Biocompatibility of the Simulated Martian Environment for Terrestrial Organisms

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Abstract

Biocompatibility of the Martian environment for Terrestrial organisms is of particular interest for Astrobiology and space exploration, and is being studied in recent publications and space missions.

In that sense, we propose to study adaptation and survival strategies of microorganisms in a simulated Martian environment. This will imply the study of the ability of bacteria to produce and maintain spores and their mechanisms of adaptation to those unfavorable environments. This experiment could allow the collection of precious information for biocompatibility of the Martian environment for Terrestrial organisms, and figuring out the probabilities and limitations for life to be distributed among planets of Solar System.

1 Introduction

This report presents the experimental plan of studies to be conducted at Mars Desert Research Station (MDRS) on the ability of bacteria to produce and maintain spores in simulated Martian environment.

Mars, today, lacks a substantial atmosphere or global dipolar magnetic field and so receives negligible protection from cosmic rays, especially solar UV radiation [3]. Certain bacteria with the ability to produce dormant stages, such as the Bacillus endospores, are capable of withstanding most of the parameters of the Martian environment and of surviving very long periods of time [4]. Because of their high resistance against different environmental extremes, Bacillus subtilis spores are a biological model system which has been studied in several space experiments.

The survivability of bacterial spores of Bacillus subtilis will be investigated after exposure to the MDRS station external environment, to simulate different subsets of the Martian environment (temperature variations, solar UV radiations, shielding by protecting materials rocks).

The interference of Martian soil components and the intense and nearly unfiltered Martian solar UV radiation with spores of B. subtilis will be tested. Different types of Mars soil analogues will be used to determine on one hand their potential toxicity alone or in combination with solar UV (phototoxicity) and on the other hand their UV protection capability. Two sets of samples will be placed outside of the MDRS station to simulate the UV radiation climate of Mars and Earth.

After exposure of samples outside of the MDRS station for 10 days, spores will be recovered and their survival will be analyzed at the MDRS by Colony Forming Unit (CFU) counting, together with parallel samples from the corresponding ground control experiment performed in the laboratory.

2 Cultivation and sporulation

Spores are obtained by cultivation under vigorous aeration in liquid Schaeffer sporulation medium [5], and purified stored [2, 6].

Spores are obtained by cultivation under vigorous aeration in Schaeffer sporulation medium containing (per litre) 16.0 g Difco nutrient broth, 2.0 g KCl, and 0.7 g MgSO4 7H2O for 4 days at 37°C until a sporulation frequency >90% is reached as judged by phase-contrast microscopy. Sporulated cultures are harvested by centrifugation (10000 g, 20 minutes, 4°C) and treated with MgSO4 (2.5 mg/ml), lysozyme (200 mg/ml), and DNAse I (2 mg/ml) for 30 minutes at 37°C to destroy the residual vegetative cells. The enzymes are inactivated by heating for 10 minutes at 80°C.

After repeated centrifugation and washing in distilled water, the purified spores (approximately 1010

spores/ml) are stored in aqueous suspension at 4°C.

3 Soil sterilization, grinding and mixing

B. subtilis spores are mixed with a powder made of 3 samples of different soils (approximately 500mg of powder) surrounding the MDRS station [4].

The mixtures (about 5*107 spores per sample) are exposed to 3 different places surrounding MDRS station, in quartz absorption cuvettes covered with UV transparent film and exposed for 10 days.

In parallel, the corresponding sample control is made of powder and spores mixtures maintained at 37°C for 10 days at MDRS station.

4 Recovery

To recover spores after exposure, samples are transferred into glass vials: 1mL of sterile phosphate buffer solution (PBS) is added to each sample, and the sample tubes are vortexed for 1min to dislodge cells into suspension in the fluid [3].

5 CFU counting

Serial 10-fold dilutions are plated on solid nutrient broth medium. From these dilutions, 50 μL are pipetted and spread on nutrient agar plates. After overnight growth at 37°C the number of colony-forming units (CFU) is counted [4].

The surviving fraction of the spores is determined from the quotient:

$$\frac{N}{N_0}$$

where N is the number of CFU of the sample and N_0 that of corresponding controls.

6 Conclusions and perspectives

The chemical composition and the low pressure of the Martian atmosphere result in an UV radiation climate which is quite different from that of the Earth, and combined with low temperature on surface, make Martian environment unfavorable for life. However, studies showed Martian conditions fit with environmental range allowing growth or survival of microorganisms [1].

The present study tend to (i) built a model for studying the survival of Terrestrial bacteria in Mars environments and to (ii) confirm previous studies in simulated Martian environments, where solar UV radiation showed deleterious effects on bacteria, but where thin layers of clay, rock or meteorite material were shown to be successful in UV-shielding, if they are in direct contact with the spores [4, 6].

7 Material

- *Bacillus subtilis* subsp. *Subtilis*
- NB agar
- Difco nutrient broth
- KCL
- MgSO4
- Lysosyme
- Cuvette quartz
- AeraSeal^{TM} film
- Balance
- Incubator
- Autoclave
- Petri boxes, faclons tubes, 200μL pipettes Shaker

About the author

Frédéric Peyrusson will assume the role of biologist and medic of the MDRS crew. After a master degree of Health Engineering and a master degree of Pharmaceutical Sciences, Frédéric started his PhD in 2013, in Bacteriology, at Université catholique de Louvain, Belgium. His topic deals with persistence of Staphylococcus aureus, especially the ability and survival strategies of the bacteria to survive in particularly unfavorable environments.

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