Symbiosis, 7 (1989) 37–50
Balaban Publishers, Philadelphia/Rehovot

The Resynthesis of Thalli of *Dermatocarpon miniatum* Under Laboratory Conditions

E. STOCKER-WORGÖTTER and R. TÜRK

Institut für Pflanzenphysiologie, Universität Salzburg
Hellbrunnerstraße 34, A-5020 Salzburg, Austria
Tel. 0043 662 8044/5553

Received September 7, 1988; Accepted October 23, 1988

Abstract

The resynthesis of thalli of the lichen *Dermatocarpon miniatum* (L.) Mann was achieved under laboratory conditions. Three different media, agar plates, silica gel and soil substrate, were used for cultivation. On agar slants developmental stages with a loose contact between the symbionts and long substrate hyphae were formed. Cultivation on silica gel was restricted to the formation of soredia-like lichen primordia. Resynthesis stages of fully developed *Dermatocarpon miniatum*-thalli only occurred on soil substrate from the natural habitat of the lichen.

Keywords: *Dermatocarpon miniatum*, laboratory culture, resynthesis

1. Introduction

Resynthesis experiments with foliose lichens have rarely been carried out. One of the best known species in this respect is *Xanthoria parietina*, which first had been resynthesized under laboratory conditions by Bonnier (1886, 1889). However, Bonnier's experiments have not been reproduced until recent times. Bubrick and Galun (1986) succeeded in cultivating *Xanthoria parietina* on agar plates. They achieved an axenic spore to spore resynthesis after 8–12 months.

Several resynthesis experiments of Ahmadjian (e.g. Ahmadjian, 1966; Ahmadjian and Heikkilä, 1970; Ahmadjian et al., 1978; Ahmadjian and Jacobs, 1982, 1985) attempted both crustose lichens (*Endocarpon pusillum*, *Staurothele clopima*, *Lecidea albocaerulescens*) and fruticose lichens (*Cladonia cristatella* and *Usnea strigosa*).

Ten years ago, Jahns and collaborators began to study the development of several lichen species in their natural environment (e.g. Jahns et al., 1978; Jahns and Ott, 1982; Schuster et al., 1985; Ott, 1987a, b). Their long-term investigations of both soredia-producing lichens and lichens with fruiting bodies provided some fundamental knowledge of lichen life cycles in the natural habitat. Thus in many cases exists a possibility to compare lichen development under laboratory conditions with investigations in the natural environment.

Although foliose lichens are wide-spread and well studied in many respects (systematically and physiologically), there are only few observations on their juvenile growth and development. After cultivating crustose lichens from spores and algae (Stocker-Wörgötter and Türk, 1987; Stocker-Wörgötter and Türk, 1988a) and diverse experiments on the growth of foliose and fruticose lichens from soredia (Wörgötter, 1985; Stocker-Wörgötter and Türk, 1988b) we directed our attention to the foliose lichen *Dermatocarpon miniatum*. This lichen is well suited for resynthesis studies: It produces a great number of mycobiont spores from perithecia and contains a fast growing green alga (*Hyalococcus dermatocarponis*, Ahmadjian, 1967) in its thallus lobes.

Our main interest in this study was to observe morphogenetic stages of the juvenile thallus development of *Dermatocarpon miniatum* under controlled laboratory conditions. The recombination of the lichen symbionts was conducted on agar slants, silica gel and soil substrate. Lichenization and thallus differentiation were investigated by light microscopy and macrophotography.

2. Materials and Methods

Specimens of the lichen *Dermatocarpon miniatum* (L.) Mann were collected on the calcareous rocks of the Nonnberg in the city of Salzburg, Austria. The lichens were treated immediately after collection or after storage at –23°C.

Cultivation of the isolated lichen components

The mycobiont was isolated from spores of the numerous perithecia, which had been formed in well-developed lichen thalli. The fruiting bodies were fixed by means of petroleum jelly to the top cover of a petri dish. The bottom cover contained the agar plate. To prevent contamination of the agar surface it is more favourable to use the inverted-spore-discharge-method. The petri dish was kept with its top cover down and in this way the spores were allowed to discharge up onto the agar surface.

The scattered and germinated spores were transferred to agar slants (BBM-Solution, Bischoff and Bold, 1963, + 2% agar) in test tubes. These mycelium stock cultures could be preserved for up to 6 months for resynthesis experiments. To study mycobiont growth on a substrate with organic substances like carbohydrates, a series of mycobiont cultures were conducted on Sabouraud-glucose-agar (Lallemant, 1985).

The photobiont was determined as *Hyalococcus dermatocarponis* (Warén, 1920; Ahmadjian, 1967). Algal cells were isolated either by the micropipette technique (Ahmadjian, 1973) or by squashing small pieces of the algal layer under the light microscope to guarantee the isolation of the right alga.

The algae were transferred to Bristol-Agar (Bristol-Solution, modified by Deason and Bold, 1960; + 1.5% agar). The photobiont cultures were conducted both on solid and in liquid Bristol-medium. Both separated symbionts were incubated in a culture chamber at 20°C + −2°C under a 14:10 hours light-dark cycle and an illumination of 60–100 μE $m^{-2}s^{-1}$ from fluorescent lamps (type Philips 65W/29RW).

Resynthesis cultures

Resynthesis cultures were generally conducted on soil substrate from the habitat of the lichen. The soil was soaked with distilled water, then filled in glass petri dishes (100×15 mm) and autoclaved for 3 hr on two successive days.

Algae from the liquid medium were transferred to the soil substrate by means of a pipette. The hyphae from the stock culture were then inoculated on the algal colonies. To simulate the possible natural way of lichen recombination, algal cells and fungal spores were brought together. Moist thallus fragments with perithecia were fixed to the top cover of the petri dishes. The cover with the fruiting bodies was then placed over the bottom half of the dish which contained the soil substrate and areas with algal colonies. By this method the spores soon came into direct contact with the photobiont.

To study the early stages of lichen synthesis a series of resynthesis experiments were conducted on sterile agar slants (1000 ml BBM-solution, Bischoff and Bold, 1963 + 40 ml soil extract of soil from the lichen habitat, prepared by the method of Esser, 1976 + 20 g agar) and silica gel substrate (BBM-solution + soil extract as described above, mixed with Silicagel G n. Stahl, Fa. Merck at a ratio of 2:1, Galun et al., 1972).

All resynthesis cultures were incubated in the culture chamber at the same day-night-cycle and illumination as described above. The intervals of rewa-

tering of the soil substrate varied from 4 to 6 weeks. The developmental stages were examined by a Reichert Polyvar light microscope. The macrophotographs were taken by an Olympus camera on Agfapan 25 films by means of bellows and a 40 mm Zeiss magnifying lens.

3. Results

Cultivation of the isolated lichen components

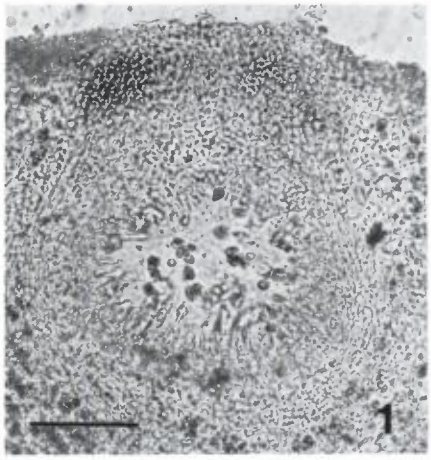
Adult thalli of *Dermatocarpon miniatum* produce numerous pear-shaped perithecia, which contain the asci and the fungal spores (Fig. 1). After ca. 24 hr the discharged spores began to germinate (Fig. 2). One unicellular, egg-shaped spore generally produces 1–2 germ tubes. Usually, 8–16 spores, the content of one or two asci, were scattered together. In this respect, most of the developing mycelia consisted of hyphae from 8–16 spores (Fig. 3).

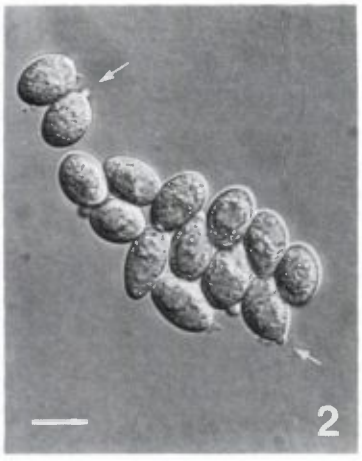
The mycobiont of *Dermatocarpon miniatum* grew very slowly. During the first days of incubation a limited growth rate of the germination tubes could be observed. After 4 weeks in culture only a few branched hyphae appeared (Fig. 4). After 2 months of cultivation on BBM-Agar, no further growth and development of the lichen fungus took place.

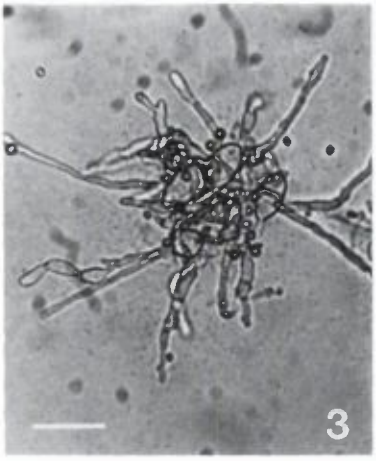
A well developed mycelium was formed only on the nutrient rich Sabouraud-agar after an incubation time of 4 weeks. Initially, these mycelia consisted of thin, short hyphae. Finally, after a period of 4 weeks a dense fungal network with a remarkable vertical development was formed (Fig. 5). Thallus-like structures did not occur.

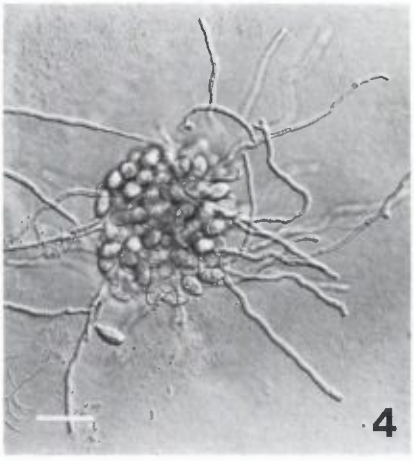
Figures 1–6.

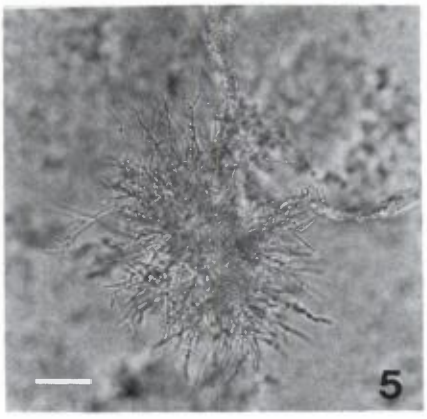
1) *Dermatocarpon miniatum*, section through a perithecium with asci and spores, bar = 100 μm.
2) *D. miniatum*, beginning of spore germination (arrows) after 24 hr on agar substrate; interference contrast. bar = 10 μm.
3) Germinated spores of *D. miniatum* after a cultivation period of 10 days on BBM-agar. bar = 25 μm.
4) *D. miniatum*, in culture for one month on BBM-agar; no fungal network had been formed; interference contrast. bar = 25 μm.
5) *D. miniatum*, mycelium cultivated on Sabouraud agar; a dense fungal network had developed after an incubation time of one month. bar = 25 μm.
6) *D. miniatum*, isolated photobiont, cultivated on Bristolagar; interference contrast. bar = 10 μm.

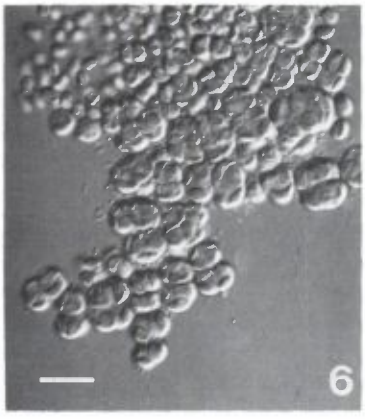

1


2


3


4


5


6

Figures 7–12.

7) *Dermatocarpon miniatum*, early fungal-algal stage after 2 days of cultivation on agar substrate; interference contrast. bar = 10 μm.

8) *D. miniatum*, algal colonies between fungal hyphae; very loose contact after 1–2 weeks in culture; interference contrast. bar = 10 μm.

9) and 10)) *D. miniatum*, early lichen associations after one and 2 1/2 months of cultivation. Fig. 9; bar = 10 μm, Fig. 10: bar = 10 μm.

11) Thallus primordia of *D. miniatum* on silica gel substrate, in culture for 3 months. bar = 500 μm.

12) Soredia-like lichen stages of *D. miniatum* from silica gel substrate after a cultivation period of 4 months. bar = 100 μm.

When algal cells were transferred to the agar surface of the mycobiont cultures, hyphal growth of the germ tubes could be accelerated for a time interval of up to one week. It was only a short-term effect, which declined during further development. Fungal growth could also be influenced by algal colonies which had no direct contact to the hyphae. We assume that the fungus could derive profit from the carbohydrates which were possibly delivered by the algae to the substrate.

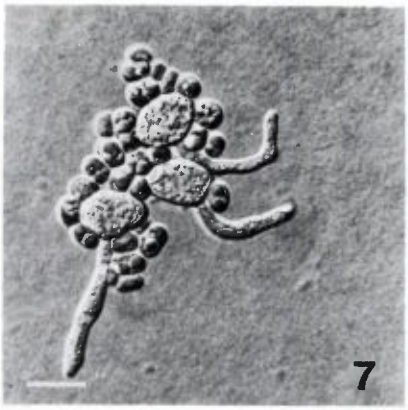
Photobiont cultures

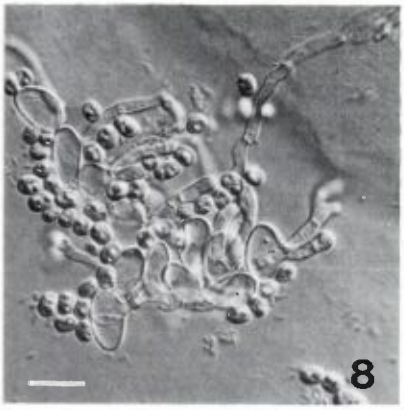
The photobiont was cultivated both on solid and in liquid Bristol-medium. Both media allowed the same high rate of cell division. After one month of cultivation some algal colonies were transferred to new media for further cultivation. The growth of *Hyalococcus dermatocarponis* was faster than that of *Trebouxia* (the most common lichen photobiont).

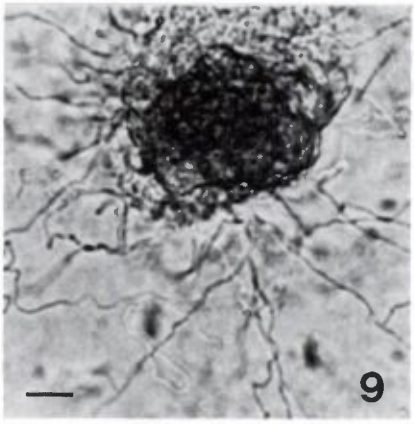
The isolated photobiont of *Dermatocarpon miniatum* on agar had a bright green colour and formed a nearly fluid layer on solid surfaces. Reproduction occurred by vegetative cell division. The algal cells appeared as single cells or in clusters of four cells which were connected by a gelatinous matrix. The photobiont cells have an oval-cylindrical shape with a parietal, plate-like chromatophore (Fig. 6). The *Hyalococcus*-cultures were kept for resynthesis experiments over a period of 2 years.

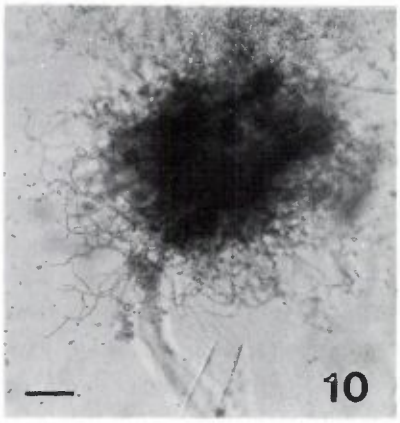
Resynthesis cultures

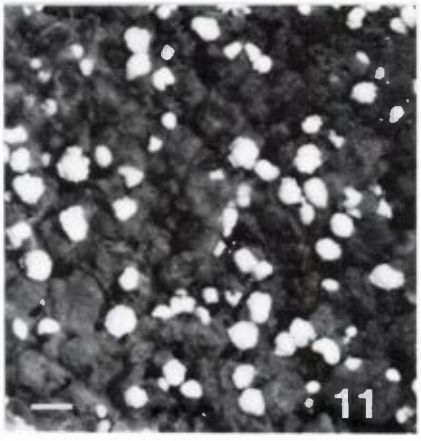
Long-term resynthesis experiments were conducted on agar slants, silica gel and on soil substrate from the lichen habitat. The early stages of cellular interaction between the symbionts were studied on agar plates. These were first inoculated with a few photobiont cells and later on the mycobiont (as hyphal fragments from the stock culture or directly from spores, Fig. 7) was

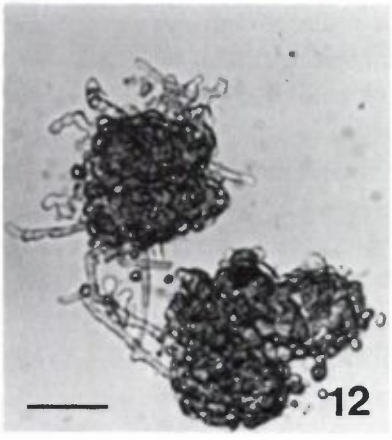

7


8


9


10


11


12

Figures 13–18.

13) Two-month-old stage of *Dermatocarpon miniatum* on soil substrate; many algal cells were integrated by fungal hyphae. bar = 25 μm.

14) *D. miniatum*, developmental stage after 3 months of cultivation on soil substrate; interference contrast. bar = 50 μm.

15) Thallus primordia of *D. miniatum* on soil substrate after a cultivation period of 4 months. bar = 500 μm.

16) Thallus initials with a rim of white fungal network (arrows) on soil substrate after 5 months in culture. bar = 500 μm. (*D. miniatum*)

17) Fully developed young thalli of *D. miniatum* on soil substrate after 7 months in culture; note the dark rim (arrows). bar = 1 mm.

18) Section through a young thallus after 7 months of cultivation on soil substrate. bar = 100 μm.

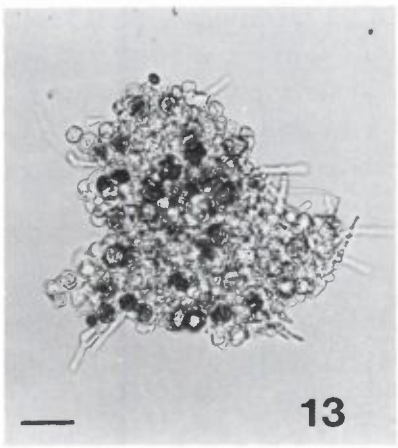
added. Figure 7 shows a very early fungal-algal stage after 2 days of cultivation, where the fungal spores had already begun to germinate. After 2 weeks of incubation on BBM-agar the fungal hyphae had enveloped several algal colonies (Fig. 8). But on agar-substrate the contact between the symbionts was very loose.

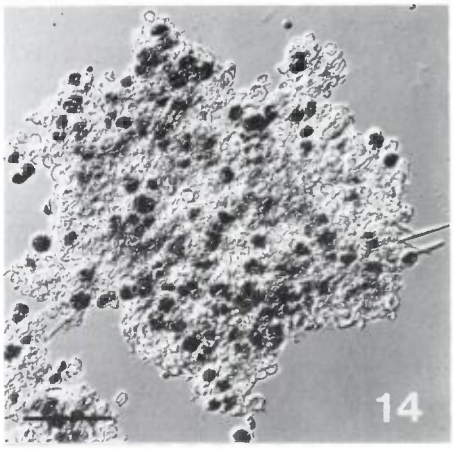
After an incubation time of 3–4 weeks small resynthesis stages appeared, which were now visible with the unaided eye on agar, silica gel and soil substrate. Algal cells had been encircled (Figs. 9, 10) and soredia-like structures were formed. After one month of cultivation resynthesis stages were also available from soil cultures. Developmental stages on agar (Fig. 10) showed a smooth and loose structure, whereas the stages on soil substrate formed a considerably more compact, but still undifferentiated tissue (Figs. 13, 14). The primordia on soil (Fig. 13) formed shorter substrate-hyphae than the culture stages from the agar plates (Fig. 10). On soil the algae had been integrated in a tight fungal network and only a few fungal hyphae on the margin were free from algal contact.

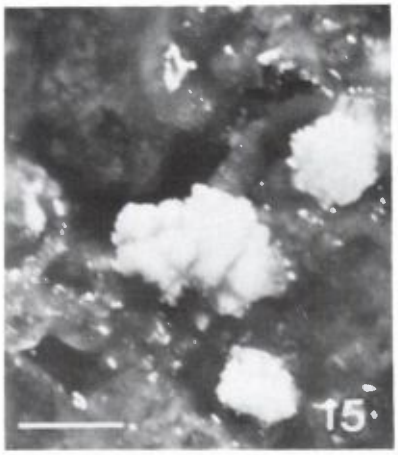
After 8 weeks of cultivation the cultures on agar slants maintained a soft and non-compact habitus. Thus, *Dermatocarpon miniatum* could not be cultivated on agar plates beyond the early stages of lichenization. This was caused by the high moisture of the agar substrate and by its favourable nutritive properties.

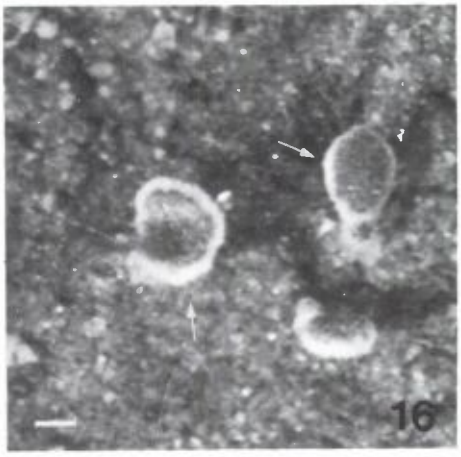
On soil substrate 2-3-months-old cultures showed the beginning of development of a fungal pseudoparenchyma, in which a great number of the available algal cells were integrated (Figs. 13, 14).

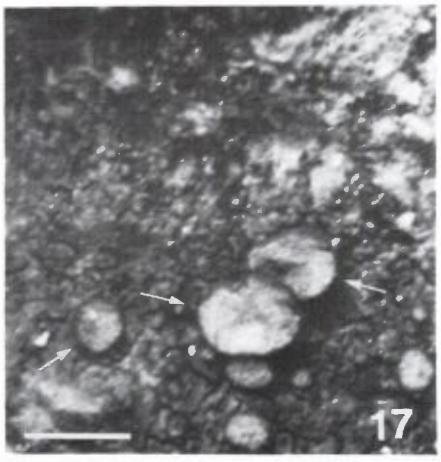
After 3–4 months thallus primordia were formed, which were distinctly delimitated from the thin algal film covering the soil substrate (Fig. 15).

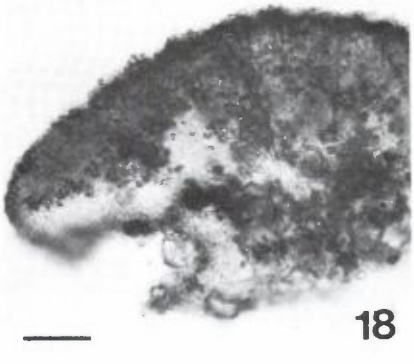

13


14


15


16


17


18

Similar primordia were also formed on silica gel substrate (Figs. 11, 12), but cultures on silica gel were restricted to these developmental stages (Fig. 12). Our results indicate that the silica gel-substrate had, in contrast to the agar plates, a lack of nutritive properties for lichen development.

Further development of *Dermatocarpon miniatum* stages could be obtained only on soil substrate. After 5 months of cultivation small thallus initials were formed with a slender transparent cortex layer. Cortex development occurred especially on the edge of the developing thalli. The primordia were surrounded by a rim of white hyphal network, which probably originated from the formation of the lower cortex (Fig. 16).

Fully developed thallus lobes were formed after a cultivation period of 6–7 months (Fig. 17).

In many respects thalli obtained in the laboratory were similar to thalli from the natural environment. Like natural thalli the resynthesized lobes had a thin upper cortex layer and a relatively thick bottom cortex layer (Fig. 18). Both thalli from culture and the natural habitat were surrounded by a dark coloured rim. Cultured lobes deviated from natural lobes only by the consistency of the thallus structure, which was more compact under natural conditions. The size of cortex, algal layer and medulla were nearly identical.

4. Discussion

The resynthesis of thalli of *Dermatocarpon miniatum* from its isolated myco- and photobiont reveals a further possibility to study the life cycle of a foliose lichen which is comparable to the well studied *Xanthoria parietina* (Bonnier, 1887, 1889; Bubrick and Galun, 1986; Ott, 1987a, b, c).

In contrast to Lallemant and co-workers (Lallemant and Bernard, 1977; Lallemant, 1985), who obtained thallus-like or even highly differentiated mycelia in their mycobiont cultures with some foliose lichens, the *Dermatocarpon miniatum* fungus did not form similar structures. On BBM-agar the *Dermatocarpon* mycobiont only formed germ tubes and rarely branched mycelia. On Sabouraud-agar, which was preferentially also used by Lallemant (Lallemant and Bernard, 1977; Lallemant, 1985) we obtained compact mycelia with vertical extension, but no layered or thallus-like structures.

Ahmadjian (e.g. Ahmadjian, 1966; Ahmadjian and Heikkilä, 1970; Ahmadjian et al., 1980; Ahmadjian and Jacobs, 1981, 1982, 1985) conducted most of the mycobiont cultures on nutrient deficient media and on nutrient

rich media such as Lilly-and-Barnett-agar and malt-yeast extract substrate. He did not obtain such highly differentiated fungal thalli (Ahmadjian, 1980) as observed by Lallemant. The different results show that obviously not every isolated lichen fungus is able to form these highly differentiated layers without the photobiont. On the other hand Lallemant's experiments indicate that even organic media alone can stimulate some lichen fungi to further differentiation.

It was of great interest that the growth of the *Dermatocarpon miniatum* fungus was influenced by algal colonies on the substrate, even when there was no direct contact between fungal hyphae and algal cells. Although the acceleration of fungal growth by the presence of algae on the substrate alone was limited to a time interval of a few days, it was a remarkable effect which occurred in all investigated cultures. Ostrovsky and Denison (1980) found that the percentage of ascospore germination of *Xanthoria parietina* was higher on lichen extract agar than on bark extract agar. This indicates that spore germination is also stimulated by an extract which contains lichen algae and not only by a favourable substrate. Lallemant and Bernard (1977) found the development of the *Lobaria-laetevirens* fungus to be completely dependent on the presence of the algal partner. For the *Lobaria-pulmonaria* mycobiont the presence of the algal colonies was also favourable for growth, but not obligatory. These examples seem to indicate that there exist different gradations of coevolution between lichen symbionts.

As a result of their carbohydrate transfer experiments Hill and Ahmadjian (1972) postulate a close relationship between *Stichococcus*-and *Hyalococcus*-algae. Our experiments to cross the *Dermatocarpon* fungus with *Stichococcus* algae from *Endocarpon pusillum* (unpublished results) on an agar substrate have not been successful to date.

Our experiments show that silica gel and soil substrate are well suited substrates for resynthesis cultures with lichens commonly growing on soil and related substrates. In agreement with Ahmadjian et al. (1980) the contamination of soil cultures is almost negligible. Although the long-term cultures had to be opened for observation and macrophotography, infections rarely occurred. The contamination with mites which is often a problem in lichen cultures could be avoided by the use of lichen material which was washed very carefully and kept frozen for some time. The cultures on silica gel were partially successful for the development of *Dermatocarpon miniatum*. The development was restricted to the formation of primordia.

Although *Dermatocarpon miniatum* is a wide-spread lichen, its growth and development in the natural environment seem to be still uninvestigated. The studies of Janex-Favre and Wagner (1986) mainly consider the ontogeny of the fruiting structures (e.g. pycnidia) of *Dermatocarpon miniatum* and related species. As *Dermatocarpon miniatum* reproduces sexually by fungal spores, it is not clear by which strategy the fungal spores can associate with the right algal partner in the natural environment.

Acknowledgements

We thank Dr. W. Ruetz (Teisendorf, Germany) for valuable discussions and critical revision of the manuscript. The investigations were supported by a grant from the Bundesministerium für Wissenschaft und Forschung.

REFERENCES

Ahmadjian, V. 1966. Artificial reestablishment of the lichen *Cladonia cristatella*. *Science* **151**: 199–201.

Ahmadjian, V. 1967. A guide to the algae occurring as lichen symbionts: isolation, culture, cultural physiology and identification. *Phycologia* **6**: 127–160.

Ahmadjian, V. and Heikkilä, H. 1970. The culture and synthesis of *Endocarpon pusillum* and *Staurothele clopima*. *Lichenologist* **4**: 259–267.

Ahmadjian, V. 1973. Methods of isolating and culturing lichen symbionts and thalli. In: *The Lichens*. V. Ahmadjian and M.E. Hale, eds. Academic Press, New York, pp. 653–659.

Ahmadjian, V., Jacobs, J.B., and Russell, L.A. 1978. Scanning electron microscope study of early lichen synthesis. *Science* **200**: 1062–1064.

Ahmadjian, V. 1980. Separation and artificial synthesis of lichens. In: *Cellular Interactions in Symbiosis and Parasitism*. C. Layton, B. Vook, P.W. Pappas and E.D. Rudolph, eds. Ohio State University Press, pp. 3–25.

Ahmadjian, V., Russell, L.A., and Hildreth, K.C. 1980. Artificial reestablishment of lichens. I. Morphological interactions between the phycobionts of different lichens and the mycobionts of *Cladonia cristatella* and *Lecanora chrysoleuca*. *Mycologia* **72**: 73–89.

Ahmadjian, V. and Jacobs, J.B. 1981. Relationship between fungus and alga in the lichen *Cladonia cristatella* Tuck. *Nature* **289(5794)**: 169–172.

Ahmadjian, V. and Jacobs, J.B. 1982. Artificial reestablishment of lichens. III. Synthetic development of *Usnea strigosa*. J. Hattori Bot. Lab. **No. 52**: 393–399.

Ahmadjian, V. and Jacobs, J.B. 1985. Artificial reestablishment of lichens. IV. Comparison between natural and synthetic thalli of *Usnea strigosa*. *Lichenologist* **17(2)**: 149–165.

Bischoff, H.W. and Bold, H.C. 1963. Some soil algae from enchanted rock and related species. Univ. Texas Publ. No. 6318, *Phycological Studies IV*: 1–95.

Bonnier, G. 1886. Recherches expérimentales sur la synthése des lichens dans un milieu privé de germes. *Compt. Rend. Acad. Sci. (Paris)* **103**: 942–944.

Bonnier, G. 1887. La constitution des lichens. *J. de Bot. (Paris)* **1**: 1–5.

Bonnier, G. 1889. Recherches sur la synthèse des lichens. *Ann.Sci. nat. Bot. (Paris)*, Ser. VII **9**: 1–34.

Bubrick, P. and Galun, M. 1986. Spore to spore resynthesis of *Xanthoria parietina*. *Lichenologist* **18(1)**: 47–49.

Deason, T.R. and Bold, H.C. 1960. Exploratory studies of Texas soil algae. Univ. Texas Publ. No. 6022. *Phycological Studies* **I**: 1–70.

Esser, K. 1976. *Kryptogamen: Blaualgen, Algen, Pilze, Flechten.* Springer-Verlag, Berlin, p. 25.

Galun, M., Marton, K., and Behr, L. 1972. A method for the culture of lichen thalli under controlled conditions. *Arch. Mikrobiol.* **83**: 189–192.

Hill, D.J. and Ahmadjian, V. 1972. Relationship between carbohydrate movement and the symbiosis in lichens with green algae. *Planta* **103**: 267–277.

Jahns, H.M., Herold, K., and Beltman, H.A. 1978. Chronological sequence, synchronisation and induction of fruit bodies in *Cladonia furcata var. furcata* (Huds.) Schrad. Nova Hedwigia. **30**: 469–526.

Jahns, H.M. and Ott, S. 1982. Flechtenentwicklung an dicht benachbarten Standorten. *Herzogia* **6**: 201–241.

Janex-Favre, M.C. and Wagner, J. 1986. Recherches ontogéniques et structurales sur les pycnides des Dermatocarpacées (Pyrenolichenes). *Bull. Soc. Mycol. Fr.* **2**: 161–182.

Lallemant, R. and Bernard, T. 1977. Obtension de cultures pures des mycosymbionts du *Lobaria-laetevirens* (Lightf.) Zahlbr. et du *Lobaria-pulmonaria* (L.) Hoffm.: le role des gonidies. *Rev. bryol. et lichénol.* **43:** 303–308.

Lallemant, R. 1985. Le développement en cultures pures in vitro des mycosymbiotes des lichens. *Can. J. Bot.* **63:** 681–703.

Ostrovsky, A. and Denison, W.C. 1980. Ascospore discharge and germination in *Xanthoria parietina. Mycologia* **72:** 1171–1179.

Ott, S. 1987a. The juvenile development of lichen thalli from vegetative diaspores. *Symbiosis* **3:** 57–74.

Ott, S. 1987b. Sexual reproduction and developmental adaptations in *Xanthoria parietina. Nord. J. Bot.* **7:** 219–228.

Ott, S. 1987c. Reproductive strategies in lichens. In: *Progress and Problems in Lichenology in the Eighties.* E. Peveling, ed. Cramer, Gebr. BorntraegerVerlagsbuchhdlg. Berlin, Stuttgart, *Bibliotheca Lichenol.* **25:** 81–93.

Schuster, G., Ott, S., and Jahns, H.M. 1985. Artificial cultures of lichens in the natural environment. *Lichenologist* **17:** 247–253.

Stocker-Wörgötter, E. and Türk, R. 1987. Die Resynthese der Flechte *Verrucaria macrostoma* unter Laborbedingungen. *Nova Hedwigia* **44:** 55–68.

Stocker-Wörgötter, E. and Türk, R. 1988a. Licht- und elektronenmikroskopische Untersuchungen von Entwicklungsstadien der Flechte *Endocarpon pusillum* unter Kulturbedingungen. *Pl. Syst. Evol.* **158:** 313–328.

Stocker-Wörgötter, E. and Türk, R. 1988b. Culture of the cyanobacterial lichen *Peltigera didactyla* from soredia under laboratory conditions. *Lichenologist* **20:** 369–375.

Warén, H. 1920. Reinkulturen von Flechtengonidien. *Finska Vet. Soc. Förh.* **61:** 1–79.

Wörgötter, E. 1985. Licht- und rasterelektronenmikroskopische Untersuchungen über die Entwicklung und das Wachstum von Flechten verschiedener Wuchsform unter Kulturbedingungen. Ph.D. Dissertation, University of Salzburg.