AN ANALYSIS OF POTENTIAL PHOTOSYNTHETIC LIFE ON MARS

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Background: Three primary requirements for life —water, organic (and chemical) materials and energy—are found within the near-surface of Mars [2,3,4, 9,11]. 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 [4,5,6,8,10] or once every ten thousand years [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 thawing [5,6,7,12]. Potential Martian habitats sufficient to protect and sustain such cyanobacteria for long periods include the north and south polar ice caps [4,5,10] as well as various sedimentary 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. *Synechocystis* sp. WT 6803 is a particularly adaptive form of cyanobacteria. We believe a bacterium similar to this strain would have the best chance for survival in the Martian conditions described above. However, while extremophiles—such as *Synechocystis*—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 this bacteria's adaptability to the effects of such conditions by putting it under various stresses similar to those it would experience on Mars. This project studied how *Synechocystis* responded to some of these various stresses, including Martian simulant soil, UV radiation, low pressure and Martian atmospheric composition.

Methods: The *Synechocystis* was tested within the Martian soil simulant by making an extract for the bacteria to grow in. To create this Martian simulant soil extract (MSSE), Martian simulant soil (from the JSC) was placed in de-ionized water and mixed thoroughly overnight. The mixture was then centrifuged and the heavy, non-transparent soil was removed. This created a transparent extract—essential for visually testing growth within the solution. After sterilizing the MSSE in an autoclave 10 different mixtures were created (in triplicate) for the *Synechocystis* to grow in by adding various stock solutions (see **Figure 1**). Using stock solutions allowed us to determine if any elemental deficiencies (for cyanobacterial growth) existed within the Martian simulant soil. The solutions were inoculated with a washed, active culture of *Synechocystis* and grown in a shaking incubator under fluorescent light. The experiment was performed with multiple concentrations of MSSE to ensure the bacteria were not lysed by excessive mineral content.

To determine growth within the various solutions the samples were visually analyzed after a sufficient number of days and the presence of macroscopic cell growth was recorded by color.

The *Synechocystis* was also tested under low pressure, ultraviolet (UV) radiation and an atmosphere high in carbon dioxide (CO_2) in order to simulate Martian conditions. This was done within the Andromeda Chamber, which was capable of simultaneously dropping the pressure to 400 mBar, creating an atmosphere of 95% CO_2 and 5% Hydrogen, and irradiating the sample with UV light. The *Synechocystis* was prepared by growing an active culture in BG-11. The samples were then put (in triplicate) within the chamber beneath a xenon lamp. The chamber was sealed with these beakers and then slowly lowered to 400 mBar to avoid boiling. After, CO_2 was gradually added—ensuring the temperature within the chamber did not vary far from room temperature—until the composition was 95% CO_2 and 5% Hydrogen. The *Synechocystis* samples were kept within the Andromeda Chamber for 50 straight hours under these conditions to provide sufficient time for changes in growth and any adaptations caused by the various stresses. As a control, three beakers of the same content as the others were put in an anaerobic glove box under a fluorescent light over the same 50 hour timeframe. This glove box was kept at an identical atmosphere (95% CO_2 and 5% Hydrogen) as the Andromeda Chamber.

A liquid sample was taken from each beaker directly after removing it from the Andromeda Chamber (and glove box) and a serial dilution was performed to count the number of viable cells in each culture. This was done by plating diluted samples on BG-11 agar plates, counting the number of colony forming units that arose after they were allowed to grow for a number of days and dividing by the dilution factor to find the original number of viable cells within the liquid culture.

A liquid sample was also taken from each beaker directly after removing it from the chamber (and glove box) and immediately frozen with dry ice. These frozen samples were brought back to the lab, where the absorption by the various photosynthetic pigments within the *Synechocystis* could be measured with a spectrophotometer. This provided an analysis of potential photosynthetic adaptations caused by the various stresses endured within the chamber.

Results: Of the various MSSE solutions only the sample with added sodium nitrate ($NaNO_3$) displayed *Synechocystis* growth (see **Figure 1**). Considering the chemical composition of the other solutions compared to this one it is apparent that sufficient nitrogen was lacking within the Martian soil simulant for the cyanobacteria to grow. Therefore, we conclude that the Martian simulant soil is nitrogen deficient. This concurs with compositional analysis of the Martian simulant soil by the Johnson Space Center [1].

The cyanobacteria samples exposed to various stresses within the Andromeda Chamber remained active and viable. The colony counts as well as the spectrophotometer analysis both turned out inconclusive as there was not sufficient correlation between the results from the Andromeda and glove box samples. However, considering the lack of distinct results between the two tested environments, this is an indication that the *Synechocystis* had little difficulty surviving the various stresses within the Andromeda Chamber for the 50 hours of exposure.

Implications for Mars: Considering the apparent lack of nitrogen in previous analyses of Martian soil [1] it seems that nitrogen-fixing bacteria—that can draw nitrogen from the little available N_2 in the Martian atmosphere—would be more likely to survive the Martian near-surface than other organisms [4]. However, limited soil has been analyzed thus far due to a lack of missions to Mars [1] and non-gaseous nitrogen sources are possible [4].

The *Synechocystis* displayed no macroscopically adverse effects from the exposure to the pressure, atmosphere and radiation within the Andromeda Chamber for a short time period. Further analysis will provide a better understanding of the growth restraints and pigment adaptations that will likely occur with added stress. Regardless, the various Martian conditions tested seem to have had minimal negative effect on the cyanobacteria—making the possibility of potential photosynthetic life on Mars, within the right environment, still feasible.

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Solution (concentration):	Pure M.S.S.E.	$NaNO_3$ (10mL/L)	$CaCl_2$ (1mL/L)	Citric Acid (1mL/L)	$FeNH_4$ Citrate (1mL/L)
Growth?	✗	✓	✗	✗	✗
Solution (concentration):	EDTA (1mL/L)	K_2HPO_4 (1mL/L)	Mag. Sulfate (1mL/L)	$NaCO_3$ (1mL/L)	Trace Metals (1mL/L)
Growth?	✗	✗	✗	✗	✗

Figure 1. Synechocystis was added to Martian soil simulant extract (MSSE) as well as a combination of MSSE and nine stock solutions. Indicated growth was shown in all six of each of the samples tested.