An Experimental Assessment on the Effects of Variations in Sulfate Concentrations on Sulfate Reducing Bacteria in Simulated Martian Conditions

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Introduction

Significant sulfate mineral exposures have been identified on the Martian surface, including ferric sulfates, Ca sulfates, and Mg-sulfates [1-7]. Liquid water in the form of brines may form and remain stable on the Martian surface or in the shallow subsurface for extended lengths of time [3; 8-11]. 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 investigated further as possible "special regions": regions where terrestrial organisms are likely to replicate and/or regions that have a high potential for the existence of extant Martian life forms [12]. For these reasons, the replication capabilities of sulfate reducing bacteria (SRB), which utilize sulfates as metabolic energy sinks (SO_4^{2-} as terminal electron acceptor) were investigated under various sulfate concentrations. Future work will investigate SRB responses to sulfate concentrations under Martian surface and subsurface conditions.

Background

Mars surface:

- 133-303 K
- Sulfates – Explorer and Viking missions [1-7]
  - Ca
  - Mg
  - Fe (II)
  - Fe (III)
- Possible stable sulfate brines [3; 8-11]

MEPAG* SR-SAG2** and Planetary Protection:

- Sulfate brines need further investigation as possible special regions [12]

Objectives:

1. Characterize survival of psychrophilic or spore forming sulfate and iron reducers
2. Constrain survivability limits (Figure 1)

* Mars Exploration Program Analysis Group
** Second (2014) Special Regions Analysis Group

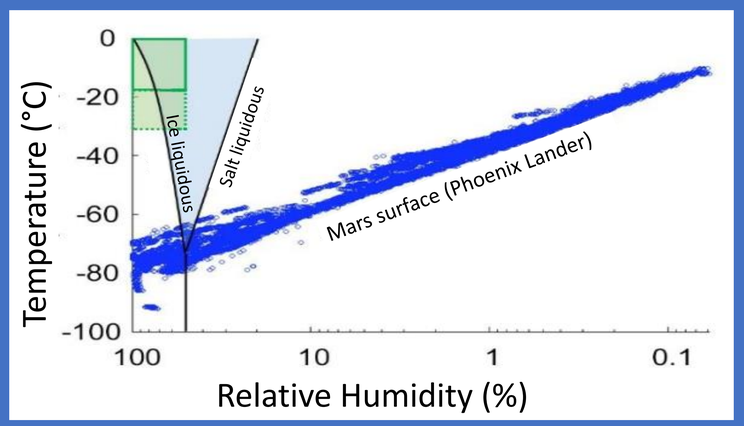


Figure 1: *Blue swath*: Martian surface conditions as measured by the Phoenix lander. *Light blue triangle*: brine stability of ferrous sulfate, calcium perchlorate, and magnesium perchlorate. *Green solid box*: current terrestrial microbe metabolism constraints. *Green dashed box*: current terrestrial microbe survival constraints. (Chevrier et al., 2012).

Methods

Three organisms from DSMZ:

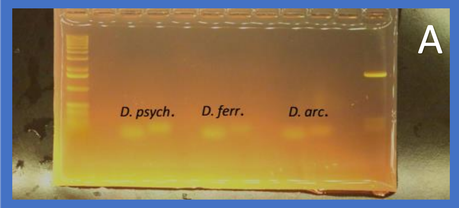
1. *Desulfotalea psychrophila* – anaerobic, sulfate reducer, psychrophile
2. *Desulfotomaculum arcticum* – anaerobic, sulfate reducer, spore former
3. *Desulfuromusa ferrireducens* – anaerobic, iron reducer, psychrophile

Culture Solutions

DSMZ Optimal Media	
+0.1% $CaSO_4$	+10% $Fe^{3+}_2(SO_4)_3$
+10% $MgSO_4$	+20% $Fe^{3+}_2(SO_4)_3$
+18% $MgSO_4$	+30% $Fe^{3+}_2(SO_4)_3$
+10% $Fe^{2+}SO_4$	+40% $Fe^{3+}_2(SO_4)_3$
+14% $Fe^{2+}SO_4$	+48% $Fe^{3+}_2(SO_4)_3$

Procedure:

1. Incubated minimum 6 months at optimal temperature
2. Analysis in varied combinations:
   i. DNA purification (MoBIO Kits)
   ii. Polymerase Chain Reaction (PCR)
      a. Generic bacterial primers (16sr1 + 27F)
      b. Sulfate-reducer specific primers (DsrAF5 + Dsr1m-RC; & DsrAF5 + DsrAF4-RC)
   a. Agar gel electrophoresis
3. Phase contrast and gram staining microscopy



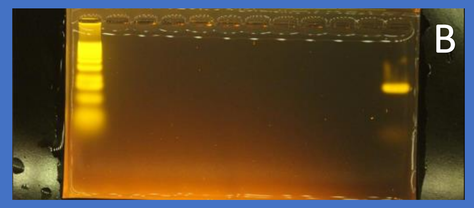


Figure 2 (Above). Agar gel wells loaded left to right: optimal media cultures of *D. psychrophila*, *D. ferrireducens*, and *D. arcticum*. Primers employed: Left: DsrAF5 + Dsr1m-RC; Right: 16sr1 +27F. **A:** Gel ran February 10, 2017. **B:** Gel ran February 12, 2017.

Results

Agar gels:

- Inconsistent gel banding (Figure 2).
- Some DNA banding, though rare

Qubit fluorometry – miniscule DNA (<25 µg/mL) yields common

Sulfide precipitation

- Evidence for biotic sulfate reduction (Figure 3) [15]

Microscopy

- Unsuccessful gram staining – rare incorporation of stain
- Brownian motion observed in some samples
- Possible *D. arcticum* spores observed (Figure 4)

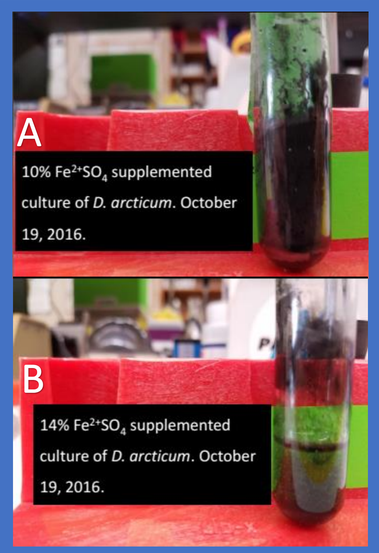


Figure 3 (Above): *D. arcticum* +10% $Fe^{2+}SO_4$ (**A**) and +14% $Fe^{2+}SO_4$ (**B**) cultures after 9 months of incubation. Black precipitate was not present at inoculation.

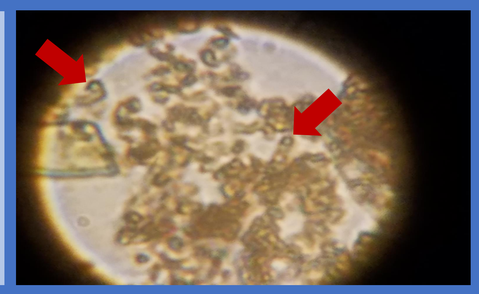


Figure 4: Phase contrast microscopy image of possible *D. arcticum* spores at 1000x magnification. Arrows indicate objects interpreted to be spores, observed in both $MgSO_4$ cultures (10% and 18%) and the 10% $Fe^{2+}SO_4$ culture.

Discussion

Likely little or no culture growth. Possible explanations:

- DSMZ optimal media insufficient
- DSMZ initial cultures insufficient
  - Supported by no significant turbidity upon delivery
- Inadequate DNA quantity
  - Supported by fluorometry

Future Work

- Different media as suggested by:
  a. Postgate (1985)
  b. Bernardez and Lima (2015)
- XRD, FTIR, and SEM
- Additional organisms (*E. coli*, psychrophiles, mesophiles etc.)
- Martian atmosphere (Figure 5)
  - 6 mbar CO_2
  - Temperatures down to 213 K

Figure 5: The *Pegasus Astrobiology Chamber* at the Arkansas Center for Space and Planetary Sciences, the University of Arkansas.

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