Interaction between host and symbiont in *Paramcium bursaria* symbiosis - Establishment of Japanese axenic symbiont and Effect of host extract -

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The freshwater protozoan green paramecium, *Paramecium bursaria* , has *Chlorella* -like green algae in its cytosol. The organism has been used as a subject for research on encosymbiosis because host cell and symbiont can be separated and reconstructed from the partners. The properties of symbiont not only differed from those of free-living *Chlorella* sp., but also there were different between European and American symbiont. However, Japanese symbiont has been difficult to establish axenic strains, and the properties were unknown.

To establish axenic algal strains from such Japanese *P. bursaria,* the host cell was crushed and applied to algal agar medium that makes nitrate the only source of nitrogen. All algal colonies, which were grown until size recognized with naked eye were contaminated with various bacteria. However, at short period of incubation axenic micro colonies were discovered though a microscope. Thus these colonies were streaked onto the agar medium including various organic nitrogen compounds. As a result, the axenic symbiont was grown onto the bacto-peptone-supplemented medium, and their axenically was confirmed by DGGE-PCR, and various tests of bacterial contamination. Thus I succeeded in establishing the axenic strains of Japanese symbiont for the first time. In addition, from results of utilization of nitrogen, Japanese axenic symbionts were maintained in the medium supplemented amino acid, such as **L**-serine, as only source of nitrogen. Furthermore, nitrate assimilation-related enzymes were examined, the activity of nitrate reductase of Japanese symbiont was not, and that is differed from those of European and American symbiont. Therefore, Japanese symbiont was depended on the host rather than European and American symbiont.

To investigate relationship via substance(s) between host and symbiont, effect of the host extract on photosynthesis of symbiont was examined using for ^{14}C labeled CO_2. As a result, it is quite phenomenon that the host extract enhanced carbon fixation of symbiont at increased concentration. Whatever the reproducible and confirmational experiments, such as the host extract was removed carbonate, were repeated, carbon fixation of symbiont was enhanced. Thus, I considered that some kind of substance(s) in the host extract, which was defined host factor enhanced carbon fixation of symbiont. The host extract enhanced carbon fixation of free-living chlorellas, and this effect was not species specificity. Furthermore, from results of heat treatment, ultrafiltration and ODS treatment of the host extract, it had an assumption that the host factor was heat-stable, low molecular weight and water-soluble substance(s). While release of photosynthate by symbiont were not increased by the host extract as reported in marine symbiosis, but at low pH released photosynthate was increased as well as European and American symbiont.