

Table S1. Primers and Vectors

Organism	Primers	Description	Sequence/ Accession number
<i>E. coli</i>	XTZ004	Primers for cloning <i>C. thermocellum</i> AdhE into pEXP5-NT/CT TOPO plasmids	AAGCTTCATTTCTTCGCA
<i>E. coli</i>	XTZ006		ATGACGAAAATAGCGAAT
<i>E. coli</i>	XTZ002	Primers for cloning <i>T. saccharolyticum</i> AdhE into pEXP5-NT/CT TOPO plasmids	TCTTAAGCTATGCACCG
<i>E. coli</i>	XTZ005		ATGGCAACGACAAAAAC
<i>C. thermocellum</i>	XSH0059	Primers for constructing the <i>C. thermocellum</i> vectors pSH016 and pSH019	CATAATATATAAGAAACCCCATCTATTTAG TTATTTGTTTGGTCAC
<i>C. thermocellum</i>	XSH0060		TTCCCACGATACCTGCTCCTGTGTATAGG
<i>C. thermocellum</i>	XSH0061		GCACAGAAAAGCTAATAGTAGTGAAAAA ATCAG
<i>C. thermocellum</i>	XSH0062		GTGACCAAACAATAACTAAATAGATGGGG GTTTCTTTTAATATTATG
<i>C. thermocellum</i>	XSH0063		AGGAGCAGGTATCGTGGGAATAGGCATG
<i>C. thermocellum</i>	XSH0064		CCTCCAAGCCACTGAACTACTGGGCCAG
<i>C. thermocellum</i>	XSH0065	C	GTAGTTCAGTGGCTTGGAGGAGCTCTTTTAT C
<i>C. thermocellum</i>	XSH0066		CTATTAGCAGTTTTCTGTGCATTTTGTCTTTC
<i>T. saccharolyticum</i>	X12435	Primers for constructing the <i>T. saccharolyticum</i> vectors pCP14 and pCP14*	TTTCGACTGAGCCTTTCGTTTTATTGATGCC TGGCTTTGAAAGCGGGTCACTCCAGTA
<i>T. saccharolyticum</i>	X12436		TCTTACCTATCACCTCAAATGGTTCGCTGGG TTTTCTTAAGCTATGCACCGTATGCT
<i>T. saccharolyticum</i>	X12437		TCCCGAGCGCCTACGAGGAATTTGTATCGCA CAAGTCGAAAGAAATAAATAACGGCTC
<i>T. saccharolyticum</i>	X12438		CCGTCAGTAGCTGAACAGGAGGGACAGCTG ATAGAGATGGCAGGATGAAATCAAGAGAA
<i>T. saccharolyticum</i>	X12438		
Organism	Vectors	Description	
<i>C. thermocellum</i>	pSH016	Integration vector	KP245915
<i>C. thermocellum</i>	pSH019	Integration vector, pSH016 with D494G mutation in AdhE.	N/A
<i>T. saccharolyticum</i>	pCP14	Integration vector	KP851973
<i>T. saccharolyticum</i>	pCP14*	Integration vector, pCP14 with G544D mutation in AdhE	N/A

Table S2. End product of strains

LL number	final pH	final pressure (psig)	hydrogen	co2	cellobiose	glucose	formate	lacticAcid	aceticAcid	ethanol	succinate	pyruvate	MOPS	malate	measured CO2 corrected with Henry's law	ethanol	cellobiose	ethanol yield
			psig						mmoles						grams	grams	grams	g ethanol/g cellobiose
LL1004-1	7.0	7.5	1.6	0.7	0.00	0.00	0.32	0.02	0.88	0.84	0.00	0.00	1.98	0.01	0.98	0.039	0.25	0.15
LL1004-2	7.1	7.1	1.3	1.0	0.00	0.00	0.43	0.02	0.85	0.89	0.00	0.00	1.99	0.02	1.45	0.041	0.25	0.16
LL346-1	6.9	11.0	1.9	1.3	0.00	0.00	0.31	0.18	1.03	0.60	0.00	0.00	1.99	0.02	1.82	0.028	0.25	0.11
LL346-2	7.0	11.1	1.9	1.3	0.00	0.00	0.30	0.17	1.03	0.61	0.00	0.00	1.97	0.02	1.78	0.028	0.25	0.11
LL350-1	6.5	4.1	0.4	0.9	0.00	0.00	0.23	0.08	0.26	1.20	0.00	0.05	2.02	0.05	1.28	0.055	0.25	0.22
LL350-2	6.6	4.5	0.3	1.1	0.00	0.00	0.17	0.06	0.18	1.15	0.00	0.06	1.98	0.06	1.49	0.053	0.25	0.21
LL1111-1	7.0	8.9	1.5	1.3	0.03	0.00	0.00	1.42	0.68	0.05	0.02	0.04	1.99		1.78	0.002	0.25	0.01
LL1111-2	7.0	7.5	1.5	0.8	0.00	0.00	0.03	1.24	0.74	0.03	0.00	0.00	2.02	0.01	1.16	0.002	0.25	0.01
LL1025-1	4.8	11.6	1.7	2.9	0.00	0.00	0.04	0.11	0.85	1.35	0.00	0.00	1.96	0.03	4.04	0.062	0.25	0.25
LL1025-2	4.8	11.4	1.7	2.8	0.00	0.00	0.03	0.05	0.80	1.39	0.00	0.00	1.94	0.03	3.86	0.064	0.25	0.26
LL1049-1	5.3	6.5	0.2	1.4	0.00	0.00	0.20	0.00	0.11	2.33	0.00	0.00	1.99	0.00	2.01	0.107	0.25	0.43
LL1049-2	5.4	6.3	0.2	2.9	0.01	0.00	0.22	0.00	0.10	2.29	0.00	0.00	1.96	0.01	4.05	0.105	0.25	0.42
LL1040-1	5.6	7.0	0.2	1.5	0.00	0.00	0.02	0.00	0.03	2.43	0.00	0.00	1.98	0.04	2.14	0.112	0.25	0.45
LL1040-2	5.7	6.5	0.2	2.9	0.01	0.00	0.03	0.00	0.03	2.42	0.00	0.00	1.96	0.05		0.111	0.25	0.44
LL1076-1	4.9	3.8	0.7	0.4	0.25	0.00	0.01	0.64	0.31	0.07	0.00	0.00	1.98	0.00	0.56	0.003	0.25	0.01
LL1076-2	4.7	8.9	0.1	0.7	0.00	0.13	0.15	0.73	0.30	0.01	0.04	0.00	1.14	0.00	0.93	0.000	0.25	0.00
LL1160-1	7.2	8.74	1.5	1.5	0.00	0.00	0.25	0.28	0.73	0.76	0.00	0.02	1.99	0.03	2.07	0.035	0.25	0.14
LL1160-2	7.1	8.87	1.6	1.4	0.00	0.00	0.24	0.27	0.75	0.76	0.00	0.02	1.99	0.03	1.99	0.035	0.25	0.14
LL1161-1	7.2	6.55	1.0	1.2	0.00	0.00	0.31	0.16	0.46	1.32	0.00	0.02	2.00	0.02	1.73	0.061	0.25	0.24
LL1161-2	7.17	6.0	0.9	0.8	0.00	0.00	0.24	0.12	0.35	1.27	0.00	0.00	1.53	0.02	1.43	0.058	0.25	0.23
LL1193-1	4.6	10.21	1.5	2.5	0.00	0.00	0.02	0.48	0.70	1.17	0.00	0.00	1.94	0.00	3.42	0.054	0.25	0.21
LL1193-2	4.7	10.79	1.6	1.5	0.00	0.00	0.03	0.41	0.73	1.17	0.00	0.01	1.98	0.00	2.11	0.054	0.25	0.21
LL1194-1	5.0	11.9	1.7	3.2	0.00	0.00	0.03	0.05	0.79	1.72	0.00	0.00	1.95	0.02	4.50	0.079	0.25	0.32
LL1194-2	5.0	11.88	1.7	1.7	0.00	0.00	0.04	0.08	0.78	1.73	0.00	0.00	1.98	0.02	2.42	0.079	0.25	0.32

Table S3. *E. coli* negative control

REACTION	sample	specific activity	SD
		U/mg of protein	
	(-) ^a before heat ^b	0.09	0.00
ADH-NADH	(-) Eluent 1	0.19	0.01
	(-) Eluent 2	0.00	0.07
	(-) before heat	0.04	0.00
ADH-NADPH	(-) Eluent 1	0.24	0.05
	(-) Eluent 2	0.37	0.00
	(-) before heat	0.02	0.00
ALDH-NADH	(-) Eluent 1	0.35	0.01
	(-) Eluent 2	0.15	0.02
	(-) before heat	0.02	0.00
ALDH-NADPH	(-) Eluent 1	0.15	0.05
	(-) Eluent 2	0.05	0.05

^a (-) refers to the cell extracts of the *E. coli* expressing the pNT-CALML3 plasmid control plasmid.

^b "Heat" refers to heat treatment as described in materials and methods.

Table S4. ADH and ALDH activity during the AdhE purification process

Source of AdhE	Strain description	Cofactor	Fraction	Total activity		Specific activity		Ratio ADH:ALDH
				ADH	ALDH	ADH	ALDH	
				U ⁻³		U/mg of protein		
<i>C. thermocellum</i> LL1004	Wild-type	NADH	Cell extract	295.1	148.4	14.4	7.2	2.0
			Eluent 1	47.8	21.8	31.9	14.5	2.2
			Eluent 2	40.9	18.7	30.1	13.7	2.2
			Eluent 3	39.7	19.1	40.1	19.3	2.1
<i>C. thermocellum</i> LL346	Ethanol-tolerant	NADH	Cell extract	35.5	89.9	0.9	2.4	0.4
			Eluent 1	3.2	13.3	1.3	5.5	0.2
			Eluent 2	3.2	9.8	2.2	6.8	0.3
			Eluent 3	1.2	8.5	2.3	16.9	0.1
<i>C. thermocellum</i> LL350	Moderate-ethanol-producer	NADH	Cell extract	237.8	163.1	11.0	7.5	1.5
			Eluent 1	45.5	26.4	39.5	22.9	1.7
			Eluent 2	34.9	28.3	33.5	27.2	1.2
			Eluent 3	19.4	16.5	44.8	38.1	1.2
<i>C. thermocellum</i> LL350	Moderate-ethanol-producer	NADPH	Cell extract	131.7	ND	6.1	ND	ND
			Eluent 1	37.0	ND	32.1	ND	ND
			Eluent 2	30.7	ND	29.4	ND	ND
			Eluent 3	19.9	ND	45.9	ND	ND
<i>T. saccharolyticum</i> LL1025	Wild-type	NADH	Cell extract	285.8	3.0	7.8	0.1	94.4
			Eluent 1	177.5	6.9	8.9	0.3	25.9
			Eluent 2	26.9	5.1	9.6	1.8	5.3
			Eluent 3	9.4	8.3	20.7	18.2	1.1
<i>T. saccharolyticum</i> LL1049	High-ethanol-producer	NADPH	Cell extract	5.8	0.9	0.4	0.1	6.6
			Eluent 1	4.4	3.5	5.2	4.1	1.2
			Eluent 2	4.8	3.9	9.5	7.7	1.2
			Eluent 3	3.9	3.9	11.6	11.8	1.0
<i>T. saccharolyticum</i> LL1040	High-ethanol-producer	NADPH	Cell extract	4.8	1.7	0.1	0.0	2.8
			Eluent 1	5.0	2.1	0.6	0.3	2.4
			Eluent 2	4.2	2.1	4.4	2.2	2.1
			Eluent 3	4.6	3.4	9.8	7.2	1.4

Table S5. Gel densitometry of affinity-purified AdhE proteins

Sample	Peak No. ^a	Area	Percentage
LL1004 AdhE	1 (AdhE)	12190.5	73.9
	2	4295.7	26.1
LL346 AdhE	1 (AdhE)	23898.4	82.8
	2	1344.0	4.7
	3	3626.8	12.6
LL350 AdhE	1 (AdhE)	9227.0	86.8
	2	1398.6	13.2
LL1025 AdhE	1 (AdhE)	15188.9	78.9
	2	3012.4	15.6
	3	1058.9	5.5
LL1040 AdhE	1 (AdhE)	2882.6	78.7
	2	779.3	21.3
LL1049 AdhE	1 (AdhE)	5338.7	89.2
	2	645.7	10.8

^a Peak numbers refer to the order of visible gel bands starting from the top, with 1 being the largest in protein weight

Table S6. Enzyme activity comparison between native AdhE and His-tagged AdhE

Reaction-cofactor	Sample ^a	specific	SD
		activity	
		U/mg of protein	
ADH-NADH	WT <i>C. thermocellum</i> AdhE	3.41	0.08
	WT <i>C. thermocellum</i> AdhE-his	5.28	0.07
	WT <i>T. saccharolyticum</i> AdhE	15.60	0.12
	WT <i>T. saccharolyticum</i> AdhE-his	14.01	0.10
ALDH-NADH	WT <i>C. thermocellum</i> AdhE	2.94	0.01
	WT <i>C. thermocellum</i> AdhE-his	2.58	0.01
	WT <i>T. saccharolyticum</i> AdhE	0.01	0.02
	WT <i>T. saccharolyticum</i> AdhE-his	0.03	0.01

^a The assays were done with *E. coli* cell extracts expressing AdhE without purification

Table S7. Enzyme assay raw data

Reaction type	Cofactor type	Location in paper	Sample ^a	Inhibition ^b	Protein stock conc. ug/ml	NADH/NADPH decrease			Reaction			specific activity umol/min/mg	Avg. specific activity ^c umol/min/mg	SD/ umol/min/mg
						baseline ^c								
						1st ^d AU/min	2nd AU/min	avg. AU/min	1st AU/min	2nd AU/min	avg. AU/min			
Purified AdhE samples														
ADH	NADH	Table S4	LL1004 CE		2052.2	0.06	0.07	0.07	2.24	2.21	2.23	14.3801		
			LL1004 E1		149.9	0.01	0.01	0.01	0.37	0.35	0.36	31.8621		
			LL1004 E2		135.6	0.02	0.03	0.03	0.32	0.33	0.32	30.0824		
		Table 3	LL1004 E3-1		98.7	0.01	0.01	0.01	0.31	0.29	0.30	40.1314	42.23	3.13
			LL1004 E3-2		77.03	0.03	0.03	0.03	0.29	0.30	0.29	46.7465		
			LL1004 E3-3		98.7	0.01	0.01	0.01	0.31	0.29	0.30	40.1314		
		Table S8	LL1004 E3-4	Ethanol	56.1	0.02	0.03	0.02	0.20	0.19	0.20	41.8981		
			LL1004 E3-4		56.1	0.02	0.03	0.02	0.10	0.11	0.10	19.7920		
			LL1004 E3-3	NAD	98.7	0.02	0.01	0.01	0.10	0.10	0.10	11.9280		
		Table S4	LL346 CE		3777.6	0.08	0.09	0.09	0.34	0.35	0.35	0.9406		
			LL346 E1		243.2	0.00	0.01	0.01	0.03	0.03	0.03	1.3205		
			LL346 E2		145	0.00	0.01	0.01	0.03	0.03	0.03	2.2148		
		Table 3	LL346 E3-1		50.4	0.03	0.03	0.03	0.04	0.04	0.04	2.3047	4.02	1.48
			LL346 E3-2		71.25	0.02	0.01	0.01	0.04	0.04	0.04	4.8208		
			LL346 E3-3		70.7	0.01	0.01	0.01	0.03	0.03	0.03	4.9289		
		Table S8	LL346 E3-3	NAD	70.7	0.00	0.01	0.00	0.02	0.03	0.02	3.9528		
			LL346 E3-3		70.7	0.01	-0.02	0.00	0.04	0.04	0.04	8.3115		
			LL350 CE	Ethanol	2165.4	0.10	0.09	0.09	1.83	1.84	1.84	10.9819		
		Table S4	LL350 E1		115.28	0.02	0.03	0.03	0.36	0.36	0.36	39.5043		
			LL350 E2		104.3	0.02	0.02	0.02	0.27	0.28	0.27	33.4761		
			LL350 E3-1		43.3	0.04	0.03	0.03	0.17	0.18	0.18	44.7998		
		Table 3	LL350 E3-2		31.37	0.00	0.01	0.01	0.10	0.10	0.10	40.6657	42.67	7.46
			LL350 E3-3		62.9	0.00	0.01	0.00	0.24	0.24	0.24	51.5121		
			LL350 E3-4		43.5	0.01	0.03	0.02	0.13	0.12	0.12	33.7085		
		Table S8	LL350 E3-4	Ethanol	43.5	0.01	-0.01	0.00	0.06	0.06	0.06	18.8491		
			LL350 E3-3	NAD	94.37	0.01	0.01	0.01	0.05	0.05	0.05	5.6475		
			LL1025 CE		3665.3	0.09	0.11	0.10	2.17	2.21	2.19	7.7979		
		Table S4	LL1025 E1		2000.1	0.06	0.09	0.08	1.36	1.39	1.38	8.8754		
			LL1025 E2		281.96	0.02	0.02	0.02	0.22	0.22	0.22	9.5503		
			LL1025 E3-1		45.38	0.01	0.00	0.01	0.08	0.07	0.08	20.7032		
		Table 3	LL1025 E3-2		120.41	0.02	0.00	0.01	0.16	0.17	0.17	17.8705	17.43	2.45
			LL1025 E3-3		97.5	0.02	0.04	0.03	0.15	0.13	0.14	15.9082		
			LL1025 E3-4		36.21	0.01	0.02	0.02	0.06	0.06	0.06	15.2280		
		Table S8	LL1025 E3-5	Ethanol	69.1	0.00	0.00	0.00	0.05	0.05	0.05	8.7709		
			LL1025 E3-1	NAD	45.38	0.00	0.00	0.00	0.04	0.04	0.04	10.2296		
			LL1049 E3-1		168.4	0.03	0.04	0.03	0.05	0.02	0.04	0.4463		
		Table 3	LL1049 E3-2		55.15	0.01	0.00	0.00	0.01	0.01	0.01	2.2797	0.66	1.97
			LL1049 E3-3		171.2	0.08	0.02	0.05	0.01	0.03	0.02	-2.0355		
			LL1049 E3-4		33.2	0.01	0.01	0.01	0.02	0.02	0.02	1.9552		
		Table 3	LL1040 E3-1		183.98	0.00	0.02	0.01	0.01	0.00	0.01	-0.1708	0.01	1.34
LL1040 E3-2			355.2	0.05	0.01	0.03	0.03	0.06	0.05	0.6540				
LL1040 E3-3			47.37	0.02	0.01	0.02	0.03	0.03	0.03	2.4521				
Table S3	(-) before heat		6739.6	0.02	0.03	0.03	0.06	0.06	0.06	0.0910				
	(-) E1		176.5	0.02	0.01	0.01	0.02	0.02	0.02	0.1897				
	(-) E2		110.3	0.02	0.02	0.01	0.00	0.00	0.00	-0.8673				
additional data ^e	(-) E3-1		1516	0.02	0.02	0.02	0.04	0.09	0.06	0.4219				
	(-) E3-2		1202.4	0.02	0.03	0.02	0.00	0.02	0.01	-0.1703				
	(-) E3-3		487	0.02	0.01	0.02	0.03	0.03	0.03	0.3003				
Table 3	(-) CE after heat		1716.44	0.10	0.10	0.10	0.15	0.15	0.15	0.3686				
	LL1004 E3-1		210.8	0.02	-0.01	0.01	0.01	0.04	0.02	1.0761				
	LL1004 E3-2		98.7	0.01	0.01	0.01	0.03	0.03	0.03	2.8383				
Table 3	LL346 E3-1		154.6	0.07	0.01	0.04	0.03	0.03	0.03	-0.2121	0.03	0.34		
	LL346 E3-2		50.4	0.03	0.04	0.03	0.04	0.04	0.04	0.2711				
	LL350 CE		2165.4	0.09	0.12	0.10	1.07	1.07	1.07	6.0805				
Table S4	LL350 E1		115.28	0.02	0.02	0.02	0.30	0.28	0.29	32.1251				
	LL350 E2		104.3	0.02	0.04	0.03	0.24	0.27	0.25	29.4144				
	LL350 E3-1		43.3	0.00	0.02	0.01	0.15	0.17	0.16	45.9360				
Table 3	LL350 E3-2		62.9	0.03	0.05	0.04	0.21	0.22	0.22	37.5858	42.30	4.28		
	LL350 E3-3		43.5	0.03	0.01	0.02	0.15	0.16	0.16	43.3843				
	LL350 E3-3	Ethanol	43.5	0.03	0.01	0.02	0.05	0.06	0.05	9.9272				
Table S8	LL350 E3-4	NADP	94.37	0.03	0.02	0.03	0.06	0.05	0.05	4.0546				
	LL1025 E3-1		97.5	0.05	0.05	0.05	0.06	0.08	0.07	2.6630				
	LL1025 E3-2		45.38	0.03	0.03	0.03	0.04	0.04	0.04	2.2002				
Table S4	LL1029 CE		1513.87	0.02	0.01	0.01	0.06	0.05	0.05	0.3823				
	LL1049 E1		84.8	0.01	0.01	0.01	0.04	0.05	0.04	5.1568				
	LL1049 E2		50.46	0.02	0.01	0.01	0.05	0.05	0.05	9.4787				
Table 3	LL1049 E3-1		33.2	0.01	0.01	0.01	0.04	0.04	0.04	11.6487	12.70	2.18		
	LL1049 E3-2		34.33	0.01	0.01	0.01	0.05	0.05	0.05	15.2061				
	LL1049 E3-3		55.15	0.02	0.00	0.01	0.05	0.06	0.06	11.2373				
Table S8	LL1049 E3-3	Ethanol	55.157	0.04	0.03	0.04	0.06	0.06	0.06	4.7074				
	LL1049 E3-3	NADP	55.157	0.01	0.01	0.01	0.03	0.03	0.03	3.8774				
	LL1040 CE		6301.2	0.01	0.00	0.01	0.04	0.04	0.04	0.0755				
Table S4	LL1040 E1		821.68	0.03	0.01	0.02	0.05	0.06	0.06	6.6095				
	LL1040 E2		95.29	0.01	0.00	0.01	0.04	0.04	0.04	4.4314				
	LL1040 E3-1		47.37	0.01	0.00	0.00	0.04	0.04	0.04	9.7508				
Table 3	LL1040 E3-2		37.69	0.02	0.02	0.02	0.06	0.07	0.06	16.1710	12.96	4.54		
	LL1040 E3-1	Ethanol	47.37	0.02	0.00	0.01	0.03	0.03	0.03	5.1928				
	LL1040 E3-1	NADP	47.37	0.01	0.01	0.01	0.02	0.02	0.02	4.8610				
Table S3	(-) before heat		6739.6	0.02	0.02	0.02	0.04	0.04	0.04	0.0419				
	(-) E1		176.5	0.03	0.03	0.03	0.03	0.03	0.03	0.2439				
	(-) E2		110.3	0.02	0.02	0.02	0.02	0.02	0.02	0.3717				
additional data ^e	(-) E3-1		450.6	0.01	0.00	0.01	0.02	0.02	0.02	0.4579				
	(-) E3-2		1516	0.04	0.02	0.03	0.05	0.06	0.05	0.2208				
	(-) E3-3		1202.4	0.05	0.05	0.05	0.07	0.06	0.06	0.1392				
Table 3	(-) CE after heat		1716.44	0.08	0.07	0.07	0.12	0.11	0.11	0.3284				
	LL1004 E3-1		110.8	0.02	0.01	0.01	0.03	0.03	0.03	2.8383				
	LL346 E3-1		154.6	0.07	0.01	0.04	0.03	0.03	0.03	-0.2121				
Table 3	LL346 E3-2		50.4	0.03	0.04	0.03	0.04	0.04	0.04	0.2711	0.03	0.34		
	LL350 CE		2165.4	0.09	0.12	0.10	1.07	1.07	1.07	6.0805				
	LL350 E1		115.28	0.02	0.02	0.02	0.30	0.28	0.29	32.1251				
Table S4	LL350 E2		104.3	0.02	0.04	0.03	0.24	0.27	0.25	29.4144				
	LL350 E3-1		43.3	0.00	0.02	0.01	0.15	0.17	0.16	45.9360				
	LL350 E3-2		62.9	0.03	0.05	0.04	0.21	0.22	0.22	37.5858				
Table 3	LL350 E3-3		43.5	0.03	0.01	0.02	0.15	0.16	0.16	43.3843	42.30	4.28		
	LL350 E3-3	Ethanol	43.5	0.03	0.01	0.02	0.05	0.06	0.05	9.9272				
	LL350 E3-4	NADP	94.37	0.03	0.02	0.03	0.06	0.05	0.05	4.0546				
Table S8	LL1025 E3-1		97.5	0.05	0.05	0.05	0.06	0.08	0.07	2.6630	2.43	0.33		
	LL1025 E3-2		45.38	0.03	0.03	0.03	0.04	0.04	0.04	2.2002				
	LL1029 CE		1513.87	0.02	0.01	0.01	0.06	0.05	0.05	0.3823				
Table S4	LL1049 E1		84.8	0.01	0.01	0.01	0.04							

Table S8. Inhibition of purified AdhE activity by ethanol, NAD⁺ or NADP⁺

Source of AdhE	Strain description	Cofactor	ADH activity			ALDH activity	
			Control	Ethanol ^a	NAD(P) ⁺ ^b	Control	NAD(P) ⁺
<i>C. thermocellum</i> LL1004	Wild-type	NADH	42.23	19.79	11.93	18.02	5.13
<i>C. thermocellum</i> LL346	Ethanol-tolerant	NADH	4.02	8.31	3.95	13.88	12.73
<i>C. thermocellum</i> LL350	Moderate-ethanol-producer	NADH	42.67	18.85	5.65	31.50	2.97
		NADPH	42.30	9.93	4.05	ND ^c	ND
<i>T. saccharolyticum</i> LL1025	Wild-type	NADH	17.43	8.77	10.23	10.63	1.10
<i>T. saccharolyticum</i> LL1049	High-ethanol-producer	NADPH	12.70	4.71	3.88	11.13	5.95
<i>T. saccharolyticum</i> LL1040	High-ethanol-producer	NADPH	12.96	5.19	4.86	5.73	3.62

^a Ethanol concentration was 1 M.

^b NAD(P)⁺ concentration was 2.35 mM. NAD(P)⁺ inhibition was measured with the significantly linked cofactor, i.e. NADH for LL1004, LL346 and LL1025 AdhE; NADPH for LL1040 and LL1049 AdhE. LL350 AdhE behaved similarly to inhibition to both cofactors, thus both measurements were presented.

^c Not determined, this enzyme was not significantly NADPH-linked in ALDH activity.