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Fungal Spores Viability on the International Space Station

I. Gomoiu[1] · E. Chatzitheodoridis[2] · S. Vadrucci[3] ·
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Abstract In this study we investigated the security of a spaceflight experiment from two points of view: spreading of dried fungal spores placed on the different wafers and their viability during short and long term missions on the International Space Station (ISS). Microscopic characteristics of spores from dried spores samples were investigated, as well as the morphology of the colonies obtained from spores that survived during mission. The selected fungal species were: *Aspergillus niger*, *Cladosporium herbarum*, *Ulocladium chartarum*, and *Basipetospora halophila*. They have been chosen mainly based on their involvement in the biodeterioration of different substrate in the ISS as well as their presence as possible contaminants of the ISS. From biological point of view, three of the selected species are black fungi, with high melanin content and therefore highly resistant to space radiation. The visual inspection and analysis of the images taken before and after the short and the long term experiments have shown that all biocontainers were returned to Earth without damages. Microscope images of the lids of the culture plates revealed that the spores of all species were actually not detached from the surface of the wafers and did not contaminate the lids. From the adhesion point of view all types of wafers can be used in space experiments, with a special comment on the viability in the particular case of iron wafers when used for spores that belong to *B. halophila* (halophilic strain). This is encouraging in performing experiments with fungi without risking contamination. The spore viability was lower in the experiment for long time to ISS conditions than that of the short experiment. From the observations, it is suggested that the environment of the enclosed biocontainer, as well as the species'specific behaviour have an important effect, reducing the viability in time. Even the

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spores were not detached from the surface of the wafers, it was observed that spores used in the long term experiment lost the outer layer of their coat without affecting the viability since they were still protected by the middle and the inner layer of the coating. This research highlights a new protocol to perform spaceflight experiments inside the ISS with fungal spores in microgravity conditions, under the additional effect of possible cosmic radiation. According to this protocol the results are expressed in terms of viability, microscopic and morphological changes.

Keywords Fungal spores · Spore viability · Fungal experiment on international Space Station · ISS

Introduction

Microorganisms sense their environment through a variety of sensors and receptors that serve to integrate the different signals into the appropriate cellular response that is optimal for survival purposes. In the space environment, factors such as microgravity, temperature, and solar UV or cosmic radiation vary significantly, especially at different altitudes and under the influence of the Earth's magnetic field. This might impose a significant effect on the physiology and pathogenesis of microorganisms or induce mutations (Nickerson et al. 2004; Horneck et al. 2010; Gomoiu et al. 2013; Wang et al. 2014). Fungi and bacteria are microorganisms that can cause severe health problems to astronauts and contamination to spacecraft. The activation of opportunistic pathogens has been already observed in the closed environments of spacecraft used in long-duration missions, increasing the risk of infectious diseases (Ilyin 2005). Gram-positive and Gram-negative bacteria, as well as fungi, were found on the surfaces inside the spacecraft, in water reservoirs aboard the ISS (La Duc et al. 2004), and in the KIBO module of ISS (Satoh et al. 2011). Spores of *Aspergillus niger*, *Cladosporium herbarum*, and *Ulocladium botrytis* have already been identified on different surfaces (mostly of equipment), and in air samples from the orbital station Mir (Novikova et al. 2006).

On board experiments have been also performed with microorganisms belonging to different bacteria (Wang et al. 2014) and with ascomycetes (Horneck et al. 2010), one experiment with the filamentous fungus *Ulocladium chartarum* (Gomoiu et al. 2013), while other experiments were performed in simulated microgravity conditions with cyanobacteria, green algae, with *Bacillus subtilis*, *Staphylococcus aureus*, two species of archaea. Flight-grown roots of soybean infected with *Phytophtora sojae* have shown more disease symptoms, and root tissues were more extensively colonized in space relative to the ground controls Ryba-White et al. 2001).

The case of inactivation or lethality of bacteria, archea, fungi and lichens has also been tested. The first lethality experiments with fungal spores were based on samples of *Penicillium roqueforti* during Gemini IX and XII missions. Lethality was found to be similarly high with conidia of the fungus *Aspergillus ochraceus*, with bacterial cells of *Deinococcus radiodurans*, and with spores of *Bacillus subtilis* (Horneck et al. 2010). Less affected were the halophilic archea species *Halorubrum chaoviatoris* when they were embedded in salt crystals (Mancinelli et al. 1998). Lichens species, such as *Rhizocarpon geographicum* and *Xanthoria elegans*, as well as Antarctic black fungi and cryptoendolithic communities, seem to cope very well with the real space environment (Brandt et al. 2015; Onofri et al. 2012).

The hypothesis of Panspermia postulates the propagation of microscopic forms of life among planets by solar radiation pressure (Horneck et al. 2012). Since 1903, new discoveries

have been reported, such as that of Martian meteorites that have provided evidence for the transfer of rock fragments from Mars to Earth in the solid state, at moderate shock pressures and temperatures (Horneck et al. 2008), in which microbial communities possibly could have been found inhabiting niche environments in subsurface rocks (Chatzitheodoridis et al. 2014; Marschall et al. 2012; Boston et al. 1992). Space exposure experiments have also demonstrated a high survivability capacity of bacterial spores in space, mostly when they are protected against solar radiation (Horneck et al. 1994; Stöffler et al. 2007). The increased spore γ-radiation resistance is correlated with the low level of spore core water which may reduce the amount of hydroxyl radicals formed (Nicholson et al. 2000).

Several techniques exist to test the viability of microorganisms (Kell et al. 1998; Breeuwer and Abee 2000; Gracias and McKillip 2004; de Vera et al. 2014). However, not all are reliable as the traditional methods. For example, fluorescein diacetate (FDA) is a rapid method to assess viability, which was applied on fungal spores of *Aspergillus niger*, *Rhizopus stolonifer*, *Penicillium citrinum*, and *Fusarium oxysporum*. The addition of NaCl or $MgCl_2$ to the staining solution increased the fluorescence intensity of *A. niger* spores but nonviable spores were distinguished (Yang et al. 1995). Additionally, culture-independent methods based on a combination of fluorescent dyes, such as LIVE/DEAD viability assay kits, are used to quantify viable cells (Olsson-Francis and Cockell 2010). Traditional methods include the direct colony count methods, which are still considered as the most robust to measure survivability in pure cultures (Horneck et al. 2008), and which were successfully applied on dry spore samples.

In this study we investigated the security of a spaceflight experiment from spreading of dried fungal spores placed on the different wafers and the viability of fungal spores during missions of short and long exposure periods on the International Space Station. This was performed using traditional methods, i.e., by investigating the microscopic characteristics of spores and, subsequently, the morphology of the colonies of these spores after the mission. The selected fungal species: *Aspergillus niger*, *Cladosporium herbarum*, *Ulocladium chartarum*, and *Basipetospora halophila*, were sent as dried spores samples to short and long spaceflight missions to the International Space Station. They have been chosen mainly based on their involvement in the biodeterioration of organic and inorganic substrates, covered with organic deposits from ISS (Gomoiu et al. 2013). Fungi of these species are expected to be possible contaminants of the ISS. Three of the selected species are black fungi, constituting an additional reason to choose them due to their high melanin content, and therefore high resistance to space radiation (Onofri et al. 2008).

Material and Methods

Biological Material

The biological material that was used for the spaceflight mission consisted of the four fungal strains *Aspergillus niger CM*-1, *Basipetospora halophila CM*-1, *Cladosporium herbarum CM*-1, and *Ulocladium chartarum CM*-1, which are deposited in the Microbial Culture Collection of the Institute of Biology, Bucharest, Romania, and are available to the scientific community. *Aspergillus niger CM*-1 was isolated from a contaminated phonograph cylinder. *Basipetospora halophila CM*-1was isolated from litter in Grota Miresei Lake, Romania. *Cladosporium herbarum CM*-1 and *Ulocladium chartarum CM*-1 were isolated from Romanian mural paintings, mainly from areas where vegetal residues were present on the intonaco layer (the

support of pictorial layer). The identification of fungal species has been made according to their macroscopic colonial characteristics, but also by their microscopic hyphal features (McLaughlin et al. 2001). Three species were cultivated for 7–10 days in the laboratory on YGC substrate medium containing 5 mg/ml yeast extract, 20 mg/ml glucose, 0.1 mg/ml chloramphenicol, and 20 mg/ml agar. Then, the spores were harvested. *Basipetospora halophila* needs about 50 days of incubation because is a halophilic species.

Spaceflight Experiment Setup

Samples of dried spores were prepared, attached on silicon, polycarbonate, and iron wafer substrates by dropping spore suspensions, and left them dry. Each wafer contained 10^6–10^8 spores/10 mm^2. After drying, the wafer substrates with the samples were placed in small, double-celled culture plates of 30 mm diameter that were hermetically closed with Teflon gaskets. Living cultures where prepared only in larger culture plates, of 60 mm diameter. All culture plates were placed in biocontainers, which are large plastic boxes with compartments on either side, both hermetically sealed with rubber gaskets and a large number of screws (Fig. 1a, c).

For the short spaceflight experiment, culture plates with living cultures of *U. chartarum* (Gomoiu et al. 2013) and with dried spores of *A. niger*, *B. halophila*, *C. herbarum*, and *U. chartarum* were sealed and assembled into three polycarbonate biocontainers (BC#1–BC#3, Pedeo Techniek, Belgium). In the initial experiment, each biocontainer contained: a) four large, 60 mm circular culture plates, two on either side of each biocontainer (Fig. 1a) with living cultures; b) eight small, 30 mm circular culture plates, four on either side of each biocontainer, with dried spores (Fig. 1a) on the different substrates (silicon, iron, polycarbonate).
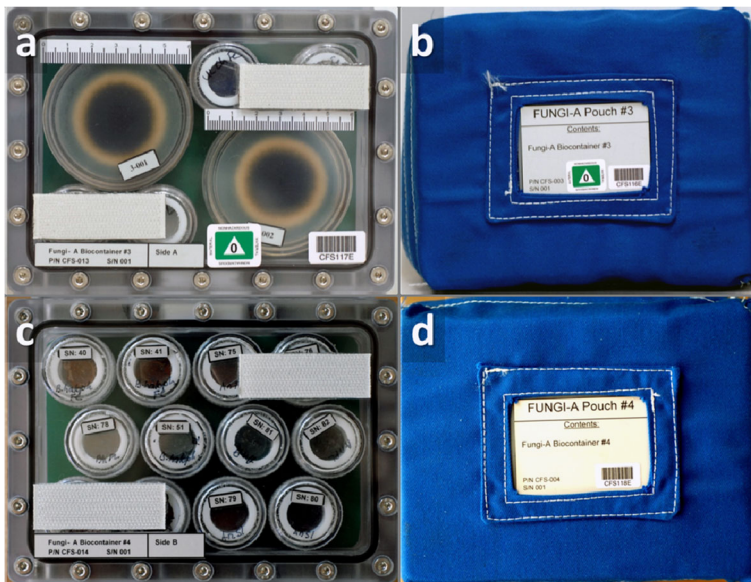

Fig. 1 Short and long time spaceflight experimental setups. **a** Biocontainer #3 with *Ulocladium chartarum* cultures and spore samples; **b** Pouch 3 used for the up- and download in the Space Shuttle, as prepared for the short spaceflight experiment; **c** Biocontainer #4 with all dried spore samples; **d** Pouch #4 used for the up- and download in the Space Shuttle, as prepared for the long spaceflight experiment. All biocontainers have two sides, both with the same arrangement of samples

For the long spaceflight experiment, twenty four small (30 mm) culture plates were placed and sealed in biocontainer BC#4, twelve in either side (Fig.1c), containing all four species suspended on the three different substrates.

All four biocontainers were equipped with a programmable, miniature-sized temperature logger (Smart Button, from ACR Systems Inc.), as well as passive radiation loggers (SCK CEN), to monitor the ionizing radiation exposure over the duration of the space mission. For protection during up- and download, every biocontainer was placed in a pouch of protective foam and of NOMEX fabric (Fig. 1b, d). All biocontainers were stowed in a stowage compartment in the Columbus module of ISS and kept there until return. Pictures were taken by the crew on flight days 5 and 9 only for biocontainers BC#1–BC#3. All biocontainers remained sealed during the experiment and were returned to Earth after 14 days for the short spaceflight experiment, and after 5 months for the long spaceflight experiment. Ground control biocontainers were prepared respecting exactly the same procedure and keeping the same conditions with those of the spaceflight, and were placed in the laboratory.

During the short spaceflight to ISS with Shuttle STS-133, as well as onboard ISS, BC#1–BC#3 were exposed to temperatures of 21.5 ± 2 °C. Respectively, the BC#4 which performed the long spaceflight to ISS (returned later with Shuttle STS-135), as well as its on board stay, was exposed to temperatures of 21.8 ± 3 °C. The total absorbed dose of ionizing radiation recorded during the flight was about 150 μGy per day for each of the samples (background corrected).

Biocontainer Contamination Investigation

The possible spreading of the spores, and therefore the contamination of the culture plates and the biocontainer lids during the duration of the experiment, was investigated by light microscopy. A trinocular Leica DMLM microscope with maximum magnification of ×1000 was used, inspecting the lids of the microcapsules where the small culture plates were placed. This was performed for the space experiment and the ground control. In parallel, the lids were sampled for possible contamination by washing; the samples were then inoculated on YGC medium, and incubated at 26 °C.

Conidial Viability Investigation

The rate of spore germination was used as a measure of viability. A spore was considered to be viable if the length of its germ tube was twice the diameter of the propagule or, if conspicuous swelling of the spore was visible (Herlinda 2010). Viability of the spores was assessed by washing 10 times each wafer, followed by inoculation of decimal dilutions onto 60 mm culture plates with YGC substrate medium. Spores of *B. halophila* have been inoculated onto the medium prepared both with distilled and with Baia Baciului water, because the strain was isolated from the Romanian salt lake. After 10 days of incubation, fungi have been counted in Colony Forming Units (CFUs). In case of *Basipetospora halophila* the incubation time was prolonged to 50 days.

Electron Microscopy

For the morphological investigation of the spores taken from the short and the long spaceflight experiments, the wafer substrates with not fixed dried suspensions were mounted on

aluminium stubs and sputter-coated with gold or platinum to avoid charging during investigation under the scanning electron microscope (SEM). Two SEM instruments were used in this study. The first was a Jeol 6380LV, which was operated in high vacuum, at a working distance of 10 mm, and an acceleration voltage of 20, 25, or 30 kV—depending on resolution requirements at the different magnifications. The second instrument was a LEO 1530 Gemini FE-SEM, operated at a working distance of 7 mm and an accelerating voltage of 5 or10 kV. Secondary electron images (SE) of spores were captured with both instruments.

Morphology of Colonies

In order to analyze the morphology of the colonies obtained after the short and the long spaceflight experiments, mainly their growth and the possible development of microcolonies as has been found in experiment with living cultures *of U. chartarum* (Gomoiu et al. 2013), spores of different variants were inoculated on YGC substrate medium in large culture plates (60 mm), sealed and assembled into polycarbonate biocontainers to have the same experimental conditions and to obtain more information about the growth of fungi in biocontainers used for space missions. The growth of the colonies was evaluated using images taken during the experiment in different instances between the 5th and the18[th] day, using similar procedures as for the *U. chartarum* colonies of the short time experiment (Gomoiu et al. 2013).

Results

Inspection of the Biocontainer and Capsule Lids after the Flight Missions

The visual inspection and analysis of the images taken before and after the short and the long term experiments has shown that all biocontainers were returned to Earth without damages. Unpacking has also shown that all microcapsules were at their original positions, on sides A and B. Temperature and radiation sensors were also at their original position and in a good shape. During transportation, the registered temperature profiles have shown that colonies remained in the required temperature range of 21–22 °C. The only worth noting observation is that after the long mission all iron wafers were oxidized. A high degree of oxidation occurred on the iron wafers that were covered with the spores of the moderately halophilic *B. halophila*. This was most probably due to chemical reactions between the iron ions and the salts of the nutrient used for the growth of the colonies obtained in the laboratory for preparation of the dried spores' samples.

Microscope images of the lids of the capsules revealed that the spores of all species were actually not detached from the surface of the wafers and did not contaminate the lids. Also the inoculation of the samples on the YGC medium did not develop any colony, meaning that no spores were detached from the surface of the wafers to contaminate the microcapsules. Similarly, microscopic inspection of the lids of the biocontainers themselves resulted to the same conclusion.

Viability of Spores after the Short Spaceflight Mission

After the short spaceflight mission, all dried spores from all types of substrate produced cultures that indicated high viability (Fig. 2a). The highest viability was measured on the

polycarbonate (plastic) wafers (95.5–96.5 % for ground controls and 95.7–96.7 % for space-flight), followed by the silicon wafer (93.2–96.3 % for ground controls and 93.4–96.2 % for spaceflight). Iron wafers have shown slightly lower values, both for the ground controls and for the spaceflight, 90.7–94.4 % and 90.6–94.3 % respectively.

Specifically the dried spores of *B. halophila* seem to be more sensitive than the spores from the sporulated colonies that are cultivated in laboratory conditions, for samples of the same period of time. The viability of dried spores for all fungal strains investigated here is similar to the viability of living colonies, irrespective of the substrate.

Viability of Spores after the Long Spaceflight Mission

The viability of the long spaceflight mission showed different results between strains (Fig.2b). *A. niger* spores showed a high viability, even after 5 months on board the ISS (91.5–95 %), as
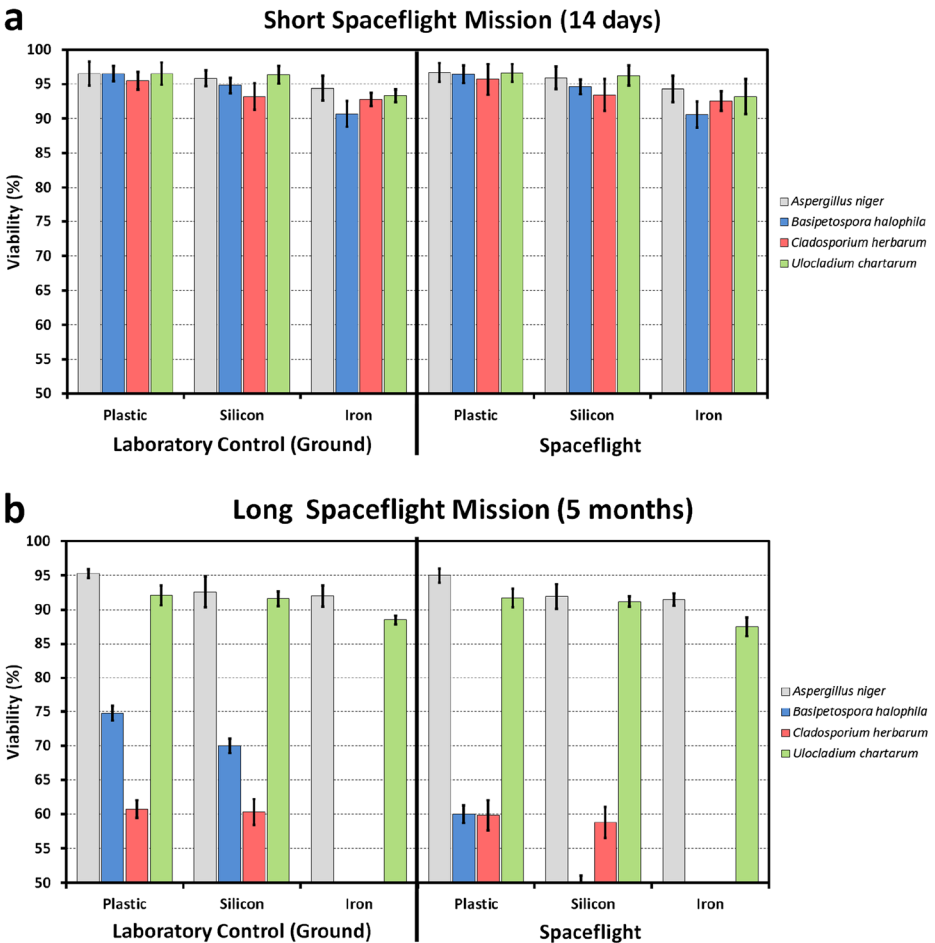

Fig. 2 Viability of fungal spores after the **(a)** short, 14 day spaceflight mission, and **(b)** after the long, 5 month spaceflight mission. Coloured bars are the mean value of 10 viability assessments (in %) and the black vertical lines are their standard deviation. Results are after 50 days of incubation time for *Basipetospora halophila*, and 10 days for the other strains

well as their laboratory controls on Earth (92–95.3 %; Fig. 2b). The highest value was observed from strains that are taken from the plastic substrate, and the lowest from the iron substrate. Moreover, viability values were only slightly lower than those found after the short time stay on board the ISS. The high oxidation of the iron wafers did not affect the viability of the spores of *A. niger* significantly because this strain is well-known for its high resistivity to heavy metals. Similar behavior but with slightly lower viability values was shown by *U. chartarum*. *B. halophila* showed higher viability values than *C. herbarum*, however, still lower that *A. niger* and *U. chartarum*. Both were not viable after retrieval from the iron wafers.

Microscopic Characterization of Spores after the Short Spaceflight Mission

After the short spaceflight mission, ground (Fig. 3a) and flight (Fig. 3b) dried spore samples of *A. niger* look similar. They have a round shape with thick, ruffled surface coats, and a connection structure to the next spore that keeps them in a chain.

Dried spore samples of *C. herbarum* contain secondary ramoconidia with up to four distal hila, and they had ellipsoidal to cylindrical-oblong shapes, with intercalary conidia of up to three distal hila. The terminal conidia were small and ellipsoidal without distal hilum (Fig. 3d).
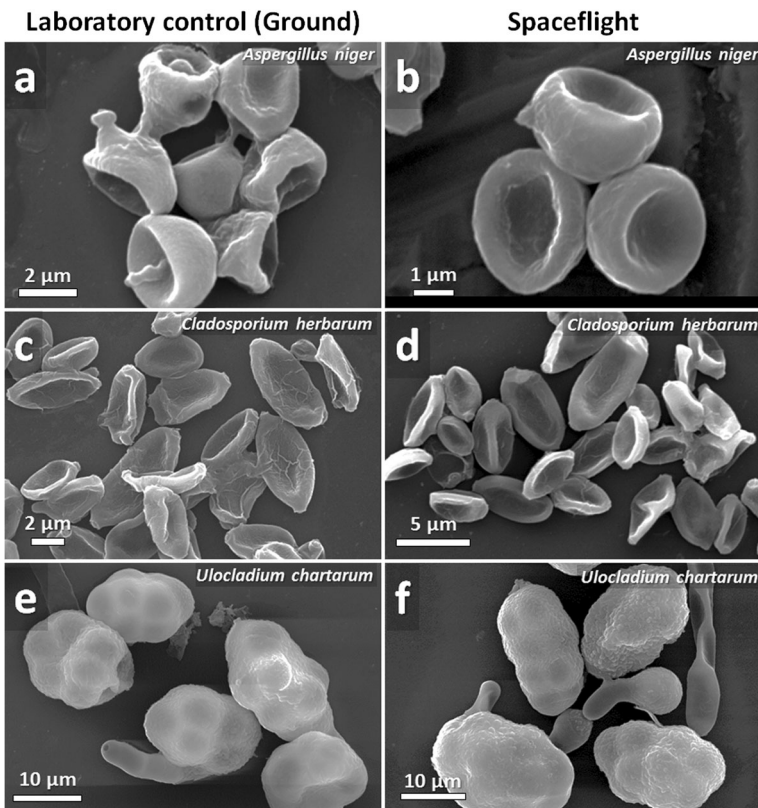

Fig. 3 Secondary electron (SE) microphotographs of dried spores from three fungal species after the short spaceflight mission (*right images*), compared to their respective ground controls (*left images*). **a**, **b** *Aspergillus niger*; **c**, **d** *Cladosporium herbarum*; **e**, **f** *Ulocladium chartarum*

They were covered by ornamentation, showing irregularly reticulate structures. Spores looked also similar to those of the ground control after the short mission (Fig. 3c).

U. chartarum spores are multi-celled due to their transverse and longitudinal septation, have an obovoid (narrowest at the base) rough-walled shape, and most of them become verrucose. *U. chartarum* spores appeared with uniform, knobby surfaces, and with a bud at one end. We did not find any changes either in flight (Fig. 3f) or in the ground (Fig. 3e) samples after the short mission. This species was able to germinate when condensation takes place, either during the photo sessions or the transportation. We estimate that condensation during the short mission was lower than during the long mission.

Microscopic characterization of spores after short space mission revealed that they look similar with dried spores samples of the mentioned species in the laboratory conditions, possibly due to short time mission and experimental conditions (inside the ISS).

Microscopic Characterization of Spores after the Long Time Mission

SEM images of *A. niger* spores revealed that spores are covered by an outer layer, which appears as ornaments, both on the ground (Fig. 4a) and the flight experiment (Fig. 4b). On FIB-SEM cross sections we found that this layer is made by granular material grouped in
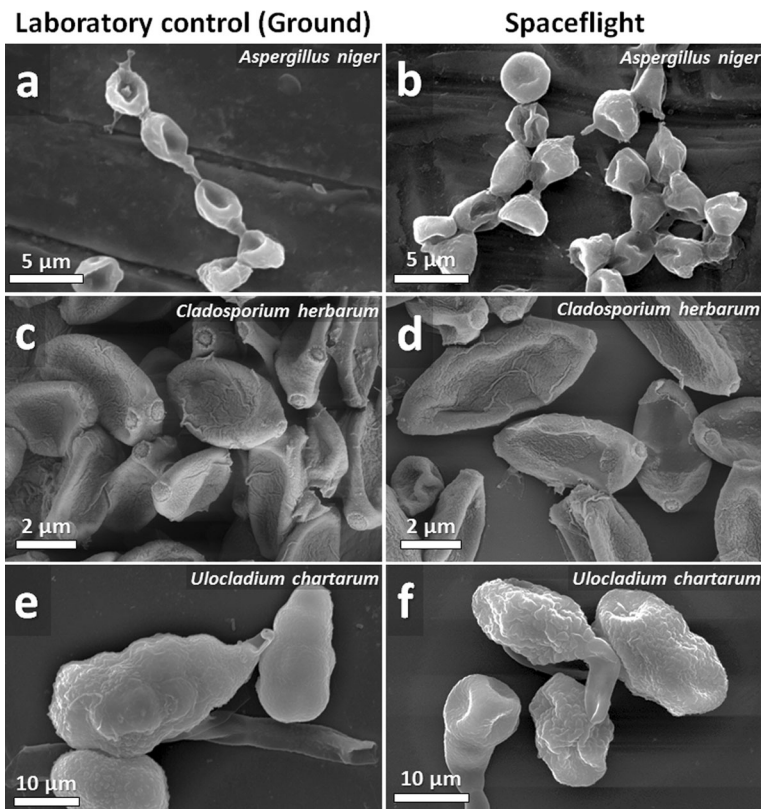

Fig. 4 Secondary electron (SE) microphotographs of dried spores from three fungal species after the long spaceflight mission (*right images*), compared to their respective ground controls (*left images*). **a**, **b** *Aspergillus niger*; **c**, **d** *Cladosporium herbarum*; **e**, **f** *Ulocladium chartarum*

different shapes, such as filamentous, round, and irregular (unpublished results). Although a partial detachment or loss of the outer layer of the spores was observed, still, their viability was not affected.

SEM examination of *C. herbarum* of both the long flight mission and the respective ground control showed that the secondary ramoconidia, as well as the conidia, contained micro-cracks, and breaking or partial detachment of reticulate surface ornamentation. These were more evident in the samples of the flight experiment (Fig. 4d) rather than those of the ground experiment (Fig. 4c), possibly an important reason for their loss of viability.

Some spores of *U. chartarum* seemed to lose their knobble surface during the germination process, confirming the ongoing morphological change (Fig. 4e, f); however, this did not affect their viability. Germination has been also put in evidence as a consequence of condensation.

Morphology of Colonies

The aerial mycelium showed similar characteristics for all spore variants that were isolated from the short and the long trip experiments on ISS, and small differences in comparison to their laboratory controls. The observed differences in the morphology of the colonies show mostly irregular margins, submerged mycelium extending at lengths outside the margin of the colony, and smaller diameters of the hyphae (Table 1). These observations suggest that the growth conditions (large culture plates, sealed and assembled into polycarbonate biocontainers) have an influence on the morphology of the colonies. Similar results have been obtained in the experiment with living cultures of *U. chartarum* (Gomoiu et al. 2013).

Table 1 Morphology of the colonies obtained from dried spores sample after short and long spaceflight mission

Characteristic	*Aspergillus niger*	*Basipetospora halophila*	*Cladosporium herbarum*	*Ulocladium chartarum*
Aerial mycelium	Dense and short	Dense and short	Dense and short	Dense and short
Colour	Black	White	Olive to brown	Green to olive, black in the centre and light cream towards the margin
Texture	Velvety	Cottony	Velvety	Slightly velvety and pitted
Margin[x]	Slightly irregular	Slightly irregular	Slightly irregular	Slightly irregular
Diameter[xx]	Medium	Low	Medium	medium
Exudates drop	Small and few	No exudate	No exudate	Small and few
Exudates colour	Colourless	-	-	Colourless
Submerged mycelium[xxx]	Extending outside the margin of the colony	Extending outside the margin of the colony	Extending outside the margin of the colony	Extending outside the margin of the colony
Development of micro colonies	No	No	No	Yes, numerous

[x] = Colonies margins at laboratory control were regular

[xx] = Colonies diameters in laboratory control were larger (*A. niger* and *U. chartarum* about 45 mm at 10 days of incubation; *C. herbarum* about 30 mm at 10 days of incubation; *B. halophila* about 20 mm at 30 days of incubation

[xxx] = Submerged mycelium in laboratory control did not extend the margin of the colony

Discussion

Spreading of Fungal Spores during Space Experiments

The biological material was represented by four filamentous fungi species *A. niger*, *B. halophila*, *C. herbarum*, and *U. chartarum*. The reasons for choosing those species were their high melanin content in the cell coat that makes them very resistant to radiation (i.e., *A. niger*, *U. chartarum*), and their role in the biodeterioration in general, particularly the deterioration of materials on ISS and of the astronauts' food spoilage as well as the identification, for some of them, in air samples from the orbital station.

During the short and the long space experiments on board the ISS, spores were not detached from their wafers to contaminate the lids. Even if their surfaces are glossy or mat, all spores were strongly attached on the surface of the wafers using the ornaments of their external walls, preventing their spreading on the lids, either of the small plates or of the biocontainers. Hydrophobines have an important role in the cell wall architecture and mediate attachment to the substrate (Girardin et al. 1999; Fontaine et al. 2010; van Veluw et al. 2013). The quasi-absence of air convection, as well as the isolated environment of the biocontainers (Gomoiu et al. 2013), has additionally contributed in preventing the spread of fungal spores. In normal growth conditions, either in the laboratory samples or in Petri dishes where the environmental air has access to the cultures, convections have an important role in spreading the fungal spores (Mims and Mims 2004; Bhattacharyya et al. 2015). The surface of polycarbonate and silicon wafers was not degraded or altered in any way in both experiments showing that they could be successfully used in microbial experiments onboard ISS.

We suggest that, from the adhesion point of view, all types of wafers can be used in space experiments with a special comment on the viability in the particular case of iron wafers when used for spores that belong to the halophilic species tested. The fact that fungal spores, although dry, were not spread around in microgravity is very important for space experiments because bacteria, yeast, and fungi could be cultivated in the same biocontainer without risking contamination.

Viability of Spores

The present study demonstrates that the viability of fungi differs depending on the strain and the duration of the experiment, a conclusion that is in agreement with the results obtained by Górny et al. (2007). In this experiment the viability of spores was found to be similar both for short flight and ground samples, while it was markedly lower in the long exposure experiment to ISS conditions than that of the short experiment for the species *B. halophila* and *C. herbarum*. From the observations, it is suggested that the environment of the enclosed biocontainer, as well as the species' biology have an important effect, reducing the viability in time. This study also revealed high survivability on silica and iron wafer substrates. It has been already observed in previous studies that spores of *A. niger* show reduced degrees of survivability on copper surfaces compared to aluminium (Weaver et al. 2010); the viability and germination of *Aspergillus* species in general is not inhibited in other extreme conditions such as elevated hydrostatic pressure and low temperature (Damare et al. 2008). Also, the impact of O atoms has no effect on the viability of *A. niger* spores and no etching of the coatings of the spores occurs (Raballand et al. 2008). Now it is demonstrated that the same structure of spores from samples from ground and flight experiments sustains similar viability

both after experiments of short and long exposure times to space conditions. Conidia of *Aspergillus* and *Ulocladium* species and lichens are known to be resistant, surviving drought, high temperatures, and UV radiation due to a melanized cell wall that is also thick (Wyatt et al. 2013; Ambiga et al. 2013; Meessen et al. 2013; Sarantopoulou et al. 2011; De Nicolás-Santiago et al. 2006). Conidia of *A. niger* additionally contain genes that are involved in the synthesis of protective compounds, including compatible solutes and protective hydrophilic proteins (Van Leeuwen et al. 2013, Van Leeuwen et al. 2013). Compatible solutes are assumed to protect conidia against drought and heat stress (the disaccharide trehalose and the polyols manitol, glycerol, erithreitol, arabinitol) and do not affect the function of the proteins and membranes when they are accumulated in high concentrations inside the cell (Krijgsheld et al. 2013).

Strong chemical reactions between iron ions and the salts that covered the spores of *B. halophila* and formed by condensation in the humid atmosphere significantly oxidized the iron wafers during the long time experiment, resulting to reduced or complete loss of viability.

Microscopic Characterization of Spores

It was observed that during the long experiment on board ISS, the outer layer as a part of the spore coat was detached or completely lost without however affecting the viability of the spores since they were still protected by the middle and the inner layer of the coating.

All fungal spores that are used in the short and the long spaceflight experiments are covered by a rodlet layer. Krijgsheld et al. (2013) observed that in *Aspergillus* sp. the hydrophobin gene RodA becomes active during sporulation, so RodA protein is produced by sterigmata and diffuses to its outer surface and of the conidial surface that form the rodlet layer. Latgé et al. (1988) demonstrated also a rodlet layer on the conidial surfaces of *C. herbarum*. Hydrophobic proteins found on the spore surface of *Cladosporium* (Sesartic et al. 2013) make them more sensitive to the toxicity of iron ions, in accordance with our results.

Ornamentation showing irregularly reticulate structures has been put in evidence after the short and the long exposure time experiment. It was more evident on FIB-SEM cross sections that after the long mission experiment ornamentations lose some of the irregularly reticulate structures. We suggest that it was due to the long exposure of the spores in culture plates integrated in biocontainer completely isolated from convection currents. Characteristic ornamentation of the surface of the spores has a taxonomic value (Heikkilä et al. 1988), but we can also add physiological value that could explain the rate of germination and the spore's viability after the spaceflight experiments. Condensation started at the early state of the germination only in the case of *U. chartarum* spores, where we found germination tubes emerging from one of the cells (Fig. 5). Spores of *C. herbarum* started to swell but germination did not take place. Conidia of *C. herbarum* under SEM had surfaces with loosely irregularly reticulate structure or embossed stripes, probably caused by the diminishing turgor and the shriveling of the young conidia. Bensch et al. (2012) reported similar results for the different species of *Cladosporium*, including *C. herbarum*.

Morphology of Colonies

The morphological examination of the colonies obtained from the spores that were used for the short and the long spaceflight experiments were originally made using the naked eye, then, microscopic characterization was also performed. They did not show major changes most
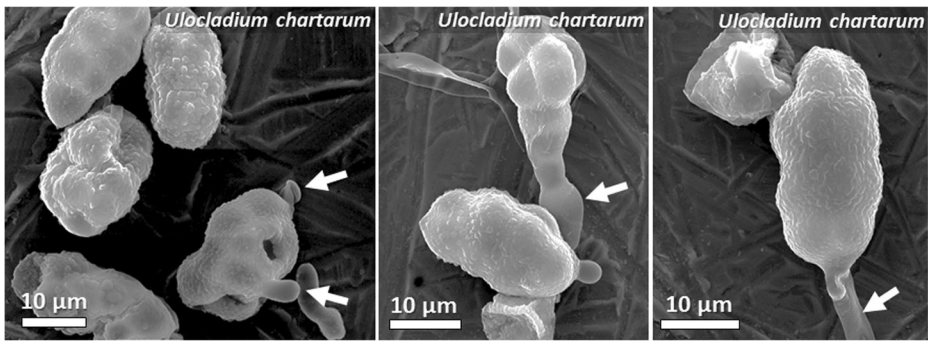
Fig. 5 Secondary electron (SE) microphotographs showing *Ulocladium chartarum* spores after the long experiment, from which germination tubes emerge at an early stage (see arrows). The substrate is iron

probably do to the absence of changes in the DNA. Past reports of experiments performed by isolating mutants have shown changes in the shape and the size of the colonies (Radha et al. 2012), disordered polarized growth (Cai et al. 2014), low spore production (Baracho and Baracho 2003), or highly increased spore production (Leonard et al. 2013).

The morphology of the colonies and their microscopic characteristics are considered as essential tools for fungal identification (Didier et al. 2014; Ogórek et al. 2012; Afzal et al. 2013) as well as for the characterization of mutants. Nowadays, for the identification of new fungal species polyphasic taxonomy uses different methods, such as morphology, physiology, metabolite production and, very important, molecular data (Silva et al. 2011).

Dry Spores as Valuable Model in Astrobiological Research

In the field of astrobiology this research highlights a new protocol to perform spaceflight experiments with fungal spores in microgravity conditions, also under the additional effect of possible cosmic radiation. According to this protocol the results are expressed in terms of viability, and of microscopic and morphological changes.

To perform similar space experiments, plastic, silicon and iron wafer substrates can be successfully used. However, when iron wafers are used as substrates for spores belonging to halophilic strains, such as *Basipetospora halophila*, iron oxidation effects should be taken into account.

The fact that dried fungal spores are not spread around in microgravity conditions is very important for subsequent space experiments, indicating that also experiments using bacteria, yeasts, and fungi could be performed in the same biocontainer without any risk of contamination.

In the future, the ESA exposure facility EXPOSE with different microorganisms can be also performed without any contamination risk.

Finally, our results suggest that the spores of *A. niger*, *C. herbarum*, *U. chartarum*, and *B. halophila* are resistant also for the long spaceflight experiment. We agree with the hypothesis of Kawaguchi et al. (2013) that aggregated cells of *Deinococcus* could sustain "massapanspermia" and therefore, it is suggested that this can be tested to be true also with aggregated spores of *A. niger* and *U. chartarum*. This could be evidence that the life on Earth came from outer space most probably as aggregated bacteria cells and aggregated fungal spores.

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