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Difference of Cell-viability and Spore Discharge Capability between Two Lichen Species, *Letharia columbiana* (Nutt.) Thoms. and *L. vulpina* (L.) Hue.

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Lichens are symbiotic associations of fungal (mycobiont) and algal (photobiont) partners. They are widespread all over the world and reproduced by sexually produced ascospores and/or asexual propagules (isidia, soredia and thallus fragments). These reproductive tools are important as taxonomic markers, therefore there are many cases where we can find the fertile species and its non-fertile (sorediate and/or isidiate) counterpart species. When we are able to culture fertile species and its non-fertile one and compare the physiological properties of these cultures, it is thought that the difference between them plays a significant role in the recognition of lichen species. It is interesting to clarify physiological properties of these lichen cultures.

We established the method of lichen tissue culture from thallus segments [1]. We also investigated the induction factors of tissue cultures as well as their growth factors and found that induction of tissue culture was based on cell-viability of the tested thalli [2, 3]. In the present paper, we studied cell-viability by induction of tissue culture and discharge of ascospores in two similar lichen species, *Letharia vulpina* (L.) Hue and its non-isidiate/sorediate and frequently fertile counterpart species, *Letharia columbiana* (Nutt.) Thoms.

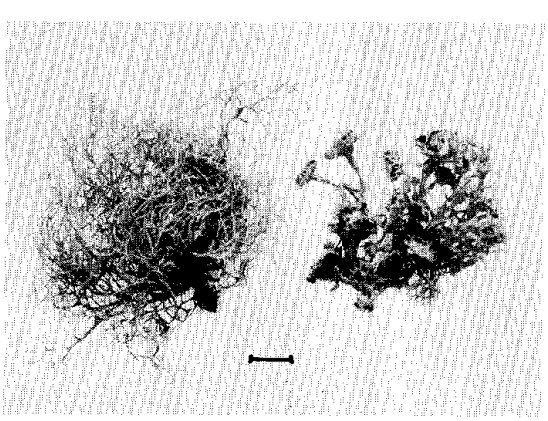

Fig. 1 *Letharia vulpina* (Left) and its non-isidiate/sorediate, frequently fertile counterpart species *Letharia columbiana* (Right). Bar, 1 cm.

Letharia columbiana and *L. vulpina* are beautiful gold-colored lichens and abundantly distributed in high mountainous areas of North America [4]. Specimens of *Letharia columbiana* (**Fig. 1**) were collected at Highway 4, north of Tamarack, Calaveras, California, U.S.A. (Kroken specimen no. 23) and Mt. Whistler, British Columbia, Canada (no. c822-16). Those of *Letharia vulpina* (**Fig. 1**) were collected at Highway 4, east of Camp Connell, Calaveras, California, U.S.A. (Kroken no. 13) and Buse Hill, British Columbia, Canada (no. c827-20). After the collection, the specimens were stored at −25°C for 9 months and voucher specimens were deposited in the herbarium of Nippon Paint Co. Ltd., Osaka, Japan.

A fragment (1 cm in length) was cut off from the tip of a thallus of each specimen. According to the Yamamoto method [1], each thallus fragment was homogenized in a mortar with sterilized water, and small segments of 150 to 500 μm in size were selected by a two-filter system. Each segment was inoculated onto an agar-plate of 5 m*l* malt-yeast extract medium in the test tube, and cultured at 15°C in the dark for 6 months. The mycobiont hyphae projecting from small segments in each test tube were observed every week after inoculation. Colony formation rate (CFR=the number of test tubes with colony formation × 100/ the number of uncontaminated test tubes) and average number of weeks until first appearance of mycobiont hyphae (IP) were measured.

We found the widespread cell-viability in vegetative thalli of lichen species belonging to the same family, Usneaceae [2] and a reduction of cell-viability after storage of thalli of some species after collection [3]. These observations indicate that cell-viability of thalli used in experiments can be evaluated by the CFRs and IPs for the induction of tissue culture. In induction experiments of tissue culture of *Letharia*

Table 1. Colony formation from the thallus segments of *Letharia* species by the tissue culture method within two weeks after collection.

Species	Locality	CFR*1	IP*2
Letharia columbiana	Canada	0	nd*3
	U.S.A.	9	9.0
Letharia vulpina	Canada	100	4.9
	U.S.A.	100	8.3

*1 CFR : Colony formation rate=the number of test tubes with colony formation × 100/ the number of uncontaminated tested tube.

*2 IP : Average number of weeks until first appearance of mycobiont hyphae.

*3 nd : No data.

Table 2. Effect of storage period at −25°C on spore discharge.

Species	DR*1 NSP*2					
	3 Months		6 Months		9 Months	
Letharia columbiana	2/2	27±17	1/1	33	0/2	0
Letharia vulpina	3/6	104±65	0/4	0	0/2	0

*1 DR : The number of apothecium discharging spores/ the number of tested one.

*2 NSP : Average number of discharged packets per apothecium discharging spores and standard deviation.

lichens collected from U.S.A. and Canada, the CFRs and IPs of *L. vulpina* were higher and smaller respectively than those of *L. columbiana* (**Table 1**). Thus, isidiate/sorediate thalli of *L. vulpina* have higher cell -viability than those of their non-isidiate/sorediate counterpart species, *L. columbiana*. Both tissue cultures of *L. columbiana* and *L. vulpina* grew well on MY medium at 15°C in the dark, therefore it is assumed that both mycobionts had the same capability of growth.

According to the Ahmadjian's method [5], an apothecium of each specimen was cut off from a thallus, submerged in sterilized water, and then the apothecium was fixed with silicon grease (Toray Silicone Co., Ltd., Tokyo, Japan) onto the inside of a top cover of a Petri dish (60 mm in diameter) containing 5 m*l* plain agar-medium. After observing spore discharge onto the agar-plate from each apothecium under the microscope (× 40), the number of discharged spore packets was counted. Spores were usually discharged as a packet consisting of several spores. Discharge ratio (DR, percentage of the number of apothecium discharging spores to the number of tested one) and NPS (the number of discharged spore packets per apothecium) were measured in each dish.

Garrett [6] found that spore discharge of lichens ceased after nine to ten months (or often sooner) of storage of the samples. Almost all the tested apothecia of *L. columbiana* thalli stored at −25°C for 3 to 6 months discharged ascospores. Only half of the tested apothecia of *L. vulpina* thalli after 3-month storage discharged ascospores while none of the tested apothecia stored for 6 months showed any spore discharge as shown in **Table 2**. This result indicates that the capability of spore discharge of frequently fertile species, *L. columbiana* is superior to that of *L. vulpina*. We also found that the discharged spores of both species could germinate inspite of the −25°C storage.

Apothecia of *L. columbiana* can survive and discharge ascospores after long storage at cold winter-like temperatures. Also, vegetative cells of *L. vulpina* thallus have such high viability as to enable all small segments to grow in culture tubes. These results suggest that the reproduction of each species is mainly carried out by its well-developed structure (either apothecia sexually producing ascospores or vegetative diaspores) on the thallus as impressed by the external appearance of each species.

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