BACTERIAL DIVERSITY OF Fe/Mn & WHITE ROCK COATINGS IN KÄRKEVAGGE: A POTENTIAL MARS ANALOGUE. R. C. Sheehan[1,2], C. L. Marnocha[2], & J. C. Dixon[2,3], [1]Central Connecticut State University, 1615 Stanley Street, New Britain, CT 06050, sheehanryc@my.ccsu.edu, [2]Arkansas Center for Space and Planetary Sciences, 202 Field House, University of Arkansas, Fayetteville, AR 72701, [3]Department of Geosciences, 113 Ozark Hall, University of Arkansas, Fayetteville, AR 72701.

Introduction: Kärkevagge is a region in the Swedish lapland which is defined by a 5km valley created by glacial processes. Kärkevagge exhibits a low mean annual temperature of -2°C, low precipitation of below 900 mm annually, an acidic environment, and a mineralogy primarily dominated by sulfate and iron [1]- aspects which make the region a well-rounded analogue for the Martian landscape.

Rock coatings are of particular interest as Martian biosignatures. Rock coatings provide UV protection from the harsh Martian atmosphere, would preserve biosignatures past the longevity of the organisms, and are, unlike subterranean environments, accessible by rover.

The region is host to a variety of rock coatings and weathering rinds. Iron-rich weathering rinds, Fe/Mn films, silican and alumina glazes, sulfate crusts, and heavy metal skins are found in the valley [2]. The rock coatings focused on currently in this project are Fe/Mn skins, white coatings composed of alumina glaze with iron and silicon, and sulfate crusts.

Microbial activity is capable of influencing geological formations [3]. Through cataloguing the microbial diversity in the rock coatings and understanding the relationship between microbes and the mineralogy on rock coatings, the potential application of rock coatings as biomarkers for life on the Martian landscape can be advanced.

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Methods: Rock coatings were fragmented and crushed. Microbial DNA was exctracted from crushed coating samples using PowerSoil® DNA Isolation Kit from MoBio (Carlsbad, CA) according to manufacturer protocols. Isolated DNA was mixed with GoTaq® Green Master Mix from Promega (Madison, WI) and universal forward and reverse primers for PCR amplification. To specifically amplify the bacterial 16S rDNA genes, 533-forward universal (5'-GTG CCA GCC GCC GCG GTA A-3') and 1492-reverse universal (5'-GGT TAC CTT GTT ACG ACT T-3') primers were used. The thermocycler was programmed for an initial denaturing of 5 min at 94°C and 35 cycles of 1 min at 94°C, 45 sec at 47°C, & 1 min at 72°C, with a final extension of 7 min at 72°C. DNA fragments of the amplified products were purified using ethanol and sodium acetate then cloned into the pSC-A cloning vector and transformed into *E. coli* cells using StrataClone PCR Cloning Kit from Agilent Technologies (Santa Clara, CA) per manufacturer protocol. The transformed cells were plated on LB-ampicillin media and incubated for no more than 20 hours. A minimum of 24 positive colonies were randomly selected from plates, with DNA fragments amplified using the M13 -20 forward and M13 reverse primers. PCR products were then sent to Functional Bioscences (Madison, WI) for purification and sequencing using the T7 universal primer.

Sequences were compared to the BLAST nucleotide database. The highest % matches were further analyzed for their isolate source and physiology.

Table 1. Bacterial isolate matches from an Fe/Mn coating (sample #1 from the H site). Nearest isolate match indicates highest identification match excluding uncultured submissions from the BLAST database. The origins of both the nearest isolate match excluding uncultured submissions and the nearest isolate match from the entire database are provided.

Sample	Nearest Isolate Match	Nearest Isolate Source	Uncultured Isolate Source
H1-02	Firmicutes bacteria	grassland soil	urban aerosol
H1-03	Firmicutes bacteria	soil	*Homo sapien* skin, popliteal fossa
H1-04	Firmicutes bacteria	soil	*Homo sapien skin*, popliteal fossa
H1-05	Firmicutes bacteria	soil	soil
H1-06	Unclassified Mn-oxidizer	rice field soil	*Homo sapien* skin, antecubital fossa
H1-08	Firmicutes bacteria	soil	soil
H1-09	Chloroplast	green algae	endolithic microbe in dolomite
H1-10	Actinobacteria	soil	soil
H1-11	Firmicutes bacteria	contaminated groundwater	*Homo sapien skin, antecubital fossa*
H1-12	Betaproteobacteria	forest soil	rice paddy soil
H1-14	Chloroplast	plant	plant
H1-15	Firmicutes bacteria	?	*Loxodonta africana* feces
H1-16	Betaproteobacteria (Np-reducer)	deglaciated granite sand	sediment laced with Np
H1-17	Firmicutes bacteria	soil	various species earthworm gut
H1-19	Alphaproteobacteria	acidic coal mine	volcanic rock
H1-21	Firmicutes bacteria	alkaline soil	alkaline soil
H1-22	Firmicutes bacteria	brown algae	brown algae
H1-23	Alphaproteobacteria	acidic peat bog	Arctic glacier ice

H1-24	Firmicutes bacteria	caverns	caverns
H1-26	Alphaproteobacteria	acidic soil	Arctic glacier ice

Results and Discussion: Of the 27 colonies sequenced from the H1 (white coating) sample, 20 provided usable DNA sequences (the colony was in a state which allowed for sequencing and the sequence provided an isolate match rather than an entire plasmid genome). Of the 27 colonies sequenced from the L2 (Fe/Mn coating) sample, 25 provided usable DNA sequences. The highest identification matches from cultured submissions and uncultured/environmental submissions from the BLAST database (Table 1) were used as references for prospective environmental tolerations and physiology of microbial life found in the sample. All isolate matches provided a %match to their respective sample DNA fragments of ≥ 90%.

For sample L2, 3 different species of *Bacillus* were identified as isolate matches. The sources of these isolate matches do not display a wide variation- soil, beans, and marine solar salterns. Diversity for this sample was very limited (a Simpson Diversity Index of 0.84), which may be an indication of a larger sample size required.

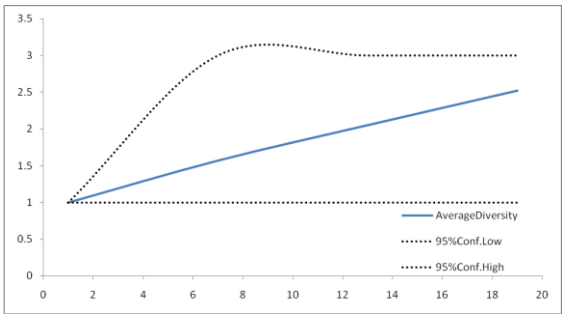

Figure 1. Rarefaction curve for sample L2 illustrate the need for a larger sampling size. This rarefaction curve compares species richness (y-axis) to the sample size (x-axis). As the curve never levels off (the first order derivative never reaches zero), it is an indication that the sampling size was not sufficient to capture the true diversity of the system.

Sample H1 displayed a higher variety in its isolate matches (a Simpson Diversity Index of 0.36). In addition to common soil, groundwater, and cavern-dwelling samples, isolate sources from H1 ranged many extreme environments.

Some matches display psychrotolerance, coming from source environments of glacier ice or deglaciated granite sand. Other isolate matches are thermophiles- one isolate source being the lava flow of the Hnaushahraun volcano in Iceland.

Other isolate matches originated from alkaline soil or acidic environments, such as acidic coal mines and peat bogs. Similar environments exhibit the oxidation of pyrite to jarosite and sulfuric acid. Microbial metabolism is generally considered to facilitate such mineralogical processes [4].

A chloroplast clone originated from endolithic microbes found within dolomite. Organisms in such environments have been notably difficult to discover [5], lending credibility to rock coatings and other endolithic environments potentially housing life in the Martian landscape which would remain unidentified without close examination.

Other isolate matches exhibit unique physiological attributes. Manganese-oxidizers and neptunium-reducers are present among the isolate matches. Heavy metal interactions similar to these might be an important source of biomineralization in certain rock coatings.

Even the ubiquitous *Bacillus* genus exhibits influence over mineralogy. The bacteria have demonstrated facilitation of iron redox cycling [6], which would affect Fe/Mn skins and white coatings. *Bacillus* endospores have even retained viability and exhibited positive growth when exposed to full Martian conditions [7], making the genus of further interest as a biosignature specimen.

Further phylogenetic analysis of the bacterial diversity of rock coating samples will be required. In the future, the phylogenetic analysis will be expanded to include archael diversity. Mineralogical analysis of rock coating by FT-IR & XRD and culturing experiments to reproduce biomineralization will provide insight to specific microbe-mineral interactions facilitating rock coating evolution.

Conclusions: Rock coatings of different morphologies provide distinct bacterial phylogenies. Such a correlation provides evidence for the influence of microbiota on variable rock coating evolution. The wide-ranging environmental tolerations and physiologies of the isolate matches lend evidence to microbiota as analogues to organisms which would potentially survive in the Martian landscape. The survivability of the organisms in such a range of environments prove their relevance to astrobiology on Mars.

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