Analysis of DNA damage in the radiation resistant microbe *Deinococcus radiodurans* R1 exposed to space 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 deinococcal cell-aggregates with different thickness were exposed to space (space samples) for about one year (space samples). 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

Intact DNA (%) of the cell-aggregates with 100 μm-thickness exposed to space was less than 1% and all cells were dead. Pyrimidine dimer was major DNA damage caused by UV. On the other hand, DNA damage in those with 1000 μm-thickness was similar between the ground references and the space samples (Fig. 1). The result indicates that UV affected only the surface of the cell-aggregates. Intact DNA (%) in the ground references and the space samples (UV> 170 nm) with 500 μm-thickness were about 54%, and that in space samples (UV> 120 nm) with 500 μm-thickness was 46%. Although a significant difference is not recognized between the two samples, UV with shorter wavelength tended to induce more damage in DNA. 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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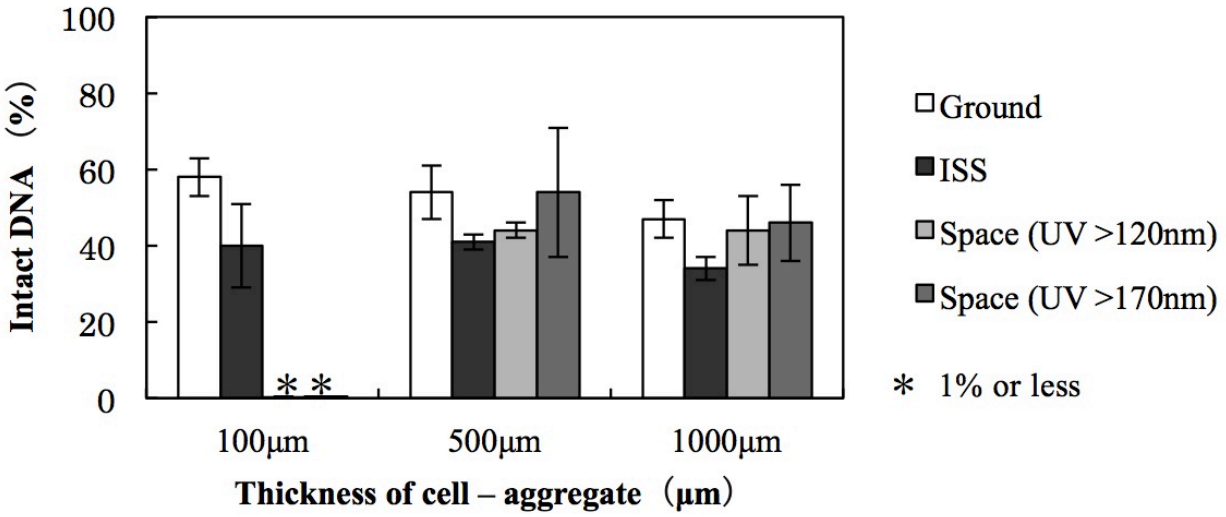

Figure 1 Percentage of Intact DNA

