Molecular adaptation strategies of microorganisms to different space and planetary UV climate conditions

Short titel: ADAPT

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ADAPT team

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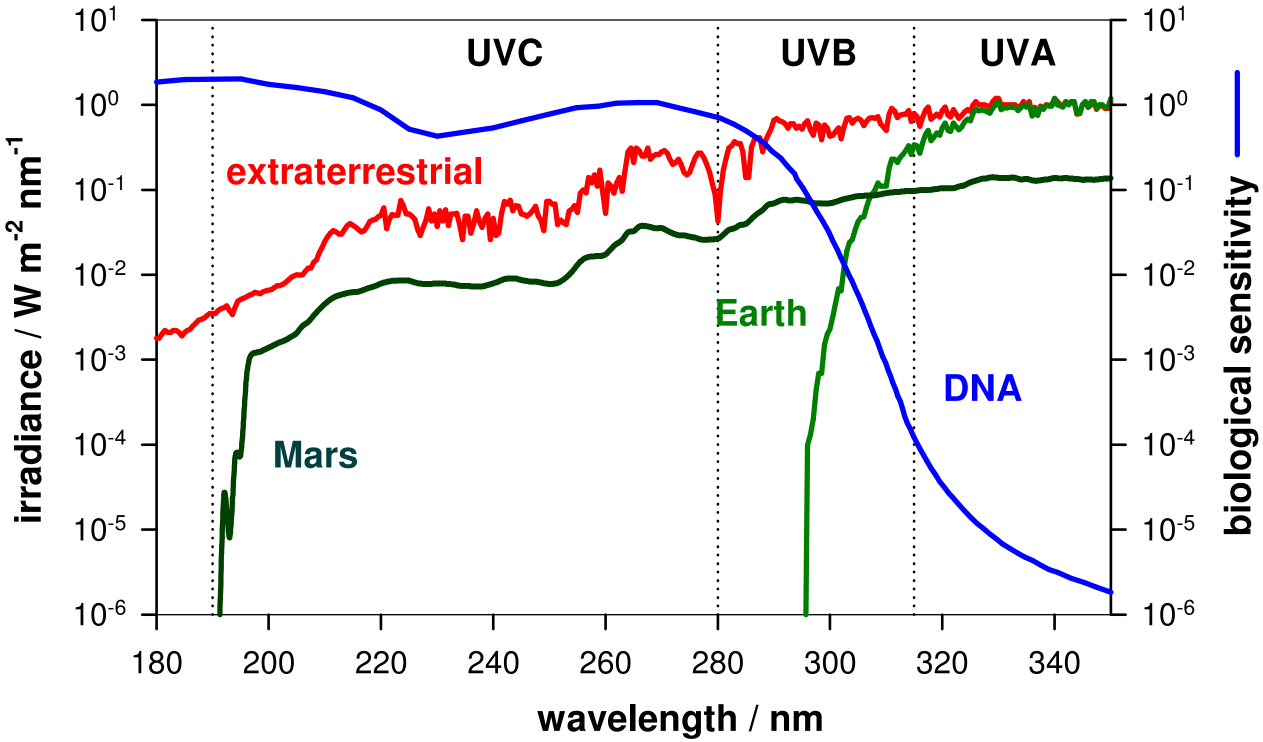
Rationale

- Evolution is a stochastic process driven by mutations and directed by natural selection which causes a population to adapt to their environment.
- Energy-rich solar UV radiation was a major driving force for the early evolution of life on earth.
- All terrestrial organisms have developed different UV protection mechanisms, i.e. amongst others several enzymatic pathways for the repair of UV-induced DNA damage, the synthesis of screening pigments, the formation of mucilage envelopes.

UV climate on Mars and Earth



Solar spectral irradiance in earth orbit, on the surface of earth and on the surface of mars compared to the generalized action spectrum for DNA damage

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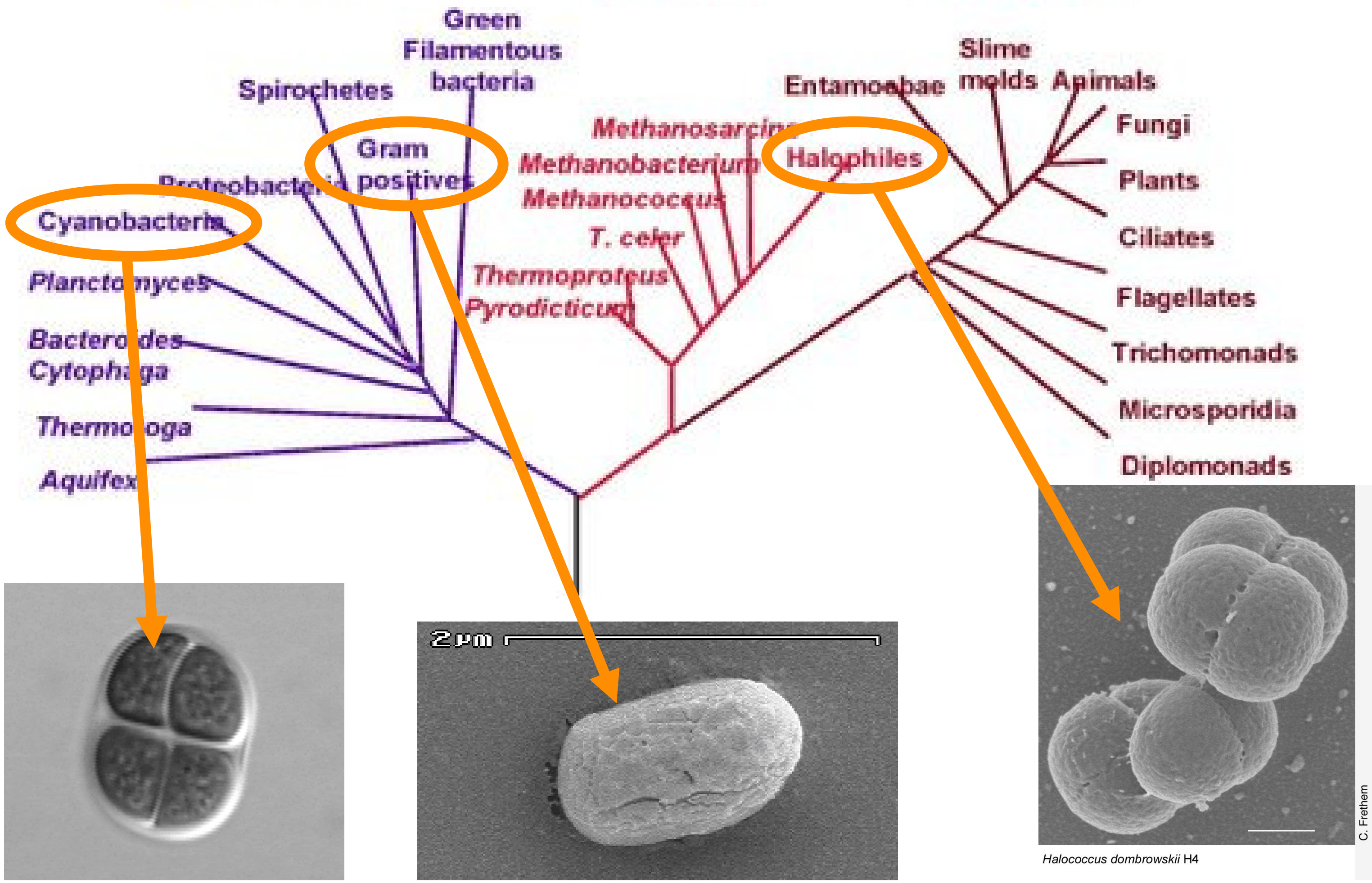
Hypothesis

- The protection mechanisms developed against UV radiation can also contribute at least partially to a higher resistance against other deleterious physical and chemical parameters, like desiccation, ionizing radiation, oxidizing agents.

Questions addressed by ADAPT

- What is the maximal genetic adaptation to a mars-like UV radiation climate achievable in a laboratory experiment?
- How is the UV radiation resistance correlated to the resistance or sensitivity against other deleterious environmental factors?
- What are the DNA damages induced by the different UV qualities?
- Which genes are involved in the repair of UV-induced DNA damages and in the overall adaptation to other deleterious environmental factors in space?
- Do the 3 selected microorganisms for ADAPT originating from very distinct terrestrial habitats and different parts of the phylogenetic tree develop similar strategies to cope with UV and other environmental strssors?

Selected model organisms

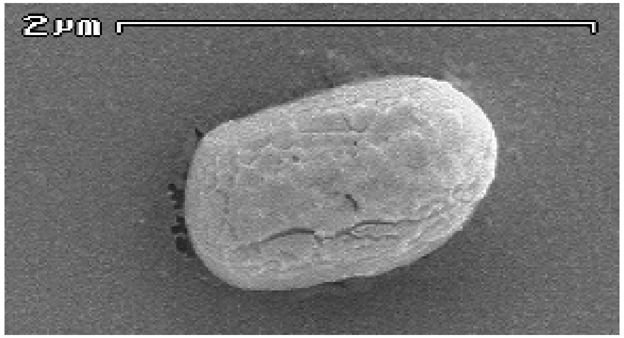


Halococcus dombrowskii H4

Selected model organisms I:

Bacillus subtilis spores

- ubiquitous soil bacterium
- intensely studied in space
- mutants with different DNA repair capacity
- genome completely sequenced
- resistant against desiccation, temperature extremes, UV and ionizing radiation by a dehydrated, highly mineralized core enclosed in a thick protective envelope and by SASPs binding to the DNA

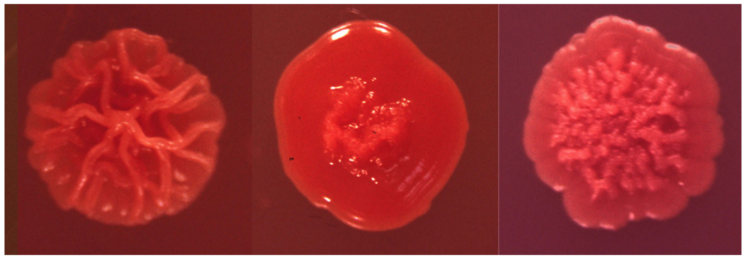


Selected model organisms II:

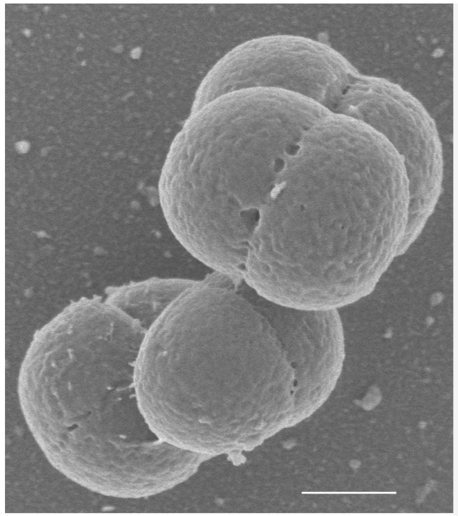
Halococcus dombrowskii

- halophilic archaea from Permian Alpine rock salt of an age of about 250 Ma
- resistant against desiccation, low temperature and low pressure
- resistant against UV radiation by pigments ?

Rock salt from the salt mine near Bad Ischl/Perneck



Red pigmented haloarchaeal colonies of approx. 1 cm diameter each



C. Fretham

Halococcus dombrowskii H4

Selected model organisms III:

Gloeocapsa sp.

- colonial cyanobacterium with a thick gelatinous sheath
- isolated from rock surfaces in Antarctica
- photosynthetic
- dessication resistant
- resistant against UV radiation by mycosporine-like amino acids (MAAs)?

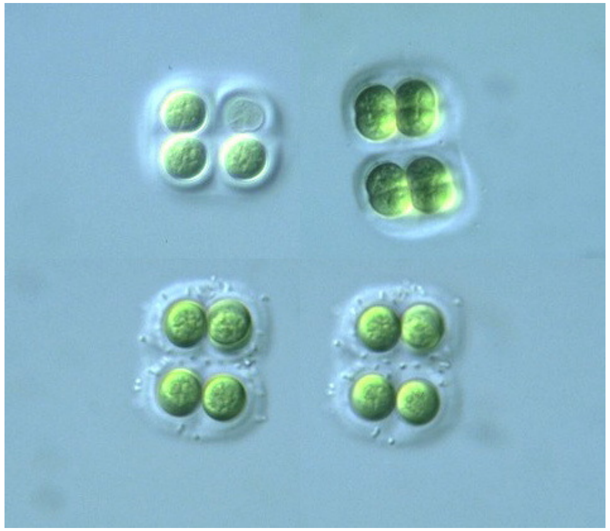
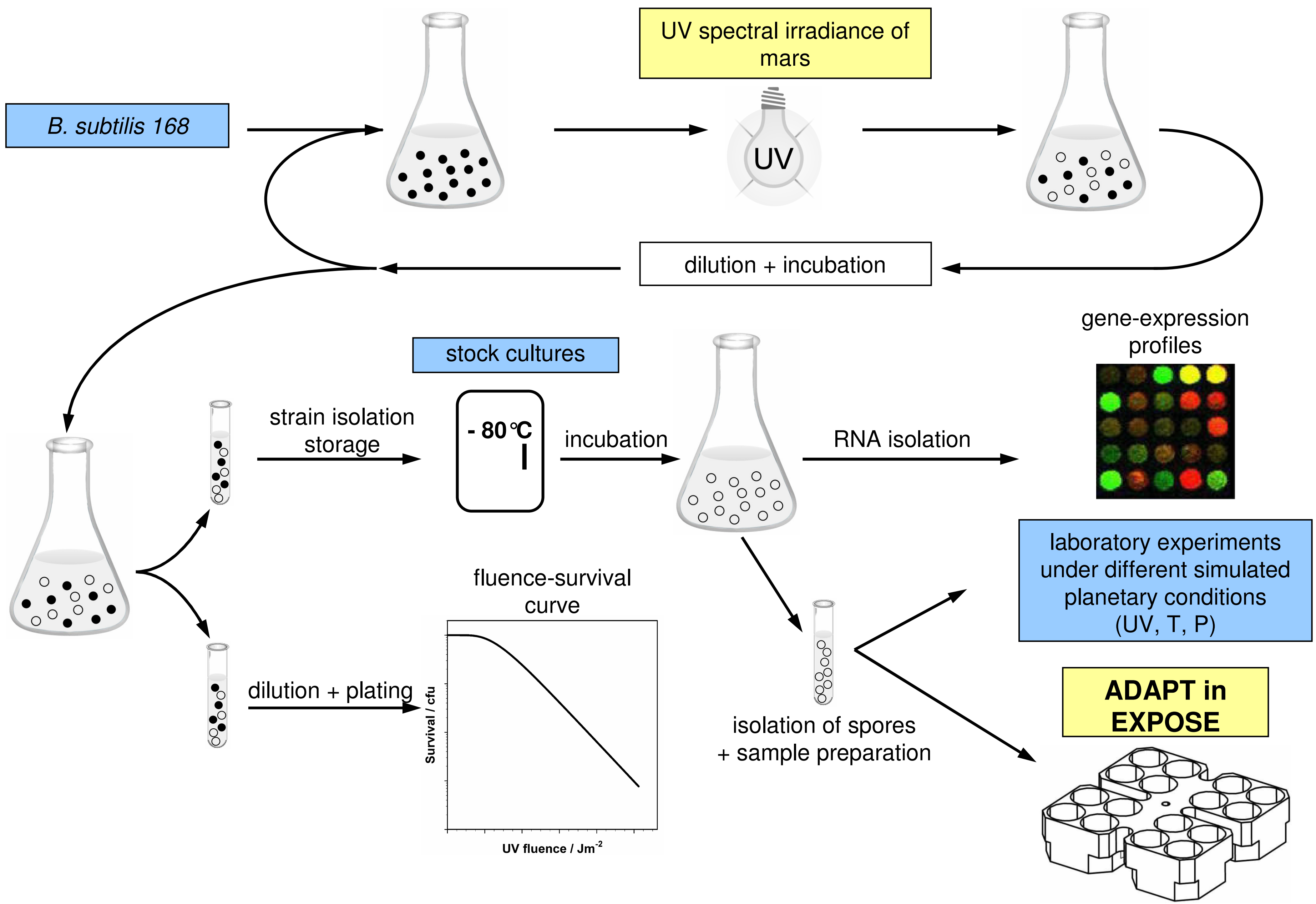
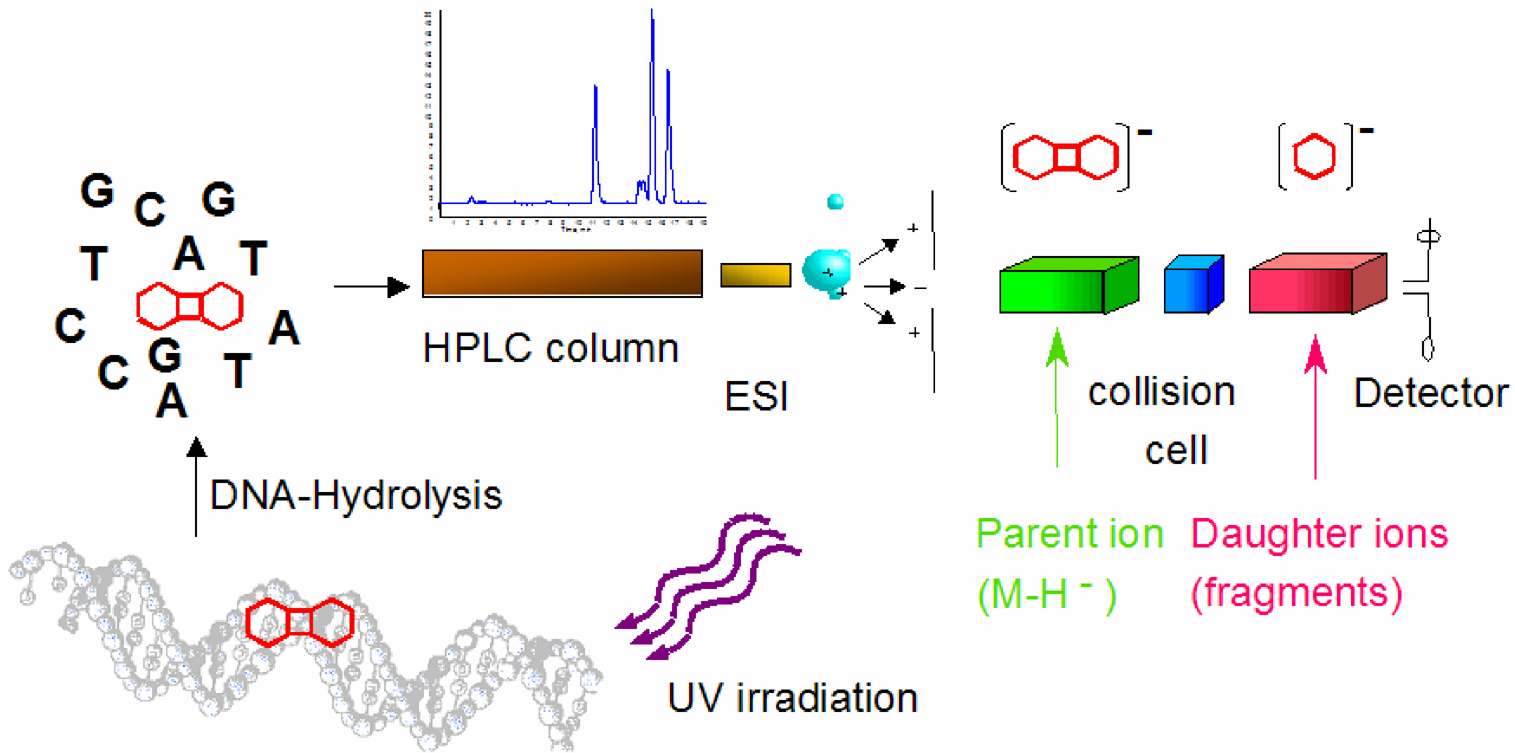


Foto: Y. Tsukii

Scheme for the adaptation of *B. subtilis* to UV radiation of different quality

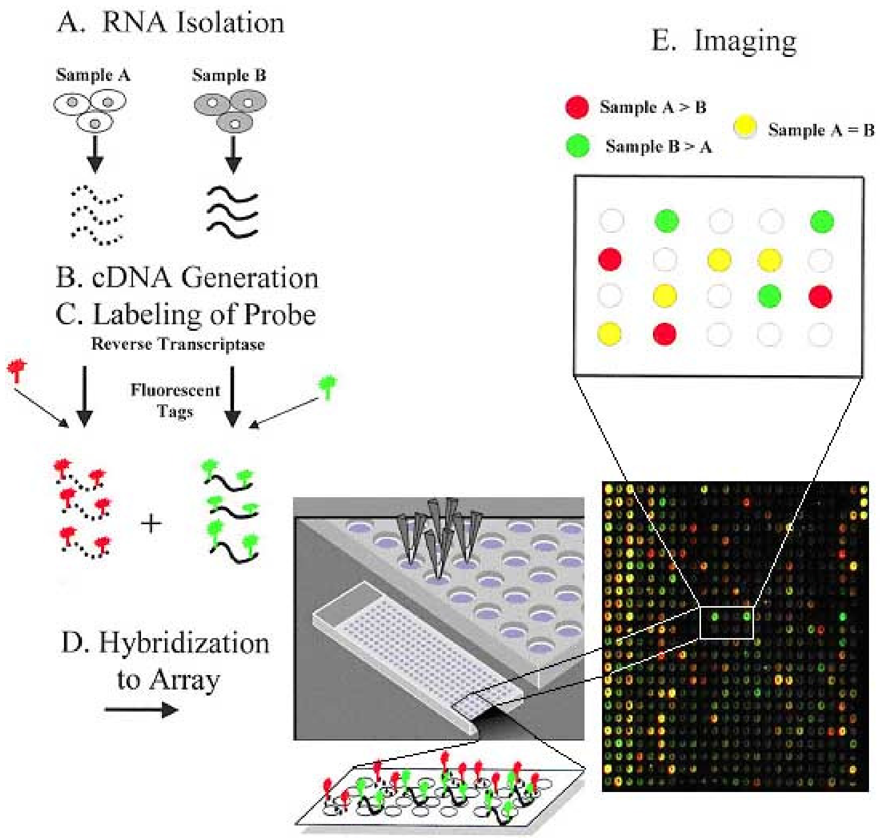


Analysis of UV-induced DNA damage



HPLC-MS/MS method

Analysis of UV-induced changes in gene expression



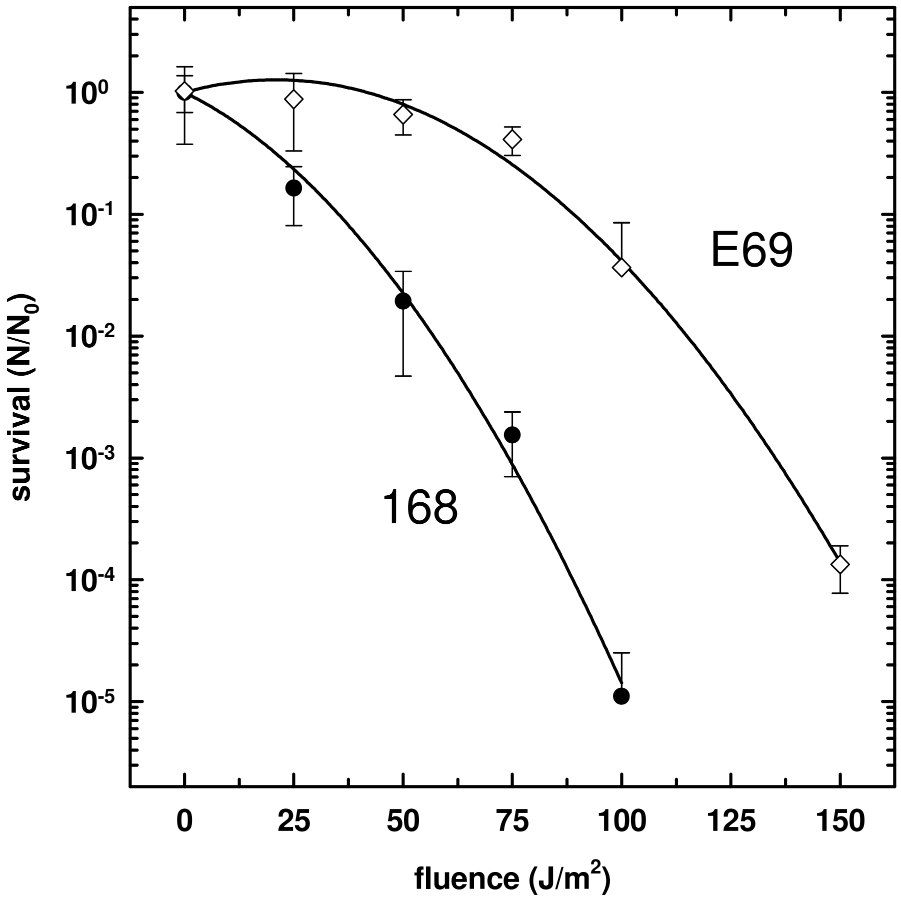
Microarray technology for gene expression profiling

UV-Resistence of *B. subtilis* cells of the UV adapted population E69 compared to strain 168

10^7 cells / ml PBS

UVC 254 nm

$F_{10}(E69) \cong 2.9 \times F_{10}(168)$

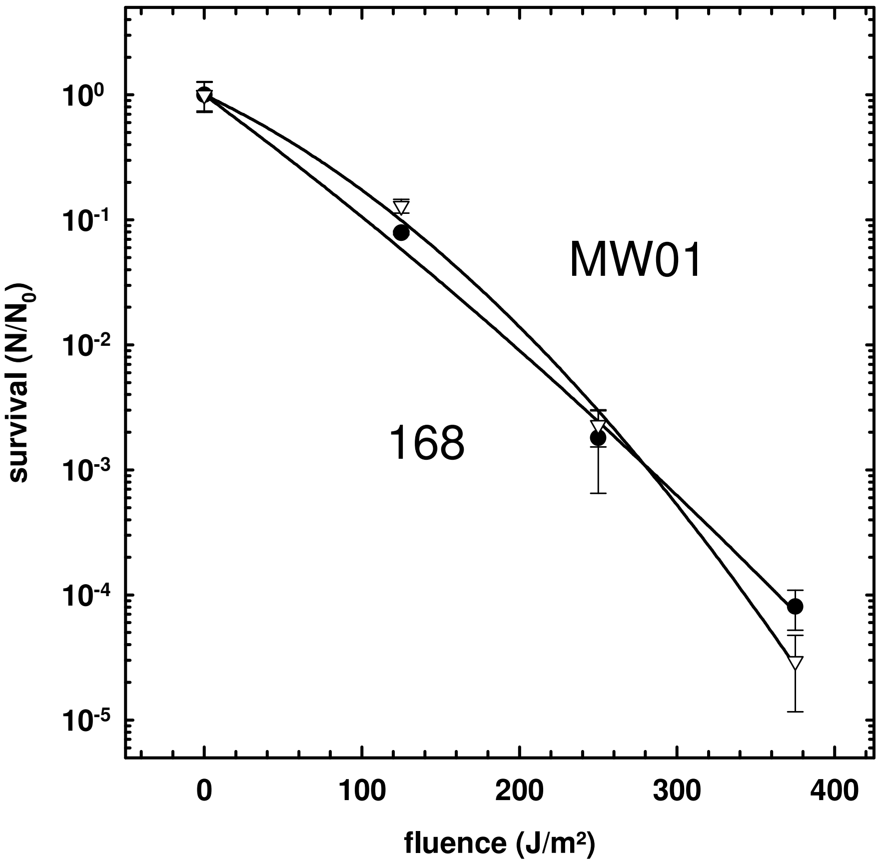


UV-Resistence of *B. subtilis* spores of strain MW01 compared to strain 168

10^7 spores / ml H_2O

UVC 254 nm

$F_{10}(MW01) \cong F_{10}(168)$

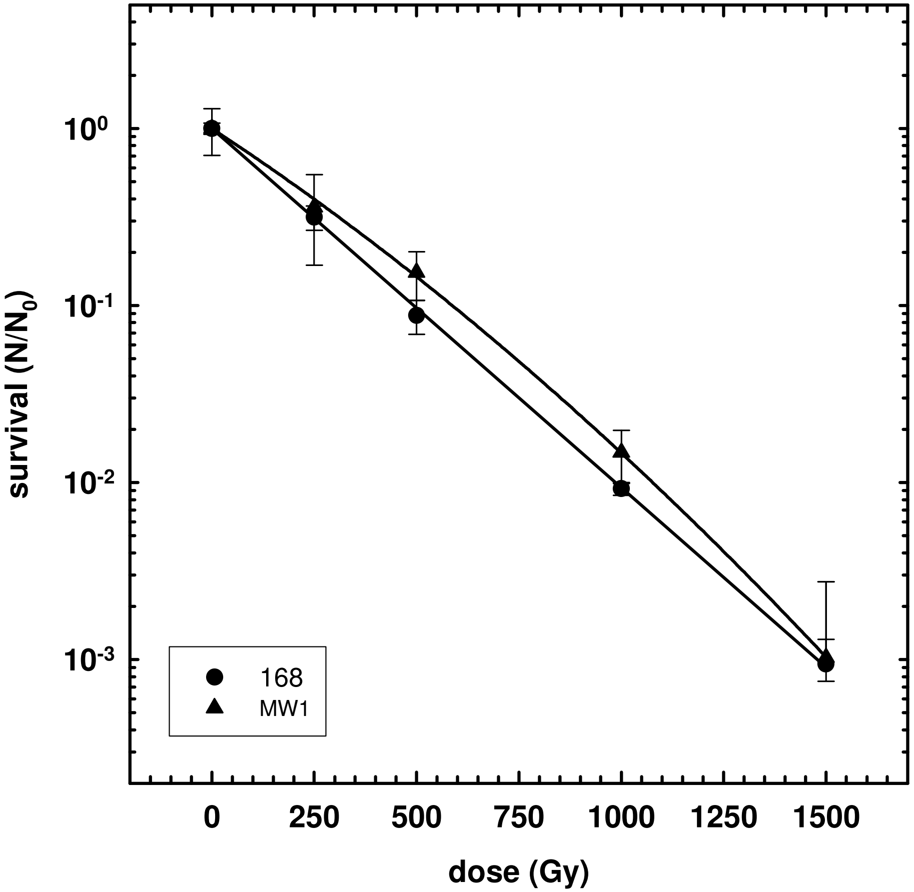


X-ray resistence of *B. subtilis* spores of strain MW01 compared to strain 168

10^7 spores / ml H_2O

150 keV X-rays

$F_{10}(MW01) \cong F_{10}(168)$



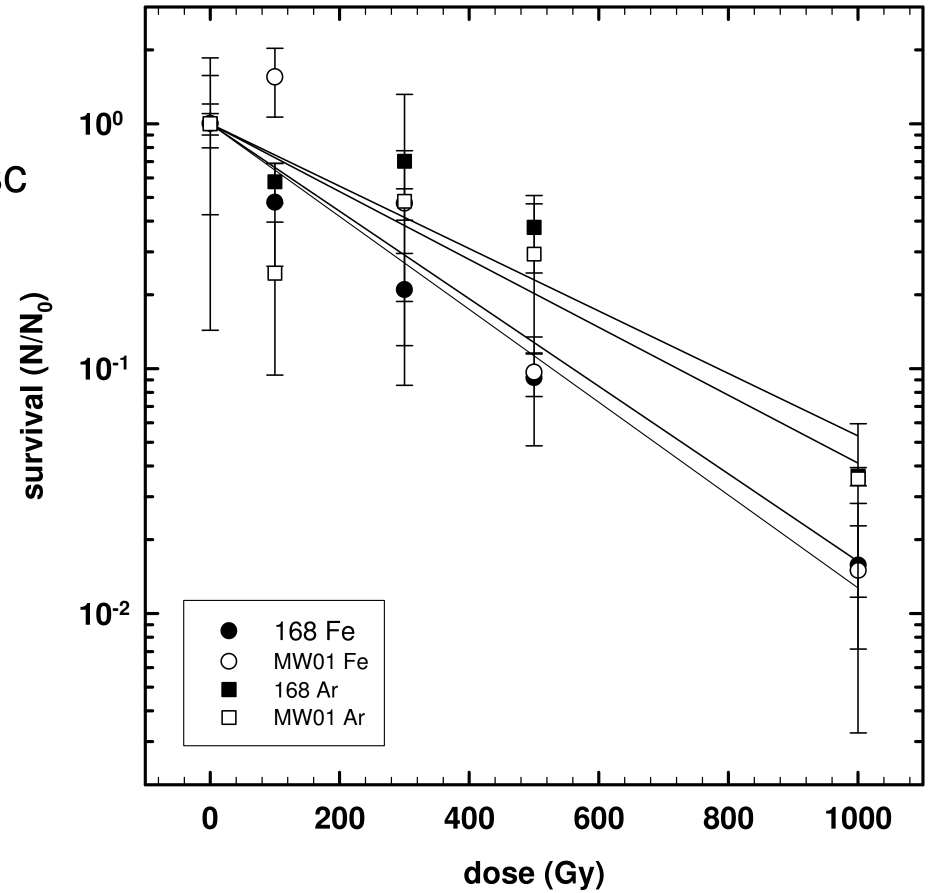
Resistence of *B. subtilis* spores of strain MW01 compared to strain 168 against heavy ions

10^7 spores / 7 mm ϕ quartz disc

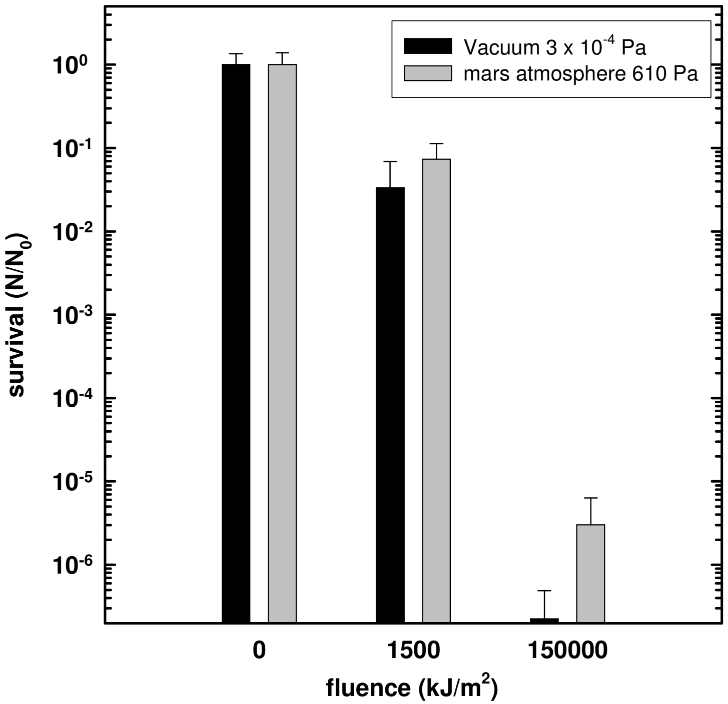
Fe: 500 MeV/n // 200 keV/µm

Ar: 500 MeV/n // 90 keV/µm

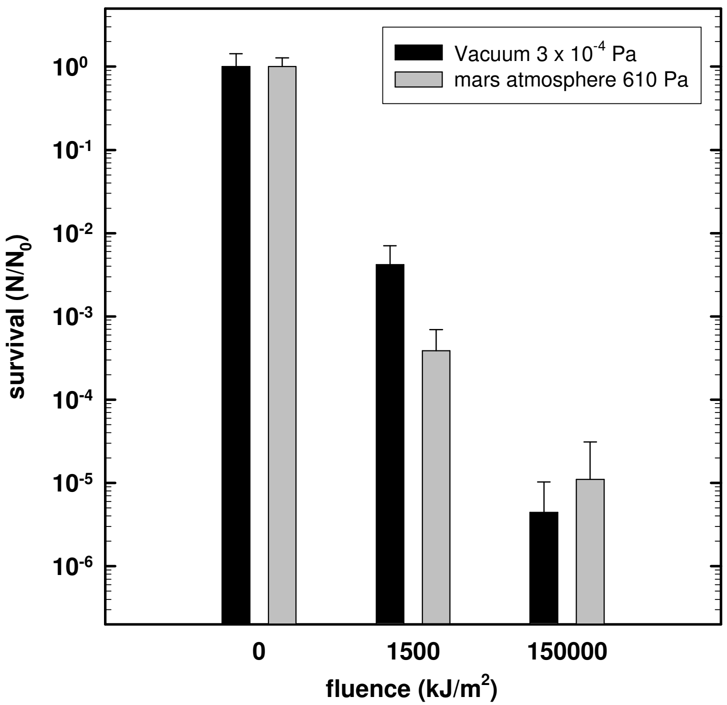
$F_{10}(MW01) \cong F_{10}(168)$



Results of the EST of ADAPT for *B. subtilis* spores of strain MW01 compared to strain 168



168



MW01

Sample preparation for *Halococcus dombrowskii*

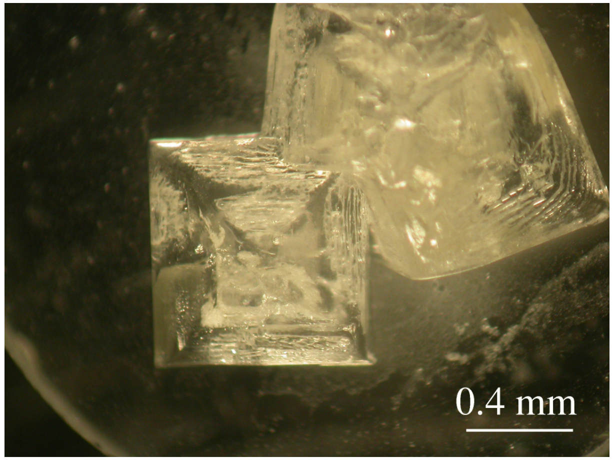


Fig. 1

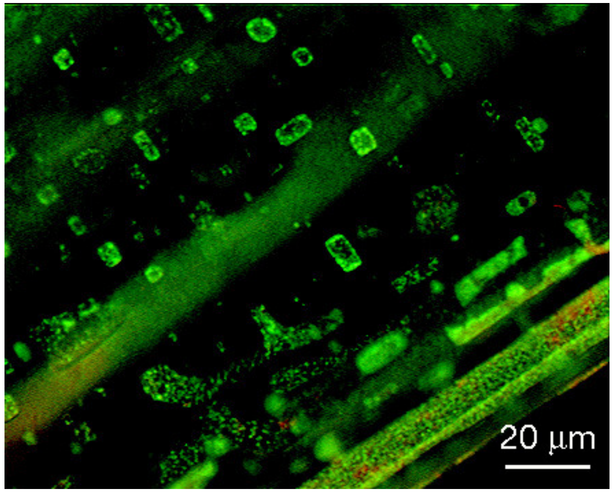


Fig. 2

Cultures of *Halococcus dombrowskii* were dried onto 11 mm ϕ quartz discs (**Fig.1**). Flat halite (NaCl) crystals of 0.8 mm thickness were formed, containing fluid inclusions.

Halococcus cells accumulated preferentially in the mostly rectangular fluid inclusions, as shown with green cells (**Fig. 2**), which were pre-stained before embedding in halite.

Laboratory experiments with *Halococcus dombrowskii*

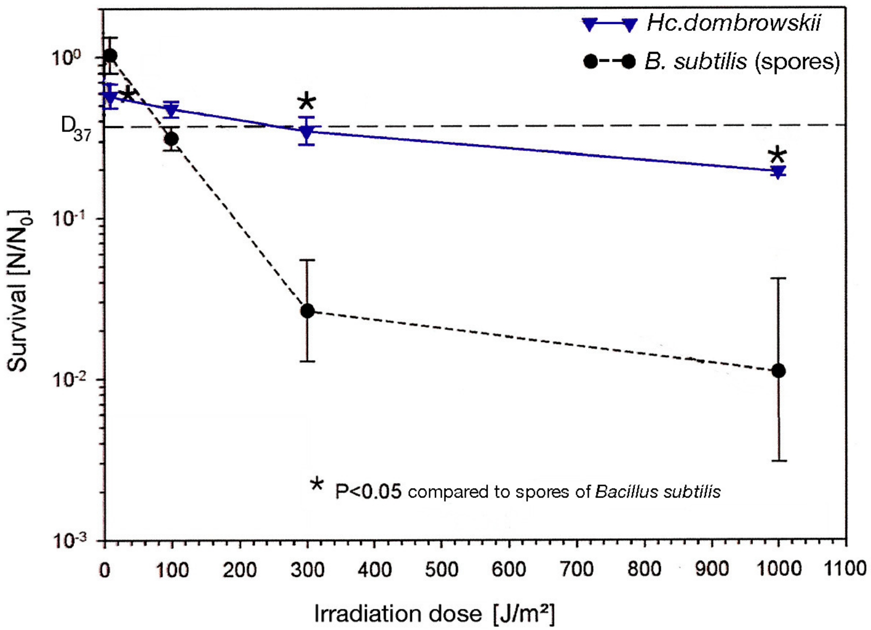


Fig. 3: Survival following irradiation with UVC (254 nm).

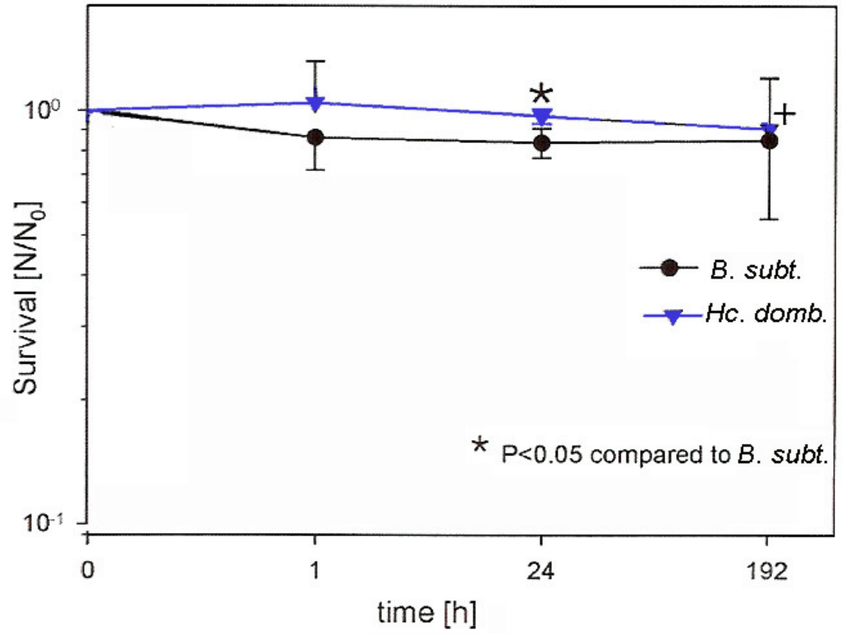


Fig. 4: Survival following exposure to vacuum (10^{-5} Pa).

Halite-embedded *Halococcus dombrowskii* survived exposure to UVC with a D_{37} value of 265 J/m^2 (**Fig. 3**), but exposure in liquid medium reduced D_{37} to about 100 J/m^2 (not shown). Its resistance to UVC was thus similar to that of *Bacillus* spores (**Fig. 3**).

Both *Hc. dombrowskii* and *Bacillus* spores were not susceptible to desiccation at 10^{-5} Pa for up to 8 days (**Fig. 4**).

Laboratory experiments with *Halococcus dombrowskii*

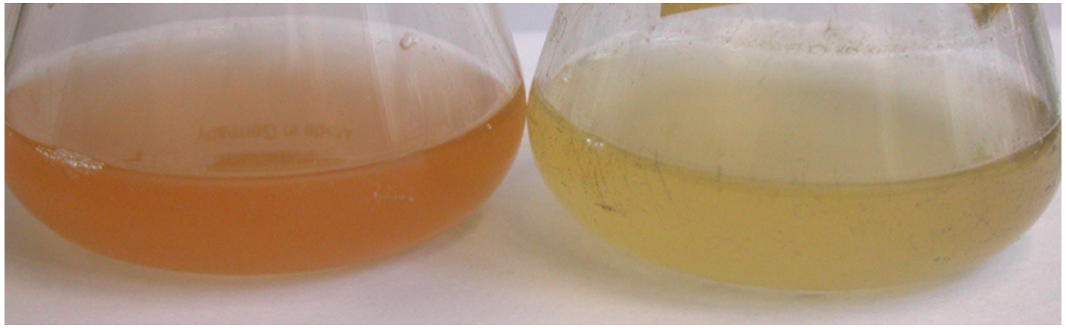


Fig. 6: Liquid cultures of *Hc. dombrowskii;* left, M2 medium, causing normal pigmentation; right, MGM medium, causing reduced pigmentation.

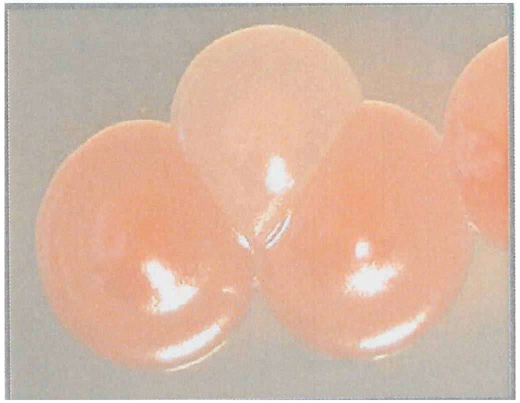


Fig. 5: Colonies of *Hc. dombrowskii* on agar. Middle: colony with reduced pigmentation.

Pigmentation of wild-type *Hc. dombrowskii* is reddish-pink; spontaneous mutants (**Fig. 5**) or treatments with various inhibitors of carotenoid synthesis lead to nearly colourless strains (**Fig. 6**). The influence of pigmentation on the resistance to space radiation is being investigated.

Thank you

for your attention!

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