Mutation analysis of the *rpoB* gene in the radiation-resistant bacterium, *Deinococcus radiodurans* R1 exposed to space

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To investigate the microbial viability and their DNA damage, the radiation-resistant bacteria *Deinococcus* spp. have been exposed at the Exposure Facility of the International Space Station (ISS) in Tanpopo mission since May 2015 [1,2]. The Exposure Panels (EPs) harboring dried-deinococcal cells returned to the ground on October 2016 after about one-year exposure. We analyze the survival rate and DNA damage of dried deinococcal cells using pulsed-field gel electrophoresis, quantitative-PCR and mutation assay. The antibiotic rifampicin binds the RNA polymerase β-subunit, which is encoded by the *rpoB* gene, and inhibits the initial step of transcription. Certain mutations in the *rpoB* gene confer rifampicin resistance [3]. Based on these characteristics of the *rpoB* gene, we determined mutant frequency and the mutation spectrum in the *D. radiodurans rpoB* gene exposure to space. From these mutation data, we estimated major DNA damage induced by the space environment.

D. radiodurans R1 cell-suspension was dropped in the wells of aluminum plates and was dried under vacuum (vacuum-dried). The dried cells were exposed to space, stored in ISS cabin or in the ground laboratory. After exposure experiment, the cells recovered from each well were used to inoculate 10 ml of mTGE medium and cultured until $OD_{590\ nm}$ reached between 0.7 and 3.0. The cell suspension was plated on mTGE agar containing 50 μg/ml rifampicin to determine the number of rifampicin resistant cells (Rif^R), and on mTGE agar without rifampicin to determine the total number of viable cells.

We also determined the sequences of the *rpoB* gene extracted from Rif^R. The rifampicin resistant mutation frequencies of the space exposed *D. radiodurans* R1 cells and those of the ground control were comparable (Fig. 1). The result suggested that the effect of UV for dried-cells exposure to space was less. Further, we will report and discuss the rifampicin-resistant spectra in the *rpoB* gene in rifampicin-resistant cells exposure to space, stored in ISS or in ground laboratory.

[1] Yamagishi, A. et al., (2007) *Bio. Sci. Space* 21: 67–75.
[2] Kawaguchi et al., 2016, *Astrobiology* 16 : 363–376.
[3] Campbell, E. A. et al., (2001) *Cell* 104: 901–912

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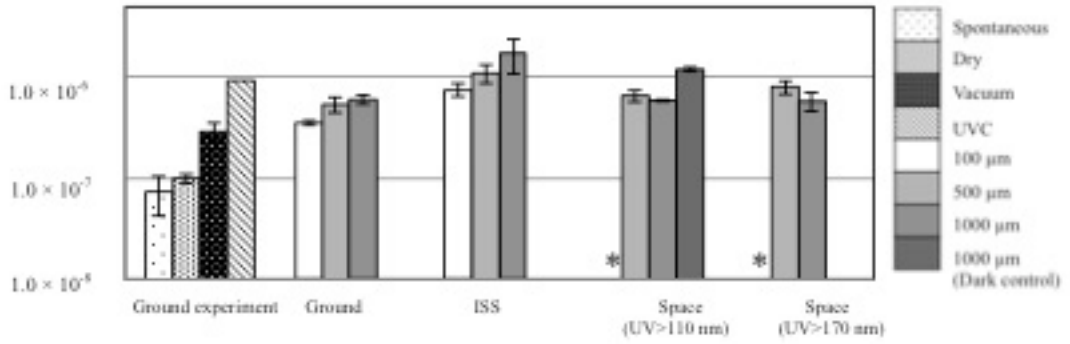
Fig.1 Mutation frequency of the *rpoB* gene in *Deinococcus radiodurans* R1



*Because samples exposed to the space were dead, mutation couldn't be analyzed.

