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LEWIS AND CLARK FUND FOR EXPLORATION AND FIELD RESEARCH IN ASTROBIOLOGY
ENVIRONMENTAL RELEVANCE OF LIPID BIOMARKERS IN HOT SPRING MARS ANALOGUES.

Final Report

Project Report

I collected one round of summer samples at both Lassen Volcanic National Park and Yellowstone National Park to study the unique community composition, lipid biomarker profile, and environmental parameters of acidic, sulfidic Mars-analogue hot springs. I was scheduled to sample again two more times in March and August for my winter and summer samples respectively however we had to cancel both field expeditions due to the public health crisis of COVID-19. I extracted these samples we were able to attain for both lipid composition and DNA. We sequenced these samples with the DNA Technologies and Expression Analysis Cores at UC Davis and are now studying their metagenomes for an upcoming paper. Our environmental samples were analyzed with the Summons Lab at MIT and revealed several interesting hopanoid biomarkers. These data will appear in a paper we hope to submit to Geobiology in the coming months and will help elucidate the environmental relevance of lipid biomarkers in the hot spring Mars analogs present in Lassen Volcanic National Park and Yellowstone National Park.

Upper Sulphur Works is low temperature and an acidic, sulfidic spring in Lassen Volcanic National Park near 40°27'09.5" N 121°32'13.3" W, California, U.S.A. (25.2 ºC; 2.9 pH). The mat itself is flocculent with a white layer of elemental sulfur on top, an acid-tolerant algal layer below the sulfur layer, and just below the algal layer is a lower purple-red layer from which we isolated Rhodopila globiformis LVNP (Fig. A).

Gibbons River Spring is similarly an acidic, sulfidic spring located near 44º41’02.9” N 110º44’37.9031” W, Yellowstone National Park, Wyoming, U.S.A. (39.6 ºC; 3.4 pH). The Yellowstone mat sits on a bed of sphagnum moss with layers of elemental sulfur, acid-tolerant algae, and a flocculent purple-red layer coating the top of the moss (Fig.B).

For LVNP samples, 2 cores were collected in August 2019 at each time point. For YNP samples, biomass was collected from the top layer of the mat in August 2019. Cores were ~ 12 inches in length and divided into 3 distinct 4-inch segments. The color of the mat core confirmed this segment zone from top (photosynthetic biomass), middle (transitional zone between photic zone and deeper sediment biomass), and sediment (deep and dark biomass). Temperature, pH, and water chemistry were collected at each sampling time. The core samples were frozen on ice immediately upon collection from mat and ultimately stored at -80 ºC until extraction for DNA. Samples designated for lipid extraction were then lyophilized for 4 days. The samples were separated into top, middle, and sediment sections and then homogenized by mortar and pestle. We then size sorted by a 2 mm sieve. The total biomass <2 mm weighed ~ 34 g and the total biomass >2 mm was ~ 10 g for each sample.

Lipids were extracted by a modified Bligh-Dyer protocol from lyophilized core biomass. Samples were weighed, transferred into solvent-cleaned glass centrifuge tubes, and extracted by ultrasonication in a monophasic ratio of water: methanol: chloroform (4:10:5). Samples were then centrifuged at 4,500 x g for 10 min and the supernatant was carried into new solvent-cleaned centrifuge tubes. An equal amount of dichloromethane and water were then added to the pooled extract supernatant and the samples were subsequently physically agitated to mix thoroughly. Once layers separated, the organic phase was then drawn off into a new solvent-cleaned centrifuge tube. We then repeated the aqueous phase extraction three more times with dichloromethane, pooled the organic fractions, and dried down the solvent under a gentle stream of nitrogen (at ~40ºC). A quantification standard was then added to the samples and dried down again at ~40ºC under a stream of nitrogen.

Large cyclic lipids were treated with a Rohmer degradation redox procedure that yields both methylated and unmethylated BHPs and then analyzed as their acetate derivatizes by gas chromatography-mass spectrometry (GC-MS) according to Jahnke et al., 1992. Sample (1/10 µL) was injected with a helium carrier gas at a constant flow rate and separation was performed on a DB-5 column (~30 m in length). Hopanoids were identified from their precise mass measurements and fragmentation patterns and by comparison of relative retention time and mass spectra to previously published analytical data (Parenteau et al., 2014).

Lipids were extracted by Soxhlet reaction from 2.5 g of lyophilized core sample. For each core depth (top, middle, and sediment), the sample was extracted with an 80:10 mixture of dichloromethane to methanol ratio over continuous heat (215 ºC). The extraction was ongoing and monitored for 7 days per sample. At the end of the extraction, the pooled total lipid extract was then transferred to a new tube and then reacted the samples with reactive copper granules for 5 days (with stir bars for agitation, at room temperature) to remove excess sulfides in our total lipid extract remaining from the extraction process. We then centrifuged the samples at 4,500 x g for 20 min and transferred the copper- and sulfide-less supernatant to a new tube. We then dried down our samples over a gentle stream of nitrogen. Once the TLE was dry we then resuspended the sampled in methanol and filtered the samples with solvent safe filters and Hamilton glass syringes. We finally dried the sample down over a gentle stream of nitrogen again and stored the dried sample at -20 ºC until it could be transported for analysis at the Summons Lab in Cambridge, MA. Liquid chromatography–mass spectrometry (LC-MS) was performed as previously described (Talbot et al., 2003, 2007) by Thomas Evans at the Summons Lab (MIT). We identified a number of extended 3-MeBHPs and their derivatives with this method.