac3319KO* and *cac0437KO* in comparison to the parental strain ATCC 55025.

Locus tag	Gene	Gene annotation	Fold change			
			<i>cac3319KO</i>		<i>cac0437KO</i>	
			AG	SG	AG	SG
Cell division						
<i>cac1692</i>	<i>ftsA</i>	Cell division protein FtsA	-1.4	-7.7	1.7	-1.6
<i>cac1693</i>	<i>ftsZ</i>	Cell division protein FtsZ	-1.2	-3.9	1.7	1.1
<i>cac0498</i>	<i>ftsX</i>	Cell division protein FtsX	1.4	-1.1	2.5	3.1
<i>cac0497</i>	<i>ftsE</i>	Cell division ATP-binding protein	UN	-1.2	3.7	4.2
<i>cac1251</i>	<i>ftsW</i>	Cell cycle protein FtsW	-1.3	-3.2	1.7	1.3
<i>cac3459</i>		Cell division GTPase FtsZ	1.3	1.5	5.9	18.5
Ribosomal protein						
<i>cac1257</i>	<i>rpl21</i>	50S ribosomal protein L21	-1.4	UN	1.3	2.1
<i>cac1259</i>	<i>rpl27</i>	50S ribosomal protein L27	-1.5	1.1	2.0	2.6
<i>cac1733</i>	<i>rpmB</i>	50S ribosomal protein L28	-3.4	1.1	1.5	5.7
<i>cac1759</i>	<i>rplS</i>	50S ribosomal protein L19	-1.5	-1.5	2.2	3.1
<i>cac2888</i>	<i>rpmE</i>	50S ribosomal protein L31	-2.0	UN	-2.1	-1.1
<i>cac3098</i>	<i>rplM</i>	50S ribosomal protein L13	-1.7	-2.5	1.6	3.1
<i>cac3103</i>	<i>rplQ</i>	50S ribosomal protein L17	-1.7	-2.8	1.4	2.8
<i>cac3114</i>	<i>rplO</i>	50S ribosomal protein L15	-1.5	-2.6	-1.5	1.3
<i>cac3115</i>	<i>rpmD</i>	50S ribosomal protein L30	-1.6	-3.3	-4.0	1.2
<i>cac3117</i>	<i>rplR</i>	50S ribosomal protein L18	-1.4	-3.0	1.1	2.5
<i>cac3118</i>	<i>rplF</i>	50S ribosomal protein L6	-1.1	-3.1	-1.4	2.1
<i>cac3121</i>	<i>rplE</i>	50S ribosomal protein L5	-1.3	-3.0	-1.2	2.4
<i>cac3122</i>	<i>rplX</i>	50S ribosomal protein L4	-1.3	-3.0	-2.1	1.8
<i>cac3123</i>	<i>rplN</i>	50S ribosomal protein L14	-1.3	-3.2	-2.9	-1.2
<i>cac3125</i>	<i>rpmC</i>	50S ribosomal protein L29	-2.5	-4.4	-3.7	-1.1
<i>cac3126</i>	<i>rplP</i>	50S ribosomal protein L16	-1.3	-2.8	-1.5	2.2
<i>cac3128</i>	<i>rplV</i>	50S ribosomal protein L22	-1.5	-1.8	-1.9	2.7
<i>cac3130</i>	<i>rplB</i>	50S ribosomal protein L2	-2.4	-3.3	1.1	2.9
<i>cac3131</i>	<i>rplW</i>	50S ribosomal protein L23	-1.7	-3.3	-2.5	1.1
<i>cac3132</i>	<i>rplD</i>	50S ribosomal protein L4	-1.3	-3.2	-2.9	-1.2
<i>cac3133</i>	<i>rplC</i>	50S ribosomal protein L3	-1.3	-3.9	-1.1	2.2
<i>cac3145</i>	<i>rplL</i>	50S ribosomal protein L7/L12	-1.3	-1.3	1.5	2.5
<i>cac3146</i>	<i>rplJ</i>	50S ribosomal protein L10	-1.3	-1.3	1.2	2.8
<i>cac3147</i>	<i>rplA</i>	50S ribosomal protein L1	-1.8	-1.5	UN	2.3
<i>cac3148</i>	<i>rplK</i>	50S ribosomal protein L11	-1.7	-1.7	1.2	2.6
<i>cac3717</i>	<i>rpmI</i>	50S ribosomal protein L9	1.2	-1.6	1.1	1.1
<i>cac1274</i>	<i>rpsT</i>	30S ribosomal protein S20	-1.9	-2.2	2.6	2.3
<i>cac1288</i>	<i>rpsU</i>	30S ribosomal protein S21	-2.0	-1.5	-5.1	-4.3
<i>cac1755</i>	<i>rpsP</i>	30S ribosomal protein S16	-1.1	1.8	3.0	6.4
<i>cac1787</i>	<i>rpsB</i>	30S ribosomal protein S2	-1.7	-1.1	1.3	2.4
<i>cac1807</i>	<i>rpsO</i>	30S ribosomal protein S15	-4.2	1.2	-1.1	2.1

<i>cac3097</i>	<i>rpsI</i>	30S ribosomal protein S9	-1.8	-2.0	1.6	3.6
<i>cac3105</i>	<i>rpsD1</i>	30S ribosomal protein S4 A	-1.4	-3.5	1.1	2.7
<i>cac3106</i>	<i>rpsK</i>	30S ribosomal protein S11	-1.1	-3.7	1.2	2.3
<i>cac3107</i>	<i>rpsM</i>	30S ribosomal protein S13	-1.4	-2.8	1.7	3.7
<i>cac3116</i>	<i>rpsE</i>	30S ribosomal protein S5	-1.7	-3.0	-1.3	3.2
<i>cac3119</i>	<i>rpsH</i>	30S ribosomal protein S8	-1.3	-2.4	-1.1	2.5
<i>cac3120</i>	<i>rpsZ</i>	30S ribosomal protein S14 type Z	UN	-2.3	-2.7	1.6
<i>cac3127</i>	<i>rpsC</i>	30S ribosomal protein S3	-1.3	-3.4	-1.3	2.3
<i>cac3129</i>	<i>rpsS</i>	30S ribosomal protein S22	-1.4	-2.8	-1.3	2.0
<i>cac3134</i>	<i>rpsJ</i>	30S ribosomal protein S10	-1.3	-4.4	1.1	2.6
<i>cac3139</i>	<i>rpsG</i>	30S ribosomal protein S7	-2.3	-1.6	1.4	3.1
<i>cac3140</i>	<i>rpsL</i>	30S ribosomal protein S12	-2.4	-1.9	1.6	4.0
<i>cac3722</i>	<i>rpsR</i>	30S ribosomal protein S18	-1.7	-1.1	1.1	2.1
<i>cac3724</i>	<i>rpsF</i>	30S ribosomal protein S6	-1.5	-1.6	UN	1.6

RNA-seq of parental and HK engineered strains can be downloaded from ArrayExpress (<https://www.ebi.ac.uk/arrayexpress/>) with the access number E-MTAB-7341. Gene annotation is based on NCBI designation. Fold change refers to differences in gene expression between parental and histidine kinase inactivated strains. Fold changes ≤ -2 indicates significantly down-regulation, while fold changes ≥ 2 indicates significantly up-regulation. Abbreviations: AG, acidogenesis; SG, solventogenesis; UN, unchanged.

Table S6. Comparison of butanol production from glucose by various solventogenic clostridia strains and processes.

Strain*	Gene manipulation	Butanol titer (g·L ⁻¹)	Yield (g·g ⁻¹)	Productivity (g·L ⁻¹ ·h ⁻¹)	Process	References
Enhanced butanol production						
ATCC 824	<i>pta</i> ::intron, <i>buk</i> ::intron, <i>adhE1</i> - decreasing acids formation	18.4	0.28	0.33	Batch fermentation	Reference 1
ATCC 824	<i>Δcac1502</i> , <i>Δupp</i> , <i>Δptb</i> , <i>Δbuk</i> , <i>ΔctfAB</i> , <i>ΔldhA</i> , <i>ΔrexA</i> , <i>ΔthlA</i> :: <i>atoB</i> , <i>Δhbd</i> :: <i>hbd1</i> - eliminating byproducts acetone, butyric acid and lactate	9–10	0.35	na	Chemostat culture;	Reference 2
JB 200	Adaptation of ATCC 55025 in FBB	19.4	0.21	0.25	Batch fermentation	Reference 3
BA 101	Chemical mutagenesis	18.6	0.32	0.38	Batch fermentation	Reference 4
ATCC 824	<i>groESL</i> - increasing butanol tolerance	17.1	na	na	Fed-batch fermentation	Reference 5
ATCC 824	<i>Δbuk</i> ; <i>ctfAB</i> , <i>adc</i> and <i>sadh</i> - producing isopropanol (4.8 g·L ⁻¹), no acetone	14.6	0.23	na	Batch fermentation	Reference 6
ATCC 824	<i>pfkA</i> and <i>pykA</i> - increasing NADH and ATP	13.1	0.16	0.18	Batch fermentation	Reference 7
		19.1	0.21	0.19	Fed-batch fermentation	
Decoupled butanol production from sporulation						
ATCC 824	<i>sigF</i> ::intron - blocking sporulation prior to asymmetric septum formation	12.4	0.21	0.10	Batch fermentation;	Reference 8
ATCC 824	<i>sigE</i> ::intron - blocking sporulation prior to asymmetric septum formation	11.8	na	na	Batch fermentation;	Reference 9
	<i>sigG</i> ::intron - blocking sporulation during endospore maturation	11.8	na	0.12	Batch fermentation;	
ATCC 824	<i>spoIIE</i> ::intron - blocking sporulation prior to asymmetric division	9.6	0.17	0.08	Batch fermentation;	Reference 10
ATCC 824	<i>Δpks</i> - decreasing sporulation and granulose accumulation	~12	na	na	Batch fermentation;	Reference 11
ATCC 55025	<i>cac3319</i> ::intron, <i>cac0323</i> ::intron	20.6	0.24	0.61	Batch fermentation with cells immobilized in FBB	This study
Fermentation with <i>in situ</i> butanol recovery						
ATCC 824		35.3–64.0	0.35 (ABE)	0.30 (ABE)	Pervaporation	Reference 12
B3	Adaptive strain from CGMCC 5234	150.6	0.23	0.38	Gas stripping	Reference 13
JB 200	Adaptation of ATCC 55025 in FBB	172	0.24	0.35	Gas stripping	Reference 3
CC101	Adaptation of BA 101 in FBB	212–232	na	na	VSVP	Reference 14

ATCC 55025	<i>cac3319::intron, cac0323::intron</i>	441.9 (Solvent: 624.9)	na	na	VSVP	This study
ATCC824	<i>Δcac1502, Δupp, Δptb, Δbuk, ΔctfAB, ΔldhA, ΔrexA, ΔthlA::atoB, Δhbd::hbd1</i>	550 (Solvent: 610) ⁺	0.35	14	Continuous extractive distillation	Reference 2

na – not available or applicable

*All strains belonged to the *C. acetobutylicum* species except for BA 101 and CC101 belonged to *C. beijerinckii* species.

⁺ Butanol and solvent titers in the solvent rich phase after phase separation; all other values were the concentrations in the condensate prior to phase separation.

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