SUPPLEMENTARY INFORMATION FOR:

Adaptive Thermogenesis as a Survival Strategy of Bacillus subtilis under Simulated Martian Stressors

Andrea Hidalgo-Arias[1,2], Sandra Martinez-Gonzalez[1], Maria Martinez-Carrancho[1], Jesús Sobrado[1], Eva García-Lopez[1], Cristina Cid[1]*

1 Centro de Astrobiología (CAB), CSIC-INTA, Torrejón de Ardoz, Madrid, Spain.
2 Escuela de Doctorado de la Universidad Autónoma de Madrid. Centro de Estudios de Posgrado, Madrid, Spain.

***Correspondence:** Cristina Cid, Centro de Astrobiología (CSIC-INTA), Ctra. Ajalvir, km 4, 28850 Torrejón de Ardoz, Madrid, Spain
E-mail: cidsc@cab.inta-csic.es
ORCID: 0000-0001-5128-4558

This file includes:

I. Supplementary Tables. S1-S2.

II. Supplementary Figures. S1-S5.

1 Supplementary Tables

Table S1. Environmental conditions of Earth, Mars and the MARTE chamber.

Environmental conditions	EARTH	MARS	MARTE CHAMBER
Temperature (°C)	56.7° - (-89.2°) [1]	10° (Summer) - 100° (In winter at the poles) [2,3]	10°C daytime; -15°C nighttime
Pressure (mbar)	1,013.25	7-20	20
Atmosphere	78% N_2; 20% O_2; 1% H_20; 0.93% Ar; 0.04% CO_2 [4]	95% CO_2; 2.7% N_2; 1.6% Ar; 0.14 O_2; 0.13% H_20; 0.08% CO	100% CO_2
Essential elements	C, H, N, O, P, S [5]	C, H, N, O, P, S [6]	C, H, N, O, P, S
Solvent	Water [7]	Brines [8]	TB culture medium
Source of energy	Solar radiation 550W/m^2 [9]	Solar radiation 550W/m^2 [10]	6h: Xenon lamp (666 W/m^2) + Deuterium lamp (5.11 W/m^2) 18h: Nighttime (no radiation sources)

SUPPLEMENTARY REFERENCES FOR TABLE S1

1. Zhang, T. *et al.* A global dataset of daily maximum and minimum near-surface air temperature at 1 km resolution over land (2003–2020). *Earth Syst. Sci. Data* **14**, 5637–5649 (2022).

2. Forget, F., Hansen, G. B. & Pollack, J. B. Low brightness temperatures of Martian polar caps: CO_2 clouds or low surface emissivity? *J. Geophys. Res.* **100**, 21219–21234 (1995).

3. Aerts, J., Röling, W., Elsaesser, A. & Ehrenfreund, P. Biota and Biomolecules in Extreme Environments on Earth: Implications for Life Detection on Mars. *Life* **4**, 535–565 (2014).

4. Grinspoon, D. H. Earth: Atmosphere. in *Encyclopedia of Planetary Science* 198–201 (Kluwer Academic Publishers, Dordrecht, 1997). doi:10.1007/1-4020-4520-4_112.

5. Maret, W. & Blower, P. Teaching the chemical elements in biochemistry: Elemental biology and metallomics. *Biochem Molecular Bio Educ* **50**, 283–289 (2022).

6. McGlynn, I. O., Fedo, C. M. & McSween, H. Y. Soil mineralogy at the Mars Exploration Rover landing sites: An assessment of the competing roles of physical sorting and chemical weathering. *J. Geophys. Res.* **117**, 2011JE003861 (2012).

7. Pohorille, A. & Pratt, L. R. Is Water the Universal Solvent for Life? *Orig Life Evol Biosph* **42**, 405–409 (2012).

8. Martín-Torres, F. J. *et al.* Transient liquid water and water activity at Gale crater on Mars. *Nature Geosci* **8**, 357–361 (2015).

9. Chen, C. J. *Physics of Solar Energy*. (Wiley, 2011). doi:10.1002/9781118172841.

10. Cockell, C. S. *et al.* Habitability: A Review. *Astrobiology* **16**, 89–117 (2016).

Table S2. Identification of proteins in *Bacillus subtilis* with significant differences in expression according to the 2D-DIGE analysis.

File: Table S2.xlsx

2 Supplementary Figures

Figure S1. 3D technical diagram of the MARTE simulation chamber.

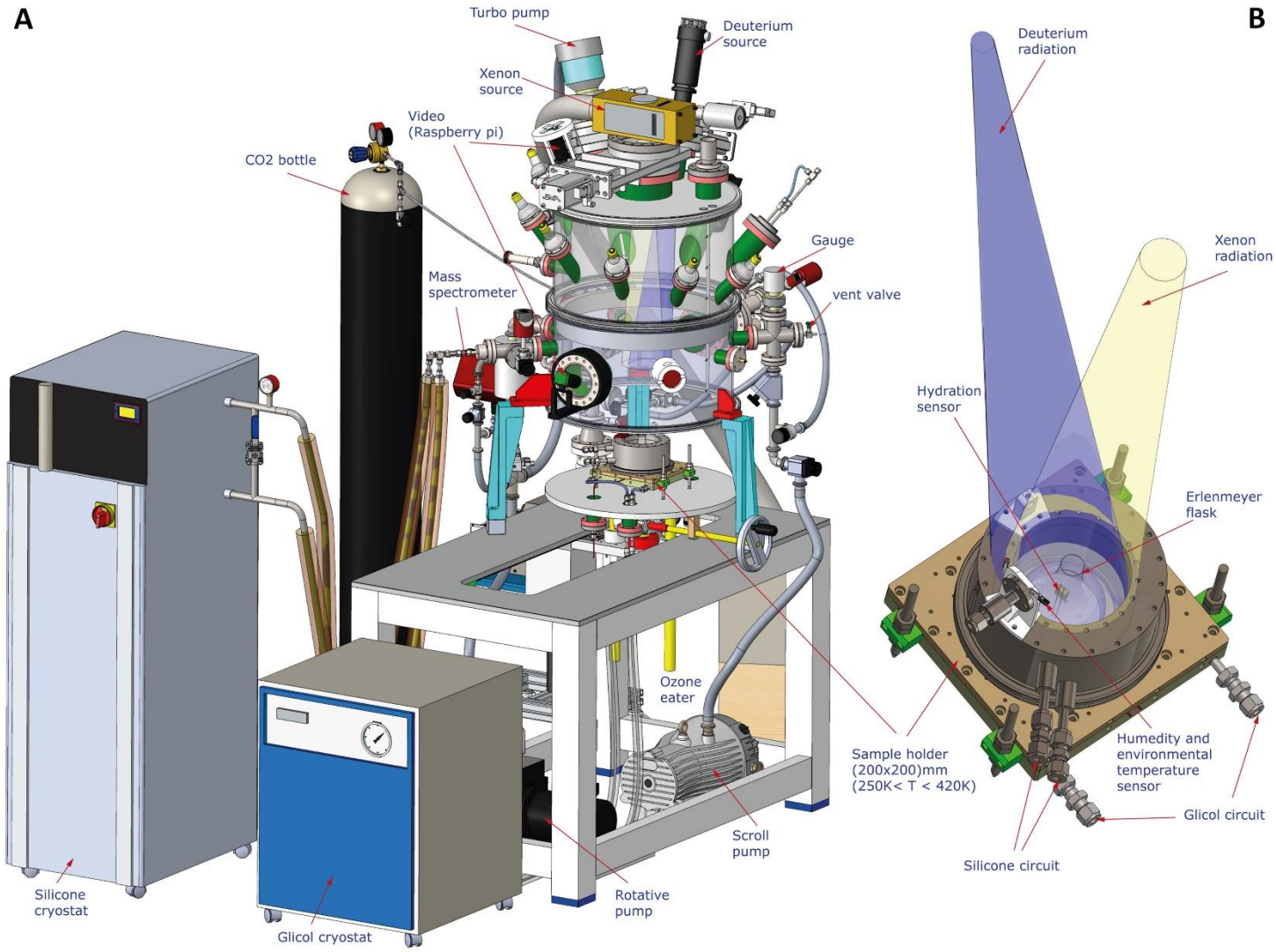
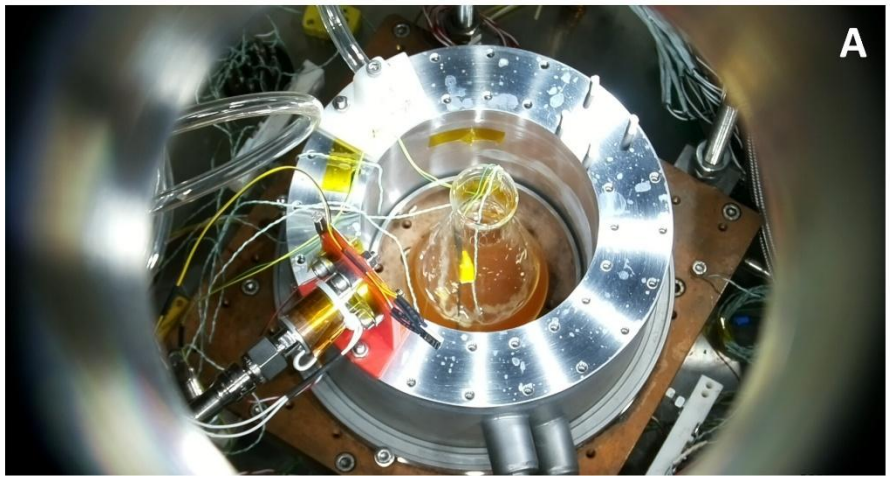


Figure S2. Phase change observed over the course of one sol. (A) When the liquid culture is introduced into the MARTE chamber, bubbling initially occurs due to the pressure drop. (B) Later, during the night, a phase change is observed as the medium freezes. During the following sunset, the sample thaws and releases bubbles again. This same process repeats at sunrise and sunset throughout the five sols.



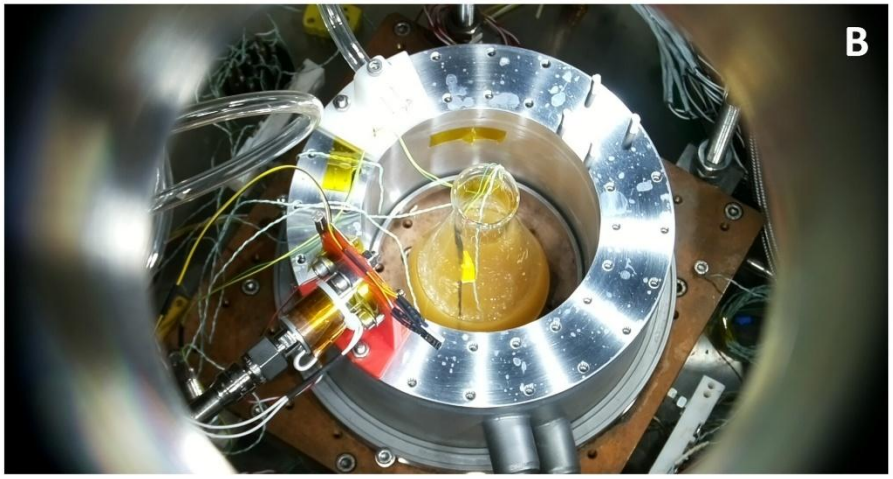


Figure S3. Still image from video 1. The image shows the moment when the culture medium is bubble-forming.

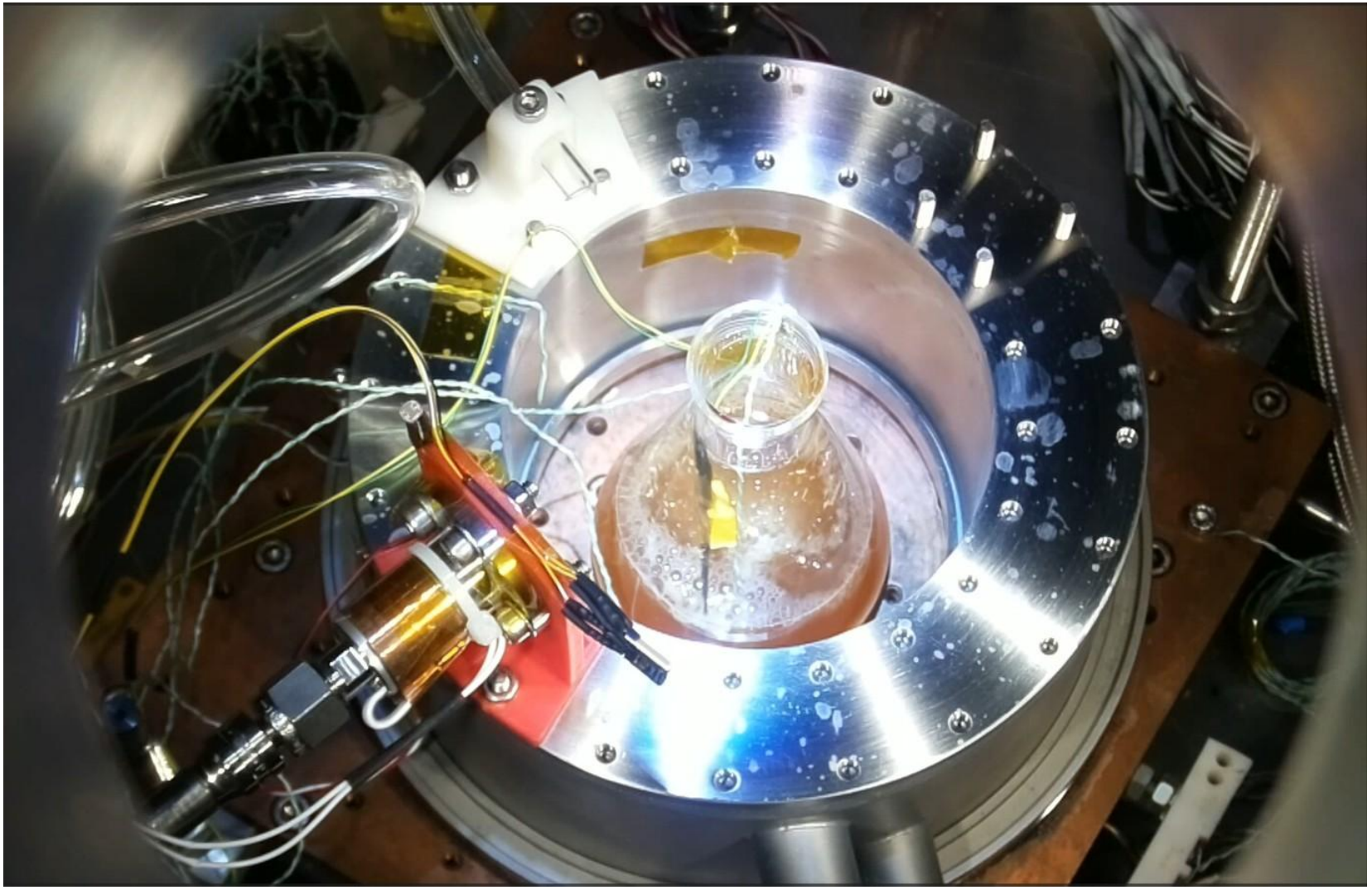


Figure S4. Temperature and electrical resistance over one sol. The graph shows sample temperature (black line) and electrical resistance (red line) throughout a sol. Chamber temperature reaches 10°C during the daytime (yellow-shaded area) and drops to -15°C at night. A sharp decrease in resistance occurs as the sample thaws, followed by a gradual increase as it refreezes at sunset.

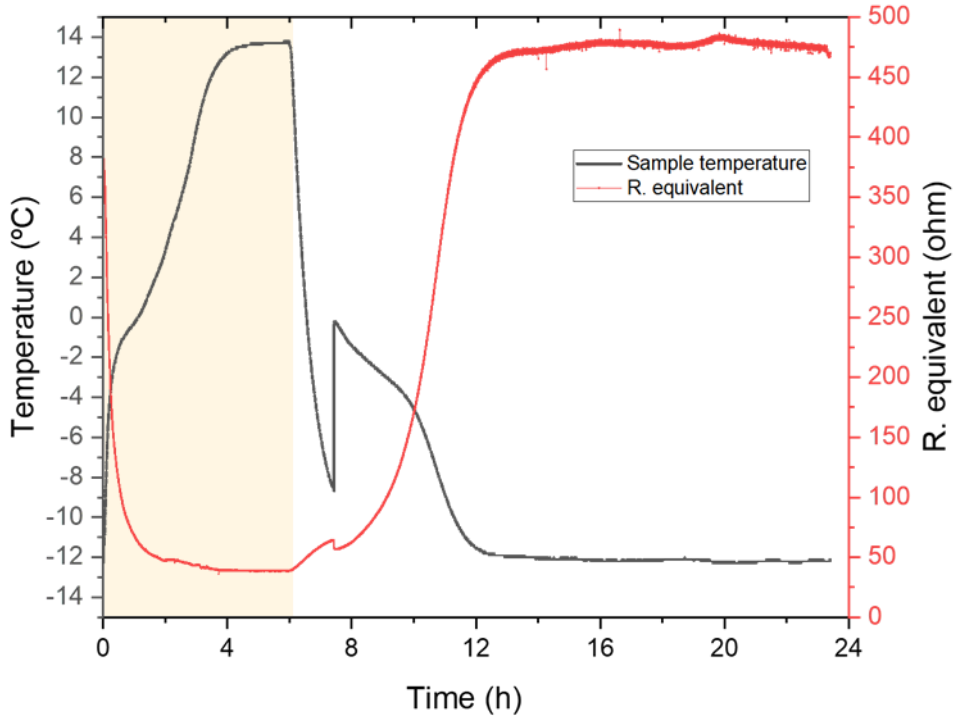


Figure S5. 2D-DIGE gel images. Representative 2D-DIGE gel of protein extracts from cultures grown under (A) standard conditions (labeled with Cy3 fluorochrome) and (B) Martian conditions (labeled with Cy5). (C) Gel stained with Coomassie Blue for overall protein visualization. Selected spots were excised, digested with trypsin, and identified by MALDI-TOF MS.