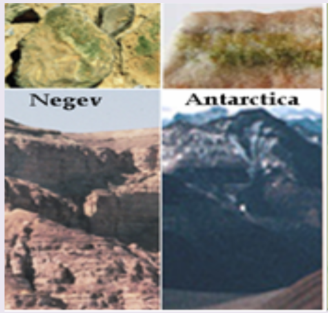
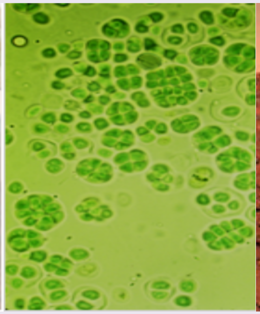
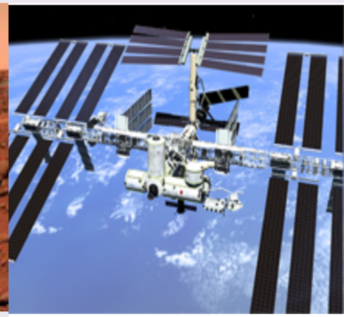





Desert cyanobacteria under space and Martian conditions : Insight into the limit of life as we know

Daniela Billi [1], Cyprien Verseux[1,2], Mickael Baquè[1,3], Claudia Fagliarone[1], Lynn Rothshild[2] and Jean-Pierre de Vera[3]

[1]University of Rome Tor Vergata, Dept. Biology, Rome, Italy
[2]NASA Ames Research Center, Earth Sciences Division, Moffett Field, California USA
[3]German Aerospace Center, Institute of Planetary Research, Berlin, Germany

5th workshop of the Italian Society of Astrobiology-15-17 September, Trieste

HOW TO INVESTIGATE THE LIMIT OF CHROOCOCCIDIOPSIS SURVIVAL ?

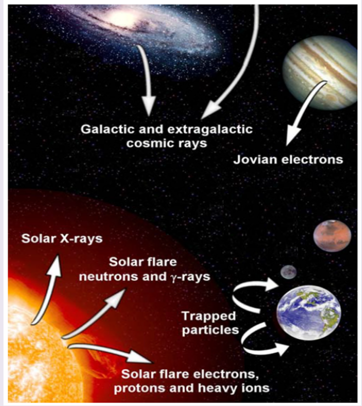
- **Field samples** (hot and cold deserts)

- **Laboratory-dried samples**

- **Irradiation facilities**
- High and low LET radiations (STARLIFE project))

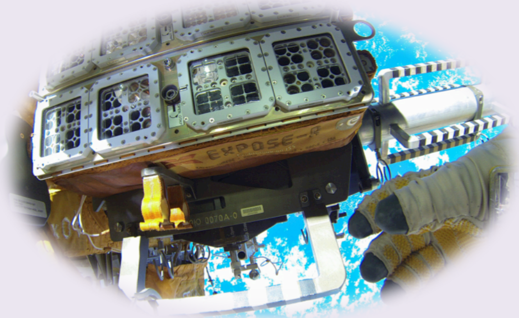
- **Martian and space simulations**

 - temperature cycles,
- UV 254 nm
- UV200-400 nm
- space vacuum
- Mars atmosphere



- **Space mission**
- EXPOSE-R2 (July 2014- May 2016)

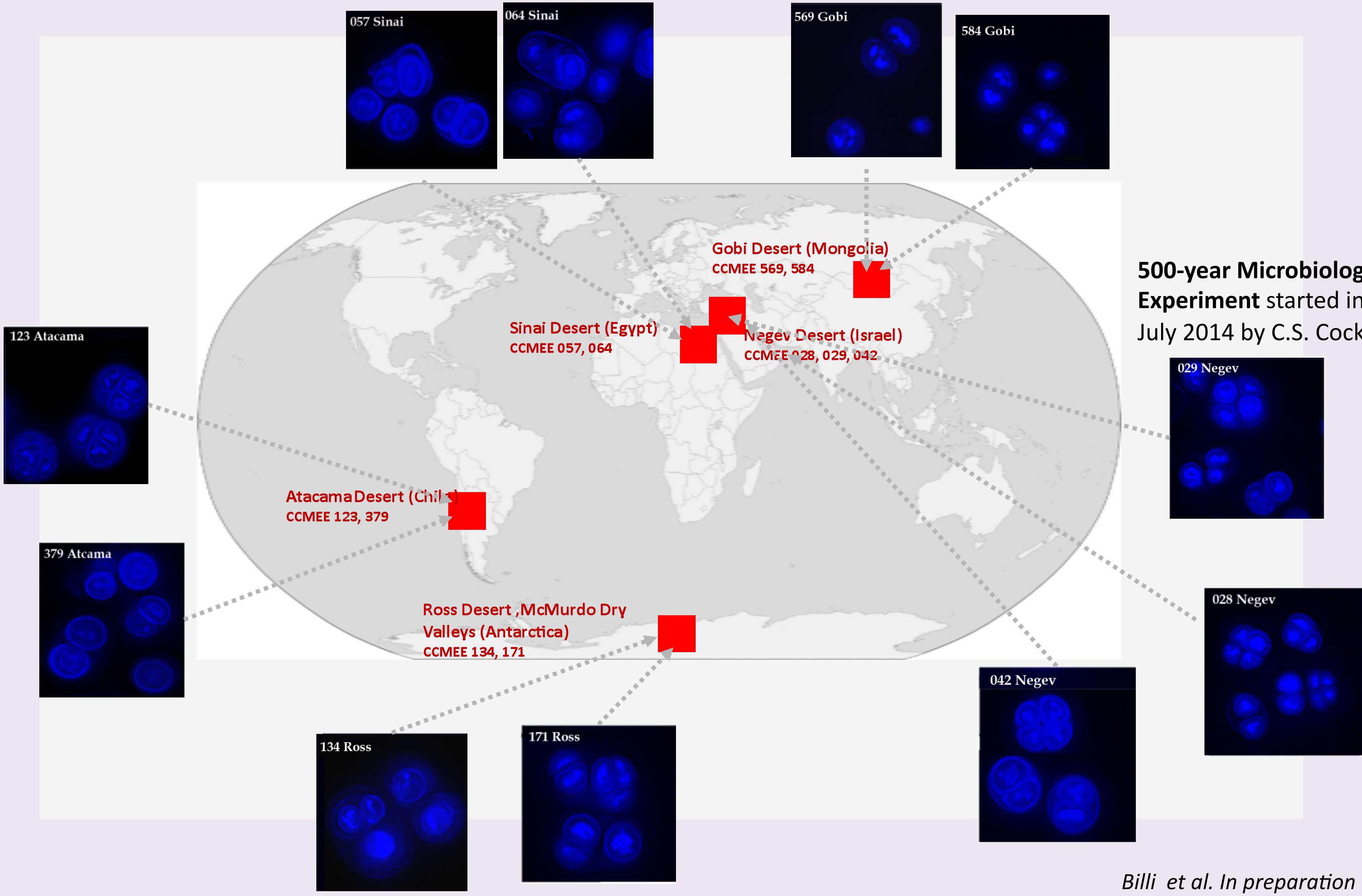
-BOSS (Biofilm Organisms Surfing Space)
-BIOMEX (BIOlogy and Mars Experiment)
- EXPOSE-E
- EXPOSE-R



STARLIFE An international project aimed at studying astrobiological model systems in their response to major components of the galactic cosmic radiation.
(PIs Ralf Moller and Stefan Letho)

ABSENCE OF LIQUID WATER : Desert *Chroococcidiopsis* strains survive prolonged desiccation

- How long is a prolonged period ? At least for 4 years



500-year Microbiology Experiment started in July 2014 by C.S. Cockell

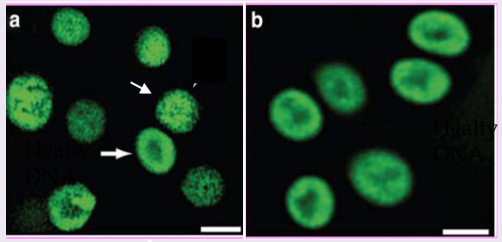
Billi et al. In preparation

Desert *Chroococcidiopsis* strains survive 4 years of air-dried storage by escaping / limiting DNA damage and oxidative stress

Single-cell level analysis of DNA damage

- TUNEL analysis showed dried cells with a signal comparable to that of healthy hydrated cells, suggesting the absence of genome fragmentation.

4 years-dried cella control cells

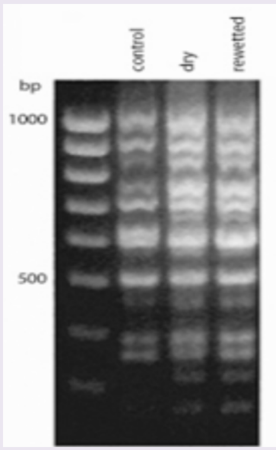


Billi D (2009) Extremophiles 13:49-57

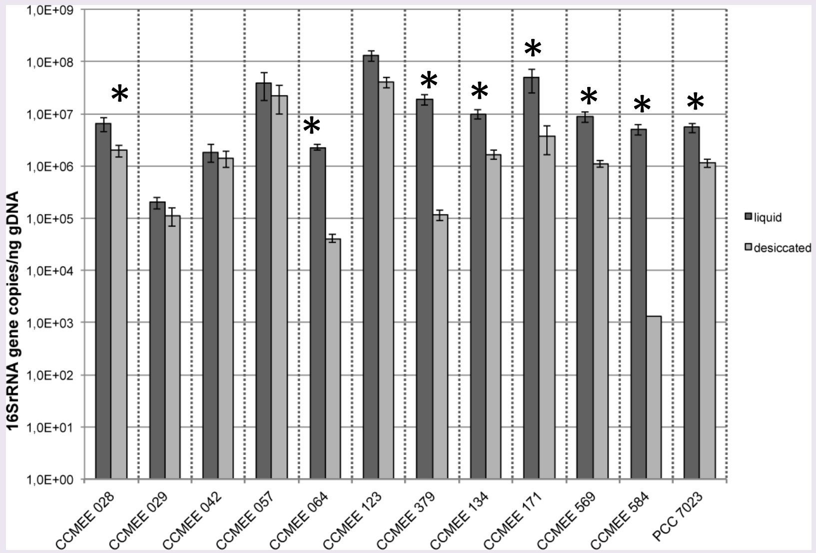
PCR-based asssays of DNA damage

- Qualitative: genome PCR fingerprinting with HIP-derived primes
- Quantitative: Real-time PCR of a target gene (1027 nt fragment of the 16S rDNA)

Genome PCR fingerprinting



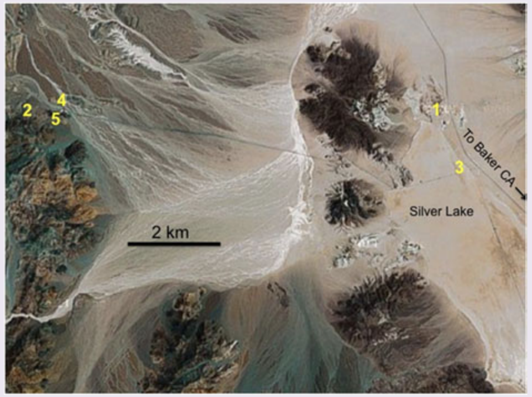
Real-time quantitative PCR of DNA damage in 11 desert strains



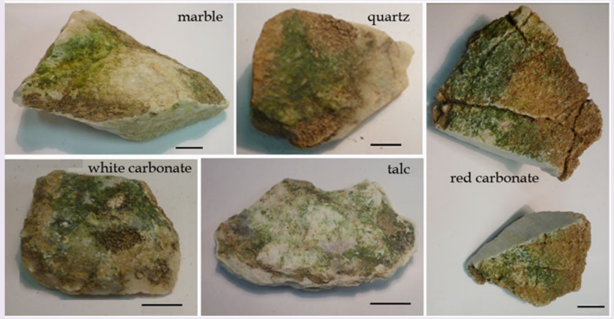
*= significative DNA damage

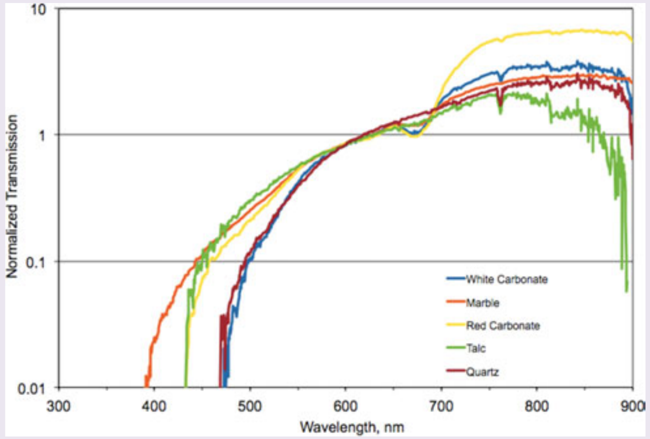
Billi et al. In preparation

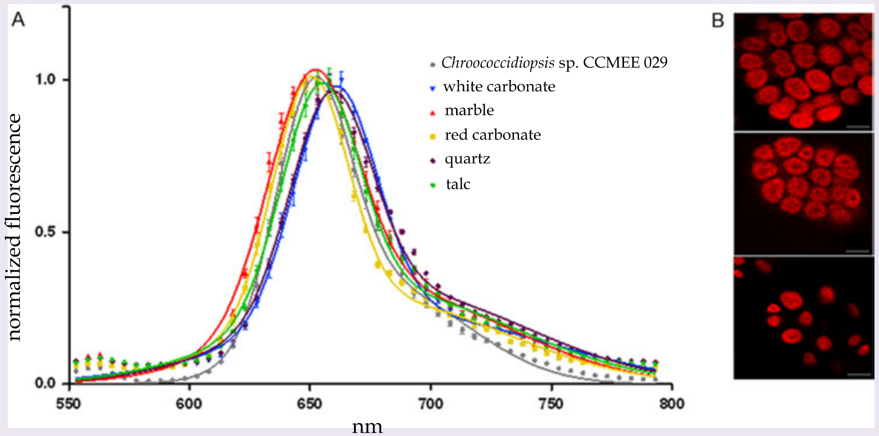
LIGHT : *Chroococcidiopsis* dominates hypolithic communities under rocks with different light transmittance



Sampling sites in the vicinity of Silver Lake, CA







Smith HD, Baqué M, Duncan AG, Lloyd CR, McKay CP, Billi D (2014)
Int. J. Astrobiology, 13: 271-277

Relevance to astrobiology
Red-coated carbonate having the highest IR transmission might in principle support near IR chlorophylls with implications for exotic photosynthesis.

IONIZING RADIATION: *Chroococcidiopsis* survive 15 kGy of X-rays, 24kGy of gamma-rays and at least 2 kGy of heavy iones

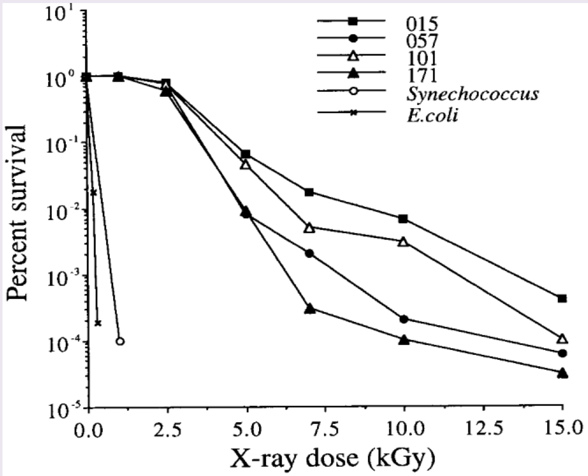
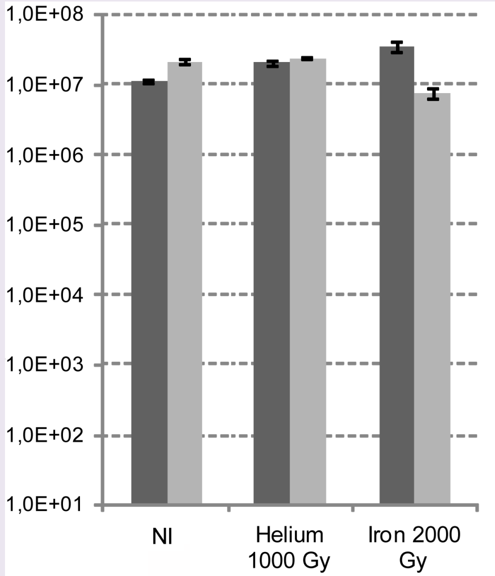


FIG. 2. Representative survival curves for four *Chroococcidiopsis* strains and controls. The values are means based on two independent trials with three replicates per trial.

Billi et al. (2000) Appl. Environ. Microbiol. 66:1489-1492



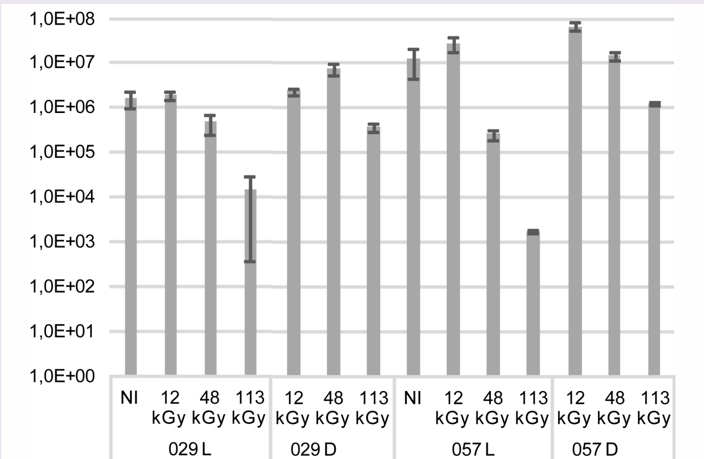
Heavy ions

Survival to 1000 Gy He, 2000 Fe

Damaged DNA : real-time qPCR (*black bars*)

Damaged membranes : PMA-PCR (*grey bars*)

DNA damage



CCMEE 029 : hydrated and dreid cells survive up **to 12 kGy, of** of gamma rays

CCMEE 057 hydrated and dreid cells survive up **to 12 kGy;**
dried cells up to **24 kGy** of gamma rays

Verseux et al. in preparation

NEW INSIGHTS FROM THE EXPOSE –R2 MISSION

BOSS: Biofilm Organisms Surfing Space

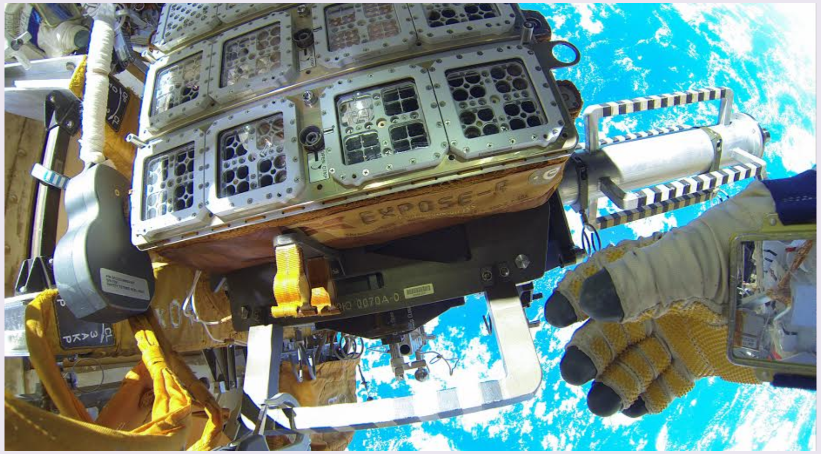
Aim: testing if microbial biofilms are more resistant to the environmental conditions in space and on Mars than planktonic counterparts

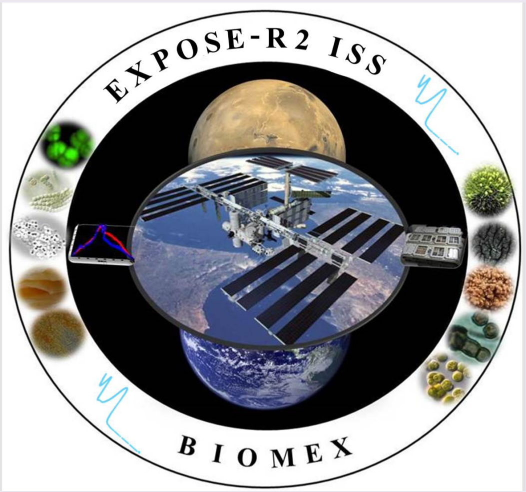
EXPOSE- R2 space mission (ESA ILSRA 2009)

Outside the ISS (July 2014 –May 2016)

Science Team Coordinator : Petra Rettberg

BOSS_Cyano : *Chroococcidiopsis* (PI D. Billi)





BIOMEX : BIOlogy and Mars-EXperiment

Aim: testing the resistance and stability of biomolecules and the endurance of extremophiles under space and Mars-like conditions (with Lunar and Mars analogues).

EXPOSE- R2 space mission (ESA ILSRA 2009)

Ou side the ISS (July 2014 –May 2016)

Science Team Coordinator : Jean-Pierre de Vera

BIOMEX_Cyano : *Chroococcidiopsis* (PI D. Billi)

Ground-based simulations @ DLR from 2011 to 2013

- Compare the resistance of dried planktonic cells vs dried biofilms
- Survival in the presence of dried planktonic cells mixed with minerals

EXPOSE-R2 EVT Part 1 Exposure Experiments

Test parameter	performed
Vacuum 10^{-5} Pa	1 h, pressure: $3.86 \times 10^{-3} \pm 0.12$ Pa 7 d, pressure: $8.50 \times 10^{-5} \pm 0.12$ Pa
Mars atmosphere (CO_2 gas composition) 10^3 Pa	1 h, pressure: $6.08 \times 10^2 \pm 0.12$ Pa 7 d, pressure: $6.00 – 6.6 \times 10^2 \pm 0.12$ Pa
Temperature -10 °C to +45 °C	66 cycles 8 h each, 2 h at -10°C ± 1°C, 2 h at +45°C ± 1°C, 2 h each for cooling and heating
Temperature max and min -25 °C and +60 °C	- 25°C ± 0.5°C, 1 h + 60°C ± 0.5°C, 1 h
Irradiation 254 nm Hg low pressure lamp @ 80 μWcm^{-2}	
0 J/m^2	0 J/m²
10 J/m²	9.6 J/m²
100 J/m²	96 J/m²
1000 J/m²	1000 J/m²
10000 J/m²	10000 J/m²

EVT part 2 run 1

Test parameter	Duration
Irradiation	50 d
Polychromatic 200-400 nm, SOL2000 @1271,2 Wm$^{-2}_{200\text{-}400nm}$	
dark	0
$1,4 \times 10^3$ kJ/m²	18 min
$1,4 \times 10^4$ kJ/m²	3 h
$1,4 \times 10^5$ kJ/m²	30 h
$4,5 \times 10^5$ kJ/m²	99 h
$6,8 \times 10^5$ kJ/m²	148 h

EVT part 2 run 2

Test parameter	Duration
Irradiation	28 d
Polychromatic 200-400 nm, SOL2000 @1271 Wm$^{-2}_{200\text{-}400nm}$	
dark	0
$5,5 \times 10^2$ kJ/m²	7min 12 sec
$5,5 \times 10^3$ kJ/m²	1 h 12 min
$1,4 \times 10^5$ kJ/m²	30 h
$2,7 \times 10^5$ kJ/m²	60 h
$5,5 \times 10^5$ kJ/m²	120 h

SVT run 1

	Test parameter	Duration
Space	Vacuum 10^{-5} Pa +	38 d
	UV irradiation, polychromatic 200-400 nm, Fluence tbd from EVT 2 or Mission calculation, max	SOL2000
	5.7×10^5 kJm^{-2}	125 h
Mars	Simulated CO_2 Mars atmosphere 10^3 Pa +	38 d
	UV irradiation, polychromatic 200-400 nm, Fluence tbd from EVT 2 or Mission calculation, max	SOL2000
	5.7×10^5 kJm^{-2}	125 h*
	Control experiment, 1 atm air, dark, room temperature	38 d

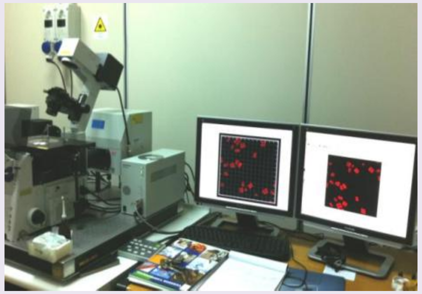
SVT run 2

	Duration
Vacuum 10^{-5} Pa +	28 d
UV irradiation, polychromatic 200-400 nm, Fluence tbd from EVT 2 or Mission calculation, max	SOL2000
$5,5 \times 10^5$ kJm^{-2}	120 h
Simulated CO_2 Mars atmosphere 10^3 Pa +	28 d
UV irradiation, polychromatic 200-400 nm, Fluence tbd from EVT 2 or Mission calculation, max	SOL2000
$5,5 \times 10^5$ kJm^{-2}	120 h
Control experiment, 1 atm air, dark, room temperature	28 d

BOSS- Experimental approach

Confocal Laser Scanning Microscopy

1. biofilm 3-D organization and damage to top layers cells
2. photosynthetic pigment autofluorescence
3.. membrane integrity (Sytox-Green stianing)
4. Composition of EPS (molecular probes)

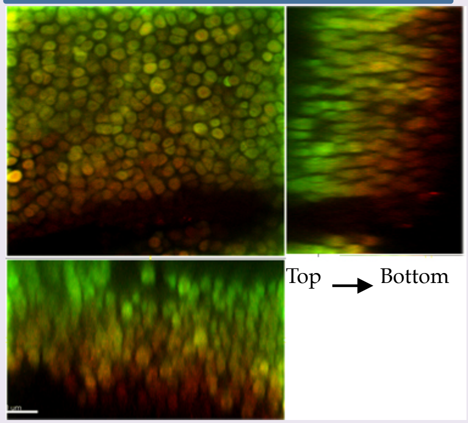


Qualitative PCR and Real-time qPCR
(PCR-stop assays)

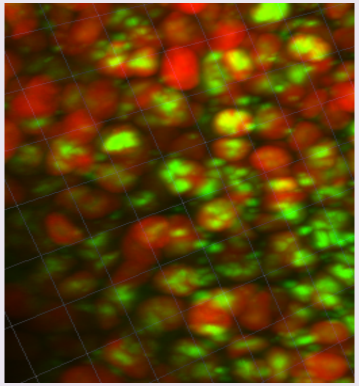
1-DNA integrity :

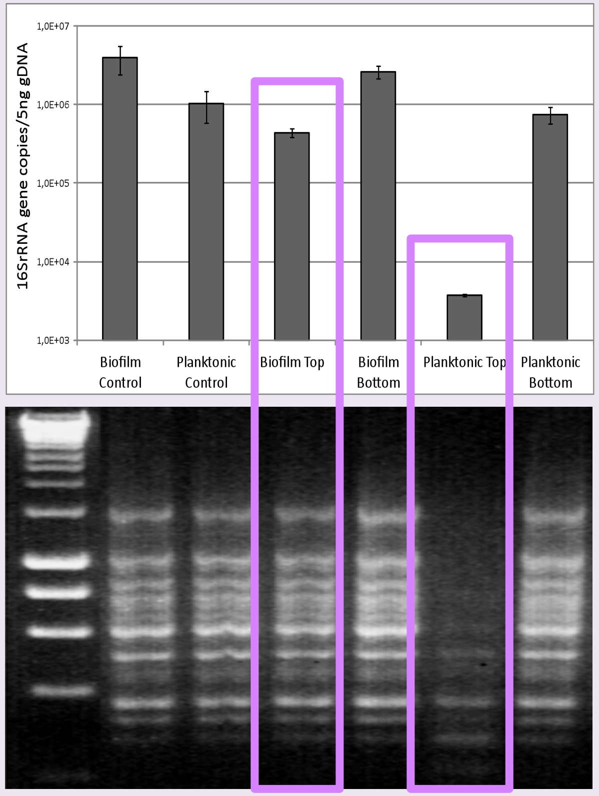
- Qualitative genome-PCR fingerprint
- Quantitave real-time qPCR

Biofilm exposed to 10 kJ/m² UVC

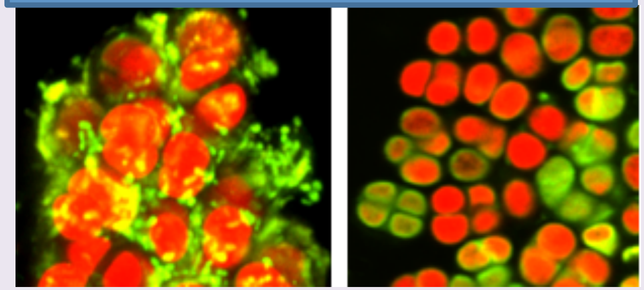


Biofilm top layer cells with damaged dots-like nucleoids

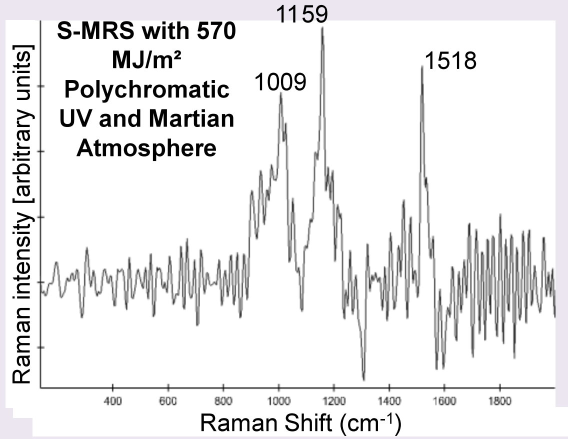




Biofilm EPS staining **Planktonic**



BIOMEX- Experimental approach



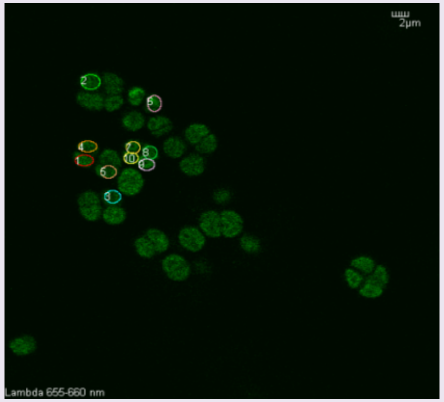
Confocal Laser Scanning Microscopy
and Qualitative PCR

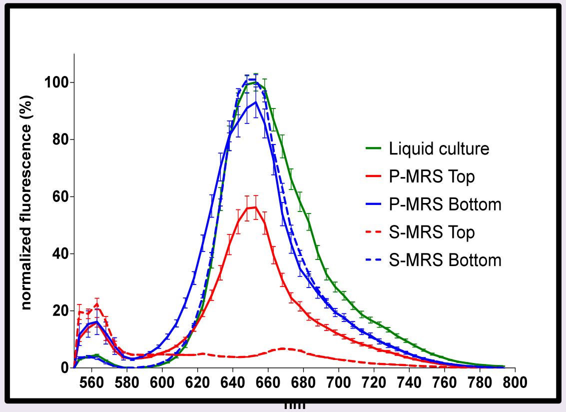
1. photosynthetic pigment autofluorescence (Lambda scan with Confocal Laser Scanning Microscopy)
- beta catorotene (Raman Spectroscopy)
2. DNA damage (PCR-based assay)
3. Survival (CFA)

Focusing on the protection provided by Mars and lunar regoliths

F

**S-MRS with 570 MJ/m²
Polychromatic UV and
Martian Atmosphere
~2 % coverage**

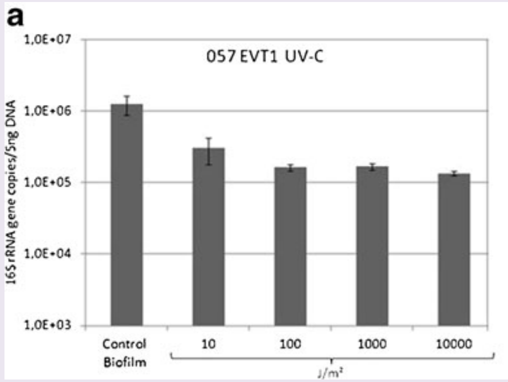


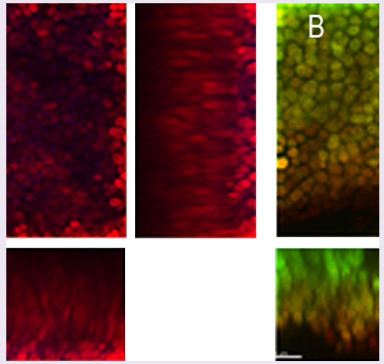


UVC RADIATION: dried biofilms are more resistant than dried layers of planktonic cells (comparable thinkness) and survive at least 10 kGy

Tested doses: 10, 100, 1.000. 10.000 J/m^2

- In biofilm top layers cells are damaged and protect bottom layers
- Biofilm survival is enhanced comapared to dried layers of plankyonic cells due to the presence of esopolysccahrides (EPS)





Baqué et al. (2013) Orig Life Evol Biosph 3:377-89.

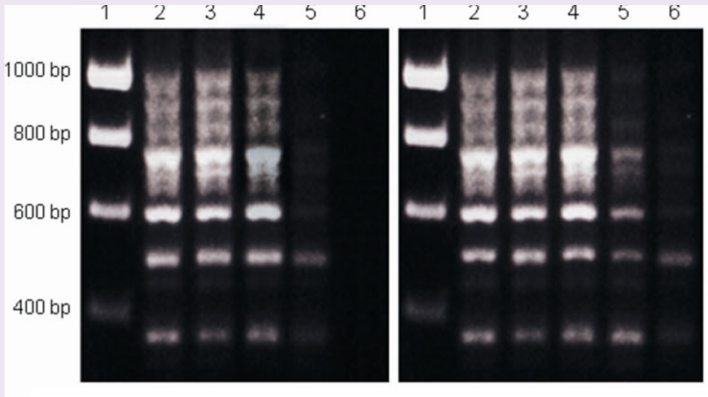
- In dried layers of planktonic cells DNA damage is limted by the presence of minerals (either lunar or Mars simulants)

No minerals

With minerals

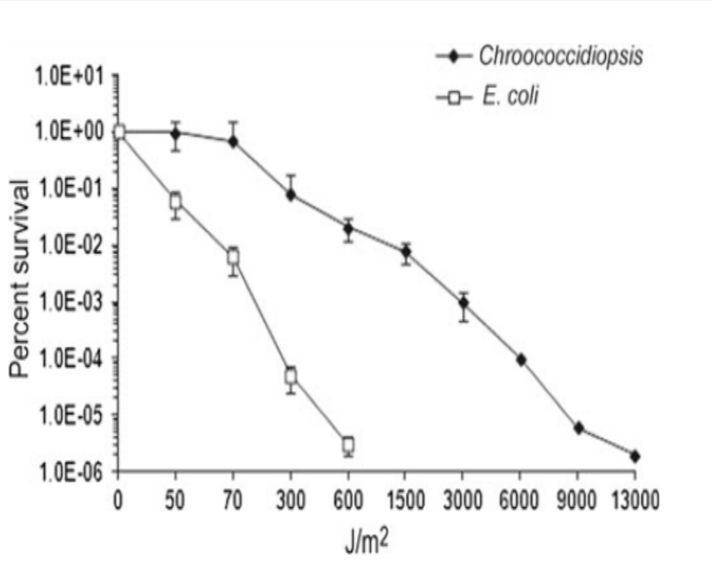
C

C



Baqué et al. (2014) Orig Life Evol Biosph 44:209–22.

UVC RADIATION: hydrated *Chroococcidiopsis* cells survive 13 KJ/m^2 of UVC radiation

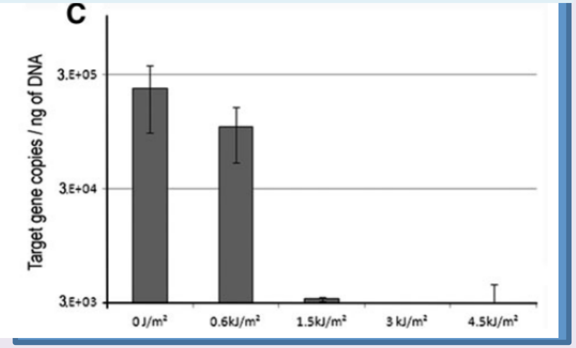


Chroococcidiopsis sp. CCMEE 029 D_{10} 300 J/m^2

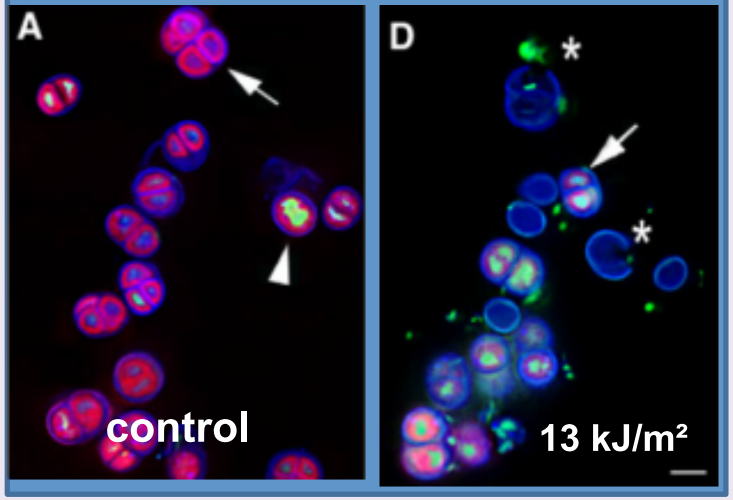
B. subtilis spores D_{10} of 303 J/m^2

D. radiodurans D_{10} of 660 J/m^2;

RT-qPCR of target gene shows extensive DNA damages



Membrane damages showed by DAPI/Sytox Green staining



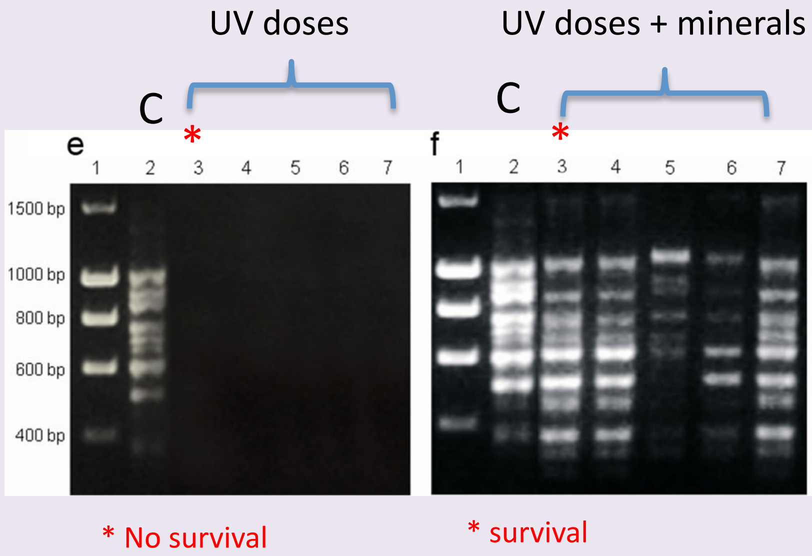
Baqué et al. (2013) Extremophiles 17:161-169

UV$_{200\text{-}400nm}$ **RADIATION** : enhanced survival of dried biofilms of *Chroococcidiopsis* CCMEE 029 compared to dried planktonic and monalyers

Tested doses

1.5×10^3 kJ/m^2 (dose reaching Mars' surface at the equator in one day)
1.5×10^4 kJ/m^2
1.5×10^5 kJ/m^2
5.0×10^5 kJ/m^2
5.5×10^5 kJ/m^2 (12 months in LEO)

- **Dried planktonic layers** survive **1.5×10^3 kJ/m^2** only in the presence of minerlas.
-
- No survival at the doses higher than 1.5×10^3 kJ/m^2 of polychromatic UV radiation, in spite of the relatively preserved genomic DNA integrity.



Baqué et al. (2014) Orig Life Evol Biosph 44:209–22.

- **Dried biofilms** survive **1.5×10^3 kJ/m^2**, but not higher doses.

- Monolayer of strain CCMEE 029 tolerated **3×10^4 J/m^2** of simulated Mars flux (Cockell et al. 2005).

SPACE and MARTIAN simulations : biofilms survival

$UV_{200\text{-}400nm}$ radiation and vacuum

$UV_{200\text{-}400nm}$ radiation and Mars atmosphere

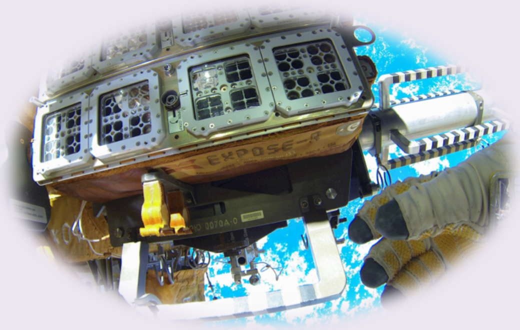
SVT run 2

		Duration
Space 0.1% ND	Vacuum 10^{-5} Pa +	28 d
	UV irradiation, polychromatic 200-400 nm, Fluence tbd from EVT 2 or Mission calculation, max	SOL2000
	5.5 x 10^2 kJ/m^2 5,5 x 10^5 kJm^{-2}	120 h
Mars O.1%ND	Simulated CO_2 Mars atmosphere 10^3 Pa +	28 d
	UV irradiation, polychromatic 200-400 nm, Fluence tbd from EVT 2 or Mission calculation, max	SOL2000
	5.5 x 10^2 kJ/m^2 5,5 x 10^5 kJm^{-2}	120 h
	Control experiment, 1 atm air, dark, room temperature	28 d

- Dried biofilms (10 cell layers) survive polychromatic UV $_{200\text{-}400}$ nm (5×10^5 kJ/m2) only with the 0.1 % ND filter (5×10^2 kJ/m^2) combined with space vacuum

- Dried planktonic cells mixed with lunar mineral do not survive 5×10^2 kJ/m^2 but survived space vacuum (dark control of the space misison)

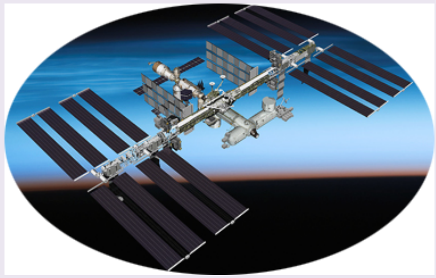
- Dried planktonic cells with Mars minerals do not survive 5×10^5 kJ/m^2 polychromatic UV irradiation (with or without ND filter), but survive Martian atmosphere (dark control of the space misison)



SURVIVAL RECORDS OF CHROOCOCCIDIOPSIS (CCMEE 029 and CCMEE 057)

- It survives at least 4 years of desiccation
- Hydrated cells survive 15 kGy X-rays
- Dried cells survive 24 kGy gamma-rays (Mars surface rays 50-150 mGy/year)
- Dried cells survive heavy ions (highest dose tested 2 kGy- Fe)

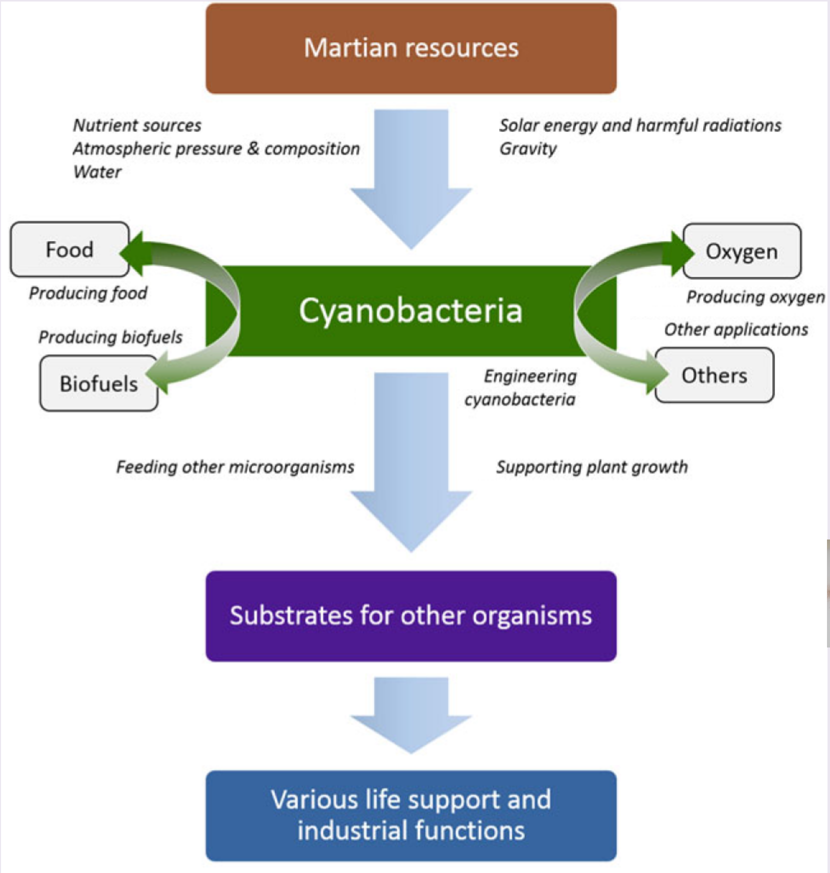
- Hydrated cells withstand up to 13 kJ/m² of UVC
- Dried biofilms survive 10 kJ/m² of UVC (highest dose tested)
- Dried cells mixed with minerals survive 1.5×10^3 kJ/m^2 full UV (dose reaching Mars' surface at the equator in one day)
- Dried biofilms cells survive 5×10^2 kJ/m^2 UV200-400 nm (dose for 12 months in space with 0.1 ND filter)

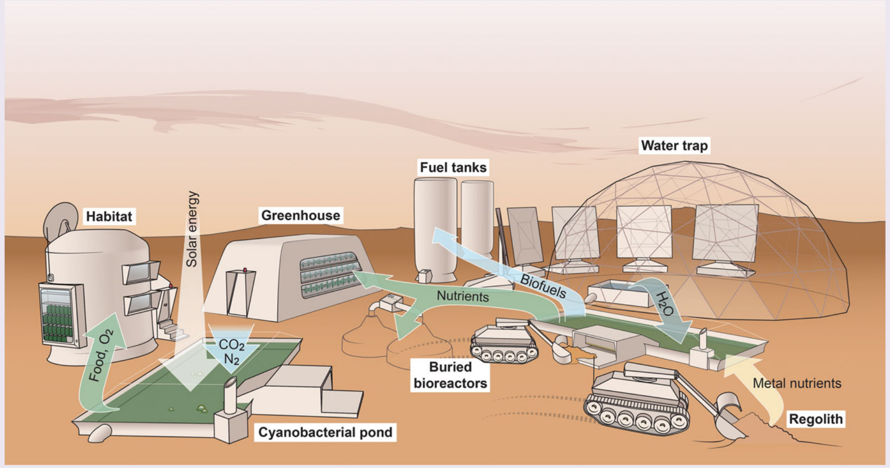


In space:

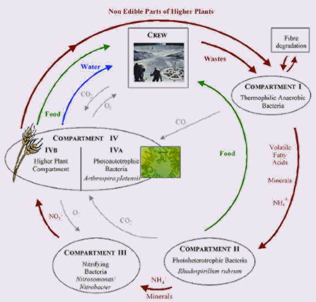
- EXPSOE-E: *Chroococcidiopsis* sp. 029 survived after 548 days in space as part of microbial epilithic community (5.15×10^5 kJ/m^2 UV dose).
- EXPOSE-R (2009-2011): *Chroococcidiopsis* sp. 029 survived when inoculated into impact-shocked gneiss.

Sustainable life support on Mars – the potential roles of cyanobacteria

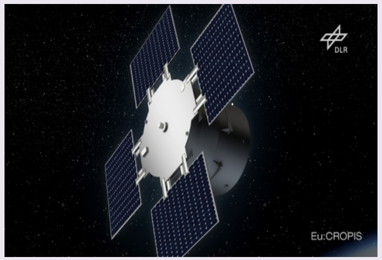


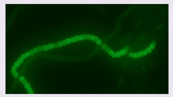


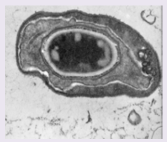
Verseux et al. (2015) Int. J. Astrobiol.



Eu:CROPIS – Growing tomatoes in space



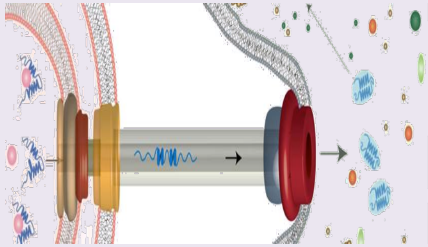




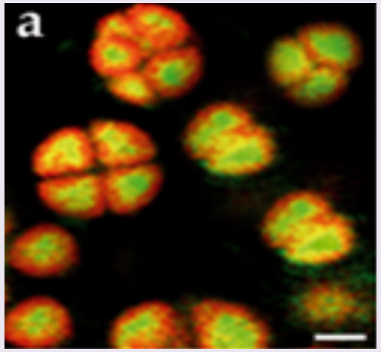
Germination of *B. subtilis* spore with sucrose secreted by *Anabaena* PCC 7120

BLSS- Take advantage of the transformability of desert strains of *Chroocococcidiopsis* and of the availability of plasmids replicating in *Chroocococcidiopsis*

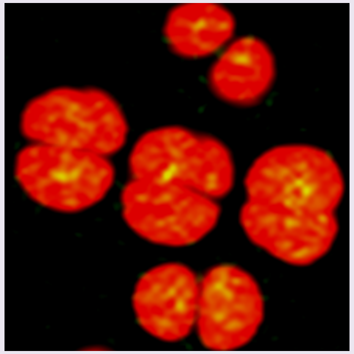
Protein (GFP) secretion

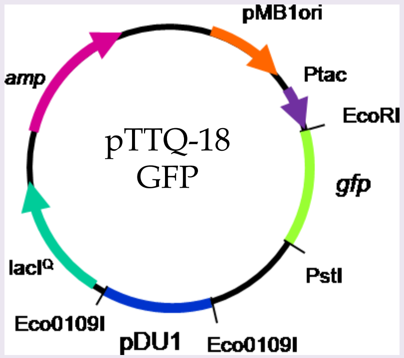


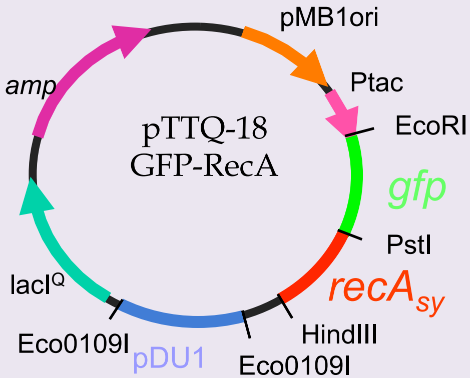
GFP expression

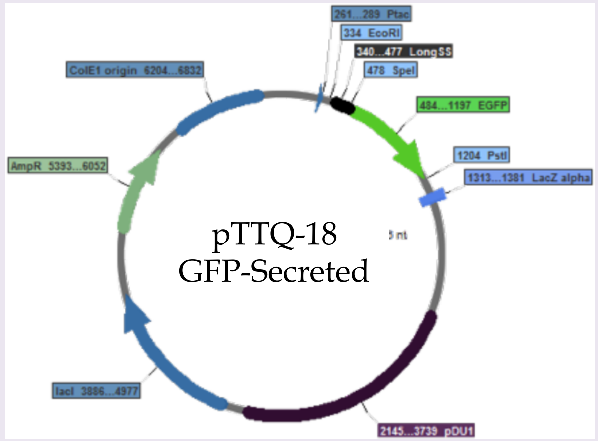


GFP-tagging



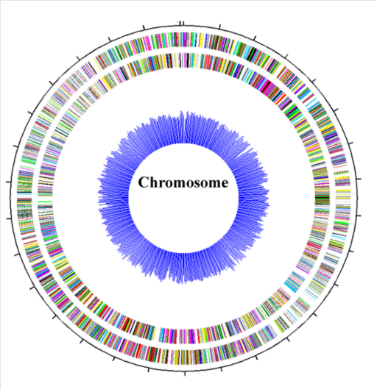


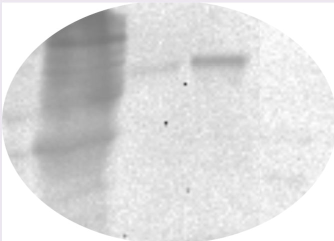




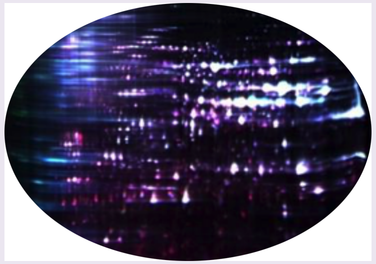
Verseux et al. in preparation
Billi (2012) Orig Life Evol Biosph 42: 235-245

"OMICS" APPROACH TO DECIPHER CHROOCOCCIDIOPSIS RESISTANCE TO EXTREME CONDITION

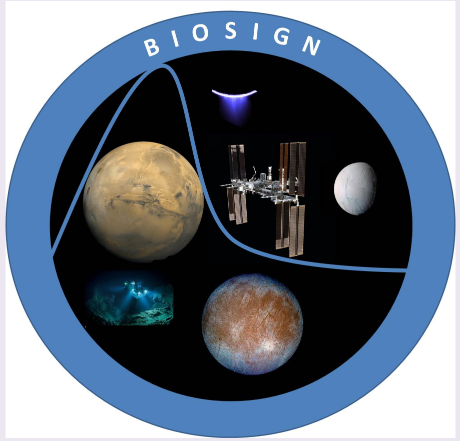




Oxidative damage to proteins



Differential Gel Electrophoresis



ILSRA 2014

Next generation RNA sequencing

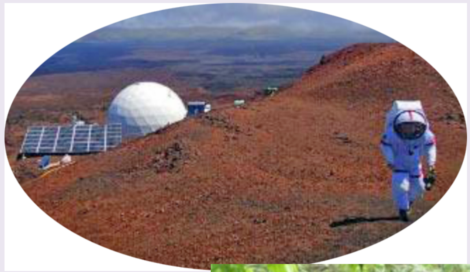
Laboratoty-dried samples

ENEA-Casaccia, Italy
Gamma rays
(5 kGy)

THANK YOU FOR THE ATTENTION ….and..thanks to theTor Vergata Astrobiology team

CENTRO DE ASTROBIOLOGÍA
Asociado al NASA Astrobiology Institute

Cyprien Verseux



Micakel Baqué

DLR

Andrea Ianneo

Samples ready for the EXPOSE-R2, May 2014

Riccardo Cifariello

Claudia Fagliarone

Extra Veicolar Activity, October 22, 2014

GRANTS:

ASI
MAE
PNRA