GROWTH OF SULFATE-REDUCING BACTERIA IN SULFATE BRINES AND THE ASTROBIOLOGICAL IMPLICATIONS FOR MARS. C. L. Marnocha[1], V. F. Chevrier[1], and D. M. Ivey[1,2], [1]Arkansas Center for Space and Planetary Sciences, 202 Field House, University of Arkansas, Fayetteville, AR 72701, [2]Department of Biological Sciences, 601 Science and Engineering, University of Arkansas, Fayetteville, AR 72701; cmarnoch@uark.edu.

Introduction: 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 [1,2]. The combination of widespread sulfates on the surface of Mars (Fig. 1) [3,4,5,6] with evidence of recent water activity suggests that the infiltration of these stable brine solutions may be the source of the water activity on Mars at present [3,6]. Recent work has suggested that saturated brines could be stable under martian conditions [1,2] thus providing a source of liquid water but at the expense of very high concentrations and very low temperatures (down to 205 K for saturated ferric sulfate).

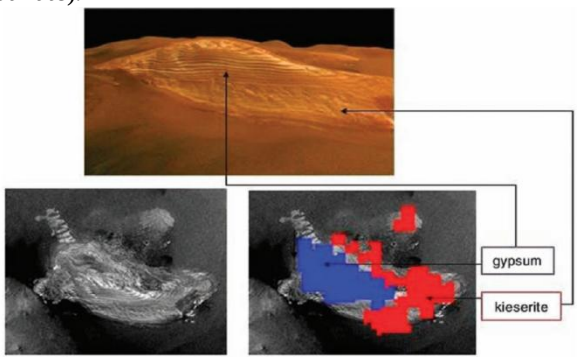

Figure 1. A sulfate-rich layer within Valles Marineris, containing kieserite and gypsum imaged by Mars Express.

The martian subsurface could provide a haven for microbial life, protected from UV radiation and utilizing sulfate brines as a liquid medium and energy source through sulfate-reduction. However, the range of conditions in which sulfate reduction can occur is not very well constrained, especially in terms of concentration and temperature. Therefore, we have cultured a variety of sulfate-reducing bacteria in varying concentrations of a number of sulfate brines as a preliminary step in the assessment of survivability of sulfate-reducing bacteria in simulated martian conditions. 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?

Methods: Magnesium, ferrous, ferric, and calcium sulfates were selected because of their presence on Mars and their capacity to significantly lower the freezing point of water. Sulfate-reducing bacteria (Table 1) 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 presence of precipitates.

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 compensate for the intrinsic absorbance of ferrous and ferric sulfate in this range.

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

A LIVE/DEAD BacLight™ Bacterial Viability Kit (Invitrogen) was used to stain samples for cell counts and cell viability. A green-fluorescent nucleic acid stain labeled all cells, while a red-fluorescent nucleic acid stain labeled only cells with damaged membranes. Samples were viewed under a fluorescence microscope using GFP and rhodium filters.

Table 2. Salt concentrations (in wt.%) used for culturing sulfate-reducing bacteria.

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

Results: All samples exhibited some turbidity, with the ferrous and ferric sulfate samples containing a precipitate and the gypsum samples showing evidence of recrystallization. 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 (listed as Ca) 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 absorption 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 Pierce BCA reagent. Magnesium sulfate at 18 wt.% also showed a very high protein concentration, possibly due to contamination (described in Table 4).

Table 4. Total cell counts and viability (as percentage of live cells vs. dead cells) for all samples, except magnesium sulfate at 18 wt.%, which appeared to have been contaminated.

Salt	Wt.%	Live (%)	Total Count (cells/mL)
Mg	5	15	8.40×10^8
Mg	10	67	3.84×10^8
Fer	5	-	7.68×10^7
Fer	10	55	2.25×10^8
Fer	17	-	1.32×10^8
Frc	10	75	1.87×10^8
Frc	30	82	1.21×10^8
Frc	48	9	5.43×10^8
Ca	~0.2	12	6.26×10^8

To overcome the interference between the Fe-sulfates and protein determination assay, direct cell counts were performed on all samples (Fig. 2). Nearly all samples showed cell counts (Table 4) in the 10^8 cells/mL range, suggesting concentrated brines significantly limit growth in Earth-like conditions. Cell viability was strongest in the magnesium sulfate at 10 wt.%, ferrous sulfate at 10 wt.%, and ferric sulfate at 10 and 30 wt.% samples, with live cell percentages over 50% compared to those with compromised cell membranes.

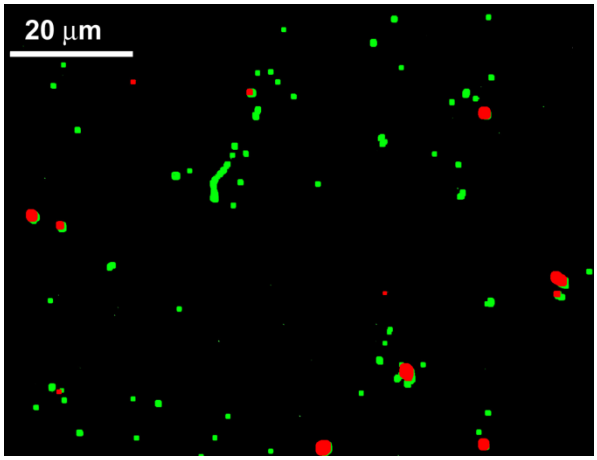

Figure 2. Ferric sulfate at 10 wt.%, with live cells shown in green and cells with damaged cell membranes shown in red.

Discussion: 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 an environment 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. Our preliminary experiments have shown that sulfate-reducing bacteria are able to survive in highly concentrated brine solutions capable of maintaining liquid water at low temperatures.

Future Work: Future work will consist of FT-IR analysis of precipitates and comparison between nutrient-rich/-limited samples and concentration of sulfate salts. Additionally, we will investigate the effect of temperatures below 273 K and lower water activity of concentrated brines at various atmospheric compositions for each of the salts at their varying concentration and growth media.

References: [1] Chevrier, V. F., and T. S. Altheide (2008), Geophys. Res. Lett., 35, L22101. [2] Altheide, T.S., et al. (2009), Earth Planet. Sci. Lett., 281. [3] Bibring, J.-P., et al. (2005), Science, 307. [4] Wang, A., et al. (2006), Mars, J. Geophys. Res., 111. [5] Gendrin, A., et al. (2005), Science, 307. [6] Squyres, S.W., et al. (2004), Science, 306.