Effect of heavy-ion irradiation on survival of *Medakamo hakoo*

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We developed an artificial photosymbiosis of microalgae with heterotrophic organisms using fish as the host. This system enables the development of fish that grow faster by receiving nutrients from the symbiotic algae and can be applied in fisheries. *Medakamo hakoo* (unicellular green algae) artificially introduced into the early embryos of medaka *Oryzias latipes* (freshwater fish) are temporarily viable in the host but disappears over time (unpublished data). Therefore, we irradiated *M. hakoo* with heavy-ion beams to introduce random genomic mutations, generating the mutant strains that can be maintained in the body of the fish for long periods. Therefore *M. hakoo* cells were initially irradiated with heavy-ion beams using carbon and argon nuclides; the resulting viability was measured, and cell morphology was observed. We aimed to determine whether heavy-ion beam irradiation effectively produces mutant *M. hakoo* strains.

M. hakoo cells (OD_{750} = approximately 0.7) were statically cultured in MGRL liquid medium[?)] was aliquoted 200 μL into 200 μL volume PCR tubes, which were irradiated with C ion beams (23 keV/μm; 0, 12.5, 25, 50, 100, 150 Gy) and Ar ion beams (189 keV/μm; 0, 10, 25, 50, 75, and 100 Gy). The cells were kept in the dark for at least 3 huors after irradiation, and then 500 cells were seeded on three MGRL gellan gum plates and incubated at 22°C at 80 μmol/m^2/s (16 hours light, 8 hours dark). We counted the number of colonies on the plates, C, after 26 days, and, Ar, after 27 days ($n = 3$; one replicate of Ar 0 Gy was excluded from calculation and set as $n = 2$ due to fungal contamination). The colony survival rate was calculated using the following formula and is shown in Fig. 1.

Colony survival rate (%) = (Average number of colonies at each Gy)/(Average number of colonies at 0 Gy)

The OD_{750} values were measured after 8 days, and the OD survival rate was determined using the following formula and shown in Fig. 1.

OD survival rate (%) = (Average values of OD_{750} at each Gy)/(Average values of OD_{750} at 0 Gy)

Both the colony and the OD survival rates decreased with irradiation dose. The colony survival rate was lower after Ar irradiation than after C irradiation when compared at the same dose.

Liquid culture samples were microscopically examined 14 days after irradiation to examine the effect of heavy-ion beam irradiation on cell morphology. Several hypertrophied cells with 4.4–6.1 μm in major axis

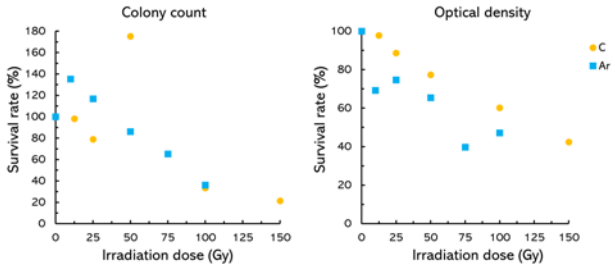

Fig. 1. Survival rate of *M. hakoo* after heavy-ion irradiation. Left: Colony count. Right: Optical density. Dots represent survival rate at each irradiation dose.

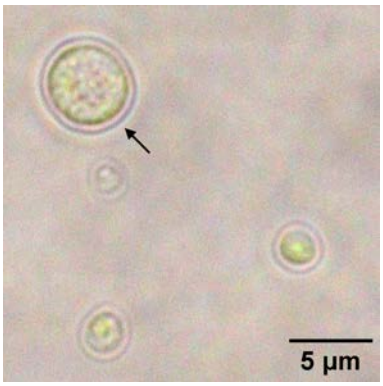

Fig. 2. Abnormal cell morphology after irradiation with 150 Gy with a C ion beam. Arrow indicates abnormally larger cell (6.1 μm major axis).

(wild type: 2.4 μm on average) were observed in the cells irradiated with 150 Gy of C ion beam shown in Fig. 2. No abnormalities in cell shape were observed in the other irradiated samples.

The survival rates of both nuclides decreased with increasing irradiation dose, suggesting that introducing mutations into the *M. hakoo* genome via heavy-ion beam irradiation is dose-dependent. The results of the colony viability assays suggested that Ar irradiation more strongly affected the viability and genome than C irradiation. However, cell morphology was abnormal only after 150 Gy of C irradiation, suggesting that the phenotype of the cells was affected only by high-dose C irradiation. These results indicate that heavy-ion beam is an effective tool for producing *M. hakoo* mutant strains.

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Reference

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