ALKALINE HYPERSALINE LAKES AS ANALOGS FOR ANCIENT MICROBIAL HABITATS ON MARS. G. D. McDonald[1], A. I. Tsapin[1], M. C. Storrie-Lombardi[1], K. H. Nealson[1], K. L. F Brinton[1], H. Sun[1], K. Venkateswaren[1], I. Tsapin[2], J. Melack[3], R. Jellison[3], [1]Jet Propulsion Laboratory, MS 183-301, 4800 Oak Grove Dr., Pasadena, CA 91109, Gene.D.McDonald@jpl.nasa.gov, [2]Moscow State Univ. Moscow,Vorobievy Gory 117, Moscow, Russia, [3]Univ. Calif. Santa Barbara, Santa Barbara, CA

Introduction: As the climate of ancient Mars became colder and drier with time, open bodies of water would have entered a regime in which evaporation exceeded input from precipitation or runoff. This would have resulted in increases in salinity and perhaps pH [1-3]. The last open water on Mars was most likely found in alkaline hypersaline lakes, and these lakes would have been the last surface aquatic habitats for life on Mars. It follows, then, that the biomarkers most likely to be found in ancient sedimentary basins on Mars are those left by organisms adapted to high salt and high pH environments. We have begun to investigate the nature of biological diversity and adaptation to these environments, and the potential for biomarker preservation in them, using Mono Lake as a terrestrial analog environment.

Organic Geochemistry: Our inital work on the organic geochemistry of Mono Lake has been centered on two classes of biomarker compounds, amino acids and fatty acids. These compounds contain both acidic and basic functional groups, and thus should be particularly sensitive to extreme pH.

Fatty acids. We have extracted total phospholipid fatty acids from near-shore surface sediments collected in fall and spring. The two samples have similar fatty acid profiles, with significant amounts of branched-chain saturated fatty acids present. These fatty acids are typically signatures of Gram positive and anaerobic Gram negative bacteria. This signature is stable between fall and spring samples. We have also tentatively identified two methoxy fatty acids in the fall sediment sample. These unusual fatty acids may represent an adaptation to extreme pH [4,5]. The estimated bacterial cell densities from fatty acid analysis are approximately 10^9 cells per gram sediment.

We have also analyzed a core sample, taken in the middle of the lake from approximately 32 meters depth. This sample shows a much greater contribution from monounsaturated fatty acids, which are probably contributed primarily by algae. The branched-chain fatty acids are also present, although at lower relative abundances. The estimated cell density in this core sample is approximately 10^{11} cells per gram.

Mole % fatty acids in Mono Lake sediments

	Fall	Spring	Core
11:0	<1	<1	<1
2OH10:0	<1	<1	<1
12:0	6.11	3.48	<1
13:0	<1	<1	<1
2-OH12:0	<1	<1	<1
3-OH12:0	<1	<1	<1
14:0	8.62	6.38	6.3
i15:0	14.04	16.55	5.2
a15:0	10.48	17.65	6.3
15:0	3.28	2.29	<1
2-OH14:0	<1	<1	<1
3-OH14:0	<1	<1	<1
i16:0	3.33	4.88	1.8
16:1d9	1.56	<1	16.5
16:0	31.6	39.31	27.8
i17:0	1.61	1.64	3.1
a17:0	2.54	1.64	2.9
17:0cyc	<1	<1	<1
17:0	1.47	<1	<1
2-OH16:0	<1	<1	<1
18:2	<1	<1	4.1
18:1d9c	<1	<1	13.4
18:1d9t	<1	<1	9.1
18:0	3.68	4.95	3.7
19:0cyc	<1	<1	<1
19:0	<1	<1	<1
20:0	1.44	1.22	<1

Amino acids. We have obtained the amino acid compositions and D/L ratios for Mono Lake water and sediment samples. The fall surface sediment had D/L ratios of 0.01-0.32, while the spring sediment had D/L ratios of 0.01-0.27. In Mono Lake water filtered through a 0.2-µm membrane, D/L for the free amino acid fraction was 0.01-0.4, while D/L for the bound

fraction was significantly higher, 0.25-0.48. Most of the increase in D/L was seen in the hydrophilic amino acids.

Spectroscopic studies. We have identified a putative biochemical Raman signature in the Mono Lake core sample using a UV resonance Raman system at 248 nm excitation wavelength. This signature consists of a broad band at around 1600 cm^{-1}. The chromophore is not extractable in 2:1 methylene chloride:methanol, acetone, or water, but disappears upon heating the sample to 500ºC in air for 2 hr. Our tentative conclusion is that this chromophore is contained in insoluble high molecular weight humic-type structures, although mineral matrix effects have not been completely ruled out.

Microbial Diversity: We have examined the total bacterial population (as judged by epifluorescence microscopy), cultivable aerobic heterotrophs, and sulfur-reducing facultative anaerobes of Mono Lake nearshore sediment. The number of non-autofluorescent microbial cells (as seen by DAPI fluorescence) was $5.6x10^9$ cells/g of wet sediment. Up to $1x10^7$ colony forming units (CFU) per ml were obtained using a defined medium based on Mono Lake water. Amylase-, lipase-, and protease-producers (determined by plate assays) were found at CFU of $4.0x10^5$, $1.5x10^5$ and $2.1x10^6$ per g, respectively. Sulfur (polysulfide) reducing bacteria were found at CFU of $5.0x10^3$ per g.

Based on morphology and physiology (enzyme activity, NaCl and pH tolerance), 27 isolates were characterized. Using phenotypic characterization, only 4 strains were identified. Fatty acid methyl ester analysis yielded six identifications. Sequence analysis of nearly complete sequences of 16S rRNA yielded 8 known species (*Paracoccus marcusii, Bacillus agaadherens, Bacillus cochnii, Pseudomonas pseudoalcaligenes, Pseudomonas putida, Halomonas variabilis,* and species of *Aeromonas* and *Shewanella*). By 16S rRNA analysis, the isolates fell into three main phylogenetic groups: Gram positive, alpha proteobacteria, and gamma proteobacteria. Seven of the gamma proteobacterial isolates are closely related to each other and fall within the *Pseudomonas* clade.

We have also cloned about 100 16S rRNA genes from the microbial community in Mono Lake nearshore sediments. Preliminary analysis of 16S gene sequences using the ARB computer program is being carried out.

In addition, we have estimated the microbial diversity in the water column of Mono Lake. We analyzed water samples collected near the shore and also in the middle of the lake. Members of the order *Actinomycetales* were readily cultured from the near-shore water column and tufa-associated samples. The 16S rRNA analyses confirmed the presence of alkaliphilic and alkalotolerant species of the order *Actinomycetales* in these samples. 16sRNA analyses showed that in the water column samples taken near the shore the most dominant microbial forms were *Actinomycetes*.

We have also cloned from the same water column 16S rRNA genes from Protobacteria (alpha and beta subdivisions) and Rickettsiales. We have so far been unable to amplify 16S rRNA genes using PCR primers specific for Archaea from water column samples

In addition, we have detected and isolated photosynthetic cyanobacteria from inside the mineral matrix of tufa from Mono Lake. These communities provide an especially useful model for the study of biosignature formation. A continuous biotic zone beneath the surface of large rock boulders is a defined target for automated in situ life detection. Bacterial activities result in physical and chemical alterations of the rock matrix, which are likely to survive over geological time scales under appropriate conditions. Also, the rock matrix serves as a mechanism to immobilize organic biosignatures, preventing their dispersal.

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