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Regeneration of Juvenile Thalli from Transplanted Soredia of *Parmotrema clavuliferum* and *Ramalina yasudae*

Yoshiaki Kon[1]* and Yoshihito Ohmura[2]

[1] Tokyo Metropolitan Hitotsubashi High School, Higashikanda 1–12, Chiyoda, Tokyo, 101–0031 Japan
[2] Department of Botany, National Museum of Nature and Science, Amakubo 4–1–1, Tsukuba, Ibaraki, 305–0005 Japan
* E-mail: spwy2ww9@herb.ocn.ne.jp

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Abstract Transplantation experiments were performed using soredia of *Parmotrema clavuliferum* and *Ramalina yasudae*, which are common lichens growing at low altitude areas of Japan. Soredia of *P. clavuliferum* were attached to adhesive tapes on a plastic plate, and the plate was fastened to a tree trunk. After 12 months, more than half of the soredia adhered to the tapes were differentiated into planiform lobes that very rarely formed cilia-like structures along the margins. For the transplantation experiment using *Ramalina yasudae*, thallus fragments bearing soredia were excised from the tips of the thallus, and the thalli were fastened to a tree trunk using a nylon mesh. After 15 months, the transplanted soredia showed a marked secretion of gelatinous matrix on their surfaces, and some differentiated into protuberance forms. After 18 months of transplantation, some of the thalli differentiated into fruticose forms.

Key words : foliose lichen, fruticose lichen, transplantation, vegetative reproduction.

Introduction

Lichen is a symbiotic organism consisting of a fungus and an alga (and/or cyanobacterium). Lichens have two main modes of reproduction: one is mycospore-based reproduction and the other is vegetative reproduction. In mycospore-based reproduction mode, germinating mycelia from the mycospore need to encounter an appropriate alga nearby to form a new lichen thallus. However, vegetative reproduction is not necessary to find algal partner in the field because the diaspore has an appropriate alga from the beginning. Therefore, it is generally considered that vegetative reproduction is a more advantageous ecological strategy than mycospore-based reproduction (Ott, 1987a).

Lichens reproduce with different types of symbiotic vegetative diaspores, such as fragments of thallus, isidia, schizidia, and soredia, which are defined by morphological and ontogenetic differences (Büdel and Scheidegger, 2008). Regeneration from a diaspore into thallus in a field was confirmed with various lichens such as *Hypogymnia physodes* (L.) Nyl., *Leptogium saturninum* (Dicks.) Nyl., *Lobaria pulmonaria* (L.) Hoffm., *L. scrobiculata* (Scop.) P. Gaertn., *Menegazzia terebrata* (Hoffm.) A. Massal., *Parmelia sulcata* Taylor, *Parmotrema tinctorium* (Nyl.) Hale, *Physcia tenella* (Scop.) DC., *Platismatia glauca* (L.) W. L. Culb. & C. F. Culb., *P. norvegica* (Lynge) W. L. Culb. & C. F. Culb., *Sticta fuliginosa* (Dicks.) Ach., *Usnea antarctica* Du Rietz, *U. filipendula* Stirt., *Xanthoria parietina* (L.) Beltr., and more (Schuster, 1985; Schuster *et al*., 1985; Ott, 1987a, 1987b, 2004; Hilmo and Ott, 2002; Honegger, 1996; Scheidegger, 1995; Scheidegger *et al*., 1995; Zoller *et al*., 2000; Kon and Kashiwadani, 2005). However, in the experiments with the traditional methods of transplantation, i.e., direct sowing onto a substrate or securing with gauze or nylon mesh, resulted in the loss of many transplanted diaspores. For example, ca. 95% of soredia were

lost during the transplantation of *Usnea antarctica* using the direct sowing method (Ott, 2004), and ca. 80% of soredia were lost during the transplantation of *L. pulmonaria* using the gauze method (Scheidegger, 1995).

The purpose of this study is to develop a more efficient method of transplantation for the regeneration of thalli from soredia using *Parmotrema clavuliferum* (Räsänen) Streimann and *Ramalina yasudae* Räsänen, which are common foliose and fruticose lichens growing at low altitude areas of Japan.

Materials and Methods

Collection data for the voucher specimens used in this transplantation experiment are as follows:

(1) *Parmotrema clavuliferum*. Japan, Honshu, Pref. Chiba, Fujibayashi, on tree trunk, 100 m alt., April 24, 2005, Y. Kon 0504241.

(2) *Ramalina yasudae*. Japan, Honshu, Pref. Chiba, Kiyosumi, on rock, 60 m alt., August 31, 2004, Y. Kon 0408311.

These specimens are preserved in the National Museum of Nature and Science, Tokyo, Japan (TNS).

Soredia of *P. clavuliferum* were detached from the thallus using a sterile needle, and they were collected onto a cartridge paper. These soredia were attached to the surface of circular adhesive tape (7 mm diam.). We attached six sets of the adhesive tapes to a plastic plate (4×7×0.5 cm) (Fig. 1A). The plastic plate was fixed by using stainless steel nails on the east side trunk of *Cryptomeria japonica* (L. f.) D. Don at a height of 1.8 m from the ground. The transplantation experiment was performed between March 12, 2006 and March 31, 2007 at the specimen collection site.

We excised 2 mm long thallus fragments bearing soredia from the tips of *R. yasudae*. The fragments were fixed using a 1.3 mm nylon mesh (2×2 cm in size) with staples attached to the east side trunk of *Castanopsis sieboldii* (Makino) Hatus. ex T. Yamaz. & Mashiba, at a height of 1.0 m from the ground; the experiment was conducted between March 13, 2005 and September 17, 2006 at the specimen collection site. Although we performed the transplantation experiment, similar to that for *P. clavuliferum*, using adhesive tapes on a plate for the soredia of *R. yasudae* on east side trunk of *Cryptomeria japonica* at a height of 1.0 m from the ground at the specimen collection site between March 13, 2005 and September 17, 2006, regenerations from the soredia were not observed in this experiment (see Results and Discussion).

Transplanted samples were periodically observed (3, 6, 9, and 12 months after transplantation for *P. clavuliferum*; 6, 12, 15, and 18 months after transplantation for *R. yasudae*) using a bright-field microscope and a scanning electron microscope (SEM) (JEOL JSM-5410LV). Samples used in the SEM analysis were naturally dried, and coated with gold in a vacuum at 20 mA for 90 s, and observed at 5–15 kv.

Results and Discussion

Regeneration of Parmotrema clavuliferum

The original soredia of *P. clavuliferum* were globular in form (ca. 40 μm diameter) and consisted of few algal cells surrounded by exposed hyphae without a cortex (Fig. 1B). Three months after transplantation, the soredia started to secrete small amounts of a gelatinous matrix onto their surfaces (Figs. 1C and 1D). Six months after transplantation, no notable change was observed in the soredia. Nine months after transplantation, small cylindrical primordia developed from the margin of a clump of tissue (Fig. 1E). The protuberance resembled a juvenile isidium that formed on the upper surface of *P. tinctorium* (see Kon and Kashiwadani, 2005). Twelve months after transplantation, the cylindrical primordia grew laterally and differentiated into planiform lobes up to 200 μm in diameter (Fig. 1F). It should be noted that cilia-like structures formed very rarely along the margins of lobes (only two lobes).

Results of our soredia transplantation experi-

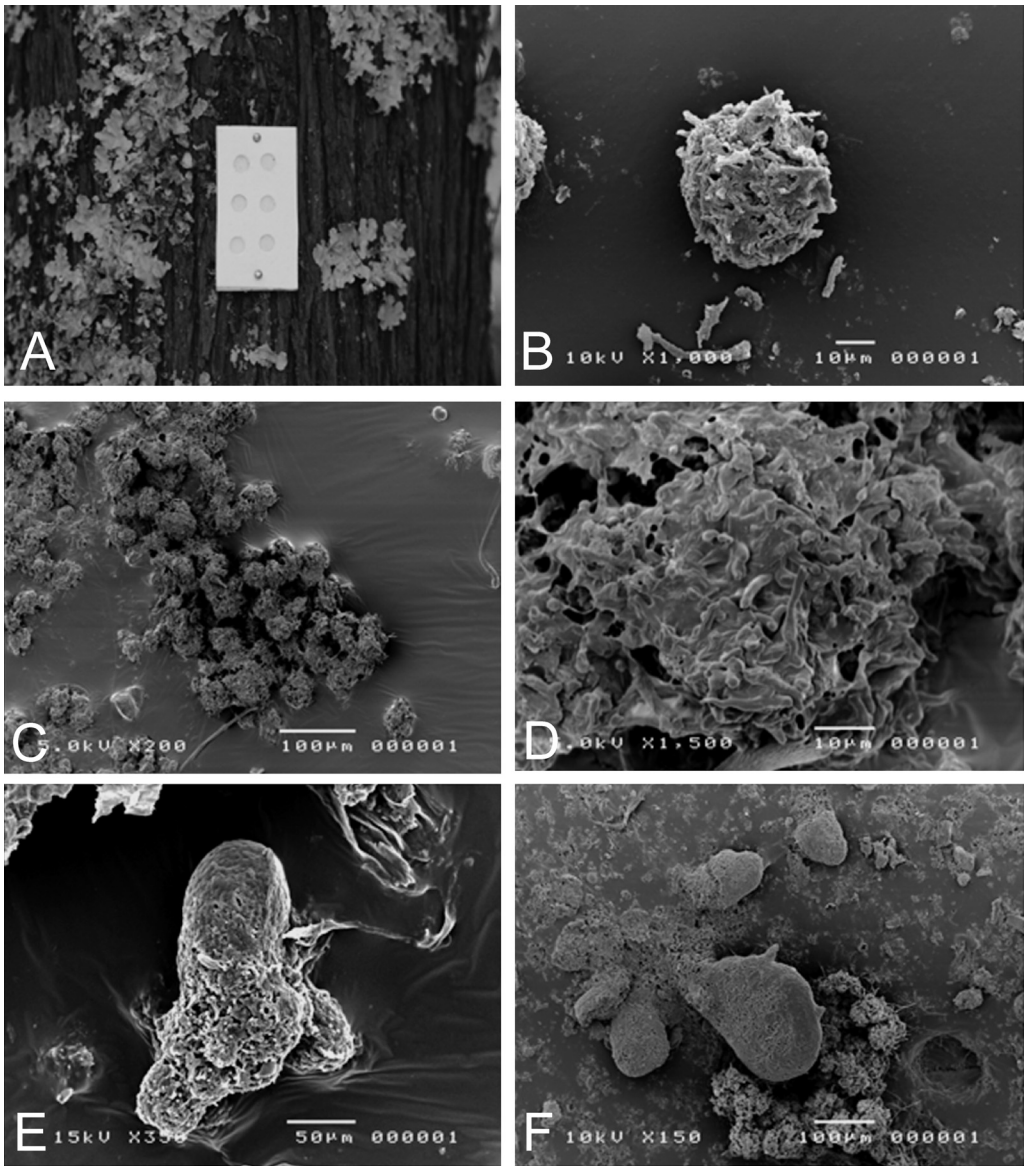

Fig. 1. *Parmotrema clavuliferum*, early developmental stages from soredia. A. An apparatus used in the transplantation experiment of soredia; soredia were attached to a plate using adhesive tape. B. Soredium detached from original thallus. C and D. Three months after transplantation. Gelatinous matrix was produced and covered the surface of the fungal hyphae. E. Nine months after transplantation. Cylindrical primordium emerged from a clump of undifferentiated tissue. F. Twelve months after transplantation. Cylindrical primordia differentiated into planiform lobes. B–F. Scanning election microscopy (SEM) photographs.

ments with *P. clavuliferum* showed dramatic differentiation in 12 months. Before the differentiation period, the developmental processes to form a planiform lobe were the same in both *P. clavuliferum* and other foliose lichens (e.g., Schuster, 1985; Schuster *et al*., 1985; Ott, 1987a, 1987b; Hilmo and Ott, 2002; Honegger, 1996; Scheidegger, 1995; Scheidegger *et al*., 1995; Zoller *et al*., 2000; Kon and Kashiwadani, 2005).

Regeneration of Ramalina yasudae

The original soredia of *R. yasudae* were globular in form (ca. 130 μm in diameter) (Fig. 2A). Most of the soredia were corticated on the surface, but others were without a cortex. The corticated soredia of this species differentiate from two origins; one is the fragmentation of the upper cortex of the thallus, and the other is the decorticated soredia consisted of exposed medulla and photobiont (see Ohmura *et al*., 2008). However,

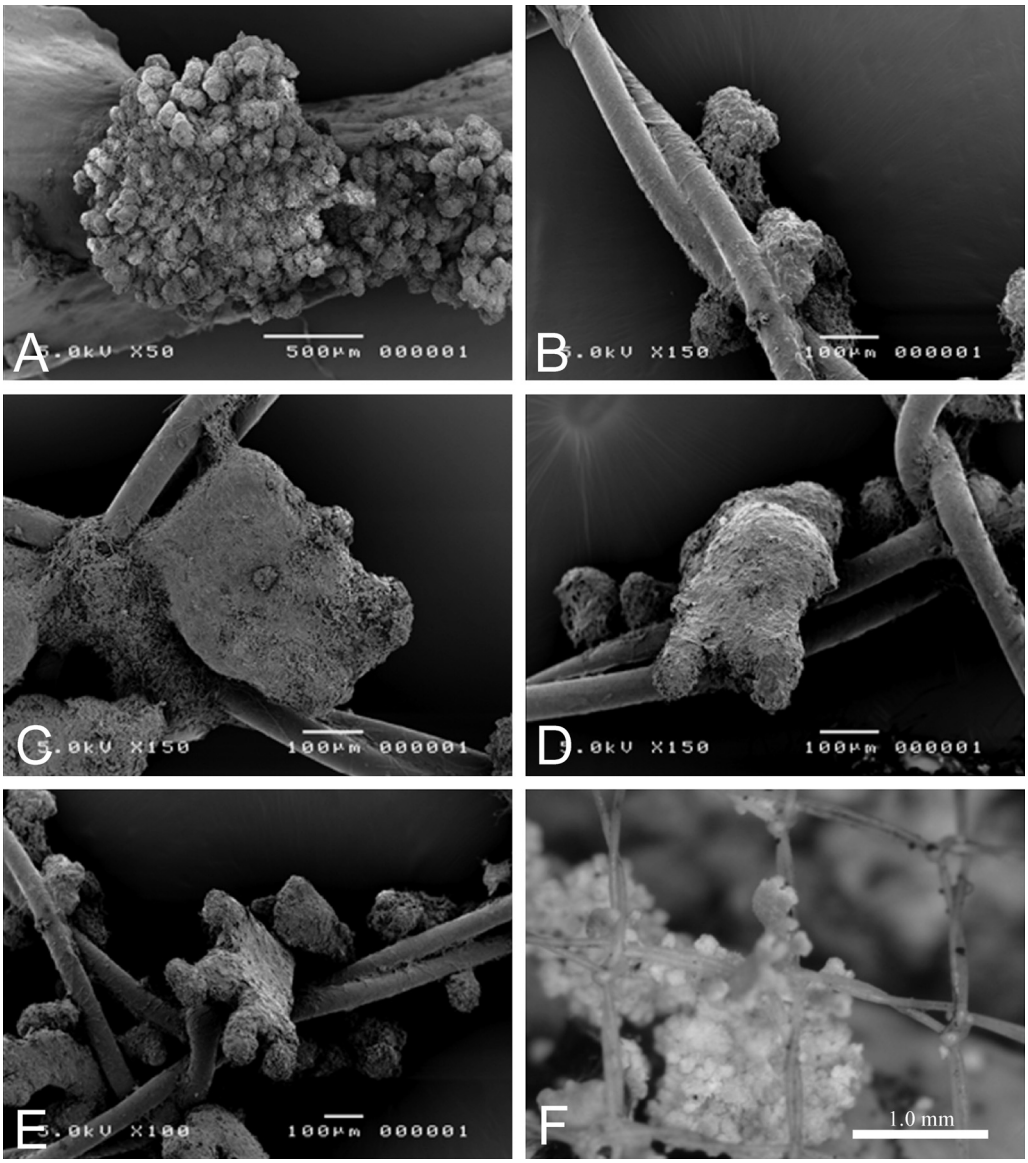

Fig. 2. *Ramalina yasudae*, early developmental stages from soredia. A. Soredia on original thallus. B. Fifteen months after transplantation. Transplanted soredia secreted gelatinous matrix on the surface and began differentiating into protuberance forms. Anchoring hyphae, which secured the protuberances on the nylon fibers, formed in this stage. C–F. Eighteen months after transplantation. Various morphologies of the developmental stages, i.e., planiform lobes (C), two protuberances on the thallus (D), and fruticose forms bearing four protuberances (E), were observed. F. Normal photograph of the developed thalli. A–E. Scanning electron microscopy (SEM) photographs.

the origin of the soredia used for this experiment may have developed from the latter origin because decorticated soredia were observed in the mass of soredia (Fig. 2A).

Soredia on thallus fragments were successfully differentiated into fruticose forms by the method using a nylon mesh stapled to the trunk of *Castanopsis sieboldii*. The details are as follows. No remarkable morphological change was observed 12 months after transplantation. However, after 15 months, the transplanted soredia showed a marked secretion of a gelatinous matrix onto their surfaces, and some differentiated into the protuberance forms of thalli (230 μm in length and 130 μm in diameter) (Fig. 2B). The protuberances developed anchoring hyphae that secured the protuberances to the nylon fibers. Eighteen months after transplantation, various morphologies of the thallus, including protuberances, planiform lobes, and branched fruticose forms, were observed (Figs. 2C–F). The protuberances were up to 400 μm in length and 300 μm in diameter. The planiform lobes were up to 300 μm in diameter, and the fruticose thalli were up to 700 μm in length. The fruticose thallus consisted of four protuberances on the tip (Figs. 2E–F). The initial stage of the fruticose thallus might be developed from the thallus with two protuberances at the tip (Fig. 2D).

All developing thalli were anchored to the nylon mesh by bundles of fungal hyphae (Figs. 2B–E). However, whether the bundle will differentiate into a holdfast of this species is uncertain from our data.

In other fruticose lichens, the transplanted soredium of *Usnea filipendula* differentiated into a basal tissue in 3 to 4 months, and the first outgrowths appeared after 10 months (Schuster, 1985). The transplanted soredium of *U. antarctica* slowly increased the mass and formed a basal tissue in the first year, and the first tips emerged from the basal tissue in the third year (Ott, 2004). The anatomical structures of their tips were the same as typical *Usnea* and consisted of a central axis, medulla, and cortex. In our transplantation experiment using *R. yasudae*, the fruticose form of the thallus was observed 18 months after transplantation, but these thalli never contained a central axis.

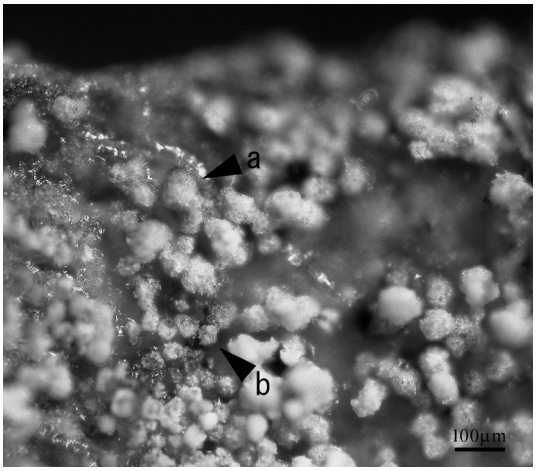

Fig. 3. Transplanted soredia of *Parmotrema clavuliferum* on adhesive tape after 12 months. More than half of the soredia on the tape differentiated into small lobes (a), and the rest remained as soredia (b).

Soredia transplantation method

Juvenile thalli successfully developed from transplanted soredia of *Parmotrema clavuliferum* and *Ramalina yasudae* in our experiments, but the transplantation method differed depending on species. Our method using adhesive tape is more efficient and easier for adhering soredia than other known methods, e.g., using gauze or pieces of tree bark (Schuster *et al.*, 1985; Zoller *et al.*, 2000). Almost all of the transplanted soredia remained on the tape throughout the experiment because of the adhesive quality of the tape used to attach the lichen fragments (see Ohmura *et al.*, 2009). More than half of the remaining soredia developed into small lobes after 12 months (Fig. 3). The transplantation method using adhesive tape is successful for *P. clavuliferum* but not for *R. yasudae*. Although soredia of *R. yasudae* were attached to adhesive tape on a plate and fastened to the trunk of *Cryptomeria japonica* as in the *P. clavuliferum* experiment, regenerations from the soredia into thalli were not observed after 18

months. The reason for the unsuccessful development of *R. yasudae* using this method was not clear. Although the development of *R. yasudae* thalli from soredia was successful with the conventional transplantation method using mesh, this method may result in the failure of more than half of the soredia in the experiment, and few soredia may develop into juvenile thalli.

Our method using adhesive tape improved the efficiency of surviving rate of diaspores, and this may be useful for further field experimental studies in ecology and morphogenesis. However, diaspore transplantation methods still need further improvement and should be optimized depending on species. In addition, further studies are needed to determine if juvenile thalli can develop into mature thalli using these methods.

References

Büdel, B. and Scheidegger, C. 2008. Thallus morphology and anatomy. In: Nash, T. H. III (ed.), Lichen Biology. Second Edition. pp. 40–68. Cambridge University Press, Cambridge.

Hilmo, O. and Ott, S. 2002. Juvenile development of the cyanolichen *Lobaria scrobiculata* and the green algal lichens *Platismatia glauca* and *Platismatia norvegica* in a boreal *Picea abies* forest. Plant Biology 4: 273–280.

Honegger, R. 1996. Experimental studies of growth and regenerative capacity in the foliose lichen *Xanthoria parietina*. New Phytologist 133: 573–581.

Kon, Y. and Kashiwadani, H. 2005. Lobule formation from isidia *Parmotrema tinctorum*. Bulletin of the National Science Museum, Series B 31: 27–131.

Ohmura, Y., Kawachi, M., Kasai, F., Sugiura, H., Ohtara, K., Kon, Y. and Hamada, N. 2009. Morphology and chemistry of *Parmotrema tinctorum* (Parmeliaceae, lichenized Ascomycota) transplanted into sites with different air pollution levels. Bulletin of the National Museum of Nature and Science, Series B 35: 91–98.

Ohmura, Y., Moon, K. H. and Kashiwadani, H. 2008. Morphology and molecular phylogeny of *Ramalina pollinaria*, *R. sekika*, and *R. yasudae* (Ramalinaceae, lichenized Ascomycotina). Journal of Japanese Botany 83: 156–164.

Ott, S., 1987a. Reproductive strategies in lichens. Bibliotheca Lichenologica 25: 81–93.

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

Ott, S., 2004. Early stages of development in *Usnea antarctica* Du Rietz in the south Shetland island, northern maritime Antarctica. Lichenologist 36: 413–423.

Scheidegger, C. 1995. Early development of transplanted isidioid soredia of *Lobaria pulmonaria* in an endangered population. Lichenologist 27: 361–374.

Scheidegger, C., Frey, B. and Zoller, S. 1995. Transplantation of symbiotic propagules and thallus fragments: methods for the conservation of threatened epiphytic lichen populations. In: Scheidegger, C., Wolseley, P. A. and Thor, G. (eds.), Conservation Biology of Lichenised Fungi. pp. 41–62. Mitteilungen der Eidgenössischen Forschungsanstalt für Wald, Schnee und Landschaft, Birmensdorf.

Schuster, G. 1985. Die Jugendentwicklung von Flechten ein Indikator für Klimabedingungen und Umweltbelastung. Bibliotheca Lichenologica 20: 1–206, abb. 1–112.

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

Zoller, S., Frey, B. and Scheidegger, C. 2000. Juvenile development and diaspore survival in the threatened epiphytic lichen species *Sticta fuliginosa*, *Leptogium saturninum* and *Menegazzia terebrata*: conclusions for in situ conservation. Plant Biology 2: 496–504.