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Lobule Formation from Isidia in *Parmotrema tinctorum*

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Abstract Vegetative diaspores (isidia) of *Parmotrema tinctorum* were kept in nylon bags. These nylon bags were stapled on the trunks of *Cryptomeria japonica*. Morphological changes of isidia were observed every three months after stapling in the natural field. Several protuberances were formed on the isidia six months after stapling. Isidia with protuberances at this stage were anchored by thin bundles of hyphae to the nylon bags. These protuberances developed into dorsiventral lobules with rhizines 12 months after stapling and the lobules were stratified as seen in the natural thalli of *P. tinctorum*. Lecanoric acid and atranorin were produced in the newly developed lobules.

Key words : Isidia, lobules formation, *Parmotrema tinctorum*.

Introduction

In contrast to the sexual reproduction of the mycobiont which requires re-lichenization with a suitable photobiont, the asexual reproduction by soredia, isidia or schizidia are regarded as ideal adaptations to lichens which are composed of fungal and algal partners. Unfortunately, however, we have only a limited knowledge on the development of these vegetative propagules into thalli either in the field or under culture. Scheidegger (1995) reported early development of isidioid soredia in *Lobaria pulmonaria* and Schuster *et al*. (1985) also reported early growth and development of soredia for *Hypogymnia physodes* and *Physcia tenella* in the natural environment. Stocker-Wörgötter & Türk (1988) also succeeded producing young thalli from soredia of *Peltigera didactyla*. However, development of true isidia has been observed only in *Peltigera praetextata*, a cyanobacterial lichen (Stocker-Wörgötter & Türk, 1995).

Parmotrema tinctorum (Nyl.) Hale is a common foliose lichen containing *Trebouxia* (a green alga) as the photobiont. It is widely distributed in temperate and tropical areas in the world, being easily recognized by the e-ciliate rounded lobes with dense isidia and by the distinct C+ red reaction in the medulla. It has the potential for two divergent strategies of reproduction, *i.e.* sexual, through ascospores ejection, and vegetative by the symbiotic propagules, isidia. However, fertile specimens are found only rarely in luxuriant populations and they are absent in most localities in Japan. Therefore, dispersal by ascospores may play only limited role in reproduction in most populations. In contrast, isidia are regularly found even in small thalli in small populations and may play major role in dispersal.

In the present paper, the successful development of juvenile lobules from isidia will be reported for *Parmotrema tinctorum*. The structure of both young and mature isidia was also observed.

Materials and Methods

Isidia used for the present study were collected from *Parmotrema tinctorum* growing on the trunks of trees at Mt. Kiyosumi, Chiba Prefec-

Fig. 1. Nylon bag stapled on the trunks of *Cryptomeria japonica*. Scale＝10 mm.

ture. They were cut off with a knife from thalli and kept in bags about 5×5 cm in size which were made of 150 μm meshed nylon. Each bag contained about 30 cylindrical or branched isidia (ca. 100–150 μm in length). These nylon bags were stapled on the trunks of *Cryptomeria japonica* D. Don growing near by the collecting site (Fig. 1). Developmental stages of isidia were investigated by light microscopy and scanning electron microscopy every three months after stapling. For light microscopic observation, sections were made by using a freezing microtome and washed with acetone solution in order to remove the secondary substances such as lecanoric acid and atranorin. Naturally dried specimens were cemented on specimen-holders, sputter coated with about 100 Å gold, and examined at 15 kV in a JEOL JSM-5410LV scanning electron microscope. Lobules newly formed on the isidia were chemically tested by the standardized thin-layer chromatographic method (Culberson, 1972).

Results and Discussion

Formation of isidia

In the initial stage, isidia appear to be small verrucae on the upper surface in *P. tinctorum*. In this stage (Fig. 2B), the verrucae are filled with algal cells, which are mostly the same as those of the medulla in size and have cortex extended from the upper cortex of the thallus. As isidia grow a little further (Fig. 2C) and become short cylindrical projections, algal cells increase in number. It is noteworthy that algal cells formed inside of the isidial projections are smaller in size comparing those derived from or in the thallus. In the later stages, these projections become distinct cylindrical isidia (Fig. 2D), which can be recognized even with the naked eye. In this stage, algal cells are concentrically distributed below the cortex and medullary hyphae of the thallus penetrate into the central part of isidia (Fig. 2A). However, no distinct stratification is observed.

Formation of juvenile thalli from isidia

Nylon bags staled on the trunks of *Cryptomeria japonica* contained about 30 isidia in various stages of development. However they were mostly cylindrical or coralloid. In the present study, cylindrical isidia were mainly observed. Cylindrical isidia were about 75 μm in diameter and 100–200 μm long (Fig. 3A).

The cut end of isidia was exposed when the nylon bags were stapled (Fig. 3A). Three months

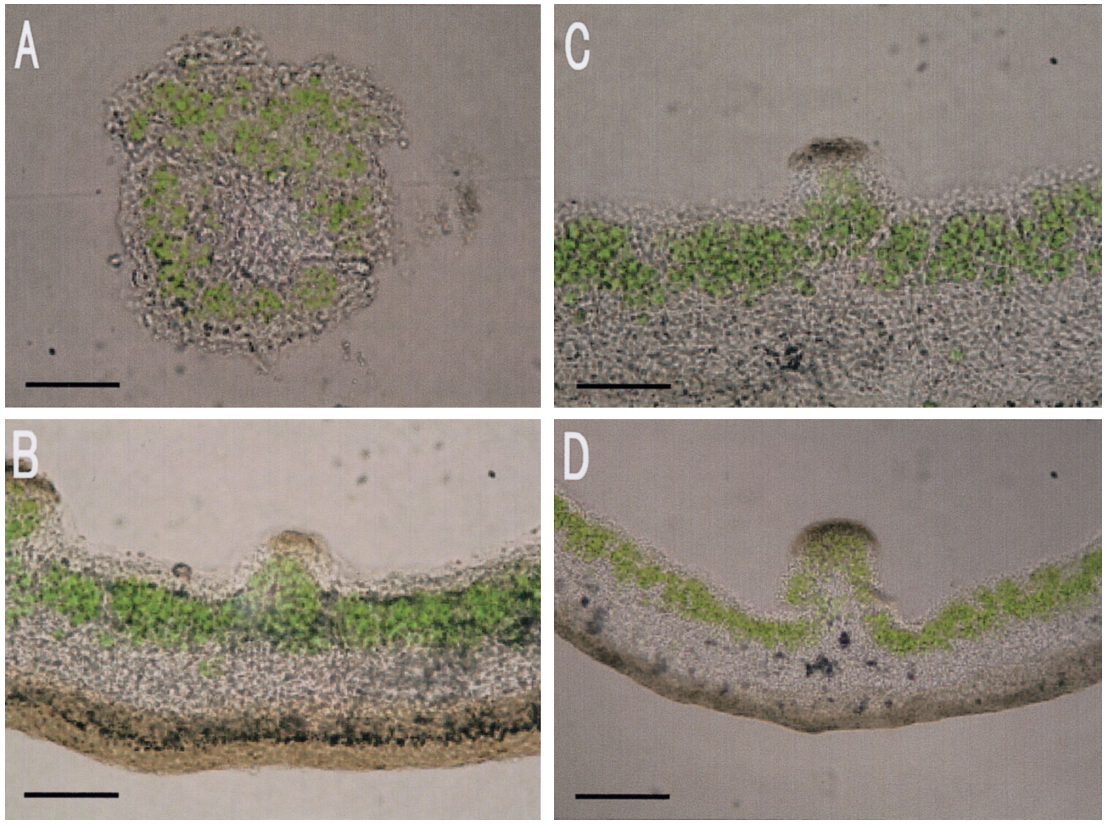

Fig. 2. Inner structure of isidia of *Parmotrema tinctorum*. A: Cross-section of mature isidia formed on the thalli of *P. tinctorum*. Algae are distributed concentrically under the cortex of the isidia and hyphae are situated in the center of isidia. B–D: Longitudinal section of young and mature isidia of *P. tinctorum*. B: Juvenile isidia. C: Young isidia. D: Mature isidia. (A–C scales = 50 μm, D scale = 100 μm).

after stapling, it was covered by mycobiont hyphae which seemed to adhere each other with gelatinous substances (Fig. 3B). In the present study, isidia of *P. tinctorum* did not seem to be fixed on 150 μm nylon mesh.

Six months after stapling, a clear morphological changes of isidia were observed. In this stage, each isidium produces several verrucae or protuberances 20–30 μm broad and 50–60 μm long (Fig. 3C). There is no distinct boundary between isidia and verrucae or protuberances and cortex of isidia is spontaneously joined to those of verrucae or protuberances. On some of them, bundles of hyphae tightly anchoring to the substratum (nylon fiber) were observed (Fig. 3C). They are comparable with anchoring hyphae reported in the early stages of development of isidioid soredia of *Lobaria pulmonaria* by Scheidegger (1995).

Twelve months after stapling, protuberances developed into dorsiventral lobules, which were pale gray and 0.2–0.35 mm broad and 0.2–0.3 mm long (Fig. 3D).

The cortex was often disintegrating at the basal parts of lobules as well as on the isidia (Fig. 3D). In this stage distinct rhizines were observed on the lower surface of the lobules (Fig. 3E). At present, however, it is uncertain whether these rhizines have derived directly from anchoring hyphae mentioned above or not. It should be noted here that lobules in this stage were clearly stratified, upper and lower cortices, algal layer and medulla being recognized (Fig. 3F), being quite similar to thalli of *P. tinctorum* in nature,

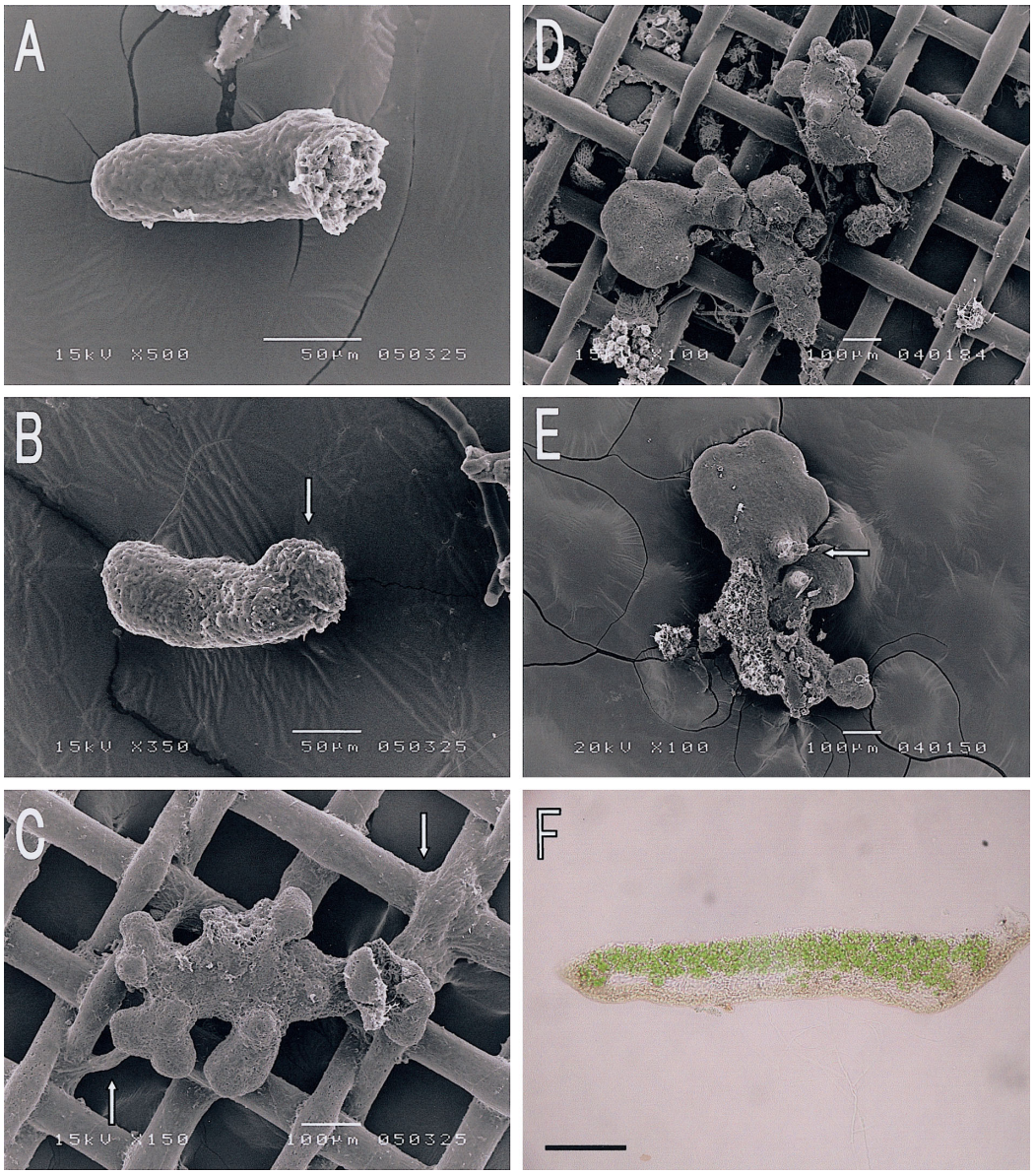

Fig. 3. Scanning electron micrography of developmental stages of lobule formation observed in the field and a lobule. A: Cylindrical isidium removed from a thallus. B: Isidium three months after stapling. The cut end (arrow) is covered with hyphae. C: Verrucae and protuberances formed on the isidia six months after stapling, showing bundles of hyphae anchoring to the substratum (arrow). D: Lobules formed on the isidia 12 months after stapling. E: Lobules 12 months after stapling, showing rhizines (arrow) on the lower surface. F: Cross-section of lobules formed on isidia 12 months after stapling. Stratified composition is similar to that of original thalli of *P. tinctorum*, though the medulla is rather thin. Scale＝100 μm.

though the medulla was rather thin. In addition, secondary products of newly formed lobules were tested by the TLC method and lecanoric acid and atranorin, constant components in *P. tinctorum*, were demonstrated on the chromatograms.

Stocker-Wörgötter & Türk (1995) observed that isidia transformed into soredial primordial at

first and then small lobules were differentiated from fused soredial clumps in *Peltigera praetextata*. In *P. tinctorum*, however, such developmental process was not observed in any stages and verrucae or protuberances were formed directly on isidia and they developed into small lobules. When Scheidegger (1995) investigated early development of isidioid soredia of *Lobaria pulmonaria*, he reported that apical and lateral pseudomeristematic growth zones were formed six months after transplantation and then the growth zones further differentiated into small lobes which have a stratified thallus as seen in foliose lichens. In *P. tinctorum*, however, verrucae or protuberances were formed in the early stages and they directly developed into lobules. Thus, verrucae or protuberances can be considered to be equivalent to pseudomeristems observed by Scheidegger (1995). It is noteworthy, on the other hand, these verrucae or protuberances resemble very much those observed on the periphery of the transplanted fragments of *P. tinctorum* (Kon *et al*., 2003).

Isidia of *P. tinctorum* are cylindrical and algal cells are concentrically distributed inside of the cortex and protuberances formed on isidia seem to have a similar inner morphology as isidia. In contrast, lobules developed from isidia are clearly stratified as mention above, though the medulla is rather thin (Fig. 3F). Therefore, disposition of algal cells and fungal hyphae should be changed, while dorsiventral lobules are developing, although such process has not been observed in the present study.

It is now obvious that isidia removed from the thallus commonly produce many protuberances and juvenile lobules in six to twelve months after detaching from the thallus. As having been suspected by most lichenologists, thus, isidia can be considered to play a major and important role in dispersal in *P. tinctorum*. Since regeneration of isidia has never been observed while they adhere onto the thallus, formation of verrucae or protuberances on isidia observed through the present study seems to be triggered by detaching from the thallus.

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