植物研究雑誌
J. Jpn. Bot.
67: 342–346 (1992)

A Phycobiont Newly Isolated from *Megalospora sulphurata* (Lichenes)[1)]

Taka-aki IHDA, Tatsuya OKAMOTO, Taketo NAKANO and Zennoske IWATSUKI
Botanical Institute, Faculty of Science, Hiroshima University,
Kagami-yama, Higashi-hiroshima-shi, Hiroshima-ken 724, JAPAN

地衣類ヤマトクロコボシゴケ（*Megalospora sulphurata*）の共生藻[1)]

飯田高明，岡本達哉，中野武登，岩月善之助
広島大学理学部植物学教室　724 東広島市鏡山 1−3

(Received on May 11, 1992)

The phycobiont was isolated from a crustose lichen, *Megalospora sulphurata* Meyen collected in Ryukyu, Japan. This alga was identified as *Dictyochloropsis reticulata* (Tsche.-Woess) Tsche.-Woess (Chlorophyta), which is a phycobiont known for the first time in the genus *Megalospora*. Zoosporogenesis of this alga occurred in cultures kept in the dark more than three hours, and zoospores began to move following light initiation.

Megalospora Meyen is a crustose lichen genus growing mainly on tree bark. Tschermak-Woess (1984) recently reported a species of unicellular green alga, *Dictyochloropsis symbiontica* Tsche.-Woess var. *pauciautosporica* Tsche.-Woess as phycobiont from *Megalospora atrorubicans* (Nyl.) Zahlbr. subsp. *australis* Sipm. and *M. gompholoma* (Müll. Arg.) Sipm. subsp. *gompholoma*. However, phycobionts of other species of *Megalospora* have never been reported until now. In this study, we isolated phycobionts from a Japanese specimen of *M. sulphurata* collected from the Ryukyu Islands.

Materials and Methods

A fresh corticolous specimen of *Megalospora sulphurata* (Ihda no. 240) was collected at 460 m alt., Mt. Omoto, Ishigaki Island, Ryukyu, southern Japan (Fig. 1). The specimen was brought to the laboratory and used for isolation of phycobionts. The lichen specimen is deposited in the herbarium of Hiroshima University (HIRO).

Phycobionts were isolated by the method of Nakano (1988). They were cultured on 1N BBM (modified by Bischoff and Bold 1963) agar slants under standard conditions (20±1°C, 2000 lux, 12h light/12h dark). After about three weeks, colonies of phycobionts were observed, and only bacteria-free colonies were transferred to BBM agar slants. Algal material was cultured under standard conditions for identification, and observations of life cycle and morphological features were made by using the light microscope after about two weeks.

Unialgal or bacteria-free strains (cult. no. TI 105) are deposited in the Botanical Institute, Faculty of Science, Hiroshima University (CCHU).

Results and Discussion

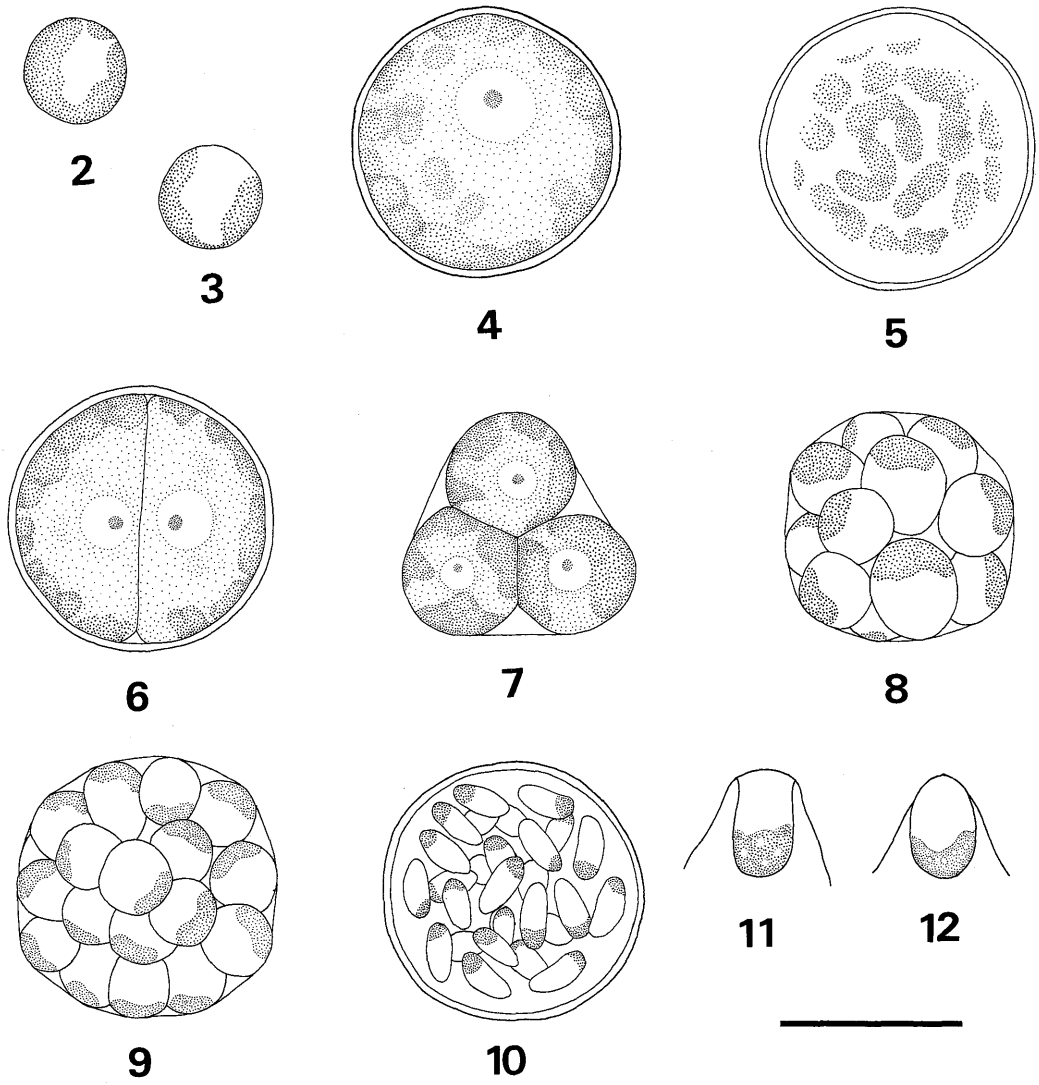
Vegetative cells of phycobionts in the thallus of *M. sulphurata* were unicellular, green and irregular in shape. Shape of chloroplast was indistinct because of the influence of mycobiont hyphae. In addition to vegetative cells, autosporangia with four spores were observed several times. In the log phase of pure cultures of phycobionts, the shape of cells and form of chloroplasts were distinct. We identified this alga as *Dictyochloropsis reticulata* (Tsche.-Woess) Tsche.-Woess in the light of the original description and figures by Tschermak-Woess (1984).

Dictyochloropsis reticulata (Tsche.-Woess) Tsche.-Woess, Pl. Syst. Evol. **147:** 317, fig. 5, a–k. 1984. Figs. 2–18.

Colonies rough, light-green in log phase of culture, slightly deep-green in stationary phase. Vegetative cells solitary, spherical to subspherical in log phase of culture, 9–16 μm in diameter, not markedly enlarging in stationary phase of cultures, with walls about 0.5 μm thick, without gelatinous matrices. Cells uninucleate. Chloroplasts parietal, with a network of interlacing strands, without pyrenoid. Asexual reproduction by zoospores (16–64 in a sporangium), aplanospores (16–64) and autospores (2–8). Zoospores ellipsoidal to slightly oviform, 2–3 μm wide and 3–5 μm long, without cell wall, without stigma, with biflagella of equal length and width. Each flagellum occurred on side of cell behind the anterior end.

Dictyochloropsis reticulata was first described as *Myrmecia reticulata* by Tschermak-Woess (1951). It was isolated from a lichen *Catillaria chalybeia* (Borr) Mass. Later, Tschermak-Woess (1984) revised this species and transferred it to the genus *Dictyochloropsis* on the basis of morphological characters of chloroplasts and zoospores, which were different from those of the genus *Myrmecia*. This species has been isolated from the following eight lichen species: *Bacidia nanipara* Lett. (Zeitler 1954), *Phlyctis argena* (Spreng.) Flot. (Tschermak-Woess 1969), *Sarcogyne regularis*

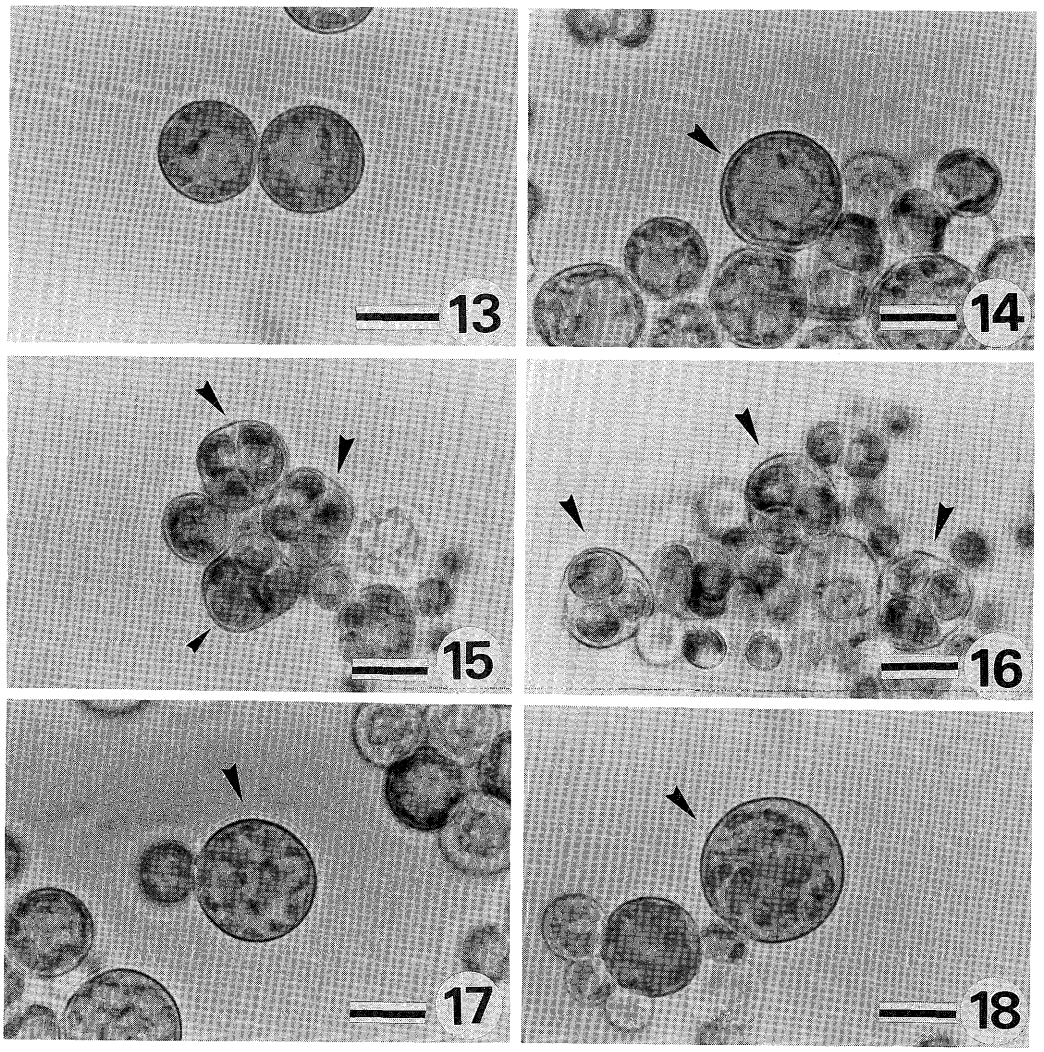
Fig. 1. *Megalospora sulphurata* Meyen. Scale = 1 mm.



Figs. 2–12. *Dictyochloropsis reticulata* (Tsche.-Woess) Tsche.-Woess. 2, 3. Young vegetative cells. 4. Median optical section of mature vegetative cell. 5. Surface view of the same cell of Fig. 4. 6. First division of protoplast. 7. Autosporangium with 4 spores. 8. Aplanosporangium with 16 spores. 9. Aplanosporangium with 32 spores. 10. Zoosporangium. 11, 12. Zoospores. Scale = 10 μm.

Körb., *Lobaria pulmonaria* (L.) Hoffm. var. *pulmonaria*, *L. pulmonaria* (L.) Hoffm. var. *meridionalis* (Vain.) Zahlbr., *L. amplissima* (Scop.) Forss. and *L. laetevirens* (Lightf.) Zahlbr. (Tschermak-Woess 1978), and *Brigantiaea ferruginea* (Müll. Arg.) Kashiw. et Kurok. (Takeshita et al. 1991). On the other hand, this species was also isolated as a free-living alga from bark of *Aesculus hippocastanum* and rock (Geitler 1965), bark of some woods (Tschermak-Woess 1978) and bark of *Cephalotaxus harringtonia* (Nakano et al. 1991).

According to Tschermak-Woess (1988), Takeshita et al. (1991) and the results of this study, the lichens containing *Dictyochloropsis* algae as the phycobionts are concentrated in three groups. They



Figs. 13–18. *Dictyochloropsis reticulata* (Tsche.-Woess) Tsche.-Woess. 13. Mature vegetative cells. 14. Mature vegetative cell of maximum size (arrowhead). 15, 16. Autosporangia with 4 spores (arrowhead). 17, 18. Zoosporangia. Scales = 10 μm.

are 1) Lobariaceae (*Lobaria* and *Pseudocyphellaria*), 2) genera previously treated as the members of Lecideaceae (*Bacidia, Brigantiaea, Catillaria, Catinaria* and *Megalospora*) and 3) genera of Caliciales (*Chaenotheca* and *Chaenothecopsis*). A considerable number of lichens belonging to these three groups may have *Dictyochloropsis* algae as phycobionts. *Dictyochloropsis reticulata* is a phycobiont known for the first time in the genus *Megalospora*. This is also the first record of phycobionts isolated from *Megalospora sulphurata*. At present, *D. reticulata* is isolated from nine lichen species.

Takeshita et al. (1991) reported that zoospores of this species could be observed only during a restricted period (about one hour) in early morning. In this study, we observed that zoosporogenesis occurred under the dark condition after more than three hours in log phase of cultures and zoospores began to move as soon as light was

initiated. The zoospore seems to have a photoreceptor as in *Chlamydomonas reinhardtii* reported by Melkonian and Robenek (1980).

We wish to express our thanks to Dr. H. Kashiwadani of National Science Museum for his valuable comments on the manuscript. Thanks are also due to Dr. J. M. Glime of Michigan Technological University for correction of the English text.

References

Bischoff H. W. and Bold H. C. 1963. Phycological Studies IV: Some soil algae from Enchanted Rock and related algal species. 95 pp. Univ. Texas Publ., Austin.

Geitler L. 1965. Notizen über einige wenig bekannte Grünalgen und eine Chytridiale. Österr. Bot. Z. **112:** 603–609.

Melkonian M. and Robenek H. 1980. Eyespot membranes of *Chlamydomonas reinhardtii*: A freeze-fracture study. J. Ultrastruct. Res. **72:** 90–102.

Nakano T. 1988. Phycobionts of some Japanese species of Graphidaceae. Lichenologist **20:** 353–360.

———, Handa S. and Takeshita S. 1991. Some corticolous algae from the Taishaku-kyo Gorge, western Japan. Nova Hedwigia **52:** 427–451.

Takeshita S., Handa S. and Nakano T. 1991. Phycobionts of *Briganteaea ferruginea* (Lichens). J. Jpn. Bot. **66:** 147–151.

Tschermak-Woess E. 1951. Über wenig bekannte und neue Flechtengonidien II. Eine neue Protococcale. *Myrmecia reticulata,* als Algenkomponente von *Catillaria chalybeia*. Österr. Bot. Z. **98:** 412–419.

——— 1969. Über wenig bekannte und neue Flechtengonidien IV. *Myrmecia reticulata* – der Algenpartner in *Phlyctis argena* und seine systematische Stellung. Österr. Bot. Z. **116:** 167–171.

——— 1978. *Myrmecia reticulata* as a phycobiont and free-living – free-living *Trebouxia* – the problem of *Stenocybe septata*. Lichenologist **10:** 69–79.

——— 1984. Über die weite Verbreitung lichenisierter von *Dictyochloropsis* und die systematische Stellung von *Myrmecia reticulata* (Chlorophyta). Pl. Syst. Evol. **147:** 299–322.

——— 1988. The algal partner. In Galun M. (ed.). CRC Handbook of Lichenology. **1:** 39–92. CRC Press, Inc. Boca Raton.

Zeitler I. 1954. Untersuchungen über die Morphologie, Entwicklungsgeschichte und Systematik von Flechtengonidien. Österr. Bot. Z. **101:** 453–487.

Endnote

[1)]Contribution from the Phytotaxonomical and Geobotanical Laboratory, Hiroshima University, N. Ser. No. 427.

要 旨

痂状の地衣類ヤマトクロコボシゴケ（*Megalospora sulphurata*）は樹皮上に生育し，熱帯から温帯まで広く分布している．現在まで本種の共生藻に関する報告は無い．今回，沖縄県石垣島から本種の標本を得，共生藻を分離・培養し，分類学的検討を行った結果，単細胞性の緑藻 *Dictyochloropsis reticulata* を得た．今までにクロコボシゴケ属（*Megalospora*）の他の2種から *Dictyochloropsis symbiontica* var. *pauciautosporica* が報告されているので，今回の結果は，クロコボシゴケ属の共生藻として2番目の種の報告となる．また，培養された *D. reticulata* の遊走子は3時間以上の暗期を経て形成され，明期直後に活動を開始することが認められた．