Analyzing the Ability of Chroococcidiopsis and Anabaena to Terraform a Martian Environment

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Abstract: Implementing plants on Mars has been a challenge as the autotrophic life does not adapt well to the environmental stressors on the planet. Scientists have been comparing plant life to prokaryotic cyanobacteria to see which species can live under the martian conditions (i.e. low pressures, high levels of carbon dioxide, limited amounts of water, increased radiation, and fluctuating temperatures). Systematic literature review was conducted to find the most practical organism that could adapt to a martian surface. Results indicated cyanobacterial species *Chroococcidiopsis* and *Anabaena* have the ideal traits for the first pioneer organisms on Mars. To incorporate all of their characteristics, a synergistic effect was proposed. *Chroococcidiopsis* and *Anabaena* would take advantage of the resources in a martian environment and alter them to resemble Earth-like atmospheric and soil conditions. To further the implementation of the bacterial species, a symbiotic relationship was proposed between *Chroococcidiopsis* and *Anabaena* with a member of the plant family Gramineae. Data showed plant *S. cereale* of the Gramineae family was able to produce the highest biomass while growing in martian regolith, proving this plant specie suitable for the martian soil. The paper explores the possibility of a synergistic effect between two resilient cyanobacteria to terraform a martian environment and later create an ecosystem where plant life could grow on Mars.

Key Terms: *Anabaena, Chroococcidiopsis,* cyanobacteria, ecopoiesis, Mars, nitrogen fixation, symbiosis, terraformation

Mars Environmental Components

The martian environment is relentless and polar compared to the conditions organisms experience on Earth. Low pressure, extreme temperatures, low moisture, high levels of carbon dioxide, and high UV flux are characteristics of the martian environment that have never been experienced by organisms on Earth (Thomas, 2006). The atmospheric pressure on Mars is 600 Pascals (Pa), which was found to be 0.6% of pressure on Earth (Makishima, 2017). Despite the fact Mars is 1.52 times farther from the Sun than Earth, the lack of greenhouse gases allows the light flux to be 5000 times greater than the average amount required to promote photosynthesis (Verseux et al., 2015). The Curiosity Rover searching the surface of Mars estimated water to be on the planet in the formation of lakes approximately 3.3-3.8 billion years ago due to flat mudstones (Makishima, 2017). Set of experiments from the Sample Analysis at Mars (SAM) of

Curiosity Rover measured atmospheric gases to be carbon dioxide (CO_2): 0.960(± 0.007), argon-40 (^{40}Ar): 0.0193(± 0.001), nitrogen (N_2): 0.0189(± 0.0003), oxygen 1.45(± 0.09) x 10^{-3}, and $^{40}Ar/^{36}Ar$: 1.9(±0.3) x 10^3 (Mahaffy et al., 2013).

Since nitrogen is an important aspect of ecosystems on Earth, scientists have been isolating its location and origin on Mars. A recent experiment from SAM found nitric oxide (NO) and hydrogen cyanide (HCN) to be present in small amounts at the Gale Crater on Mars (Stern et al., 2015). The same experiment discussed how NO was 'produced by thermal decomposition of martian sediments" through lightning impacts that fixed nitrogen (Stern et al., 2015). This is promising since nitrogen needs to be implemented in any new environment with goals of ecopoiesis. Ecopoiesis is the process of habitating and engineering a planet to sustain life and liquid water; originally founded by Bob Haynes and Chris McKay in connection with Carl Sagan in 1970 (Boland et al., 2014). To understand the extent to which ecopoiesis needs to take place, scientists researched which martian factors supported life on the planet. Sustaining life on a planet requires liquid water, carbon, free energy, and a "position in the habitable zone of its solar system" (Morely, 2016). The basic elements bacteria require to stay alive (O, N, P, C, H, S, K, Mg, Na, Fe, Ca) have all been detected on Mars in the soil or atmosphere (Verseux et al., 2015). Certain organisms obtain nutrients from organic matter, yet it was not detected on the surface of Mars (Wieger Wamelink et al., 2014). Many studies also reveal the surface soil on Mars is not toxic to living organisms (Morely, 2016). Even if it was, nitrogen-fixing bacteria have been used to detoxify metal levels in soil, proving the soil would not be a problem for organisms (Wieger Wamelink et al., 2014). Clearly all of the nutrients and micronutrients organisms need to survive are present on Mars, showing the environment is the only restricting factor (Verseux et al., 2015).

Current Testings

The mission towards colonization on Mars is a major topic of discussion when contemplating solar system exploration. To bring life to an uninhabited planet, tests must be undertaken to determine the best solution for an ecopoiesis of Mars.

Early scientists, including Bob Haynes and Chris McKay, presented their proposal of dark material on the Mars pole to isolate greenhouse gases and allow conditions where liquid water may be on the martian surface (Boland et al., 2014). This theory has been researched, but implementation of organisms to create greenhouse gases has become the most practical solution.

As the Mars mission allowed scientists to move from the theoretical aspect onto the experimental level, they tested various types of microorganisms to find the ideal pioneer organism for Mars. One experiment called Biofilm Organisms Surfing Space (BOSS) was exposed to Low Earth Orbit to mimic the martian conditions while growing various cyanobacterial films and planktonic cultures (Baqué, et al., 2013). Another research mission on the International Space Station (ISS), called EXPOSE, used nano satellites orbiting areas of high space radiation, thus allowing experiments to mimic harsh martian conditions (Billi et al., 2013). National Aeronautics and Space Administration (NASA) completed three EXPOSE missions, all testing different types of fungi, lichens, plant, cyanobacteria, and other possible pioneer organisms for the martian environment (Rettberg et al., 2013). Since self-sufficiency will be a priority on the mission, support systems are being created to find the most efficient way for astronauts to live on the surface of Mars. Versatile cyanobacteria species are being targeted for these bioregenerative life support systems (BLSS) (Verseux et al., 2015).

Cyanobacteria

Scientists made efforts to experiment and record different species of plants in replicated Mars environments. An experiment conducted by Wieger Wamelink, a senior ecologist at Wageningen University & Research, proved various plant species to adequately grow in martian regolith under Earth conditions. Germination percentage, formation of leaves, and dry weight were all the highest with the plants grown in the martian regolith when compared to the growth of plant species in lunar or earth soils under the same conditions (Wieger Wamelink et al., 2014). However, studies found plants are sensitive to high carbon dioxide levels, low pressure, and increased radiation (Kanervo et al., 2005). An Earth-like environment would be created on Mars

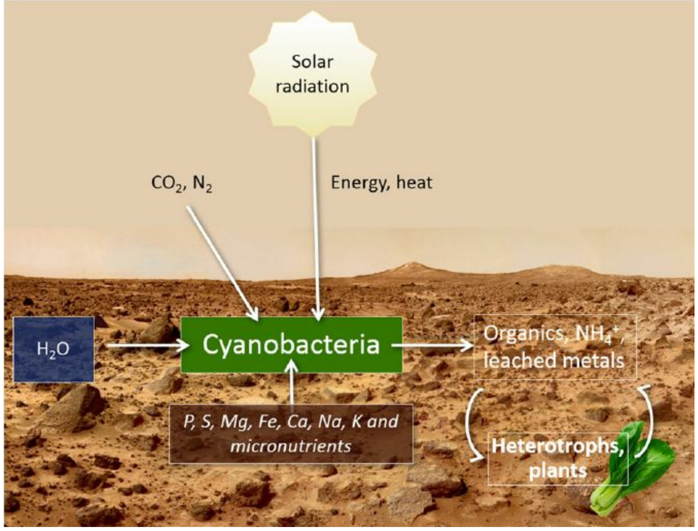


Figure 2: This figure shows the plausibility of cyanobacteria using resources on Mars and creating valuable waste products for other life (Verseux et al., 2015).

first since plants show they can grow well in martian soil only, not the atmospheric conditions of the planet.

To counter the demanding requirements of plant life, cyanobacteria- a type of extremophile- has been recognized for their capability to terraform the martian environment for other organisms. Extremophiles are organisms including bacteria, archaea, and eukarya that can thrive in hostile and inhospitable environments (Rampelotto, 2013). Cyanobacteria are the most complex and versatile prokaryote with the ability to carry out oxygenic photosynthesis (Sciuto & Moro, 2015).

The source of oxygen in oxygenic photosynthesis: $\mathbf{2H_2O \rightarrow 4H^+ + 4e^- + O_2}$

These phototrophic prokaryotes were the first microorganisms on Earth to adapt to surface conditions. One theory in 1905 founded by a Russian biologist and botanist, Mereschkowsky, described how cyanobacteria led to the development of the first photosynthetic eukaryote through a symbiotic event. This idea of eukaryotic evolution is called the Endosymbiotic Theory and proves the importance of cyanobacteria (Sciuto & Moro, 2015). Additional studies demonstrate cyanobacteria were the main cause for the Great Oxidation Event- the first appearance of oxygen among the Earth’s surface approximately 2.3 billion years ago (Lyons et al., 2014). Cyanobacteria have also adapted to survive in hyper-saline and hyper-alkaline ecosystems (Kanervo et al., 2005). Cyanobacteria can handle heavy metal content and use this tolerance to recharge the soil with less metal content, reducing the toxic chemicals and adding micronutrients (Padhy et al., 2016). While cyanobacteria were important for early Earth formation, they are just as necessary in nature today as they continue to be the main food web producers (Chapman, 2010). Cyanobacteria are recorded to produce seven times the amount of oxygen a year when compared to trees in Southeastern California (Verseux et al., 2015). Cyanobacteria show lasting viability and photosynthetic tolerance under extraterrestrial conditions from early life to current day food cycles.

Cyanobacteria have the ability to form symbiotic relations with plants and increase plant growth. These microorganisms improve plant productivity by promoting vitamins, amino acids, polypeptides, and antifungal substances (Mohsen et al., 2016). Cyanobacteria produce plant hormones called phytohormones that increases the number of roots in a plant (Mohsen et al., 2016). One study conducted over two winter seasons showed the increase of foliar cyanobacterial spray (consisting of either *Anabaena* or *Nostoc* cells) on lettuce plants greatly increased the height, total yield, chlorophyll a content, and head weight of lettuce plants when compared to the control (Mohsen et al., 2016). One well known symbiotic relationship between bacteria and plant life is the *Anabaena-Azolla* relationship. *Anabaena* cells have shown to multiply in *Azolla* leaf cavities until the cells can longer fill the space (Hill, 1977). Another study showed growing rice plants (*Oryza sativa)* in fly ash and nitrogen-fixing cyanobacteria (*Anabaena*) increased the plant shoot length, dry weight, number of seeds, leaf area, and chlorophyll a content (Padhy et al., 2016). Metabolic activities of leaves and the properties of soil improved under the cyanobacterial additions in *Oryza sativa* (Padhy et al., 2016). While fly ash differs from martian regolith, cyanobacteria still can improve plant growth significantly despite the soil type.

Importance of Nitrogen

Originally, the only source of fixed nitrogen (as NOx) on Earth was produced by lightning discharges through reaction with carbon dioxide (Grula, 2005). This required prokaryotes to evolve autonomous mechanisms to fix their own nitrogen (Grula, 2005). Nitrogen fixation is the process of converting atmospheric nitrogen into ammonia and nitrate using nitrifying bacteria (Mancinelli & Banin, 2003). The main enzyme used to break down atmospheric nitrogen is nitrogenase (Verseux et al., 2015). Nitrogen could not be produced earlier due to inhibition of the two trace metals Molybdenum (Mo) and Vanadium (V) that nitrogen fixation relies on (Lyons et al., 2014). These trace metals convert gaseous nitrogen to available ammonia (NH_3^+) that nitrogenase can continue to break down (Lyons et al., 2014). As trace metal concentrations increased, nitrogen fixation and oxygen production were able to begin (Lyons et al., 2014). All living organisms were then able to build proteins, nucleic acids, and metabolic molecules (ATP and NADPH), which slowly evolved the first life on Earth (Grula, 2005).

Some filamentous cyanobacteria evolved to generate fixed nitrogen using heterocysts (SSERC Bulletin, 2007). Heterocysts have a thick membrane impermeable to oxygen, allowing them to convert atmospheric nitrogen to ammonia or nitrate (NO_3^-) (Sciuto & Moro, 2015). The evolutionary heterocyst cell fixed nitrogen in the presence of oxygen without the previously required nitrogenase enzyme. Nitrogen is a necessary mineral because it’s reactive forms of nitrate and ammonia are vital for plant growth (Wieger Wamelink et al., 2014). Since plants cannot fix their own nitrogen, they require a symbiotic relationship with microorganisms embedded in their roots to achieve their nitrogen supply (Verseux et al., 2015). Plants need fixed nitrogen for protein and nucleic acid synthesis (Stern et al., 2015).

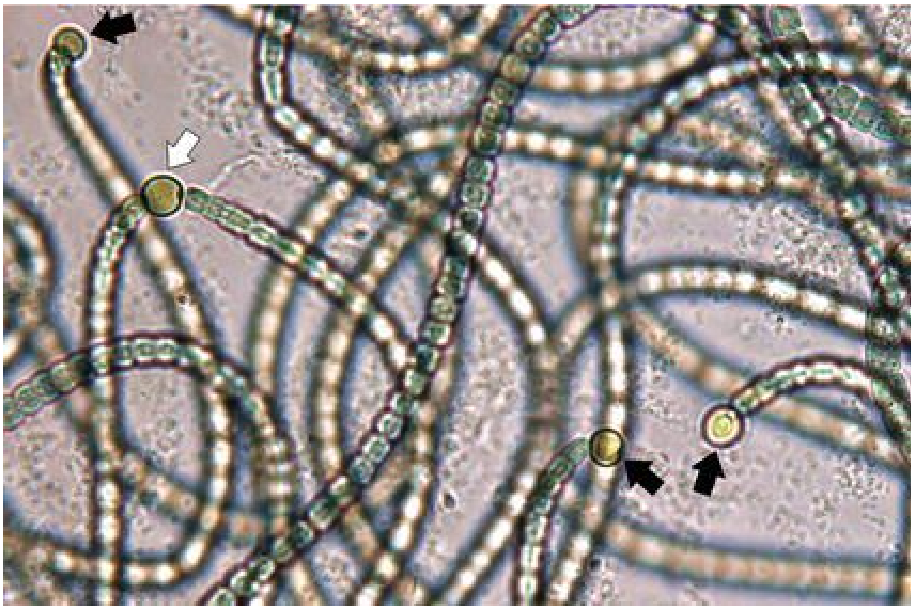
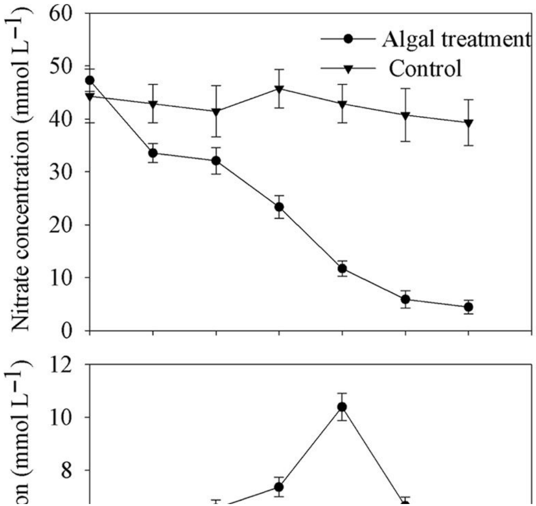


Figure 3. Light microscopy picture of a filamentous cyanobacterium belonging to the order Nostocales, exhibiting herterocytes both at the ends of filaments (terminal heterocytes, *black arrows*) and inside the filaments (intercalary heteorcytes, *white arrow*) (Sciuto & Moro, 2015).

One study in Lake Taihu investigated how cyanobacterial blooms affected the nitrogen cycle; specifically the process of nitrogen fixation and denitrification. Denitrification reduces nitrate to nitrite and then releases gaseous nitrogen to the atmosphere. Atmospheric nitrogen levels, light, and presence of plants all affect denitrification. Chen, a professor at Xidian University in China, and his team (2012) implemented nitrate in experimental aquatic tanks and there was a clear breakdown of nitrites, proving conventional denitrification took place in these



cyanobacterial blooms. Results demonstrated denitrification as nitrates reduced into nitrogen gas, nitrous oxide, nitric oxide, and nitrite (Figure 4). The team's formulated equation takes into account that the nitrate to nitrite conversion in cyanobacteria requires only ⅓ of hydrogen gas that is needed in a nitrate to gaseous nitrogen conversion.

Equation: $N_C = (\Delta_{nitrate} - \frac{2}{3}\Delta_{nitrite})/D_c$

Chen and his team concluded if nitrates are not present in martian soil, nitrogen removal rates would be dependent on nitrogen fixation as it is on Earth (Chen et al., 2012).

Chroococcidiopsis

Chroococcidiopsis genus are targeted cyanobacteria for the Mars mission because they survive in the extreme environments of the McMurdo Dry Valleys in Antarctica and the Academa Desert (Baqué, et al., 2013). These locations parallel the freezing and scorching

Figure 4. Temporal variations of nitrate, nitrite and dissolved oxygen concentrations in submerged tanks with $4 X 10^9$ cells L^- cyanobacteria added and control tanks in the field experiment. Data are mean ± SD (n=3) (Chen et al., 2012).

Figure 5. Two examples of cyanobacteria: *Anabaena* sp. PCC7120 and *Chroococcidiopsis* sp. CCMEE 029 (Verseux et al., 2015).

temperatures on Mars. Since *Chroococcidiopsis* live in terrestrial sites similar to the environment on Mars, they have high resistance to simulated space conditions (Verseux et al., 2015). The phototrophic desert strains of *Chroococcidiopsis* have a high desiccation tolerance, giving them tolerance to UV flux (Billi et al., 2013). *Chroococcidiopsis* maintain viability under direct UV flux longer than other species (i.e. *Bacillus subtilis*) at approximately 1-5 minutes without soil covering (Cockell et al., 2005). These dried *Chroococcidiopsis* cells use protection mechanisms to maintain subcellular components such as genomic DNA and their plasma membrane (Billi et al., 2013). *Chroococcidiopsis* absorb the ionizing radiation in the top cell layer, while protecting DNA and other crucial cellular components in the lower layers (Baqué et al., 2013). The cells' repairing mechanisms regenerate the damage done to their plasmids after desiccation once mixed with water (Verseux et al., 2015). These adaptations to radiation prove UV radiation would not be a limiting factor to potential Mars habitation (Cockell et al., 2005).

While *Chroococcidiopsis* cells have many built-in environmental advantages, they grow slowly when compared to other bacteria species (Verseux et al., 2015). *Chroococcidiopsis* cells also lack the capability of denitrification that other species possess (Billi et al., 2013). Combination with another species would be necessary for *Chroococcidiopsis* since the species alone cannot conduct denitrification or nitrogen fixation.

Anabaena

Anabaena is a photosynthetic blue-green algae, classified as cyanobacteria, that grows in freshwater and makes its own chemical energy (Morely, 2016). *Anabaena* has the ability to biologically retrieve nutrients directly from the martian rocks (Verseux et al., 2015). *Anabaena* has high biomass production rates and an ability to fix nitrogen (Verseux et al., 2015). This high biomass means there will be plenty of organic matter left after the *Anabaena* communities die off. However, it was seen *Anabaena* cells did not form heterocysts until stimulated by nitrogen depletion (Hill, 1977). Studies also found *Anabaena* can be genetically engineered to produce sucrose (Verseux et al., 2015). The ability to be genetically engineered gives the species an advantage because scientists could manipulate the cells to adapt to harsher conditions.

Current Situation

Since plants cannot independently grow in the current martian conditions (*i.e.* high levels of CO_2, limited liquid water, extreme temperatures), cyanobacteria would be the ideal organisms on Mars to start the first step of ecopoiesis: terraformation. However, scientists have not found a single cyanobacterial species that exhibits all of the traits required to live on the surface of Mars. A synergistic effect between two species would solve this because their combined characteristics would allow them to terraform the environment with all the necessary components. *Chroococcidiopsis* and *Anabaena*, in the phylum of cyanobacteria, have shown desirable traits under martian conditions. The investigation of these cyanobacteria reveals they have the majority of characteristics needed for an ecopoiesis of Mars; potentially outweighing other bacterial species. While cyanobacteria have the capability of converting a martian environment to an Earth-like one, they cannot solely provide a food source for the crew. It is known plants form symbiotic relationships with bacteria, so the second step of the terraformation would be to implement plant life. With this in mind, the cyanobacterial species selected for the synergistic effect must successfully terraform a martian environment enough to make it habitable for plants.

The question remains; will *Chroococcidiopsis* and *Anabaena* be the most effective first step in terraforming a martian environment to make it habitable for potential plant life, thus creating a food supply on Mars?

Purpose

Investigating the characteristics of *Chroococcidiopsis* and *Anabaena* for a potential synergistic effect between the two species leading to terraformation of Mars surface. Then exploring if a symbiotic relationship between cyanobacteria and plant life could be beneficial to both species in the harsh environment on Mars.

Alternative Hypothesis

Chroococcidiopsis and *Anabaena* will terraform a martian environment through a synergistic effect.

Null Hypothesis

Chroococcidiopsis and *Anabaena* will not terraform a martian environment through a synergistic effect.

Method

Systematic literature review was conducted for this research study. The method collected data from multiple perspectives and gathered them to compare their findings. The systematic literature review sources were found through peer-reviewed articles online. The goal of the data is to investigate how each cyanobacterial species could contribute to the terraformation of Mars. Surveys were not appropriate because it was irrelevant to collect data on people in this project. Furthermore, secondary data analysis would not be necessary because the majority of research came from multiple sources. There was not one sole source or database that described all factors relevant to this study. The selected research design was most appropriate because it allowed comparison to other studies, various findings, and acknowledged gaps in the scientific field.

Explanation of Data Collection

The majority of peer reviewed articles were obtained from online databases. The frequently used computerized databases were the Elsevier, Cambridge University Press, National Center for Biotechnology Information (NCBI), ScienceDirect, Taylor & Francis Online, Google Scholar, Scientific Research, and Springer sites. Keywords used to find articles were: cyanobacteria, ecopoiesis, terraforming, martian atmosphere, nitrogen fixation, and symbiosis. Additional information was gathered on the NASA website regarding the most recent experiments on the ISS. The time frame of articles was not taken into account because old articles could prove accurate as long as current day articles showed the same results. No outdated articles were used, however, and any papers dating before 2000 were checked for accuracy with present day articles to guarantee applicability. The time frame of data collection continued through the entire year to guarantee all new articles published on this subject were analyzed. No data was collected experimentally through lab work. The data tables were constructed through Google Docs and the graphs were plotted on Microsoft Excel. The tables used multiple papers to show a trend in the data. The graph only referenced one paper, but the author sent his 841 data plots from the 20 different experiments in the paper, allowing the data to be constructed in a different way than it was presented in the author's published peer reviewed paper. The data from the graph is an average of all 20 experiments by plant species with standard deviation error bars and later explanation of the coefficient variation values.

Results

Table 1. This table compares the maximum time two bacterial species (*Chroococcidiopsis* sp. 029 v. *Bacillus subtilis*) can survive exposure to UV flux under varying soil height. References: (Billi et al., 2016; Cockell et al., 2005).

Bacteria species	Height of soil needed for complete radiation protection	Maximum time elapsed under UV flux until loss of viability
Bacillus subtilis	1.0-mm	8 hours
Chroococcidiopsis sp. 029	3.0-mm	1.5 years

Table 2. This table shows the growth of two bacterial species (*Chroococcidiopsis* sp. 029 v. *Bacillus subtilis*) under UV flux and martian soil. References: (Billi et al., 2016; Cockell et al., 2005).

Bacteria species	Reduction of viability (%)	Time elapsed under UV flux
Bacillus subtilis	99.9	15 secs
Chroococcidiopsis sp. 029	1	5 minutes

Table 3. This table shows the comparison of bacterial species and their characteristics required to survive in a martian environment. References in the table.

Properties	Bacterial species	References
Extreme cold temperature resistance	*Chroococcidiopsis, Synechocystis*	(Kanervo et al., 2005)
Extreme hot temperature resistance	*Chroococcidiopsis, Anabaena*	(Thomas et al., 2006; Morely, 2016)
Fast growth rates	*Anabaena, Pseudomonas, Nostoc, Cyanothece*	(Hill, 1977; Thomas et al., 2006; de Vera et al., 2014; Verseux et al., 2016)
Nitrogen fixation	*Cyanothece, Arthrospira, Anabaena*	(Verseux et al., 2016; SSERC Bulletin, 2007)
UV flux resistance	*Bacillus, Chroococcidiopsis, Arthrospira*	(Cockell et al., 2005; Baqué et al., 2013; Verseux et al., 2016)
Desiccation resistance	*Chroococcidiopsis, Anabaena*	(Billi et al., 2013)
CO_2 tolerance	*Anabaena, Synechocystis, Plectonema boryanum*	(Kanervo et al., 2005; Verseux et al., 2016; Thomas et al., 2006)
Denitrification	*Pseudomonas aeruginosa, Alcaligenes, Paracoccus*	(Boland et al., 2014; Thomas et al., 2006)
Low pressure	*Synechocystis, Serratia*	(Kanervo et al., 2005;

	liquefaciens	Verseux et al., 2016)

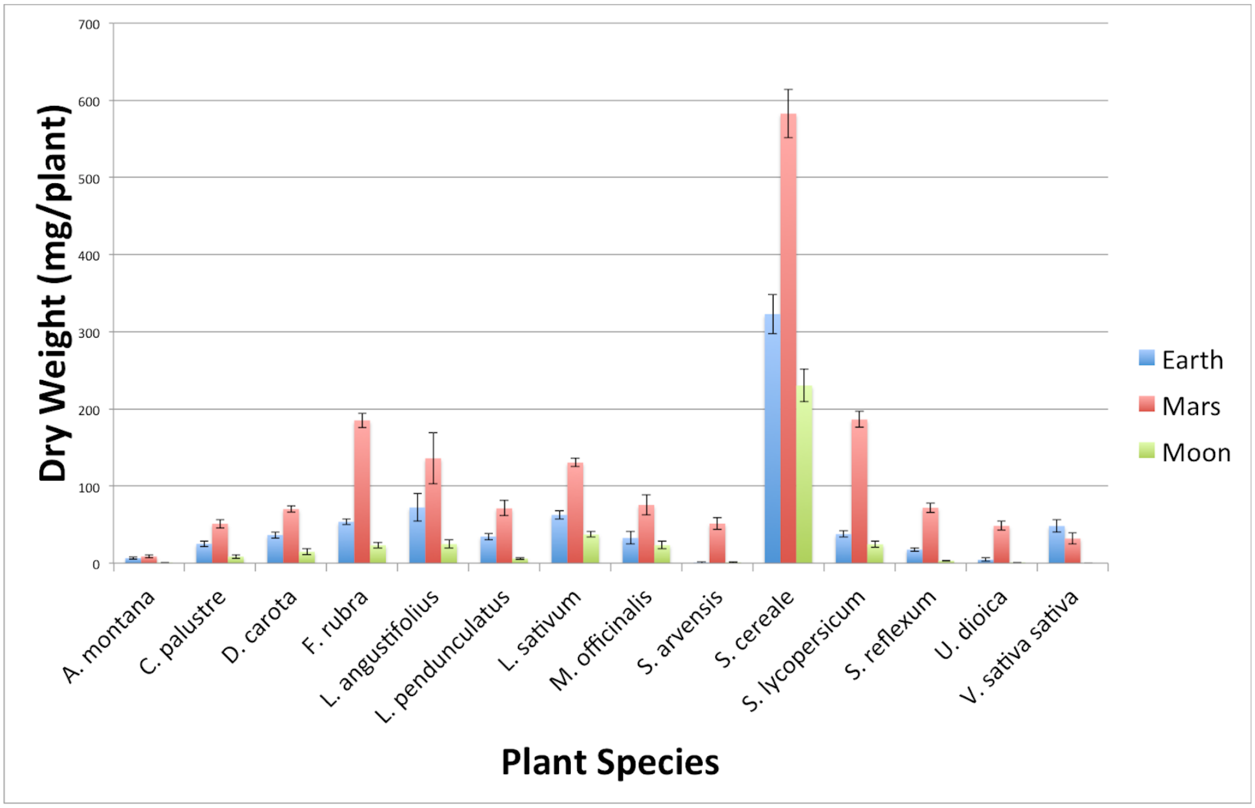


Figure 1. The Effect of Soil Type On Plant Average Dry Weight, This graph demonstrates the success of various plant species in lunar, martian, and earth soils under Earth-like conditions (Wieger Wamelink et al., 2014).

Data Analysis

Both *Chroococcidiopsis* and *Bacillus* showed complete viability under a layer of martian regolith. *Chroococcidiopsis* cells required less protection than the *Bacillus* cells, but both adequately grew under high levels of ionizing radiation. *Chroococcidiopsis* could survive in those conditions for over a year with the minimal protection, while *Bacillus* could only last 8 hours (Table 1).

Direct UV flux resulted in higher viability rates in the cyanobacterial species *Chroococcidiopsis* than the bacterial species *Bacillus.* Viability was lost 99.9% after 15 seconds for *Bacillus* and the cells lost all ability to function. However, *Chroococcidiopsis* only lost 1% viability after 5 minutes (Table 2).

The cyanobacterial species *Chroococcidiopsis* and *Synechocystis* have cold temperature resistance, while *Chroococcidiopsis* and *Anabaena* are resistant to extreme hot temperatures. *Chroococcidiopsis, Nostoc, Cyanothece,* and *Pseudomonas* show the ability to grow at a fast rate and create quick, organic biomass when they die off. *Cyanothece, Arthrospira,* and *Anabaena* developed specialized cells called heterocysts, allowing them to fix nitrogen in an environment. *Bacillus, Chroococcidiopsis,* and *Arthrospira* possess resistance to UV flux, allowing them to live under extreme radiation while keeping viability in their cells. *Chroococcidiopsis* and *Anabaena* show their cells can live in a dried state for periods of time and regenerate once water is mixed with their colonies. *Synechocystis, Anabaena,* and *Plectonema boryanum* have built in tolerance to carbon dioxide and can survive the high concentrations, while other species found the increased carbon dioxide levels toxic. *Pseudomonas, Alcaligenes,* and *Paracoccus* adapted to break down nitrogenous compounds and release nitrogen back to the atmosphere through denitrification. *Synechocystis* and *Serratia liquefaciens* can handle pressure lower than that on Earth without losing photosynthetic properties (Table 3).

The plant species grew the best and produced the highest dry weight in martian soil. The plant species grew the worst in the lunar soil and grew average in the Earth soil. *S. cereale* had the most success in all three soil types and had significantly higher dry weights than the other species. *V. sativa sativa* had the lowest growth rate with the lowest average biomass. Standard deviation was calculated for all 20 dry weight averages and are seen in the error bars. The coefficient variations were also calculated, but not plotted anywhere on the graph. The plant species with the higher dry weights had coefficient variations close to 0, proving their individual values were close to their mean values. This validates the dry weight averages from the 20 experiments. For example, in martian soil *S. cereale* was p= 0.2400954, *F. rubra* was p=0.2241547, and *S. lycopersicum* was p=0.248577. There were 14 variables in the experiment, allowing the coefficient variation to be close to 0 instead of 1.

Discussion

After comparing 13 bacterial species in Table 3, it is clear that a single species cannot accomplish all of the requirements needed to survive and terraform Mars. All of the bacterial species presented have thick membranes, allowing them to possess many of the characteristics needed to survive the harsh conditions. The *Chroococcidiopsis* cells take advantage of their thick membranes by surviving the longest under high levels of radiation. This is seen in Tables 1 and 2 as *Chroococcidiopsis* outlive *Bacillus* by years under a layer of protective soil. The increased level of radiation was a major concern when regarding life on Mars and this data proves it will not be a problem as long as the cyanobacteria is protected by soil. The fast growth rates seen in *Anabaena, Pseudomonas, Nostoc,* and *Cyanothece* eventually will enrich soil as they die and become dead, organic matter for other organisms to feed on (Table 3). Cyanobacterial species such as *Chroococcidiopsis* and *Anabaena* developed desiccation resistance, allowing them to function without water for extended periods of time, which is needed on Mars since there is no liquid water available. The implementation of the nitrogen cycle on Mars is a priority, and Table

3 shows the types of bacterial species that conduct nitrogen fixation and denitrification on Earth. Scientists have found many bacterial species to adapt to one or two of the martian environmental stressors, but in order to terraform a martian environment, a synergistic effect clearly must be applied.

The first part of the research question asks if *Chroococcidiopsis* and *Anabaena* will be able to terraform a martian environment. In Table 3 it is evident *Chroococcidiopsis* and *Anabaena* have the most characteristics when compared to the other species. This supports the hypothesis that these two cyanobacterial species will effectively terraform a martian environment. Combining the two species would be better than implementing just one because each species lacks a couple characteristics needed to enrich the martian atmosphere. For example, *Chroococcidiopsis* cannot fix nitrogen, meaning they would not be able to enrich the soil with the nitrogenous compounds plants require. *Anabaena,* on the other hand, is unable to handle the extreme radiation levels as well as *Chroococcidiopsis* without soil protection. The goal of the synergistic effect is to implement two bacterial species that would be able terraform a martian environment longer than other species before dying off.

The reason for a synergistic effect is to later symbiotically add plants to the martian environment and create a food supply on Mars. *Anabaena* has the ability to carry out symbiotic relations with plant species of the Gramineae family as shown previously in *Oryza sativa*. Plant life is seen to have the highest biomass averages in martian soil (Figure 1), showing their ability to grow on Mars once the environment assimilates as an Earth-like one through the cyanobacterial synergism. *S. cereale* showed to be the most successful when comparing other edible plants grown in martian soil in Figure 1. *S. cereale* is a part of the Gramineae family, showing how a symbiotic relationship with *Anabaena* could potentially resemble the same benefits *Oryza sativa* has with *Anabaena* (Mia, 2017).

Conclusion

Adverse conditions create problems for many plant species, so extremophiles, such as cyanobacteria, would be the most valuable organism to grow on Mars. A synergistic effect between *Chroococcidiopsis* and *Anabaena* would be the most effective considering they exhibit various traits needed to survive the martian environment, guaranteeing a combination of their characteristics along with their phototrophic abilities. The synergism would create an Earth-like environment for the plant species. Terraforming the environment with *Chroococcidiopsis* and *Anabaena* first would allow plant species to grow better than if they were implemented without the ecopoiesis. After *Chroococcidiopsis* and *Anabaena* nutrienize the soil and enrich the atmosphere with necessary gases, a symbiotic relationship could be formed with a plant, thus providing a small scale ecosystem on Mars. Plant *S. cereale* proved to grow the best in martian regolith, suggesting a symbiotic relationship could be formed with a species in the Gramineae family.

Limitations

Scientists have not conducted experiments to test a synergistic effect between cyanobacterial species, so there is no quantitative data available online. However, many authors stated a synergism would be the best way to incorporate all of their traits in their papers' discussions. Even though these scientists did not take a further step to experiment with a synergistic effect, there was an abundant amount of information regarding various cyanobacterial and bacterial species experimented individually under martian conditions. The lack of quantitative data consequently did not provide enough information for paired T tests or graphs focusing on bacterial growth under martian conditions. Instead this study compared each bacterial characteristic individually and proposed the synergistic effect between two highly versatile cyanobacterial species, *Chroococcidiopsis* and *Anabaena*.

There is also no quantitative data or experimentation showing a symbiotic effect between plants and cyanobacteria under the martian environment, even though various papers encourage this in their further work sections. Instead, this study only analyzed the effect of cyanobacteria on plant biomass under Earth conditions, not martian conditions. Despite the lack of experimentation on this proposal, many authors encourage future research on synergism or symbiosis; and the quantitative data gathered proves it would be a vital path to investigate when determining life on Mars.

Further Work

While the combination of *Chroococcidiopsis* and *Anabaena* would terraform an environment and fix nitrogen for plant life, an ideal ecopoiesis would include the process of denitrification. In the beginning it would not be necessary, but for long term goals of agriculture on Mars, a denitrifying species would need to be implemented in the synergistic relationship. Experiments found *P. aeruginosa* under varying pressure and elevated levels of carbon dioxide could still consume nitrates and nitrites through denitrification, while their growth rates stayed consistent under all atmospheric conditions and different soil analogs (Hart et al., 2000). Other possible denitrifying bacterial species are exhibited in Table 2.

As inferred previously, addition of one or more bacterial species to the synergism allows more characteristics to terraform the environment. Conducting research regarding three or more bacterial species that carry various adaptations to the martian environment would maximize the terraformation process and would be worth experimenting more on.

Most bacterial species have the capability of being genetically altered, which could enable a single species to have all of these desired characteristics. Further experimentation on manipulating bacterial species could lead scientists to a cell that exhibits all of the traits needed to survive and change the martian environment.

Experimentation with Gramineae plant species and cyanobacteria under martian factors could give scientists answers to how symbiotic relations work under restricting martian conditions. It is seen plants of the Gramineae family have high growth rates in martian regolith (*S. cereale* in Figure 1), so an investigation of how these plants could live under martian environmental conditions would be of value. Researching how different plant species form symbiotic relations with *Chroococcidiopsis* and *Anabaena* should also be looked into.

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