Effect of heavy-ion irradiation on survival rate of *Synechocystis* sp. PCC 6803

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The unicellular cyanobacterium *Synechocystis* sp. PCC 6803 is a widely used model organism in prokaryotic studies. It was the first photosynthetic microorganism to have its genome sequenced,[1)] and its well-established transformation techniques have advanced research of cyanobacterial genetics and metabolism. Heavy-ion beam irradiation can generate useful mutants in such model organisms, enabling rapid identification of causal genes, but there are few reports of such applications in cyanobacteria. Here, we report survival rates and morphological observations of *Synechocystis* sp. PCC 6803 irradiated with iron (Fe) ions (LET: 796 keV/μm) and carbon (C) ions (LET: 23 keV/μm).

The cyanobacterium was cultivated in BG-11 medium. For Fe-ion irradiation, the cyanobacteria were concentrated approximately 16-fold to an OD_{730} of 2.27, and 19 μL aliquots were dispensed into 100 μL 8-strip tubes (Takara). For C-ion irradiation, the cyanobacteria were adjusted to an OD_{730} of 3.73, and 100 μL aliquots were dispensed into 100 μL 8-strip tubes (Takara) for irradiation. As cyanobacteria are microorganisms, relatively high-dose conditions were used for Fe ions, specifically 0, 25, 100, 200, 400, 800, 1000, and 2000 Gy. Additionally, since an increasing trend in survival rate was observed at low doses, irradiation experiments were also conducted under C ion beam conditions of 0, 25, and 150 Gy to confirm the survival rate increase. Survival rates at each dose were determined using two methods: colony counting on agar medium and turbidity measurement in liquid medium. The cell concentration of the 0 Gy samples was determined using a hemocytometer, and 800 cells per dose were seeded onto BG-11 agar medium. Both agar and shaking cultures were incubated at 25°C under continuous light conditions of 30 μmol/m^2/s.

OD_{730} was measured 7 days post-irradiation to generate survival curve data. Survival rates were calculated by dividing the OD_{730} of each dose by the OD_{730} of the 0 Gy sample for the respective. Each condition was performed in triplicate ($n = 3$), and the averages were plotted. In Fe-irradiated samples, survival rates increased at 25 Gy but gradually decreased with higher doses (Fig. 1(A)). Survival rates were also calculated based on colony counts, which showed a decline in survival with increasing doses, similar to liquid culture samples. However, the survival rates derived from colony counts decreased more sharply than those based on OD_{730} (Fig. 1(A)). No colonies were observed at doses of 1000 Gy and 2000 Gy on agar medium. For C irradiation, the survival curve based on OD_{730} measurements 7 days post-irradiation indicated increased survival at 25 Gy (Fig. 1(B)). Furthermore, in some of the 800 Gy, 1000 Gy, and 2000 Gy iron-irradiated samples, granule-like structures were observed inside the cyanobacteria that were not originally observed (Fig. 2).

It is well known that heavy-ion beam irradiation of

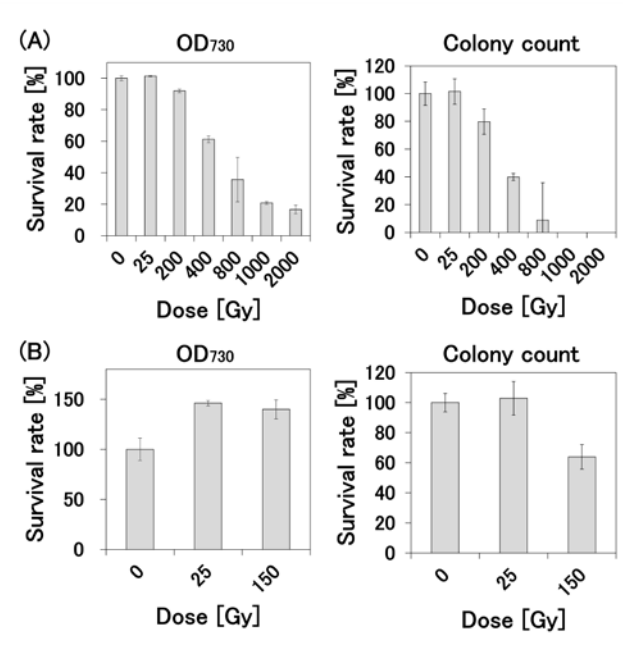

Fig. 1. (A) Survival curves for Fe-ion irradiation. (B) Survival curves for C-ion irradiation. $n = 3$, bars: SE.

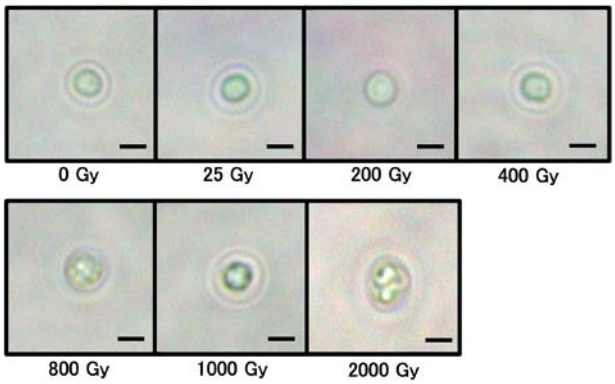

Fig. 2. Morphology of Fe-ion irradiated cells. bars: 2 μm.

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microorganisms often achieves a survival rate of approximately 10%, which is effective for obtaining desired mutants. For *Synechocystis* sp. PCC 6803, Fe-ion irradiation at high doses ranging from 800 to 2000 Gy was suggested to be effective. Additionally, a similar increase in survival rate was observed with 25 Gy of C-ion irradiation, compared to the results with Fe ion beams. These results suggest that the survival rate of *Synechocystis* sp. PCC 6803 is dose-dependent.

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Reference
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