

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 Panel (EP) harboring dried-deinococcal cells was returned to the ground on August 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. Rifampicin is an antibiotic that binds to RNA polymerase β -subunit encoded by *rpoB* gene, thus inhibiting the initial step of transcription. Certain *rpoB* mutations confer rifampicin resistance to bacteria [3]. On this basis, we determined the mutant frequency and mutation spectrum in the *rpoB* gene of *Deinococcus radiodurans* that was exposed to space. From these 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 dried under vacuum (vacuum-dried). The dried cells were exposed to space, stored in the ISS cabin or in the ground laboratory. After exposure experiment, the cells recovered from each well were used to mix with 10 ml of mTGE medium and cultured until OD_{590 nm} reached between 1.1 and 3.0. The culture was plated on mTGE agar supplemented by 50 μ g/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 DNA sequences of the *rpoB* gene extracted from Rif^R.

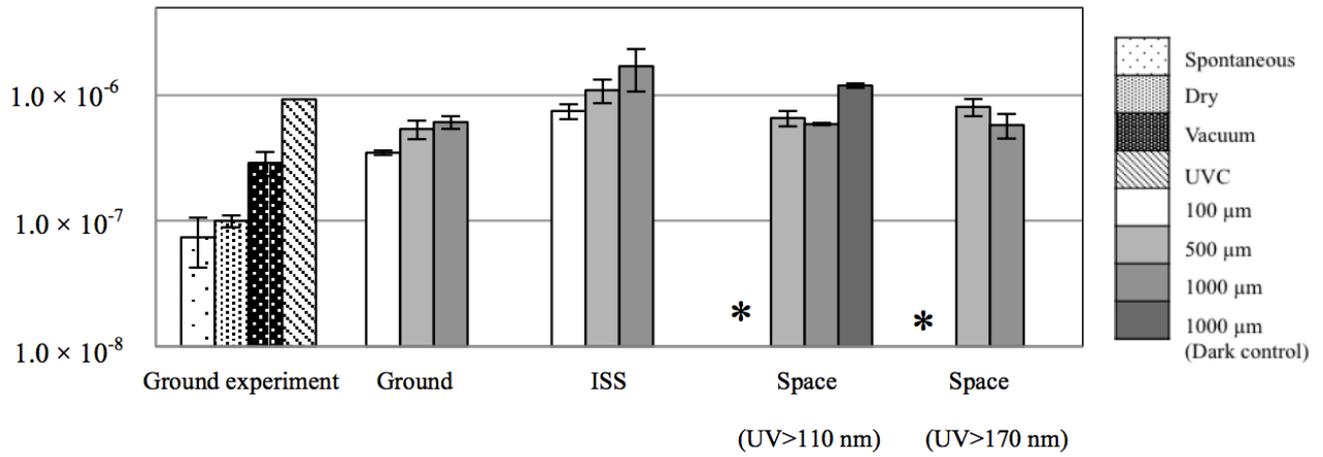
The mutant frequencies of space exposed cells and ground control were comparable (Fig. 1). The result suggested that the effect of UV on mutation induction was marginal in dried deinococcal cells exposed to space for about one year. Further, we will report and discuss the mutation spectra of the *rpoB* gene in rifampicin-resistant cells obtained from samples exposed to space, stored in the ISS cabin or in the ground laboratory.

[1] Yamagishi, A. et al., (2007) *Bio. Sci. Space* 21: 67–75.

[2] Kawaguchi, Y. et al., (2016) *Astrobiology* 16: 363–376.

[3] Campbell, E. A. et al., (2001) *Cell* 104: 901–912.

Keywords: *Deinococcus radiodurans* R1, DNA damage, Tanpopo mission, mutation analysis, *rpoB* gene, rifampicin



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

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