

Isotopic Biosignatures in Geothermal Springs as Analogues for Early Earth: Examples from Western Canada. A.L. Brady¹, C.E. Sharp², S.E. Grasby⁴, P.F. Dunfield³ and G.F. Slater¹, ¹School of Geography and Earth Sciences, McMaster University, 1280 Main St. West, Hamilton, Ontario, Canada L8S 4K1 bradyal@mcmaster.ca, ²Energy Bioengineering Group, University of Calgary, 2500 University Drive N.W., Calgary, Alberta, Canada T2N 1N4 ³Department of Biological Sciences, University of Calgary, 2500 University Drive N.W., Calgary, Alberta, Canada T2N 1N4, ⁴Geological Survey of Canada, 3303 33rd Street N.W., Calgary, Alberta, Canada T2L 2A7.

Introduction: Carbon and energy sources (e.g. CH₄, CO, H₂) derived from the deep sub-surface are potential drivers of surface communities found in terrestrial geothermal ecosystems, making them relevant analogues for early Earth. Observations of potential spring deposits on Mars also highlight the importance of geothermal springs as astrobiology targets to understand extinct spring associated communities [1,2]. Hot springs are ideal locations in which to investigate isotopic biosignatures that have the potential for long-term preservation in the geological record as many springs harbor both photosynthetic and chemotrophic communities. The abundance of hot spring environments in western Canada [3] presents an opportunity to investigate biomarkers associated with thermophilic microbial systems and to better characterize photosynthetic but potentially non-cyanobacteria dominated systems. Source water temperatures of springs collected predominantly from B.C. and the Yukon range from 30 to ~ 85°C, pH ~ 6.3 – 7.2 and exhibit variations in geochemistry and gas composition. 16S rRNA pyrosequencing of microbial mats and sediments from these previously uncharacterized springs revealed communities that include members of bacterial phyla such as *Cyanobacteria*, *Proteobacteria*, *Chloroflexi*, as well as Archaea.

Microbial phospholipid and glycolipid fatty acid (PLFA and GLFA) analysis was used to further characterize microbial metabolic capabilities and to identify ¹³C biosignatures of *in situ* autotrophy and/or heterotrophy. PLFA and GLFA profiles show variation between and within spring systems. Ubiquitous straight chain fatty acids, predominantly 16:0, are present in all samples (> 50% in some cases) but profiles show noticeable differences in the contribution of branched and monoenoic PLFA. In particular, high proportions of iso- and anteiso-branched PLFA indicative of gram-positive heterotrophic bacteria were present, ranging from ~ 10 to 50% of total PLFA. A dominance of iso-over anteiso- is particularly prevalent in samples with environmental temperatures > 50°C reflecting adaptations of membrane fluidity at high temperatures. GLFA profiles are likewise dominated by straight chain fatty acids and the specific fatty acids identified are comparable to those in the PLFA fraction. However, the abundance of compounds varies. In some cases, fatty

acids present in the PLFA fraction have decreased in abundance (e.g. i/a15:0) in the GLFA fraction and others were more prevalent in the GLFA fraction. While glycolipids may be produced by cyanobacteria, they are often classified as storage products [4]. As such, the GLFA detected may represent compounds with greater potential for long-term preservation as compared to PLFA from the same community.

PLFA $\delta^{13}\text{C}$ values show variability both within individual spring samples (e.g. PLFA ranges from -29 to -40‰) and between (16:0 varies from -16.1 to -39.4‰ in different springs). Large $\Delta^{13}\text{C}_{\text{org-PLFA}}$ offsets indicative of autotrophic synthesis (~ 6‰) are found in combination with smaller offsets associated with heterotrophic bacteria (~ 2-3‰). PLFA $\delta^{13}\text{C}$ values from mats with *Cyanobacteria* and *Chloroflexi* members are enriched relative to cyanobacteria dominated mats from other geothermal systems suggesting potential differences in dominant metabolisms and/or carbon sources.

Investigation into the identity and isotopic composition of organic components in geothermal communities builds our knowledge of the types of metabolisms present and associated signatures. Understanding the roles and relative contributions of different metabolisms (e.g. photosynthetic, chemolithoautotrophic) that lead to the formation of biosignatures that may be preserved in the geological record is of fundamental importance to reconstructing life in ancient geothermal systems.

References: [1] Allen C.C. and Oehler D.Z. (2008) *Astrobiology*, 8, 1093-1112. [2] Grasby S.E., Hutcheon I., Krouse H.R. (2000) *Appl. Geochem.*, 15, 439-454. [3] Grasby S.E., Richards B., Sharp C.E., Brady A.L., Jones G.M., Dunfield P.F. (2013) *Can. J. Earth Sci.*, 50, 94-108. [4] Kates M. (1964) *Adv. Lipid Res.*, 2, 17-90.