AN ANALYSIS OF POTENTIAL PHOTOSYNTHETIC LIFE ON MARS

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Background: Three primary requirements for life —water, organic (or 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 we can learn much about this bacteria's resistance 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 these various stresses, including Martian Soil Simulant, UV radiation, low pressure and Martian atmospheric composition.

Methods: To test *Synechocystis* within the Martian soil simulant we created an extract for the bacteria to grow in. To create this Martian simulant soil extract (MSSE) we placed Martian simulant soil in de-ionized water and mixed them thoroughly overnight. We then centrifuged the mixture and removed the heavy, non-transparent soil. This created a transparent extract—essential for visually testing growth within the solution. After sterilizing the MSSE in an autoclave we created 10 different mixtures (in triplicate) for the *Synechocystis* to grow in by adding various stock solutions (see Figure 1). Using stock solutions allowed us to determine what elemental deficiencies, if any, existed within the Martian simulant soil. We then inoculated all of the solutions with a washed (to avoid nutrient carryover into the solutions), active culture of *Synechocystis* and let them grow in a shaking incubator under fluorescent light.

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

We also tested *Synechocystis* 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 our sample with UV and visible light. We prepared the *Synechocystis* by growing an active culture and adding 20mL to a 1L beaker with 400mL of BG-11 (*Synechocystis'* preferred media) directly before putting the sample within the chamber. We put 3 beakers (the experiment was done in triplicate) directly under the beam of a xenon lamp to provide UV radiation. We put 3 other beakers in the chamber but out of the direct beam of radiation in order to test only visible light. We also covered the 3 visible light samples with borosilicate glass to aid in only allowing visible light through. We then sealed the chamber with these 6 beakers and slowly lowered the chamber to 400 mBar to avoid boiling. We also slowly added CO_2—ensuring the temperature within the chamber did not vary far from room temperature—until the composition was 95% CO_2 and 5% Hydrogen. We kept the *Synechocystis* samples within the Andromeda Chamber for 50 straight hours at these conditions to provide sufficient time for changes in growth and any adaptations caused by the various stresses. As a

control, we took 3 beakers of the same content as the others and put them in an anaerobic glove box under a fluorescent light over the same 50-hour time-frame. The glove box was kept at an identical atmosphere (95% CO_2 and 5% Hydrogen) as the Andromeda Chamber.

To determine the viability of the cells we took a liquid sample from each beaker directly after removing it from the Andromeda Chamber (and glove box) and performed a serial dilution for the purpose of counting the number of viable cells in each culture. This was done by plating diluted sample on BG-11 agar plates, counting the number of colony forming units (CFUs) 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.

To find any adaptations we took a liquid sample of each beaker directly after removing it from the chamber (and glove box) and immediately froze them on dry ice. We then brought these frozen samples back to our lab, concentrated the *Synechocystis* and measured the absorption by the various photosynthetic pigments with our spectrophotometer. This allowed us to determine any photosynthetic adaptations caused by the various stresses endured within the chamber.

Results: Of the various MSSE solutions only the sample with added sodium nitrate displayed *Synechocystis* growth. 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 initially appeared to be active and viable. However, further analysis of viable cell counts and pigment adaptations will be performed.

Implications for Mars: Considering the apparent lack of nitrogen in previous analyses of Martian soil [1] it seems more likely that such organisms as nitrogen-fixing bacteria—which can draw nitrogen from the limited N_2 in the Martian atmosphere—would be able to survive the Martian near-surface[4]. However, limited Martian 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* has shown no macroscopically adverse effects from the exposure to the pressure, atmosphere and radiation stress within the Andromeda Chamber. Further analysis will provide a better understanding of the growth restraints and pigment adaptations that likely occurred. However, 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, more feasible.

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Solution:	Pure M.S.S.E.	$NaNO_3$	$CaCl_2$	Citric Acid	$FeNH_4$ Citrate
Growth?	✗	✓	✗	✗	✗
Solution:	EDTA	K_2HPO_4	Mag. Sulfate	$NaCO_3$	Trace Metals
Growth?	✗	✗	✗	✗	✗

Figure 1. Synechocystis was added to Martian soil simulant extract (MSSE) as well as a combination of MSSE and 9 stock solutions. Any indicated growth was shown in all six of each of the samples tested.