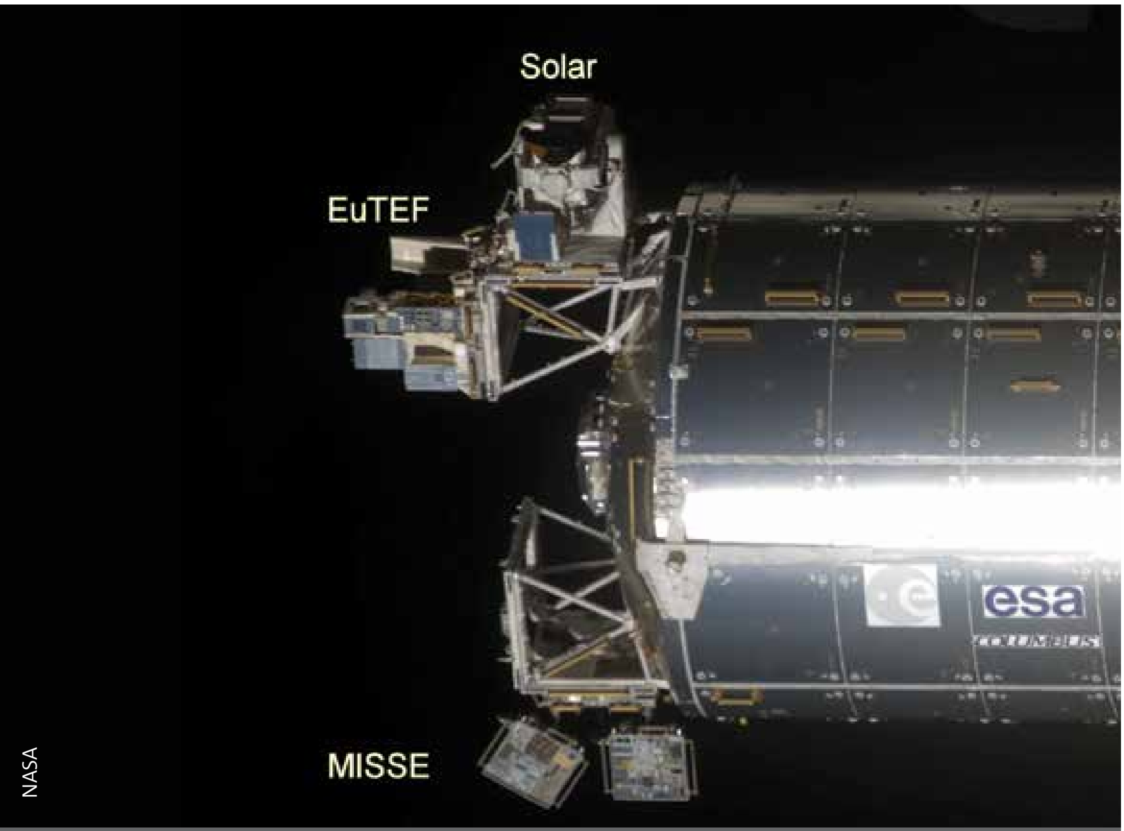
→ SPACE FOR LIFE

human spaceflight science newsletter

Issue 7 | April 2015



↑ The Columbus External Payload Facility (left top and bottom), showing the location of three external payloads in June 2008 including ESA's Solar facility and European Technology Exposure Facility (EuTEF) as well as two panels from NASA's Materials ISS Experiment (MISSE)

In this issue:

→ MILESTONES IN ASTROBIOLOGY

The results of the Expose-R facility have recently been published together in a special issue of the International Journal of Astrobiology. For this reason this edition of the Space for Life Newsletter has been dedicated to ESA's astrobiology research and the results that have been published. This edition will even present some of the results that have been published related to the Expose-R2 series of follow-on experiments that are currently on orbit.

ESA Research in Astrobiology

Astrobiology is a vitally import area of research for ESA, covering research in biology in testing the boundaries of survival of terrestrial life, and organic chemistry which is testing the effect of the space environment on organic chemicals which could form the basis of life itself. The wealth of results coming from the Expose-R facility have been built on an abundance of other results coming from previous space missions funded by ESA.

Neighbouring planets such as Mars and Jupiter's moons Europa and Ganymede are targets for the search for life beyond Earth, and organisms living in extreme conditions on Earth can provide vital information in this search such as the type of habitats where life could be sustained related to radiation levels, salinity, pressure, temperature variation etc.

This research can shed light on the survivability of different organisms on other planets, how they may possibly build resistance when exposed to such harsh conditions, and help determine planetary protection measures in order to restrict possible contamination of another planetary body by surface probes sent from Earth.

Astrobiology is therefore vital in helping to answer important questions with relation to how life started on our planet and the potential for life existing throughout the universe.

Previous Research

ESA-funded research has made major improvements in our knowledge of astrobiology following successful research undertaken previously on the 11-month EURECA mission (1992 – 1993), the Biopan facility on five separate two-week Foton missions between 1994 – 2007, and the 18–month duration Expose-E experiments on the ISS EuTEF external payload (Feb 2008 - Sept 2009). The Expose-R research has now added to this wealth of important data.


NASA

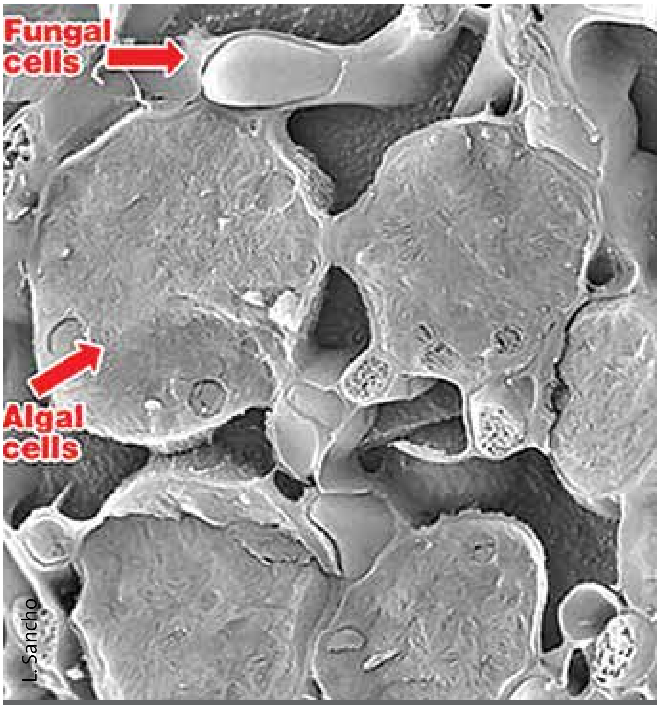
↑ The European Retrievable Carrier (EURECA) following deployment in August 1992

One of the main focuses in the search for living organisms on other planets and the possibilities for transfer of life between planets started with bacterial spores, due to the organism's simplicity and robustness to stay alive under adverse conditions, opening the possibility of it surviving an interplanetary journey exposed to the harsh space environment.

This has now developed to encompass more advanced organisms following the results of ESA experiments on the Foton-M2 and Foton-M3 missions in 2005 and 2007 respectively. As part of these astrobiology experiments using ESA's Biopan (an exposure facility attached outside the hull of Foton) it was discovered that some regular plant seeds, lichens (during Foton-M2 and Foton-M3) and water bears or tardigrades (during Foton-M3) could survive the open space environment during approximately two weeks of exposure to open space conditions.


ESA/S. Corvaja

↑ Biopan exposure facility (with ESA logo) installed on the side of Foton-M3 during launch preparations in 2007



L.Sancho

↑ Microscopic image of lichen showing fungal and algal cells

This was a significant step forward as these organisms are far more advanced on an evolutionary scale than bacteria. Lichens are not actually single organisms but an association of millions of algal cells, which cooperate in photosynthesis and are held in a fungal mesh. The algal cells and the fungus have a symbiotic relationship, with the algal cells providing the fungus with food and the fungus providing the alga with a suitable living environment for growth. In fact lichens can be considered as very simple ecosystems. Analysis post flight showed a full rate of survival and an unchanged ability for photosynthesis. Water bears or tardigrades, which are known to survive under conditions that would kill most organisms, could also survive exposure to space, if protected against UV.


Willow Gabriel and Bob Goldstein

↑ Scanning Electron Microscope image of tardigrades or water bears, now known to survive the harsh conditions of open space

Results from the EuTEF-Expose-E experiments

Following on from the Tardigrades In Space (TARDIS) experiment on Foton-M3, major astrobiology research on Columbus took place on EuTEF's Expose-E platform which included five astrobiology experiments. These experiments not only covered a wider sample of organisms, they also provided a greater exposure time, more than 1.5 years.

One part of the samples was exposed to open space conditions while the other part was exposed to modified conditions (low pressure, atmosphere mainly of CO_2 and an altered UV-spectrum) to simulate conditions on Mars.

From the Expose-E experiments on EuTEF, the halophilic archaea *Halococcus dombrowskii* seemed tolerant to the space conditions (the ADAPT experiment) as lots of cells were still alive, their morphology was unchanged, and their enzymatic activities were still measurable.

In the Lichens and Fungi (LIFE) experiment two samples resisted full solar irradiation (one lichen and one black fungus) whereas almost all the samples survived with different levels (from almost 2.5% - 100%) if shielded but for from UV light.


DLR

↑ De-integration of Lichens (LIFE) flight samples at DLR. Lichens are multicellular, macroscopic organisms


DLR

↑ Flight sample carrier of experiments ADAPT and PROTECT after de-integration

In the PROTECT experiment which was dealing with planetary protection issues, bacterial spores of *Bacillus subtilis* and *Bacillus pumilus* were able to survive a simulated journey to Mars if protected against solar UV-C. At the surface of Mars, they may also survive for several years if shielded in a shadowed spot against direct solar irradiation.

In the SEEDS experiment hundreds of plant seeds (of *Arabidopsis* and tobacco) were exposed to space conditions. On studying the germination of the seeds on return to Earth, a remarkable quantity of survivors was found in both species. Seeds that had been shielded against solar UV, but were exposed to the other conditions of space (vacuum, cosmic radiation, freezing/thawing, micro-g) survived at even higher rates.

↑ *Xanthoria elegans*, a lichen known as one of the best known survivors in open space.

The quantity of different organisms we know of can survive the open space environment is growing, and the conditions under which they can survive, (length of time, degree of shielding etc.) is also being elucidated in more depth to move us closer to answering the important questions being posed by astrobiology. Expose-R is the latest in a long line of ESA research facilities in this area producing valuable results, with more to come with the future conclusion of Expose-R2 currently on orbit.

With the future of human spaceflight looking towards exploration beyond low-Earth orbit, the potential for expanding this type of research to the more demanding radiation environment outside of Earth's magnetic field is growing.

Furthermore if the potential for automated, in-situ analysis were developed, this would also provide the opportunity for time-dependent measurements to be taken, and open up the potential for free-flying missions or even piggy-backing onto other satellite missions where the stringent safety requirements associated with human spaceflight would not be a factor.

→ INTRODUCTION TO THE EXPOSE-R FACILITY:

From Life in Space to Life on Mars

The Expose-R facility has been a key exponent of ESA's research in astrobiology since 2009, having completed 22 months of exposure on its first set of experiments and now undertaking a follow-up set of experiments since August 2014. We take a brief look at the facility and the types of conditions that are offered since this versatile astrobiology platform started operations.

NASA/Roscosmos

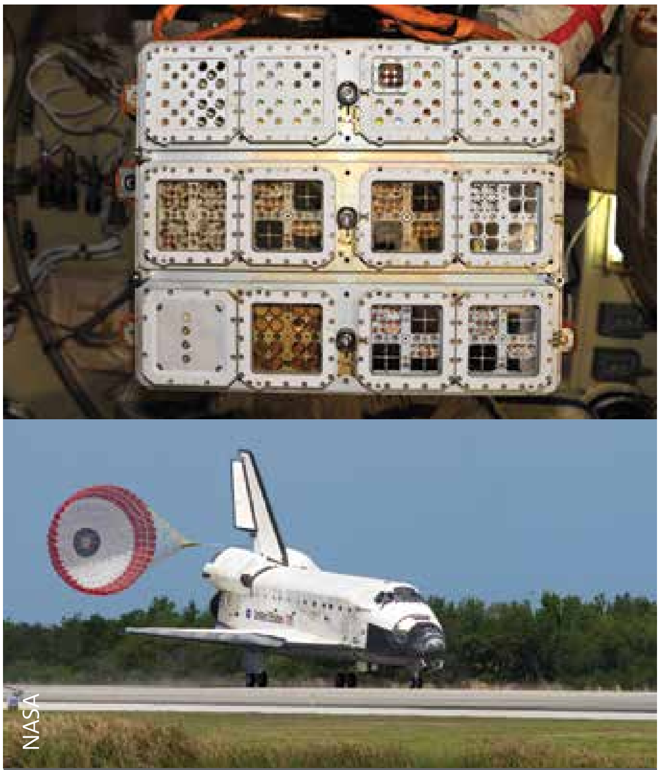
↑ The Expose-R facility following installation on the outside of the Zvezda Service Module on the ISS in March 2009


NASA/Roscosmos

↑ The Expose-R facility pictured on the outside of the Zvezda Service Module in November 2009

ESA's research in astrobiology is carried out by exposing different samples in orbit where, away from the protective environment of Earth, the organisms or organic chemicals can be exposed to full solar UV, vacuum, cosmic rays, weightlessness and perpetual temperature variations as the International Space Station continuously passes between areas of direct sunlight and the cold darkness of Earth's shadow.

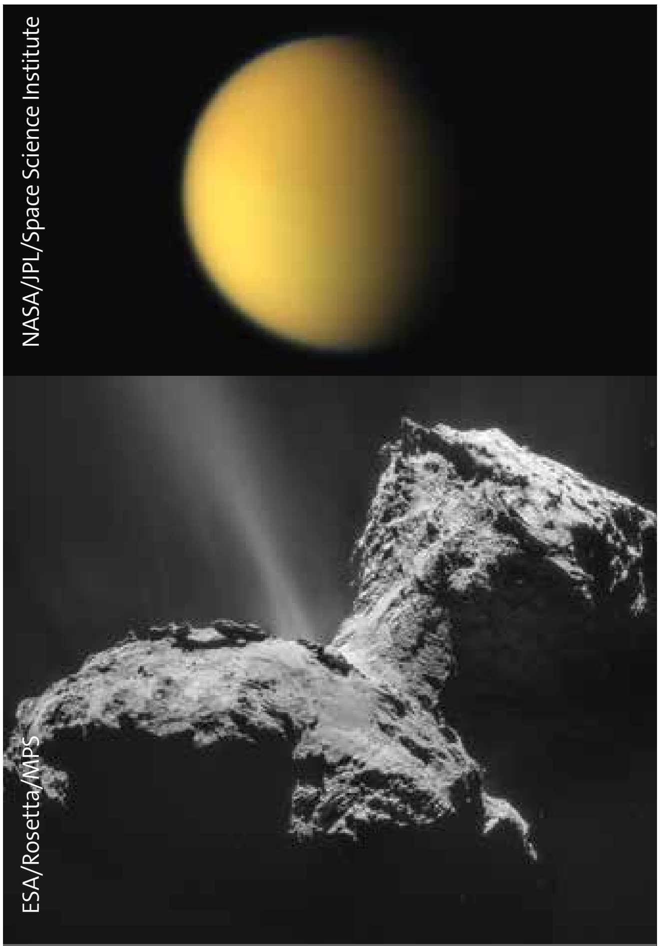
Expose-R was first installed outside the Russian segment of the ISS during a spacewalk in March 2009. The facility (and its samples) remained exposed outside the ISS for almost two years, being retrieved during a spacewalk in January 2011 prior to returning the sample trays back to Earth on the final Shuttle Discovery flight (STS-133). This made Expose-R the second longest astrobiology mission in Low-Earth Orbit. Since August 2014 the facility is again outside the ISS, loaded with a follow-up series of experiments/samples. This second run is known as Expose-R2. (Detailed article in November 2014 newsletter).


NASA

↑ Top: Expose-R back inside the ISS in January 2011 following retrieval during a Russian EVA
Bottom: Landing of STS-133 Shuttle *Discovery*, which returned the samples for Expose-R to Earth

Expose-R, which was built by Kayser-Threde GmbH (now OHB System AG) in Germany and RUAG Space in Switzerland, hosted a suite of nine astrobiology experiments (eight from ESA and one from IBMP, Moscow) to study biological processes in the simulated climate of planets and the probabilities, and limitations for life to be distributed among the bodies of our Solar System. The two chemical experiments were aiming to study processes of chemical evolution including stability of biomolecules (such as amino acids) and analogues to complex organic compounds detected in, for example, interstellar space, comets and the atmosphere of Titan.

The Expose-R payload also included active (R3D-R) and passive radiation detectors which provided vital data for interpreting the results from the experiments as well as improving our knowledge of the radiation environment in Low-Earth Orbit. The radiation instrumentation and the results of the experiments are covered in detail in separate articles in this newsletter.


NASA/JPL/Space Science Institute

ESA/Rosetta/MPS

↑ Top: Natural colour composite of Titan taken during ESA/NASA/ASI Cassini-Huygens mission in April 2005
Bottom: Comet 67P/Churyumov–Gerasimenko photographed in January 2015 during ESA's Rosetta Mission
Experiments in organic chemistry were studying compounds detected on comets and the atmosphere of Titan

Sample Accommodation

The experiments were accommodated in three special sample trays each with four sample locations so 12 locations in total. These were loaded with a total of 1220 samples (1062 biological samples, 130 chemical samples and 28 passive radiation dosimeter packages) plus the R3D-R instrument.

ESA/NASA/Roscosmos

↑ Top: Empty Expose-R facility prior to sample tray insertion
Middle: Example of sample tray (for Expose-R2 experiments) prior to installation inside the Expose-R facility
Bottom: Sample carrier from one of the Expose-R2 experiments (PSS)

The 'open space' samples were placed behind magnesium fluoride (MgF_2) windows which allow all wavelengths from deep ultraviolet to far infrared through. For simulated Mars conditions samples were placed behind quartz windows to filter out the necessary UV wavelengths. Additional neutral density filters were also used for some samples to reduce the quantity of each different wavelength of UV getting through (to either 1%, 0.01% and 0.0001%, no filter =100%).

ESA/NASA/Roscosmos

↑ Roscosmos cosmonaut Oleg Artemyev with Expose-R2 payload prior to its installation outside the ISS in August 2014

The samples were arranged in layers with the top layer in each sample location exposed to full solar UV while the lower one or two layers were kept in darkness, shielded from solar UV. For some experiments, the sun-protected lower layers simulated samples buried in the soil of a planetary body or meteorite. Some of the samples were exposed to vacuum by venting the sample compartments at the start of the exposure period, while others were kept in an inert (argon) atmosphere to stop sample metabolism and to maintain pressure.

The experiment environment was monitored by nine thermometers, four UV sensors and one radiometer, complementary to the investigator-provided R3D-R instrument. Data was acquired every 10 seconds and transmitted regularly to the Microgravity User Support Center (MUSC) at DLR, in Cologne, Germany, from where the data was forwarded to the science teams.

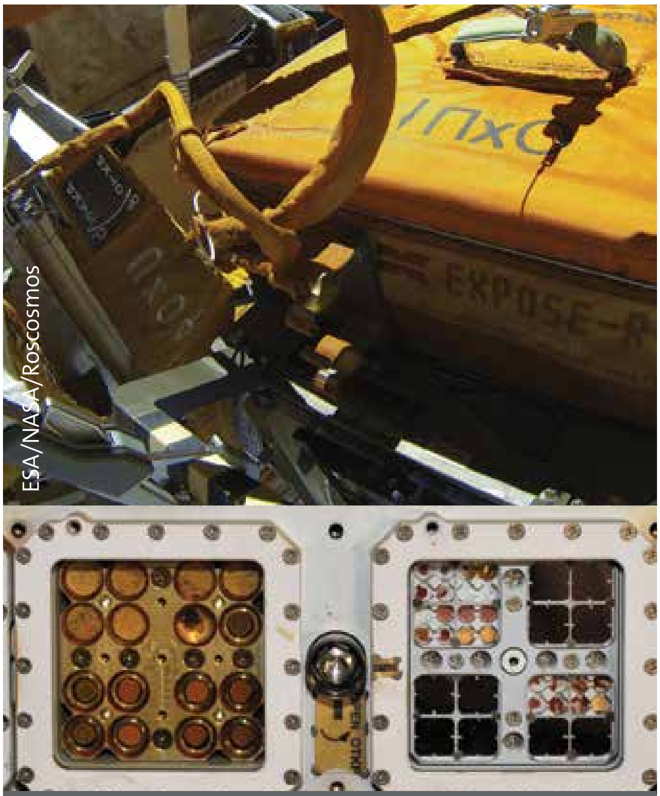
The data recorded in orbit was used by the science teams for post-flight analysis purposes and also provided the parameters for the Mission Ground Reference experiment. This took place in the Planetary and Space simulation facilities at DLR in Cologne, Germany. This ground reference simulation used identical hardware and samples and simulated a variety of space conditions.

Overcoming Adversity: Modelling Additional Data

During the Expose-R mission a computer failure occurred on the ISS which prohibited the data storage capabilities for significant periods of time. This problem was successfully resolved by filling the gaps with environmental data acquired from modelling. Using the geometrical position of Expose-R on the ISS (known) and the changing position and attitude of the ISS versus the Sun (also known), the solar irradiation and ensuing temperature changes could be reconstructed to a very high degree.

The results also had to take into account some contamination consisting of a brownish film that unexpectedly emerged in orbit on the inside of the top windows of the Expose-R sample locations exposed to vacuum, which had the effect of acting like a filter. The contamination appears to be built from volatile compounds, originating from the Expose-R facility and its test samples which developed into a film on the window surface on exposure to solar UV. The chance of this happening in the future can possibly be reduced by having volatile compounds released first, before allowing solar UV to reach the windows. This two-step procedure is now being tested on Expose-R2. After installation, the Expose-R2 facility was immediately evacuated, protected by a sun shield. Only weeks later, during another spacewalk, the sun shield was removed. Hopefully, the UV rays hitting the windows will have no contaminants to act upon this time.

Taking these factors into consideration, overall the Expose-R facility produced a significant amount of results. The interested reader can find them collectively (15 research papers) in a 142-page special edition of the *International Journal of Astrobiology*, Vol. 14, Issue 1.


ESA/NASA/Roscosmos

↑ Top: The Expose-R2 payload with Sun-shield still attached
Bottom: Example of brown window staining (left sample carrier) during Expose-R mission which appears to be built from volatile compounds originating from the Expose-R facility and its contents

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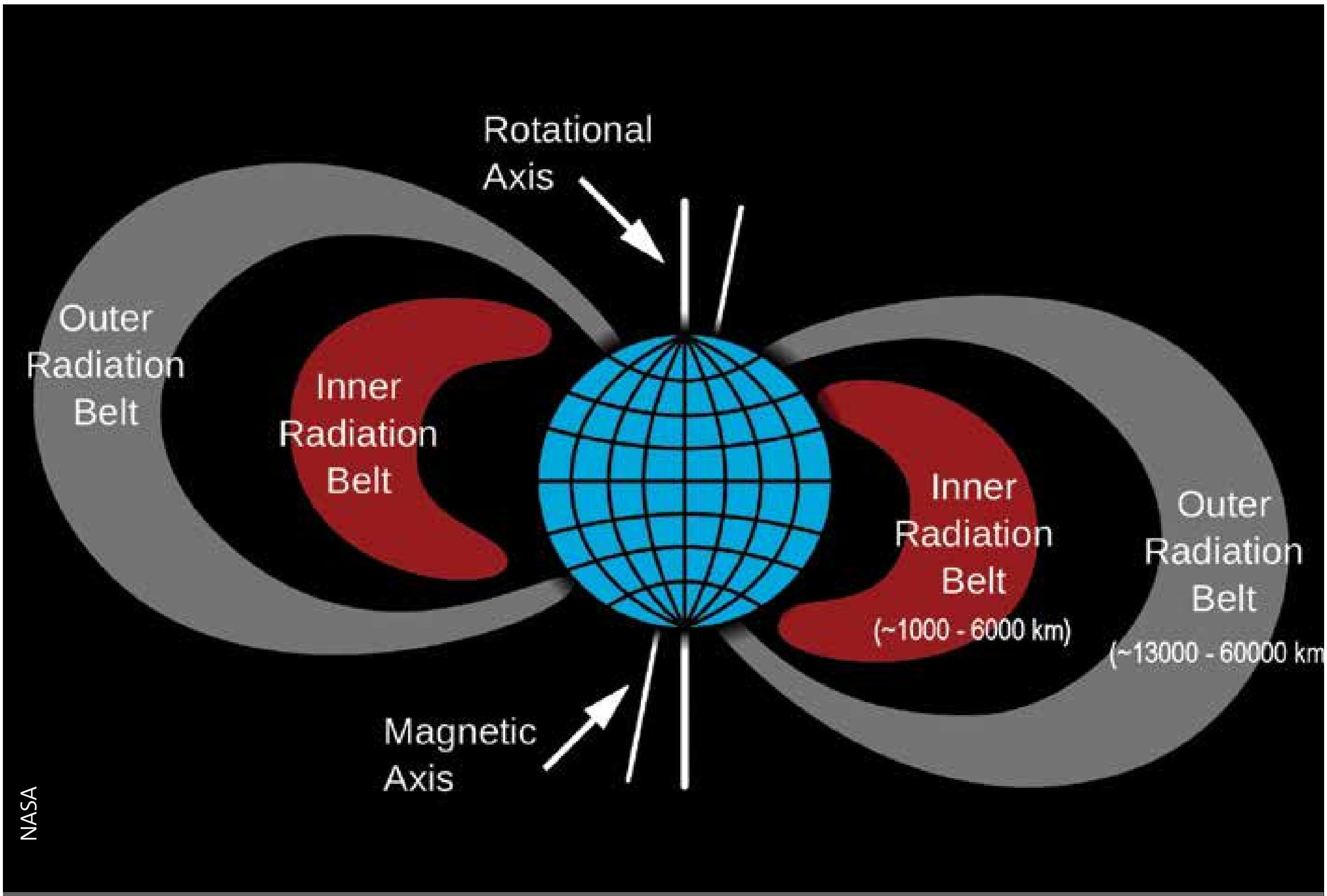
→ RADIATION RESEARCH ON EXPOSE-R:

Mapping radiation for two years on the Expose-R facility

The radiation environment in Low-Earth Orbit is very complex and constantly changing. As part of the Expose-R2 mission two different sets of radiation detectors were installed in the facility. The first was an active radiation detector to take time-dependent measurements during the course of the mission. The second were sets of passive detectors to measure the overall radiation dose. Not only do these measurements increase our knowledge of the radiation environment in Low-Earth Orbit, they also provide vital data to help with analysis for the different experiments in astrobiology and organic chemistry. We take a look at some of the key findings from the instruments during the mission.

Due to Earth's magnetic field, terrestrial life is protected from the harmful radiation coming from the Sun and also from the more exotic galactic and cosmic radiation that finds its origins in supernova explosions, collisions between stars, pulsars and black-holes. The spectrum of radiation in low-Earth orbit is wide: UV, X-rays, and cosmic particles: electrons, neutrons, protons and heavy ions (cosmic rays). The levels of these different types of radiation can vary for a number of reasons. The 11-year solar cycle (we are currently in the 24th solar cycle since 1755) causes variations, for example with increased solar flare activity. Although the Sun keeps a steady rhythm with its activity going up and down every 11 years, it cannot fully be predicted what the Sun will do during the next 11 years. There is too much variability. Other natural events such as galactic supernovas can have a similar unpredictable influence on the radiation levels in LEO. Considering the orbit of the ISS, there



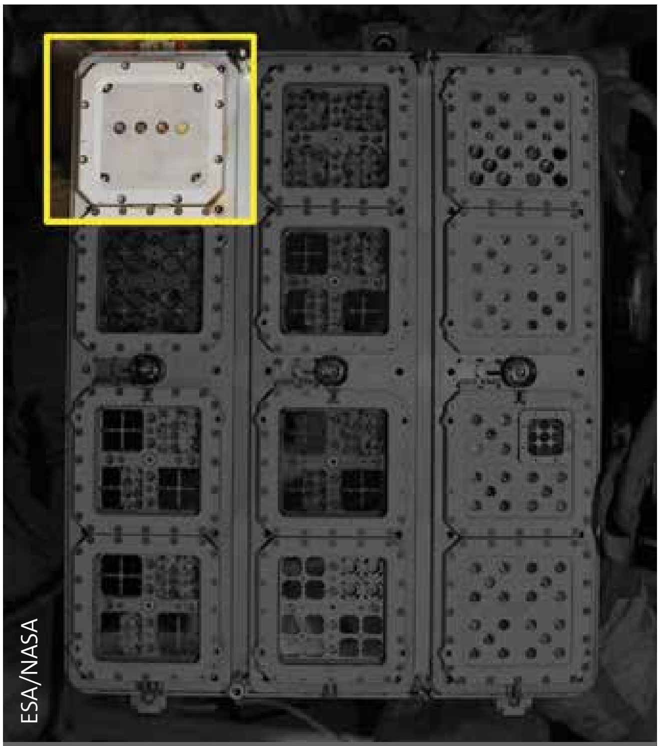
↑ Schematic of the Inner and Outer Radiation Belts around Earth

is the additional daily effect of increased radiation when the Station passes through the South Atlantic Anomaly where Earth's magnetic field is weaker and the ISS encounters increased radiation doses for a short period of time. With all these different elements the radiation environment around our planet is therefore a too complex environment to fully predict.

Undertaking this research covers different purposes. For the Expose-R experiments it provides quantitative data about a component of the space environment that could have affected the samples during their time in orbit. It also helps to improve our knowledge of the radiation environment in Low-Earth Orbit.

The Radiation Risks Radiometer Dosimeter (R3D), which is under the scientific lead of Dr. T. Dachev from the Space Research and Technologies Institute of Bulgarian Academy of Sciences and Dr. M. Lebert from the Department of Biology, at the Friedrich-Alexander-University in Erlangen, Germany is the active radiation detector used during the Expose-E, -R and -R2 missions, active implying that it was taking time-dependent measurements (every 10 seconds) to monitor and record fluctuations in the radiation environment during the course of the mission.

This data was supplemented by passive thermoluminescent detectors, which were analysed post-flight to provide a measure of overall absorbed dose (no time resolution).



↑ The Radiation Risks Radiometer-Dosimeter (R3D-R) active radiation monitor highlighted in its location on the Expose-R payload in January 2011

Three different types of ionizing radiation were detected and quantified with the R3D-R (the R3D instrument that flew on Expose-R) instrument: galactic cosmic rays, protons from the inner radiation belt in the region of the South Atlantic anomaly and energetic electrons from the outer radiation belt.

Two distinctive periods occurred during the EXPOSE-R mission. Between March 2009 to January 2010 there was a period of very low solar activity connected with an unusual deep minimum of solar cycle 23. From January to August 2010, solar and geomagnetic activities increased as solar cycle 24 progressed and had a significant effect on electron flux and dose rates variations.

Galactic Cosmic Radiation

Galactic Cosmic Radiation provided dose rates between 0.03 and 20–25 µGy per hour (lowest readings at the magnetic equator, highest at high latitudes). This provided average daily absorbed dose rates of 81.4 µGy. This is actually lower than readings taken inside the ISS (March–June 2009) which can be attributed to secondary particles generated when primary particles pass through the walls of the ISS. Higher daily dose rates were seen in the first period due to a decreased interplanetary magnetic field caused by the unusual deep solar minimum at the end of the sunspot cycle 23.

Protons from the Inner Radiation Belt

Protons from the inner radiation belt provided dose rates which varied from 10-15 to 2649 µGy per hour, giving the daily averaged absorbed dose rate of 506 µGy per day (ranging from 326 to 704 µGy per day).

This is much higher than similar readings taken inside the ISS (March–June 2009) which gave an averaged absorbed dose rate of about 105 µGy per day due to increased shielding of the ISS walls. Altitude had an influence. An increase in flying altitude of the ISS from about 350 to 370 km caused an increase in the average daily dose rate from 400 to 600 µGy per day. Conversely, Shuttle dockings caused a temporary drop in dose measurements, apparently because the Shuttle itself acted as a radiation shield.

Electrons from the Outer Radiation Belt

Electrons from the Outer Radiation Belt provided the fewest measurements and provided an averaged absorbed dose of 89 µGy per day (ranging between 0.64 and 2348 µGy per day). The dose rate of 2348 µGy per day is much higher than the other sources, but can only be measured behind very thin shielding (as was the case for the R3D-R).

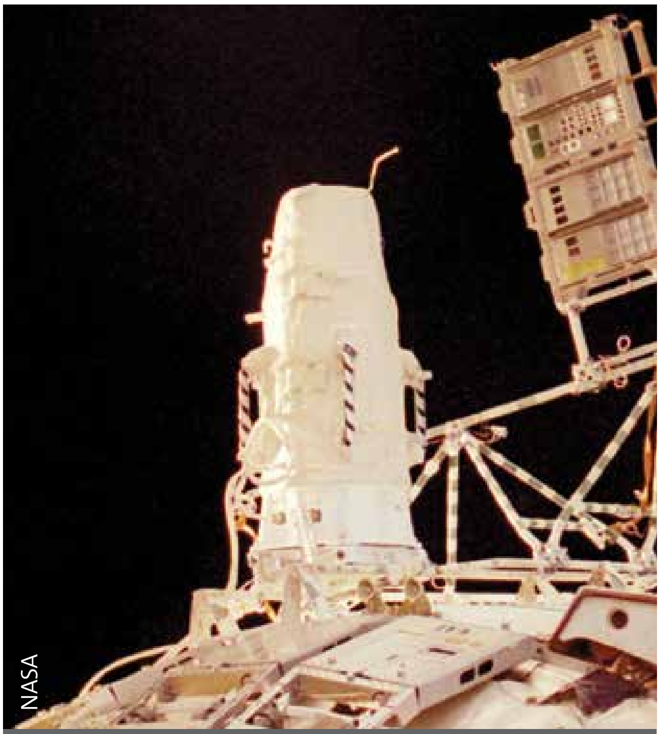
There was low Outer Radiation Belt activity from 1 March 2009 and 1 March 2010 due to low solar and magnetic activity. From 1 April – 20 August 2010 increased activity was observed (significantly on 6 and 7 April with further high measurements afterwards) with the highest dose rate of 21,131.6 µGy per hour obtained from a single measurement.

The high variations in April–May 2010 were connected with the second largest fluence of electrons recorded in the history of the Geostationary Operational Environmental Satellites (GOES) which have been taking measurements since the 1970's.

Total Measurements

When compared to measurements taken by the R3D-E during the Expose-E mission (which exposed a similar suite of astrobiology experiments from February 2008 – September 2009), the hourly and daily average dose rates during the Expose-R mission were higher for proton and electron measurements due to reduced shielding in the vicinity of the R3D-R instrument but lower for Galactic Cosmic Radiation.

The three different components measured provided a total daily dose rate of 675.4 µGy per day which is close to measurements taken with the Matroshka facility outside the ISS from January 2004 to August 2005.


NASA

↑ The Matroshka payload which was located outside the ISS in 2004/2005

The levels measured will be taken into account for the Expose-R samples as the Inner and Outer Radiation Belt doses measured will decrease with shielding thickness surrounding the samples and with a small increase in Galactic Cosmic Radiation dose due to secondary particles. As mentioned in the second article there was also a loss of information due to a computer crash on the ISS which required modelling and estimation of certain environmental parameters.

Thermoluminescent detectors

The small passive thermoluminescent dosimeters were located beneath the Expose-R sample carriers close to the samples of interest. The dosimeters were extracted at DLR in Cologne. A subsection of these were analysed at DLR while the remainder were distributed to the science teams at the Institute of Atomic and Subatomic Physics of the Vienna University of Technology in Austria and the Institute of Nuclear Physics in Krakow, Poland for analysis. The dose rate varied across sample locations due to shielding effects and the average dose rate varied between approximately 276 and 381 µGy per day. These values reflect the high shielding of the Expose-R facility as they are close to ISS interior measurements in the Russian segment of the ISS and also inside Columbus and, as such, the biological samples were predominantly exposed to galactic cosmic heavy ions and protons and not electrons.

Data from the active R3D-R instrument is a factor of two higher than the passive detectors. This can readily be explained by the very thin shielding in front of the R3D detector.

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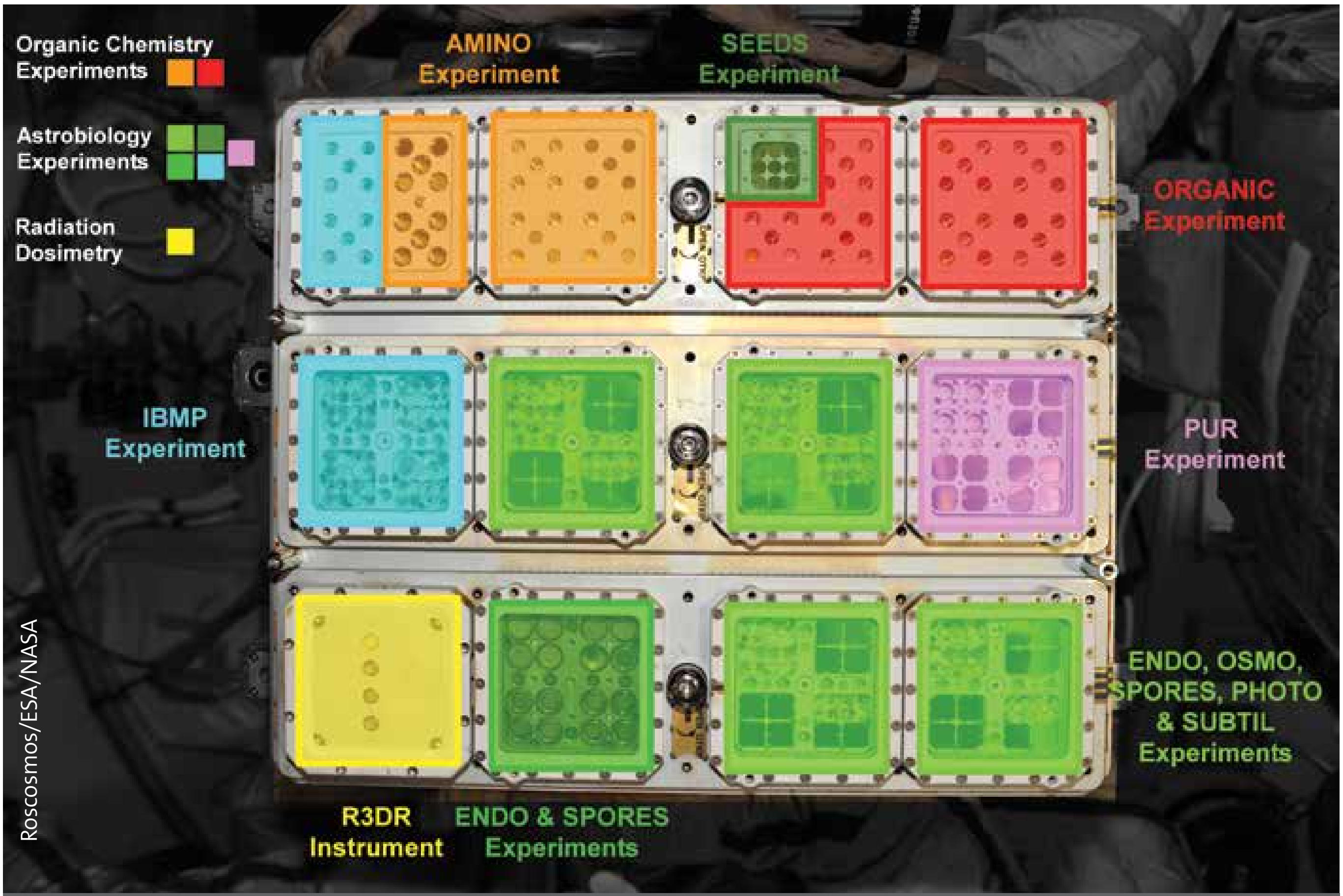
→ BIOLOGY RESEARCH ON EXPOSE-R

Safety in Clusters

The Expose-R facility has produced some very interesting results in astrobiology with respect to the survivability of different organisms in the open space environment. This is very significant considering the length of exposure of samples being 22 months making it the second longest set of astrobiology exposure experiments ever undertaken. Solar ultraviolet radiation seems the most harmful factor in space and a significant amount of organisms could survive the open space environment if shielded from solar UV as would be the case if just below the surface of a meteoroid, on early Earth or a planet like Mars. There were, however, some cluster forming organisms that could also survive exposure to solar UV indicating safety in numbers as in multi-layered cells, the outer layers acted as a UV shield for the inner layers.

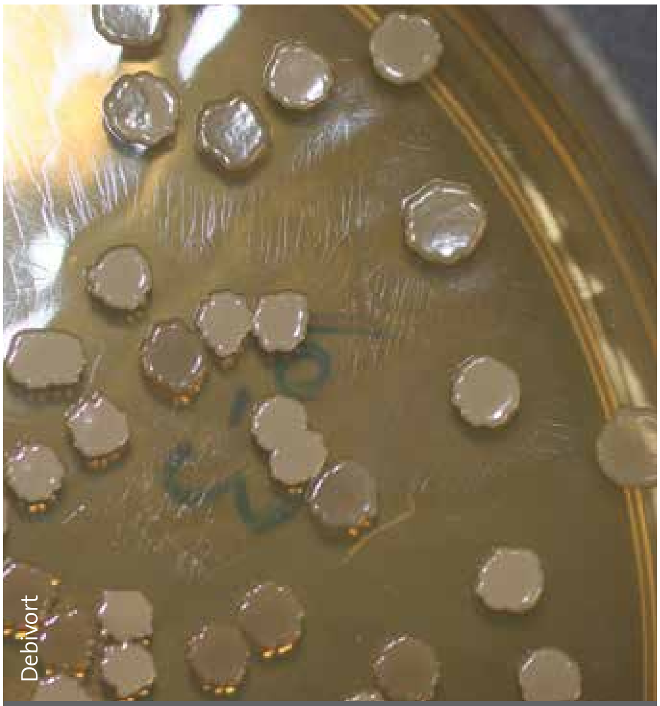


Roscosmos/ESA/NASA

↑ Locations of the different experiments on Expose-R

A true multi-user facility, Expose-R hosted a collection of nine different ESA experiments plus one guest experiment from the Institute of Biomedical Problems (IBMP) in Moscow. From that total group of ten, seven experiments were focussed on the impact of the harsh space conditions on terrestrial organisms. The test samples were subjected to extra-terrestrial conditions in Low Earth Orbit to study the probabilities and limitations for life to be distributed among the bodies of our solar system.

Research into the limits of survivability of different organisms has been on-going for many years with the list of different organisms and the conditions under which they can survive in open space ever growing as the research in this area expands. Organisms such as dormant spores of the *Bacillus subtilis* bacterium (found in soil and the gastrointestinal tract of humans for example) can survive for years in open space, but only if protected from solar UV. Different blue green algae (cyanobacteria) and halophiles, which thrive in salt-rich environments, were also shown to be able to survive for (at least) weeks in space, as were complex, multi-cellular organisms such as lichens, water bears (tardigrades) and plant seeds.



↑ Colonies of *B. subtilis* grown on a culture dish in a molecular biology laboratory

The organisms that were being studied within the different Expose-R astrobiology experiments were pre-selected on Earth and already known as extremophiles, capable of thriving under the most extreme conditions on Earth. This included organisms that can thrive in salt crusts and organisms that can bridge periods of extreme dryness in a desiccated state. The latter include fungal spores, plant seeds, and lichens.

The Harsh Environment of Space and Earth

There are five different space factors that samples are exposed to during such research: unfiltered solar light, space vacuum, cosmic radiation, weightlessness and freezing/thawing cycles. From that set of five, the major limiting factor is full-spectrum solar UV radiation, which the vast majority of organisms is not able to cope with. The solar UV spectrum can be divided into three sections: UV-A (315–400 nm), UV-B (280–315 nm), and UV-C (200–280 nm), with the shorter wavelengths (UV-B and UV-C) having the most detrimental effect on organisms exposed to it. That said, the penetration depth of UV is extremely small. If a thin layer of shielding is afforded, as is the case just under the surface of Mars or a meteoroid, a great deal more organisms would actually be able to survive.

Space vacuum is another harmful factor causing dehydration/desiccation and severe biological damage if cells cannot protect themselves. This is one reason for selecting organisms which can adapt to arid conditions for long periods.

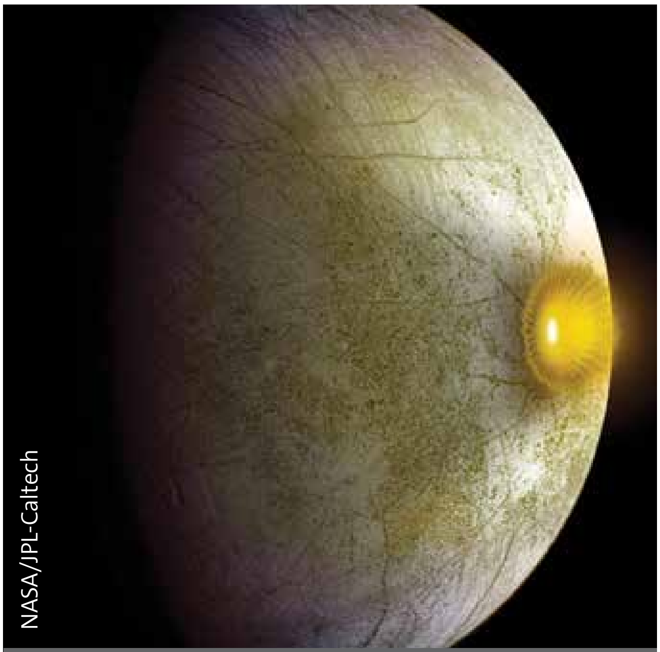
↑ The Great Salt Lake in Utah photographed from the ISS in July 2014 is typically 3-5 times saltier than the ocean. The northern arm of the lake (red colour) typically has twice the salinity of the rest of the lake due to the railroad causeway crossing the lake restricting water flow. Each half of the lake hence accommodates different algal and bacterial species which cause the colour difference

The ability to cope with high concentrations of salt and/or desiccation seems to be a good predictor of protection from UV radiation damage going on previous research. This has been shown in microbes on Earth such as with organisms living in salt crusts formed following evaporation (common example of which is the Great Salt Lake in Utah). Salt formations on the surface of other planets could therefore potentially be an important place to search for life where water is limited.

Surviving in Space: Expose-R Experiments and Results *

It is known that rock fragments can escape from planetary bodies, and that interplanetary transfer of matter has occurred several times during the history of our Solar System. We have, for example, found several chunks of the Moon and Mars here on Earth which arrived as meteorites. If a large enough asteroid, meteoroid or comet hits a life-sustaining planet, could rocks ejected into space by the impact offer enough protection to embedded spores to make interplanetary transfer feasible (the theory of Lithopanspermia)?

In addition, could an endolithic lifestyle (endoliths are organisms that live inside rocks or pores in rocks) provide suitable shelter against the UV radiation on early Earth or other planets lacking oxygen with a higher UV radiation dose than on Earth today? Organisms on the Earth's surface prior to the rise in oxygen would have received a UV radiation dose up to 1000 times more damaging to DNA than today.


NASA/JPL-Caltech

↑ Artist's concept of high-speed collision between an asteroid and Jupiter's moon Europa. Clay-type minerals have been found on its surface from data analysis from NASA's Galileo mission. The pattern of minerals suggests a shallow angle strike by an asteroid which could typically carry organic compounds

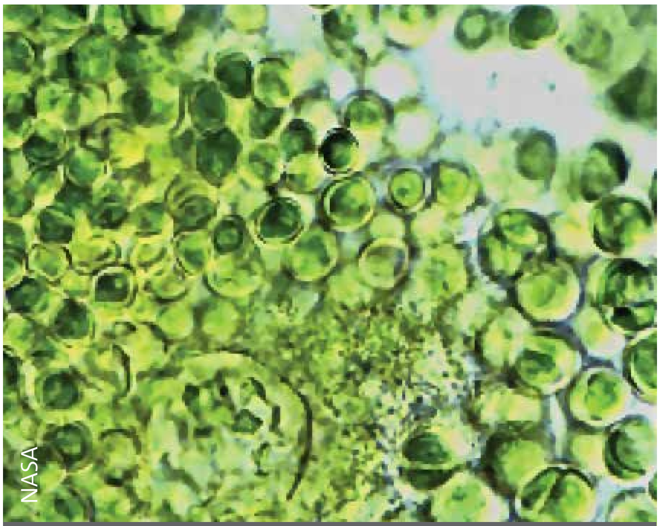
These were key elements at the heart of the ESA-funded SPORES, PHOTO, ENDO and OSMO astrobiology experiments that took place on the Expose-R facility**.

The experiments were set up in the Expose-R sample trays as can be seen in the above diagram. As previously described, the uppermost samples were either located behind magnesium fluoride (MgF2) windows to allow all wavelengths from deep ultraviolet to far infrared through, or quartz windows with the addition of neutral-density filters to simulate Martian conditions. The lower layer dark samples were shielded from solar UV – for comparison. Samples were also either exposed to space vacuum or kept at normal pressure in an inert gas (argon) environment. The samples were subjected to temperatures moving up and down between -25 deg C and +45 deg C during orbital flight.


Keisotyo

↑ *Trichoderma* colony in nature on decaying wood. *Trichoderma longibrachiatum* showed viability when exposed to open space conditions including solar UV when exposed for 22 months

Different organisms were exposed during the experiments though we focus on spores of *Bacillus subtilis*, the fungus *Trichoderma longibrachiatum*, the microorganism *Halorubrum chaoviator* and the cyanobacteria *Synechococcus* and *Chroococcidiopsis*, which have proven their high resistance to real and/or simulated outer space conditions, with additional mention of the IBMP samples.


NASA

↑ A photomicrograph of Chroococcidiopsis, enlarged 100 times

Surviving Solar UV

Spores of the fungus *Trichoderma longibrachiatum* (from the SPORES experiment) survived exposure to the full spectrum of solar UV irradiation or the Martian equivalent spectrum for the 22 months of exposure. This was at a reduced, but not significant, survival rate compared to the Sun-shielded vacuum samples, which had about a 30% survival rate. *Aspergillus* fungal spores (from the IBMP experiments) also remained viable after UV exposure. This was probably due to the fact that the spores form clusters, and the outer layers of spores may have shielded the inner cells.

For the remaining organisms, solar UV-radiation seems to be the primary factor in killing these organisms and as such they would not survive extended exposure on the surface of for example early Earth or Mars. None were viable when exposed to solar UV with only a very limited survival in certain organisms when the solar UV was attenuated to extremely low levels. For *Bacillus subtilis* (the SPORES experiment) the exposed solar UV vacuum samples were completely inactivated even when mixed with simulated Martian meteorite powder. The length of time exposed also seems to be significant for *Synechococcus* (the OSMO experiment). The samples on Expose-R that were placed behind filters to receive a lower UV dose had a lower survival rate than *Synechococcus* samples exposed to an approximately five times greater total dose of UV-B and UV-C on the earlier short-duration (two-week) Biopan missions.

Surviving Beneath the Surface

In addition to *Trichoderma longibrachiatum* and *Aspergillus* spores mentioned above, all the other samples mentioned showed viability/survival at different levels when shielded from solar UV either in the lower (dark) layers of samples, or embedded into different simulated meteorite materials.

Synechococcus and *Halorubrum chaoviator* dark samples (from the OSMO experiment) exposed to vacuum had around a 90% survival rate compared to the ground controls. The samples are extremely desiccation resistant which is most likely related to their salt tolerance, which protects them from dehydration and desiccation.

The cyanobacterium *Chroococcidiopsis* (from the ENDO experiment), which is one of the most tolerant to extremes of all of the known cyanobacteria, was viable when embedded within impact-shocked gneiss after exposure to the intense UV radiation environment for 22 months. Impact-shocked gneiss is a crystalline, low-porosity metamorphic rock, which was chosen as the protective rock habitat within the ENDO experiment. The gneiss samples were 5 mm thick discs which allows visible light transmission sufficient to support photosynthetic growth (required by *Chroococcidiopsis* for energy).

Bacillus subtilis (from the SPORES experiment) survived in the lower layers with even greater survival when additionally covered with meteorite material.

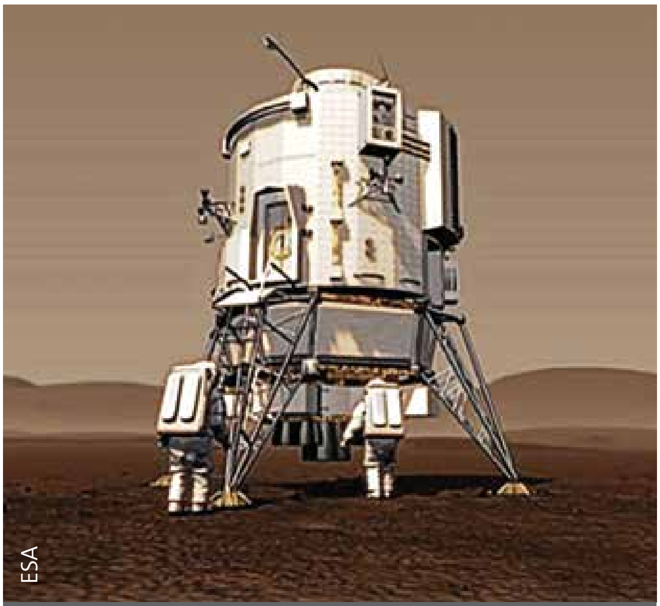
The results of all these experiments suggest that the protection afforded by meteorite material or within impact-shocked rocks is adequate to preserve viability of different organisms during interplanetary travel or beneath the surface, or in impact craters, on early Earth or Mars or other planets lacking a sufficient atmospheric UV radiation shield.

The results of the ENDO experiment (using *Chroococcidiopsis*) further demonstrated that phototrophic microorganisms i.e. organisms that use solar light as a primary energy source, could have colonized early land masses if just under the surface where enough light could penetrate for photosynthesis. In addition organisms such as *Chroococcidiopsis* have the potential to be used in applications as part of surface habitats for future human exploration missions to, for example, Mars, as part of biological ecosystems within life support systems for oxygen production or food production to aid in the formation of soil.

The survivability of these organisms also provides interesting information with respect to planetary protection issues as sending probes to different planets carries the risk of accidentally biologically ‘contaminating‘ a foreign planetary body in advance of being able to analyse it in its native state.

Biological Dosimeters: The PUR Experiment

Outside of the pure astrobiology experiments there was an additional experiment that was a cross-over between astrobiology and radiation dosimetry. Due to the complexities of the radiation environment in space there are different standards of radiation measurement for different purposes. One important standard of measurement within radiation dosimetry is the biologically effective radiation dose which in layman’s terms equates to the health risk to biological tissue from exposure to different types of radiation.


ESA

↑ Organisms that can survive certain aspects of the open space environment could find their way into future exploration missions to for example Mars where they could become part of life support systems or within surface habitats or ‘greenhouses’ aiding in creating oxygen and soil

On Earth biological dosimeters are already used within this area with the bacteriophage T7 (a bacterial virus) and uracil (which is one of the four nucleic acid bases of RNA) being two components that have shown that they can be used as dosimeters as they have a tolerance to extreme conditions and have a stable relationship with UV radiation exposure for determination of the effective radiation dose.

The Phage and Uracil Response (PUR) experiment was studying the use of the bacteriophage T7 and uracil as biological UV dosimeters in open space.

Measuring the decrease in optical density of the samples compared to pre-flight provided an assessment of the ultraviolet damage to phage DNA nucleoprotein and uracil and is proportional to the biologically effective radiation dose. There was no significant change in the phage and uracil samples before and after flight for the lower layer (dark) samples not exposed to UV radiation.

Uracil proved to be more sensitive to solar UV radiation. It was completely destroyed when exposed behind windows with 100% transparency.

Both phage T7 and uracil showed that biological dosimeters used on Earth can also be suitable for use in Low-Earth orbit (and beyond) with uracil showing a greater sensitivity. This increased sensitivity is a positive, though could become a drawback if pondering a much longer mission as a dosimeters which are too sensitive run the risk of becoming saturated.

Having such biological dosimeters will be very helpful to determine accumulated radiation levels and their effect on biological material for example for future astrobiological research and potentially for astronauts on future human spaceflight missions outside of Low-Earth Orbit.

** The results have taken into account the computer failure and window staining that was discussed in article 2 'Introduction to the Expose-R facility'.*
*** The ESA experiment package also included the SEEDS and SUBTIL experiments. A paper about the SEEDS experiment is awaiting publication whilst the SUBTIL experiment did not produce any publishable results.*

The SPORES experiment was under the scientific lead of G. Horneck, DLR (DE). The PHOTO experiment was under the scientific lead of J. Cadet, CEA (FR). The ENDO experiment was under the scientific lead of C. Cockell, UKCA (UK) and the OSMO experiment was under the scientific lead of R. Mancinelli, NASA Ames (US).

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→ ORGANIC CHEMISTRY RESEARCH ON EXPOSE-R:

The Forerunners of Life from Titan to Early Earth

↑ The 8% of solar electromagnetic radiation which is composed of UV in low-Earth orbit is crucial for organic chemistry

Life as we know it is, without exception, based on complex organic (carbon-based) compounds such as proteins and nucleic acids (DNA and RNA). Cells, plants or animals: they are all fabricated from that same molecular toolbox and have apparently evolved over time from some simple, common ancestor. What this LUCA (Last Universal Common Ancestor) looked like we don't know, but it must have been the end product of a long process of chemical evolution. Chemical evolution concerns the natural development from non-life into life, whereas Darwinian evolution deals with the development from simple life to complex life. Chemical evolution was probably started with the formation of carbonaceous molecules in the core of giant red stars. These molecules were ejected from there whereafter the chemical evolution continued, for example, in the atmosphere or on the surface of a planet. ESA-funded research in organic chemistry is helping to improve our knowledge of chemical reactions during space exposure, probing the questions of how the space environment affects different organic compounds, and how they are broken down and rebuilt in different forms. This may one day help to discover the key to where, and how, along this complex chain of events chemistry turns into biology, or help look for the signatures of life on other planets.

The radiation of stars plays a key role in the evolution of organic molecules in astrophysical environments and in planetary atmospheres. The 8% of solar electromagnetic radiation outside of Earth's atmosphere which is composed of UV is crucial for organic chemistry since UV drives most chemical evolution, either for breaking down organic structures or for building new compounds with an increase in molecular complexity.

The Amino and Organics Experiments

Two ESA organic chemistry experiments were part of the Expose-R set of experiments. The ORGANIC experiment under the scientific lead of Prof. Pascale Ehrenfreund (University of Leiden, NL) concentrated on the evolution of carbon polymers relevant to the processes occurring in the interstellar medium and circumstellar clouds.

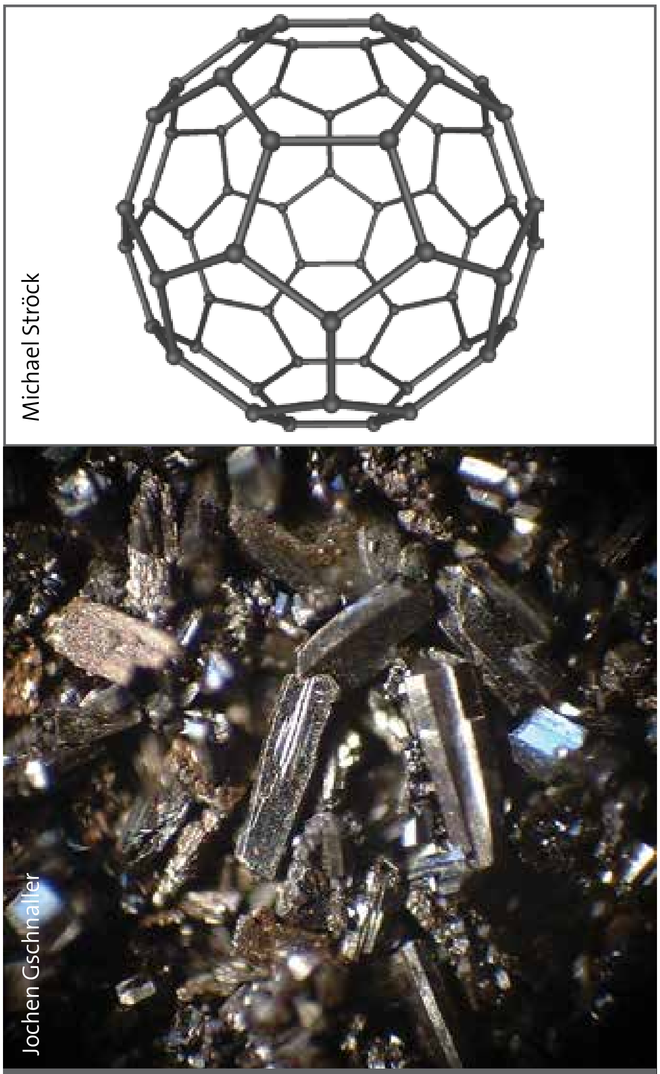
The AMINO experiment under the scientific lead of Prof. Hervé Cottin (LISA, Paris, FR) was actually composed of three individual experiments dealing with the effect of Solar UV on a variety of chemical substances which have a relevance to chemical evolution in (1) the atmosphere of Titan; (2) in comets, carbonaceous meteorites and micro-meteorites; and (3) to the RNA-world hypothesis (explained later) on early planet Earth.

Sample Accomodation

The organic chemistry samples on Expose-R were kept behind magnesium fluoride (MgF_2) windows (hardware set-up shown in previous article) for full UV exposure, accompanied by reference samples in darkness, shielded from UV exposure. Gaseous samples (simulating an atmosphere) were kept in closed cells while solid samples were either kept in closed cells (where gaseous products produced after UV exposure could be analysed post-flight), or in open (vacuum-exposed) cells. Again as with the biology experiments, results had to take into account window contamination and partly modelled environmental data.

Organic Chemistry in the Interstellar Medium

Polycyclic aromatic hydrocarbons (PAHs) have been identified in many space environments and are estimated to make up approximately 15% of the cosmic carbon as well as being present in meteorites, moons and possibly in comets.


Michael Ströck

Jochen Gschnaller

↑ C_{60} fullerene. Top: A 3D model of a C_{60} molecule, also called a "Buckyball"
Bottom: C_{60} Buckminsterfullerene, crystallized. From the Leopold-Franzens-Universität Innsbruck

Their abundance and stability in space makes them prime components in organic chemistry research.

Fullerenes (specific molecules made of carbon) constitute only a small fraction of cosmic carbon, but their recent detection in planetary nebula also provides an important research element in organic chemistry. Fullerenes are also produced in nature for example being formed by lightning discharges in the atmosphere.

The ORGANIC experiment exposed fourteen samples in thin films: 11 polycyclic aromatic hydrocarbons, and three fullerenes. The results confirmed the stability of polycyclic aromatic hydrocarbons according to their molecular structure. The most stable structures were the thin films of fullerenes (C_{60}, C_{70} and a $C_{60}/C_{70}/C_{84}$ mixture) and the compact regular polycyclic aromatic hydrocarbons (perylene, coronene, ovalene, circobiphenyl, dinaphto-coronene, dicoronylene). This was followed by non-compact polycyclic aromatic hydrocarbons (tetracene, chrysene, dibenzo-octacene and tetrabenzo-heptacene) that exhibit some minor level of degradation. Polycyclic aromatic hydrocarbons that include an atom that is not carbon or hydrogen in their molecular structure such as diphenanthrothiophene ($C_{28}H_{16}S$), are the most fragile and show the highest degradation rate with exposure.

These results confirm ground tests that have been undertaken, which can only partially simulate in-orbit environmental conditions. The results therefore support our understanding of how UV in space interacts with organic molecules.

Meteorites and Small Bodies of the Solar System

Organic molecules that were delivered from space to Earth via carbonaceous meteorites and comets could have triggered the origin of life on the Earth.

For this part of the Amino experiment a selection of organic molecules (amino acids and a dipeptide) were exposed in pure form or as a mixture. Some samples were embedded in meteoritic powder and all have been detected in the Murchison meteorite and other carbonaceous meteorites found on Earth.

The amino acids alanine, glycine, aminoisobutyric acid, aminobutyric acid and valine were quite resistant to solar UV radiation, since about 60% of the compounds were recovered. The presence of the meteorite powder moderately increases the rate of compound survival for all these amino acids and in the case of aspartic acid, the increase in survival rate was dramatic being 45% higher (from just over 20% survival without meteorite powder). The dipeptide dileucine is entirely degraded when exposed in free form, but still present when associated with meteorite powder.

This confirms that some of the UV-exposed organic compounds could survive transport in interstellar space when exposed to UV, even more so if not fully exposed but embedded in appropriate mineral matter. They could also resist UV radiation on or beneath the surface of Mars though exposure time and depth of protection within the Martian soil are important factors.

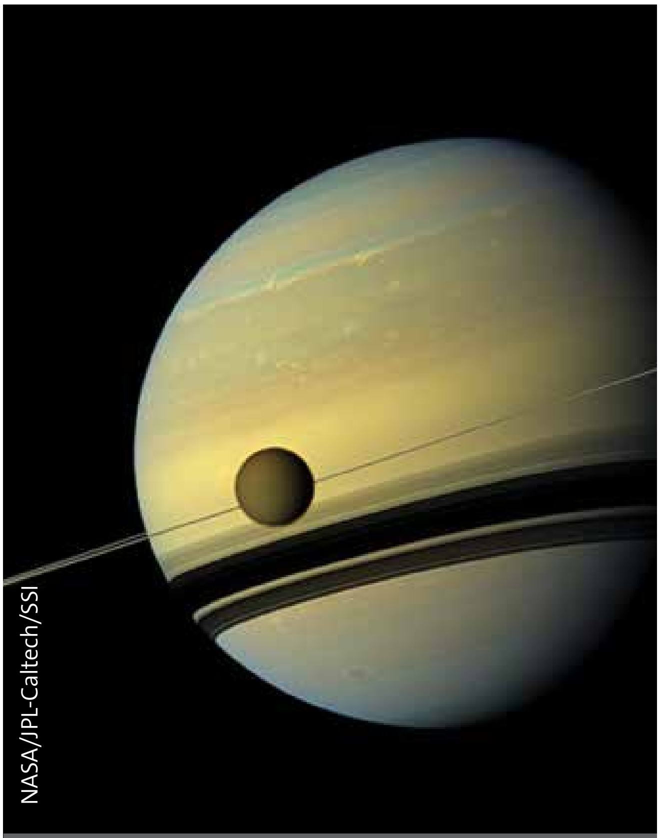
United States Department of Energy

↑ Fragment of the Murchison meteorite and isolated individual particles (shown in the test). When the meteorite fell to Earth in September 1969 more than 100 kg of fragments were found with individual mass up to 7 kg

Chemical Processes in Titan's atmosphere

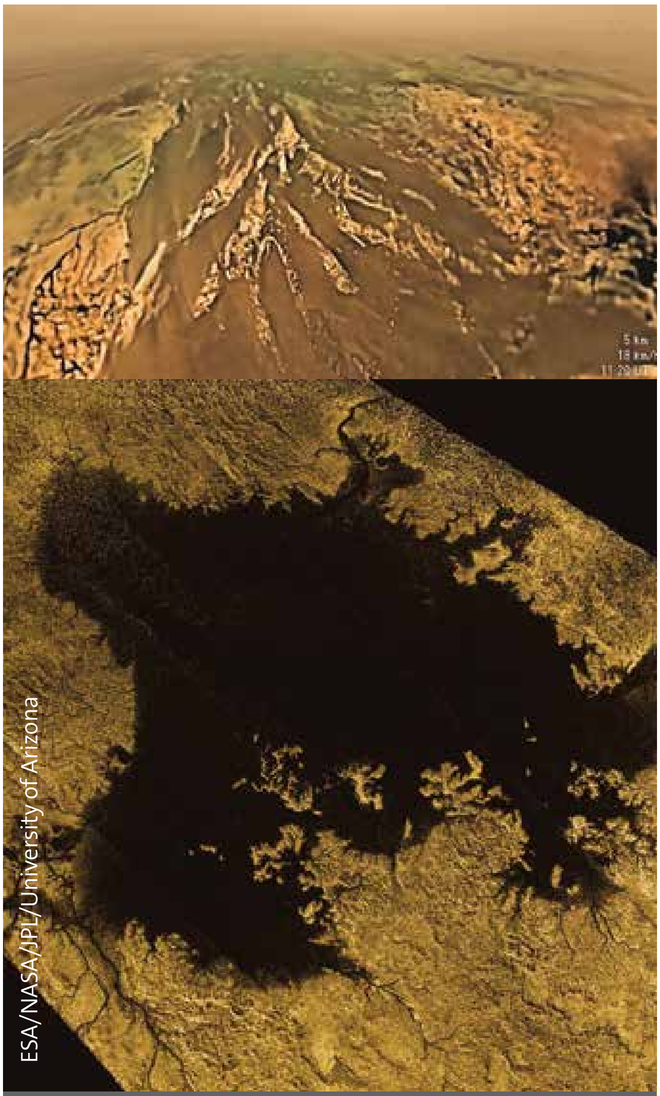
Titan is Saturn's largest moon. It is the only natural satellite known to have a dense atmosphere and the only object other than Earth where clear evidence of stable bodies of surface liquid has been found.

Solar UV initiates a complex chemistry in Titan's atmosphere that turns lightest gaseous organic compounds such as methane (CH_4) into heavy and solid organic aerosols. Methane is the most abundant hydrocarbon found in its atmosphere (and in fact all the atmospheres of Jupiter, Saturn, Uranus and Neptune).



NASA/JPL-Caltech/SSI

↑ Natural colour view of Titan and Saturn from NASA's Cassini spacecraft. The Cassini-Huygens mission is a cooperative project of NASA, ESA and ASI



ESA/NASA/JPL/University of Arizona

↑ Top: Image taken from ESA's Huygens probe in January 2005 on its descent to Titan's surface as part of the ESA/NASA/ASI Cassini-Huygens mission
Bottom: A false-colour image of Ligeia Mare, the second largest known body of liquid on Titan, taken from the Cassini satellite. It measures roughly 420 km x 350 km and is filled with liquid hydrocarbons, including methane

This part of the Amino experiment was looking at the chemical breakdown of methane under solar UV exposure within simulated Titan atmospheric (nitrogen and methane) gas mixtures. Solid samples were also exposed (simulating the organic aerosol haze in Titan's atmosphere) though results for the solid samples have not been as yet published.

For the UV exposed gas samples the levels of methane decreased by 22% due to chemical breakdown of methane under UV exposure which leads to the formation of saturated hydrocarbons (i.e. hydrocarbons saturated with hydrogen). These included ethane (C_2H_6), propane (C_3H_8), isobutene (C_4H_{10}), and n-pentane (C_5H_{12}). Ethane and propane have also been detected in Titan's and Saturn's atmospheres.

The breakdown of methane was lower than expected though could be due to presence of an organic film on the inside of the sample windows in line with aerosols found on Titan which could act as a UV filter. The experiment could also not replicate the production of unsaturated hydrocarbons found in Titan's atmosphere though this is probably due to lower pressure on Titan compared to inside the experiment cells.

This experiment has therefore provided a significant amount of positive data which provides a solid basis for future space research in this area.

The Potential for Life on Early Earth

The 'RNA World' hypothesis proposes that self-replicating ribonucleic acid (RNA) molecules were the precursors to all current life on Earth.

Water, carbon monoxide, sulphur dioxide, methane and dinitrogen molecules present in the atmosphere of the primitive Earth might have reacted to form such compounds as ammonia, hydrogen cyanide, formaldehyde, acetonitrile, cyanogen and cyanoacetylene with cosmic rays, ionizing reactions, electric discharges and other processes leading eventually to the formation of the elementary building blocks of life i.e. amino acids and nucleic acid bases. Substantial amounts of nucleic acid bases (building blocks of DNA and RNA) and amino acids have been found in meteorites.

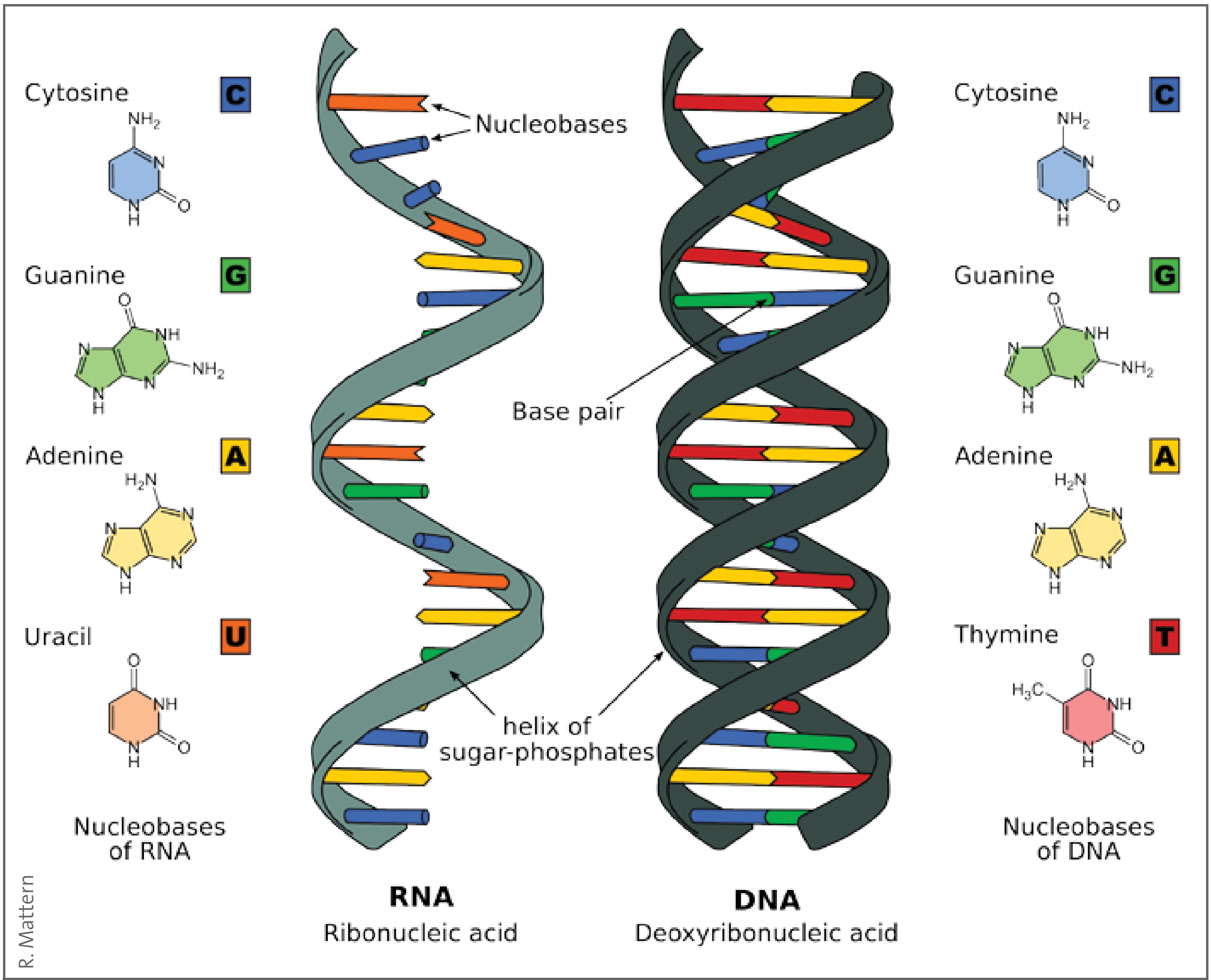
This part of Amino experiment was looking at the stability of RNA molecules at the surface of the primitive Earth or other planets not protected from energetic UV (by an ozone layer for instance). There is evidence that primitive oceans on Earth were highly saline and salt is also abundant on planets such as Mars and Jupiter's moon Europa. Salt crystals have also shown to play a protective role against thermal degradation in RNA molecules.

Small functional RNAs were exposed either alone or with sodium chloride. In the UV-exposed top layer of Expose there was a strong degradation of RNA. Sodium chloride was found to induce a slight protective effect. There wasn't any degradation in the lower layer (dark) samples.

The degradation that RNA suffers under the influence of solar UV tells us that the only means for RNA to remain viable in open space prior to entering a life-supporting environment would be if shielded from solar UV. RNA would also most likely not survive on the surface of Early Earth. However with life on Earth probably starting in seawater, which acts as a filter against UV, if there was RNA in the seas and oceans it would have been safely protected.

Future Research Possibilities

A limitation of the Expose-type experiments is that the test samples can only be studied before and after the flight. If changes are introduced during the exposure period in space, which last from 1 to 2 years on Expose, nobody can tell when, and at which speed, these changes were introduced. We can only look at the end product. New capabilities for exposure platforms are moving towards automated in-situ diagnostics. This will enable the scientists to follow the space-induced changes over time. Enabling the exposure of samples as ice mixtures in conditions to simulate organic chemistry in the outer Solar System and dense molecular clouds would be a further step forward as would be experiments outside of low-



R. Mattern

↑ Comparison of a single-stranded RNA and a double-stranded DNA with their corresponding nucleobases

Earth orbit in order to remove the protection from the vast array of galactic cosmic rays and solar wind radiations due to Earth's magnetic field.

Both the Amino and Organic experiments clearly showed the robustness of many of the organic compound samples to the conditions in open space for 22 months, specifically to UV exposure, providing more information on how different hydrocarbons and amino acids can survive a high degree of UV exposure with a high degree of stability, and how more complex compounds can be formed in the atmosphere of Titan under UV exposure. This has improved our knowledge and the outlook is very positive for further research in this area.

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↑ Scientist at DLR exposing different samples for the BIOMEX and BOSS experiments to different forms of radiation

→ RESULTS FROM THE EXPOSE-R2 EXPERIMENTS

BIOMEX Experiment Shows Interesting Results Prior to Flight

The BIOlogy and Mars EXperiment (BIOMEX) is one of the three ESA experiments on the Expose-R2 astrobiology payload which was installed outside the ISS in August 2014. Even before its launch into orbit there was a lot of attention focussed around this experiment. Many associated scientific papers had already been published, which led to extensive television coverage in Germany and Italy after Expose-R2 became active on orbit.

The ground research has been throwing up some surprising findings on how particular organisms are able to cope with conditions in open space (i.e. not inside the ISS), pointing towards the fact that in some cases exposure to more extreme, extraterrestrial environmental conditions (i.e. vacuum and UV radiation instead of only UV), increases the chance of survival. Not only have the ground tests help finalise the sample selection for the now on-going Expose-R2 mission, the data is also feeding into future research missions, such as ExoMars, for the search for life on different planetary bodies, and presents the potential for use in biotechnology applications related to biological life support systems or even extraterrestrial agriculture and soil production.

The ground tests for astrobiology experiments simulate as far as possible the conditions that will be experienced by samples in space and help to de-select any organism that are unlikely to survive under true space conditions. These ground tests in themselves provide a lot of new information, and pose lots of questions, on how these terrestrial organisms are equipped to survive under the most extreme extra-terrestrial conditions. What mechanisms do they employ to cope? Are they equipped with special pigments, a different intracellular structure, a special physiology? Etc. BIOMEX is one of the Expose-R2 experiments delving into these very questions.

ESA/NASA/Roscosmos

↑ Roscosmos cosmonaut Oleg Artemyev with Expose-R2 payload prior to its installation outside the ISS in August 2014

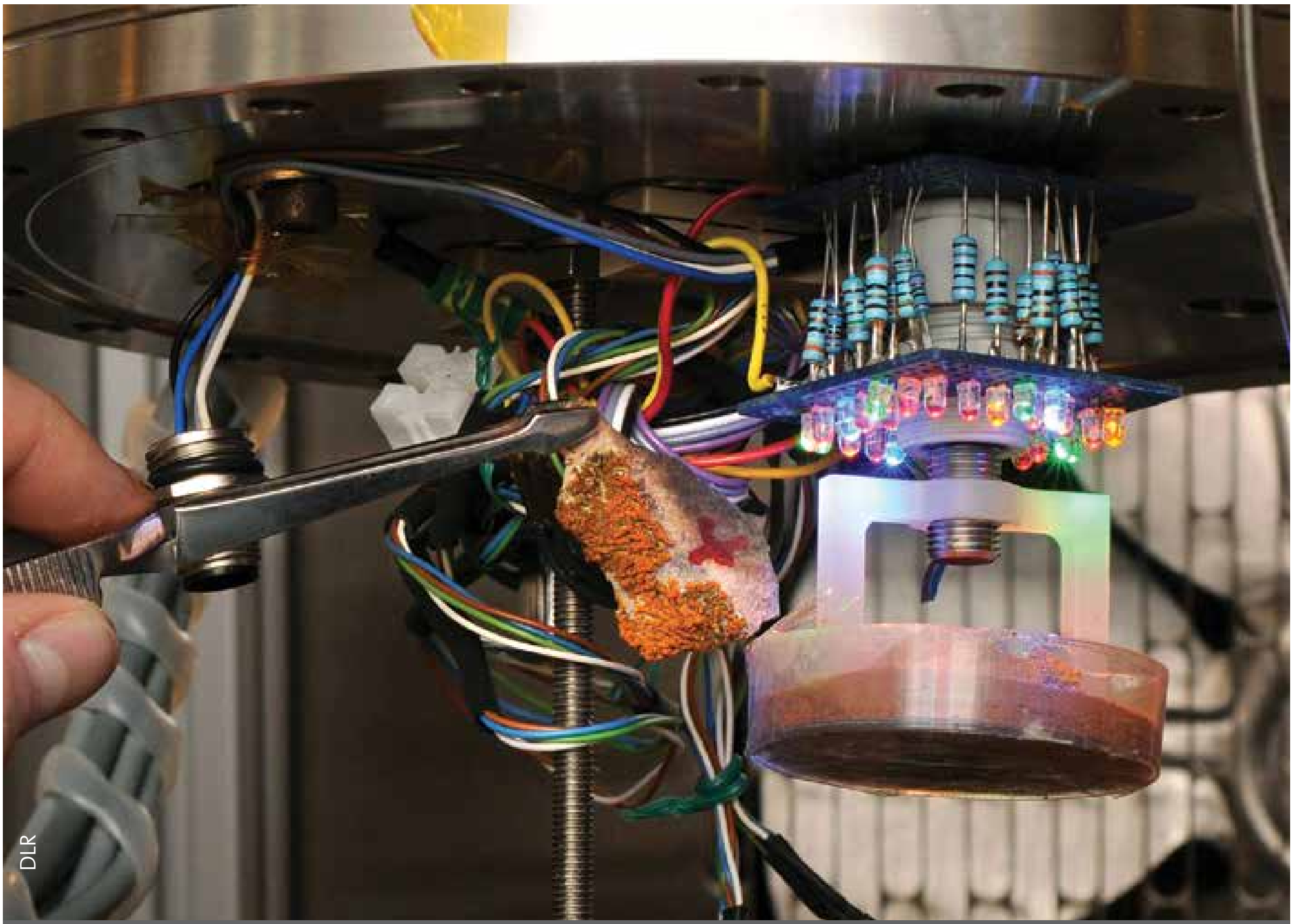
DLR

↑ Raman microscope with spectrometer

Looking for Biological Signatures of Life

The prime objective of BIOMEX, under the scientific lead of Dr. Jean-Pierre de Vera of DLR's Institute of Planetary Research in Berlin, is to evaluate to what extent simple organisms and the components thereof (biomolecules) are resistant to, and can maintain their stability under, space and Mars-like conditions; therefore a variety of pigments and cell components are under investigation. This will help to establish a biosignature database (based on techniques such as Raman spectroscopy) of organisms, associated compounds and combinations of compounds, providing unique information which will help in the future to search for the signs of existing or extinct extra-terrestrial life.

Raman spectroscopy is one of the techniques used as part of ground tests to determine biological signals in organisms slated for possible in-orbit exposure. It is a non-destructive *in situ* technique for identifying organic compounds and mineral products, and is well-established in areas like pharmacy, biology, and mineralogy. ESA's ExoMars mission in 2018 will feature the first planetary lander for which a Raman spectrometer is part of the instrumentation. It is a powerful tool that provides unique information about the overall chemical composition of investigated microbial samples. In addition, it allows the detection of biochemicals as possible signature of extinct or fossilized life.



↑ Lichen on a piece of rock (the lichen is orange) under investigation in DLR's Mars simulation facility

Testing the Limits of Different Organisms

The secondary objective of BIOMEX is to investigate the endurance of extremophiles (organisms that survive extreme conditions on Earth) to the space environment, focusing on their interactions with simulated Martian minerals, which in itself can lead to molecular changes that can contribute to life detection strategies. Biominerals (minerals produced by living organisms) may serve as biomarkers of life even if just a remnant of a producing organisms which vanished long before.

The different organisms tested on ground as part of the BIOMEX experiment included lichens, fungi, blue-green algae, (cyano-bacteria), green algae, archaea, bryophytes (such as the common liverwort), bacteria, yeasts as well as different pigments (such as chlorophyll, ß-Carotene and melanin). The organisms range from those which thrive in the Antarctic and in deserts to organisms which survive in high saline environments. The simulated Martian soil samples used in BIOMEX replicated Early acidic Mars and Mars at a later epoch.

Simulating Space Conditions

The ground tests prior to flight took place at the Planetary and Space Simulation facilities (PSI) at the Institute of Aerospace Medicine at DLR in Cologne, Germany. The tests simulated space and Martian conditions including space vacuum, Mars atmosphere, UV-C radiation, and temperature cycles

↑ Cyanobacteria of the genus *Nostoc* (here imaged under an optical microscope) are among the organisms under investigation as part of the BIOMEX experiment and extremes. The in-flight conditions cannot be simulated completely on ground, though a fair indication can be provided prior to flight in simulation tests.

Results

Lichens

Lichens are in fact more of a mini-ecosystem than an organism. They are a symbiotic partnership consisting of a fungus (known as the mycobiont) and an alga (known as the photobiont). Some lichens tolerate extreme environmental conditions including high levels of ultraviolet radiation (as can be found at high altitudes on mountain tops), infrequent water supply, extreme drought, heat and cold. The photosynthetic capacity of the algae is crucial for the nutrition of both organisms. Previous experiments have shown good survival of selected lichens to space conditions, including when exposed to solar UV.

Ground tests were undertaken on many different organisms including the lichen species *Circinaria gyrosa* and *Buellia frigida*. *Circinaria gyrosa* naturally grows in steppe-like and continental deserts of the Northern hemisphere and *Buellia frigida* is a maritime lichen found especially in continental Antarctica.

The symbiotic partnership is itself a key protective mechanism in both lichen species as the algal photobionts undergo much more cellular damage if exposed without the associated fungal mycobionts. The damage is even more extreme when the photobionts are metabolically active (not-dormant


DLR

↑ Dr. Jean-Pierre de Vera, the scientific lead of the BIOMEX experiment, collecting polar lichens in the Antarctic


Australian Antarctic Data Centre

↑ Two Antarctic lichens *Buellia frigida* (dark colour) and *Candelariella flava* (yellow colour) or dehydrated), having a destructive and lasting effect on chlorophyll (photosynthesis), and causing DNA- and protein-disruption, and enzyme inactivation.

Desiccation (being dormant and dehydrated) from being exposed to vacuum combined with UV exposure actually increases the survivability of these organisms compared to non-desiccated samples exposed to UV but not vacuum, similar to findings on different organisms in the Expose-R biology article in this newsletter. Another interesting result from these ground tests is that freezing also protected the lichens against the damaging effect of solar UV when compared to room temperature samples exposed to solar UV.

It therefore seems that the mechanisms that these organisms employ to go into a dormant state for protection against extreme stressors on Earth also inadvertently provide protection against UV-C radiation (not even present on Earth), if the protective mechanisms are triggered in space to protect against for example freezing or vacuum.

That said even though certain mechanisms maybe common to certain species of lichen, there are bound to be differences between species. Lichens come in different species-specific colours, dependent on their pigmentation: brown, green, yellow, orange, grey, purple, white and black. Some species, for instance contain the pigment melanin, which is also found in the human skin, and is the primary determinant of skin colour and protects against UV.

Blue Green Algae

The cyanobacteria used in the BIOMEX experiment belong to the species of *Chroococcidiopsis*, a photosynthetic desiccation-/radiation-tolerant microscopic organism, able to thrive in extreme deserts on Earth like the Atacama desert in Chile and the Dry Valleys in Antarctica. Its natural habitats on Earth represent the closest analogues to a possible Martian habitat. Previously it has shown resistance to real space conditions when shielded from solar UV both on the 18-month Expose-E mission and 22-month Expose-R mission (discussed in the biology article within the ENDO experiment) and its resistance is enhanced when in a dry, non-metabolic state.

Mgarten

↑ Kombucha including the culture

With the ENDO experiment showing the survivability of *Chroococcidiopsis* when embedded in impact-shocked gneiss ground, tests in advance of Expose-R2 also confirmed increased survivability when embedded in lunar and Martian soil types when exposed to solar UV and vacuum conditions. *Chroococcidiopsis* DNA and pigments were still detectable (with ß-carotene as the dominant feature in the spectrum using Raman spectroscopy) not only making them promising molecular biomarkers for detecting life, for example, on the Moon and Mars, but also indicating that this cyanobacterium is suitable for the Expose-R2 mission.

Chroococcidiopsis has the potential to contribute to the development of biological in situ resource utilization processes on future exploration missions. Results from ground tests on this blue-green algae are also feeding into analysis techniques for the future ExoMars mission, determining optimal parameters using Raman spectroscopy to determine the presence of such microorganisms and associated minerals on other planetary bodies.

Kombucha

The Kombucha culture, used in the BIOMEX experiment is a symbiotic culture of bacteria and yeast, known for centuries in Asia as a fermenter in drinks. It has potential positive health effects with respect to digestion and the immune system. As a probiotic for plants it could help in food supply techniques or form part of advanced biological life support systems in space and on other planetary bodies even with respect to soil generation on other planets.

During fermentation the bacteria produce cellulose fibres (which may have practical implications in nanobiotechnology and biomedicine). During the ground tests the culture showed a high survival rate when exposed to space conditions when mixed with simulated lunar soil. Results show that bacterial cellulose, which provides a protection for the inhabiting microorganisms against simulated space conditions including UV, may be a biosignature for the search for life.

Future

The research has provided numerous pieces of information which will improve our chances of detecting life on other planetary bodies whether extant or extinct. This may also feed into strategies for planetary protection. The findings will also help with follow-on geo-biological studies on the Moon and as preparation and support for future Mars exploration missions.

This area of research could further have an impact in many different areas. Biotechnology is an obvious choice with the potential for using extremophiles within life-support systems for human space exploration (e.g. the MELiSSA project) and in situ resource utilization on the Moon and Mars.

The potential that techniques such as Raman spectroscopy hold as well provide great potential benefits for *in situ* observation of samples over time, allowing the observation of behaviour of organisms and compounds to their surroundings. Technology such as Raman spectrometers, will also serve as reference for future exposure experiments on the Moon surface regarding the stability of data already gathered.

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