Supplementary Information

Simultaneous, high-precision measurements of δ^2H and $\delta^{13}C$ in nanomole quantities of acetate using ESI-Quadrupole-Orbitrap MS

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Supplementary Information

$\delta^{13}C$ and $\delta^{2}H$ values of acetate measured by EA-IRMS:

Carbon isotope compositions of three acetate working standards were measured on a Thermo-Fisher Flash combustion Elemental Analyzer (EA) coupled to a Thermo Scientific Delta-V isotope-ratio mass spectrometer (IRMS) at Caltech. Carbon isotope ratios of combusted CO_2 were calibrated to the VPDB scale using a single CO_2 reference gas (-12.04‰) measured before and after sample peaks. International reference materials valine (USGS74, 9.3‰), glycine (USGS64, -40.81‰), sucrose (NIST8542, -10.47‰), and caffeine (IAEA600, -27.77‰) were used to correct for further instrumental fractionations associated with combustion.

Hydrogen isotope compositions were measured on a Thermo thermal-conversion (TC) EA coupled to a Thermo Finnigan Delta Plus XP IRMS at Indiana University, Bloomington. Samples were weighed into silver capsules and dried in a glass vacuum desiccator for 6 hours. The desiccator was flushed with dry nitrogen gas before being opened. Silver capsules were quickly crushed and sealed in a Costech Zero-Blank autosampler, which was flushed with helium. The TC/EA was set to a furnace temperature of 1100°C, a GC oven temperature of 40°C, and a flow rate of 70 mL/min. Samples were calibrated to the VSMOW scale by analyzing USGS77 (polyethylene powder) and C_{36} n-alkane #2 provided by Arndt Schimmelman (Indiana University).

Description of working standards:

Table S1: Acetate working standards measured by EA-IRMS

Standard	$\delta^{13}C$ (VPDB)	$\delta^{2}H$ (VSMOW)	Source
AcSt (Sodium acetate)	-19.2 ± 0.1‰	-127 ± 1.9‰	Malinckrodt
AcA (Sodium acetate)	-26.5 ± 0.1‰	-153 ± 2.0‰	Fisher Scientific
AcB (Sodium acetate)	-34.6 ± 0.1‰	-95 ± 1.1‰	Allied Chemicals

Effect of concentration on isotopic accuracy

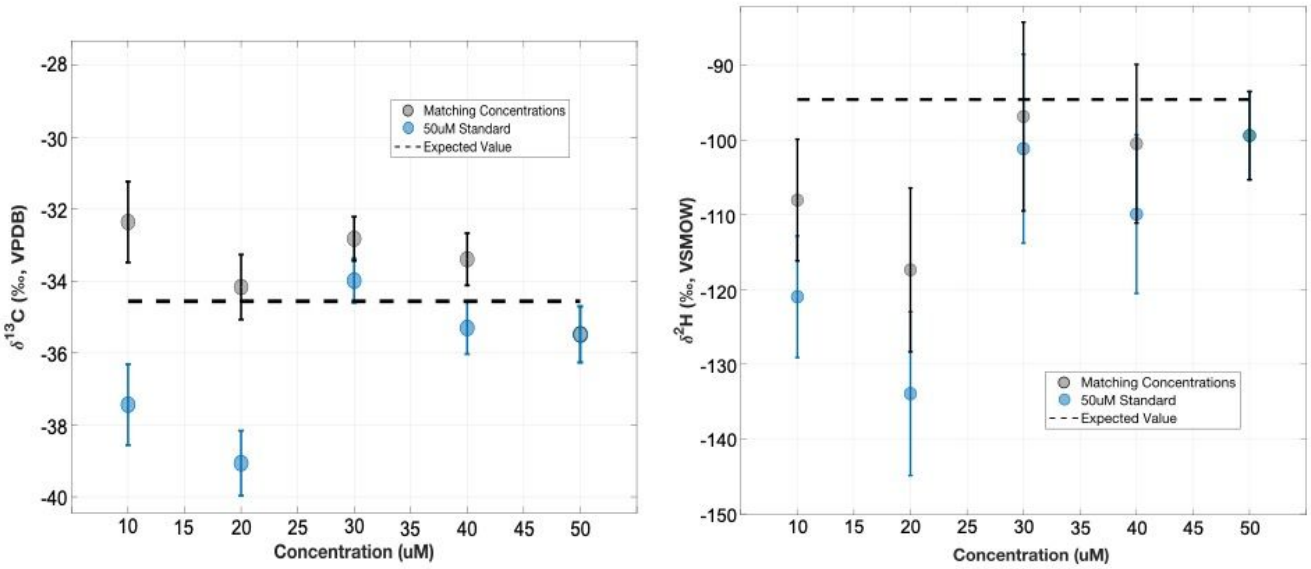

Figure S1: An acetate standard (AcB) was measured against working standard AcSt across a range of concentrations. AcSt was either kept at a constant 50 µM concentration (blue) or was diluted to match the concentration of AcB (black). Accuracy decreased when the solutions were < 30 µM, potentially due to the larger influence of background acetate at low concentrations.

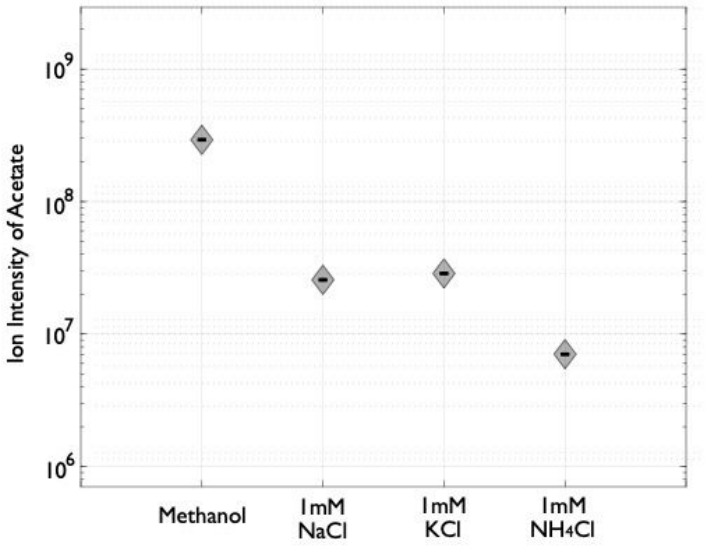

Figure S2: Ionization yield of 50µM acetate in pure methanol versus in 1 mM solutions of NaCl, KCl and NH_4Cl. Addition of inorganic salts reduces the ionization of acetate by more than 10-fold.

Bacterial Cultures:

Table S2: Bacterial strains grown in this study

Species	**Strain**	**Source**	**Source Location**
Sporomusa ovata	DSM 2662	Volker Müller	Goethe University Frankfurt, Germany
Acetobacterium woodii	DSM 1030	Volker Müller	Goethe University Frankfurt, Germany
Clostridium pasteurianum	LMG 3285	DSMZ	Brunswick, Germany

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Table S3: Growth medium composition for cultures analyzed in this study.

Component	A. woodii & S. ovata	C. pasteurianum	units
KH2PO4	0.2	2.15	g/L
NH_4Cl	0.25	--	g/L
$Na_2HPO_4 \cdot 7H_2O$	--	9.16	g/L
NH_4SO_4	--	3	g/L
NaCl	1.16	--	mg/L
$MgSO_4 \cdot 7H_2O$	1.45	--	mg/L
$CaCl_2 \cdot 2H_2O$	0.11	0.013	g/L
KCl	0.5	--	g/L
$KHCO_3$	6	--	g/L
$Na_2S \cdot 9H_2O$	0.3	--	g/L
Na_2SeO_3	17	--	µg/L
$Na_2WO_4 \cdot 2H_2O$	33	--	µg/L
$FeSO_4 \cdot 7H_2O$	2	--	mg/L
$FeCl_3 \cdot 6H_2O$	2027	2027	µg/L
H_3BO_3	30	30	µg/L
$MnCl_2 \cdot 4H_2O$	100	100	µg/L
$CoCl_2 \cdot 6H_2O$	190	190	µg/L
$NiCl_2 \cdot 6H_2O$	24	24	µg/L
$CuCl_2 \cdot 2H_2O$	2	2	µg/L
$ZnCl_2$	68	68	µg/L
Riboflavin	100	100	µg/L
Biotin	30	30	µg/L
Thiamine HCl	100	100	µg/L
L-Ascorbic acid	100	100	µg/L
d-Ca-pantothenate	100	100	µg/L

Folic acid	100	100	µg/L
Nicotinic acid	100	100	µg/L
4-aminobenzoic acid	100	100	µg/L
pyridoxine HCl	100	100	µg/L
Lipoic acid	100	100	µg/L
Thiamine pyrophosphate	100	100	µg/L
Cyanocobalamin	10	10	µg/L

Culture Conditions:
Acetobacterium woodii and *Sporomusa ovata* were both grown in 25 mL balch tubes with 10 mL of media under 20 psi 80:20 H_2:CO_2 at pH 7 and 30ºC. Cultures were shaken at 180 rpm and grew to completion over one day. Media aliquots were taken in stationary phase, filtered through a 0.2 µm filter, and frozen.

Clostridium pasteurianum was grown in 1L sealed culture bottles with 100 mL of media under an N_2 atmosphere at 37ºC. Cultures were not shaken. Media aliquots were taken in end-log phase, filtered through a 0.2 µm filter, and frozen.