SOME SPECIES OF *TREBOUXIA*, A GENUS OF LICHENIZED ALGAE, ISOLATED FROM TAIWAN FRUTICOSE LICHENS[1]

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ABSTRACT

Six species of the phycobionts, *Trebouxia*, have been successfully isolated and cultured from Taiwan fruticose lichens, mainly from the family of *Cladoniaceae*. It has been reported that the algal symbionts isolated from fruticose lichens (i. e. *Stereocaulon*, *Cladonia*, *Pilophorus*) (Ahmadjian 1960) all belonged to Group I. But based on this investigation, Taiwan fruticose lichens contain phycobionts *Trebouxia* from both Group I and Group II. It has been further assumed that distinct species of lichens, contain the same phycobionts, (at least, at the species level), but many forms of *Trebouxia* which differ both morphologically and physiologically, occur in the same species of lichens.

INTRODUCTION

In the last issue of Taiwania (Wang-Yang 1968), some experimental observations on the algal symbionts of four Taiwan lichens were reported. A morphological study was given. This is a continuation of the work on the algal symbionts of Taiwan lichens, mainly from the family *Cladoniaceae*. This study contributes to the more thorough understanding of the specificity of lichens with regards to their algal symbionts.

The main criterion used to identify the isolated cultured phycobionts is its cellular organization as reported in previous studies (Wang-Yang 1965, 1968). The characteristics of colonial variations and other physiological aspects are used as secondary taxonomic criteria of the algal symbionts.

MATERIALS AND METHODS

Fresh specimens of the following fruticose lichens[3] collected from Ali-Shan, and of *Cladonia rangiferina* collected from Yu-Shan, were used. In the following list of the lichens from which successful algal isolations have been made, the number refers to the culture.

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(3) Specimens of the *Cladonia* genus and *Usnea orientalis* were determined by S. Kurokawa, National Science Museum, Japan.
Specimens of the *Stereocaulon* genus were determined by I. M. Lamb, Director of Farlow Herbarium, Harvard University, Cambridge, Mass., U. S. A.

Cladonia aggregata (SW.) Ach. 628
Cl. cornuta (L.) Schaer. 636, 646, 651
Cl. furcata (Huds.) Schrad. 627, 649
Cl. mitis (L.) Ach. 635
Cl. rangiferina (L.) Ach. 144
Cl. sp. 639
Stereocaulon chlorocaproids Zahbl 654
St. sorediiferum Hue 652
Usnea orientalis Mot. f. *esorediosa* Asahina 647

The same methods of isolation of single algal cells and culture media employed, were followed as previously described (Wang-Yang, 1968). The percentage of colonies obtained from phycobiont isolations from fruticose lichens ranged from 0 to 100. Stock cultures of the algal symbionts are being maintained in the culture collection of algae in our Botany Department.

RESULTS

Descriptions of the isolated algal symbionts:

A. Phycobionts isolated from the genus *Cladonia*:

1. Phycobionts of *Cladonia aggregata* (Cult. Coll. No. 628)

Trebouxia impressa Group II (Ahmadjian 1960:681)

Vegetative cells are spherical (Pl. 2, Fig. 1), and the sizes of mature vegetative cells range from 18.5×18.5 microns to 23.0×23.0 microns. A gelatinous sheath is barely visible around cells grown in all culture media. Aplanospores numbered 8 to 64 per sporangium. Motile round and flattened zoospores are abundantly produced. Vacuolar formation is produced in old cultures. The thickening of the cell wall appears in old mature cells. The color of the algal colony is green. The colony is compact and its surface is covered with small knob-like elevations (Pl. 1, Fig. 1). Approximately, the same growth rate occurs both in the light and dark under the same culture conditions.

2. Phycobionts of *Cladonia cornuta* (Cult. Coll. 636, 646, 651)

Trebouxia impressa Group II. (Ahmadjian 1960:681)

Vegetative cells are spherical, and the size of mature vegetative cells range from 18.0×18.0 to 22.0×22.0 microns. The chromatophore is central and has a smooth margin. A thin gelatinous sheath is produced only in mineral solutions. Aplanospores numbered 8 to 64. Only rounded zoospores are produced. The color of the colony is green. The colony is compact and has a rugose surface. Granular substances are produced in the cells of old cultures.

3. Phycobionts of *Cladonia furcata* (Huds.) Schrad. (Cult. Coll. No. 627, 649)

Trebouxia impressa Group II. (Ahmadjian 1960:681)

Most of the vegetative cells are spherical but a few are ellipsoidal. The sizes

of mature vegetative cells range from 8.5×8.5 microns to 11.0×11.0 microns (Cult. No. 649). The cells range from 20.0×20.0 to 27.0×27.0 microns (Cult. No. 627). Rounded and flattened zoospores are abundantly produced. A gelatinous sheath is present around cells in culture No. 649 but absent in culture No. 627. Aplanospores numbered 8–64 in a sporangium. The color of the colonies are green. The colony has a rugose surface and an irregular margin when the colony was grown under light (Cult. No. 627) (Pl. 1, Fig. 3). The colony developed a vermiform surface when grown in the dark (Pl. 1, Fig. 4). The color of the colonies of Cult. No. 649 are yellowish-green. The colonies are firm and have a rugose surface.

4. Phycobionts of *Cladonia mitis* (Cult. Coll. No. 635)

Trebouxia sp. Group II.

Vegetative cells are spherical and the size of the mature cells range from 17.5× 17.5 to 19.5×19.5 microns. Rounded and flattened zoospores are produced. The color of the colony is green.

5. Phycobionts of *Cladonia rangiferina* (Cult. Coll. No. 144)

Trebouxia glomerata Group I. (Pl. 3, Figures 1–8) (Ahmadjian 1960:681)

The vegetative cells are mostly egg-shaped (Pl. 2, Fig. 3), some however, are spherical or ellipsoidal (Pl. 2, Fig. 2). Cells have an average diameter range of 14.0× 18.0 microns to 20.0×27.0 microns. The chromatophore has deep marginal invaginations and extends to the cell wall. No gelatinous sheath could be seen on any cells grown under any of the cultural conditions used in this study. The number of aplanospores produced are more than 32. Rounded and flattened zoospores are abundant. The color of the colony is green. The colony has a vermiform surface when grown under light (Pl. 1, Fig. 5). The colony had a ridged surface when grown in the dark (Pl. 1, Fig. 6).

6. Phycobionts of *Cladonia* sp. (Cult. Coll. No. 639)

Trebouxia gelatinosa Group II. (Ahmadjian 1960:681)

Vegetative cells are spherical and the size of the mature cells range from 18.0× 18.0 to 20.0×20.0 microns. Numerous rounded and flattened zoospores are produced. Some cells are in groups. The color of the colony is green. The colony is watery, has an irregularly crenate margin and is vermiform in the center. A gelatinous sheath is present under all cultural conditions.

B. Phycobionts isolated from the genus *Stereocaulon*:

1. Phycobionts of *Stereocaulon chlorocaproids* Zahbl. (Cult. Coll. No. 654)

Trebouxia species Group I.

The color of the colony is dark green. The colony is watery, has a curved margin, with a high convex, rugose surface. A gelatinous sheath is produced. The vegetative cells are ellipsoidal. The size of the mature cells range from 8.5×12.5 to 12.0×22.0 microns. Rounded and flattened zoospores are produced even on solid medium.

2. Phycobionts of *Stereocaulon sorediiferum* Hue. (Cult. Coll. No. 652)

Trebouxia arboricola Group II. (Ahmadjian 1960:681)

The vegetative cells are spherical, the size of the mature vegetative cells range from 8.0×8.0 to 10.0×10.0 microns. No zoospores were produced in any cultural conditions used. A sheath is obscure or absent when grown in mineral solutions.

3. Phycobionts isolated from *Usnea orientalis* (Cult. Coll. No. 647)

Trebouxia impressa Group II. (Ahmadjian 1960:681)

Vegetative cells are spherical and the size of the mature cells range from 17.5× 17.5 to 19.5×19.5 microns. Rounded and flattened zoospores are produced. The color of the colony is green.

DISCUSSION

1. The results show that different genera and species of lichens contain the morphologically identical phycobionts (e. g. lichens of *Cladonia aggregata, Cl. cornuta, Cl. furcata, Usnea orientalis*) and these differ from each other only in their colonial features.
2. Different specimens of the same species of lichens contain the morphologically identical phycobionts but their physiological features were sometimes different (e. g. of *Cladonia cornuta*).
3. Fruticose lichens contain not only phycobionts of *Trebouxia* Group 1, but also contain *Trebouxia* Group II. Previous studies have all claimed that *Trebouxia* Group II were isolated only from foliose and crustose lichens (Ahmadjian 1960). From this investigation, it is interesting to note that *Trebouxia* Group II has also been isolated from fruticose lichens.
4. There can be no doubt that different species of lichens, and different specimens of the same lichen (species), contain the same phycobionts, at least, at the species level. It can be stated that many distinct forms of *Trebouxia*, both morphological and physiological, are found in the many thousands of lichen species which contain this algal genus as a phycobiont.

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Explanation of Figures

Plate 1.

Fig. 1. A compact colony of phycobionts from *Cladonia aggregata*, with small knob-like elevations on its surface, colony grown in the light; Photographed after 3 months growth. ×1.5 natural size.

Fig. 2. A colony of phycobionts from *Cladonia aggregata* grown in the dark. Colony was watery and had knob-like projections on its surface. Photographed after 3 months growth. ×1.5 natural size.

Fig. 3. Rugose surface and irregular margin of the colony of phycobionts from *Cladonia furcata*. The colony was grown in the light; Photographed after 3 months growth. ×1.5 natural size.

Fig. 4. Vermiform surface of the colony of phycobionts from *Cladonia furcata*. The colony was grown in the dark; Photographed after 3 months growth. ×1.5 natural size.

Fig. 5. Vermiform surface of the colony of phycobionts from *Cladonia rangiferina*. The colony was grown in the light; Photographed after 3 months growth. ×1.5 natural size.

Fig. 6. Ridged surface of the colony of phycobionts from *Cladonia rangiferina*. The colony was grown in the dark; Photographed after 3 months growth. ×1.5 natural size.

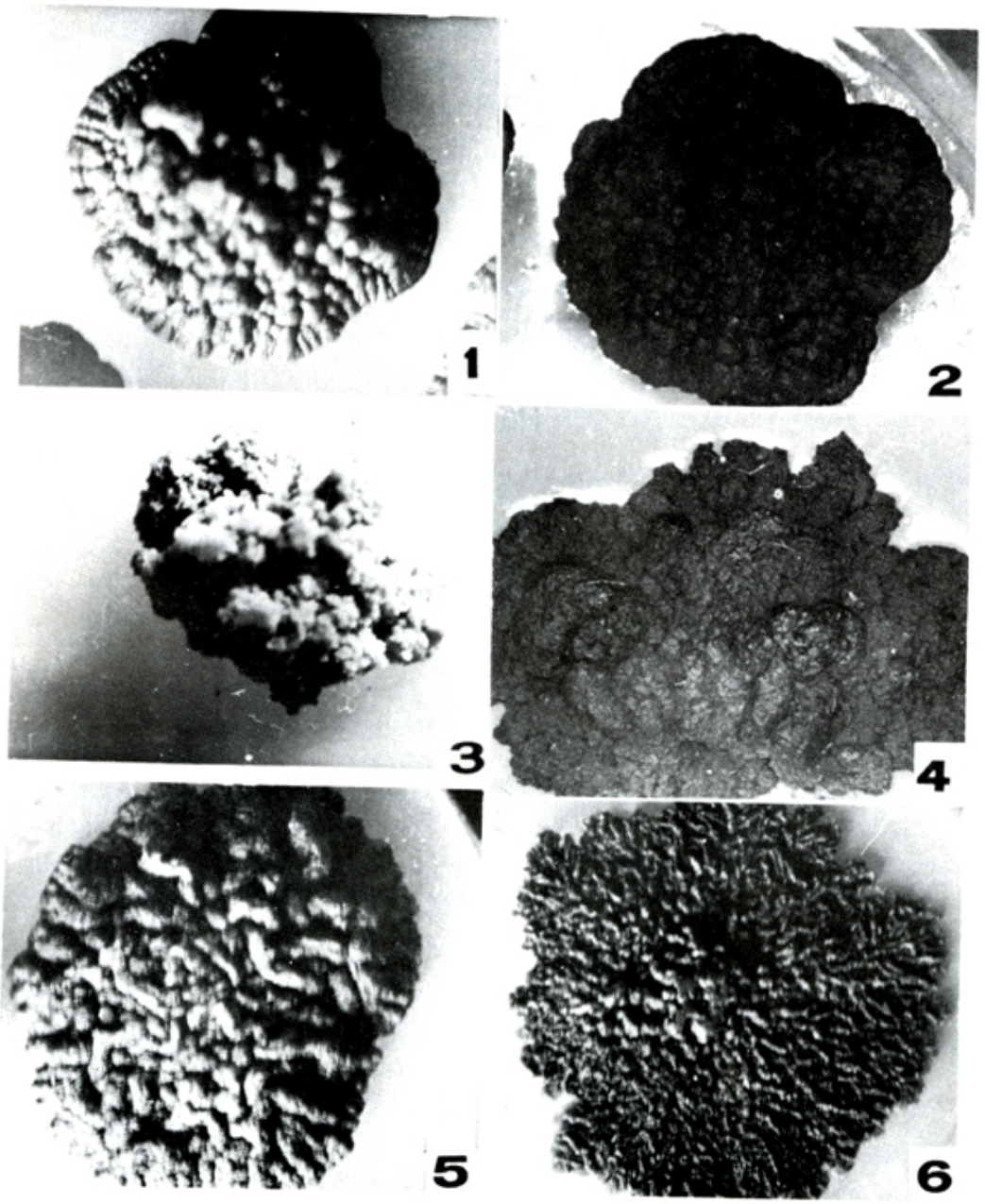
Plate 2.

Fig. 1. Vegetative cell of *Trebouxia* Group II, showing distinctive nucleus. ×1200.

Fig. 2. Ellipsoidal shaped vegetative cells of *Trebouxia* Group I. ×950.

Fig. 3. Egg-shaped vegetative cells of *Trebouxia* Group I. ×750.

Plate 1.



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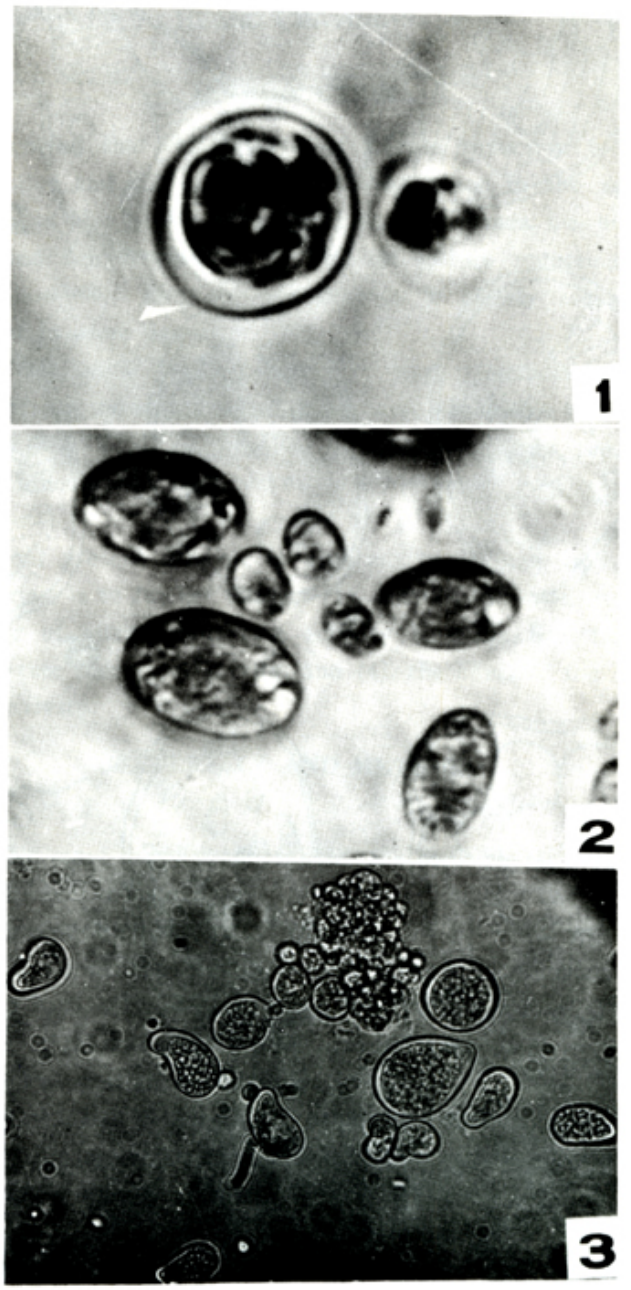

Explanation of Figures

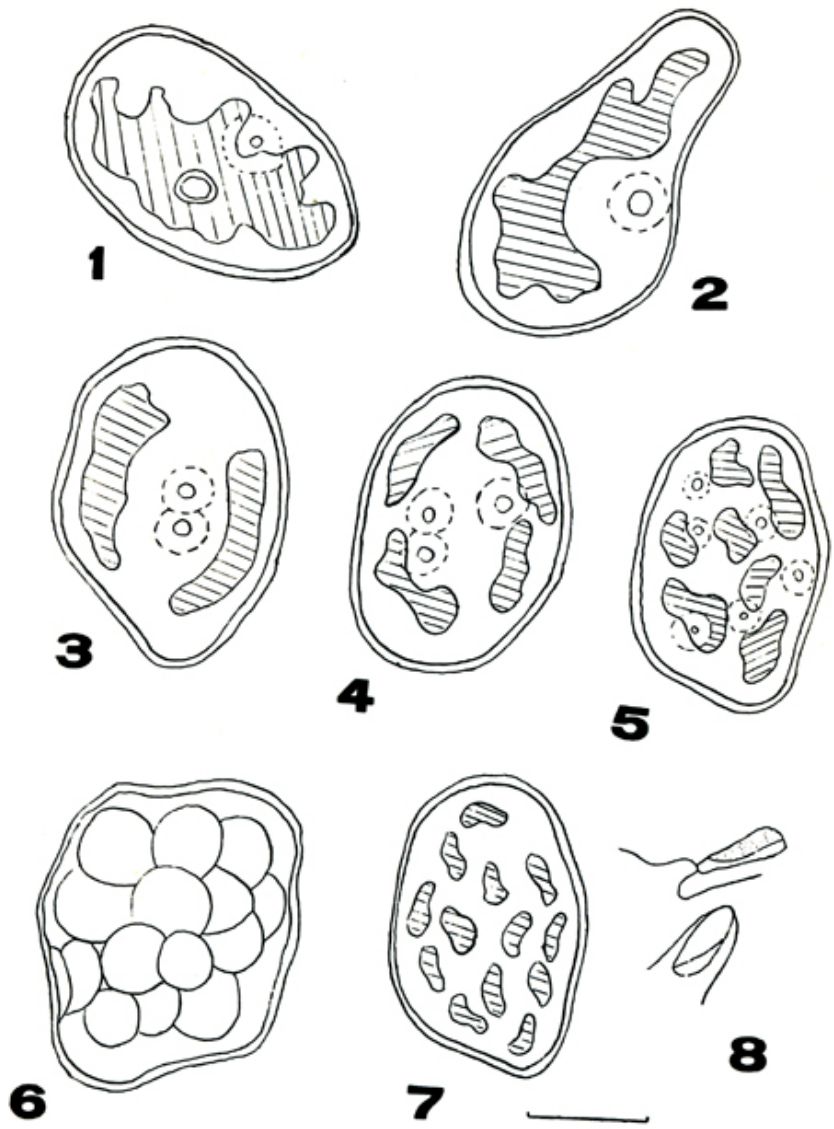

Plate 3. Camera lucida drawings of *Trebouxia* Group I.

Fig. 1. Ellipsoidal-shaped vegetative cell with large central chromatophore containing 1 pyrenoid, and its nucleus.
Fig. 2. Egg-shaped vegetative cell with incised chromatophore.
Fig. 3-5. Stages in the division of the chromatophore.
Fig. 6. Aplanosporangium.
Fig. 7. Zoosporangium.
Fig. 8. Zoospores.
Unit=10 microns