ISOLATION AND CULTIVATION OF THE VEGETATIVE CELLS OF *PORPHYRA HAITANENSIS*

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Abstract

This research deals mainly with the use of the vegetative cells of a marine red alga *Porphyra haitanensis* (laver) as seeds and their culture into young thalli for further cultivation in the sea. The experimental process was as follows. Enzymatically isolated vegetative cells were attached to rope substrates and cultured in the laboratory for a month until they became about 0.2—0.5 cm long sporelings and were then attached (7—15 sporelings per cm of rope) to nets for removal to the open sea for cultivation. A month after culturing, the thalli reached a maximum length of 50 cm (average of 20—30 cm). The thalli grew faster as the water temperature dropped from 21°C to 17°C. It was proved that the vegetative cells isolated from a small thallus about 5 cm long could grow up into normal thalli after being kept frozen for a year. The results of this study show that vegetative cells can be used as new seeds to simplify the production of laver sporelings. This technique is a significant advance in the field of *Porphyra* culture.

INTRODUCTION

The conventional method of cultivating *Porphyra* is to grow filaments in clam shells resulting in the production of conchospores which are then cultured in tanks of seawater in the laboratory for four to five months. When the conchospores are fully mature, they are collected onto rope nets, and then cultured in the sea. This is not only a complex procedure, but also time consuming, and economically not so feasible. Many attempts have been made to improve on this method. Zhao et al. (1984) tried to isolate vegetative cells from the thallus of *P. yezoensis* by mechanical disruption. Tang (1982) succeeded for the first time in isolating vegetative cells of *P. suborbiculata* with enzymes. M. Polne-Fuller et al. (1984) reported their work of vegetative propagation of *P. perforata* in the laboratory. Both *Porphyra*, *P. yezoensis* and *P. suborbiculata* have in their life cycles asexual monospores which can develop into thalli directly. Wang et al. (1984, unpublished) isolated and cultured the vegetative cells from the thalli of *P. haitanensis* and cultured them into 6 cm long young thalli in the laboratory. Instead of obtaining conchospores, the authors tried to culture vegetative cells directly into sporelings for cultivation in the open sea in an experiment carried out from September 1984 through January 1985.

MATERIALS AND METHOD

P. haitanensis used in this study were obtained from two counties of Fujian Province, China. Small thalli about 5 cm long were collected from Jingjiang County in October 1983 and larger ones about 30 cm long were collected from Lianjiang County in October 1984. Both small and large thalli had been kept frozen at $-10°C$ before they were used. Usually, the frozen thalli were immersed in seawater to restore them to their normal condition for the experiments. For the small thalli, the

whole plants were dissociated with enzymes. The large thalli were cut into apical, middle, basal and rhizoid portions (Fig. 1), and, except for the apical portions, which were discarded because they were mature, the other three parts were separately dissociated with enzymes to determine their respective growth potentials. The treatment of the parent plants and dissociation and collection of vegetative cells were described in a separate paper by Wang et al. (1984, unpublished). The mass of individual cells was diluted by adding MES medium to some extent to facilitate the cell collection. Polyvinyl ropes wound around glass plates served as substrates for the vegetative cells (Figs. 2, 3). The rope plates were thoroughly washed and kept dry before use. The cell suspension was carefully dropped with a Pasteur pipette to the rope plates and allowed to stand for 1.5 to 3 hours. The MES medium was slowly added with a pipete from the edge of the rope until the rope plates were covered by the medium. The rope plates with cells were then kept still for three days to allow the cells to attach themselves onto the ropes.

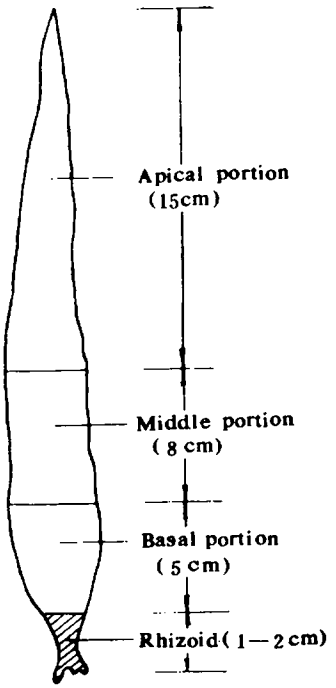


Fig. 1 The four parts of large thallus

The culture conditions for the vegetative cells were as follows. The rope plates with the cells were placed under natural light with an intensity of 1,000 Lux (12 L: 12 D) at a temperature of 20°C ± 2°C. Ten days after cell attachment onto the ropes, the MES medium was renewed, and another 10 days later the MES medium was replaced with the N–P medium (NO_3–N 4 ppm and PO_4–P 0.4 ppm).

When the young thalli on the rope plates were about 0.2—0.5 cm long, the ropes with thalli attached were unwound and formed into nets. The young thalli attached to the rope nets were then transported to the open sea for cultivation.

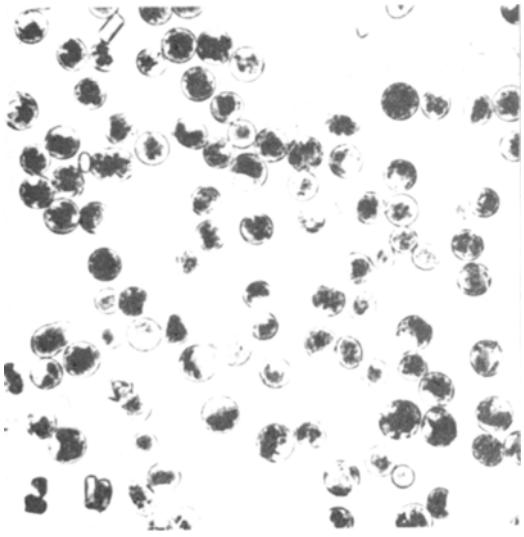


Fig. 2 Individual vegetative cells isolated from the thalli of *Porphyra haitanensis*

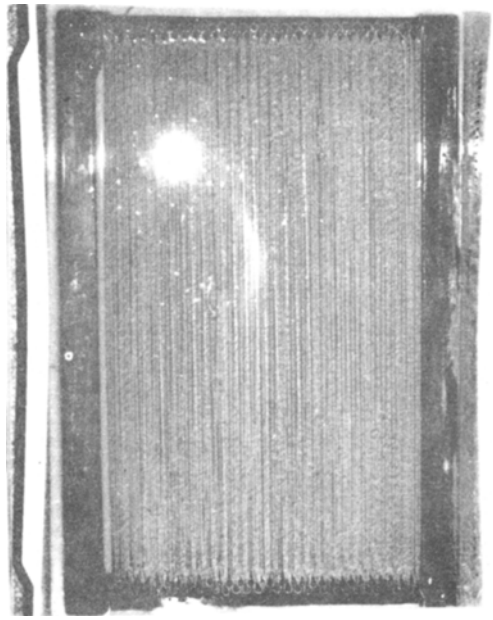


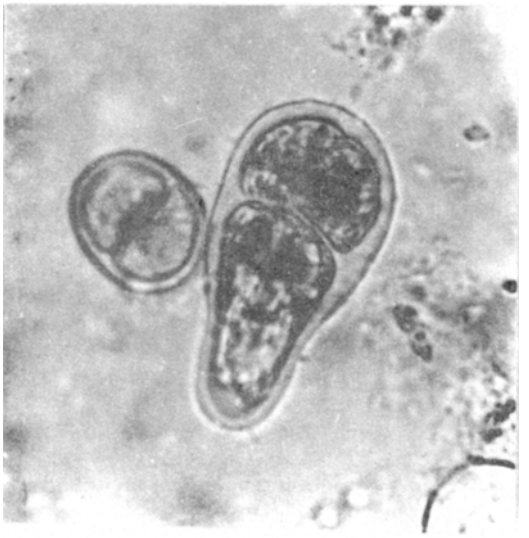
Fig. 3 Polyvinyl rope as substrate to which the isolated vegetative cells are attached

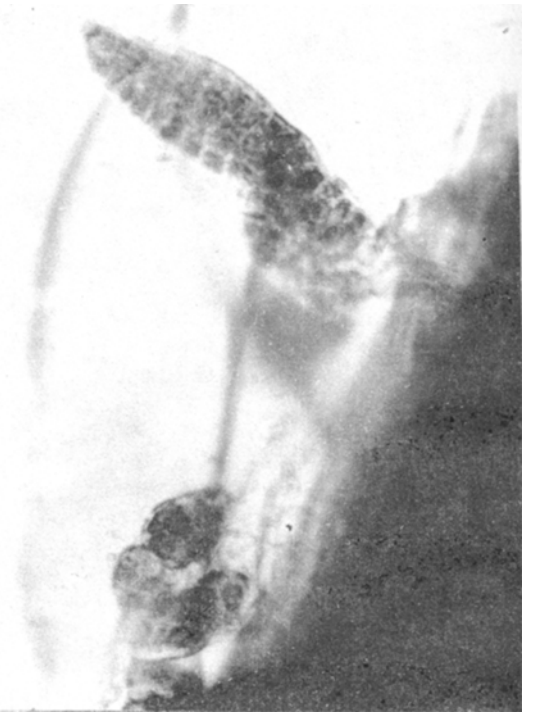
RESULTS

1. Formation of the young thallus: The formation of young thalli derived from the vegetative cells of small thalli was observed. After the vegetative cells were collected onto the ropes, the young thalli were usually visible to the naked eye within 30 days. At the initial stage very tiny dark dots appeared on the ropes. Microscopic observation revealed normal and abnormal young thalli grown from vegetative cells, and filaments grown from carpospores. The normal thalli had rhizoids attached firmly to the substrate(Fig.5), and a distinctive form, with only one cell at the tip of the thallus (Fig.4). Abnormal thalli usually had no normal rhizoids so that they could not attach to the ropes and easily fell off in the course of changing the medium.

2. The relationship of parent thalli and formation of the young thallus: Observations on the growth of young thalli from different parent plants showed that cells from small thalli (5 cm in length) had the greatest growth rate. The maximum density reached to 28 young thalli per centimeter of rope, with an average of 15 / cm. Most young thalli were normal and had well developed rhizoids. The ratio of normal young thalli to filaments was 2:1. In the case of the large thalli (30 cm in length), the number of young thalli derived from the cells of the middle portion of the thallus was small, and only few had normal rhizoids. Most cells could form sexual cells——carpospores and sperms. The ratio of young thalli to filaments was one to four. The young thalli derived from the cells near the rhizoid portion were often abnormal has had weaker differentiation ability and a decrepit appearance. The vegetative cells from the basal portion of the thalli showed a greater tendency to develop into young and normal thalli with rhizoids.

3. Growth observations. After three days of culturing with running seawater in the laboratory, the germinating thalli were moved to the open sea. The growth of the thalli and the change in water temperature are shown in Fig.10. At the beginning stage, the young thalli were about 0.2—0.5 cm in length. After culturing in the sea for about one month, they reached up to 30 cm in length, with a





Figs. 4—5 Germination and growth of the vegetative cells cultured in the laboratory

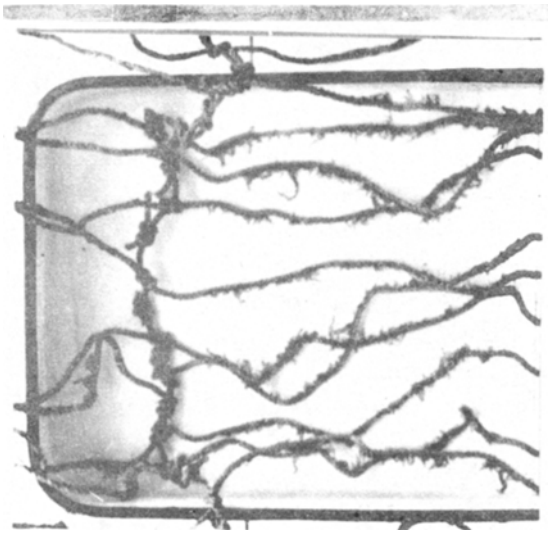


Fig.6 Young thalli cultured in the sea for 9 days

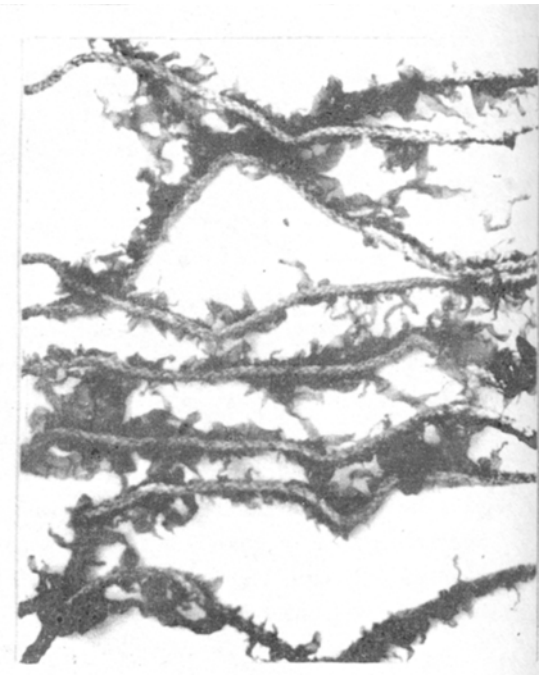


Fig.7 Young thalli cultured in the sea for 17 days

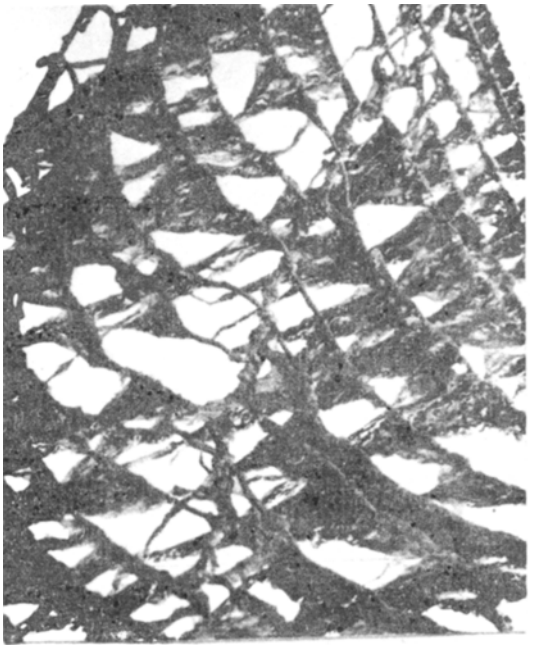


Fig.8 The mature thallus grown on the rope net for 30 days

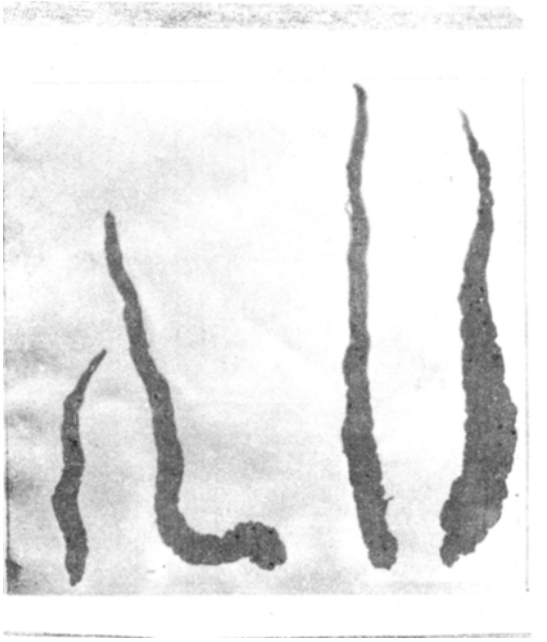


Fig.9 The mature specimen of *P. haitanensis* collected from the rope net after having been cultured in the sea for 30 days

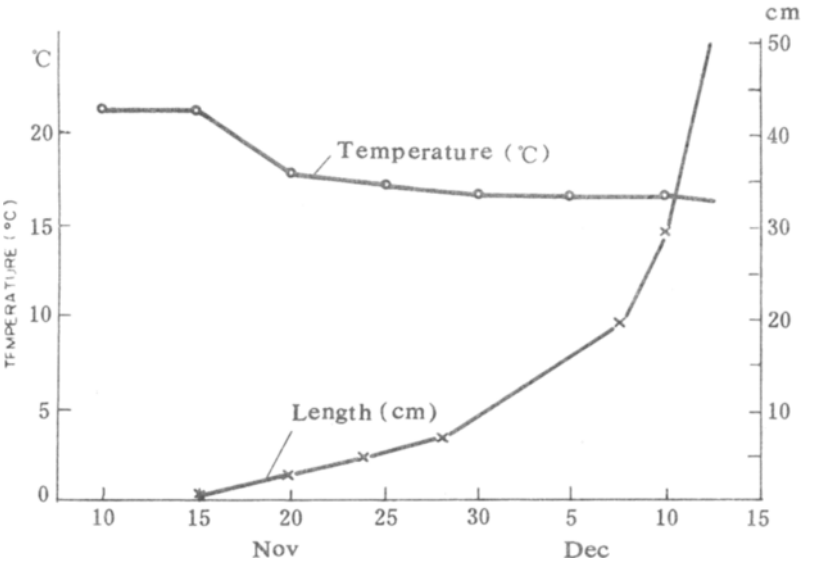


Fig.10 Relationship between temperature change and thallus growth

maximum size of 50 cm (Figs. 6—9). The color and shapes of the thalli were normal and similar to those developed from conchospores. They were dioecious, that is, they had separate male and female thalli. The water temperature showed a gradually declining tendency from the beginning temperature of 21°C to 17°C at the end. It was shown that the young thalli were able to adapt to temperature changes. As the temperature dropped, the thalli would gradually grow larger (Fig.10).

DISCUSSION

Many scientists have made efforts to improve and simplify the conventional method of growing *Porphyra* from vegetative cells instead of from conchospores. But, so far only a few of the methods were successful and most are still in the experimental stages. In this study the authors investigated, for the first time, the use of the vegetative cells of *Porphyra haitanensis* in *Porphyra* farms. The research showed that the small thalli of *Porphyra* (about 5 cm in length) can be used as the source of vegetative cells. From late August through early September the isolated vegetative cells can be collected and cultured in the laboratory and moved to the open sea for further cultivation. It is apparent that the eventual replacement of the conventional method of cultivating *Porphyra* with the simplified method which uses vegetative cells for cultivation to commercial size *Porphyra* will be of great economic importance.

Porphyra haitanensis is a dioecious red alga, with sperm and carpogonia appearing on different thalli. The thalli from vegetative cells also have a dioecious state. Since *P. haitanensis* has no asexual monospores in its life cycle, it is confirmed that the newly formed thalli developed from vegetative cells, rather than from monospores. *P. yezoensis* and *P. suborbiculata* in their life cycles have monospores which can directly develop into thalli. If these *Porphyra* monospores were used as material for vegetative cells, it would be hard to distinguish the origin of the newly formed thalli.

The use of vegetative cells from thalli as seed material is operationally advantageous. Unlike the cell and tissue culture of higher plants, strict sterile conditions are usually not necessary in the course of isolation, collection and cultivation of the vegetative cells of *P. haitanensis*. There is no callus stage in the course of culturing. As long as suitable growth conditions exist, it will usually take about 50 days for the vegetative cells to develop into visible young thalli.

The origin of the vegetative cells is important depending on the nature of the vegetative cells used. This study indicated that the immature small thalli were much superior to the mature larger thallli as source material for breeding. Since most cells of the immature small thallus have not yet developed into sexual cells, these cells will soon develop into young thalli, with only a few cells developing into filaments. On the other hand, since the cells from the upper and middle portions of the large thalli showed a higher degree of differentiation and development, most of these cells develop into filaments, and fewer cells develop to normal thalli. Vegetative cells from the basal portion of the large thalli showed relatively high vitality and so can also be a source of vegetative cells.

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