INVESTIGATION OF THE SPATIAL RELATIONSHIPS OF BACTERIA ASSOCIATED WITH ROCK VARNISH. A. C. Corcoran[1], D.R. Noguera[1] and K.R. Kuhlman[2], University of Wisconsin-Madison (3201 Engineering Hall, 1415 Engineering Drive, acrocoran@wisc.edu), [2]Affiliation for second author (402 Engineering Research Building, 1500 Eng. Research Bldg., Madison, WI).

Introduction: Understanding the characteristics of life in terrestial environments analogous to the martian environment influences the search for life on Mars [1-4]. Especially important is the search for life in niche environments that may harbor life in the arid and high UV radiated martian environment [1-4]. Rock varnish is a 10-500 μm thick coating with nanostratigraphic layering composed of approximately 70% clay minerals cemented together by 30% oxides and hydroxides of manganese and iron [3-4]. Rock Varnish forms in arid to hyperarid region and may exist as a UV shield for the organisms within it [1] and is a niche environment in areas where it is difficult for organisms to exist even in soil such as the Yungay reagion of the Atacama Desert [3]. Rock coatings that resemble varnish have been observed on Mars making this an environment worth investigating [1].

The goal of the research is to assess the spatial relationships between rock varnish from Martian analog environments and microbial communities that inhabit them. Fluorescence in Situ Hybridization (FISH) was used in order to determine these spatial relationships. FISH is the use of dye tagged oligonucleotide probes that target the the small or large subunit rRNA of an organism. The hybridization of this probe with the rRNA of organisms then yields information on the microbial ecology of samples in various environments [5]. The microbial ecology data available is the type of organism as well as its spatial relationship to other organisms as well as its immediate environment.

Due to low ribosomal content and high autofluouresnce, a variation of FISH with Catalyzed Reporter Deposition(CARDFISH) in order to vizualise cells in situ [6]. Using CARDFISH the spatial relationships of cells to the varnish can be established. Additionally identification of the community that inhabits this niche environment illustrates how terrestrial lifeforms survive in this niche envrionment.

Sample Processing: The field areas evaluated in this study were Cima Volcanic Field in the Mojave Desert California and an area near Darwin California. Samples were collected and shipped back to the University of Wisconsin-Madison for molecular analysis.

Varnish was ground off of the parent rock and then analyzed in several different ways. DNA was extracted form the Varnish and then the 16S or 18S rRNA was sequenced to determine the community composition of both samples. This rRNA data was then classfied to an 80% confidence level using the Ribosomal Database Project [7]. Also Phospholipid fatty acids (PLFA) analysis was performed in order to determine cell count and realtive community composition.

Community composition information from sequencing and PLFA anaylsis was then used to determine the rRNA target sites for probes for CARDFISH. This was performed on ground varnish in order to optimize a protocol for later CARDFISH on cross sections of the varnish.

CARDFISH replaces the fluorescent tag on the 5' end of the oligonucleotide probe used in traditional FISH with Horseradish Peroxidase(HRP). The HRP tag is reacted with fluorescently tagged tyramide. This process yields fluorescent data on the number and type of cell within the sample when analyzed by confocal microscopy.

Results: PLFA analysis yielded cell counts of $6.69\ E^{7}$ (cells/gram)to $1.54E^{8}$(cells/gram) indicating that a significant amount of life does exist in this niche environment. The 16S and 18S sequence data discoverend in this study represents a significant increase in the total known sequences from varnish environments. In the Ribosomal Database Project there are 167 sequences found in Rock Varnish studies. 141 16S seqeunces were discovered in varnish from this research. This is resprents a signficant expansion in 16S knowledge of Bacteria and Archaea and 18S knowledge for Eukaraya. The organisms found include Cyanobacteria, Alphaproteobacteria and Thermoprotei.

The CARDFISH results show several successful hybridizations and were able to get around much of the autofluoresnce that would typically plague this kind of research. Figure 1 is an image of a piece of rock varnish that has been hybridized with the bacteria targeting probes EUB338 I, II, III. This represents a breakthrough in terms of obtaining successful in situ results from rock varnish. A probe targeteing Archaea was also successfully hybrized in these samples to give an idea of microbial community structure.

The CARDFISH results from these images provide the community compostion and spatial relationships to help understand this terrestrial niche environment. The organisms that survive in rock varnish may yield clues as to which environments organisms would survive on Mars.

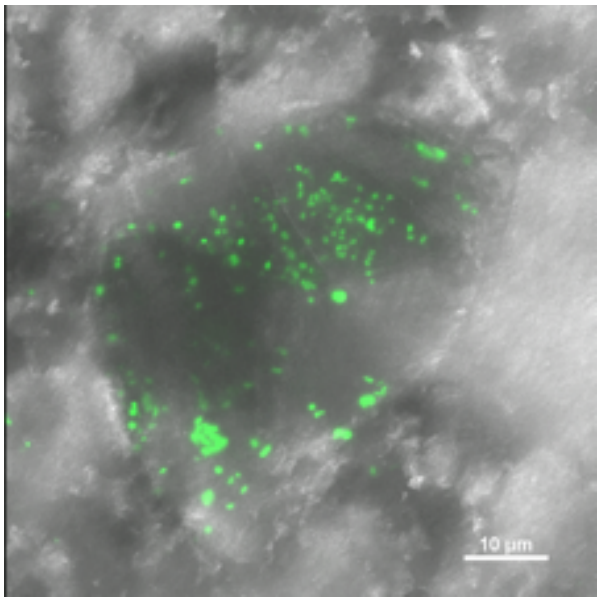

Figure 1. Transmitted light and 488nm image of Rock Varnish from the Cima Volcanic field in the Mojave desert. Sample has been hybridized with

References: [1]DiGregorio, B.E. (2002), Rock varnish as a habitat for extant life on Mars, in Instruments, Methods, and Missions for Astrobiology IV, edited by R. B. Hoover et al., Proc. SPIE Int. Soc. Opt. Eng., 4495, 120–130. [2]Northup, D.E, Snider, J.R. Spilde, M.N. Porter, M.L. van de Kamp, J.L. Boston, P.J. Nyberg, A.M. and Bargar, J.R. (2006) Diversity of rock varnish bacterial communities *GEOPHYSICAL RESEARCH*, 115, G02007 [3]Kuhlman, K.R., W.G. Fusco, from Black Canyon, New Mexico *JOURNAL OF* M.T. La Duc, L.B. Allenbach, C.L. Ball, G.M. Kuhlman, R.C. Anderson, I.K. Erickson, T. Stuecker, J. Benardini, J.L. Strap, and R.L. Crawford Diversity of microorganisms within rock varnish in the Whipple Mountains, California. *Applied and Environmental Microbiology*, **72**(2): 1708-1715. [4] Kuhlman, K.R., P. Venkat, M.T. La Duc, G.M. Kuhlman, and C.P. McKay (2008) Biodiversity of the Microbial Community Associated with Rock Varnish at Yungay, Atacama Desert, Chile. *Journal of Geophysical Research-Biogeosciences*. [5]Amann, R.I. (1995) In situ identification of micro-organisms by whole-cell hybridization with rRNA-targeted nucleic acid probes, in *Molecular microbial ecology manual*, A.D.L. Akkermans, J.D. van Elsas, and F.J. de Brujin, Editors. Kluwer Academic Publishers: Dordrecht, The Netherlands. 1-15.[6] Pernthaler, A., J. Pernthaler, and R. Amann (2002) Fluorescence in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria. *Applied and Environmental Microbiology*, **68**(6): 3094-3101. [7]Wang, Q, G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. *Appl Environ Microbiol.* 73(16):5261-7