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Studies of symbiotic association between green paramecia and their symbiotic algae using feedless culture strain of *Paramecium bursaria*

<u>Hiroshi Hosoya</u> (Department of Biological Science, Faculty of Science, Kanagawa University, Kanagawa, Japan)

The green paramecium, Paramecium bursaria, is a unicellular protist, which harbors hundreds of symbiotic algae in its body. It is widely known as a research target for eukaryotic cell-cell symbiosis. Many experiments have been conducted and their results have been also reported for the purpose of clarifying phototaxis, photosynthesis of symbiotic algae and cell proliferation, using Paramecium bursaria. However, in each researcher, various kinds of microorganisms including bacteria are used as food when culturing the ciliate. Further, lettuce infusion is used as a medium, but the composition of lettuce leaf is not always constant. Due to these multiple reasons, common culture conditions have not been established among Paramecium bursaria researchers. This gives a significant problem on the reproducibility of the results of various experiments using Paramecium bursaria. Therefore, in our laboratory, it has been investigated whether Paramecium bursaria collected from the field can be cultivated without feeding. As a result, a "feedless culture strain (KUNY-2)" was established. This strain was isolated from the field in 2015, cultivated with feeding until 2017, and then has been cultivated without feeding until now. Interestingly, it was revealed that bacteria were always present in the culture medium of KUNY-2, even under the condition that the prey bacteria were not fed. Therefore, the composition of bacteria in Paramecium bursaria was analyzed at each time point before and after start of culture without feeding. As a result, it has been clarified that a certain type of bacteria is always detected in the body of Paramecium bursaria. Symbiotic algae in Paramecium bursaria can be easily isolated from the host and cultured. In addition, by co-culturing this cloned symbiotic algae with the host, the endosymbiotic relationship can be simply re-established. Then, Liquid culture of isolated symbiotic algae was carried out, and bacteria in the symbiotic algae were also examined. As a result, it became clear that the types of bacteria detected in each of the host and the symbiotic algae are different. Here, we discuss the role of bacteria in establishment of symbiosis between Paramecium bursaria and symbiotic algae.

2pmrlccelldivision@gmail.com (Hiroshi Hosoya)