ANAEROBIC CULTURING EXPERIMENTS OF SULFATE CRUSTS, Fe/Mn SKINS, AND ALUMINUM GLAZES FROM KÄRKEVAGGE, SWEDISH LAPLAND. R. L. Mickol[1] and C. L. Marnocha[1] [1]Arkansas Center for Space and Planetary Sciences, 202 Old Museum Building, University of Arkansas, Fayetteville, AR 72701; rmickol@uark.edu.

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 compatible with martian mineralogy have not been studied from a geomicrobiological perspective in such detail, particularly with respect to astrobiology on Mars.

In this work, we attempt to culture and analyze anaerobic organisms. Because anaerobic organisms do not require oxygen, we present rock coatings as potential biosignatures for Mars that warrant more in-depth investigation.

Study Site: Kärkevagge is a glacially-eroded U-shaped valley in Swedish Lapland. The upper valley walls are made up of garnet mica schist, while the lower valley walls are predominately quartz mica schist. The two units are divided by thin beds of marble, with finely disseminated pyrite found throughout the valley [3, 4].

Rock coatings are ubiquitous in the valley. Nomenclature herein used to describe coatings are derived from chemical analysis by Dixon et al. [4] and Dorn's [2] classification system. The three coating types used in this study are sulfate crusts (jarosite, gypsum), Fe/Mn films (goethite, hematite), and aluminum glazes (basaluminite and alunite) [4].

Methods: Representatives from the three coating types were sampled in summer 2010 and summer 2012 from transects along the eastern and western valley walls.

Two types of anaerobic culture media were prepared following the procedure of Kendrick and Kral [5]. Ten milliliters of each medium were added to each of 22 tubes, and the tubes were sterilized via autoclave. Eleven samples consisting of all three coating types from both 2010 and 2012 were selected for culturing, with two replicates per sample per medium. Rock coating samples were weighed out and placed in a Coy anaerobic chamber with the sterilized media. The rock coating samples were placed in the corresponding test tubes within the anaerobic chamber, restoppered, and removed from the chamber. A sterile solution of 2.5% sodium sulfide was added to the media following removal from the chamber and the tubes were pressurized with 200 kPa of H_2 gas. Tubes were incubated at room temperature for three months with methane production analyzed via gas chromatography after two weeks, and again after five additional weeks.

In a supplementary experiment, a second set of tubes was prepared to serve as additional controls. Two types of anaerobic culture media were prepared as above [5]. Ten milliliters of one medium were added to each of twenty tubes, and the tubes were sterilized via autoclave. Samples of each of the three coating types were added to four separate tubes containing the medium. For each coating type (as well as the control tubes), one tube contained a sterile solution of 2.5% sodium sulfide, and was pressurized to 200 kPa with H_2. Additionally, one tube contained sodium sulfide, but was not pressurized, and another tube was pressurized, but did not contain sodium sulfide. The final tube did not contain either sodium sulfide or pressurized hydrogen gas. The above procedure was repeated for Fe/Mn skins and jarosite crusts for the second medium. Tubes were incubated at room temperature for 2 weeks.

Tubes were sampled for bacterial growth using a standard methylene blue staining procedure. Tubes showing high concentration of bacteria were further analyzed via live/dead stain using fluorescent microscopy.

Results: In the first experiment, two weeks following preparation, tubes were analyzed for methane production via gas chromatography. No methane was present in any samples, nor was any methane detected five weeks later.

Liquid media from tubes from both experimental sets, chosen at random, were analyzed for bacterial growth. Microscopy was used to observe cell morphology and arrangement. Culture from tubes exhibiting turbidiity were stained with methylene blue and observed with a brightfield microscope. A control tube containing solely medium, 2.5% sodium sulfide and pressurized to 200 kPa with H_2, showed no growth, as expected. All other tubes analyzed (five in total) showed growth in varying amounts (Figs. 1-2). Additionally, two tubes under aerobic conditions also showed growth (Fig. 3).

Tubes with growth seen under methylene blue staining were futher analyzed. Culture media was stained with Live/Dead stain (Life Technologies) and observed with a fluorescence microscope (Fig. 4).

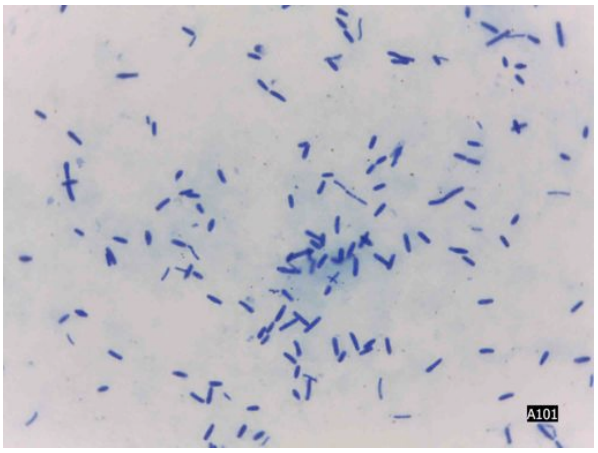

Figure 1. Image of bacteria sampled from anaerobic Fe/Mn film in anaerobic culture medium, magnification = 1000x.

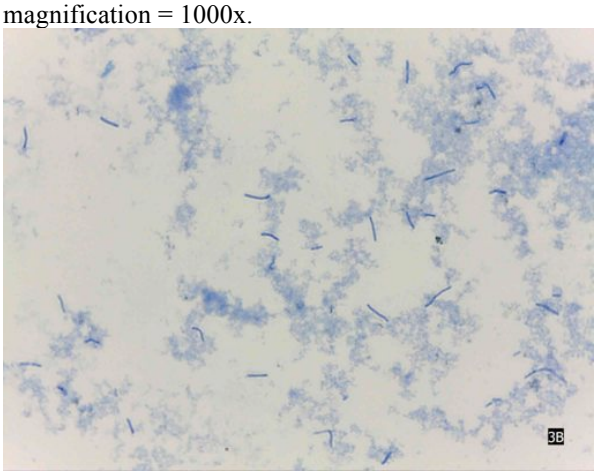

Figure 2. Image of bacteria sampled from anaerobic jarosite crust in anaerobic culture medium with 2.5% sodium sulfide and pressurized with H_2, magnification = 1000x.

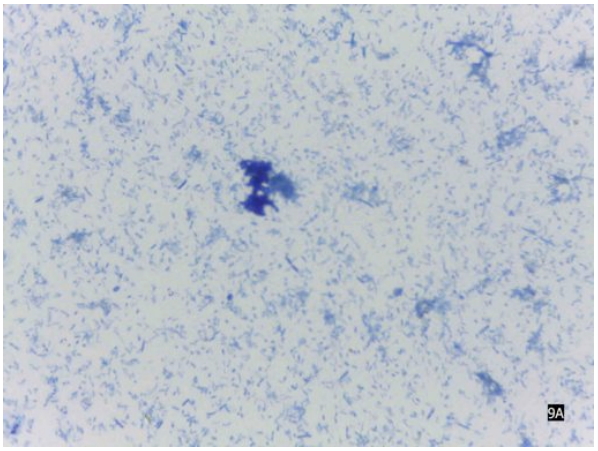

Figure 3. Image of bacteria sampled from aerobic Fe/Mn film in anaerobic culture medium with 2.5% sodium sulfide and pressurized with H_2, magnification = 1000x.

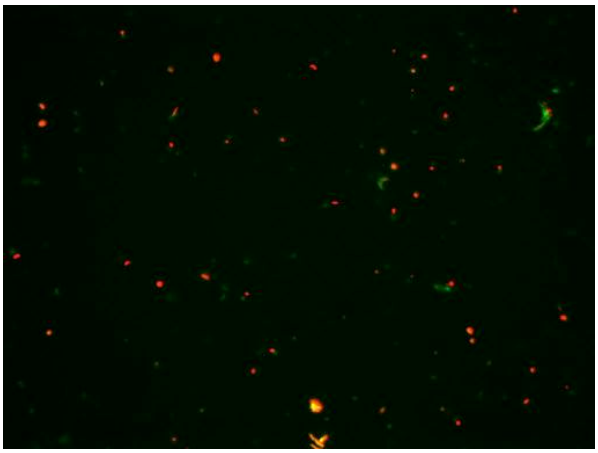

Figure 4. Image of live/dead stain of bacteria sampled from anaerobic Fe/Mn film in anaerobic culture medium, magnification = 400x.

Discussion and Conclusions: This experiment complements the study of microbial diversity in rock coatings found in Kärkevagge, Sweden. The ability for rock coating microbes to withstand anaerobic conditions furthers the potential for coatings to be abodes for life on Mars and serve as potential biosignatures. Recent sequencing analyses have revealed the presence of anaerobic bacteria in the rock coatings, including sulfate-reducing bacteria (Marnocha and Dixon, LPSC XLIV, this conference).

Tubes were analyzed for methane production as an indicator of methanogen presence. The lack of detection of methane does not necessarily rule out methanogen presence in both our rock coating samples, or in the rock coatings in Kärkevagge.

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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] Dixon, J.C. et al. (2002) *Bull. Geol. Soc. Am., 114*, 226-238. [4] Dixon, J.C. et al. (1995) *Geogr. Ann. A., 77*, 259-267 [5] Kendrick, M.G. and Kral, T.A. (2006) *Astrobiology*, 6, 546–551.