Supplemental Materials for

Ethanol Metabolism Dynamics in *Clostridium ljungdahlii* Grown on Carbon Monoxide

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Table S1 Primers used in the qPCR.

Gene locus	**Primer**	**Sequence (5’-3’)**
CLJU_RS09865/CLJU_c20110	aor1-F	GCACCGCTTACAGGAACTATA
	aor1-R	ACTGGTGAATCAGCCTTATCC
CLJU_RS01170/CLJU_c20210	aor2-F	TAGCAGCAGATGGATTGTCAC
	aor2-R	CAGGTACACCGTATGAGTCACA
CLJU_RS08095/CLJU_c16510	adhE1-F	ATCAGTTGGTGGTGGCTCAG
	adhE1-R	TTCCTGCGGATGTTGCTACT
CLJU_RS08100/CLJU_c016520	adhE2-F	GATCCAACCCTTGCTACA
	adhE2-R	GATGCTCATACATTACCCAC
CLJU_RS01185/CLJU_c12780	ack-F	ATTTACAGCAGGACTTGGAG
	ack-R	GTGCTTATTTCTAGTGCCTC
CLJU_RS01190/ CLJU_c12770	pta-RT-1	AGAAGGGAATAACGCCAGAA
	pta-RT-2	TATGAACCGCACCTGAAACC
CLJU_RS18530/CLJU_c37630	folD-RT-1	AACCAGTGGCAGATGCTATA
	folD-RT-2	CGTTTGCTCCAACTCTTACT
CLJU_RS18520/CLJU_c37610	metF-RT-1	AAAGATGCAGGATTAGAACC
	metF-RT-2	GCCACCTGTAATATCCCAAC
CLJU_RS18540/CLJU_c37650	fhs-RT-1	AGCAAAGACCCAATACTCCT
	fhs-RT-2	TTTCCAAGACCTGGCATC
CLJU_RS00045/CLJU_c00080	16S rRNA-F	AGAGTTTGATCCTGGCTCAG
	16S rRNA-R	TACGGCTACCTTGTTACGACT

Table S2 The expression profiles of the genes located in the central metabolic pathways during fermentation grown on CO of *C. ljungdahlii*.

ORF	**Gene and Protein**	**RPKM[c]**			
		Sta. t =96 h	Exp. t =48 h	Change fold[a]	No.[b]
	Wood-Ljungdal pathway				
CLJU_RS04480/ CLJU_c09090	NAD(P)/FAD-dependent oxidoreductase	4222	243	**-17.3**	1
CLJU_RS04485/ CLJU_c09100	nitrate reductase subunit beta	4464	259	**-17.2**	1
CLJU_RS04490/ CLJU_c09110	*cooS*, carbon-monoxide dehydrogenase catalytic subunit	4109.5	299.5	**-13.7**	1
CLJU_RS18490/ CLJU_c37550	*cdhC*, CO dehydrogenase/CO-methylating acetyl-CoA synthase complex subunit beta	4282.5	14842.5	**3. 5**	8
CLJU_RS18495/ CLJU_c37560	carbon monoxide dehydrogenase	4657	15325	**3.3**	8
CLJU_RS18500/ CLJU_c37570	acetyl-CoA synthase /synthase complex subunit gamma	5221.5	10347	**2.0**	8
CLJU_RS18505/ CLJU_c37580	acetyl-CoA synthase /synthase complex subunit delta	4042.5	10479	**2.6**	8
CLJU_RS18510/ CLJU_c37590	carbon monoxide dehydrogenase	3177	9011	**2.8**	8
CLJU_RS18515/ CLJU_c37600	*lpdA*, dihydrolipoyl dehydrogenase	4924	10186.5	**2.1**	7?
CLJU_RS18520/ CLJU_c37610	*metF*, 5,10-methylenetetrahydrofolate reductase	4580.5	10922	**2.4**	6
CLJU_RS18525/ CLJU_c37620	hypothetical protein, 5,10-methylene-tetrahydrofolate	4516	11381	**2.5**	5
CLJU_RS18530/ CLJU_c37630	*folD*, dehydrogenase/5,10-methylene-tetrahydrofolate cyclohydrolase	4306	11045	**2.6**	4
CLJU_RS18535/ CLJU_c37640	sugar ABC transporter substrate-binding protein	8777	23098.5	**2.6**	
CLJU_RS18540/ CLJU_c37650	*fhs*, formate-tetrahydrofolate ligase	11884.5	33969	**2.9**	3
CLJU_RS18545/ CLJU_c37660	carbon monoxide dehydrogenase	4673.5	8737.5	**1.9**	8
CLJU_RS18550/ CLJU_c37670	*cooS*, carbon-monoxide dehydrogenase catalytic subunit	10396.5	10251	**1.0**	8
CLJU_RS04405/CLJU_c08930	*fdh2*,formate dehydrogenase subunit alpha	405	206.5	**-2.0**	
CLJU_RS09825/CLJU_c20040	*fdh3*, formate dehydrogenase H subunit alpha selenocysteine-containing	1740.5	2409.5	**1.4**	
CLJU_RS03440/CLJU_c06990	*fdh1*, formate dehydrogenase H subunit alpha selenocysteine-containing	1301.5	527	**-2.5**	2
CLJU_RS03445/CLJU_c07000	*moeA*, molybdopterin molybdenumtransferase MoeA	344.5	195	**-1.8**	2
CLJU_RS03450/CLJU_c07010	*mobB*, molybdopterin-guanine dinucleotide biosynthesis protein B	332	204.5	**-1.6**	2
CLJU_RS03455/CLJU_c07020	*fdhD*, sulfurtransferase FdhD	187	116	**-1.6**	2
CLJU_RS03460/CLJU_c07030	NADH-quinone oxidoreductase subunit NuoE	640.5	903	**1.4**	2
CLJU_RS03465/CLJU_c07040	NADH dehydrogenase	622	764.5	**1.2**	2
CLJU_RS03470/CLJU_c07050	2Fe-2S iron-sulfur cluster binding domain-containing protein	964	821	**-1.6**	2
CLJU_RS03475/CLJU_c07060	4Fe-4S dicluster domain-containing protein	1094.5	830.5	**-1.3**	2
CLJU_RS03480/CLJU_c07070	4Fe-4S dicluster domain-containing protein	1702.5	1121.5	**-1.5**	2
CLJU_RS03485/CLJU_c07080	4Fe-4S dicluster domain-containing protein	2756	1290.5	**-2.1**	2

	AdhE and AOR pathway				
CLJU_RS08095/CLJU_c16510	*adhE1*, bifunctional acetaldehyde-CoA/alcohol dehydrogenase	266	4916.5	**18.5**	11
CLJU_RS08100/CLJU_c16520	*adhE2*, bifunctional acetaldehyde-CoA/alcohol dehydrogenase	0	7	**0**	
CLJU_RS09865/CLJU_c20110	*aor1*, aldehyde ferredoxin oxidoreductase	36.5	21.5	**-1.7**	
CLJU_RS09915/CLJU_c20210	*aor2*, aldehyde ferredoxin oxidoreductase	16796.5	4249	**-4.0**	12
	Acetate biosynthesis pathway				
CLJU_RS06260/CLJU_c12770	*pta*, phosphate acetyltransferase	691.5	2110	**3.1**	9
CLJU_RS06265/CLJU_c12780	*ack*, acetate kinase	825	2241.5	**2.7**	10

Genes are listed in order of old and new ORF numbers; genes located in the same operon are assembled by same shading; ORF: Open reading frame; [a]: Data represent the values change fold of PRKM during exponential phase, as compared to those during stationary phase. The positive values represent the increased change fold during exponential phase. The negative values represent the decreased change fold during exponential phase; [b]: The number represents the correlation functional genes participating in the metabolic reactions in Figure 6; [c]: RPKM is the abbreviation of Reads per Kilobase per Million mapped reads.

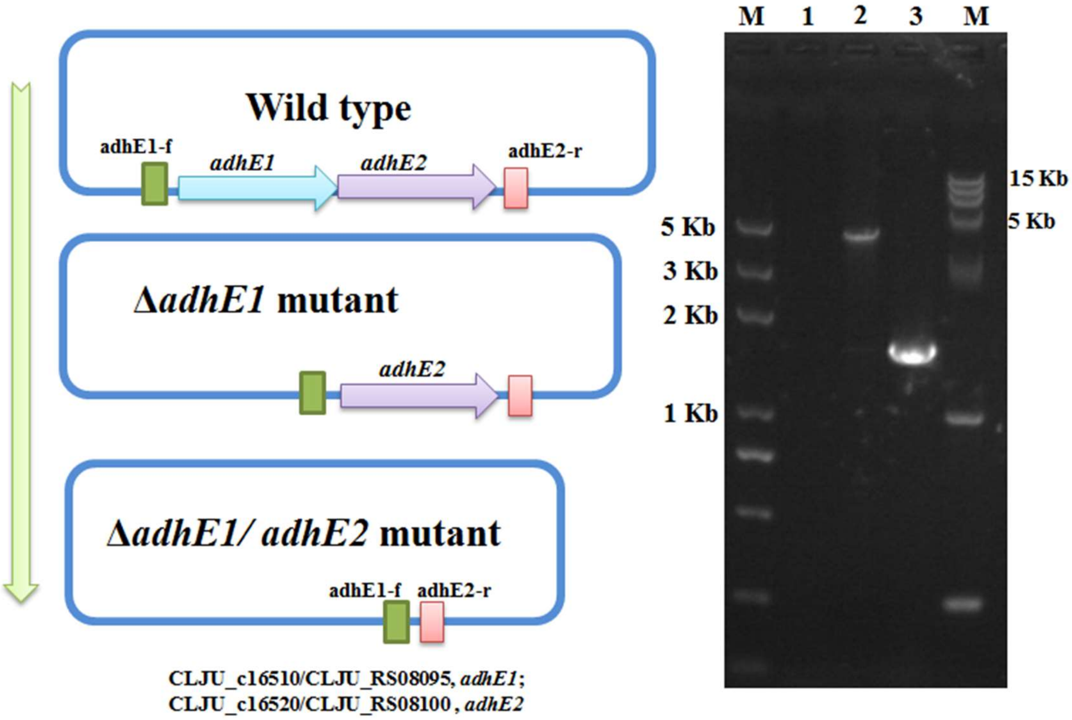

Fig. S1 The schematic process of *adhE* deletion and PCR results of *C. ljungdahlii. adhE1* (1791269-1793881) and *adhE2* (1794033-1796666) are adjacent in the genome. The deletion fragments are located 1791272-1793770 (*ΔadhE1*) and 1791272-1796633 (*ΔadhE1+2*), respectively (Köpke et. al. 2010, Proc Natl Acad Sci 107:13087-13092. doi: 10.1073/pnas.1004716107). The PCR results and sequencing results showed that we obtained correct mutants. Left: adhE1-f and adhE2-r are primer names (Table S1), which used in the following PCR test. Right: M, marker; lane 1: wild type; lane 2: *ΔadhE1*; lane 3: *ΔadhE1+2;* The fragment of WT is too long to obtain.

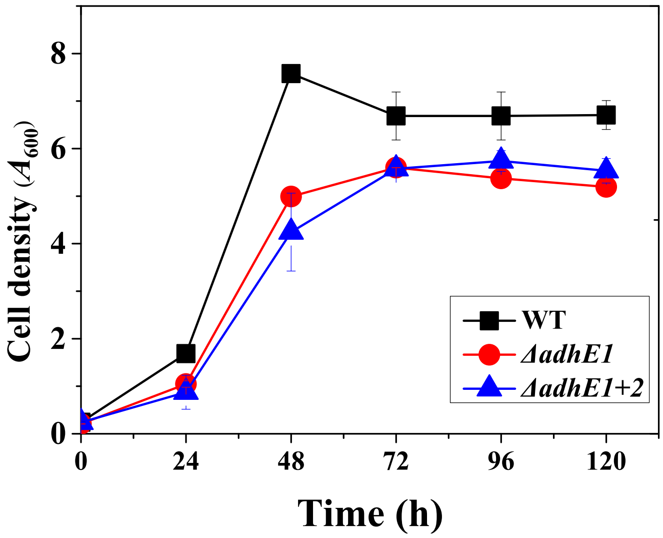

Fig. S2 Growth profiles of *C. ljungdahlii* WT and *adhE* gene deletion mutants on YTF medium.

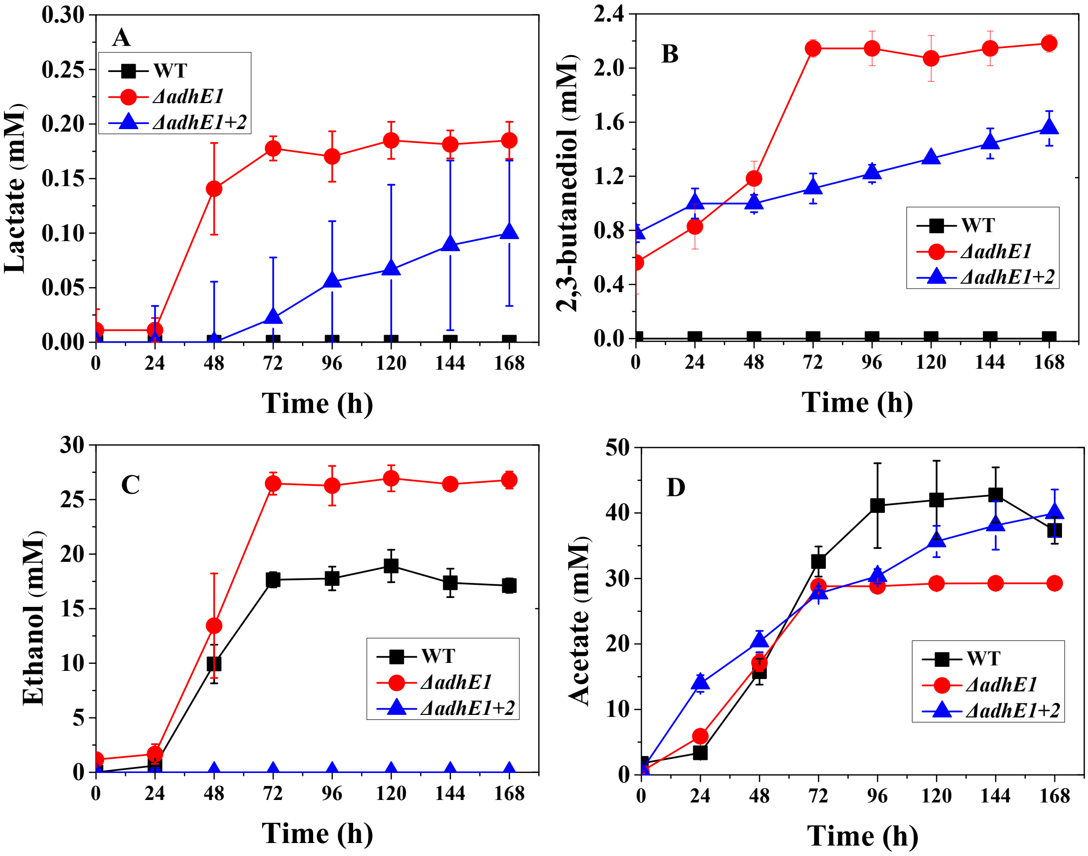

Fig. S3 Product profiles of *C. ljungdahlii* WT and *adhE* deletion mutants grown on fructose. (A): Lactate; (B): 2,3-butanediol; (C): Ethanol; (D): Acetate.

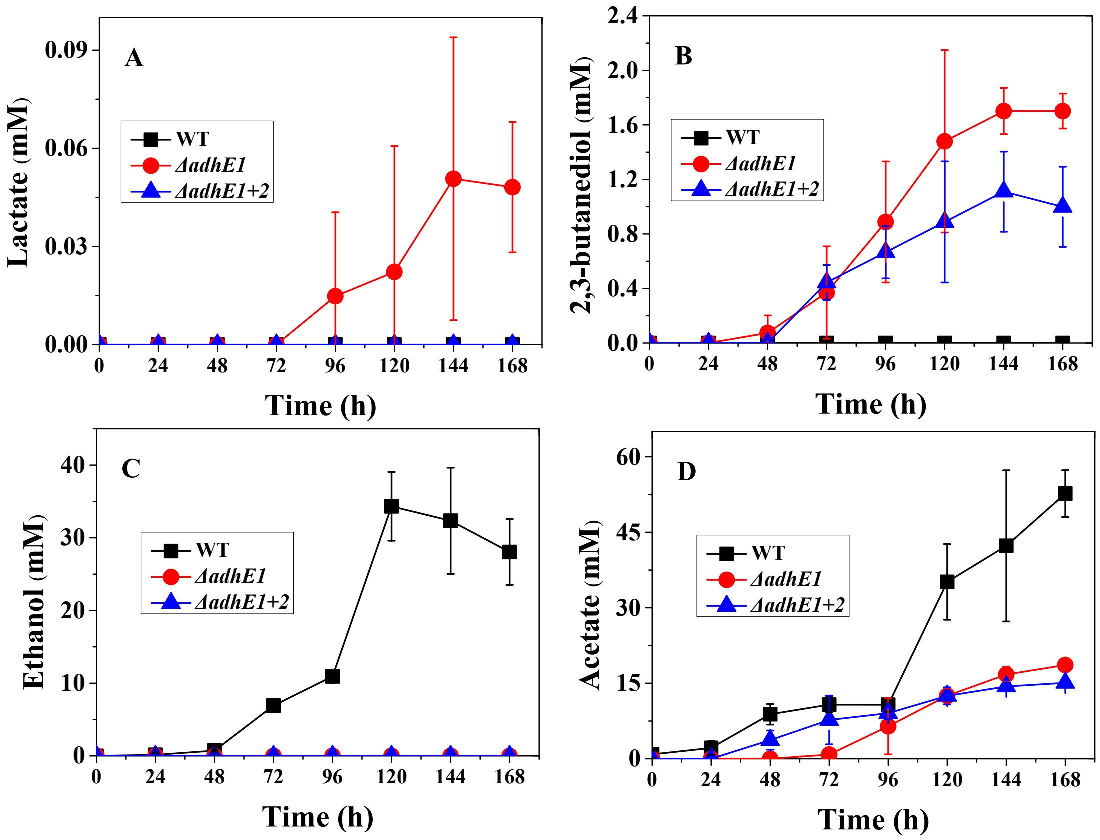

Fig. S4 Product profiles of *C. ljungdahlii* WT and *adhE* deletion mutants grown on CO:CO$_2$ (80:20, v/v). (A): Lactate; (B): 2,3-butanediol; (C): Ethanol; (D): Acetate.

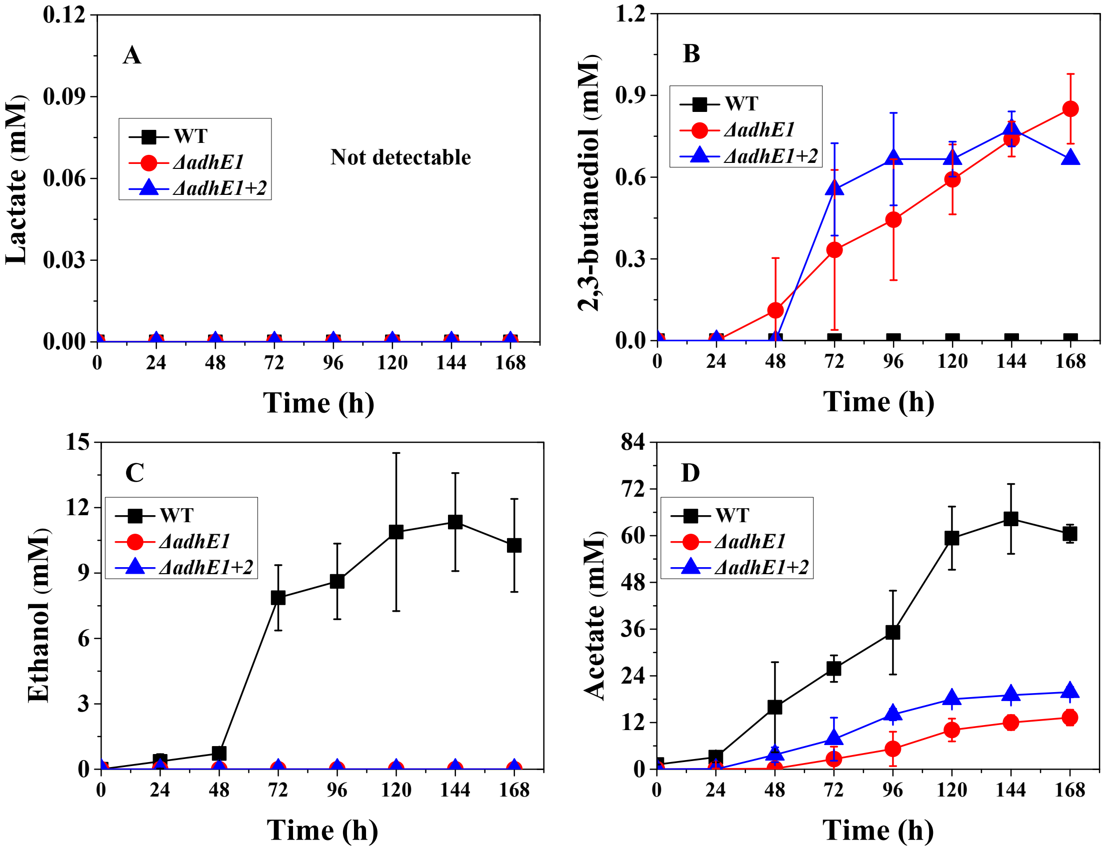

Fig. S5 Product profiles of *C. ljungdahlii* WT and *adhE* deletion mutants grown on CO_2:H_2 (60:40, v/v). (A): Lactate; (B): 2,3-butanediol; (C): Ethanol; (D): Acetate.

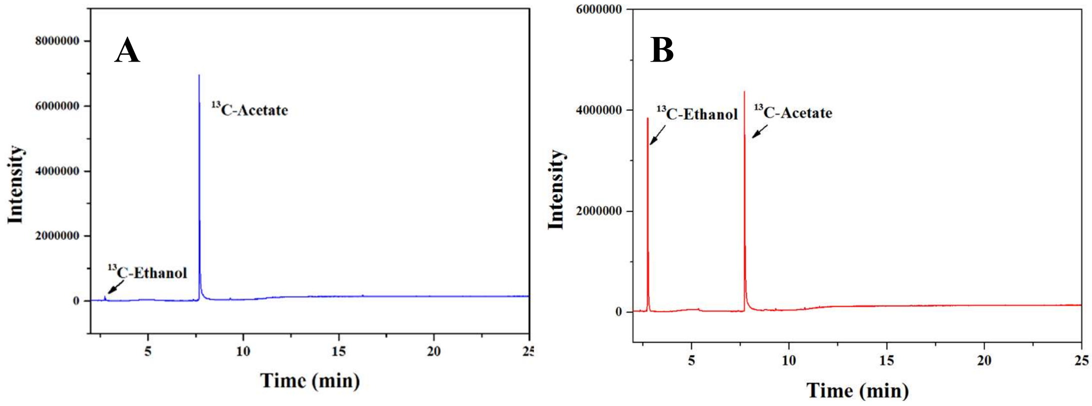

Fig. S6 Detection of ^{13}C labeled products after incubation of *C. ljungdahlii* WT cells in fresh medium with ^{13}C-acetate (A) or ^{13}C-ethanol (B) for 12 h under anaerobic condition.

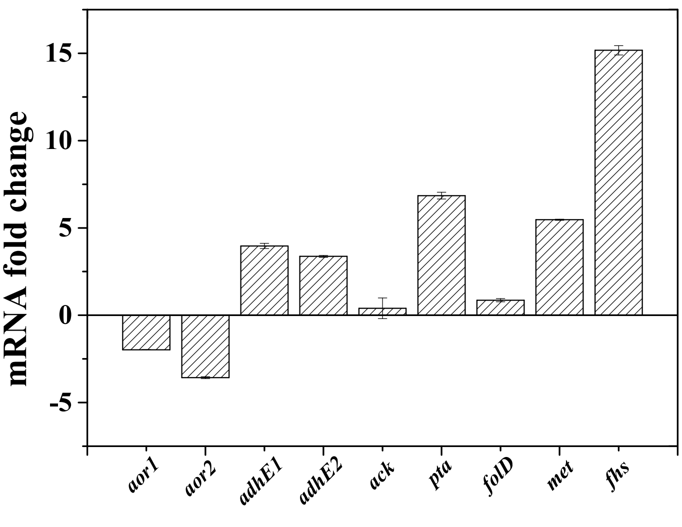

Fig. S7 Comparison of gene expression levels of *C. ljungdahlii* between exponential and stationary growth phases.