Isolation method of marine red alga *Agardhiella subulate*

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Agardhiella subulate, a red macroalga, is commonly known as seaweed and it is consumed as aquatic food. This sea vegetable is attracting increasing attention as an emerging source of bioactive natural products.[1] Breeding new cultivars with properties such as high yield, high environmental adaptability, or high concentrations of constituents with human health benefits is desirable to enhance its value. In this study, we attempted to establish mutagenesis in *A. subulate* using an argon (Ar) ion beam as mutagen to meet future demands. First, we observed the growing point of the red algae.

The *A. subulate* were cultured with NORI SEED (Daiichi Seimo Co., Ltd.), a highly enriched culture solution. A culture medium was prepared using 80 μL of NORI SEED diluted with 300 mL of autoclaved sea water. Microplantlets were cultivated for a month in a stirred 300 mL marine flask with the culture medium. The flask was placed at 25°C under a 12 h photoperiod, with light intensity of 50 μmol photons $\cdot$ m^{-2}s^{-1} and the culture medium in the flask was replaced every week. Each microplantlet was inoculated into a well of a 12-well plate containing 2 mL of fresh culture medium.

For a morphometrical evaluation, we took photographs of growing tips of the algal thalli every day. From the comparison of the branching patterns in each image, the curved structure was observed in the same position (Fig. 1c, arrow head), whereas a difference was found in the newly elongated tissue (Fig. 1c, white bar). This result indicates that cell division and cell elongation occurred in this region and there were growing points in the tips of the algal thallus.

To develop an isolation method for the mutant line, we applied a heavy-ion-beam irradiation system to *A. subulate*. The microplantlets were replaced into 15 mL conical tubes with fresh 2 mL of culture medium at each irradiation condition. The tubes containing microplantlets were placed in the automatic sample irradiation apparatus[2] and each of them was irradiated with a heavy-ion beam. Heavy-ion-beam irradiation was performed using Ar (LET = 188 keV/μm) at 6 irradiation doses of 1, 2, 4, 8, 16, and 32 Gy generated via RIKEN RI Beam Factory.[3] Four hours after irradiation, each microplantlet was inoculated into a well of the 12-well plate containing 2 mL of fresh culture medium for each irradiation condition. The plates were placed at 25°C under a 12 h photoperiod with light intensity of 50 μmol photons $\cdot$ m^{-2}s^{-1} and the culture medium in the dishes was replaced every week. After monthly culture, the morphological mutants were selected with microscopic observation. The most front edge of the algal thallus was cut off with 5 mm length and replaced into a well of a 6-well plate containing 5 mL of fresh culture medium. Mutant lines were established by repeating these processes four times (Fig. 2a). According to the comparison between control and mutant lines, significant differences were observed in shape and growth ratio (Figs. 2b and 2c). These phenotypes were stably observed at each isolation process. These results suggest that the possibility of chimericalness in the apical meristem has been excluded. This method will be useful in the future to screen valuable mutants with human benefits.

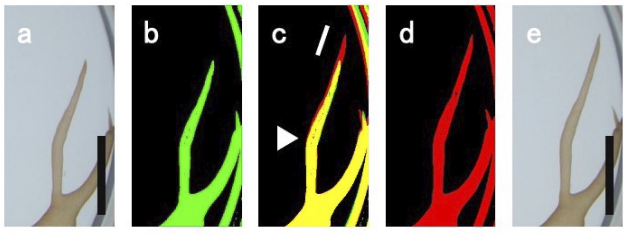

Fig. 1. Photographs of growing front edges of algal thalli. (a) Control branching pattern of red alga. (b) Binarized image of (a). (c) Merged data. The arrow head represents the bended structure of thalli. The white bar represents the difference in the length. (d) Binarized image of (e). (e) One day after branching pattern of red alga. Scale bars represent 2.5 mm.

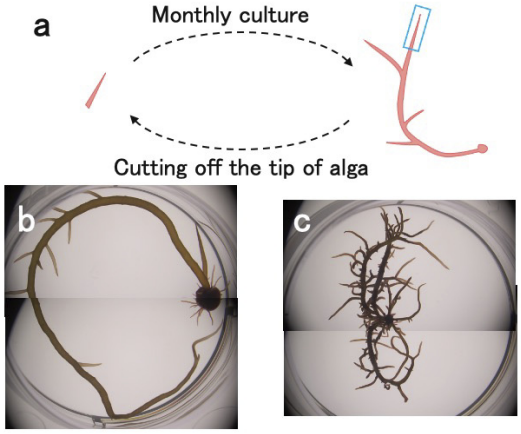

Fig. 2. Mutant isolation method and isolated mutant alga. (a) Diagram of the mutant isolation method. (b) A whole-body figure of the control alga. (c) A whole-body figure of the mutant alga.

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References
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