LIFE – Lichens and Fungi Experiment

Survival of black fungi in Space preliminary results

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Lord Kelvin wrote in 1871 *“Should the time come when this earth comes into collision with another body, … many great and small fragments carrying seeds of living plants and animals would undoubtedly be scattered through space. Hence, … we must regard it as probable … that there are countless seed-bearing meteoric stones moving about through space …”*

Svante Arrhenius "The Propagation of Life in Space," *Die Umschau*, **7**, 481 (1903)

The transfer of viable organisms by means of lithopanspermia implies three different scenarios :

1) Safe escape from the parent planet

2) Survival in space

3) Survival during landing

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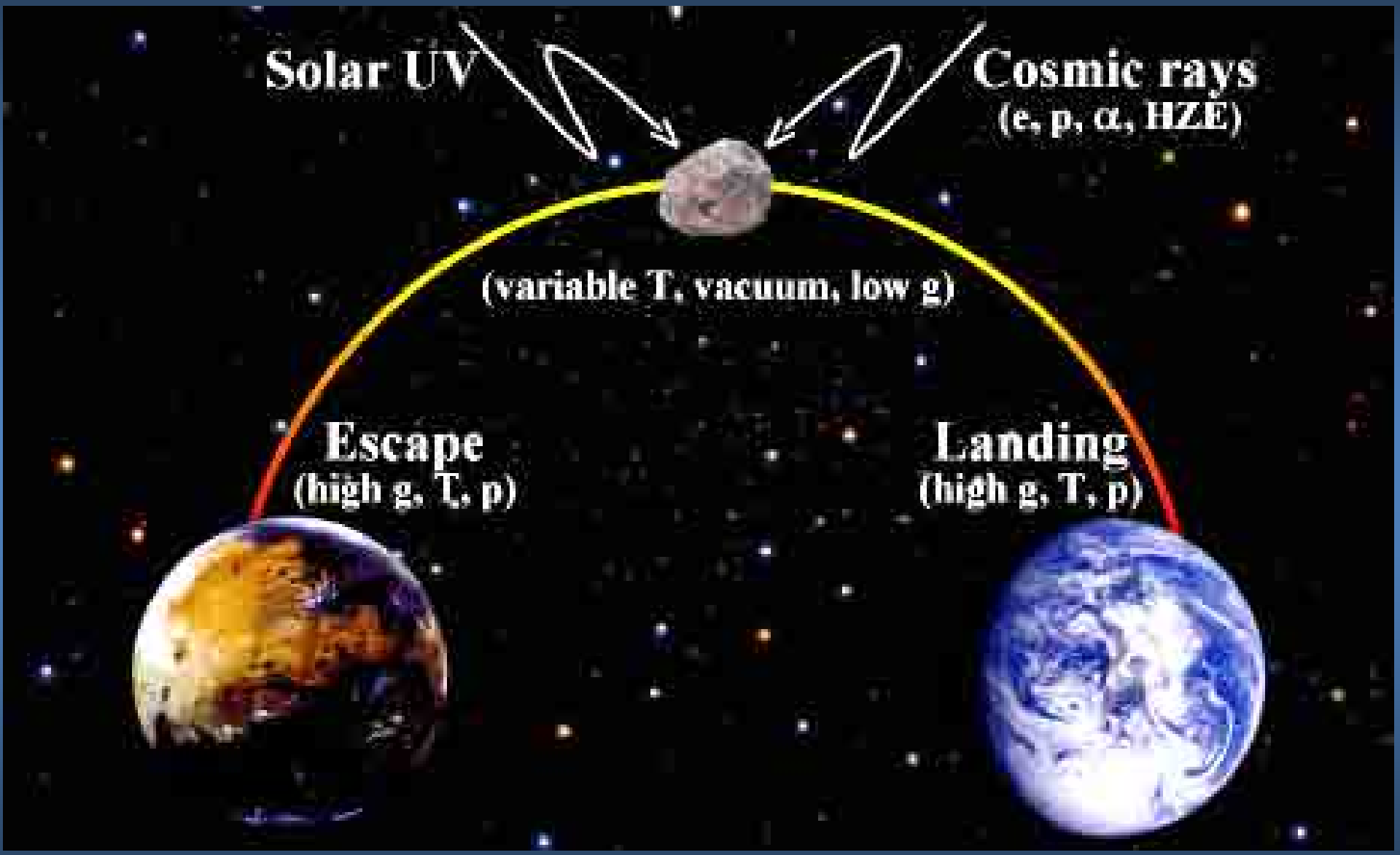
Solar UV
Cosmic rays
(e, p, α, HZE)
(variable T, vacuum, low g)
Escape
(high g, T, p)
Landing
(high g, T, p)

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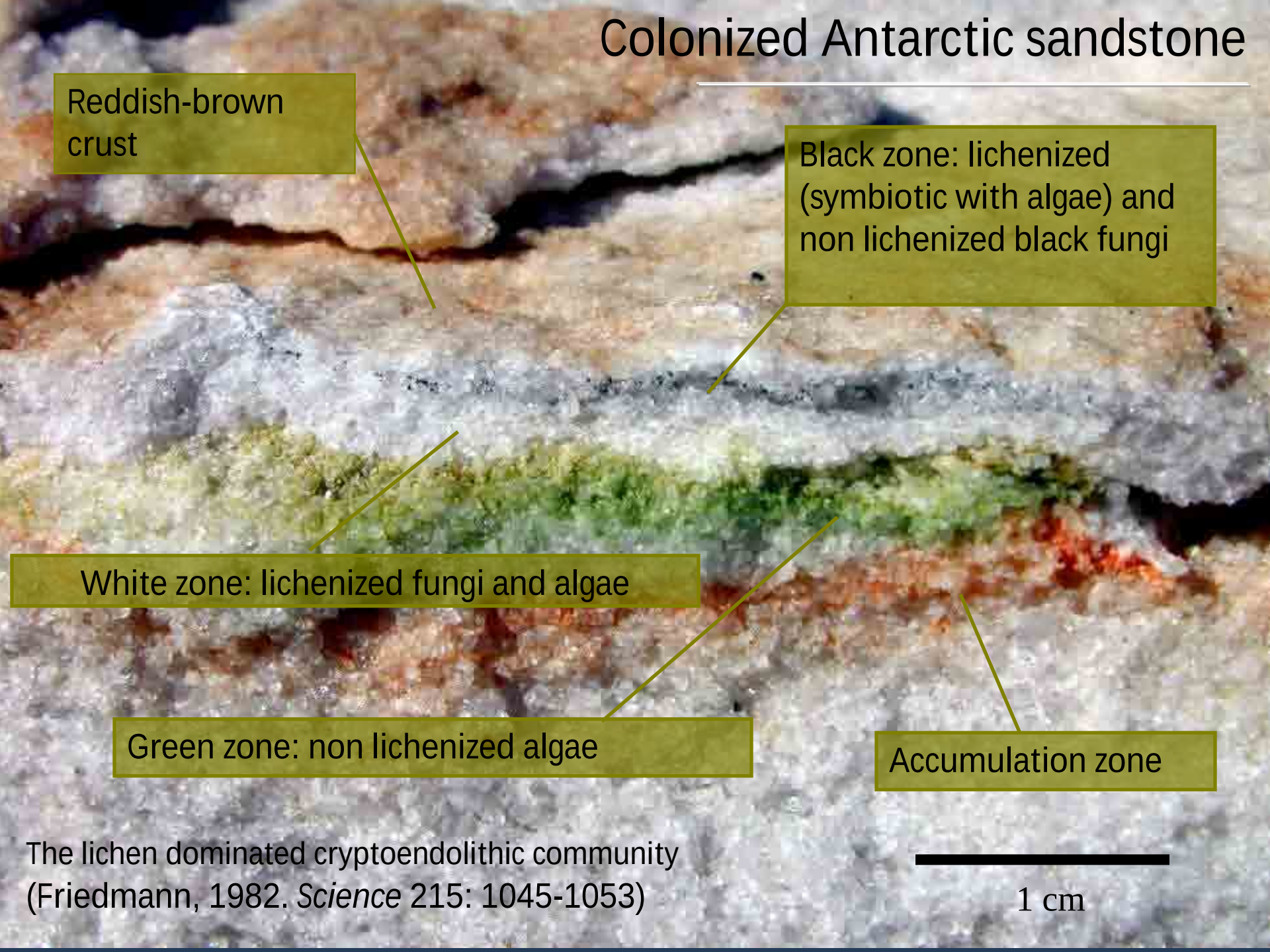
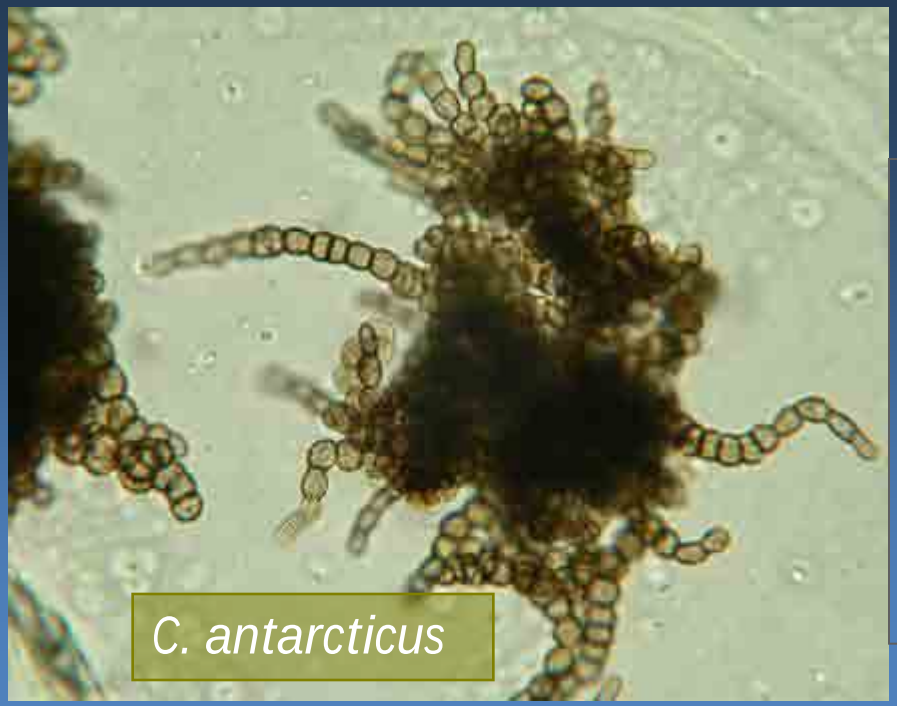
Colonized Antarctic sandstone

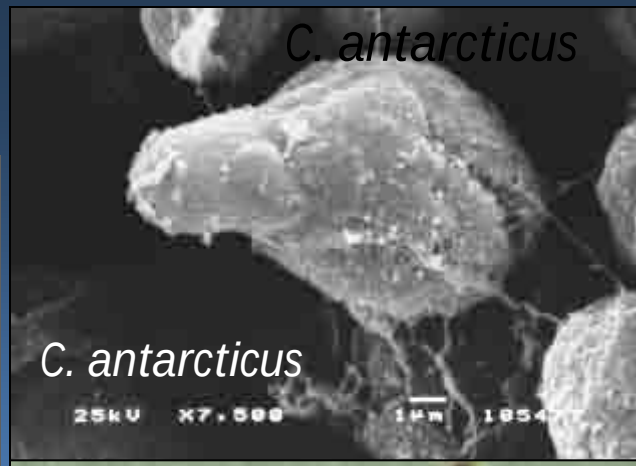


The lichen dominated cryptoendolithic community
(Friedmann, 1982. *Science* 215: 1045-1053)

Battleship Promontory



Antarctic rock black fungi belonging to the genus *Cryomyces*



Selbmann *et al.*, 2005 *Stud. Mycol*. 51: 1-32

How do these organisms survive?

Tolerance to extreme:

- Desiccation
- low temperatures and high thermal fluctuations
- UV radiation

The high resistance showed by Antarctic black fungi to the different stress factors, makes them very good models to investigate long term resistance to conditions as harsh as ***space conditions***.

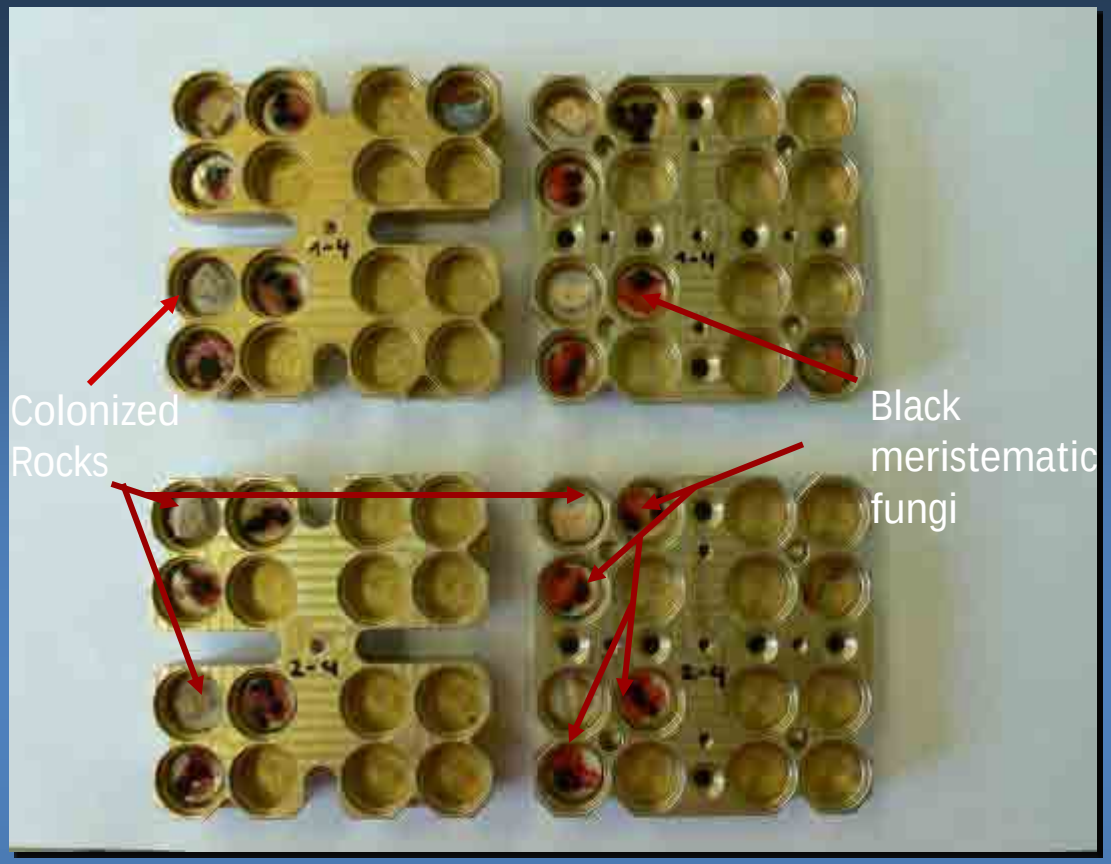
The hypothesis of transfer of living organisms within meteoric rocks suggested to extend the experiments to the whole cryptoendolithic community.

LIFE - Resistance of endolithic fungi and lichens to space conditions

EXPERIMENT DESCRIPTION:

Long term exposure (~1.5 years) of cryptoendolithic Antarctic communities, Antarctic black rock fungi, epilithic lichens, and mycobiont, to real space and simulated environmental Mars conditions on International Space Station (ISS).

LIFE carriers



Samples preparation

Fragment of colonized Antarctic sandstone and dried colonies on agar of black meristematic fungi are glued on sterile teflon disks (11 mm Ø)

Colonized rock fragments

Cryptoendolithic black fungi

Tray assembly

Sample composition & layout

Type of Sample carriers: ROSE Type-3 (16 well)

Carrier #1

Pocket type: vented (Vacuum)
Atmosphere: Space Vacuum
UV range: >110 nm (MgF_2)
(½ screened with 0.1% MgF_2 neutral density filter)
Temperature range: no temperature control

Carrier #2

Pocket type: not vented
Atmosphere: 6 mbar CO_2 (Mars) prepared on ground, valves closed, remain closed during the flight
UV range: >200 nm (Suprasil + long pass cut off filter)
(½ screened with 0.1% Suprasil neutral density filter)
Temperature range: no temperature control

Because of the long period of exposition on ISS, 0.1% neutral density filter are chosen as optimal within the previously tested value of 0.01% and 1%.

LIFE samples

	Space dark	Space UV	Space UV 0.1%	Mars dark	Mars UV	Mars UV 0.1%
Cryomyces antarcticus	98	114	122	226	242	250
Cryomyces minteri	101	117	125	229	245	253
Colonized Rocks	97	113	121	225	241	249

Goals:
-Viable vs dead
-How many are viable (%)?
-Biodiversity in rock samples

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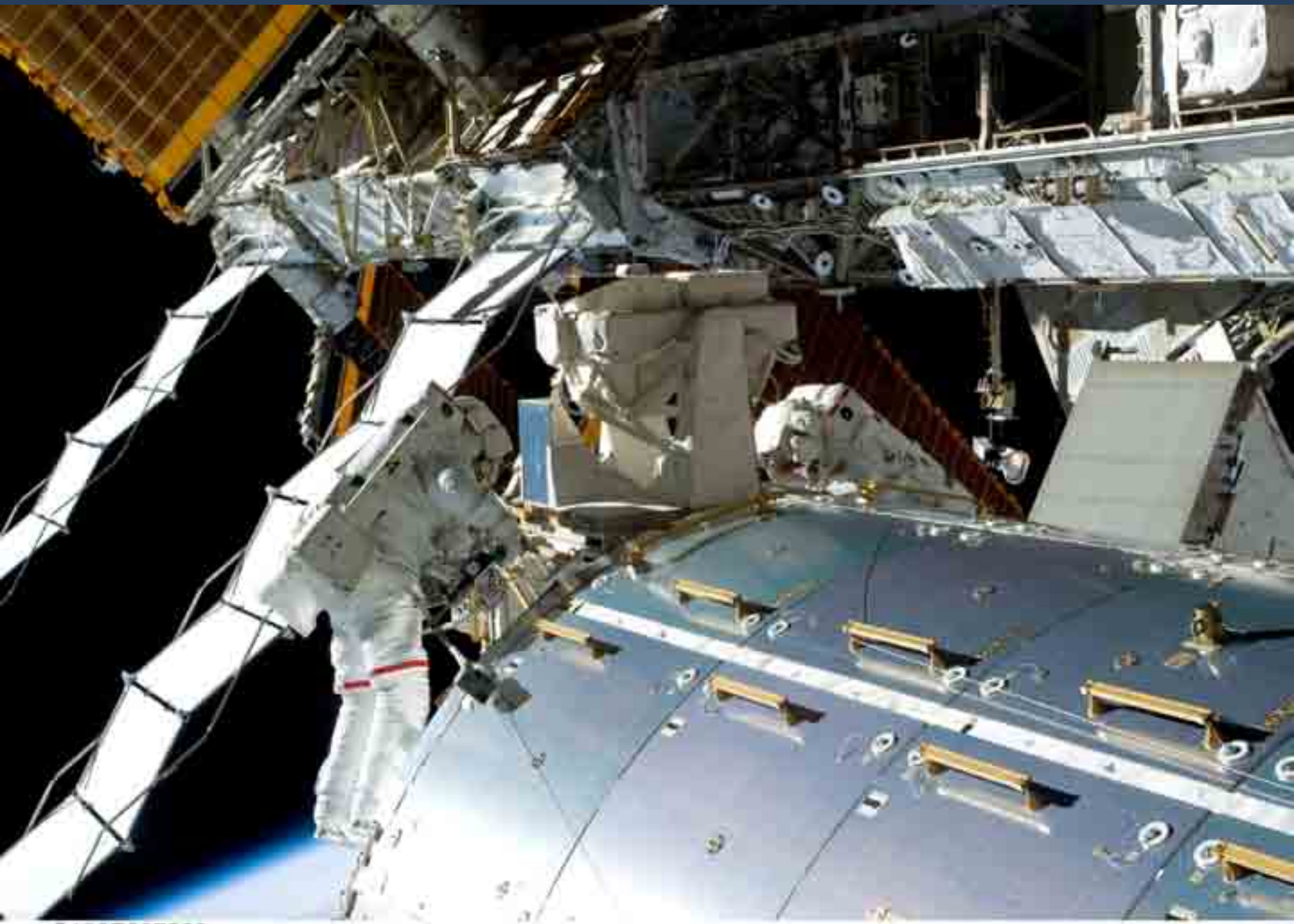

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esa

How can we evaluate the viability of fungi?

Culture-dependent analysis
=*cultivation on agar-media*

Culture-independent analysis

Propidium MonoAzide assay

- A protocol that allows an evaluation of live and dead cells:
- Once inside the cells, PMA intercalates into the DNA and can be covalently cross-linked to it, which strongly inhibits PCR amplification. By using PCR after PMA treatment, the analysis of microbial communities can theoretically be limited to cells with intact cell membranes.

DNA Extraction: MAXWELL 16

•This protocol works with Bacteria and now we know that is a reliable protocol also for Fungi and for other eukaryotic organisms.

Q-PCR

- *quantitative real time polymerase chain reaction* (QPCR/qPCR) is used to amplify and simultaneously quantify a targeted DNA molecule.
- It enables both detection and quantification
- its key feature is that the amplified DNA is detected as the reaction progresses in *real time*, a new approach compared to standard PCR, where the product of the reaction is detected at its end.
- This technique, when used in combination with the PMA assay, can be used to calculate total viable cell population.

Analysis of Fungal diversity in sandstone samples

- Perform PCR of ITS regions with ITS1F and TW13 primer set

- Purify PCR products

- Insert PCR purified products in a pCR4-TOPO vector
  Chemical transformation conducted with OneShot TOP10 chemically competent *Escherichia coli* from the TOPO-TA cloning kit

- Perform RFLP for colony screening

- Insert sequencing

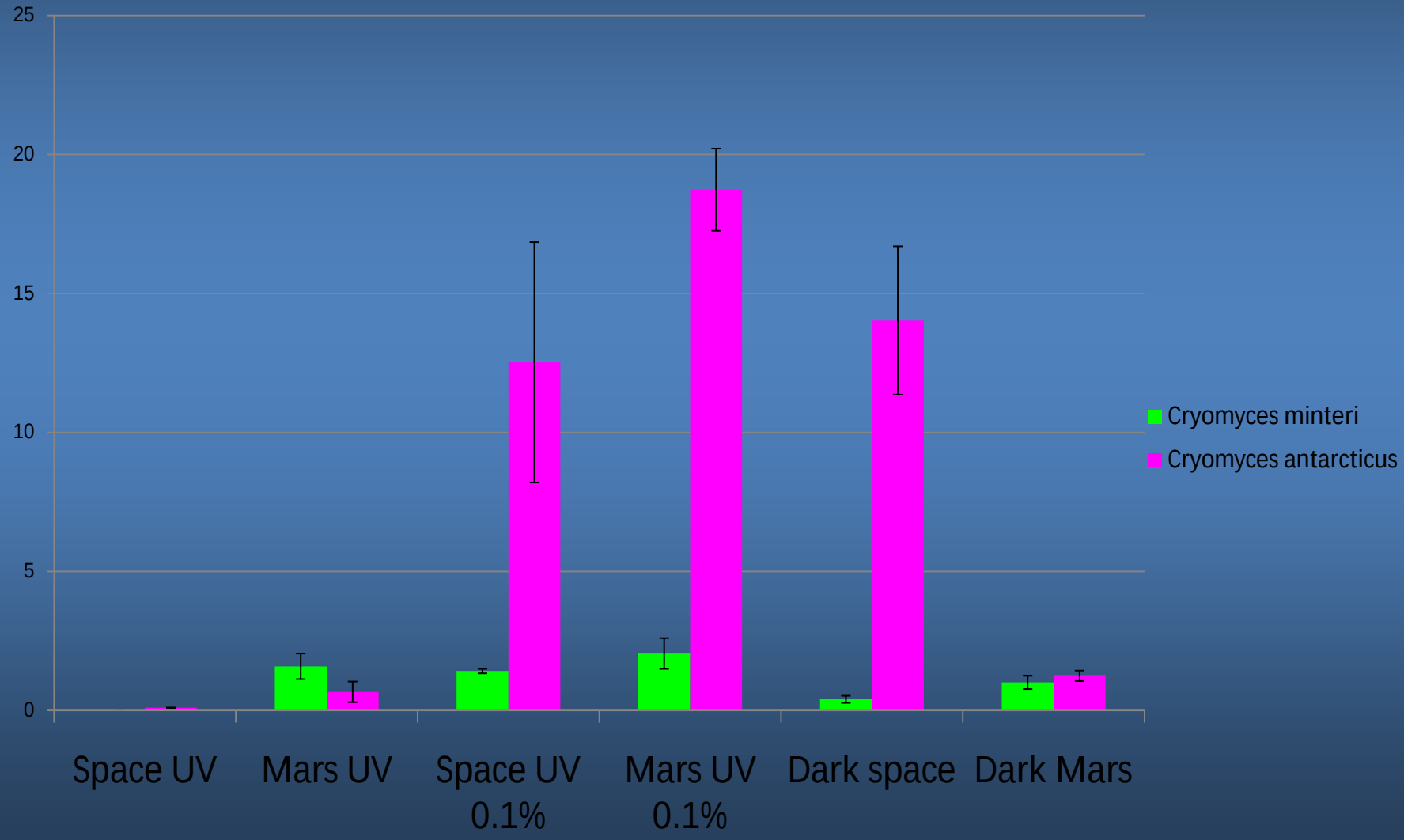
Preliminary results

Culture-Dependent analysis

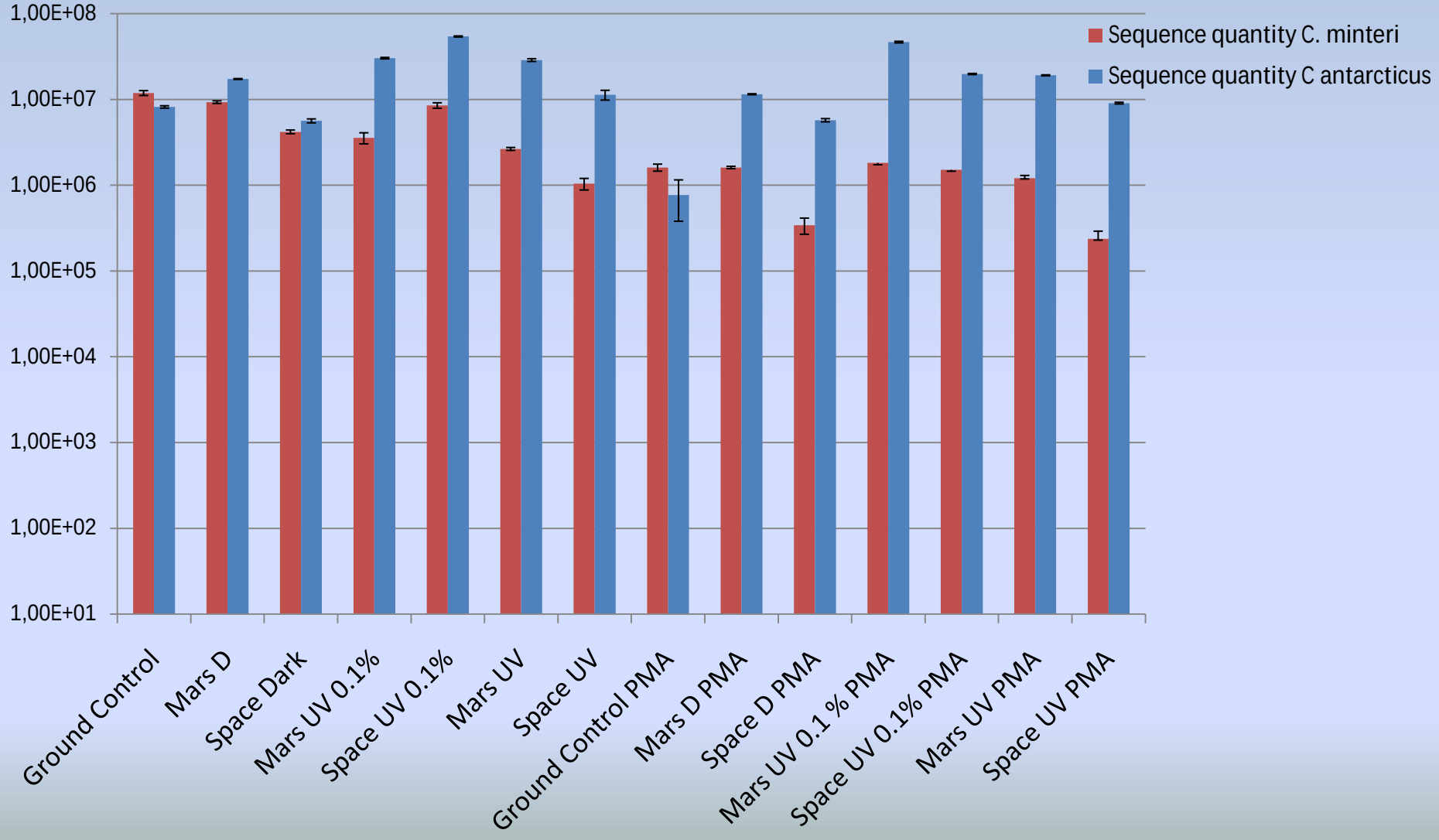
Cryomyces antarcticus

Cryomyces minteri

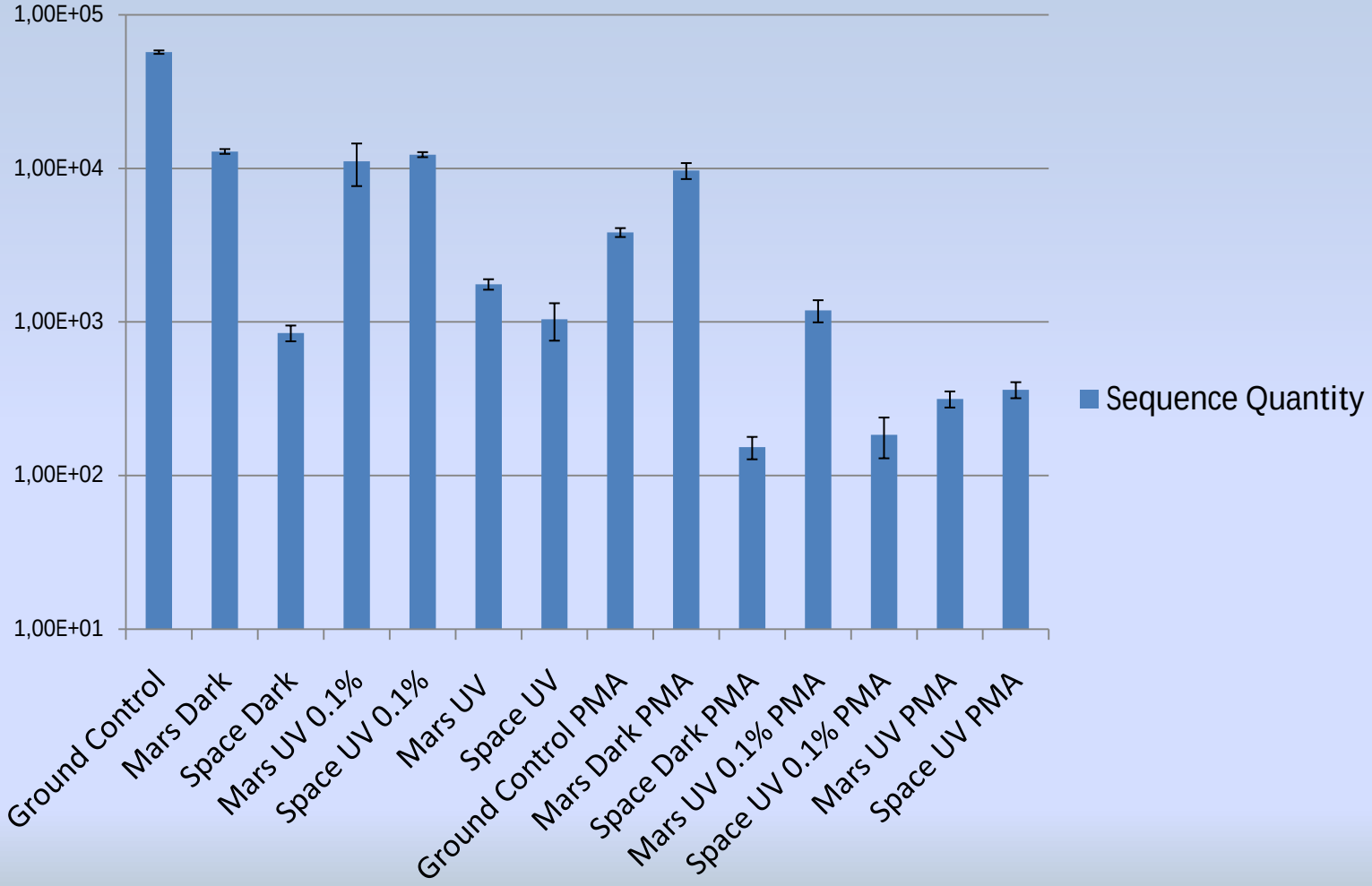
Percentage of survival measured by cultural-dependent methods



Cryomyces antarcticus (CCFEE 515) and *Cryomyces minteri* (CCFEE 5187)



Colonised Rocks: fungal viability analysis



Fungal DNA in rocks from space

- Cladosporium sp.
- Bullera sp.

DNA from living fungi

- Cladosporium sp.
- Candida sp.?

Conclusions

- **Cryptoendolithic meristematic black fungi are alive**
- **Fungi in rock community can resist for 1.5 years in Low Earth Orbit space conditions**
- **Transfer of life through meteorites between planets could be possible!**

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Thanks for your attention…