Physiological Characterization of Symbiotic *Chlorella* Isolated from Japanese *Paramecium bursaria*. — Their Nitrogen Utilization and Photosynthesis —

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Green ciliate *Paramecium bursaria* has a symbiotic association with green alga *Chlorella*. This endosymbiosis is not an absolute one and thus symbiotic algae can be isolated and cultured independently. Previous studies were performed using European and American *P. bursaria* and their symbiotic *Chlorella*. Recently, axenic strains of Japanese symbiotic *Chlorella* have been established. Some specific characteristics of the Japanese symbiotic algae on nitrogen utilization were revealed, however, the details of its physiological characteristics were still unclear.

Study on nitrogen utilization of the Japanese symbiotic *Chlorella* was carried out and the addition of Arg, Ala, Ser, Gly, Asn and Gln caused an algal remarkable growth. Amino acid transport by *Chlorella* spp. was measured using radiolabeled amino acids, and the results indicated that free-living *Chlorella* could import only Arg. In contrast, the Japanese symbiotic *Chlorella* could import all 20 amino acids via at least three amino acid transport systems. Amino acid transport by the Japanese symbiotic alga was inhibited by Ca^{2+} up to approximately 80%, which is known as an activator of amino acid transport. The inhibition was recovered by sugars including maltose which was released by the symbiotic alga. Sugars increased amino acid transport at low concentration (for Glc, EC_{50} = 3μM), although the symbiotic alga could not import these sugars. In addition, non-metabolizable sugars also activated amino acid transport. Therefore, it is considered that sugars act as a signal.

To investigate the relationship between host and symbiotic algae, the effect of *P. bursaria* extract on algal photosynthesis was previously evaluated, suggesting the existence of algal carbon fixation-enhancing factor (host factor) in the extract. The factor was identified as a mixture of K^+, Ca^{2+} and Mg^{2+}. An amount of fixed carbon was changed with the concentrations of the cations. The concentrations of the cations in green *Paramecium* were more suitable for algal carbon fixation than those in non-symbiotic *Paramecium*. Oxygen evolution by the symbiotic alga was rapidly stopped when the extracellular cations were removed, therefore the alga required the extracellular cations for its photosynthesis.

From the existence of the constitutive amino acid transport systems of the Japanese symbiotic alga and the effects of Ca^{2+} and sugars on the amino acid uptake, amino acid transport between host and symbiotic *Chlorella* and its possible regulatory system were discussed. In addition, from the low adaptation of the symbiotic alga to change in extracellular condition, degeneration of the symbiotic alga via the *Paramecium* endosymbiosis was also discussed.