HighRad on BIOPAN 6, Foton M3

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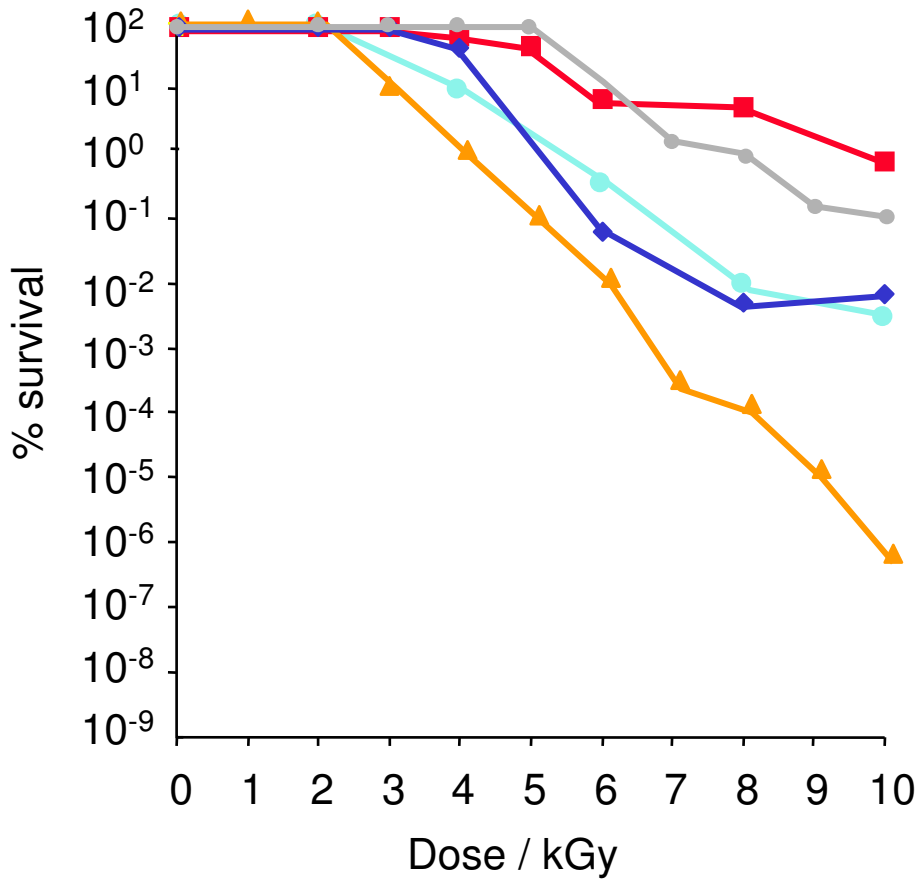
Questions addressed by HighRad

- Can highly radiation resistant non-spore forming microorganisms like the Archeon *Thermococcus gammatolerans* and the Bacterium *Deinococcus radiodurans* survive under space conditions concerning extreme desiccation by space vacuum and simulated Martian UV radiation characterized by biologically deleterious energy-rich short UV wavelengths not existing on present Earth?

- Are there toxic and/or phototoxic effects of Martian soil analogues on these organisms under space conditions?

- What is the spectrum of UV-induced DNA photoproducts after exposure to space conditions?

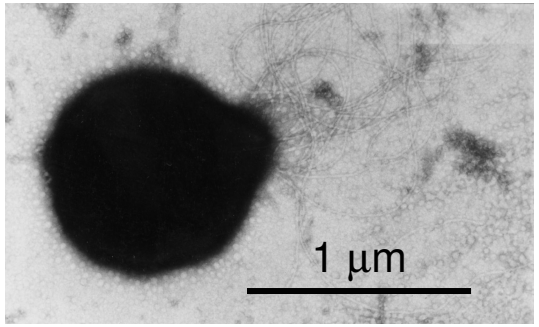
Resistance to ionizing radiation



Strain	D_{37} (kGy)
P. abyssi	2,70
Strain EJ1	3,40
Strain EJ2	4,30
Th. Gammatolerans	5,70
D. radiodurans	5,73 (0_2)

Part 1: *Thermococcus gammatolerans*

- Most radioresistant hyperthermophilic archaeon known to date
- Growth temperature: 55 °C – 95 °C (optimal at 88 °C)
- Optimal pH: 6
- Optimal NaCl concentration: 20 g/l
- G+C mol% = 54,5
- Strictly anaerobic
- Chemoorganotrophic
- Yeast extract, tryptone and peptone as carbon and energy sources for growth
- Elementar sulphur or cystein required for growth



Thermococcus gammatolerans

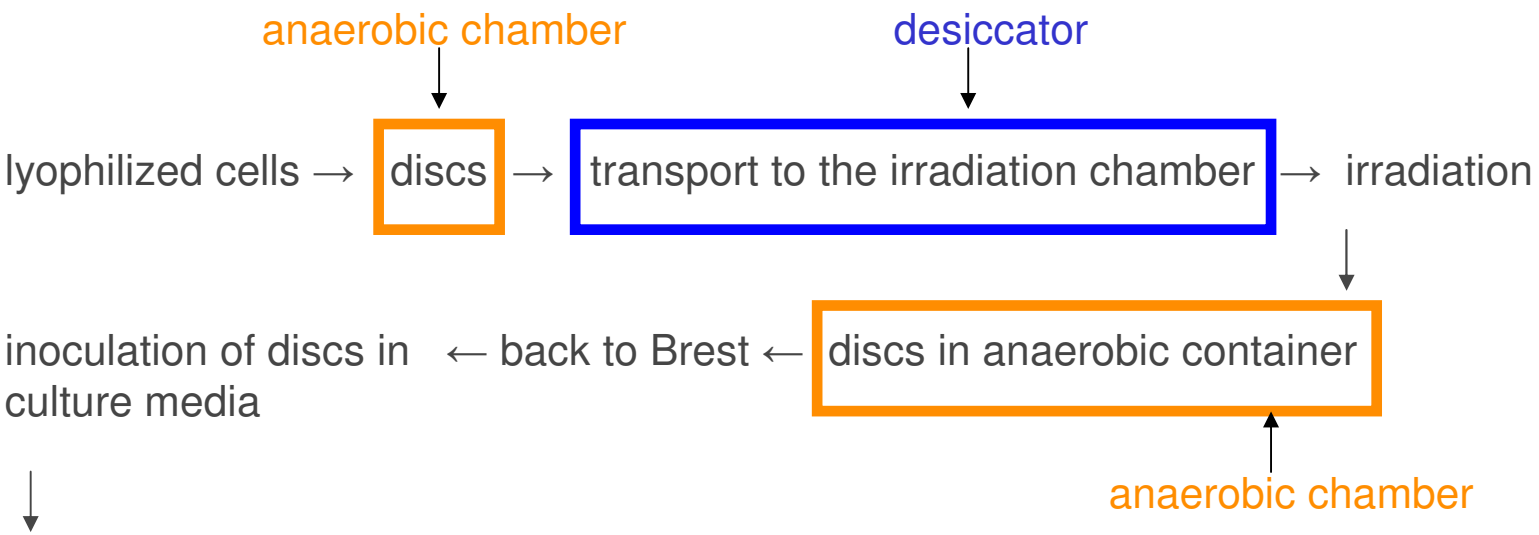
Pre-tests for the planned flight experiment

- Desiccation test: no survival

- Optimal conditions of lyophilisation:
  - mineral medium (growth medium without organic components)
  - Stationary growth phase
  - 10^{10} cells/vial
  - Survival: 40% in average

- Oxygen sensitivity: only 50% survival after 1 day of exposure

UV exposure experiments at DLR



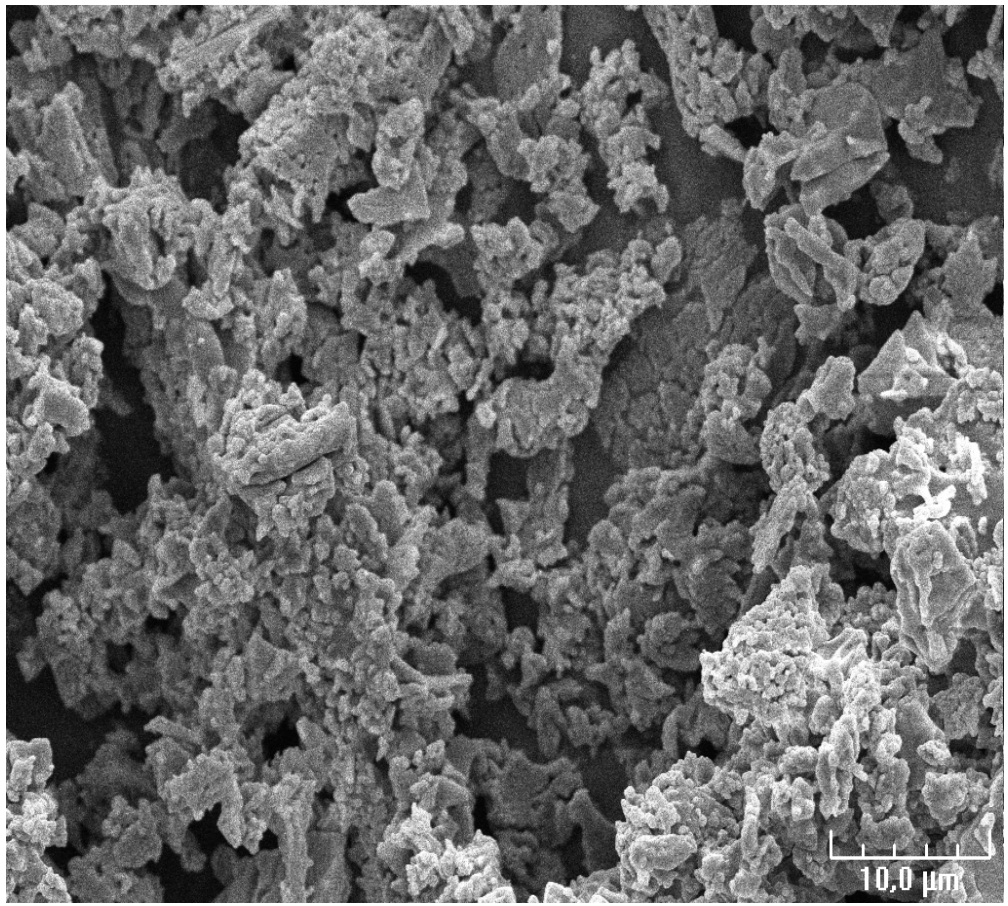
2 independent experiments with 5 replicates each

Results

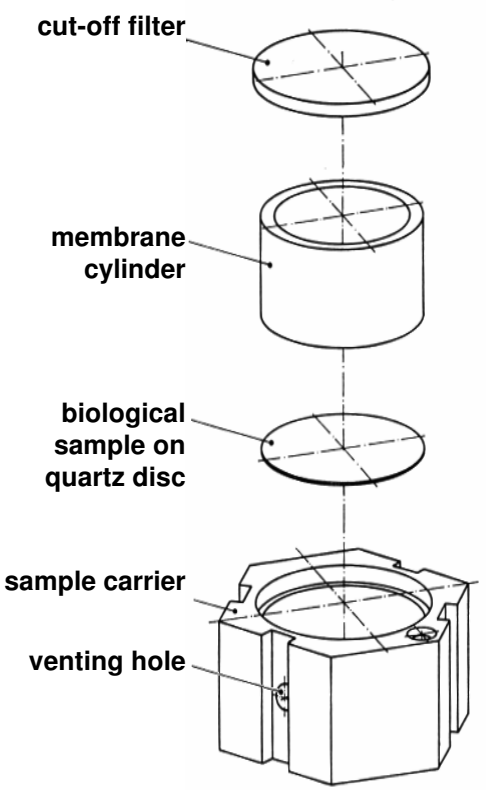
fluences	growth (+) or no growth (-) for 5 replicates					
50 J/m²	+	+	+	+	+	Exp 1
	+	+	+	-	-	Exp 2
100 J/m²	+	+	+	+	+	Exp 1
	+	+	+	-	-	Exp 2
1000 J/m²	+	+	-	-	-	Exp 1
	+	+	-	-	-	Exp 2
5000 J/m²	+	+	+	-	-	Exp 1
	+	+	+	-	-	Exp 2

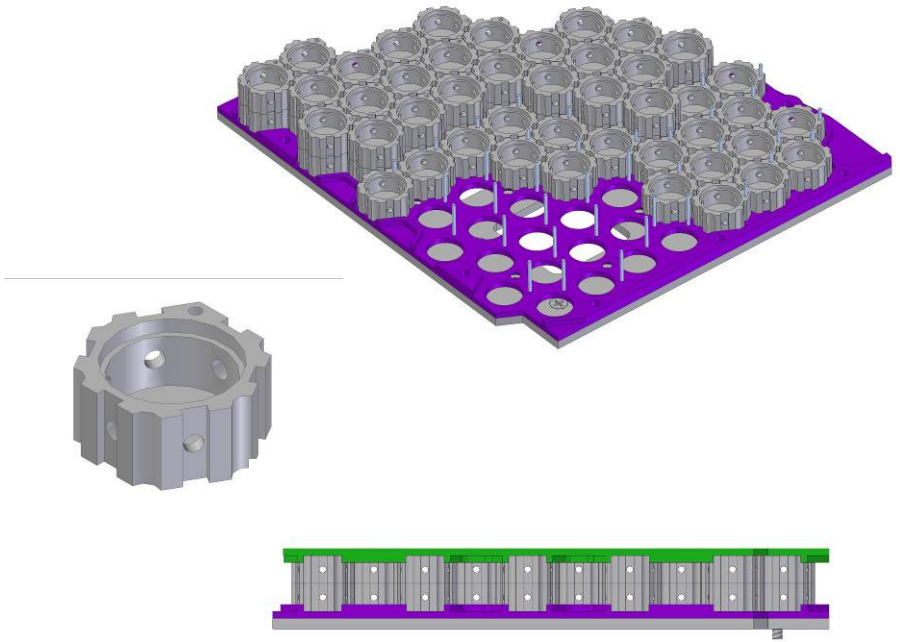
UVC radiation (254 nm)

SEM of lyophilized *Thermococcus gammatolerans*



The HighRad sample carrier for exposure to space conditions





Conclusions from the pre-tests with *Thermococcus gammatolerans*

- *Thermoccocus gammatolerans* resists to lyophilisation (cold temperature, vacuum), but it is then very sensitive to oxygen.

- The cell deposit procedure does not allow to estimate the cell density on the discs prior to UV exposure or to obtain a monolayer of cells.

- However, perhaps because of the protection given by multilayers of cells, lyophilized cells of *Thermococcus gammatolerans* seem to resist to UV doses up to 5000 J/m^2.

~ For the FOTON M-3 flight experiment only cells of *Deinococcus radiodurans* were used.

Part 2: *Deinococcus radiodurans*

gram positive

complex cell wall

pigmented

chemoorganotroph

generation time about 80 min

temperature optimum 30 °C

no spore former

non mobile

occurs mainly in tetrads and diplococci

genome sequenced

desiccation resistant

resistant against oxidative and cross-linking agents

- extremely radiation resistant (ionising and UV radiation)

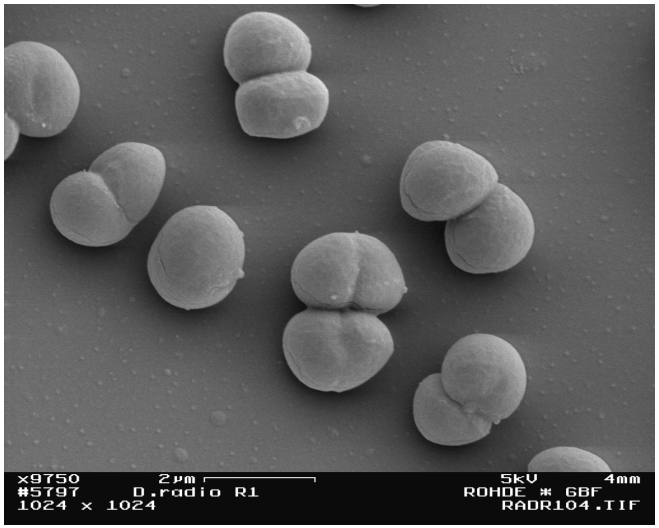


Foto: Rohde, GBF, D

Sample preparation

- immobilization of 1 x 10^7 *Deinococcus radiodurans* cfu's mixed with 0.3 mg of the Martian soil analogue MRS07

  MRS07:47.7 % Montmorillonite, 9.9 % Kaolinit, 21.3 % Hematite (+ SiO_2), 13.0 % Anhydrite, 7.1 % Magnesiumsulfate, 1.0 % Sodiumchloride, 58 wt% of the minerals grainsize < 2 µm, 42 wt% ~ 0.2 mm

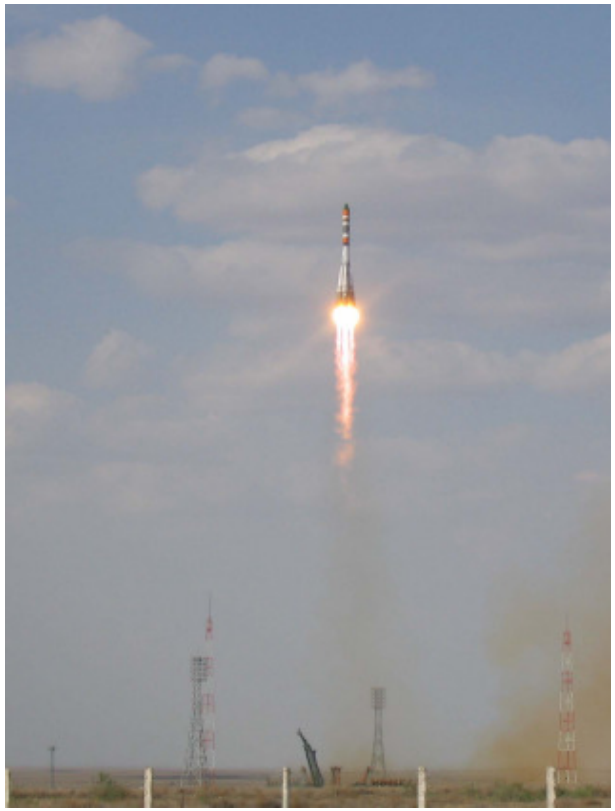
- 1 set of samples for
  - UV + vacuum exposure in space
  - vacuum exposure in space

- flight, ground, laboratory controls storage at ambient T and at 4 °C

HighRad Mission Biopan 6 on Foton M3

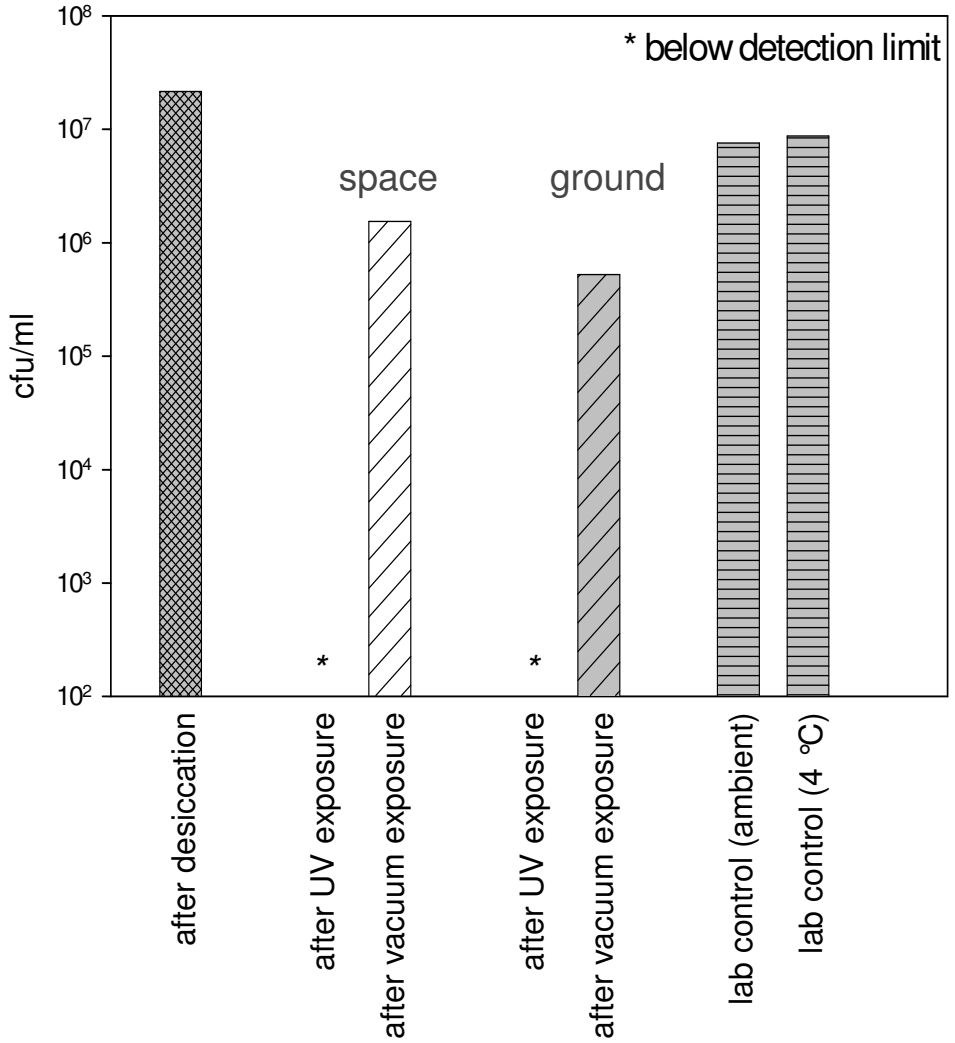
Launch 14.09.2007 in Baikonur

Landing 26.09.2007 in Kasachstan

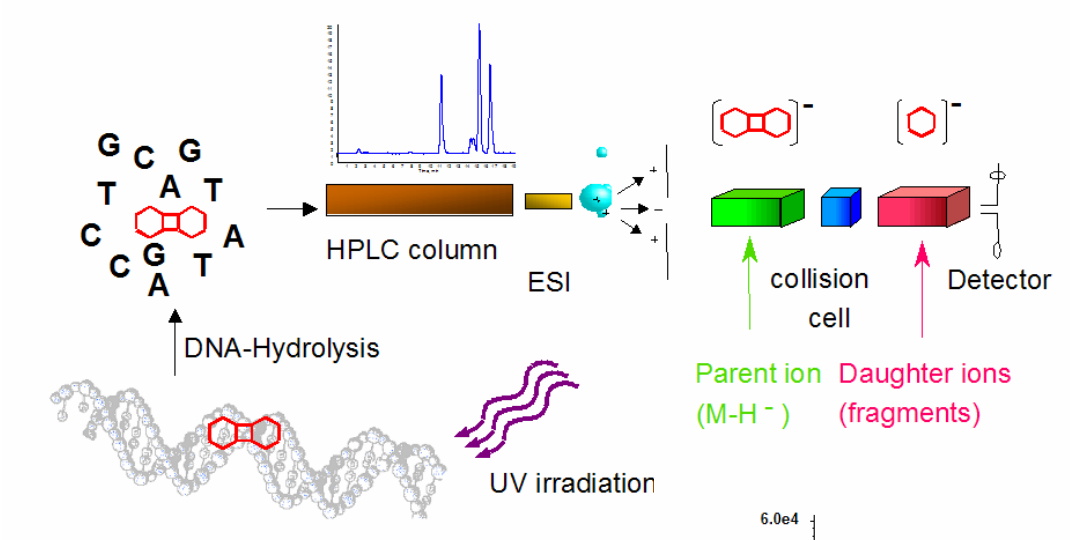


HighRad results Survival

- *Deinococcus radiodurans* cells did not survive the exposure to space vacuum and simulated Martian UV radiation (8.729 MJ/m^2 ,~ 22 SCh).
- *Deinococcus radiodurans* cells were inactivated by space vacuum by a factor of ~ 10.
- Comparable results were obtained in the ground simulation experiment.

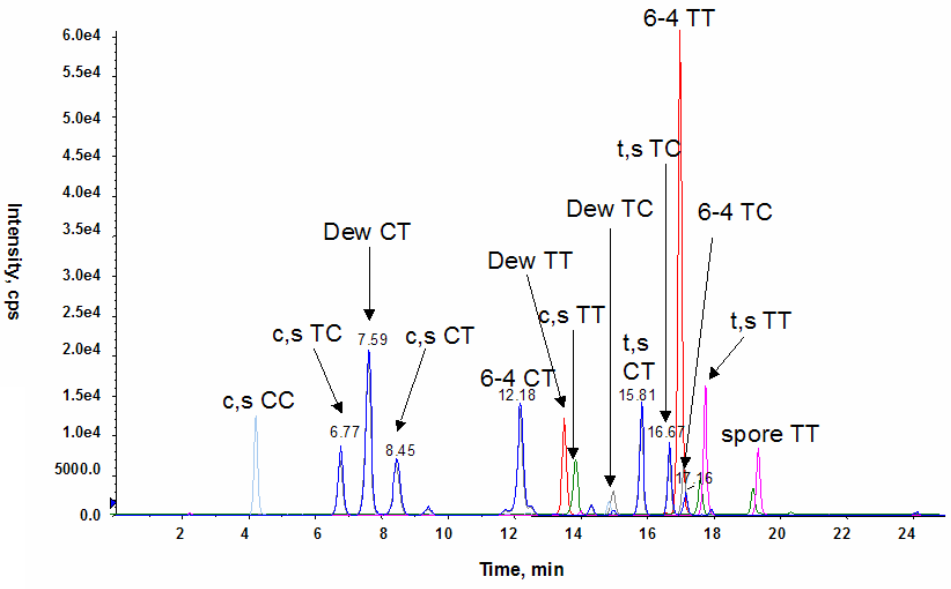


DNA analysis - photoproducts



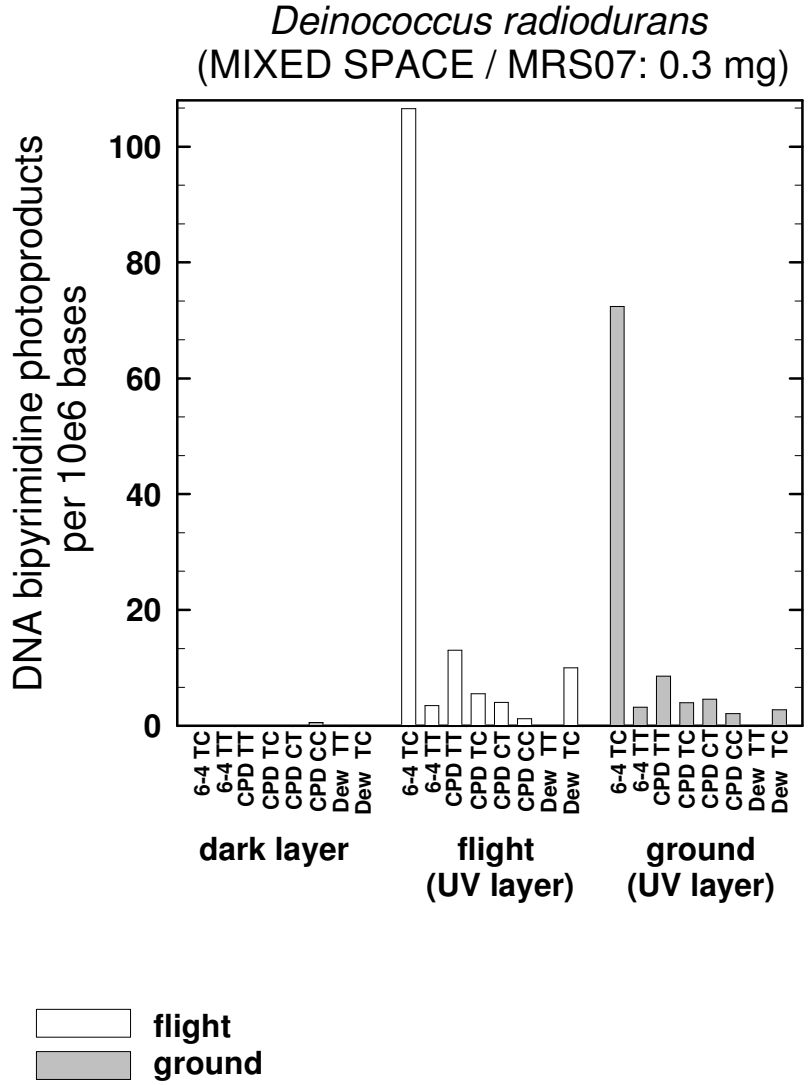
HPLC/MS+MS

Mass spectrogram of the standards of the different UV-induced pyrimidine dimers



HighRad results
DNA photoproducts

- Simulated Martian UV radiation induced the different types of pyrimidine dimers.
- The main photoproduct is 6-4 TC.
- Comparable results were obtained in the ground simulation experiment.



Thank you
for your attention!

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