

EXPOSE-R2 on the International Space Station (2014–2016): Results from the PSS and BOSS Astrobiology Experiments

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Abstract

EXPOSE facilities were ESA multiuser facilities mounted outside the International Space Station for astrobiology experiments. Between 2008 and 2016, three series of experiments were conducted involving chemical and biological samples to test their resistance and evolution in the space environment in low Earth orbit. In this *Astrobiology* special collection, results from two experiments of the EXPOSE-R2 campaign (2014–2016) are presented: Biofilm Organisms Surfing Space (BOSS) relating to biology and Photochemistry on the Space Station (PSS) dealing with astrochemistry. Key Words: Biochip—Biofilms—Photochemistry—ISS—Mars simulation. *Astrobiology* 19, 975–978.

Introduction

BYOND THE MORE POPULAR space exploration missions (as exemplified, for instance, by the Mars Curiosity rover and the Rosetta probe and its lander Philae), space is a privileged tool for the study of astrobiology. Early experiments that involved the exposure of microorganisms to space conditions date back to the Gemini and Apollo missions in the 1960s and 1970s. Supporting hardware and devices have since been significantly improved, and at present, the exterior of the International Space Station (ISS) is regularly used as a laboratory for conducting chemical or biological astrobiology studies in outer space. This environment with its unique combination of different types of radiation, solar and cosmic, is particularly challenging to simulate in ground facilities (Cottin *et al.*, 2017).

The EXPOSE-R2 mission was the third to employ the ESA EXPOSE facility, which is situated outside the ISS and has been in use since 2008. EXPOSE-E was set on the EUTEF facility outside the European Columbus module from February 2008 until August 2009 (Rabbow *et al.*, 2012). EXPOSE-R was mounted on an external platform of the Russian service module Zvezda from March 2009 until March 2011 (Rabbow *et al.* 2015). EXPOSE-R2 was also mounted outside the Zvezda module and was shipped to the ISS on July 23, 2014, with four international astrobiological experiments: BIOMEX, BOSS, PSS, a series of Russian samples from IMBP, and also a dosimetry experiment R3D-R2. Exposure of the samples to low Earth orbit solar UV lasted for 469 days. Samples were returned

to Earth in March and June 2016. A Mission Ground Reference (MGR) experiment with the same hardware as in space and a complete set of samples was performed at the German Aerospace Center (DLR) in Cologne in parallel to the space mission (Rabbow *et al.*, 2017).

In this special collection of articles in *Astrobiology*, the main results of the BOSS and PSS experiments are presented. The results of the BIOMEX experiment were published in the February 2019 issue of the journal (de Vera *et al.*, 2019).

The BOSS (Biofilm Organisms Surfing Space) Experiment

Microorganisms account for over 99% of all living matter on Earth. They generally exhibit two distinct modes of behavior. The first is the free-floating, or planktonic, form in which single cells float or swim independently from each other in a liquid medium. The second is an attached state in which cells are closely packed and firmly attached to each other and usually form a solid surface. A change in behavior is triggered by many factors, including quorum sensing, as well as other mechanisms that vary between species. When a microbial cell switches modes, it undergoes a phenotypic shift in behavior in which large suites of genes are up- and down-regulated. Most microorganisms on Earth are organized into biofilms and microbial mat communities. Biofilms are among the oldest clear signs of life on Earth. They might also be the first forms of life to be detected on other planets and moons of our Solar System.

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A biofilm is a structured community of microorganisms that is adherent to a surface and encapsulated within a self-developed matrix of extracellular polymeric substances (EPS). Bacteria that live in a biofilm usually have significantly different properties from free-floating bacteria of the same species, as the dense and protected environment of the film allows them to cooperate and interact in various ways. One benefit of this environment is increased resistance to different chemical and physical agents, as the dense extracellular matrix and the outer layer of cells protect the interior of the community.

In the space experiment BOSS, the tested hypothesis was that the biofilm form of life with microorganisms embedded and aggregated in their EPS matrix is suited to support long-term survival of microorganisms under harsh environmental conditions as they exist in space and on Mars, and it is superior to the same bacteria in the form of planktonic cultures. The tested environmental parameters in the long-term exposure experiment on the ISS were space vacuum, simulated martian atmosphere, and pressure alone and in combination with extraterrestrial and Mars-like solar UV radiation. Different bacteria were selected for the space experiment. In this special collection, results are presented for (i) *Deinococcus geothermalis*, a polyextremophilic bacterium with a high resistance against desiccation and radiation comparable to the closely related well-known *Deinococcus radiodurans*; (ii) *Gloeocapsa* sp., a cyanobacterium isolated from limestone after long-term exposure to space in the previous EXPOSE-E space experiment ADAPT; and (iii) desert strains of the cyanobacterium *Chroococcidiopsis* spp. All microorganisms were exposed in the form of planktonic cells and biofilms. In parallel to the space experiment, a MGR experiment was performed with identical samples according to the parameters obtained from the ISS in planetary and space simulation facilities on the ground (Rabbow *et al.*, 2017).

Panitz *et al.* (2019 in this issue) show that *Deinococcus geothermalis* cells remained viable and culturable in the desiccated state and were thus able to survive space and Mars-like conditions and tolerate high extraterrestrial UV radiation for more than 2 years. Culturability decreased, but was better preserved, in the biofilm consortium than in planktonic cells. These results are correlated to differences in genomic integrity after exposure. However, cultivation-independent viability markers such as membrane integrity, ATP content, and intracellular esterase activity remained nearly unaffected, indicating that subpopulations of the cells had survived in a viable but nonculturable state.

Wadsworth *et al.* (2019 in this issue) demonstrate that cell aggregates of *Gloeocapsa* sp., which consist of groups of metabolically inactive cells that do not form structured layered biofilms, conferred protection against space conditions in comparison to planktonic cells. Furthermore, the experiment demonstrated that the cyanobacterial cell aggregates provided a microhabitat for a smaller bacterial co-cultured species that also survived in space. This observation shows that viable cells can “hitchhike” through space within the confines of larger protecting cells or cell aggregates.

Billi *et al.* (2019 in this issue) show that (i) the photosynthetic pigments in different *Chroococcidiopsis* spp. desert strains that were exposed to space and Mars-like conditions were better preserved in cells in the bottom layer of the samples than in cells in the top layer, (ii) the DNA in

biofilm cells was less damaged than that in planktonic cells, and (iii) the survival measured by cultivation was higher in biofilm samples than in planktonic cells.

These findings support the hypothesis that the biofilm form of life supports the long-term survival of microorganisms under the harsh environmental conditions in space and on Mars to a higher degree than does the planktonic lifestyle.

The Photochemistry on the Space Station (PSS) Experiment

The radiation of stars plays a crucial role in the evolution of organic molecules in astrophysical environments and planetary atmospheres. In the Solar System, UV photons emitted by the Sun drive most of the chemical evolution that occurs, either for the building of new compounds with an increase in the molecular complexity or the destruction of the most elaborated structures. Thus, solar UV initiates exceptionally complex chemistry in Titan’s atmosphere that turns the lightest gaseous organic compounds, such as methane, into heavy and solid organic aerosols. It can also erase the organic traces of past life at the surface of planets, such as Mars, and destroy organic molecules present on meteorites. In the interstellar medium, the UV radiation field (in addition to cosmic rays) is also responsible for the chemical evolution and the extraordinary diversity of organic molecules detected, either in the gaseous or in the solid phase (for instance, in icy mixtures condensed on interstellar grains). A heritage of this complex chemistry could be found in cometary material, although a significant fraction of the interstellar material is expected to be reprocessed in the solar nebula. The goal of the PSS experiment was to improve our knowledge of the chemical nature and evolution of organic molecules involved in extraterrestrial environments with astrobiological implications. This is built upon similar experiments previously conducted in low Earth orbit and the extensive experience in this field of most of the team members (both for the science and technical sides of the project).

The PSS experiment is a follow-up of the PROCESS (Cottin *et al.*, 2012) and AMINO (Cottin *et al.*, 2015a) experiments conducted on EXPOSE-E and EXPOSE-R, respectively, with an improved hardware that allows for better simulation of the martian environment as well as the exposure of aptamers and antibodies, which will help prepare future generations of biochip-based space instruments (Cottin *et al.*, 2015b).

Baratta *et al.* (2019 in this issue) report on the evolution of organic samples prepared by 200 keV He⁺ irradiation of N₂:CH₄:CO icy mixtures deposited at 17 K, on vacuum UV transparent MgF₂ windows. Such material made by irradiation of ice mixtures could be present in interstellar clouds or comets, from which they could be expelled on dust particles and reach Earth as interplanetary dust particles (IDPs). Results show that the nitriles and amines moieties contained in relatively large IDPs (>20–30 μm) could have survived in the interplanetary medium; hence they could have reached prebiotic Earth.

Stalport *et al.* (2019 in this issue) report on the stability of organic compounds in the martian environment: chrysenes, adenine, and glycine—pure or deposited on an iron-rich amorphous mineral phase—were exposed to solar UV,

under a filter selected to simulate UV spectrum reaching the surface of the Red Planet. Measurements showed that all exposed samples were partially degraded, and quantum efficiencies of photodecomposition were calculated in the 200–250 nm wavelength range. None of the tested organics are stable under low Earth orbit solar UV radiation conditions. Interestingly, the presence of an iron-rich mineral phase increases their degradation.

Coussot *et al.* (2019a and 2019b in this issue) report on studies aimed to test whether spaceflight conditions might have influences on the performances of antibodies or aptamer-based biochips for future space instrumentation. Such innovative analytical instruments are under development to detect signatures of present or past life on planetary bodies. They are based on high affinity and specificity molecular recognition to detect target compounds at sub-nanomolar concentrations. The results presented suggest that cosmic radiation has no significant effect on the aptamers or antibodies recognition ability. However, repeated temperature cycling seems to alter the mobility of its fluorescein dye for aptamers, which could be interpreted as a loss of recognition, as the absence of the target, or as an alteration of the fluorescent dye properties.

References

- Baratta, G.A., Accolla, M., Chaput, D., Cottin, H., Palumbo, M.E., and Strazzulla, G. (2019) Photolysis of cometary organic dust analogs on the EXPOSE-R2 mission at the International Space Station. *Astrobiology* 19:1018–1036.
- Billi, D., Staibano, C., Verseux, C., Faglierone, C., Mosca, C., Baqué, M., Rabbow, E., and Rettberg, P. (2019) Dried biofilms of desert strains of *Chroococcidiopsis* survived prolonged exposure to space and Mars-like conditions in low Earth orbit. *Astrobiology* 19:1008–1017.
- Cottin, H., Guan, Y.Y., Noblet, A., Poch, O., Saiagh, K., Cloix, M., Macari, F., Jérôme, M., Coll, P., Raulin, F., Stalport, F., Szopa, C., Bertrand, M., Chabin, A., Westall, F., Chaput, D., Demets, R., and Brack, A. (2012) The PROCESS experiment: an astrochemistry laboratory for solid and gaseous organic samples in low Earth orbit. *Astrobiology* 12: 412–425.
- Cottin, H., Saiagh, K., Guan, Y.Y., Cloix, M., Khalaf, D., Macari, F., Jérôme, M., Polienor, J.-M., Bénilan, Y., Coll, P., Fray, N., Gazeau, M.-C., Raulin, F., Stalport, F., Carrasco, N., Szopa, C., Bertrand, M., Chabin, A., Westall, F., Vergne, J., Da Silva, L.A., Maurel, M.-C., Chaput, D., Demets, R., and Brack, A. (2015a) The AMINO experiment: a laboratory for astrochemistry and astrobiology on the EXPOSE-R facility of the International Space Station. *Int J Astrobiol* 14:67–77.
- Cottin, H., Saiagh, K., Nguyen, D., Grand, N., Bénilan, Y., Cloix, M., Coll, P., Gazeau, M.-C., Fray, N., Khalaf, D., Raulin, F., Stalport, F., Carrasco, N., Szopa, C., Chaput, D., Bertrand, M., Westall, F., Mattioda, A., Quinn, R., Ricco, A., Santos, O., Baratta, G., Strazzulla, G., Palumbo, M.E., Le Postollec, A., Dobrijevic, M., Coussot, G., Vigier, F., Vandenebeele-Trambouze, O., Incerti, S., and Berger, T. (2015b) Photochemical studies in low Earth orbit for organic compounds related to small bodies, Titan and Mars. *Current and Future Facilities*, Bulletin de la Société Royale des Sciences de Liège 84:60–73.
- Cottin, H., Kotler, J.M., Billi, D., Cockell, C., Demets, R., Ehrenfreund, P., Elsaesser, A., d'Hendecourt, L., van Loon, J.J.W.A., Martins, Z., Onofri, S., Quinn, R.C., Rabbow, E., Rettberg, P., Ricco, A.J., Slenzka, K., de la Torre, R., de Vera, J.-P., Westall, F., Carrasco, N., Fresneau, A., Kawaguchi, Y., Kebukawa, Y., Nguyen, D., Poch, O., Saiagh, K., Stalport, F., Yamagishi, A., Yano, H., and Klamm, B.A. (2017) Space as a tool for astrobiology: review and recommendations for experimentations in Earth orbit and beyond. *Space Sci Rev* 209:83–181.
- Coussot, G., Le Postollec, A., Faye, C., Baqué, M., Vandenebeele-Trambouze, O., Incerti, S., Vigier, F., Chaput, D., Cottin, H., Przybyla, B., Berger, T., and Dobrijevic, M. (2019a) Photochemistry on the Space Station—antibody resistance to space conditions after exposure outside the International Space Station. *Astrobiology* 19:1053–1062.
- Coussot, G., Le Postollec, A., Incerti, S., Baqué, M., Faye, C., Vandenebeele-Trambouze, O., Cottin, H., Ravelet, C., Peyrin, E., Fiore, E., Vigier, F., Caron, J., Chaput, D., Przybyla, B., Berger, T., and Dobrijevic, M. (2019b) Photochemistry on the Space Station—aptamer resistance to space conditions: particle exposure from irradiation facilities and real exposure outside the International Space Station. *Astrobiology* 19: 1063–1074.
- de Vera, J.-P., Alawi, M., Backhaus, T., Baqué, M., Billi, D., Böttger, U., Berger, T., Bohmeier, M., Cockell, C., Demets, R., de la Torre Noetzel, R., Edwards, H., Elsaesser, A., Faglierone, C., Fiedler, A., Foing, B., Foucher, F., Fritz, J., Hanke, F., Herzog, T., Horneck, G., Hübers, H.-W., Huwe, B., Joshi, J., Kozyrovska, N., Kruchten, M., Lasch, P., Lee, N., Leuko, S., Leya, T., Lorek, A., Martínez-Frías, J., Meessen, J., Moritz, S., Moeller, R., Olsson-Francis, K., Onofri, S., Ott, S., Pacelli, C., Podolich, O., Rabbow, E., Reitz, G., Rettberg, P., Reva, O., Rothschild, L., Garcia Sancho, L., Schulze-Makuch, D., Selbmann, L., Serrano, P., Szewzyk, U., Verseux, C., Wadsworth, J., Wagner, D., Westall, F., Wolter, D., and Zucconi, L. (2019) Limits of life and the habitability of Mars: the ESA space experiment BIOMEX on the ISS. *Astrobiology* 19:145–157.
- Panitz, C., Frösler, J., Wingender, J., Flemming, H.-C., and Rettberg, P. (2019) Tolerances of *Deinococcus geothermalis* biofilms and planktonic cells exposed to space and simulated martian conditions in low Earth orbit for almost 2 years. *Astrobiology* 19:979–994.
- Rabbow, E., Rettberg, P., Barczyk, S., Bohmeier, M., Parpart, A., Panitz, C., Horneck, G., von Heise-Rotenburg, R., Hoppenbrouwers, T., Willnecker, R., Baglioni, P., Demets, R., Dettmann, J., and Reitz, G. (2012) EXPOSE-E: an ESA astrobiology mission 1.5 years in space. *Astrobiology* 12: 374–386.
- Rabbow, E., Rettberg, P., Barczyk, S., Bohmeier, M., Parpart, A., Panitz, C., Horneck, G., Burfeindt, J., Molter, F., Jaramillo, E., Pereira, C., Weiß, P., Willnecker, R., Demets, R., and Dettmann, J. (2015) The astrobiological mission EXPOSE-R on board of the International Space Station. *Int J Astrobiol* 14:3–16.
- Rabbow, E., Rettberg, P., Parpart, A., Panitz, C., Schulte, W., Molter, F., Jaramillo, E., Demets, R., Weiß, P., and Willnecker, R. (2017) EXPOSE-R2: the astrobiological ESA mission on board of the International Space Station. *Front Microbiol* 8, doi:10.3389/fmicb.2017.01533.
- Stalport, F., Rouquette, L., Poch, O., Dequaire, T., Chaouche-Mechidal, N., Payart, S., Szopa, C., Coll, P., Chaput, D., Jaber, M., Raulin, F., and Cottin, H. (2019) The Photo-

chemistry on Space Station (PSS) experiment: organic matter under Mars-like surface UV radiation conditions in low Earth orbit. *Astrobiology* 19:1037–1052.

Wadsworth, J., Rettberg, P., and Cockell, C.S. (2019) Aggregated cell masses provide protection against space extremes and a microhabitat for hitchhiking co-inhabitants. *Astrobiology* 19:995–1007.

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Abbreviations Used

BOSS = Biofilm Organisms Surfing Space
 EPS = extracellular polymeric substances
 IDPs = interplanetary dust particles
 ISS = International Space Station
 MGR = Mission Ground Reference
 PSS = Photochemistry on the Space Station