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The Effects of Water Content on the Resynthesis, Growth of Mycobiont and Photobiont and Lichen Substances Production

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Abstract The effect of water content was observed with respect to lichen fibril formation rate, growth rate of mycobiont and photobiont and production of lichen substances. Low water content caused rather rapid re-differentiation of fibrils but inhibited the growth of photobiont. In addition, low water content seems to accelerate the production of lichen substances.

Key words: lichen, agar concentration, resynthesis, lichen substances.

Introduction

Artificial synthesis and culture of lichens have increased rapidly in recent years. Lichens belonging to the genera *Cladonia* and *Usnea* were synthesized artificially in flasks and tubes (Ahmadjian *et al.*, 1980, 1981, 1985). As previously reported by Kon *et al.* (1990, 1993), thalli of *Usnea* species can be routinely induced from their growing tissues *in vitro* and the induced thalli are quite similar to natural ones.

Ahmadjian *et al.* (1980) reported that dry condition as an environmental factor is quite important to induce the lichen thalli artificially. Kon *et al.* (1990) reported that the temperature was an important factor as the culture condition for the lichen thalli to re-differentiate. The water content was changed by different agar concentration in the medium. The effect of water content is observed with respect to the fibril formation rate, the growth of mycobiont and photobiont, and the production of lichen substances.

Material and Methods

Usnea confusa subsp. Asah. *kitamiensis* (Asahina) Asahina was collected at Tanzawa, Kanagawa Pref. in May 1987. Growing tissues were obtained from living thalli by the method described by Yamamoto (1985). Agar was added to the normal malt-yeast extract medium in 1, 2, 3, 4 and 5% in concentration. The water content (W) in cultures and the medium was calculated by the following expression.

Water content = fresh weight − dry weight/fresh weight × 100(%)

Fibril formation rate was calculated as a percentage of number of test tubes, in which fibrils or at least one fibril were formed, to ten test tubes tested. Each set of ten test tubes was incubated under a 12/12 hr light (C.13 μmol/m^2/s)-dark cycle at 18°C, pH 5.8.

The method of measuring the growth rate of the mycobiont is as follows. The mycobiont is mildly ground down and floated on distilled water. Part of it (c. 1 mg) was planted in the normal MY medium in a test tube. The mycobiont was separated from the medium seven weeks later and the dry weight was weighed.

The growth of the photobiont was measured as follows. The photobiont cultured in a test tube containing BBM medium is floated on distilled water. Part of it (c. 0.000135 ml) was planted and cultured on the normal MY medium of plastic petri dishes under light condition (13 μmol/m^2/hr.). Packed Cell Volume of the photobiont was measured six weeks later. pH and temperature were the same as in the case of mycobiont.

Secondary products of each synthetic culture and natural lichen were determined and quantified by TLC (Culberson, Elix, 1990) and HPLC methods described by Kon *et al.* (1993).

Aluminum sheets were used as plugs of test tubes in most cultures, while polypropylene caps were also used for some other test tubes.

Results and Discussion

Figure 1 shows relations between agar concentration and formation rate of the lichen fibrils observed 66 days after inoculation. It appeared to indicate that the lichen fibrils formation varies in accordance with the agar concentration. That is, the fibril formation rates are higher, when the agar concentrations are higher. It is noteworthy that fibrils formation rates are much higher, when the polypropylene stopping was used instead of the aluminum stopping as shown in parentheses in the Table 1.

Table 2 shows the water contents in mixed cultures of mycobiont and photobiont on the medium of different agar concentrations. The water contents in mixed cultures of mycobiont and photobiont are lower when agar concentrations are higher. It is apparent that the water contents in culture vary depending on the agar concentration in the medium. On 66 days after inoculation, water contents in the medium are much lower when polypropylene caps are used for aluminum sheets as shown in parentheses in the Table 2, even though the agar concentrations are the same at the beginning of the inoculation. The difference of water content in the medium may be probably caused by the size of slit between cap and test tubes.

Judging from the above-mentioned result, the water contents in mixed cultures depend on the amount of water contained in the medium.

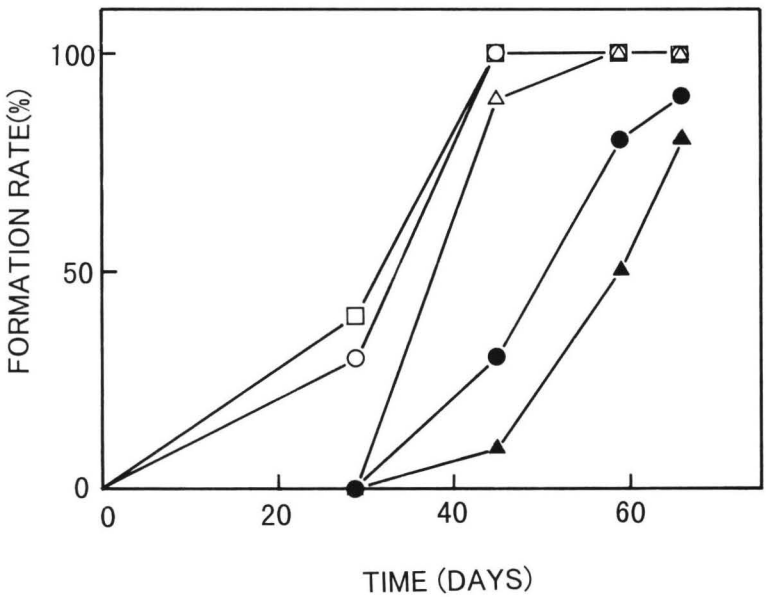

Fig. 1 Lichen formation rate (percentages of number of test tubes, in which fibrils, or at least a fibril, were formed, to ten test tubes) of regenerated fibrils on the medium with various agar concentration when aluminum stopping was used.
▲: 1% ●: 2% △: 3% ○: 4% □: 5%

Table 1. Average number of fibrils formed in a test tube on 66 days after inoculation.

Agar conc.	1	2	3	4	5
Average number of fibrils	3.5±1.7	7.3±6.0 (>100)	14.5±6.7 (>100)	23.2±10.3 (>100)	36.2±15.0

* Data which were measured when polypropylene caps were used were shown in ( ).

Table 2. Water content of mixed culture and agar on 66 day after inoculation.

Water content (%)	Agar conc. (%)				
	1	2	3	4	5
Mixed culture	89.2±0.47	87.5±0.37 (83.9±0.74)	85.0±0.91 (81.8±1.05)	83.3±0.80 (79.7±1.12)	80.2±1.19
Agar	98.6±0.19	97.1±0.35 (95.9±0.38)	95.8±0.20 (94.9±0.39)	94.6±0.47 (93.7±0.25)	93.5±0.73

* Data which were measured when polypropylene caps were used were shown in ( ).

Table 3. Lichen substances produced in cultured tissues.

Content (μg/mg. dr. wet)	Agar conc. (%)				
	1	2	3	4	5
Salazinic acid	ND	ND (ND)	ND (ND)	ND (ND)	ND
Norstictic acid	0.2	0.2 (22.5)	2.5 (43.5)	6.0 (31.0)	26.0
Usnic acid	ND	ND (6.8)	0.2 (19.4)	1.0 (19.5)	6.8

*Data which were measured when polypropylene caps were used were shown in ( ).

It is also noted here that the rate of the re-differentiation of the lichen fibrils depends on the water contents included in mixed cultures. When culturing by using polypropylene caps, higher rates of the re-differentiation of the lichen fibrils were obtained as mentioned above. It was probably caused by higher degrees of evaporation through larger slits between caps and test tubes. The results mentioned above may indicate that drier conditions of the medium give higher rates of re-differentiation of lichen fibrils.

Table 3 shows the amount of lichen substances produced by growing tissues under a different concentration of agar. Salazinic, norstictic and usnic acids are detected in *U. confusa* which grows in the natural condition. Salazinic acid was detected in none of growing tissues. However, the contents of usnic acid and norstictic acid increased as the density of agar rises. Especially, the contents of both acids were higher when polypropylene caps were used. That is, when culturing in the density of agar with a high lichen fibril formation rate, the synthesis of lichen substances was active.

Culberson (1992), Hamada (1993) and Kinoshita (1993) are reporting that mycobiont cultured under the dry conation or the high osmotic condition actively synthesizes lichen substances. Their reports also seem to support the results of the present study.

Figure 2 is a result of examining the influence of the concentration of agar on the growth of myocobiont and photobiont. The growth of mycobiont was enhanced when the amount of moisture of the medium decreased. On the other hand, that of photobiont shows tendency to repress on the medium with low moisture condition.

The results mentioned above is corresponding to that reported by Kon *et al.* (1993). That is, the best culture condition of the re-differentiation of the lichen fibrils is corresponding to the best culture condition of the growth of mycobiont. In the medium of high water content, the growth of mycobiont is slow. On the other hand, the growth of photobiont is relatively good. Mycobiont surrounding photobiont or

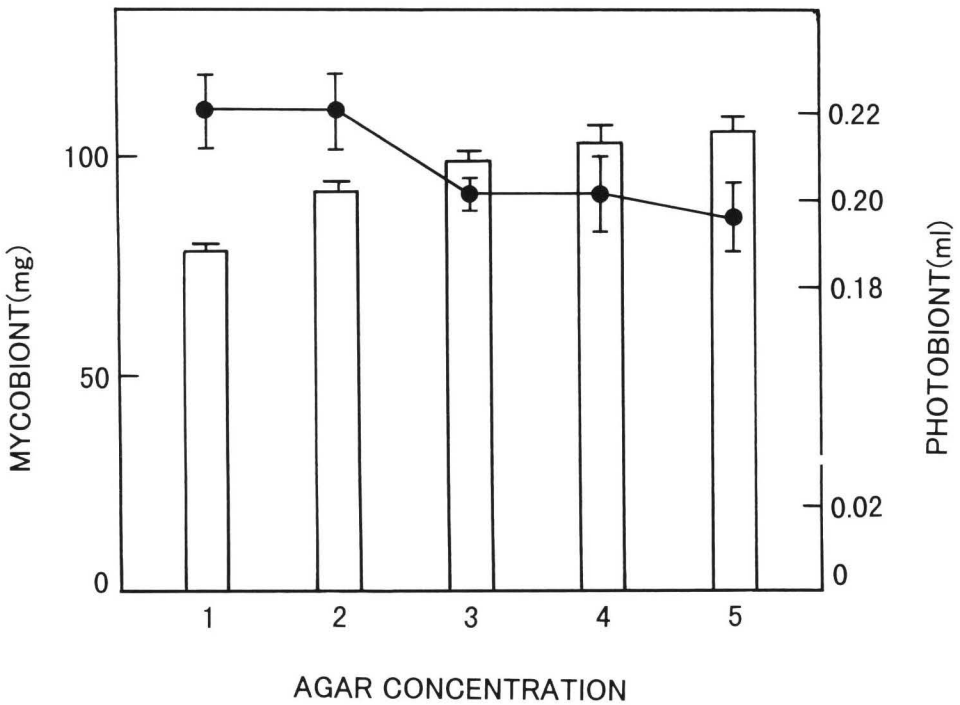

Fig. 2 Growth rate of isolated mycobiont and photobiont of *U. confusa* subsp. *kitamiensis* cultured on the medium with various agar concentration. Each column represents the mean±S.D. of 10 determinations of mycelia (left) and, each plot represents the mean±S.D. of 3 determinations of photobiont (right). Vertical bars give±S.D. where larger than the symbols.

soredial compound is formed in the early stage of development of lichen fibrils (Kon, 1990). Photobiont which proliferates actively can not be surrounded by mycobiont on the medium of high water content. Therefore, it is suggested that soredial cluster formation and the formation of the lichen fibrils did not advance in the medium of high water content.

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