Insights into Microbial Functioning using Raman Stable Isotope Probing (R-SIP) at the Single Cell Level

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Introduction

Raman spectroscopy is a rapid, noninvasive technique that measures the inelastic scattering of light following the illumination of a sample with a monochromatic laser beam. This technique can determine the chemical composition of a sample as a result of molecular vibrations based upon bonding configuration, generating unique Raman fingerprints.

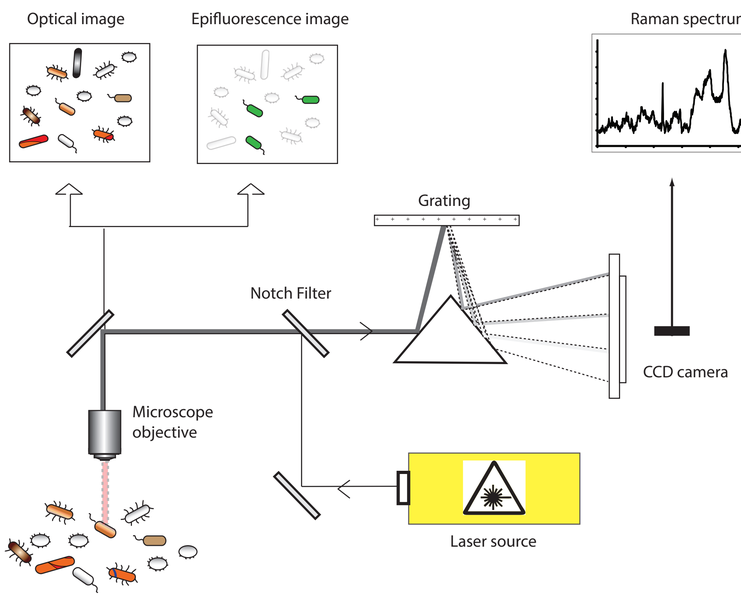
One useful property of Raman spectroscopy is that heavy stable isotopes exhibit unique Raman spectra compared to their natural abundance counterparts, due to a 'red-shift' in Raman peaks towards longer wavelengths, and this can be exploited by microbial ecologists to study microbial processes through Raman Stable Isotope Probing (R-SIP).

Here we show the application of R-SIP for detecting isotopically-labelled substrate incorporation into microbial cells in the lab, expanding the range of previously used stable isotopes.

Methods

A schematic diagram of a Raman microspectrometer (Fig 1.) shows a monochromatic laserbeam (532 nm) was used to probe single and clusters of microbail cells dried onto CaF_2 slides. Spectra from single cells was collected in 60-120s. Mapping of cells took place using a x-y motorised stage with 0.2 µm steps and 0.5s exposure per point.

Fig 1.



Previous work using Raman Stable Isotope Probing has been limited to using ^{13}C labelled substrates. To expolore if this technique could be expanded to a wider range of isotopes, we conducted cellular labelling experiments using ^{13}C-glucose, ^{15}N-Ammonium chloride, ^{2}H-water (deuterium oxide), and ^{18}O-water. One µl of log phase *Escherichia coli* was transfered to M9 minimal media containing the isotopically labelled substrate. The labelled culture was then grown up to stationary phase, harvested and washed before drying on a slide for sampling and imaging.

Fig 2.

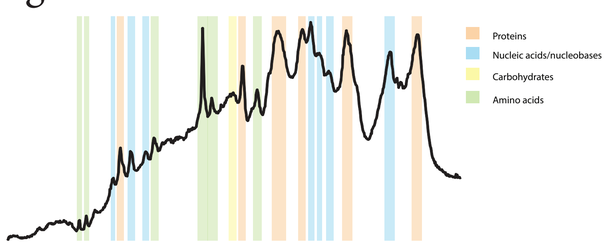


Fig 3.

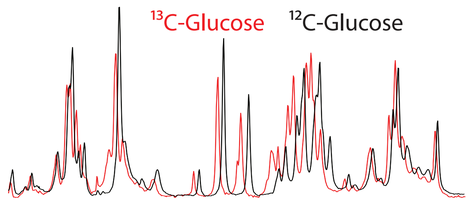


Fig 2. Shows a typical Raman spectrum from *E. coli*, showing major peaks assigned to proteins, nucleic acids, carbohydrates, and amino acids. Fig 3. illustrates the 'red-shift' experienced when a heavy ^{13}C stable isotope of glucose (red) is compared to a lighter ^{12}C isotope (black).

Results

Fig 4.

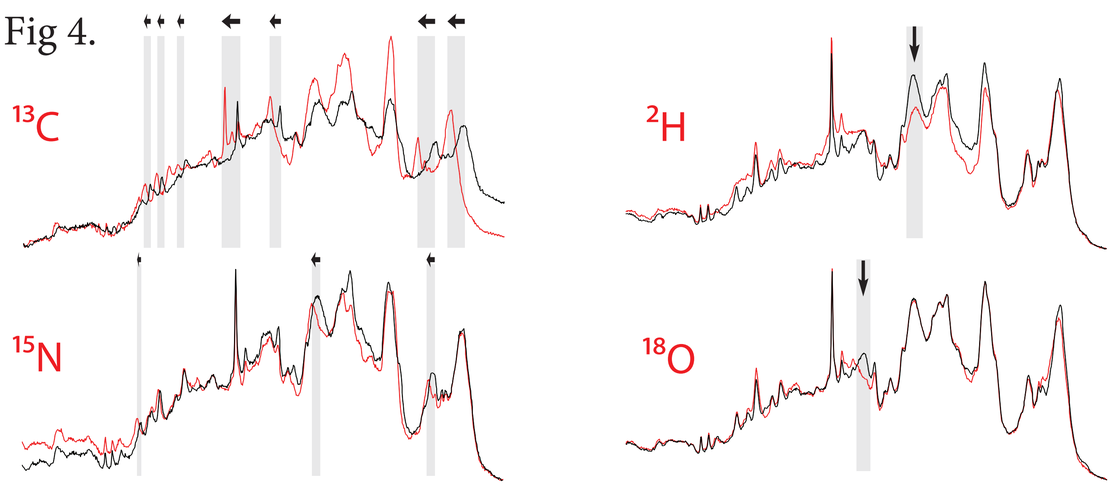
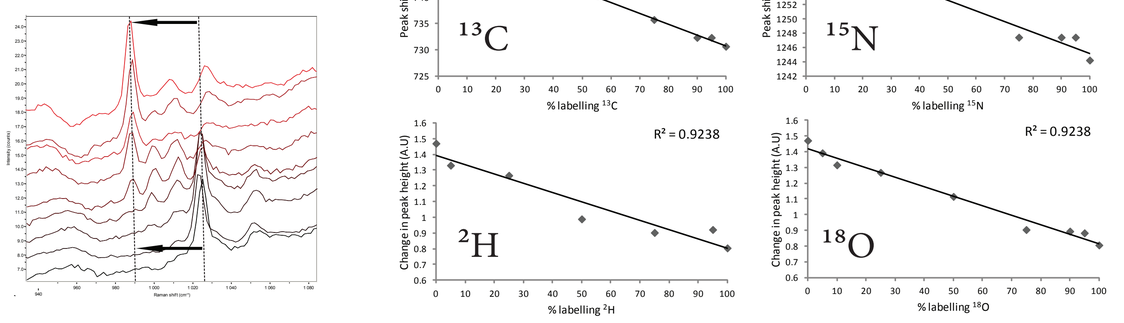


Fig 4. shows the Raman red-shift associated with isotope uptake into *Escherichia coli* biomolecules with heavy isotopes (**red**) compared to their more abundant and lighter stable isotopes (**black**). Heavy isotopes ^{13}C, and to a lesser extent ^{15}N show 'red-shifts' whereas ^{2}H, and ^{18}O show a quantitiavie downwards shifts in peak intensity in C-O stretching (^{18}O) and Amide III random coil (^{2}H). Calibration curves in Fig 5 shows the quantitative nature of this technique.

Fig 5.



The figures below show the incorporation of stable isotopes into natural microbial communities. Fig 6. shows the result of a Raman map across a natural mixed-species biofilm from Movile Cave, Romania, after labelling with ^{13}C-Methane, where yellow shows isotopically labelled cells, blue unlabelled cells and red Eukaryotic cells. Fig 7. shows single cell mapping of a soil biofilm grown on a glass slide.

Fig 6.

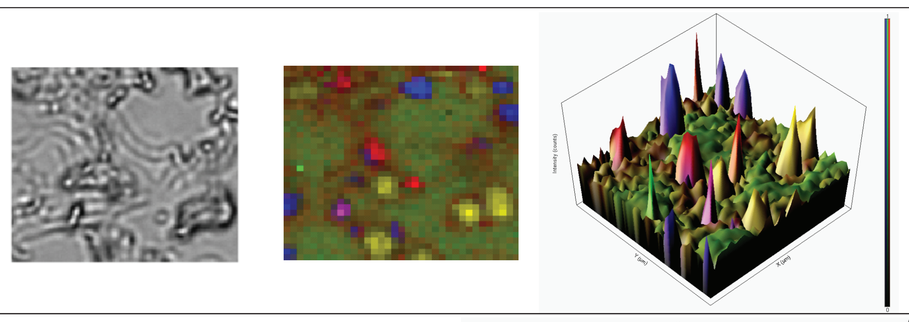
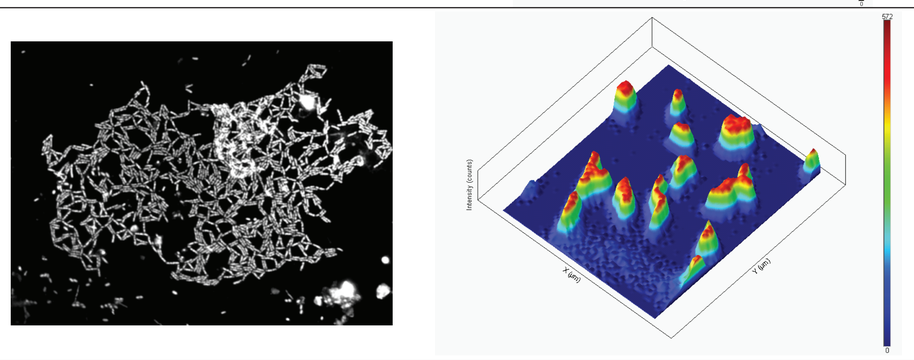


Fig 7.



Conclusions

Raman Stable Isotope Probing (R-SIP) is a versatile and powerful tool for investigating microbial functioning at the single-cell scale. The toolbox of isotopes that can be used for this technique is potentially diverse, and may have many applications for studying different ecological pathways in natural microbial ecosystems. The ability to probe communities at the single-cell level, and even in live, actively growing communities such as biofilms may broaden the scope of this technique even further.

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