Supplementary information

Combining Raman and FT-IR spectroscopy with quantitative isotopic labelling for differentiation of *E. coli* cells at community and single cell levels

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Abstract

There is no doubt that the contribution of microbially-mediated bioprocesses towards maintenance of life on earth is vital. However, understanding these microbes *in situ* is currently a bottleneck as most methods require culturing these microorganisms to suitable biomass levels so that the microbial phenotype can be measured. The development of new culture-independent strategies such as stable isotope probing (SIP) coupled with molecular biology has been a break through towards linking gene to function, whilst circumventing *in vitro* culturing. In this study we have combined Raman spectroscopy and Fourier transform infrared (FT-IR) spectroscopy, as metabolic fingerprinting approaches, with SIP to demonstrate the quantitative labelling and differentiation of *Escherichia coli* cells. *E. coli* cells were grown in minimal medium with fixed final concentrations of carbon and nitrogen supply, but with different ratios and combinations of $^{13}C/^{12}C$ glucose and $^{15}N/^{14}N$ ammonium chloride, as the sole carbon and nitrogen sources respectively. The cells were collected at stationary phase and examined by Raman and FT-IR spectroscopies. The multivariate analysis investigation of FT-IR and Raman data illustrated unique clustering patterns resulting from specific spectral shifts upon the incorporation of different isotopes, which were directly correlated with the ratio of the isotopically labelled content of the medium. Multivariate analysis results of the single-cell Raman spectra followed the same trend, exhibiting a separation between *E. coli* cells labelled with different isotopes.

Supplementary information

Tables

Table S1. Details of minimal medium with varying ratios and combinations of ^{13}C-glucose and ^{15}N-ammonium chloride examined in this study. The concentrations of the labelled compounds are presented as percentage ratios of the total glucose and ammonium chloride in the medium.

Growth conditions	^{13}C-glucose %	^{15}N-ammonium chloride %
1	0	0
2	0	10
3	0	20
4	0	30
5	0	40
6	0	50
7	0	60
8	0	70
9	0	80
10	0	90
11	0	100
12	10	0
13	20	0
14	30	0
15	40	0
16	50	0
17	60	0
18	70	0
19	80	0
20	90	0
21	100	0
22	50	50
23	100	50
24	50	100
25	100	100

Table S2. Major FT-IR spectral shifts in wavenumbers detected due to ^{13}C and ^{15}N incorporation.

Unlabelled (cm^{-1})	^{15}N (Δ cm^{-1})	^{13}C (Δ cm^{-1})	Assignment
2961	0	-10	Fatty acids (CH_3 asym. str)
2924	0	-8	Fatty acids (CH_2 asym. str)
2855	0	-6	Fatty acids (CH_2 asym. str)
1655	0	-39	Amide I (C=O)
1547	-15	-12	Amide II (C-N, N-H)
1398	0	-27	Fatty acids and amino acids (COO^-)
1242	-4	-6	Amide III (C-N, N-H)

Supplementary information

Table S3. Major Raman spectral shifts in wavenumber shifts detected due to ^{13}C and ^{15}N incorporation.

Unlabelled (cm^{-1})	^{15}N (Δ cm^{-1})	^{13}C (Δ cm^{-1})	Assignment
783	-5	-14	Cytosine, uracil (C=O, C-N and ring deformation)
1003	0	-36	Phenylalanine (ring breathing)
1250	-21	-14	Amide III (C-N stretch, N-H bend)
1336	-17	-17	Adenine (C-N stretch, C-H and N-H bend)
1574	-8	-47	Nucleic acids
1661	0	-36	Amide I, unsaturated lipids (C=O)

Figures

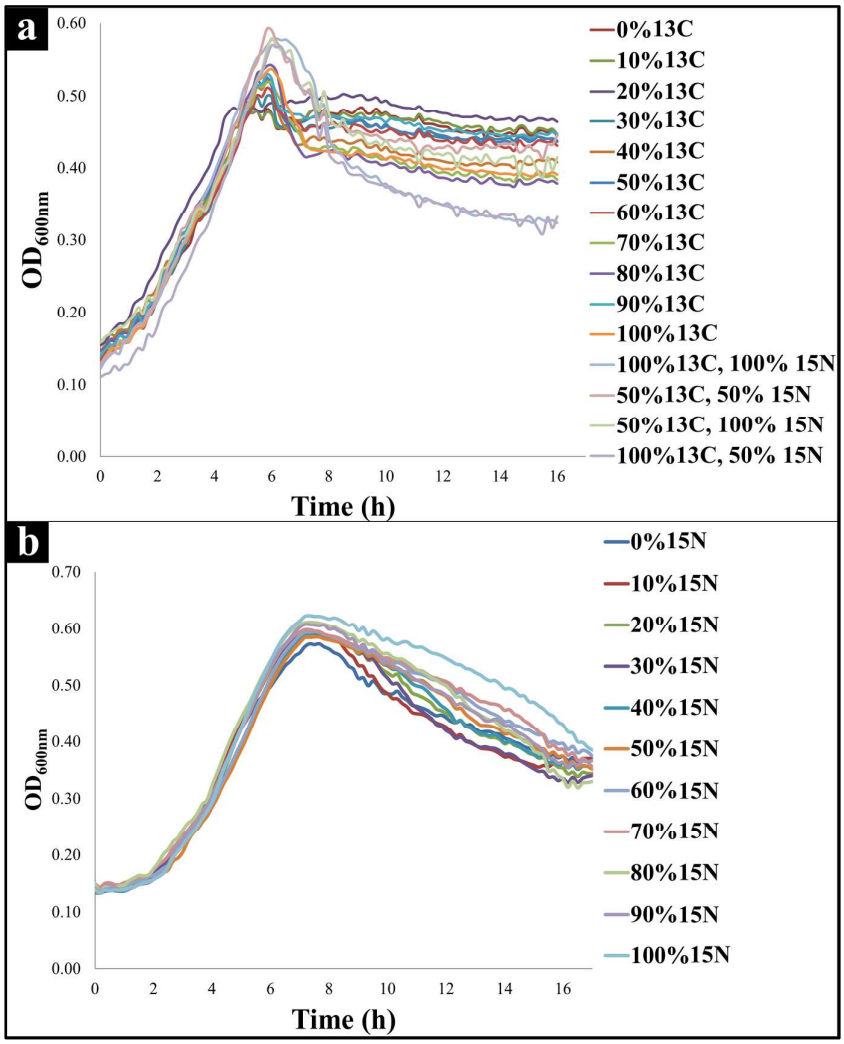

Figure S1. Growth profiles of *E. coli* K-12 on defined medium with varying ratios of; **a)** $^{13}C/^{12}C$ glucose and combinations of ^{15}N ammonium chloride, **b)** $^{15}N/^{14}N$ ammonium chloride. Each growth profile is average of 10 biological replicates.

Supplementary information

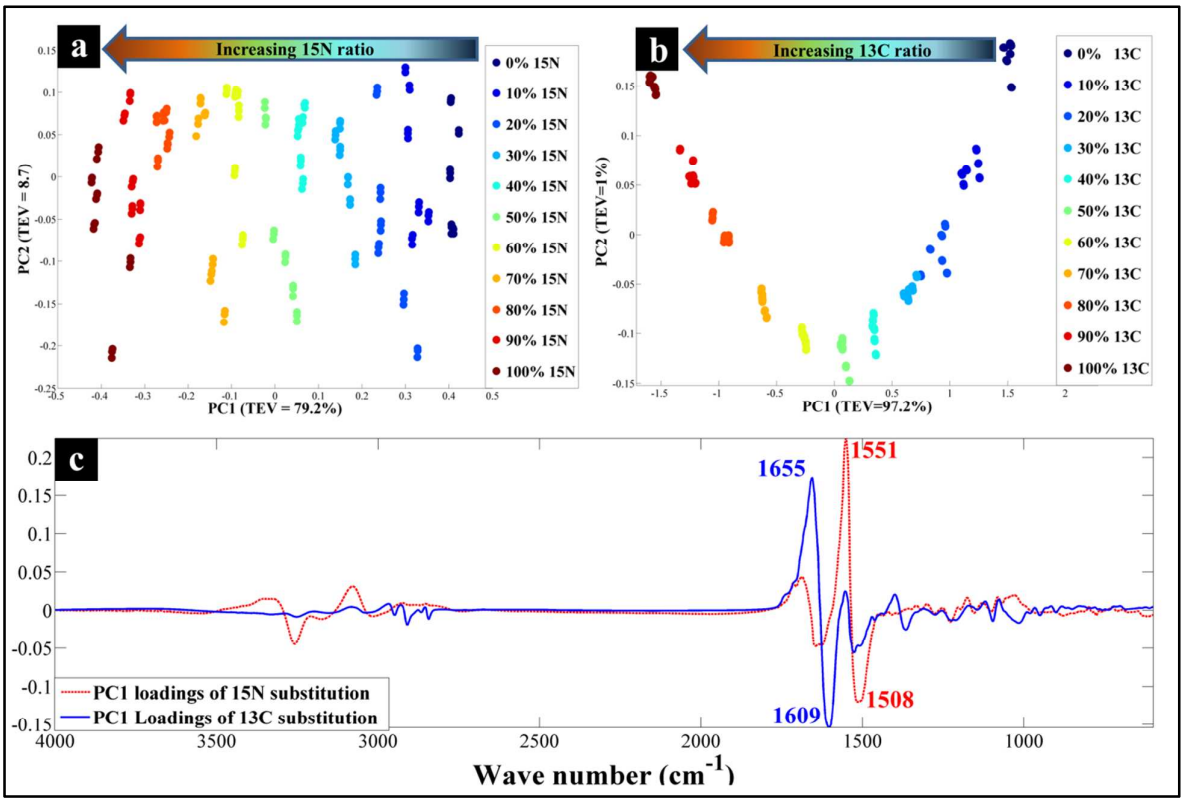

Figure S2. PCA scores plots and PC1 loadings plots of FT-IR spectral data of *E. coli* cells grown on ^{15}N-ammonium chloride **(a, c)** and ^{13}C-glucose **(b, c)** substitution conditions. PC1 loadings plot of ^{15}N substitution conditions (c, dashed red line) demonstrates the shift in the amide II (1551 cm^{-1}) to lower wavenumber as the major variable, whilst the PC1 loadings of ^{13}C substitution (c, blue line) suggests amide I (1655 cm^{-1}) as the most significant variable affecting the clustering pattern of the data.

Supplementary information

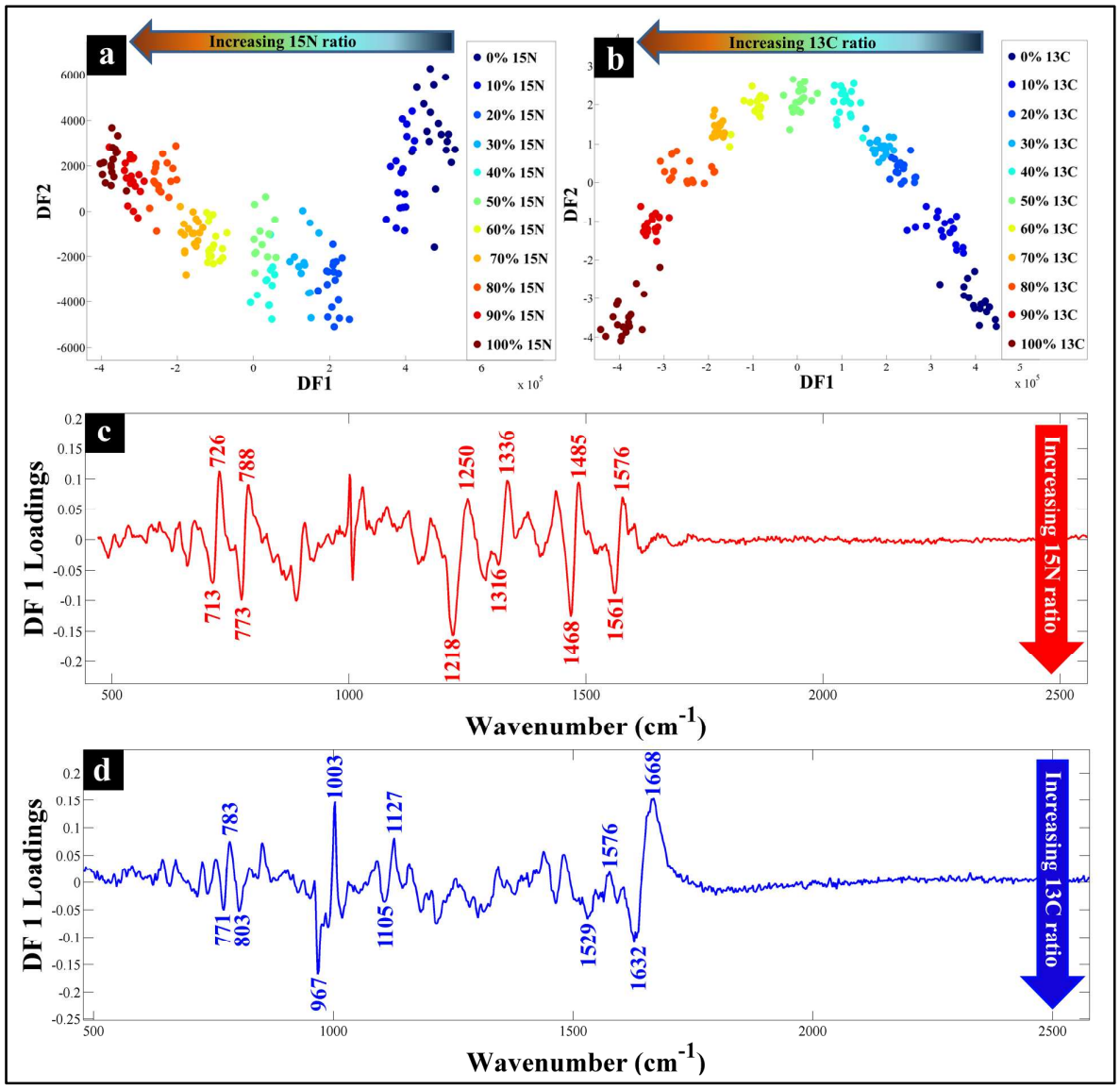

Figure S3. PC-DFA scores plots and DF1 loadings plots of Raman spectral data of *E. coli* cells grown on ^{15}N-ammonium chloride **(a, c)** and ^{13}C-glucose **(b, d)** substitution conditions. DF1 loadings plots of ^{15}N and ^{13}C substitution conditions (c, d) demonstrates the significant spectral bands shifted to lower wavenumber upon the incorporation of the heavy isotopes.