

**PYROSEQUENCING ANALYSIS OF BACTERIAL COMMUNITIES IN ROCK COATINGS FROM SWEDISH LAPLAND.** C. L. Marnocha<sup>1</sup> and J.C. Dixon<sup>1,2</sup> Arkansas Center for Space and Planetary Sciences, 202 Old Museum Building, University of Arkansas, Fayetteville, AR 72701, <sup>2</sup>Department of Geosciences, 113 Ozark Hall, University of Arkansas, Fayetteville, AR 72701; cmarnoch@uark.edu.

**Introduction:** Rock coatings have been observed on Mars since Viking [1]. Terrestrial rock coatings can protect microbes from UV radiation and preserve microfossils as evidence of past life [2-4]. Because of these observations, rock varnish has become a target of astrobiological research. Other coating types, however, have been largely ignored, despite their compatibility with martian mineralogy.

A preliminary study by the authors [5] analyzed sequences from PCR-amplified 16S rDNA genes isolated from total genomic DNA from rock coatings in Kärkevagge, Sweden. These observations suggested that additional sampling was needed in order to obtain better representation of bacterial communities present in the coatings.

Rock coatings used in this study include: Fe/Mn films, composed of goethite and hematite, sulfate crusts composed of jarosite and gypsum, and aluminum glazes dominated by basaluminite with traces of gypsum and alunite (Fig. 1) [6]. Instrumentation aboard the Mars Exploration Rovers (MER) and Mars Reconnaissance Orbiter (MRO) have detected the presence of goethite, hematite, jarosite, gypsum, and alunite [7-9].



**Fig. 1. Rock coatings used in this study. A: Fe/Mn film; B: Sulfate (jarosite) crust; C: Aluminum glaze**

The observation of these minerals make the coatings found in Kärkevagge an exciting target for astrobiological studies. In this study, we have assessed the bacterial diversity of three coating types using 454 pyrosequencing.

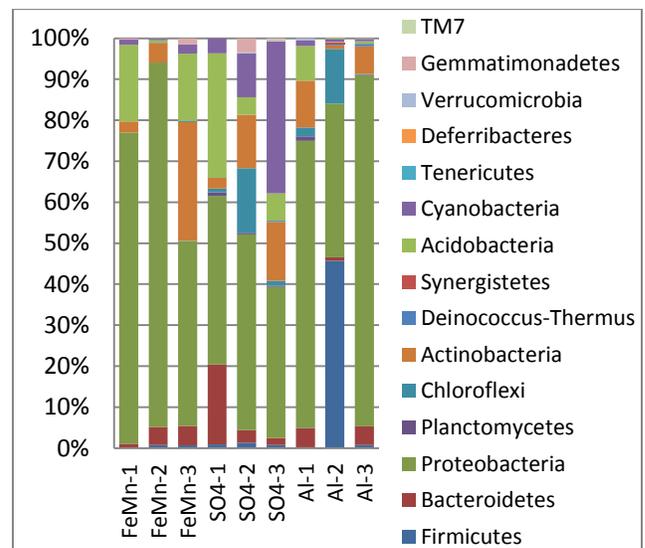
**Study Site:** Kärkevagge is a glacially-eroded U-shaped valley in Swedish Lapland. The elevation of the floor ranges from 600 meters above sea level (masl) at the valley mouth to 800 masl at the valley head. The upper valley walls are predominately garnet mica schist, while the lower valley walls are quartz mica schist. Separating the two units are thin beds of marble and pyrite is finely disseminated throughout the valley. The mean annual temperature is  $-2^{\circ}\text{C}$  with total annual

precipitation of  $\sim 800$  mm, between 50-75% of which comes in the form of snow [10].

**Methods:** Representatives from each coating type were sampled in August 2012 along transects in the valley. These samples were then subsampled and three subsamples of each coating type from different transects were then sent to Research and Testing Lab (RTL) in Lubbock, TX. RTL performed DNA extraction and 454 pyrosequencing using 28F-519R bacterial 16S assay.

All subsequent analyses were performed using *mothur* software [11].

**Results:** Pyrosequencing yielded showed the presence of representatives across 15 different phyla between the total nine samples. Ten of those phyla were represented in all coating samples. Within the Proteobacteria, all classes were represented except for zeta-proteobacteria (Fig. 2). The dominant phylum for five coating samples is  $\alpha$ -proteobacteria, with other samples dominated by Actinobacteria, Acidobacteria, Firmicutes, and Cyanobacteria.



**Fig. 2. Percent distribution of phyla for each sample. FeMn are Fe/Mn films, SO4 = sulfate crusts, Al = aluminum glazes.**

Unweighted UniFrac  $P$ -values [12] indicated that all coating communities differed significantly from one another ( $P < 0.01$ ). Additionally, communities within a given coating type (e.g. each of three Fe/Mn film samples) were also significantly different from one

another ( $P < 0.01$ ). At a genetic distance of 0.03, 32 OTUs were shared between the three coating types (Fig. 3).

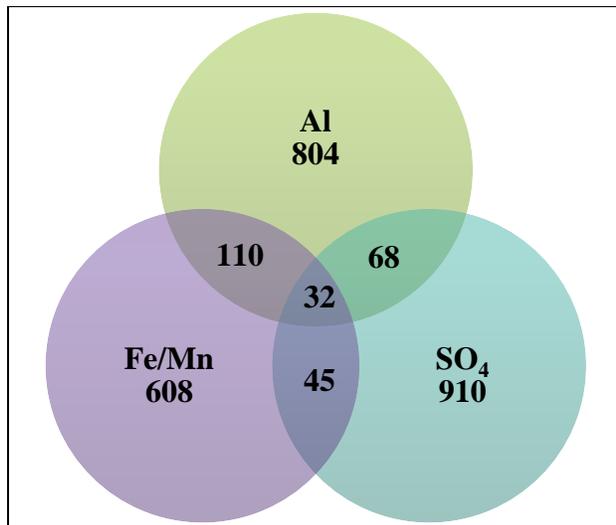


Fig. 3. Venn diagram of OTUs by mineralogy type. OTUs were generated at a genetic distance of 0.03.

Metabolisms of represented taxa include photosynthesis, sulfate reduction, sulfur oxidation and reduction, sulfide oxidizers, Fe oxidation and reduction, Mn reduction, and nitrogen fixation.

Acidophiles were by and large the most commonly represented extremophilic taxa. Psychrophiles, thermophiles, alkaphiles, halophiles, and radiation-resistant species were also detected, many of which had multiple environmental tolerances (e.g. *Acidosoma sibirica*, which is psychrophilic and acidophilic).

**Discussion and Conclusions:** The sequencing reveals distinct bacterial communities between sample sites within the same coating type, as well as distinct communities between coating mineralogy.

Extremophilic representatives and the presence of metabolisms that utilize Fe/S redox reactions reinforce Kärkevagge as a strong mineralogical and limited environmental analog to Mars. Organisms found in the rock coatings of Kärkevagge have the potential to participate in biologically-controlled and biologically-influenced biomineralization [13]. Thus, biomineralization may play an important role in the genesis of rock coatings [14-16].

Biomineralization in the generation of rock coatings further enforce rock coatings as an important astrobiological target on Mars. Instruments such as ChemCam can use LIBS to do chemical analysis on potential rock coatings using its capacity to analyze at depth on the micron scale. Rock coatings are often layered on the same micron scale [10]. Identification of

rock coatings using ChemCam may then lead the way to additional analysis by instruments such as MAHLI, APSX, and SAM in order to search for biosignatures.

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