BACTERIAL DIVERSITY OF Al- RICH 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 defined by a 5km valley created by glacial processes. Kärkevagge exhibits a low mean annual temperature of -2°C, low annual precipitation of approximately 900 mm annually, acidic water chemistry, and a mineralogy primarily dominated by sulfates and iron-bearing minerals [1]- attributes 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, are capable of preserving evidence of an organism after their expiration, and are, unlike subterranean environments, accessible by rover.

Kärkevagge is host to a variety of rock coatings and weathering rinds of diverse mineralogies. Iron-rich weathering rinds, Fe/Mn films, silica 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 white coatings composed of alumina glaze with iron and silicon.

Microbial activity is capable of influencing geological formations [3]. Reduction and oxidation of minerals can be accomplished as a byproduct of biological processes [4]. More complicated mineralogical processes through biological means can achieve further complex geological formations [5].

Through cataloguing microbial diversity within sample rock coatings from the region 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. Two different archael primer sets were also applied to the extracted DNA to amplifial archael rDNA identifier segments. No usable DNA sequences were provided from archael primer sets. 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 aligned against the Basic Local Alignment Search Tool (BLAST) database. The highest % matches were further analyzed for their isolate source and physiology.

Table 1. Bacterial isolate matches from an Al-rich 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	grassland soil	aerosol
H1-03	Firmicutes	soil	human skin
H1-04	Firmicutes	soil	human skin
H1-05	Firmicutes	soil	soil
H1-06	Unclassified Mn-oxidizer	rice field soil	human skin
H1-08	Firmicutes	soil	soil
H1-09	Chloroplast	green algae	dolomite microbe
H1-10	Actinobacteria	soil	soil
H1-11	Firmicutes	contaminated groundwater	human skin
H1-12	Betaproteobacteria	forest soil	rice paddy soil
H1-14	Chloroplast	plant	plant
H1-15	Firmicutes	?	elephant feces

H1-16	Betaproteobacteria (Np-reducer)	deglaciated granite sand	Np-enriched sediment
H1-17	Firmicutes	soil	earthworm gut
H1-19	Alphaproteobacteria	acidic coal mine	volcanic rock
H1-21	Firmicutes	alkaline soil	alkaline soil
H1-22	Firmicutes	brown algae	brown algae
H1-23	Alphaproteobacteria	acidic peat bog	Arctic glacier ice
H1-24	Firmicutes	caverns	caverns
H1-26	Alphaproteobacteria	acidic soil	Arctic glacier ice

Results and Discussion: Of the 27 colonies sequenced from the H1 sample, 20 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%.

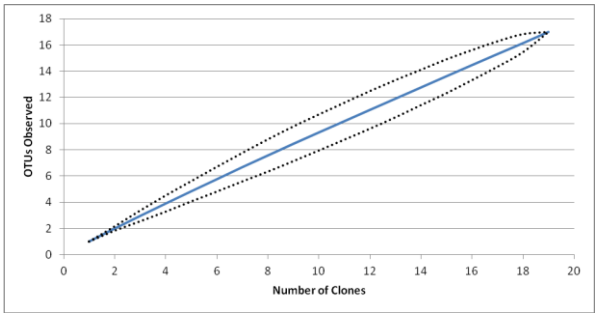

Figure 1. Rarefaction curve for sample H1 illustrates the need for a larger sampling size. This rarefaction curve compares operational taxonomic units (y-axis) to the sample size (x-axis). Species richness is described by the number of isolate matches with unique phylogenetic origins (OTU's). As the curve remains linear throughout (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 high 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.

Isolate matches displayed a wide variety of temperature tolerance- including both psychrophiles and thermophiles. Psychrotolerant matches originated from glacier ice and deglaciated granite sand. One isolate match was sourced from the lava flow of the Hnaushahraun volcano in Iceland.

Other isolate matches originate 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 has been suggested as an important mechanism in the formation of secondary minerals such as jarosite in acid mine drainage [6].

A chloroplast clone originated from endolithic microbes found within dolomite. Organisms in such environments have been notably difficult to discover [7]. Subterranean environments have been posited as potential sources of Martian life would be suitable to house endolithic microbes.

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.

The diverse physiologies displayed in the reference isolate matches provide a variety of biomineralization processes which may generate unique mineralogies indicative of biogenesis. Isolate sources offer conditions similar to the Martian landscape, providing relevance of the isolate match organisms to potential Martian lifeforms.

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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