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LISA: Mars and the limits of life

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Abstract. We describe the results of the first tests made on LISA, a simulator of planetary environments designed and built in Padua, dedicated to the study of the limit of bacterial life on the planet Mars. Tests on the cryogenic circuit, on the UV illumination and on bacterial coltures at room temperature that shall be used as references are described.

Key words. Astrobiology – Planets and satellites: individual: Mars – Methods: laboratory – Instrumentation: miscellaneous

1. Introduction

During 2004 and 2005, financed by the university of Padua, we designed and built a simulator of planetary environments (LISA=Laboratorio Italiano di Simulazione Ambienti). This is the first, and at present the only, environmental simulator of its kind in Italy. Its versatility makes it able to reproduce a wide range of controlled and stable situations, from "warm" (human body or terrestrial conditions) to "cold" (from Antarctica to Mars) with six different samples that may be exposed to the same environment simultaneously. A full description of the simulator has been presented by Galletta et al. (2006).

The simulator is currently used as a research tool for Astrobiology or Planetary Geology in extreme conditions of temperature, pressure and atmosphere, not achievable in other ways in terrestrial laboratories. In particular, we want to reproduce the Martian environments, with a group of biologists interested in the study of bacterial metabolism in harsh environments.

The planet Mars is a privileged place in the Solar System on which to search for lifeforms, past or present. In fact, for several years it has been known that Mars had water on its surface(Nyquist et al. 2001; Head et al. 1999). In 1976 the Viking 1 and 2 probes landed on Mars and performed experiments aimed at the detection of life. They produced data whose interpretation has been doubtful or ambiguous (see Burgess 1978). Subsequently, the analysis of

Send offprint requests to: G. Galletta meteorites such as ALH 84001 (Mc Kay et al. 1996) with possible traces of fossil bacteria, have reopened the debate.

While waiting for new experiments, it is necessary to understand the possibilities of finding life in the present Martian conditions. The surface is not protected like that of the Earth by an atmosphere with an ozone layer: the ground pressure is 6 mbar and the daily UV flux is 361 kJ/m^2, compared to 1000 mbar and 39 kJ/m^2 on the Earth (Cockell et al. 2000). The consequent lack of greenhouse effect produces a mean temperature between -70 and -10°C at a latitude of 20° north (the landing site of Viking 1 and Pathfinder) depending on the Sun's elevation above the horizon. From radiometric data of the probes orbiting around Mars, the temperature can locally reach 27°C while at the polar caps in the winter can fall to -143°C. These cold temperatures and these excursions are impossible on the Earth and must be simulated in the laboratory.

If life was born on Mars billions of years ago, it may have found suitable conditions beneath the surface, where temperature and pressure increase with depth. A stable environment may exist under the duricrust or the dust cover, with annual thermal stability (< 10°C) at a depth of 10 cm, with temperatures between -70 e -40°C and shielded from UV rays. Water may be liquid at depths betweeen 150 m (0,7 atm) and 8 km (600 atm), according to the different models of the Martian surface. Terrestrial lifeforms exist (barophiles) that are able to live at those pressures. Occasionally, the arrival on the surface of some bacteria can determine the extinction or mutations of more UV resistant species.

Before reaching the red planet, we may explore more economically what kind of lifeforms may live in such an extreme environment. LISA satisfies all these conditions because it is a relatively easy way to study what happens in the environmental conditions on the planet Mars. In doing the biological experiments, we must take into account some caveats: 1) life on a planet different from that on Earth may use a completely different combination of nucleic acids and amino acids with respect to the first terrestrial lifeforms; 2) we don't have (yet) either Martian bacteria or Martian soils for the test. We use terrestrial surrogates!

2. Tests of experimental conditions

The sample to be studied (ground, chemical substances or bacterial cultures) is deposited in containers similar to Petri dishes, but specifically produced in aluminium by the mechanical laboratories of the Asiago Astronomical Observatory, that can be cooled or warmed by contact. In order to guarantee experimental sterility, the culture containers are hermetically closed in steel vessels (reaction cells) of about 250 cc. These vessels are connected with the exterior via a pipe equipped with mechanical filters of suitable porosity (based on the dimensions of the biological samples used). These filters allow the passage of gases through the pumps but prevent the spread of the microorganisms or particles in the cryostat chamber.

The vessels containing the biological samples are cooled by contact with a large aluminium dish that is at the top of a small reservoir of about 2000 cc, filled from a larger reservoir of Ranger Air Liquide at a pressure of 2 bar. A PT100 temperature sensor connected with an electric valve opens or close the liquid nitrogen flux of the Ranger reservoir. If needed, an electric resistance between the dish and the reservoir is open, in a feedback circuit that keeps the vessels temperature fixed within ±0.5°.

The thermal efficiency of LISA was studied by cooling the simulation chamber with a large flux of liquid nitrogen and measuring the inner temperatures of the chamber and of the vessels at different points. We measured with several PT100 sensors the temperature of the reservoir, the dish and the vessels, at the onset and the end of the flux of liquid nitrogen, calculating how fast the temperature changes in the different parts of LISA. The results are shown in Figure 1. The goal temperature selected was -80 °C, maintained by the feedback circuit(valve+electric resistance). The target temperature has been reached by the floor of the vessels in ~80 min, starting from a room temperature of 25 °C. The cal-

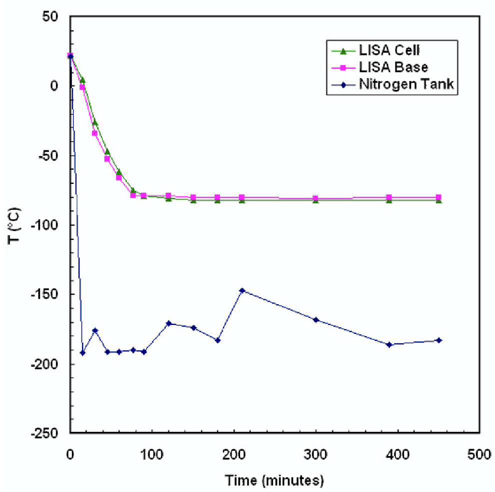

Fig. 1. Decrease of Temperature in the different portions of the Simulator

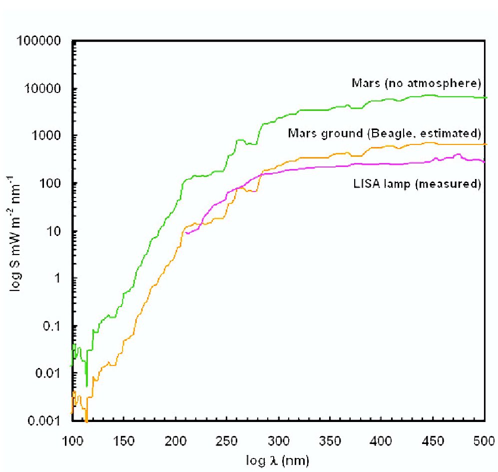

Fig. 2. Flux of LISA lamp compared with that of Mars (outside the atmosphere and estimated on the ground).

culated cooling rate of the dish and vessels is not linear and is approximated by a gradient of -0.78±0.06 °/min. When swithched off, the system (dish plus vessels) keeps its temperature within 1°C for about 20 minutes, starting to increase thereafter.

In the upper part of the vessels quartz windows are located, allowing the passage of radiation, including UV rays longer than 160 nm.

Table 1. Martian and LISA atmosphere

Gas	Mars	LISA
CO_2	95.32%	95.5%
N_2	2.7%	3%
Ar	1.6-1,7%	1.6%
O_2	0.13-0.2%	0.13%
CO	0.08%	0.07%
H_2O	210 ppm	absent
NO	100 ppm	absent
Ne	2.5 ppm	300 ppm
HDO	0.85 ppm	absent
Kr	0.3 ppm	300 ppm
Xe	0.08 ppm	300 ppm

Different types and thicknesses of UV filters may be placed over the windows to simultaneously evaluate the sample response to various doses of radiation.

To test the performances of the UV illumination system, we estimated the UV flux received by the samples using a photospectrometer with a sensor, placed on the dish at the location of the vessels. It registered how much UV was falling on the vessels after passing through the quartz windows. The values were taken in different positions and calibrated using various UV filters with passbands of 20 μm. The measured flux is shown in Figure 2 and compared with the solar flux received by Mars outside the atmosphere and estimated at ground level (Cockell et al. 2000).

The Martian atmosphere is composed mainly of CO_2, with a small percentage of N_2 (see Table 1). Its complete reproduction is very hard, and we used a mixture of gases ordered from a specialised factory, whose composition is shown in the third column of the Table. This mixture is quite expensive, so for the majority of the tests described here we used simply CO_2 gas.

To test the procedure for biological samples, the biologists of our team cultured different bacterial strains in their laboratories. These were then placed in LISA and irradiated by the UV lamp with different exposure times by initially covering all the quartz windows with aluminium diaphragms, and then opening them

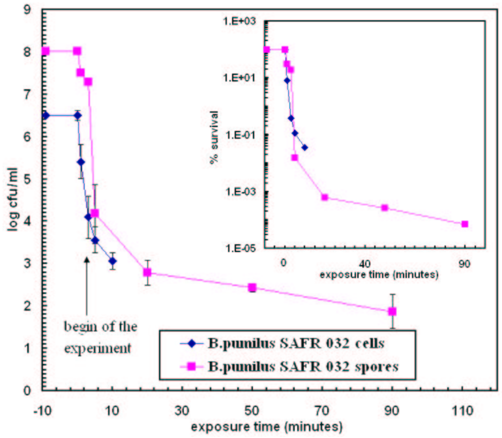

Fig. 3. Survival curves of *Bacillus pumilus* (spores and vegetative cells) at room temperature (25°C), 7 mbar of CO_2, vs. time, irradiated with the lamp whose flux is shown in Figure 2. In the inset, the percentage of survival is shown.

in nested intervals. At the end of the experiments, all the six bacterial samples were kept at the same atmospheric pressure and temperature conditions but received different doses of radiation. Then the pressure and eventually the temperature were brought to normal conditions and the bacterial sample, extracted from the vessels, analysed in order to estimate the degree of deactivation or the metabolic variations. The first experiments performed are "by reference" at room temperature. The survival curves exposed in Figure 3 demonstrate the higher survival capability of spores than vegetative cells obtained from the same bacterial strain. One of them is shown in Figure 3. We used cultures of *Deinococcus radiodurans*, particularly resistant to ionizing radiations such as gamma rays, *Bacillus nealsonii* and *Bacillus pumilus*.

3. Conclusions

The LISA simulator has been tested with the conditions of the planet Mars and is now starting biological experiments to understand what kind of (terrestrial) lifeforms may survive on the surface or sub-surface. LISA may also be used for the testing of materials and samples in conditions similar to those of Antarctica or colder environments. These tests have shown that LISA is able to simulate the environmental conditions on Mars at various latitudes.

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