INTERACTIONS OF MINERALS AND MICROBES IN EVAPORITE ENVIRONMENTS: CAN WE USE THIS INFORMATION TO IDENTIFY POTENTIAL EXTRATERRESTRIAL LIFE?  . R. L. Brigmon1 C. Yeager1 and P. A. Morris2, 1Savannah River National Laboratory, 227 Gateway, Bldg 999W, Aiken, So. Carolina, 29808, r03brigmon@srnl.gov, 2Dept of Natural Science, University of Houston-Downtown, Tx., 77002, smithp@uhd.edu.

Introduction: Traditional analysis of evaporite environments have either focused on the geology or the halophilic organisms. It is relatively rare that the two have been combined and even rarer that both disciplines have been incorporated in comparing evaporite sites. The depositional sites vary in pH, temperature and the influence of springs and as a result the mineralogy is variable. Evaporite environments have been identified from as early in Earth’s history as the Archean. Planetary scientists use this knowledge for evaluating planetary materials. For example, carbonates and sulfate minerals have been identified from Mars meteorites and from Mars surface by both lander, rover and orbital missions. The Mars Phoenix mission in 2008 found gypsum, a calcium sulfate.

An analysis of modern evaporite environments indicate that all possess halophilic tolerant macro- and microorganisms, besides being responsible for diverse biogeochemical and metabolic interactions in evaporite environments select mineral surfaces such as chloride and sulfate minerals for as a substrate and are actively involved in the precipitation of some of the minerals such as calcium carbonate. In the following paragraphs we will describe this association and address the issue that the existence of some evaporite minerals can be considered potential indicators of extraterrestrial life.

Methods: Samples were collected with either 15 or 50 ml sterile polycarbonate tube from a diversity of sites at each geographical locality, Dead Sea, Israel, Storrs Lake, and Mono Lake, California. All samples were obtained at depths from 0.1 - 0.5 m and kept cold (~ 3° C) until laboratory processing or were preserved in a 5% formalyn solution. Salinity, ion concentrations, molecular data analysis were analyzed; preserved specimens were imaged using and environmental scanning electron microscope (ESEM). Acridine Orange (AO) was used to examine microorganisms in biofilms with a Zeiss 510 laser confocal scanning microscope (LCSM) (Carl Zeiss, Oberkochen, Germany). For LCSM samples were heat fixed onto a microscope coated with 2% gelatin in PBS and redryed, and then stained with AO and rinsed with PBS.

Results: ESEM Microbial biofilm were evident in the mineral encrusted sate as observed in the Dead Sea Samples from the Jordan side in Figure 1.

Figure 1. Microorganisms encrusted on clay surface in Dead Sea sediment sample

Figure 2 demonstrates a similar sample from the Jordan side of Dead Seas as Figure 1 only the LCSM technique allows a three dimensional view of the biofilm and clays. The arrows point to microorganisms embedded in the clay material. In some cases just under the surface.

Figure 2. LCSM of bacteria on clay surface embedded in detritus.

Cyanobacteria-like microorganisms as well as bacteria were also observed with LCSM in the biofilm of the Jordanian Dead Sea clay samples (Figure 3).
Cyanobacteria-like microorganisms were observed with LCSM.

A three dimensional view of Figure 3 with LCSM is show in Figure 4. Cyanobacteria-like microorganisms were observed with LCSM embedded in the biofilm several microns in depth.

Between 138 and 165 16S rRNA gene sequences were analyzed from each of the four distinctive colored layers (pink, green, purple, gray) of a Storrs Lake biofilm (Fig 5). The compositions of the 16S rRNA gene libraries from each of the layers were quite similar.

Each of the libraries were comprised largely of Proteobacteria (65-79%) and Bacteroidetes (19-33%) sequences. Sequences representing the Planctomycetes, Firmicutes, Chloroflexi, and TM7 phyla were also present at very low levels.

Surprisingly, we did not detect any cyanobacterial sequences, though cyanobacterial cells were readily visualized via microscopic analysis of the biofilm material. Differential DNA extraction efficiencies between cyanobacteria and other bacteria could lead to under-representation of these phototrophs in these libraries. Alternatively, the abundance of cyanobacteria in the biofilm community could be less than 1-2% of the total microbial population; therefore, representative 16S rRNA gene sequences would not necessarily be detected with the moderately sized clone libraries that we analyzed (though at this abundance the large, obvious cyanobacterial cells could easily be detected via microscopy).

Table 1 Storrs Lake 16S rRNA Gene Sequence Types

<table>
<thead>
<tr>
<th>Sequence designation</th>
<th>% of library</th>
<th>Phyla</th>
<th>Closest cultured relative</th>
</tr>
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<tbody>
<tr>
<td>SLG-29</td>
<td>21%</td>
<td>Bacteroidetes</td>
<td><em>Gracilimonas tropica</em></td>
</tr>
<tr>
<td>SLG-19</td>
<td>16%</td>
<td>γ-proteobacteria</td>
<td><em>Marinobacter algicola</em></td>
</tr>
<tr>
<td>SLG-46</td>
<td>23%</td>
<td>γ-proteobacteria</td>
<td><em>Halomonas variabilis</em></td>
</tr>
<tr>
<td>SLG-48</td>
<td>8%</td>
<td>α-proteobacteria</td>
<td><em>Stappia meyeri</em></td>
</tr>
<tr>
<td>SLG-69</td>
<td>4%</td>
<td>α-proteobacteria</td>
<td><em>Rhodobium marinum</em></td>
</tr>
</tbody>
</table>

Table 1 lists the most common 16S rRNA gene sequence types that were identified from the Storrs Lake biofilm. Each of these organisms are haloophilic.

**Discussion:** Microorganisms and their physiological and environmental adaptations can influence or enhance mineral precipitation by producing biofilms. Depending on water chemistry, pH, influence of spring systems a suite of minerals will be precipitated that can be influenced by microbial growth and metabolism.

**Conclusions:** Each site has number of halophilic microbes recorded. There was no clear separation between water column and microbes associated with sediments Each site, because of local environmental constraints, is different from another site, pH will influence mineral precipitation, but microorganism have strong influence (Dead Sea vs. Storrs Lake). Microorganism, based on morphology, appear to have preferential mineral/detrital substrates. Joint microbial and geochemical analyses are needed better to elucidate the interactions and relationships determining the ecology at these sites.