



Conference Abstract

Studying the isotopic composition of microbial methane with a genetically-tractable methanogen

Jonathan Gropp^{‡,§}, Markus Bill^l, Daniel A Stolper[‡], Dipti D Nayak[§]

[‡] Department of Earth and Planetary Science, University of California Berkeley, Berkeley, CA, United States of America

[§] Department of Molecular and Cell Biology, University of California Berkeley, Berkeley, CA, United States of America

^l Energy Geosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA, United States of America

Corresponding author: Jonathan Gropp (ja.gropp@gmail.com)

Received: 24 Jun 2023 | Published: 16 Oct 2023

Citation: Gropp J, Bill M, Stolper DA, Nayak DD (2023) Studying the isotopic composition of microbial methane with a genetically-tractable methanogen. ARPHA Conference Abstracts 6: e108567.

<https://doi.org/10.3897/aca.6.e108567>

Abstract

Nearly all biogenic methane is produced by a group of microorganisms called methanogenic archaea (or methanogens). Methanogens can use a variety of substrates, such as $H_2 + CO_2$, acetate, and methylated compounds, for methanogenesis. Previous studies have shown that the stable carbon and hydrogen isotopic compositions of methane produced by methanogens can vary drastically depending on the substrate composition and concentration in the environment. For instance, the concentration of H_2 in the environment has a substantial impact on the isotopic composition of methane derived from hydrogenotrophic methanogenesis (reduction of CO_2 to methane using H_2 as the electron donor) (Valentine et al. 2004, Penning et al. 2005). While there is substantial empirical data on isotopic signatures of methane from different substrates and under different conditions, the physiological and molecular features that control these values are not as well understood. To address this, we are using the metabolically diverse and genetically tractable methanogen, *Methanosarcina acetivorans* as a model system to uncover key cellular processes that control the stable bulk isotopic composition of methane (i.e., $^{13}C/^{12}C$ and D/H ratios), and the distributions of the “clumped” $^{13}CH_3D$ and $^{12}CH_2D_2$ isotopologues.

The methanogen *M. acetivorans* grows on a wide variety of compounds such as acetate, methanol, methylamines, and methylsulfides. We found that the methylotrophic pathways

(for methanol and trimethylamine) and the acetoclastic pathway have large and similar primary hydrogen isotopic effects (α of ~ 0.45). These data are in contrast to previous findings and imply a minor isotopic exchange between CH_4 and H_2O (Valentine et al. 2004, Gruen et al. 2018). Focusing first on the methylotrophic pathway, we generated mutants of two key enzymes in the methylotrophic pathway: a) methyl coenzyme M reductase (Mcr) that catalyzes the last step in methanogenesis and b) methyltransferases that catalyze the first step in methylotrophic methanogenesis from methanol (Mta). A mutant with reduced Mcr expression had no observable change in the hydrogen isotopic effect relative to the wild-type, validating the initial observation of minimal H_2O - CH_4 hydrogen isotopic exchange. One of the Mta mutants, which only expressed a specific methyltransferase isoform, had a smaller carbon isotopic effect relative to the other isoforms (α of ~ 1.074 vs. ~ 1.080). Since the isoforms are thought to be identical in structure, the different isotopic effects could result from differential expression of each isoform, or from different kinetic properties. By combining our genetic approaches with traditional and high-resolution isotopic analytical methods, we aim to develop a quantitative understanding of the mechanisms that control the isotopic compositions of biological methane. Our preliminary results show that *M. acetivorans* would be an ideal candidate for such research, which could help in understanding methanogens' physiology in natural environments in past, present, and future Earth.

Keywords

methane, isotopes, CRISPR/Cas9, clumped

Presenting author

Jonathan Gropp

Conflicts of interest

The authors have declared that no competing interests exist.

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