

## Supporting information:

### **Advantages of using biologically generated $^{13}\text{C}$ -labelled multiple internal standards for stable isotope-assisted LC-MS based lipidomics**

Malak A. Jaber,<sup>a,b,\*</sup> Bruna de Falco,<sup>b,\*</sup> Salah Abdelrazig,<sup>b</sup> Catharine A. Ortori,<sup>b</sup> David A. Barrett,<sup>b</sup>  
Dong-Hyun Kim<sup>b,\*</sup>

<sup>a</sup> Faculty of Pharmacy and Medical Sciences, University of Petra, Amman, Jordan

<sup>b</sup> Centre for Analytical Bioscience, Advanced Materials and Healthcare Technology Division, School of Pharmacy, University of Nottingham, University Park, Nottingham, NG7 2RD, United Kingdom

\* Corresponding author, e-mail address: [Dong-hyun.Kim@nottingham.ac.uk](mailto:Dong-hyun.Kim@nottingham.ac.uk)

\* Shared first authorship

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## **Materials**

D-Glucose, peptone, M9 minimal salts, magnesium sulphate heptahydrate, calcium chloride dihydrate, thiamine hydrochloride, propidium iodide and fluorescein diacetate were supplied by Sigma-Aldrich (St. Louis, USA). Bacto Agar was bought from Scientific Laboratory Supplies (Nottingham, UK), and the yeast extract from OXOID (Basingstoke, UK). The yeast nitrogen base without amino acids, L-methionine, L-histidine, L-leucine, uracil and the complete supplement mixture were bought from Formedium (Norfolk, UK).

## **Biosynthesized <sup>13</sup>C-Internal Standard extraction**

10 mL (OD<sub>600</sub>=1) of cellular suspension (for *A. platensis* samples 5 mg of lyophilised powder) were suspended in 2.5 mL methanol and vortexed for 5 min, then 1 mL de-ionised H<sub>2</sub>O and 1.25 mL chloroform were added and vortexed for 5 min at 4°C. Samples were then centrifuged for 10 min at 13000 g and the chloroform phases were dried under vacuum at room temperature. The dried samples were reconstituted using 200 µL of LC-MS grade isopropanol and stored at -80°C until analysis.

## **Lipids extraction from plasma samples**

50 µL of plasma were extracted with 500 µL of chloroform/methanol (1:2) and 100 µL of IS mixture, vortexed for 1 minute and mixed with 500 µL of water. The samples were vortexed for 5 minutes and additional 500 µL of chloroform were added to the solutions, vortexed and centrifuged at 13000 g for 10 minutes at 4°C. The lower chloroform phase (400 µL) was collected, and a second extraction was performed on the plasma samples by adding 500 µL of chloroform, vortexed for 5 minutes and centrifuged at 13000 g for 10 minutes at 4°C. The lower phase (400 µL) was combined with the previous extract. The organic phase was dried under vacuum at room temperature and reconstituted using 100 µL LC-MS grade isopropanol and stored at -80°C until further analysis.

## **LC-MS lipidomics analysis**

For LC analysis the mobile phases used were: (A) acetonitrile:ammonium acetate (40:60, final concentration of 10 mM) and (B) isopropanol:acetonitrile:ammonium acetate (80:10:10, final concentration of 10 mM). The gradient was as follows: 0 min: 30% B, flow rate 300  $\mu$ L, 2 min: 35% B, flow rate 300  $\mu$ L, 8 min: 100% B, flow rate 300  $\mu$ L, 17 min: 100% B, flow rate 500  $\mu$ L, 18-20 min: 20% B, flow rate 300  $\mu$ L. Column and autosampler temperatures were 45 and 4°C respectively. Sample injection volume was 10  $\mu$ L.

For MS analysis data were acquired simultaneously in a full scan ion mode at  $m/z$  100–1500, resolution of 70,000, automatic gain control<sup>45</sup> target of 3E6 and maximum ion injection time of 100 ms. Data were acquired in a tandem MS/MS mode at data-dependent MS/MS scans on top 5 precursor ions per scan, resolution of 17,500, AGC target of 1E5, maximum ion injection time of 50 ms, scan range from 200 to 2,000  $m/z$  and electrospray voltage of 4 kV. The capillary and heater temperatures were maintained at 256 and 413 °C, respectively. Sheath, auxiliary and sweep gas flow rate (arbitrary units) were adjusted to 48, 11 and 2, respectively. The raw data for LC-MS/MS were acquired and visualised with Xcalibur 2.2 software (Thermo Scientific, Hemel Hempstead, UK). The MS performance was assessed at each run using quality control samples (QC) interspaced between every 3-10 samples based on the total number of analysed samples. To obtain QC sample, an aliquot (10  $\mu$ L) of each biological sample was pooled together.

The list of labelled and unlabelled lipid species alongside their proposed identity and their expected RT identified using LipidSearch<sup>TM</sup> software was introduced as a database into Trace Finder software (Thermo Fisher Scientific, Waltham, MA) used to integrate and extract peaks areas of lipid pairs from the LC-MS/MS raw data based on mass error of 5 ppm, RT window of 30 s and S/N of 10. The performance was assessed by comparing the CV% across all metabolites and replicates.

**Table S1.** LipidSearch parameter used for lipid identification.

<b>Search parameter</b>	<b>Settings</b>
Database	Q-Exactive
Search type	Product
Experiment type	LC-MS
Precursor tolerance	5 ppm
Product tolerance	5 ppm
Intensity threshold	1.0%
Execute Quantitation	on
m/z tolerance (quantitation)	-/+ 5.0 ppm
RT range (min.) (quantitation)	-/+ 0.5
Top rank filter	On
Main node filter	Main isomer peak
m-Score threshold	5.0 (1.0) *
c-Score threshold	2.0
FA priority	On
ID Quality Filter	A, B, C and D
Target class	ALL lipid classes
Ion adducts (pos)	H <sup>+</sup> , Na <sup>+</sup> and NH <sub>4</sub> <sup>+</sup>
Ion adducts (neg)	H <sup>-</sup> and CH <sub>3</sub> COO <sup>-</sup>
S/N	2

\*m-Score threshold was selected as 1 when the lipids profile of different species was explored in order to include as much as possible of that species lipidome to get a better comparison between the studied species.

**Table S2.** LipidSearch parameters used for alignment.

<b>Search parameter</b>	<b>Setting</b>
Exp type	LC-MS
Alignment method	mean
R.T. Tolerance	0.5 min
Calculate unassigned peak area	On
Filter type	New filter
Top rank filter	On
Main node filter	Main isomer peak
m-Score threshold	5.0 (1.0) *
ID quality filter	A, B, C and D

\*m-Score threshold was selected as 1 when the lipids profile of different species was explored in order to include as much as possible of that species lipidome to get a better comparison between the studied species.

**Table S3.** List of lipid standards from SPLASH LipidoMix standard solution used for normalisation.

Lipid Standard	Adduct	Polarity	m/z	RT (min)
ChE 18:1(d <sub>7</sub> )	[M+NH <sub>4</sub> ] <sup>+</sup>	+	675.6772	14.50
DG 15:0-18:1(d <sub>7</sub> )	[M+NH <sub>4</sub> ] <sup>+</sup>	+	605.5837	9.70
	[M-H+CH <sub>3</sub> CO <sub>2</sub> H] <sup>-</sup>	-	646.5637	9.70
LPC 18:1(d <sub>7</sub> )	[M+H] <sup>+</sup>	+	529.3986	5.61
	[M-H+CH <sub>3</sub> CO <sub>2</sub> H] <sup>-</sup>	-	587.4052	5.61
LPE 18:1(d <sub>7</sub> )	[M+H] <sup>+</sup>	+	487.3517	5.74
	[M-H] <sup>-</sup>	-	485.3371	5.74
MG 18:1(d <sub>7</sub> )	[M+NH <sub>4</sub> ] <sup>+</sup>	+	381.3697	6.82
	[M-H+CH <sub>3</sub> CO <sub>2</sub> H] <sup>-</sup>	-	422.3497	6.82
PA 15:0-18:1(d <sub>7</sub> )	[M+NH <sub>4</sub> ] <sup>+</sup>	+	685.5500	9.16
	[M-H] <sup>-</sup>	-	666.5089	9.16
PC 15:0-18:1(d <sub>7</sub> )	[M+H] <sup>+</sup>	+	753.6126	8.99
	[M-H+CH <sub>3</sub> CO <sub>2</sub> H] <sup>-</sup>	-	811.6192	8.99
PE 15:0-18:1(d <sub>7</sub> )	[M+H] <sup>+</sup>	+	711.5657	9.04
	[M-H] <sup>-</sup>	-	709.5511	9.04
PG 15:0-18:1(d <sub>7</sub> )	[M+NH <sub>4</sub> ] <sup>+</sup>	+	759.5868	8.66
	[2M-H] <sup>-</sup>	-	1482.099	8.66
PI 15:0-18:1(d <sub>7</sub> )	[M+H] <sup>+</sup>	+	830.5763	8.58
	[M-H] <sup>-</sup>	-	828.5618	8.57
PS 15:0-18:1(d <sub>7</sub> )	[M+H] <sup>+</sup>	+	755.5555	8.78
	[M-H] <sup>-</sup>	+	753.5410	8.78
SM d18:1-18:1(d <sub>9</sub> )	[M+H] <sup>+</sup>	+	738.646	8.82
	[M+NH <sub>4</sub> ] <sup>+</sup>	+	829.7977	13.09
TG 15:0-18:1(d <sub>7</sub> )-15:0				

**Table S4.** Lipid ions identified in *P. pastoris* and their <sup>13</sup>C-enrichment based on their labelling pattern and the labelling percentage of the fully labelled isotopologues.

Number	Lipid ion ID	Class	Number of carbon atom in the ion	Polarity	Labelling percentage of fully labelled ions	Expected <sup>13</sup> C-labelling degree based on the fully labelling percentage obtained
1	Cer(d17:0/16:0)+H	Cer	33	+	0.00	0.00
2	Cer(d17:0/18:0)+H	Cer	35	+	0.00	0.00
3	Cer(d18:0/18:0)+H	Cer	36	+	68.99	98.97
4	Cer(d18:0/18:0)-H	Cer	36	-	74.95	99.20
5	Cer(d18:1/16:0)+H	Cer	34	+	72.16	99.04
6	Cer(d18:1/18:0)+H	Cer	36	+	67.49	98.91
7	Cer(d18:1/28:5)+H	Cer	46	+	59.71	98.89
8	Cer(d18:2/16:0)+H	Cer	34	+	68.10	98.88
9	Cer(d18:2/16:0+O)+H	Cer	34	+	73.76	99.11
10	Cer(d18:2/16:0+O)-H	Cer	34	-	56.83	98.35
11	Cer(d18:2/18:0)+H	Cer	36	+	66.00	98.85
12	Cer(d18:2/18:0+O)+H	Cer	36	+	61.05	98.64
13	Cer(d18:2/18:0+O)-H	Cer	36	-	60.28	98.60
14	Cer(d24:0/18:0+O)+H	Cer	42	+	53.92	98.54
15	Cer(d24:1)-H	Cer	24	-	0.00	0.00
16	CerG1(d18:2/16:0+O)+H	CerG1	40	+	63.71	98.88
17	CerG1(d18:2/18:0+O)+H	CerG1	42	+	59.28	98.76
18	CerG1(d18:2/18:1)+H	CerG1	42	+	63.43	98.92
19	DG(15:0/18:2)+NH <sub>4</sub>	DG	36	+	68.15	98.94
20	DG(16:0/14:0)+NH <sub>4</sub>	DG	33	+	0.19	82.71
21	DG(16:0/16:0)+H	DG	35	+	0.00	0.00

22	DG(16:0/16:0)+ NH <sub>4</sub>	DG	35	+	0.05	80.26
23	DG(16:0/17:0)+ NH <sub>4</sub>	DG	36	+	0.00	0.00
24	DG(16:0/18:1)+ NH <sub>4</sub>	DG	37	+	66.08	98.89
25	DG(16:1/16:1)+ NH <sub>4</sub>	DG	35	+	69.35	98.96
26	DG(16:1/18:1)+ NH <sub>4</sub>	DG	37	+	65.74	98.87
27	DG(16:1/18:2)+ NH <sub>4</sub>	DG	37	+	66.28	98.89
28	DG(16:1/18:3)+ NH <sub>4</sub>	DG	37	+	66.51	98.90
29	DG(17:0/18:1)+ NH <sub>4</sub>	DG	38	+	65.99	98.91
30	DG(17:1/18:1)+ NH <sub>4</sub>	DG	38	+	65.86	98.91
31	DG(17:1/18:2)+ NH <sub>4</sub>	DG	38	+	64.67	98.86
32	DG(17:1/18:3)+ NH <sub>4</sub>	DG	38	+	60.47	98.69
33	DG(18:0/16:0)+H	DG	37	+	0.00	0.00
34	DG(18:0/16:0)+ NH <sub>4</sub>	DG	37	+	0.03	80.16
35	DG(18:0/16:0)+ NH <sub>4</sub>	DG	37	+	0.00	0.00
36	DG(18:0/17:0)+ NH <sub>4</sub>	DG	38	+	0.31	85.87
37	DG(18:0/18:0)+H	DG	39	+	0.00	0.00
38	DG(18:0/18:1)+ NH <sub>4</sub>	DG	39	+	61.15	98.75
39	DG(18:0/20:0)+ NH <sub>4</sub>	DG	41	+	0.12	84.84
40	DG(18:1/14:0)+ NH <sub>4</sub>	DG	35	+	69.65	98.97
41	DG(18:1/18:1)+H	DG	39	+	62.23	98.79
42	DG(18:1/18:1)+ NH <sub>4</sub>	DG	39	+	65.19	98.91
43	DG(18:1/18:2)+ NH <sub>4</sub>	DG	39	+	64.55	98.88
44	DG(18:1/19:4)+H	DG	40	+	0.00	0.00
45	DG(18:1/24:0)+ NH <sub>4</sub>	DG	45	+	56.02	98.72
46	DG(18:2/17:3)+ NH <sub>4</sub>	DG	38	+	65.96	98.91
47	DG(18:2/18:2)+ NH <sub>4</sub>	DG	39	+	63.39	98.84
48	DG(18:3/18:2)+H	DG	39	+	0.22	85.51
49	DG(18:3/18:2)+ NH <sub>4</sub>	DG	39	+	63.43	98.84
50	DG(18:3/18:3)+H	DG	39	+	0.00	0.00
51	DG(18:3/18:3)+ NH <sub>4</sub>	DG	39	+	61.35	98.76
52	DG(20:1/18:2)+ NH <sub>4</sub>	DG	41	+	42.30	97.92
53	DG(20:1/18:3)+ NH <sub>4</sub>	DG	41	+	58.38	98.70
54	DG(37:4)+H	DG	40	+	0.00	0.00
55	dMePE(16:0/18:1)-H	dMePE	41	-	67.25	99.04
56	dMePE(16:0/18:2)-H	dMePE	41	-	66.30	99.00
57	dMePE(16:2/18:2)-H	dMePE	41	-	61.74	98.83
58	dMePE(18:1/18:1)-H	dMePE	43	-	57.62	98.73
59	dMePE(18:1/18:2)-H	dMePE	43	-	59.13	98.79
60	dMePE(18:1/18:3)-H	dMePE	43	-	42.32	98.02
61	dMePE(18:3/18:2)-H	dMePE	43	-	62.63	98.92
62	dMePE(33:2)-H	dMePE	40	-	62.38	98.83
63	dMePE(35:2)-H	dMePE	42	-	27.78	97.00
64	FA(18:2)-H	FA	18	-	81.27	98.85
65	LPC(16:0)+CH <sub>3</sub> COO	LPC	24	-	70.67	98.56
66	LPC(16:0)+H	LPC	24	+	75.42	98.83
67	LPC(16:1)+H	LPC	24	+	73.20	98.71
68	LPC(17:1)+H	LPC	25	+	70.65	98.62
69	LPC(18:0)+H	LPC	26	+	75.68	98.93
70	LPC(18:1)+H	LPC	26	+	71.68	98.73
71	LPC(18:2)+H	LPC	26	+	74.85	98.89
72	LPC(18:3)+H	LPC	26	+	71.73	98.73
73	LPC(20:5)+H	LPC	28	+	0.00	0.00
74	LPC(37:6)+H	LPC	45	+	0.00	0.00
75	LPE(16:0)-H	LPE	21	-	77.04	98.77
76	LPE(18:1)-H	LPE	23	-	74.95	98.75
77	LPE(18:2)+H	LPE	23	+	0.00	0.00
78	LPE(18:2)-H	LPE	23	-	73.84	98.69
79	LPE(18:3)+H	LPE	23	+	81.58	99.12



80	LPE(18:3)-H	LPE	23	-	75.42	98.78
81	MG(14:0)+H	MG	17	+	0.00	0.00
82	MG(14:0)+ NH <sub>4</sub>	MG	17	+	0.00	0.00
83	MG(16:0)+H	MG	19	+	0.00	0.00
84	MG(16:0)+ NH <sub>4</sub>	MG	19	+	0.00	0.00
85	MG(16:0)+ NH <sub>4</sub>	MG	19	+	0.00	0.00
86	MG(18:0)+H	MG	21	+	0.00	0.00
87	MG(18:0)+H	MG	21	+	0.00	0.00
88	MG(18:0)+ NH <sub>4</sub>	MG	21	+	0.00	0.00
89	MG(18:0)+ NH <sub>4</sub>	MG	21	+	0.00	0.00
90	MG(18:0)+ NH <sub>4</sub>	MG	21	+	0.00	0.00
91	OAHFA(32:1)-H	OAHFA	32	-	65.32	98.68
92	OAHFA(36:1)-H	OAHFA	36	-	62.48	98.70
93	OAHFA(36:3)-H	OAHFA	36	-	66.16	98.86
94	OAHFA(38:6)-H	OAHFA	38	-	0.00	0.00
95	PA(16:0/18:1)-H	PA	37	-	61.20	98.68
96	PA(16:0/18:2)-H	PA	37	-	71.50	99.10
97	PA(16:0/18:2)-H	PA	37	-	66.79	98.91
98	PA(18:1/18:1)-H	PA	39	-	68.40	99.03
99	PA(18:1/18:2)-H	PA	39	-	68.01	99.02
100	PA(18:3/18:2)-H	PA	39	-	68.09	99.02
101	PC(15:2/18:2)+H	PC	41	+	64.95	98.95
102	PC(16:0/18:1)+H	PC	42	+	59.86	98.79
103	PC(18:1/19:2)+H	PC	45	+	58.22	98.80
104	PC(30:1)+H	PC	38	+	64.31	98.84
105	PC(32:0)+H	PC	40	+	68.47	99.06
106	PC(32:1)+H	PC	40	+	64.98	98.93
107	PC(32:2)+H	PC	40	+	62.62	98.84
108	PC(32:3)+H	PC	40	+	60.84	98.77
109	PC(32:4)+H	PC	40	+	62.52	98.83
110	PC(33:1)+H	PC	41	+	65.17	98.96
111	PC(33:3)+H	PC	41	+	59.94	98.76
112	PC(33:4)+H	PC	41	+	58.93	98.72
113	PC(33:5)+H	PC	41	+	65.64	98.98
114	PC(34:2)+H	PC	42	+	59.62	98.78
115	PC(34:3)+H	PC	42	+	59.50	98.77
116	PC(34:4)+H	PC	42	+	58.66	98.74
117	PC(34:5)+H	PC	42	+	59.42	98.77
118	PC(34:6)+H	PC	42	+	60.85	98.82
119	PC(35:1)+H	PC	43	+	63.66	98.96
120	PC(35:3)+H	PC	43	+	0.00	0.00
121	PC(35:4)+H	PC	43	+	57.08	98.70
122	PC(35:5)+H	PC	43	+	57.43	98.72
123	PC(35:6)+H	PC	43	+	56.04	98.66
124	PC(36:1)+H	PC	44	+	56.15	98.70
125	PC(36:2)+H	PC	44	+	57.38	98.75
126	PC(36:3)+H	PC	44	+	58.42	98.79
127	PC(36:3)+H	PC	44	+	62.65	98.94
128	PC(36:4)+H	PC	44	+	57.91	98.77
129	PC(36:5)+H	PC	44	+	56.81	98.72
130	PC(36:6)+H	PC	44	+	56.12	98.70
131	PC(36:7)+H	PC	44	+	0.00	0.00
132	PC(37:2)+H	PC	45	+	44.07	98.20
133	PC(37:4)+H	PC	45	+	54.74	98.67
134	PC(38:2)+H	PC	46	+	57.23	98.79
135	PC(38:3)+H	PC	46	+	57.06	98.79
136	PC(38:4)+H	PC	46	+	55.96	98.75
137	PC(38:5)+H	PC	46	+	50.57	98.53

138	PC(38:6)+H	PC	46	+	47.08	98.38
139	PE(16:0/16:0)+H	PE	37	+	71.28	99.09
140	PE(16:0/16:1)+H	PE	37	+	64.89	98.84
141	PE(16:0/18:1)+H	PE	39	+	65.37	98.92
142	PE(16:0/18:1)-H	PE	39	-	63.79	98.85
143	PE(16:0/18:3)+H	PE	39	+	65.32	98.91
144	PE(16:0/18:3)-H	PE	39	-	64.04	98.86
145	PE(16:0e)+H	PE	21	+	81.76	99.05
146	PE(16:1/16:1)+H	PE	37	+	64.72	98.83
147	PE(16:1/16:1)-H	PE	37	-	61.63	98.70
148	PE(16:1/18:1)-H	PE	39	-	63.60	98.85
149	PE(16:1/18:3)+H	PE	39	+	64.17	98.87
150	PE(16:1/18:3)-H	PE	39	-	63.03	98.82
151	PE(16:2/18:3)+H	PE	39	+	65.42	98.92
152	PE(17:0/18:1)+H	PE	40	+	58.21	98.66
153	PE(17:1/16:0)-H	PE	38	-	68.55	99.01
154	PE(17:1/18:1)+H	PE	40	+	62.77	98.84
155	PE(17:1/18:1)-H	PE	40	-	62.38	98.83
156	PE(17:1/18:2)+H	PE	40	+	63.00	98.85
157	PE(18:0/18:1)+H	PE	41	+	66.80	99.02
158	PE(18:0/18:1)-H	PE	41	-	66.74	99.02
159	PE(18:0p)+H	PE	23	+	94.42	99.75
160	PE(18:1/18:1)+H	PE	41	+	64.53	98.94
161	PE(18:1/18:1)-H	PE	41	-	63.07	98.88
162	PE(18:1/18:2)+H	PE	41	+	65.17	98.96
163	PE(18:1/18:2)-H	PE	41	-	62.13	98.85
164	PE(18:2/17:2)-H	PE	40	-	61.25	98.78
165	PE(18:2/17:3)+H	PE	40	+	61.95	98.81
166	PE(18:2/17:3)-H	PE	40	-	60.45	98.75
167	PE(18:2/18:2)+H	PE	41	+	64.95	98.95
168	PE(18:2/18:2)-H	PE	41	-	61.74	98.83
169	PE(18:3/18:2)+H	PE	41	+	63.60	98.90
170	PE(18:3/18:2)-H	PE	41	-	59.06	98.72
171	PE(18:3/18:3)+H	PE	41	+	60.89	98.80
172	PE(18:3/18:3)-H	PE	41	-	58.57	98.70
173	PE(32:1e)+H	PE	37	+	91.11	99.75
174	PE(34:0e)+H	PE	39	+	0.00	0.00
175	PE(35:4)+H	PE	40	+	66.58	98.99
176	PE(37:0p)+H	PE	42	+	50.96	98.41
177	PE(37:1p)+H	PE	42	+	62.33	98.88
178	PE(37:2)-H	PE	42	-	27.78	97.00
179	PE(37:5)-H	PE	42	-	25.88	96.83
180	PE(42:2p)+H	PE	47	+	53.74	98.69
181	PE(43:10)+H	PE	48	+	0.00	0.00
182	PE(45:11)+H	PE	50	+	0.00	0.00
183	PE(45:12)+H	PE	50	+	0.00	0.00
184	PE(45:13)+H	PE	50	+	0.00	0.00
185	PE(47:10)+H	PE	52	+	0.00	0.00
186	PE(47:12)+H	PE	52	+	0.00	0.00
187	PE(47:13)+H	PE	52	+	0.00	0.00
188	PG(16:0/18:1)+ NH <sub>4</sub>	PG	40	+	66.22	98.97
189	PG(16:0/18:1)-H	PG	40	-	69.19	99.08
190	PG(16:0/18:2)+ NH <sub>4</sub>	PG	40	+	66.72	98.99
191	PG(16:0/18:2)-H	PG	40	-	63.59	98.87
192	PG(16:0/18:3)-H	PG	40	-	65.63	98.95
193	PG(18:1/18:2)-H	PG	42	-	62.58	98.89
194	PG(18:1/18:3)-H	PG	42	-	62.48	98.89
195	PG(18:3/18:2)-H	PG	42	-	62.12	98.87

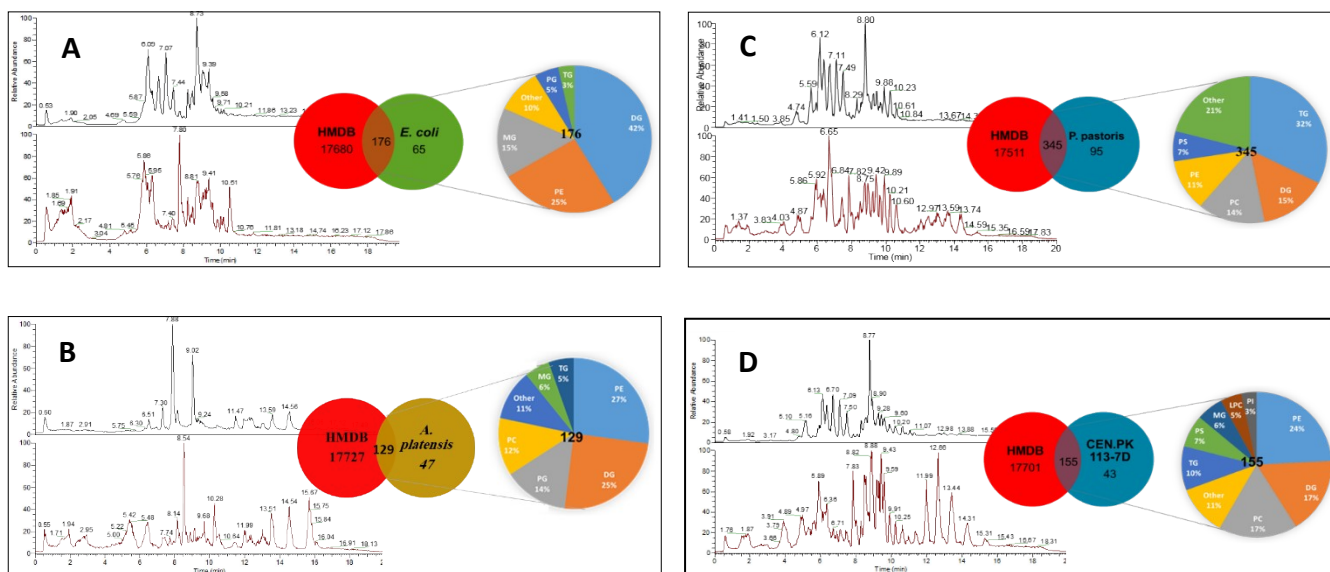
196	PG(28:0/16:0)+H	PG	50	+	0.00	0.00
197	PG(28:0/18:1)+H	PG	52	+	0.00	0.00
198	PG(28:0/18:2)+H	PG	52	+	0.00	0.00
199	PI(16:0/16:1)+ NH <sub>4</sub>	PI	41	+	66.56	99.01
200	PI(16:0/16:1)-H	PI	41	-	63.51	98.90
201	PI(16:0/18:1)+H	PI	43	+	61.05	98.86
202	PI(16:0/18:2)+H	PI	43	+	63.66	98.96
203	PI(16:0/18:2)+ NH <sub>4</sub>	PI	43	+	63.75	98.96
204	PI(16:0/18:2)-H	PI	43	-	60.84	98.85
205	PI(16:0/18:3)+ NH <sub>4</sub>	PI	43	+	63.54	98.95
206	PI(16:0/18:3)-H	PI	43	-	60.81	98.85
207	PI(16:1/18:3)-H	PI	43	-	59.03	98.78
208	PI(17:0/18:1)-H	PI	44	-	59.21	98.82
209	PI(17:1/16:0)-H	PI	42	-	61.97	98.87
210	PI(17:1/18:1)-H	PI	44	-	59.49	98.83
211	PI(18:0/18:1)+ NH <sub>4</sub>	PI	45	+	64.18	99.02
212	PI(18:0/18:1)-H	PI	45	-	58.55	98.82
213	PI(18:1/18:1)-H	PI	45	-	59.66	98.86
214	PI(18:1/18:2)-H	PI	45	-	58.60	98.82
215	PI(18:2/18:2)+ NH <sub>4</sub>	PI	45	+	59.15	98.84
216	PI(18:2/18:2)-H	PI	45	-	58.05	98.80
217	PI(18:3/18:3)+ NH <sub>4</sub>	PI	45	+	64.73	99.04
218	PI(18:3/18:3)-H	PI	45	-	59.31	98.85
219	PI(36:3)+NH <sub>4</sub>	PI	45	+	59.70	98.86
220	PS(16:0/16:1)-H	PS	38	-	48.72	98.13
221	PS(16:0/18:1)+H	PS	40	+	68.79	99.07
222	PS(16:0/18:1)-H	PS	40	-	69.38	99.09
223	PS(16:0/18:2)-H	PS	40	-	62.82	98.84
224	PS(16:0/18:3)-H	PS	40	-	63.38	98.87
225	PS(18:1/18:1)-H	PS	42	-	64.63	98.97
226	PS(18:1/18:2)-H	PS	42	-	70.00	99.15
227	PS(36:1)-H	PS	42	-	1.38	90.31
228	PS(36:4)-H	PS	42	-	0.00	0.00
229	PS(36:4)-H	PS	42	-	0.00	0.00
230	PS(37:3)-H	PS	43	-	0.00	0.00
231	PS(37:4)-H	PS	43	-	0.00	0.00
232	PS(38:1p)-H	PS	44	-	68.99	99.16
233	PS(39:4)-H	PS	45	-	0.00	0.00
234	PS(39:6)-H	PS	45	-	6.45	94.09
235	PS(40:1)-H	PS	46	-	1.05	90.56
236	PS(40:4)-H	PS	46	-	1.11	90.68
237	PS(40:5)-H	PS	46	-	1.06	90.59
238	PS(42:1)-H	PS	48	-	0.00	0.00
239	SM(d41:5)+H	SM	46	+	0.00	0.00
240	So(d17:1)+H	So	17	+	0.00	0.00
241	So(d18:0)+H	So	18	+	84.08	99.04
242	So(d18:1)+H	So	18	+	79.99	98.77
243	So(d20:0)+H	So	20	+	73.85	98.50
244	So(d20:1)+H	So	20	+	78.68	98.81
245	TG(12:0/18:2/18:2)+NH <sub>4</sub>	TG	51	+	53.47	98.78
246	TG(12:0/18:2/20:5)+H	TG	53	+	0.00	0.00
247	TG(14:0/18:3/20:4)+H	TG	55	+	0.00	0.00
248	TG(15:0/16:0/18:1)+ NH <sub>4</sub>	TG	52	+	54.34	98.83
249	TG(15:0/16:0/18:2)+ NH <sub>4</sub>	TG	52	+	55.17	98.86
250	TG(15:0/16:1/18:3)+ NH <sub>4</sub>	TG	52	+	50.97	98.71
251	TG(16:0/14:0/16:0)+ NH <sub>4</sub>	TG	49	+	5.48	94.24
252	TG(16:0/14:0/18:1)+ NH <sub>4</sub>	TG	51	+	57.73	98.93
253	TG(16:0/14:0/20:4)+H	TG	53	+	0.00	0.00

254	TG(16:0/16:0/16:0)+ NH <sub>4</sub>	TG	51	+	1.41	91.99
255	TG(16:0/16:0/18:1)+ NH <sub>4</sub>	TG	53	+	56.21	98.92
256	TG(16:0/16:0/18:3)+H	TG	53	+	0.00	0.00
257	TG(16:0/16:1/18:1)+ NH <sub>4</sub>	TG	53	+	55.08	98.88
258	TG(16:0/16:1/18:2)+ NH <sub>4</sub>	TG	53	+	54.90	98.88
259	TG(16:0/17:0/18:1)+ NH <sub>4</sub>	TG	54	+	54.35	98.88
260	TG(16:0/17:1/18:1)+ NH <sub>4</sub>	TG	54	+	54.71	98.89
261	TG(16:0/17:1/20:4)+H	TG	56	+	1.02	92.14
262	TG(16:0/18:1/18:1)+ NH <sub>4</sub>	TG	55	+	54.06	98.89
263	TG(16:0/18:1/20:4)+H	TG	57	+	0.00	0.00
264	TG(16:0/18:2/18:3)+ NH <sub>4</sub>	TG	55	+	50.82	98.78
265	TG(16:0/20:5/20:5)+H	TG	59	+	0.10	88.96
266	TG(16:1/12:0/18:1)+ NH <sub>4</sub>	TG	49	+	59.05	98.93
267	TG(16:1/14:0/17:1)+ NH <sub>4</sub>	TG	50	+	56.16	98.85
268	TG(16:1/14:0/18:1)+ NH <sub>4</sub>	TG	51	+	57.83	98.93
269	TG(16:1/16:1/16:1)+ NH <sub>4</sub>	TG	51	+	55.54	98.85
270	TG(16:1/16:1/17:1)+ NH <sub>4</sub>	TG	52	+	53.89	98.82
271	TG(16:1/16:1/18:2)+ NH <sub>4</sub>	TG	53	+	54.04	98.85
272	TG(16:1/16:1/18:3)+ NH <sub>4</sub>	TG	53	+	52.65	98.80
273	TG(16:1/16:2/18:3)+ NH <sub>4</sub>	TG	53	+	47.29	98.60
274	TG(16:1/17:1/18:1)+ NH <sub>4</sub>	TG	54	+	54.40	98.88
275	TG(16:1/17:1/18:2)+ NH <sub>4</sub>	TG	54	+	52.89	98.83
276	TG(16:1/17:3/18:2)+ NH <sub>4</sub>	TG	54	+	48.34	98.66
277	TG(16:1/17:3/18:3)+ NH <sub>4</sub>	TG	54	+	46.18	98.58
278	TG(16:1/18:1/18:1)+ NH <sub>4</sub>	TG	55	+	53.47	98.87
279	TG(16:1/18:1/18:2)+ NH <sub>4</sub>	TG	55	+	52.73	98.84
280	TG(16:1/18:1/18:3)+H	TG	55	+	0.00	0.00
281	TG(16:1/18:2/18:3)+ NH <sub>4</sub>	TG	55	+	50.32	98.76
282	TG(16:1/18:2/20:5)+H	TG	57	+	0.00	0.00
283	TG(16:1/18:3/18:3)+ NH <sub>4</sub>	TG	55	+	48.32	98.69
284	TG(16:2/18:3/18:3)+ NH <sub>4</sub>	TG	55	+	49.27	98.72
285	TG(17:0/18:1/18:1)+ NH <sub>4</sub>	TG	56	+	53.25	98.88
286	TG(17:1/17:2/17:2)+ NH <sub>4</sub>	TG	54	+	50.02	98.73
287	TG(17:4/18:3/21:5)+H	TG	59	+	0.00	0.00
288	TG(18:0/16:0/16:0)+ NH <sub>4</sub>	TG	53	+	0.33	89.78
289	TG(18:0/16:0/18:0)+ NH <sub>4</sub>	TG	55	+	0.06	87.50
290	TG(18:0/16:0/18:1)+ NH <sub>4</sub>	TG	55	+	55.30	98.93
291	TG(18:0/16:1/18:1)+ NH <sub>4</sub>	TG	55	+	54.06	98.89
292	TG(18:0/18:0/18:0)+ NH <sub>4</sub>	TG	57	+	0.00	0.00
293	TG(18:0/18:0/18:1)+ NH <sub>4</sub>	TG	57	+	51.06	98.83
294	TG(18:0/18:1/18:1)+ NH <sub>4</sub>	TG	57	+	53.09	98.90
295	TG(18:0/18:1/18:2)+ NH <sub>4</sub>	TG	57	+	52.89	98.89
296	TG(18:0/18:1/20:4)+H	TG	59	+	0.00	0.00
297	TG(18:1/14:0/14:0)+ NH <sub>4</sub>	TG	49	+	65.05	99.13
298	TG(18:1/17:1/18:1)+ NH <sub>4</sub>	TG	56	+	52.89	98.87
299	TG(18:1/17:1/18:2)+ NH <sub>4</sub>	TG	56	+	52.67	98.86
300	TG(18:1/17:1/20:5)+H	TG	58	+	0.81	92.04
301	TG(18:1/18:1/18:2)+ NH <sub>4</sub>	TG	57	+	52.43	98.87
302	TG(18:1/18:1/19:2)+ NH <sub>4</sub>	TG	58	+	50.74	98.84
303	TG(18:1/18:1/19:3)+H	TG	58	+	0.00	0.00
304	TG(18:1/18:1/19:5)+ NH <sub>4</sub>	TG	58	+	0.64	91.66
305	TG(18:1/18:2/18:2)+ NH <sub>4</sub>	TG	57	+	50.10	98.79
306	TG(18:1/18:2/18:3)+H	TG	57	+	0.00	0.00
307	TG(18:1/18:2/18:3)+ NH <sub>4</sub>	TG	57	+	49.27	98.77
308	TG(18:1/18:2/20:3)+ NH <sub>4</sub>	TG	59	+	48.49	98.78
309	TG(18:1/18:2/22:0)+ NH <sub>4</sub>	TG	61	+	50.57	98.89
310	TG(18:1/18:3/18:3)+H	TG	57	+	0.00	0.00
311	TG(18:1/18:3/19:5)+ NH <sub>4</sub>	TG	58	+	0.00	0.00

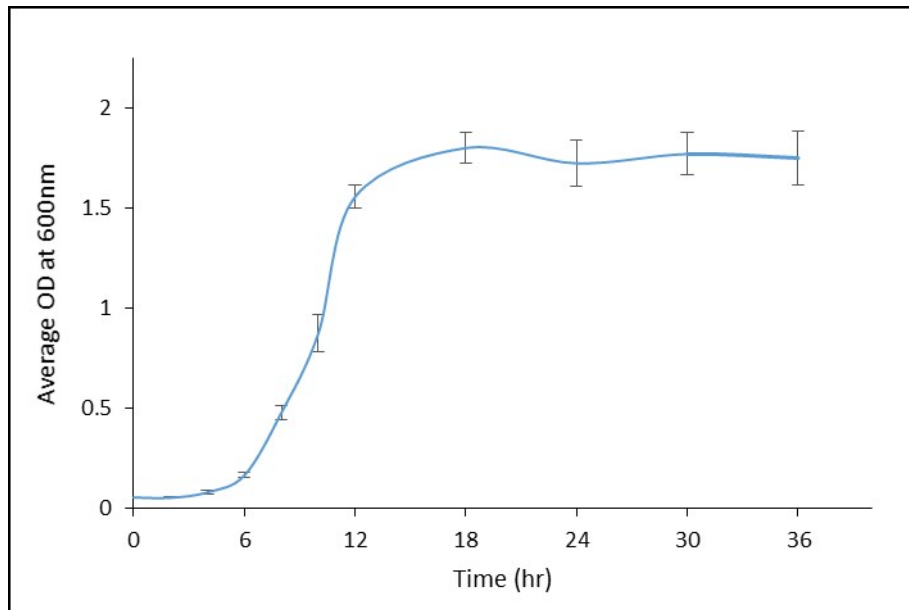
312	TG(18:1/18:3/22:0)+ NH <sub>4</sub>	TG	61	+	47.34	98.78
313	TG(18:1/18:3/24:0)+ NH <sub>4</sub>	TG	63	+	45.07	98.74
314	TG(18:1/20:4/22:0)+H	TG	63	+	0.00	0.00
315	TG(18:1/20:4/22:1)+H	TG	63	+	0.00	0.00
316	TG(18:2/17:1/18:2)+ NH <sub>4</sub>	TG	56	+	50.67	98.79
317	TG(18:2/17:3/17:3)+ NH <sub>4</sub>	TG	55	+	0.00	0.00
318	TG(18:3/17:1/18:2)+ NH <sub>4</sub>	TG	56	+	50.09	98.77
319	TG(18:3/17:2/18:2)+ NH <sub>4</sub>	TG	56	+	47.53	98.68
320	TG(18:3/17:3/17:3)+ NH <sub>4</sub>	TG	55	+	2.95	93.79
321	TG(18:3/17:3/18:2)+ NH <sub>4</sub>	TG	56	+	45.19	98.59
322	TG(18:3/17:3/18:3)+ NH <sub>4</sub>	TG	56	+	42.22	98.47
323	TG(18:3/17:3/20:5)+H	TG	58	+	0.00	0.00
324	TG(18:3/18:2/18:2)+ NH <sub>4</sub>	TG	57	+	47.59	98.71
325	TG(18:3/18:2/18:2)+ NH <sub>4</sub>	TG	57	+	1.45	92.84
326	TG(18:3/18:2/18:3)+ NH <sub>4</sub>	TG	57	+	47.07	98.69
327	TG(18:3/18:3/18:3)+H	TG	57	+	0.00	0.00
328	TG(18:3/18:3/18:3)+ NH <sub>4</sub>	TG	57	+	45.90	98.64
329	TG(18:3/18:3/20:5)+H	TG	59	+	0.05	87.89
330	TG(18:4/16:1/16:1)+H	TG	53	+	0.00	0.00
331	TG(18:4/16:1/18:3)+H	TG	55	+	0.00	0.00
332	TG(18:4/16:2/18:3)+H	TG	55	+	0.00	0.00
333	TG(18:4/17:1/18:1)+H	TG	56	+	0.13	88.81
334	TG(18:4/17:2/18:3)+H	TG	56	+	0.00	0.00
335	TG(18:4/18:2/18:3)+ NH <sub>4</sub>	TG	57	+	0.00	0.00
336	TG(18:4/18:3/18:3)+H	TG	57	+	0.00	0.00
337	TG(18:4/18:3/18:3)+ NH <sub>4</sub>	TG	57	+	0.00	0.00
338	TG(19:0/18:1/18:1)+ NH <sub>4</sub>	TG	58	+	43.47	98.57
339	TG(19:1/16:0/18:3)+H	TG	56	+	0.00	0.00
340	TG(19:1/18:1/18:1)+ NH <sub>4</sub>	TG	58	+	46.29	98.68
341	TG(19:1/18:1/18:2)+ NH <sub>4</sub>	TG	58	+	41.30	98.49
342	TG(19:1/18:1/18:3)+ NH <sub>4</sub>	TG	58	+	51.67	98.87
343	TG(19:3/18:2/18:3)+ NH <sub>4</sub>	TG	58	+	0.80	92.02
344	TG(19:4/18:2/18:3)+H	TG	58	+	0.00	0.00
345	TG(19:4/18:3/18:3)+ NH <sub>4</sub>	TG	58	+	0.00	0.00
346	TG(19:5/18:2/18:3)+H	TG	58	+	0.00	0.00
347	TG(20:0/18:1/18:1)+ NH <sub>4</sub>	TG	59	+	41.18	98.51
348	TG(20:0/18:1/18:3)+ NH <sub>4</sub>	TG	59	+	37.58	98.35
349	TG(20:1/18:1/18:1)+ NH <sub>4</sub>	TG	59	+	0.00	0.00
350	TG(20:1/18:2/18:2)+ NH <sub>4</sub>	TG	59	+	46.05	98.69
351	TG(20:3/18:2/18:2)+ NH <sub>4</sub>	TG	59	+	41.89	98.54
352	TG(20:5/17:1/18:2)+H	TG	58	+	0.00	0.00
353	TG(24:3/18:1/18:3)+H	TG	63	+	0.00	0.00
354	TG(24:4/16:0/16:0)+ NH <sub>4</sub>	TG	59	+	0.00	0.00
355	TG(54:11)+ NH <sub>4</sub>	TG	57	+	0.00	0.00
356	TG(55:10p)+H	TG	58	+	0.00	0.00
357	TG(55:11)+ NH <sub>4</sub>	TG	58	+	0.44	91.07

**Table S5. The VIP values of significantly changing lipid species before and after normalisation on data acquired on three separate days.**

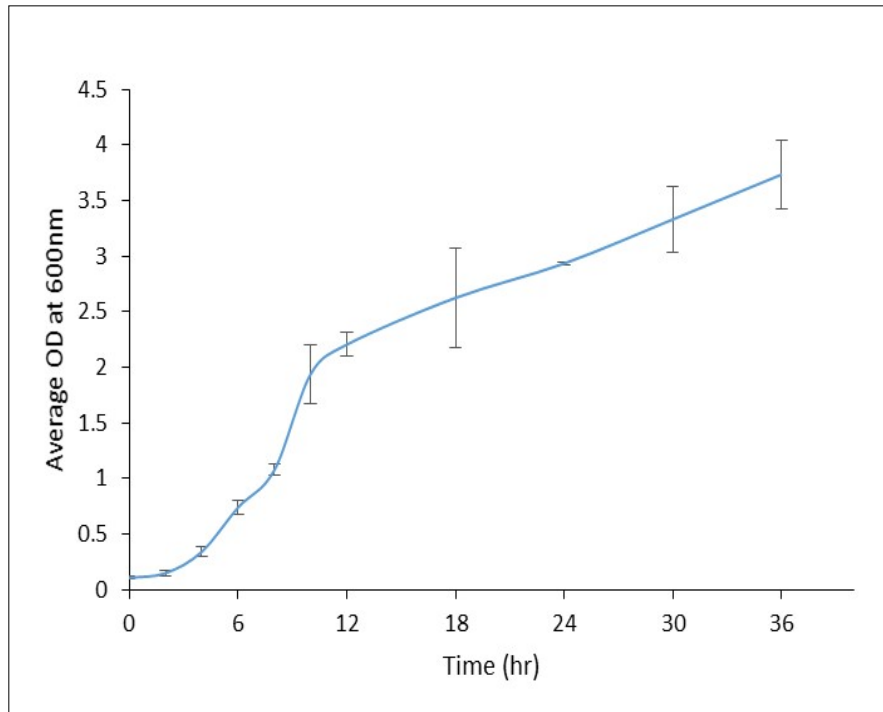
Lipid species	Raw	TIC	<sup>13</sup> C-IS
FA(18:2)-H	1.4852	2.2246	0.0010
LPC(16:0)+H	6.0870	0.2225	0.5849
LPC(16:0)+Na	1.2142	0.8755	0.9661
LPC(18:0)+H	2.2400	0.5722	2.0485
PC(34:1)+H	2.0250	3.3773	0.0493
PC(34:2)+H	2.9357	5.6058	0.0062
PC(36:2)+H	2.2591	3.8371	0.0224
PC(36:3)+H	1.0649	3.0939	0.0014
PC(36:4)+H	1.7327	2.2890	0.0002
PC(38:4)+H	1.2105	1.5941	1.3170
TG(16:0)/(16:0)/(16:1)+NH <sub>4</sub>	1.2865	0.8146	0.1529
TG(16:0)/(18:1)/(18:1)+NH <sub>4</sub>	4.1338	2.9869	0.0268
TG(16:0)/(18:2)/(18:2)+NH <sub>4</sub>	2.9488	2.2587	0.0085
TG(16:1)/(16:1)/(18:1)+NH <sub>4</sub>	1.6273	1.5207	0.0033
TG(18:1)/(18:2)/(18:2)+NH <sub>4</sub>	1.1443	0.8173	0.0056



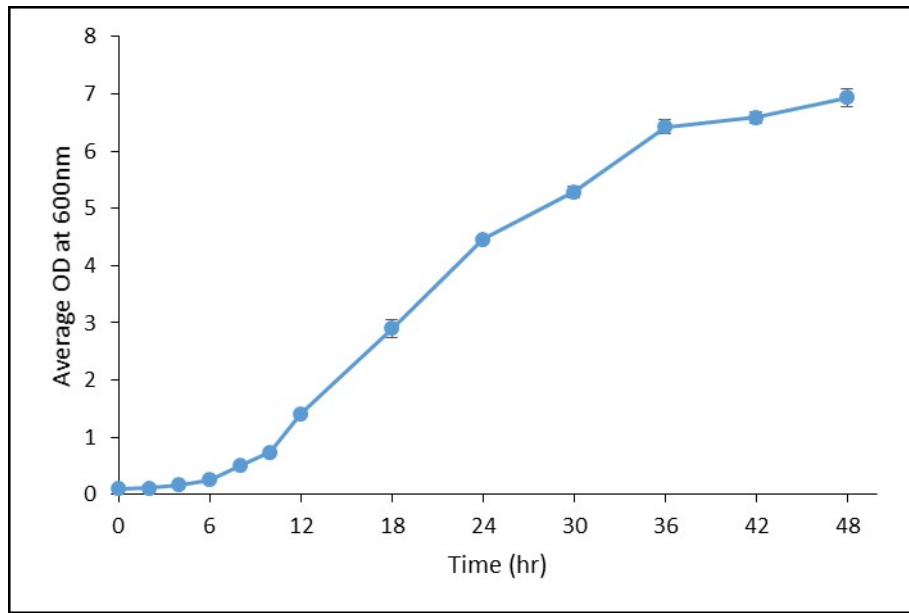
**Figure S1.** Total ion current (TIC) chromatograms of all microorganism extracts with ESI in negative (in black) and positive mode (in red). A) *E. coli*; B) *A. platensis* (spirulina); C) *P. pastoris*; D) *S. cerevisiae* CEN.PK113-7D. The intersections of the lipids dataset from HMDB and each strain are represented by circles. The pie charts show the total number of lipids (in the centre) extracted from each strain and identified by LipidSearch in positive and negative mode that are in common to HMDB.



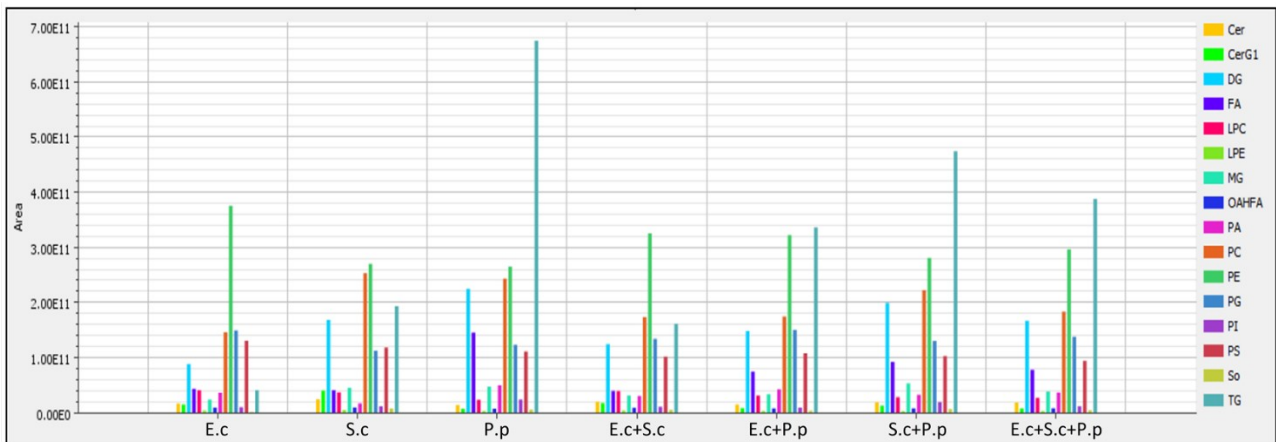
**Figure S2.** Growth curve of *E. coli* MG1655 in minimal media (n=3). The OD<sub>600</sub> of the culture was measured every 2 h for 36 h to investigate the growth rate of *E. coli* in minimal media. This media can support *E. coli* growth and the cells enter the stationary phase after ~12 h of inoculation.



**Figure S3.** Growth curve of *S. cerevisiae* CEN.PK 113-7D in selected minimal media (n=3). The OD<sub>600</sub> was measured every 2 h for 36 h to investigate the growth rate of *S. cerevisiae* in minimal media. This media can support *S. cerevisiae* growth and the cells enter the stationary phase after ~12 h of inoculation.

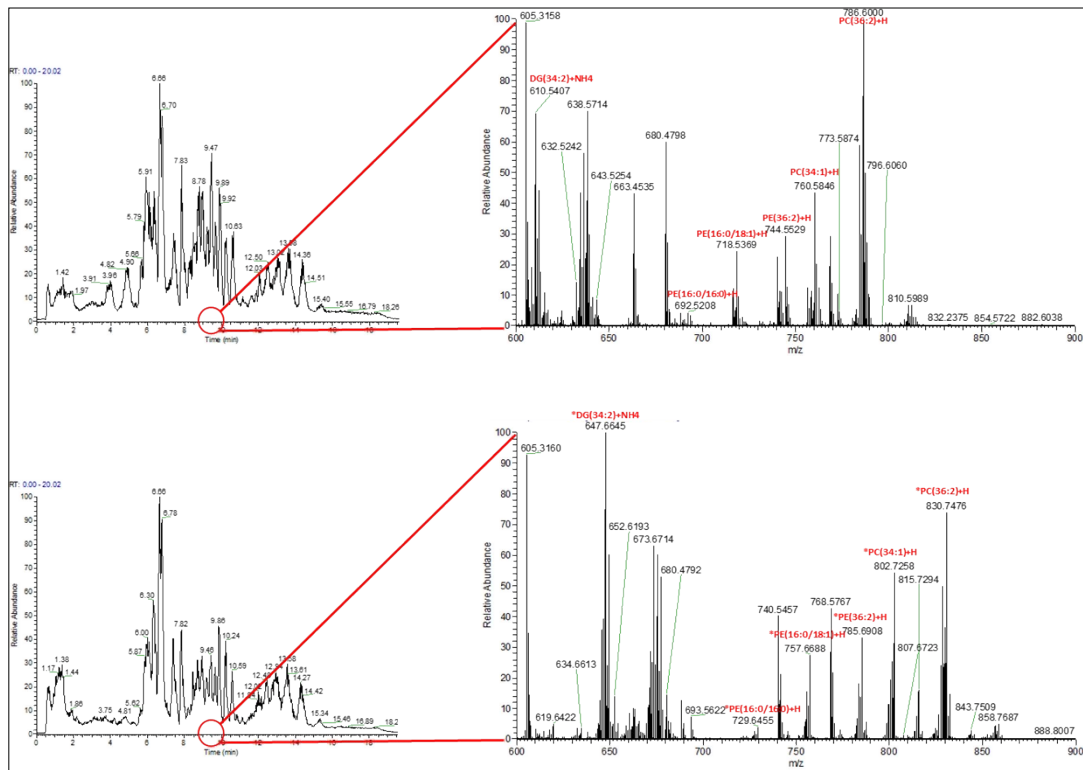


**Figure S4.** Growth curve of *P. pastoris* NCYC175 in selected minimal media (n=3). The OD<sub>600</sub> of the culture was measured every 2 h for 48 h to investigate the growth rate of *P. pastoris* in minimal media. This media can support *P. pastoris* growth and the cells enter the stationary phase after ~36 h of inoculation.

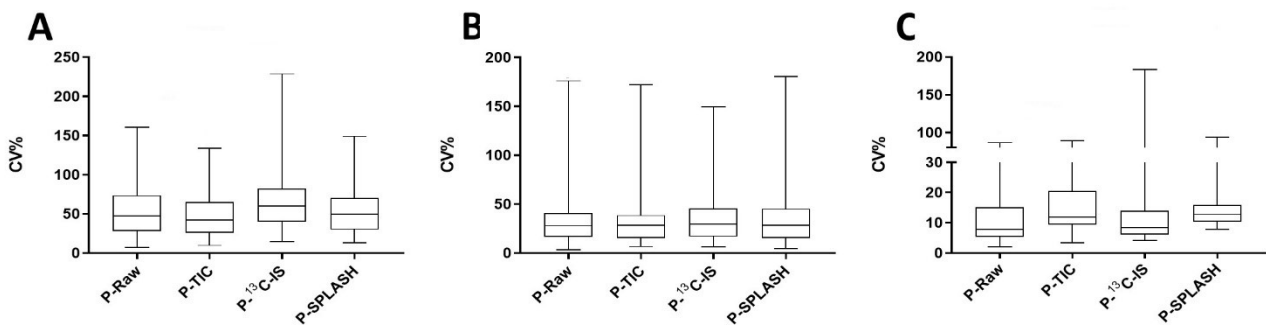


**Figure S5.** Lipid class profiles of *E. coli* MG1655, *S. cerevisiae* CEN.PK 113-7D and *P. pastoris* NCYC 175, and different combinations of these species. Abbreviations: E.c- *E. coli* extract, S.c- *S. cerevisiae* CEN.PK extract, P.p- *P. pastoris* extract.





**Figure S6.** Comparison of LC-MS chromatogram (left side) and full scan MS spectra (right side) of recorded unlabelled (top) and labelled (bottom) *P. pastoris* extract in positive mode between 9-10 min within the  $m/z$  range of 600-900.



**Figure S7.** Coefficient of variance distributions for normalization of different set of samples using different methods presented by box and whisker plots. Data normalized by TIC (P-TIC),  $^{13}\text{C}$ -IS (P- $^{13}\text{C}$ -IS), and by SPLASH® solution (P-SPLASH) were compared to raw un-normalized data. **(A)** Common ions (112) between human plasma samples and  $^{13}\text{C}$ -yeast extract ( $n=6$ ); **(B)** Common ions (132) between mouse plasma samples and  $^{13}\text{C}$ -yeast extract ( $n=6$ ); **(C)** Common ions (102) between pooled plasma samples and  $^{13}\text{C}$ -yeast extract.