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Distribution and diversity of chlorophyll *d* containing cyanobacteria

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Almost all oxygenic photosynthetic organisms (land plants, algae and cyanobacteria) contain chlorophyll (Chl) *a* as their major pigment, and utilize visible light (400-700 nm in wavelength) for photosynthesis. This spectral range of radiation is called as photosyntherically active radiation (PAR), which has been thought to be necessary for oxygenic photosynthesis. *Acaryochloris* is a genus in the Cyanobacteria, which contains chlorophyll (Chl) *d* as the predominant pigment. They can utilize far-red light (700-750 nm) in addition to photosynthetically active radiation (PAR: 400-700 nm) for oxygenic photosynthesis. To reveal the ecological significances of this organism and the Chl *d*-based photosynthesis, the basic ecological studies on *Acaryochloris* were required. In this study, we aimed to investigate the distribution, diversity and amounts of *Acaryochloris* spp. using molecular biological methods.

The polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) was used to detect epiphytic *Acaryochloris* cells from macroalgae. However, *Acaryochloris* cells were hardly detected from most samples of didemnid ascidian and sponge which were collected from the coasts of the Republic of Palau, because of predominant cyanobacterial symbionts existing in these invertebrates. Then, I designed and used the *Acaryochloris*-selective primer set to detect *Acaryochloris* cells and analyzed phylogenetic diversity of them. By using this selective detection method, many phylotypes of *Acaryochloris* were detected in invertebrate samples collected from Palau and macroalgae collected from the coast of Japan and South Africa. Moreover, *Acaryochloris* phylotypes were also detected from pebbles, sands and seawater samples collected from Japanese coast. These results revealed that *Acaryochloris* spp. could randomly attach to various substrates, and they were widely distributed from tropical to subarctic region. It was also revealed that many and diverged phylotypes of *Acaryochloris* were distributed in environments. Phylogenetic analyses demonstrated that these phylotypes were diverged into three phylogenetic subgroups which were different one another at least in species level.

The detection and quantification methods for Chl *d* from environmental samples were also developed by employing high performance liquid chromatography, and I determined the amounts of Chl *d* extracted from macroalgal samples. As a result, certain amounts of Chl *d* were detected from most macroalgae, and the ratio of Chl *d* to Chl *a* was approximately 1% on average.

In conclusion, it was revealed that certain amounts of *Acaryochloris* spp. were globally distributed around the coastal environments, and the ratio of Chl *d*/Chl *a* reached up to approximately 1% in the seaweed beds. It suggested that Chl *d* could contribute about 1% of the primary production in seaweed bed area, which has been neglected from the estimation of carbon cycle at the coastal environments.

Keywords: chlorophyll *d*, cyanobacteria