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Photobionts of Japanese *Sphaerophorus* (Lichenes)*

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Abstract

There is one previous report that *Sphaerophorus* has exclusively *Trebouxia* as photobiont. Our results are in contradiction to that report. We isolated the photobionts from four species of *Sphaerophorus* (calicialean lichen) collected in Japan. These were identified as a unicellular green alga, *Dictyochloropsis symbiontica* Tsch.-Woess. This is the first record at the species level on the photobiont of *Sphaerophorus*. There is one previous report on lichens of the Caliciales and two families of Lecanorales (Lobariaceae, Lecideaceae) having this species as their photobionts. Our results support those observations.

Keywords: *Dictyochloropsis*, lichen photobiont, *Sphaerophorus*, lichen

1. Introduction

Sphaerophorus Pers. (Sphaerophoraceae, Caliciales) is a fruticose lichen genus growing in Japan mainly on tree bark. Six species have been found from

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alpine areas to lowland areas in Japan (Yoshimura 1974): They are *Sphaerophorus diplotypus* Vainio, *S. formosanus* (Zahlbr.) Asah., *S. fragilis* (L.) Pers., *S. meiophorus* (Nyl.) Vainio, *S. melanocarpus* (Sw.) D.C. and *S. turfaceus* Asah. Ihda et al. (1992) reported that a considerable number of lichens belonging to Caliciales might have the unicellular green alga, *Dictyochloropsis* as photobiont. However, the photobionts of *Sphaerophorus* have never been isolated and investigated at the species level until now. Tibell (1984) mentioned that *Sphaerophorus* have exclusively *Trebouxia* as photobiont, and that *Scytonema* occurs in cephalodia of *S. stereocauloides*. In this study, we isolated photobionts from eight Japanese specimens of four *Sphaerophorus* species, and investigated these taxonomically.

2. Materials and Methods

Fresh specimens of four species of *Sphaerophorus* were collected from various areas in Japan. These were used to isolate photobionts from their thalli (Fig. 1). The specimens were: *Sphaerophorus fragilis* (L.) Pers.: Hokkaido, Kami-shihoro-cho, Tengu-dake (ca. 1900 m alt.), on rock, Ihda 163 (culture no. TI 80), coll. T. Ihda. *S. diplotypus* Vainio: Shikoku, Ehime-ken, Omogo-mura, Omogo-kei (ca. 700 m alt.), on bark of *Quercus* sp., Ihda 23 (TI 22), coll. T. Ihda. *S. meiophorus* (Nyl.) Vainio: Honshu, Mie-ken, Miyagawa-mura, Ohdai-gahara-san, Shakaga-take (ca. 1600 m alt.), on bark of *Fagus crenata*, Ihda 252 (TI 186), coll. T. Ihda. *S. melanocarpus* (Sw.) DC.: Honshu, Mie-ken, Miyagawa-mura, Ohdai-gahara-san, Shakaga-take (ca. 1600 m alt.), on bark of *Fagus crenata*, Ihda 260 (TI 185), Ihda 194 (TI 103), Ihda 247 (TI 194): on bark of *Rhododendron degonianum* ssp. *heptamerum* var. *hondoense*, Ihda 501 (TI 612), coll. T. Ihda. Honshu, Mie-ken, Nansei-cho, Oniga-jo (ca. 30 m alt.), with mosses on rock, Ihda 502 (TI 613), coll. H. Doei. (1994). These lichen specimens are deposited in the herbarium of Hiroshima University (HIRO.)

Photobionts were isolated by the methods of Nakano (1988). They were cultured on 1N BBM (modified by Bischoff and Bold 1963) agar slants under standard conditions (20°C, 35 μmole photons $m^{-2}sec^{-1}$, 12h light/12h dark). After about three weeks, many colonies of photobionts and a few free-living algae were observed. Bacteria-free and unialgal photobionts were transferred to BBM agar slants by micropippet methods (Ahmadjian, 1967). These axenic strains were cultured on BBM plates under standard conditions for 2–4 weeks. Identification, and observations on life cycle and morphology were made using light microscopy.

Axenic strains are deposited in the Institute of Biological Science, Faculty of Science, Hiroshima University (CCHU).

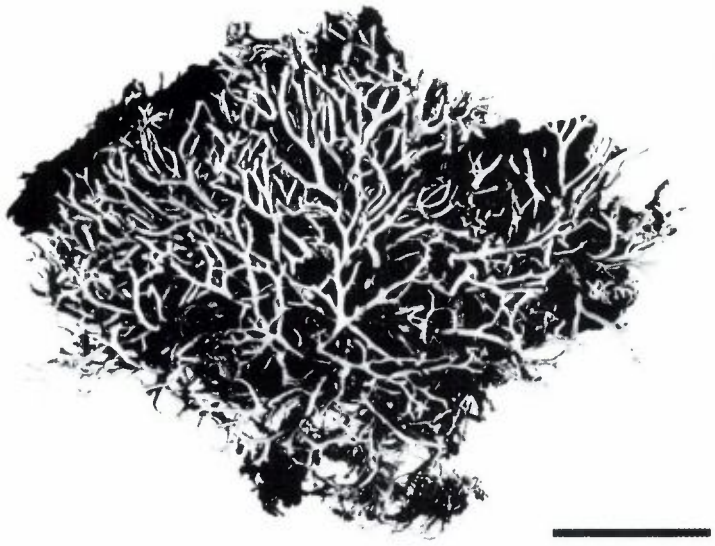


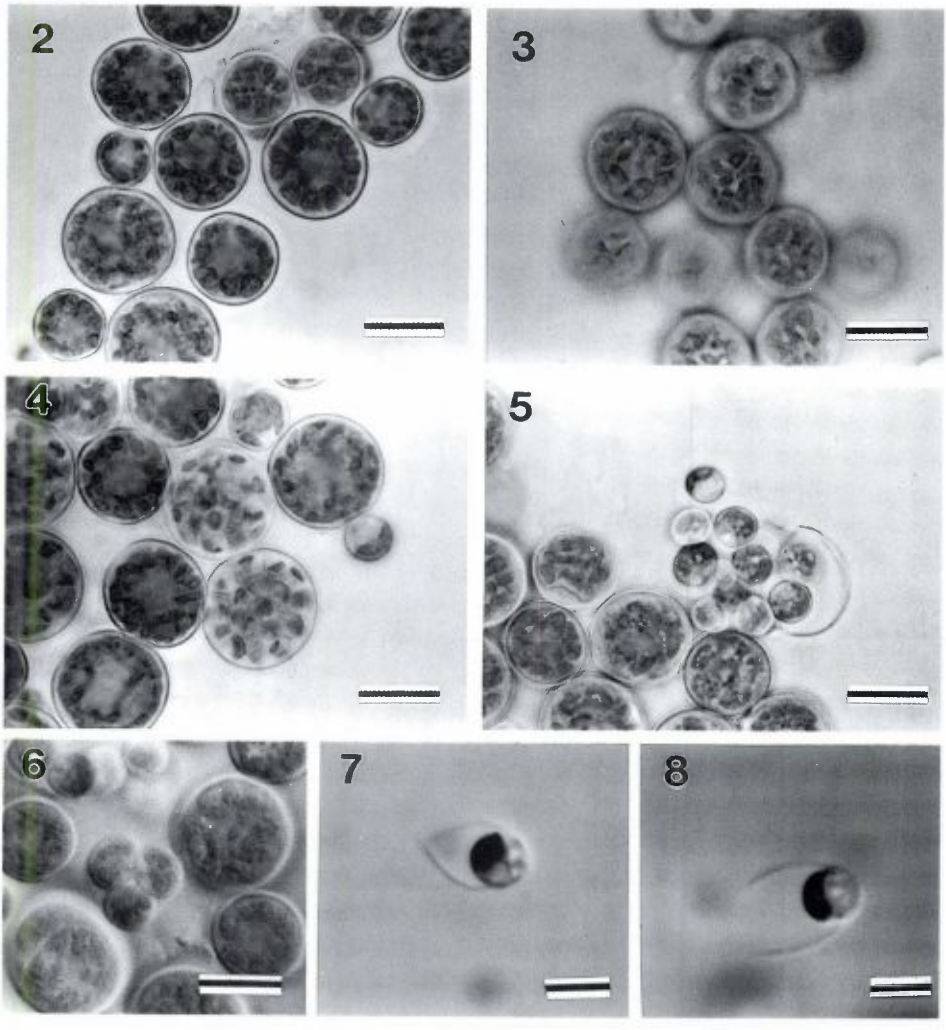
Figure 1. *Sphaerophorus meiophorus* (Nyl.) Vainio. Scale = 1 cm.

3. Results

Photobionts in the thalli of *Sphaerophorus* were unicellular, green in color and irregular in shape. In log phase cultures, all photobionts were unicellular green algae. The chloroplast was parietal and reticulate. This alga conforms with *Dictyochloropsis symbiontica* Tsch.-Woess (Tschermak-Woess, 1980).

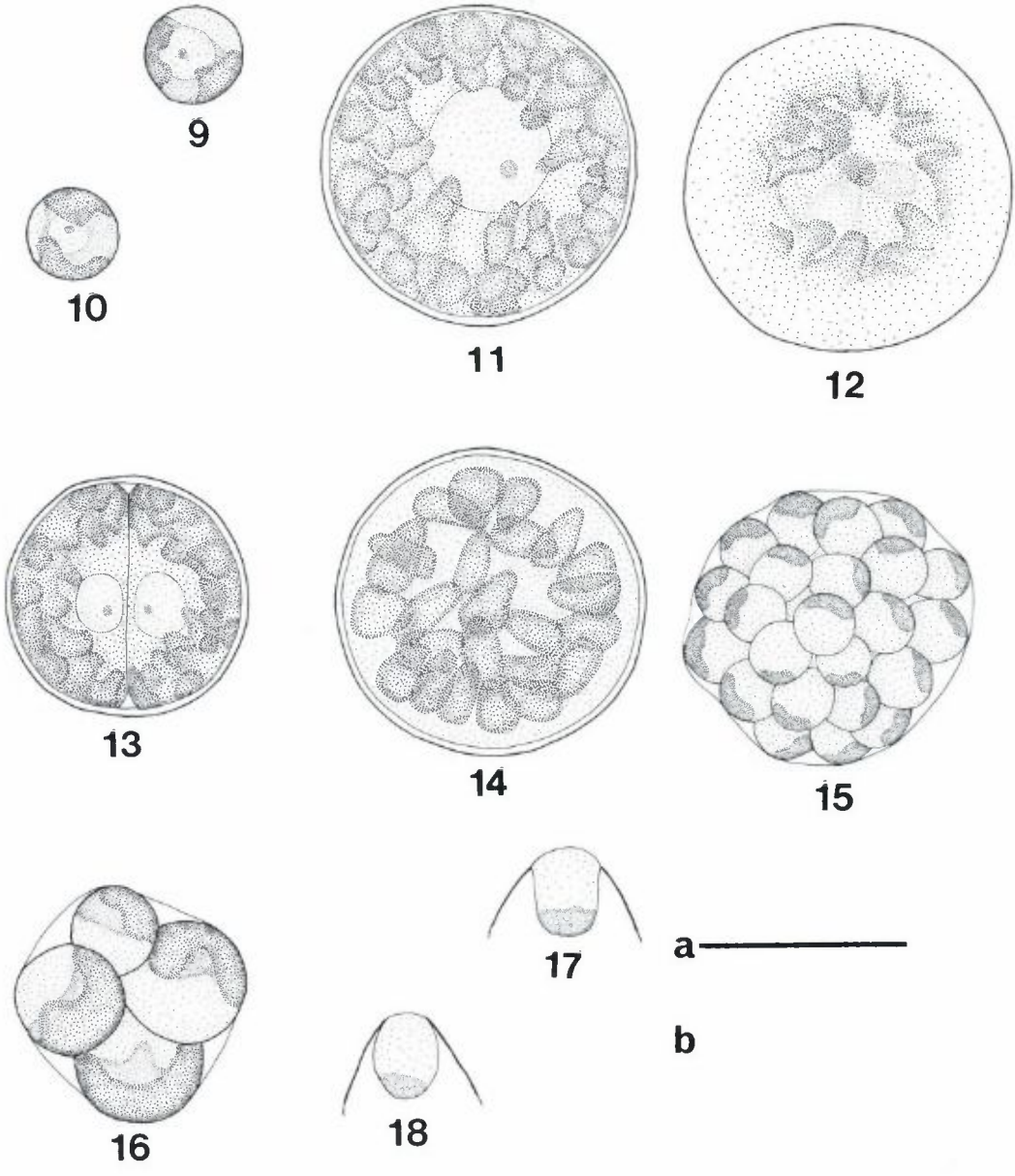
Colonies rough, light-green in log phase cultures, deep green in stationary phase. Vegetative cells solitary, spherical to subspherical in log phase, 10–19 μm in diameter, not markedly enlarging in stationary phase, with walls about 0.5 μm thick, without gelatinous matrices. Cells uninucleate. Chloroplasts parietal, with multilayer reticulate structure, lacking pyrenoid.

Reproduction asexual, by zoospores (16–32 in a sporangium), aplanospores (16–32) and autospores (4–8). Zoospores, ellipsoid to ovoid, 2–3 μm wide, 3–5 μm long, with two flagella of equal length, lacking a cell wall. Flagella emerged on each side of the anterior end of the zoospore. Stigma and contractile vacuoles not observed. See Figs. 2–18.



Figures 2–8. *Dictyochloropsis symbiontica* Tsch.-Woess. 2. Median optical sections of vegetative cells. 3. Surface view of mature vegetative cells. 4. Zoosporangia. 5. Rupture of aplanosporangia and aplanospores. 6. Autosporangium. 7, 8. Zoospores. Scale = 10 μm (Figs. 2-6), scale = 2 μm (Figs. 7, 8).

In 2–3 day cultures, chloroplasts of young vegetative cells were parietal and formed a single layer (Figs. 9, 10). In 5–7 day culture, chloroplasts were reticulate and multilayered structure that developed in the direction of central



Figures 9–18. *Dictyochloropsis symbiontica* Tsch.-Woess. 9, 10. Young vegetative cells. 11. Median optical section of mature vegetative cell. 12. Surface view of the same cell of Fig. 11. 13. First protoplast division (first sporogensis). 14. Zoosporangium. 15. Aplanosporangium. 16. Autosporangium. 17, 18. Zoospores. Scale a = 10 µm (Figs. 9–16), scale b = 2 µm (Figs. 17, 18).

portion of the cells (Figs. 2, 3, 11, 12). We also observed division of protoplast in the mature vegetative cells (Fig. 13). After about 2–3 week cultures, we observed many zoosporangia forming (Figs. 4, 14), but aplano- and autosporangia were rarely observed. Zoospore release occurred after three hours in the dark in log phase cultures (Figs. 7, 8, 17, 18). In four week cultures (stationary phase), we observed aplano- and autosporangia (Figs. 5, 6, 15, 16). However, zoosporangia were not observed.

4. Discussion

Dictyochloropsis symbiontica was first described by Tschermak-Woess (1980), as an isolate from the lichen, *Chaenothecopsis consociata*. She also isolated this species as a free-living alga from bark of *Picea abies*. Later, Tschermak-Woess (1984) described *D. symbiontica* var. *pauciautosporica* Tsch.-Woess as a photobiont of *Megalospora gompholoma*, *M. atrorubicans* and *Pseudocyphellaria aurata*. Recently, this species has been isolated as a free-living alga on the bark of *Juniperus rigida* and *Neolistsea aciculata* in Japan (Handa and Nakano, 1988). This is the first record of *D. symbiontica* as a photobiont of *Sphaerophorus*.

Tschermak-Woess (1984) noted that *D. symbiontica* var. *pauciautosporica* formed fewer autospores than *D. symbiontica* var. *symbiontica* isolated from *Chaenothecopsis consociata* and *Pseudocyphellaria aurata*. We judged that the eight strains isolated from five species of *Sphaerophorus* belonged to *D. symbiontica* var. *symbiontica* on the basis that they formed many autospores in log phase culture.

Takeshita et al. (1991) reported that zoospores of *Dictyochloropsis reticulata* isolated from *Brigantiaea ferruginea* could be observed only during a restricted period (about one hour) in the early morning. Ihda et al. (1992) reported that zoosporogenesis of *D. reticulata* isolated from *Megalospora sulphurata* occurred after more than three hours in the dark in log phase cultures, and zoospores began to move as soon as light was initiated. In this study, zoospores of *D. symbiontica* also began to move on light initiation after dark conditions for more than three hours. A dark period for zoosporogenesis could be a requirement of all species of *Dictyochloropsis*.

Ihda et al. (1992) reported that lichens containing *Dictyochloropsis* as photobionts are in three groups on the basis of the list of Tschermak-Woess (1988), Takeshita et al. (1991) and their own results. These are two families of Lecanorales (Lobariaceae, Lecideaceae) and Caliciales. *Sphaerophorus* belongs to the Caliciales. Therefore, our results support their opinions.

Tibell (1984) mentioned that *Trebouxia* is exclusively a photobiont of *Sphaerophorus*. Our results are in contradiction to those observation. There have been several reports about a wide range of photobionts occurring in the genus level of the Caliciales. Four genera of green algae, *Trebouxia, Trentepohlia, Stichococcus* and *Dictyochloropsis* isolated from *Chaenotheca* (Raths, 1938; Tschermak-Woess, 1978, 1995). Tibell (1984) reported that some species of *Chaenotheca* had vicariant photobionts in different areas (Tibell, 1980, 1982).

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