Robust strains isolated by heavy-ion beam irradiation and endurance screening in the green algae, *Haematococcus pluvialis*

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Haematococcus pluvialis is an egg-shaped or spherical unicellular green microalga ranging from 20-50 μm in diameter, belonging to the green algal genus Volvocales (Chlorophyceae). After endospore formation, the alga releases zoospores with two flagellars that grow and become the nonmotile palmela cell. The green cell becomes a cyst following exposure to an environmental stress factor such as strong light or low nutrients, and accumulates astaxanthin, which exerts a strong antioxidative effect, in the cell[1]. If it is possible to obtain a strain with high tolerance to environmental stress (i.e., a robust strain), it seems that the percentage of cell death is decreased, and it is therefore possible to improve productivity. In this study, we attempted to isolate robust strains of *H. pluvialis* using heavy-ion beam irradiation and endurance screening.

Heavy-ion beams of carbon (C), argon (Ar) or iron (Fe) were irradiated on *H. pluvialis* at various doses (C: 0-200 Gy; Ar: 0-100 Gy; Fe: 0-75 Gy), and the number of colony forming units was counted (Fig. 1). The LETs were 22.5 keV/μm, 309 keV/μm, and 790 keV/μm, for C, Ar, and Fe, respectively; thus, the number of colonies formed changed in the order of LETs in *H. pluvialis,* and the overkill reported with *Arabidopsis* did not occur [2].

The cell culture irradiated with the heavy-ion beam of each ion species and dose was inoculated on an agar plate (Fig. 1). To isolate robust strains, the plates were subjected to endurance screening, which included incubation for 3-12 months until the nutrient medium dried up. Most of the cells were died to emit white fluorescence under UV irradiation.

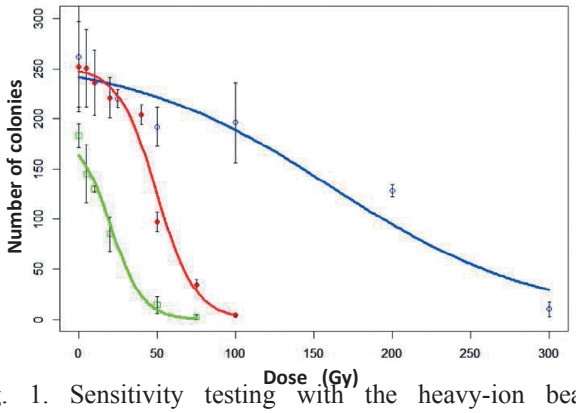

Fig. 1. Sensitivity testing with the heavy-ion beam. Following heavy-ion beam irradiation of *H. pluvials* with each dose of the C ion (○), Ar ion (●) and Fe ion (□), 3,000 cells from each culture were inoculated on an agar plate (n=4 or 5).

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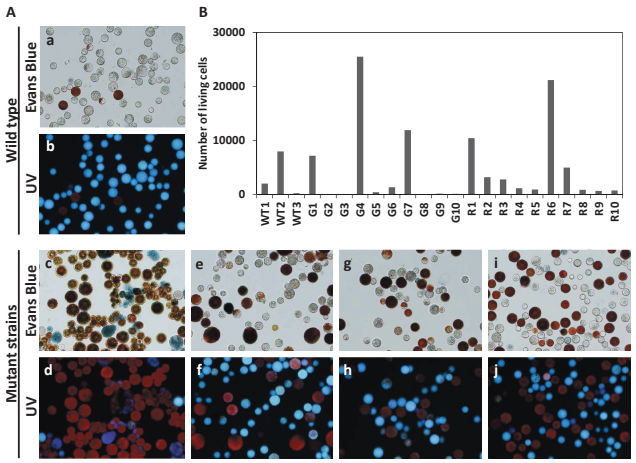

Fig. 2. Microscopic observation (A) and number of living cells (B) in robust strains. WT (a, b), G4 (c, d), G7 (e, f), R1 (g, h), R6 (i, j) in A. Number of living cells (total cell no. × survival rate) in B. Dead cells are stained with Evans blue and emit the white fluorescence under UV irradiation.

After selecting robust strains that were still alive as the red or green cells from dead cells emitting white fluorescence through the use of microscopic observation, they were cultivated with continuous light, and each set of 10 strains maintained a red or a green character. The red strain is a candidate for high astaxanthin accumulation and the green strain is a candidate for high proliferation with chlorophylls. The culture scale was increased to the BBM liquid medium in 100-mL flasks under continuous light (125 μmol photons·m^{-2}·s^{-1}), and sodium acetate solution (final concentration: 45 mM) was added to induce astaxanthin accumulation. The survival rate of the wild type strain (WT1-3) at 7 days post-inoculation was 0-6.2% under these conditions (Fig. 2Aa,b and B). The number of living cells, as determined by cell count, and survival rate were high in G4 (91.8%), G7 (74.9%), R1 (47.2%), and R6 (61.7%) (Fig. 2Ac-h and B). Isolates G4, G7, R1, and R6 indicated high chlorophyll and carotenoid production rates, and G7 and R1 showed conspicuously high carotenoid production (data not shown).

The heavy-ion beams irradiated into the four robust strains were the Ar ion beam at 25 Gy, and C ion beam at 100 Gy. Under these irradiation conditions, approximately 200 colonies formed on an agar plate (Fig. . It is thought that the irradiation conditions are suitable for the variant construction of *H. pluvialis*.

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References

1) Wayama et al., Plos One **8**(1), e53618 (2013).
2) Kazama et al., Plant Biotechnol. **25**(1), 113-117 (2008).