A FURTHER ANALYSIS OF POTENTIAL PHOTOSYNTHETIC LIFE ON MARS

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Background: Three primary requirements for life-water, essential elements and energy-are found within the near-surface of Mars [2,3,4, 8,10]. Shielded from the various stresses imposed by the Martian surface environment, it is believed that life could find the perfect niche within meters of the surface [2,4,5,6]. Simple photosynthetic organisms could theoretically receive enough energy from the sun at certain periods—whether once every year [4,5,6,9] or once every ten thousand vears [4,13]—to thaw out from a frozen, dormant state and reproduce until they refreeze. This theory is based upon examples set by extremophiles-many of them photosynthetic cyanobacteria-currently living within the Antarctic and Arctic ice caps on Earth that hibernate through frozen conditions and remain viable upon Potential Martian habitats thawing [5,6,7,12]. sufficient to protect and sustain such cyanobacteria for long periods include the north and south polar ice caps [4,5,9] as well as various sedimenttary rock formations [13].

Introduction: As a combined result of these arguments, we theorize that photosynthetic organisms, under the right conditions and with the properly adapted surroundings, have the necessary requisites for life and can still exist on Mars. In particular, terrestrial psychrophilic (or psychrotolerant) nitrogen-fixing organisms provide the best analog for potential Martian life due to the planet's surface temperature as well as soil and atmospheric composition [11]. These nitrogenfixing bacteria have the ability to grab atmospheric nitrogen (N_2) and subsequently are not limited by nitrogen availability within their surrounding soil-ideal for Martian surface terrain that appears to be devoid of soluble nitrogen [11]. However, while such extremophiles have been studied within their diverse, severe environments on Earth [7,12], there is no specific terrestrial environment that can properly simulate Martian conditions. Therefore, we feel much can be learned about the bacterial adaptability to such conditions by putting them under various stresses similar to those it would experience on Mars. To perform these tests we acquired masses of cyanobacteria from the McMurdo Dry Valleys in Antarctica and attempted to isolate nitrogen-fixing strains. This project studied how these bacteria responded to some these various stresses, including Martian Regolith Simulant, high salinity, UV radiation, low pressure and Martian atmospheric composition.

Methods: Bacteria were grown under lamps at a temperature of 12°C within a cold water bath and refrigerator. This proved to be a fairly optimal temperature for growth, although the cyanobacteria would still grow (albeit slower) at 4°C.

Viability of the bacteria after exposure to various stresses was either tested visually or with fluorometry. Visual tests involved analyzing day by day macroscopic green growth or microscopic bacterial growth (or movement). A fluorometer was used to test viability by measuring the fluorescence given off by the photosynthetic apparatus within the bacteria upon exposure to instantaneous light stress.

Nitrogen-fixing strains were isolated from clumps of Antarctic matter by growing them in BG-11_o. BG-11_o is the common recipe for BG-11 without NaNO₃ in order to remove all nitrogen from the medium. This promoted the growth of nitrogen-fixing bacteria capable of using N_2 from the atmosphere.

The bacteria were tested within the Martian Regolith Simulant by making an extract for the bacteria to grow in. To create this Martian Regolith Simulant extract (MRSE), Martian Regolith Simulant (from the JSC, [1]) was placed in deionized water and mixed thoroughly overnight. The mixture was then centrifuged and the heavy, non-transparent soil was removed. This created a transparent extract. After sterilizing the MRSE in an autoclave we inoculated washed, active cyanobacteria samples into liquid MRSE or onto MRSE agar plates (made with 1.5% Noble Agar). Growth is tested visually as well as with fluorometry. **Results:** Nitrogen-fixing strains have been successfully isolated from the Antarctic cyanobacterial masses by using BG-11_o. Filamentous nitrogen-fixing strains show clear heterocysts (locations of nitrogen fixation).

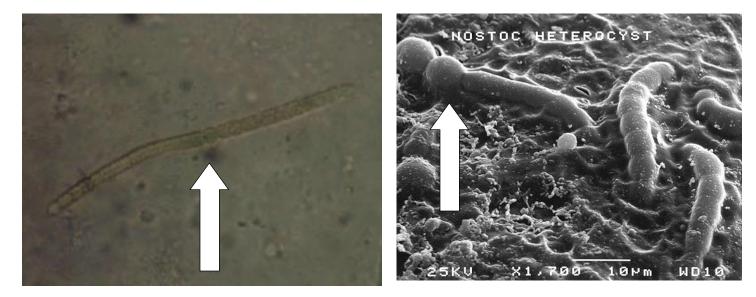
The nitrogen-fixing strains appear to be growing well in the MRSE according to visual tests. Further tests will be performed (including fluorometry) to prove their adaptability to this Martian Regolith Simulant.

Future Work: These nitrogen-fixing cyanobacteria will be tested under various other stresses such as high salinity, UV radiation, Martian atmospheric composition and possibly low pressure.

Implications for Mars: The Antarctic nitrogen-fixing cyanobacteria appear to grow well in Martian Regolith Simulant. This reaffirms the belief that such possible photosynthetic life on Mars will likely be able to fix nitrogen if Martian soil is devoid of nitrogen (as tests to this point have shown). Further tests on these bacteria under potential Martian conditions will provide an added basis for the possibility of photosynthetic life on Mars. Such analyses will hopefully convince Mars researchers to consider the possibility for photosynthetic life on the red planet during its continued exploration. Instrumentation including fluorometry could provide a straightforward way to test for such photosynthetic life.

References: [1] Allen, C.C. (1998) 29th Lunar and Planetary Science Conference, Abstract 1690. [2] Arrieta, R.T. (1997) From the Atacama to Makalu, pp. 215-238. [3] Christensen, P. R. et al. (2000) J. Geophy. Res., 105, 9623. [4] Clark, B.C. (1998) J.Geophy. Res. Planets, 103, 28545. [5] Clifford, S.M. et al (2000) Icarus, 144, 210. [6] Gilichinsky, D.A. (2001) Astrobiology, The Quest for the Conditions of Life, pp. 125-148. [7] Gordon, D.A. et al. (2000) Microbial Ecol., 39, 197. [8] Malin, M.C., and Edgett, K.S. (2000) Science, 288, 2330. [9] McKay, C.P. (1997) Exobiology: Matter, Energy, and Information in the Origin and Evolution of Life in the Universe, pp. 219-227. [10] McKay, D. S. et al. (1999) The Fifth Annual Conference on Mars, Abstract 6211. [11] Sakon, J.J., and Burnap, R.L. (2004) 35th Lunar and Planetary Science Conference, Abstract 1943. [12] Tang, E.P.Y. and Vincent, W.F. (1999) New Phytol., 142, 315. [13] Thomas, D.J. and Schimel, J.P. (1991) Icarus, 91, 199.

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Figures: These pictures display Antarctic filamentous nitrogen-fixing bacteria. The left one shows a cyanobacterium at 400x magnification. The right one shows a cyanobacterium at 1700x magnification. Arrows in both pictures point to the heterocysts that allow nitrogen-fixation in each bacterium.