Orig Life Evol Biosph (2012) 42:253–262
DOI 10.1007/s11084-012-9282-5

ASTROBIOLOGY

LIFE Experiment: Isolation of Cryptoendolithic Organisms from Antarctic Colonized Sandstone Exposed to Space and Simulated Mars Conditions on the International Space Station

Giuliano Scalzi · Laura Selbmann · Laura Zucconi · Elke Rabbow · Gerda Horneck · Patrizia Albertano · Silvano Onofri

Received: 5 December 2011 / Accepted: 5 April 2012 /
Published online: 12 June 2012

Abstract Desiccated Antarctic rocks colonized by cryptoendolithic communities were exposed on the International Space Station (ISS) to space and simulated Mars conditions (LiFE—Lichens and Fungi Experiment). After 1.5 years in space samples were retrieved, rehydrated and spread on different culture media. Colonies of a green alga and a pink-coloured fungus developed on Malt-Agar medium; they were isolated from a sample exposed to simulated Mars conditions beneath a 0.1 %T Suprasil neutral density filter and from a sample exposed to space vacuum without solar radiation exposure, respectively. None of the other flight samples showed any growth after incubation. The two organisms able to grow were identified at genus level by Small SubUnit (SSU) and Internal Transcribed Spacer (ITS) rDNA sequencing as *Stichococcus* sp. (green alga) and *Acarospora* sp. (lichenized fungal genus) respectively. The data in the present study provide experimental information on the possibility of eukaryotic life transfer from one planet to another by means of rocks and of survival in Mars environment.

Paper presented at the 11th European Workshop on Astrobiology—EANA 11, 11th–14th July 2011, Köln, Germany

Patrizia Albertano died on March, 14th 2012. She fully contributed to the manuscript with her research, and writing, until her death.

G. Scalzi (✉) · L. Selbmann · L. Zucconi · S. Onofri
Department of Ecological and Biological Sciences (DEB), University of Tuscia,
Largo dell'Università snc, 01100 Viterbo, Italy
e-mail: scalzig@gmail.com

E. Rabbow · G. Horneck
German Aerospace Centre, Institute of Aerospace Medicine, Linder Hoehe, 51170 Köln, Germany

P. Albertano
Department of Biology, University of Rome "Tor Vergata", via della Ricerca Scientifica snc,
00133 Rome, Italy

Keywords Antarctic colonized rocks · EXPOSE-E · International space station · Lithopanspermia · Lichens and Fungi Experiment

Introduction

The Lithopanspermia hypothesis suggests that impact-ejected rocks could transfer living organisms through space from one planet to another (Horneck et al. 2010). This scenario implies that rock-embedded organisms need to survive (i) the ejection into space within rock fragments, (ii) the journey through space for a long time (hundreds up to thousands or millions of years) and (iii) the landing on another planet (Gladman et al. 1996; Mileikowsky et al. 2000; Horneck et al. 2008; Nicholson 2009).

Despite an ever-expanding understanding of the limits of life in the Earth's most extreme environments, little remains known about life's potential to survive when removed from Earth and the all-encompassing biosphere that harbors it. Scientific literature has numerous reports on microbial survival and proliferation in the most inhospitable environments that our planet has to offer (Rothschild and Mancinelli 2001; Venkateswaran et al. 2001; Canganella and Wiegel 2011). All the while, reports addressing the uppermost limits of microbial survival in near-Earth orbit (Horneck et al. 2010), in the interplanetary (Mileikowsky et al. 2000) or interstellar space are scarce (Valtonen et al. 2009). Early studies of long-term survival in outer space have dealt with prokaryotes, mostly bacterial endospores (e.g., Horneck et al. 1994). Later on, studies were extended to eukaryotes exposed to either simulated space conditions or space during short-term flights, such as the experiments in the framework of ESA's Biopan missions (Sancho et al. 2007; de la Torre et al. 2010; Raggio et al. 2011). The lichens *Rhizocarpon geographicum, Xanthoria elegans* and *Aspicilia fruticulosa* were exposed to space environment for about 10 days; long term experiments in space on eukaryotic test organisms have been done for the first time within this LIFE experiment. To ascertain the uppermost extreme limits of life, it is important to start examining a wider spectrum of microbiota, including those rock-embedded communities from extreme environments such as Antarctic deserts, and expose them to space in their integrity, i.e. in their natural substrate.

Cryptoendolithic communities are among the most resistant life forms ever encountered, originally discovered in the extremely cold, hyper-arid Dry Valleys of Antarctica (Friedmann 1982). In structural cavities of Antarctic sandstones lichenized and non-lichenized fungi and algae (cryptoendoliths), bacteria, cyanobacteria, form the lichen-dominated cryptoendolithic community (Friedmann 1982). Their extreme environment is considered the most similar terrestrial environment to Mars surface (Wynn-Williams and Edwards 2000; Onofri et al. 2004); it is characterized by very low temperatures, ranging from −20 to −50 °C in winter, with annual average temperature below 0 °C, wide thermal fluctuations, frequent freeze/thaw cycles, dryness due to the lack of snow or ice cover and rare precipitations (<100 mm water equivalent per year), high salt concentrations, low nutrient availability and high radiation including UV (Onofri et al. 2007). Molecular studies of environmental DNA extracts from these communities suggested the identity of an endolithic fungal phylotype with adjacent epilithically growing *Buellia* spp. (>97 % similarity of SSU rDNA sequences; de la Torre et al. 2003). The same authors highlighted the microbial biodiversity in these communities comprising lichen mycobionts, free-living fungi, algae, as well as *Actinobacteria*, *Alphaproteobacteria*, *Gammaproteobacteria* and some other unidentified bacterial phylotypes. Some new genera and species of black meristematic fungi have also been described, but most taxa have not been formally described (Selbmann et al. 2005; Selbmann et al. 2008). The increasing knowledge about diverse eukaryotes thriving in such harsh

conditions suggested to test the survival of a whole rock community in a space flight experiment under space and simulated Martian conditions.

In this LIFE experiment, fragments of sandstone colonized by cryptoendolithic communities from the McMurdo Dry Valleys (Antarctica) were flown in the European EXPOSE-E facility attached to the exterior of the ISS. Their suitability was tested before flight in ground-based Experiment Verification Tests (Onofri et al. 2009). The samples were exposed for 18 months to the environment of space in Low Earth Orbit (LEO) and to simulated Mars conditions. Once returned to Earth they were subjected to both, molecular and cultivation-based assays to estimate survival and viability (Onofri et al. 2012) including growth ability.

Materials and Methods

Colonized Sandstone

A sample of colonized porous sandstone of the Beacon Supergroup was collected by L. Zucconi at Battleship Promontory (76°54′37.6″S 160°55′27.5″E), Southern Victoria Land, Antarctica in January 2004; it was stored under sterile conditions at −20 °C. The samples were individually inspected under the stereomicroscope along the lengthwise section of the substrate, where they presented the typical colored bands, indicating the presence of active and living lichen dominated microbial cryptoendolithic community (Friedmann 1982; Nienow and Friedmann 1993; Friedmann and Weed 1987). Well colonized sandstone fragments (11 mm wide, maximum 6 mm thick, on average 437 mg) including remarkable portions of microbial biomass were removed under sterile conditions by striking the sandstone sample lengthwise with a rock hammer. Excised fragments were dehydrated at room temperature and glued, using Wacker silicone glue RTV-S 69, on sterile teflon disks (12 mm diameter). These fragments were successively accommodated in different trays of the EXPOSE-E facility.

EXPOSE-E Facility

The European Space Agency (ESA) has recently developed the multi-user research facility EXPOSE-E as an exterior partition of the ISS (Rabbow et al. 2009) (Fig. 1). This facility is designed to foster and promote astrobiology research including studies on the survival of, and genotypic and phenotypic changes in, model organisms (plant seeds, bacterial spores, black meristematic fungi, lichens, and cryptoendolithic communities) when exposed to the outer space environment.

The EXPOSE-E facility is a multi-user facility having a box-shaped structure, accommodating samples in three different and separate compartments, called trays (Fig. 1). Each tray is subdivided in four sample carriers composed of 16 wells disposed on two levels, to face either outward towards space and radiations or kept beneath at the same conditions but in the darkness. The LIFE samples were exposed either in Tray 1 that was vented with access to space vacuum or in Tray 2 that was sealed and filled with a simulated Mars gas mixture. The carriers were covered by an optical filter system to control intensity and spectral range of solar UV irradiation. The conditions to which the Antarctic sandstone fragments were exposed are listed in Table 1. During the 1.5 year lasting mission the rock samples experienced space vacuum (10^{-7} to 10^{-4} Pa) (Horneck et al. 2010), cosmic radiation (≤190 mGy) (Berger et al. 2012) and the full spectrum of solar extraterrestrial electromagnetic radiation (for space exposed samples with a long pass cut-off at 110 nm, and for simulated Mars condition samples with a long pass cut off at 200 nm). The UV (200–400 nm) fluences reached

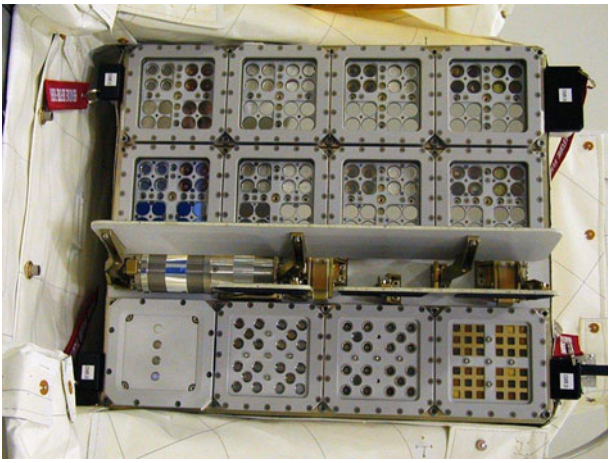

Fig. 1 EXPOSE-E Facility before space flight

9.19×10^5 Jm^{-2} (below a 0.1 % transmission neutral density filter) and 6.34×10^8 Jm^{-2} (100 % transmission insolated samples). All fluences were calculated for the biologically active UV range of 200 nm <λ> 400 nm for the whole mission and depended on the orientation of the ISS to the sun. Temperature varied between −21.5 °C and +59.6 °C (Rabbow et al. 2012; Onofri et al. 2012).

Cultivability Tests

Treated samples (only one third of the whole sample analyzed, meanly 140 mg), ground controls (mean weight 91,2 mg) and fresh colonized rocks (weight about 500 mg) were gently homogenized in sterile physiological solution (0.9 % NaCl w/v) and plated on MA (Malt Agar, incubated at 15 °C for 3 months. The MA medium was selected because it gave best growth for colonies from fresh colonized rock samples. Each test was performed in triplicate.

The organisms that were able to grow were isolated and stored in pure culture at 10 °C.

Table 1 Exposure conditions during the 1.5 year lasting LIFE experiment

Sample specification	Parameter
Space Dark	Space vacuum (10^{-7} to 10^{-4} Pa)
Space 0.1 % insolated	Space vacuum and solar UV (>110 nm) screened with a MgF_2 neutral density filter of 0.1 % transmission
Space 100 % insolated	Space vacuum and solar UV (>110 nm)
Mars Dark	1000 Pa CO_2 gas mixture
Mars 0.1 % insolated	1000 Pa CO_2 and solar UV (>200 nm) screened with a Suprasil neutral density filter of 0.1 % transmission
Mars 100 % insolated	1000 Pa CO_2 gas mixture and solar UV (>200 nm)
Ground Control	Dark, room temperature, 1 atm

DNA Extraction and Sequencing

DNA was extracted from colonies grown for 6 months at 10 °C, using Nucleospin Plant Kit (Macherey Nagel, Düren, Germany) according to the user's manual. Polymerase Chain Reactions (PCR) were prepared in PCR tubes adding 12.5 μl of 2× BioMix (catalog no. 25011, BioLine GmbH, Luckenwalde, Germany), 5 μM of each primer (1 μl each), 40 ng of template (1 μl), to a final volume of 25 μl by adding nuclease free water (9.5 μl). Internal Transcribed Spacers (ITS) were amplified using ITS1 and ITS4 primers (Table 2) for fungi with the following PCR protocol: an initial denaturation step at 95 °C for 2 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and an extension at 72 °C for 30 s. At the end of the last cycle, an additional extension at 72 °C for 5 min was performed. The algal Internal Transcribed Spacer was amplified using ITS1T and ITS4T primers (Table 2) with the following protocol: initial denaturation at 94 °C for 2 min, 30 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, and an extension at 72 °C for 2 min; an additional extension at 72 °C for 7 min was performed. Algal SSU was amplified with a PCR spanning the whole rRNA Small Subunit region using primers Euk A and Euk B (Table 2) with the following protocol: an initial denaturation at 94 °C for 3 min, 30 cycles of denaturation at 94 °C for 45 s, annealing at 55 °C for 1 min, and extension at 72 °C for 3 min, and a final extension at 72 °C for 5 min. The DNA sequences were assessed by direct sequencing of the PCR product (Macrogen, South Korea).

Sequence assembling was performed by using the software ChromasPro v1.41 (2003–2007 Conor McCarthy School of Health Science, Griffith University, Southport, Queensland, Australia). Sequences were compared in the public domain (NCBI) using BLASTn algorithm.

Results

The first test in the LIFE experiment was to observe the effects of dehydration. The growth ability of the flight samples, i.e. Antarctic sandstones hosting cryptoendolithic communities that were exposed to different conditions in space was compared to that of the dehydrated Ground Control (GC) and a fresh sample of sandstone stored at −20 °C. Table 3 shows the colonies formed after cultivation on MA. The highest colony numbers were obtained from the fresh samples: 52±10.5 algae, 1±0 black fungi, 90±27 pink fungi and 12.6±2.0 yeasts.

Table 2 Nuclear SSU and ITS primers used for algal and fungal DNA amplification and sequencing (Katana et al. 2001; Moro et al. 2009)

Name	Sequence 5′-3′	Organism
EukA	AACCTGGTTGATCCTGCCAGT	Algae
898-919R	TAAATCCAAGAATTTCACCTCT	Algae
1936-57R	GGTAGGAGCGACGGGCGGTGTG	Algae
CHLORO F	TGGCCTATCTTGTTGGTCTGT	Algae
EukB	TGATCCTTCTGCAGGTTCACCTAC	Algae
ITS1T	GGAAGGATCATTGAATCTATCGT	Algae
ITS4T	GGTTCGCTCGCCGCTACTA	Algae
ITS1	TCCGTAGGTGAACCTGCGG	Fungi
ITS4	TCCTCCGCTTATTGATATGC	Fungi

Table 3 Growth studies of the LIFE experiment after the 1.5 years lasting space mission: Colonies formed after cultivation on MA (Flight samples and controls)

Exposure conditions	Colony formation				Total colonies/mg
	Algae	Black fungi	Filamentous pink fungi	Yeasts	
Space Dark	0	0	3[a]	0	0.021
Space 0.1 % insolated	0	0	0	0	0
Space 100 % insolated	0	0	0	0	0
Mars Dark	0	0	0	0	0
Mars 0.1 % insolated	1[a]	0	0	0	0.007
Mars 100 % insolaed	0	0	0	0	0
Ground Control	0	0	20.5±3.5	0	0.18
Fresh sample (dark,−20 °C, 1 atm)	52±10.5	1±0	90±27.0	12.66±2.0	0.29

[a] Total CFU obtained from 3 replicates

The GC sample, which was stored for 1.5 years in the laboratory, showed only 20.56±3.5 colonies of pink fungi.

Three fungal colony with a pale pink mycelium (Fig. 2) composed of appressed hyphae of 4 μm in width were obtained from a flight sample that was exposed to space vacuum, but kept in the dark (Table 3: Space Dark).

One colony of a green unicellular alga (Fig. 3) grew from a flight sample that was exposed to simulated Mars atmosphere and the Martian spectrum of solar radiation >200 nm beneath a suprasil neutral density filter (Table 3: Mars 0.1 % insolated). All other flight samples did not result in any colonies after incubation (Table 3).

Macroscopic observations and microscopic morphological analyses showed that both, the pink fungus from the Space Dark sample as well as the green unicellular alga from the Mars 0.1 % insolated sample have features identical to the same microorganisms isolated from the fresh and the GC samples. In order to get a better understanding on which genera or species belong to the two survivors in Space and Mars condition, additional molecular investigation has been performed.

The sequences obtained through PCR amplification and sequencing of ITS rDNA portions, gave low identity (94 %), with E-value=0, in NCBI GenBank with the lichenized genus *Acarospora* A. Massal. The closest related sequence was that of *Acarospora rosulata* (accession number GU184116.1). Based on this data the fungus was identified at the genus level only.

To further identify the green alga, the same analyses (PCR amplification of ITS rDNA portions and sequencing) showed that the ITS sequences matched with different algal genera with similarities ranging from 95 % to 97 %, E-value=0. They belonged to the Class *Trebouxiophyceae*, 5 of them showing similarities to the Order *Chlorellales,* and 1, the genus *Stichococcus*, that is nested within a moderately supported cluster comprising the weakly supported order *Prasiolales*, (97 % identity) (Neustupa et al. 2007). SSU sequencing showed a similarity ranging from 94 % to 96 %, E-value=0, with different algal species in the genus *Stichococcus*, the closest related sequence (96 %) was *S. deasonii* UTEX 1706 (accession number DQ275460). This strain grew mostly as single cells or four-celled short uniseriate and unbranched, straight or sigmoid, filaments. However, the morphology of the

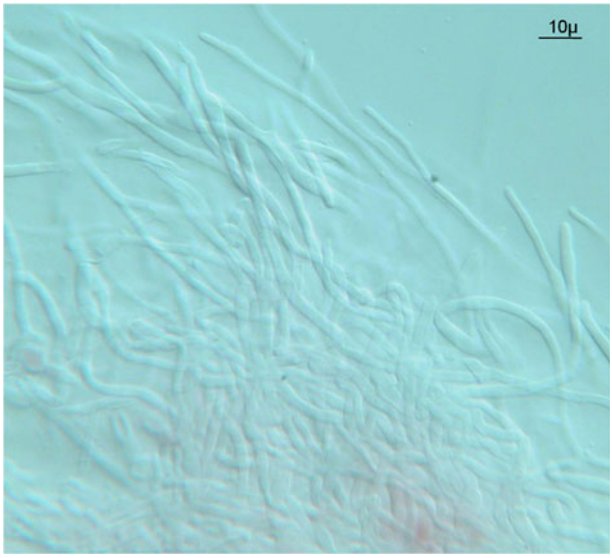

Fig. 2 Hyphal network of the mycelium isolated from a rock exposed for 1.5 years to space vacuum (10^{-7} to 10^{-4} Pa) in the dark (Space Dark sample), seen at the light microscope. Usually lichenized fungi, when separated from their photobionts, lose all their morphological peculiarities and grow as common mycelia

cylindrical cells, 5 μm wide and 10 μm long on average, with broadly rounded poles, does not completely match the morphological description of the genus *Stichococcus* Nägeli (1849), in which only one unlobed parietal chloroplast is usually present, while our strain was almost always characterized by two chloroplasts.

Discussion

LIFE is the first experiment where cryptoendolithic microorganisms within their natural substratum have been exposed for long time to space and simulated Martian conditions in

Fig. 3 Green alga isolated from colonized sandstone exposed to simulated Mars conditions in space beneath a Suprasil neutral density filter with a cut off at 200 nm and 0.1 % transmission (Space 0.1 % insolated). Phylogenetic analyses indicate that the alga belongs to the genus *Stichococcus*

Low Earth Orbit. After exposure to the conditions applied during the LIFE experiment in outer space, a few cryptoendolithic organisms grew after recovery, namely in one Space Dark sample (3 fungal colonies) and in one Mars 0.1 % insolated sample (1 green algal colony). The pink lichenized fungus seemed to belong to the genus *Acarospora* and the green alga seemed to belong to the genus *Stichococcus*. Due to the scarcity of algal ITS and SSU sequences in nucleotide databases, it was only possible to address the alga to genus level. Considering the generic level of the data obtained, an extended study based on morphology and multi-gene molecular phylogeny is in progress.

All other flight samples did not result in any growth under the conditions tested. Isolation in culture from fresh samples produced mainly pink fungal colonies, and green algae, besides few yeasts and black fungi. From GC only a lower number of pink fungal colonies have been obtained. 1.5 years of desiccation of GC samples justify the decrease of CFUs observed, as well as the absence of yeasts, black fungi and green algae (Table 3). It is interesting to note that although no algal growth was observed in the ground control samples, one algal colony was isolated from the Mars 0.1 % insolated sample (Table 3). This fact could be due to (i) prolonged desiccation in Earth atmosphere could be more harmful than Mars condition simulated in space (ii) the green alga was absent in the GC sample analysed, due to the not uniform distribution of the organisms composing the community within the Antarctic sandstone fragments. Pink fungi and the green alga, isolated from samples exposed on the exterior of ISS, are micro- and macroscopically identical to the ones obtained from fresh samples and GC, belonging to the same species living in the same cryptoendolithic community. Besides, *Acarospora* and *Stichococcus* are known to be members of lichen dominated cryptoendolithic microbial community, colonizing porous sandstone of the Beacon Supergroup (Nienow and Friedmann 1993). Antarctic cryptoendolithic organisms have been suggested as good study model for survival in space because of their ability to cope with the complex interplay of extremely low temperatures, thermal fluctuations, arid phases and high radiation intensities (Selbmann et al. 2005, 2011; Ruisi et al. 2007). Moreover, it has been assumed that organisms living within rocks could enter a cryptobiotic state after being ejected into space (Onofri et al. 2012).

This feature is possibly aided by the presence of abundant extracellular polymeric substances (EPS) in many endolithic species. EPS production allows the community to survive long dry periods, it may be abundant in fungi (Selbmann et al. 2005), in lichen thalli (de Vera et al. 2004; De los Rios et al. 2005), and in the whole cryptoendolithic community (De los Ríos et al. 2004). Previous studies ascertained that the presence of sugars such as glucose or buffer salts on the surface of *Bacillus subtilis* spores act as chemical protectants, highly increasing their survival in space (Horneck et al. 1994). Furthermore Mancinelli and Klovstad (2000) showed that under 1 mm of Martian analog soil a *B. subtilis* monolayer did not exhibit loss of viability after exposure to 12.3 kJm^{-2} of UV radiation from deuterium source, and some protection was even afforded by a layer 12 μm thick. The few rock-colonizing organisms that were isolated after retrieval of EXPOSE-E could have been survived because of: (i) the ability of cryptoendolithic organisms to tolerate desiccation and radiation and (ii) the shielding of even a small layer of rock material thereby preventing loss of viability after simulated Martian UV irradiation. However, the study showed also that only a few microorganisms out of the rock colonizing community survived after 1.5 years in space or in a simulated Martian climate while most of them died. Although they are adapted to cope with extreme dryness and cold in the Antarctic desert, they were damaged by the much more hostile conditions of space or simulated Mars.

Nevertheless extremophilic endolithic Eukarya composing this Antarctic community may be suitable model organisms to investigate the resistance of life in the framework of

Lithopanspermia. After astrobiology research has centered—at least in the past decades—almost exclusively on prokaryotic microorganisms, these recent findings of the LIFE experiment allow us to select models from a wider spectrum of living organisms.

Acknowledgments We thank the staff at the European Space Agency for the provision and operations of the EXPOSE-E facility and Thomas Berger for the cosmic ray dosimetry data. We also thank to the Italian National Program of Antarctic Researches and Italian National Antarctic Museum "Felice Ippolito" for funding collection of Antarctic samples and strains and samples analyses.

References

Berger T, Hajek M, Bilski P, Vanhavere F, Horwacik T, Körner C, Reitz G (2012) Measurements of the dose due to ionizing radiation within the EXPOSE-E experiment applying passive radiation detectors. Astrobiology 12 (in press)

Canganella F, Wiegel J (2011) Extremophiles: from abyssal to terrestrial ecosystems and possibly beyond. Naturwissenschaften 4:253–279

De la Torre JR, Goebel BM, Friedmann EI, Pace NR (2003) Microbial diversity of cryptoendolithic communities from the McMurdo Dry Valleys, Antarctica. Appl Environ Microbiol 215:3858–3867

De la Torre R, Sancho L, Horneck G, de los Ríos A, Wierzchos J, Olsson-Francis K, Cockell CS, Rettberg P, Berger T, de Vera JPP, Ott S, Martinez Frías J, Melendi PG, Lucas MM, Reina M, Pintado A, Demets R (2010) Survival of lichens and bacteria exposed to outer space conditions—results of the Lithopanspermia experiments. Icarus 208:735–748

De los Ríos A, Wiezchos J, Sancho LG, Ascaso C (2004) Exploring the physiological state of continental Antarctic endolithic microorganisms by microscopy. FEMS Microbiol Ecol 50:143–152

De los Rios A, Sancho LG, Grube M, Wierzchos J, Ascaso C (2005) Endolithic growth of two Lecidea lichens ingranite from continental Antarctica detected by molecular and microscopy techniques. New Phytol 165:181–190

De Vera JP, Horneck G, Rettberg P, Ott S (2004) The potential of the lichen symbiosis to cope with the extreme conditions of outer space—II: germination capacity of lichen ascospores in response to simulated space conditions. Adv Space Res 33:1236–1243

Friedmann EI (1982) Endolithic microorganisms in the Antarctic cold desert. Science 215:1045–1053

Friedmann EI, Weed R (1987) Microbial trace-fossil formation, biogenous, and abiotic weathering in the Antarctic Cold Desert. Science 236:645–652

Gladman BJ, Burns JA, Duncan M, Lee P, Levison HF (1996) The exchange of impact ejecta between terrestrial planets. Science 271:1387–1392

Horneck G, Bucker H, Reitz G (1994) Long-term survival of bacterial spores in space. Adv Space Res 14:41–45

Horneck G, Stöffler D, Ott S, Hornemann U, Cockell CS, Moeller R, Meyer C, de Vera JP, Fritz J, Schade S, Artemieva NA (2008) Microbial rock inhabitants survive impact and ejection from host planet: first phase of lithopanspermia experimentally tested. Astrobiology 8:17–44

Horneck G, Klaus DM, Mancinelli RL (2010) Space microbiology. Microbiol Mol Biol Rev 74:121–156

Katana A, Kwiatowski J, Spalik K, Zakrys B, Szalacha E, Szymanska H (2001) Phylogenetic position of *Koliella* (Chlorophyta) as inferred from nuclear and chloroplast small subunit rDNA. J Phycol 37:443–451

Mancinelli RL, Klovstad M (2000) Martian soil and UV radiation: microbial viability assessment on spacecraft surfaces. Planet Space Sci 48:1093–1097

Mileikowsky C, Cucinotta F, Wilson JW, Gladman B, Horneck G, Lindegren L, Melosh J, Rickman H, Valtonen M, Zheng JQ (2000) Natural transfer of viable microbes in space, Part 1: from Mars to Earth and Earth to Mars. Icarus 145:391–427

Moro CV, Crouzet O, Rasconi S, Thouvenot A, Coffe G, Batisson I, Bohatier J (2009) New design strategy for development of specific primer sets for PCR-based detection of *Chlorophyceae* and *Bacillariophyceae* in environmental samples. Appl Environ Microbiol 75:5729–5733

Nägeli C (1849) Gattungen einzelliger Algen, physiologisch und systematisch bearbeitet. Neue Denkschriften der Allg. Schweiz Ges Gesammten Naturwissenschaften 10(7):i–viii, 1–139, pls I–VIII

Neustupa J, Elias M, Sejnohova L (2007) A taxonomic study of two Stichococcus species (Trebouxiophyceae, Chlorophyta) with a starch-enveloped pyrenoid. Nova Hedwig 84:51–63

Nicholson WL (2009) Ancient micronauts: interplanetary transport of microbes by cosmic impacts. Trends Microbiol 17:243–250

Nienow JA, Friedmann EI (1993) Terrestrial lithophytic (rock) communities. In: Friedmann EI (ed) Antarctic microbiology. Wiley, New York, pp 343–412

Onofri S, Selbmann L, Zucconi L, Pagano S (2004) Antarctic microfungi as models for exobiology. Planet Space Sci 52:229–237

Onofri S, Selbman L, de Hoog GS, Grube M, Barreca D, Ruisi S, Zucconi L (2007) Evolution and adaptation of fungi at boundaries of life. Adv Space Res 40:1657–1664

Onofri S, Barreca D, Selbmann L, Isola D, Rabbow E, Horneck G, de Vera JP, Hatton J, Zucconi L (2009) Resistance of Antarctic black fungi and cryptoendolithic communities to simulated space and Martian conditions. Stud Mycol 61:99–109

Onofri S, De la Torre R, De Vera JP, Ott S, Zucconi L, Selbmann L, Scalzi G, Venkateswaran KJ, Rabbow E, Sanchez Iñigo FJ, Horneck G (2012) Survival of rock-colonizing organisms after 1.5 year in outer space. Astrobiology 12 (in publ.)

Rabbow E, Horneck G, Rettberg P, Schott JU, Panitz C, L'Afflitto A, von Heise-Rotenburg R, Willnecker R, Baglioni P, Hatton J, Dettmann J, Demets R, Reitz G (2009) EXPOSE, an astrobiological exposure facility on the international space station—from proposal to flight. Orig Life Evol B 39(6):581–598

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, Reitz G (2012) EXPOSE-E, an ESA Astrobiology mission 1.5 years in space. Astrobiology 12 (in press)

Raggio J, Pintado A, Ascaso C, De la Torre R, De los Ríos A, Wierzchos J, Horneck G, Sancho LG (2011) Whole lichen thalli survive exposure to space conditions: results of Lithopanspermia experiment with Aspicilia fruticulosa. Astrobiology 4:281–292

Rothschild LJ, Mancinelli RL (2001) Life in estreme environments. Nature 409:1092–1101

Ruisi S, Barreca D, Selbmann L, Zucconi L, Onofri S (2007) Fungi in Antarctica. Rev Environ Sci Biotechnol 6:127–141

Sancho LG, De la Torre R, Horneck G, Ascaso C, De los Rios A, Pintado A, Wierzchos J, Schuster M (2007) Lichens survive in space: results from the 2005 LICHENS experiment. Astrobiology 7:443–454

Selbmann L, De Hoog GS, Mazzaglia A, Friedmann EI, Onofri S (2005) Fungi at the edge of life: cryptoendolithic black fungi from Antarctic desert. Stud Mycol 51:1–32

Selbmann L, De Hoog GS, Zucconi L, Isola D, Ruisi S, Gerrits van den Ende AHG, Ruibal C, De Leo F, Urzì C, Onofri S (2008) Drought meets acid: three new genera in a dothidealean clade of extremotolerant fungi. Stud Mycol 61:1–20

Selbmann L, Isola D, Zucconi L, Onofri S (2011) Resistance to UV-B induced DNA damage in extreme-tolerant cryptoendolithic Antarctic fungi: detection by PCR assays. Fungal Biol 115:937–944

Valtonen M, Nurmi P, Zheng JQ, Cucinotta FA, Wilson JW, Horneck G, Lindegren L, Melosh J, Rickman H, Mileikowsky C (2009) Natural transfer of viable microbes in space from planets in the extra-solar systems to a planet in our Solar System and vice-versa. Astrophys J 690:210–215

Venkateswaran K, Satomi M, Chung S, Kern R, Koukol R, Basic C, White D (2001) Molecular microbial diversity of a spacecraft assembly facility. Syst Appl Microbiol 24:311–320

Wynn-Williams DD, Edwards HGM (2000) Antarctic ecosystems as model for extraterrestrial surface habitats. Planet Space Sci 48:1065–1075