Supplementary data to Baumgart et al. (2011)

A. TABLE S1. Oligonucleotides used in this study

Oligonucleotide	Sequence (5' → 3') and properties[a]
acn-A-fw-EcoRI	TATATAGAATTCGCATGTTCTGGCCTGCATGAATG
acn-B-rev	**CCCATCCACTAAACTTAAACA**AGTCACAGTGAGCTCCAATTCTAAC
acn-C-fw	**TGTTTAAGTTTAGTGGATGGG**TACGTGCTGCGTCAGATGGCTG
acn-D-rev-BamHI	TATATAGGATCCGTTGGTGGCATTCAGTGGGCTTC
acn-upstream	TCTTTTTCCAAAGTCGGAAACTG
icd-A-fw-EcoRI	GCGCGAATTCGCATCCTCGAAGACCTCGCAG
icd-B-rev	**CCCATCCACTAAACTTAAACA**GTCGGTGCGGGTCCAGATGATC
icd-C-fw	**TGTTTAAGTTTAGTGGATGGG**AACGAGATCGTTGACGCACTG
icd-D-rev-BamHI	GCGCGGATCCGGCTTGTTGAAGTTGGCTGAGTAC
Icd-upstream	GGTGCAGCCACCAAGGTGATCTTGG
acn-RBS-BamHI-fw	TATATAGGATCCAAGGAGATATAGATTTGGAGCTCACTGTGACTGAAAG
acn-BamHI-rv	TATATAGGATCCCGTATTAGAAAGGACCCCACCAC
acn-NdeI-fw	GACCATATGTTGGAGCTCACTGTGACTGAAAG
acn-NheI-STOP-rv	GACGCTAGCTTACTTAGAAGAAGCAGCCATCTG
gltA-RBS-NheI-fw	GCGCGCTAGCGAGATATACTAATGTTTGAAAGGGATATCGTGGC
gltA-NheI-rv	GCGCGCTAGCTTAGCGCTCCTCGCGAGGAACCAAC
icd-NdeI-fw	GCGCCATATGGCTAAGATCATCTGGACCC
icd-NheI-STOP-rv	GCGCGCTAGCTTACTTCTTCAGTGCGTCAACGATC
gltA-fw	CCTGAGGTTGCGTTATAGGGTGGCG
gltA-rv	CATTATGACAGACGAAGTGGCTTCG
gltA-seq-f	GTAAGGAGCACCCTTGCGTGCGCGG
gltA-seq-b	CAGGACCAGCTGAACCCACTCGATGAGG
citH-NdeI-fw	GCGCCATATGAGCAGCTCAACACTTCTCCTG
citH-NheI-rv	GCGCGCTAGCTTATGCGCTGAGTGGCACGACG
sequenz_citH_fw	ACTGGCACTTGCAGTGAACTTCC

[a]In some cases oligonucleotides were designed to introduce recognition sites for restriction endonucleases(recognition sites underlined). Complementary sequences used for overlap extension PCR are written in bold letters.

B. Growth of *C. glutamicum* wild type and Δ*acn*-1 on CGXII minimal medium with L-glutamine or L-glutamate supplementation

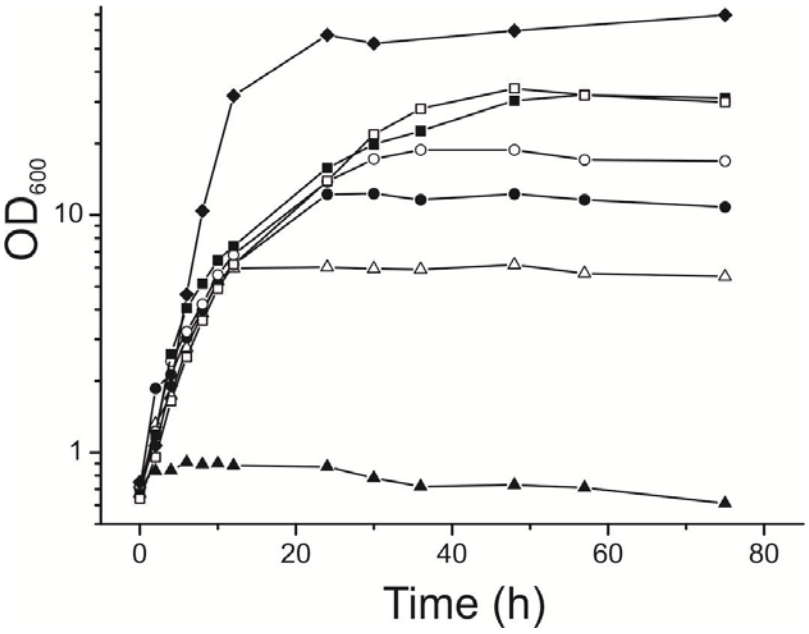


Fig. S1. Growth of *C. glutamicum* wild type and Δ*acn*-1 on CGXII minimal medium with L-glutamine or L-glutamate supplementation. Cells of *C. glutamicum* wild type (♦) or Δ*acn*-1 (all other symbols) were cultivated in CGXII minimal medium with 4% (w/v) glucose and the following supplementations: ▲, without supplementation; Δ, 5 mM L-glutamine; ●, 20 mM L-glutamine; ○, 40 mM L-glutamine; ■, 100 mM L-glutamine; □ and ♦, 100 mM L-glutamate. A representative result of three independent experiments is shown.

C. Complementation studies with *C. glutamicum* Δ*acn*-1

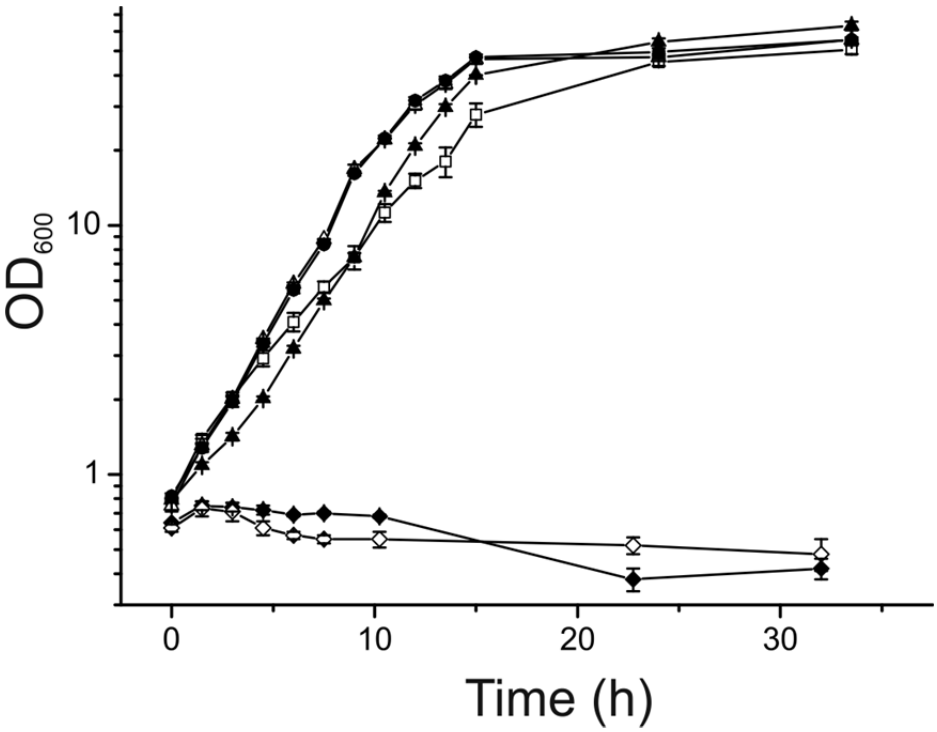


Fig. S2. Complementation studies with *C. glutamicum* Δ*acn*-1. Cells were cultivated in CGXII medium with 4% (w/v) glucose, 25 µM kanamycin and with or without 20 µM IPTG. These media were not supplemented with L-glutamate and L-glutamine. Δ, wild type with pAN6 –IPTG; ●, wild type with pAN6 + 20 µM IPTG; ♦, Δ*acn*-1 with pAN6 –IPTG; ◊, Δ*acn*-1 with pAN6-acn –IPTG; □, Δ*acn*-1 with pAN6-acn-gltA –IPTG; ▲, Δ*acn*-1 with pAN6-acn-gltA + 20 µM IPTG. The plasmid pAN6-acn contains only the aconitase gene under control of the *tac* promoter, the plasmid pAN6-acn-gltA the genes for aconitase and citrate synthase, each with a separate ribosomal binding site. The wild type cultures with the control plasmid pAN6 grew almost identically in the absence and presence of IPTG, therefore the growth curves cannot be distinguished. As the *tac* promoter of the vector pAN6 is leaky, complementation of strain Δ*acn*-1 by pAN6-acn-gltA occurs also without IPTG addition. Addition of 20 µM IPTG leads to increased expression and improved growth of this strain. The data shown represent average values and standard deviations of three independent cultures

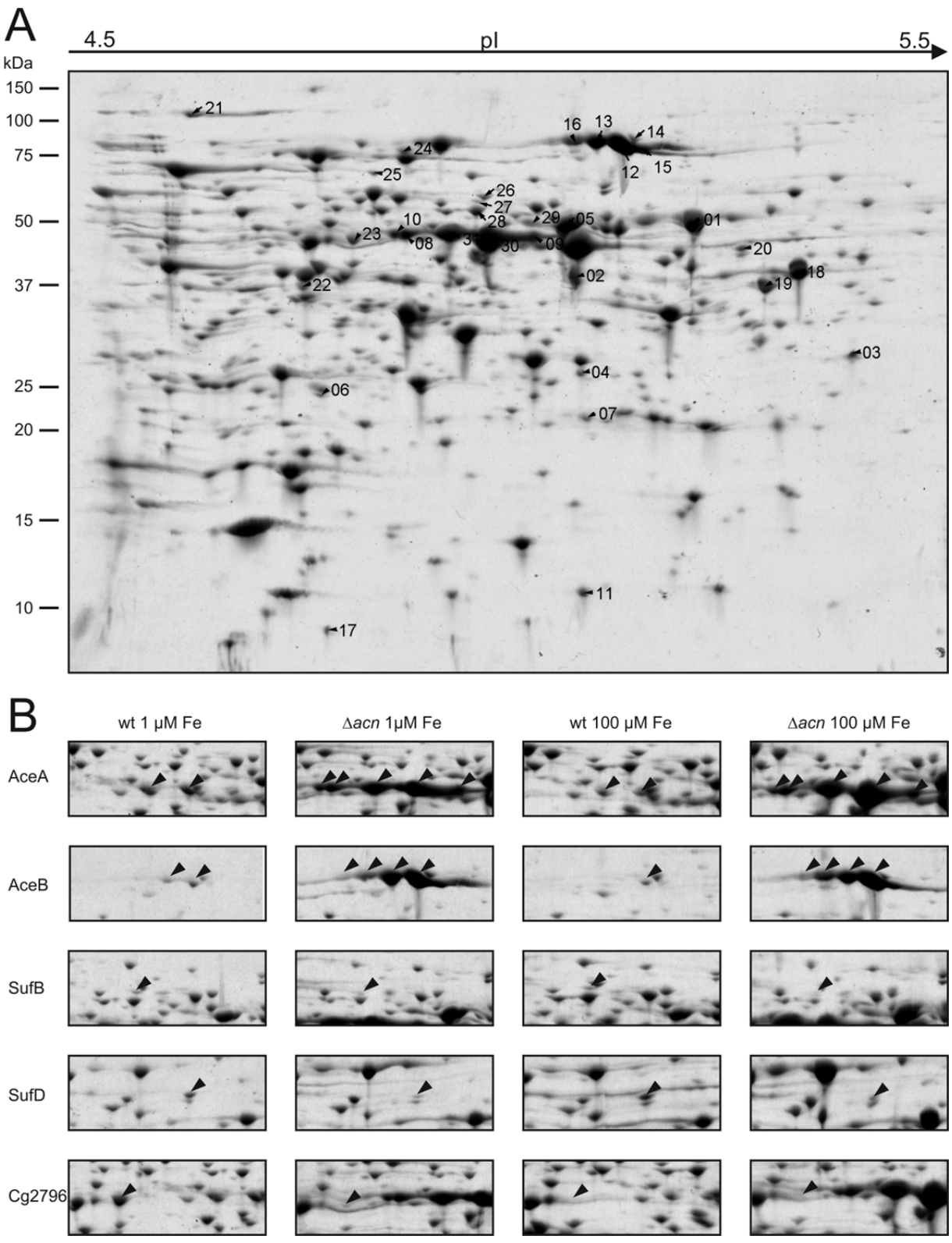
D. Proteome analysis of *C. glutamicum* Δ*acn*-1.

Methods. In order to analyse changes in the proteome due to an aconitase deletion, *C. glutamicum* wild type and the Δ*acn*-1 mutant were cultivated as described in 50 ml CGXII medium supplemented with 4% (w/v) glucose and 100 mM sodium glutamate until an OD_{600} of ~5 was reached. The cells were harvested, washed once in PBS and resuspended in 1 ml PBS containing Complete EDTA-free protease inhibitor (Roche Diagnostics, Mannheim, Germany). 1 ml of this cell suspension was mixed with 250 mg zirconia-silica beads (0.1-mm diameter; Roth, Karlsruhe, Germany) and the cells were mechanically disrupted by 3×30 s bead beating. Intact cells and cell debris were removed by centrifugation at 16000 g for 15 min at 4°C and the cell-free extract was subjected to ultracentrifugation (1 h, 150,000 g, 4°C). 300 µg protein of the resulting supernatant was used for 2D gel electrophoresis. Three gels starting from independent cultures were performed for each condition and strain. The gels were analysed using the software Delta 2D (Decodon, Greifswald, Germany). Proteins showing differential expression were identified by peptide mass fingerprinting using an Ultraflex III TOF/TOF mass spectrometer (Bruker Daltonics, Bremen, Germany). The MASCOT software was used to compare the peptide mass patterns obtained with those of all proteins from the theoretical *C. glutamicum* proteome. The molecular weight search (MOWSE) scoring scheme with a cut-off value of 45 was used for unequivocal identification of proteins.

Results. As described in the background section, several bacterial aconitases were shown to have not only an enzymatic, but also a regulatory function. To get hints if aconitase from *C. glutamicum* is also involved in regulation and to gain more insight into the global effects of the absence of aconitase, a comparative 2D gel analysis was performed. Cells of the wild type and the mutant strain Δ*acn*-1 were cultivated either under iron starvation (1 µM Fe) or iron excess (100 µM Fe) conditions in CGXII glucose minimal medium supplemented with 100 mM L-glutamate. Significant differences in the protein pattern of wild type and Δ*acn*-1 mutant were observed and the relevant proteins were analysed by peptide mass fingerprinting using MALDI-TOF mass spectrometry (Fig. S1). In total, 13 proteins were identified which showed a more than 1.75-fold increased level in the Δ*acn*-1 strain and 13 proteins with a more than 1.75-fold decreased level in at least one of the tested conditions, which are listed in Table S2. For comparison, this table also shows the mRNA ratios of the corresponding genes, which were obtained from DNA microarray data presented in detail in the next section. The most drastic changes were observed for the key enzymes of the glyoxylate cycle, isocitrate lyase

(*aceA*) and malate synthase (*aceB*), whose protein levels were increased ~6-fold and >10-fold in the Δ*acn*-1 strain, respectively (Fig. S1B). Interestingly, both proteins were present in several spots, which might be an indication for posttranslational modifications. Besides isocitrate lyase and malate synthase, four other enzymes involved in the metabolism of acetate or ethanol showed increased protein levels in the Δ*acn*-1 mutant, which were acetate kinase (*ackA*), phosphotransacetylase (*pta*), alcohol dehydrogenase (*adhA*), and acetaldehyde dehydrogenase (*ald*). A common characteristic of the corresponding six genes is that all of them are activated by the transcriptional regulator RamA in the presence of acetate. SufB and SufD, two proteins which are part of the only known Fe-S cluster assembly and repair machinery of *C. glutamicum*, showed 3- to 4-fold decreased levels in the Δ*acn*-1 strain, both under iron excess and iron limitation (Fig. S1B). One protein, a putative 2-methylcitrate dehydratase (cg2796), was identified whose level differed in the wild type and the Δ*acn*-1 mutant dependent on the iron supply of the medium. The protein level was higher in the mutant than in the wild type under iron excess, but lower under iron limitation.

Fig. S3 (next page). Comparative 2D gel analysis of *C. glutamicum* Δ*acn*-1 and wild type under iron starvation and iron excess. Cells were cultivated in CGXII minimal medium with 4% (w/v) glucose, 100 mM L-glutamate and either 1 µM or 100 µM $FeSO_4$ and harvested in the exponential growth phase. 300 µg cell extract protein was used for each 2D gel. A is the fusion of all 12 gels. The numbers indicate proteins (and the corresponding genes) which were differentially expressed and identified by MALDI-TOF MS analysis. 01, Cg2840 (*actA*); 02, Cg3047 (*ackA*); 03, Cg0876 (*sigH*); 04, Cg2091 (*ppgK*); 05, Cg3096 (*aldA*); 06, Cg1209 (*phnA*); 07, Cg0404; 08, Cg2560 (*aceA*); 09, Cg2560 (*aceA*); 10, Cg2560 (*aceA*); 11, Cg0963; 12, 13, Cg2559 (*aceB*); 14, Cg3079 (*clpB*); 15, 16, Cg2559 (*aceB*); 17, Cg0215 (*cspA*); 18, Cg1791 (*gapA*); 19, Cg3107 (*adhA*); 20, Cg1763 (*sufD*); 21, Cg1737 (*acn*); 22, Cg2240 (*thiF*) & Cg2530 (*treX*); 23, Cg2796 (*prpD3)*; 24, Cg1290 (*metE*); 25, Cg2185 (*proS*); 26, Cg1366 (*atpA*); 27, Cg1764 (*sufB*); 28, Cg3119 (*cysJ*); 29, Cg3096 (*aldA*); 30, Cg2560 (*aceA*); 31, Cg2560 (*aceA*) & Cg3048 (*pta*). B shows sections of the fusion images of the four respective conditions (wt 1 µM Fe, Δ*acn* 1 µM Fe, wt 100 µM Fe, Δ*acn* 100 µM Fe) for selected proteins.


A
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pI
5.5
kDa
150
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37
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04
06
07
11
17
B
wt 1 µM Fe
Δacn 1µM Fe
wt 100 µM Fe
Δacn 100 µM Fe
AceA
AceB
SufB
SufD
Cg2796

TABLE S2. Differentially expressed proteins in *C. glutamicum* Δ*acn*-1 compared to the wild type identified by comparative 2D gel analysis and MALDI-TOF MS

Protein spot no.[a]	Locus tag	Gene and annotated function	Protein ratio 1 µM Fe[b]	Protein ratio 100 µM Fe[b]	mRNA ratio 1 µM Fe[c]	mRNA ratio 35 µM Fe[c]
13	cg2559	*aceB*, malate synthase	4.67	19.43	608.19	65.20
12	cg2559	*aceB*, malate synthase	7.32	17.59		
16	cg2559	*aceB*, malate synthase	3.17	17.54		
15	cg2559	*aceB*, malate synthase	11.73	11.67		
10	cg2560	*aceA*, isocitrate lyase	4.46	14.13	6615.86	192.64
8	cg2560	*aceA*, isocitrate lyase	3.62	6.75		
30	cg2560	*aceA*, isocitrate lyase	2.41	4.23		
9	cg2560	*aceA*, isocitrate lyase	2.32	2.39		
31	cg2560	*aceA*, isocitrate lyase	2.42	6.05		
31	cg3048	*pta*, phosphotransacetylase	2.42	6.05	4.99	6.31
2	cg3047	*ackA*, acetate kinase	1.76	1.9	4.52	5.45
11	cg0963	hypothetical protein	3.43	3.44	n.c.[e]	2.57
23	cg2796	*prpD3*, putative 2-methylcitrate dehydratase	0.33	2.98	7.56	8.89
19	cg3107	*adhA*, Zn-dependent alcohol dehydrogenase	2.1	2.62	2.82	n.c.
1	cg2840	*actA*, CoA transferase	1.52	2.46	n.c.	n.c.
5	cg3096	*aldA*, aldehyde dehydrogenase	1.7	2.28	5.53	3.22
29	cg3096	*aldA*, aldehyde dehydrogenase	1.78	2.17	5.53	3.22
14	cg3079	*clpB*, probable ATP-dependent protease (heat shock protein)	3.91	2.05	n.c.	2.15
18	cg1791	*gapA*, glyceraldehyde-3-phosphate dehydrogenase	1.35	2	2.53	2.27
4	cg2091	*ppgK*, polyphosphate glucokinase	1.32	1.87	n.c.	n.c.
3	cg0876	*sigH*, RNA polymerase sigma factor	1.06	1.76	n.c.	n.c.
24	cg1290	*metE*, 5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	0.45	0.86	0.39	0.14
25	cg2185	*proS*, prolyl-tRNA synthetase	0.46	0.48	n.c.	0.34
7	cg0404	protein of nitroreductase family	0.36	0.46	0.31	0.13
21	cg1737	*acn*, aconitase	n.e.[d]	n.e.	0.00	0.01
22	cg2240	*thiF*, enzyme involved in thiamine biosynthesis	0.46	0.43	0.44	0.34
22	cg2530	*treX*, trehalose metabolism	0.46	0.43	n.c.	n.c.
26	cg1366	*atpA*, F_1F_0-ATP synthase, subunit □	0.25	0.39	0.27	0.20
20	cg1763	*sufD*, Fe-S cluster assembly protein	0.3	0.37	3.10	3.32
28	cg3119	*cysJ*, probable sulfite reductase (flavoprotein)	0.53	0.30	5.45	n.c.
6	cg1209	*phnA*, uncharacterized Zn-ribbon-containing protein	0.66	0.27	n.c.	n.c.
27	cg1764	*sufB*, Fe-S cluster assembly protein	0.34	0.24	3.74	3.48
17	cg0215	*cspA*, cold-shock protein	0.42	0.16	0.31	n.c.

[a]Protein numbers corresponding to the spots indicated in Fig. S1.

[b]Protein ratios of the 2D gel analysis (Δ*acn*-1 vs. wild type) of the normalised spot volumes.

[c]The mRNA ratios (Δ*acn*-1 vs. wild type) derived from DNA microarray analyses were added for comparison. The entire transcriptome results are given in Tables S3 and S5.

[d]n.e., not evaluable.

[e]n.c., not changed.

E. Transcriptome analysis of *C. glutamicum* wild type and Δ*acn*-1 using DNA microarrays.

Methods. The preparation of RNA and the synthesis of fluorescently labelled cDNA was performed as described (Möker et al. (2004) Mol. Microbiol. 54:420-438). Custom-made DNA microarrays for *C. glutamicum* ATCC13032 printed with 70mer oligonucleotides were obtained from Operon (Cologne, Germany) and are based on the genome sequence entry NC_006958 (Kalinowski et al. (2003) J. Biotechnol. 104:5-25). Hybridisation and stringent washing of the microarrays was performed according to the instructions of the supplier. Hybridization was carried out for 16–18 h at 42°C using a MAUI hybridisation system (BioMicro Systems, Salt Lake City, USA). After washing the microarrays were dried by centrifugation (5 min, 520 g) and fluorescence was determined at 532 nm (Cy3-dUTP) and 635 nm (Cy5-dUTP) with 10 µm resolution using an Axon GenePix 6000 laser scanner (Axon Instruments, Sunnyvale, USA). Quantitative image analysis was carried out using GenePix image analysis software and results were saved as GPR-file (GenePix Pro 6.0, Axon Instruments). For data normalization, GPR-files were processed using the BioConductor/R-packages limma and marray. Processed and normalized data as well as experimental details (MIAME) were stored in the in-house microarray database for further analysis (Polen and Wendisch (2004) Appl. Biochem. Biotechnol. 118:215-232).

Using DNA microarray technology, the genome-wide mRNA concentrations of *C. glutamicum* wild type were compared with those of the mutant strain Δ*acn*-1. The strains were cultivated in CGXII minimal medium with 4% (w/v) glucose and 100 mM sodium glutamate and harvested in the exponential growth phase at an OD600 of ~5 for RNA isolation. Five microarray experiments were performed with cells cultured under iron starvation (1 µM $FeSO_4$) and three with cells grown under standard iron conditions (35 µM $FeSO_4$), each starting from an independent culture. Another set of DNA microarray experiments was performed to compare gene expression of the wild type carrying the *citH* expression plasmid pAN6-citH immediately before and 20 min after addition of 50 mM sodium citrate and 5 mM $CaCl_2$ to a culture at an OD_{600} of about 5 growing in CGXII minimal medium with 4% (w/v) glucose, 50 µg ml^{-1} kanamycin and 1 mM IPTG. A similar comparison was performed for the wild type carrying pAN6 (control strain). Each of these comparisons was performed three times starting from independent cultures.

To filter for differentially expressed genes and reliable signal detection in each of the comparisons, the following quality filter was applied: (i) flags ≤ 0 (GenePix Pro 6.0), (ii)

signal/noise ≥5 for Cy5 (F635Median/B635Median, GenePix Pro 6.0) or Cy3 (F532Median/B532Median, GenePix Pro 6.0), (iii) ≥ two-fold change between both strains or conditions, respectively, and (iv) significant change ($P < 0.05$) in Student's t-test (Excel, Microsoft).

Results. To complement the comparison of the wild type and the Δ*acn*-1 strain at the mRNA level and thus get additional information about global changes in gene expression due to the absence of aconitase, transcriptome comparisons were performed using whole-genome DNA microarrays based on 70-mer oligonucleotides. Three independent experiments were performed with RNA from cells cultivated under standard iron conditions (35 µM $FeSO_4$) and five experiments with RNA from cells grown under iron limitation (1 µM $FeSO_4$). For cultivation CGXII minimal medium containing 222 mM glucose and 100 mM L-glutamate was used. The cells were harvested in the exponential growth phase at an OD_{600} of about five. As the growth rates were quite different (~0.4 h^{-1} for the wild type, ~0.2 h^{-1} for Δ*acn*-1), a significant number of changes was expected.

For the cells grown under standard iron conditions, more than 800 genes showed an mRNA ratio ≥2 or ≤0.5, which are listed in **Table S3 (Excel file)**. As there is such a large number of altered genes, only the ones most relevant for our studies will be discussed here. Table S4 shows those genes which were more than 10-fold up- or downregulated. The strongest upregulation was observed for *aceA* and *aceB* coding for isocitrate lyase and malate synthase (193-fold and 65-fold, respectively), which is consistent with the proteome comparison. The mRNA levels of *pta* (coding for phosphotransacetylase) and *ackA* (coding for acetate kinase) were also increased 6.3- and 5.5-fold, respectively. Three TCA cycle genes were upregulated: *gltA* (coding for citrate synthase) 3.2 times, *fumC* (coding for fumarase) 2.6 times, and *mdh* (coding for malate dehydrogenase) 2.9 times. In addition, 28 genes coding for regulatory proteins had a significantly altered mRNA level, such as *citAB* (2.6- and 2.2-fold upregulated), *ramA* (2.1 fold upregulated), or *ramB* (2.9 fold downregulated). Interestingly, the genes of the *suf* operon (cg1759-1765) involved in Fe-S cluster formation and repair were 2.6- to 4-fold upregulated in the Δ*acn*-1 mutant, whereas the protein levels of two of the corresponding proteins, SufB and SufD, were found to be decreased (compare Table S1). This indicates that there might be a posttranscriptional regulatory link between aconitase and the synthesis of the Suf proteins, a hypothesis which is subject of further investigations. The most significantly downregulated gene in the Δ*acn*-1 strain was *acn*, confirming the deletion of this gene. In addition the mRNA level of *malE* was decreased about 60 times. This gene codes for

malic enzyme, which catalyses the oxidative decarboxylation of malate to pyruvate with the concomitant reduction of $NADP^+$.

For the comparison of the two strains under iron starvation conditions similar results were obtained. About 600 genes in total had an at least two-fold altered mRNA level, but the fraction that was more than ten-fold changed was larger than in the experiment with excess iron **(Table S5, Excel file)**. A file with all genes which were more than two-fold regulated is supplied as Table S6.

TABLE S4. Genes with a ≥10-fold changed mRNA level in *C. glutamicum* Δ*acn*-1 compared to the wild type cultivated under standard iron conditions (35 µM Fe).[a]

Locus tag	Gene and annotated function	Ratio	p-value
cg2560	*aceA*, isocitrate lyase	192.6	0.002
cg2559	*aceB*, malate synthase	65.2	0.000
cg2430	hypothetical protein	26.6	0.005
cg0077	hypothetical protein	14.0	0.043
cg1292	flavin-containing monooxygenase 3	10.7	0.001
cg1405	siderophore-interacting protein	10.0	0.000
cg0825	*fabG*, 3-ketoacyl-(acyl-carrier-protein) reductase	0.099	0.003
cg2937	ABC-type dipeptide/oligopeptide/nickel transport system, secreted component	0.099	0.007
cg1435	*ilvB*, acetolactate synthase I large subunit	0.098	0.002
cg0621	putative integral membrane protein	0.097	0.001
cg1169	Na^+-dependent transporters of the SNF family	0.097	0.000
cg2119	*pfkB*, 1-phosphofructokinase protein	0.095	0.000
cg0831	sugar ABC transporter, permease protein	0.093	0.004
cg1230	hypothetical protein	0.088	0.000
cg0624	secreted oxidoreductase	0.087	0.001
cg1231	*chaA*, Ca^{2+}/H^+ antiporter	0.086	0.001
cg2925	*ptsS*, enzyme II sucrose protein	0.078	0.001
cg0506	spermidine/putrescine transport system, ATPase component	0.077	0.001
cg0622	cobalt transport system, ATPase component	0.076	0.000
cg3431	*rnpA*, ribonuclease p	0.066	0.000
cg0623	cobalt transport system, permease component	0.065	0.000
cg0756	*cstA*, putative carbon starvation protein A	0.063	0.003
cg0507	ABC transport system permease protein	0.062	0.001
cg0957	*fas-IB*, fatty acid synthase	0.055	0.000
cg0508	iron/thiamine transport system, secreted component	0.046	0.000
cg0812	*dtsR1*, acetyl/propionyl-CoA carboxylase beta chain	0.044	0.000
cg3335	*malE*, malic enzyme	0.017	0.001
cg1737	*acn*, aconitase	0.008	0.000

[a]Cells were grown in CGXII minimal medium with 4% (w/v) glucose, 100 mM glutamate, and 35 µM $FeSO_4$ and harvested in the exponential growth phase at an OD_{600} of about 5. The ratio represents the mean value from three independent DNA microarray experiments.

TABLE S6. Genes with a ≥10-fold changed mRNA level in *C. glutamicum* Δ*acn*-1 compared to the wild type under iron starvation (1 µM Fe).[a]

Locus tag	Gene and annotated function	Ratio	n	p-value
cg2560	*aceA*, isocitrate lyase	6615.9	5	0.004
cg2559	*aceB*, malate synthase	608.2	5	0.003
cg3141	*hmp*, flavohemoprotein	291.1	5	0.005
cg2430	hypothetical protein	129.3	3	0.035
cg1179	sensory box/GGDEF family protein	83.5	3	0.032
cg1055	*menG*, ribonuclease activity regulator protein RraA	60.5	4	0.018
cg1180	glycosyltransferase, probably involved in cell wall biogenesis	60.4	3	0.034
cg1182	hypothetical protein	57.4	3	0.037
cg2999	putative ferredoxin reductase	50.6	4	0.037
cg0797	*prpB1*, probable methylisocitric acid lyase	33.5	5	0.013
cg2797	hypothetical protein	28.2	4	0.041
cg2380	hypothetical protein	22.1	3	0.046
cg3274	site-specific recombinases, DNA invertase Pin homolog-fragment	21.8	4	0.025
cg0662	FAD/FMN-containing dehydrogenase	21.1	4	0.031
cg2518	putative secreted protein	18.6	4	0.021
cg0798	*prpC1*, citrate synthase	18.2	4	0.020
cg2311	SAM-dependent methyltransferase	17.7	5	0.009
cg2250	putative secreted lipoprotein	17.7	4	0.043
cg2674	Alkylhydroperoxidase, AhpD family core domain	16.1	4	0.018
cg1349	membrane protein containing CBS domain	11.7	4	0.030
cg1744	*pacL*, cation-transporting ATPase	11.6	4	0.011
cg0105	hypothetical protein	11.6	4	0.026
cg1330	similar to GTP pyrophosphokinase	11.2	4	0.046
cg2676	ABC-type dipeptide/oligopeptide/nickel transport systems, permease component	11.0	5	0.000
cg2186	hypothetical protein	0.098	5	0.040
cg1379	*ssuB*, aliphatic sulfonates ATP-binding ABC transporterprotein	0.097	3	0.034
cg1977	putative secreted protein	0.092	4	0.002
cg3431	*rnpA*, ribonuclease p	0.087	5	0.000
cg1435	*ilvB*, acetolactate synthase I large subunit	0.082	5	0.000
cg0848	*wbbL*, putative rhamnosyl transferase WbbL	0.079	5	0.000
cg1782	tnp13b(IS*Cg13b*), transposase	0.078	4	0.012
cg0621	putative integral membrane protein	0.078	3	0.002
cg0811	*dtsR2*, acetyl/propionyl CoA carboxylase, beta subunit	0.073	5	0.000
cg1836	secreted solute-binding protein, aminodeoxychorismate lyase-like	0.066	3	0.037
cg0405	cobalamin/Fe^{3+}-siderophores transport system, secreted component	0.065	4	0.000
cg3404	ABC-type cobalamin/Fe^{3+}-siderophores transport system, secreted component	0.065	5	0.000
cg0573	*rplL*, 50S ribosomal protein L7/L12	0.062	4	0.035
cg0623	cobalt transport system, permease component	0.059	4	0.006
cg2894	drug resistance-related transcriptional repressor	0.058	5	0.003
cg0957	*fas-IB*, fatty acid synthase	0.055	5	0.001
cg1578	acyltransferase family, membrane protein	0.053	4	0.008
cg0756	*cstA*, putative carbon starvation protein A	0.045	5	0.000
cg0508	iron/thiamine transport system, secreted component	0.038	3	0.036
cg0471	conserved secreted protein	0.034	4	0.024
cg0809	*maf*, Maf-like protein	0.030	5	0.002
cg0810	hypothetical protein	0.016	3	0.025
cg2925	*ptsS*, enzyme II sucrose protein	0.010	4	0.015
cg3335	*malE*, malic enzyme	0.009	4	0.004
cg2893	permease of the major facilitator superfamily	0.004	4	0.001
cg3226	putative L-lactate permease	0.003	3	0.017
cg0812	*dtsR1*, acetyl/propionyl-CoA carboxylase beta chain	0.003	5	0.001
cg0896	hypothetical protein	0.003	4	0.010
cg1737	*acn*, aconitase	0.001	5	0.008

[a]Cells were grown in CGXII minimal medium with 4% (w/v) glucose, 100 mM glutamate, and 1 µM $FeSO_4$ and harvested in the exponential growth phase at an OD_{600} of about 5. The ratio represents the mean value from n independent DNA microarray experiments.

F. Experimental design for testing the influence of suddenly increased cytoplasmic citrate levels on *C. glutamicum*.

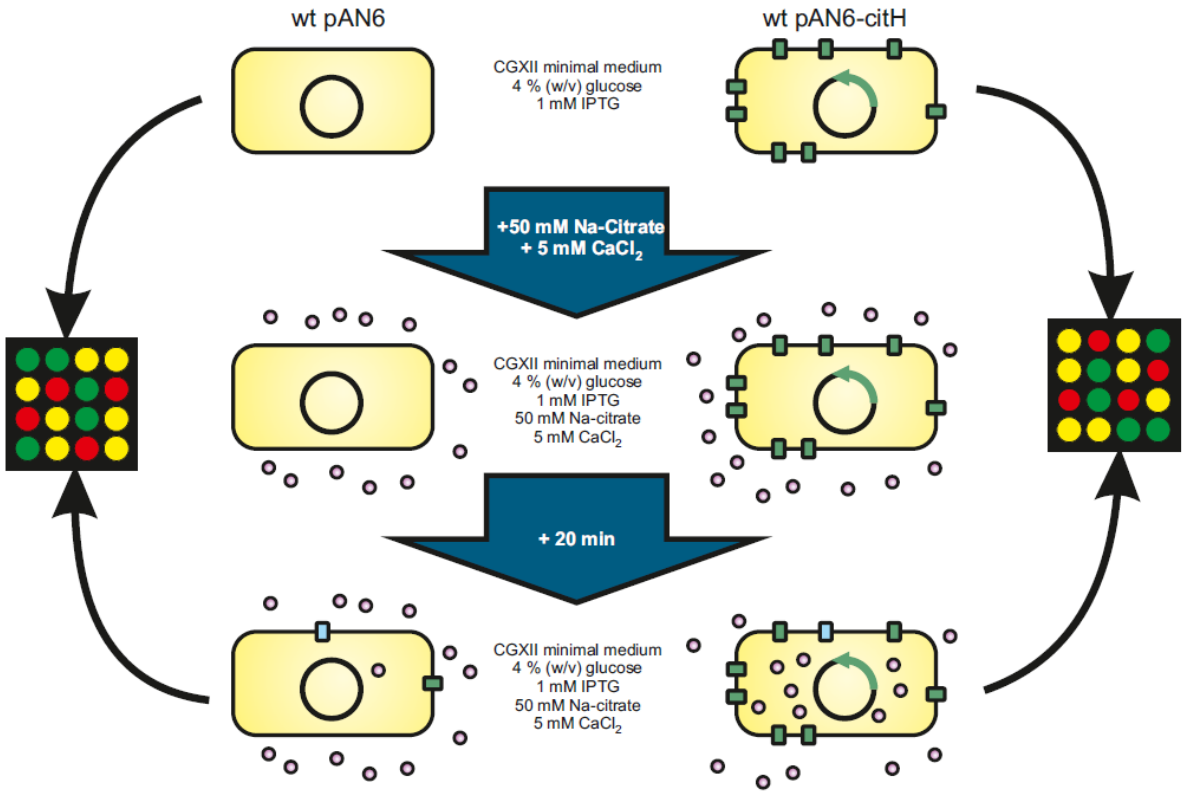

Fig. S4. Two derivatives of *C. glutamicum* wild type were used, one carrying the *citH* expression plasmid pAN6-citH (black circle with green arrow) and the other one carrying the control vector pAN6 (black circle). The presence of the citrate carrier CitH in the cytoplasmic membrane is indicated by the green rectangles. Both strains were cultivated in CGXII minimal medium with 4% (w/v) glucose and 1 mM IPTG. At an OD_{600} of about five, samples were taken for transcriptome analysis and the medium was supplemented with 50 mM trisodium citrate (indicated as purple circles) and 5 mM $CaCl_2$. After 20 min both strains should have synthesized the two citrate uptake systems CitH (green rectangles) and TctCBA (blue rectangles) due to the extracellular citrate stimulus. At this time point the second samples for transcriptome analysis were taken. The results of the transcriptome analysis are presented in **Tables S7 and S8 (Excel files)**.