SULFATE-REDUCING BACTERIA AS A MODEL FOR LIFE IN THE MARTIAN SUBSURFACE.
C. L. Marnocha[1], V. F. Chevrier[1], and D. M. Ivey[1,2], [1]Arkansas Center for Space and Planetary Sciences, 202 Old Museum Building, University of Arkansas, Fayetteville, AR 72701, [2]Department of Biology, 601 Science and Engineering, University of Arkansas, Fayetteville, AR 72701; cmarnocha@uark.edu.

Introduction: The combination of widespread sulfates on the surface of Mars (Fig. 1) [1,2,3,4] with evidence of recent water activity [5,6] suggests that the infiltration of these stable brine solutions may be the source of the water activity on Mars at present [1,4]. Experimental studies have shown that sulfate solutions can effectively lower the freezing point of water and that sulfate brine solutions can remain stable in martian conditions [7,8]. Thus, saturated brines could be stable under martian conditions [7,8] thus providing a source of liquid water but at the expense of very high concentrations (molalities up to several mol kg^{-1}) and very low temperatures (down to 205 K for saturated ferric sulfate [7]).

In the search for life in the subsurface of Mars, where it would likely be found due to its protective properties against UV irradiation, sulfate-reduction is one possible metabolic mechanism. However, the range of conditions in which sulfate reduction can occur, especially in terms of concentration and temperature is not very well constrained. Therefore, we propose experiments with sulfate-reducing bacteria as a possible model for life on Mars, exposed to various sulfate solutions at various concentrations and temperatures.

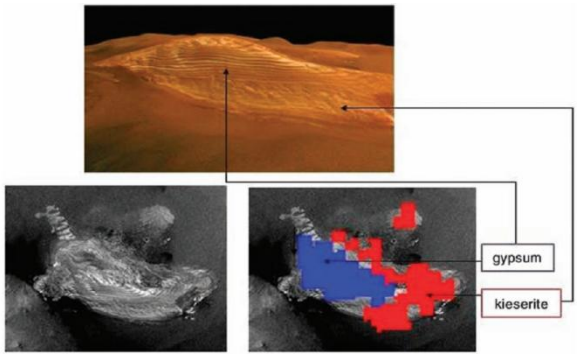

Figure 1. A sulfate-rich layer within Valles Marineris, containing kieserite and gypsum imaged by Mars Express.

The two ultimate goals of this study are to determine the following: 1. Can sulfate-reducing bacteria survive in Mars-like conditions; and 2. What biosignatures are produced by metabolically active sulfate-reducing bacteria in martian conditions?

To experimentally demonstrate the ability of sulfate-reducing bacteria to survive and proliferate in martian conditions, we will subject a variety of sulfate-reducing bacteria to conditions ranging from those considered ideal to increasingly more hostile and Mars-like. Bacteria will be cultured on solutions of magnesium, ferrous, ferric, and calcium sulfates, all of which have been shown to be predominant sulfates on the martian surface [5]. Temperature will be decreased to the eutectic temperature of the sulfate solutions under a low pressure simulated CO_2 atmosphere. Additionally, cultures will be subjected to diurnal and UV cycles, and ultimately grown under a layer of simulated martian regolith to determine if this provides any protection from UV irradiation.

Preliminary results of this study examined the survivability of several species of sulfate reducing bacteria in sulfate solutions at various concentrations, but grown in otherwise optimal conditions. A list of model species used in this study are shown in Table 1.

Table 1. Literature data for sulfate-reducing bacteria to be used in this study. Temp. indicates range found in literature, * indicates that this range has not yet been determined.

Organism	*Energy Source*	*Temp. (C°)*	*Source*
Desulfotalea psychrophila*	H_2 (Chemo-lithotroph)	-1.8 – 19	Arctic marine sediments
Desulfotalea arctica	H_2 (Chemo-lithotroph)	-1.8 – 26	Arctic marine sediments
Desulfobacter psychrotolerans	Acetate	-6 – 26.3	North Sea sediments
Desulfotomaculum reducens	Metal-reducing heterotroph	*	Heavy metal contam. seds
Desulfotomaculum arcticum	Organics, H_2, amino acids, alcohols	26 – 46.5	Cold fjord sediments, Svalbard

Methods: Sulfate-reducing bacteria were grown in four different sulfate salt solutions at varying concentrations up to the eutectic (Table 2). Two replicates of cultures were made in both nutrient-rich and nutrient-limited media. Nutrient-rich media consisted of MS medium, a trace mineral solution, and CO_2 and H_2 gas. Nutrient-limited tubes contained only CO_2 and H_2 gas. Samples were incubated at 37°C for 5 months and were examined for turbidity and presense of precipitates.

Table 2. Concentrations of salts given in weight percent for magnesium, ferrous, and ferric sulfate.

$MgSO_4$	$FeSO_4$	$Fe_2(SO_4)_3$	$CaSO_4$
5	5	10	~0.2
10	10	30	-
18	17	48	-

Cell growth was quantified by protein concentration. Pierce BCA Protein Determination Assay was used to colorimetrically determine protein concentration by measuring absorbance at 562 nm. Background absorbance was measured for ferrous and ferric sulfate without the protein determination reagent added to compenstate for the intrinsic absorbance of ferrous and ferric sulfate in this range.

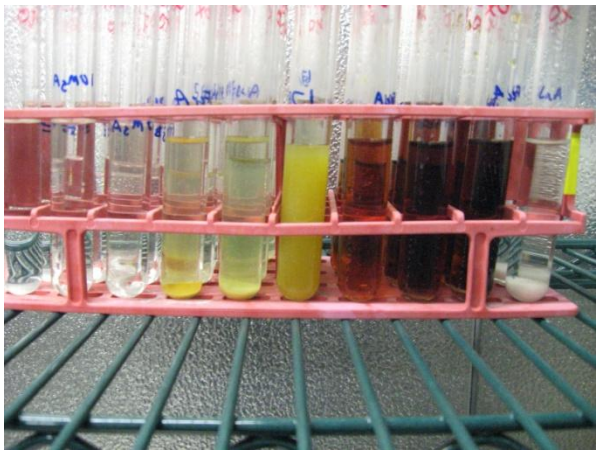

Figure 2. From left to right: Magnesium sulfate at 5, 10, 18 wt.%, ferrous sulfate at 5, 10, 17 wt.%, ferric sulfate at 10, 30, 48 wt.%, and gypsum at ~0.2 wt.%.

Results: All samples exhibited some turbidity, with ferrous and ferric sulfate samples containing a precipitate and the gypsum samples showing evidence of recrystallization (Fig. 2). Infrared spectroscopy and X-ray diffraction (XRD) will be performed on the solid phase of the samples to determine the mineralogical composition of precipitates.

Magnesium sulfate and gypsum samples at all weight percents, both nutrient-limited and nutrient-rich samples showed significant growth in the form of protein concentration (Table 3).

Table 3. Protein concentration for $MgSO_4$ and $CaSO_4$ samples at varying concentrations, both in nutrient-rich (indicated by +) and nutrient-limited (-) cultures. Gypsum's ($CaSO_4$) concentration is at saturation at 37°C (~0.2 wt%).

Sulfate	wt.%	Nutrient	Protein (ug/ml)
Mg	5	+	1088
Mg	10	+	1204
Ca	0.2	+	904
Mg	5	-	437
Mg	10	-	1213
Ca	0.2	-	812

Despite compensating for the natural absorbtion of ferrous and ferric sulfate by taking background measurements, samples still showed anomalously high protein concentrations, most likely because of interference between the iron in the samples with the the Pierce BCA reagent. Magnesium sulfate at 18 wt.% also showed a veryhigh protein concentration.

Because of interference with the ferrous and ferric sulfate samples, additional methods will be employed to quantify growth of these cultures, possibly through direct cell count in order to minimize any interference resulting from the constituents of the cultures.

Discussion: Apparent growth in all samples, regardless of nature and concentration of sulfate used, , and presence of nutrients, shows the durability of such microbes and suggests that further experimental should be pursued in order to push the limits of survivability for these species, in particular with respect to low temperature.

Following an analysis of uninoculated sulfate solutions for abiotic reactions producing precipitates, precipitates from cultures will be analyzed for their mineralogical content to provide a starting point for determining what biosignatures might be present on Mars to indicate the presense of metabolically active life.

Conclusions: An early Mars may have been warmer and wetter than today and perhaps much more hospitable to life as we know it. But the subsurface of Mars provides a habitat that, at present, may protect microbial life from harsh conditions at the surface. Conditions in the subsurface, nonetheless, are still not typically ideal environments for many microorganisms. However, extremophilic bacteria utilizing sulfate-reduction for metabolism could be capable of withstanding current conditions in the martian subsurface.

Growth of sulfate-reducing bacteria on high concentrations of sulfates indicates the hardiness of such bacteria and their ability to survive in high salinity. Future work with this study will gradually subject these bacteria to conditions closer resembling the conditions in the martian subsurface, with the fundamental goal being to show that the conditions on Mars do not necessary preclude life.

References: [1] Bibring, J.-P., et al. (2005), Science, 307, 1576-1581. [2] Wang, A., et al. (2006), Mars, J. Geophys. Res., 111, E02S17. [3] Gendrin, A., et al. (2005), Science, 307, 1587-1591. [4] Squyres, S.W., et al. (2004), Science, 306, 1709-1714. [5] Malin, M.C. and K.S. Edgett (2000), Science, 288, 2330-2335. [6] Malin, M. C., et al. (2006), Science, 314, 1573-1577. [7] Chevrier, V. F., and T. S. Altheide (2008), Geophys. Res. Lett., 35, L22101. [8] Altheide, T.S., et al. (2009), Earth Planet. Sci. Lett., 281, 69-78.