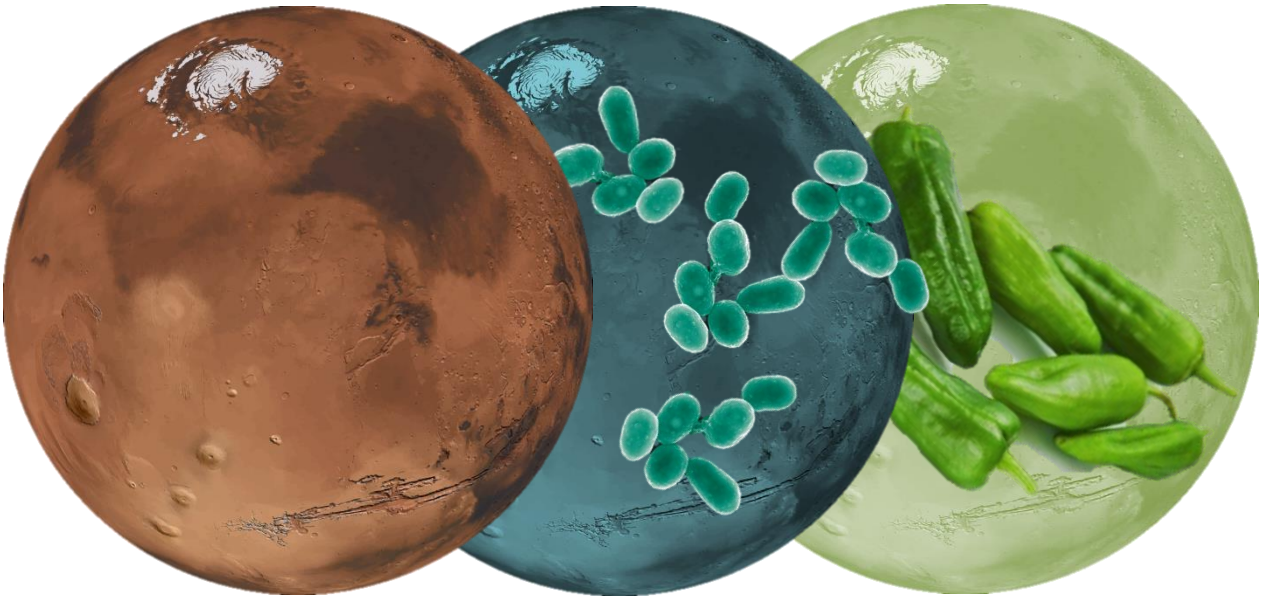
High School Students for Agricultural Science Research

Volume 8

"Project Mars"



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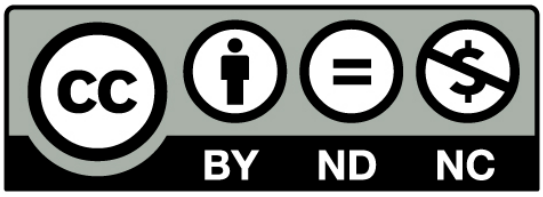

Index

Simulating Martian soil as part of an educational project in Astrobiology with high school students

Noemí Castillo Tejada[1], *Candela del Castillo Puertas*[1], *Pablo Delgado Alaminos*[1], *Nuño Gutiérrez Martín*[1], *Alberto López Galindo*[2], *Concepción Jiménez de Cisneros Vencelá*[2], *Antonio Quesada Ramos*[1]

[1]IES Zaidín Vergeles, Granada

[2]Instituto Andaluz de Ciencias de la Tierra. UGR-CSIC.

Summary

As a part of an educational project devoted to study the possibility that terrestrial microorganisms can survive in a Martian environment, it has been proposed to produce a martian soil analogue from volcanic terrestrial rocks. Volcanic scoria have been analysed by X-ray fluorescence and X-ray diffraction at the Instituto Andaluz de Ciencias de la Tierra (UGR-CSIC). Chemical and mineral composition of our samples are similar to rocks studied by rover vehicles in Mars. Therefore, they can be an acceptable material to simulate Martian soil.

KEYWORDS: Mars, soil, analogue, volcanic scoria, tephrite, basanite, minerals, X-ray fluorescence, X-ray diffraction.

Introduction

Analogue research is an important tool to approach distant places in the universe. This research focuses both on environments and materials. For example, the study of extreme environments and its life forms helps to assess the possibility of life in exoplanets. On the other hand, laboratory analogues such as rocks and soil simulants provide tools to research other planets conditions in our laboratories.

Mars is the closest planet in the Solar System where life could have appeared. In the past the environment in the Red Planet was similar to that of the Earth, with a thick atmosphere, mild temperatures and oceans and rivers of liquid water. As our planet billions years ago, it was a good scenario for life. At present, life is almost impossible in Mars.

In our high school we are carrying out an educative project whose main objective is to assess if terrestrial microorganisms could survive at the present environment conditions of Mars. To do this it is important to dispose of a Martian soil simulant. Several Martian soil simulants have been developed; one of the most widely used is a weathered volcanic ash from Mauna Kea, Hawaii (Stevens et al. 2018). As a part of our project, we have decided to prepare our own analogue from terrestrial rocks. Therefore, it is essential know Martian rocks composition.

The composition of Mars surface is mainly basaltic. Two main spectral regions were identified from Thermal Emission Spectrometer data from Mars Global Surveyor. One of them had a basaltic composition dominated by plagioclase and clinopiroxene. The other one had and andesitic composition dominated by plagioclase and volcanic glass. The basaltic composition is confined to older surfaces and the more silicic composition is concentrated in younger northern regions (Bandfield et al., 2000). Both regions are shown in figure 1.

Volcanic scoria is dark volcanic rock; it has low density because of the numerous vesicles formed when gases disolved in magma come out when lava erupts. It is part of ejected material from volcanoes. It is basaltic or andesitic in composition with a high percentage of volcanic glass. The similarity of these rocks to those that form the main regions in Mars has made us to consider them as possible analogues of Mars regolith. Furthermore, they are easy to obtain as they are sold for gardening.

To assess if volcanic scoria could be considered a good candidate for Martian soil analogue it is necessary to study both, chemical and mineralogical composition of our samples and compare our results with data obtained from rovers in Mars.

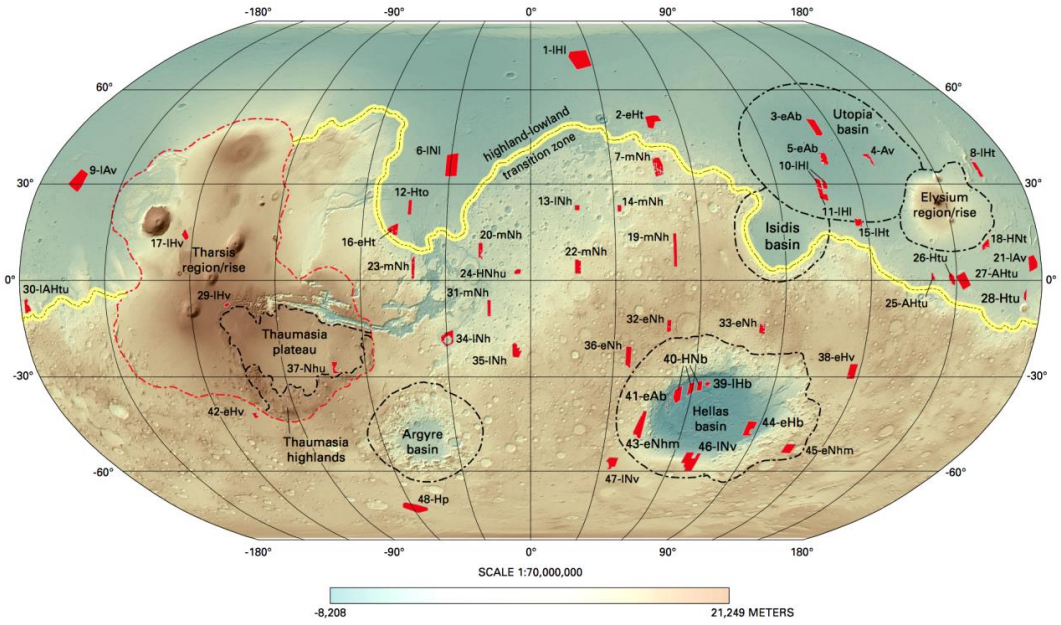

Figure 1. Mars topography. Two main regions identified by Mars Global Surveyor.

Material and Methods

Volcanic scoria was adquired from a general store. Fragments had a size between 12 and 18 mm and they were dark (NZV sample) or purplish red (RZV sample). Figure 2 shows both samples; their vesicular aspect was observed with a stereomicroscope (Figure 3).They were

washed to eliminate organic matter and separated into two batches: one with red stones and other with black fragments.

Figure 2. Black volcanic scoria (NZV) and red volcanic scoria (RZV).

Figure 3. Vesicles in volcanic scoria in RZV and NZV samples (20X).

In order to know the composition of the samples, they were studied at the Instituto Andaluz de Ciencias de la Tierra (IACT - CSIC). Major element oxide concentrations were measured with X-ray fluorescence analysis. Known the composition, the TAS diagram, total alkali oxides (Na2O and K2O)/silica content (SiO2) has been used to assign names to our rocks.

The mineralogy of the samples was determined with X-ray diffraction analysis. Our results were compared to those from Martian rocks obtained from literature.

Results

The chemical compositions of NZV and RZV samples were assessed with X-ray fluorescence. Results are shown in table 1. The chemical composition is very similar in both rocks with small differences for some oxides like MgO, Na_2O and K_2O. The elements with the main differences are Cl, S and Rb.

Table 1. Differences in composition of volcanic rocks used as simulants of Martian soil.

Elemento	NZV	RZV	Elemento	NZV	RZV
SiO2 (%)	41,950	41,851	Rb (PPM)	47	29
Al2O3 (%)	13,766	13,876	Sr (PPM)	929	975
Fe2O3 (%)	13,828	13,821	Y (PPM)	26	27
MnO (%)	0,229	0,229	Zr (PPM)	271	275
MgO (%)	8,734	7,891	Nb (PPM)	98	102
CaO (%)	11,879	12,038	Mo (PPM)	0	0
Na2O (%)	3,760	4,431	Pd (PPM)	0	0
K2O (%)	1,854	1,625	Ag (PPM)	0	0
TiO2 (%)	2,654	2,706	Cd (PPM)	0	0
P2O5 (%)	0,683	0,741	In (PPM)	0	0
F (PPM)	0	0	Sn (PPM)	0	0
S (PPM)	176	109	Sb (PPM)	0	0
Cl (PPM)	1021	687	Te (PPM)	0	0
Sc (PPM)	0	0	I (PPM)	0	0
V (PPM)	276	271	Cs (PPM)	0	0
Cr (PPM)	622	694	Ba (PPM)	694	719
Co (PPM)	67	57	La (PPM)	0	0
Ni (PPM)	445	448	Hf (PPM)	0	0
Cu (PPM)	130	148	Ta (PPM)	0	0
Zn (PPM)	147	129	W (PPM)	0	0
Ga (PPM)	25	18	Pb (PPM)	0	0
Ge (PPM)	0	0	Bi (PPM)	0	0
As (PPM)	0	0	Th (PPM)	0	0
Se (PPM)	0	0	U (PPM)	0	0
Br (PPM)	0	0	H2O (%)	0,03	0,19

Figure 4 shows the elemental composition of NZV and RZV samples. Selected data have been represented in order to compare them with avalaible values from Mars. Figure 5 shows the elemental composition of soils at three regions on Mars. Gusev Crater was studied by Mars Exploration Rover *Spirit*, Meridiani Planum by *Opportunity* and Gale Crater was analysed by *Curiosity*.

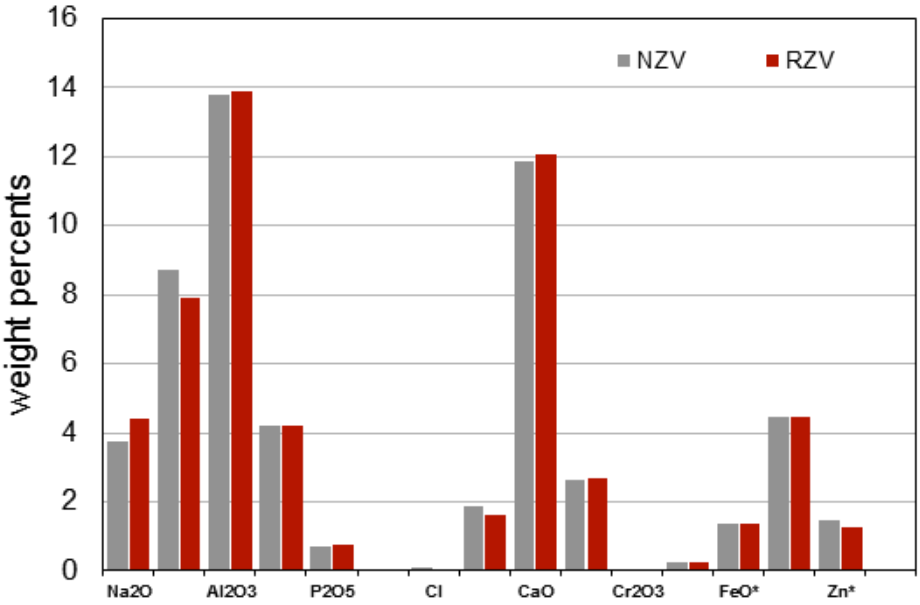

Figure 4. Elemental composition of NZV and RZV samples. Concentrations of silicon dioxide and iron oxide are divided by 10; nickel, zinc and bromine levels were multiplied by 100. SO_3 and Cr_2O_3 have not been determined.

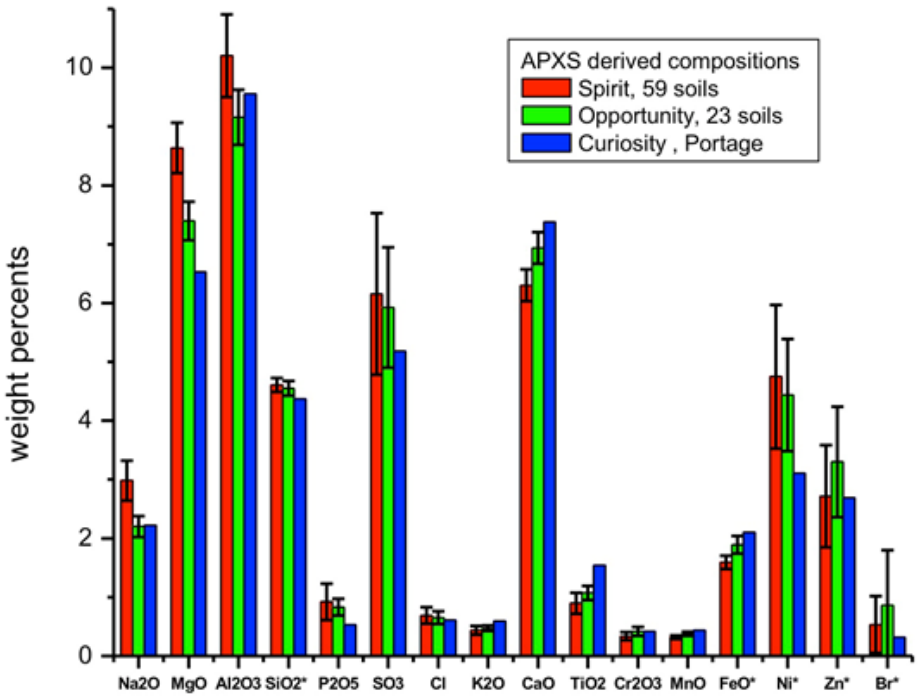

Figure 5. Martian soil composition data from three different areas

Regarding the main components, SiO_2 and MgO concentrations are similar both in Martian soils and our samples; the percentage of Al_2O_3 is higher in volcanic scoria but Fe_2O_3 and MnO concentrations are lower than in Mars soils.

Volcanic rocks can be identified using their chemical composition. The TAS classification (total alkali-silica diagram) is based on the relationships between the combined alkali content (Na_2O and K_2O) and silica content (SiO_2). The chemical composition of NZV and RZV samples points to tephrite/basanite. Figure 6 shows the TAS graphic for different rocks analysed in Mars (from McSween et al, 2006), and their comparison with NZV and RZV samples. Results indicate that these samples are similar to some Martian samples.

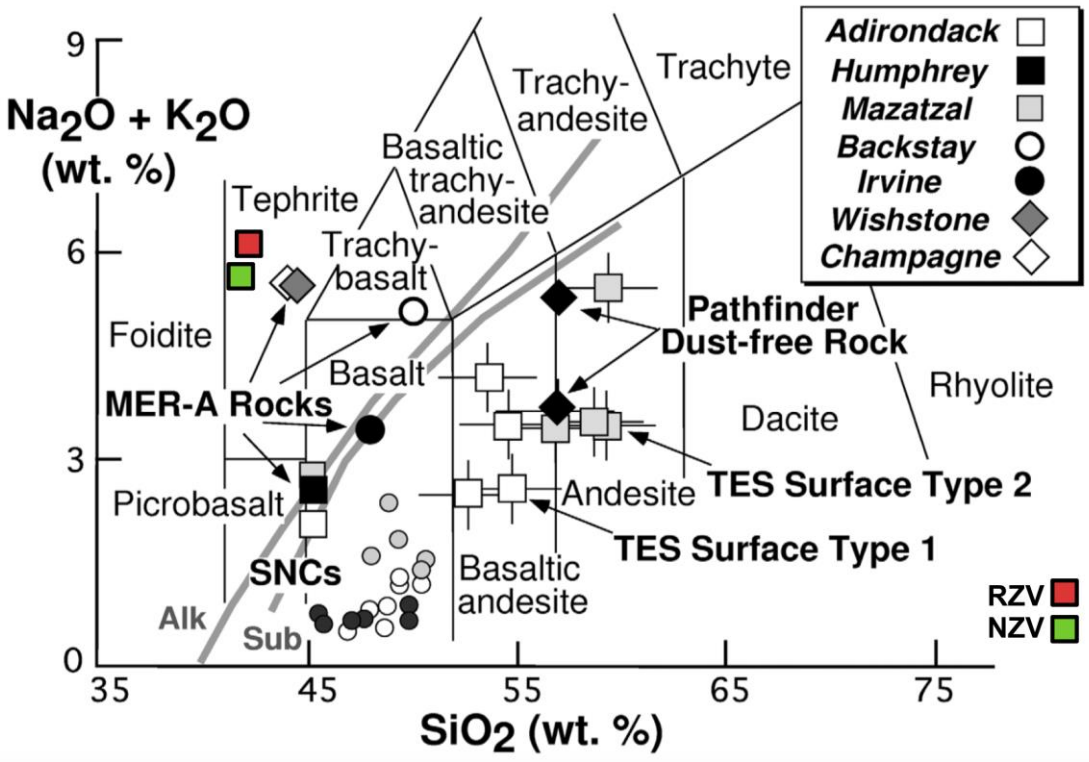

Figure 6. Alkalis versus silica classification diagram for volcanic rocks from Mars and RZV and NZV samples. Martian rocks Wishstone and Champagne are tephrites, close in composition to our samples (graphic taken from McSween et al, 2006). RZV and NZV are indicated by a red and green square, respectively.

Mineral composition of NZV and RZV samples were determined by X-ray diffraction and are shown in figures 7 and 8. The major crystallographic peaks for NZV sample denote the presence of diopside, nepheline, microcline, olivine and labradorite. The major crystallographic peaks for RZV show the presence of the same minerals plus hematite. The red color of this sample is due to this ferric mineral. Both diagrams show an amorphous component due to volcanic glass. The mineralogical composition is compatible with tephrite/basanite.

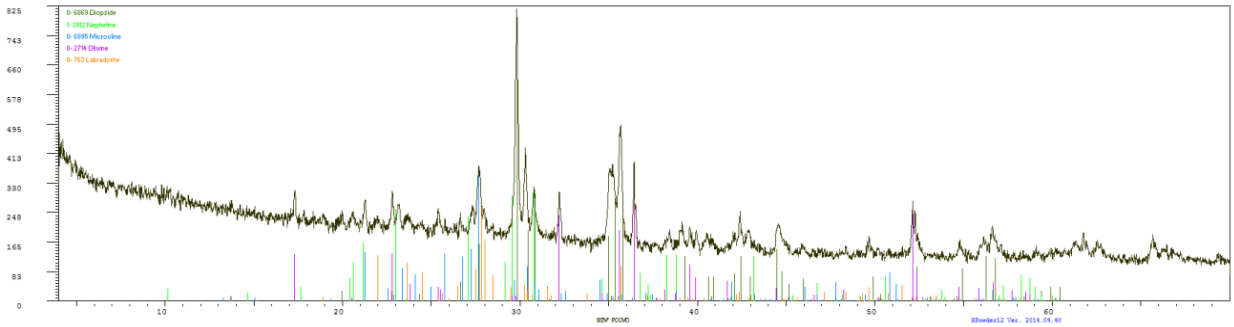

Figure 7. X-ray diffractogram of NZV sample. Major crystallographic peaks are compatible with diopside, nepheline, microcline, olivine and labradorite.

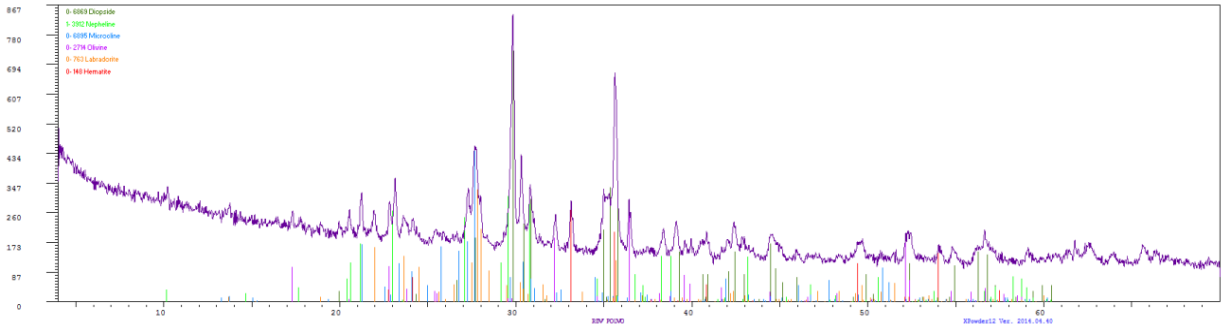

Figure 8. X-ray diffractogram of RZV sample. Major crystallographic peaks are compatible with same minerals in NZV sample plus hematite.

Discussion

The starting point to obtain an analogue of a Martian soil is to study what kind of rocks there are on the surface of Mars and compare their mineral and chemical composition with similar terrestrial rocks.

Data from Thermal Emission Spectrometer, in the Mars Global Surveyor, revealed two different regions in low albedo regions of Mars characterised by different types of volcanic rocks. Basaltic composition was typical in older regions while basaltic andesite was abundant in younger northern places (Banfield et al., 2000). Later research established that the composition of the rocks in both regions was mainly basaltic, but with different degree of weathering (Yen et al, 2005; McSween et al., 2006).

Volcanic scoria is a vesicular volcanic rock, mostly basaltic or andesitic in composition. It is easy to get from stores as it is used in gardening. So, this material was selected as a good candidate to prepare our Martian soil analogue. In a commercial sample we found two main types of fragments based on their colour: black (NZV sample) and reddish (RZV sample). Both of them were analysed in order to know the mineral and chemical composition and the results were compared to those obtained by rovers in Mars.

The chemical composition of NZV and RZV samples was studied by X-ray fluorescence. Both samples had similar composition and they have been identified as tephrite/basanite. Similar rocks have been found on Mars: chemical analysis of relative unaltered rocks at Columbia Hills indicate that presence of tephrite.

The first X-ray diffraction analysis of Martian soil was performed on 2012 at Rocknest, a sand patch in Gale Crater by the rover Curiosity. The fraction of sand studied contained 55% of crystalline material and 45% X-ray amorphous material. The crystalline component was basaltic, composed of plagioclase, feldspar, olivine and the clinopiroxenes augite and pigeonite. This component is similar to that inferred for Martian basalts across the planet (Blake et al., 2013).

X-ray diffraction in NZV and RZV samples revealed the presence of diopside (a clinopiroxene), nepheline (feldspathoid), microcline (potassium feldspar), olivine (nesosilicate) and labradorite (plagioclase) with amorphous material. Mineral composition of both types of rock confirms our results with X-ray fluorescence analysis. Differences between black and red samples were due to the presence of hematite in the red one. Iron oxides are relevant minerals on Mars (Rull & Cuadros, 2018).

Our analysis show that chemical and mineralogical composition of NZV and RZV samples is similar to that of tephrites, rocks identified on Mars. In conclusion volcanic scoria, mainly the purplish red sample with hematite can be a good candidate for a Martian soil simulant.

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Now we are the martians: *Bacillus megaterium*

Manal Mansour, Antonio Zamora, Elena Rodríguez, Paula Duro

IES Zaidín Vergeles, Granada

Summary

The project was basically to see if our terrestrial bacteria would be able to survive on Mars and create colonies so that we could start a new life on the red planet. We began our experiments with the simplest life we know, bacteria, because in that way we could see that problems are much more easily than if we used more complex organisms.

Mars has got some circumstances such as its low atmospheric pressure, radiation, etc. So, in relation to those changes we have done experiments in which we studied the reaction of our 6 bacteria to those factors.

As we have already said, Mars has a much lower pressure than Earth, so the first thing we did was to create an environment where the pressure was lower than on Earth itself to see if our microorganisms were able to survive.

If we look at the surface of the red planet, it is very different from what we know, and therefore we decided to analyze it, making a Martian analog, once we knew how that soil worked and the substances that could be there, we decided to subject our bacteria to experiments on the tolerance of chlorates in different media, solid and liquid.

In addition, on Mars, the temperature is more extreme and therefore more dangerous, so we decided to investigate if this would be a factor that would make problems, we subjected the bacteria to very different temperature cycles (-80°C, night temperature on Mars and 20°C, temperature of the day.)

The last experiment we did had to do with that atmosphere that can give us problems colonizing Mars, because it is very light and it doesn't protect the planet from the Sun. Knowing this, we decided to expose our bacteria to ultraviolet radiation, which is what they would receive from our star.

KEYWORDS: *Bacillus megaterium, bacteria, survival, Mars, tolerance, growth.*

Introduction

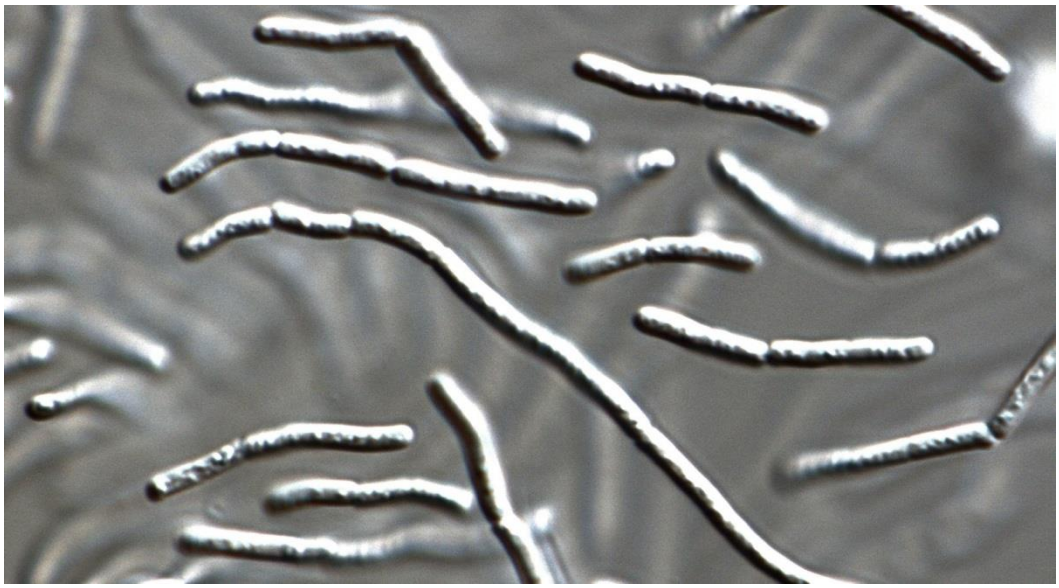
We wanted to assess the possibility that one day we could inhabit the red planet, that one which has caused us curiosity for so long, Mars. And we have done it through some small candidates to be our first colonizers of that planet, our bacteria: *Escherichia coli, Bacillus megaterium, Bacillus subtilis, Halomonas sp, Pseudomonas putida, Pseudomonas stutzeri*.

Mars has got very different conditions than the Earth, and the most important and obvious is the tenuous and weak atmosphere that it has. This makes the Sun's radiation much higher, the pressure changes, etc. Our bacteria are accustomed and adapted to live in terrestrial conditions and therefore, when subjected to experiments where you totally change their environment, they haven't always been good enough to survive.

Mars is a hostile planet in terms of life, it is a difficult planet to cultivate and colonize, even though we continue to see it as our primary option, so if an experiment gives us bad results for that process we don't stop, we continue until we find the solution, and that's what our project is about, to find solutions to the problems in Mars.

Material and Methods

- Microorganisms. *Bacillus megaterium* is a rod-like, Gram positive bacterium. It is mainly aerobic and it forms spores in adverse conditions. It is one of the biggest known bacteria, with a cell length of up of 4 µm and a diameter of 1.5 µm. It grows at temperatures from 3°C to 45°C, with the optimum around 30°C. It is an ubiquitous bacterium.



Bacillus megaterium. Image courtesy of Juan de Dios Alché Ramirez (EEZ, CSIC).

- Low atmospheric pressure: For this experiment, we have created a vacuum chamber with a glass canister that has been connected to a tube that was coupled to a vacuum trap to extract as much air as possible.

  In a second experiment, we used a vacuum pump that we connected to a desiccator and a vacuum gauge that allowed us to measure the pressure throughout the circuit.

Figure 2. Equipment used for generating a low-pressure atmosphere

- Chlorate tolerance in a solid medium: Different amounts of potassium chlorate were added to the culture medium to achieve concentrations of 0M, 0.05M, 0.1M and 0.2M; on the plates, we added the bacteria and we let it incubate at a temperature of 30 °C.
- Chlorate tolerance in a liquid medium (KClO3): We use the spectrophotometer, an instrument that measures the amount of light absorbed after crossing a sample. So, as the number of microorganisms increases in liquid culture, its turbidity increases and by measuring its optical density we estimate the number of bacteria present.

Figure 3. Spectrophotometer Shimadzu UV-120-02 used

- Temperature changes: We prepare tubes with 3 grams of our analog of the soil of Mars and inoculate them with 500 microliters (0.5 ml) of liquid culture of each of our microorganisms. They were then subjected to alternating cycles of freezing at -80 ° C and thawing at room temperature.
- Ultraviolet radiation: We placed, on Petri plates, 100 microliters of each culture. We let them be absorbed, then cover half a plate with aluminum foil and irradiate for one minute with an UV light lamp at 254 nm.

Results

Similarities between our martian soil analogue and the real martian soil.

In our laboratory it has been prepared an analogue of Martian soil from Earth volcanic rocks. The first objective was to see how similar was our analogue to Martian soil. Our samples were analyzed at the Instituto Andaluz de Ciencias de la Tierra (IACT, CSIC). After numerous tests we can conclude that it can be considered as an analogue of Mars soil since the composition of both is similar.

Bacterial tolerance to low atmospheric pressure.

Two batches of Petri plates were prepared. In each one of them 5 microliters of the different cultures of the bacteria were inoculated. One was placed in the container and the air was extracted while the other was left outside. Both were grown at room temperature.

The results show how *Bacillus megaterium* showed a remarkable improvement in its development after the first days, when it was seen as one of the best adapted, unlike in the previous ones.

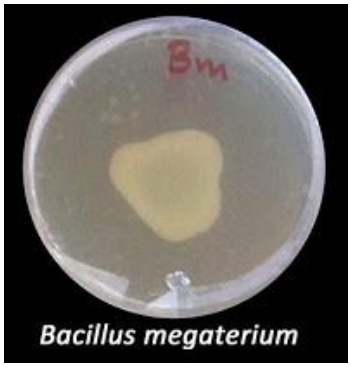


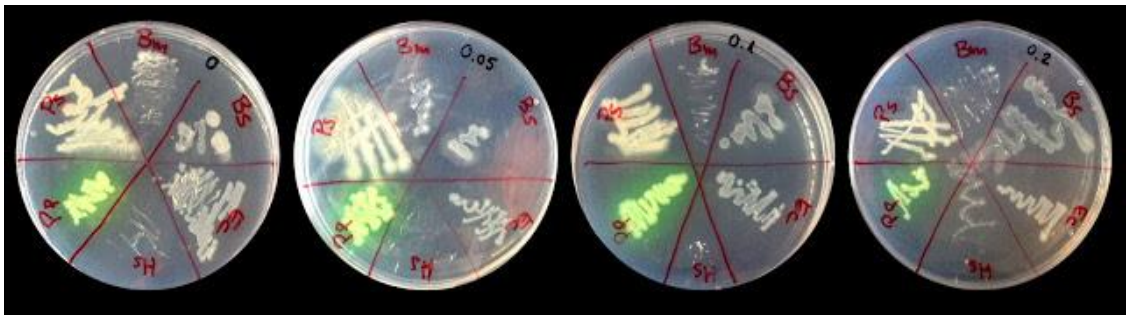
Figure 4. Growth of *B. megaterium* on LB plate under low pressure conditions

We have tested in a second experiment if our bacteria would survive in an environment with low atmospheric pressure. We used the vacuum pump that we have described before. Even though it wasn't very effective, we managed to reduce the pressure to one third of the atmospheric pressure.
After the experiment, *B. megaterium* didn't grow much, but enough to see that it survived, which was what we were looking for with this experiment.

Chlorate tolerance in a solid medium.

We started our project making the bacteria grow in different concentrations of potassium chlorate ($KClO_3$). The concentrations of this substance were of 0M, 0.05M, 0.1M and 0.2M.

We can appreciate that as the concentration grows, the growth decreases.



Results after the first 24 hours.

Results after the first 48 hours.

Figure 5. Growth on LB plates in the presence of different concentrations of $KClO_3$. **Bm** indicates *Bacillus megaterium*

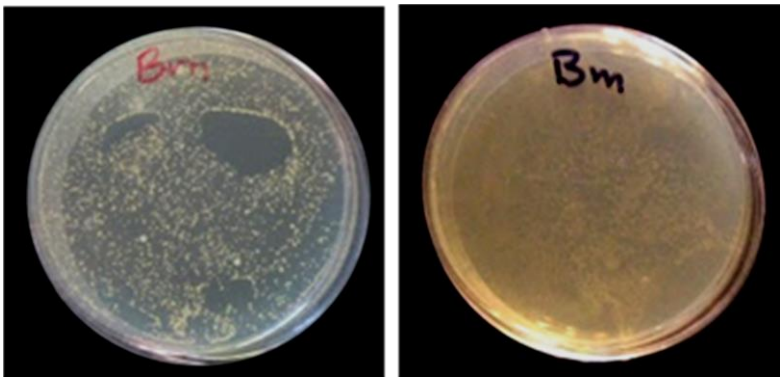
Chlorate tolerance in a liquid medium ($KClO_3$).

This time we grew the bacteria in LB medium with the same concentrations of potassium chlorate as the last experiment. Using a spectrophotometer to measure its growth and these were the results we got:

$KClO_3$	optical density
0 M	1.53
0.2 M	0.663
0.5 M	0.055

Tolerance to temperature changes.

During the experiment we were able to observe that the bacterium *Bacillus megaterium* has grown a lot and also it grew a number of bacteria not belonging to the same.



1 x (1 week -80°C + 1 week 20°C) 3 x (1 week -80°C + 1 week 20°C)

Figure 6. Growth after different cycles of temperature changes.

Ultraviolet radiation.

Regarding the last experiment, the bacteria was exposed to UV radiation, 254 nm during 1 minute. The results show that *B. megaterium* is able to survive despite the radiation.

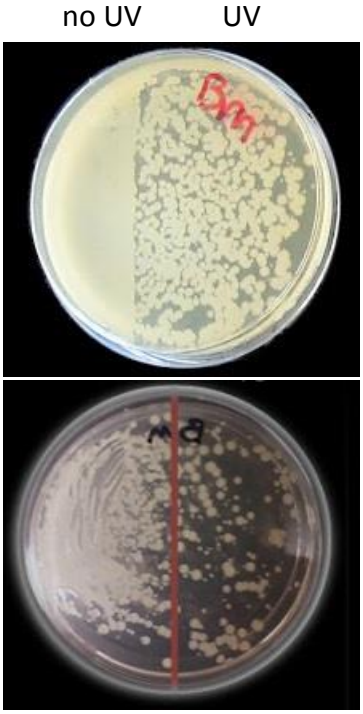


Image from the results we got at our high school.
254 nm during 1 minute. Hand UV lamp

Image from experiments at the Experimental Station of Zaidín.
254 nm during 1 minute. UV transilluminator

Figure 7. Effect of UV irradiation on growth

Discussion

After all the experiments carried out, we concluded that *Bacillus megaterium* would not be the best candidate to colonize Mars since we see how, exposing it to independent physical agents among them, its survival is not the greatest among all the bacteria that are candidates to inhabit the red planet.

B. Megaterium has endured all the experiments even in a mild way. The big problem is that, on Mars, they would face all these factors together and randomly, it is a hostile terrain where the bacteria are not adapted and therefore these agents are deadly.

The conclusion is that our bacteria is not the most resistant and maybe in the future we could modify its DNA so that they are surviving to other conditions. We could also make a terrain on Mars created by us thanks to the information obtained in the experiments about tolerance to all these external agents regarding our bacteria, this would cause the bacteria to end up probably surviving those conditions and living in a completely normal way, and, maybe, they would endure the normal conditions of Mars.

The characteristics of this land would be:

- The low pressure would not be a big problem since during the experiment we could see that the bacteria grew without any problem, which means that they support low pressures.
- About chlorates and perchlorates, we know that *Bacillus megaterium* did not tolerate these experiments well, so, in our analog, we should have a minimum amount of these salts.
- We saw that the temperature changes of Mars (15-20°C during the day and -80°C during the night) would not disprove the survival of our bacteria on the red planet, so in our analogue we could leave it the same.
- With regard to ultraviolet radiation there would be no problem, since, in fact, it was the one that best survived the radiation.

Acknowledgements

We want to thank our teacher of Biology and Geology, Antonio Quesada, for having the initiative of this project and getting it done. Thanks to Manuel Espinosa for helping us and offering us his methods and knowledge.
And thanks to the Zaidín Experimental Station because they have helped us to know more and learn.
Everyone has made this project more dynamic and fun, we have learned to grow bacteria, make experiments, etc.
Thank you very much because without you it would not have been possible.

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We have used the information from our blog http://biolabzv.blogspot.com/
And from other websites: https://en.wikipedia.org/wiki/Bacillus_megaterium

Pseudomonas putida colonizing Mars

Pablo Delgado Alaminos, Alejandro Sánchez Morales, Enrique Arias Rey

IES Zaidín Vergeles. Granada

Summary

The goal of this project is to see if there could be microbial life in the fourth planet of the solar system, Mars. For that, a series of experiments have been done that recreate the environment of Mars to see if some bacteria could survive there. In these experiments we have simulated four of the main characteristics of this planet, the low pressure, the high presence of chlorates, large temperature changes and high ultraviolet radiation. In general, the results have been quite good for the bacterium that our group studies, *Pseudomona putida*, since it has survived and grown in all the experiments carried out.

Keywords: *Pseudomonas putida* (*P. putida*), Mars, Chlorates, Low pressure, Temperature changes, Ultraviolet radiation, Survival, Contamination and Inoculation.

Introduction

A long time ago, Mars was very similar to Earth. It had rivers and lakes and the atmosphere was very different. In this conditions, life could have evolved there. At present, conditions are completely different. There is not liquid water, but high concentrations of chlorates and perchlorate could keep water in liquid state in depth. The atmosphere is very thin and it has not oxygene; consequently, high levels of radiation reach the surface of Mars. In conclusion, Mars is an inhospitable planet. We want to know if some terrestrial bacteria could be able to survive in this environment.

Our group has worked on the bacterium *P. putida*, this microorganism belongs to the phylum *Proteobacteria*, to the order *Pseudomonadales*, to the family *Pseudomonadaceae* and to the genus *Pseudomonas*.

This cane-shaped species is characterized by its great degradation to organic compounds and its wide catabolic potential (energy production), in liquids they grow abundantly forming a ring and a bluish-green sediment that is quite easy to recognize naked eye.

With this bacterium the results of our tests have been quite good thanks to its salt tolerance, resistance to temperature changes, at low pressure and ultraviolet radiation.

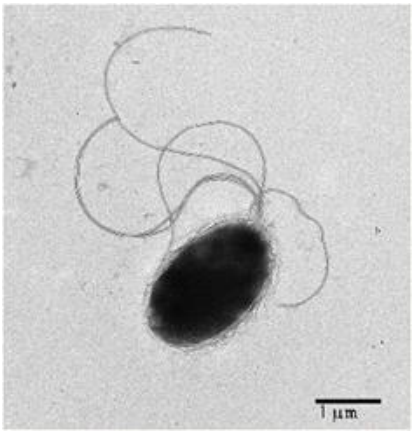

Figure 1. *Pseudomonas putida* observed with an electron microscope (Wikipedia).

Material and Methods

Growth at low atmospheric pressure

To carry out this experiment we used a device which simulated the pressure of the atmosphere of Mars, absorbing the air from the container where *P. putida* was. This homemade instrument consists of a vacuum pump, a vacuum gauge to measure the pressure and a desiccator where the microorganisms have been placed, as it is not prepared professionally it has faults in the ducts, since a little air is introduced which makes the pressure is one third to that of the Earth; while the pressure of Mars is 30 times lower than what we have achieved, but even so it is a good simulation to check the possible existence of life in this created environment. We can know that we have this pressure because before performing this experiment we used balloons which were empty and when the pressure diminished where they were, they were inflated due to making our artificial atmosphere dimmer and creating a negative pressure that made these inflate.

Tolerance to Chlorates

The material used in this test has been four Petri dishes, in which we have placed different concentrations of potassium chlorate. In one there was no chlorate, and in the remaining three the amount of this salt was 0.05M, 0.1M 0.2M (M = molar concentration of chlorate in the serum of the plate).

After preparing everything incubated for 24 hours and 48 hours at a constant temperature of 30 degrees Celsius to see the differences in survival and growth.

This experiment was done on a solid medium, but since there may also be groundwater on Mars with these salts, we have done another experiment in a liquid medium.

In that trial the microbiologist Manuel Espinosa had prepared a culture in which our bacteria were in a liquid LB medium and we used a spectrophotometer to know how many

microorganisms had survived in this medium with different concentrations of potasium chlorate. We could estimate the concentration of microorganisms measuring the turbidity of the cultures. Higher the number of microorganisms, higher the turbidity of the cultures.

Survival to Temperature Changes

For this experiment we have prepared recipients with 3 grams of sterilized Martian soil analogue which was inoculated with our microorganism. Then it was exposed to temperature changes from -80ºC to 20ºC. One month later, we took 100 mg of simulated martian soil and added 500 microliters of sterile LB culture medium.

From this new solution we have extracted 100 microliters which were deposited in a Petri dish to verify that the microorganisms have managed to survive these temperature changes.

Resistance to Ultraviolet Radiation

In the experiment we tested the resistance of the bacterium *P. putida* to ultraviolet radiation. We used an apparatus that irradiated enough to recreate the environment of Mars. To do this *P. putida* were poured along with a little serum in a petri dish, using a dropper; Then the drop of this serum was dissolved in the plate when using small glass beads, moving them on the plate. Once this was done they were exposed to a 254 nm uv radiation for one minute, but only half of the plate, since the other half was covered with aluminum foil to observe the differences between how they would grow with radiation and without radiation. As the results obtained in the experiment were not conclusive they were repeated al the Estación Experimental del Zaidin.

Results and Discussion

Low Pressure

P. putida has been able to grow at low atmospheric pressure. Our device kept pressure to 1/3 of atmospheric pressure in Earth and microorganisms were able to grow inside it. (Figure 2).

Figure 2. *P. putida* grown at low atmospheric pressure.

Tolerance to Chlorates

The results obtained have been very good in this experiment, because in the crops we can see that the *P. putida* grows a lot and keeps the quantity of microorganisms in the different concentrations of chlorates, which means that the *P. putida* has a great resistance to these salts.

Another thing that must be highlighted is that yellowish and greenish pigmentation that has and that the difference from the others, it comes from the pioverdin pigment. This is produced by these *Pseudomonas* and some others when there is little iron in the middle.

If we look at our bacteria at 24 hours (Figure 3) and 48 hours (Figure 4) after being prepared in this medium, we observe that it has grown a little, this is normal because by carrying in that environment only two days it still has the necessary nutrients to grow.

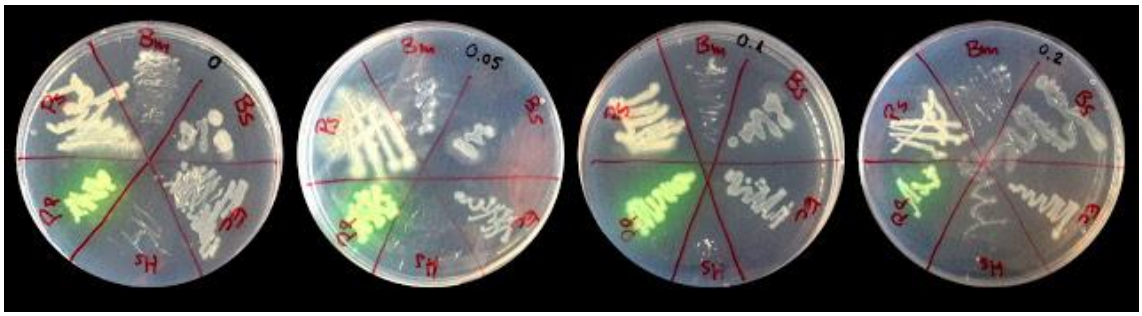

Figure 3. *P putida* (fluorescent yellow) in chlorate tolerance 24 hours after being prepared.

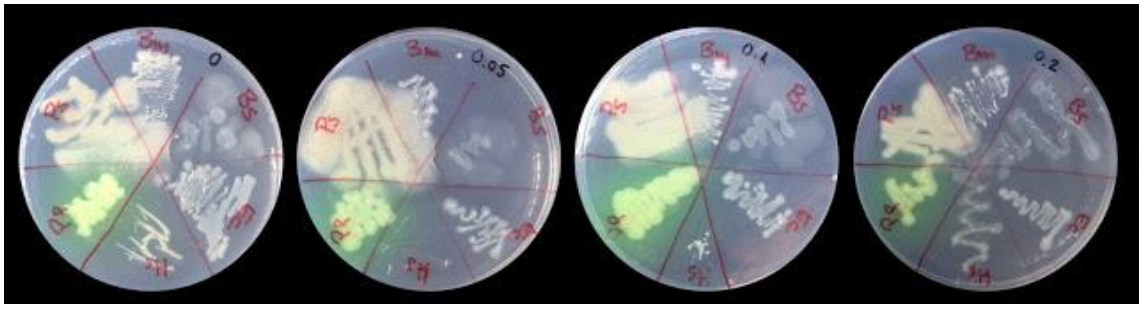

Figure 4. *P putida* (fluorescent yellow) in chlorate tolerance 48 hours after being prepared.

This experiment is the one that has been done in solid medium, if we see the results in the liquid medium we can see that these are not very different.

According to the data obtained from the spectrophotometer *P. putida* has had quite similar results when there is 0.5 M of potassium chlorate (Table 1) and when this salt is not present, this tells us that these microorganisms could perfectly survive in a hypothetical sea or underground ocean on Mars, where the chlorates were abundant. This brine would have to be underground due to the low atmospheric pressure of the red planet.

Table 1. Resistance of *P. putida* to chlorates

$KClO_3$	optical density
0 M	1.53
0.2 M	0.663
0.5 M	0.055

Survival to Temperature Changes

It can be seen that *P. putida* has managed to grow quite a lot; It has spread throughout the plaque and practically no individual colonies are distinguished, since they have been able to survive enough bacteria so they are close together. It is also true that there are about three small places where they have not managed to grow, although this is not so relevant. (Figure 6) The *Pseudomonas* have adapted a whitish color like most other bacteria, is the most common color.

Figure 6. *P. putida* subjected to temperature changes.

When two months later we saw how our bacteria were subjected to these changes in temperature, we saw that they were completely different, they had grown more, which did not make sense since, being subjected to such drastic temperature changes, they should perish (Figure 7). Another new characteristic that we can observe is that yellowish tone that it has; As we could not understand why this was so, we did another experiment depositing these *P. putida* bacteria in another sterilized petri dish. In the results we observed that this yellowish tone still exists (Figure 8), which leads us to conclude that the plaque was contaminated due to an external agent, probably from human hands, since there is a bacterium in them that adopt that color and it is also It is quite likely that the pipette or the plate itself has been in contact with us more than necessary.

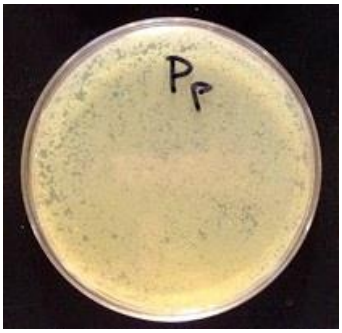

Figure 7. *P. putida* after two months exposed to temperature changes.

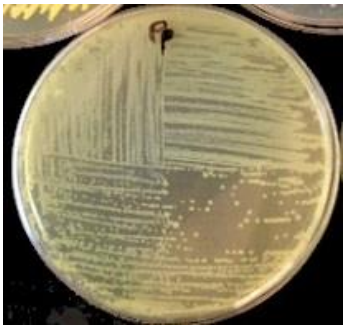

Figure 8. Passing part of the previous bacteria to another Petri dish.

Resistance to Ultraviolet Radiation

In this experiment in which we have exposed *P. putida* to ultraviolet radiation, the results have not been convincing because bacteria have grown more or less uniformly throughout the plaque (Figure 9). What should have happened would be that in one half of the plate they had grown normal, since they were protected by aluminum foil and in the other half they had grown less, since they were exposed to ultraviolet radiation.

Figure 9. *P. putida* subjected to radiation (first experiment)

What we have understood that has happened is that when we spilled a drop of serum with our bacteria this was not distributed well by the plate so it did not dry well and did not yield good results. A few days later when Manolo Espinosa repeated the experiment, more convincing results were obtained (Figure 10).

Figure 10. *P. putida* subjected to radiation (second experiment)

It can be seen that in the irradiated part they have managed to survive a lot less bacteria than in the non-irradiated part, this is normal since the ultraviolet radiation kills the bacteria and any other organism. We can also see that apart from growing less they have also grown with many individual colonies, since by surviving fewer bacteria always grow in this way, more separated from each other because there is less space between them.

All the experiments we have done throughout this project show that *P. putida* have managed to grow or simply survive in the difficult conditions of Mars. Knowing this we have considered our project as a conclusion that these microorganisms would be perfectly capable of surviving on the red planet, especially under the ground, which is where less UV radiation arrives and therefore there is a greater chance that they will not perish.

Acknowledgments

We want to thank the teacher of IES Zaidín Vergeles, Antonio Quesada Ramos, who is the one who has prepared the most experiments, since he has done it in the IES Zaidín Vergeles, the place where almost all the experiments have been done. We also thank Manuel Espinosa for being the director of the project and for coming to our center and helping us with the experiments of the bacteria, his help has been fundamental. Finally, we thank the Experimental Station of Zaidín and CSIC for having helped us both in biology (with our bacteria) and in geology (by analyzing the rocks to know if they were a good analogous soil of Mars), respectfully.

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Life of *Pseudomonas stutzeri* on Mars

Tania Castillo Tejada, Gabriela Fernanda Cedillo Quesada, Alba Espigares Lorente, Carla Heras Domene, Isabel Morell Fernández

IES Zaidín Vergeles. Granada

Summary

We are going to test the resistance of *Pseudomonas stutzeri* in a simulated Mars environment. For discovering how much it can tolerate we have made some experiments, that are going to reveal if our bacteria would be able to live in Mars. Our results show that this microorganism is a good candidate to colonize the Red Planet.

Keywords: *Pseudomonas stutzeri*, life, Mars, bacterial colony, bacteria.

Introduction

We have done this research to carry out the mystery of life on Mars considering the possibility of the survival of different bacteria and microorganisms in that environment. In our case, we have been able to discover several facets of the bacterium, *Pseudomonas stutzeri*, where we have tested it to a variety of experiments so that we can check their capacity to survive in the different conditions of the planet Mars.

The atmosphere of Mars is much more tenuous than the terrestrial one and with a totally different composition. It is very abundant in carbon dioxide and very poor in oxygen. Atmospheric pressure is one hundredth of what exists on our planet.

An important factor for the possibility of life on Mars is the presence of liquid water, something that is impossible on the surface of Mars given the low temperatures prevailing. However, there are structures that resemble water courses and whose morphology has even changed in a relatively short period of time. That is why it is proposed that in the subsoil of Mars there could be liquid water, but the only possibility that this happens is as part of brines, more or less concentrated solutions of mineral salts in water. This could be possible because in Mars the presence of chlorates and perchlorates in the soil has been detected, substances that in solution could cause a decrease in the freezing point of water and favor the liquid state despite of low temperatures.

As a consequence of its tenuous atmosphere, temperatures on Mars surface are very low and this is an adverse factor for life. In relation to this factor, regions closed to the equator or Mars have temperatures that oscillate between 15 and 20 ° C during the day although they would fall to -80 ° C during the night. Anyway, there is possibility of existing liquid water.

Another factor related to Mars atmosphere is the absence of oxygen and ozone. So, it can not filter high energy radiation coming from the Sun and the space. This radiation, for example, ultraviolet, would be letal for microorganisms at the surface of Mars.

Material and Methods

In the first session we met the microorganisms which we have worked and we learnt about basic techniques of work in microbiology. The microorganisms that we were going to study in our classroom were: *Bacillus subtilis*, *Bacillus megaterium*, *Pseudomonas putida*, *Pseudomonas stutzeri*, *Escherichia coli* y *Halomonas sp*. Our group worked with *P. stutzeri*.

Pseudomonas stutzeri is a denitrifying bacterium distributed in the environment. Some strains are able to fix dinitrogen, and other participate in the degradation of pollutans or interact with toxic metals. These characteristics make this microorganism very interesting for studying its tolerance to Mars conditions.

The objective of the first experiments was to study the possibility of the bacteria survival in low pressure environments. At first, we prepared a simple vacuum chamber with a glass canister to which we connected a tube coupled to a vacuum trap to extract as much air as possible. Two batches of Petri dishes were prepared, in each of which 5 microliters of the different cultures of the bacteria were inoculated. One was placed in the container and the air was extracted while the other was left outside. Both were grown at room temperature.

We also used a vacuum desiccator to which we have connected a pump that extracts the air and a vacuum gauge. We reduce the pressure inside the container to a third of the existing outside although this value is still far from the pressure that is on the surface of Mars. We incubate the 48h batteries inside the chamber at room temperature.

From a microbiological point of view and from the perspective of our project it is important to value the possibility that our bacteria they could survive in the presence of chlorates and perchlorates. Our experiment has consisted in study the growth of our bacteria in plates with increasing concentrations of potassium chlorate ($KClO_3$). To the culture medium that we use (LB) it has been added the sufficient amount of this salt for achieve finals

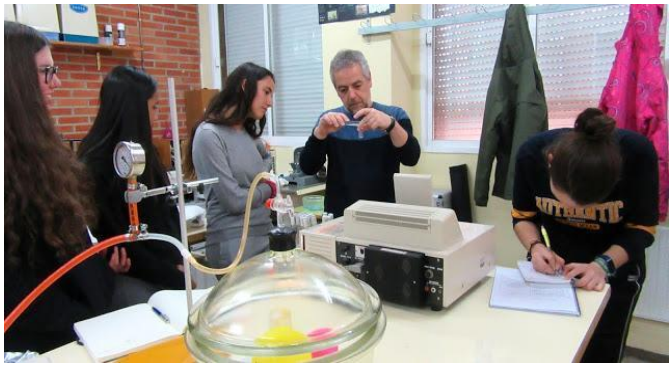
concentrations of 0.05M, 0,1M and 0.2M. And on these plates we have inoculated our bacteria and we put them to inoculate at a temperature of 30ºC.

We also evaluated the growth of bacteria in liquid media. We had cultures of our six microorganinsms in liquid medium with different concentrations of potassium chlorate. We were taught to measure the growth of bacteria using a spectrophotometer. The basis of this technique lies in measuring the turbidity of the medium. As the number of microorganisms increases, so the turbidity does. So, knowing the optical density of the liquid culture we can estimate the concentration of microorganisms.

To simulate the survival to temperature conditions around the equator or Mars we prepared tubes with three grams of our analog of the soil of Mars and incubated them with 500 microliters (0.5 ml) of liquid culture of each one of our microorganisms. Next, they underwent alternating cycles of freezing at -80 ° C and thawing at room temperature for some time.

Then we extracted 100mg of material from each of the six tubes we had previously inoculated with the bacteria into six eppendorf tubes. At the high school laboratory we added 500 microliters of sterile LB culture medium, mixed well and plated 100 microliters in each of the Petri dishes with solid LB.

To study the tolerance of microorganisms to ultraviolet radiation we have evaluated the survival of microorganisms to ultraviolet radiation. To do this, we have placed 100 microlitres of liquid culture on Petri dishes and we have allowed them to be absorbed. Then we have covered half a plate with aluminum foil and irradiated for one minute with a UV light lamp at 254 nm. We have repeated this experiment with tubes containing a simile of sterile Martian soil. We have left them incubating for several days. As the results were not conclusive, the experiment was repeated at the Estación Experimental del Zaidín.



Results and Discussion

The first experiment that we did in this research was to assess if microorganisms could survive at low atmospheric pressure, at the conditions found on Mars. We used a

homemade device and all the bacteria grew but air had entered into the recipient. We repeated the experiment with a different design with the help of a vacuum pump.

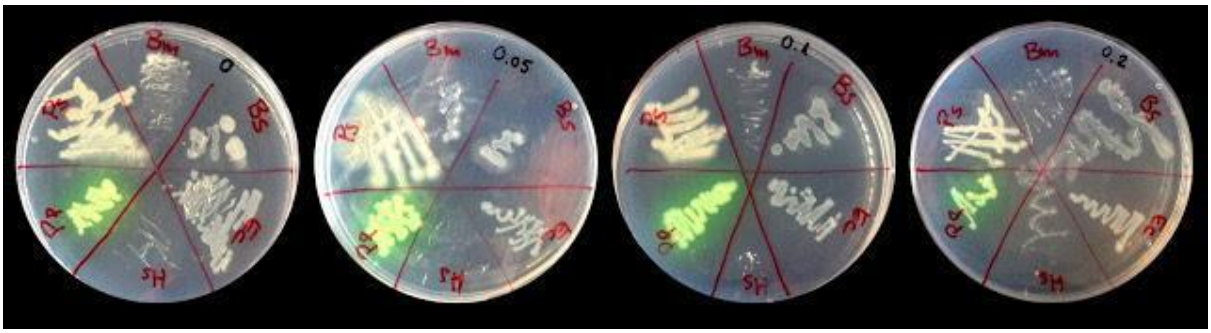
We inoculated Petri dishes with our bacteria and put them for 48 hours inside a vacuum desiccator connected to a vacuum pump. *Pseudomonas stutzeri* grew in those conditions.

The pressure we obtained was not enough because on Mars it is 30 times smaller. Although we were not able to get an atmospheric pressure similar to Mars, our results are important because they confirm that *P. stutzeri* can grow at low atmospheric pressure, at least one third of our planet's.

Liquid water in Mars near the surface of the planet only can exists if there are salts forming solutions so that the freezing point was several degrees below 0ºC. Chlorates and perchlorates are able to decrease the freezing point of water and they have been detected in Mars.

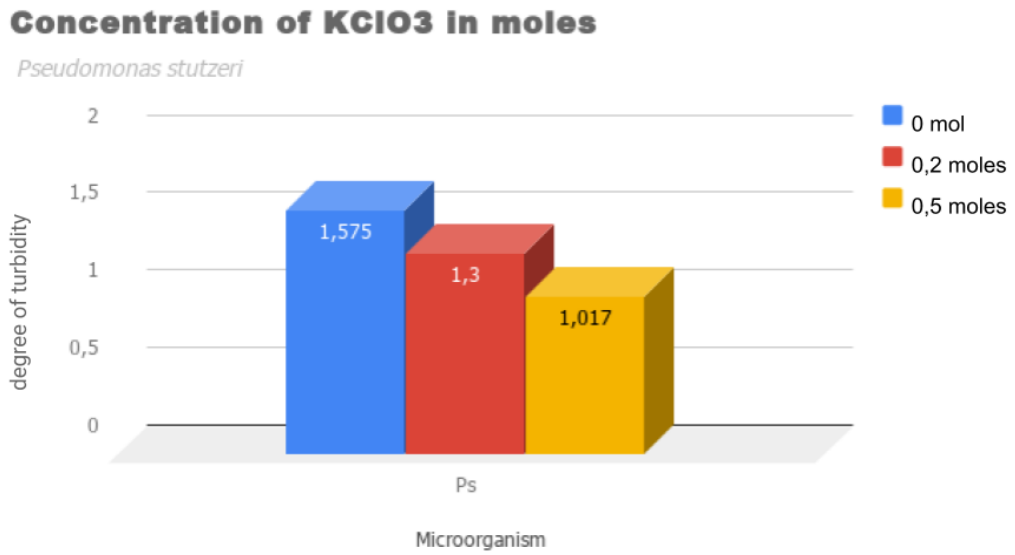
We prepared some Petri dishes with different concentrations of potassium chlorate (0M, 0,05M, 0,1M and 0,2M). After 40 hours of incubation, *P. stutzeri* grew in all the plates. So, we can conclude that our bacteria survive in the presence of chlorates.

24h



48h

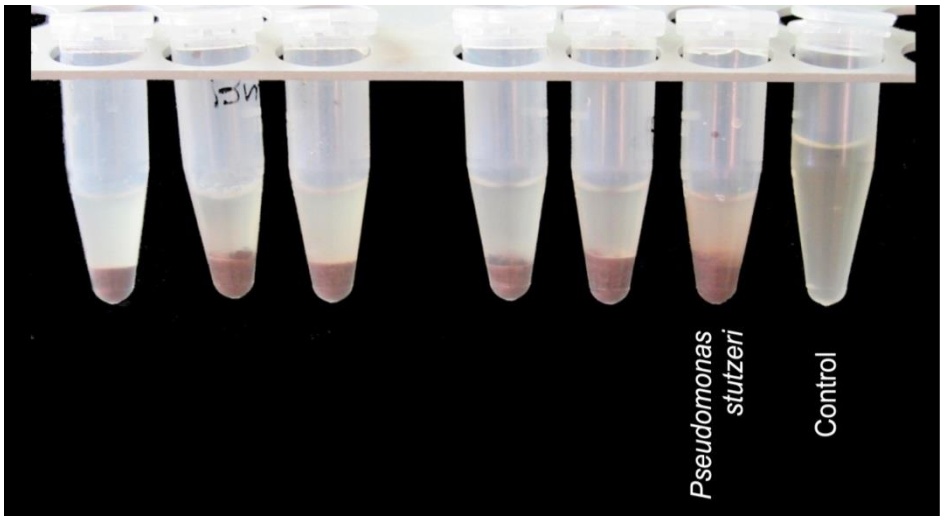
We also studied tolerance to chlorates in liquid medium with the help of the spectrophotometer. As we increase the concentration of $KClO_3$ the optical density of the cultures decreases. But our results show that *P. stutzeri* is able to survive at concentrations of potassium chlorate as high as 0,5M.



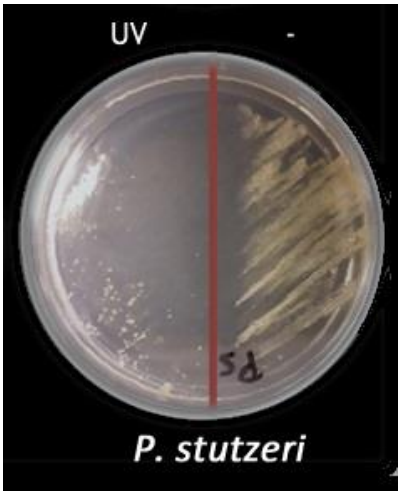
We have studied the survival of microorganisms to extreme temperature changes because in Mars equator, it can oscillate from 15-20 ºC during the day to -80 ºC during the night.

We can ensure that, even these extreme temperature changes, bacteria survive and grow and this is a big step as we could observe in Petri dishes after a month of changes from -80ºC to room temperature at the Zaidín Experimental Station.

But we put them in conditions of temperature at the Equator of Mars, which is like the best area in terms of temperature for the day. We should do the same but placing them in the conditions of other regions of Mars and also adapting to the different seasons of the year. That is, we could see the minimum and maximum temperatures that occur in the polar ice caps, where the minimum are about -150ºC. And so try to see if the microorganisms would survive.



However, to say if a bacteria is more or less resistant, we would have to know the number of cells from which we started. For example, if we have got 2.000 colonies and we put 2,000 cells of *P. stutzeri*, their survival would be 100%. I we had one million of cells of *P.stutzeri* at the beginning and recovered 20,000, only 2% of the initial population would have survived. We also study the survival adding liquid medium to soil samples and incubating them. We could observe that the eppendorf tube with *P. stutzeri* was the one with the largest turbidity. So, *P. stutzeri* resists temperature changes at least for a month.



Finally, *P. stutzeri* was sensible to ultraviolet radiation. We irradiated half a Petri dish with ultraviolet light (254 nm) for one minute; the other half plate was a control, covered with aluminum foil, so it hasn't receive uv light. Only few colonies were observed in the irradiated area.

In conclusion, our experiments show that *P. stutzeri* could survive to low atmospheric pressure, to the presence of chlorates and in conditions of temperature like those in Mars equator for a time. However we have not tested all these conditions simultaneously. In case it survives, it would live in the underground, protected of radiation from space.

Acknowledgments

We want to thanks several people but, in particular to Manuel Espinosa, for cooperating and his help in these experiment, to Antonio Quesada for guiding us at all times from the beginning to the end and to whole the team of the *Zaidin Experimental Station.*

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Escherichia coli to the Red Planet

Alejandro Ortiz Minaeva, Lucca Caetano Landim, Pablo Cardaldas Fornieles, Nuño Gutiérrez Martín, Gerardo González Rachidi

IES Zaidín Vergeles. Granada

Summary

In the IES Zaidín Vergeles High School, a scientific project has been developed with an educational purpose that encourages scientific research by putting into practice the scientific method. The main objective has been to find results that show us if some bacteria are able to grow in an environment to which they are not used and to check if the existence of life on Mars is possible.

This project helps us to study the Earth at the high school comparing it with Mars, and the objective, apart from learning, is to see if our bacterium, *Escherichia coli*, is able to survive in the conditions of Mars.

Keywords: *Escherichia coli*, pollution, atmospheric pressure, Mars, radiation, life of Mars, chlorates, bacteria.

Introduction

At present the existence of life is not known in Mars. The conditions in this planet are so different from Earth that they make difficult to think that there could be life similar to that of the Earth.

The main goal of our project is to verify the capacity of survival of terrestrial bacteria in Mars unusual environmental conditions. In our case, we are going to work with *Escherichia coli*. If we obtain a complete interpretation of our experiments, we can lead to very important conclusions about the possible existence of life in Mars, and in the future, settling of terrestrial life on the Red Planet.

We know the adversities *Escherichia coli* has to face in Mars but not the behavior of this bacterium in that extreme environment. We do not know the resistance of our bacterium to the red planet conditions and this is the main goal of our research: to verify and investigate with the experiments prepared during the project if *E. coli* would survive there. We will investigate the resistance to conditions similars to Mars related to the atmosphere, temperature, salts presents in soil or radiation.

We will propose hypothesis about the existence of past, current or future life in the red planet. And especially if *E. coli* would be able to inhabit Mars.

Material and Methods

1. Microorganism.

Escherichia coli is a Gram negative, facultative anaerobic, rod-shape bacterium. Most *E. coli* strains are harmless and they live in the gut, where they produce vitamins and prevent colonization of the intestine with pathogenic bacteria. It grows very easily and it is a prokaryotic model organism, used in biotechnology and microbiology. Under favorable conditions, it takes up to 20 minutes to reproduce.

2. Obtaining a Martian soil analogue.

To prepare the analog of Martian soil, the general composition was studied from the available information. Commercial volcanic material was acquired for use in gardening, which was analyzed. To see its resemblance to the Martian soil we went to the IACT (Andalusian Institute of Earth Sciences) to see if its mineralogical and geochemical composition was similar to those of Mars. There, our rocks were subjected to techniques used for their analysis; X-ray diffraction and fluorescence of X-rays.

3. Low pressure conditions.

We manufacture a vacuum chamber with a glass canister that has been connected to a tube that was coupled to a vacuum trap to extract as much air as possible. The tolerance to low pressure was tested putting 5 microliters of *E.coli* culture in a Petri dish inside the recipient. Another device was made. We have used a vacuum pump that we have connected to a desiccator and a vacuum gauge that allows us to measure the pressure throughout the circuit. Our pump is not very efficient, yet we manage to reduce the pressure within the system to one third of the atmospheric Earth pressure.

4. Chlorates tolerance.

Our first experiment was to test the growth of our bacteria on plates with increasing concentrations of potassium chlorate ($KClO_3$). A sufficient amount of this salt has been added to the culture medium to achieve final concentrations of 0.05M, 0.1M and 0.2M. And on these plates we have inoculated our bacteria and we have put it to incubate at a temperature of 30ºC.

Our second experiment is to assess the growth of bacteria in liquid media. For this, cultures have been prepared in liquid medium to which different amounts of potassium chlorate have been added until reaching the necessary concentrations. Bacterial growth was measured by spectrophotometry. The basis of this technique is that as the number of microorganisms in a liquid culture increases the turbidity of the same so by measuring the optical density of those can estimate the number of bacteria.

5. Temperature changes.

We have prepared tubes with 3 grams of our Martian soil analog that had been sterilized. They have been placed 200 microliters of culture of our microorganism *E.coli*. This tube underwent cycles of freezing and defrost at the Estación Experimental del Zaidín in which temperature alternated between 20ºC (room temperature) and -80ºC.

6. Tolerance to ultraviolet radiation.

The survival of our microorganism to ultraviolet radiation has been assessed. For this we have placed 100 microlitres of liquid culture on Petri dishes and we have allowed them to be absorbed. Then we have covered half a plate with aluminum foil and irradiated for one minute with a UV light lamp at 254 nm.

Results

1. Analogue of the martian soil.

Our first objective was to assess if Earth volcanic rocks resembled those of the red planet in composition. Our samples were analysed at the Andalusian Institute of Earth Sciences (IACT-CSIC). Our rocks were determined as tephrite/basanite. These are two types of volcanic rocks that are projected in the same field at the modal classification level, differing because the basaite has more than 10% of normative olivine. Both are formed when the volcanic magma cools and they are useful for our project since their composition is quite similar to that of rocks studied by rovers at the Gusev crater on Mars.

2. Growth at low pressure.

E. coli grew into the atmosphere at low pressure, less than with normal Earth pressure as expected. The pressure to which we have subjected it is not exact to that of Mars but it is useful to know that *E.coli* is capable of growing with low pressure. A pressure inside the vessel that is equivalent to 1/3 of the atmospheric pressure on Earth has been reached. Even so, the pressure on Mars is 30 times lower.

3. Chlorates tolerance.

Our bacterium has managed to grow at solutions of potassium chlorate ($KClO_3$) of 0.05M, 0.1M and 0.2M; both, un liquid medium and in solid medium placed in Petri dishes.

In liquid cultures, we can know how many bacteria there are in the medium measuring the optical density with a spectrophotometer. The values were: 0M = 1,535; 0,2M = 1,316; 0,5M = 0,785. These results are shown in the graphic.

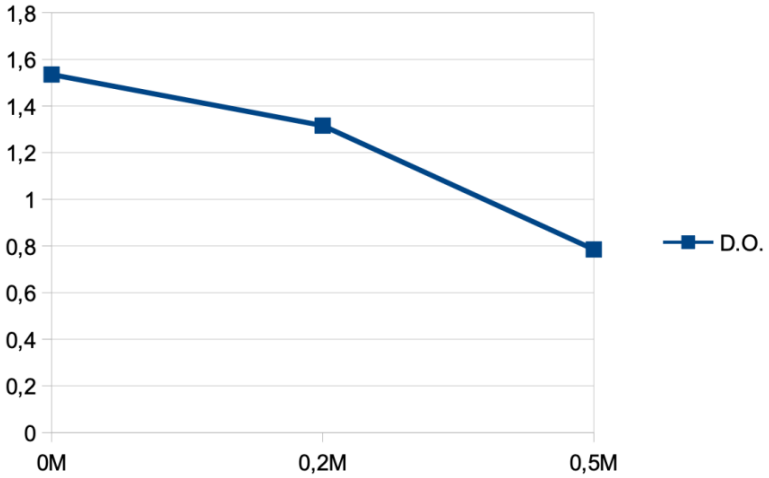

Figure 1. Survival of *E. coli* grown in the presence of different potassium chlorate concentrations

Obviously the more potassium chlorate there are fewer bacteria survival, but our bacteria are able to survive chlorates and on the surface of Mars.

4. Temperature changes.

E. coli has managed to survive temperature changes that oscillate between 20 degrees with freezing periods of -80 degrees. We have counted the colonies in the Petri dish. With the data we have calculated the percentage of bacteria that have survived and is:
1,500 (the bacteria that have survived) divided by 1,000,000,000 (the bacteria that were at the beginning) = a little more than 0%.

5. Tolerance to ultraviolet radiation

E.coli has also survived to exposure to ultraviolet radiation. The effect it has had has been little, as far as we can appreciate colonies in the plate in the part irradiated. In conclusion, our microorganism can resist dosis of uv radiation similar to that we have tested.

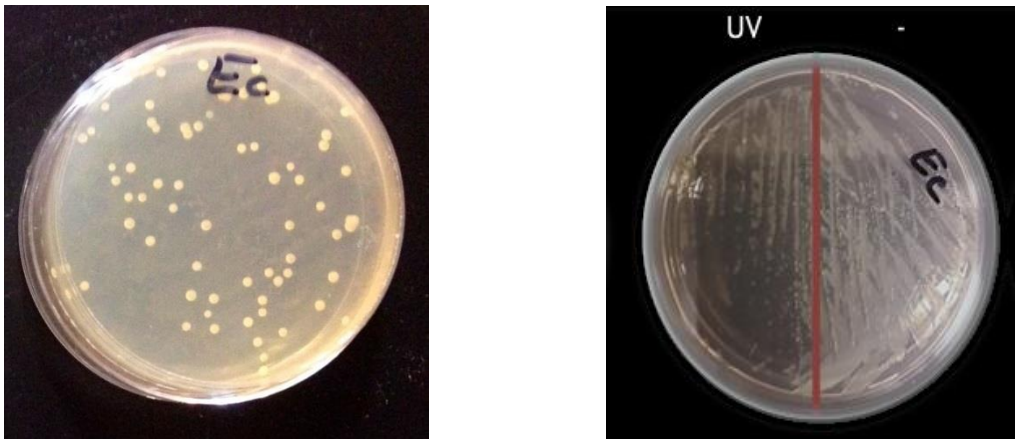

Figure 2. Survival of *E. coli* after temperature changes (left) and UV exposure (right)

Discussion

The results of our experiments conclude that *E. coli* can grow in conditions of low atmospheric pressure as low as one third of the terrestrial one. It can resist change of temperature from -80 ºC to 20 ºC. It can grow up to concentrations of 0,2 M potassium chlorate in the culture medium. And it survives when subjected to ultraviolet radiation on the surface. It survival will be higher in the soil as it stops uv radiation. However we have not studied the survival of *E. coli* to several conditions simultaneously.

Nevertheless, Mars could be a good candidate to host *E. coli* bacterium since it is capable of growing in similar soil, subjected to ultraviolet light in soil depth, is resistant to chlorates and perchlorates. The only thing that could not possibly hold out for sure is the temperature changes because more than 99% of E. coli died in the experiments. And we have not experienced with a pressure as low as that of Mars. More experiments are needed.

Acknowledgements

This work has helped us to learn to make scientific reports, more things about the life of the researchers and their scope of work. All this developes our skills at the high school. That is why we would like to thank our institute and the Zaidín Experimental Station for letting us be part of this project. Also Manuel Espinosa who has guided us throughout the project apart from showing us the facilities of EEZ together with Juan de Dios Alché. And especially to thank our teacher Antonio Quesada for guiding us and helping us throughout the course and for giving classes so pleasantly looking for new ways for us to learn, like this project.

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Bacillus subtilis in the Red Planet

Noemí Castillo, Julia Martínez, Naomi Vargas, Gema López, Candela del Castillo.

IES Zaidín Vergeles. Granada

Summary

Mars is a neighboring planet about which information is limited, and we could once need it to our survival. From this approach, we have created a martian soil analogous in our classroom, in which we have proved the survival of a bacterium: *Bacillus subtilis*. We have submitted it to the environmental conditions of Mars: extreme temperature cicles, ultraviolet radiation, low atmospheric pressure and coexistence with potassium chlorate. The results revealed us a difficult - but not impossible- survival for our bacteria, which could be determined with the prolongation of our experimentation.

KEYWORDS: Bacteria, *Bacillus subtilis*, Mars, martian soil, analogous, survival, extreme temperature, terraforming.

Introduction

One of the great characteristics that human beings have, is curiosity; the curiosity about that we don't know. And martian terrain characteristics and our compatibility with it, is one of those unknown topics.

That is the greatest motivation why we are going to simulate Mars in our classroom using a microorganism, *Bacillus subtilis*, to prove its survival as a living being in the conditions of the red planet.

As we all know, our planet resources are in extreme danger and it would disappear if we do not take environmental precautions. So this could be too the beginning of a much bigger project: the terraforming of the red planet for our own survival.

Material and methods

Bacillus subtilis is a rod shaped, Gram positive bacterium, found in soil and in the gastrointestinal tract of ruminants and humans. It is a facultative anaerobe. It can form an endospore, a structure that allows it to survive in extreme environmental conditions as temperature and desiccation. It is considered the best studied Gram positive bacterium and a model organism.

We have grown microorganisms in LB solid and liquid culture medium.

We have prepared a soil from volcanic rocks to simulate that of Mars. We have simulated Mars low atmospheric pressure with a desiccator connected to a vacuum pump. Tolerance to chlorates has been tested both in solid and liquid medium adding potassium chlorate to LB medium to get the required concentration. Tolerance to extreme Mars temperatures has been tested alternating the microorganisms to temperatures from -80°C to room temperature. Survival to ultraviolet radiation has been studied exposing cultures to a UV lamp (254 nm) for a minute.

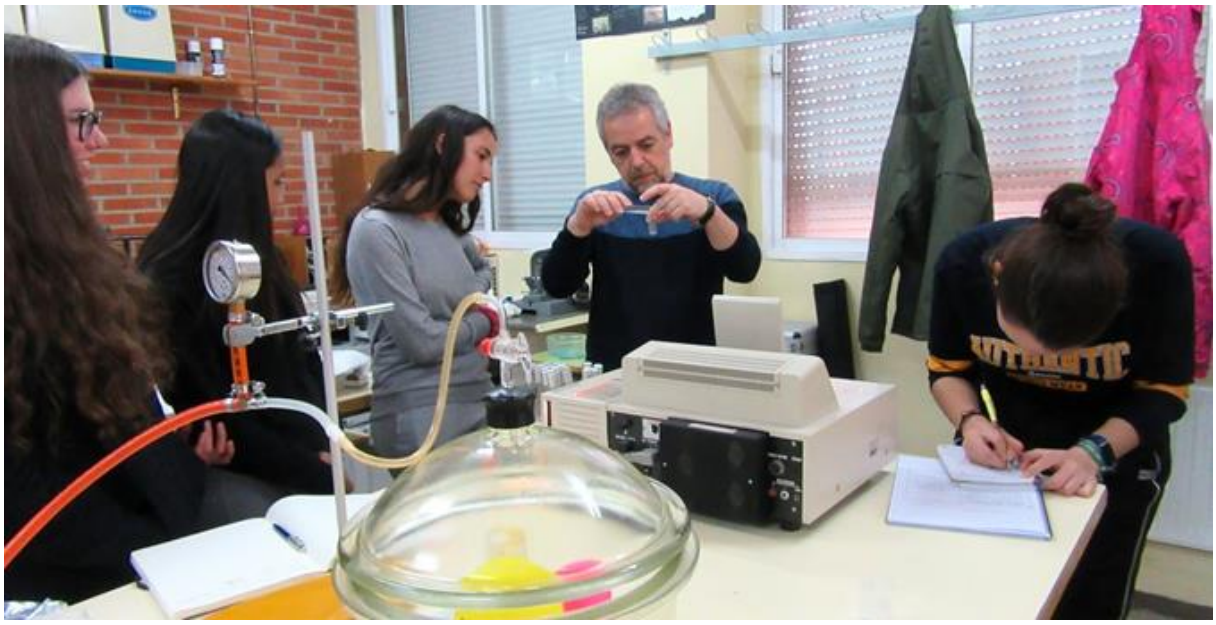

Figure 1. Some of the instruments used in this research

Results

In the first experiments we conducted, we tested the strength and survival of our bacterium, *Bacillus subtilis*, in a simulation of what would be a Martian atmosphere, through vacuum techniques and exposure to extreme temperature changes. What we were able to observe was growth on the plates, indicating an adequate adaptation and development in these media. These were the first indications of a possible development of our bacterium on Mars.

The next experiment was the exposure of several bacteria to different concentrations of potassium chlorate. In all of them we were able to observe a noticeable decrease in their rate of growth, since these are not used to exposure to these salts in Earth environments. One of the best adapted was ours, its development against the three concentrations of chlorate are quite similar, which can give us a slight idea of the chance of survival they have on a Martian surface.

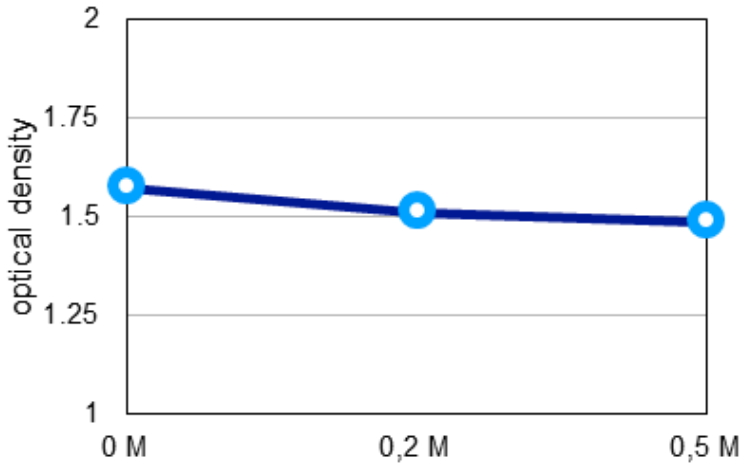

Figure 2. Survival of *B. subtilis* at different potassium chlorate concentrations

After a month of temperature changes in the environment of the bacteria, we detect a great resistance to drastic temperature changes and it reproduction was not disrupted, which is very important on Mars. It experimented with 20°C what the day would mean on Mars and -80°C what is estimated of a night on Mars. These characteristics make Bacillus subtilis a good candidate for living on Mars.

Two months later, new samples were taken for further temperature experiments, but in the process we had a problem: the Petri dish turned from whitish color to a yellowish tone, we contaminated the soil or the culture medium.

We subjected *B. subtilis* to ultraviolet radiation, because exposure in the red planet is much higher than here, on Earth. This radiation causes the near-total extermination of bacterial colonies, and this would really hurt the ability of him to survive on Mars.

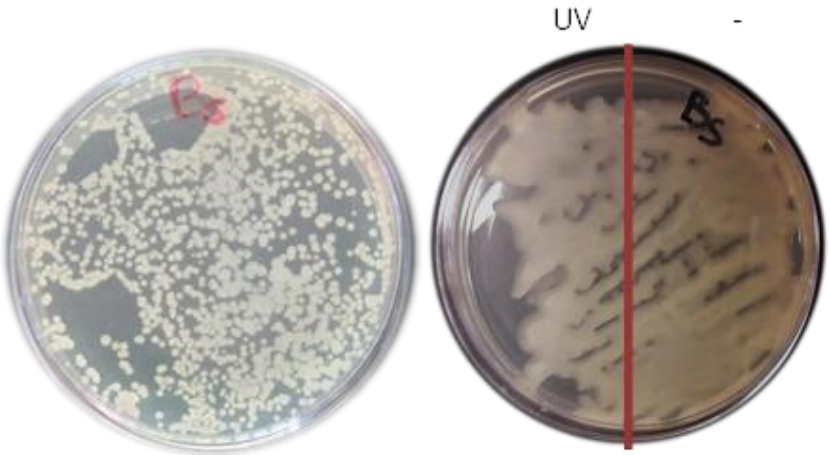

Figure 3. Survival of *B. subtilis* after several rounds of temperature changes (left) and after irraditating with UV light one side of the plate (right).

Discussion

In the first experiment, where we proved the resistance of *Bacillus subtilis* in a simulation on Mars atmosphere, the bacteria survived. It means that *B. subtilis* has the capacity to adapt to other atmospheric pressures.

At the same time in this experiment, we exposed the bacteria to hot and cold temperature cicles. The contrast of Mars' nocturne and diurnal temperature is very high, so that's an important point to the survival of the bacteria there.

In the experiment where we exposed the bacteria to potassium chlorate the results were catastrophic for most bacteria: the number of colonies declined considerably. However, *Bacillus subtilis* showed the highest survival rate, so there is a rare possibility that it could survive on Mars.

In the experiment were we exposed the bacteria to ultraviolet radiation the amount of bacteria decreased a lot. So we conclude that it can't adapt to this condition. Mars' radiation is an important fact, so this could this could be an inconvenient for life there.

In the first experiments that we carried out the results were positive: *B. subtilis* was resistant enough. The experiment with the rest showed that the number of bacteria went down as time goes by. That means that bacteria in Mars would die in a short period of time.

Our results demonstrate that, considering all the aspects of Mars' environment, it would be really difficult that our bacteria survived there. This isn't a good news because the base of our experiment was to consolidate its survival in that extreme conditions.

Acknowledgements

The achievement of this investigation has been posible thanks to, first at all, our teacher of natural sciences: Antonio Quesada Ramos, belonging to the Institute Zaidín Vergeles, across whom we have gained access to Manuel Espinosa, investigator of EEZ, who has guided and supervised the project on a voluntary basis.

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Growing plants on Mars: not so easy

Antonio Quesada Ramos[1], *Carmelo Ruiz*[2], *Manuel Espinosa-Urgel*[2], *José Manuel Palma*[2]

[1]IES Zaidín Vergeles. Granada; [2]Estación Experimental del Zaidín, CSIC. Granada

Summary

Future manned missions to Mars or the colonization of the red planet require systems to provide food, oxygen and water for human beings. Plants can produce them in case they could be cultivated in Mars. We have carried out a project with High School Students to assess the capacity of terrestrial plants to grow in a soil with nearly similar features to that of Mars. We have prepared that simulant soil from volcanic scoria, with a chemical and mineralogical composition similar to soils studied by rovers. Organic matter and microorganisms were eliminated. *Arabidopsis thaliana* and pepper (*Capsicum annuum)* seeds were cultivated in this soil. Plants showed a lower development when they were cultivated in our simulant soil and irrigated only with distilled water. The addition of a nutrient solution had inconsistent effects on our plants; while the growth of pepper was improved, Arabidopsis plants had a decline in the development and, eventually, they died. Growing plants successfully in Mars requires to study both nutritional requirements and tolerance to potential toxic substances.

KEYWORDS: Mars, soil analogue, volcanic scoria, *Arabidopsis thaliana*, *Capsicum annuum*, *Pseudomonas putida*, *Pseudomonas stutzeri*.

Introduction

Mars is an astrobiological target not only by its similarity or proximity to our planet, but because of the fact that billions years ago Mars had liquid water and an environment similar to that of the Earth in which life appeared. Nevertheless, at present life is almost impossible to exist in the red planet. The scarcity of liquid water, extreme low temperatures, a low pressure atmosphere with high levels of carbon dioxide, a gravity that is one third of that in the Earth, the high levels of radiation on the surface, and a lack of organic nutrients are factors that make very difficult to survive in Mars.

Because of this interest, manned mission to Mars and, furthermore, the hypothetical colonization of the red planet require systems to provide mainly oxygen, water and food for the metabolic needs of humans beings,. On the Earth, these functions are basically facilitated by plants; either through CO2 absorption and O2 emission, water purification through transpiration, waste product recycling via mineral nutrition or as a food source, plants key an important role in this context (Monje *et al*., 2003; Wolff *et al*., 2014).

Future human missions to Mars generate a special interest in the study of the response of plants to its extreme conditions. Accordingly, the investigation of the plant growth in

conditions similar to those of Mars could indicate the feasibility of cultivating them in that planet. Wamelink *et al*. (2014) have investigated the possibility of growing plants in a Mars simulant soil. They propose that all essential minerals for the growth of plants appear to be present in sufficient quantities with the exception of nitrogen in reactive forms (NO3, NH4). In fact, in their experiments, several species of plants were able to germinate and complete their life cycle for a period of 50 days without the addition of nutrients.

The main objective of this research was to assess the capacity of terrestrial plants for growing in a soil as similar as possible to that of Mars. In our laboratory we have studied the growth of *Arabidopsis thaliana* and pepper (*Capsicum annuum*) in a Mars simulant soil. We have prepared the analogue from volcanic scoria, with a mineralogical and chemical composition similar to that of some soils of Mars. Organic matter has been eliminated by washing the rocks in running water. As there are no known life forms in martian soil, pots were sterilized to eliminate microorganisms.

Material and methods

Mars soil simulant

Material similar to the Martian regolith was prepared from volcanic scoria adquired from a commercial brand used in gardening ("Greda volcánica", Batllé). Chemical and mineralogical composition of these rocks were analysed by X-ray fluorescence and X-ray diffraction at the Instituto Andaluz de Ciencias de la Tierra (CSIC, Granada, Spain). The results showed that our samples had similar characteristics to some soils studied in Mars by rovers (Castillo Tejada et al, 2019). The chemical composition of the soil can be consulted in that reference in this same volume.

To prepare the soil for plant culture and growing, rocks were crushed with an iron mortar and washed with tap water at least for two hours and then dried in an oven at 80ºC. Pots with 750 g of the so obtained soils containing volcanic fragments of different sizes (from fine powder to about 0.125 mm3) were prepared. Then, they were sterilized by autoclaving (two cycles, 121ºC, 15 minutes each).

Plant species

We have studied germination and growth of two plant species in our Mars soil simulant under different conditions.

Arabidopsis thaliana, cv. Columbia is a small flowering plant used as a model organism in plant biology and it has been used by several authors in experiments on Astrobiology (Richards et al., 2006; Wolff et al. 2014). It has not agronomic significance (it is rather a weed) but it offers advantages for basic research in genetics and molecular biology. It has a rapid life cycle: about six-eight weeks from germination to mature seeds. A. thaliana produces very small seeds; it provided an advantage for our research as its internal nutrient pool is quickly metabolized and the plant becomes totally dependent of light and whatever

is available in the soil. Columbia ecotype has been used for these experiments since is the one which is commonly reported as wild type for in most laboratories.

The second plant used has important agricultural and nutritional values. Pepper (*Capsicum annuum* L. cv. Padrón) receives its name from the municipality of Padrón (A Coruña) where they are widely cultivated since the XVII century. These are small peppers, about 5 cm long, with elongated conic shape and a colour ranging from bright green to yellowish green while they are still unripe, which is the common consumed appearance. Peppers are not only important as a food, but the fruits are also an excellent source of health-related compounds, such as ascorbic acid (vitamin C), carotenoids (pro-vitamin A), and other antioxidants such as tocopherols, flavonoids and capsaicinoids (Wahyuni et al.2013). Peppers seeds were surface-sterilized with NaClO at 5% (w/v) for five minutes, and then rinsed several times with deionized water.

Figure 1 shows a comparison between seeds from *A. thaliana* and pepper, variety Padrón.

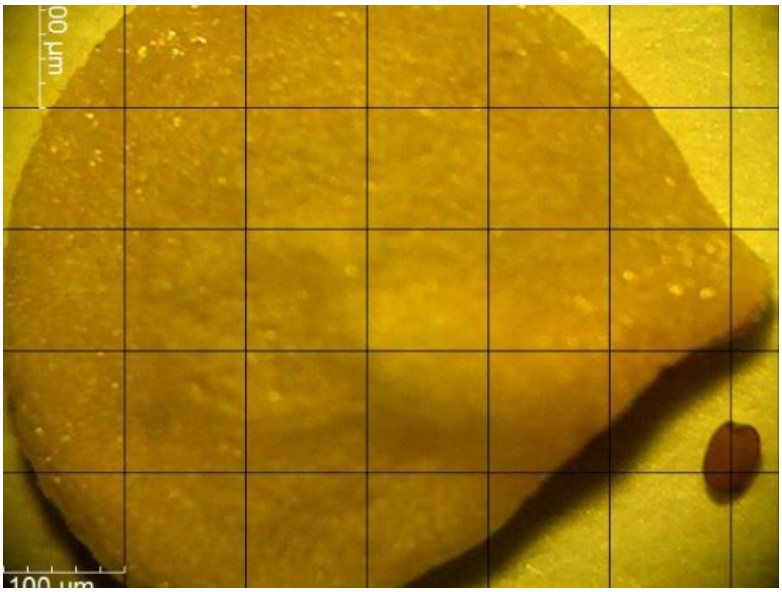

Figure 1. Comparative size of *Capsicum annum* and *Arabidopsis thaliana* (lower right corner) seeds observed through a stereomicroscope. The scale is shown on the lower left corner.

Microorganisms

In Mars, it has been proposed the presence in the soil of toxic substances, like heavy metals and metalloids, that could prevent plants from thriving. Furthermore, Martian regolith is poor in assimilable nitrogen like nitrates or ammonia and so volcanic soils are. In order to improve the conditions of our Mars-simulating soil to grow plants, it has been considered the addition of microorganisms to our samples. For that purpose, species of the genus *Pseudomonas* are specially interesting as they are able to metabolize toxic compounds from soils, and even to fix dinitrogen.

Pseudomonas putida is a Gram negative, saprotrophic soil bacterium. It has a diversified metabolism which allows it to degrade organic compounds and it also has a great capacity to tolerate heavy metals and metalloids (Canovas et al., 2003). In fact, it has been used in bioremediation as this microorganism is able to degrade environmental pollutans.

Pseudomonas stutzeri is a nonfluorescent denitrifying bacterium widely distributed in the environment. It has been proposed as a model organism for denitrification studies. Some strains are able to fix dinitrogen, and others participate in the degradation of pollutans or interact with toxic metals (Lalucat et al., 2006).

The tolerance of *P. putida* and *P. stutzeri* to environmental conditions similar to those of Mars have been previously studied at our laboratory (Castillo-Tejada *et al.*, 2019; Delgado-Alaminos *et al*., 2019).

Experimental design

The main objective of this research was to assess the capacity of *Arabidopsis thaliana* and *Capsicum annuum* to grow in a sterile soil similar to that of Mars and to study simultaneous treatments that could improve the thriving of plants. For each plant species, we applied four treatments (T1-T4) and prepared four pots with the same quantity (750 g) of Mars soil simulant. In the case of *Arabidopsis*, nine groups of non sterilized seeds (2-3 each group) were planted in every pot. Six sterilized seeds of *Capsicum* were planted in every pot.

- Treatment **T1**: Seeds were deposited directly over the soil and pots were only irrigated with distilled water to prevent the potential interferences with nutrients that might be present in the water; in this case, plants only had available for their growth the mains contained in the Mars soil simulant.

- Treatment **T2**: Seeds were deposited directly over the soil and and pots were watered with a nutrient solution. We used the modified Hewitt nutrient solution described in Tortosa et al. (2018), diluted 1:2 with distilled water.

- Treatment **T3**: Previous to planting, seed were inoculated with two Pseudomonas species. 100 ml of a liquid culture of bacteria containing either *P. putida* or *P. stutzeri* were added to seeds from both plant species. Some of them were treated with. Plants were only irrigated with distilled water as in T1.

- Treatment **T4**: Previous to planting, seed were inoculated as in T3 and then supplied with the nutrient solution as in T2.

After sowing, pots were covered with aluminum foil during three days for A. thaliana, and for five days in case of C. annuum seeds. Then, the cultures were maintained for 15 weeks and pictures were taken after 1, 4, and 8 weeks and at the end of the experiment. Plants were illuminated for 16 hours every day with compact fluorescent lamps: 2x 8W, 2700K lamps (Phillips) and 2x 20W 6400K lamps (ROHS). Plants were watered as corresponding to each treatment three times a week. At the end of the experiment, the presence of microorganisms in the soil where inoculated seeds were planted was assessed. For this, 50 µl were taken from the pots and spread in Petri dishes with TSA (tripticase soy agar) medium. Plates were incubated at 28ºC for two days.

Results

Almost all the seeds of *A. thaliana* and *C. annuum* germinated during the first week. However, the germination and growth response to the analogue of the Martian soil was different in both plant species. Figure 2 shows the evolution of *A. thaliana* plants throughout fifteen weeks in the four treatments (T1-T4). Although *Arabidopsis* has a rapid life cycle that takes about six-eight weeks from germination to mature seeds, growth of the plants in this assay was very slow. Under the best conditions (T1 and T3), the plant height was about 1 cm and plants did not reach the flowering stage.

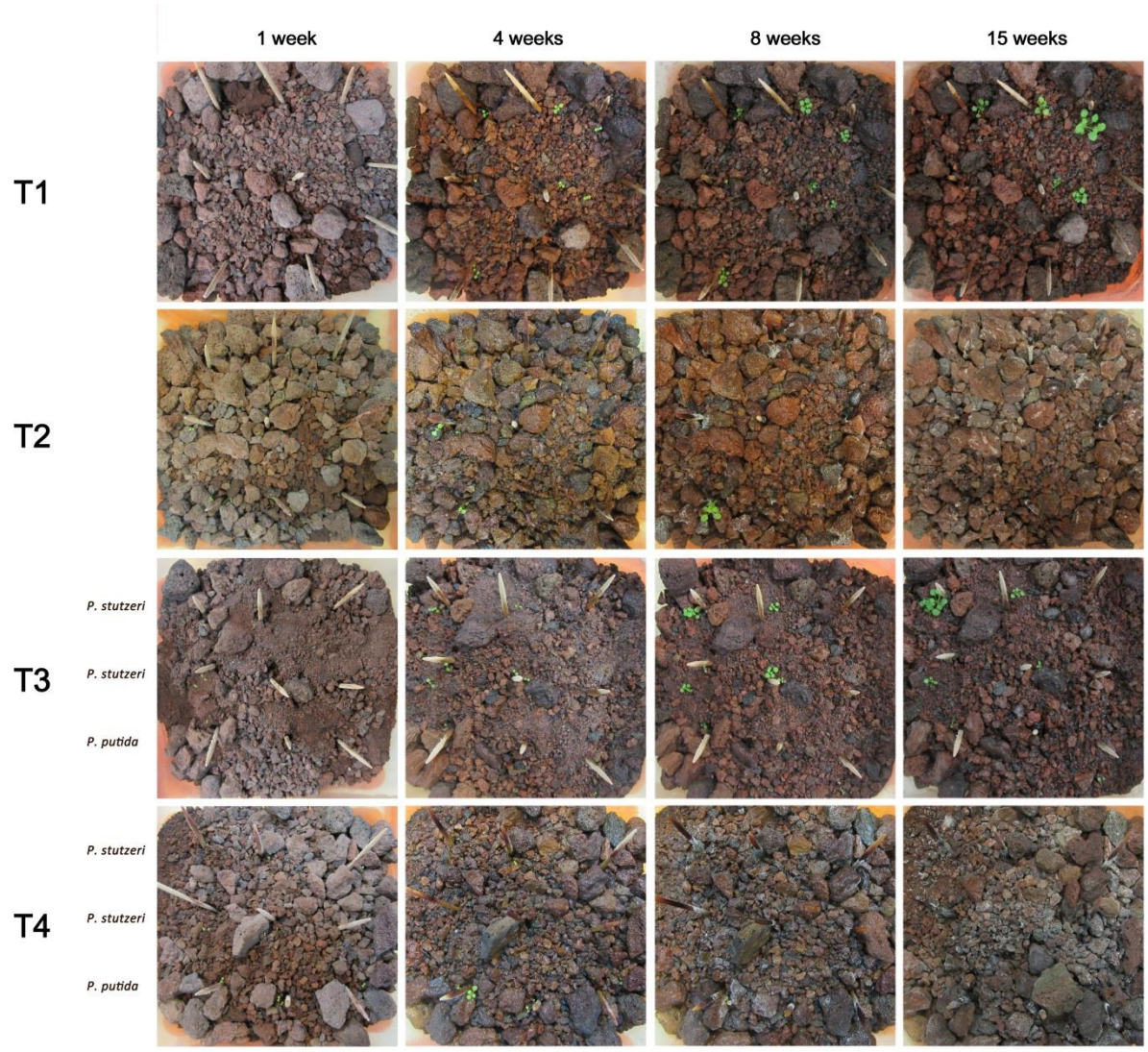

Figure 2. Growth of *Arabidopsis thaliana* in a Mars soil simulant under different treatments (T1-T4).

Differences in the response of *A. thaliana* to different treatments were observed. Thus, it was found that plants irrigated only with distilled water had a higher percentage of survival. On the contrary, all the plants supplied with nutrient solution were not able to grow until the end of the experiment. The addition of microorganisms did not have any obvious effect either in the growth or the survival of plants (Figure 3).

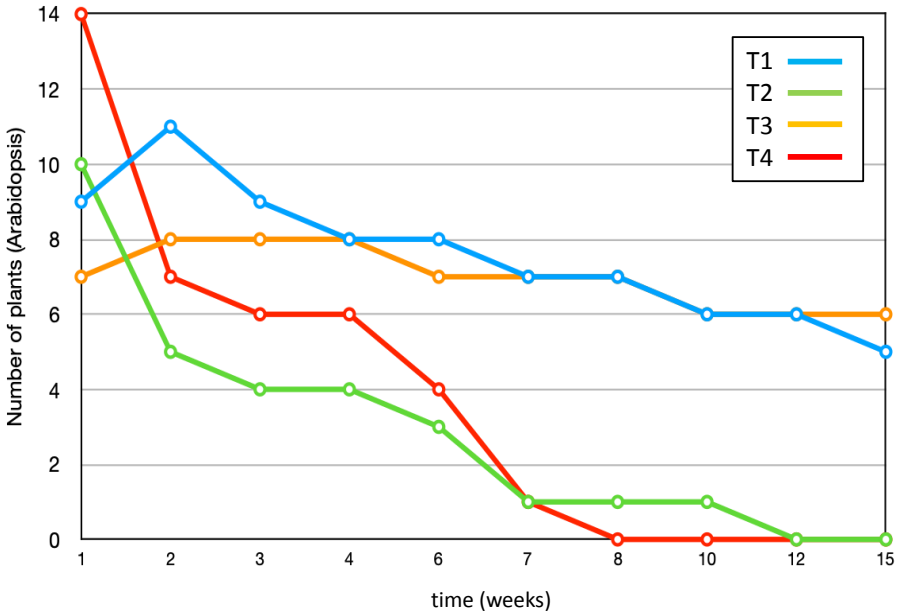

Figure 3. Survival of *A. thaliana* plants in a Martian-like soil under different treatments (T1-T4).

The presence of microorganisms in the soil simulant was monitored at the end of the experiment. Figure 4 shows colonies grown after liquid samples taken from the soil of cultivation were spread onto TSA Petri dishes. There was bacterial growth in all the samples, the nutrient solution showing the higher proliferation of microorganisms. Although bacteria could not be totally identified, some colonies of *P. putida* were recognizable by the presence of pioverdine in the dishes.

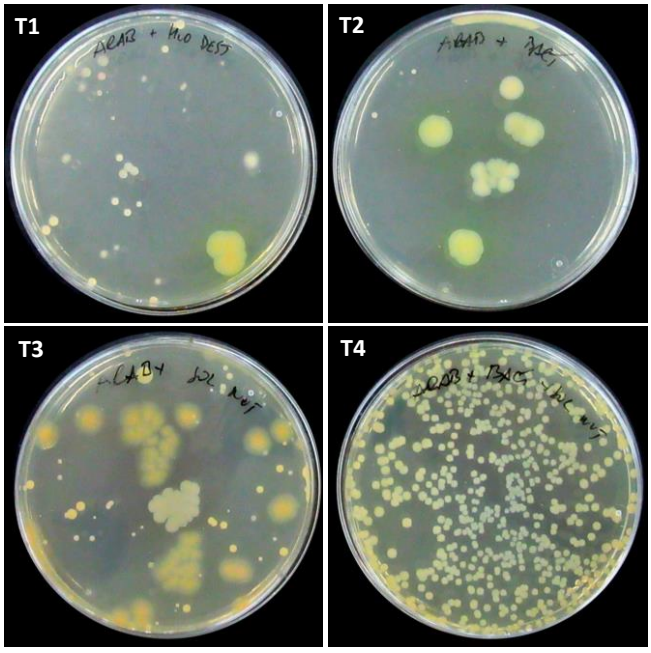

Figure 4. Bacterial growth after incubation of liquid samples obtained from soils of treatments T1-T4 appplied to *Arabidopsis thaliana*.

On the other hand, almost all pepper seeds germinated. Figure 5 shows the evolution of pepper plants in the martian soil simulant. Plants experienced a development decline when they were irrigated only with distilled water. From six plants germinated in every pot only two developed real leaves after 15 weeks, both in pots with and without microorganisms.

It was also observed that nutrient solutions favoured the plants growth, but bacteria did not have a great influence on the growth of pepper plants.

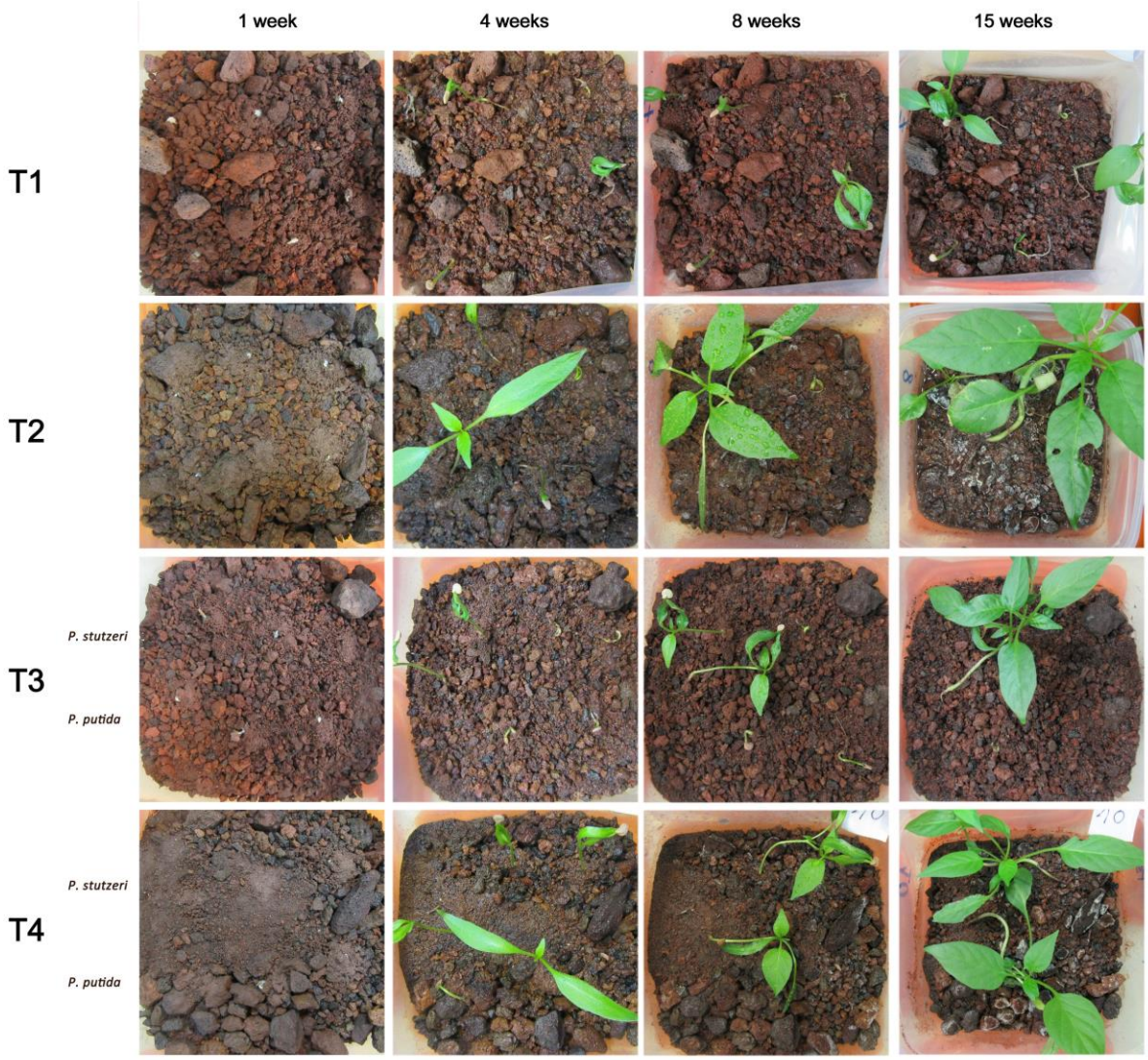

Figure 5. Growth of *C. annuum* plants in a Mars soil simulant under different treatments (T1-T4).

As with *A. thaliana*, there were also differences between the concentration of microorganisms in the soil where pepper plants grew after fifteen weeks of cultivation (Figure 6). The higher number of colonies was observed in dishes inoculated with soil water from pots treated with microorganisms.

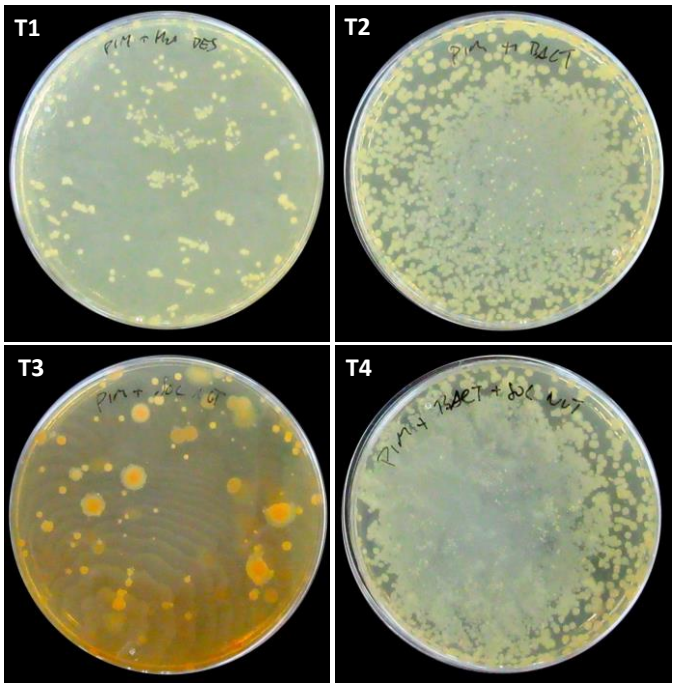

Figure 6. Bacterial growth after incubation of liquid samples obtained from soils of treatments T1-T4 appplied to *C. annuum*.

Discussion

Environmental conditions are harsh for life in Mars. An atmosphere with low pressure and high concentration of carbon dioxide, low temperatures, high levels of radiation, scarcity of liquid water and a soil that lacks of organic material and contains toxic compounds like perchlorates and iron oxides seems to draw an impossible scenario for growing plants in the red planet.

In this research we have studied the potential effect of the martian regolith on the germination and development of two plant species: *Arabidopsis thaliana* and *Capsicum annuum*. We have simulated a martian soil from volcanic scoria and have eliminated its organic matter and microorganisms. Chemical composition of soil is similar to that indicated by analyses made in Mars; also as in Mars, our soil lacks assimilable forms of nitrogen but has certain levels of iron oxides.

Our experiments showed that our soil simulant, and probably the soil of Mars, are perhaps inappropriate for growing plants. We have observed a lack of development in plants in that soil only irrigated with distilled water. Our soil lacks essential nutrients for plants like nitrogen and has elements like iron or aluminum oxides which in high concentrations might be toxic and probably inhibited plant growth. These results disagree with those described by Wamelink *et al*. (2014) who reported that plants were able to germinate, grow and flower in a Mars regolith simulant handled by the NASA. The lack of nutrients probably causes the observed lack of development both in *A. thaliana* and *C. annuum*. However, the addition of the same nutrients to the pots through the supplementation of nutrient solution showed inconsistent results. *Arabidopsis* plants died in those cases, while peppers improved their

development. Probably some compound of the nutrient solution is toxic for Arabidopsis per se or combined with some chemical present in the soil.

Microorganisms and organic matter are essential compound of soils. We have not observed an improvement in the development of plants grown in soil added with both species of Pseudomonas. However, we have noticed the presence of fungi on pots treated with nutrient solution, revealing the presence of organic matter, probably with a positive effect on pepper plants.

From our research we can conclude that it is not easy to cultivate plants neither in a martian soil analogue nor in Mars. Every plant has specific nutritional requirements and it is necessary to study them as well as their tolerance to possible toxic substances present in those soils in order to be successful in a future hypothetical colonization of the Red Planet.

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