Mem. S.A.It. Vol. 92, 94

Memorie della

Unravelling the molecular basis of an anhydrobiotic cyanobacterium revival after exposure to extreme dryness, Mars-like UV flux and space vacuum: Implications for future missions beyond low Earth orbit

C. Fagliarone[1], C. Mosca[1], A. Napoli[1], B.Gallego Fernandez[1], and D. Billi[1]

University of Rome Tor Vergata, Department of Biology, Laboratory of Astrobiology and Molecular Biology of Cyanobacteria, Rome, Italy

Abstract. Desert cyanobacteria of the genus *Chroococcidiopsis* possess a remarkable resistance to desiccation and radiation, a requisite needed for using the current available expose facilities in low Earth orbit. Investigating their capability to cope with the exposure to real space conditions, Mars-like simulations in space or on the ground as well as to laboratory-simulations of icy moons, will contribute to understand the limits of life and assess the potential habitability of Mars and icy moons. When the accumulated damages exceeded the repair mechanisms, mainly DNA repair pathways, new insights can be inferred on biomarker detectability. Clearly the investigation of the molecular responses to non-Earth conditions is constrained by the difficulties in performing real-time monitoring. When such an approach will be available, especially for platforms beyond the low Earth orbit, it will have implications to develop support human space exploration, for instance using cyanobacteria as a chassis for space synthetic biology or life support systems.

Key words. Cyanobacteria – space – Mars simulations – biosignatures – DNA repair

1. Desert cyanobacteria as astrobiological model system

Deserts members of the genus *Chroococcidiopsis* dominate the most extreme arid habitats in hot and cold deserts, where they colonize the last refuges for life at the interface between stones and soil or within porous rocks, persisting in a desiccated state for most of the time (Billi 2018). These cyanobacteria are characterized by a pronounced capability of withstanding the lethal effects of extreme dryness; for example, *Chroococcidiopsis* sp. CCMEE 029 was reported to survive 4 years of air-drying (Billi 2009; Fagliarone et al. 2017). The molecular bases of the capability of avoiding/limiting the lethal effects of water removal remain to be fully deciphered. However, for *Chroococcidiopsis* sp. CCMEE 029 the avoidance of protein oxidative damage was reported as a first line of defense against desiccation (Fagliarone et al. 2017) as well as the accumulation of sucrose and trehalose, two disaccharides known to substitute water thus

stabilizing dried cells (Fagliarone et al. 2020). As a byproduct of desiccation tolerance, desert strains of *Chroococcidiopsis* are radiation tolerant. Hydrated cells can cope with 15 kGy of gamma rays (Billi et al. 2000) and $13kJ/m^2$ of UVC radiation (Baqué et al. 2013b). The radioresistance treshold of this strain was further extended when dried cells survived 24 kGy of γ-radiation and 2 kGy of iron ions (Verseux et al. 2017). The outstanding capability to persist under extreme desiccation and high radiation doses makes *Chroococcidiopsis* a model organism for astrobiology experiments, considering that the current available space facilities allow the exposure of only dried cells (Cottin et al. 2017).

2. Planetary and Space Laboratory Simulations

Deserts members of the genus *Chroococcidiopsis* were exposed to space and planetary laboratory simulations known as Experiment Verification Tests (EVTs) and Scientific Verification Tests (SVTs) that were performed at the Planetary and Space Simulation Facilities (DLR-Institute of Aerospace Medicine in Köln). The aim was to optimize samples preparation and analysis protocols before the ESA EXPOSE-R2 space mission. In the Biolfilm Organisms Surfing Space (BOSS) space experiment the hypothesis was tested that biofilm-forming bacteria are more resistant to space and Martian conditions than their planktonic counterparts (Cottin & Rettberg 2019). While the BIOMEX (BIOlogy and Mars EXperiment) space experiment investigated the survival of selected extremophiles and the stability/degradation of their biological components (pigments, cell wall components, etc.), when mixed with Martian and lunar mineral analogues (de Vera et al. 2019). EVTs included the exposure to a Mars-like atmosphere, space vacuum temperature cycles, temperature UVC up to $10kJm^{-2}$ and polychromatic UV (200–400 nm) radiation from 1.4×10^3 to $6.8 \times 10^5 kJm^{-2}$. SVTs were performed by exposing the samples to $5.7 \times 10^5 kJm^{-2}$ of polychromatic UV (200–400 nm) radiation, attenuated with 0.1% ND filter a dose expected after 1 year in LEO, under space vacuum and Mars conditions (de Vera et al. 2019). When dried biofilms of *Chroococcidiopsis* sp. 029 (about 50 μm) were exposed to EVTs and SVTs, an enhanced performance of biofilms was reported compared to planktonic counterparts, being able to cope with $5 \times 10^2 kJm^{-2}$ of UV polychromatic radiation (corresponding to 500 MJ m^{-2} attenuated with 0.1% ND filter) combined with space vacuum or Martian atmosphere (Baqué et al. 2013a). When EVTs and SVTs were performed on dried cells of *Chroococcidiopsis* sp. 029 in thin layers (about 20 μm) mixed with Martian or Lunar mineral simulants, colony-forming ability was retained after $10kJm^{-2}$ of UVC, whereas cells mixed with Lunar mineral simulant formed colonies only after the lowest polychromatic UV dose tested, e. g., $1.5 \times 10^3 kJm^{-2}$, while colony-forming ability was lost after $5 \times 10^2 kJm^{-2}$ in combination with space vacuum (Baqué et al. 2014, 2016). DNA was also detectable in dried cells mixed with Martian regolith simulants after exposure to $5.7 \times 10^2 kJm^{-2}$ of polychromatic UV (corresponding to $570MJm^{-2}$ with 0.1% ND filter) combined with a Mars-like atmosphere, although colony-forming ability was lost (Baqué et al. 2016). Therefore, since *Chroococcidiopsis* sp. 029 withstood ground-based simulations it was implemented in the ESA selected BOSS and BIOMEX experiments performed from 2014-2016 using the ESA Expose facility installed outside the International Space Station. Recently the investigation of the recovery of dried biofilms of *Chroococcidiopsis* sp. 029 exposed to $1.5 \times 10^3 kJm^{-2}$ of a Mars-like UV flux, after 7 years of air-dried storage, revealed the accumulation of different types and/or amounts of DNA lesions that were repaired by the over-expression of genes encoding proteins of repair pathways of UV-induced DNA damage (Mosca et al. 2019) The resistance of deserts strains of *Chroococcidiopsis* to space radiation was further investigated in the frame of the STARLIFE project, by using radiations mimicking single components of the cosmic radiation, e. g., accelerated heavy ions and high doses of X- and γ-rays (Moeller et al.

2017). When dried *Chroococcidiopsis* cells were irradiated with heavy ions and gamma rays, survival occurred after 2 kGy of iron ions and 24 kGy of gamma -rays (Verseux et al. 2017). In addition, dried *Chroococcidiopsis* exposed to 113 kGy of gamma rays retained a carotenoid Raman peaks that lost only 15-20% of its intensity compared to non-irradiated control (Baqué et al. 2020). Recently, two *Chroococcidiopsis* strains, one form the Negev Desert and one from the Dry Valleys in Antarctica, were exposed to liquid-water vein systems simulated with a climatic chamber using salt/ice mixtures as expected for the icy crusts of Europa. Both strains survived the exposure to 258 K in NaCl solution, probably as they migrated in the liquid veins between ice grain boundaries. They also survived at 258 K in Na2SO4 and MgSO4-salty-ice samples that is a temperature well below the eutectic temperature of the solutions, where liquid veins should not exist anymore. Moreover, both strains survived the exposure at 233 K in each salty-ice sample, whereas there were no survivors at 203 K (Cosciotti et al. 2019).

3. Expose-R2 space mission

Space provides a unique research field for astrobiology, thanks to a combination of extreme parameters that cannot be simulated on the ground, i.e., low pressure, temperature fluctuations, short ultraviolet wavelengths and complex cosmic ionizing radiation (Rabbow et al. 2017). Hence desert strains of Chroococcidiopsis were exposed to space and Mars-like conditions in low Earth orbit (LEO) during the EXPOSE-R2 space mission (2014-2016). Post-flight analyses of *Chroococcidiopsis* BIOMEX samples retrieved from top-layer carriers showed that Mars and Moon regolith simulants provided sufficient UV-radiation shielding, thus favoring cell survival (Billi et al. 2019b, 2020). While post-flight analyses of *Chroococcidiopsis* BOSS samples retrieved from top-layer carriers showed an enhanced biofilm endurance compared to planktonic counterparts, a feature related to the UV-shielding provided by the top-layer cells that protected the lower layer cells of the biofilm (Billi et al. 2019a). Instead, post-flight analyses of *Chroococcidiopsis* BIOMEX and BOSS samples retrieved from bottom-layer carriers did not reveal any reduction in survival or extensive DNA damage compared to air-dried ground controls, at least according to the detection limit of the PCR-stop assays used to detect DNA lesions (Billi et al. 2019b,a).

4. Future BIOSIGN space mission

The future space ESA selected space experiment BIOSIGN (BIO-SIgnatures and habitable Niches) project will be carried out using the new ESA Expose facility outside the ISS in order to expand the knowledge on the limits of life, to identify potentially habitable niches in the solar system (with particular emphasis on Mars, Enceladus and Europe) and to establish a biomarker database for life research (de Vera & The Life Detection Group of BIOMEX/BIOSIGN 2019). In the BIOSIGN space experiment, a selection of psychrophils, barophiles, halophiles, radiophiles and xerophiles, including desert strains of *Chroococcidiopsis*, will be exposed to planetary simulated conditions and in LEO, thus implementing the results achieved during the BIOMEX (BIOlogy and Mars EXperiment) and BOSS (Biofilm Organisms Surfing Space) experiments. In particular, since the results obtained for *Chroococcidiopsis* in the BOSS_Cyano and BIOMEX_Cyano experiements have shown a greater resistance of biofilms compared to the planktonic counterparts as well as the protective role played by simulants of Martian regolith, for the BIOSIGN_Cyano *Chroococcidiopsis* biofilms will be grown in the presence of Martian regolith simulants and natural terrestrial substrates. In addition, BIOSIGN_Cyano will use spectrometric and spectroscopic techniques together with PCR and CLSM techniques used in BIOMEX_Cyano to evaluate the integrity of genomic DNA and photosynthetic pigments. Finally, by taking advantage of sequenced genome of *Chroococcidiopsis* sp. 029, the expression of DNA repair genes will be

monitored during the recovery from planetary simulated conditions and in LEO.

5. Conclusions

The capability of desert strains of *Chroococcidiopsis* to cope with space and Mars-like condition in LEO has extended our knowledge on the limit of life under non-Earth conditions (Billi 2020). Ultraviolet radiation was confirmed to be the most harmful factor to microorganisms in space (Horneck et al. 2010). Indeed, the UV dose received by *Chroococcidiopsis* samples during the EXPOSE-R2 mission was ranging from $2.19 \times 10^2 kJ/m^2$ to $3.19 \times 10^2 kJ/m^2$ (depending on the position within the facility and the presence of a 0.1% neutral filter), corresponded to approximately 4h-8h of an average UV flux of Mars (Cockell et al. 2000). Results suggested that a life form might have indeed survived on Mars if shielded from UV, for instance by Martian dust, or if occurring as biofilm. This has implications for the hypothesis that, during Mars's climatic history, desiccation- and radiation- tolerant life-forms could have survived in habitable niches and protected niches while transported (Westall et al. 2013). Considering the radioresistance of desert strains of *Chroococcidiopsis* , the total dose of 0.5 Gy of ionizing radiation received during the EXPOSE-R2 space mission did not affect cell survival. Based on the dose of $76 mGy/year$ measured by the Curiosity rover at Gale Crater's surface (Hassler et al. 2014), dried *Chroococcidiopsis* cells would survive on Mars more than half a decade. During the ground-based simulations, when radiation doses exceeded the repair potential upon rehydration, relevant indications were inferred on biomarker detectability. For example, the permanence of a strong Raman carotenoid signal in dried *Chroococcidiopsis* exposed to 113 kGy of gamma rays, revealed the detectability of remains of a pigment-containing life form, after a radiation dose accumulated in about 1.5 million years on the Martian surface or in 13 million years at 2 m depth. This suggested that biomarker detection under ionizing radiation might exceed timescales of 13 million years and might be enhanced in dried cells protected by minerals. Post-flight analysis performed after the retrieval and rehydration on the ground of dried *Chroococcidiopsis* cells highlighted a key role of DNA repair pathways. Clearly the evaluation of the cellular responses to the space environment is constrained by the difficulties of performing real-time monitoring. When such a possibility will be made available for astrobiology space experiments, it will have implications for several application in supporting human space exploration, for instance using astrobiology relevant microorganisms as a chassis for space synthetic biology, e.g., endowing it with new abilities, such us the production on-demand of compounds or by using it to feed synthetic biology-enhanced cells for bioprinting of biomaterials or life support system (Rothschild 2016; Menezes et al. 2015).

Acknowledgements. Research was supported by the Italian Space Agency (BOSS_Cyano grant 053-R.0; BIOMEX_ Cyano grant 051-R.0; BIOSIGN_Cyano grant 2018-15-U.0; Life in Space grant 2019-3 U.0).

References

Baqué, M., Napoli, A., Fagliarone, C., et al. 2020, Life, 10

Baqué, M., Scalzi, G., Rabbow, E., Rettberg, P., & Billi, D. 2013a, Origins of Life and Evolution of Biospheres, 43, 377

Baqué, M., Verseux, C., Böttger, U., et al. 2016, Origins of Life and Evolution of Biospheres, 46, 289

Baqué, M., Verseux, C., Rabbow, E., de Vera, J.-P. P., & Billi, D. 2014, Origins of Life and Evolution of Biospheres, 44, 209

Baqué, M., Viaggiu, E., Scalzi, G., & Billi, D. 2013b, Extremophiles, 17, 161

Billi, D. 2009, Extremophiles, 13

Billi, D. 2018, International Journal of Astrobiology, 18, 483

Billi, D. 2020, Challenging the Survival Thresholds of a Desert Cyanobacterium under Laboratory Simulated and Space Conditions (John Wiley & Sons, Ltd), 183–195

Billi, D., Mosca, C., Fagliarone, C., et al. 2020, International Journal of Astrobiology, 19, 53–60
Billi, D., Staibano, C., Verseux, C., et al. 2019a, Astrobiology, 19, 1008
Billi, D., Verseux, C., Fagliarone, C., et al. 2019b, Astrobiology, 19, 158
Billi, D., Wright, D. J., Helm, R. F., et al. 2000, Applied and Environmental Microbiology, 66, 1680
Cockell, C. S., Catling, D. C., Davis, W. L., et al. 2000, Icarus, 146, 343
Cosciotti, B., Balbi, A., Ceccarelli, A., et al. 2019, Life, 9
Cottin, H., Kotler, J. M., Billi, D., et al. 2017, Space Sci. Rev., 209, 83
Cottin, H. & Rettberg, P. 2019, Astrobiology, 19, 975
de Vera, J.-P., Alawi, M., Backhaus, T., et al. 2019, Astrobiology, 19, 145
de Vera, J.-P. & The Life Detection Group of BIOMEX/BIOSIGN. 2019, A Systematic Way to Life Detection: Combining Field, Lab and Space Research in Low Earth Orbit (Springer), 111
Fagliarone, C., Mosca, C., Ubaldi, I., et al. 2017, Extremophiles, 21, 1
Fagliarone, C., Napoli, A., Chiavarini, S., et al. 2020, Frontiers in Astronomy and Space Sciences, 7, 31
Hassler, D., Zeitlin, C., Wimmer-Schweingruber, R., et al. 2014, Science, 343
Horneck, G., Klaus, D. M., & Mancinelli, R. L. 2010, Microbiology and Molecular Biology Reviews, 74, 121
Menezes, A. A., Montague, M. G., Cumbers, J., Hogan, J. A., & Arkin, A. P. 2015, Journal of The Royal Society Interface, 12, 20150803
Moeller, R., Raguse, M., Leuko, S., et al. 2017, Astrobiology, 17, 101
Mosca, C., Rothschild, L. J., Napoli, A., et al. 2019, Frontiers in Microbiology, 10, 2312
Rabbow, E., Rettberg, P., Parpart, A., et al. 2017, Frontiers in Microbiology, 8, 1533
Rothschild, L. 2016, Biochemical Society Transactions, 44, 1158
Verseux, C., Baqué, M., Cifariello, R., et al. 2017, Astrobiology, 17, 118
Westall, F., Loizeau, D., Foucher, F., et al. 2013, Astrobiology, 13, 887