Europlanet TA Scientific Report

PROJECT LEADER

Project number: ***20-EPN2-069***
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TA Facility visited: TA2 Facility 6 – DLR Planetary Simulation Laboratory (PASLAB)

Project Title: Challenging the adaptability of an anhydrobiotic cyanobacterium to Mars-like conditions

Scientific Report Summary.
(plain text, no figures, maximum 250 words, to be included in database and published)

Different studies reported the endurance of cyanobacteria to Mars-like conditions; however, little is known about the cellular and molecular mechanisms responsible for this resistance. The further combination of Martian UV fluxes and perchlorate ions at concentrations found on the surface of Mars increases the challenges for survival. Under this context, this study aimed to investigate the adaptability and cellular responses of metabolically active biofilms of *Chroococcidiopsis* CCMEE 029 to Martian surface-like conditions combined with perchlorate ions.

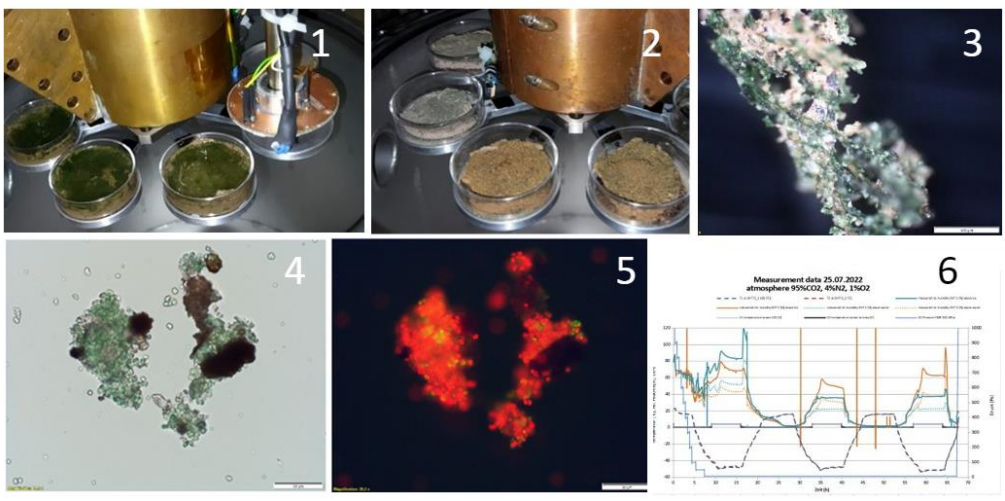
Biofilms obtained from cells mixed with two different Martian regolith analogs and 2.4 mM of perchlorate ions on top of an agarized regolith-based medium were exposed to unprotected Mars-like conditions for 3 days. Parameters consisted of a Mars-like atmosphere (95% CO_2, 4% N_2, 1% O_2) constant pressure of 700 Pa, periodic photosynthetically active radiation (PAR, 400-700nm, 3W/ m^2/s) and UV (4W/m^2/s) irradiation for 16 h followed by 8 h of dark with diurnal cycling of relative humidity and temperature from 75% to 0% and +15ºC to -50ºC respectively. The photosynthetic yield was followed during the exposure with the Mini-PAM analyzer integrated into the Martian simulation chamber. Post-exposure analyses of cell-viability assessment, CFU capacity, and pigment autofluorescence and morphology will be performed. Proteomics analyses are ongoing in collaboration with Dr. Peter Lasch from the Robert Koch Institute, Berlin (Doellinger et al. 2020).

Overall, this study will contribute to extending our appreciation of the limits of life as we know it, from the habitability of Mars to future management of Life Support and *In-Situ* Resource Utilization systems.

Full Scientific Report on the outcome of your TNA visit

We encourage you to add figures to your report, which should be approx. 1 page of text plus figures.

The aim of this study was to investigate the response of metabolic active biofilms of the cyanobacterium *Chroococcidiopsis* CCMEE 029 to Mars surface-like conditions and perchlorate ions using the Martian Simulation Chamber (MSC) in DLR, Berlin. Prior TNA visit, samples were prepared by mixing 029 cells in the log phase with JSC-Mars-1A or PMRS Martian regolith analog and plated on top of agarized regolith-based medium (consisting of 0.2 g/ml regolith in ddH_2O with 17 mM $NaNO_3$) supplemented with 2.4 mM of perchlorate ions. Perchlorate was provided as a mixture of 40% $Mg(ClO_4)_2$ and 60% $Ca(ClO_4)_2$ as reported by the Phoenix Lander (Hecht et al., 2009). Plates were incubated at 25°C under a photon flux density of 40 µmol/m²/s allowing biofilm to form. A few days before the visit samples were sent to the Planetary Simulation Laboratory (PASLAB), DLR, Berlin, while biological triplicates were kept in the laboratory in Rome as timepoint 0 to allow the evaluation of the traveling effect on the samples. The visit lasted 7 days with a weekend in the middle, of which the first day was used for experimental setup and calibration, 6 days for exposure of both experimental sets (3 days for JSC-Mars-1A biofilms and 3 days for PMRS biofilms), and a final day for data collection. On the first day, a test biofilm was used to calibrate the MSC, in addition, the timepoint 1 samples (after travel and before exposure) were transported to the Robert Kotch Institute (RKI) (Berlin) where proteomic analyses were performed in collaboration with Dr. Peter Lasch using the Easy Extraction and Digestion (SPEED) protocol for protein extraction (Doellinger et al., 2020). On this day I was welcomed and introduced to the teams and the different facilities of both institutes. On the second day (Friday 22.07.2022) we started to run the first experimental set (4 biological replicates of PMRS biofilms, Fig. 1). The final setup plan consisted of 3 days of exposure to a Martian-like atmosphere (95% CO_2, 4% N_2, 1% O_2) and pressure (700 Pa), with diurnal cycling of 16 h PAR (3 W/ m²/s**)** and UV (4W/m²/s) irradiation and 8 h of dark, relative humidity from 75% to 0,1%, and temperatures of 15ªC to -50ºC. Continuous monitoring of photosynthesis yield was analyzed by the Mini-PAM device integrated into the MSC, while daily measurements were taken of the control samples kept in the laboratory incubator using an external Mini-PAM. On the 5th day (Monday 25th) we observed that the experiment failed during the weekend due to an increase in sample volume from low temperatures blocking the MSC's rotor. The exposure was equivalent to 1 day of Martian surface conditions. Then we decided to expose only 2 biological samples of each experimental set for 3 days with the same parameters and measurements. To avoid similar failure, biofilms were cut on the borders. On the 7th day (Thursday 28th) the experiment ended (Fig 2), and samples (exposed and controls) were taken to the RKI for proteomics analysis. From all exposed samples and controls I stored aliquots at -20ºC in glycerol and evaluate the CFU capacity by performing serial dilutions and drop platting in BG-11 agarized medium. We also analyzed some of the failed exposed samples under stereo and fluorescent microscopes (Fig. 3 and 4-5). On the last day (Friday 29th) I completed all the measurements and collected all data (Fig. 6, preliminary data).



- Give details of any publications arising/planned (include conference abstracts etc)

Results aim to be presented at the next EANA conferences and be submitted for publication in an Astrobiology-relevant journal.

- Host confirmation

Please can hosts fill in/check this table confirming the breakdown of time for this TA project:

Dates for travel to accommodation for TA visit (if physical visit by applicant)	Start Date of TA project at facility	Number of lab/field days spent on TA Visit pre-analytical preparation	Number of days in lab/field site for TA Visit	Number of days spent in lab for TA Visit data analysis	End Date of TA project at facility	Dates for travel home (if physical visit by applicant)
Departed: 29-07-2022 Arrived: 21-07-2022	21-07-2022	[1]	7	[1]	29-07-2022	Departed: 29-07-2022 Arrived: 21-09-2022

The host is required to approve the report agreeing it is an accurate account of the research performed.

Host Name	**Dr. Mickael Baqué**
Host Signature	
Date	**15.08.2022**

- Project Leader confirmation

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Project Leader Name	**Beatriz Gallego Fernández**
Project Leader Signature	
Date	**15/08/2022**