

UV Resistance and Salt Screens: The Search for Microbial Life in the Martian Environment

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The search for life on Mars is a daunting task that requires that we limit our efforts to areas with the maximum potential for high-yield discoveries. Evidence of ancient hypersaline environments on Mars as discovered by the Opportunity MER and the existence of halophilic micro-organisms on Earth strongly suggests we include these environments in our search for extraterrestrial life. UV radiation incident upon the surface of Mars has often been cited as a limiting environmental challenge for life. Recent experimental evidence has shown that salts can reduce the sterilizing effects of ionizing radiation through abiotic processes. This research proposal seeks to test this hypothesis in a field setting to help determine the optimal geological settings for biological field sampling in a Martian-type environment.

LITERATURE REVIEW

The evidence gathered by the Mars Exploration Rover (MER) Opportunity¹ suggesting the presence of hypersaline environments at some point in Martian history at Meridiani Planum has given astrobiologists a strong starting point to look for life on the red planet. Halophilic organisms are prolific on Earth, showing great diversity in survival mechanisms and environmental tolerances². Studies on the model halophilic archeon *Halobacterium* sp. strain NRC-1 have shown the organism to be highly tolerant to a range of environmental conditions including desiccation, high vacuum, UV-C and gamma irradiation^{3,4}. Viable halophiles have been shown to survive for 250 million years in brine inclusions within salt crystals on Earth⁵, signaling the possibility that microorganisms could exist within halites on Mars.

The Martian environment is known to be a range of challenging environmental conditions, the most biologically relevant being the presence of high incident levels of UV radiation, which result in the rapid formation of DNA lesions which, if left unrepaired, can result in cell lethality. The high levels of UV radiation reaching the surface of Mars in the absence of an ozone layer has often been cited as the most critical parameter for bacterial survival^{6,7}.

Recent evidence from my work indicates that the composition and concentration of salts in the surrounding environment plays a role in reducing the amount of DNA damage caused by exposure ionizing radiation. Gamma irradiation was used to generate oxidative damages *in vitro* using puc19 plasmid DNA and *in vivo* using *Halobacterium* cells. A variety of salts/metals were used to suspend both the plasmid DNA and whole cells to measure the effects of each compound on oxidative damages induced by exposure to ionizing radiation. Halides, specifically bromide and to a lesser extend chloride, were shown to be excellent scavengers of the DNA-damaging hydroxyl radicals thereby reducing the overall damage to DNA. DNA damage is responsible for the bulk of the lethality of oxidizing agents. Halides are also common elements in hypersaline environments on Earth in locations such as Great Salt Lake in Utah, and on Mars at Meridiani Planum. Thus, evaporate deposits which include bromide and chloride are excellent locations to search for microbes in high-radiation

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environments. I am interested in expanding these studies to UV-C radiation. A portion of the cellular damages induced by UV irradiation is caused by photooxidation reactions, implying that the same salts that help to reduce the oxidative damage to DNA induced by gamma irradiation may also provide protection from the effects of UV irradiation.

High and low salt environments in the MDRS study area have previously been identified by Expedition One and Expedition Gamma crewmembers⁸. The Salt Wash and Brushy Basin Member sites in the Morrison Formation are of particular interest as high-salt environments due to the presence of evaporites. The Tunuck Member site in the Mancos Shale Formation is of interest as a potential low-salt site with deposits indicative of past water modifications. These sites are proposed here for use as sampling sites to test the hypothesis that the geological/minerological setting can provide an abiotic means of shielding halophilic microbes from UV radiation. This study will help to identify the criteria important for site selection for future Mars biological sampling areas by investigating the interplay between geological setting and biological survival upon exposure to UV radiation.

This work strongly adheres to the biological experimentation program for the MDRS as outlined in "A Systematic Approach to Studies at the Mars Analog Research Stations"⁹ focusing on the capacity for microbial life to thrive in the area, spatial distributions of life, and microbial diversity within microhabitats.

PROBLEM STATEMENT

This project seeks to isolate optimal locations to search for microbial life on Mars based on the ability to survive exposure to the types of radiation which reach the surface of Mars. The hypothesis being tested is that the presence of salts and/or minerals will help to increase resistance to UV-C radiation through abiotic shielding mechanisms.

METHODOLOGY

1. 10 sampling sites will be chosen (5 high-salt, 5 low-salt) based on previous MDRS Expeditions (specifically Expeditions One and Beta) and/or geological analyses of Expedition Gamma.
2. Digital photographs of each sample site will be taken to document the local environment of each sample site. Care will be taken to find samples of similar granule size to reduce the variation in UV shielding effects caused by large particle size.
3. 10 samples will be collected (5 from high-salt environments, 5 from low-salt environments) 3 cm wide x 3 cm high x 0.5 cm deep. Each sample will be placed into a sterile 50 mL tube.
4. Each sample will be split into quarters by volume into 4 sterile 50 mL tubes (one control non-hydrated, one experimental non-hydrated, one control hydrated, one experimental hydrated) upon return to the Habitat. Two milliliters of water or 15% (w/v) saline solution will be added to each of the 'Hydrated' sample tubes depending if the sample is from a non-salt or high-salt environment respectively. These tubes will be incubated at room temperature for 15 min with periodic gentle shaking by hand to re-hydrate samples.
5. Experimental sample will be placed one at a time in separate Petri plates. These samples will be exposed to 100 J/m² of UV-C radiation (254 nm) using a handheld UVP Pen-Ray lamp
6. Control samples will be left un-irradiated.

7. A series of volumes of each sample (10, 100, and 400 uL) will be plated onto the appropriate agar plate (LB agar for low-salt samples, LB saline agar for high-salt samples). Both control and experimental samples will be plated.
8. The plates will be incubated at 37 °C until good colony growth is observed.
9. Colony growth will be enumerated and survival ratio calculated as (N/No) where N is the number of observed colonies from the experimental sample and No is the number of observed colonies from the control sample.

REQUIRED RESOURCES

1. Digital camera**
2. Ruler**
3. Petri plates* (80)
4. 50mL sterile tubes* (40)
5. 50mL tube rack*
6. Agar* (30 g)
7. NaCl* (27 g)
8. Tryptophan (15 g)
9. Yeast extract* (7.5 g)
10. Water* (1.6 L)
11. Scoopula*
12. Weigh paper*
13. Scale*
14. Pipettor (200 uL)*, and sterile pipette tips*
15. Tea lights*
16. Ethanol (95 %)**
17. Glass spreader (can be make from glass Pasteur pipette)*
18. Incubator (37 °C)*
19. UV protection face-shield**
20. UV source ** UVP Pen-Ray lamp; 228.6 mm x 9.5 mm, UVP Inc.

PROPOSED ANALYSES

Comparisons of geological composition of both high-salt and low-salt environments of similar rock particle size will allow testing of the hypothesis that salts and/or minerals in the surrounding regolith can reduce the sterilizing effects of UV-C radiation on microbial life. The survival ratio calculations will be examined marking any differences between the survival ability of microbes in the high-salt versus low-salt environments. The use of a control sample which has been removed from the original geological context will allow for a more accurate measurement of the effect of the mineralogical/geological environment on UV-C resistance.

The mix of previous geological and biodiversity analyses with this test of environmental resistance will help to narrow the scope of future field experimentation sites to search for potential microbial life on Mars.

* denotes materials located at the MDRS

** denotes materials to be supplied by myself

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