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Introduction

Limits of Life and the Habitability of Mars: The ESA Space Experiment BIOMEX on the ISS

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Abstract

BIOMEX (BIOlogy and Mars EXperiment) is an ESA/Roscosmos space exposure experiment housed within the exposure facility EXPOSE-R2 outside the Zvezda module on the International Space Station (ISS). The design of the multiuser facility supports—among others—the BIOMEX investigations into the stability and level of degradation of space-exposed biosignatures such as pigments, secondary metabolites, and cell surfaces in contact with a terrestrial and Mars analog mineral environment. In parallel, analysis on the viability of the investigated organisms has provided relevant data for evaluation of the habitability of Mars, for the limits of life, and for the likelihood of an interplanetary transfer of life (theory of lithopanspermia). In this project, lichens, archaea, bacteria, cyanobacteria, snow/permafrost algae, meristematic black fungi, and bryophytes from alpine and polar habitats were embedded, grown, and cultured on a mixture of martian and lunar regolith analogs or other terrestrial minerals. The organisms and regolith analogs and terrestrial mineral mixtures were then exposed to space and to simulated Mars-like conditions by way of the EXPOSE-R2 facility. In this special issue, we present the first set of data obtained in reference to our investigation into the habitability of Mars and limits of life. This project was initiated and implemented by the BIOMEX group, an international and interdisciplinary consortium of 30 institutes in 12 countries on 3 continents. Preflight tests for sample selection, results from ground-based simulation experiments, and the space experiments themselves are presented and include a complete overview of the scientific processes required for this space experiment and postflight analysis. The presented BIOMEX concept could be scaled up to future exposure experiments on the Moon and will serve as a pretest in low Earth orbit. Key Words: EXPOSE-R2—BIOMEX—Habitability—Limits of life—Extremophiles—Mars. Astrobiology 19, 145–157.

1. Results from Previous Spaceflight and Ground-Based Experiments

Previous experiments in spaceflight and ground-based studies, which were performed before the BIOMEX (BIOlogy and Mars EXperiment) proposal submission to ESA, showed that, in particular, microcolonies of bacteria, meristematic black fungi, and symbiotic associations of microorganisms such as lichens are able to survive and be reactivated after simulated and direct space experiments (Tarasenko *et al.,* 1990; Horneck *et al.,* 1994; de Vera *et al.,* 2003, 2004a, 2004b, 2007, 2008, 2010; de la Torre Noetzel *et al.,* 2007; Sancho *et al.,* 2007; Onofri *et al.,* 2008, 2010; Olsson-Francis *et al.,* 2009; de la Torre *et al.,* 2010; de Vera and Ott, 2010). Bacteria strains such as *Bacillus subtilis* and *Deinococcus radiodurans* have shown a certain radiation and vacuum tolerance (Horneck, 1993; Horneck *et al.,* 1994, 2001; Rettberg *et al.,* 2002, 2004; Möller *et al.,* 2007a, 2007b, 2007c; Pogoda de la Vega *et al.,* 2007; Wassmann *et al.,* 2012; Panitz *et al.,* 2014). Gram-negative endophytic bacteria and cyanobacteria survived a 14-day shuttle flight (within the shuttle interior) and exhibited enhanced plant colonizing activity in microgravity (Tarasenko *et al.,* 1990). During the BIOPAN 5 and 6 experiments, the lichens *Rhizocarpon geographicum* and *Xanthoria elegans* were analyzed after exposure to space conditions of about 11–14 days coupled with parallel tests in ground-based facilities. These results have led to the conclusion that the tested symbiotic eukaryotic associations of alga and fungi in the lichen were not seriously damaged, and nearly 70–100% of the tested lichens survived. The lichens were physiologically active and able to germinate and grow. Furthermore, investigations on the mutation rate of photoproducts on the DNA have shown that the mycobiont (the fungal symbiont) is practically unaffected by UV radiation and that the algal symbiont is more sensitive (de Vera *et al.,* 2003, 2004a, 2004b, 2007, 2008, 2010; de Vera, 2005; de la Torre Noetzel *et al.,* 2007; Sancho *et al.,* 2007; de la Torre *et al.,* 2010; de Vera and Ott, 2010). Cyanobacteria, as has been shown by analysis on akinetes (resting-state cells of cyanobacteria), were also able to survive the low Earth orbit and simulated extraterrestrial conditions (Olsson-Francis *et al.,* 2009), while vegetative cells of *Chroococcidiopsis* sp. CCMEE 029 survived prolonged desiccation periods (Billi, 2009; Fagliarone *et al.,* 2017) and a few minutes of exposure to an attenuated Mars-like UV flux and 4 h of exposure to a Mars-like UV flux (Cockell *et al.,* 2005). Numerous species mentioned in this study were even able to survive simulated catastrophes as induced by asteroid impact simulations (Horneck *et al.,* 2001, 2008; Stöffler *et al.,* 2007; Meyer *et al.,* 2011). Mars simulation tests with methanogenic archaea have also shown a remarkable level of survival and demonstrated physiological activity during exposure to Mars-like environmental conditions (Morozova and Wagner, 2007; Morozova *et al.,* 2007, 2015; Schirmack *et al.,* 2014). The same has been observed for meristematic black fungi during a ground-based experiment in the facilities at the German Aerospace Center (DLR) Cologne named EVT (Experiment Verification Test), which was performed for the Lichens and Fungi Experiment (LIFE) on EXPOSE-E (Onofri *et al.,* 2008) and after the final space experiment (Onofri *et al.,* 2012, 2015). In other ground-based experiments, we were able to show that *Paenibacillus* sp. caused biocorrosion of anorthosite rock (Lytvynenko *et al.,* 2006). In total, we can presume that a wide variety of different microorganisms, even from higher evolutionary advanced levels than those of archaea or bacteria, are able to resist and survive space and Mars-like conditions for a period of time (at least for 1.5 years). However, because of the limited capacity of the space exposure facilities, further work with replicates and other samples is still needed to finally answer questions on the degree of Mars' habitability or the kind of space and Mars-like environmental conditions that are limiting factors in reference to the most important vital functions of life (de Vera *et al.,* 2014; Schulze-Makuch *et al.,* 2015).

The BIOMEX results presented here further advance our knowledge and address pressing questions as mentioned above

TABLE 1. SELECTED SAMPLES FOR BIOMEX

BIOMEX selected samples for spaceflight	
Archaea	*Methanosarcina* sp. strain SMA-21 (terrestrial permafrost) (GFZ/AWI Potsdam)
Bacteria	*Deinococcus radiodurans* wild type and crtI or crtB (nonpigmented) (DLR Cologne)
	Biofilm containing *Leptothrix, Pedomicrobium, Pseudomonas, Hyphomonas, Tetrasphaera* (TU Berlin)
	Cyanobacterium *Nostoc* sp. strain CCCryo 231-06 (Fraunhofer IZI-BB)
	Cyanobacterium *Gloeocapsa* OU-20 (Astrobiology Center Edinburgh)
	Cyanobacterium *Chroococcidiopsis* sp. CCMEE 029 (Uni Roma)
Alga	Green alga *Sphaerocystis* sp. CCCryo 101-99 (Fraunhofer IZI-BB)
Lichens	*Circinaria gyrosa* (INTA)
	Buellia frigida (Antarctic lichen) (H-H-Uni Düsseldorf)
Fungi	Cryptoendolithic Antarctic black fungus *Cryomyces antarcticus* CCFEE 515 (Uni Viterbo)
Bryophytes	*Grimmia sessitana* (alpine samples) (Uni Potsdam)
	Marchantia polymorpha L. (Uni Potsdam)
Biomolecules	Pigment Chlorophyll (H-H-Uni Düsseldorf)
	Pigment *beta*-Carotene (H-H-Uni Düsseldorf)
	Pigment Naringenin (H-H-Uni Düsseldorf)
	Pigment Quercitin (H-H-Uni Düsseldorf)
	Pigment Parietin (H-H-Uni Düsseldorf)
	Pigment Melanin (H-H-Uni Düsseldorf)
	Cellulose (H-H-Uni Düsseldorf)
	Chitin (H-H-Uni Düsseldorf)
Biofilm	Kombucha biofilm containing: Yeasts: *Saccharomyces ludwigii, Schizosaccharomyces pombe, Zygosaccharomyces rouxii, Zygosaccharomyces bailii, Brettanomyces bruxellensis;* Bacteria: *Paenibacillus* sp. IMBG221, *Acetobacter nitrogenifigens, Gluconacetobacter kombuchae* sp. nov., *Gluconacetobacter xylinum* (NAS Ukraine)
Substrates/Minerals	Agar (as a substitute for Murein) (H-H-Uni Düsseldorf)
	Minerals lunar analog mixture (MfN Berlin)
	Minerals P-MRS: Early acidic Mars analog (Mixture of Fe_2O_3, montmorillonite, chamosite, kaolinite, siderite, hydromagnesite, quartz, gabbro, and dunite) (MfN Berlin)
	Minerals S-MRS: Late basic Mars analog (Mixture of hematite, goethite, gypsum, quartz, gabbro, dunite) (MfN Berlin)
	Silica discs (glass) (Astrobiology Center Edinburgh)

Gray shaded cells indicate the samples for which results are available and included in this special collection.

Table 2. Mars and Lunar Analog Mineral Mixtures

Component	*P-MRS (wt %)*	*S-MRS (wt %)*	*LRA (wt %)*
Gabbro (Groß-Bieberau, Germany)	3	32	-
Dunite—Olivine Fo_{96} (Åheim, Norway)	2	15	5.7
CPx—Diopside (Kragerö, Norway)	-	-	8.9
OPx—Hyperstehn (Egersund, Norway)	-	-	5.7
Anorthosite—Plagioclase (Larvik, Norway)	-	-	66.8
Quarzite (Bayerischen Wald, Germany)	10	3	-
Apatite (Minas Gerais, Brasil)	-	-	1.1
Hematite (Cerro Bolivar, Venezuela)	5	13	-
Illmenite (Flekkefiord, Norway)	-	-	1.1
Iron (Fe)	-	-	1.3
Montmorillonite (Hallertau, Germany)	45	-	-
Chamosite (Nucic, Czech Republic)	20	-	-
Kaolinite (Hirschau, Germany)	5	-	-
Siderite (Hüttenberg, Austria)	5	-	-
Hydromagnesite (Albaner Berge, Italy)	5	-	-
Goethite (Salchendorf, Germany)	-	7	-
Gypsum (Nüttermoor, Germany)	-	30	-
Volcanic slag (Aeolian islands, Italy)	-	-	9.4

P-MRS: phyllosilicatic martian regolith = early acidic. S-MRS: sulfatic martian regolith = late basic. LRA: lunar regolith analog.

to an extended degree in comparison to previously executed space experiments, which, for the most part, were more restricted and focused on investigating the likelihood of an interplanetary transfer of life as is formulated in the lithopanspermia hypothesis (Richter, 1865; Thomson, 1894; Arrhenius, 1903; see also Lee *et al.,* 2017). Accordingly, these new BIOMEX experiments in space were intended to address new questions in planetary research and improve future space exploration goals. Nevertheless, it is clear that the results obtained by BIOMEX could also be used to evaluate previously performed space experiments in reference to lithopanspermia.

2. Sample Selection

As a consequence of results obtained in previous space experiments that engendered a significant number of still-open questions, a proposal named BIOMEX (ILSRA-2009-0834) was submitted in 2009. This was in response to the ESA international research announcement for research in space life sciences at the International Space Station (ISS)—ILSRA-2009—and BIOMEX was successfully selected. The proposal included replicate exposure of known species used in previous space experiments such as the reassessed Antarctic

FIG. 1. Mars analog pellets integrated in the EXPOSE-R2 hardware.

fungus *Cryomyces antarcticus,* the cyanobacterium *Chroococcidiopsis* sp., the lichen *Circinaria gyrosa* (formerly known as *Aspicilia fruticulosa* before reclassification; Sohrabi *et al.,* 2013), and a set of new, preselected organisms for further preflight experiments (see Table 1). After survival of these organisms was shown, the samples were incorporated into the BIOMEX experiment, integrated into the final EXPOSE-R2 hardware, and sent to space on the ISS.

This new sample set was chosen systematically and comprised a selection from archaea, bacteria, and eukaryotes, which represent the three main domains of the tree of life. Most of these organisms were collected from Mars analog habitats distributed on different continents, which include the Alps (climatic and geomorphologic Mars analogy: gullies, polygons, temperatures below 0°C, dryness, and elevated UV irradiation), the steppe highlands of Central Spain (characterized by extreme insolation, high temperature contrasts, and arid summers Crespo and Barreno, 1978]), and regions in the Arctic and Antarctica. The aim of selecting a wide variety of species was to identify which are able to demonstrate the limits of life with regard to the applied space and Mars-like conditions in low Earth orbit, as well as further our understanding of the kind of species for which Mars could be habitable.

2.1. Biological samples

A set of organisms was tested, which were embedded in, or grown on, Mars (optionally lunar) analogs and other terrestrial minerals. Bacteria, biofilms of bacteria and yeast species, cyanobacteria, archaea, lichens, snow/permafrost algae, meristematic black fungi, and bryophytes of mostly alpine and polar habitats of desiccation- and radiation-resistant strains were chosen because some of these organisms are thought to be among the oldest on Earth (Wang *et al.,* 1999; Schidlowski, 2001; Campbell *et al.,* 2003; Yuan *et al.,* 2005) and, over time, have evolutionarily adapted to different environmental conditions. Some of these organisms could even be Mars-relevant because they use Mars-atmosphere resources such as CO_2 to form methane, a trace gas found remotely in the martian atmosphere (Formisano *et al.,* 2004; Mumma *et al.,* 2009) and *in situ* at Gale Crater by way of the rover Curiosity (Webster *et al.,* 2015). The processes that lead to extreme variations in the methane concentration of the martian atmosphere, in particular the potential for abiogenic origin (Lefèvre and Forget, 2009), have recently been reviewed (Yung *et al.,* 2018). In previous studies, some of the organisms studied in the BIOMEX experiments have also exhibited a high resistance under simulated and real space conditions or simulated martian conditions. Others, like the newly selected bryophytes, provide insights on the resistance capabilities of an evolutionarily younger life-form. Details on the selected species are listed in Table 1.

2.2. Mars and lunar analog mineral mixtures

With the BIOMEX experiment, our goal was to analyze the effects of a space environment that approaches as closely as possible Mars-like environmental conditions and includes the use of Mars analog mixtures that could serve as a substrate or an embedding matrix for biological samples (see Table 2 and Figs. 1 and 2). These Mars analog mineral mixtures mimic the regolith cover from early and late evolutionary stages of Mars (Böttger *et al.,* 2012). The components of the mixtures were developed in the Museum für Naturkunde (MfN) Berlin (Germany) in the context of the Helmholtz-Alliance ''Planetary Evolution and Life'' proposal and based on several observational studies (Bibring *et al.,* 2005, 2006; Poulet *et al.,* 2005; Chevrier and Mathé, 2007). It is important to test the effects of space and the martian environment on minerals in parallel biological investigations. A welcome consequence of this space experiment would be that the investigated samples would also be tested for viability and space-resistance capacity and provide valuable data if used in a replicate space experiment with regard to the probability of lithopanspermia in the Earth-Mars system. The lithopanspermia hypothesis has also been investigated in previous space experiments on FOTON/BIOPAN some years ago and on the EXPOSE-E mission on the ISS. However, replicates are still needed. Besides the Mars analog mineral mixtures, the MfN also provided a lunar regolith analog (see Table 2 and Figs. 1 and 3) for investigation into the influence of the lunar surface material on organisms, which could be relevant for life-support systems such as the selected and tested cyanobacteria (not part of this special collection of articles).

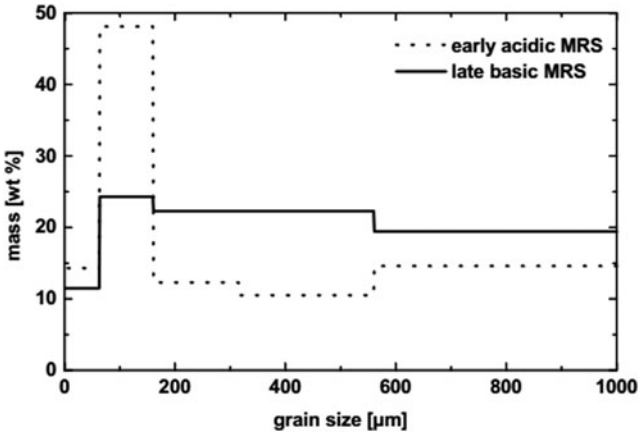

FIG. 2. Grain size distribution of martian regolith analog P-MRS (early acidic MRS) and S-MRS (late basic MRS).

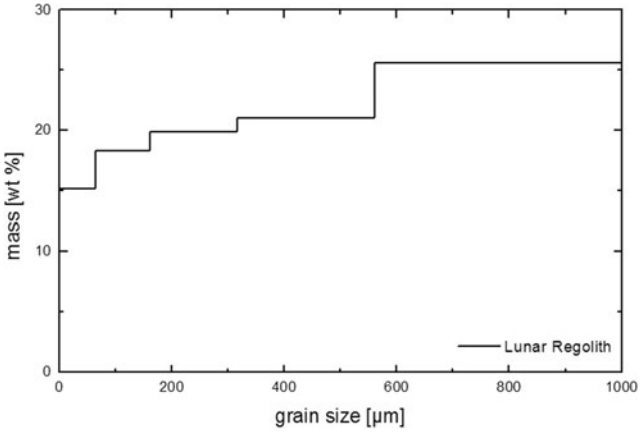

FIG. 3. Grain size distribution of lunar regolith analog material LRA.

Table 3. Experiment Verification Tests (EVTs)

EXPOSE-R2 EVT part 1	*BIOMEX Experiment*
Test parameter	*performed*
Vacuum 10^{-5} Pa	7 d, pressure: 3.5×10^{-2} $\pm$0.12 Pa
Mars atmosphere (CO_2 gas composition) 103 Pa	7 d, pressure: 6.5×10^{2} $\pm$0.12 Pa
Temperature −10°C to +45°C	48 cycles 8 h each
Temperature max and min −25°C and +60°C	−25°C $\pm$ 0.5°C, 1 h +60°C $\pm$ 0.5°C, 1 h
Irradiation 254 nm Hg low-pressure lamp @ 56 μW/cm^2	0 s → 0 J/m^2 18 s → 10.1 J/m^2 2 min 59 s → 100.2 J/m^2 29 min 46 s → 1000.2 J/m^2 4 h 57 min 37 s → 9999.9 J/m^2
EXPOSE-R2 EVT part 2 (run 1+2)	*BIOMEX Experiment*
Run 1 Irradiation 200–400 nm SOL2000 @ 1,271.2 W/m$^2_{200–400nm}$	0 s → dark 18 min → 1.4×10^{3} kJ/m^2 3 h → 1.4×10^{4} kJ/m^2 30 h → 1.4×10^{5} kJ/m^2 99 h → 4.5×10^{5} kJ/m^2 148 h → 6.8×10^{5} kJ/m^2
Run 2 Irradiation 200–400 nm SOL2000 @ 1,271.2 W/m$^2_{200–400nm}$ (as for a 12-month mission duration)	0 s → dark 432 s → 5.5×10^{2} kJ/m^2 (0.1% ND filter) 1 h 12 min → 5.5×10^{3} kJ/m^2 (1.0% ND filter) 30 h → 1.4×10^{5} kJ/m^2 60 h → 2.7×10^{5} kJ/m^2 120 h → 5.5×10^{5} kJ/m^2
Gluing test	>24 h vulcanization, glue: Wacker-silicone

ND: neutral density.

2.3. Ground-based environmental and space simulations

Many preflight tests were performed to discern whether the chosen samples (Fig. 4) are able to resist extreme conditions with reference to space and martian environments. After an array of experiments on Mars-like regolith and desiccation tests, the organisms were exposed to Experiment Verification Tests (EVTs) and Scientific Verification Tests (SVTs) (Rabbow *et al.,* 2017). In the EVTs, a number of parameters were tested individually on the selected samples. Among these tests, vacuum, a low-pressure Mars-like CO_2 atmosphere, extreme temperature cycles from far below zero to more than 40°C, and UVC irradiation were applied (see Table 3). The SVT experiments were conducted inside hardware with conditions that approached those of the space environment at the ISS (see Table 4).

2.4. Radiation conditions in space

For several of the BIOMEX experiments, we used different neutral density filters (Fig. 5) as covers below the sample window or as cutoff filters, which allowed for a radiation spectral range such as that present at the surface of Mars. For the more protected samples, doses varied between 4.13×10^{1} kJ/m^2 and 6.5×10^{3} kJ/m^2 (see Fig. 5 for details). Samples such as the cyanobacteria of the genera *Nostoc, Gloeocapsa,* and *Chroococcidiopsis;* the green snow/permafrost alga *Sphaerocystis;* and the fungus *Cryomyces antarcticus* were partly shielded by special MgF_2 neutral density filters with transmission of 0.1% for space conditions and quartz filters with transmission of 0.1% for Mars-like conditions (for detailed description of the EXPOSE-R2 facility and the space mission, see Rabbow *et al.* [2017]). These allowed for an approximation of martian subsurface radiation conditions, representing a thin soil cover with a significantly reduced amount of transmission. The reason for these filters, which covered select samples, was also to mimic those conditions that occur in natural habitats where organisms primarily colonize endolithic niches or fissures and cracks in rocks or soils or are embedded by shielding snow or ice. Some of these organisms occur naturally as endoliths, such as *Chroococcidiopsis* sp. and *Cryomyces antarcticus*. Furthermore, the neutral density filters with 0.1% transmission

Table 4. Scientific Verification Tests (SVTs)

SVT	*Duration*	*Pressure*	*Atmosphere*	*Temperature* (T)	T *extremes*	*Irradiation*
Tray 1	December 2013–January 2014, 38 d	vacuum pressure at 4.1×10^{-5} Pa		*T* cycles between −25°C (16 h in the dark) and +10°C (8 h during irradiation)		The upper layers of each tray: $UVR_{200–400nm}$ with 1271 Wm^{-2} (5.7×10^{5} kJ m^{-2}) for 5924 min The lower layers of the trays were kept in the dark
Tray 2			Mars atmosphere (95.55% CO_2, 2.7% N_2, 1.6% Ar, 0.15% O_2, and ~370 ppm H_2O at 1 kPa)	−23°C		

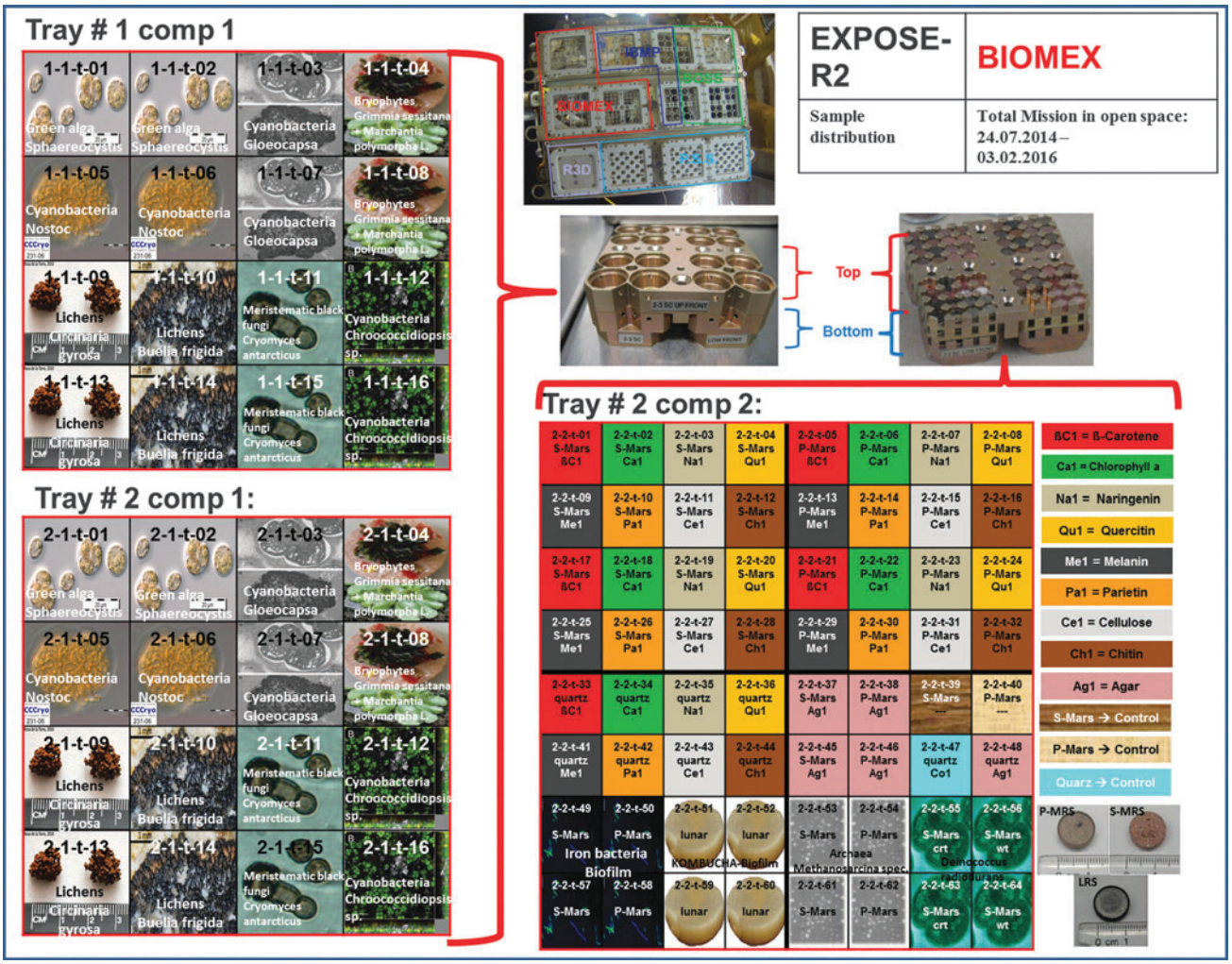

FIG. 4. Visual table of the sample distribution within the EXPOSE-R2 hardware.

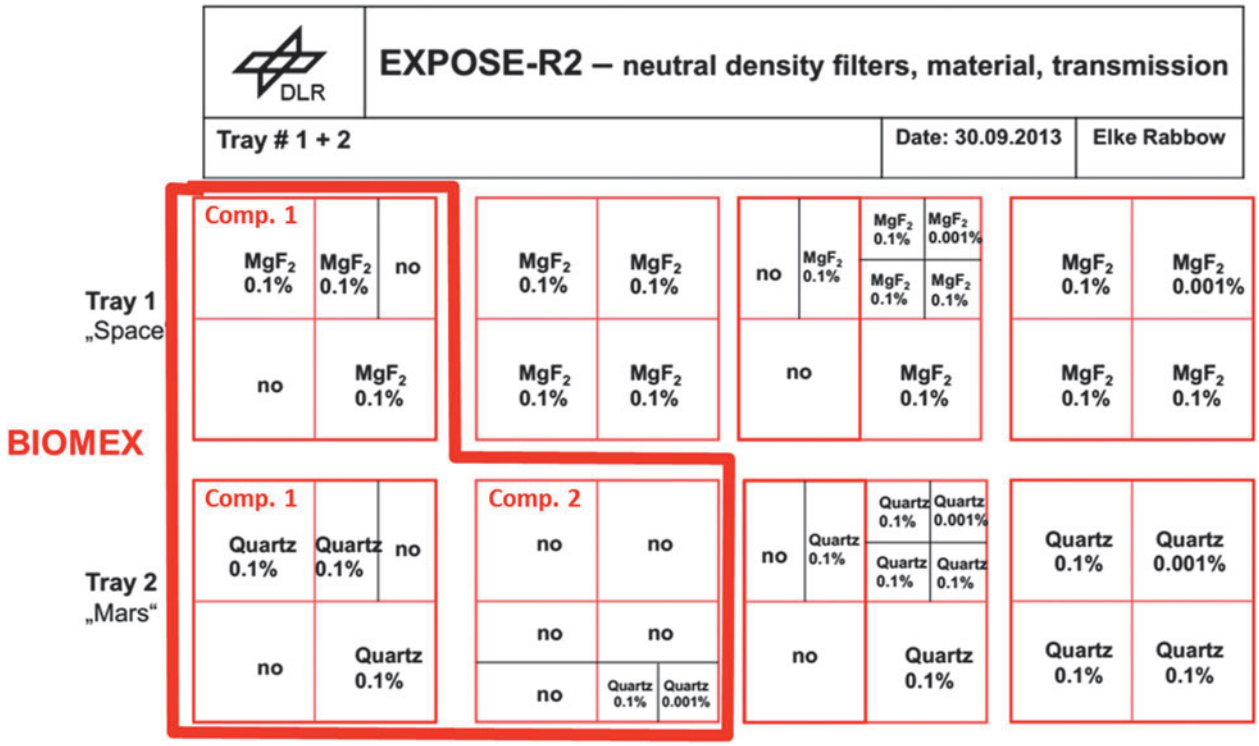

FIG. 5. The distribution of neutral density filters and the values of transmission depending on the used material.

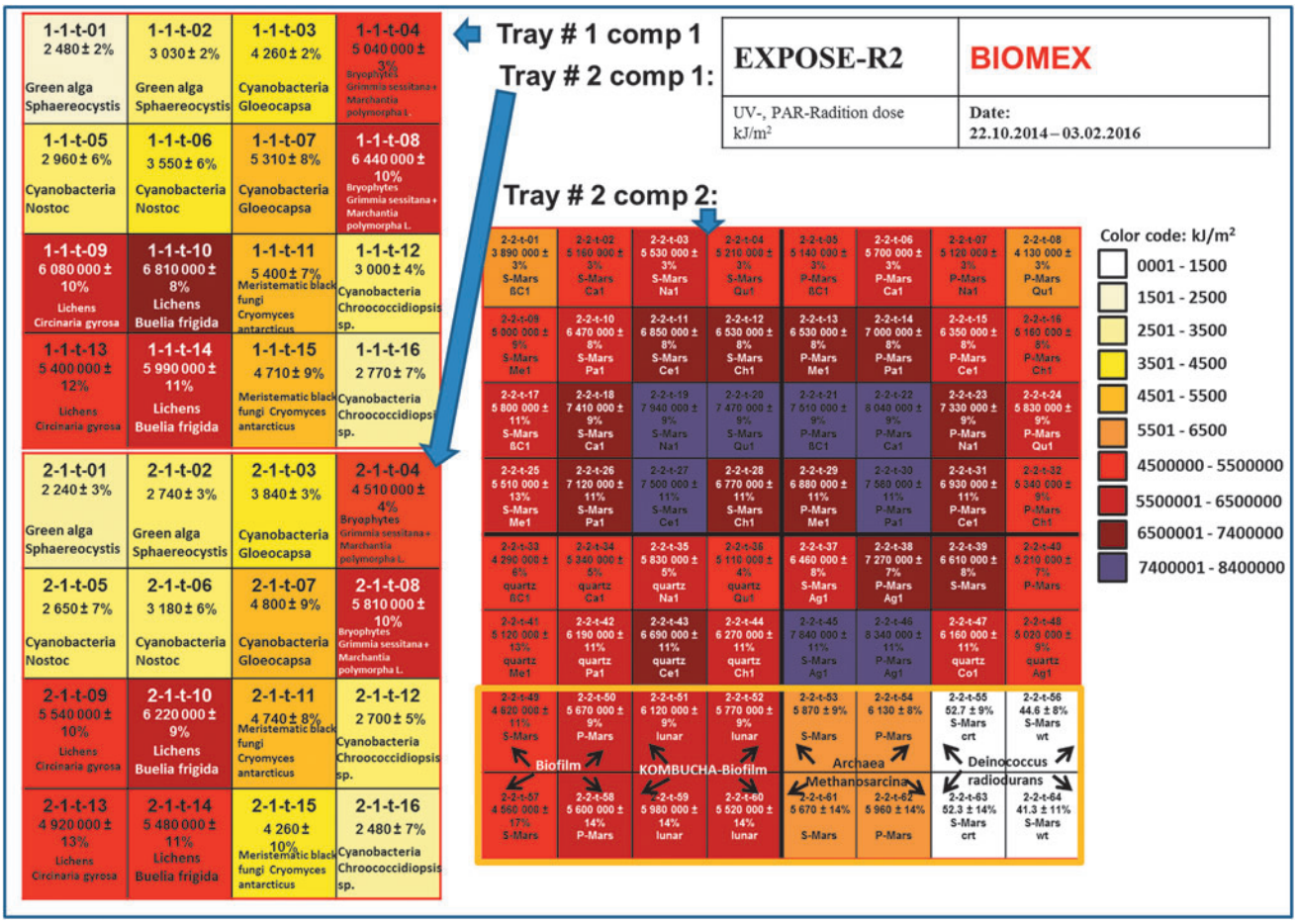

FIG. 6. Final UV/PAR radiation dose distribution on the BIOMEX compartments within EXPOSE-R2 after exposure on the ISS (data provided by RedShift).

TABLE 5. RESULTS LISTED ACCORDING TO THE TOPICS ''LIMITS OF LIFE'' AND HABITABILITY OF LIFE

Sample category / domain	species	Results on limits of life			Results on Habitability of Mars		
		selection tests	EVT/SVT	space	selection tests	EVT/SVT	space
Archaea	*Methanosarcina* sp.strain SMA-21 (terrestrial permafrost) (GFZ/AWI Potsdam)	±			+		
Bacteria	Cyanobacterium *Chroococcidiopsis sp.* CCMEE 029 (Uni Roma)			+			+
Fungi	Cryptoendolithic Antarctic black fungus *Cryomyces antarcticus* CCFEE 515 (Uni Viterbo)		+	±		+	±
Lichens	*Circinaria gyrosa* (INTA) *Buellia frigida* (Antarctic lichen) (H-H-Uni Düsseldorf)			± ±			± ±
Bryophytes	*Grimmia sessitana* (alpine samples) (Uni Potsdam)		±			±	
Biofilm	<u>*KOMBUCHA* Biofilm containing:</u> <u>Yeasts:</u> *Saccharomyces ludwigii, Schizosaccharomyces pombe, Zygosaccharomyces rouxii, Zygosaccharomyces bailii, Brettanomyces bruxellensis*; <u>Bacteria:</u> *Paenibacillus* sp. IMBG221, *Acetobacter nitrogenifigens*, *Gluconacetobacter kombuchae* sp. nov., *Gluconacetobacter xylinum.*(NAS Ukraine)			±			±

(+) Survival / metabolically active / growth capacity, (±) partly survival, more damaged

were additionally applied to the samples of the methanogen archaeon *Methanosarcina soligelidi* SMA 21. In this case, these filters were used because of the specific nature of this organism's original habitat, which is situated within permafrost-affected soils and protected by soil particles with different grain sizes. *Deinococcus radiodurans* was covered by neutral density filters with a transmission of 0.01%. The measured and calculated data with regard to the final doses the samples experienced, which included UVA, UVB, UVC, PAR (photosynthetically active radiation), and Lyman alpha, are represented in Fig. 6 and were kindly provided by ESA via computations completed by the company RedShift Design and Engineering BVBA.

Significant variation was observed in the dose of UV among samples that were placed within sample sites not protected by filters. The observed variations could be explained by the dependence of the sample position in the hardware, which would have been exposed to a variety of shadowing effects during the orbit of the ISS. When filters were not used in the BIOMEX experiments, the final doses varied between 4.5×10^6 and 8.4×10^6 kJ/m^2. Specific organisms exposed without any neutral density filters include the epilithic lichens *Circinaria gyrosa* and *Buellia frigida,* the epilithically living bryophytes *Grimmia* sp. and *Marchantia polymorpha,* the iron bacteria biofilm, and the kombucha biofilm. Biomolecules exposed on the surface and embedded in the Mars analog mineral pellets also endured the same direct space conditions without any neutral density filters.

3. Overview of Results within This Special Collection

The results presented in this special collection are arranged to provide an overview of each step of the processes involved in the BIOMEX experiment, that is, from selecting

TABLE 6. DETAILED RESULT LIST EXPLAINING THE CLASSIFICATION SHOWN IN TABLE 5

Sample category / domain	species	Results on limits of life			Results on Habitability of Mars		
		selection tests	EVT/SVT	space	selection tests	EVT/SVT	space
Archaea	*Methanosarcina* sp.strain SMA-21 (terrestrial permafrost) (GFZ/AWI Potsdam)	Decrease of CH_4 production rate on the used Mars-analog high concentration of perchlorate (not applied in BIOMEX space exposure)			CH_4 production rate on the used Mars-analog minerals stable (but decrease on high concentations of perchlorates / not applied in BIOMEX space exposure)		
Bacteria	Cyanobacterium *Chroococcidiopsis* sp. CCMEE 029 (Uni Roma)			Survival and recovery on Mars analog minerals, low DNA-damage particularly in the more protected endolithically dark control areas			Survival and recovery on Mars analog minerals, low DNA-damage particularly in the more protected endolithically dark control areas
Fungi	Cryptoendolithic Antarctic black fungus *Cryomyces antarcticus CCFEE 535* (Uni Viterbo)		On Mars-analog S-MRS with and without direct irradiation no significant decrease in growth and no DNA damage. On (non)irradiated samples with P-MRS slight decrease of growth and slight increase of DNA damage. Original sandstone material and LRA under simulated space and Mars conditions slightly affect the growth	On irradiated S-MRS 40 % still growing, on irradiated P-MRS 79 % growing. In Martian atmosphere without irradiation on S-MRS about 55 % growth and on P-MRS about less than 20 % growth .In all cases within both MRS significant membrane damage. On LRA no significant changings in growth and less damaged membranes		On Mars-analog S-MRS with and without direct irradiation no significant decrease in growth or increase of DNA damage and on (non)irradiated samples with P-MRS slight decrease of growth and slight increase of DNA damage.	On irradiated S-MRS 40 % still growing, on irradiated P-MRS 79 % growing. In Martian atmosphere without irradiation on S-MRS about 55 % growth and on P-MRS about less than 20 % growth.In all cases within both MRS significant membrane damage.
Lichens	*Circinaria gyrosa* (INTA) *Buellia frigida (antarctic lichen) (H-H-Uni Düsseldorf)*			Quick moderate-high recovery of the PSII activity in the space dark control (vacuum and Mars atmosphere), where also morphology and DNA stability was observed. But irradiated samples under the same conditions were significantly affected. In the cultivation assay only the space exposed algal symbionts are still able to grow and form colonies. These results indicate that the lichen Buellia frigida is partly able to survive low earth orbit conditions.			Quick moderate-high recovery of the PSII activity in the space dark control (vacuum and Mars atmosphere), where also morphology and DNA stability was observed. But irradiated samples under the same conditions were significantly affected. The post-exposed lichen symbionts show after LIVE/DEAD staining viability rates of up to 23.6% for the algal and up to 10.5% for the fungal symbiont. This means Mars is less habitable for Buelia frigida.
Bryophytes	*Grimmia sessitana (alpine samples) (Uni Potsdam)*		The mosses were still vital after doses of radiation expected during the EXPOSE-R2 mission on the ISS. This earliest extant lineage of land plants is highly resistant to extreme abiotic simulated space and Mars-like conditions.			The mosses were still vital after doses of radiation expected during the EXPOSE-R2 mission on the ISS. This earliest extant lineage of land plants is highly resistant to extreme abiotic simulated space and Mars-like conditions.	
Biofilm	*KOMBUCHA Biofilm containing: Yeasts: Saccharomyces ludwigii, Schizosaccaromyces pombe, Zygosaccharomyces rouxii, Zygosaccharomyces bailii, Brettanomyces bruxellensis; Bacteria: Paenibacillus sp. IMBG221, Acetobacter nitrogenifigens, Gluconacetobacter kombuchae sp. nov., Gluconacetobacter xylinum.(NAS Ukraine)*			After returning to Earth, the space-flown bacterial-yeast community recovered in two months. Within the UV-irradiated samples, a degradation of DNA, changes in the cellular membranes, and an inhibition of cellulose synthesis were observed. After a series of culture experiments, the revived communities restored partially their composition and the associated activities.			After returning to Earth, the space-flown bacterial-yeast community recovered in two months. Within the UV-irradiated samples, a degradation of DNA, changes in the cellular membranes, and an inhibition of cellulose synthesis were observed. After a series of culture experiments, the communities restored partially their composition and the associated activities.

S-MRS: sulfatic martian regolith. P-MRS: phyllosilicatic martian regolith. LRA: lunar regolith analog.

samples for simulation experiments to the final exposure experiments in space. Results from pretests on the chosen methanogenic archaeon of the genus *Methanosarcina* at the preselection level are reported in Serrano *et al.* (2019). This archaeon was chosen because of its relevance with regard to its potential for being metabolically active on Mars. Therefore, the first tests were designed to use different substrates that contained magnesium perchlorate to establish a Mars-relevant perchlorate environment and mineral mixture before attempting the next selection step of applying atmospheric and radiation-related environmental conditions within the preflight experiments EVTs and SVTs. With regard to the EVTs and SVTs, we present the results obtained by analysis of the fungus *Cryomyces antarcticus* (Pacelli *et al.,* 2019) and the moss *Grimmia* sp. (Huwe *et al.,* 2019). Results obtained after space exposure are shown from a series of analyses on the biofilm kombucha (Podolich *et al.,* 2019), the cyanobacterium *Chroococcidiopsis* (Billi *et al.,* 2019), the cryptoendolithic Antarctic fungus *Cryomyces antarcticus* (Onofri *et al.,* 2019), and the lichens *Buellia frigida* (Backhaus *et al.*, this issue) and *Circinaria gyrosa* (de la Torre *et al.*, not part of this issue). A rough summary of the different studies is given in Table 5, and more details are shown in Table 6. Survival, physiological activity, and growth capacity were detected in all organisms tested. However, life's vital functions decreased from slight to significant, and the reader is directed to specific articles in this collection for detailed discussion of these findings. Several of the selected archaea, bacteria, and heterogenic multilayered biofilms formed by a multitude of species were found to be the most resistant to simulated or direct space and Mars-like conditions. Less resistance and a significant decrease in cell numbers and vitality with regard to the Mars-like environment were shown for multicellular life-forms such as the tested fungus *Cryomyces antarcticus* (Onofri *et al.,* 2019) and the lichens *Buellia frigida* (Backhaus *et al.,* 2019) and *Circinaria gyrosa* (de la Torre Noetzel *et al.*, 2019). The bryophyte *Grimmia* sp. was an exception (Huwe *et al.,* 2019), but further analysis after space exposure might show whether this specific moss is also resistant to the conditions in space. Actual results in reference to the bryophytes were shown only for the preflight selection mode of EVT and SVT. Our results so far indicate that present Mars seems to be habitable for archaea and bacteria over longer timescales. However, a clearer understanding of the limits of life would be achievable with the implementation of extended space exposure experiments on the Moon, for example, with similar space exposure facilities as those used in the present study (see de Vera *et al.,* 2012).

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Abbreviations Used

BIOMEX = BIOlogy and Mars EXperiment
DLR = German Aerospace Center
EVTs = Experiment Verification Tests
ISS = International Space Station
MfN = Museum für Naturkunde
PAR = photosynthetically active radiation
SVTs = Scientific Verification Tests