

BACTERIAL DIVERSITY IN DESERT VARNISH

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Abstract

We report here results of a culture-independent technique analysis of bacterial diversity in a rock desert varnish coating from Death Valley, California. DNA was extracted from varnish coatings, and using PCR to amplify 16S rRNA, both Archaea and Bacteria rRNA were amplified from coatings and surrounding soils. Clone libraries of bacterial- and archaeal-specific PCR product from desert varnish were then constructed and phylogenetic analyses, using full-length sequences, were done. This study indicates that there are a wide variety of prokaryotic microorganism in varnish coatings including non-thermophilic crenarchaeota, and possibly autophototrophic primary producers. If DNA from recent or ancient microbes is ever found on Mars, then understanding the mechanisms for preservation of DNA in extreme Earth environments is essential.

Background

Desert varnish is a dark coating that forms on rocks and archaeological monuments in arid and semi-arid regions throughout the world. Varnish-like coatings may also exist on Mars, as suggested by observations at Viking and Mars Pathfinder landing sites.¹⁻³ Rock coatings found in arid deserts such as the Atacama, the Gobi, the Great Victoria, the Namib, the Negev, the Sonoran, and the Mojave are formed in some of the most hostile conditions on Earth. Long periods of draught alternating with infrequent periods of heavy rain, intense UV radiation, and high temperatures often exceeding ~60°C are typical. Desert varnish is normally composed of oxygen, silicon, and aluminum with lesser amounts of iron, manganese, potassium, calcium, titanium, magnesium, sodium, sulphur, phosphorous, barium, carbon, and nitrogen. Some organic compounds have been previously identified from rock coatings including amino acids^{4,5}, and lipids⁶. The process of varnish formation is a complex depositional process and is not fully understood. It has been argued that its genesis may be either biological⁷⁻¹⁵, inorganic^{16,17}, or as a result of both organic and inorganic reactions interacting on rock surfaces¹⁸. The inorganic chemistry of varnish coatings has been well analyzed, however the organic components have been less studied. The presence of labile compounds such as serine⁴, presents a paradox that may be answered by its sequestration in or on clays or in an amorphous silicate matrix. While DNA may also be entombed in silica or

complexed by Si-O-C bonds, it is possibly also complexed with oxides of iron¹⁹ and or manganese²⁰.

Previous Culture Based Results

Previous culture based studies have found primarily Gram-positive bacteria²¹. Hungate (1987) found 78 out of 79 Gram-positive bacteria obtained by plating from the Negev Desert. The predominant genera were *Bacillus*, *Geodermatophilus*, *Arthrobacter*, and *Micrococcus* and are similar to microorganisms cultured from the American southwest. Amino acid analyses supports the likelihood of an association with Gram-positive bacteria⁴. However, culture-based techniques tend to underestimate microbial diversity.

Culture independent results

We first presented preliminary results of culture independent analyses of desert varnish in the spring of 2003²². Another preliminary study soon followed from another area of the Mojave Desert and also found microbial DNA²³. We continue to sequence Bacteria, Archaea, and Eukarya from soils and rock coatings.

DNA was extracted from 500 mg of varnish using a Fast Soil DNA extraction kit (Bio101) and Beadbeater (Savant). Polymerase Chain Reaction (PCR) to amplify bacterial 16S ribosomal RNA (rRNA) genes was performed using DNA from soil and varnish as template with primers 27f and 1492r²⁴. PCR cycling parameters were 30 cycles of 95°C for 0.5 min, 55°C for 1.5 min, and 72°C for 2.5 min. Clone libraries of resulting PCR product were constructed using a TOPO TA Cloning kit (Invitrogen) and screened by amplified ribosomal DNA restriction analysis²⁵. Clones were divided into groups that shared the same RFLP pattern, and plasmid from selected clones in each group was sequenced with the same primers used for amplification by the UW-Seattle Biochemistry DNA Sequencing Facility. Resulting full length sequences were checked for chimeras using the CHIMERIA_CHECK program provided by the Ribosomal Database Project-II (RDP-II)²⁶, which identified that almost all of the sequences were chimeric in nature. Sub-sections of these sequences that did not appear to be chimeric in nature were determined using the CHIMERA_CHECK and SEQUENCE_ALIGNER tools provided by RDP-II. These sub-sections of sequences were assigned to a taxonomic group using the SEQUENCE_MATCH tool provided by RDP-II and by BLAST²⁷; if this analysis indicated similarity to more

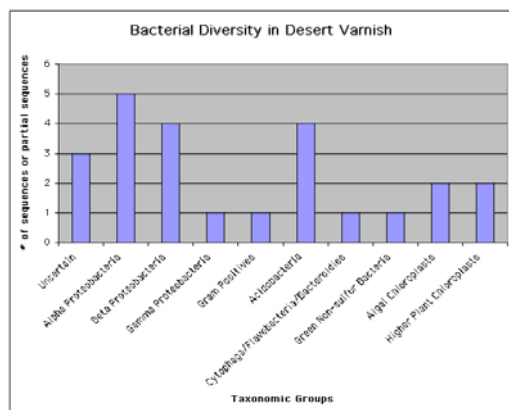


Figure 1. Bacterial diversity in desert varnish from the western region of Death Valley, California.

than one group, the sequence was designated as “uncertain” taxonomic affiliation (Figure 1). Archaeal sequences were not chimeric, and phylogenetic analysis inferred them to be closely related to non-thermophilic soil crenarchaeota¹⁸.

This study indicates that there is a diverse population of microorganisms on or in varnish surfaces. The presence of 16S rRNA sequences similar to those of autotrophic organisms suggests that primary producers may be present (Figure 1). SEM was used to determine the presence of microbes on the surface of the varnish coatings. Few bacteria were observed however, suggesting that the DNA is derived from within the coating but not precluding that it is a result of enhancing bacteria from outer surfaces.

Conclusions and Applications to Mars

Microbial DNA is sequestered in rock coatings in harsh desert environments. Analyses of rock coatings from other diverse locations needs to be done and may provide clues as to the role of microbes and varnish formation and could also suggest how DNA might be preserved on other planets such as Mars.

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