BACTERIAL COMMUNITY STRUCTURE OF SULFATE CRUSTS, Fe/Mn SKINS, AND ALUMINA COATINGS FROM KÄRKEVAGGE, SWEDISH LAPLAND.

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Introduction: Rock coatings, such as desert rock varnish, have long been suspected as potential safe havens for microbial life on Mars [1,2]. Desert varnish in particular has been a focus of geomicrobiologists and astrobiologists for its distinctive morphology, mineralogy, and microbial communities. Other rock coating types, however, have not been studied from a geomicrobiological perspective in such detail, particularly with respect to astrobiological implications.

In this work, we describe the community structure and phylogeny of the bacterial isolates from sulfate crusts, Fe/Mn skins, and Al-rich coatings, and how communities such as these may be involved in the genesis of the rock coatings. We present rock coatings as a potential biosignatures for Mars that warrant more in-depth investigation.

Study Site: Kärkevagge is a glacially-eroded U-shaped valley in Swedish Lapland. The elevation of the floor ranges from 600 masl at the valley mouth to 800 masl at the valley head. The mean annual temperature is -2°C with total annual precipitation of ~800 mm, between 50-75% of which comes in the form of snow [3]. Water chemistry in the valley is divided between zones of dominant bicarbonate, sulfate, and mixes between the two ions, with pH of streams, springs, and lakes ranging from 4.5 to 8.4 [4].

Rock coatings are ubiquitous in the valley. Chemical and limited mineralogical analysis by Dixon and colleagues [5] allowed for coating types to be described using nomenclature and categorization by Dorn [2]. Coatings types include silica and alumina glazes, sulfate crusts of jarosite and gypsum, Fe/Mn and other heavy metal skins. Early work [6] suggested the presence of microbes in the rock coatings, but only recently [7] have these coatings been studied for the influence the micro biota may have on their genesis.

Methods: Representatives from three coating types (jarosite crusts, Fe/Mn skins, and Al-rich white coatings) were sampled in summer 2010 from multiple sites along the valley walls of Kärkevagge, with an emphasis on the eastern side of the valley. Samples for DNA analysis were stored at -20°C. DNA was extracted from aseptically pulverized coatings using PowerSoil DNA Isolation Kit from MoBio (Carlsbad, CA) according to manufacturer's protocols. Following extraction, 16S rDNA genes were amplified via PCR using universal 533-forward and 1392-reverse primers. Archaeal primers were also attempted, but unsuccessful. Amplicons were cloned a pSC-A vector using the StrataClone PCR Cloning Kit (Agilent Technologies). After plating and incubation, positive colonies were randomly selected and further PCR-amplified using an M13 -20 forward and M13 reverse primer set. These samples were submitted to Functional Biosciences (Madison, WI) for Exo/SAP cleanup and sequencing using the T7 primer.

Returned sequences that passed a quality threshold were checked for chimeras using Bellerophon and non-chimeric sequences were aligned using both BLAST and greengenes for downstream analysis. BLAST alignment results were used to determine phyla distribution of the communities for each rock coating as well as to identify the environments of nearest neighbors. Greengenes alignments were used with mothur to generate rarefaction curves, estimates of diveristy and richness, and operational taxonomic units (OTUs) shared across coating types.

Results: Figure 1 shows a Venn diagram of unique and shared OTUs across coating types, at an OTU clustering distance of 0.05. Assuming all sequences represent unique OTUs generates a diagram with no shared OTUs between coating types (figure not shown). It should be noted that Fe/Mn unique isolates number 40, but at the 0.05 clustering distance, are reduced to only 2 OTUs.

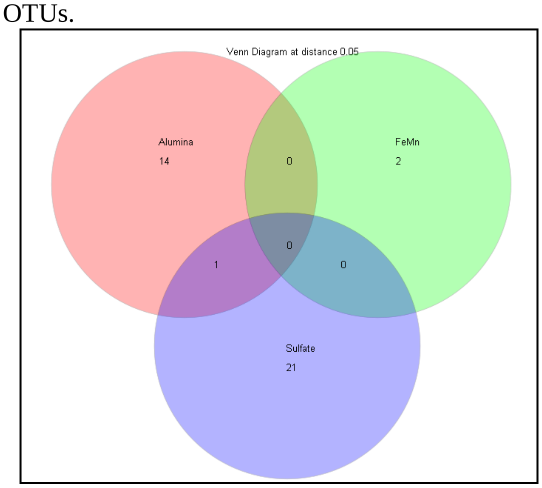

Fig. 1. Venn diagram of shared OTUs between rock coating types. Consists of non-chimeric isolates from 2 sulfate crusts, 2 Fe/Mn skins, and 1 Al-rich white coating. Isolates are clustered into OTUs at a pairwise distance of 0.05.

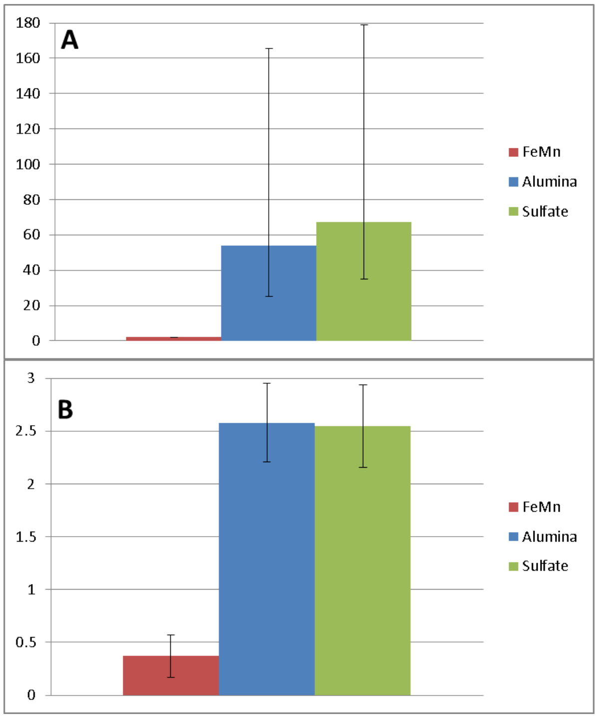

Fig. 2. A: Chao1 richness estimator, C: Shannon index of diversity. All values were taken at a distance of 0.05.

This is no more evident than in the Shannon diversity index and chao1 richness estimate shown in figure 2. Whether sulfate crusts or alumina coatings are more diverse compared to one another is more ambiguous.

Nearest neighbors came primarily from soil environments, with acid mine drainage also making up a significant proportion of environments of nearest neighbors. It should be noted that a marine saltern and marble slurry isolates are matches only to the Fe/Mn skin clones, which were all from the Bacillus genus.

Discussion and Conclusions: Perhaps the most striking result of this investigation is the distinctness of the community structure between rock coating types. In some cases, two different coating types were sampled along the same transect in the valley, but contain markedly different bacterial communities. Similarly, water chemistry between some sample sites remains similar, if not the same, yet results in communities with minimal or no OTUs shared between them. The distinctiveness of mineralogy and community structure between coatings may provide stronger and more recognizable signals of possible biological activity on Mars, in contrast to what would most likely be sterile regolith surrounding. In this scenario, evidence of past life may also be preserved in a rock coating.

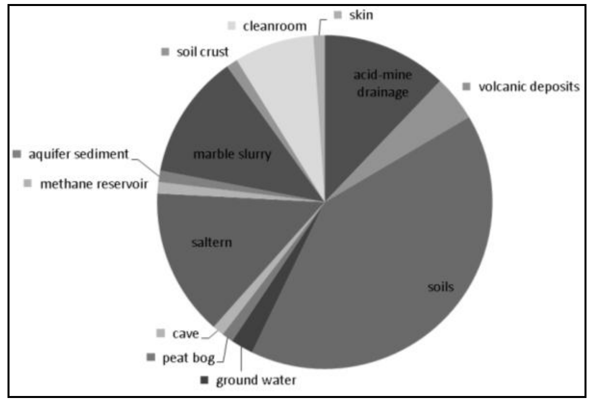

Figure 3. Environments of nearest neighbors to the isolates from the five rock coatings described in this study. The nearest neighbors were identified through the BLAST database including incultured submissions.

The Al-rich white coating and sulfate coatings contain by far the richer bacterial communities, while the Fe/Mn skins contained all Bacillus-type clones for both samples. However, dominant Bacillus-type bacteria is not unexpected for a Fe-rich mineral accumulation, given the association of the Bacillus taxa with Fe-mobilization. Many of these environments suggest a large range of environmental stresses tolerated by the microbial communities within the various rock coatings in Kärkevagge, including tolerance to salinity, extreme temperatures, acidity, and alkalinity.

Prior to the bacterial characterization of the rock coatings, it had been suggested that the geochemistry of the valley was akin to a coal mine or a sulfidic mine tailing pile [8]. For the K2 sample in particular, 8 out of 15 clones had nearest BLAST neighbors from coal mine and sulfidic mine tailing sites. These environments and the secondary minerals produced there have long been known to be facilitated by microbial physiology. These two similar geochemical environments with some shared bacterial community makeup suggests that the rock coatings from Kärkevagge may owe their genesis to bacterial metabolism.

References:

[1] Krinsley, D. et al. (2009) *Astrobiology*, *9*, 551-562. [2] Dorn, R. (1998) No. 6 in Developments in Earth Surface Processes Series, 429 pp. [3] Thorn, C.E. et al. (1999) *Permafrost Periglac.*, *10*, 317-330. [4] Campbell, S.W. et al. (2001) *Geogr. Ann. A.*, *83*, 169-178. [5] Dixon, J.C. et al. (2002) *Bull. Geol. Soc. Am.*, *114*, 226-238. [6] Dixon, J.C. et al. (1995) *Geogr. Ann. A.*, *77*, 259-267. [7] Marnocha, C.L. (2011). *XLII LPS*, Abstract #1598. [8] Darmody, R.G. et al. (2001) *Geomorphology*, *41*, 53-62.