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 deposits 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 (SO₄²⁻) 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.

Methods

Three organisms from DSMZ:
1. Desulfotalea psychrophila – anaerobic, sulfate reducer, psychrophilic
2. Desulfotomaculum arcticum – anaerobic, sulfate reducer, spore former
3. Desulfuromonas ferrireducens – anaerobic, iron reducer, psychrophilic

Culture Solutions

<table>
<thead>
<tr>
<th>DSMZ Optimal Media</th>
<th>+10% Fe³⁺(SO₄)₃</th>
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<tbody>
<tr>
<td>+0.1% CaSO₄</td>
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<tr>
<td>+10% MgSO₄</td>
<td>+20% Fe³⁺(SO₄)₃</td>
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<tr>
<td>+18% MgSO₄</td>
<td>+30% Fe³⁺(SO₄)₃</td>
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<tr>
<td>+10% Fe²⁺SO₄</td>
<td>+40% Fe³⁺(SO₄)₃</td>
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<tr>
<td>+14% Fe²⁺SO₄</td>
<td>+48% Fe³⁺(SO₄)₃</td>
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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

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)

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:
• Postgate (1985)
• Bernardae and Lima (2015)
• XRD, FTIR, and SEM
• Additional organisms (E. coli, psychrophiles, mesophiles etc.)
• Martian atmosphere (Figure 5)
  – 6 mbar CO₂
  – Temperatures down to 213 K

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.

Figure 3 (Above). D. arcticum +10% Fe²⁺SO₄ (A) and +14% Fe²⁺SO₄ (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 MgSO₄ cultures (10% and 18%) and the 10% Fe²⁺SO₄ culture.

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

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References