Supplementary Material to

The key to acetate: Metabolic fluxes of acetic acid bacteria under cocoa pulp fermentation simulating conditions

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1) List of reactions

Table S1: List of reactions and their corresponding annotated genes

Abbreviation	Enzyme	EC Number	Gene (*Acetobacter pasteurianus* IFO3283-01)[a]	Gene (*Acetobacter pasteurianus* 386B)[b]
ACO	Aconitase	4.2.1.3	APA01_25120	APA386B_1323
ALS	α-Acetolactate synthase	2.2.1.6	APA01_03810, APA01_03830, APA01_19270	APA386B_835, APA386B_836, APA386B_1863
ALDC	α-Acetolactate decarboxylase	4.1.1.5	APA01_03800	APA386B_1862
ADH1	Alcohol dehydrogenase, membrane-associated, quinone-dependent	1.1.5.5	APA01_00250 APA01_00860 APA01_00870 APA01_03250 APA01_04650 APA01_07070 APA01_08590 APA01_12700 APA01_13900 APA01_15620 APA01_22100 APA01_23220 APA01_24830	APA386B_1948 APA386B_1574, APA386B_1575
ADH2	Alcohol dehydrogenase (NAD(P) dependent)	1.1.1.1 1.1.1.2	n.a.	APA386B_1507, APA386B_2362, APA386B_496
ALDH1	Aldehyde dehydrogenase, membrane-associated, quinone-dependent	1.2.99.3	n.a.	APA386B_1211, APA386B_2542, APA386B_2544
ALDH2	Aldehyde dehydrogenase (NAD(P) dependent)	1.2.1.3 1.2.1.4	APA01_23770 APA01_22400 APA01_22090 APA01_20540 APA01_20430	APA386B_909 APA386B_151
CS	Citrate synthase	2.3.3.1	APA01-03600, APA01_10670	APA386B_2584
ENO	Enolase	4.2.1.11	eno	APA386B_2618
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase	1.2.1.12	APA01_03800	APA386B_1520
GPDH	Glucose 6-phosphate dehydrogenase	1.1.1.49	APA01_10420, APA01_06250	APA386B_729
GLPDH	Gluconate dehydrogenase	1.1.1.44	APA01_23130	APA386B_1154
RuPI	Ribulose 5-phosphate epimerase	5.1.3.1	APA01_08750	APA386B_2378
RPI	Ribose 5-phosphate isomerase	5.3.1.6	APA01_23160	APA386B_1157
TKT1	transketolase	2.2.1.1	APA01_00390, APA01_23110	APA386B_1152, APA386B_1521
TAL	transaldolase	2.2.1.2	APA01_23120	APA386B_1153
TKT2	transketolase	2.2.1.1	APA01_00390,	APA386B_1152,

			APA01_23110	APA386B_1521
FBP	Fructose 1,6-bisphosphatase	3.1.3.11	glpX	APA386B_993
FBA	Fructose-bisphosphate aldolase	4.1.2.13	APA01_02600	APA386B_1752
ICDH	Isocitrate dehydrogenase	1.1.1.42 1.1.1.41	APA01_06250, APA01_26420	APA386B_2121 APA386B_2558
PC	Pyruvate carboxylase	6.4.1.1	APA42C_25210	n.a.
PDC	Pyruvate decarboxylase	4.1.1.1	APA01_23490	APA386B_1186
PDH	Pyruvate dehydrogenase	1.2.4.1 2.3.1.12	APA01_05950	APA386B_2083, APA386B_2084, APA386B_2737. APA386B_2738, APA386B_2085, APA386B_2736, APA386B_2735, APA386B_2271
ACS	Acetyl-CoA synthetase	6.2.1.1	APA01_03620	
PHM	Phosphohexomutase, phosphoglucomutase	5.4.2.2	APA01_40080, APA01_26780	APA386B_513
AarC	Succinyl-CoA : acetate CoA transferase	3.1.2.1	APA01_10710	APA386B_2589
SDH	Succinate dehydrogenase	1.3.99.1	APA01_00320, APA01_00310	APA386B_1513 - APA386B_1516
FH	Fumarate hydratase	4.2.1.2	APA01_24940, APA01_14580	APA386B_321, APA386B_1305
ME	Malic Enzyme”	1.1.1.40	APA01_01110	APA386B_1600
OGDC	α-ketoglutarate dehydrogenase	1.2.4.2 1.8.1.4 2.3.1.61	APA01_07660, APA01_07670	APA386B_2269, APA368B_2270
MQO	Malate dehydrogenase, malate : quinone oxidoreductase	1.1.5.4	APA01_01110, APA01_11550	APA386B_2675
PEPC	Phosphoenolpyruvate carboxylase	4.1.1.31	APA01_16650[c]	APA386B_587, APA386B_588
PGK	Phosphoglycerate kinase	2.7.2.3	pgk	APA386B_1519
PGM	Phosphoglycerate mutase	5.4.2.1	APA01_23500, APA01_25860, APA01_22500	APA386B_1895, APA386B_2495
PPDK	Pyruvate phosphate dikinase	2.7.9.1	APA01_19100	APA386B_814
PYK	Pyruvate kinase	2.7.1.40	APA01_06160	APA386B_2108
TPI	Triose phosphate isomerase	5.3.1.1	APA01_12250	APA386B_2746

[a]Azuma Y, Hosoyama A, Matsutani M, Furuya N, Horikawa H, Harada T, Hirakawa H, Kuhara S, Matsushita K, Fujita N, Shirai M. 2009. Whole-genome analyses reveal genetic instability of *Acetobacter pasteurianus*. Nucleic Acids Res 37(17):5768–5783. (Accession: www.biocyc.org)

[b]Illeghems K, de Vuyst L, Weckx S. 2013. Complete genome sequence and comparative analysis of *Acetobacter pasteurianus* 386B, a strain well-adapted to the cocoa bean fermentation ecosystem. BMC Genomics 14(1):526. (Accession: DDBJ/EMBL/GenBank, Accession Numbers: HF677570-HF677577)

[c]This gene is annotated with activity for 4.1.1.31 and 4.1.1.32 (phosphoenolpyruvate carboxykinase). However, there is now experimental evidence for phosphoenolpyruvate carboxykinase.

2) Cultivation profiles of *A. pasteurianus* NCC 316 and *A. ghanensis* DSM 18895 grown in cocoa pulp simulation medium

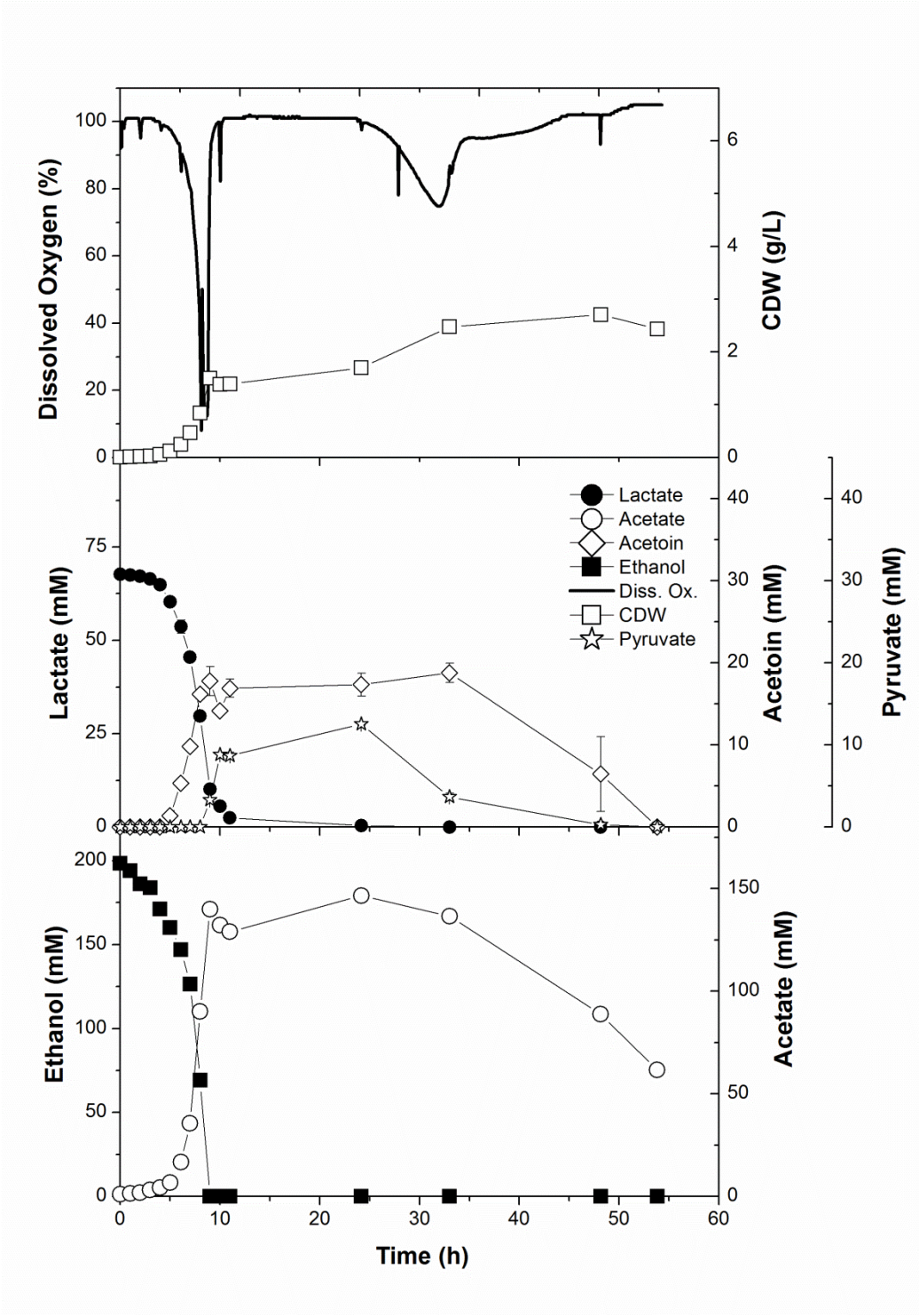

Figure S1: Cultivation profile of *A. pasteurianus* NCC 316 in cocoa pulp simulation medium.

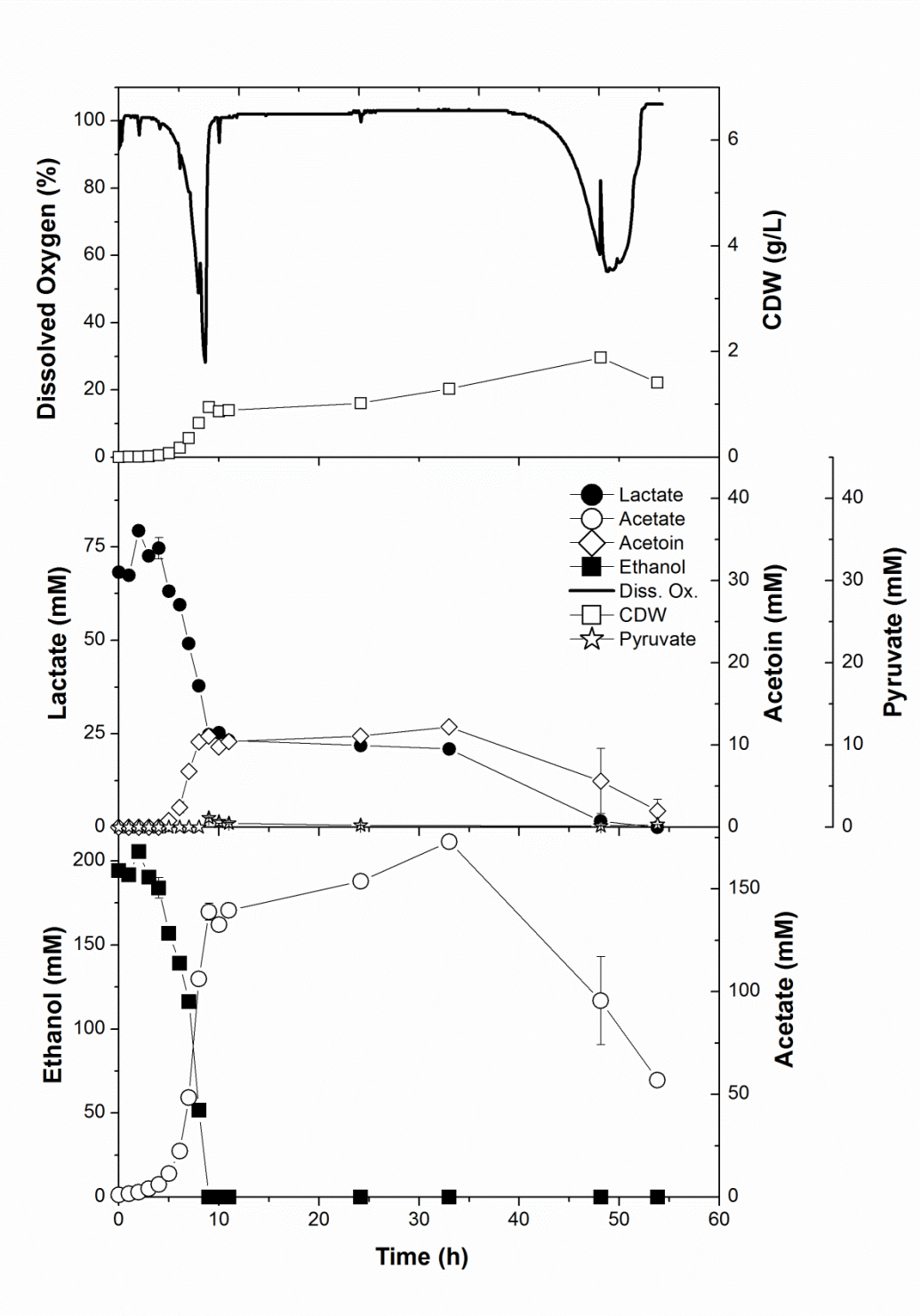

Figure S2: Cultivation profile of *A. ghanensis* DSM 18895 in cocoa pulp simulation medium.

3) Degree of *de novo* synthesis of amino acids by *A. pasteurianus* NCC 316 and *A. ghanensis* DSM 18895 grown in cocoa pulp simulation medium

Table S2: Mass isotopomer distributions of the carbon backbones of proteinogenic amino acids in cell pellets of *A. pasteurianus* NCC 316 and *A. ghanensis* DSM 18895 grown in cocoa pulp simulation medium with [U-^{13}C] lactate and [U-^{13}C] ethanol. The sum of fractional labeling, the degree of *de novo* synthesis, and the degree of amino acid uptake were derived from these mass isotopomer distributions.

Amino acid family	Amino acid	Mass isotopomer fraction ± standard deviation[a] (%)										Sum of fractional labeling (%)	Degree of *de novo* synthesis [b] (%)	Degree of amino acid uptake (%)
		m+0	*m*+1	*m*+2	*m*+3	*m*+4	*m*+5	*m*+6	*m*+7	*m*+8	*m*+9			
A. pasteurianus NCC 316														
Pyruvate	Ala	83.4 ± 0.8	3.8 ± 0.1	1.9 ± 0.1	10.9 ± 0.6							13.4 ± 0.7	12.3 ± 0.7	87.7 ± 0.7
	Leu	86.1 ± 0.7	6.0 ± 0.1	0.1 ± 0.0	0.8 ± 0.0	0.9 ± 0.0	6.1 ± 0.6					8.5 ± 0.6	7.4 ± 0.6	92.6 ± 0.6
	Val	39.6 ± 0.1	2.5 ± 0.0	3.1 ± 0.2	3.3 ± 0.2	4.3 ± 0.0	47.3 ± 0.3					54.4 ± 0.5	53.3 ± 0.5	46.7 ± 0.5
Serine	Ser	94.8 ± 0.8	3.4 ± 0.1	0.2 ± 0.2	1.6 ± 0.5							2.8 ± 0.7	1.7 ± 0.7	98.3 ± 0.7
	Gly	95.2 ± 0.9	3.4 ± 0.3	1.4 ± 0.5								3.1 ± 0.7	2.0 ± 0.7	98.0 ± 0.7
Glutamate	Glu	64.6 ± 0.6	4.9 ± 0.1	23.9 ± 1.0	2.4 ± 0.2	2.3 ± 0.4	1.9 ± 0.3					15.8 ± 1.2	14.7 ± 1.2	85.3 ± 1.2
Aspartate	Asp	82.1 ± 3.3	4.2 ± 0.0	6.6 ± 1.8	2.9 ± 0.8	4.3 ± 0.8						10.8 ± 2.3	9.7 ± 2.3	90.3 ± 2.3
	Thr	84.8 ± 2.3	4.1 ± 0.1	5.3 ± 1.2	2.3 ± 0.5	3.5 ± 0.5						8.9 ± 1.5	7.8 ± 1.5	92.2 ± 1.5
	Lys	16.9 ± 0.3	4.5 ± 0.1	32.9 ± 1.4	33.6 ± 1.4	5.3 ± 1.1	3.5 ± 0.8	3.4 ± 0.6				38.3 ± 3.2	37.2 ± 3.2	62.8 ± 3.2

	Ile	62.3 ± 1.7	5.4 ± 0.0	27.6 ± 1.9	2.0 ± 0.0	1.5 ± 0.1	1.2 ± 0.1					15.7 ± 1.0	14.6 ± 1.0	85.4 ± 1.0
aromatic	Tyr	32.8 ± 0.4	3.8 ± 0.2	0.8 ± 0.2	1.1 ± 0.1	1.4 ± 0.1	3.7 ± 0.7	8.1 ± 1.1	4.0 ± 0.1	10.5 ± 1.1	33.8 ± 2.7	55.4 ± 5.1	54.3 ± 5.1	44.6 ± 5.1
	Phe	85.3 ± 0.3	8.4 ± 0.0	0.4 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.5 ± 0.0	0.8 ± 0.1	0.4 ± 0.0	1.0 ± 0.1	2.9 ± 0.3	6.1 ± 0.5	5.0 ± 0.5	95.0 ± 0.5
						A. ghanensis DSM 18895								
Pyruvate	Ala	83.4 ± 1.1	3.8 ± 0.1	2.1 ± 0.3	10.7 ± 0.8							13.4 ± 1.0	12.3 ± 1.0	87.7 ± 1.0
	Leu	67.3 ± 1.4	6.4 ± 0.1	0.3 ± 0.0	2.1 ± 0.0	3.0 ± 0.0	20.8 ± 1.3					25.9 ± 1.3	24.8 ± 1.3	75.2 ± 1.3
	Val	27.6 ± 2.3	1.8 ± 0.2	2.9 ± 0.2	3.3 ± 0.1	5.5 ± 0.2	58.8 ± 2.6					66.7 ± 2.9	65.6 ± 2.9	34.4 ± 2.9
Serine	Ser	45.7 ± 3.4	6.9 ± 0.2	6.5 ± 0.3	40.9 ± 2.9							47.5 ± 2.9	46.4 ± 2.9	53.6 ± 2.9
	Gly	57.0 ± 2.6	4.8 ± 0.2	38.2 ± 2.4								40.6 ± 2.5	39.5 ± 2.5	60.5 ± 2.5
Glutamate	Glu	45.3 ± 4.5	4.5 ± 0.3	32.1 ± 1.3	6.0 ± 0.8	7.6 ± 2.2	4.6 ± 3.1					28.0 ± 2.5	26.9 ± 2.5	73.2 ± 2.5
Aspartate	Asp	72.1 ± 6.4	4.1 ± 0.2	13.0 ± 1.3	5.9 ± 2.3	4.9 ± 3.1						16.9 ± 5.5	15.8 ± 5.5	84.3 ± 5.5
	Thr	74.6 ± 5.7	4.0 ± 0.2	11.7 ± 1.3	5.3 ± 2.0	4.4 ± 2.7						15.2 ± 4.9	14.1 ± 4.9	85.9 ± 4.9
	Lys	15.6 ± 1.3	4.1 ± 0.3	29.6 ± 1.9	30.4 ± 1.6	9.2 ± 0.9	6.7 ± 1.9	4.3 ± 2.3				41.8 ± 6.0	40.7 ± 6.0	59.3 ± 6.0
	Ile	57.2 ± 1.1	5.4 ± 0.1	29.1 ± 0.0	3.5 ± 0.3	3.4 ± 0.5	1.5 ± 0.4					19.0 ± 1.0	17.9 ± 1.0	82.1 ± 1.0
aromatic	Tyr	31.5 ± 1.4	3.6 ± 0.4	0.9 ± 0.3	1.3 ± 0.5	1.5 ± 0.4	4.8 ± 1.1	7.7 ± 1.0	3.8 ± 0.2	11.7 ± 0.7	33.3 ± 5.8	57.4 ± 8.4	56.3 ± 8.4	43.7 ± 8.4
	Phe	84.7 ± 0.5	8.7 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	1.0 ± 0.1	2.9 ± 0.4	6.4 ± 0.8	5.3 ± 0.8	95.8 ± 0.8

[a]Labeling pattern of proteinogenic amino acids, obtained when ([$^{13}C_3$]lactate and [$^{13}C_2$]ethanol were fed. Generally, the data represent mass isotopomer distributions in the M-57 fragments, which contain the entire carbon backbone. Only in the case of leucine and isoleucine, the M-85 fragments (C_2-C_5) were considered.

[b] de novo synthesis = sum of fractional labeling – 1.1 %

4) Estimation of the metabolic origin of the produced amino acids

The metabolic origin of the individual amino acids was calculated using the following equations:

$$\psi_{Lac} = \frac{SFL_{Lac} - 1.1}{SFL_{Lac} - 1.1 + SFL_{Eth} - 1.1} \cdot 100 \quad (1)$$

$$\psi_{Lac} = \frac{SFL_{Lac} - 1.1}{SFL_{Lac} - 1.1 + SFL_{Eth} - 1.1} \cdot 100 \quad (2)$$

ψ_{Lac} Degree of synthesis from lactate

ψ_{Eth} Degree of synthesis from ethanol

SFL_{Lac} Sum of fractional labeling of amino acids in cells grown on [$^{13}C_3$] lactate and [$^{12}C_2$] ethanol (Table S2)

SFL_{Eth} Sum of fractional labeling of amino acids in cells grown on [$^{12}C_3$] lactate and [$^{13}C_2$] ethanol (Table S3)

The mass isotopomer distributions of the amino acids and the sum of fractional labeling are listed in the Tables S1 and S2.

Table S3: Mass isotopomer distributions of the carbon backbones of proteinogenic amino acids in cell pellets of *A. pasteurianus* NCC 316 and *A. ghanensis* DSM 18895 grown in cocoa pulp simulation medium with [U-^{13}C] lactate and [U-^{12}C] ethanol. The sum of fractional labeling was derived from these mass isotopomer distributions.

Amino acid family	Amino acid	Mass isotopomer fraction ± standard deviation[a] (%)										Sum of fractional labeling (%)
		m+0	*m*+1	*m*+2	*m*+3	*m*+4	*m*+5	*m*+6	*m*+7	*m*+8	*m*+9	
						A. pasteurianus NCC 316						
Pyruvate	Ala	84.5 ± 0.7	3.4 ± 0.0	1.3 ± 0.1	10.8 ± 0.7							12.8 ± 0.7
	Leu	89.7 ± 0.7	5.3 ± 0.0	0.5 ± 0.0	0.7 ± 0.0	4.5 ± 0.7	2.3 ± 0.0					7.6 ± 0.6
	Val	38.9 ± 0.4	2.5 ± 0.1	3.1 ± 0.2	3.4 ± 0.2	4.3 ± 0.1	47.9 ± 0.7					55.0 ± 0.9
Serine	Ser	94.6 ± 1.1	3.4 ± 0.2	0.3 ± 0.1	1.7 ± 0.7							3.0 ±0.8
	Gly	95.0 ± 1.0	3.4 ± 0.3	1.6 ± 0.6								3.3 ± 0.8
Glutamate	Glu	81.1 ± 0.6	5.6 ± 0.2	10.5 ± 0.8	1.7 ± 0.2	0.5 ± 0.0	0.6 ± 0.0					7.4 ± 0.5
Aspartate	Asp	86.9 ± 1.8	4.4 ± 0.3	3.0 ± 0.8	1.9 ± 0.2	3.7 ± 0.5						7.6 ± 1.1
	Thr	88.6 ± 1.1	4.3 ± 0.2	2.5 ± 0.5	1.6 ± 0.1	3.1 ± 0.3						6.6 ± 0.7
	Lys	16.9 ± 0.5	4.7 ± 0.2	34.6 ± 0.4	35.3 ± 0.2	3.4 ± 0.4	2.2 ± 0.3	2.9 ± 0.3				37.0 ± 1.1
	Ile	62.3 ± 1.8	5.5 ± 0.0	28.7 ± 1.8	1.6 ± 0.0	0.9 ± 0.0	1.0 ± 0.0					15.2 ± 0.8
aromatic	Tyr	31.6 ± 1.1	3.8 ± 0.2	0.8 ± 0.4	1.3 ± 0.6	1.6 ± 0.6	3.8 ± 1.0	8.2 ± 1.0	4.0 ± 0.3	10.3 ± 1.1	34.6 ± 4.5	57.4 ± 7.6
	Phe	85.2 ± 0.6	8.3 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.5 ± 0.1	0.8 ± 0.2	0.4 ± 0.1	1.0 ± 0.1	2.9 ± 0.2	6.3 ± 0.6

		A. ghanensis DSM 18895										
Pyruvate	Ala	85.0 ± 0.7	3.6 ± 0.1	1.3 ± 0.1	10.1 ± 0.4							12.1 ± 0.5
	Leu	67.3 ± 1.2	4.8 ± 0.2	1.6 ± 0.0	2.2 ± 0.1	15.8 ± 1.6	8.3 ± 0.4					23.8 ± 1.8
	Val	26.7 ± 1.2	1.8 ± 0.1	3.1 ± 0.2	3.4 ± 0.2	5.3 ± 0.1	59.7 ± 1.5					67.6 ± 1.8
Serine	Ser	49.4 ± 1.9	4.6 ± 0.1	46.1 ± 1.8								48.6 ± 2.3
	Gly	56.4 ± 1.9	4.8 ± 0.2	38.8 ± 1.8								41.2 ± 1.8
Glutamate	Glu	71.4 ± 0.7	7.1 ± 0.8	17.0 ± 0.6	2.8 ± 0.5	1.1 ± 0.1	0.5 ± 0.1					11.3 ± 0.8
Aspartate	Asp	93.9 ± 2.3	5.8 ± 0.9	6.6 ± 0.6	1.7 ± 0.4	2.0 ± 0.5						8.1 ± 1.3
	Thr	85.1 ± 2.1	5.6 ± 0.7	5.9 ± 0.6	1.6 ± 0.3	1.9 ± 0.5						7.4 ± 1.2
	Lys	15.6 ± 0.4	4.6 ± 0.0	34.1 ± 0.8	35.4 ± 0.1	5.9 ± 0.5	2.6 ± 0.4	1.7 ± 0.3				47.7 ± 1.4
	Ile	55.8 ± 0.9	5.4 ± 0.0	33.1 ± 0.2	3.0 ± 0.4	1.8 ± 0.2	0.9 ± 0.2					18.5 ± 0.7
aromatic	Tyr	31.5 ± 0.6	3.6 ± 0.1	0.7 ± 0.1	1.2 ± 0.2	1.4 ± 0.2	4.9 ± 1.1	8.0 ± 0.9	3.6 ± 0.0	11.7 ± 1.2	33.5 ± 4.0	57.6 ± 6.6
	Phe	85.7 ± 0.5	8.4 ± 0.1	0.4 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	0.6 ± 0.0	0.4 ± 0.0	0.9 ± 0.0	2.6 ± 0.5	5.7 ± 0.6

[a]Generally, the data represent mass isotopomer distributions in the M-57 fragments, which contain the entire carbon backbone. Only in the case of leucine and isoleucine, the M-85 fragments (C_2-C_5) were considered.

Table S4: Mass isotopomer distributions of the carbon backbones of proteinogenic amino acids in cell pellets of *A. pasteurianus* NCC 316 and *A. ghanensis* DSM 18895 grown in cocoa pulp simulation medium with [U-^{12}C] lactate and [U-^{13}C] ethanol. The sum of fractional labeling was derived from these mass isotopomer distributions.

Amino acid family	Amino acid	Mass isotopomer fraction ± standard deviation[a] (%)										Sum of fractional labeling (%)
		m+0	*m*+1	*m*+2	*m*+3	*m*+4	*m*+5	*m*+6	*m*+7	*m*+8	*m*+9	
						A. pasteurianus NCC 316						
Pyruvate	Ala	95.3 ± 0.2	3.9 ± 0.1	0.7 ± 0.1	0.1 ± 0.0							1.8 ± 0.1
	Leu	89.9 ± 0.6	9.8 ± 0.5	0.2 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0					2.0 ± 0.1
	Val	94.5 ± 0.0	5.3 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0						1.1 ± 0.0
Serine	Ser	97.0 ± 0.0	3.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0							1.0 ± 0.0
	Gly	97.8 ± 0.0	2.2 ± 0.0	0.0 ± 0.0								1.1 ± 0.0
Glutamate	Glu	74.8 ± 0.7	5.4 ± 0.0	17.6 ± 0.3	1.3 ± 0.2	0.8 ± 0.2	0.1 ± 0.0					9.6 ± 0.4
Aspartate	Asp	90.7 ± 1.8	4.4 ± 0.1	4.3 ± 1.3	0.5 ± 0.3	0.1 ± 0.0						3.7 ± 0.9
	Thr	91.9 ± 1.2	4.1 ± 0.1	3.5 ± 0.9	0.4 ± 0.2	0.0 ± 0.0						3.2 ± 0.7
	Lys	88.9 ± 1.2	7.2 ± 3.1	0.5 ± 0.2	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.1					2.7 ± 0.5
	Ile	93.0 ± 0.3	6.2 ± 0.1	0.7 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0					1.6 ± 0.1
aromatic	Tyr	88.9 ± 1.2	9.0 ± 0.2	0.8 ± 0.5	0.6 ± 0.5	0.6 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.7 ± 0.4
	Phe	90.6 ± 0.1	8.9 ± 0.1	0.4 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.1 ± 0.1

					A. ghanensis DSM 18895							
Pyruvate	Ala	94.8 ± 0.7	4.0 ± 0.2	0.9 ± 0.3	0.3 ± 0.2							2.2 ± 0.5
	Leu	80.2 ± 1.1	18.9 ± 1.2	0.8 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0					1.4 ± 0.3
	Val	93.7 ± 0.3	5.6 ± 0.1	0.5 ± 0.2	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0					1.5 ± 0.1
Serine	Ser	96.5 ± 0.0	3.4 ± 0.2	0.0 ± 0.0	0.1 ± 0.0							1.2 ± 0.1
	Gly	97.6 ± 0.1	2.3 ± 0.0	0.0 ± 0.0								1.2 ± 0.1
Glutamate	Glu	60.5 ± 6.3	6.3 ± 0.1	25.1 ± 2.0	4.0 ± 1.5	3.4 ± 1.8	0.8 ± 0.9					17.2 ± 4.0
Aspartate	Asp	81.2 ± 5.3	5.5 ± 0.4	9.8 ± 2.3	2.8 ± 1.8	0.7 ± 0.8						9.0 ± 3.4
	Thr	83.2 ± 4.5	5.2 ± 0.3	8.6 ± 2.0	2.4 ± 1.6	0.6 ± 0.7						8.0 ± 0.1
	Lys	77.5 ± 3.8	8.8 ± 1.2	6.9 ± 2.5	2.0 ± 1.1	0.5 ± 0.4	0.1 ± 0.0	4.2 ± 7.1				5.5 ± 1.6
	Ile	90.2 ± 0.5	7.5 ± 0.1	2.0 ± 0.3	0.3 ± 0.1	0.0 ± 0.0	0.0 ± 0.0					4.2 ± 0.2
aromatic	Tyr	68.4 ± 2.4	9.7 ± 0.4	1.2 ± 0.3	1.0 ± 0.3	1.0 ± 0.3	0.4 ± 0.6	0.3 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.6 ± 1.1
	Phe	90.1 ± 0.3	9.0 ± 0.1	0.5 ± 0.1	0.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.3 ± 0.1

[a]Generally, the data represent mass isotopomer distributions in the M-57 fragments, which contain the entire carbon backbone. Only in the case of leucine and isoleucine, the M-85 fragments (C_2-C_5) were considered.

Table S5: Metabolic origin of amino acids during the first growth phase of *A. pasteurianus* NCC 316 and *A. ghanensis* DSM 18895 on cocoa pulp simulation medium. The amino acids are grouped according to their biosynthetic families {Stephanopoulos 1998 #347}. The relative fraction of de-novo synthesis was calculated from the sum of fractional labeling (SFL) in the respective amino acid. Therefore, experimental mass isotopomer data were corrected for the natural occurrence of isotopes in the derivatization residue and in non-carbon atoms {Yang 2009 #107}.

			***A. pasteurianus* NCC 316**		***A. ghanensis* DSM 18895**	
Amino acid family	**Amino acid**	**Precursors**	**Degree of synthesis from lactate ± standard deviation (%)**	**Degree of synthesis from ethanol ± standard deviation (%)**	**Degree of synthesis from lactate ± standard deviation (%)**	**Degree of synthesis from ethanol ± standard deviation (%)**
Pyruvate	Ala	Pyr	94 ± 1	6 ± 1	91 ± 3	9 ± 3
	Leu	Pyr, AcCoA	87 ± 2	13 ± 2	89 ± 1	11 ± 1
	Val	Pyr	100	0[a]	100	0[a]
Serine	Ser	3PG	100	0[a]	100	0[a]
	Gly	3PG	100	0[a]	100	0[a]
Glutamate	Glu	aKG	42 ± 2	58 ± 2	39 ± 6	61 ± 6
Aspartate	Asp	OAA	72 ± 8	28 ± 8	47 ± 12	52 ± 12
	Thr	OAA	72 ± 7	28 ± 7	48 ± 12	52 ± 12
	Lys	OAA, Pyr	96 ± 1	4 ± 1	89 ± 4	11 ± 4
	Ile	OAA, Pyr	100 ± 0	0[a]	92 ± 1	8 ± 1
aromatic	Tyr	PEP, E4P	99 ± 2	1 ± 2	97 ± 1	3 ± 2
	Phe	PEP, E4P	100	0[a]	100	0[a]

5) Mass isotopomer distributions of selected amino acid fragments

Table S6: Mass isotopomer distributions (%) and the corresponding standard deviations of the carbon backbones of proteinogenic amino acids in cell pellets of *A. pasteurianus* NCC 316 and *A. ghanensis* DSM 18895 grown in cocoa pulp simulation medium with [3-^{13}C] lactate and [U-^{13}C] ethanol.

Amino acid	Fragment	Labeled substrate					
		[U-^{13}C] lactate			[U-^{13}C] ethanol		
		m+0	*m*+1	*m*+2	*m*+0	*m*+1	*m*+2
Ala							
Val	M-57						
Val	M-residue (*m/z* = 302)	46.9 ± 0.6	3.8 ± 0.0	49.3 ± 0.5	97.5 ± 0.0	2.5 ± 0.0	0.0 ± 0.0
Glu							
Glu							
Asp							
Asp	M-residue (*m/z* = 302)	90.5 ± 1.3	2.8 ± 0.2	6.8 ± 1.0	94.8 ± 1.0	2.6 ± 0.2	2.5 ± 0.8
Tyr	M-residue (*m/z* = 302)	38.4 ± 0.3	4.2 ± 0.0	57.4 ± 0.3	97.8 ± 0.0	2.2 ± 0.0	0.0 ± 0.0
Ala							
Val	M-57						
Val	M-residue (*m/z* = 302)	33.6 ± 1.2	4.2 ± 0.0	62.2 ± 1.2	97.3 ± 0.0	2.7 ± 0.0	0.0 ± 0.0
Glu							
Glu							

Asp							
Asp	M-residue (*m/z* = 302)	89.5 ± 1.3	3.8 ± 0.5	6.7 ± 0.8	90.9 ± 1.5	3.8 ± 0.4	5.4 ± 1.1
Tyr	M-residue (*m/z* = 302)	36.9 ± 0.9	4.2 ± 0.1	58.9 ± 0.8	97.7 ± 0.0	2.2 ± 0.0	0.1 ± 0.0

6) Contribution of lactate and ethanol to the major fermentation products.

The contribution of lactate and ethanol to the formation of the final product acetate was estimated by using the following equations:

$$v_{Lac\rightarrow Ac} = \frac{SFL_{Ac(Lac)} - 1.1}{SFL_{Ac(Eth+Lac)} - 1.1} \cdot 100\% \quad (3)$$

$$v_{Eth\rightarrow Ac} = \frac{SFL_{Ac(Eth)} - 1.1}{SFL_{Ac(Eth+Lac)} - 1.1} \cdot 100\% \quad (4)$$

$$v_{Lac\rightarrow Ac} + v_{Eth\rightarrow Ac} = 100\% \quad (5)$$

$v_{Eth\rightarrow Ac}$ Flux of ethanol into acetate.

$v_{Lac\rightarrow Ac}$ Flux of lactate into acetate.

$SFL_{Ac(Lac)}$ Sum of fractional labeling of acetate produced by cells grown on [$^{13}C_3$] lactate and [$^{12}C_2$] ethanol (Tables S5, S6).

$SFL_{Ac(Eth)}$ Sum of fractional labeling of acetate produced by cells grown on [$^{12}C_3$] lactate and [$^{13}C_2$] ethanol (Tables S5, S6).

$SFL_{Ac(Eth)}$ Sum of fractional labeling of acetate produced by cells grown on [$^{13}C_3$] lactate and [$^{13}C_2$] ethanol (Tables S5, S6).

The contribution of lactate and ethanol to the formation of the final product acetoin was calculated analogously.

Table S7: Experimental mass isotopomer distributions of the carbon backbones of acetate and acetoin (% of total pool ± standard deviation) and sum of fractional labeling (SFL) in cultures of *A. pasteurianus* NCC 316 grown in cocoa pulp simulation medium

Analyte	**Mass isotopomer fraction / SFL**	**Labeled substrate**		
		[$^{13}C_3$] lactate, [$^{13}C_2$] ethanol	**[$^{13}C_3$] lactate**	**[$^{13}C_2$] ethanol**
Acetate	*m*+0	95.5 ± 0.1	95.4 ± 0.1	9.4 ± 0.5
	m+1	2.8 ± 0.0	3.7 ± 0.1	11.1 ± 0.1
	m+2	1.7 ± 0.1	0.9 ± 0.0	79.6 ± 0.7
	(SFL)	85.1 ± 0.8	2.8 ± 0.1	84.0 ± 0.9
Acetoin (M-43)	*m*+0	0.6 ± 0.2	0.3 ± 0.2	94.7 ± 0.5
	m+1	0.9 ± 0.1	0.9 ± 0.1	4.5 ± 0.1
	m+2	7.8 ± 0.6	8.6 ± 0.5	0.7 ± 0.4
	m+3	16.1 ± 0.1	15.7 ± 0.1	0.0 ± 0.0
	m+4	74.6 ± 0.4	74.4 ± 0.5	0.0 ± 0.0
	(SFL)	89.6 ± 1.0	89.6 ± 0.9	1.4 8 ± 0.1

Table S8: Experimental mass isotopomer distributions of the carbon backbones of acetate and acetoin (% of total pool ± standard deviation) and sum of fractional labeling (SFL) in cultures of *A. ghanensis* DSM 18895 grown in cocoa pulp simulation medium

Analyte	**Mass isotopomer fraction / SFL**	**Labeled substrate**		
		[$^{13}C_3$] lactate, [$^{13}C_2$] ethanol	**[$^{13}C_3$] lactate**	**[$^{13}C_2$] ethanol**
Acetate	*m*+0	93.4 ± 0.5	93.7 ± 0.1	9.2 ± 0.4
	m+1	3.1 ± 0.0	5.3 ± 0.1	11.0 ± 0.2
	m+2	3.5 ± 0.4	1.0 ± 0.0	79.8 ± 0.6
	(SFL)	85.2 ± 0.7	5.1 ± 0.5	82.4 ± 1.1
Acetoin (M-43)	*m*+0	0.6 ± 0.2	0.3 ± 0.0	94.7 ± 0.5
	m+1	0.9 ± 0.1	0.9 ± 0.1	4.5 ± 0.1
	m+2	7.8 ± 0.6	8.6 ± 0.5	0.7 ± 0.6
	m+3	16.1 ± 0.1	15.7 ± 0.1	0.0 ± 0.1
	m+4	74.6 ± 0.4	74.4 0.5	0.0 ± 0.4
	(SFL)	90.8 ± 0.8	90.7 ± 0.9	1.5 ± 0.5

7) Estimation of the labeling patterns of precursors.

The specific carbon mass isotopomer distributions of precursors was estimated from the ^{13}C labeling patterns of the respective amino acid. The real mass isotopomer distribution of a proteinogenic amino acid is a linear combination of the labeling ^{13}C patterns of the produced amino acid and the assimilated amino acid:

$$I_{meas} = I_{AA,prod} \cdot \psi + I_{nat} \cdot (1 - \psi)$$

ψ	Degree of synthesis of amino acid
I_{meas}	Measured ^{13}C mass isotopomer distribution
$I_{AA,prod}$	^{13}C mass isotopomer distribution of amino acid fragment, produced from precursor
I_{nat}	^{13}C mass isotopomer distribution of assimilated amino acid (natural labeling)

The ^{13}C mass isotopomer distribution of the amino acid is mapped to the respective precursor:

$$I_{AA,prod} \rightarrow I_{prec}$$

I_{prec}	^{13}C mass isotopomer distribution of precursor

Table S9: Estimated ^{13}C mass isotopomer distributions (%) and corresponding standard deviations of precursors derived from proteinogenic amino acids of *A. pasteurianus* NCC 316 grown in cocoa pulp simulation medium

Precursor (carbon atom)	Amino acid (fragment)	Mass isotopomer fraction	Labeled substrate	
			$[^{13}C_3]$ lactate	$[^{13}C_2]$ ethanol
OAA (C_1-C_2)	Aspartate (*m/z* = 302)	*m*+0	21.6	69.0
		m+1	0.9	7.1
		m+2	69.5	23.9
PEP (C_1-C_2)	Tyrosine (*m/z* = 302)	*m*+0	0.0	97.8
		m+1	5.3	2.2
		m+2	94.7	0.0
Pyr (C_1-C_2)	Valine (*m/z* = 302)	*m*+0	2.1	97.0
		m+1	5.3	2.9
		m+2	92.5	0.0

Table S10: Estimated ^{13}C mass isotopomer distributions (%) and corresponding standard deviations of precursors derived from proteinogenic amino acids of *A. ghanensis* DSM 18895 grown in cocoa pulp simulation medium

Precursor (carbon atom)	Amino acid (fragment)	Mass isotopomer fraction	Labeled substrate	
			$[^{13}C_3]$ lactate	$[^{13}C_2]$ ethanol
OAA (C_1-C_2)	Aspartate (*m/z* = 302)	*m*+0	44.9	61.6
		m+1	12.7	37.4
		m+2	42.4	1.0
PEP (C_1-C_2)	Tyrosine (*m/z* = 302)	*m*+0	0	97.4
		m+1	0.5	2.4
		m+2	94.7	0.1
Pyr (C_1-C_2)	Valine (*m/z* = 302)	*m*+0	0	85.1
		m+1	5.3	4.2
		m+2	94.8	10.7

8) Metabolic fluxes of *A. pasteurianus* NCC 316

The stoichiometric model that was used for flux estimation is given in Table S10. Experimental mass isotopomer data from four parallel labeling experiments were considered in the flux estimation approach.

Table S11: Input model for OpenFLUX. The known extracellular fluxes from Table S9 and precursor drain to biomass were normalized to the cumulative uptake flux of lactate and ethanol.

RxnID	rxnEq	rxnCTrans	rates	rxnType	basis	deviation
R1	Eth_Ex = Eth	ab = ab		F	71.96	0.844
R2	Lac_Ex = PyrPEP	abc = abc		F	28.04	0.844
R3	Glu_Ex = Glu	abcde = abcde		F		
R4	Asp_Ex = Asp	abcd = abcd		F		
R5	Ala_Ex = Ala	abc = abc		F		
R6	Val_Ex = Val	abcde = abcde		F		
R7	Tyr_Ex = Tyr1	abcde = abcde		F		
R8	GAP + DHAP = G6P	abc + def = abcdef		F		
R9	GAP = DHAP	abc = abc		F		
R10	3PG = GAP	abc = abc		F		
R11	PyrPEP = 3PG	abc = abc		F		
R12	GAP + GAP + GAP = P5PE4P	abc + def + ghi = abcdefghi		F		
R13	AcCoA + OAA = Icit	ab + cdef = fedbac		F		
R14	Icit = AKG + CO2	abcdef = abcde + f		F		
R15	AKG = 0.5 Suc + 0.5 Suc + CO2	abcde = 0.5 abcd + 0.5 dcba + e		F		
R16	Suc = OAA	abcd = abcd		F		
R17	PyrPEP + CO2 = OAA	abc + d = abcd		F		
R18	PyrPEP = Ac + CO2	abc = bc + a		F		
R19	PyrPEP = AcCoA + CO2	abc = bc + a		F		
R20	PyrPEP = Acn1 + CO2	abc = bc + a		F		
R21	Acn1 + PyrPEP = Acn + CO2	ab + cde = abde + c		F		
R22	CO2 = CO2_Ex	a = a		F		
R23	Eth = Ac	ab = ab		F		
R24	Eth = AcCoA	ab = ab		F		
R25	Ac = Ac_Ex	ab = ab		F	63.82	2.341

R26	Acn = Acn_Ex	abcd = abcd	F	9.71	0.900
R27	PyrPEP = PyrPEP_B		B	0.21	0.004
R28	3PG = 3PG_B		B	0.05	0.001
R29	P5PE4P = P5PE4P_B		B	0.43	0.008
R30	GAP = GAP_B		B	0.09	0.002
R31	OAA = OAA_B		B	0.36	0.007
R32	AKG = AKG_B		B	0.08	0.001
R33	AcCoA = AcCoA_B		B	1.65	0.030
R34	G6P = G6P_B		B	0.17	0.003
R35	PyrPEP = Ala	abc = abc	F		
R36	Ala = PyrPEP	abc = abc	FR		
R37	PyrPEP + PyrPEP = Tyr1 + CO2	abc + def = abcef + d	F		
R38	PyrPEP + PyrPEP = Val + CO2	abc + def = abefc + d	F		
R39	AKG = Glu	abcde = abcde	FR		
R40	OAA = Asp	abcd = abcd	FR		
R41	Glu = AKG	abcde = abcde	R		
R42	Asp = OAA	abcd = abcd	R		
R43	Val = ValB		B	0.27	0.005
R44	Asp = AspB		B	0.31	0.006
R45	Glu = GluB		B	0.34	0.006
R46	Tyr1 = TyrB		B	0.09	0.002
R47	Ala = AlaB		B	0.39	0.007
R48	Val = ValX	abcde = abcde	S		
R49	Asp = AspX	abcd = abcd	S		
R50	Glu = GluX	abcde = abcde	S		
R51	Ala = AlaX	abc = abc	S		
R52	Ac = AcX	ab = ab	S		
R53	Tyr1 = Tyr302X + TyrRX	abcde = ab + cde	S		
R54	Asp = Asp302X + AspRX	abcd = ab + cd	S		
R55	Val = Val302X + ValRX	abcde = ab + cde	S		
R56	Glu = Glu330X + GluRX	abcde =bcde + a	S		

excludedMetabolites
Lac_Ex
Eth_Ex
Glu_Ex
Asp_Ex
Val_Ex

Ala_Ex
3PG_B
PyrPEP_B
GAP_B
P5PE4P_B
OAA_B
AKG_B
AcCoA_B
G6P_B
CO2_Ex
Ac_Ex
Acn_Ex
ValB
AspB
GluB
AlaB
Tyr_Ex
TyrB
simulatedMDVs
ValX#11111
AlaX#111
AspX#1111
GluX#11111
Tyr302X#11
Glu330X#1111
Asp302X#11
Val302X#11
(Ac#11)
inputSubstrates
Lac_Ex
Eth_Ex
Val_Ex
Ala_Ex
Asp_Ex
Glu_Ex
Tyr_Ex

RxnID = Reaction Identification, rxnCTrans = Carbon Transition, rates = Known Reaction Rates, rxnType = Reaction Type (F=irreversible, FR=forward reaction of a reversible reaction, R=backward reaction, S=excluded from the metabolite balance, B=excluded from the isotopomer balance), basis = Invariant Flux Basis, deviation = experimental error, excludedMetabolites = metabolites excluded from the balance model, simulatedMDVs =

simulated mass isotopomer distribution vectors, inputSubstrates = compounds that are used as substrates.

Table S12: Experimental mass isotopomer distributions of fragments used flux estimation.

Labeled substrate	Molecule	Fragment	Mass isotopomer	Mass isotopomer fraction	Error (standard deviation)
[U-^{13}C] lactate, [U-^{13}C] ethanol	Val	M-57	*m*+0	0.344	0.001
			m+1	0.089	0.000
			m+2	0.061	0.002
			m+3	0.040	0.002
			m+4	0.046	0.000
			m+5	0.421	0.003
	Ala	M-57	*m*+0	0.662	0.005
			m+1	0.160	0.001
	Asp	M-57	*m*+0	0.556	0.020
			m+1	0.203	0.006
			m+2	0.138	0.010
	Glu	M-57	*m*+0	0.433	0.004
			m+1	0.168	0.001
			m+2	0.237	0.006
	Tyr	M-residue (*m/z* = 302)	*m*+0	0.346	0.001
			m+1	0.119	0.000
			m+2	0.536	0.001
	Glu	M-159	*m*+0	0.486	0.004
			m+1	0.149	0.001
			m+2	0.239	0.007
	Asp	M-residue (*m/z* = 302)	*m*+0	0.670	0.015
			m+1	0.184	0.001
	Val	M-residue (*m/z* = 302)	*m*+0	0.406	0.002
			m+1	0.130	0.000
			m+2	0.464	0.002
[U-^{13}C] lactate	Val	M-57	*m*+0	0.339	0.003
			m+1	0.088	0.001
			m+2	0.061	0.002
			m+3	0.040	0.002
			m+4	0.046	0.001
			m+5	0.427	0.007
	Ala	M-57	*m*+0	0.671	0.005
			m+1	0.158	0.001
	Asp	M-57	*m*+0	0.586	0.011
			m+1	0.214	0.001
			m+2	0.119	0.004
	Glu	M-57	*m*+0	0.539	0.004
			m+1	0.206	0.002
			m+2	0.164	0.005
	Tyr	M-residue (*m/z* = 302)	*m*+0	0.338	0.004
			m+1	0.118	0.000
			m+2	0.544	0.004
	Glu	M-159	*m*+0	0.607	0.005

			m+1	0.184	0.003
			m+2	0.152	0.005
	Asp	M-residue (*m/z* = 302)	*m*+0	0.690	0.008
			m+1	0.186	0.000
	Val	M-residue (*m/z* = 302)	*m*+0	0.402	0.004
			m+1	0.129	0.001
			m+2	0.469	0.005
	Acetate	*m/z* = 43	*m*+0	0.952	0.001
			m+1	0.029	0.000
	Val	M-57	*m*+0	0.313	0.001
			m+1	0.144	0.002
			m+2	0.408	0.002
			m+3	0.094	0.000
			m+4	0.036	0.000
			m+5	0.006	0.001
	Ala	M-57	*m*+0	0.660	0.004
			m+1	0.241	0.003
	Asp	M-57	*m*+0	0.582	0.007
			m+1	0.260	0.004
			m+2	0.117	0.002
	Glu	M-57	*m*+0	0.541	0.005
			m+1	0.279	0.003
			m+2	0.129	0.001
[3-^{13}C] lactate	Tyr	M-residue (*m/z* = 302)	*m*+0	0.733	0.001
			m+1	0.192	0.000
	Glu	M-159	*m*+0	0.609	0.006
			m+1	0.263	0.004
			m+2	0.099	0.001
	Asp	M-residue (*m/z* = 302)	*m*+0	0.718	0.003
			m+1	0.205	0.003
	Val	M-residue (*m/z* = 302)	*m*+0	0.694	0.000
			m+1	0.191	0.000
			m+2	0.115	0.001
	Acetate	*m/z* = 43	*m*+0	0.951	0.001
			m+1	0.038	0.001
	Val	M-57	*m*+0	0.729	0.000
			m+1	0.184	0.000
			m+2	0.072	0.000
			m+3	0.012	0.000
			m+4	0.002	0.000
			m+5	0.000	0.000
	Ala	M-57	*m*+0	0.738	0.001
			m+1	0.174	0.001
	Asp	M-57	*m*+0	0.603	0.011
			m+1	0.218	0.003
[U-^{13}C] ethanol			*m*+2	0.130	0.007
	Glu	M-57	*m*+0	0.497	0.005
			m+1	0.192	0.001
			m+2	0.205	0.002
	Tyr	M-residue (*m/z* = 302)	*m*+0	0.733	0.000
			m+1	0.192	0.000
	Glu	M-159	*m*+2	0.558	0.005
			m+0	0.172	0.000
			m+1	0.194	0.002
	Asp	M-residue	*m*+2	0.716	0.006

	(*m/z* = 302)			
		m+0	0.191	0.000
Val	M-residue (*m/z* = 302)	*m*+1	0.731	0.000
		m+0	0.194	0.000
		m+1	0.075	0.000

The simulated and experimental mass isotopomer distributions of are presented in Figure S3:

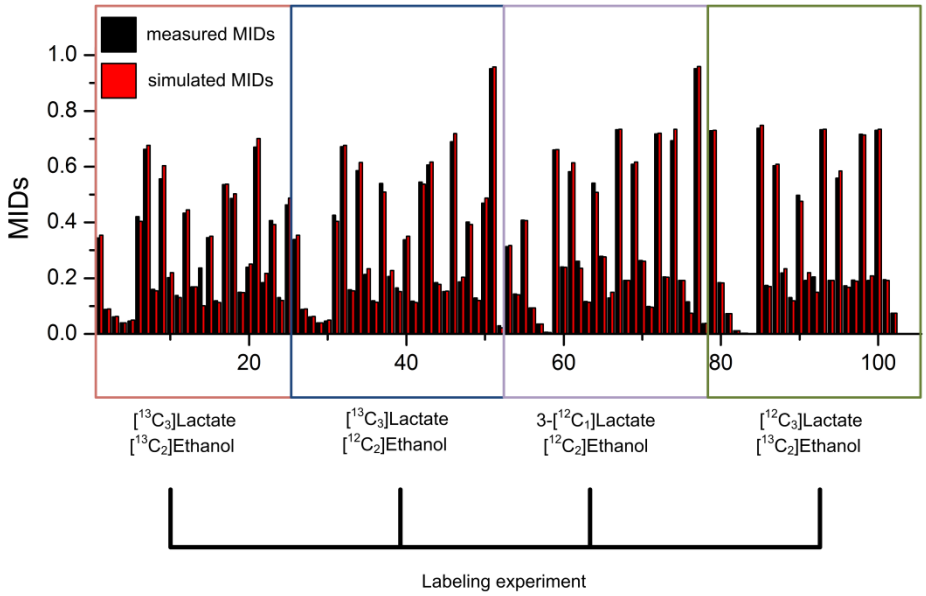

Figure S3: Simulated and experimental mass isotopomer distributions (MIDs).