

Table S1. Strains, plasmids and primers used in this study.

	Relevant characteristics ^a	Source
Strains		
<i>Clostridium acetobutylicum</i> ATCC 824	Wild type strain	American Type Culture Collection (ATCC)
<i>Escherichia coli</i> TOP10	F ⁻ , <i>mcrA</i> , Δ(<i>mrr-hsdRMS-mcrBC</i>), φ80 <i>lacZ</i> Δ <i>M15</i> , Δ <i>lacX74</i> , <i>recA1</i> , <i>araD139</i> , Δ(<i>ara-leu</i>)7697, <i>galU</i> , <i>galK</i> , <i>rpsL</i> , (Str ^R), <i>endA1</i> , <i>nupG</i>	Invitrogen GmbH, Karlsruhe, Germany
<i>C. acetobutylicum glcG::int</i> (1224)	Group II intron inserted at 1224./1225. bp of <i>fer</i> (CAC0570), Erm ^R	This study
<i>C. acetobutylicum glcCE::int</i> (193)	Group II intron inserted at 193./194. bp of <i>glcCE</i> (CAC0386), Erm ^R	This study
<i>C. acetobutylicum glcG::int</i> (1224)- <i>glcCE::int</i> (193)	Group II intron inserted at 1224./1225. bp of <i>glcG</i> (CAC0570) and at 193./194. bp of <i>glcCE</i> (CAC0386), Erm ^R	This study
Plasmids		
pMTL007	Clostridial expression vector for expression of ClosTron containing Erm RAM, Cm ^R , IPTG-inducible <i>fac</i> promoter	Heap <i>et al.</i> (2007) [40]
pMTL007C-E2	Clostridial expression vector for expression of ClosTron containing Erm RAM, Cm ^R .	Heap <i>et al.</i> (2010) [39]
pAN2	Plasmid harboring φ3T I methyltransferase gene of <i>B. subtilis</i> phage φ3 <i>I</i> to methylate shuttle plasmids before their introduction into <i>C. acetobutylicum</i> , Tet ^R	Heap <i>et al.</i> (2007) [40]
pMTL007C-E2- <i>glcG</i>	ClosTron plasmid retargeted to <i>C. acetobutylicum glcG</i> (CAC0570) gene, Cm ^R .	This study
pMTL007C-E2- <i>glcCE</i>	ClosTron plasmid retargeted to <i>C. acetobutylicum glcCE</i> (CAC0386) gene, Cm ^R .	This study
Primers		
EBS universal	CGAAATTAGAACTTGC GTTCAGTAAAC	Heap <i>et al.</i> (2010) [39]
<i>glcG</i> ClosTron mutant primers	<i>glcG</i> -IBS:AAAAAAGCTTATAATTATCCTTAGA AGGCGTTAAGGTGCGCCCAGATAGGGTG <i>glcG</i> -EBS1d:CAGATTGTACAAATGTGGTGATA ACAGATAAGTCGTTAAGAATAACTTACCTTT CTTTGT	This study
	<i>glcG</i> -EBS2:TGAACGCAAGTTTCTAATTTTCGAT TCCTTCTCGATAGAGGAAAGTGCT	
	<i>glcCE</i> -IBS:AAAAAAGCTTATAATTATCCTTAC CACCCAATGAGGTGCGCCCAGATAGGGTG <i>glcCE</i> -EBS1d:CAGATTGTACAAATGTGGTGAT AACAGATAAGTCAATGAGTCTAACTTACCTT TCTTTGT	This study
<i>glcCE</i> ClosTron mutant primers	<i>glcCE</i> -EBS2:TGAACGCAAGTTTCTAATTTTCGG TTGGTGGTCGATAGAGGAAAGTGCT	
<i>glcG</i> gene check primers	CAC0570F:ACTTGCTGCATTGCGATCTG CAC0570R:CTTCAGTTCCAAAAAC	This study
	CAC0386F:ACGTGATATAAGAATCAAACCTTC C	
<i>glcCE</i> gene check primers	CAC0386F:CTTGGAAGTCTGACATACTATG	This study
Intron II probe primers	Intron II-F:CGCGACTCATAGAATTATTTC Intron II-R:ATACTCAGGCCTCAATTAACC	This study

^a Cm^R, chloramphenicol/thiamphenicol resistance gene; Tet^R, tetracycline resistance gene; Erm^R, erythromycin resistance gene.

Table S2. Glucose consumption, cell density and product yields of the *C. acetobutylicum* wild type, mutants *glcG::int(1224)* and *glcCE::int(193)* in the phosphate-limited continuous fermentation.

Growth phase	Strains	Glucose consumed(mM)	OD ₆₀₀	Acetone (mM)	Ethanol (mM)	Butanol (mM)	Acetate (mM)	Butyrate (mM)
Acidogenesis	WT	172	4.7	0.2	5.3	6	13	73
	<i>glcG::int(1224)</i>	183	4.6	0.15	4.4	4.6	44.7	66.3
	<i>glcCE::int(193)</i>	174	4.3	0.13	3.96	3.3	39.9	62.4
Solventogenesis	WT	153	4	20	7.3	45	18	23
	<i>glcG::int(1224)</i>	147.8	4	28	6.6	54	26.7	23.1
	<i>glcCE::int(193)</i>	131	4	19.6	4.5	31.6	26.7	20.7

Table S3 Upregulated genes of *C. acetobutylicum* wild type and the mutant *glcCE::int(193)* grown on glucose and cellobiose via DNA microarray.

Groups	ORF	Annotated function	Expression ratio
wild type grown on cellobiose compared to that grown on glucose	CAC0383	PTS, cellobiose-specific component	IIC 5.78
	CAC0384	licB PTS system, cellobiose-specific component	4.66
	CAC0385	licC PTS system, beta-glucosidase	4.84
	CAC0386	PTS, cellobiose-specific component	IIC 2.91
<i>glcCE::int(193)</i> grown on cellobiose compared to the wild type	CAC2951	lacC tagatose-6-phosphate kinase	4.09
	CAC2952	gatY tagatose-1,6-diphosphate aldolase	4.04
	CAC2953	lacB galactose-6-phosphate isomerase	4.65
	CAC2954	lacA galactose-6-phosphate isomerase	4.27
	CAC2955	lacR lactose phosphotransferase system repressor	4.52
	CAC2956	PTS, galactitol/fructose specific IIC component	5.77

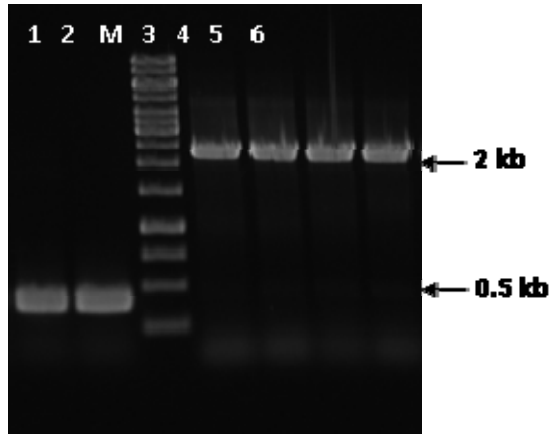


Figure S1. PCR verification of *C. acetobutylicum* *glcG::int(1224)*, *glcCE::int(193)* and *glcG::int(1224)-glcCE::int(193)*. M, marker; lane 1, CAC0570F and CAC0570R primers, wild type genome DNA; lane 2, CAC0386F and CAC0386R primers, wild type genome DNA; lane 3, CAC0570F and CAC0570R primers, *glcG::int(1224)* genome DNA; lane 4, CAC0386F and CAC0386R primers, *glcCE::int(193)* genome DNA; lane 5, CAC0570F and CAC0570R primers, *glcG::int(1224)-glcCE::int(193)* genome DNA; lane 6, CAC0386F and CAC0386R primers, *glcG::int(1224)-glcCE::int(193)* genome DNA

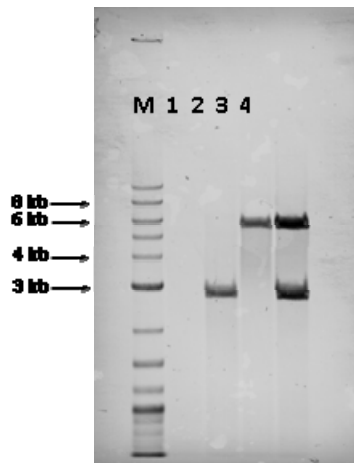


Figure S2. Southern hybridization to demonstrate the presence of intron in the constructed *C. acetobutylicum* mutants. M, Marker; lane 1, The wild type (negative control); lane 2, The mutant *glcG::int(1224)*; lane 3, The mutant *glcCE::int(193)*; lane 4, The mutant *glcG::int(1224)-glcCE::int(193)*.

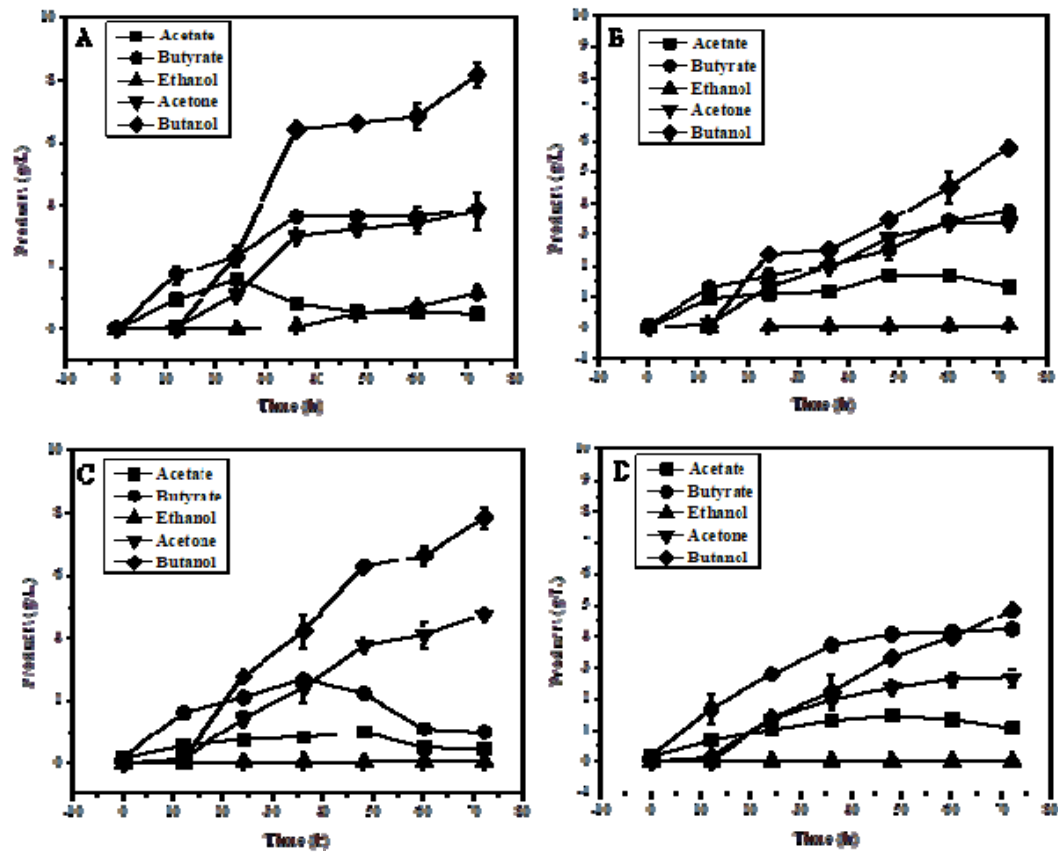


Figure S3. Product concentrations of the *C. acetobutylicum* wild type(A), *glcG::int(1224)*(B), *glcCE::int(193)*(C), and *glcG::int(1224)-glcCE::int(193)* (D) in the batch culture with glucose as the sole carbon source.

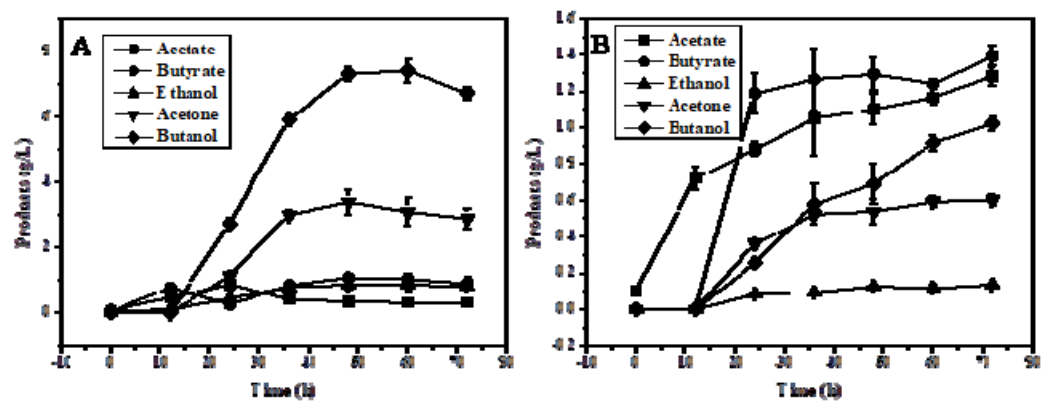


Figure S4. Product concentrations of the *C. acetobutylicum* wild type (A) and *glcCE::int(193)* (B) in the batch culture with cellobiose as the sole carbon source.