Estimation of hydrogen isotope enrichment in proteins and lipids in a microbial community

Ben Bowen*(BPBowen@lbl.gov)[2], Curt Fischer[1], Chongle Pan[3], Bob Hettich[3], Trent Northen[2], **Jillian F. Banfield**[1,2]

[1]University of California, Berkeley, CA 94720, USA. [2]Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA.[3]Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA.

In the biosynthesis of lipids and amino acids, microorganisms may preferentially use one of the hydrogen stable isotopes (^{2}H and ^{1}H or D and H). This results in different fractions of hydrogen stable isotopes in synthesized biomass versus water in the growth medium. Isotope preference partially stems from the physiology of the microorganisms. Autotrophic microorganisms, for example, preferentially incorporate ^{1}H during lipid biosynthesis, possibly resulting from reactions involving NAD(P)H that forms isotopically light acetyl-CoA. Stable isotope fractionation is traditionally measured using isotope ratio mass spectrometry. However, it is challenging to measure and identify unknown molecules in a complex sample using this technique. Here we present a new approach for measuring hydrogen fractionation in complex samples of proteins and lipids from an acidophilic microbial community in acid mine drainage.

The analyses targeted coexisting bacteria and archaea grown in a 4 atom% ^{2}H-enriched medium. To measure average hydrogen fractionation in amino acids, the community proteome was digested using trypsin and measured using 2-dimension liquid-chromatography coupled with high-resolution tandem mass spectrometry analysis on an Orbitrap Velos. The Sipros algorithm (code.google.com/p/sipro) was used to search each MS^2 spectrum against all peptide sequences in the AMD protein database at every ^{2}H atom% level in 1% precision. The ^{2}H enrichment and sequence of 4,477 unique peptides from 36,090 spectra were identified. Further, to more precisely quantify isotopic enrichment, a MATLAB program was developed to find the optimum ^{2}H values which best fit the isotopic distributions of identified peptides in MS and MS^2 spectra. The results indicated that the microbial community has an average ^{2}H depletion of -360 ‰ relative to water in the growth medium for proteins. We applied a similar technique to the analysis of lipids identified using LCMS, and the lipids were found to be isotopically light by -260 ‰ relative to water in the growth medium.

This difference between -260 ‰ ^{2}H fractionation in lipids and -360 ‰ in proteins suggests that additional factors, that do not involve NAD(P)H, are also contributing to ^{2}H/^{1}H fractionation. Further, autotrophic bacteria sharing 94% 16S rRNA gene sequence identity displayed statistically significant differences in protein hydrogen isotope fractionation. This suggests these microbes have different metabolic traits determined by their distinct ecological niches. In addition, it was found that heterotrophic, archaeal members of the community had isotopically light protein and were significantly different from coexisting bacteria. Potentially, this could be attributed to metabolite transfer from autotrophs and unknown aspects of fractionation associated with iron reduction. Differential fractionation of hydrogen stable isotopes into metabolites and proteins may reveal trophic levels of members of microbial communities. The approach developed here

provided insights into the metabolic characteristics of organisms in natural communities, and may be applied to analyze other systems.

This work was supported by Genome Sciences Program in Carbon Cycling (contract number DE- SC0004665).