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Culture studies on artificially induced aplanospores and their development in the marine alga *Boergesenia forbesii* (Harvey) Feldmann (Chlorophyceae, Siphonocladales)

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When the vegetative thallus of the gigantic unicellular marine alga, *Boergesenia forbesii*, is either placed in concentrated sea-water (×2.0) or subjected to mechanical stimulation (pinching with a pincette or probing with a fine glass needle), its protoplasm changes swiftly into numerous aplanospores. Most of these aplanospores develop into normal new plants in culture.

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Introduction

Little has been reported so far concerning the reproduction of *Boergesenia forbesii*. Feldmann (1938) observed material from Madagascar which was preserved in formalin, and presented some figures and statements about aplanospores. He mentioned that "Dans certains individus, le contenu de l'utricule se fragmente en un grand nombre de corps sphériques de taille très variable pouvant aller de 10 μ de diamétre jusqu'à plus de 300 μ et constitués par des masses de protoplasme dense très riches en amidon et entourés d'une membrane mince. . . . On peut les considérer comme des aplanospores susceptibles de donner naissance à de nouveaux individus". However, he observed neither the process of formation of the aplanospores nor their development in living material.

In the same year, Iyengar (1938) described the reproduction of this species. He observed asexual reproduction by means of quadriflagellate zoospores, and he also mentioned that "Under unfavourable conditions, plenty of cysts are formed inside the vesicle. These cysts grow into new plants when conditions become more favourable again". However, he did not give any figures.

We have cultured the vegetative thalli of *Boergesenia forbesii* and obtained "aplanospores" or "cysts" such as Feldmann (1938) and Iyengar (1938) described. The present paper deals with aplanospore formation and development.

Materials and Methods

The materials used were collected around Cape Ayamaru at the northeastern part of Amami-ôshima Island, Japan (Fig. 1). They were growing on the sandy bottoms of littoral pools of coral reefs. The collections were carried out in May and June of 1971. The materials were kept at about 13 C and brought to the laboratory. After being carefully washed with filtered (Tôyô filter paper *No. 4*) and sterilized (15 min. autoclaving at 120 C) sea-water, algal thalli were put one by one into glass vessels which contained 200–300 ml of sea-water. No nutriment was added. These vessels were kept at 25 C, 1000 lux, 14 hr light, 10 hr dark. Toshiba fluorescent lamp *DSDL* was used as an artificial light source.

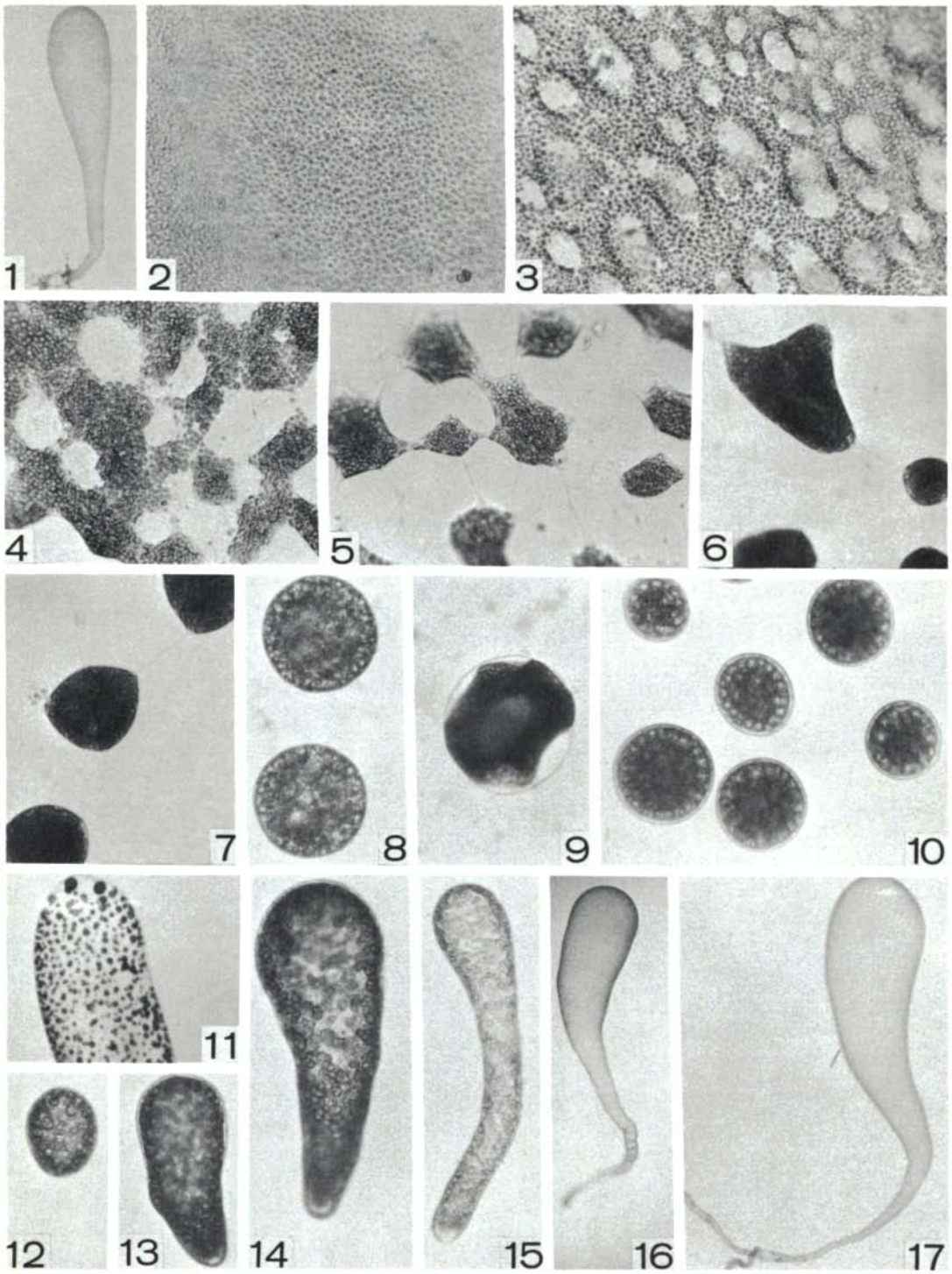
When the thalli were placed in the concentrated sea-water (×2.0) for half an hour, or were stuck with a fine glass needle (100–200 μm diam., 1.0–1.5 mm long), or pinched with a pincette, their protoplasm changed into many aplanospores within 6–9 hours. Then the basal portion of the vesicle was cut off with scissors, and a quantity of aplanospores were pipetted into a watch glass or hollow glass slide that contained filtered and sterilized sea-water. By horizontal rotation of the glass, the aplanospores were readily collected at the central part of the bottom. After the aplanospores were washed five times, they were transplanted into glass tubes that contained 15 ml of Provasoli's *ES*–medium (Provasoli, 1966). The glass tubes

were kept on a slant for 10–12 hr, while the aplanospores sank, scattered evenly, and adhered to the inner lateral surface of the tubes. The sporelings were cultured at 30 C, 1000 lux, 12 hr light, 12 hr dark. The culture medium was changed every two weeks. The cultures were not axenic, but were strictly unialgal.

Observations and Results

When the vegetative thalli were placed in concentrated sea-water or subjected to the mechanical stimuli mentioned above, the protoplasm changed into numerous aplanospores. In the vegetative thallus, the polygonal, flat-discoidal chloroplasts were distributed parietally and densely along the inner wall of the vesicle (Fig. 2). Initiation of aplanospore formation was recognized first of all by the formation of a network of chloroplasts (Fig. 3). Within half an hour to an hour after a vesicle was stimulated with concentrated sea-water or stuck with a glass needle, perforations occurred and the chloroplasts formed a network. The perforations soon enlarged, and the network broke down into dark green irregularly shaped masses of protoplasm (Fig. 4, 5). Each mass was covered with a thin membrane and connected to other masses by fine strands (Fig. 6). The masses then became spherical (Fig. 7). Within 4–6 hours, many dark green balls of various sizes were formed in a vesicle. At this stage, the fine connecting strands still remained (Fig. 7). After 6–9 hours, these masses became spherical, the fine strands disappeared, and new cell walls formed (Fig. 8). Though the cell walls were very thin, their presence was confirmed by means of plasmolysis with concentrated sea-water (Fig. 9). The existence of a newly-formed cell wall indicated the completion of aplanospore formation. At this stage, the chloroplasts were distributed over the entire cell surface, enclosing a large central vacuole. As long as the aplanospores remained within a vesicle, they never germinated. Their cell walls became thicker (Fig. 10), and they remained within the mother vesicle for a long period (20–30–50 days) (Fig. 11). In the culture vessels, the cell wall of mother vesicles did not break down spontaneously. No openings were formed to liberate the aplanospores. However, when a vesicle was broken down artificially and the liberated aplanospores were transplanted into fresh medium and kept under favourable conditions, as mentioned above, they rapidly increased their volume and began to germinate (Fig. 12). No period of dormancy was observed in the fresh medium. A germination tube formed on the side away from the light. Cell contents remained within the original body (Fig. 13, 14). The mode of germination was of the so-called immediate filamentous type (Inoh, 1947). After 3–5 days, the germlings became multinucleate, and the chloroplasts developed into numerous polygonal flat-discoids and arranged themselves reticularly. Each chloroplast had a pyrenoid. After 8–10 days, the germling became a curved, non-septate siphonous body with a filamentous rhizoid which was produced by the continuous elongation of the germination tube (Fig. 15). The upper portion of a germling was an initial of a primary vesicle. After 20 days, annular constrictions appeared at the basal portion of the vesicle and the rhizoid (Fig. 16). After 60 days, the thalli attained the height of 20–30 mm (Fig. 17). When thalli thus obtained were placed in concentrated sea-water or given the same mechanical stimuli mentioned above, they again formed numerous aplanospores. We have obtained three generations using these methods.

FIG. 1. Vegetative thallus; ×1.4. FIG. 2. Chloroplasts in the vegetative thallus; ×160. FIG. 3. Perforations in the arrangement of the chloroplasts, 30–60 min after the thallus was stimulated with a fine glass needle; ×160. FIG. 4. The perforations enlarged and broken down into irregular shaped protoplasmic masses, after 1–2 hr; ×160. FIG. 5. Irregularly shaped protoplasmic masses, connected with fine strands, after 2–3 hr; ×160. FIG. 6. Protoplasmic mass covered with a thin membrane and connected with fine strands, after 3–4 hr; ×160. FIG. 7. Nearly spherical protoplasmic masses, fine strands still remaining, after 4–6 hr; ×160. FIG. 8. Spherical protoplasmic masses; strands have already disappeared, after 6–9 hr; ×160. FIG. 9. Plasmolysed aplanospore whose cell wall is clearly visible, after 6–9 hr; ×160. FIG. 10. Thickened cell walls of aplanospores in the mother vesicle, after 5 days; ×160. FIG. 11. A part of a vesicle that contains aplanospores; ×6.0. FIG. 12. Aplanospore germinating, after 24 hr; ×100. FIG. 13. Germinating aplanospore issuing its germination tube away from the light source, after 2 days; ×100. FIG. 14. Germling becoming clavate, siphonous body, after 5 days; ×120. FIG. 15. Clavate, siphonous germling with filamentous rhizoid, after 8 days; ×100. FIG. 16. Juvenile thallus from aplanospore with basal portion and rhizoid annularly constricted, after 20 days; ×20. FIG. 17. Thallus from aplanospore, after 60 days; ×3.0.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Discussion

The aplanospores which were obtained by means of the above methods are like those described by Feldmann (1938) and Iyengar (1938). The development and formation of aplanospores of *Valonia ventricosa* were observed by Murray (1893), Boergesen (1913), Kanda (1940), and Chihara (1959). Algae which consist of single giant cells, such as *Boergesenia* and *Valonia,* often sustain various kinds of damage in the field. Moreover, slight damage to a part of the body means the death of the entire individual. The ability to form aplanospores swiftly can thus be regarded as adaptive.

In *B. forbesii,* the aplanospores do not germinate, but remain dormant as long as they remain within the vesicle. However, as soon as they are transferred into fresh medium, they begin to germinate and develop. In culture vessels, the vesicles which have aplanospores neither break down spontaneously nor develop openings. However, in the field, it is probable that, as they grow in the littoral zone, some strong mechanical actions, such as waves, cause vesicles not only to form aplanospores, but also to break down so that aplanospores may be discharged. The liberated aplanospores may germinate and develop into new plants. In fact, in the field we do encounter some vesicles which contain aplanospores and some vesicles with broken apical portions.

Recently, Tatewaki and Nagata (1970) reported that extracellular protoplasts of the coenocytic marine alga *Bryopsis plumosa* have the ability not only to synthesize new cell walls *in vitro,* but also to develop into normal pinnate plants. A similar phenomenon was observed in the extracellular protoplasts of the present alga, *Boergesenia forbesii.*

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References

BOERGESEN, F. 1913. The marine algae of the Danish West Indies. Pt. I. Chlorophyceae. *Dansk. Bot. Arkiv.,* 1(4): 1–158.

CHIHARA, M. 1959. Studies on the life-history of the green algae in the warm seas around Japan(9). Supplementary note on the life-history of *Valonia macrophysa* Kütz. *J. Jap. Bot.,* 34(9): 257–266.

FELDMANN, J. 1938. Sur la classification de l'ordre des Siphonocladales. *Rev. Gén. Bot.,* 50: 571–598.

INOH, S. 1947. Morphogenesis of marine algae. *Hokuryukan,* Tokyo: 217.

IYENGAR, M. O. P. 1938. On the structure and life-history of *Pseudovalonia forbesii* (Harvey) Iyengar (*Valonia forbesii* Harv.). *J. Indian Bot. Soc.,* 17(2 and 3): 191–194.

KANDA, C. 1940. Studies on the genus *Valonia* from Palao. *Kagaku-nanyô,* 3(2): 107–116.

MURRAY, G. 1893. On *Halicystis* and *Valonia. Phycol. Mem.,* Pt. II: 47–52.

PROVASOLI, L. 1966. Media and prospects for the cultivation of marine algae. *In* Watanabe, A. and Hattori, A. (ed.), Cultures and Collections of Algae. *Proc. U.S.-Jap. Conf. Hakone,* Sept. 1966: 63–75.

TATEWAKI, M., AND NAGATA, K. 1970. Surviving protoplasts *in vitro* and their development in *Bryopsis. J. Phycol.,* 6: 401–403.