Whole Lichen Thalli Survive Exposure to Space Conditions: Results of Lithopanspermia Experiment with Aspicilia fruticulosa

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Abstract

The Lithopanspermia space experiment was launched in 2007 with the European Biopan facility for a 10-day spaceflight on board a Russian Foton retrievable satellite. Lithopanspermia included for the first time the vagrant lichen species Aspicilia fruticulosa from Guadalajara steppic highlands (Central Spain), as well as other lichen species. During spaceflight, the samples were exposed to selected space conditions, that is, the space vacuum, cosmic radiation, and different spectral ranges of solar radiation (λ ≥ 110, >200, >290, or >400 nm, respectively). After retrieval, the algal and fungal metabolic integrity of the samples were evaluated in terms of chlorophyll a fluorescence, ultrastructure, and CO2 exchange rates. Whereas the space vacuum and cosmic radiation did not impair the metabolic activity of the lichens, solar electromagnetic radiation, especially in the wavelength range between 100 and 200 nm, caused reduced chlorophyll a yield fluorescence; however, there was a complete recovery after 72 h of reactivation. All samples showed positive rates of net photosynthesis and dark respiration in the gas exchange experiment. Although the ultrastructure of all flight samples showed some probable stress-induced changes (such as the presence of electron-dense bodies in cytoplasmic vacuoles and between the chloroplast thylakoids in photobiont cells as well as in cytoplasmic vacuoles of the mycobiont cells), we concluded that A. fruticulosa was capable of repairing all space-induced damage. Due to size limitations within the Lithopanspermia hardware, the possibility for replication on the sun-exposed samples was limited, and these first results on the resistance of the lichen symbiosis A. fruticulosa to space conditions and, in particular, on the spectral effectiveness of solar extraterrestrial radiation must be considered preliminary. Further testing in space and under space-simulated conditions will be required. Results of this study indicate that the quest to discern the limits of lichen symbiosis resistance to extreme environmental conditions remains open. Key Words: Astrobiology—Lichens—Panspermia—Chlorophyll fluorescence—CO2 exchange—Ultrastructure. Astrobiology 11, xxx–xxx.

1. Introduction

The development of space technology has opened the gates to experiments in astrobiology, a relatively new and interdisciplinary field of research that aims to achieve a better understanding of the processes that led to the origin, evolution, and distribution of life on Earth or elsewhere in the Universe (Horneck, 1995). Richter (1865) and Arrhenius (1903) first proposed the panspermia theory, which speculates about the transfer of life between planets. Although panspermia theory has little more than an idea and there is no evidence that it has occurred, the various steps required for the transfer of organisms from one planet to another have been the focus of experimental testing (Cockell, 2008). This technical availability has led to several studies, either under simulated space conditions (Buecker and Horneck, 1970; Mancinelli and Klovstad, 2000; De la Torre et al., 2003) or in spaceflight experiments (Horneck, 1993; Fajardo-Cavalzos et al., 2005; Sancho et al., 2007; De los Rı´os et al., 2010; Horneck et al., 2010). One of the main objectives of these astrobiological experiments has been to test whether different kinds of organisms can survive in the extremely hostile conditions of...
interplanetary space with particular attention to the space vacuum that causes dehydration of the samples and the high intensity of cosmic rays and solar extraterrestrial UV radiation, the latter being especially harmful to DNA. The survival capacity of exposed organisms is an interesting feature that can indirectly support or deny the old panspermia theory and recent revisions (Friedmann et al., 2001; Fajardo-Cavalzos et al., 2005).

In view of the harsh conditions of space, the choice of suitable test organisms is of great importance. Some authors, such as Horneck (1993), Horneck et al. (1994, 2001), and Mancinelli et al. (1998), have worked with bacterial endospores and halophiles, respectively, because of their known exceptionally high resistance to harsh terrestrial climatic conditions, while, in recent years, lichens have also been tested (Sancho et al., 2007, 2008; De la Torre et al., 2010). Lichens are symbiotic organisms (green algae or cyanobacteria, or both, with a fungus) that are able to colonize a wide range of habitats around the world (Kappen, 1988), including harsh environments such as deserts (Lange et al., 1994; Pintado et al., 2005), high mountains (Sancho and Kappen, 1989; Reiter et al., 2007), and polar regions (Green et al., 1998; Pannewitz et al., 2003). In Antarctica, they often are the dominant organisms in terrestrial ecosystems and have been reported as far south as 86 29’S (Siple, 1938; Øvstedal and Lewis Smith, 2001). Under particularly severe conditions in continental Antarctica (De los Ríos et al., 2005), lichens survive inside rocks, where they form endolithic communities. Lichens, as poikilohydric organisms, are only active when wet; when dehydrated and inactive, they can be highly tolerant to extreme conditions of light, temperature, and drought (Kappen and Valladares, 2007). Because of these characteristics, lichens have been successfully used in experiments under simulated space conditions (De la Torre et al., 2003, 2007; De Vera et al., 2003, 2004), and they have been launched into space in the experiment Lichens (Sancho et al., 2007) and Lithopanspermia (Sancho et al., 2008; De la Torre et al., 2010) in order to better understand the effects of extreme space conditions on multicellular eukaryotic organisms.

The experiment Lithopanspermia included for the first time the globoid lichen *Aspicilia fruticulosa* (Eversm.) Flagey (Fig. 1A) from Guadalajara steppes, central Spain, a habitat characterized by high temperature contrasts, high frost incidence, and very dry summers (Crespo and Barreno, 1978). The species was suggested for this space study because it is characterized by high temperature contrasts and long periods of dryness. In the previous Lichens experiment, the two crustose lichens (*the alpine species Rhizocarpon geographicum* (L.) DC. and *Xanthoria elegans* (Link) Th. Fr.) were studied with chlorophyll fluorescence and microscopy analysis (Sancho et al., 2007). Here, we not only used the same techniques but also added CO2 exchange measurements for the first time. We were able to measure accurately the physiological resistance of the algal cells in terms of CO2 assimilation rates, and for the first time we also used dark respiration to evaluate the fungal physiological state after exposure. The combination of chlorophyll fluorescence, fungal and algal cellular ultrastructure, and CO2 exchange measurements provided additional information about the survival capacity of the lichen symbiosis in space.

2. Material and Methods

2.1. Experimental design

Thalli of the globoid lichen *A. fruticulosa* growing on claylike red soil were collected near Zacarejas (Guadalajara, Spain) at 1240 m above sea level (Fig. 1B). Intact lichen samples 6–7 mm in diameter were mounted in the hardware of the space experiment Lithopanspermia (designed by INIA, Spanish Aerospace Establishment, Madrid, Spain), which was included in the Biopan facility of ESA (Demets et al., 2005). The experiment Lithopanspermia was part of the Biopan-6 mission, during which the samples were exposed to selected conditions in space for 10 days. The hardware characteristics and space conditions are described in detail in De la Torre et al. (2010).

During the 10-day spaceflight all samples were fully exposed to the space vacuum (about 10−6 Pa) and cosmic ionizing radiation (4–100 mGy, depending on mass shielding). In addition, some samples (flight sun-exposed samples) were exposed to solar extraterrestrial electromagnetic radiation, whereas others (flight dark samples) were protected from this radiation by a shield but otherwise still exposed to space. Another set of samples (Earth controls) was kept on Earth and stored at room temperature under dry and dark conditions. The flight sun-exposed samples were exposed beneath an optical filter system that provided different UV radiation environments. One sample was exposed to the full spectrum of solar extraterrestrial UV and visible radiation (λ > 110 nm) by use of a MgF2 filter; a second sample had a SIO synthetic quartz filter that allowed transmission of wavelengths at λ ≥ 200 nm. A third sample had a long-pass filter that allowed exposure to λ ≥ 290 nm and simulated Earth’s UV conditions. A fourth sample was exposed to radiation of wavelengths longer than 400 nm, the zero-UV radiation treatment. Limitations of the hardware in space meant that the number of samples for each flight sun-exposure condition was n = 1. However, because the different radiation exposures (for flight sun-exposed samples) showed no apparent differences in their physiological response, we consider the number of samples exposed to space conditions for each experiment (sun exposed and flight dark) as n = 4.
The number of Earth control samples was \( n = 8 \). The experimental design, which is a compromise between achieving a useful result and enough information to plan future research, went through several reviews and was approved by ESA before the flight.

2.2. Chlorophyll fluorescence analysis

All thalli were reactivated after the flight in a growth chamber at 10°C and 100 μmol photon m\(^{-2}\) s\(^{-1}\) photon flux density (PFD) for 12/12 h dark/light photoperiod. They were moistened twice daily by spraying with bottled mineral water. Chlorophyll a fluorescence of the reactivated samples was measured with a photosynthesis yield analyzer (Mini-PAM, Walz Company, Germany) as described by Sancho et al. (2007). The chosen parameter for the metabolic evaluation was the potential maximum photosystem II quantum yield (\( F_{v}/F_{m} \); Schreiber et al., 1994), where \( F_{m} \) is the maximum fluorescence after a saturation pulse of actinic light (photosynthetic active radiation, 400–700 nm) and \( F_{v} = (F_{m} - F_{o}) \) is
2.3. CO₂ exchange

After an additional period of 72 h reactivation, CO₂ exchange was measured at 20°C and 400, 800, 1200 μmol photon m⁻² s⁻¹ PFD. The exchange system was a four-channel CO₂/H₂O absolute nondispersive infrared gas analyzer. The cuvette of this new gas exchange system is small (3 cm long and 4 cm width) and allows rapid and accurate (±0.1%) measurements on single samples of low biomass. Temperature and relative humidity inside the cuvette were controlled, and the air flow was regulated at 600 mL min⁻¹.

A standard procedure was used. Samples were first hydrated by 20 min of mineral water immersion to ensure complete saturation. Samples were then placed in the cuvette and dark respiration (DR) was measured. The samples were then illuminated with a LED light source 3040-L at 400 μmol photon m⁻² s⁻¹ PFD until the net photosynthetic rate (NP) reached a constant value, and this was repeated at 800 and 1200 μmol photon m⁻² s⁻¹ for 5 min each. Net CO₂ exchange at the highest PFD was taken as the maximum CO₂ net assimilation value (A_max). Measurements were made over a 5-day period.

2.4. Statistical analysis

Comparison of means of controls with a significance level of p ≤ 0.05 was performed by one-way analysis of variance. Comparisons of the data of the flight dark samples and the Earth controls were performed with Duncan’s multiple range test (p ≤ 0.05). Statgraphics version 5.1 was used.

2.5. Microscopy analysis

2.5.1. Low-temperature scanning electron microscopy.

Lichen thalli of A. fruticulosa were examined with a LTSEM after the gas exchange measurements. Small lichen fragments were fixed onto the specimen holder of the cryotransfer system (Oxford CT1500), plunged into liquid nitrogen, and then transferred to the scanning electron microscope (SEM) via an air-lock transfer device. The frozen specimens were cryo-fractured in the preparation unit and transferred directly via a second air lock to the microscope cold stage, where they were etched for 2 min at −90°C. The following beam conditions for etching were used: acceleration potential of 2 kV, probe current of 300 Pa, beam diameter of 70 nm, and aperture size of 120 μm. After ice sublimation, the etched surfaces were gold sputter coated with 200 Å thick layer in the preparation unit. Samples were subsequently transferred onto the cold stage of the SEM chamber. Fractured and etched surfaces were observed under DSM960 Zeiss SEM at −135°C under 15 kV acceleration potential, 10 mm working distance, 200 pA probe current.

2.5.2. Transmission electron microscopy.

After the gas exchange measurements were made, small lichen fragments of A. fruticulosa were fixed in glutaraldehyde and osmium tetroxide solutions, dehydrated in a graded ethanol series, and embedded in Spurr’s resin following the protocol described by Ascaso and Galván (1976), Ascaso (1978), and De los Ríos and Ascaso (2002). Ultrathin sections were post-stained with lead citrate (Reynolds, 1963) and observed in a Zeiss EM910 transmission electron microscope operating at 80 kV.

2.6. Thin layer chromatography

A few fragments of some A. fruticulosa samples were taken for thin layer chromatography (TLC) assays to look for lichen substances. The extraction was made following Huneck and Yoshimura (1996), and samples were run on Merck silica gel 60 F254 pre-coated glass-backed TLC plates (layer thickness 0.25 mm) 20×20 cm. Solvent system C was (170 mL toluene/30 mL acetic acid) prepared according to Lumbsch (2002).

3. Results

3.1. Chlorophyll a fluorescence

Preflight Fv/Fm for all samples (flight sun-exposed samples, flight dark samples, and Earth controls) ranged between 636 and 753 (values are 1000 times actual yield; Figs. 2–6 and Table 1). These values were taken as the reference level for an intact healthy thallus (Demmig-Adams et al., 1990). After spaceflight, the Fv/Fm value of all flight sun-exposed samples that were measured subsequent to 3 h reactivation was significantly reduced compared to the pre-flight data. The lowest value (49% of the preflight value) was observed for the sample that was exposed to the full spectrum of extraterrestrial solar UV and visible radiation (λ ≥ 110 nm, Fig. 2). All other flight sun-exposed samples had a less pronounced, but reduced, initial Fv/Fm value after 3 h...
of reactivation (84% for the samples exposed to $\lambda \geq 200$ nm; 77% for those exposed to $\lambda \geq 290$ or $\lambda \geq 400$ nm of solar radiation) (Table 1 and Figs. 3–5). Fv/Fm values gradually increased with longer reactivation periods until maximum values were reached at 72 h, some of which were identical to the preflight level, some nearly identical. For example, the most affected sample, that is, the one exposed to $\lambda \geq 110$ nm, reached an Fv/Fm value of 633, which is very close to the preflight value of 636. This high capacity of recovery is an indication that damage to the photosystems was reversible within 3 days of reactivation. The flight dark samples reached, after 3 h, a 90% of Fv/Fm in relation to the preflight value. At 48 h it was 95%, and at 72 h it rose until 98% of the preflight record (percentages are averages of the four replicates). The Earth control samples showed a small reduction (to 90%) in their mean Fv/Fm value at 3 h of reactivation; then the value dropped to 89% at 24 h, rose to 90% at 48 h, and finally reached 95% of the preflight yield at 72 h of reactivation (Fig. 6). Differences between flight dark sample data and Earth control data at each time of the recovery period were not significant ($p \leq 0.05$). For both flight dark samples and Earth controls no significant differences
(p ≤ 0.05) were found between preflight Fv/Fm and that subsequent to recovery at 72 h.

3.2. CO₂ exchange

The CO₂ exchange results of the flight samples are shown in Fig. 7 as DR (negative values) and NP (positive values) at increasing PFD. The horizontal bars represent the mean of both Earth control and flight dark samples, which were not significantly (p ≤ 0.05) different. Therefore, they were grouped together as “controls” with regard to sun exposure. However, we recognize that the flight dark samples, unlike the Earth controls, were exposed to cosmic rays, the space vacuum, and temperature fluctuations during the spaceflight. The dashed line is the mean Aₘₐₓ of the control, and the solid line is the mean DR of the control. Table 2 shows the numerical results of the CO₂ exchange measurements. The Aₘₐₓ of the flight sun-exposed samples and flight dark samples ranged between 1.1 and 1.7 μmol CO₂ kg dw⁻¹ s⁻¹, and were close to the 1.6 ± 0.6 μmol CO₂ kg dw⁻¹ s⁻¹ of the Earth control samples. For net photosynthesis of the four flight sun-exposed samples, we obtained a mean 1.4 ± 0.2 μmol CO₂ kg dw⁻¹ s⁻¹, which suggests little effect due to the different radiation treatments. The standard deviation of the flight sun-exposed samples is even lower than the standard deviation of the other two treatments,

<table>
<thead>
<tr>
<th>Flight λ ≥ 110 nm n = 1</th>
<th>Flight λ ≥ 200 nm n = 1</th>
<th>Flight λ ≥ 290 nm n = 1</th>
<th>Flight λ ≥ 400 nm n = 1</th>
<th>Flight dark n = 4</th>
<th>Earth control n = 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preflight 636</td>
<td>726</td>
<td>753</td>
<td>735</td>
<td>694.0 ± 38.0a</td>
<td>688.0 ± 44.1a</td>
</tr>
<tr>
<td>3 h</td>
<td>312</td>
<td>611</td>
<td>579</td>
<td>563</td>
<td>625.8 ± 81.2a</td>
</tr>
<tr>
<td>24 h</td>
<td>410</td>
<td>680</td>
<td>687</td>
<td>644</td>
<td>640.0 ± 42.2b</td>
</tr>
<tr>
<td>48 h</td>
<td>548</td>
<td>711</td>
<td>710</td>
<td>662</td>
<td>661.8 ± 32.2abc</td>
</tr>
<tr>
<td>72 h</td>
<td>653</td>
<td>726</td>
<td>719</td>
<td>710</td>
<td>683.8 ± 25.3ab</td>
</tr>
</tbody>
</table>

Mean value ± standard deviation. Different letters in the same column mean significant differences according to Duncan’s multiple range test (p ≤ 0.05).

FIG. 7. Aₘₐₓ values (positive) and DR values for all flight samples.
which again supports that the different radiation treatments had no effect. The DR of the flight dark samples and Earth controls were statistically identical (Table 2) and were also identical to DR values of the flight sun-exposed samples exposed to solar radiation of \( \lambda \geq 110 \) and \( \lambda \geq 400 \) nm. However, flight sun-exposed samples exposed to either \( \lambda \geq 200 \) or \( \lambda \geq 290 \) nm showed slightly elevated DR of 1.7 or 1.5 \( \mu \text{mol CO}_2 \text{ kg}^{-1} \text{ s}^{-1} \), respectively. Except for these two samples, all other flight samples showed similar metabolic activity, without clear differences between the different space exposure conditions.

### 3.3. Microscopy analysis

**Figures 8 and 9** show the appearance of photobiont and mycobiont cells of the Earth control lichens. The photobiont chloroplast thylakoid membranes showed a normal appearance, but the pyrenoids (P) were rather poor regarding the number of pyrenoglobuli (Pg). It seems that there is a tendency toward swelling and curling thylakoids. The appearance of cytoplasmic lipid storage bodies (Sb) and mitochondria (m) was normal (Fig. 8). Vacuoles were not observed in photobiont cells. The mycobiont cells (Fig. 9) had well-preserved mitochondria (m) and concentric bodies (cc).

**Figs. 8–9**

The photobiont cells of the flight dark samples (Fig. 10) had more pyrenoglobuli than the Earth control. The presence of dense bodies in the cytoplasm vacuoles (V) and between the thylakoids (head of arrow) probably indicates stress or some degree of senescence. Starch granules were present (s).

**Figs. 10–11**

Mycobiont cells exhibited vacuoles (Fig. 11) containing irregular dense bodies (V).

**Algal pyrenoids** had dense matrix and numerous thylakoid membranes in flight samples exposed to solar radiation at \( \lambda \geq 110 \) nm (Fig. 12). Electron-dense membranous complex-like lipidic structures with a very dense appearance were frequently observed inside the chloroplast (head of arrow). Vacuoles were seen in the algal cytoplasm (V), either empty or with dense bodies (indicating stress). Mitochondria did not appear well defined in these cells. In the mycobiont cells (Fig. 13), we observed a lack of concentric bodies, and the mitochondria seemed to be in rather good shape. All the vacuoles (V) contained very electron-dense deposits. Figure 14, obtained by low-temperature scanning electron microscopy, shows the integrity of the algal (white arrows) cells walls in the flight sample exposed to \( \lambda \geq 110 \) nm solar radiation.

**Figs. 13–14**

The algal cells of the flight samples exposed to solar radiation of \( \lambda \geq 200 \) (Fig. 15) had pyrenoids with dense matrix and many thylakoid membranes. Starch was observed inside the chloroplast. Vacuoles with electron-dense bodies were frequent in the cytoplasm (V) of photobiont cells, which probably indicates cellular stress.

### 3.4. Thin layer chromatography

Two different and simple thalli of *A. fruticulosa* collected from the same locality were analyzed by TLC, and no lichen substances were found.

### 4. Discussion

In outer space, organisms are exposed to a complex matrix of extreme environmental conditions, which consist of high vacuum, extraterrestrial solar UV radiation, and a wide range of temperatures (Nicholson et al., 2005). The high vacuum conditions are extremely dehydrating, an effect that has been considered to be one of the most lethal factors of the space environment (Sancho et al., 2007). Whereas most organisms are severely damaged by this treatment, the flight dark samples of *A. fruticulosa* that were exposed to the vacuum of space for 10 days did not show any physiological impairment as indicated by unchanged chlorophyll a activity, respiration, and net photosynthesis. A comparable high resistance to the desiccating conditions of the space vacuum was observed in previous spaceflight experiments for the lichens *Rhizocarpon geographicum* (L.) DC and *Xanthoria elegans* (Link) Th. (Sancho et al., 2007, 2008), for tardigrades (Jönsson et al., 2008), as well as for spores of the bacterium *Bacillus subtilis* (reviewed in Horneck et al., 2010).

Although the physiological state of the flight dark samples did not differ from that of the Earth controls, they did show some ultrastructural changes, such as the presence of dense bodies in the cytoplasm of photobiont cells as well as in vacuoles of the mycobiont cells, all probably a consequence of the exposure to the space vacuum. We also saw more
pyrenoglobuli in the flight dark samples than in the Earth control samples.

Transmission electron microscopy pictures show the consequences of dehydration to be a peripheral location of pyrenoglobuli inside the pyrenoid (Ascaso and Galván, 1976; Ascaso, 1978), a decrease in the number of pyrenoglobuli per pyrenoid area (Ascaso et al., 1986), or a weak staining of parts of the proteinaceous pyrenoid matrix (Ascaso, 1978; Brown et al., 1987). The desiccation tolerance of lichen species from arid habitats has been fully reviewed and discussed by Kappen and Valladares (2007) and in other works (Lange et al., 1997, 2006; Pintado et al., 2005; Del Prado and Sancho, 2007). The role of lichenic sugar alcohols in the extreme desiccation tolerance of these organisms in outer space was discussed also in Sancho et al. (2007).

In addition to the dehydrating effect of the space vacuum, organisms in space must cope with exposure to cosmic radiation and intense solar UV radiation. From the recovery

FIGS. 8–13. TEM images of photobiont (8, 10, 12) and mycobiont cells (9, 11, 13) from Earth control (8 and 9), flight dark sample (10 and 11), and flight sample exposed to solar radiation at $\lambda \geq 110$ nm (12 and 13) treatments. Pg, pyrenoglobuli; P, pyrenoid; Sb, algal cells cytoplasmic storage bodies; m, mitochondria; N, nucleus; cc, fungal cells concentric bodies; V, vacuoles; s, starch granules.
data of chlorophyll $a$ activity (Figs. 2–6), we concluded that (i) the interval between 100 and 200 nm of solar UV radiation was probably the most harmful part of the solar spectrum for the samples, and (ii) the photosystem II activity of all UV- or visible-exposed specimens fully recovered at the end of the reactivation period of 3 days and reached values identical or very close to those of the preflight samples, which shows that their metabolism was not irreversibly damaged. The lack of metabolic damage in the flight dark samples (Fig. 6) also confirmed that solar extraterrestrial UV radiation was the most harmful parameter in space rather than vacuum, cosmic radiation, or extreme temperatures. Three days of reactivation were sufficient for the full recovery of the photosystem II system of space-exposed samples and showed that solar extraterrestrial UV and cosmic radiation were not limiting factors for the photosynthetic performance of *A. fruticulosa* after 10 days of spaceflight. This observation was further confirmed by the CO$_2$ exchange
measurements. After the recovery period of 72 h and an additional 72 h reactivation, the UV-exposed flight samples had $A_{\text{max}}$ values that were almost identical to those of the Earth controls (Table 2).

Many authors (Millanes and Vicente, 2003; Solhaug et al., 2003) have suggested that the lichen substances are a source of effective protection against UV radiation. The lack of these substances in A. fruticulosa points to other mechanisms in the lichen morphology and anatomy for protection under UV radiation. This could be the structure of the upper cortex (Gauslaa and Solhaug, 2001). The role of the thick and dense fungal cortex in lichens as a protective factor to high UV exposures was demonstrated in field measurements by De la Torre et al. (2002). It seems that algae populations inside the lichens are extremely well protected by this compact layer that works as a protective screen. Although different ultrastructural changes have been observed in the experiment, it appears that the photosystems are well protected as one of the most important structures inside the lichen photobionts.

Vacuoles with dense electron bodies were frequently observed in the cytoplasm of photobiont cells of thalli exposed to wavelengths of $\lambda \geq 110$, 200, and 290 nm, but not in the thalli exposed to a wavelength of $\lambda \geq 400$ nm. These electron-dense bodies could possibly represent a lipid accumulation as a consequence of the loss of lipid from the cellular membranes, which indicates some degree of senescence (Ascaso et al., 1986). The integrity of algal cell walls can be seen as a positive feature. The relationship between metabolic data and ultrastructural analysis carried out by electron microscopy shows interesting features that must be carefully assessed. Disorganization of the thylakoid lamellae inside the pyrenoids, which could be the result of the loss of lipids from the thylakoid membranes, was observed in response to spaceflight. The majority of pyrenoid tubules became collapsed and their lumen almost entirely lost with the consecutive random relocalization of the pyrenoglobuli. The collapse of the tubules could be due either to the withdrawal of water from the lumen of the tube or to a change of the configuration of the dehydrated pyrenoid protein structure (Ascaso et al., 1988). More affected pyrenoids were present in cells from the flight dark samples and from the flight sun-exposed sample that suffered the full solar spectrum ($\lambda \geq 110$ nm), as well as those for the ranges of $\lambda \geq 200$ and $\lambda \geq 290$ nm, where the thylakoid membranes had lost their integrity. The presence of dense bodies between the thylakoid membranes in the flight dark samples and the sample exposed to $\lambda \geq 110$ nm could be lipid loss from the thylakoid membranes. In all these cases the next step could probably be total disorganization of the pyrenoid matrix. Similar electron-dense deposits were observed in R. geographicum after the Lichens spaceflight (De los Ríos et al., 2010). The appearance of the thylakoid membranes inside the pyrenoid was normal in the Earth controls and in the visible-exposed ($\lambda \geq 400$ nm) flight sample, which showed pyrenoglobuli that were also well attached to the thylakoid membranes. It appears that ultrastructural changes were reversible or, at least, that the cellular integrity was sufficiently high to maintain a properly working lichen metabolism. Sancho et al. (2007) and De los Ríos et al. (2010) showed that ultrastructural damage on cells of R. geographicum and X. elegans from high mountain environments did not necessarily indicate a metabolic failure. Although microscopy images provide many examples of the different types of treatments, it is difficult to know exactly the percentage of cells affected because we cannot evaluate the entire population. The analysis of the ultrastructural damage observed by microscopy and their consequences in lichen metabolism is, as mentioned earlier, revealing several responses to the effects of space radiation exposure, While the results presented here constitute a good first approach, these new observations should be investigated in detail.

5. Conclusions and Implications

The main advantage of A. fruticulosa with respect to other lichen species previously included in space experiments is its unattached and compact morphotype, which allows individual thalli to be studied rather than fragments attached to the substrate. This also affords the opportunity to compare the physiological performance of selected thalli within a larger population, which clearly increases our current knowledge in relation to lichen symbiosis resistance to outer space conditions.

That we had only one experimental measurement of the flight sun-exposed samples exposed to different UV ranges of extraterrestrial solar radiation encourages future simulated and real flight experiments that will complement promising preliminary results presented here. More work with simulated space conditions on A. fruticulosa is currently underway with the intent to reinforce these results. If we consider our experiment to have been one of survival/nonsurvival, however, it should be viewed as a success in that all the flight sun-exposed samples survived space conditions.

The new experiment BIOMEX (Biology and Mars Experiment, ESA-ILSRA 2009-0834), which is coordinated by DLR (German Aerospace Research Center, Berlin) and will take place on the Expose-2R facility of the International Space Station, has as its main objective study of the resistance and survival of A. fruticulosa as a test system, in comparison with other selected organisms (lichens, fungi, algae, bryophytes, bacteria, biofilms, cyanobacteria, archaea), when exposed to real space and simulated martian conditions. After a long-term exposure (December 2011 to May 2013), degradation of the cell surfaces of A. fruticulosa, both in contact with terrestrial, lunar, and Mars analog minerals and as a complete system, before and after the flight, will be studied by INTA in coordination with UCM and other institutions. We hope to obtain information about which type of basic traces should be of significance when searching for signs of life in extraterrestrial habitats—in particular on the Moon and on Mars.

Whether the panspermia theory is a real possibility or just speculation seems to be a more open question than ever. The current availability of good experimental designs through the development of suitable technology opens new gates to researchers of many disciplines that are interested in this issue. Lichens have proven to be exceptionally suitable organisms for experiments in astrobiology. The endurance of different types of lichens from different habitats, the possibility of lichen propagules that resist atmospheric entry, and long-term exposure experiments are the next challenges that these amazingly resistant symbiotic organisms should afford investigators in pursuit of answers to the remaining questions in radiation exposure biology.
FRUTICOSE LICHEN SURVIVED SPACE EXPOSURE

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Author Disclosure Statement

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Abbreviations

DR, dark respiration; LTSEM, low-temperature scanning electron microscope NP, net photosynthetic rate; PFD, photon flux density; SEM, scanning electron microscope; TLC, thin layer chromatography.

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AU3: Assays correct as written?
AU4: Sentence correct as written?
AU5: Arrows correct as written?