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**<sup>1</sup>HOW WOULD A LANDING PARTY SAMPLE LIFE ON MARS?  
METHODS TESTING AT THE MARS DESERT RESEARCH STATION,  
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**ABSTRACT**

Considerable evidence exists to suggest that conditions permissive for some type of microbial life might exist on Mars. Strategies for detecting putative lifeforms present considerable challenges, and are necessarily based on assumptions regarding their chemistry and ecology. The Mars Desert Research Station (MDRS) provides an analog setting for testing methods and hypotheses under simulated Martian operational conditions. We had three main objectives during our rotation; first, to test sample collection methods under simulation; second, to evaluate the human impact on the near-habitat microflora, and finally, to integrate the entire crew into biology activities. In the future, there should be a broad science mission for MDRS involving both biology and geology, in order to provide for sustained scientific achievement.

**KEYWORDS:** Desert microbiology, Microbial sampling, Exobiology

**INTRODUCTION**

What sort of life might we look for on Mars? The Viking Lander missions were designed to look for metabolic activity, such as photosynthesis and respiration, based on assumptions regarding Earth organisms (1,2). This mission was a remarkable technical achievement, despite the negative but somewhat ambiguous conclusions. It is more reasonable to start with only the most general assumptions, namely that any organism would be carbon-based and would operate under chemical and thermodynamic principles.

If life on Mars and Earth shared a common origin, by somehow seeding each other during early planetary history, then Martian organisms, fossil or extant, may well be based on some form of nucleic acids and proteins familiar on Earth. Also, it is possible that the chemical options for functional life are restricted, and an independent origin would yield the same result. On the other hand, Martian life may have evolved a completely novel biochemistry, with different molecules for genetic storage and expression, and different structural and catalytic polymers. In any case, some type of cell surface or membrane must bound such organisms, in order to enclose the living chemistry

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from the outside environment. Energy-yielding metabolism might be chemically exotic, based on available compounds for oxidation/reduction (3). Organisms might be dormant for millions of years, and only metabolize and reproduce when conditions of temperature, water, and nutrient availability are permissive (4,5).

Regardless of Martian biochemistry, there must be more than one kind, and the principle of natural selection would apply. If life originated on Mars around the same time that it did on Earth, then genetic variants must have arisen; those most successfully adapted to its particular niche would have persisted. Given the massive geological changes, and the resulting selection pressures, which have taken place since planet formation, numerous lineages would have arisen in any extant life. Probably these various types exist in communities, and together participate in an ecosystem. All types must be able to colonize new areas, in order to escape frequent habitat destruction.

These more general assumptions can be used to guide strategies for the next generation of Martian life searches. Analog testing of any strategy in representative Earth environments is essential to developing a mission that can answer questions regarding the presence of past or present Martian life. MDRS provides such an opportunity in a desert setting. The biology goals during the two-week Rotation 5 were designed to take advantage of the unique possibilities of this environment:

1. To test sample collection and detection methods under closed simulation
2. To evaluate the human impact on the near-habitat microflora
3. To integrate all crew members into biological research activities

## **SAMPLE COLLECTION STRATEGIES**

All samples were collected by crewmembers wearing simulated pressure suits, which included bulky gloves. Therefore, manipulation was difficult and required preparation of all tools and sampling devices prior to leaving the “Hab”. In all, three different detection and sampling strategies were explored.

### **Detection Direct: Attachment of Microorganisms to a Sampling Unit under Dry Conditions**

While the chemical nature of the outer surface of any Martian organism is unknown, it likely includes a complex mixture of polymers bearing regions of positive and negative charges, and non-polar, hydrophobic regions. Earth microorganisms are a particular example of this structural arrangement. Attachment of a organism to a sampling device makes no assumptions, other than charge interactions, and does not require metabolic activity of any kind. Use of a glass microscope slide has been a common technique for retrieving microbes from aquatic environments, and it is of considerable interest to determine whether attachment can take place under completely dry conditions. The area chosen for this determination was waypoint 105, an area of rocky potholes about 500 M south of the Hab, which is covered by a luxuriant growth of

unidentified bronze-yellow lichen (Figure 1). A sample of this lichen eluted from a small pebble revealed both rounded algal cells and fungal mycelium under the microscope.

### Methods

Prior to the EVA to this site, microscope slides were attached to conspicuous bright pink plastic tape telltales and alcohol sterilized. They were placed in a Ziploc plastic bag, likewise sterilized with alcohol, for transport. At the site, several locations were chosen for placement in direct contact with rock or sand. The slides were imbedded in the sand or if against rock, covered with another rock to keep them in place. The pink telltales were visible to identify the sites. After five days, the slides were retrieved for microscopic examination by pulling on the telltales with a large forceps. They were then placed in alcohol-washed collection bags. Unfortunately, many of the slides were broken, including all of the ones in a sandy area, perhaps as a consequence of a severe windstorm that had occurred during the period of attachment. No rain fell during this period. However, some slides were available for inspection.

### Results

Under wet mount, slides that had been in direct contact with the lichen-coated rock showed the same algal and mycelial cells originally observed at that site. Clearly it is possible for at least some microorganisms to attach to a solid substrate under completely dry conditions. In the future, this method should be improved by design of a holder to insert fragile slides into compacted regolith, and by use of plastic slides or a flexible, transparent membrane that can be mounted on a microscope slide in the laboratory.

### **Sample Collection: Windblown Dust**

If life exists on Mars today, there must be some sort of dispersal mechanism to allow transfer of viable forms to new habitats. An obvious candidate for this is windblown dust. Major storms lasting months and covering significant portions of the planet are known to exist. On Earth, dust is known to transfer microorganisms thousands of miles, such as between the Sahara Desert and the Caribbean (6,7). The goals for our rotation were first, to establish a method for collecting windblown dust at MDRS, and second, to profile any microorganisms present in the sample.

### Methods

The collection devices used consisted of cylindrical, clear plastic sample vials attached to 60 cm bamboo garden stakes with duct tape. Holes (~4mm) were drilled around the rim to allow dust entry from any direction. The interiors of the vials were sterilized with alcohol, holes were covered with tape, and plastic snap lids were placed on top. The collectors were bundled for transport by pedestrian and ATV EVA teams. The collectors were installed at previously established windy sites, and retrieved for inspection.

Collected material was suspended in 2 ml of sterile, distilled H<sub>2</sub>O and transferred to a sterile conical centrifuge tube with a transfer pipet. After tabletop settling of the suspension, 10  $\mu$ l of the aqueous slurry was streaked onto LB agar plates. After incubation at 30°C for 1-2 days, colonies were observed and counted.

## Results

The first attempts at collecting dust were not successful. Wind conditions in the MDRS area are quite variable; the first installation of collectors at waypoint 102 contained no visible sediment after two days, probably due to the dead calm conditions. The second attempt at the same site, a small, exposed hill, suffered the opposite problem. A severe windstorm blew over the collectors, which were found to be empty. One problem with the collector design is the difficulty in placing the bottom in compacted regolith, especially by pressure-suited crewmembers. Even pounding the stakes with a geology hammer, and bracing the bottom with nearby rocks and sand, did not provide adequate stability. Finally, a collector was taped to the flagpole immediately outside the Hab in a second windstorm. While this technique did succeed in collecting a sizeable sample, the collector design should be improved so that they can be left in remote areas, and collectors should be left in place long enough to ensure adequate wind transfer.

The material collected settled out into three obvious zones: at the bottom was a coarse (0.5-2mm) gray layer. Above that was a medium to fine-grained deep rusty red layer. A zone of non-settling, gray-white fines blended with the aqueous supernatant. This layer was sampled for plating. After incubation, many colony types appeared, as distinguished by color and morphology. One type was unique to this sample, and not found in samples taken directly from the soil around the Hab. These results and their limitations will be discussed in greater detail below.

## **Detection by Growth: Winogradsky (Ecosystem) Columns prepared with MDRS Soil Samples**

Since most microorganisms in soil samples are undescribed and not readily culturable, a column growth arrangement provides a setting for enrichment growth under natural conditions. In general, the sample is placed in a closed glass or plastic tube, which is then allowed to incubate under specified conditions of temperature, light, etc (8). Organisms that require oxygen will proliferate at or near the top air space, while anaerobes will only grow near the bottom. If the organisms are pigmented, colored layers can be observed in the column. The value of this technique is that nothing need be known about the nutritional requirements of the organisms, since the environment in which they are found presumably supplies what is required. Modern versions of this technique have ports through which gasses and other metabolic products can be sampled.

In a desert environment, the types of organisms present in a sample may depend on the availability of water. Therefore we collected samples from areas which were

always dry (any rain would immediately run off), intermittently wet (from the dry bottom of an obvious rivulet), and always wet (mud from a streambed).

### Methods

The columns used were flat-bottomed glass tubes (2 cm x 15 cm) with plastic caps. The soil samples had been collected in alcohol-sterilized plastic sample vials with snap-cap lids. Samples of each (10 g) were placed in the tubes and sufficient sterile, deionized water was placed in each to thoroughly wet the soil, with a substantial aqueous layer above the streambed sample. The columns were then incubated on a sunny windowsill in Chicago, IL, for three months.

### Results

Each sample differed in color and texture, probably because water plays a major role in flushing soluble and finely pulverized minerals. The dry sample was pale, pinkish gray, with a fines layer at the top. The wet sample was gray-brown and coarse. The intermittently wet sample was very finely textured and deep rusty red.

After undisturbed incubation under natural light-dark daily cycles, different types of presumed biological activity were observed in each column. The Dry sample showed three colored layers in the top cm of the soil. Just below the water, a yellowish-white layer appeared over time, with a pinkish layer just below. Below that a dark gray-brown layer appeared. No gas bubbles were ever observed. The bottom portion of the column showed no visible changes. A microbial mat was seen floating on the aqueous layer.

The Wet sample is quite different. A purple-black zone appeared near the bottom, under somewhat anaerobic conditions. A cluster of bubbles 1-2 mm in diameter appeared in the upper half of the column. The surface of the soil was greenish, and floating clumps of green material were dispersed throughout the aqueous layer.

The Intermittently Wet sample showed several types of apparent growth at the top 2 mm of the column. Just below the liquid surface, separate streaks of reddish-purple and green black appeared after two months. Several kinds of colonies, mostly viscous and mucoid, grew on the glass wall above the liquid surface. Some of these colonies were pale pink.

While none of the presumed biological growth regions has been further characterized, it seems likely that each sample region contains different types of microbial populations. Selection for different organisms may well be a consequence of the differing chemical compositions present in the soil. This point should be explored further, and a more sophisticated column that permits direct sampling should be employed to analyze metabolic activity *in situ*.

## HUMAN IMPACT ON THE NEAR-HAB MICROFLORA

Humans obviously disturb the microbial environment, both by altering soil and water chemistry, and by releasing human-associated microbes. While it is definitely not clear that any Earth microorganism would survive and reproduce if released on Mars, the question is of considerable interest for the ultimate development of agriculture. The objective for this study was to determine the extent of variation in the kinds of microorganisms that could be cultured adjacent to the Hab following construction and five rotations. It was assumed that the extent of disturbance varies with distance from the Hab.

### Methods

Two directions were chosen for sampling: relatively undisturbed (U), southwest of the main airlock (197° from geographic north), and contaminated (C), across the leach field (111° from geographic north). Surface soil samples (0.5 ml) were taken at 1, 5, and 20 meters from the Hab. Distances were measured with a steel tape. Prior to sampling, alcohol-sterilized plastic snap-cap micro-centrifuge vials with volume markings were labeled and placed in Ziploc bags. At the designated distance the appropriate vial was removed from the bag with a large forceps and the sample collected from the surface.

For analysis, each sample was resuspended in 1 ml sterile H<sub>2</sub>O, vortexed 15 sec, and allowed to settle on the workbench for 30 min. Soil was then pelleted by centrifuging in an Eppendorf micro-centrifuge at maximum speed for 15 sec. A 20- $\mu$ l portion of each supernatant was transferred to 190  $\mu$ l sterile H<sub>2</sub>O, from which serial 10-fold dilutions were prepared. Each dilution was streaked onto LB plates with a 10- $\mu$ l plastic inoculation loop. All plates were transferred to an incubator at 33°C for 1-3 days. Colonies differing in color and morphology were counted separately from the dilution providing the most accurate count. The pH of the original supernatants was estimated with narrow-spectrum pH paper.

### Results

Collection of these samples was nontrivial, due to the major windstorm taking place on the day this was carried out. Sustained wind speeds of greater than 40 mph with gusts up to 56 mph were recorded while we were on EVA. Dr. Vladimir Pletser assisted with the collection and measurements, and Dr. William Clancey made still and video recordings. While it was frequently difficult to stand during wind gusts, it was essential to collect samples on that day to allow time for microbial growth before the end of our rotation. Exploration parties on Mars may well be under the same kind of constraints.

Soil pH varied somewhat among the samples, as shown in Table 1. The highest pH was recorded in the sample presumably least affected by Hab occupation, the 20-m sample in the undisturbed direction. Samples in the contaminated direction were somewhat more acidic.

After incubation of the plates, several colony types were observed, whose distribution varied among the samples. Some were evident in all samples, and some were unique to particular samples or directions. The colony types observed are as follows:

- A: Round, bright orange
- B: Small, white, cusped, like a molar tooth
- C: White, glossy
- D: Large, white, puckered
- E: Flat, bright yellow
- F: Flat, translucent
- G: Rounded, yellow
- H: Large, white opaque

The distribution of colony types is shown in Table 2. The most abundant type is shown in boldface, and any unique to that sample is underlined. Orange colonies (A) were present in all samples, while C, E, and F types were only found in the undisturbed direction. The distinctive cusped colonies, B, were only present in the 20-m undisturbed sample, where they were the dominant form. The contaminated direction samples uniquely produced colonies of types D and G. The windblown dust sample, discussed above, produced colonies of types **A**, **C**, **F**, **G**, and H, which was unique to this sample. None of these organisms has been identified at present.

While these results suggest varying patterns of microbial populations surrounding the Hab, the methods employed in this study are crude and by no means identify all types present. It is to be expected that most microorganisms in a sample cannot, in fact, be cultured. LB plates are rich medium which no doubt exclude many autotrophic forms. Many microbes may well remain trapped within crevices in individual soil particles.

## **INTEGRATION OF THE CREW INTO BIOLOGICAL RESEARCH**

All members of Crew 5 participated in this work, which could not have been accomplished without their help. They helped with sample collection, detector installation, field measurements, and photography. In order for biology to succeed in an analog mission, all crewmembers should feel connected to a successful outcome. The biologist is obligated to explain procedures carefully, and to provide properly packaged materials so that other crewmembers can carry out their assignments without confusion or difficulty. Crew 5 members, who came from different professional backgrounds, made many valuable suggestions for improving both equipment and procedures. Finally, scientific results are interesting and exciting, and everyone should share in the pleasure as well as the work.

## **CONCLUSIONS**

Analog field testing is essential for the design of an effective experimental strategy for searching for life on Mars. The work done during Rotation 5 provides encouragement for the approaches to sampling methods attempted, and suggests

improvements for further methods and equipment design. Some improvements could be made in the Hab itself. While every attempt was made to carry out the culturing procedures under sterile conditions, construction aspects in the building left holes open to the outside through which dust readily penetrated. A simple and inexpensive solution would be a “clean workstation” or glovebox apparatus in which to carry out sterile operations.

Further improvements in equipment and procedures are suggested by the experience of the conditions encountered in this environment, such as high winds, compacted soil, and limited power availability. For example, the initial attempt at collecting soil samples near the Hab was aborted when one of the sample-collecting bags was blown away in the very high wind. Dr. Pletser, who had astronaut training, suggested that all bags and tools should be tethered to the person who was not using them; it is much easier for a person to remove objects from a partner’s pockets than from his own. After this procedure was implemented, collection took place without further problems.

In future missions, there should be a broad, overall science mission that takes advantage of the uniqueness of Martian analog research and of the remote desert site. Possible components of such a program might include:

- Development and testing of life detection methods
- Study of soil chemistry and mineral formations in relation to microbial communities
- Miniature instrumentation development and testing
- Development of field data recording methods

A general program would not preclude other initiatives, but provide a framework for recruitment of collaborators. Consistent research progress would also attract funding, and, best of all, contribute to successful exploration of Mars.

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## TABLES AND FIGURES

**Table 1. Estimated pH Values of Near-Hab Soil Samples**

Distance from Hab, m			
	1	5	20
<b>Undisturbed</b>	7.8	7.5	>8.4
<b>Contaminated</b>	7.5	7.5	7.2

**Table 2. Summary of Colony Types in Near-Hab Soil Samples**

Distance from Hab, m			
	1	5	20
<b>Undisturbed</b>	A, C, E, F	A, C, F, G	A, <u>B</u>
<b>Contaminated</b>	A, D, G	A, C, D	A, C, D



Figure 1 View of waypoint 105 showing lichen-coated rock and a telltale from an inserted microscope slide sampler. The backpack and GPS unit provide scale.