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PLURISPORE DEVELOPMENT OF *CLADOSIPHON OKAMURANUS* TOKIDA (CHORDARIACEAE, PHAEOPHYTA)

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Abstract

Development of plurispores and effects of temperature on their attachment to substrata were studied using mature plurilocular sporangia of *Cladosiphon okamuranus* Tokida. Development of plurispore showed the disc type, a large number of assimilatory filaments were found to develop from the middle or central part of a disc. These compact and parallel assimilatory filaments formed pseudoparenchyma (multiaxial) with abundant lateral branches. The attachment and development of a plurispore were affected significantly by temperature. Suitable seeding temperature was found to be 17-26º and 26℃ being optimum for successful attachment. At a temperature over 26ºC, the plurispore could not attach tightly to a substratum or developed poorly.

Introduction

Cladosiphon okamuranus Tokida (Chordariaceae, Phaeophyta) is commercially known as "mozuku". The species is widely used as a sea vegetable in Amami-ohshima, Kagoshima Prefecture and Ishigaki Island, Okinawa Prefecture, Japan. The mature alga is ramified, tender, filiform and medium-dark brown in color. The length can exceed 50 cm with a maximum diameter of 3.0-4.0 mm.

The earlier reports on this species were mainly about the ecology and the seedling growth (Shinmura 1977, Shinmura and Yamanaka 1974a, b, c, 1975, 1976). Since the beginning of this century medicinal value of the species has been investigated. It has been reported that extracellular polysaccharides extracted from the species have displayed some anticoagulant, antitumour and antiviral characteristics (Tako 2003, Shibata *et al*. 2003, Tako *et al.* 2000, Wang and Zhang 2002, Wang *et al*. 2003, 2004, Zhang *et al*. 2004). At present, the alga is considered as an important marine medicinal plant in Japan.

The history of the artificial cultivation of the species dates back to early 1970s. However, the production is yet to meet the ever increasing demand. Development of improved techniques are of paramount importance for increased farm-level production. The purpose of the present study was to investigate the development of plurispores and determine optimum temperature for successful attachment and development into sporophytes.

Materials and Methods

Unialgal culture: Sporophytes of *Cladosiphon okamuranus* were collected from Okinawa Island in 2004. Unialgal cultures were obtained from plurilocular sporangia pipetted onto glass slides filled with Provasoli medium (Provasoli and Pintner, 1980) containing 100 µg/l VB1, 0.2 µg/l VB12 and 1 µg/l biotin. The seawater collected from Yellow sea was filtrated through a 0.45 µm acetate cellulose membrane and sterilized by boiling. Since the diameter of the discs appeared to be about 20 µm, the medium was replenished at an interval of four days. The cultures were illuminated with white fluorescent light of approximately 30 µmol/m^2/s at 23℃. Photoperiod was set to 12L: 12D. The growth was monitored routinely with an inverted microscope. At the

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end of the culture period (25 days), many discs (diameter of 300-700 μm) with lots of assimilatory filaments were found to appear and then the light intensity was reduced for storage to 15 ± 5 μmol/m^2/s at 17ºC.

Temperature test: Discs with equal diameter were pipetted from glass slide stored at 17ºC and placed onto new slides and cultured at different temperatures (15, 17, 20, 23, 26 and 28ºC) with light intensity of 30 μmol/m^2/s (photoperiod set was 12L: 12D). Each treatment group consisted of five replications. At first the medium was replenished after 24h, then in every two days. Four days later, the number of discs in each slide was measured and the IDD (initial density of discs: average number of discs in each treatment/basal area of the glass slide) was calculated. At the same time, 15 discs with equal initial diameter were randomly sampled from each treatment for further investigation.

Expanded culture: The discs stored at 17ºC in glass slide were selected and cultured in 500 ml flasks with the same medium and culture conditions as in the "unialgae culture". The medium was not replenished during the culture period. The flasks were stored at 17ºC and with a photoperiod of 12L: 12D. When the flask surface was completely covered by the discs (Light intensity: 15 ± 5 μmol/m^2/s).

Seed culture-nets: Culture-nets (18×1.6 m) composed of vinyl chlorides were used as adhesive substrates and glass jar (55×40×50 cm) was used as the vessel. The alga was inoculated at the 1:100 (vol.:vol.), i.e., each jar contained 0.88 L alga solution in 88 L culture medium. Photoperiod was 12L: 12D and temperature was set according to the result of "Temperature test". Aerated water was provided continuously. Culture-nets were reversed once in two days in order to ensure even distribution of seeds.

Culture in the field: When culture-nets were completely covered with brown discs they were transported to the farm. Field cultivation was carried out in Qingdao, China from the beginning of July in 2005. During the transition, the nets were provided with saturated humidity and at the farm they were placed in the 1~3 m deep water. Growth and developmental stages of the sporophytes of *Cladosiphon okamuranus* were monitored routinely.

Results and Discussion

Development of plurispores: The plurispore on germination developed into a disc of radiating cells. Peripheral cells in the disc were variform, while middle or central ones were round. The disc gradually expanded by the growth of peripheral cells, while unbranched hairs were found to develop from round cells in the centre (Fig. 1a). When the disc was about 150 μm in diameter, assimilatory filaments developed from the middle region (Fig.1b,c). With the enlargement of the disc, more and more assimilatory filaments were found to develop parallel to each other (Fig. 1d-f) giving the alga a multiaxial nature. The disc at a certain size (about 1 mm) stopped growing, when assimilatory filaments produced numerous lateral branches (Fig. 1g). The compact median assimilatory filaments of the germlings transformed into an elongated laterally branched sporophyte of pseudoparenchymatous nature (Fig. 1f). About 25-35 days later, sporophyte was found to be about 1.0 cm in length (Fig. 1g). Assimilatory filaments could divaricate into more filaments (Fig. 1h). Plurilocular sporangia were shown to locate close to the apical part of some assimilatory filaments (Fig. 1i).

The assimilatory filaments of the germling developed hairs at the tip (Fig. 1h). During the hair formation, the apical cells of the assimilatory filaments were found to be longer, thinner and empty looking than the basal ones. The hair formation might be due to deficiency of phosphorus or other elements in the medium.

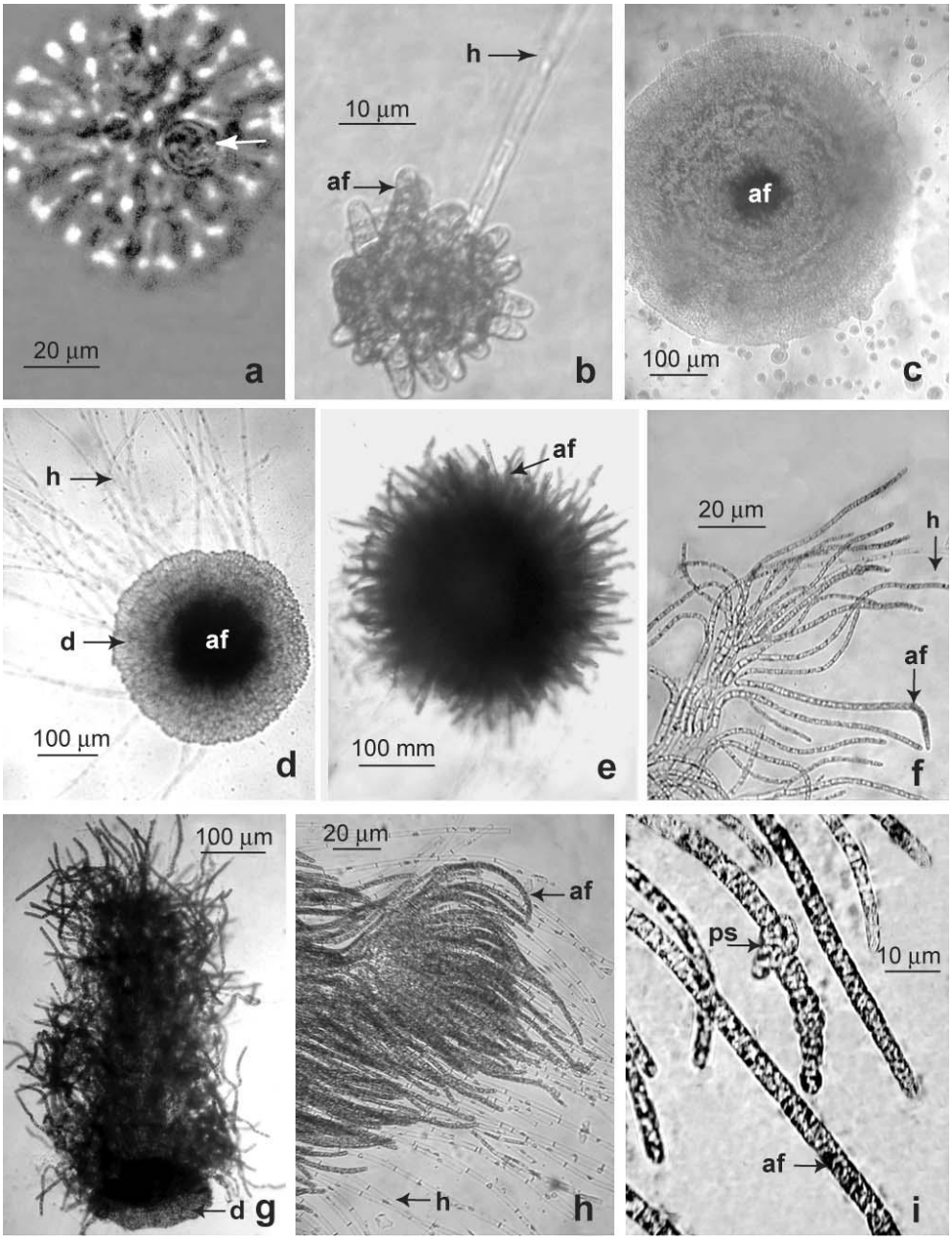

Fig.1. Development stages of plurispore of *Cladosiphon okamuranus*. (a) hair cells (arrow) formed from the middle part of the disc, (b) globoid of hairs and assimilatory filaments, (c) tiny discs around the large parent disc (1100 µm) at 26ºC, (d) disc with hairs and assimilatory filaments, (e) maximum disc, (f) pseudoparenchyma developed multiaxial from the basal cells of assimilatoty filaments, (g) sporophyte of about 1 cm long, (h) outermost part of longitudinal section of cultured germling with hairs from assimilatory filaments, (i) plurilocular sporangia located near the top of assimilatory filaments.

af: assimilatory filament, d: disc, h: hair, ps: plurilocular sporangium.

During investigating plurispore development of the species, the method of pipetting mature plurilocuar sporangia was used, which made the research much easier, accurate, and made it possible to photograph throughout plurispore development. The method, in one hand, helped to investigate the life history of the species effectively, on the other, it confirmed the feasibility to culture the species successfully with sea-water from other region.

Effects of temperature on the attachment and development of plurispores: The number of plurispores attached to the substrate was severely affected by temperature. As shown in Fig 2, IDD showed a gradient of 23 > 26 > 20 > 28 > 17ºC. At 15ºC, no discs were found. At 28ºC, most of the discs could not attach tightly and the development of the assimilatory filaments engendering from discs were found to be poor. Therefore, suitable seeding temperature is 17-26ºC. Shinmura (1974c) reported that suitable culture condition for the attachment of plurispores was 20-25ºC.

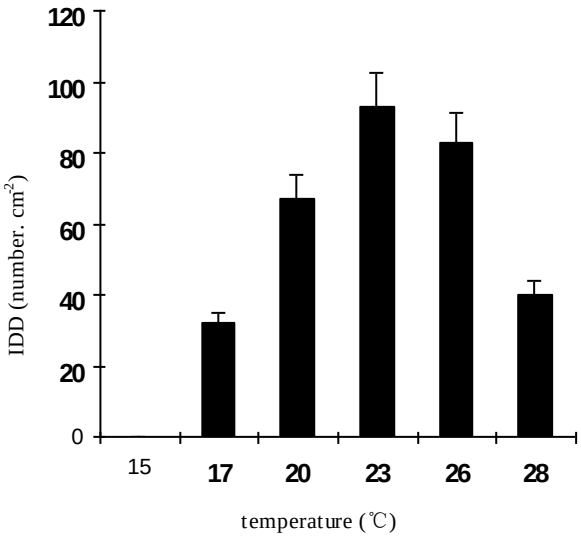

Fig. 2. Effect of temperature on attachment of plurispore.

Results further revealed that the growth of discs increased as the temperature increased from 17 to 26℃ (Fig. 3). The maximum diameter of disc was considerably higher at 26ºC than those at lower temperatures (1100, 670, 470 and 470 μm at 26, 23, 20 and 17ºC, respectively). Therefore, it could be inferred that the suitable temperature for the growth of germlings is between 20 and 26℃. However, Shinmura (1974c) reported that 25-30ºC was the suitable water temperature for plurispore development.

In addition, at 26℃ there were plenty of small discs around the large discs (Fig.1c). It was observed that the small discs were developed from plurispores released from the mature plurilocular sporangia, therefore it could be deduced that 26℃ is optimum temperature for seeding, a feature not known earlier.

Culture in the field: The raft culture technique was used in culturing this alga. Field culture was carried out in Qingdao, China. According to the results of temperature test, plurispores could develop to normal sporophytes only within a range of 17-26℃ indicating that culturing the species in the farms could be done only in the summer.

Though assimilatory filaments of the transported seedlings were found to be lost due to friction, numerous hairs appeared from discs within 24 hours. Two days later, assimilatory

filaments engendered from discs. At eight days time, the number of assimilatory filaments was significantly high (Fig. 4a). Twenty days later, the basal cells of many assimilatory filaments arranged parallelly and formed multiaxial pseudoparenchyma (Fig. 4b). The length of the 28 days old germlings could be up to 2 cm and at this stage branched germlings could also be found (Fig. 4c). Thirty six days later, the sporophyte became 6 cm long (Fig. 4d).

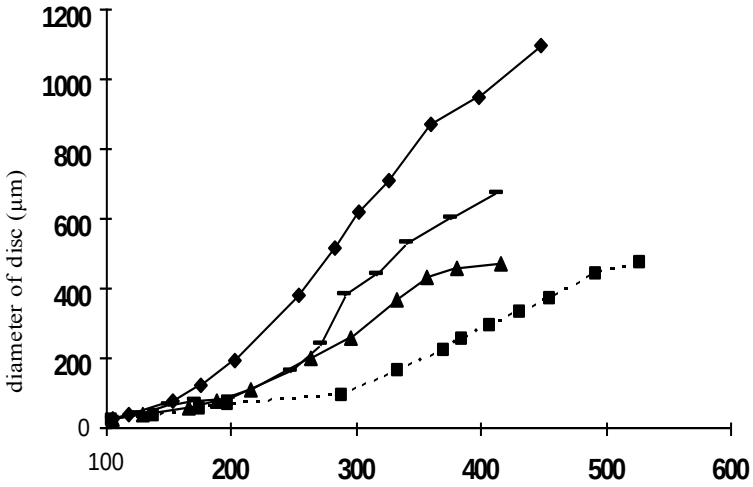

Fig. 3. Effect of temperature on growth of plurispores.

--■-- 17ºC, --▲-- 20ºC, --—-- 23ºC, --♦-- 26ºC.

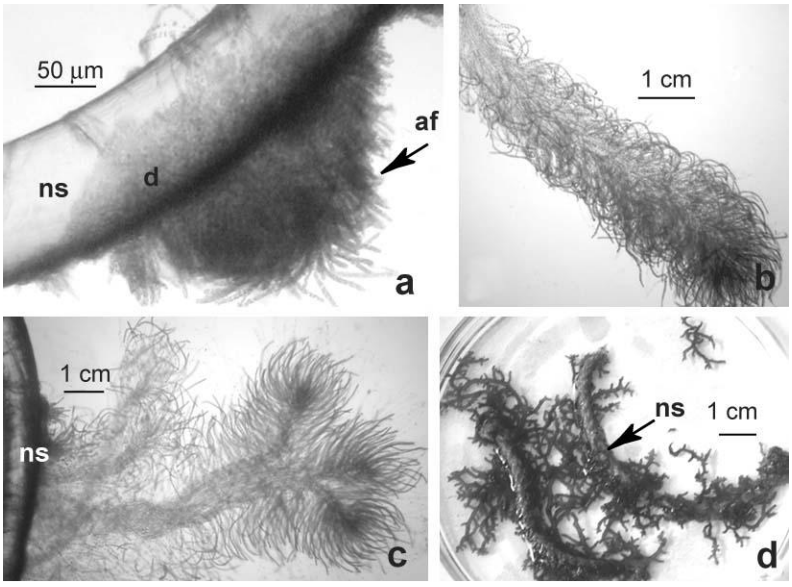

Fig. 4. *Cladosiphon okamuranus* cultured in the field. (a) eight days old germlings with abundant assimilatory filaments on a net string, (b) a multiaxial germlings enlarged, (c) branched multiaxial germlings, (d) germlings of about 6 cm long on net strings. af: assimilatory filament, d: disc, ns: net strings.

Although *Cladosiphon okamuranus* has high nutritive value, until now production only in Japan cannot meet its demand. Expansion of the farm-level production is one way to solve the issue. The experiment in Qingdao has corroborated its practicality to a certain extent, but more study is needed before the system is introduced to other region successfully.

Acknowledgement

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