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Notes on Fucales 8. Regeneration from rhizoid-piece of *Pelvetia* germling to complete thallus

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Eggs of *Pelvetia wrightii* were cultured in glass vessels. Rhizoids derived from these sporelings were cut into some pieces and cultured separately. As a result, these pieces commenced successive cell divisions, and finally gave rise to new thalli like original one. The new thallus formed new rhizoids, and these rhizoids were also capable of forming new thalli when they were cut out and cultured again.

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Not many instances have been known as to the development of vegetative thalli from isolated rhizoids. In moss, a chloronema sometimes comes out from a rhizoid (BÜNNING and WETTSTEIN 1953). In *Bryopsis*, induction of the thallus from a rhizoid is experimentally known (JACOBS 1951). In *Derbesia*, a drop of protoplast gives rise to a thallus (RIETEMA 1973). In *Zonaria*, *Dictyopteris*, *Grateloupia* etc., sometimes the thallus grows up from the rhizoid in culture (KUMAGAI and INOH 1964, 1972, MURAKAMI, INOH and OHMORI 1967). As for the Fucales, an embryo-like body is formed naturally from the rhizoid of young embryos (MCLACHLAN and CHEN 1972). On the other hand, although the site of rhizoid development is determined in Fucales eggs, such as in *Fucus* and *Pelvetia*, the once determined site is stable and kept irreversibly (WHITAKER 1938, SAGA and NAKAZAWA 1974). The relation between the stability in early stages like this and the instability in later stages shall be analyzed by further instances. The author confirmed that rhizoids of *Pelvetia* sporelings bore thalli, and it is dealt with in this paper.

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Material and Methods

In November, 1974, *Pelvetia wrightii* was collected from the beach of Charatsunai, Muroran, Hokkaido. Fertilized eggs were obtained according to ABE's method (ABE 1970). The eggs were sown on slide glass plates as 1 egg for 1 plate, and they were cultured being placed in petri dishes with 150 m*l* PESI medium (TATEWAKI 1969), at 14°C, under white light of about 2000 lux, alternating 14 hr light and 10 hr dark. The culture medium was renewed monthly. Thus, in half a year, several thalli of about 5 cm in length grew up from a single egg. These young thalli adhered to the slide glasses by a number of 1 to 5 mm long rhizoids. Under a microscope, the rhizoids were observed to consist of two differential

parts, i.e. the thick brown part filled with plastids, and the slender transparent part wanting in plastids.

The transparent parts were cut into about 3 mm long pieces for the experimental materials. Five pieces were cultured in test tubes containing 10 m*l* PESI medium under the same conditions mentioned above. Two months later, 2 to 3 mm long thalli grew up from these pieces. These thalli were transferred to the petri dishes containing 150 m*l* PESI medium. Thus, 40 dishes were prepared. These were then cultured being divided equally into four different conditions: 1) 14°C with 14 hr photoperiod, 2) 14°C with 10 hr photoperiod, 3) 10°C with 14 hr photoperiod and 4) 10°C with 10 hr photoperiod. The culture medium was renewed monthly.

Result

The transparent rhizoid piece (Figs. 1A, 2A) gradually became brown with an increase in plastids in 2 or 3 days. After a week, the rhizoid piece, filled with plas-

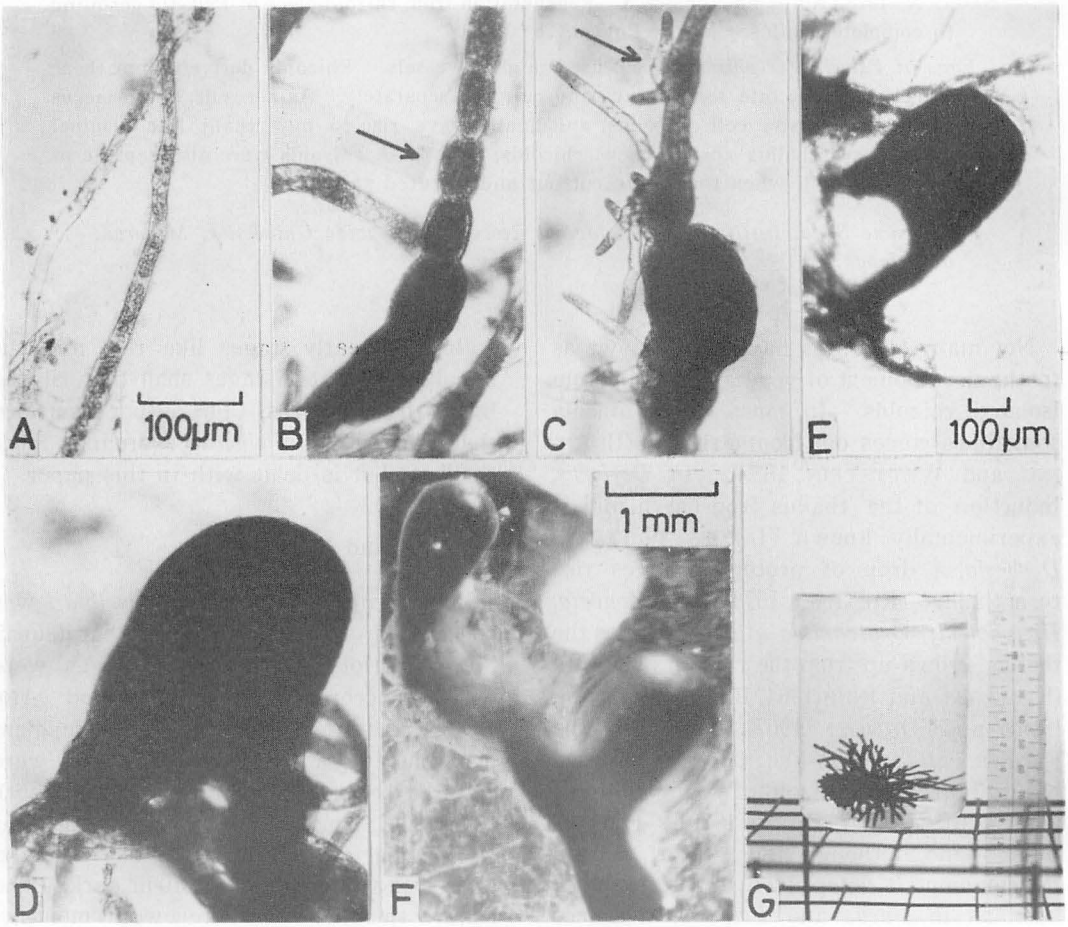

Fig. 1. Development of *Pelvetia* rhizoid piece. A, two pieces, a transparent one yet without cell divisions on the left, and a brown dividing one on the right, cultured for a week. B, rhizoid cells containg full of protoplasm being divided transversely (arrowed), cultured for 2 weeks. C, initial of upright shoot, 3-week culture, rhizoids are also developing. Arrow indicates a transversely divided part. D, upright shoot in 4-week culture. E. upright shoot in 5-week culture. F, dichtomous thalli developed in 2-month culture. G, multiple thalli originated from a single rhizoid piece, 8-month culture. Use scale in A for A–D; scale in E for E; scale in F for F.

tids, underwent cell division transversely into smaller cells of about 50 μm in length and about 20 μm in width (Figs. 1A, 2B). Next, the cells became thicker gradually (Fig. 2C), and after about 10 days longitudinal divisions took place. Thus in two weeks, some of them grew up already to embryo-like bodies consisting of several tens of cells, and differentiated new rhizoids. These embryo-like bodies were further divided in various directions and grew up 3-dimentionally. Sometimes, new rhizoids came out from the parts of two or more cell rows (Fig. 1C). In 3 weeks, the embryo-like bodies grew up larger, and the largest one which bore new rhizoids was measured to be about 100 μm (Figs. 1C, 2E). Four to five weeks after the inoculation, the embryo-like bodies grew upright shoot (Figs. 1D, 2F). In 2 months, 2 to 3 mm long erect thalli were seen with naked eye. Some of them were developed into dichotomous thalli (Fig. 1F). Thus 8 months after the beginning, about 20 thalli of several centimeters in length were obtained from a single original piece cut out from the primary rhizoid, especially under the condition of 14°C, 14 hr photoperiod. In other conditions, the growth was a little delayed, but similar thalli also grew up finally.

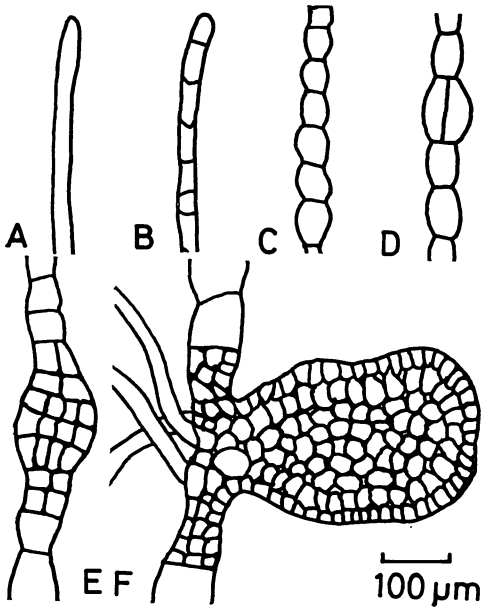

Fig. 2. A, a rhizoid piece at the beginning of culture; B, the same cultured for 1 week; C, cultured 10 days; D, cultured 2 weeks; E, cultured 3 weeks. F, cultured 5 weeks. Use scale in F for A-F.

The new rhizoids of these regenerate thalli were cut into a certain pieces again, and cultured likewise. As a result, it was found that the same embryo-like thallus developed from these again. Thus, the successive culture was possible.

Discussion

In a multicellular system, generally, the constituent cells have same chromosome configuration, but these cells of each organ are differentiated each other. Sometimes a cell which is dedifferentiated by isolation from multicellular system acquires embryonality, then it regenerates complete system. In *Pelvetia* also, dealt with here, the parenchymatous and rhizoidal cells of sporeling have probably the same chromosome configuration. However, the rhizoid pole of *Pelvetia* eggs are determined at the shaded side when the eggs are illuminated unilaterally. In this way, if the polarity is once determined, it is no more reversible. Therefore, the rhizoid pole cannot be altered to the shoot pole even if illuminated reversely (SAGA and NAKAZAWA 1974). However, as is seen in the above experiments, after the actual growth of isolated rhizoids it can develop an upright shoot. This fact shows that some changes occured in characteristics of protoplasm in the course of growth of the rhizoid. It is considered that the rhizoid-piece derived from *Pelvetia* sporeling gains embryonality by means of injury and isolation. It begins normal morphogenesis like a original sporeling.

As remarked in the result of experiments, the successive culture is possible. This will open a way of new approach to the study of fucaceous algal development.

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嵯峨直恒： Fucales ノート 8. エゾイシゲ仮根からの葉状体の再生

培養したエゾイシゲ幼体より仮根を単離しその再生を観察した。単離された糸状の仮根はまず1次元的に分裂し，ついで2次元，3次元的に分裂を行ない葉状体となった。葉状体からは新しい仮根が形成された。この仮根を再び単離すると，上記の様に再生し，葉状体と仮根を形成した。このようにして，仮根を植え継ぐことにより，エゾイシゲの継代培養が可能となった。(051 室蘭市母恋南町 1-13，北海道大学理学部附属海藻研究施設)