Isolating mutant red algae *Agardhiella subulata* using C-ion-beam irradiation

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Agardhiella subulata, commonly known as edible red algae, is a species that produces phycoerythrin,[1] which has effective antihypertensive[2] and anti-inflammatory activities.[3] Increasing the production of active ingredients by *A. subulata* has received considerable interest. New cultivars with a high yield, high environmental adaptability, or high concentrations of constituents with human health benefits should be bred for enhancing the value of *A. subulata*. We determined the appropriate C-ion beam conditions for effectively inducing the red algae mutagenesis.

Small fragments of *A. subulata* were cultured with NORI SEED (Daiichi Seimo Co., Ltd.), a highly enriched culture solution. A culture medium was prepared using 160 μL of NORI SEED diluted with 300 mL of autoclaved sea water. Small fragments were allowed to develop into regenerative buds for three weeks in a stirred 300 mL marine flask with the culture medium. The flask was placed at 25°C under a 12 hours photoperiod, with a light intensity of 50 μmol photons $\cdot$ m^{-2}s^{-1}. The culture medium in the flask was replaced every week. The regenerative buds were placed into 5 mL tubes under each irradiation condition. Heavy-ion-beam irradiation was performed using C (LET = 30 keV/μm), with eight irradiation doses of 5, 10, 20, 40, 70, 100, 200, or 400 Gy generated at the RIKEN RI-beam factory.[4] Doses above 70 Gy strongly inhibited proliferation and were unsuitable for screening.[5] The mutant lines were established at doses below 40 Gy through isolating the ion-beam-irradiated sections five times using the regenerative 5 mm tip of the algae containing the growing point.[6]

We developed a large-scale screening method for the initial selection, which was based on the length of the algae and the number of branches. We selected 38 lines from 4,800 C-ion-beam-irradiated regenerative buds (Table 1). Each mutant line was agitated for the secondary selection and selected based on the wet weight and number of lateral branches per unit. Five lines were isolated (Fig. 1), and 40 Gy of C-ion-beam irradiation was found to be the optimum dose for selecting useful mutant algae (Table 1). We plan to conduct large-scale screening using by carbon-ion-beam irradiation under the optimum condition to screen more high-growth mutant lines.

This study was performed with a commissioned research fund provided by The Fukushima Institute for Research, Education and Innovation (F-REI) (JPFR24030102).

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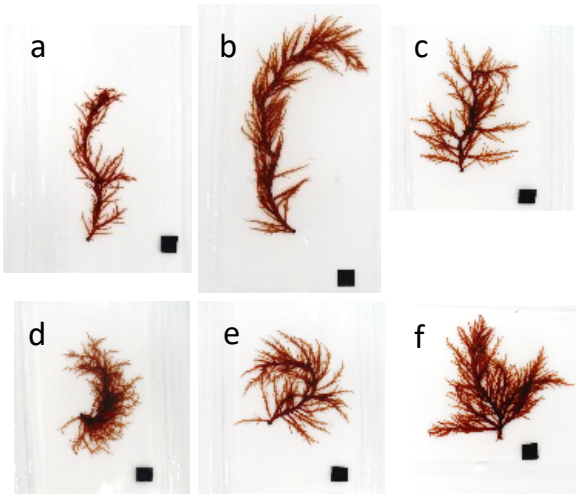

Fig. 1. Comparison of control and mutant algae. (a) A control whole-body figure. (b-f) Isolated mutant whole-body figures. All mutants have many lateral branches than control. Black block indicates 1 cm square.

Table 1. Frequencies of mutant algae established by C-ion beam irradiation.

Irradiation dose (Gy)	Irradiated regenerative buds (No.)	1st screening (No.)	Mutant frequency (%)	2nd screening (No.)	Mutant frequency (%)
5	1200	8	0.7	0	0.0
10	1200	6	0.5	0	0.0
20	1200	11	0.9	1	0.1
40	1200	13	1.1	4	0.3
Total	4800	38	0.8	5	0.1

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DOI:10.34448/RIKEN.APR.58-177