

## Analysis of mutations of *rpoB* gene in *Deinococcus radiodurans* R1 induced by simulated space conditions

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To investigate the microbial viability and their DNA damage, the radioresistant bacteria *Deinococcus* spp. have been exposed at Exposure Facility of International Space Station (ISS) in Tanpopo mission since May 2015 [1]. The Exposure Panels (EPs) harboring dried-deinococcal cells will return to the ground after about one-, two- and three-year exposure. We are going to analyze the survival rate and DNA damage of dried deinococcal cells using pulsed-field gel electrophoresis, quantitative-PCR and mutation assay. The antibiotics rifampicin binds the RNA polymerase  $\beta$ -subunit, which is encoded by *rpoB* gene, and inhibits the initial step of the transcription activity. Certain mutations in the *rpoB* gene confer rifampicin resistance [2]. Based on the above understanding, we will determine mutant frequency and the mutation spectrum for the *D. radiodurans* *rpoB* gene. From these mutation data, we will estimate major DNA damage induced by space environment. For this purpose, the mutagenic specificity of the *D. radiodurans* *rpoB* gene in simulated space conditions was investigated in this study.

The *D. radiodurans* R1 cell-suspension was dropped in the wells of aluminum plates ( $\phi$ 20 mm) and was dried under vacuum (vacuum-dried). The dried cells were exposed to vacuum ( $< 10^{-5}$  torr) or UVC<sub>254nm</sub> under the vacuum conditions. As a control, we analyzed the vacuum-dried cells without additional vacuum incubation. After exposure experiment, the cells were recovered from each well. inoculated to 10 ml of mTGE medium and cultured to show the OD<sub>590 nm</sub> to be about 4. The cell suspension was plated on mTGE agar containing 50 $\mu$ g/ml of rifampicin to determine the number of rifampicin resistant colonies (Rif<sup>R</sup>), and on mTGE agar without rifampicin to determine the total number of viable colonies.

The rifampicin-resistant mutant frequency of vacuum-dried cells was  $1.3 (\pm 0.5) \times 10^{-8}$ . The rifampicin-resistant mutant frequency of the *D. radiodurans* R1 wet cells has been shown to be about  $1.5 \times 10^{-8}$  [3]. The result suggests that the rifampicin-resistant mutant frequencies of vacuum-dried cells and wet cells are comparable for *D. radiodurans* R1. Further, we will report and discuss the rifampicin-resistant mutant frequency and mutation spectra in the *rpoB* gene of rifampicin-resistant cells following exposure to UVC<sub>254nm</sub> and vacuum ( $< 10^{-5}$  torr).

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

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

[3] Kim, M. et al., (2004) *Genetics* 166: 661-668.

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