Report of HIMAC experiments granted by "Living in Space"

Experiment / project name: Determination of the collaborative efforts and interaction of different mechanisms in the DNA repair of low and high LET radiation induced damage in *Bacillus subtilis* spores (CO-REPAIR) [HIMAC Project no. 17J422]
(A03-1) Multidisciplinary Analysis of the Effect of Low Fluence Particle Radiation on Animals and Biological Adaptations (Living in Space)
Visit duration: 2019/1/20-2/1

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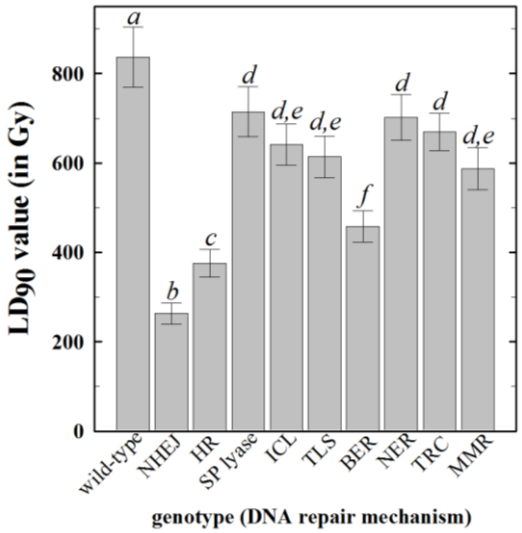
Beam time (date and heavy ions):
1) Jan. 24th, 2019 (23:00 – 30:30 (11:00 p.m. – 06:30 a.m.)), Iron Fe 500 MeV/n (LET 200 keV/µm)
2) Jan. 25th, 2019 (23:00 – 31:00 (10:00 p.m. – 07:00 a.m.)), Iron Fe 500 MeV/n (LET 200 keV/µm)

Aim and scope of experiment: Spores of *Bacillus subtilis* have been used extensively as biological dosimeters for probing terrestrial and extraterrestrial ionizing radiation for environmental and astrobiological studies. Ionizing radiation can damage cellular components though direct deposition of radiation energy into biomolecules and indirectly by generating reactive oxygen species. The biological effects of ionizing radiation are thought to arise from the formation of single- and double-strand breaks (SSB and DSB) in DNA and clustered DNA damage, e.g. two or more closely spaced lesions, including abasic sites, base lesions, SSB or DSB. CO-REPAIR is designed to provide new insights in interaction and teamwork on spore-specific and universal DNA repair mechanisms. Within the proposed research project is aimed to study the major and alternative DNA repair mechanisms (e.g., spore photoproduct lyase (SP lyase), nucleotide excision repair (NER), base excision repair (BER), recombinational repair (HR), translesion synthesis (TLS), transcription-coupled repair (TCR), interstrand cross-link (ICL) repair), mismatch repair, MMR) as single knockout cell lines as combined with an additional mutation in NHEJ. A major focus is the ***identification of the collaborative and supporting efforts of other DNA repair mechanisms in the process of NHEJ*** as the major DNA double strand break (DSB) repair pathway. Here, we study the effects of low and high LET ions. For studying the DNA repair of irradiated spores, a combination of various biochemical and molecular biological methods was used to study the spore resistance to heavy ion radiation exposure. Two in their LET differing heavy ions used to study spore survivability and analyses of the germination capability.

Methodology: For studying the impact of heavy ion exposure irradiation-induced DNA damage on spore survival, air-dried spore samples (~108 spores) were irradiated at room temperature with Helium (with an LET of 2.2 keV/µm) and Iron (highest LET; 200 keV/µm) ions. The surviving fraction of *B. subtilis* spores was determined from the quotient N/N_0, with N being the number of CFU of the irradiated sample and N0 being that of the non-irradiated controls. Spore survival was plotted as a function of heavy ion irradiation dose. The best-fit curves were used to calculate LD_{90} values, i.e., the lethal dose for 90% of the initial population, for statistical comparison. Each experiment was repeated at least three times, and the data shown are expressed as averages ± standard deviations. The treated spores were compared statistically using Student's t test. Values were analyzed in multigroup pairwise combinations, and differences with P values of ≤ 0.05 were considered statistically significant.

Results (spore survival)



Spores of all (single repair) mutant strains tested were significantly more sensitive to He ions than were wild-type spores (LD_{90}: 837±67 Gy), with the *ligD ku* (NHEJ; LD_{90}: 236±23 Gy) spores being the most sensitive.

Fig. 1 Resistance of single mutant and wild-type spores to high-energy charged He ions radiation. LD90 values are expressed as averages ± standard deviations (n = 3). Lowercase letters above the bars denote groups significantly different by ANOVA ($P \leq 0.05$). Genotypes: NHEJ: *ligD ku*; HR: *recA*; SP lyase: *splB*; ICL: *sbcDC*; TLS: *polY1,2*; BER: *exoA nfo*; NER: *uvrAB*; TRC: *mfd*; MMR: *mutSL*.

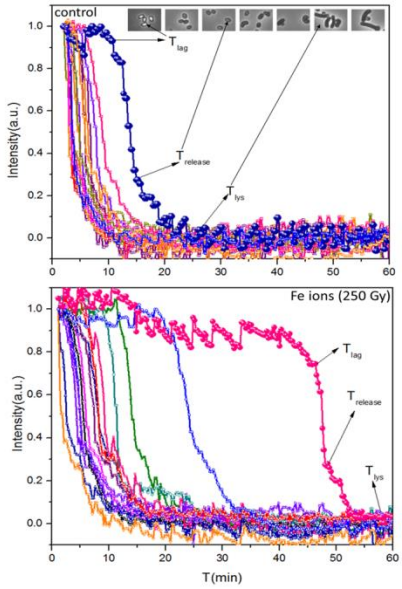
In order, from most to least sensitive, were spores of mutants in *ligD ku* > *recA* > *exoA nfo* > *mutSL* > *polY1,2* > *sbcDC* > *mfd* > *uvrAB* > *splB* >> wild-type. Repair of DSB and base modification/loss via NHEJ, HR and BER appear to be important mechanisms for spore resistance to He ion radiation and the SP lyase or NER-mediated repair of dimers plays only a lesser role.

Results (spore germination)

Spore germination involves complex signal transduction pathways and biophysical events that have been studied best in spores of *B. subtilis*. The process of an individual spore's germination is divided into different phases according to a spore's optical intensity in phase-contrast or differential interference contrast (DIC) microscopy.

Ca-DPA leakage begins after germinant addition and is probably coincident with the time of commitment, followed by the completion of rapid CaDPA release and a small decline in spore refractility due to the hydrolysis of the spore cortex PG and spore core swelling and hydration; the time when spore refractivity becomes constant is termed.

The analysis of spore populations provided information on the germination behavior of irradiated spores, these germination curves were the average behavior of many different spores whose individual behavior differ due Fe ion radiation damage.



Germination of (wild-type) *B. subtilis* spores irradiated with Fe ions.

Our results reinforced the notion that survival after high doses of ionizing radiation does not depend on a single mechanism or process, but instead is multifaceted. Despite the protection mechanisms provided by the components of the dormant spore, potentially lethal or mutagenic damage may accumulate in the spore DNA. It is finally **the accuracy with which spore DNA damage can be repaired during germination that determines the degree of radiation resistance**. Strikingly, the **complete germination and its complex signal transduction pathways are strongly effected by heavy ion radiation**, which has not reported before.

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