BACTERIAL DIVERSITY OF SULFATE ROCK COATINGS IN KÄRKEVAGGE, SWEDISH LAPLAND: A POTENTIAL MARS ANALOG. C. L. Marnocha[1] and J.C. Dixon[1,2] Arkansas Center for Space and Planetary Sciences, 202 Field House, University of Arkansas, Fayetteville, AR 72701, [2]Department of Geosciences, 113 Ozark Hall, University of Arkansas, Fayetteville, AR 72701. cmarnoch@uark.edu.

Introduction: Kärkevagge is a glacially eroded U-shaped valley in Swedish Lapland with a denudation regime characterized by massive rock slides, resultant boulder fields, slush avalanches, and debris flows (Fig. 1). One of the most striking features of the weathering regime of the valley is the abundance and diversity of rock coatings and weathering rinds. Porous, iron-rich weathering rinds, Fe/Mn films, silica and alumina glazes, sulfate crusts, and heavy metal skins are widespread in the valley (Fig. 2).

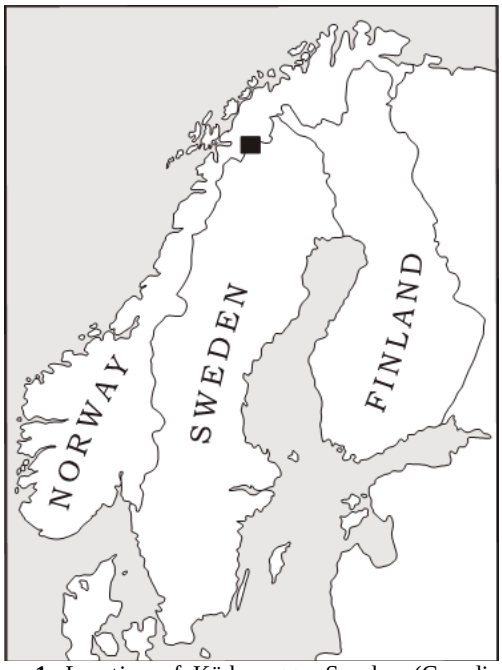

Figure 1. Location of Kärkevagge, Sweden (Coordinates: 68° 26' N, 18° 18' E)

The mineralogy, low precipitation, and low mean annual temperature of the valley make a case for it being a good terrestrial analog for Mars. Particularly, the valley is acidic and sulfate-dominanted with associated pyrite and other Fe minerals. Sulfate deposits discovered in the shallow subsurface of Mars by Mars Exploration Rover, Spirit [1] suggest a system in which fluid infiltration is a source of localized ion mobilization and transport. The mobilization of such ions and the accumulation of sulfates in terrestrial environments is often facilitated by microbes. Acid-mine drainage, for example, features organisms that precipitate acidic, oxidized sulfur states, with jarosite being a common mineral associated with this environment [2]. In addition to the mineralogical and environmental analogy to Mars, rock coatings provide a slightly endolithic environment, sheltering any life from UV exposure.

Despite detailed research on the chemistry of rock coatings, water chemistry, transport processes, and overall weathering regime of the valley, research on potential microbial influence on rock coating formation and weathering has been largely ignored. The interpretation of rock coating genesis up to this point in time has been based on inorganic processes, despite microorganisms having been observed [3]. To better understand the relationship between microbe and mineral in the rock coatings, we have begun a phylogenetic inventory of bacteria found in the rock coatings. The goal of this work is to essentially address three questions: (1) What microbes are present (phylogenetics), (2) what are they doing with respect to rock coating formation (microbe-mineral interactions), and (3) how are they doing it (gene expression). This work represents the initial phylogenetic analysis of rock coatings sampled in Summer 2010.

Figure 2. Left: Fe/Mn skin. Right: Sulfate crust (jarosite) accumulating on the underside of a rock, with a mat of orange lichens on the exposed surface.

Methods: Genomic DNA was extracted from a crushed sulfate crust sample using PowerSoil® DNA Isolation Kit from MoBio (Carlsbad, CA) according to manufacturer protocols. Bacterial 16S rDNA genes were targeted using 533-forward (5'-GTG CCA GCC GCC GCG GTA A-3') and 1492-reverse universal (5'-GGT TAC CTT GTT ACG ACT T-3') primers and amplified via PCR. Thermocycler parameters were as follows: 5' at 94°C for denaturing, followed by 35 cycles of 1' at 94°C, 45" at 47°C, and 1' at 72°C, with a 7' final extension at 72°C. Amplified PCR products were then purified and cloned into the pSC-A cloning vector using StrataClone PCR Cloning Kit (Agilent Technologies, Santa Clara, CA). Transformed *E. coli* cells were then plated on LB-ampicillin plates and incubated no more than 24 hours, per manufacturer instructions. Twenty positive colonies were randomly selected from the plates, with fragments amplified using the M13 -20 forward and M13 reverse primer set,

with binding sites on the pSC-A vector. Once amplification had been confirmed on a gel, the crude PCR products were sent to Functional Biosciences (Madison, WI) to be purified, then sequenced using the T7 universal primer.

Sequences were compared to the BLAST database and matches >96% were further analyzed for anomalies.

Table 1. Bacterial isolates from a sulfate crust (K2 signifies sample site K, and sample #2). Nearest isolate match indicates the organism with the highest identification match percentage for those with 96%+ matches and the origin of that isolate. In the BLAST search, uncultured organism submissions were not excluded.

Sample	Best Match	Isolate Source
K2-1	Chloroflexi bacterium	Acid-impacted subalpine stream sediments
K2-2	Uncultured bacterium	Hawaiian volcanic deposits
K2-3	Uncultured bacterium	Hawaiian volcanic deposits
K2-4	Uncultured bacterium	Antarctic terrestrial habitat
K2-5	Actinobacteria	Sulfidic mine waste dumps
K2-6	Actinobacteria	Sulfidic mine waste dumps
K2-8	Acidiphilium	Acidic, metal-rich mine water
K2-9	Uncultured bacterium	PCB-polluted soil
K2-10	Uncultured bacterium	Antarctic terrestrial habitat
K2-11	Uncultured bacterium	Iron Mountain, CA (case of pyrite dissolution)
K2-12	Uncultured bacterium	Pb/Zn mine tailings
K2-13	Chloroflexi bacterium	Acid-impacted subalpine stream sediments
K2-16	Uncultured bacterium	Hawaiian volcanic deposits
K2-18	Uncultured bacterium	Hawaiian volcanic deposits
K2-19	Actinobacteria	Sulfidic mine waste dumps
K2-20	*Acidiphilium rubrum*	Acidic coal mine drainage

Results and Discussion: Of the original twenty positive colonies, 16 clones had matches >96% with the BLAST database, including uncultured and environmental submissions (Table 1). A number of clones were found to be in the phyla of Chloroflexi, Actinobacteria, and other closely related groups. The closest percent-match excluding uncultured bacteria for several of the unidentified clones belonged to these groups. Chloroflexi and Actinobacteria phyla are diverse taxa, found in a broad range of environments and conditions. Chloroflexi bacteria have been found on the undersides of quartz stones in the Atacama Desert [4]. Actinobacteria make up a particularly large phyla with an ancient divergence, ranging from pathogens to soil bacteria [5].

Isolate origins included predominantly acidic environments, and often various acid-mine drainage settings. The Actinobacteria clones have their origins in sulfidic mine waste. As previously described, these environments feature oxidized pyrite to form jarosite and sulfuric acid, generally understood to be facilitated by microbial metabolism [6]. The presence of disseminated pyrite, sulfuric acid, and related bacteria in the valley strongly suggest a biological origin for jarosite crusts in the valley.

Isolate sources also show the potential for heavy metal-tolerance in the bacteria found, warranting further investigation into their metabolic byproducts and mechanisms of survival in those conditions. Further, these bacteria must also be tolerant to temperatures from 17.6°C to -26°C [7], low annual precipitation, and high salt concentrations. While such bacteria are not the most extreme in terms of survival in any single environmental stress, their "jack-of-many-trades" survivability makes them of particular interest for astrobiology on Mars.

Future studies will include an increase in the number of rock coating samples analyzed for their microbial content, with an emphasis on variety both spatially and in coating mineralogy. Future samples will be sequenced with two reads, using both the T7 and T3 primers on the pSC-A vector and then aligned to ensure better coverage and provide a more accurate isolate match in later analysis. FT-IR and XRD will also be used to analyze the mineralogy of the rock coatings and how mineralogy varies as both a function of location and microbiota.

Conclusions: Evidence of survivability alone is not substantial enough to pique interest in this environment. The nature of the rock coatings themselves are important, not only because they could provide a surface habitat on Mars protected from radiation, but also because of the their distinct morphologies. Should these rock coatings prove to have their genesis predominantly biological, rock coatings could be used as potential bioindicators on Mars, easily accessed by current and future rovers, such as Opportunity and MSL. Ultimately, this work seeks to determine the biogenacity of the rock coatings and their potential as biosignatures on Mars.

References: [1] Wang, A., et al. (2006), Mars, J. Geophys. Res., 111. [2] Sharp, M., et al. (1999), Geology, 27. [3] Dixon, J.C., et al. (1995), Geog. Ann., 77A. [4] Lacap, D.C., et al. (2010), Extremophiles. [5] Ventura, M., et al. (2007), Microbiol. Mol. Biol. R., 71. [6] Elwood Madden, M.E., et al. (2004), Nature, 431. [7] Thorn, C.E., et al. (1999), Permafrost Periglac., 10.