SUPPLEMENTAL MATERIAL

Diverse energy-conserving pathways in *Clostridium difficile*: Growth in the absence of amino acid Stickland acceptors and the role of the Wood-Ljungdahl Pathway

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Fig.S1

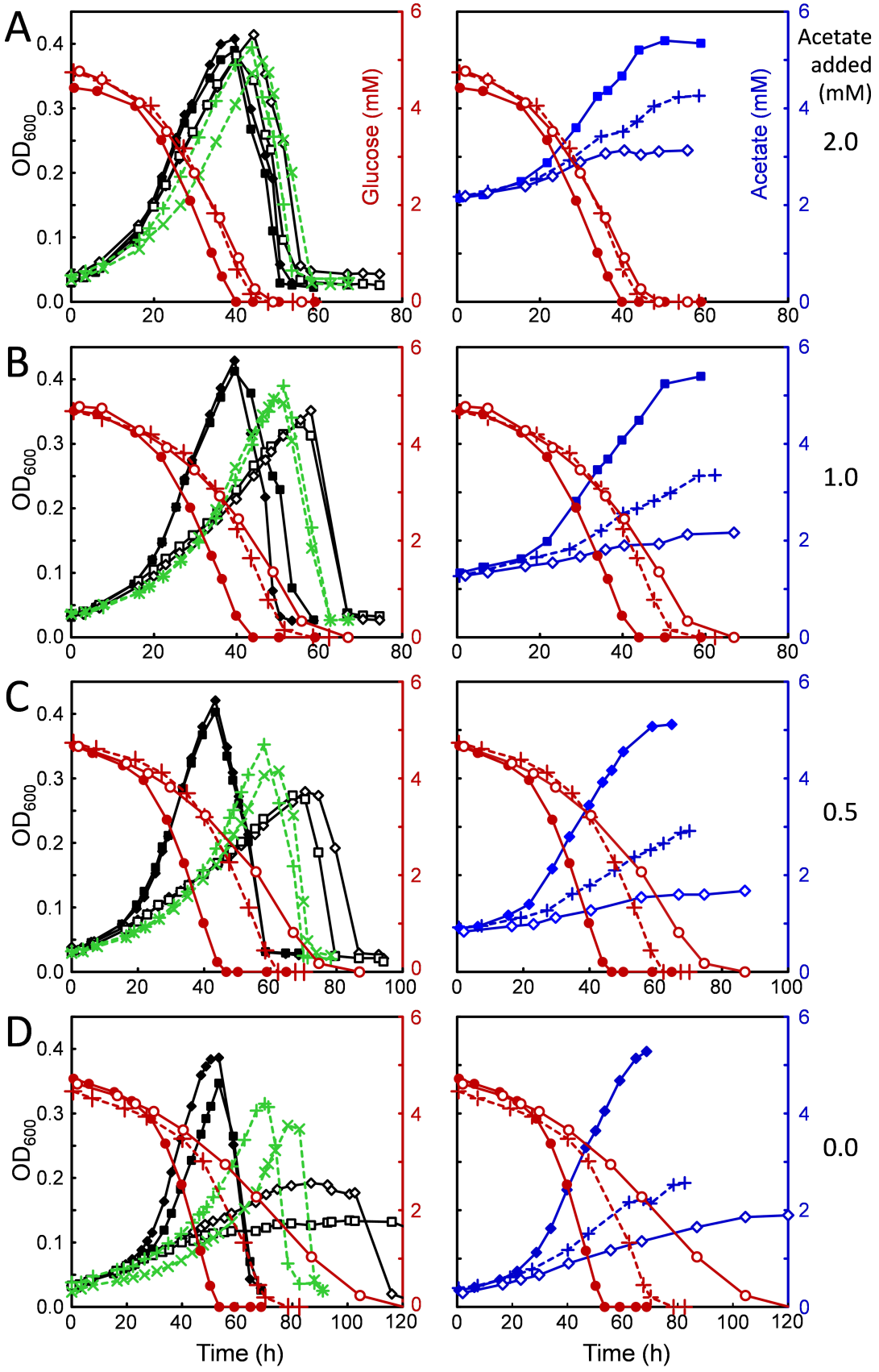
A
B
C
D
OD600
Glucose (mM)
Acetate (mM)
Acetate added (mM)
2.0
1.0
0.5
0.0
Time (h)
Time (h)

Figure S1. Complementation of the *C. difficile* Δ*acsB* mutant

Wild-type, Δ*acsB* mutant and the complemented Δ*acsB* mutant are compared for growth, glucose consumption and acetate production in glucose-only (Glc only) medium under acetate-limiting conditions. Glc only medium contained decreasing amounts of sodium acetate (**A-D**, 2.0, 1.0, 0.5 and 0 mM). Panels on the left show growth OD_{600} of WT (closed black symbols), Δ*acsB* mutant (open black symbols), and the complemented Δ*acsB* mutant (+ green symbols, dashed lines), and glucose consumption (red corresponding symbols). Panels at the right show acetate production, WT (closed blue symbols), Δ*acsB* mutant (open blue symbols), and the complemented Δ*acsB* mutant (+ blue symbols, dashed lines) from the same growths and the corresponding glucose consumption curves (in red) for comparison.

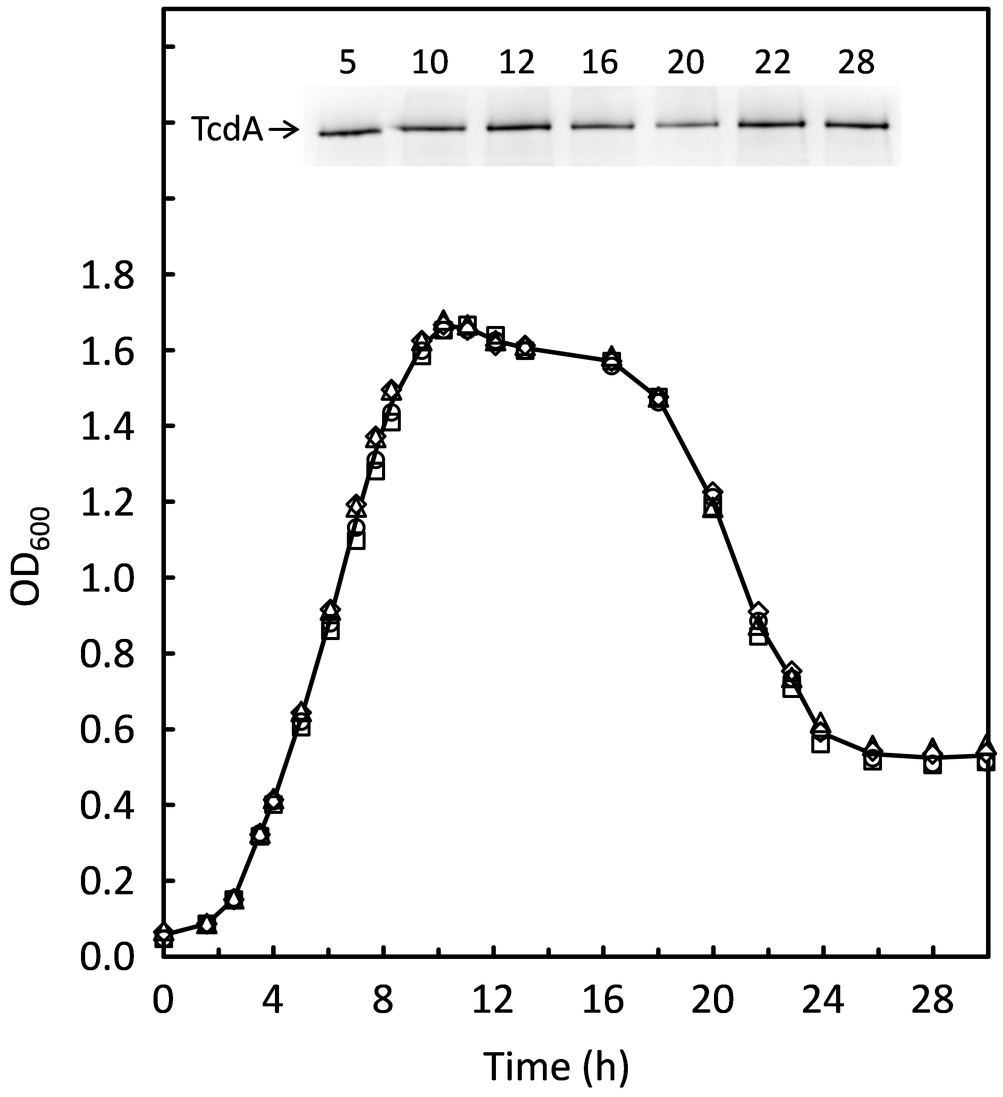

Figure S2. TcdA toxin levels in *C. difficile* during growth in BHIS medium

Growth was followed on four BHIS cultures as described under Materials and Methods. Inset; Western blot of an 8% acrylamide SDS-PAGE gel with samples removed from cultures at the times indicated (hours) using anti-TcdA as primary antibody.

TABLE S1 Evolution of H_2 during growth in BHIS

Time (hr)	*BHIS-1 H_2 (%)*	*BHIS-2 H_2 (%)*
2.3	0.42	0.56
4.6	2.06	2.04
6.8	3.84	3.99
9.2	7.12	8.11
21.4	9.46	10.97
25.9	9.96	11.70

Duplicate 10 ml BHIS day cultures (BHIS-1 and BHIS-2) were prepared as described under Materials and Methods, and monitored for growth by OD_{600} and for H_2 evolution by gas-tight, valve-equipped, syringe sampling of the headspace (17 ml total). Hydrogen in aliquots of the gas phase (0.2 ml) was quantified by GC using a 1.3 m molecular sieve 13 X column (isothermal at 40 °C) with N_2 as carrier gas, producing negative peaks by TCD detection. The cultures were grown at 37 °C and periodically shaken. The maximum amount of H_2 found was 10-12% of the gas phase by volume. Thus, the 17 ml headspace contained approximately 7.6 to 9.1 μmol H_2 evolved per ml of culture, which, in relation to other substrates and products (Table S2) represents a substantial number of reducing equivalents disposed of as H_2.

TABLE S3 Bacterial strains and plasmids

Strain or plasmid	*Relevant features*	*Source*
E. coli strains		
DH5α		Invitrogen
TOP10		Invitrogen
NEB10-beta		Biolabs
NM522		Promega
NM522(DE3)		This study
XL1-Blue		Stratagene
S17-1		ATCC 47055
SG-Ec2270	S17-1 with pSG1217	This study
SG-Ec2280	S17-1 with pMTL84151::*acsB*	This study
C. difficile strains		
CD630	Genome reference strain	ATCC BAA-1382
CD630Δ*acsB*	CD630 with in-frame deletion in the acetyl CoA synthase *acsB* gene	This study
SG-Cd113	CD630Δ*acsB* with pMTL84151::*acsB*	This study
Clostridium ljungdahlii		ATCC 55383
Plasmids		
pCR-BluntII-TOPO		Invitrogen
pMTL83151	*Clostridium* modular plasmid	N. Minton
pMTL83151::codA	pMTL83151 with modified *C. pasteurianum* fdx promoter fused to the *E. coli codA* gene and cloned into the BamHI and NcoI sites	This study
pSG1217	pMTL83151::codA with the *acsB* allele exchange cassette cloned into the PmeI site	This study
pMTL84151	*Clostridium* modular plasmid used for complementation	N. Minton
pMTL84151::*acsB*	Complementation plasmid, pMTL84151 with WLP promoter fused to the *acsB* and cloned into the BamHI and AatII sites	This study

Table S4 Oligonucleotide sequences

Name	Sequence (5'→3')	Use
fdx-codA-BamHI-A-f	*5' GTGCATGGATCCGATCGAGATAGTATATGATGCATATTC*	*fdx-codA fusion*
fdx-codA-B-r	5' GTTTGTAAAGCGTTATTCGACACATTATGAAATACACCTCCTTAAAATTTTAATC	
fdx-codA-C-f	5' GATTAAAATTTTAAGGAGGTGTATTTCATAATGTGTCGAATAACGCTTTACAAAC	
fdx-codA-NcoI-D-r	5' GGTCGACCATGGTCAACGTTTGTAATCGATGGCTTC	
acsE-PmeI-A-f	5' GTGCATGTTTAAACGATATAGCAGCTGAATACGAAGCAATGG	*acsB* allele exchange
acsB-5pdel-B-r	5' CTAATACTCCTTCAGAATCGTCAGCAGCTTGAGCCGCACCTAAAGC	
acsB-3pdel-C-f	5' GCTTTAGGTGCGGCTCAAGCTGCTGACGATTCTGAAGGAGTATTAG	
gcvH-PmeI-D-r	5' GGTCGAGTTTAAACTAATCCAGCCTCGTATTCTTTATCAC	
PL-AE-PmeI-f	5' GACGGATTTCACATTTGCCGTTTTGTAAACGAATTGCAGG	PCR of integrants and double cross-over recs
PL-AE-PmeI-r	5' AGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGG	
acsB-AE-1f	5' CTGAGTTCAACAATGTTCCTATAGCATTAGATACAGC	
acsB-AE-1r	5' GATAAAAGGAAAGTATAGCTAAATTTGCGTGCACTC	
pro-BamHI-A-f	5' GCATGGATCCGAAATAGGATAAATCGCTTGAAATAAATGAATTAAAG	native promoter-*acsB*
pro-acsB-B-r	5' GATTCATATTCCTATCCCCTTTTTTTGATTTTTAAAATCCC	
pro-acsB-C-f	5' TCAAAAAAAGGGGATAGGAATATGAATCTATATAATATAATCTTTACAGGGTCAG	
acsB-AatII-D-f	5' GCATGACGTCTTACATTACACTTTCCATAGCTAATGCTGG	