An Experimental Assessment on the Effects of Variations in Sulfate Concentrations on Sulfate Reducing Bacteria in Simulated Martian Conditions. M. Silver[1]; S. Mora[2], M. Ivey[3], and V. Chevrier[4], *[1]Arkansas Center for Space and Planetary Sciences at the University of Arkansas. 332 N Arkansas Ave., Fayetteville, AR 72701.* mmsilver@email.uark.edu, *[2]The University of Arkansas Department of Biological Sciences. 1 University of Arkansas, Fayetteville, AR 72701.* slmosque@email.uark.edu, *[3]The University of Arkansas Department of Biological Sciences. 1 University of Arkansas, Fayetteville, AR 72701.* mivey@email.uark.edu, *[4]Arkansas Center for Space and Planetary Sciences at the University of Arkansas. 332 N Arkansas Ave., Fayetteville, AR 72701. vchevrie@email.uark.edu*

Introduction:

Significant sulfate mineral exposures have been identified on the Martian surface by the Viking era missions and NASA Mars Exploration Rovers[1][2] [3][4] including, ferric sulfates[6][7], Ca sulfates, and Mg-sulfates. Investigation of Martian conditions indicates that liquid water in the form of brines may form and remain stable at the surface or in the shallow subsurface for extended lengths of time [3] [8][9][10][11]. According to the second Mars Exploration Program Analysis Group (MEPAG) Special Regions Analysis Group (SR-SAG2). these sulfate brines on the Martian surface or shallow subsurface need to be considered "special regions": regions where terrestrial organisms are likely to replicate and/or have a high potential for the existence of extant Martian life forms[12]. For these reasons, the replication capabilities of organisms which utilize sulfates as metabolic energy sinks (SO_4^{2-} as terminal electron acceptor) were investigated.

Experiment:

To assess the habitability of Martian sulfate special regions, organisms capable of enduring extreme temperatures and low water activities were chosen for study. Four extremophile sulfate reducing bacteria species representing psychrophilic, chemolithoautotrophic, heterotrophic, and sporulating organisms were investigated (Table 1), *Desulfotalea psychrophila*, *Desulfobacter psychrotolerans*, and *Desulfotomaculum arcticum*, as well as an iron-reducing organism (*Desulfuromusa ferrireducens)* to test for the combination of iron reduction and sulfate reduction in ferrous/ferric sulfates (Table 1).

Initial experiments were performed under optimal organism conditions to provide a baseline (control) to compare future work to. Optimal growth solutions for each organism were prepared as outlined in the catalog of the Deutsche Sammlung von Mikroorganismen und Zellkulturen in sterile, Nitrogen (N_2) flooded anaerobic conditions. Exponential phase cells were collected, washed with fresh growth medium, and inoculated into synthetic minimal media [13] supplemented with varying solutions of $CaSO_4$, $MgSO_4$, $Fe^{2+}SO_4$, and $Fe^{3+}{}_2(SO_4)_3$ (Table 1). Solutions were then incubated at the respective organism's ideal growth temperature for 1 month.

Cultures were purified using a suite of MOBIO DNA Isolation Kits. PCR utilizing primers based on the 16s rRNA of six phylogenetic groups of sulfate-reducing bacteria was performed to amplify culture DNA concentrations (Table 1). Gel electrophoresis was

Organism	*Energy source*	T_{Range} °C	T_{Op} °C	pH_R	*Primers used*	*Experiment sulfate supplement*
Desulfotalea psychrophila	H_2 (Chemo-lithotroph)	-1.8 – 19	10	7.3 –7.6	SRA FWD & REV DsrAF5 & Dsr1m-rc DsrAF5 & DsrAF4-RC 27F & 16sr1	*0.1% $CaSO_4$* *10% $MgSO_4$* *18 % $MgSO_4$* *10% $Fe^{2+}SO_4$* *14% $Fe^{2+}SO_4$* *10% $Fe^{3+}{}_2(SO_4)_3$* *20% $Fe^{3+}{}_2(SO_4)_3$* *30% $Fe^{3+}{}_2(SO_4)_3$* *40% $Fe^{3+}{}_2(SO_4)_3$* *48% $Fe^{3+}{}_2(SO_4)_3$*
Desulfobacter psychrotolerans	Acetate	-6 – 26.3	20	7.2 –7.4	DsrAF5 & Dsr1m-rc DsrAF5 & DsrAF4-RC 27F & 16sr1	
Desulfotomaculum arcticum	Organics, amino acids, H_2, alcohols	26 – 46.5	44	7.1 –7.5	DsrAF5 & Dsr1m-rc DsrAF5 & DsrAF4-RC 27F & 16sr1 Darc DsrA FWD & REV	
Desulfuromusa ferrireducens	Organics, amino acids, H_2, alcohols	-2 – 23	14	6.5– 7.9	DsrAF5 & Dsr1m-rc DsrAF5 & DsrAF4-RC 27F & 16sr1	

Table 1. Literature data of model microorganisms, primers used, and experimental solution sulfate supplementation. T_{Range}, T_{Op}, and pH_{Range}, refer to temperature range, optimal temperature, pH range, and optimal pH, respectively. Sulfate supplement percentages represent the percentage of total solution consisting of the specified sulfate. *Desulfuromusa ferrireducens* is the iron-reducing psychrophile used in the experiments with iron sulfates. Figure modified from Chevrier and Ivey (2014).

then performed to provide an initial, indirect molecular indication of cell number.

Purified cultures were then analyzed for double stranded DNA (dsDNA) concentrations using a Qubit high sensitivity dsDNA flourometer.

Results:

Gel electrophoresis of *D. psychrophila*, *D. arcticum*, and *D. ferrireducens* cultures subjected to media supplemented with varying sulfate concentrations has been inconclusive thus far: gels display inconsistent DNA banding of cultures. In most cases, cultures sampled for PCR and gel electrophorese display a significant level of bioturbation, despite not consistently yielding DNA bands.

Conclusions:

The presence of bioturbation in cultures and intermittent DNA banding in gels indicates metabolic activity. A lack of DNA banding in PCR gels may be a result of 1) not enough DNA in PCR amplified samples subjected to gel electrophoresis, 2) insufficient DNA shearing during PCR amplification, 3) the use of inappropriate primers, or 4) slight variations in DNA purification and PCR processes.

Future Analysis: Incubated cultures will be analyzed using 1) transcriptase qPCR of dissimilatory sulfite reductase (dsrAB) genes [14] on isolated total RNA samples, 2) nested PCR-DGGE characterizing communities of SRBs, 3) direct microscopic cell counts, and 4) most probably cell counts. PCR and gel electrophoresis work shall continue until consistent DNA banding can be achieved.

Future Work:

Once control samples have been characterized, SRBs will be subjected to a suite of varying temperatures, water activities, pressures, and gas compositions to accurately replicate Martian surface and subsurface conditions. Cultures will be analyzed as described, as well as through X-Ray diffraction and Fourier transform infrared spectroscopy to investigate the morphology and semi-quantitative chemical composition of precipitates and to identify morphologies in biominerals that may form.

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