**PRELIMINARY CHARACTERIZATION OF A MICROBIAL COMMUNITY OF ROCK VARNISH FROM DEATH VALLEY, CALIFORNIA.** K. R. Kuhlman[1], M. T. La Duc[1], G. M. Kuhlman[1], R. C. Anderson[1], D. A. Newcombe[1,2], W. Fusco[2], T. Steucker[2], L. Allenbach[2], C. Ball[2], and R. L. Crawford[2], [1]Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Grove Dr., Pasadena, CA 91109, kkuhlman@jpl.nasa.gov; [2]Environmental Research Institute, University of Idaho, Moscow, ID 83844, crawford@uidaho.edu.

**Introduction:** Rock varnish (also referred to as desert varnish in the literature because it is particularly noticeable in desert environments) is a dark, thin (typically 50-500µm thick), layered veneer composed of clay minerals cemented together by oxides and hydroxides of manganese and iron [1-4]. Some scientists suggest that varnish may provide a historical record of environmental processes such as global warming and long-term climate change. However, despite more than 30 years of study using modern microanalytical and microbial culturing techniques, the nucleation and growth mechanisms of rock varnish remain a mystery [4,5].

Rock varnish is of interest to the Mars science community because a varnish-like sheen has been reported on the rocks at the Viking Lander sites [6,7]. It therefore important for us to understand the formation mechanisms of terrestrial varnish – abiotic, biotic, or a combination of the two -- as this understanding may give us clues concerning the chemical and physical processes occurring on the surface of Mars. It is strongly believed by some in the biogeochemistry community that microbes have a role in forming rock varnish, and iron- and manganese-oxidation by microbes isolated from varnish has been extensively investigated [815-16]. Only two of these studies have investigated the microbial genetics of varnish [16,16]. These studies examined the morphological, physiological and molecular characteristics of microbes that had previously been cultured from various rock varnishes and identified the cultivars using 16S rDNA sequencing techniques. However, it is well known that most of organisms existing in nature are refractory to cultivation [17-20], so many important organisms would have been missed.

The currently described work investigates the genetics of rock varnish microbial community from a site in the Whipple Mtns., south of Death Valley, CA, near Parker, Arizona (Figure 1). We employed both cultural and molecular techniques to characterize the microorganisms found within the varnish and surrounding soil with the objectives of (a) identifying microorganisms potentially involved in varnish formation, and (b) discovering microorganisms that simply use the varnish as an extreme habitat.

**Varnish collection:** Since we are investigating the biocomplexity of the microbial communities of rock varnishes, it was critical that the samples be collected aseptically as possible. The samples were approached from the downwind direction, photographed *in situ*, picked up at arm's length using sterile gloves, placed within sterile Whirl-paks™ and sealed. Loose dirt on the undersides of the varnished rocks has proven to be a contamination problem during storage and harvesting of the varnish in the laboratory. The samples were then wrapped in protective material to prevent damage. In future collections, glass containers will be used to prevent the deposition of polymers from the Whirl-paks™ on the surface of the varnish, thus contaminating the surface for microanalytical work [21].

The varnish was harvested from the host rock in a laminar flow bench. A Dremel tool with a coarse bit that had been flame sterilized was used to grind the varnish off the host rock and into a sterile container [22]. For use as controls we also collected soil samples adjacent to some of the varnished clasts.

Figure 1. Macro photograph of a varnished clast used in our study. The underlying grid has 10 divisions per inch.

**Microbial Enumerations:** Powdered rock varnish (0.1 gram) was added to 1 milliliter of sterile double-distilled $H_20$. The samples were then stained with a stock DAPI (or Acridine Orange) (50mg/mL) solution. Samples were filtered onto 25mm Millipore Isopore 22µm pore size black polycarbonate filters with Whatman 25mm GF/F filters used for support. Fluorescing cells were counted on a Zeiss Research epifluorescence microscope equipped with an Osram

Xenon short arc photo optic lamp XBO 75W, and Chroma #31000 filter set for DAPI/Hoechst/AMCA.

The mean field (n) counted per sample was 57.16. The field standard deviation per sample was 7.48. The rock varnish had an average DAPI direct count of $9.0x10^7$ cells $gram^{-1}$ (standard deviation = 1.2E+07). There was no determinable difference between DAPI and Acridine Orange direct counts.

**Cultivable UV-resistant microorganisms:** Five distinct colony types were purified from TSA plates after exposure to the UV irradiation treatment. These strains were examined microscopically using both a standard light and a transmission electron microscope (TEM). The 16S rDNA gene of each isolated was amplified by PCR, cloned and sequenced for comparison to the RDP phylogenetic database.

We examined each cultivated strain for its resistance to UV(C) irradiation. The strains were quite resistant, and still showed growth on TSA plates after exposure for five minutes at UV(C) intensities that readily killed E. coli. Affiliations were estimated for three of the strains to genus level only using a similarity level of >98%.

*Strain RV1 (white colony).* Multiple *Arthrobacter* strains were found in a white colony (*A. polychromogenes, A. ramosus, A. oxidans, A. globifromis, and* unspeciated *A. spp.* show 99% sequence similarity with this isolate). Eppard, et al. (1996) reported the culturing of three strains highly related to *Arthobacter agilis* from rock varnish in the Mojave desert, but did not report UV resistance. [13].

*Strain RV2 (orange colony).* Various strains of *Curtobacterium flaccumfaciens* (e.g., AY273208.1) and an unidentified glacial ice bacterium (AF479342.1) show 98% sequence similarity to this isolate. Thus, this strain could be a candidate for survival in conditions such as those found in the polar regions of Mars.

*Strain RV4 (black colony).* This strain shows 16S rDNA sequence similarities of 98% (forward primers) and 99% (reverse primers) to a *Geodermatophilus obscura* originally isolated from soil samples from the Amargosa Desert of Nevada by Luedemann (1968) [20] and found in Negev desert soil and monuments by Eppard, et al., (1996) [13]. The strain's morphological and growth characteristics fit the description of *Geodermatophilus obscura* cluster I as described by Eppard, et al. (1996).

**The uncultivable microbial community**: We generated three rDNA libraries from the Death Valley rock varnish community DNA and two control libraries from soil adjacent to the varnished rock and lacking varnish. Varnish 16S rDNA libraries were prepared for Eubacteria and Archaea, and an 18S rDNA library for Eukarya. The control soil libraries were prepared for Eubacteria and Archaea. An 18S rDNA control library for Eukarya was not obtained for the soil despite six attempts. Between 100-200 clones were prepared for each library.

The clones within each library have been arranged into related subgroups through examination of their RFLP patterns, and 16S rDNA PCR products of representative members of each subgroup were sequenced.

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