



PROTECT: Investigations of the resistance of spacecraft isolates to outer space for planetary protection purposes.

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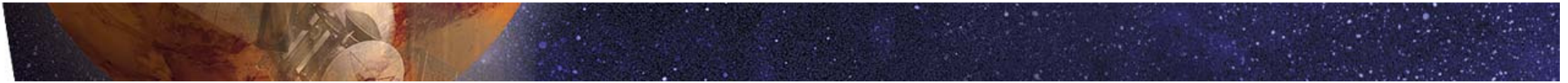
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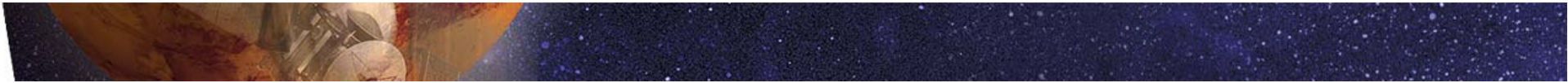
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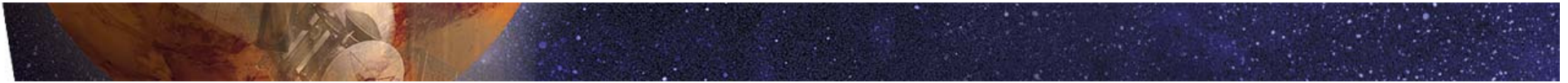
PROTECT: Investigations of the resistance of spacecraft isolates to outer space for planetary protection purposes.

Untersuchungen der Widerstandsfähigkeit von Mikroorganismen, isoliert von Raumfahrzeugen bzw. aus Raumfahrzeug-Montage-Anlagen (*spacecraft assembly facilities*) gegenüber extraterrestrischen Bedingungen



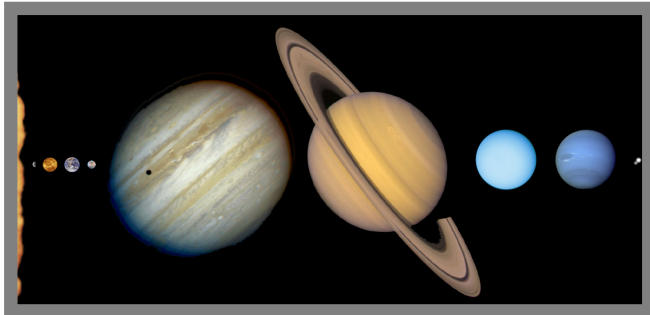
Overview

- background
- hypothesis
- questions
- methods
- preliminary data/results and short summary
- status of PROTECT
- open questions



Background

Planetary missions:

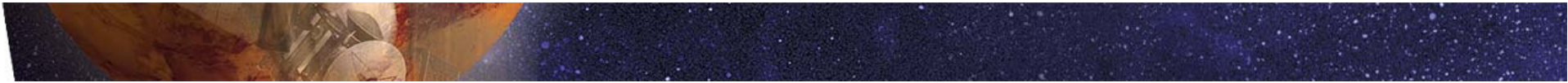


- detection of signatures of life and / or return of planetary samples to Earth

Planetary protection:



- cleaning (and sterilization) of spacecraft and components to avoid contamination with terrestrial (micro)-organisms (forward PP)
- control and monitoring of the bioburden to evaluate the success of cleaning
- determination of the microbial diversity of cultivable organisms from the spacecraft



Hypothesis

Fact:

These environmental isolates shown an elevated resistance to various physical and chemical treatments – compared to their related laboratory strains!

Hypothesis:

The conditions of the ultraclean spacecraft assembly facilities lead to a selection of the most resistant (micro-)organisms.

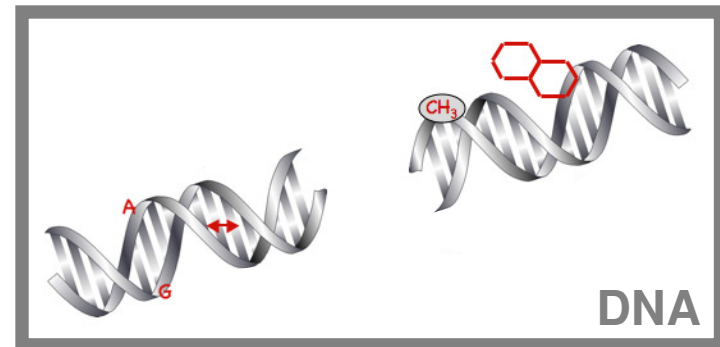
Such microorganisms survive sterilization procedures and withstand the hostile conditions of interstellar space and planetary surface (=> contamination).

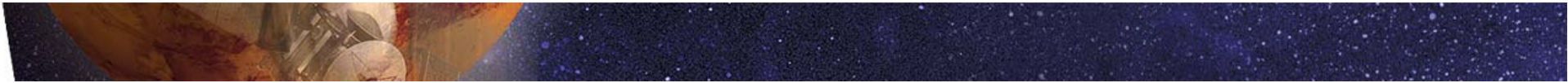
Aim of PROTECT

in-situ investigation of the resistance of these extremely resistant „spacecraft survivors“

Determination of the spore resistance to extraterrestrial environment:

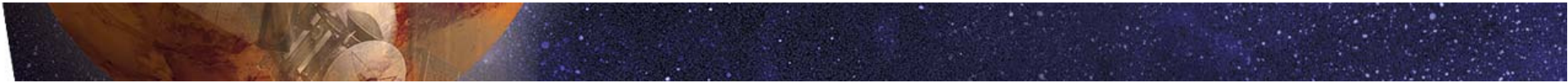
- i) degree of resistance,
- ii) types of induced cellular damage,
- iii) mechanism(s) by which spores can withstand or repair damages.





Questions

- i) How is the survival comparable of spores, isolated from spacecrafts, and the laboratory relatives after exposure to space conditions?
- ii) What are the nature and importance of the spore DNA damage caused by space conditions?
- iii) What are the rates of the damage accumulation and how do they correspond to spore inactivation rates?
- iv) What are the recovery capabilities of spores after exposure to sublethal dose of damage?
- v) What specific repair systems are activated during spore germination?



State of knowledge

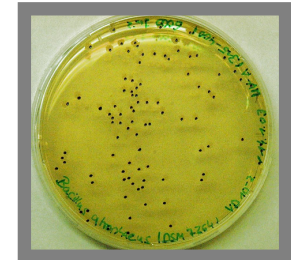
previous spaceflight experiments and ground-based studies with spores of *Bacillus subtilis* (as bacterial model system)

1972	MEED on Apollo 16 (UV, space vacuum, HZE particles)
1983	ES029 on Spacelab 1 (space vacuum, solar UV radiation)
1984-90	EXOSTACK on LDEF (6 years in LEO)
1992-93	ERA on EURECA (space vacuum, UV radiation)
1993	UV-RAD on Spacelab D2 (space vacuum, UV radiation)
1994,97,99	SURVIVAL on Biopan (space vacuum, UV radiation)
2002,05,07	MARSTOX on Biopan (space vacuum, UV radiation)
2008	SPORES on EXPOSE-R (to be flown)

Molecular biological analysis techniques

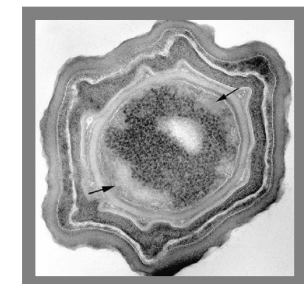
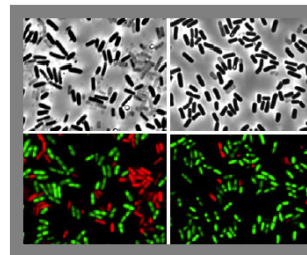
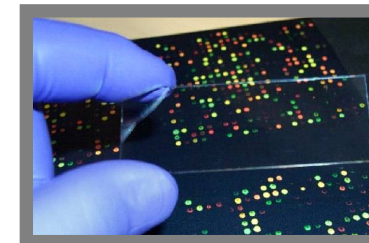
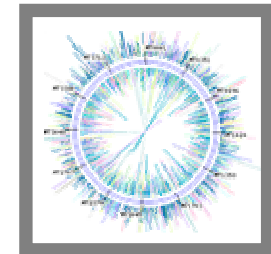
classical microbiology methods

- colony formation ability (sensitivity)



in the last ~ 15 years **progress in the molecular biology analysis techniques**

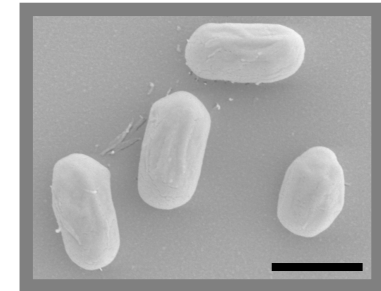
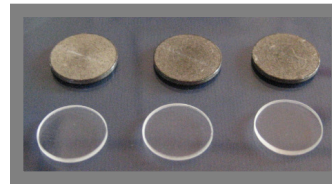
- PCR polymerase chain reaction
- DNA / gene manipulation and whole genome sequencing
- DNA microarray technology
- chromatography (HPLC-MS/MS)
- spectroscopy (Raman, FT-IR)
- fluorescence microscopy



Biological test samples

Preparatory work:

- i) standardized identification of biological strains,
- ii) definition of sterilization procedures,
- iii) biocompatibility of spacecraft-qualified material,
- iv) experiments under simulated space/planetary conditions.

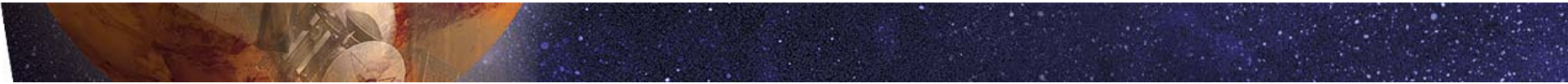


Spores of *Bacillus* sp.:

B. pumilis (SAFR-032, spacecraft isolate)

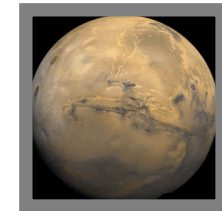
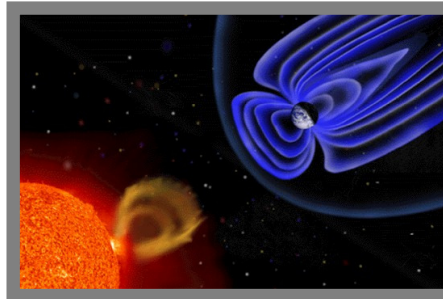
B. atrophaeus (DIN bioindicator, sterilization control)

B. subtilis (reference microorganism)*



Experimental tests: response of spores to simulated space and planetary conditions

- i) UV radiation,
- ii) space vacuum,
- iii) ionizing radiation,
- iv) temperature variations,
- v) planetary atmosphere.



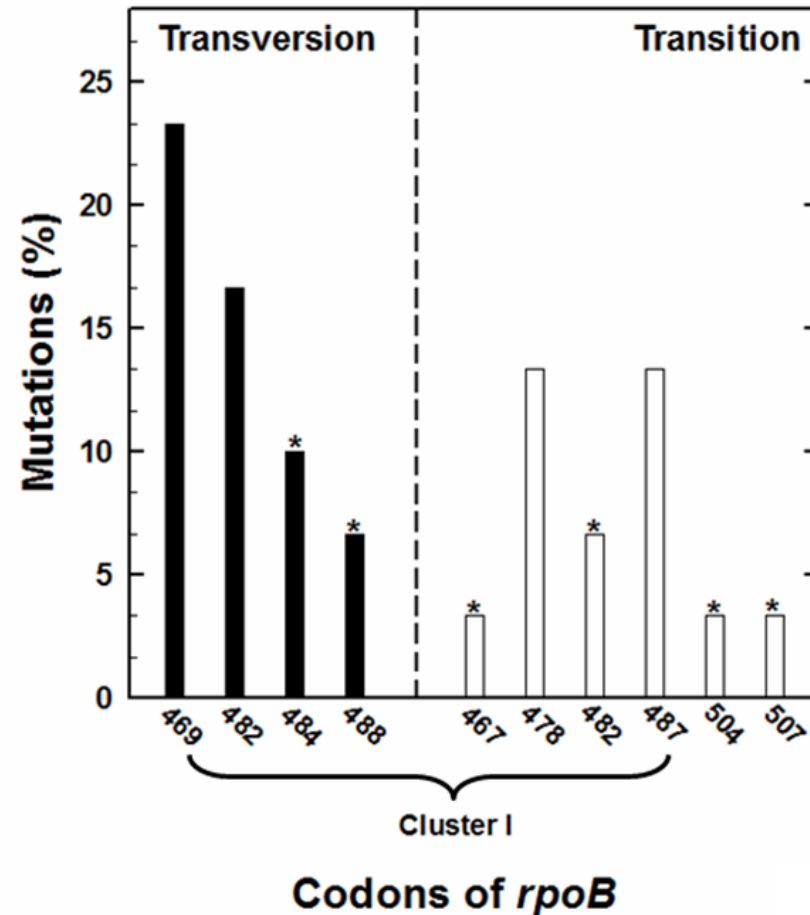
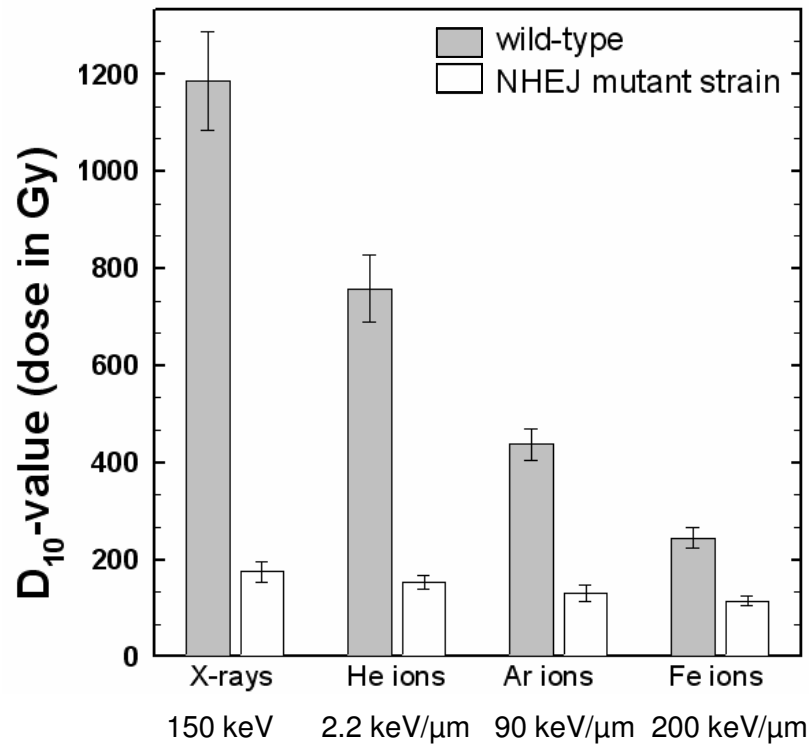
Experiment analysis

- i) spore viability,
- ii) mutagenic spectrum,
- iii) determination of the induced DNA lesions,
- iv) global gene expression (transcriptional profiling),
- v) determination of the mechanism(s) involved in the spore resistance.

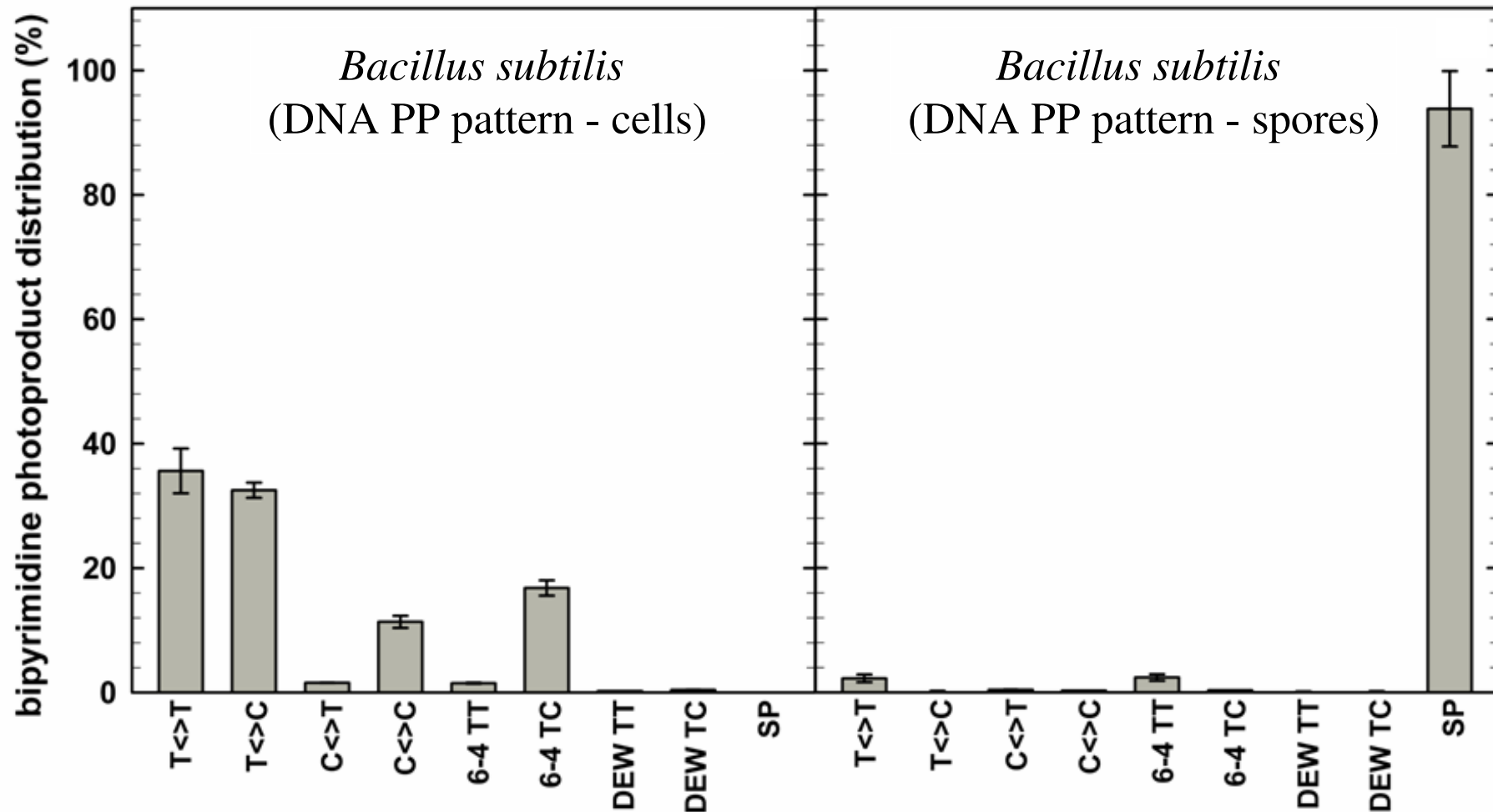


Response of *Bacillus subtilis* spores to space parameter

ionizing radiation (X-rays and selected heavy ions)



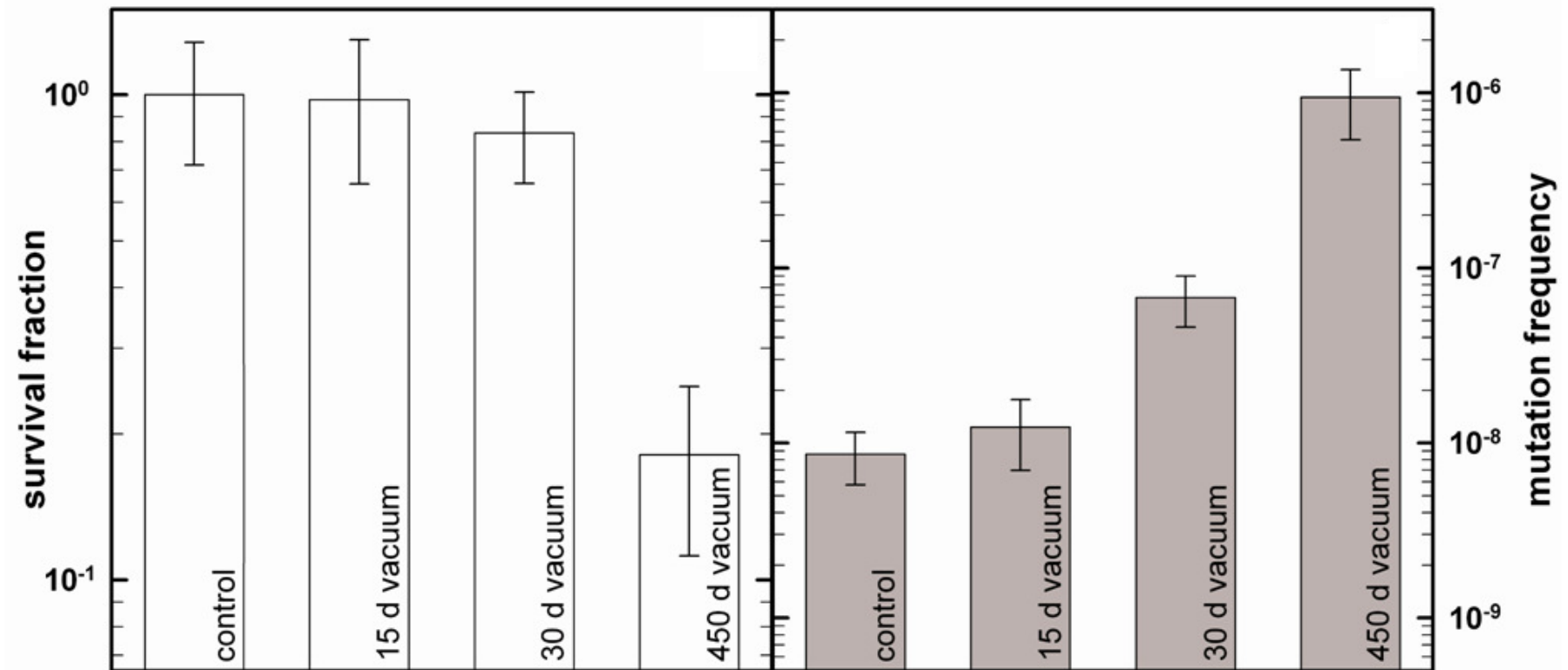
Response of *Bacillus subtilis* spores to space parameter



results published in *Int. Microbiol.* (2007)

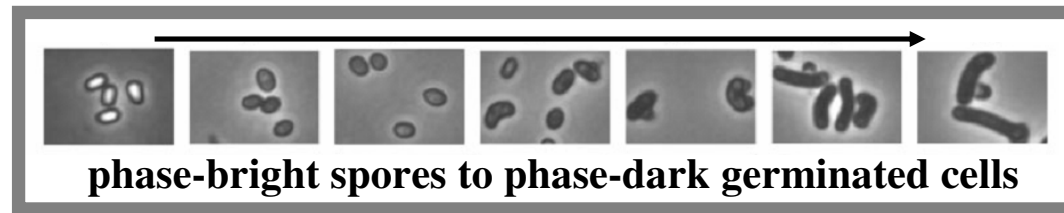
Response of *Bacillus subtilis* spores to space parameter

space vacuum ($> 10^{-7}$ Pa)

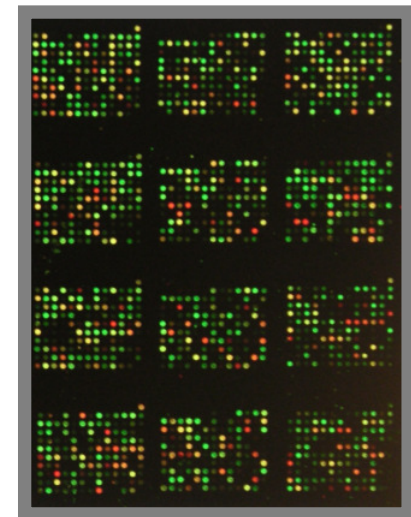


Response of *Bacillus subtilis* spores to space parameter

global gene expression during spore germination

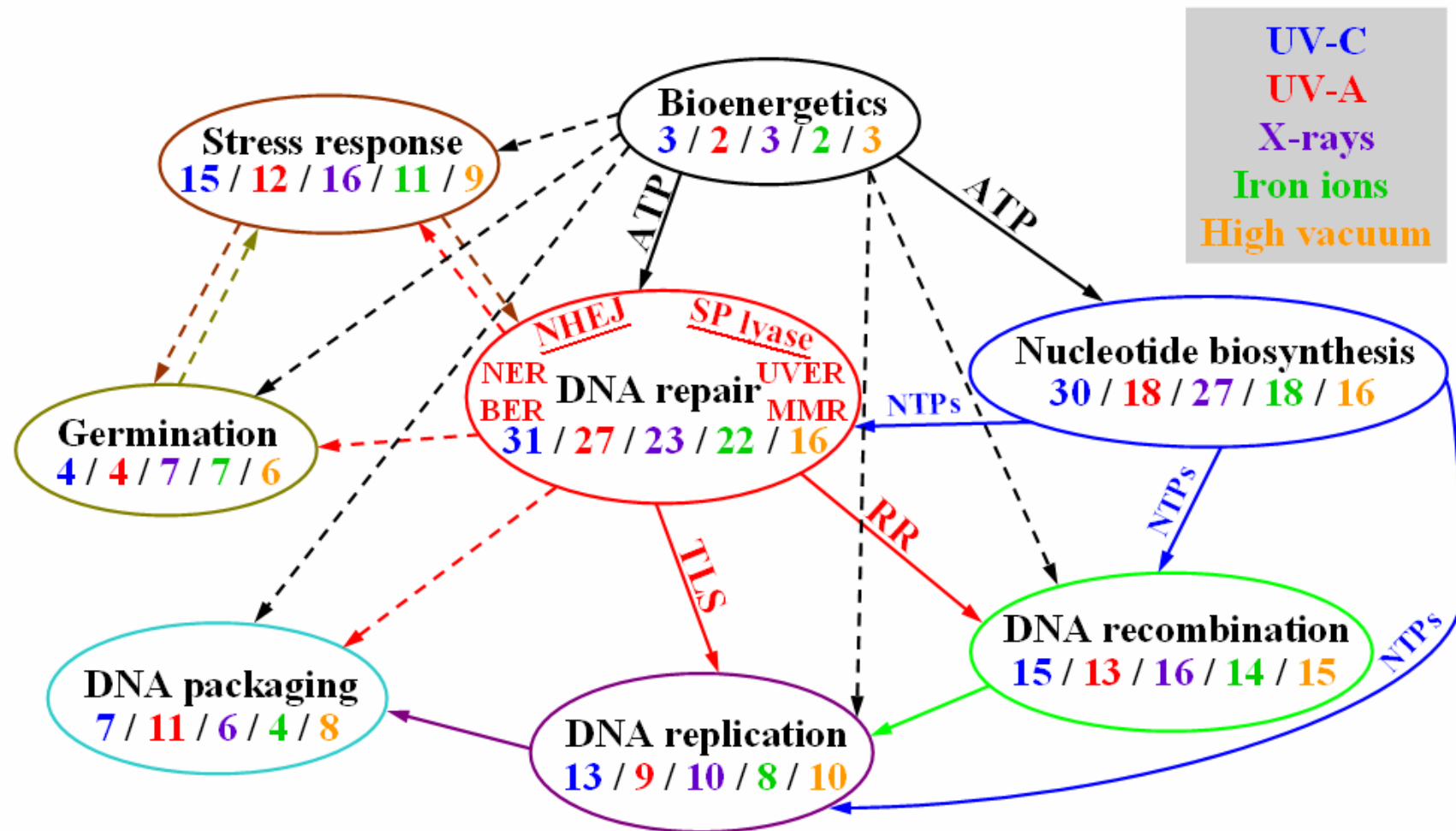


- 1) spores exposed to single space condition
- 2) return to active microbial life (germination)
- 3) analysis of the transcriptional response (mRNA)
- 4) bioinformatic interpretation of the gathered data



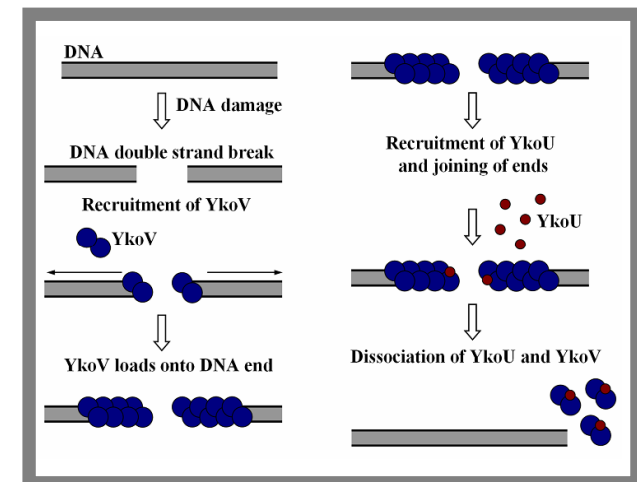
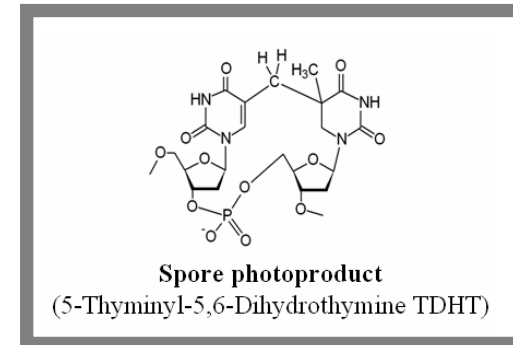
Response of *Bacillus subtilis* spores to space parameter

bioinformatics: functional affiliation of the up-regulated genes



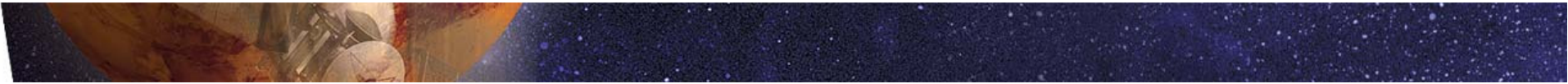
Summary of the preliminary data

- reduction values for spore inactivation
- UV radiation caused DNA lesions
- functional characterization of non homologous end joining as the spore's DNA double strand break repair mechanisms
- general “built in” transcriptional program to ensure genome restoration during spore germination



Environmental requirements of PROTECT and accommodation in 3 pockets of the EXPOSE facility

Pocket	1 (space vacuum)	2 (Mars climate)	3 (1 atm control)
Pocket type	vented	closed	closed
Atmosphere	No	Mars atmosphere	Inert gas (Ar)
Pressure	Open to space	6 mbar	1 bar
UV radiation	full spectrum	Mars UV-climate	a) full spectrum b) Mars UV-climate
UV range	> 110 nm	> 200 nm	a) > 110 nm b) > 200 nm
Galactic cosmic radiation	total flux	total flux	total flux
Temperature	Not regulated	-60°C to 5°C	tbd.
Exposure time	100-365 d	100-365 d	100-365 d



First data of the EST of PROTECT

Experimental setup:

Spores of *Bacillus subtilis* 168

1) in simulated space vacuum and

2) under CO₂ atmosphere (Mars)

irradiation: 200-400 nm UV

	space vacuum	CO ₂ atmosphere
fluence (kJ/m ²)	survival	
1 500	3.5 %	7.4 %
150 000	0.00002 %	0.0003 %

Experiment status of PROTECT

EXPOSE-Forschen auf der Internationalen Raumstation (ISS) - Windows Internet Explorer

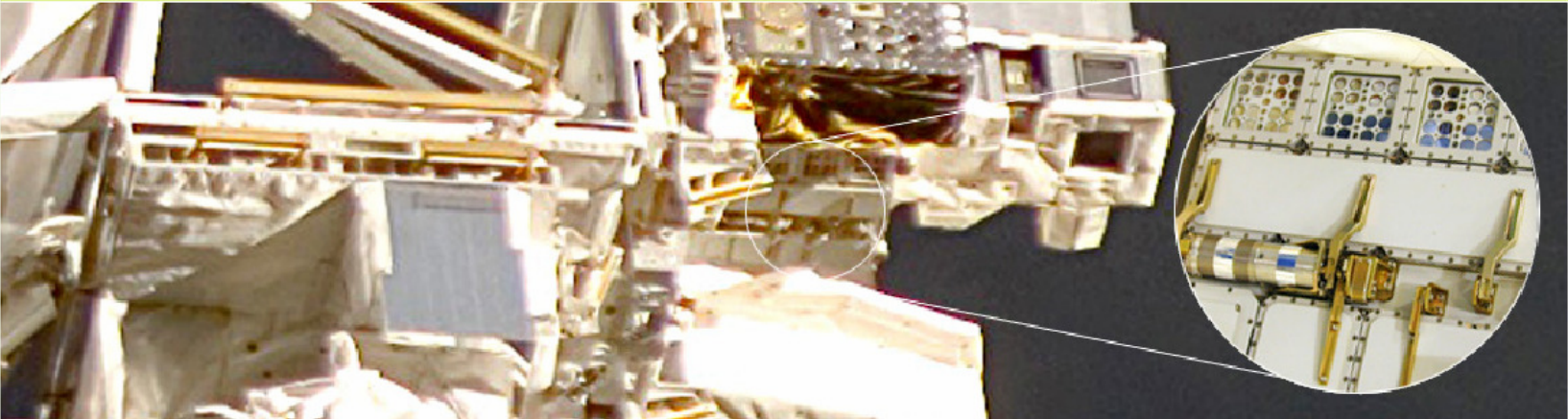
http://www.go.dlr.de/musc/expose/

DLR MUSC MICROGRAVITY USER SUPPORT CENTER

Astrobiologie auf EXPOSE

Forschen auf der Internationalen Raumstation (ISS)

VIRTUELLER KONTROLLRAUM INTERNATIONALE RAUMSTATION (ISS) European Exposure Facility EXPOSE-E TELEMETRIEDATEN EXPOSE-E



INTERNATIONALE RAUMSTATION (ISS)

Das Labor im All

14 Nationen betreiben derzeit ein gemeinsames Großforschungslabor im Weltall, die Internationale Raumstation ISS. Es ist das größte wissenschaftlich-technische Projekt in der Geschichte der Menschheit. Es beweist, dass eine friedliche internationale Nutzung des Weltraums zum Vorteil aller Partner möglich und sinnvoll ist. Seit dem „Erstbezug“ am 2. November 2000 forschen Astronauten auf der ISS, darunter auch an exzellenten Experimenten aus Deutschland. ISS Partner sind die USA, Russland, zehn Mitgliedstaaten der ESA, Kanada und Japan. Mit dem in Deutschland gebauten Raumlabor COLUMBUS ist am 11. Februar 2008 Europa dauerhaft in die ISS eingezogen.

Internet | Geschützter Modus: Aktiv

120%

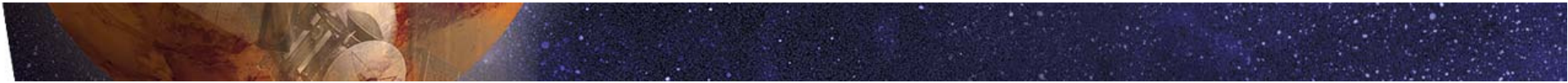
Significance of these results (PROTECT)

for planetary protection: - bioload measurements by adding modern molecular biology methods



- microbial survival / persistence after sterilization
- recommendations of new sterilization protocols (prevention of further selection of hyper-resistant cell-lines in spacecraft assembly facilities)

PROTECT: first time data on the responses of these most resistant microbial species to outer space conditions.



Outlook and open questions

What are the types of lesions caused by cosmic radiation and space vacuum (alone and in combination)?

What are the accumulation rates of DNA damage in spores exposed to space conditions?

What is the damage level for the initiation/inhibition of DNA repair during spore germination?

What are the regulatory mechanisms in the DNA restoration during germination?

Are there detection and removal mechanism(s) for induced genomic alterations?



Thank you very much for your attention!

Questions?
email to:
ralf.moeller@dlr.de

