
Isolation of fucoxanthin-deficient green mutants from the haptophyte *Tisochrysis lutea*

Shohei Kubo*¹, Tomoharu Sano², Masanobu Kawachi², Yoshihiro Hase³, Katsuya Satoh³, Yutaka Oono³, Yasushi Iwata⁴, Hiroya Araie⁵, Kohei Yoneda⁶, Yoshiaki Maeda⁶, and Iwane Suzuki^{†6}

¹Graduate School of Science and Technology, University of Tsukuba, Tennoudai 1-1-1, Tsukuba, Ibaraki 305-8572, Japan – Japan

²Biodiversity Division, National Institute for Environmental Studies, Tsukuba, Ibaraki, 305-8506, Japan – Japan

³Department of Quantum-Applied Biosciences, Takasaki Institute for Advanced Quantum Science, National Institutes for Quantum Science and Technology, 1233 Watanuki, Takasaki, Gunma 370-1292, Japan – Japan

⁴Research Institute for Advanced Electronics and Photonics, National Institute of Advanced Industrial Science and Technology, Tsukuba Central 2, Tsukuba 305-8568, Japan – Japan

⁵Department of Biosciences, Kanto Gakuin University College of Science and Technology, Mitsuura-higashi, Kanazawa-Ku, Yokohama, Kanagawa 236-8501, Japan – Japan

⁶Institute of Life and Environmental Sciences, University of Tsukuba, Tennoudai 1-1-1, Tsukuba, Ibaraki 305-8572, Japan – Japan

Abstract

Fucoxanthin is one of the light-harvesting pigments that is reported to have antioxidant, anticancer, and antidiabetic effects. However, its biosynthetic pathway is not fully understood. In the present study, we aimed to isolate and analyze fucoxanthin-deficient mutants of the haptophyte *Tisochrysis lutea* with irradiation of heavy ion beams. The cells of *T. lutea* (C8-14 strain) suspended in MA-ESM (artificial seawater) medium were irradiated with 45 to 120 Gy of carbon ion beams and then spread on agar-solidified MA-ESM. The survival rate decreased upon the irradiation dose increase, reaching approximately 10% at 65 Gy (Figure 1). We isolated two green-colored mutants, namely GR1 and GR2, after irradiation at 45 Gy and 100 Gy, respectively.

Analysis of the pigments by liquid chromatography equipped with mass spectrometry (LC-MS) revealed that the green mutants lost fucoxanthin and implied that each GR1 and GR2 strain may accumulate haptoxanthin and phaneroxanthin, respectively, that are reported fucoxanthin precursors. We subsequently determined the whole-genome sequence in both strains and found that a gene for zeaxanthin epoxidase 1 (ZEP1) and a gene for carotenoid isomerase 5 (CRTISO5) were mutated in the GR1 and GR2, respectively. ZEP1 converts haptoxanthin into phaneroxanthin, and CRTISO5 converts phaneroxanthin into fucoxanthin. The similarities between the orthologs from the diatom *Phaeodactylum tricorutum* and *T. lutea* were 68% and 75%, respectively. This is the first findings of the genes for fucoxanthin synthesis in *T. lutea*. Figure 1: Survival rate of *Tisochrysis lutea* strain C8-14 cells after irradiation of the carbon ion beams.

*Speaker

†Corresponding author:

Keywords: Haptophyte, Fucoxanthin, Heavy ion, Mutant, Genome