Taxonomic Studies on Lichenized Basidiomycetes and their Photobionts in Japan: Towards the Establishment of a Model Co-culture System of Lichen Symbiosis

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Hiroshi Masumoto

Abstract

Among about 100,000 known species of fungi, about 20% of them form symbioses with green algae or cyanobacteria. The fungus and alga that constitute the lichen thallus are called mycobiont and photobiont, respectively. In lichen symbiosis, fungi and algae are generally considered to be in a mutualistic relationship. However, symbiont interactions are still only partially understood. For example, in terms of nutrition transfer, although there is evidence that the photobiont gives photosynthates to the mycobiont, what fungi give to algae is not yet understood. In addition, how fungi and algae cooperate to form the lichen thallus and how they recognize each other are also poorly understood. Therefore, the establishment of a model co-culture system may contribute much toward elucidating the nature of lichen symbiont interactions.

In this study, I attempted to establish a model co-culture system using lichenized basidiomycetes. This is because while the growth of lichenized ascomycete is generally very slow, that of lichenized basidiomycetes is expected to be relatively faster based on the fact that they have the potential to form seasonal fruiting bodies in a short period. Between 2017 and 2019, including some specimens collected before this study, 5 genera (*Bryoclavula* gen. nov. ad int., *Dictyonema*, *Lichenomphalia*, *Multiclavula,* and *Sulzbacheromyces*) and 9 species of lichenized basidiomycetes from Japan were collected and identified. From these collections, 5 genera and 7 species of the collected lichenized basidiomycetes were established in culture, and the photobionts of all collected lichenized basidiomycetes were successfully cultured. Co-culture experiments were then carried out between the fungus with the fastest growth and its photobiont, and the culture conditions in which the thallus formation was induced were determined.

The fruiting body of the specimen *H. Masumoto 293* was similar to that of *Multiclavula*, but the thallus had an undifferentiated structure that differed from the globular thallus units typical of *Multiclavula*. Phylogenetic analyses revealed that *H. Masumoto 293* was placed in Cantharellales along with *Multiclavula*, but they were in different lineages. Therefore, this taxon was proposed as a

new species *Bryoclavula phycophila* gen. et sp. nov. ad int. The photobiont of *B. phycophila* was found to be a member of the genus *Elliptochloris*, but identification of the photobiont at species level was impossible because several undescribed *Elliptochloris* species were isolated together from the same thallus. In the genus *Dictyonema*, *D. moorei* was obtained. The photobiont of *D. moorei* was identified as a member in the genus *Rhizonema*. Pure cultures both of the genus *Dictyonema* and its photobiont *Rhizonema* were established for the first time. In the genus *Lichenomphalia*, 3 species were obtained: *L. hudsoniana*, *L. meridionalis* (new to Asia), and *L. umbelllifera* (new to Japan). Their photobionts were all identified as *Coccomyxa subellipsoidea*. A pure culture of *L. meridionalis* was established for the first time. In the genus *Multiclavula*, 3 species were obtained: *M. mucida*, *M. petricola* sp. nov. ad int., and *M. vernalis* (new to Japan). *Multiclavula petricola* is unlike other *Multiclavula* species in that it occurs on rocks. The photobiont of *M. mucida* was identified as the green alga *E. subsphaerica* with the aid of transmission electron microscopy (TEM). The photobiont both of *M. petricola* and *M. vernalis* were determined to be an undescribed species genetically related to *E. subsphaerica*. In the genus *Sulzbacheromyces*, *S. sinensis* was obtained. The photobiont of *S. sinensis* was found to be an undescribed species in the genus *Bracteacoccus*.

Among the cultured lichenized basidiomycetes, *M. mucida* showed the fastest hyphal growth. Therefore, co-culture experiments of *M. mucida* and its photobiont *E. subsphaerica* cultures were performed to determine the condition under which thallus formation was induced. The hyphae surrounded several algal cells and formed the thallus only when co-cultured on a balsa wood plate placed on corn meal agar. Through this co-culture experiment, thallus formation of *M. mucida* was successfully induced *in vitro* for the first time. At the same time, the cell condition of *E. subsphaerica* in the co-culture was compared with that in the single culture. In the single culture of the photobiont, the chloroplast did not develop, whereas in the co-culture, the chloroplast developed well, strongly suggesting that the photobiont is getting something essential from the mycobiont. The present study would contribute to reveal hitherto unelucidated relationships between fungi and algae in lichens which have been often vaguely recognized as “symbiosis”.