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Effects of Nutrition on the Physiology of Zooxanthella *Symbiodinium microadriaticum*
CCMP829

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Many marine invertebrates in tropics and subtropics have symbiotic dinoflagellates referred to as zooxanthellae. A previous study on the morphology of zooxanthellae reported two distinct morphologies and motility: one is a motile oval shape and the other is an immotile spherical shape. The oval shape can be observed when zooxanthellae are isolated from their hosts and kept in culture conditions. In contrast, the spherical shape is exclusively observed *in hospite*. Zooxanthellae acquire its motility in response to the morphological changes from spherical to oval (Kawaguchi 1944, Palao Tropical Biological Station 2: 675-679). Since this first report, yet, no inducer that is involved in the mechanism for the motility change has been discovered.

We hypothesized that inorganic nitrogen compound(s) taken up by zooxanthellae induce the changes in motility as well as morphology. Zooxanthellae *in hospite* can utilize the waste metabolites, e.g. ammonium ions (NH_4^+) from the host. In contrast, they could take nitrate ions (NO_3^-) up from seawater in a free-living motile stage. NO_3^- and NH_4^+ are simple N-containing molecules that can be ubiquitously found in natural environment, and the source for nitrogen assimilation in plants and algae.

We used *Symbiodinium microadriaticum* CCMP 829 as a model system to explore inducers for the motile changes in zooxanthellae. For zooxanthellae culture, a series of the modified K-medium was applied. NO_3^- and NH_4^+ were examined as nitrogen sources with the concentrations of 100, 250, 500 and 1000 μM for each. The K-medium without supplemental NO_3^- and NH_4^+ was used as control. Light condition was a 12 light ($20\mu\text{mol m}^{-2} \text{s}^{-1}$) and 12 dark cycle for culture. The concentrations of $\text{NO}_3^- + \text{NO}_2^-$, NO_2^- and NH_4^+ were measured using an automated water quality analyzer (QuAAtro, BRAN+LUEBBE, Germany). The background concentrations of each ion species were lower than 10 μM .

We cultured zooxanthellae for four weeks, observing motile activity of zooxanthellae. After three days from inoculation, a substantial proportion of the cells exhibited active motility under microscopic observation. Cell density and motility were estimated with a hemocytometer under a microscope to determine the ratio of motile cells to total cell number. The results showed a proportional relationship between the motility and the concentration of NO_3^- supplemented in the culture medium. The NO_3^- -dependency of the motility was not observed in a culture incubated for longer than one week.

These results suggest that NO_3^- is capable of inducing the zooxanthellae motility. We will discuss the possibility that physiological change of zooxanthellae induced by NO_3^- and necessary conditions for the induction of zooxanthellae motility under natural environments.