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SUMMARY OF DOCTORAL THESIS

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Title: The impact of host plant (*Pinus thunbergii*) on the mycelial features of the ectomycorrhizal mushroom *Rhizopogon roseolus*

(外生菌根菌ショウロの菌糸形状に及ぼす宿主クロマツの影響)

Most cytology reports on ectomycorrhiza (ECM) have been less focused on the fungal structures, especially on the exploratory organ. Chapter 2 in this this study aimed to evaluate the morphological response of explorative mycelia of ECM forming fungi with and without the occurrence of the ECM host. I assessed the mycelial aggregates of *Rhizopogon roseolus* which was inoculated with and without *Pinus thunbergii* under controlled laboratory conditions. The mycelial aggregates with the host produced strikingly complex hyphal strands relative to those without host. Light microscopy revealed that the cytology and plectology of both mycelial aggregates had the approximately similar architecture. The tubular hyphae diameter without the host was consistently larger than that with the host. This study confirmed that the septa diameter of the tubular hyphae conjunction of mycelial aggregates with the host were shorter than those without the host. In addition, partially and completely dissolved septa of the tubular hyphae were evident with and without host. I also described the thromboplerous hyphae, which have rarely been reported in vitro. These hyphae were produced in higher numbers near the fungal inoculum with and without the host. However, further investigation needs to be done regarding the morphogenesis and specific role of the thromboplerous hyphae.

The impact of host plant on the mycelial features of mycorrhiza symbiont and its characteristics has been poorly investigated. Chapter 3 in this study aimed to compare and quantify (statistically tested) some of the mycelial features of an ectomycorrhiza (ECM) forming fungus with and without the ECM host. The ECM forming fungus, *R. roseolus,* inoculated with or without *P. thunbergii* on both rich and poor nutrient media, was observed under laboratory conditions. On rich medium, fungi with the host had the highest colony diameter and consistently produced the highest hyphal length relative to fungi on other media. Thus, the host had a significant impact on the mycelia production of *R. roseolus* in both rich and poor media. Further, fungi without the host had higher hyphae anastomosis numbers per hyphal length on both poor and rich media than fungi with the host in the same medium. Anastomosis, which refers to the fusion of two parallel hyphae, was evident in all experiments. However, there was no significant impact of the host on the number of hyphal anastomoses. In addition, fungi without the host had more frequent hyphal branching both on rich and poor media than fungi with the host. The occurrence of a host only had a significant impact on the formation of the hyphal branch on poor medium. Further, a chlamydospores-like structure was identified, which had a higher diameter when formed with the host both on rich and poor media.

During the assessment of mycelial cords in chapter 3, I noted the occurrence of sclerotium on extraradical mycelia. The sclerotia were less than 0.27 mm and can be observed after 2 months of incubation. I found and suspected the small structures like spores inside the sclerotium. These structures were ellipsoid, hyaline, with the smooth

surface. I then incubated the sclerotium and these small structures on TM7 detecting medium whether they can produce the secondary mycelia of *R. Roseolus*, but no germination was observed. Interestingly, the bacterial colonies which connected to the hyphae of sclerotium were appeared. The colonies were transferred to Luria agar (LA) medium. The morphological observation of bacterial cells from TM7 and LA confirmed that they were the same as the small structures inside the sclerotium. This data is the first report on the occurence of sclerotium of *R. roseolus* on pure cultures. Further study is required to reveal the role of bacteria on the production of sclerotium of *R. Roseolus.* In addition, the investigation regarding the origin of the bacteria and mechanism of bacterial transfer to the sclerotium should be done.

While observing the mycelial features of the ectomycorrhizal (ECM) fungus, *R. roseolus* in chapter 4, I noted the formation of hyphal coils in laboratory cultures. The coiled hyphae initially formed at the hyphal tip of the ECM fungi with or without the plant host (*P. thunbergii*), both in rich and poor Modified Melin-Norkrans (MMN) media. Hyphal coils formed from the hyphal tips to the center of the hyphae, with rope-like hyphal strands fused to the extensive circles. Hyphal coils were generally round, oval, and ellipsoid in shape. They were composed of 1–5 layers of hyphae. The hyphal coil loop was consistently larger without the host than with the host in both media. Host occurrence had a significant impact on the diameter of the coil loops. In addition, the terminal part of the mature coils was melanized and separated from the unmelanized coil by a septum. The melanized coils resembled thick condensed hyphae and were detached and scattered throughout the fungal colony. The observation of morphological characteristics confirmed that the mature coil released into the mycelia is the thromboplerous hyphae. I also observed the formation of thromboplerous hyphae from hyphal coils. This is the first report on hyphal coil morphogenesis and its role in the initial development of thromboplerous hyphae.

The edible ectomycorrhizal (ECM) mushroom *R. roseolus* usually develops basidium and basidiospores in the gleba of its basidiomata. In chapter 5, I report a novel production of basidia in laboratory cultures of the edible ECM mushroom. The basidium with sterigma was observed on the old mantle structure (> six months) of the ECM between *R. roseolus* and *P. thunbergii* in a modified Melin and Norkrans (MMN) medium that was subjected to a temperature shock from 25 °C to 4 °C. The basidia were cylindrical to clavate, with prominent sterigmata and no basidiospores. In addition, branched cystidia were evident in two or three clavate-to-ovoid cells. The absence of basidiospores might indicate partial development of the basidium structure as a response to environmental stress, and incomplete life cycle of *R. roseolus.* This study suggests the possibility of obtaining the primary mycelium of *R. roseolus* from pure cultures and may be an alternative genetic source for cultivation purposes. Further observations are required to induce basidiosporogenesis of *R. roseolus* basidia in an agar medium focusing on temperature. The production of *R. roseolus* spores in laboratory cultures will contribute significantly to improve the breeding of *R. roseolus*.

The findings of the current study will add to the current knowledge regarding host impact to ECM fungi mycelial features. Furthermore, this study reveals some aspects of the fungal response in regard to fungi-plant interaction strategy on the occurrence and absence of the host. This study also provides new information on the origin and morphogenesis of some ECM fungal structures. In addition, this study showed the importance of basic mycology work which essential to clarify the life cycle of an edible ECM mushroom. The better understanding of life cycle of *R. roseolus* will greatly increase the possibility of obtaining spores in laboratory cultures and significantly improved the future works of breeding of this edible mushroom.