

## Analysis of DNA damage induced by space exposure of *Deinococcus radiodurans* R1 in Tanpopo mission

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Tanpopo mission is a Japanese astrobiology experiment addressing basic questions on the origin of terrestrial life and panspermia hypothesis (Yamagishi et al., 2009; Kawaguchi et al., 2016). We have started the space experiments at the Exposure Facility of the Japan Experiment Module on the International Space Station (ISS). Capture experiment investigates existence of terrestrial microbes in space. Exposure experiment investigates the microbial survival and DNA damage caused in space. We analyze degree and types of DNA damage in *Deinococcus radiodurans* using following methods: 1) comparison of survival fractions of mutant strains deficient in each of DNA repair systems, 2) analysis of DNA double-strand breaks using pulsed-field gel electrophoresis, 3) estimation of DNA damage using quantitative-PCR (q-PCR), 4) detection of mutation in *rpoB* gene and 5) analysis of DNA base damage using LC-MS/MS. In this work, we quantified DNA damage (double-strand breaks, single-strand breaks, hydrolysis of base, modified base, and so on) in part of the *rpoB* gene using q-PCR.

### Methods

Dried *deinococcus* cell-aggregates with different thickness were exposed to space (space samples) for about one year. The cells were also stored in the ground laboratory (ground references) and in ISS cabin (ISS references). After exposure or storage, genomic DNA was extracted from each sample and an 887-bp region in the *rpoB* gene was amplified by q-PCR. Intact DNA (%) was determined from the quotient  $N/N_0$ , where  $N$  = copy number of *rpoB* gene amplified from DNA of exposed or stored cells and  $N_0$  = copy number of *rpoB* gene amplified from freshly prepared DNA.

### Results and Discussion

Cell-aggregates with 100  $\mu$ m-thickness exposed to space all cells were dead. Intact DNA of the cell-aggregates with 100  $\mu$ m-thickness exposed to space was less than 1%. On the other hand, Survival fraction in those with 500, 1000  $\mu$ m-thickness was similar between the ground references and the space samples. The result indicates that UV affected only the surface of the cell-aggregates. Intact DNA showed a good correlation with surviving fraction. We will also report the types and degrees of DNA damage using other methods.

Yamagishi, A., et al., (2007) *Biol. Sci. Space* 21: 67–75. , Kawaguchi, Y., et al., (2016) *Astrobiology* 16: 363–376.

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