Effects of heavy-ion beam irradiation on the survival and morphology of *Cyanidioschyzon merolae*

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Cyanidioschyzon merolae (*C. merolae*) is a unicellular red alga that inhabits strongly acidic hot springs (pH 2.5, 45°C). The organism contains a single nucleus, mitochondrion, and chloroplast, with a highly compact cell diameter of approximately 2 μm. Its genome has a size of approximately 16.5 Mb[1] and comprises 5,331 identified genes,[2] making it one of the smallest eukaryotic genomes known. Notably, it is considered that *C. merolae* strongly reflects the characteristics of pre-endosymbiotic cyanobacteria-like organisms, despite being a eukaryote.[3] Because of this unique feature, *C. merolae* is positioned as an excellent model organism for studying ancient endosymbiotic events. In our research, we aim to generate mutants with novel traits by irradiating *C. merolae* with heavy-ion beams. As part of the preliminary data collection, we assessed the effects of carbon-ion (C-ion) and argon-ion (Ar-ion) beam irradiation on their survival rates and morphological characteristics.

The *C. merolae* wild-type strain 10D used for our heavy-ion beam irradiation experiments was cultured in 2 × Allen's medium (pH 2.5) under light conditions (40 W/m^2) at 42°C with shaking at 130 rpm. Suspensions of *C. merolae* (OD_{750} = 1) were irradiated with C-ion beams (23 keV/μm) at doses of 0, 12.5, 25, 50, 100, and 150 Gy, and with Ar-ion beams (189 keV/μm) at doses of 0, 10, 25, 50, 75, and 100 Gy. Following irradiation, the cells were incubated under dark conditions for over 9 hours and subsequently diluted in 2 × Allen's medium to achieve an OD_{750} of 0.04–0.06 before being cultured under standard conditions. On day 8 of cultivation, the OD_{750} values were measured, and survival rates were calculated based on the OD_{750} value of non-irradiated cells as 100%. Simultaneously, the morphologies of approximately 100 cells in 20 μL of suspension were observed to determine the percentage of morphologically abnormal cells. All measurements of survival rates and morphological abnormalities were performed in triplicate.

In the C-ion beam irradiation experiment, the survival rate remained above 40% regardless of dose (Fig. 1A). Morphological abnormality rates tended to increase with higher doses (Fig. 1B). Additionally, the morphology following C-ion beam irradiation exhibited distinct patterns depending on the dose: at relatively low doses, chloroplasts were observed protruding and forming branching structures due to mitotic abnormalities, whereas at high doses, chloroplasts were dispersed in the cytoplasm, accompanied by cellular enlargement (Fig. 2A).

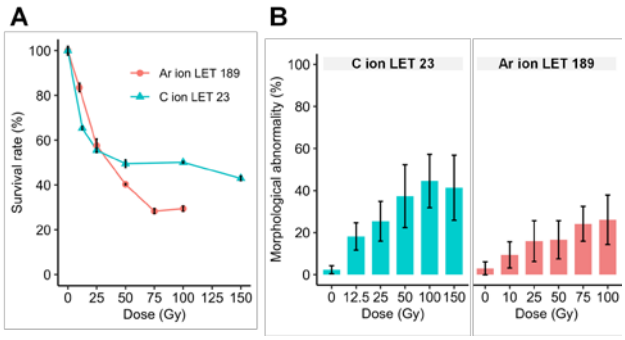

Fig. 1. Survival rate and morphological abnormality rate (A) Survival rate. (B) Morphological abnormality rate. Data shown are in means ± SD of three replicates.

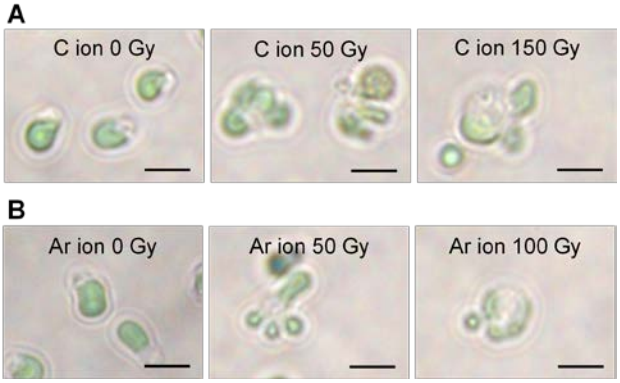

Fig. 2. Morphology after heavy-ion beam irradiation (A) C-ion beam irradiation. (B) Ar-ion beam irradiation. Scale bar: 5 μm

In the Ar-ion beam irradiation experiment, the survival rate decreased in a dose-dependent manner (Fig. 1A). The rates of morphological abnormalities and the resulting morphologies followed trends similar to those observed with C-ion beam irradiation (Fig. 1B, 2B).

These results indicate that *C. merolae* exhibits decreased survival rates and increased morphological abnormality rates as the irradiation dose increases. Furthermore, it was found that mutation-induced morphological abnormalities in *C. merolae* display consistent phenotypes irrespective of the type of irradiating ion.

Acknowledgments

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