Simple addition or depletion of uracil can regulate the switch for controlling symbiosis between cnidarian host and pyrimidine auxotroph *Symbiodinium* mutant

Y. Ishii[1], S. Maruyama[1], K. Fujimura-Kamada[2], N. Kutsuna[3,4,] S. Takahashi[2,5], M. Kawata[1], J. Minagawa[2,5]

1. Department of Environmental Life Sciences, Graduate School of Life Sciences, Tohoku University, Sendai, Miyagi, Japan

2. Division of Environmental Photobiology, National Institute for Basic Biology, Okazaki, Aichi, Japan

3. Department of Integrated Biosciences, Graduate School of Frontier Sciences, University of Tokyo, Kashiwanoha, Chiba, Japan

4. LPixel Inc., Bunkyo, Tokyo, Japan

5. Department of Basic Biology, School of Life Science, SOKENDAI (The Graduate University for Advanced Studies), Okazaki, Aichi, Japan

Symbiodinium spp. are a dinoflagellate known to sustain a stable symbiotic relationship with cnidarian animals (e.g. coral, sea anemone, jellyfish) via endosymbiosis in the gastrodermal (endodermal) cells of host cnidarians. These algae play key roles as a primary producer in the coral reef ecosystems in the oligotrophic tropical and subtropical oceans. Although recent studies including draft genome sequences of three *Symbiodinium* species (*S. minutum, S. kawagutii and S. microadriaticum*) and transcriptome experiments have suggested many candidate genes potentially possessing symbiosis-related functions, there are a lot of difficulties lying ahead for functional analysis of symbiosis-related genes due to a lack of genetic tools, i.e. readily available mutant strains and established methods of transformation. Toward developing genetic tools for coral symbiosis, we isolated uracil auxotroph *Symbiodinium* mutants using 5-fluoroorotic acid, which inhibited the growth of cells expressing *URA3* encoding orotidine-5'-monophosphate decarboxylase. After culturing *Symbiodinium* sp. strain SSB01 in the presence of 5-fluoroorotic acid, we isolated cells possessing mutated *URA3* genes and requiring uracil for growth. Sequence analyses and genetic complementation tests using yeast demonstrated that one of the mutant cell lines had a point mutation in *URA3* gene, resulting in a splicing error at an unusual exon–intron junction ('GA-AG"G"' type), and consequently, loss of the enzyme activity. This mutant could maintain a symbiotic relationship with the model sea anemone *Exaiptasia pallida* only in sea water containing uracil, demonstrating that the symbiotic status could be switched by the simple addition or depletion of a nutrient. These suggest that genetic mutation(s) in the *Symbiodinium* genome directly affect the cell growth as well as symbiotic ability.