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Definition of Exobiology Experiments for Future Mars Missions

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NASA Cooperative Agreement NCC 2-479

Progress Report
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Summary: During the past year we have concentrated on two objectives. The first objective is ongoing and is to define the experimental parameters that are necessary to conduct autonomously a mineralogical analysis of the Martian surface in situ using differential thermal analysis coupled with gas chromatography (DTA/GC). The rationale in support of this objective is that proper interpretation of the mineralogical data from the DTA/GC can be used to better describe the present and past environments of Mars, leading to a better assessment of the probability of life evolving on Mars. To meet these objectives we have analyzed a number of samples collected from nature using the DTA/GC. One of the more significant findings was that in samples of desert varnish we detected magnetite and maghemite that may serve as potential biomarkers (see below for discussion) applicable to DTA/GC analyses of Martian surface material during landed missions. The second objective follows from the first and is to better understand microbe-environment interactions by determining the response of microbes to changes in their environment, including extreme desiccation and solar UV-radiation. The rationale behind this is to develop hypotheses regarding what may have happened to life that may have arose on Mars, and microbial life that may get to the surface of Mars via spacecraft, or meteors from Earth. To accomplish this objective we have exposed microbes, collected from NaCl and gypsum-halite crystals, to the space environment aboard the ESA-German Biopan facility for 15 days. The most significant finding was that these microbes survived the exposure better than others.

Microbe Environment Interactions. The overall objective of the Biopan study is to understand the responses of osmophilic/halophilic microbes to space vacuum and solar UV-radiation. Nucleic acid bases absorb UV-radiation strongly, with maximum absorbance at 254 nm. Proteins also absorb UV-radiation with a peak at 280 nm. It has been well established that cell death by UV radiation is due primarily to its action on DNA. Among the several defects in DNA caused by radiation, the formation of thymidine dimers followed by strand breaks are the most prominent. Cellular death due to anhydrobiosis (extreme desiccation) results from lipid, protein and nucleic acid irreversible phase changes such as denaturation and structural breakage (Cox, 1993; Cox 1987; Dose et al., 1992).

One of the specific reported mechanisms of death due to anhydrobiosis in prokaryotes is the dehydration of DNA causing strand breaks (Dose et al., 1992).

Few studies have been conducted on the effects of the space environment on microbes. These studies reflect the effects of the space environment on representatives of a small number of microbial species. Microbes tested in the space environment to date include *Bacillus subtilis* spores, bacteriophage T-1, and Tobacco Mosaic Virus (reviewed in Horneck and Brack, 1992, and Horneck, 1993), and diatoms. The results of these studies revealed that *Bacillus subtilis* spores will survive for years in space if protected against high solar UV-radiation flux (Horneck, 1993), whereas viruses lose viability on the order of weeks to months (reviewed in Horneck and Brack, 1992). We have recently discovered that microorganisms inhabiting gypsum-halite crusts and NaCl crystals are extremely osmophilic and can withstand extreme desiccation (Rothschild et al., 1994). Because of this, and the fact that gypsum-halite and NaCl attenuate UV-radiation, we hypothesize that these organisms are good candidates for survival in the space environment. To that end, we exposed these organisms, in a dried state, to the space environment in Earth orbit aboard a satellite equipped with the European Space Agency's Biopan facility. Data from the Biopan experiments indicates that the osmophiles can survive in the space environment, whereas most others die within minutes. The organisms were exposed to the space environment for 2 weeks while in earth orbit aboard the ESA Biopan facility. As controls, organisms were kept in the dark and only exposed to space vacuum. Ground control time course experiments (vacuum dark, vacuum UV, and UV only exposed samples) were conducted in a space simulation facility. All samples were compared to unexposed samples. Survivability was determined by plate counts and the most probable number technique. DNA breakage was determined by labeling breaks in the DNA with ^{32}P followed by translation.

During the Biopan flight the total radiation dose to the organisms was 10^4 KJm^{-2}. *Haloarcula-* G and *Synechococcus* survived exposure to the space environment with the number of breaks in the DNA of the UV exposed samples greater than the vacuum only exposed sample. Results of the ground time course studies revealed that *Haloarcula-* G (Figure 1) and *Synechococcus* (data not shown) survived over 100 hours of exposure to UV radiation from a deuterium lamp (Intensity = 0.07 W m^{-2} at 200 nm and 0.03 Wm^{-2} at 300 nm).

The number of breaks in the DNA of *Haloarcula-* G increases with increased exposure time to UV and vacuum, with exposure to UV exhibiting a greater number of breaks than exposure to vacuum alone (Figure 1). From these data we conclude that *Haloarcula-* G isolated from a NaCl crystal & Synechococcus (Nägeli) inhabiting gypsum-halite crusts

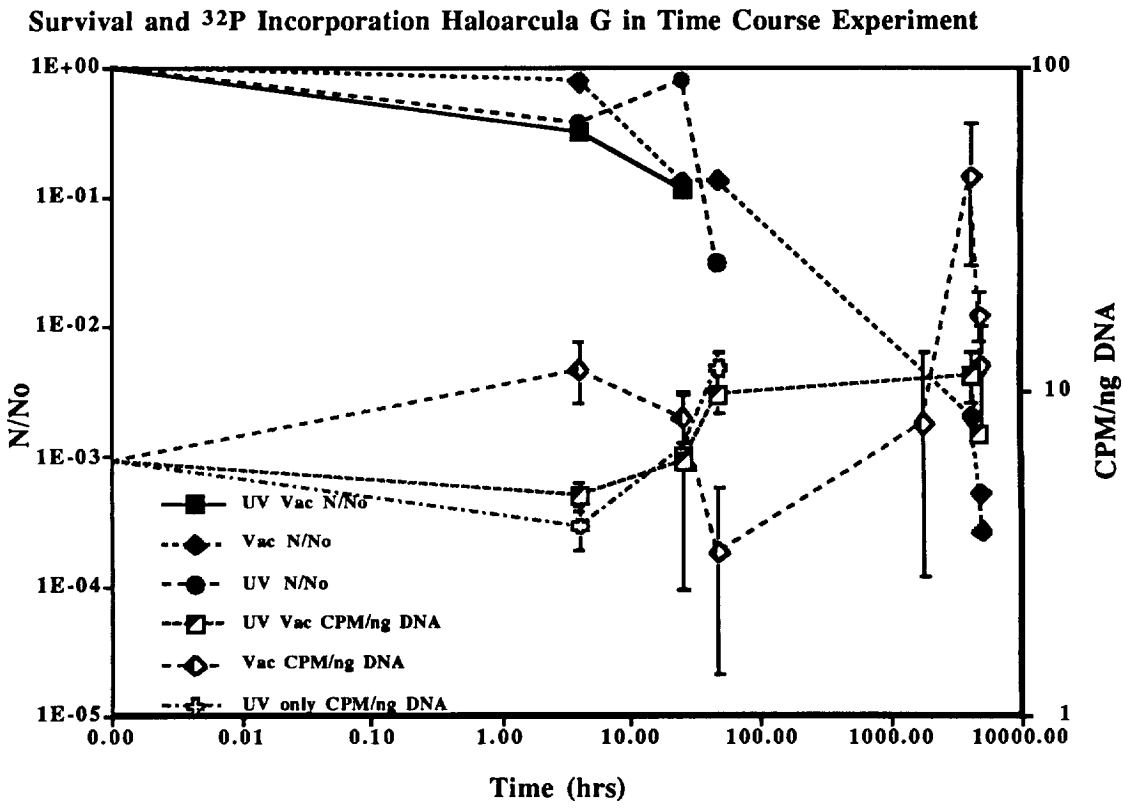

Figure 1. Survival (N/No solid symbols) and levels of ^{32}P incorporation into DNA (cpm/mg DNA) of Haloarcula-G (open symbols) over time exposed to UV & vacuum, UV in air, or Vacuum only.

have a better rate of survival when exposed to the space environment than any organism tested to date. The data also suggest that DNA damage due to UV is greater than that caused by anhydrobiosis.

Rates of nitrogen fixation, denitrification, carbon fixation and phosphate incorporation into DNA were determined *in situ* on a microbial community inhabiting an area one meter from the source of Octopus Spring, a thermal (86 °C) alkaline (pH=8.2) spring in Yellowstone National Park, Wyoming. Nitrogen fixation rates were determined using the acetylene reduction method. Rates of denitrification were determined similarly using acetylene to block the reduction of nitrous oxide to dinitrogen. Nitrogen assimilation rates were determined using $^{15}NO_3^-$ and $^{15}NH_4^+$. Carbon assimilation rates were determined using ^{14}C-acetate. Carbon fixation rates were determined using ^{14}C labeled CO_2. Phosphate incorporation was determined using $^{33}PO_4^{3-}$. The mean nitrogen fixation rate was 0.671 nmoles ethylene produced mg^{-1} protein hr^{-1}. The mean denitrification rate was

0.158 nmoles N_2O denitrified mg^{-1} protein hr^{-1}. This community assimilated NH_4^+ at a rate of 0.88 $\mu g\ hr^{-1}$, but could not assimilate NO_3^-. Results of water analyses combined with these data indicate that the community relies on nitrogen fixation for its source of fixed nitrogen. The carbon fixation and acetate assimilation studies show that the community is comprised of primarily phototrophs, secondarily chemoautotrophs and lastly heterotrophs. This is surprising because no chlorophyll containing pigments were found in extracts of the community, but a new type of carotenoid pigment appears to exist in these organisms and may be part of a here-to-fore undiscovered photo-pigment system.

Samples of soda-nitre have been collected from the Atacama desert in Chile and are currently being analyzed in the laboratory for viable microbes as well as by DTA/GC.

DTA/GC Measurements. We have analyzed a variety of substances important to exobiology using DTA/GC. The substances tested included organic compounds (proteins, amino acids), evaporites (NO_3^-, CO_3^{2-}, and NO_2^- salts), clays (montmorillonite, kaolinite, nontronite), Fe-enriched clays (Banin et al., 1993), non-clays (palagonite), and various mixtures of these substances. In addition, we have analyzed samples collected from ecosystems ranging from Antarctic endolithic rocks, evaporitic deposits occurring near thermal alkaline and acid springs in Yellowstone National Park, Wyoming, to gypsum halite evaporitic crusts that form along the intertidal. We compared the DTA/GC with several other techniques and found it to be the most appropriate as a flight instrument for the *in situ* determination of the mineralogy of the Martian surface material .

Desert varnish, a coating of manganese and iron oxides held in a clay matrix on the surface of rocks, is widespread on Earth's deserts. Its formation is thought to involve, weathering processes acting in conjunction with microbial activity. Consequently, if desert varnish similar to that found on earth is found on Mars, then the probability for life to have arisen on Mars increases.

Data from a typical analysis of a sample of desert varnish collected from Nevada is depicted in figure 2. Analyses were conducted using 30 mg of aluminum oxide as the reference material, and 30 mg of sample. Samples were removed from rocks covered with varnish with aluminum oxide grit (this was used because it would not give a DTA thermal signature nor would it react with the varnish). Samples were analyzed under vacuum using a Dupont model 1600 high temperature DTA oven equipped with a model 910 cell base. The heating rate was 10 $^oC\ min^{-1}$. The system is controlled by a Sun Sparc II workstation. The system was tested and calibrated with pure samples of the suspected unknown minerals in the sample, as well as mixtures of these minerals. Gas evolution during sample heating is sensed by a pressure sensor which triggers a valve allowing the evolved gas to expand from the oven chamber into a GC sample loop.

The first endotherm (~100 °C) depicted in figure 2 is accompanied by the production of water vapor and can be attributed to the vaporization of water adsorbed to the sample, while the second (~120-130 °C) is due to Illite. The exotherms and endotherms occurring between 140 and 320 °C are most likely due to the oxidation and transformations of oxides and oxyhydroxides of manganese. The exothermic peak observed between 320 °C and 360 °C is due to the transition of magnetite (Fe_3O_4) to maghemite (γ-Fe_2O_3). The endotherm centered around 400 °C is accompanied by the formation of water and is due to the oxidation of goethite (α-FeOOH) to hematite (α-Fe_2O_3). This exotherm may be due to the transition of γ-$Fe_2O_3 \rightarrow \alpha$-$Fe_2O_3$ (hematite). The next thermal event is a large exotherm between 420 and 560 °C and can be attributed to the transition of γ-$Fe_2O_3 \rightarrow \alpha$-$Fe_2O_3$. This exotherm is followed immediately by what appears to be two overlapping endotherms that are accompanied by the formation of water and are probably due to dehydroxylation of the Illite and kaolinite. Usually clay dehydroxyaltion endotherms are much broader, but in this case they are being somewhat masked by the γ-$Fe_2O_3 \rightarrow \alpha$-$Fe_2O_3$ exotherm. The next thermal event (~ 640 °C) is a small endotherm that is probably due to a change in the magnetic properties of hematite (Mackenzie, 1970). The endotherm occurring between 690 and 725 °C is accompanied by the formation of water and is due to the dehydroxylation of montmorillonite. The next three thermal events represent the high temperature transition reactions of montmorillonite, illite and kaolinite respectively.

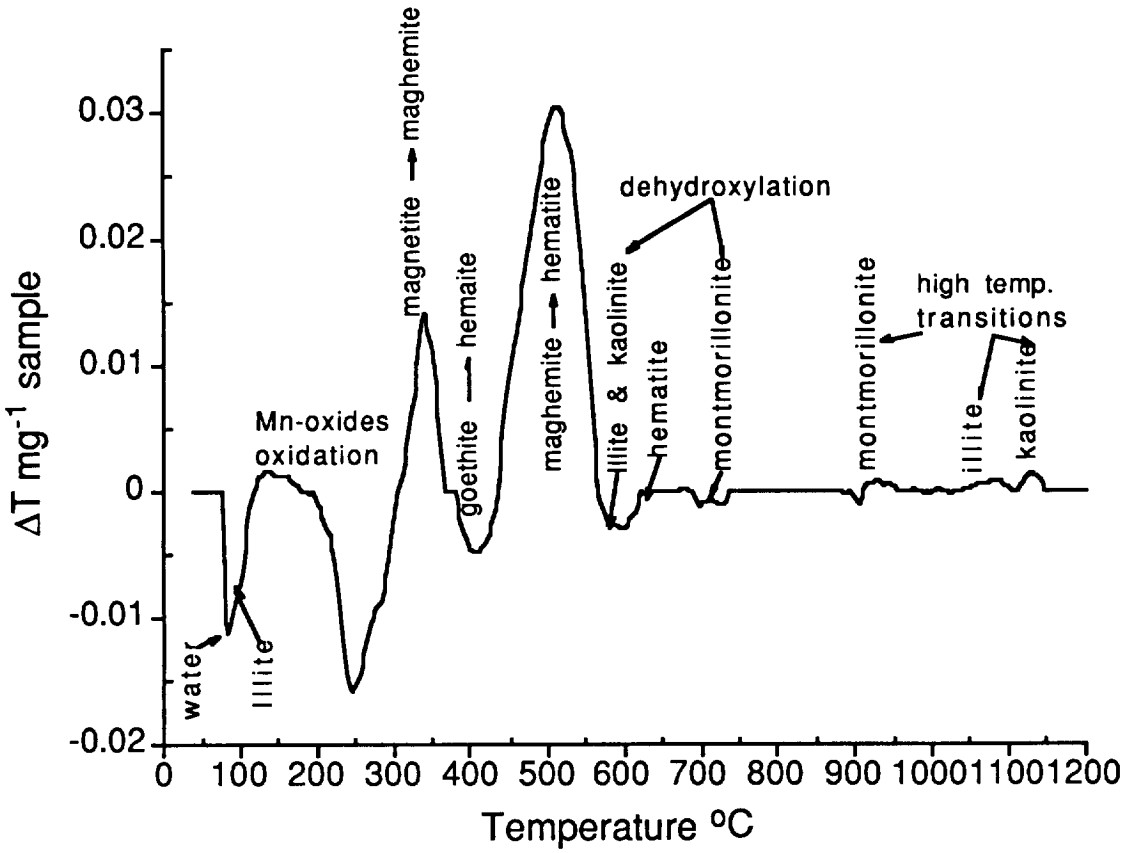

Figure 4. Results of analysis of 30 mg of desert varnish collected from Reno, Nevada by DTA. 30 mg of Aluminum oxide was used as the reference. The samples and reference were heated at 10 °C min^{-1}.The endotherms and exotherms are labeled as to the minerals and reactions that gave rise to each.

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Following is a list of presentations and publications resulting from this project during the past year:

Invited Presentations

1995, Survival of Osmophilic Microbes Exposed to the Space Environment, Institute for air and space medicine, Cologne, Germany

1995, The Nature of Nitrogen in the Environment, as part of the NASA sponsored Workshop on Nitrogen Cycling in Closed or Controlled Environments, University of California-Berkeley, Berkeley, CA.

1995, Exposure of Osmophilic Microbes to the Space Environment: Application to the Moon, Annual Meeting of the European Geophysical Society, Hamburg, Germany.

Publications

Mancinelli, R. L. 1995. The regulation of methane oxidation in soil. *Ann. Rev. Microbiol.* **49:**581-605.

Bishop, J. L, C. M. Pieters, R. G. Burns, J. O. Edwards, R. L. Mancinelli, and H. Fröschl. 1995. Reflectance spectroscopy of ferric sulfate - doped montmorillonites as Mars soil analog materials. *Icarus.* **117:** 101-119.

Banin, A., and R. L. Mancinelli. 1995. Life on Mars? I. The chemical environment. *Adv. Space Res.* **15:**(3) 163-170.

Mancinelli, R. L. and A. Banin. 1995. Life on Mars? II. Physical Restrictions. *Adv. Space. Res.,* **15:**(3)171-178.

Schwartz, D. E., R. L. Mancinelli, and M. R. White. 1995. Search for life on Mars: Evaluation of techniques. *Adv. Space Res.,* **15:**(3)202-208.

Published Abstracts

Mancinelli, R. L., M. R. White, and L. J. Rothschild. 1995. DNA integrity and survival of *Synechococcus* (Nägeli) exposed solar irradiation and vacuum in earth orbit. *PSA Abt.* **54:**11.

Rothschild, L. J., and R. L. Mancinelli. 1995. Field analyses of carbon, nitrogen, and DNA metabolism in algal mats. *PSA Abt.* **82:**16.

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Mancinelli, R. L., and M. R. White. 1995. Effects of nitrate and ammonium on methane oxidation, and denitrification in a sanitary landfill. *ASM Abt.* **N-146:**357.