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Rapid Communication

Environmental Data and Survival Data of *Deinococcus aetherius* from the Exposure Facility of the Japan Experimental Module of the International Space Station Obtained by the Tanpopo Mission

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Abstract

The Tanpopo mission has two objectives: (1) test the panspermia hypothesis and (2) test whether organic compounds may have been transferred to Earth before the origin of life. We developed an exposure panel (EP) designed to expose microbes and organic compounds to the space environment and a capture panel designed to capture high-velocity particles on the International Space Station (ISS) using aerogel contained in an aluminum container. The panels returned after 1 year of exposure at the Exposure Facility of the Japan Experimental Module, ISS. In this communication, we report the measurements of temperature, radiation dosimeter and vacuum ultraviolet dosimeter in the EP, and survival data of *Deinococcus aetherius*. The environmental data are consistent with survival data of microbes and organic compounds, which will be presented elsewhere in detail. Key Words: Temperature monitoring—Alanine VUV dosimeter—Radiation monitor. Astrobiology 18, xxx–xxx.

1. Introduction

TANPOPO IS THE JAPANESE WORD FOR DANDELION, a plant with seeds that are spread by wind. This image nicely reflects the Tanpopo mission, which was designed to examine possible interplanetary migration of microbes and organic compounds at the Exposure Facility (EF) of the Japan Experimental Module (JEM: Kibo) of the International Space Station (ISS) (Yamagishi *et al.*, 2013; Kawaguchi *et al.*, 2016). The Tanpopo mission has two scientific objectives. The first objective is to test the panspermia hypothesis. More than 100 years ago, Arrhenius proposed that spores may be distributed through space (Arrhenius, 1908), and the Tanpopo mission is designed to test this hypothesis on the ISS. The second objective is to test the potential for chemical evolutionary processes before the origin of life. Organic compounds had accumulated on Earth before the origin of life and these compounds may have been transferred to the surface of Earth by micrometeoroids. Using our apparatus, we will test for the possible presence of organics in micrometeoroids.

The Tanpopo mission consists of six subthemes as follows: capture of microbes in space (subtheme 1), exposure of microbes in space (subtheme 2), analysis of organic compounds in interplanetary dust (subtheme 3), exposure of organic compounds in space (subtheme 4), measurement of space debris at the ISS orbit (subtheme 5), and evaluation of ultralow-density aerogel developed for the Tanpopo mission (subtheme 6). The panspermia hypothesis will be addressed in subthemes (1) and (2). The possible space origin of organic compounds before the origin of terrestrial life will be addressed in subthemes (3) and (4). Ultralow-density silica aerogel specifically designed for the Tanpopo mission (Tabata *et al.*, 2015) will be evaluated in subtheme (6).

Silica aerogel comprises dried amorphous porous SiO_2, and this low-density aerogel can reduce the damage caused upon impact. The function of aerogel will be analyzed by evaluating the performance of aerogel in subthemes (1), (3), and (5). The amount of space debris smaller than 1 mm in diameter in low Earth orbit (LEO) has increased (Higashide *et al.*, 2012). To obtain the latest data on the distribution of space debris at LEO, we will capture and count the number

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of metal particles in the aerogel in subtheme (5). Because survival of microbes (subtheme 2) and organic compounds (subtheme 4) depends on environmental factors, we have analyzed and report here the measurements of temperature, the radiation dosimeter, and the vacuum ultraviolet (VUV) dosimeter in the exposure panel (EP).

2. Tanpopo Mission Apparatus

We developed two types of apparatus for the Tanpopo mission: capture panels (CPs) with aerogel to capture microparticles (Supplementary Fig. S1; Supplementary Data are available online at www.liebertonline.com/ast) and EPs for exposure of microbes and organic materials (Fig. 1). Each CP contains a silica aerogel block in an aluminum mesh container. Each EP consists of 20 exposure units, each containing microbes, organic compounds, alanine VUV dosimeters, or radiation dosimeters. The cross section of a basic exposure unit for microbes and organic compounds is shown in Fig. 1. A window with an effective diameter of 16 mm was installed to avoid cross contamination. The window was made of either magnesium fluoride or quartz to investigate VUV wavelength dependency. A metal mesh was placed at the top of the window to prevent scattering of accidentally broken windows. Although there are vents and clearances in a unit to expose a sample to a vacuum, O-rings and a metallic filter at the bottom were used to prevent the sample from leaking out of the unit. Samples were set on two plates at the top and bottom. The top plate is exposed to solar light, while the bottom is kept dark as a control. The characteristics of the plates differ depending on the research objective.

3. Tanpopo Mission Timeline

Three EPs and 36 CPs were launched by the space carrier Space-X CRS-6 in April 2015 (Supplementary Fig. S2). The 3 EPs and 12 CPs were placed on the Exposed Experiment Handrail Attachment Mechanism (ExHAM) in the ISS for the first year of operation (Kawaguchi *et al.*, 2016). The ExHAM was exposed in the depressurized air lock for about 2 weeks. On May 26, 2015, the ExHAM with panels was placed on the EF of JEM-ISS through the air lock of JEM-ISS using robotic arms.

On June 13, 2016, after about 1 year of exposure, the ExHAM was transferred to a pressurized area through the air lock. The first set of CPs was removed from the ExHAM, stored in the pressurized area of the ISS, and a new set of CPs was attached to the ExHAM for the next exposure operation. One of the three EPs used for the first-year exposure experiment was removed from the ExHAM to be returned to the ground, while the other two were left on the ExHAM for the next two exposure operations. The retrieved EP and CPs were returned to the ground in the space capsule. In October 2016, the EP was separated into exposure units, each harboring either microbe or organic compounds, and was handed over to the scientist in charge of the respective microbe or organic compound. Some units were dedicated to UV or radiation dose measurement. CPs were initially inspected for high-velocity impact, and these data will be published elsewhere.

4. Mechanical Space Thermometer

In the Tanpopo mission, the maximum temperature of the exposed panel needed to be below 80°C for microbe survival. We developed a mechanical thermometer that did not depend on electric power supply to monitor this requirement. Based on the thermal model calculation, the temperature range on the ISS orbit of the exposed panel was expected to be between −115.0°C and 32.8°C and therefore the range of the thermometer was designed to be −140°C to 80°C with 5°C accuracy.

The mechanical space thermometer (MST) consists of an indicator and a temperature sensor (Fig. 2). The indicator ($110 \times 70 \times 8.5$ mm) comprises an aluminum alloy body and a stainless steel panel with a U-shaped window to view the pointer. The temperature sensor is a heat-treated, bimetallic

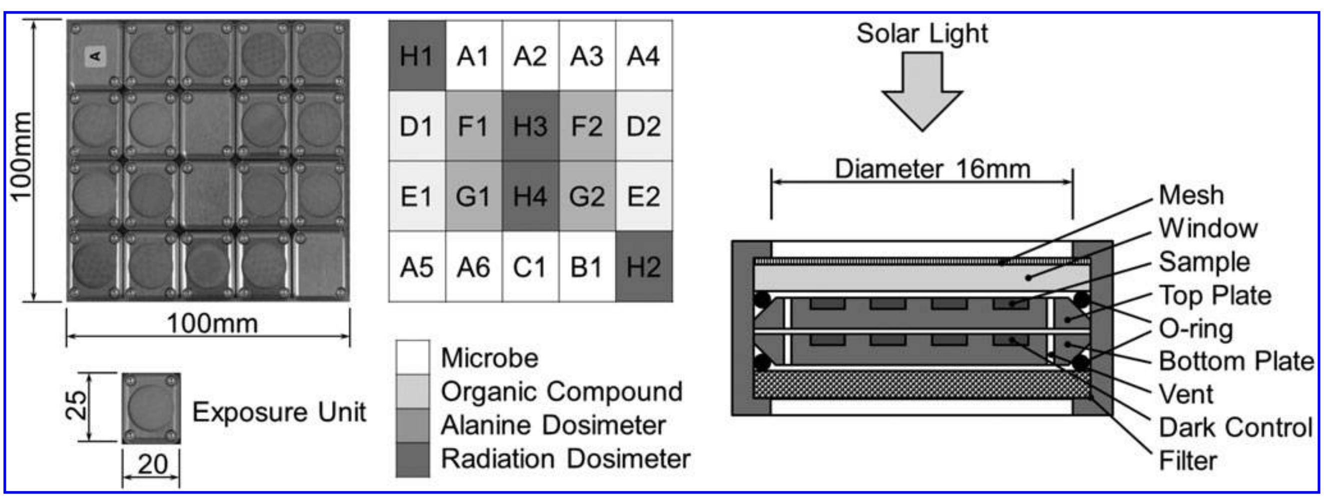

FIG. 1. The exposure panel for the Tanpopo mission. The exposure panel ($100 \times 100 \times 20$ mm) consists of 20 exposure units ($25 \times 20 \times 11.5$ mm). Each exposure unit with a window in the front contains either a bacterial (A–C) or organic sample (D, E) or an alanine VUV dosimeter (F, G). The unit without a window contains radio dosimeters (H). VUV, vacuum ultraviolet.

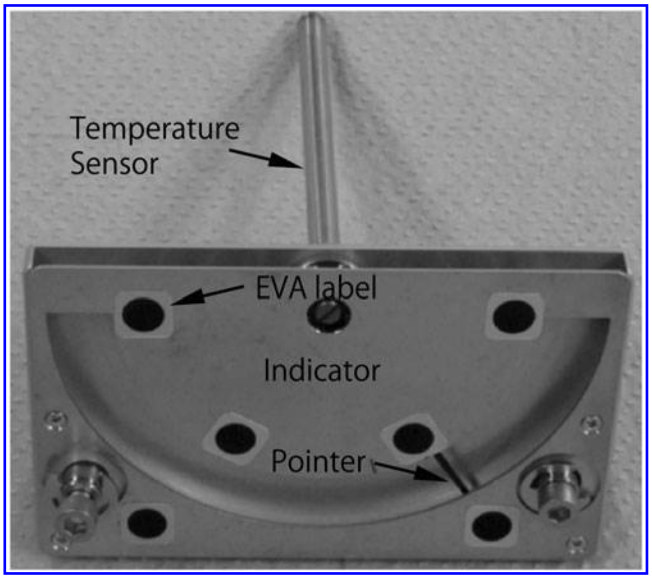

FIG. 2. The mechanical space thermometer. The mechanical space thermometer consists of an indicator and a temperature sensor and its mass is 159 g.

strip coil in a stainless steel pipe (6 mm diameter × 100 mm). The pointer is connected to a shaft that rotates with temperature change.

The MST was attached to one of the Tanpopo EPs. The indicator was video-imaged by an extravehicular video camera attached to the Kibo-EF and controlled from the ground. The temperature was estimated from six metal EVA (extravehicular activity) labels attached to the display board and the pointer angle. Five measurements with different sun beta angles, including the angle where the maximum temperature was expected from the model calculation, high positive beta and low beta, were conducted in ∼1 month during the first-year operation. Each measurement took about 6 h to monitor four revolutions of the ISS around Earth.

Table 1 shows data obtained from five measurements of the first-year experiment; the maximum and minimum temperatures were 24°C ± 5°C and –21°C ± 5°C, respectively (Hashimoto *et al.*, 2016). The high temperature resulting from the higher sun incident angle was expected in the orbit with the low beta angle. However, there are many objects interfering with sunlight, including other instruments, the ISS, solar panels, and heat radiation boards near and/or above the Tanpopo apparatus. Heat conduction, heat radiation, and shade from them affect the temperature of the Tanpopo apparatus depending on the solar beta angle. Since all their positions were not included in the model calculation, the maximum temperature and minimum temperature estimated from the measurement were different from those obtained in the model calculation. However, the maximal temperature was recorded during the orbit with low beta angle as expected from the high sunlight incident angle.

Table 1. Temperature Measurements from the Tanpopo Thermometer Onboard the Kibo-EF (Hashimoto *et al.*, 2016)

No.	*Sun beta angle (degree)*	*Observation date*	*Maximum temperature (°C)*	*Minimum temperature (°C)*
1	+74	June 2, 2015	–9 ± 5	–13 ± 5
2	+53	June 8, 2015	–11 ± 5	–21 ± 5
3	+29	June 13, 2015	16 ± 5	–1 ± 5
4	+1	June 20, 2015	18 ± 5	–3 ± 5
5	–28	July 3, 2015	24 ± 5	–2 ± 5

EF, Exposure Facility.

5. Radiation Dosimetry

5.1. Radiation dosimetry system

Aluminum oxide (Al_2O_3)-based optically stimulated luminescence dosimeters (OSLDs) (Yukihara and Mckeever, 2011) and silver-activated phosphate glass-based radiophotoluminescence dosimeters (RPLDs) (Miyamoto *et al.*, 2011) were used for radiation dosimetry outside and inside the ISS. These dosimeters have previously been used for space radiation dosimetry (Sihver *et al.*, 2016) in addition to conventional thermoluminescence dosimeters (NCRP, 2002; Berger *et al.*, 2016). We have employed the two types of luminescence dosimeters (LDs) to cross-check the dose data. Space radiation dosimetry is usually carried out with a combination of LDs and plastic nuclear track detectors (PNTDs) (Doke *et al.*, 1995). The latter are used to monitor charged particles with various linear energy transfer (LET) values. The typical PNTD, CR-39, is sensitive only to high LET particles ≥10 keV/μm in water, covering a high LET region, while LD is less sensitive to high LET particles, covering low LET particles <10 keV/μm consisting mainly of protons and He ions. However, the CR-39 PNTD is not active in vacuum conditions (Kodaira *et al.*, 2009). Because the Tanpopo experiment is operated in vacuum conditions, only LDs have been employed for radiation dosimetry. According to previous space radiation data (Kodaira *et al.*, 2014), the total absorbed dose obtained without using the CR-39 PNTD may have underestimated the dose by 5%, which corresponds to the dose of high LET particles.

The OSLD (7.2 mm diameter 0.5-mm thick; Nagase Landauer, Inc., Japan) was analyzed with a Microstar reader system (Landauer, Inc., IL), illuminating and stimulating with 525 nm LED, while the RPLD (8.5 × 8.5 × 1.5-mm thick; Chiyoda Technol Corporation, Japan) was analyzed by the standard processing protocol, including annealing and readout luminescence excited with 355 nm YAG laser, and quality controlled by Chiyoda Technol Corporation for personal dosimetry. These dosimeters were calibrated by means of a standard ^{137}Cs γ-ray source and heavy ion beams from HIMAC (Heavy Ion Medical Accelerator in Chiba). Different materials were placed on top of the dosimeter to test the space radiation shield effect (Table 2).

5.2. Radiation dosimetry results

The mean absorbed dose with the thinnest cover (0.55 g/cm^2) was 272 mGy outside and 100 mGy inside the Kibo module for the duration of the flight (Table 2). The radiation dose outside was about three times higher than the dose inside because of the well-shielded environment inside the Kibo module. As illustrated in Fig. 1, samples are shielded with a

Table 2. Measured Absorbed Dose with Three Different Shields Outside and Inside the Kibo Module

Thickness [g/cm²]	*Outside the Kibo module*		*Inside the Kibo module*		*Shields and thickness*
	OSLD [mGy]	*RPLD [mGy]*	*OSLD [mGy]*	*RPLD [mGy]*	
0.55	294.5 ± 18.0	249.0 ± 28.9	95.5 ± 1.2	104.6 ± 1.3	None
2.95	151.4 ± 2.1	162.4 ± 3.6	90.6 ± 2.3	98.2 ± 1.5	3 mm SUS316
6.23	145.7 ± 1.3	156.8 ± 4.2	93.7 ± 1.4	102.6 ± 0.7	5 mm Pb

The corresponding material thickness was achieved by placing the shield inside a holder made of 2 mm Al.
OSLDs, optically stimulated luminescence dosimeters; RPLDs, radiophotoluminescence dosimeters.

window made of SiO_2 (0.53 g/cm^2) or MgF_2 (0.63 g/cm^2). The dose follows the exponential function as shown in Supplementary Fig. S3A so that the actual dose received by samples could be estimated by fitting data with the exponential function. The mean dose (D_r [mGy]) of OSLD and RPLD as a function of material thickness (x [g/cm^2]) is expressed as

$$D_r = 242.3 \times e^{-1.3x} + 151.2.$$

Although the dose outside decreased from 0.55 to 2.95 g/cm^2 following the exponential function, the dose remained almost constant inside (Supplementary Fig. S5A). This indicates that low-energy protons with energies less than about 50 MeV trapped in the South Atlantic Anomaly would be attenuated in materials with 3 g/cm^2. The measured dose reduction outside the Kibo module strongly supports previous results (Supplementary Fig. S3B).

6. Alanine VUV Dosimeter

Since VUV radiation is the major source of chemical and biological effects at LEO, the VUV dose was estimated. In EXPOSE-E (Rabbow *et al.*, 2009) and EXPOSE-R (Rabbow *et al.*, 2015) experiments, the VUV dose was monitored by electronic UV sensors, and data were obtained every 10 s and transmitted to the ground by telemetry (Rabbow *et al.*, 2009, 2015). Because of partial loss of data (Rabbow *et al.*, 2009, 2015), the total UV flux was estimated from interpolation of transmitted data (Rabbow *et al.*, 2009). We developed an integrating type of VUV sensor using an alanine film coated on a quartz window in the Tanpopo experiment.

The alanine film absorbs VUV wavelengths shorter than 190 nm and is dissociated by the absorbed VUV light (Izumi and Nakagawa, 2011). The dissociation quantum efficiency of the alanine film with a 172-nm photon was determined to be 0.1 using a 172-nm Xe_2* excimer lamp (Izumi and Nakagawa, 2011).

Schematic diagrams of alanine dosimeters are shown in Supplementary Fig. S4. Alanine films with a diameter of 14 mm were applied to the windows using a vacuum evaporation technique. Windows were set on a rotating disk during the evaporation procedure to obtain an even thickness of alanine. Film thickness was 584 ± 8 nm as measured by the infrared absorption spectrum with an FT-IR spectrometer (Spectrum One; PerkinElmer Japan, Tokyo) and chromatography analysis with an HPLC system (SPD-10AVP; Shimadzu, Kyoto, Japan). A standard curve was obtained with a 172-nm Xe_2* excimer lamp (SUS03; Ushio, Tokyo, Japan), and irradiation was carried out in a vacuum chamber (about 10^{-3} Pa) in a laboratory. Flux of 172-nm photons was determined by a calibrated photodiode (S1227-1010BQ; Hamamatsu Photonics, Hamamatsu) to be about 10^{16} photons/m^2 (corresponding to 115 W/m^2).

Using a quartz window with 160-nm cutoff wavelength, we can limit the wavelength region of the alanine dosimeter to be at $160 < \lambda < 190$ nm. We estimated the solar radiation at $160 < \lambda < 190$ nm from the standard curve of the 172-nm dissociation experiment in the laboratory.

Irradiation doses of returned dosimeters after about 1 year of exposure on the ISS orbit were studied. Deviation of the film thickness was between −2.5% and +2.5% along the *x*-axis and was less along the *y*-axis (Supplementary Fig. S5), which was similar to the dark control dosimeter, suggesting no detectable nonhomogeneous solar radiation distribution. Remaining alanine content was estimated, and radiation doses were determined from the standard curve to be 4.29 ± 0.08 kJ/m^2 at 172 nm irradiation. The accumulated solar irradiation dose, D, at $160 < \lambda < 190$ nm after a 382-day exposure on the ISS orbit was 7.09 ± 0.13 kJ/m^2 based on the detection efficiency 0.605, while solar irradiance at the LEO was estimated from data reported by Lean (1991) and was 48.1 mW/m^2 for $160 < \lambda < 190$ nm for 382 days, corresponding to the accumulated solar irradiance D_0 1.59 MJ/m^2.

Total dose D of the alanine dosimeter in the Tanpopo experiment can be $D = \eta_1\eta_2\eta_3 D_0$. The factor η_1 is the efficiency due to the night–day cycle of the ISS, $\eta_1 = 0.50$. The factor η_2 is the efficiency of solar illumination in the daytime, which is the square of integration of cosθ for $-\pi/2 < \theta < +\pi/2$, thus $\eta_2 = 0.100$. The factor η_3 is the effect of shading and was estimated to be 0.091 from D_0 and D obtained from the alanine dosimeter in the current experiment.

It is important to note that η_1 and η_2 affect all objects rotating and orbiting around Earth, resulting in average integrated sunlight energy at the surface of the object lower than continuous solar illumination. The estimated $\eta_3 = 0.091$ corresponds to 35 equivalent solar days (ESDs) in the 382-day duration of the Tanpopo first-year mission. D_0 values from 100 nm to 400 nm are shown in Supplementary Table S1.

7. Survival Analysis of *Deinococcus aetherius*

The *D. aetherius* strain ST0316 was cultured at 30°C on mTGE agar plates supplemented with 1% glycerol for about 10 days. *D. aetherius* cells were collected from the agar plate with a sterilized platinum loop and washed with

10 mM phosphate buffer three times. Aluminum plates containing cylindrical wells (2.0 mm diameter, 2 mm or 100-μm depth) with a flat floor were used as sample folders (Kawaguchi *et al.*, 2016). Cell suspensions were dropped into wells and dried under 3.3×10^{-2} atm in a desiccator (SPD–WVGS300; SANYO, Tokyo, Japan) on a clean bench. These steps were repeated 20 to 30 times and concluded with the final drying process for more than 16 h under 3.3×10^{-2} atm in a desiccator. The wells were filled with a different amount of *D. aetherius* cells corresponding to about 100, 500, and 1000 μm cell layers. The sample in the upper aluminum plate was exposed to the space vacuum and UV irradiated through an MgF_2 window for 382 days.

After exposure and the samples' return to the laboratory, dehydrated cells were recovered from wells of aluminum plates by resuspending the cell pellet with 10 mM phosphate buffer. Aliquots of Deinococcus cells were serially diluted in sterile phosphate buffer and dropped on mTGE medium plates. Colonies were counted after incubation at 30°C for 4 weeks. The surviving fractions were determined from the quotient N/N_0, where N is the number of colony-forming units of space samples and N_0 is those at the time of sample preparation.

Surviving fractions of cell aggregate of *D. aetherius* ST exposed to space for about a year were 0, $(6.7 \pm 4.9) \times 10^{-4}$, and $(7.8 \pm 2.7) \times 10^{-4}$ ($n = 3$ or 2) for 100, 500, and 1000 μm cell layers, respectively. Although the cell layer with 100-μm thickness is not sufficient, 500 μm is sufficient to survive in space for a year. Provably, dead cells in the surface portion shielded the cells beneath from UV radiation. Space-exposed, dried *D. aetherius* samples with 500 μm^t (μm^t: abbreviates μm thickness) or thicker that were illuminated with UV radiation survived, which supports the panspermia hypothesis. Samples with 100 μm^t were dead.

8. Conclusion

From the measurement of the first-year experiment, the maximum and minimum temperatures were 24°C ± 5°C and –21°C ± 5°C, respectively. The mean absorbed dose at the thinnest cover (0.55 g/m^2) was 272 mGy outside and 100 mGy inside the Kibo module for the duration of the flight. These data are compatible with survival of microbes and organic compounds, which will be reported elsewhere. The accumulated solar irradiation dose at $160 < \lambda < 190$ nm after 1 year of exposure in the ISS orbit was 7.09 ± 0.13 kJ/(m$^2 \cdot$y), corresponding to 35 ESDs. These data will be compared with survival of microbes and organic compounds in the upper sample plates of the exposure units. Space-exposed, dried *D. aetherius* samples with 500 μm^t or thicker that were illuminated with UV radiation survived, which supports the panspermia hypothesis, while samples with 100 μm^t were dead.

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Author Disclosure Statement

No competing financial interests exist.

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Abbreviations Used

CPs = capture panels
EF = Exposure Facility
EP = exposure panel
ESD = equivalent solar day
ExHAM = Experiment Handrail Attachment Mechanism
HIMAC = Heavy Ion Medical Accelerator in Chiba
ISS = International Space Station
JEM = Japan Experimental Module
LDs = luminescence dosimeters
LEO = low Earth orbit
LET = linear energy transfer
MST = mechanical space thermometer
mTGE = Tryptone, glucose, and beef extract medium
OSLDs = optically stimulated luminescence dosimeters
PNTDs = plastic nuclear track detectors
RPLDs = radiophotoluminescence dosimeters
VUV = vacuum ultraviolet