

Supporting Information

Isotopomer analysis. Cell pellets were hydrolyzed in 6 M HCl at 100°C for 24 hrs. After air-drying overnight, the dried samples containing free amino acids were derivatized with *N*-(tert-butyl-dimethylsilyl)-*N*-methyl-trifluoroacetamide in tetrahydrofuran at 70°C for 1 hr. Isotopomer measurements were made on a GC (Hewlett-Packard, model 6890, Agilent Technologies, Palo Alto, CA) equipped with a DB5-MS column (J&W Scientific, Falsom, CA) and a mass spectrometer (MS) (5975, Agilent Technologies, Palo Alto, CA). Several groups of charged fragments were detected by GC-MS for the amino acids: the [M-57]⁺ or [M-15]⁺ group, which contains un-fragmented amino acids; the [M-159]⁺ or [M-85]⁺ group, which contains amino acids losing α carboxyl group; and the f302 group, which contains the fragments of the first two carbons from derivatized amino acids. The [M-57]⁺ peaks in leucine and isoleucine overlap with other peaks. Published algorithms were used to correct the effects of natural isotopes on the mass distributions of amino acids (1) mass fractions (i.e. M0, M1, M2... which are fractions of unlabeled, single-labeled, and double-labeled amino acids...).

Table S1. Sequences of primers described in this report.

Gene (loci number, predicted/ reported function)	Forward primer (5' → 3')	Reverse primer (5' → 3')
16S rRNA gene	GCAACGCGAAGAACCTT ACC	GGGCACCCTCGCATCTC
<i>pykA</i> (HM1_0076, pyruvate kinase)	GCCCGAATCATCTCCAT CAG	AACGCCCCGCACGAA
<i>acn</i> (HM1_0105, aconitase)	AATCAGCCTGTGGTCCC TGT	CAGAGACACCGCCCGAGT T
<i>fdxR</i> (HM1_0289, ferredoxin- NADP ⁺ reductase, FNR)	CCTGCTCCCGGTCAAAA TC	TTCTTCGCGCCGATGAA
<i>porA</i> (HM1_0807, pyruvate: Fd oxidoreductase, PFOR)	GAAGCCTGCAACCCCTA CTATAAG	GGTGAGTTTGCCGATCTC CTT
<i>acsA</i> (HM1_0951, acetyl-CoA synthetase)	TCCAAACCTGAAATCCT ATGAAGAG	AGAACTCGCGCTCCACAT CT
<i>icd</i> (HM1_1471, isocitrate dehydrogenase)	TCAACCCCGGATCGGTC	CTGCCAGCCGAGGTGTTC
<i>mdh</i> (HM1_1472, malate dehydrogenase)	CGGCTATGAGGGCATCT ACAC	CGGTCAGCTCGATCTCAA AGA
<i>ackA</i> (HM1_2157, acetate kinase)	CCCGCGTCGGTGACAT	CGTCAATCCCTCTTTTTCC ATC
<i>ppdK</i> (HM1_2461, pyruvate phosphate dikinase)	AGATGTCGTTGCCGGTA TCC	AAGCATTGGGCAGTTCT TC
<i>korC</i> (HM1_2762, KFOR, γ subunit)	GGGAAGCCCTCGAAAAA GC	TGTTTTCATCTCCTCGGTCCC T
<i>oorB</i> (HM1_2763, KFOR, β subunit)	AAAGGGACCACCGCTCC T	GCCAGGTTGCAGATGTGCG A
<i>korA</i> (HM1_2766, KFOR, α subunit)	CGGCGACCATCCTGTCA T	CCGTCAGGTTGAAGCATT CC
<i>korD</i> (HM1_2767, KFOR, δ subunit)	AAGTGTTGGGCGCTGAC G	TGCACTTGGTACATTTTTT CGG
<i>nifV</i> (HM1_0858, homocitrate synthase)	GAAGCCTATCCGCCCGA	GCTGTATTTGCCAAAGGC GA
<i>aksA</i> (HM1_2993, homocitrate synthase)	CGCTTCCCGTTCTGATAT TGA	CGCCCAGCTTTTTGGCTT
<i>pckA</i> (HM1_2773, PEP carboxykinase)	GATGCCATCTTCCACGA GGTA	CAGTCCCTGTTACGTGTGCG AAA

Table S2. Isotopomer labeling patterns of protein-based amino acids in the *Desulfovibrio vulgaris* Hildenborough (DvH) cultures grown on non-labeled lactate and [2-¹³C]acetate.^a

Amino acid	Precursor	Ion	M-57	M-159
Ala	pyruvate	M0	0.89	0.85
		M1	0.10	0.12
		M2	0.01	0.03
Leu	pyruvate	M0		0.34
	acetyl-CoA	M1	peaks	0.49
		M2	overlapped	0.14
Phe	phosphoenolpyruvate	M0	0.65	0.63
	erythrose-4-phosphate	M1	0.28	0.29
		M2	0.05	0.06
Asp	oxaloacetate	M0	0.85	0.86
		M1	0.14	0.10
		M2	0.01	0.02
Glu	α -ketoglutarate	M0	0.43	0.42
		M1	0.49	0.50
		M2	0.03	0.06
His	ribose-5-phosphate	M0	0.78	0.81
		M1	0.13	0.13
		M2	0.06	0.03

^a While DvH cannot grow using acetate as the sole carbon and energy source, DvH can grow with a mixed-substrate (lactate and acetate). When [2-¹³C]acetate and non-labeled lactate with 1:1 molar ratio were presented in DvH growth medium, a doubling time ~ 9 hours was observed during the middle-log phase. The labeling carbons were significantly enriched in its protein-based amino acids for the cultures harvested during the late-log growth phase (see Table above), indicating that labeled acetate was partially utilized for protein synthesis in the mixed-substrate culture. The labeling data presented above also illustrates that acetate \leftrightarrow acetyl-CoA \leftrightarrow pyruvate is a reversible pathway. The DvH culture condition was described in the Experimental Procedures.

Table S3. Isotopomer labeling patterns of protein-based amino acids in the *H. modesticaldum* cultures grown on [3-¹³C]pyruvate.

Amino acid	Precursor	Ion	M-57	M-159	f302	Proposed ¹³C enriched positions
Ala	pyruvate	M0	0.06	0.09	0.13	*C-C-COOH
		M1	0.89	0.80	0.85	
		M2	0.05	0.11	0.02	
Gly	serine	M0	0.70	0.72	0.28	*C-COOH
		M1	0.29	0.28	0.35	
		M2	0.01		0.37	
Val	pyruvate	M0	0.06	0.08	0.73	*C-*C-C-C-COOH
		M1	0.03	0.05	0.18	
		M2	0.87	0.78	0.10	
Leu	pyruvate acetyl-CoA	M0	0.06	0.08	0.28	*C-*C-C-C-*C-COOH
		M1	0.02	0.02	0.30	
		M2	0.15	0.27	0.42	
		M3	0.75	0.57		
Ile	pyruvate threonine	M0	0.05	0.05	0.22	*C-*C-C-C-*C-COOH
		M1	0.02	0.02	0.48	
		M2	0.27	0.34	0.29	
		M3	0.64	0.57		
Met	aspartate methyl-THF	M0	0.06	0.06	0.29	*C-S-C-*C-C-COOH
		M1	0.37	0.39	0.54	
		M2	0.52	0.52	0.17	
		M3	0.05	0.04		
Ser	3-phospho- glycerate	M0	0.09	0.09	0.75	*C-C-COOH
		M1	0.87	0.89	0.24	
		M2	0.04	0.02	0.01	
Thr	aspartate	M0	0.07	0.08	0.11	C-*C-C-COOH
		M1	0.83	0.86	0.64	
		M2	0.11	0.07	0.25	
Phe	PEP	M0	0.06	0.06	0.76	*C-*C-C-C-C-C-*C-C-COOH (clockwise)
	erythrose-4- phosphate	M1	0.01	0.01	0.23	
		M2	0.04	0.05	0.01	
		M3	0.74	0.75		
		M4	0.14	0.13		
Asp	OAA	M0	0.06	0.06	0.58	C-*C-C-COOH
		M1	0.82	0.87	0.39	
		M2	0.12	0.07	0.03	

Glu	α -KG	M0	0.07	0.07	0.71	C-*C-C-*C-COOH
		M1	0.10	0.22	0.24	
		M2	0.76	0.67	0.05	
		M3	0.07	0.05		
His	ribose-5-phosphate	M0	0.06	0.13	0.14	N-*C-N-C-C-C-C-*COOH
		M1	0.12	0.43	0.78	
		M2	0.53	0.35	0.08	
		M3	0.28	0.08		
Lys	aspartate	M0	0.07	0.07	0.91	C-*C-C-*C-C-COOH
		M1	0.05	0.04	0.09	
	pyruvate	M2	0.79	0.81	0.00	
		M3	0.09	0.06		
		M4	0.00	0.01		

Table S4. Isotopomer labeling patterns of protein-based amino acids in the *H. modesticaldum* cultures grown on ¹³C-labeled sodium bicarbonate and non-labeled pyruvate.

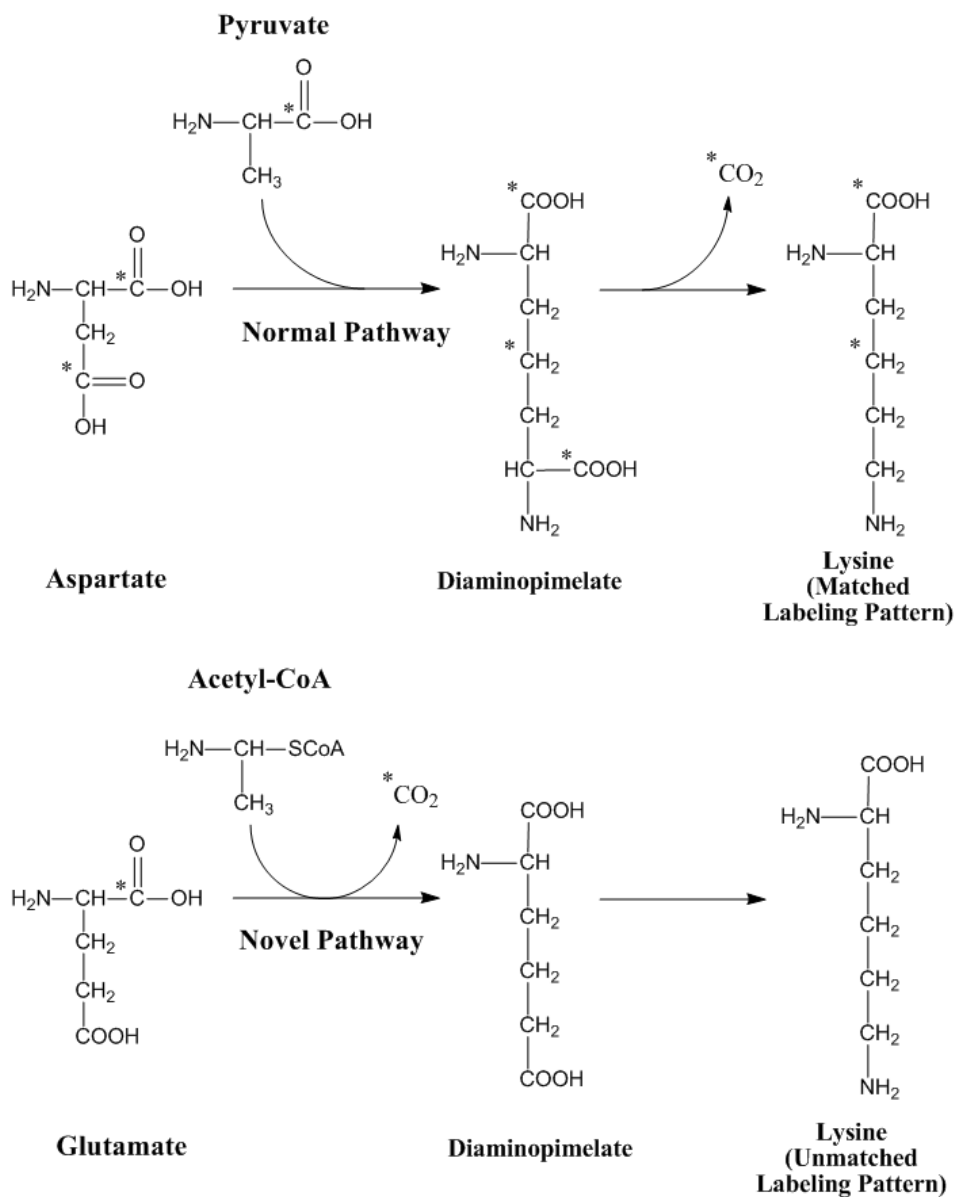
Amino acid	Precursors	Ions	M-57	M-159	f302	Proposed ¹³ C enriched positions
Ala	pyruvate	M0	0.47	0.92	0.47	C-C-*COOH
		M1	0.52	0.03	0.53	
		M2	0.01	0.05	0.00	
Gly	serine	M0	0.49	0.99	0.24	C-*COOH
		M1	0.51	0.01	0.29	
		M2	0.01		0.47	
Val	pyruvate	M0	0.43	0.93	0.51	C-C-C-C-*COOH
		M1	0.54	0.05	0.48	
		M2	0.03	0.01	0.01	
Leu	pyruvate acetyl-CoA	M0	0.74	0.92	0.80	Non-labeled
		M1	0.20	0.07	0.19	
		M2	0.02	0.01	0.01	
		M3	0.04	0.00		
Ile	pyruvate threonine	M0	0.64	0.86	0.65	Non-labeled
		M1	0.29	0.13	0.31	
		M2	0.07	0.01	0.04	
		M3	0.00	0.00		
Met	aspartate methyl-THF	M0	0.23	0.42	0.28	C-S-*C-C-C-*COOH
		M1	0.45	0.52	0.42	
		M2	0.29	0.04	0.30	
		M3	0.03	0.01		
Ser	3-phospho- glycerate	M0	0.45	0.96	0.48	C-C-*COOH
		M1	0.54	0.03	0.51	
		M2	0.02	0.01	0.01	
Thr	aspartate	M0	0.23	0.43	0.20	*C-C-C-*COOH
		M1	0.47	0.57	0.49	
		M2	0.30	0.02	0.31	
Phe	PEP erythrose-4- phosphate	M0	0.14	0.27	0.41	C-C-*C-*C-C-C-C-C-*COOH
		M1	0.32	0.45	0.59	
		M2	0.35	0.25	0.00	
		M3	0.18	0.03		
Asp	OAA	M0	0.24	0.47	0.47	*COOH-C-C-*COOH
		M1	0.46	0.52	0.53	
		M2	0.31	0.01	0.00	
Glu	α -KG	M0	0.38	0.43	0.78	C-C-C-C-*COOH

		M1	0.50	0.54	0.21	
		M2	0.11	0.02	0.01	
		M3	0.00	0.00		
Lys	aspartate	M0	0.26	0.42	0.87	C-C-*C-C-C-*COOH
	Pyruvate	M1	0.41	0.54	0.13	
		M2	0.34	0.03	0.00	
		M3	0.00	0.01		
		M4	0.01	0.00		

Table S5. List of bacteria with the citramalate pathway reported.

Species	References	CimA similarity (GSU1798)	CimA similarity (MJ1392)	Growth conditions
<i>Roseobacter denitrifican</i>	(2)	RD1_3182, 45%	RD1_1121, 40%	aerobic
<i>Methanobacterium thermoautotrophicum</i>	(3)	MTH1481, 28%	MTH723, 58%	anaerobic
<i>Methanococcus jannaschii</i>	(4)	ND	MJ1392, 100%	aerobic (<i>E. coli</i>)
<i>Leptospira interrogans</i>	(5,6), (7)	LIC11726, 26%	LIC11726, 41%	aerobic (<i>E. coli</i>)
<i>Thermoproteus neutropkilccs</i>	(8)	Tneu_0320, 45%	Tneu_0832, 55%	anaerobic, thermophilic
<i>Ignicoccus hospitalis</i>	(9)	Igni_0645, 45%	Igni_0983, 52%	anaerobic, thermophilic
<i>Geobacter sulfurreducens</i>	(10)	GSU1798, 100%	GSU1906, 41%	anaerobic
<i>Geobacter metallireducens</i>	(11)	Gmet_1879, 92%	Gmet_1265, 42%	anaerobic
<i>Serratia marcescens</i>	(12)	Spro_0745, 26%	Spro_0745, 37%	anaerobic
<i>Thermoanaerobacter</i> sp. X514	(13)	Teth514_1204 49%	Teth514_0415 45%	anaerobic, thermophilic
<i>Dehalococcoides ethenogenes</i> 195	(14)	DET0825, 53%	DET0830, 41%	anaerobic
<i>Heliobacterium modesticaldum</i>	(15) and this report	HM_1519, 55%	HM_1515, 45%	anaerobic

Fig. S1. The normal versus the novel pathway for lysine biosynthesis in the *H. modesticaldum* cultures grown on [1-¹³C]pyruvate. Predicted labeled carbons are marked by asterisks.



References in Supporting Information

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