Report of HIMAC experiments granted by "Living in Space"

(A03-1) Multidisciplinary Analysis of the Effect of Low Fluence Particle Radiation on Animals and Biological Adaptations (Living in Space)
Visit duration: July 9th - 27th, 2019

HIMAC project 17J422
Title: "Determination of the collaborative efforts of different mechanisms in the DNA repair of low and high LET irradiated *Bacillus subtilis* spores (CO-REPAIR)"

HIMAC project 19J432
Title: "Response of probiotic microorganisms to heavy ion radiation (PRO-RAD)"

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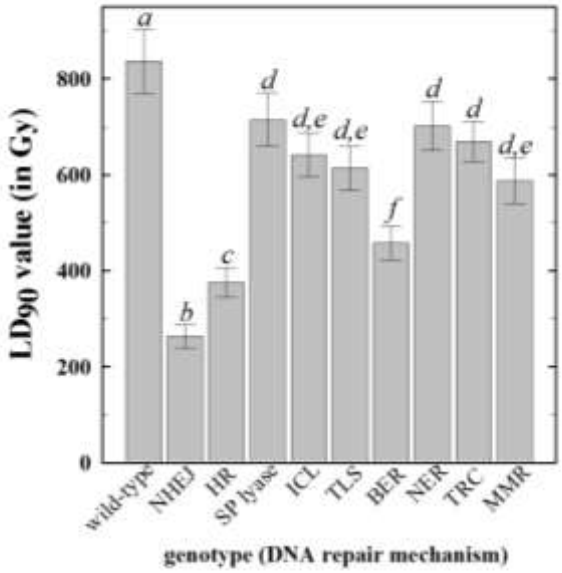
Beam time (date and heavy ions):
1) July 12th, 2019 (23:00–31:00 (11:00 p.m.–07:00 a.m.)), Helium He 150 MeV/n (LET 22. keV/µm)
2) July 18th, 2019 (23:00–30:30 (10:00 p.m.–06:30 a.m.)), Iron Fe 500 MeV/n (LET 200 keV/µm)

HIMAC project 17J422
Title: "Determination of the collaborative efforts of different mechanisms in the DNA repair of low and high LET irradiated *Bacillus subtilis* spores (CO-REPAIR)"

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.



For studying the impact of heavy ion exposure irradiation-induced DNA damage on spore survival, air-dried spore samples (~10^8 spores) were irradiated at room temperature with Helium (with an LET of 2.2 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 N_0 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. **Repair of DSB and base modification/loss via NHEJ, HE 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.

Resistance of single mutant and wild-type spores to high-energy charged He ions radiation. LD_{90} 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: *polY*1,2; BER: *exoA nfo*; NER: *uvrAB*; TRC: *mfd*; MMR: *mutSL*.

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.

HIMAC project 19J432
Title: "Response of probiotic microorganisms to heavy ion radiation (PRO-RAD)"

Aim and scope of experiment:
Spaceflight can significantly impact microorganisms, altering proliferation rates, physiology, metabolism and virulence. In the case of the species that compose the human microbiome, changes observed during space flights include a decrease in the relative abundance of beneficial microorganisms and an increase in opportunistic pathogens. Accumulating evidence suggests that long-term exposure to the space environment can negatively affect human immune function. A manned mission to Mars will present a host of challenges for the astronauts making the long journey, including the difficulty of maintaining a healthy microbiome. **Our microbiome helps us break down food, protects us from infection and bolsters our immune system**. Proactive efforts to establish a healthy microbiome are critical for the physiological and psychological well-being of the crew. Consequently, it is crucial to identify **how extended space radiation impacts probiotic microorganisms**. However, very little is known about the resilience and response of these probiotic microorganisms to space radiation. Filling this 'knowledge gap' is crucial to ensuring astronaut health for long duration space missions, and systematic studies on testing the radiation response of probiotic microorganisms can fulfill this role. Within this project we aim to determine whether the ***viability of commercially available gut probiotics and selected probiotic microbial strain are affected by the low versus high LET particle radiation*** (data analyses is currently in progress).

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Attendance at the "Living In Space" Young Researchers Summer Camp 2019 in Tokushima

By Katharina Siems, PhD student in Space Microbiology at the German Aerospace Center in Cologne at the Institute for Aerospace Medicine in the Department for Radiation Biology

I was very honored to be invited to the "Living in Space" Young Researchers Meeting in Tokushima, which took place from the 25th of July until the 26th of July 2019. Before the meeting. The meeting started on July 25th around at 14 pm. The location for the meeting was the hotel Kanponoyado Tokushima which had an astonishing view over the Tokushima bay area. After the first opening remarks, students from each research groups gave short presentations about their research and scientific goals. Presenting groups were from Gumna University, National Institute of Radiological Sciences (NIRS), Tokushima University, Kyoto University, Matsumoto University and Tsukuba University. The presented research topics were very interesting and varied from studying the effects of space conditions to zebrafish (Tokushima University) to the presentation of new methods to expose samples to simulated microgravity and radiation at the same time (Gunma University). After the short presentations by the students, two invited speakers gave a lecture. The first speaker was Dr. Hidehiko Agata and the second speaker was Dr. Keiji Fukui. In the end of this very interesting afternoon, we had dinner all together. The dinner was accompanied by the performance of a dance group, which performed a traditional dance routine from Tokushima. In the end of the performance we were all invited to join the dance. After dinner, we had a poster session, where all students got the chance to present their research. All posters that were presented by the young researches were very impressive and included future-oriented research projects ranging from the research field of human psychology to plant sciences. On the second day after breakfast, the second two invited speakers gave their lectures. The first speaker of the day was Dr. Tatsuya Aiba and the second speaker was Prof. Ryutaro Izumi. After the two talks, we got the chance to get together in small groups and discuss different questions. The questions concerned space exploration, future of mankind in the space environment, long-term space missions and also the challenges of spaceflight and which kind of technology we would need to overcome all these challenges. After the discussion, we had lunch at the hotel and the meeting was officially closed. For me, the meeting was a great experience to get together with Japanese students and exchange thoughts on science and space research.