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TISSUE CULTURE OF SOME ANTARCTIC LICHENS PRESERVED IN THE REFRIGERATOR

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Abstract: Cultured tissues were obtained for the first time from the frozen thalli of three Antarctic lichens, *Rhizocarpon flavum* DODGE & BAK., *Umbilicaria aprina* NYL., and *U. decussata* (VILL.) ZAHLBR. preserved in a refrigerator for nearly five years. The effect of temperature on their growth was determined by means of tissue culture. For comparison, cultured tissues of two Japanese *Umbilicaria* species, *U. muehlenbergii* (ACH.) TUCK. and *U. pennsylvanica* HOFFM., were used. All cultured tissues treated, except those of *U. aprina*, show better growth at 15°C than at 5°C. Only *U. aprina* (mycobiont) grows better at 5°C than at 15°C and it seems to be strongly adapted to cold temperature; it grows five times its initial weight at 5°C after 16 weeks culture, while at 15°C the same species grows twice its initial weight.

1. Introduction

Cultured tissues were obtained from the fresh vegetative lichen thalli which were usually within two weeks of collection (YAMAMOTO *et al.*, 1985, 1987a, b; YOSHIMURA *et al.*, 1987). We were able to get undifferentiated cultured tissues for the first time from the frozen lichen thalli, which had been preserved for a long period in the refrigerator. In addition, by using cultured tissues we observed the effect of temperature on the growth of cultured tissues.

Although the present paper is preliminary, it may be the first time that the cultured tissues of Antarctic lichens and the effect of temperature on their growth have been reported.

2. Materials

Materials used for this study were collected from the Syowa Station area by H. KANDA during the 24th Japanese Antarctic Research Expedition in 1983. They were frozen at −20°C and stored at the National Institute of Polar Research (Tokyo). For the comparison of the effect of temperature on growth, cultured tissues of two Japanese *Umbilicaria* species, collected in northern Honshu in 1987 by I. YOSHIMURA, were investigated. Lichen names follows KASHIWADANI (1982) for Antarctic lichens and YOSHIMURA (1974) for Japanese lichens.

Umbilicaria aprina NYL. syn. *Umbilicaria antarctica* FREY & LAMB.: Antarctic.

Standnibba, near Syowa Station, elevation 170 m, January 21, 1983, collector H. KANDA, 1011-b.

Umbilicaria decussata (VILL.) ZAHLBR.: Antarctic. Standnibba, near Syowa Station, January 21, 1983, collector H. KANDA no. 648.

Rhizocarpon flavum DODGE & BAK.: Antarctic. Yukidori Valley, Langhovde, near Syowa Station, elevation 170 m, October 1, 1983, collector H. KANDA, 1014-n.

Umbilicaria muehlenbergii (ACH.) TUCK.: Japan. Honshu. Mt. Nuidoishiyama, Shimokita Peninsula, Prov. Mutsu (Aomori-ken), August, 1987, collector I. YOSHIMURA no. 339.

Umbilicaria pennsylvanica HOFFM.: Japan. Honshu. Mt. Nuidoishiyama, Shimokita Peninsula, Prov. Mutsu, (Aomori-ken), August 1987, collector I. YOSHIMURA no. 340.

3. Methods

3.1. Induction

Cultured lichen tissues were induced with the same method described by YAMAMOTO *et al.* (1985) (Fig. 1). A: Lichen thalli stored in the freezer, were put at room temperature for about one day; then they were cut into pieces of 1 cm long or 1 cm square; B: they were then washed with city water for 1 h; C: homogenized with 5 m*l* of distilled water under sterile conditions; D: the suspensions were passed through a sterilized stainless filter with a 500 μm mesh; E: the filtrates were passed through a sterilized nylon filter with a 150 μm mesh; F: small segments from the second filtration were picked up with sterilized bomboo sticks under a binocular microscope; G: these fragments were planted onto slant media in test tubes and cultured at 15°C under shaded conditions (about 50 lx); the medium consisted of 2% w/v malt extract, 0.2% w/v yeast extract and 2% w/v agar (AHAMADJIAN, 1961, 1964).

Cultured tissues that were usually composed of fresh hyphae and algae, appeared after two to four weeks, and were transferred to a fresh medium every about six weeks under the same conditions.

3.2. Sub-culture

Cultured tissues were used to test the effect of temperature on growth. Cultured

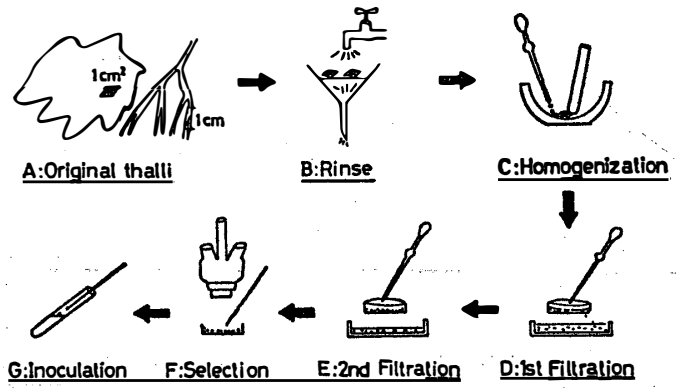

Fig. 1. Induction and culture methods of lichens.

tissues were cut into segments (about 5 mg) with a scalpel. Two segments were placed on each plate of malt yeast-medium (Petri dishes 9 cm in diam.) and cultured for four weeks at 5°C and 15°C under about 501x (Fig. 2). Every four weeks, growing segments were transferred to fresh media of the same composition and cultured under the same conditions.

4. Results and Discussion

Cultured tissues were obtained from the all species treated. The growth rate curves of each species at both temperatures are presented in Figs. 3–5. At 5°C, cultured tissues of *Rhizocarpon flavum* (both mycobionts and phycobionts) do not grow, while at 15°C they grow 13 times of the initial weight after 12 weeks (Fig. 3). Although the growth is slow, tissues of *Umbilicaria aprina* (mycobiont) grow better at 5°C than at 15°C; even after 16 weeks culture at 5°C they grew only five times the initial weight (Fig. 3).

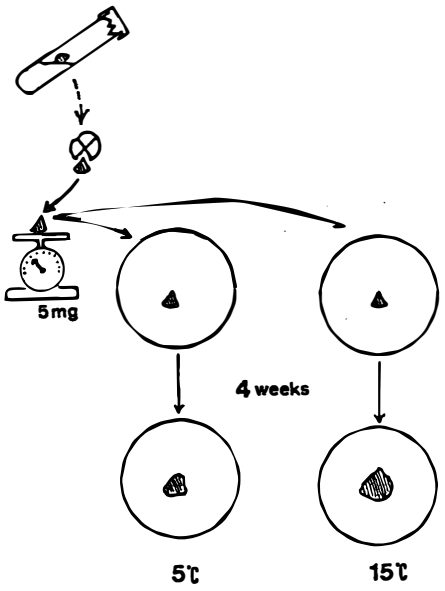

Fig. 2. Subculture method of lichen tissues.

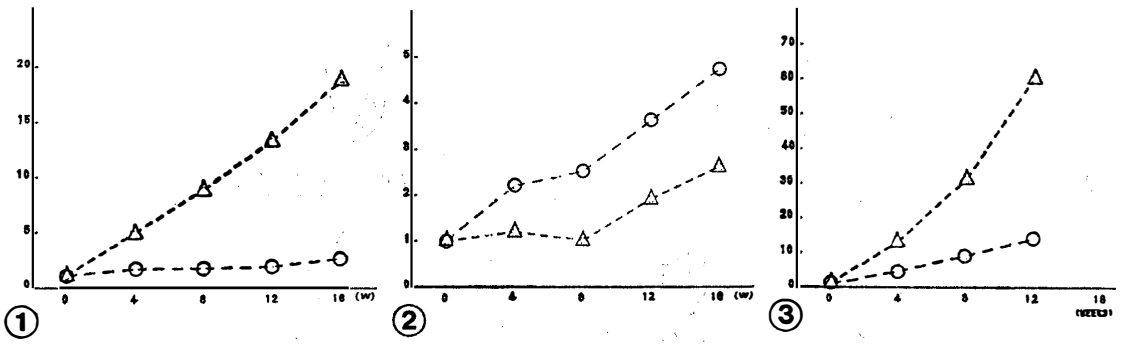

Fig. 3. Effect of temperature on tissue growth rate of Antarctic lichens. 1. Rhizocarpon flavum *(both mycobiont and phycobiont). 2.* Umbilicaria aprina *(myconbiont). 3.* Umbilicaria decussata *(mycobiont).* —○— *5°C;* —△—*15°C.*

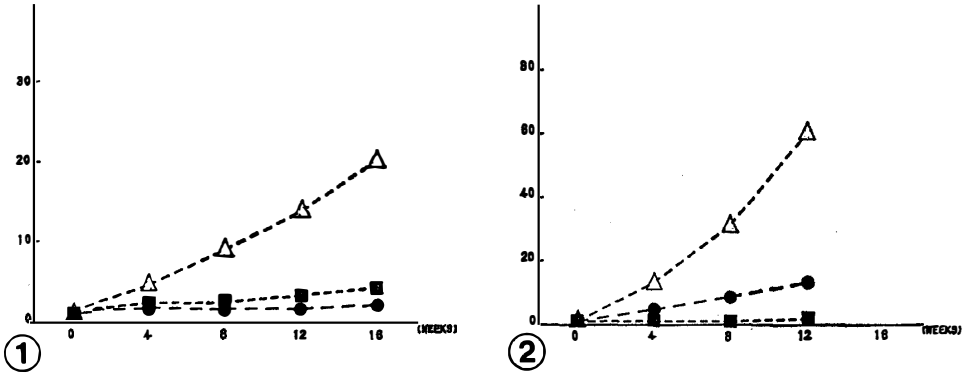

Fig. 4. Tissue growth rate of three Antarctic lichens at (1) 5°C and (2) 15°C. —●— Rhizocarpon flavum; —△— Umbilicaria decussata; —■— U. aprina.

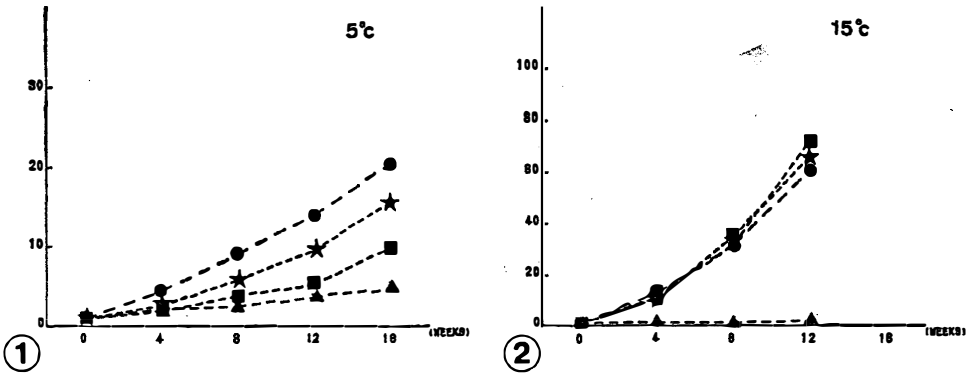

Fig. 5. Tissue growth rate of four Umbilicaria *species at (1) 5°C and (2) 15°C.* —●— U. decussata; —▲— U. aprina; —■— U. muehlenbergii; —★— U. pennsylvanica.

Cultured tissues of *Umbilicaria decussata* (mycobiont) grow well, increasing by about 60 times their initial weight after 12 weeks at 15°C; even at 5°C *U. decussata* grows well, with increases of about 14 times their initial weight after 12 weeks (Fig. 3).

Of the three Antarctic lichens, *Umbilicaria decussata* grows best and *Rhizocarpon flavum* seems not to grow at 5°C (Fig. 6). *U. decussata* grows best at 15°C, while *U. aprina* does not grow well at 15°C (Fig. 3).

Two Japanese *Umbilicaria* species were used for comparison. Cultured tissues (mycobiont) of *U. pennsylvanica* grow very well, similar to those of the Antarctic *U. decussata*. They grow nearly 100 times their initial weight at 15°C after 16 weeks culture (Fig. 5). The cultured tissues (mycobiont) of *U. muehlenbergii* grow very well and nearly as much as the Antarctic *U. decussata* (Fig. 5). Figure 5 also shows the growth of 4 *Umbilicaria* species at 5°C and 15°C. With the exception of *U. aprina*, the other three species of *Umbilicaria* grow well and show similar growth rate at 15°C. As shown in Fig. 3-(2), cultured tissues of *U. aprina* grow better at 5°C than at 15°C. Antarctic *U. aprina* (mycobiont) seems to be strongly adapted to cold temperature. As seen in Fig. 5, the growth of *U. decussata* (mycobiont) is similar to that of *Umbilicaria* species which normally grow in cool temperate and subalpine regions of Japan.

Although the present investigation is preliminary, the growth of these Antarctic

lichens responds differently to temperature. Some Antarctic species seem to be strongly adapted to cold temperature (*i.e. Umbilicaria aprina*), and some species behave similarly to subalpine lichens in the Northern Hemisphere, performing better at a moderate temperature (15°C) than at a cold temperature (5°C).

5. Conclusion

1. Frozen lichen material can be used for tissue culture.
2. Antarctic *Umbilicaria aprina* (mycobionts) seems to be well adopted to cold temperature, growing better at 5°C than at 15°C.
3. Antarctic *Umbilicaria decussata* has a growth rate similar to *Umbilicaria* species which grow in subalpine and cool-temperate regions in Japan.
4. *Umbilicaria decussata* and *Rhizocarpon flavum* exhibit better growth rates at 15°C than at 5°C.

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