Syo KUROKAWA*: Results of isolation and culture of lichen fungi and algae**

黒川 逍*: 地衣体を構成する菌類および藻類の分離と培養によって得た二・三の知見**

Lichen fungi and algae have long been considered to be cultured only with extreme difficulty. Recently, however, V. Ahmadjian (1967) summarized the results of his studies on isolation and culture of lichen fungi and algae, establishing isolation techniques of fungal and algal components of lichens. His studies have brought together a great deal of knowledge on physiology of the fungal and algal components of lichens. His studies also indicate that we will be able to make great progress in understanding or even controlling lichen symbiosis in the near future.

Some botanists believe that lichens should be considered a separate plant group, whereas some others consider that lichens should be classified under Fungi. In order to decide whether lichens are a separate group or not, the study of lichen symbiosis may yield some important facts and suggestions us concerning the association of fungi and algae. It also may give us helpful suggestions relating to the differentiation or evolution of lichens.

With these points in mind, I started to study the isolation of fungi and algae from various lichens, re-establishment of lichen association, chemical products of fungal and algal components, etc., in cooperation with Dr. S. Shibata of the Department of Pharmaceutical Sciences, University of Tokyo. Experiments have been carried out mainly by Mr. T. Komiya, a graduate student in this Department.

Algal cells were taken from fragments of lichen thalli by using a micromanipulator and transferred onto modified Bristol's medium, Detmer's medium, or Bold's medium. The cultures were incubated at 20°C under illumination with an incandescent light (1.000–2.000 lux). Isolation of fungal

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components were made from spores taken from apothecia onto various agar media in Petri dishes or test tubes. Agar media used here were dried yeast medium, soil extract agar medium, or Kaufman's modified malt extract medium. The fungal components have been cultured with some difficulty in some Japanese lichens such as species of *Anaptychia*, *Collema*, *Lobaria*, and *Peltigera*, while the algal components have been isolated and cultured without difficulty. In *Ramalina crassa* (Nyl.) Mot., both algal and fungal components have been isolated without difficulty and have been growing well. Therefore, this is the species mainly used in the experiments mentioned below.

We have been getting a number of interesting results through culture studies of algal and fungal components of *Ramalina crassa*. One of them is the lichen association reestablishment with isolated fungi and algae. Both algal cells and ascospores of *R. crassa* were cultured on an agar medium. Two or three months after incubation, a small colony composed of both fungal hyphae and algal cells was formed. In the colony, algal cells were scattered randomly among hyphae. After four or five months, algal cells were found mainly near the middle of a hyphal layer and a thin layer mainly composed of algal cells was observed. After seven month, the colony was composed of three layers; an outer layer composed of more or less densely interwoven hyphae, a central layer mainly of algal cells, and an inner layer of loosely interwoven hyphae. Even though the colony did not form any erect or ascending thalli, the inner structure resembled very much that of the thallus of *R. crassa* in nature; the outer layer corresponds to the cortex of the lichen thalli, the central layer to the gonidial layer, and the inner layer to the medulla.

Another interesting problem is the comparison of chemical products of lichens and their separate fungal and algal components. In 1949, Castle and Kubsch reported the production of usnic, didymic, and rhodocladonic acids by a fungus isolated from *Cladonia cristatella* Tuck. However, Ahmadjian (1964) and most other lichenologists denied the results obtained by Castle and Kubsch. In our study, comparison of chemical products of *Ramalina crassa* and its algal and fungal components was made by using thin layer chromatographic methods. The chromatograms were developed with a mixture of acetone and benzene (1 : 4). The results are shown in

fig. 1. It is interesting that both usnic and salacinic acids have been demonstrated on chromatograms of acetone extracts of *R. crassa* as well as its fungal component (Komiya and Shibata 1969). These substances, however, were never demonstrated in the algal component. Most other substances demonstrated in the fungal component are also identical with those in *R. crassa*, though some of them have not yet been identified with any known substances. Furthermore, substances produced by the lichen, except for an unknown sterol, were never demonstrated on chromatograms of acetone extract of the algal component. When the culture of the fungal component is maintained at 10°C, usnic and salacinic acids are produced by the fungus, as mentioned above. However, only usnic acid (no salacinic acid) was produced by the fungus when the culture was incubated at 25°C.

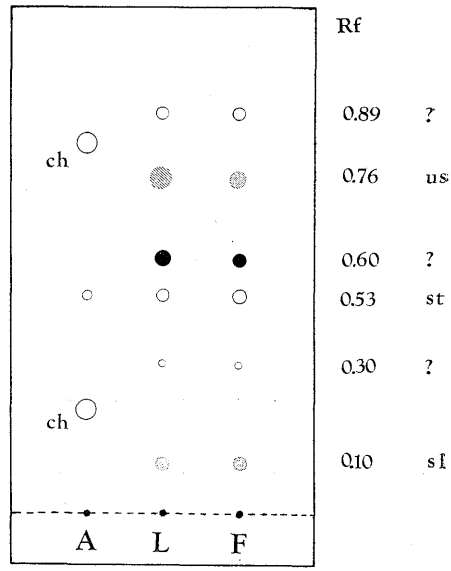

Fig. 1. Results of thin layer chromatographic tests on the acetone extracts of *Ramalina crassa* (L) and its fungal (F) and algal (A) components. ch: chlorophyll. sl: salacinic acid. st: sterol. us: usnic acid.

Comparison of chemical products of *Caloplaca aurantiaca* (Lightf.) Th. Fr. and its fungal component was also made. *C. aurantiaca* produces parietin, emosin, and an unknown anthraquinone in nature. With the thin layer chromatographic methods, these three substances were also demonstrated in acetone extract of the fungal component cultured on malt extract agar medium.

When lichen thalli of *Ramalina crassa* are kept under air containing CO_2 labeled by C^{14} under illumination, the CO_2 absorbed by the lichen thalli can be traced by its radioactivity. Sections of the thallus were pasted on glass slides and covered with a photo film. After removing it from the slides, the film was developed. Then, blackened silver grains were found

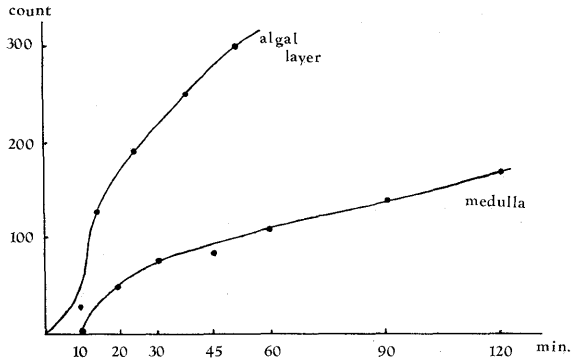

Fig. 2. CO_2 absorption by the algal layer and the medulla in *Ramalina crassa*.

at certain places on the film where it had been exposed to radioactivity of CO_2 labeled by C^{14}. Thus the distribution of absorbed atmospheric CO_2 in these lichen thalli is represented by the distribution of blackened silver grains on the film. When the thalli of *R. crassa* are kept for 10 minutes, CO_2 labeled by C^{14} is located mainly in the algal layer. Kept for 40 minutes, CO_2 is distributed in the algal layer, but hyphae surrounding algal cells also show radioactivity. Kept for 1 hour, CO_2 is distributed in the algal layer as well as in the medulla (composed of fungal hyphae). The relationships between CO_2 absorption and time period are shown in fig. 2, where CO_2 absorption is represented by numbers of blackened silver grains counted. The results of these experiments show that atmospheric CO_2 is absorbed by lichen algae quite rapidly (in 10 minutes), and CO_2 absorbed by algal cells is gradually transferred to fungal hyphae.

Comparison of sugar alcohol contained in *Ramalina crassa* and its fungal and algal components has also been made. *R. crassa* as well as the fungus and the alga isolated from it were extracted with 80% ethyl alcohol. The ethyl alcohol extracts were washed by ether in order to remove chlorophyll and fats, and then amino acids were removed by ion exchange resign. Thus the so-called "monosaccharide fraction" was obtained, but it contained mainly sugar alcohol. The fraction was dissolved in a mixture of ethyl acetate and trifluoro-acetic anhydride, following the trifluoroacetylation methods. Then, gas chromatographic testings were made. The fraction of *R. crassa*

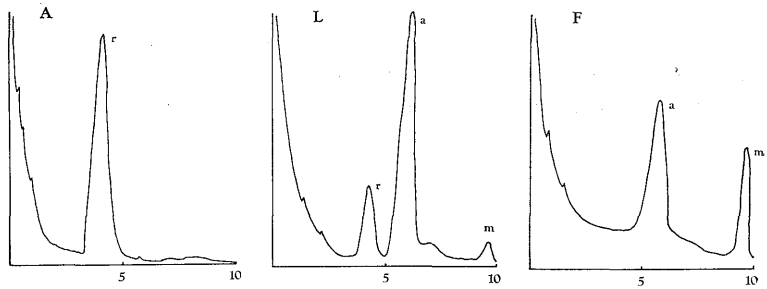

Fig. 3. Results of gas chromatographic tests on the ethyl alcohol extracts of *Ramalina crassa* (L) and its fungal (F) and algal (A) components. a: arabitol. m: mannitol. r: ribitol.

yielded ribitol (18.1%), arabitol (74.0%), and mannitol (5.1%), while the fraction of the alga yielded only ribitol (99.2%) and that of the fungus yielded arabitol (40.8%) and mannitol (33.%) (fig. 3).

Judging from the results of the experiments mentioned above, the lichen alga absorbs atmospheric CO_2 by carbon assimilation to form mainly ribitol. Then ribitol is gradually transferred to fungal hyphae in the lichen thalli. Lichen fungi may store carbohydrates transferred from algal cells as arabitol and mannitol. They also produce various lichen substances such as depsides, depsidones, anthraquinones, etc., as synthetic products of arabitol and mannitol.

References

Ahmadjian, V. 1964. Further studies on lichenized fungi. Bryologist **67**: 87–98. —— 1967. The Lichen Symbiosis. 152 pp. Waltham, Toronto. Castle, H. & Flora Kubsch. 1949. The production of usnic, didymic, and rhodocladonic acids by the fungal component in the lichen *Cladonia cristatella*. Arch. Biochem. **23**: 158–159. Komiya, T. & S. Shibata. 1969. Formation of lichen substances by mycobionts of lichens. Isolation of (+) usnic acid and salacinic acid from mycobionts of *Ramalina* spp. Chem. Pharm. Bull. **17**: 1305–1306.

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地衣体を構成する菌類および藻類の培養は非常に困難なものと考えられていたが，V. Ahmadjian の最近の研究でその方法もほぼ確立された。本論文では東大薬学部の柴田承二教授および小宮威弥氏の協力で得た分離と培養に関する 2·3 の知見を報告した。実験は主としてハマカラタチゴケ (*Ramalina crassa*) について行なった。ハマカラタチゴケから一旦分離した菌と藻を同一培地上で培養すると，7ケ月後には地衣体の構造によく似た組織の分化が認められた。ハマカラタチゴケとそれから分離した菌および藻が生産する化学物質を比較すると，藻から得られるものはもとの地衣体の生産物と異なる（ステロールを除いて）が，菌のそれは非常によく似ていて，とくにウスニン酸やサラチン酸が菌単独でも生産されることは注目に値する。C^{14} でラベルした CO_2 を使って炭酸同化作用による CO_2 の吸収をみると，地衣体内では CO_2 はまず藻細胞に吸収され，約1時間後には地衣体の菌糸にも見出されるようになる。この結果と，地衣体，藻細胞，および菌糸に含まれる糖アルコールの組成の比較から次のことが推定される。すなわち，炭酸同化作用によって地衣体に吸収された CO_2 は藻細胞のなかでリビトールとなり，比較的短時間のうちに菌糸に移動して，アラビトールやマンニトールとして貯蔵されるものと考えられる。

口堀田　満（文・画）：**しょくぶつもいきている**　23×26 cm, pp. 44（原色）；別冊 “解説書” pp. 16（単色写真入り）¥500，千趣会（大阪市北区同心町）　第一線の植物学者が幼児のための単行書を著わすことは稀である。前川博士の‘緑のこどもたち’のほかには寡聞で知らないが，本書は著者自身の創作絵画がその主要部分をなしている点において極めて特異のものである。各絵は相対する2ページにわたって1種づつ計20種あり，近景の植物の大きい図と，その後方の中・遠景が見事なパースペクティブと鮮烈な色彩で捕えられている。各図各様の大胆なポスター的手法による構成は，余白にある短かくて平明な説明文と共に，幼児に対するパターン教育用に成功しているのは勿論，成人も，いや，生植物の綿密な写生に基いてしか画けない具象性によって，専攻の植物学者もこれに学ぶことができる。絵は四季の移り変りの外に，夜景や地下の部分をあらわしたものもあり，単に高山～亜熱帯などわが国の異なる植生の紹介に止らず，生きている植物の諸器官や全体としての生活・いとなみ—生と死と・生長と繁殖と—を強い印刻力をもって楽しく教えてくれる。しかし著者の意図は単に新形式の絵本の提供にあるのではなく，次の世代にこの方面の目を開かせ，後に続くものを育てるのにあると見た。昭和45年5月以来，版を重ねているのも当然である。‘えほん自然科学館’12巻の中第7冊。　（津山　尚）