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ORIGINAL ARTICLE

BIOKIS: A Model Payload for Multidisciplinary Experiments in Microgravity

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Abstract In this paper we report about the BIOKIS payload: a multidisciplinary set of experiments and measurements in the fields of Biology (4) and Dosimetry (3) performed in microgravity. BIOKIS took advantage of the last STS-134 Endeavour mission and engineering state of the art in Space Life Science. The BIOKIS payload is compact, efficient, and capable to host experiments with different samples and science

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disciplines. Moreover, the time overlap of biological experiments and dosimetry measurements will produce more insightful information.

Keywords DAMA · BIOKIS · BIOKON · Experiment hardware · Experiment unit · BioS-SPORE · Arabidops-ISS · PHOTOEVOLUTION · TARDIKISS · HiDOSE · 3DISS · nDOSE

Abbreviations

ISS	International Space Station
ASI	Agenzia Spaziale Italiana
NASA	National Aeronautics and Space Administration
AM	Italian Air Force, International Space Station
HW	Hardware
EU	Experiment Unit
TLD	Thermo Luminescence Dosimeters
LET	Linear Energy Transfer
LEO	Low Earth Orbit
PCR	Polymerase Chain Reaction
KI	Kayser Italia

Introduction

Microgravity and radiation are the two main factors acting on biological systems during space flights. A fifty years long activity in space flights has allowed the scientific community to investigate on the effects of exposing to space conditions biological systems.

Clearly, microgravity and radiation both affect biological systems during spaceflight, hence multidisciplinary approaches are ideal candidates for scientific investigations in the frame of space life sciences.

Presently, the reduced capabilities in terms of upload/download due to the end of the Shuttle program asks for a further optimization of scientific payloads in terms of reduced dimensions, reduced crew time and power resources. Nevertheless, experimenting in microgravity has to be feasible.

On the 1st of June, the last landing of the Endeavour at NASA's Kennedy Space Center ended the STS-134 mission which lasted for 16 days.

The joint ASI-AM DAMA mission supported the selection of a set of scientific experiments to be executed in short-duration microgravity taking advantage of the microgravity environment on board the Space Shuttle docked to the International Space Station (ISS).

In the present paper we report on seven different experiments that made up the BIOKIS scientific payload. The payload hosted four experiments targeting biological working hypotheses on four different model organisms and three dosimetry measurements.

The biological experiments, namely BioS-SPORE, Arabidops-ISS, PHOTO-EVOLUTION, and *TARDIKISS*, respectively investigate on the *Saccharomyces genus* yeast, *Arabidopsis thaliana*, *Chlamydomonas reinhardtii* model organisms and *tardigrades* a model organism for space research. The latter hitting the headlines in 2009 as the organisms survived space vacuum and combined exposure to space vacuum and solar radiation (Jönsson et al. 2008).

The aim of BioS-SPORE is to assess the importance of space flight concerning generations of diversity and the birth of new species. Two different aspects are studied: yeast sporulation and germination. The molecular mechanisms of reproductive isolation under examination in the BioS-SPORE experiment are of great interest for evolutionary biology.

The main objective of the PHOTO-EVOLUTION project is to provide further insights on the tolerance of different strains of algae *Chlamydomonas reinhardtii* to extreme environmental conditions. The exploitation of oxygenic photosynthetic microalgae is particularly suited for life regenerative supporting system in manned long-term space missions, being able to produce the oxygen necessary for atmosphere revitalization, food and nutraceuticals for human consumption.

Arabidops-ISS strives to increase the understanding of gravitropism, a complex and coordinated process whose entire mechanism is still unclear. At molecular level, reactive oxygen species (ROS) and nitric oxide (NO) are implicated in the gravitropic response (Mugnai et al. 2008). The impact of microgravity on the expression profile changes of the ROS and NO related genes pathways both in *Arabidopsis thaliana* wild type and the mutant *eir1* (which exhibit pronounced deficiencies in root gravitropism) will be assessed.

TARDIKISS attempts to deepen the study of life history traits, regulation of antioxidant defences, DNA damage and its repairing mechanisms in tardigrades subjected to spaceflight stresses. Tardigrades can cope with stress due to their ability to enter a highly stable state of suspended animation, due to complete animal desiccation (anhydrobiosis) recoverable by rehydration (Nelson et al. 2010; Guidetti et al. 2011). Such features makes them ideal candidates as model for multicellular organism in microgravity. In fact, tardigrades exhibited a remarkable resistance and high survival rate to extreme space conditions in three different experiments (TARDIS: Jönsson et al. 2008, TARSE: Rebecchi et al. 2009, 2011; RoTaRad: Persson et al. 2011).

The remaining three experiments, namely HiDOSE, nDOSE, 3DISS respectively investigate on radiation in space, a crucial topic as the exposure of astronauts and instrumentation to the cosmic radiation poses a major risk to space flight.

The radiation hazard for the astronauts consists of the primary cosmic radiation (95% by hydrogen ions i.e. protons, 3.5% of alpha particles and in small part of heavier nuclei (Fig. 1), and secondary radiation produced by the primary particles interacting with shielding materials of the vehicles.

The space radiation, depending on its origin, can be classified into solar cosmic rays (SCR), galactic cosmic rays, and trapped particles in the Van Allen belts. Given the complexity of the radiation field in space environment, dose measurements should be considered an asset of any space mission. This goal is very complex and not completely achieved yet.

HiDose purpose is to evaluate the exposure dose to charged particles for biology experiments in the STS-134 mission. Thermo Luminescence Detectors (TLDs) are used because of their small size, resistance to environmental conditions and the possibility of accumulating data for long period of time without need of external power.

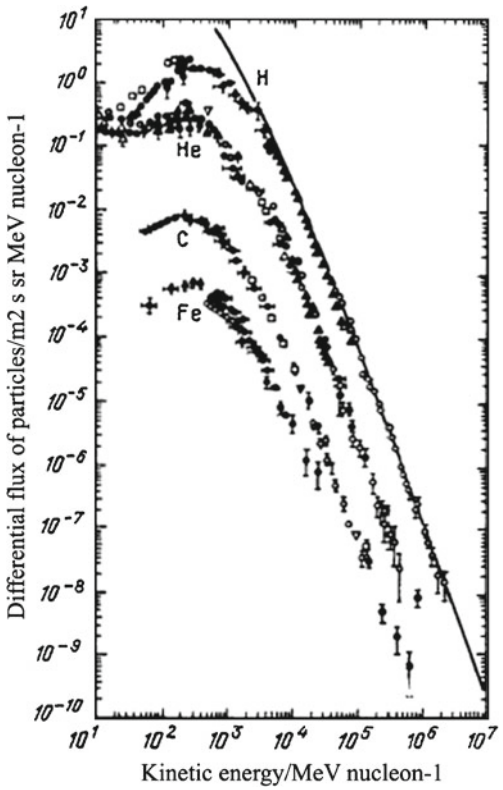

Fig. 1 Cosmic ray primary radiation (Simpson 1983)

The nDOSE measurement system, based on neutron bubble detectors and stack bismuth track detectors aims to evaluate the neutron field on the entire energy range on the ISS and it represents an important contribution to the radiation environment assessment during the STS-134 mission.

3DISS target is to investigate the primary damage, i.e. the damage suffered by the organic molecule after direct interaction with the cosmic rays. Due to the complexity of the radiation field inside the spacecraft environment, it is hard to simulate it in the laboratory and to reconstruct the actual radiation hazard. Thus, it is fundamental to perform experiments analyzing both the biological damage suffered by the tissues and the main features of the radiation causing the damage. For such purpose a dedicated diamond dosimeter covered with nucleic acid is tested.

Payload

The hardware involved in the BIOKIS payload was entirely developed and manufactured by Kayser Italia (KI) that features a long-term experience in the field of life and physical science investigations in microgravity.

KI ensured dosimeters reliability and with regard to the biological experiments, the execution of different scientific protocols. Biological sample maintaining, feeding, activation and fixation enabling re-entry analyses, was performed by means of precisely tuned fluid displacement.

The BIOKIS payload is made up by seven different experiments accommodated in two BIOKONs (BIOKIS 001, BIOKIS 002), a KI standard transportable container for experiments processing (Fig. 2).

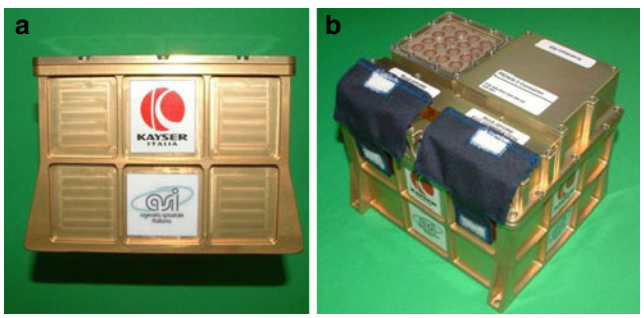

Fig. 2 BIOKON standard container. **a** BIOKISS 001 (in. dim. 16 × 9 × 13 cm; out. dim. 18 × 16 × 11 cm, weight 1.64 kg) carrying nDOSE and TARDIKISS. **b** BIOKISS 002 (in. dim. 16 × 9 × 13 cm, out. dim.18 × 16 × 14 cm, weight 2.15 kg) carrying BioS-SPORE, Arabidops-ISS, PHOTOEVOLUTION, 3DISS, HiDOSE

Table 1 Overview on the BIOKIS payload. BIOKON (Biological Container), EU (Experiment Unit)

Experiment	BIOKON	Sample/Dosimeter
TARDIKISS	BIOKIS 001	Tardigrades: 12 Petri Dishes (2 Species *Paramacrobiotus richtersi* and *Ramazzottius oberhaeuseri*, 120 specimens).
nDOSE	BIOKIS 001	Bubble dosimeters and Bismuth stack detectors.
BioS-SPORE	BIOKIS 002	*Saccharomyces*: 2 EU. 8 samples, *S. bayanus* and *S. cerevisiae* spores and cells. 4 different genotypes.
Arabidops-ISS	BIOKIS 002	*Arabidopsis thaliana* 2 EU tot. 4 samples (2 wild types, 2 mutants, 60 seeds per sample).
PHOTOEVOLUTION	BIOKIS 002	*Chlamydomonas reinhardtii:* 1 EU tot. 16 samples (1 wild type [IL] and 3 mutants [I163N, I163T, P162S] each in four replicas).
3DISS	BIOKIS 002	3 diamond dosimeters with genetic material shield. 4 bare diamond dosimeters, 7 Eppendorf caps containg *Bacillus subtilis* DNA.
HiDOSE	BIOKIS 002	3 different Thermo Luminescence Dosimeters. 8 for each type.

On the whole, a volume of approximately seven litres served to provide a number of statistically significant samples and dosimeters for each of the seven experiments. The BIOKIS 001 and BIOKIS 002 were placed into the Shuttle Middeck for the whole mission.

Hereafter, the experiments of the BIOKIS payload, their respective accommodation, the biological samples and dosimeters under investigation are reported in Table 1.

Two i-buttons data logger recorded temperature data for the BIOKIS 001 and BIOKIS 002 during the mission (Fig. 3).

BioS-SPORE Experiment

Material and Methods

The BioS-SPORE EU is composed of a main body made of semi-crystalline thermoplastic polymer, outer and internal pistons, and reservoirs linked by a fluidic pathway. The inner pistons are provided with o-rings to avoid cross-contamination. Outer pistons are provided with o-rings allowing for EU sealing (i.e. level of containment). Each EU allows performing 4 independent experiments at the same time. Liquid displacements (i.e. start of sporulation and germination) are permitted by pushing inwards the outer piston by means of a dedicated tool. This was operated in space by manual intervention of the astronaut. We took advantage of the availability of interspecies hybrid strains, obtained by crossing *S. bayanus* and *S. cerevisiae* yeasts, whose extent of sporulation varied between 10% and 20% with only very rare asci holding viable ascospores (Sebastiani et al. 2002). On Earth, cells of the parental yeast species *S. cerevisiae*, the S288c strain, and *S. bayanus*, CC6, as well as cells of the hybrid species CC189, were grown in liquid rich medium (YPD, 1% yeast extract, 2% peptone, 2% glucose) for about 18 h and then transferred in solid YPD medium to obtain aged cultures used to induce yeast sporulation in adequate medium (SPO, 0.25% yeast extract, 2% potassium acetate, 0.1% glucose). After 5–7 days, cultures were collected and the samples were treated to eliminate vegetative cells to obtain a pure preparation of spores. The pure preparation was obtained by com-

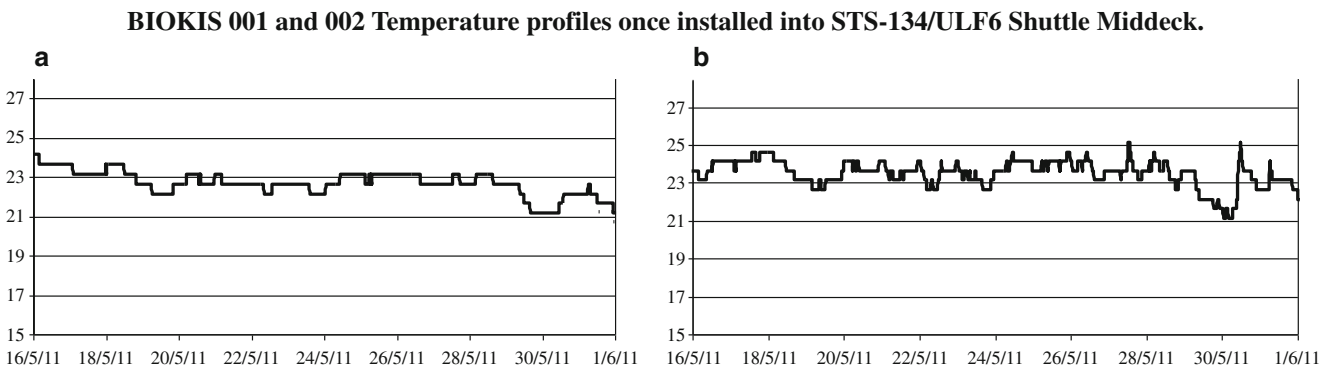

Fig. 3 BIOKIS 001 (**a**) and 002 (**b**) temperature

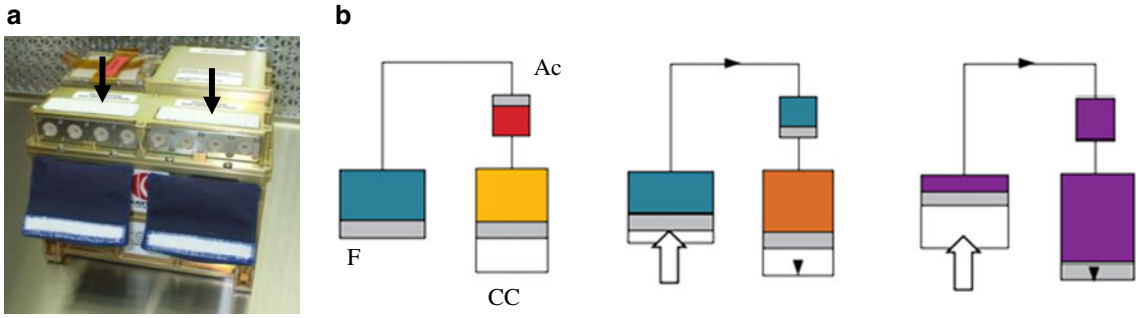

Fig. 4 **a** *Black arrows* indicate BioS-SPORE EUs integrated on top of the BIOKON 002. **b** BioS-SPORE fluidics: *CC* Culture Chamber, *F* Fixative, *Ac* Activator

bining zymoliase and other treatments, adapting the procedure by Bahalul et al. (2010). Spores viability was then evaluated by counting colony forming units after 3 days of growth on solid rich medium. Spores and cells were stored in water till hardware integration.

Results

In order to test the sporulation and germination ability of the sample within the BioS-SPORE hardware, a pre-flight test was performed on Earth simulating the following experiment on orbit. This was then considered as 1 g control with respect to the space-flight results. After 24 h on orbit, sporulation was induced in the corresponding experimental unit (Sporulation EU) by adding SPO medium in each of the 4 chambers containing the 4 different strains in cell form (Fig. 4).

Similarly, after 36 h, the activation of the Germination EU was performed by adding to the different 4 chambers YPD medium allowing germination of the spores. At the end of the mission, sporulation and germination rate were defined for each culture, by proliferative and vegetative cells count of recovered samples from the Sporulation EU and the Germination EU, respectively. We observed no significant differences on sporulation ability. On the other hand, spores of *S. bayanus* and the hybrid species were completely not able to germinate whereas *S. cerevisiae* was (Fig. 5). This is a completely new finding indicating that a species of the *Saccharomyces* genus cannot germinate in space flight, suggesting that germination is species and maybe strain dependent and paving the way to studies on the genes required for germination during space flight. Since we observed the presence of some asci of *S. cerevisiae* (Fig. 5), gravity seems to influence the complete germination process but otherwise indicates that *S. cerevisiae* spores remain viable in gravity absence conditions. The observed differences in spore viability evidence that space flight affects hybrid viability, thus formation of new species.

The differences in spore viability also show that space flight affects the ability of spores of the *S. bayanus* species to germinate. Thus the hybrid also cannot germinate in space. This is a novel finding whose molecular bases are worth further investigation. The result indicates on the possibility of transporting terrestrial species to non-terrestrial ecosystems.

Our preliminary results indicate the importance of continuing these experiments to understand the mechanisms responsible for an altered cell cycle during space flight. Further studies on this aspect hold the promise to enable the discovery of genes essential for germination in space.

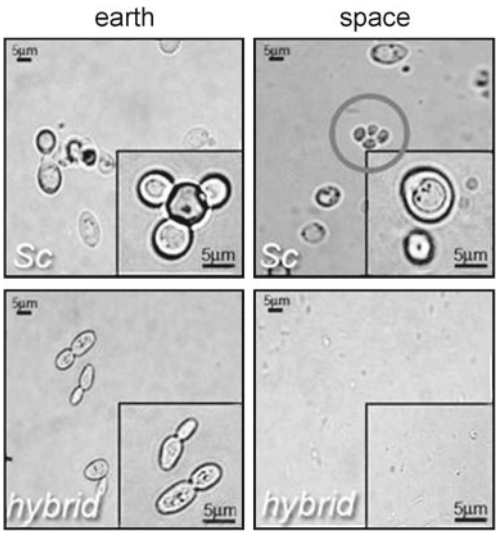

Fig. 5 Space flight influences germination and sporulation ability of *S. bayanus* and *S. cerevisiae*/*S. bayanus* hybrids. *S. cerevisiae* (Sc) and interspecies hybrid (hybrid) samples are represented. Hybrid germination ability is highly compromised in orbit. Scale bar 5 µm

PHOTOEVOLUTION Experiment

Materials and Methods

The PHOTOEVOLUTION EU is composed of a main body made of semi-crystalline thermoplastic polymer, in which 16 independent culture chambers are machined. O-rings avoid cross-contamination between the chambers and provide EU sealing (i.e. level of containment). A transparent cover made of poly methacrylate exposes *Chlamydomonas* cultures to light. Mutagenesis: directed evolution experiments and site-directed mutagenesis were performed by PCR using as a template the pSH5 plasmid, harboring the intronless *psb*A gene. The resulting DNA fragments of the *psb*A gene were used for transformation of *Chlamydomonas* chloroplast without further purification steps as previously reported (Rea et al. 2011).

Oxygen evolution: a Chlorolab-2 liquid-phase oxygen electrode system (Hansatech, UK) was used to measure photosynthetic O_2 evolution in liquid cell cultures containing equal amounts of chlorophyll (1562 μg ml^{-1}), in the presence of 10 mM $NaHCO_3$ as additional carbon source. The curves were recorded by gradual increase of the light intensity from 200 up to 900 μmol m^{-2} s^{-1}.

Photosynthetic performance: in on-ground experiments, photosynthetic performance was assessed by chlorophyll fluorescence measurements using the Fluorescence Monitoring System (FMSII, Hansatech, King's Lynn, Norfolk, UK). Fluorescence activity was recorded at every critical step of the experimental procedure: (i) arrival at the Kennedy Space Centre-NASA, (ii) before the launch (iii) after landing and (iv) at the arrival at the principal investigator's laboratory. The fluorescence emission was measured at room temperature for 2.5 s, the duration of the saturated pulse was 0.7 s.

Integration of algae in the PHOTO-I device: selected mutants (IL, I163T, I163N, P162S) were tested on-ground for their capability to tolerate the immobilization conditions on solid medium inside the PHOTO-I device by modulating cells concentration and medium content (volume). Viability was tested in the presence or absence of light, in order to determine the minimal and maximum duration times without loss of life. Strains able to survive for about one month without significant physiological changes were selected.

C. reinhardtii cultures were grown in TAP liquid medium to reach the early exponential growth phase, harvested by weak centrifugation, re-suspended to obtained a final $OD_{750} = 2$ and layered on 300 μl of 1.65% TAP agar medium previously solidified in the biocell

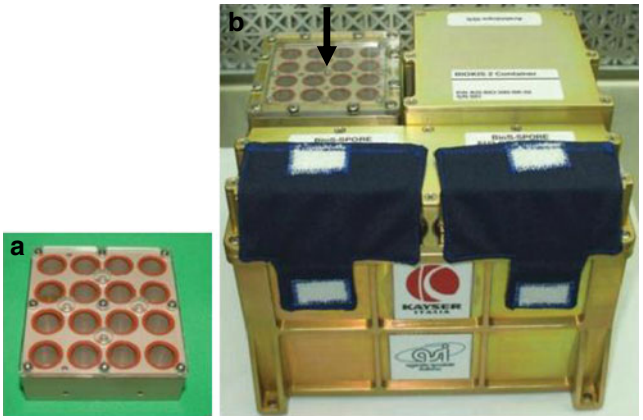

Fig. 6 **a** PHOTOEVOLUTION EU. **b** *Black arrow* indicates EU integrated on top of the BIOKON 002

of the PHOTO-I device (Fig. 6). The PHOTO-I is a passive container specifically designed by Kayser Italia. It consists of a plastic body having sixteen wells hosting the biological materials and a transparent inner cover allowing the photosynthetic reaction of the enclosed samples. O-ring provide to each well hermetic closure of the instrument, avoiding contamination and excess over dry of the solid media. After cell culture immobilization, PHOTO-I was hermetically closed by two covers, placed into the BIOKON 002 (Fig. 6) and sent to the Kennedy Space Center.

Results

A huge library of *C. reinhardtii* mutants has been produced exploiting an *in vitro* directed evolution strategy targeted to the photosynthetic reaction centre D1 protein. This protein plays a crucial role in the photosynthetic solar energy conversion and is a hot-spot for radiation-induced radical injuries. Evolution is based, among the other mechanisms, on the emerging of casual mutations which could confer advantageous traits to the host. By simulating and accelerating this process *in vitro* we isolated random mutants with pronounced tolerance to radical-generating proton or neutron irradiation. Several of these strains hosted aminoacidic substitutions near to the oxygen evolving complex, including the I163N, I163T and P162S mutations. Based on these results, we produced the corresponding site directed mutants and proved their capability to cope with free radical generating conditions by on-ground experiments (Rea et al. 2011). Oxygen evolution capacity was monitored under high fluency condition and a higher oxygen evolution capacity was demonstrated in all the mutants compared to the reference strain, IL (Fig. 7). These mutants, with the parental strain

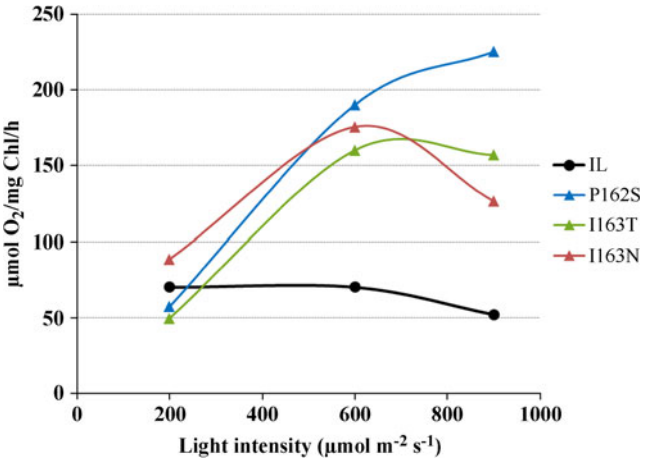

Fig. 7 Light-dependent oxygen evolution capacity of the site-directed *Chlamydomonas* D1 mutants

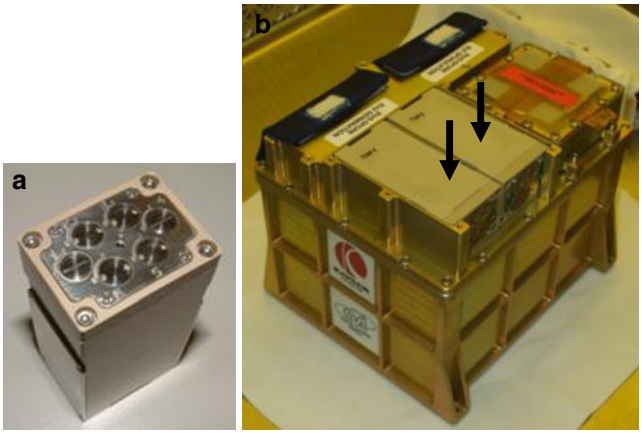

Fig. 8 **a** Arabidops-ISS EU. **b** EU during integration on top of the BIOKON 002. *Black arrows* indicate EU integrated on top of the BIOKON 002

IL, took part in the BIOKIS project enclosed in the PHOTO-I hardware.

Before flight, the experimental set-up was optimised in order to get vital cells in space (see "Materials and Methods"). After the flight, the mutants displayed a higher photosynthetic performance and a faster rate of re-growing compared to the parental strain indicating a higher capacity of stress recovering. The mutants' enhanced capability to survive to the cosmic adverse conditions has been related to the particular localization of the aminoacid substitution in the D1 structure. Besides, the mutants' improved photosynthetic performance could be related to their enhanced ability to evolve oxygen in extreme environmental conditions and/or to a different pattern of antioxidant pigments. Real-Time PCR and HPLC experiments, anti-oxidant analyses and bioinformatic studies will be helpful to elucidate the molecular mechanisms underlying the stress-tolerance to space environment. The relevance of PHOTOEVOLUTION project could be not only in the creation of new systems able to regenerate oxygen in space, but also in environmental and medical contexts. In fact, the identification of novel mutants with improved photosynthetic activity may have useful applications in the treatment of civil or industrial wastewater. Finally, the adopted experimental plan may be used to identify molecular markers of specific pathological conditions.

Arabidops-ISS Experiment

Materials and Methods

The Arabidops-ISS EU (Fig. 8) is composed of a main body made of semi-crystalline thermoplastic polymer, outer and internal pistons, reservoirs and culture chambers connected by a fluidic pathway. The inner pistons are provided with o-rings to avoid cross-contamination. Each EU allows performing 2 independent experiments. Outer pistons are provided with o-rings allowing for EU sealing (i.e. level of containment). Liquid displacements (fixation) are permitted by a release system based on preloaded springs. Spring release and consequent piston movement inwards the body were operated in space by manual intervention of the astronaut. Two EUs were previously sterilized for 1 h (two cycles of 30′) and used for the experiment. In the meantime, the medium was prepared by mixing 0.5% MS (Murashige-Skoog nutrient mix), 0.8% agar and 1% PPM (Plant Protection Mixture, Plant Cell Technology, USA). The pH of the medium was stabilized at 5.7 and then sterilized for 20′. After sterilization, the culture chambers were filled with the medium. The medium solidified at environmental temperature. Sterilized *Arabidopsis* seeds (*A. thaliana*, wt and *eir1* genotypes) were placed on the medium (two culture chambers per genotype, 60 seeds per chamber) and the culture chambers were then closed. All the procedures after sterilization were performed under laminar flow cabinet to maintain sterilization. EUs were then stored at 4°C for 2 days to perform vernalization, to obtain a simultaneous seed germination. After 2 days of vernalization, the EUs were placed into the STS-134/ULF6 Shuttle Middeck during the orbit phase. The recorded temperature profile was in the range of 23°C ± 2°C. Two days before return (r-2d), *Arabidopsis* seedlings were fixed by RNAlater injection. Fixation was manually activated by the crew. After fixation, the EUs were stored onboard at 4°C, and then placed at -80°C once back to earth until the handover to the PI.

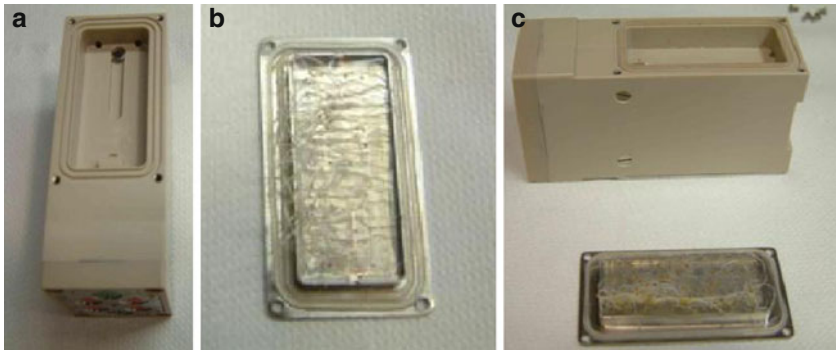

Fig. 9 **a** Arabidops-ISS EU and **b** Culture Chamber (not in real proportion compared to EU) with germinated seeds from ground experiment. **c** Seedlings recovery from fligh experiment (CC in real proportion)

Total RNA was isolated using the RNeasy® plant mini kit (Qiagen, California).

Results

No differences in the germination rate were found in both the genotypes under flight and ground conditions (Fig. 9). We also noticed the presence of long hypocotyles due to etiolation in all the conditions and genotypes. Gene expression analysis is still under investigation, and no data are currently available.

TARDIKISS Experiment

Material and Methods

We compared data obtained from three sample sets. The Flight samples (F) were compared to two control samples. The former (Ground Control, GC) was maintained in the Modena laboratory for the duration of the flight; the latter (Temperature Control, TC) was a post-flight control in which samples were exposed to the temperature profile experienced by tardigrades the days immediately before, during, and just after the flight mission. Desiccated specimens and eggs of two eutardigrade species, namely *Paramacrobiotus richtersi* and *Ramazzottius oberhaeuseri*, have been used. The former species was already used in the FOTON-M3 mission, while the latter species was used for the first time in a space mission. Six replicates of ten specimens per species and per sample set were prepared by desiccating them on paper squares under controlled laboratory conditions (see Rebecchi et al. 2009). The paper with the desiccated specimens was stored in twelve small plastic Petri dishes (1.8 cm × 1.0 cm) enveloped with parafilm and integrated within the BIOKON facility (Fig. 10), where a radiation dosimeter for neutrons (see nDOSE experiment) and a temperature data logger were also present. The temperature profile within the Biokon during the flight mission was relatively constant (from 22.0°C to 24.5°C; Fig. 2). To this day, we have completed the analysis on tardigrade survival. Animal viability was evaluated 24 h (t_{24}) and 48 h (t_{48}) from the beginning of rehydration with the protocol described in Rebecchi et al. (2009, 2011). The animal rehydration of F and GC samples was undertaken immediately after the delivery of samples in our laboratory. Specimens of *P. richtersi* were reared according to Altiero and Rebecchi (2001).

Results

Survival rate of *R. oberhaeuseri* and *P. richtersi* belonging to the three sample sets are reported in Fig. 11.

Flight tardigrades of both species showed a very high survival. The survival rate of *R. oberhaeuseri* was 98.3% (stdev = 4.1) both at t_{24} and t_{48}, while those of *P. richtersi* were 90.0% (stdev = 8.2) at t_{24} and 91.7% (stdev = 6.9) at t_{48}; for both species, significant differences between t_{24} and t_{48} survival were not recorded. No significant differences were also scored between the survivals of flight animals of the two species, both at t_{24} and t_{48}. Regarding *R. oberhaeuseri*, statistical differences were not observed ($F_{(2,15)} = 0.53$;

Fig. 10 TARDIKISS containers integrated into the BIOKIS 001

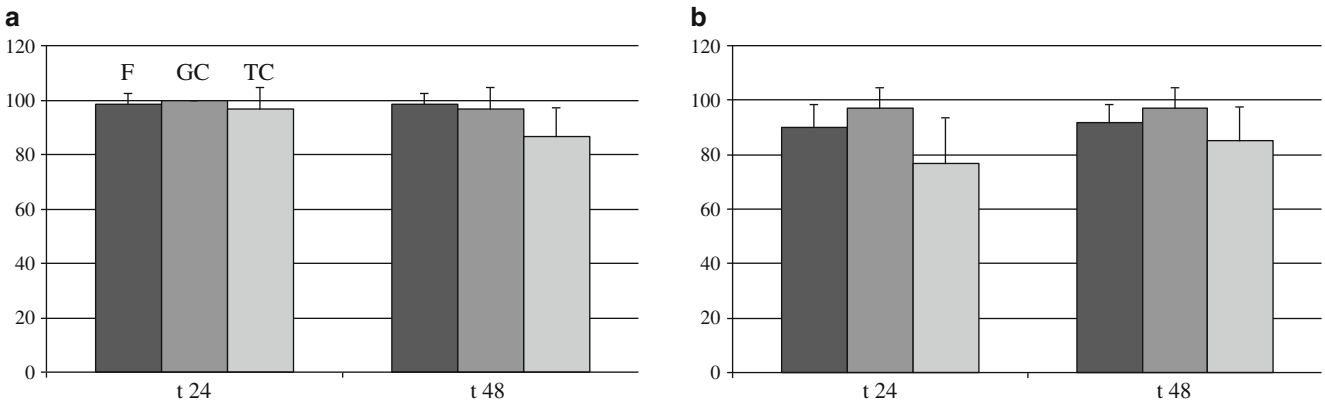

Fig. 11 Survival of *Ramazzottius oberhaeuseri* (**a**) and *Paramacrobiotus richtersi* (**b**) specimens involved in the Flight (*F*), Ground Control (*GC*) and Temperature Control (*TC*) experiments during the TARDIKISS flight on board of STS-134 space flight. Bars = standard deviations. Y axis survival rate, X axis t_{24} and t_{48} subgroups. Statistical analysis was carried out with ANOVA after arc-sine transformation of the data and Tukey post hoc test

$p = 0.599$) among the survival rates of F vs GC (100.0%; stdev = 0.0) and F vs TC (96.7%; stdev = 8.2) samples at t_{24}. Instead, significant differences in survival ($F_{(2,15)} = 4.82$; $p = 0.024$) were recorded among the three sample sets at t_{48}. In particular, F samples had a higher survival than the TC samples (86.67%; stdev = 10.33; $p = 0.034$). As regards *P. richtersi*, statistical differences ($F_{(2,16)} = 4.71$; $p = 0.025$) were recorded among the survival rates at t_{24} of F, GC (97.1%; stdev = 7.6) and TC (76.7%; stdev = 17.0) samples; the survival of GC was higher than that of TC samples ($p = 0.019$). Significant differences were not evidenced in survival rate among the three sample sets at t_{48}($F_{(2,16)} = 2.493$; $p = 0.114$).

After 48 h from the rehydration, alive specimens of *P. richtersi* from F, GC and TC samples were reared on-ground. Flight females laid eggs with normal shape that were able to hatch. Newborns exhibited normal morphology and behaviour and they laid viable eggs at sexual maturity.

The post flight high survival rate recorded for specimens of both tardigrade species of the TARDIKISS project was expected because desiccated tardigrades are very resistant to extreme physical and chemical stresses (Rebecchi et al. 2007, 2009, 2011; Jönsson et al. 2008; Altiero et al. 2011). For both species, the flight animals did not show differences in survival from on ground control ones. Only in *R. oberhaeuseri* differences were observed between F and TC samples. Therefore, we deduced that during the TARDIKISS flight, microgravity and cosmic radiation did not significantly affect survival of flown tardigrades. Forthcoming analysis of life history traits, antioxidant metabolism and eventual DNA damages using TARDIKISS samples is expected to give us further information on biochemical mechanisms of the tardigrade tolerance. Our data, further indicate that these multicellular organisms represent a useful animal tool for space research. Unfortunately, TARDIKISS was a passive project, without any possibility of changing the animal exposition to space conditions nor to analyse biochemical and physiological animal responses during the flight. To further develop the space research using tardigrades, the design and production of facilities to perform active experiments on hydrated tardigrades as well as experiments on open space are necessary. As already stated (Rebecchi et al. 2009), natural exposure to space environment, including open space, should be used without hesitation as a unique research laboratory that provides a more realistic evaluation of the mechanisms that could allow multicellular organisms to survive the synergistic action of space stressors.

HiDose Experiment

Material and Methods

HiDOSE experiment was performed using Thermo Luminescence Dosimeters (TLD) placed on the BIOKIS 002 BIOKON container (Fig. 12).

Measurements were obtained using three different types of TLD: TLD-100 (LiF:Mg,Ti), TLD-600 (^{6}LiF:Mg,Ti) and TLD-700 (^{7}LiF:Mg,Ti), previously calibrated at the NASA Space Radiation Laboratory (NSRL) at the Brookhaven National Laboratory, Upton New York. The main purpose of these ground-based measurements was to acquire TL-efficiency to apply this knowledge to the determination of dose

Fig. 12 HiDOSE TLD sensor during integration into BIOKON 002

equivalent from space radiation. Since the energy range of space radiation ranges up to a few GeV/n and its spectral composition extends from protons to Fe nuclei, evaluation of TL-efficiency is crucial to the performance of TLDs (Berger and Hajek 2008; Yasuda et al. 2006). The results of TL-efficiencies suggested that the dose equivalent from space radiation for astronauts, calculated with the calibration factor obtained exposing TLDs at 6 MeV photon beam (reference radiation obtained at LINAC of "Istituto per la Cura dei Tumori di Napoli, Fondazione Pascale") was underestimated (Pugliese et al. 2010).

The TLD used in HiDose experiment were 3.2 × 3.2 × 0.89 mm. TLDs were annealed in air at 400°C for 1 h before the exposition.

Thermoluminescent dosimeters (8 for each type) were positioned inside one of the two BIOKON passive container.

Two TLDs for each type were hosted inside a plastic box to record the radiation exposure during all the pre-launch and return phases.

The readings of these detector were subtracted from the readings of the on-flight detectors to evaluate only the radiation in orbit.

Results

Reading of TLDs was performed by an Harshaw model 3500 manual TL reader, at the Radioactivity Laboratory (LaRa) of the University of Naples Federico II. In Table 2 the preliminary results for all types of TLDs exposed on board of the Shuttle are reported. The value of the dose equivalent rate (mSv/d) is the same for TLD100 and TLD700, and highest for TLD600 that are sensitive also to neutrons.

These results will be used also to evaluate the exposure to galactic cosmic rays for biology experiments in the different positions in the BIOKON box.

Table 2 Values of dose equivalent rate

TLD	mSv	mSv/die
100	5.17 ± 0.28	0.32 ± 0.02
600	5.80 ± 0.31	0.36 ± 0.03
700	5.15 ± 0.27	0.32 ± 0.02

These preliminary results indicate that the dose equivalent rate due to space radiation exposure during the STS-134 mission is 320 μSv/die (measured by TLD 100 and TLD700) and 360 μSv (measured by TLD 600), according with the results obtained from long duration flights.

3DISS Experiment

Material and Methods

The detectors were contained in Eppendorf vials embedded in a layer of foam material within the BIOKIS 002 in a way as shown in Fig. 10. The use of tissue equivalent, biocompatible, offline dosimeters based on diamond substrates allows fabrication of versatile dosimeters to monitor astronauts, environment and experiments in space (De Sio et al. 2010). The use of naked and dehydrated genetic material (bacteria DNA and RNA) allows integrating the real radiation damage suffered due to the radiation environment inside the spacecrafts (without biological self-repairing effects). The biocompatibility of diamond allows the fabrication of integrated dosimetric biological substrate.

The aim of the DNA on Diamond Dosimeters on board the International Space Station (3DISS) experiment was to measure the dose absorbed during a space mission on ISS in order to evaluate the actual genetic damage suffered in space. Three diamond dosimeters supported DNA on top surface, four were bare dosimeters and other DNA materials were hermetically sealed in seven Eppendorf vials. The experiment tried to correlate the biological damage to the dosimetric measurement, because the substrate (diamond) for the nucleic acids was also prepared to perform dosimetric measurements at the same time.

Diamond substrates were fabricated for this experiment by the Diamond Materials, by means of a microwave-plasma-CVD reactor with ellipsoidal cavity that provides stable deposition conditions (Füner et al. 1998), to grow thick polycrystalline diamond samples with high morphological quality. Detectors were fabricated by using a standard semiconductor device process. A couple of electric contacts were made by thermally evaporating chromium (Cr) and gold

(Au) layers on both top and bottom diamond surfaces (Fig. 13).

The top gold layer was evaporated without breaking the vacuum, in order to avoid the oxidation of the chromium and the consequent change of the properties of the diamond-metal junction.

DNA of bacterial origin, DNA as plasmid pHV14 from *Bacillus subtilis* was used, offering several advantages: it is a quite small sequence of DNA (7296 base pairs), the nucleotide sequence is known, it is present at high copy number in the cell and it is isolated from *B. subtilis* strain by standard procedures.

The damage to be investigated is essentially the single and double strand breaks, formation of molecular adducts (cross-links) and the loss of purine and pyrimidine bases. It should be noted that dehydrated DNA is not able to give rise to replication and repair processes after damaging in space. This means that all the biological damage that we are going to extract after the current laboratory experiments will be the integral of the damage suffered during the whole flight and will be solely and exclusively due to impinging radiation.

In order to evaluate the integrity of DNA (single and double strand), DNA fragments characterized by different molecular weights will be separated by means of electrophoresis technique. The circular plasmid DNA has to be linearized by addition of EcoRI and respective buffers.

Results

Work is in progress to analyze the DNA from the dosimeters and the Eppendorf vials and to measure the dose integrated by the diamond dosimeters. Gathering data also from the other experiments on board the BIOKON 002 box of the BIOKIS suite of experiments will be crucial to correlate the observed DNA damage to the measured dose precisely.

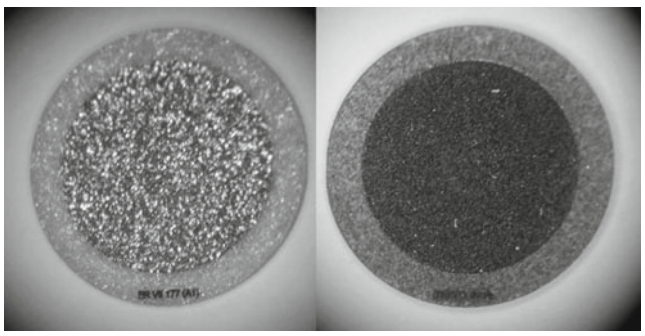

Fig. 13 Pictures of the top (*on the left*) and bottom sides (*on the right*) of one of the fabricated dosimeters

nDose Experiment

Material and Methods

The detectors were embedded in different layers of foam material within the BIOKIS 001: the bismuth stack tracks detectors were at the bottom layer, the BD-PND bubble detectors were at the middle layer and the BDT bubble detector dosimeters were at the top layer. A system of passive neutron detectors is chosen in various energy intervals to avoid interference with on board electronics: bubble dosimeters from thermal energy to 20 MeV and Bismuth stack tracks detectors over 200 MeV to 200 GeV.

Bubble Dosimeter Bubble dosimeters (BTI, Ontario Canada), calibrated against an AmBe source in terms of NCRP38 (NCRP 1971), are suitable for neutron integral dose measurements in the range of thermal energy (BDT model 0.025 eV) and fast neutron energy (BD-PND model 100 keV–20 MeV) (Bubble Technology Industries 2003) (with suitable conversion factor ambient dose equivalent H* could be obtained).

Bubble Detectors provide instant visible detection and measurement of neutron dose. Inside the detector, tiny droplets of superheated liquid are dispersed throughout a clear polymer. When a neutron strikes a droplet, the droplet immediately vaporizes, forming a visible gas bubble trapped in the gel. The number of droplets provides a direct measurement of the tissue-equivalent neutron dose. The Bubble Detector Neutron Dosimeter is the only neutron dosimeter where the response is independent of dose rate and energy, with zero sensitivity to gamma radiation. With an isotropic angular response, neutron dose can be accurately measured regardless of the direction of neutrons relative to the detector.

Bismuth Stack Detector Bismuth stack detectors, for relativistic neutrons ($E > 200$ MeV), consist of a stack of ^{209}Bi layers and Mylar foils: the fission fragments coming form the neutron interaction with bismuth layer produce tracks on the Mylar foils that become visible after a chemical attack (Cross and Tommasino 1997). The response of the detectors corresponds to bismuth fission curve.

Results

From the analysis of neutron bubble detectors the neutron component in the thermal energy range obtained with BDT detector and in the interval from 100 keV up to 20 MeV obtained with BD-PND detector (Fig. 14) has been evaluated. Average neutron dose of

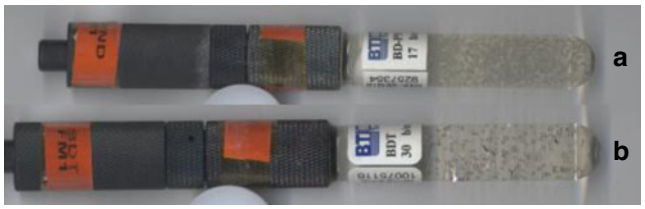

Fig. 14 BDPND (**a**) and BDT (**b**) bubble detector utilized during STS134 mission

17.2 μSv/day ± 20% has been estimated. The bismuth stack detector is still under analysis and the experimental results will be published in future papers.

Conclusion

Accurate evaluation of damage due to cosmic ray interactions with aircraft shielding is becoming more and more important because of the long-term manned space missions planned in the next future and the high number of spacecrafts for planetary explorations. We reported on the evaluation of the neutron component of the radiation environment in the particular Low Earth Orbit followed by the ISS Space Station. Secondary neutrons are high LET radiation and their energy cover more than eleven orders of magnitude and they can produce dramatic damages in the astronauts' health and the instrumentations. The method here described, based on both experimental measurements with passive detectors and MC simulations, could contribute to a better assessment of the radiation environment in Low Earth Orbit.

Overall Conclusion

The joint ASI-AM DAMA mission represented an opportunity for the Italian scientific community to embark on ambitious research ventures. The mission was completed with full scientific, technical and programmatic success. All the experiments were performed as expected and preliminary results confirmed the reliability of each experimental hypothesis. Thus, we can refer to the BIOKIS payload philosophy as a model for multidisciplinary missions.

Moreover, the time overlap of biological experiments and dosimetry measurement enhance the scientific outcome prompting collaboration among teams.

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