SUPPORTING INFORMATION FOR

Cross-platform comparison of *Caenorhabditis elegans* tissue extraction strategies for comprehensive metabolome coverage

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Protocol S-1. Exact procedures of extraction methods

(1) Mortar and pestle: We pre-chilled the acid-washed mortar and pestle with liquid nitrogen, and then manually ground the frozen worm pellet to a fine powder (~10 min), adding more liquid nitrogen as necessary to keep the sample cold. The extraction solvents were then added directly to the frozen powder in the mortar.

(2) Homogenizer: The sample was homogenized for 3 min with a T25 ultrathurax (Janke & Kunkel, Staufen, Germany) at 24,000 rpm in a 10 ml glass vial. In order to keep the homogenizer's grinding blades completely immersed inside the solvent, we increased the solvent volumes for the MeOH extraction to 3.6 ml 90 % and 2 ml 80 % MeOH for the first and second step, respectively. For the MC extraction, the first step used 3.6 ml of 2:1:0.4 methanol:chloroform:water to maintain a 2:1:1 ratio, and 2 ml of 1:1 chloroform:water in the second step.

(3) Bead-beater: The frozen pellet was split into two 2 ml tubes (StarLabs, Milton Keynes, UK), each prefilled with ~ 100 µl 0.1 mm SiO_2 beads (VWR) and pre-cooled on dry ice. We added 1ml of the extraction solvent to the frozen pellet, and started the bead-beater before the pellet had thawed. We used a FastPrep (MP Biomedicals, Illkirch, France) bead-beater, operated at 6.5 m/s for 2 times 30 seconds with a one minute ice-cooling step in between.

(4) Ball mill with metal spheroids: As for (3), we split the pellet into two 2 ml microcentrifuge tubes, each filled with one stainless steel "cone ball" (an oblate spheroid, designed to be more efficient at grinding than a spherical ball; Retsch, Leeds, UK) and the corresponding solvent. We then extracted the samples in a 'TissueLyser' (Qiagen, Crawley, UK) reciprocating ball mill (25 Hz, 8 min).

(5) Ball mill with glass beads: Exactly as for (4), but instead of a metal spheroid, we used 0.1 ml of 0.1 mm SiO_2 beads.

(6) Ball mill with stainless steel ball bearing: exactly as for (4), except the samples were not split, and were extracted in 5 ml stainless steel vessels that had been previously cooled to liquid nitrogen temperatures, using a single spherical 7 mm stainless steel ball bearing

Protocol S-2. Detailed GC-MS experimental procedures.

For GC-MS, we derivatized the samples using the two-step methoximation/silylation protocol of Fiehn *et al.*[32]. Briefly, 20 µl of 1 mM U-^{13}C-Glucose and 2,3,3-d_3-Leucine were added as quantitation standards and 10 µl of 1.5 mg/ml 27-d-myristic acid for retention time locking. In the first derivatization step, the samples were incubated with 10 µl of 40 mg/ml methoxyamine hydrochloride in anhydrous pyridine for 90 minutes at 30° C. For the second step, samples were silylated with 90 µl of MSTFA for 30 minutes at 37° C. Finally, 10 µl of 1mM 2-fluorobiphenyl in anhydrous pyridine was added as an injection standard. For GC-MS analysis, samples were injected into deactivated splitless liners of a Agilent 7890 gas chromatograph, coupled to a 5975 MSD (Agilent Technologies UK Ltd.) quadrupole mass detector, according to the Fiehn method[32]. Quality control (QC) samples, obtained from an additional 80 % MeOH mortar pestle extraction were spaced throughout the run and repeatedly injected at the end to assess both runtime and machine variation, respectively. Metabolites were assigned using the Fiehn Library[32], after deconvolution with AMDIS. As AMDIS does not integrate the baseline where a metabolite was not detected in a sample, baseline re-integration for each metabolite was performed using interactive in-house developed Matlab code.

Protocol S-3. Detailed LC-MS experimental procedures.

The extracts were redissolved in 120 µl water, centrifuged, and 100 ul transferred to autosampler vials for analysis. The remaining volumes were pooled and used as quality control (QC) samples. All samples (10µl) were injected onto a 2.1x100 mm Acquity BEH C18 column (1.7 µm) (Waters Corporation, Milford, USA) and eluted using a 25 min gradient of 100% A to 100% B (A= water, 0.1% formic acid, B= acetonitrile, 0.1% formic acid) at a flow rate of 500 µl/min and column temperature of 40° C. An Acquity UPLC system (Waters Ltd. Elstree, UK) was coupled online to an LCT Premier mass spectrometer (Waters MS Technologies, Ltd., Manchester, UK), acquiring in negative ionization mode (ESI-). Capillary voltage was 2.4 Kv, sample cone was 35 V, desolvation temperature 350° C, source temperature 120° C, and desolvation gas flow 900 L/hr. The LCT Premier was operated in V-optics mode, with a data acquisition rate of 0.1 s and a 0.01 s inter-scan delay. As lockmass, a solution of 200 pg/µl (50:50 ACN:H_20) leucine enkephalin (*m/z* 556.2771) was infused into the instrument at 3µl/min. Data was collected in centroid mode with a scan range of 50-1000 *m/z* and 3 averaged lockmass scans every 15 s. The column was conditioned using 5 QC samples, which were also injected every 10 samples throughout the run to monitor runtime variation. All extraction samples were injected in random blocks. Finally, 5 QC samples were consecutively injected in the middle of the run to assess

pure machine variation. For processing, the raw data was deconvoluted with the free xcms software package[33], which aligns features across sample groups with respect to retention time and *m/z*. All method/solvent combinations, the conditioning samples, the runtime QCs and the repeated injections were each defined as xcms sample groups. xcms was used with the recommended UPLC parameters (method: "centWave", peakwidth: 3-15 s, final rt alignment window (bw) <= 2 s) and the 'stitch' lockspray correction method[34], to account for the analyte signal gap that can occur during the lock mass scans (an uncorrected chromatogram is shown as supplementary Figure S-1). Further statistical analysis of reported features was performed in R; this stage only used features which were detected in all five replicates of at least one method/solvent combination.

Protocol S-4. Detailed NMR experimental procedures.

Samples were rehydrated in 650 µl of NMR buffer (100% $^{2}H_2O$, 1 mM sodium trimethylsilyl-2,2,3,3-$^{2}H_4$-propionate (TSP), 0.1 M phosphate buffer pH 7.0), centrifuged again to remove undissolved residues, and 600 µl transferred to 5 mm NMR tubes. All spectra were obtained on a Bruker Avance DRX600 spectrometer

with a 14.1 T magnet (Bruker Biospin; Rheinstetten, Germany) at 600.13 MHz (ambient probe temperature 300 °K) as reported previously[35]. A standard ^{1}H NOESY [recycle delay (RD)-90°-t1-90°-tm-90°-acquire] with a RD of 3.5 s, t(90°) of ~13 μs, a tm of 0.1 s, and an acquisition time of 1.3 s was acquired over 12 kHz with a resolution of 32 k data points; 128 scans were collected and summed per sample. The water signal was suppressed by pre-saturation during the RD. For peak assignment coupling constant resolved (J-Res), ^{1}H-^{1}H correlation spectroscopy (H,H-COSY) and heteronuclear ^{1}H-^{13}C single quantum bond correlation (HSQC) were used. Thirty-two assigned metabolites were then fitted with the NMR Suite 6 (Chenomx, Alberta, Canada) software package across all spectra. Statistical analysis of fitted metabolites was performed in Matlab.

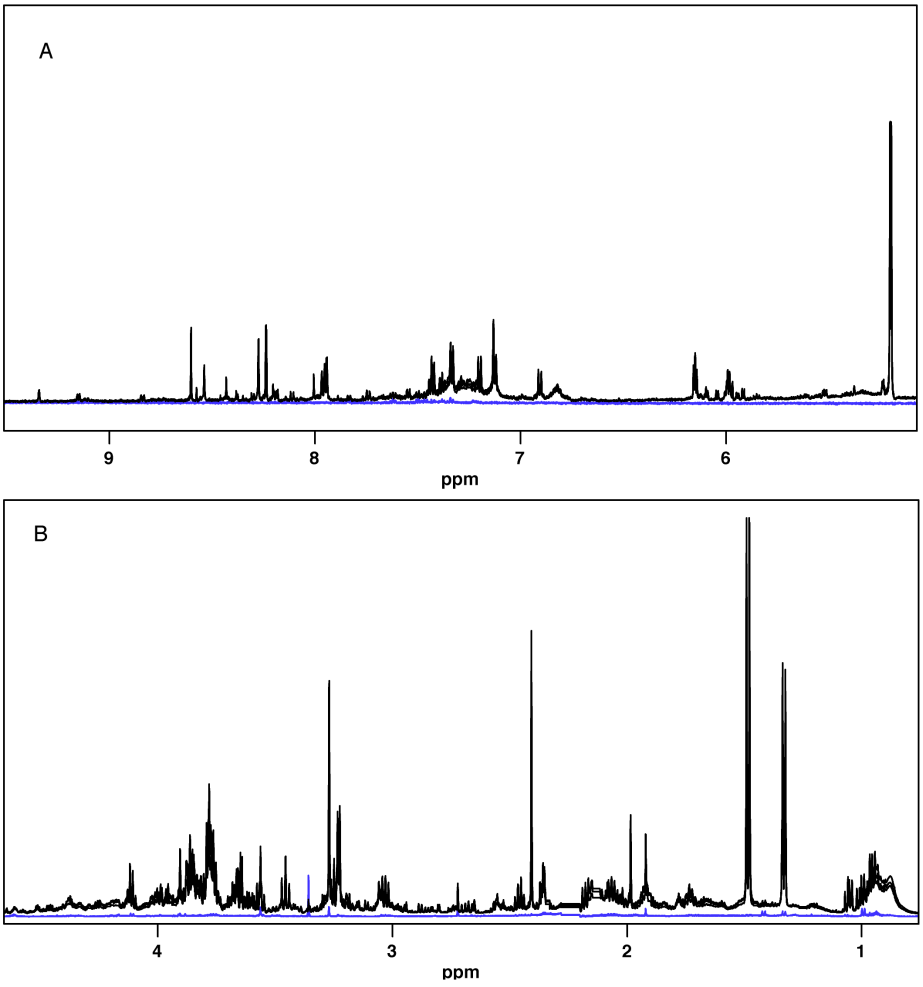

Figure S-5. Comparison of contribution of nematode and bacterial biomass to the total metabolite profile, as evaluated by 600 MHz ^{1}H NMR spectroscopy. Extracts of equal-volume worm pellets (black) and overlying supernatants (blue); n=3 for both. A: aromatic region. B: aliphatic region. NB that A has the vertical scale increased by a factor of 5 compared to B.

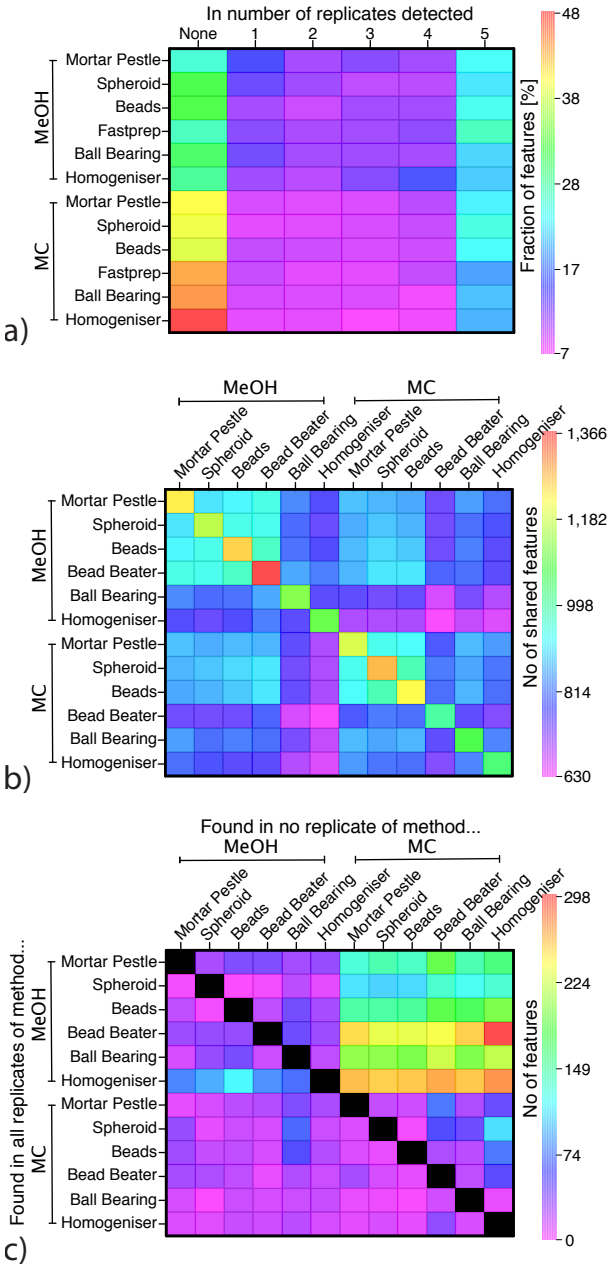

Figure S-6. Distribution of LC-MS metabolite features.

(a) Consistency of metabolic features across the extraction replicates – the color scale indicates the fraction of total number of features detected in the entire experiment. For most extraction methods, the greatest percentage was found in either no replicates, or all replicates.

(b) Color scale indicates the number of common reproducible (5 out of 5 replicates) features between methods, with the diagonal representing the number of reproducible features of each method.

(c) Color scale indicates the overlap between reproducible (5/5) and not detected features (0/5) between all methods, i.e. the number of features that are present in all replicates of one method and no replicates of another method.

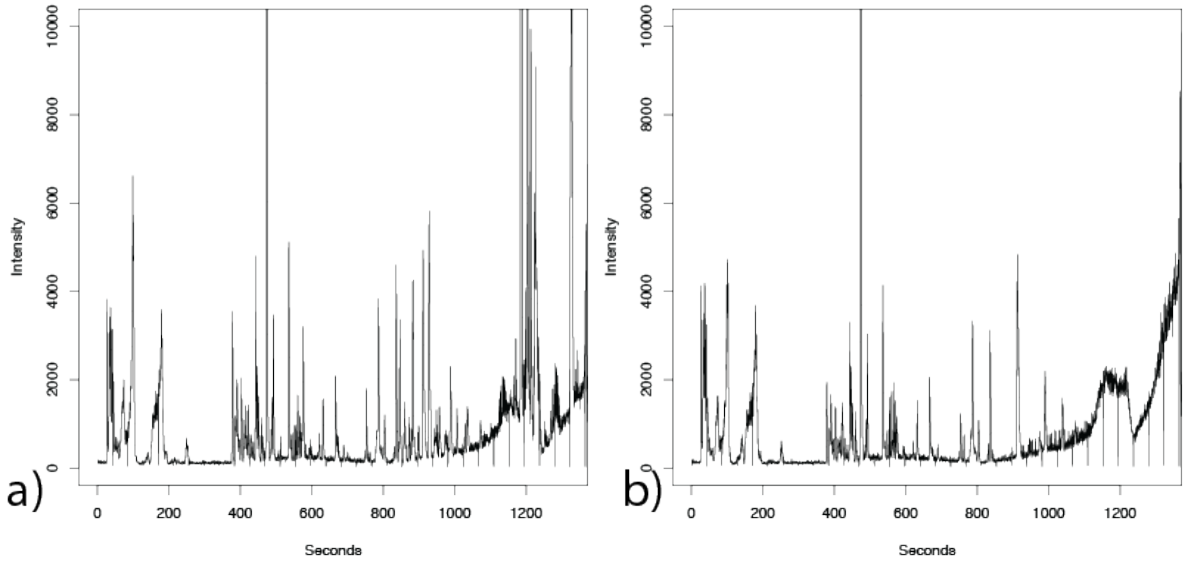

Figure S-7. LC-MS base ion peak chromatograms for a representative MeOH (a) and MC (b) extractions. Whereas the chromatogram appears to be identical up to a retention time of 700 seconds, between 800 to 1000 less features are visible in the MC sample. The periodic vertical lines are missing scans due to lock mass scans, which are corrected by using the gap filling algorithm from Benton *et al*. (Bioinformatics 2010 26:2488-2489).

TABLE S-8. Metabolites detected in C. elegans by GC-MS and/or NMR spectroscopy.

Name	PubChem Key	GC-MS	NMR	Class
Betaine	247		x	Biogenic amine
Choline	305		x	Biogenic amine
O-phosphocolamine	1015	x		Biogenic amine
Phosphocholine	1014		x	Biogenic amine
Putrescine	1045	x		Biogenic amine
Methoxytryptamine	1833	x		Biogenic amine
Phenylethanolamine	1000	x		Biogenic amine
3-aminoisobutyric acid	64956	x		Amino acid
Alanine	602		x	Amino acid
Arginine	232		x	Amino acid
Asparagine	236		x	Amino acid
Aspartate	424		x	Amino acid
Glutamate	611		x	Amino acid
Glutamine	738		x	Amino acid
Glycine	750	x	x	Amino acid
Isoleucine	791	x	x	Amino acid
Leucine	857		x	Amino acid
Lysine	866		x	Amino acid
Methionine	876	x		Amino acid
Phenylalanine	994	x	x	Amino acid
Proline	614	x		Amino acid
Pyroglutamic acid	7405	x		Amino acid
Serine	617	x	x	Amino acid
Threonine	205	x		Amino acid
Threonine	205		x	Amino acid
Tyrosine	1153		x	Amino acid
Valine	1182	x	x	Amino acid
Beta- alanine	239	x		Amino acid Derivative/Precursor
Cystathionine	834		x	Amino acid Derivative/Precursor
D-Ala-D-Ala	601	x		Amino acid Derivative/Precursor
Homoserine	779	x		Amino acid Derivative/Precursor
Hydroxy-proline	5810	x		Amino acid Derivative/Precursor
Ornithine	389	x		Amino acid Derivative/Precursor

D-(+)-Trehalose	1143	x	x	Carbohydrate
Glucose	5793		x	Carbohydrate
3-Glycerophosphate	754	x		Carbohydrate Derivative/Precursor
3-Phosphoglycerate	724	x		Carbohydrate Derivative/Precursor
D-glucose-6-phosphate	5958	x		Carbohydrate Derivative/Precursor
Gluconic acid	604	x		Carbohydrate Derivative/Precursor
Glycerol	753	x	x	Carbohydrate Derivative/Precursor
myo-Inositol	892	x		Carbohydrate Derivative/Precursor
sn-Glycero-3-phosphocholine	71920		x	Carbohydrate Derivative/Precursor
Sucrose	5988	x		Carbohydrate Derivative/Precursor
Acetate	175		x	Carboxylic acid
Citramalic acid	1081	x		Carboxylic acid
Citric acid	311	x		Carboxylic acid
Formate	283		x	Carboxylic acid
Fumarate	723	x	x	Carboxylic acid
Lactate	612	x	x	Carboxylic acid
Malate	525	x		Carboxylic acid
Phosphoenolpyruvic acid	1005	x		Carboxylic acid
Phosphoglycolic acid	529	x		Carboxylic acid
Propionate	1032		x	Carboxylic acid
Succinate	1110	x	x	Carboxylic acid
Heptadecanoic acid	10456	x		Fatty acid
Linoleic acid	3931	x		Fatty acid
Palmitic acid	985	x		Fatty acid
Stearic acid	5281	x		Fatty acid
Adenosine	191	x		Nucleoside
Guanosine	765	x		Nucleoside
Xanthosine	1189	x		Nucleoside
Adenine	190	x		Nucleoside base
Thymine	1135	x		Nucleoside base
Uracil	1174	x		Nucleoside base
Xanthine	1188	x		Nucleoside base
AMP	224	x	x	Nucleotide
ATP	238		x	Nucleotide
GTP	762		x	Nucleotide
Inosine 5-monophosphate	8582	x		Nucleotide
UMP	6030	x		Nucleotide
Pyrophosphate	1023	x		Phospate
Nicotinic acid	938	x		Vitamin or derivative
Pantothenic acid	988	x		Vitamin or derivative
Cholesterol	304	x		Steroid

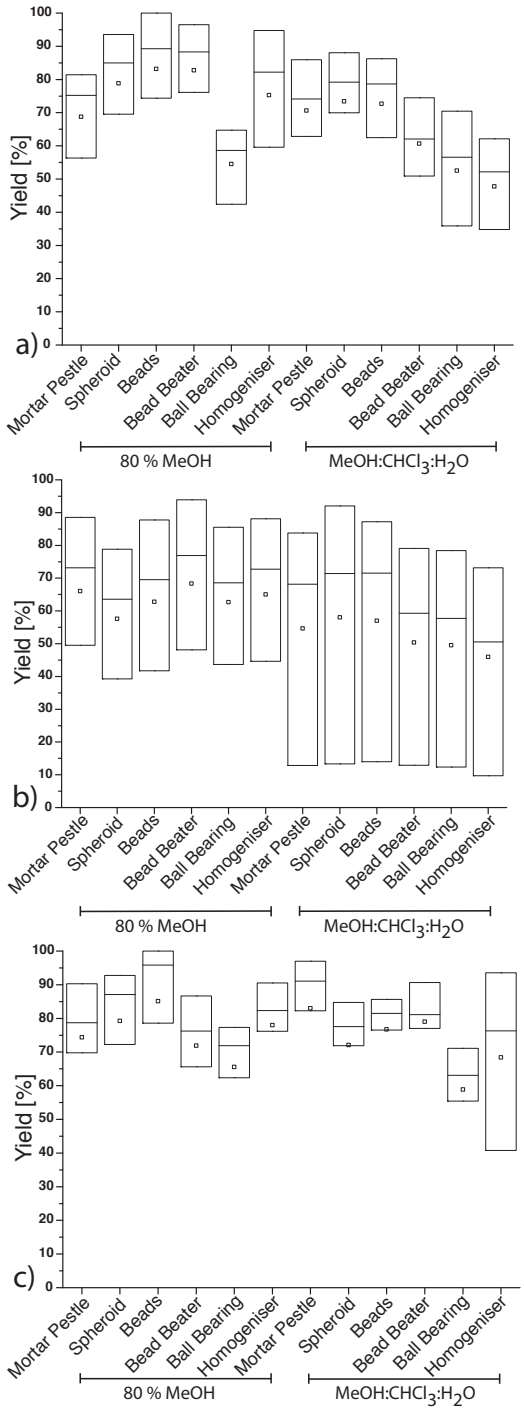

Figure S-9. Relative yield for (a) GC-MS, UPLC-MS (b) and NMR (c). The yield of a metabolite feature is expressed as a percentage of the highest signal intensity found by any of the extraction methods. The box represents the 25th and 75th percentiles, with a line at median height and a dot representing the mean.