A STUDY ON LICHENIZED FUNGI OF TAIWAN FRUTICOSE LICHENS[1]

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Abstract: Eight kinds of mycobionts of Taiwan fruticose lichens were cultivated and produced a basic type of colony. Observations on the single-spore cultures, morphological differentiation of fungal symbionts of these lichens were made. The mycobionts from fruticose lichens were compact, hard in consistency. The cultivated mycobionts rarely produced pigment and also did not produce reproductive structures. Experimental evidence shows that different algae in lichens are associated with different fungi.

INTRODUCTION

In the last issue of Taiwania I reported some experimental observations on the algal symbionts of Taiwan fruticose lichens (Wang-Yang 1970). Along with the study on the algal symbionts of these lichens, attempts were made to isolate and culture the fungal symbionts of the Taiwan fruticose lichens. Observations on single-spore cultures, morphological differentiation and a few of the physiological features of the fungal symbionts of these lichens were made. Based on these experimental evidences on both algal and fungal symbionts of these lichens, it is possible to discover if the different algae in the lichens are associated with the same fungus or with different fungi within each genus.

MATERIALS AND METHODS

A. Materials:

Freshly collected Taiwan fruticose lichens, with ascocarps, were used as the materials for the studies of mycobionts. In the following list are the lichens* from which successful fungal isolations have been made.

Baeomyces placophyllus Ach.
B. roseus Pers.
Cladonia aggregata (SW.) Ach.
Cl. cornuta (L.) Schrad.
Cl. furcata (Huds.) Ach.

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* Species of *Baeomyces* were determined by the author. Species of *Cladonia* were determined by Dr. S. Kurokawa, National Science Museum, Japan. Species of *Stereocaulon* were determined by Dr. I. M. Lamb, Farlow Herbarium, Harvard University.

Cl. rangiferina (L.) Ach.
Stereocaulon chlorocaproids Zahlbr.
St. formosanum Zahlbr.
St. sorediiferum Hue.

B. Methods:

The isolation methods for most lichen fungi used in this investigation is as follows (Ahmadjian 1961):

1. Freshly collected lichen thalli with ascocarps were washed in tap water for fifteen to twenty minutes to remove the epiphytes.

2. Fruiting bodies of lichens were broken off by forceps and affixed by means of vaseline to a petri-dish cover. The bottom half of the dish was filled with a layer of soil-extract-nutrient agar. The top half of the dish was then inverted on the bottom half.

3. Spores were forecably discharged onto the agar layer.

4. Pieces of agar substrate which contained single-spores or many spores were transferred to agar slants with organic nutrient agar (Malt-Yeast-Extract agar) after the germination of the spores.

5. Cultures of mycobionts were maintained in complete darkness at 20°C.

6. Cultures of mycobionts were kept in 250 ml. Erlenmeyer flasks with 90 ml. MEYE and were stocked in the culture room of the department.

OBSERVATIONS AND RESULTS

Species of *Baeomyces* and *Stereocaulon* emitted masses of spores within 24 hours. Species of *Cladonia* emitted spores from 48 hours to 72 hours. Germination of spores from *Baeomyces* and *Cladonia* and *Stereocaulon* were obtained within 24 hours following spore discharge.

Unsuccessful germination of spores from some species was obtained. Hyphal fragments were isolated by the micropipette method from *Cladonia rangiferina*. The mycobiont of *Cladonia rangiferina* was cultured by means of hyphal fragments growing around cells of *Trebouxia* phycobionts at 25°C. Isolated mycobionts were grown on organic nutrient agar at 20°C. Comparative growth and morphological observations were made.

1. Baeomyces placophyllus.

Unsuccessful germination of spores occurred following the discharge of a mass of spores (Pl. 1, Fig. 1). The spores were hyaline, 2-celled and with thick cell walls.

2. Baeomyces roseus

Rapid germination of spores took place after discharge. Growth, after four weeks slowed down. Germinating spores were ellipsoidal or oblong. Hyphal cells of this mycobiont were filled with oil droplets (Pl. 1, Fig. 2).

3. Cladonia aggregata:

Masses of spores were discharged. Spores were 2-celled. Rapid germination and initial growth occurred. The initial colony was pure white. The color of the older portion of the colony changed to light brown as the mycobiont colony grew older. Colony growth stopped after its size reached to 1–2 mm in diameter.

4. Cladonia cornuta

The spores discharged were muriform cells (Pl. 1, Fig. 3). Rapid germination and initial growth occurred. Colonies were white, then turned a bright yellow color. The colony produced a bright, yellow, water soluble pigment in liquid medium.

5. Cladonia furcata

Masses of spores were discharged, followed by rapid germination and initial growth. Colonies stopped their growth in size when they reached 1 mm in diameter.

6. Cladonia rangiferina

The mycobionts of this lichen were isolated by the means of the selection of hyphae. The hyphal fragments from around the *Trebouxia* phycobionts were used. The initial growth of the colony was very rapid, but slowed somewhat as colonies aged (i.e. after 3-months). The diameter of the colonies reached 1 cm. Colonies were convex with a very firm structure, and were compact (Pl. 2, Fig. 1, Fig. 2). As the mycobionts became older, the color of the entire colony changed from light pink to brown to dark brown. Portions of the branching hyphal cells were somewhat swollen (Pl. 2, Fig. 3). Light brown crystals were produced in the older portions of the colony (Pl. 2, Fig. 4).

7. Stereocaulon chlorocaproids

Many spores were discharged and germinated. Spores were very long with numerous oil droplets (Pl. 1, Fig. 4). Hyphal cells of this mycobiont were branched. Mycelia were hyaline, colonies were pure white.

8. Stereocaulon formosanum

Scattered spores with oil droplets were discharged very shortly and a few branching hyphal cells were formed. Growth was slow and stopped after one month.

9. Stereocaulon sorediiferum

Numerous spores were discharged and all germinated. Germinating spores were 4-celled and fusiform. Hyphal cells of this mycobiont were branching. Mycelia were hyaline (Pl. 2, Fig. 5 & 6).

DISCUSSION

Generally, the mycobionts from fruticose lichens are compact, and hard in consistency. The gross features of the colony are similar to the phycobionts. The isolated mycobionts did not show any reproductive structures. The experimental results show that the cultured mycobionts rarely produced pigment. Most of the mycobionts from Taiwan lichens of *Cladonia* slowed down in their rate of growth

after an initial period. Therefore, some physiological aspects of this investigation can not carried out in this part of our experimental investigation. But the early morphological characteristics have been observed. In the previous study of the algal symbionts of Taiwan fruticose lichen (Wang-Yang, 1970), a comparative growth of phycobionts was obtained. That is, the same species of the algal symbionts isolated from different lichen species (i. e. *Cladonia aggregata*, *Cladonia cornuta*, *Cladonia furcata*) showed physiological strains of phycobionts. From the results of this investigation, it is shown that different species of lichens contain the same species of phycobionts but are different in strain, their fungal symbionts are different. Experimental evidence reveals that the different algae in *Cladonia* are associated with different fungi. Two other species of lichens *Stereocaulon chlorocaproids* and *St. sorediiferum* also supports this evidence. The algal component is more commonly used in separating certain groups of species in each lichen genus. At least, from this investigation, it does seem probable that different species of fungi will produce different lichens with different strains of the same algae species.

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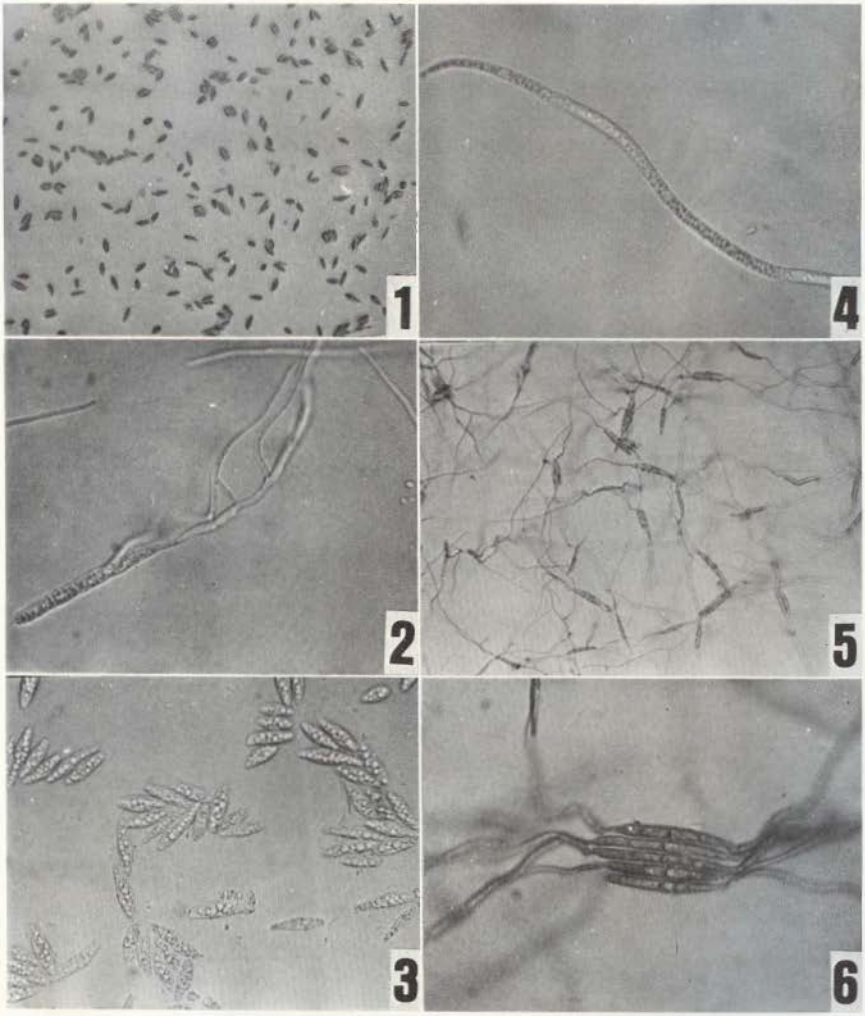

EXPLANATION OF FIGURES

Plate 1.

Fig. 1. Discharged spores of *Baeomyces placophyllus.* x100.
Fig. 2. Germinating cells from discharged spores of *B. roseus.* x150.
Fig. 3. Muriform spores of *Cladonia aggregata.* x100.
Fig. 4. Spore showing numerous oil droplets, from *Stereocaulon chlorocaproids.* x4
Fig. 5. 4-celled, fusiform spores from *St. sorediiferum.* x40.
Fig. 6. 4-celled, fusiform spores from *St. sorediiferum.* x400.

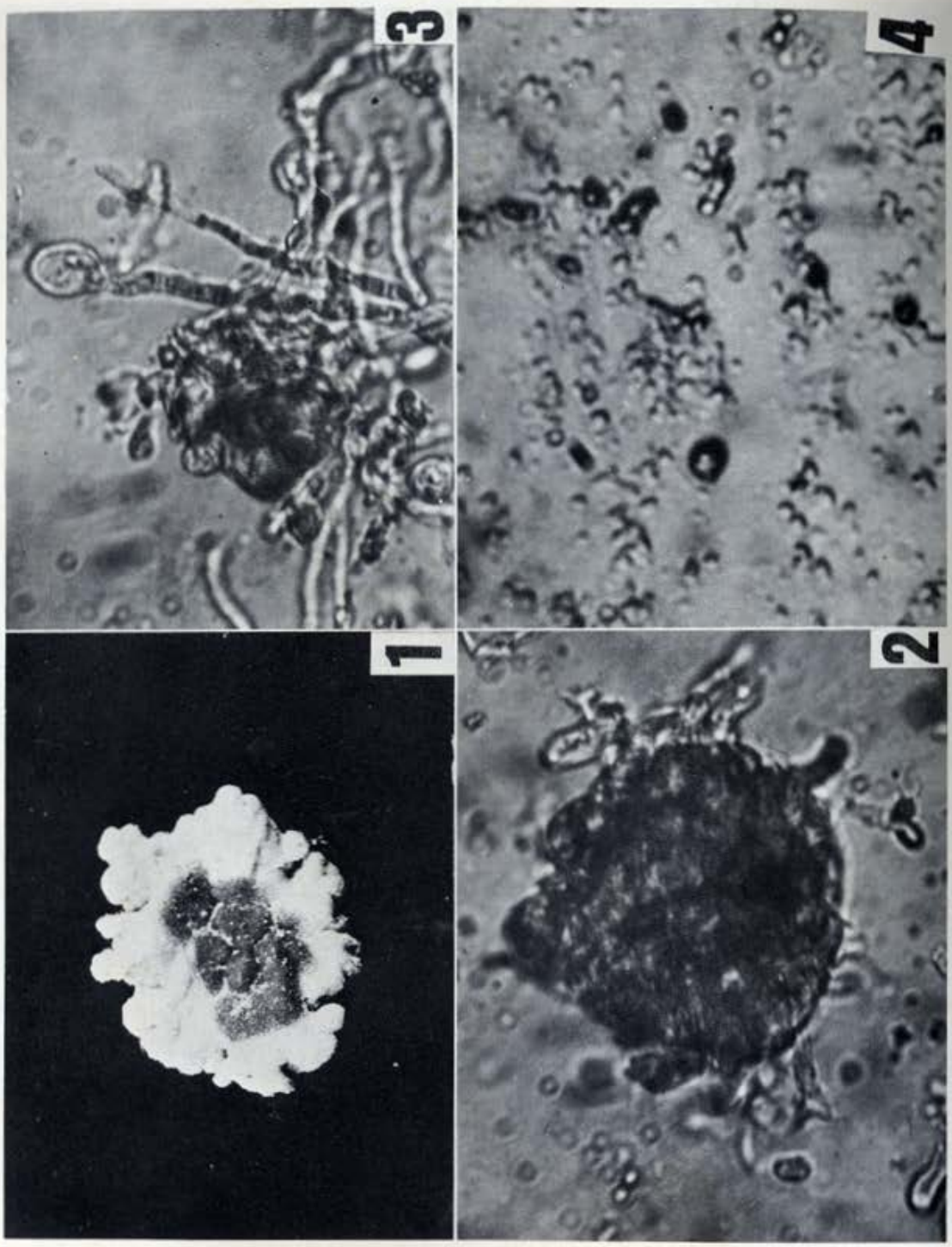

Plate 2.

Fig. 1. Two month old colony of a mycobiont of *Cladonia rangiferina.* x15.
Fig. 2. A mass of cells of the mycobiont of *Cl. rangiferina.* x150.
Fig. 3. Swollen portion of hyphal cells of mycobionts of *Cl. rangiferina.* x400.
Fig. 4. Light brown crystals produced in older portions of the mycobiont colony of *Cl. rangiferina.* x400.