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ULTRASTRUCTURE OF SPOROPHYTE OF *CLADOSIPHON OKAMURANUS* TOKIDA (ECTOCARPALES, PHAEOPHYCEAE)

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Abstract

Transmission Electron Microscopy of 35 day old culture of *Cladosiphon okamuranus* Tokida, revealed several chloroplasts and other organelles in each cell of assimilatory filaments. Each chloroplast possesses single pyrenoid and Lipid bodies while in hair cells, there were few chloroplasts clinging to plasma-membrane and many pathholes were seen in the cell wall.

Introduction

Cladosiphon okamuranus Tokida (Phaeophyta, Chordariaceae) is commercially known as "mozuku" and widely been used as a sea vegetable in many parts of Asia especially in Japan. The species is ramified, filiform, tender, medium-dark brown and its length can be up to 50 cm with a diameter of about 3.0-4.0 mm. During development it formed a disc from which hairs and assimilatory filaments develop (Zhu *et al.* 2007)

Substantial work has been carried out on the ecology, growth and development of seedlings (Shinmura 1977, Shinmura and Yamanaka 1974a,b,c, 1975, Zhang *et al.* 2004, Zhu *et al.* 2007), active ingredients and medicinal value as anti-inflamatory, anticoagulant, antitumour and antiviral (Tako 2003, Tako *et al.* 2000, Wang and Zhang 2002, Shibata *et al.* 2003, Wang *et al.* 2003) of *C. okamuranus*. But, the ultrastructure of the species has not been studied. Therefore, in the present account the ultrastructure of the species is presented.

Materials and Methods

Sporophytes of *Cladosiphon okamuranus* were collected from Okinawa Island in 2004. Unialgal cultures of the alga and developmental stages have been described by Zhu *et al.* (2007).

Germlings of 35-day old were fixed in 2.5% glutaraldehyde for 1.5 hr, followed by repeated washing for several times with 0.1 mol IL PBS. Materials were then postfixed in 1.0% OsO_4 and stained in 0.1 *mol/*L PBS. After dehydration with a graded series of ethanol, they were embedded in Epon (Polysciences, Eppelheim, Germany). Embedded materials were cut from blocks and mounted on copper grids in order to post stain with aqueous 2% uranyl acetate followed by lead citrate. The final specimens were then used for viewing and photography with H-7000 TEM.

Results and Discussion

The cells at the discoid stage of growth were surrounded by a side wall of about 0.3 µm thickness with comparatively thinner cross walls (Fig. 1). But, in the assimilatory filaments (AF), all cells had thick wall. In addition, the presence of gaps was evident through cell wall, maybe it was just through the gaps mature plurispores were released.

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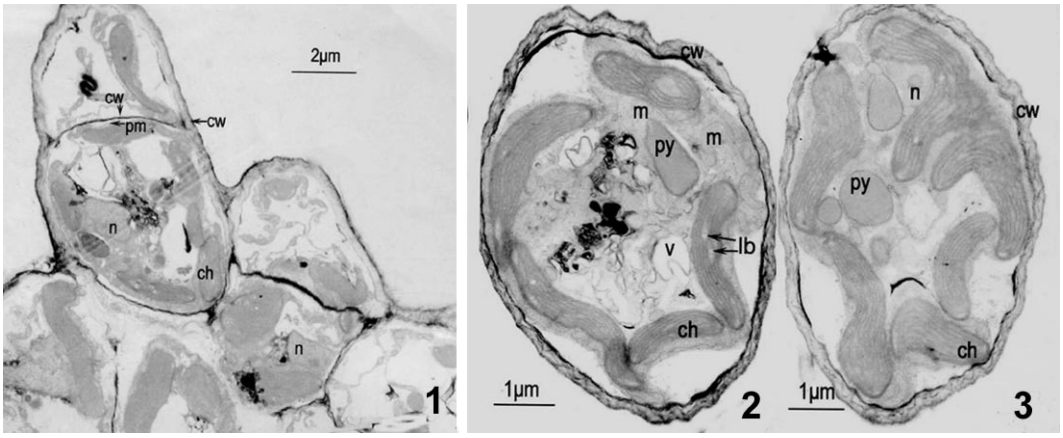
The AF cells (Figs 2-3) were of oval shaped and about 6x10 μm in size. Each possessed usually more than 3 chloroplasts. A chloroplast was often shown to have 3-10 thylakoids and a prominent pyrenoid at the inner side, projecting from the outer part of the stroma of the chloroplast. Electron-opaque material could be seen in the chloroplast. Within the pyrenoid matrix, ribosomes were absent and lamellae did not intrude.



Figs 1-3. TEM of cells of discoid stage and AF of *C. okamuranus*. 1. Cells with thin wall in discoid stage. 2-3. Cross sections of AF with many chloroplasts, (ch, chlorplast; cw, cell wall; lb, lipid body; m, mitochondrion; n, nucleus; pm, plasmamembrane; py, pyrenoid; v, vacuole).

Pyrenoids mainly contain RUBISCO, a key enzyme involved in carbon dioxide fixation. The presence or absence of a pyrenoid and the number of pyrenoids in the chloroplast have generally been believed to indicate a primitive phylogenetic status in the Phaeophyceae (Kawai and Kurogi 1992). In the study, both plurispore and AF cells at the discoid stage had pyrenoids, indicating at least to some extent that the alga is in the primitive status in phylogenetic chart.

Abundant mitochondria, with either oval or circular profiles, were visible around the chloroplast and nucleus in AF cells. The cytoplasm was filled with numerous vascuoles frequently containing fibrillar contents. The large vascuoles have specific shape, however, the small ones were globoid or oval shaped. Lipid bodies (LBs) also existed among thylakoids in groups of two to more.

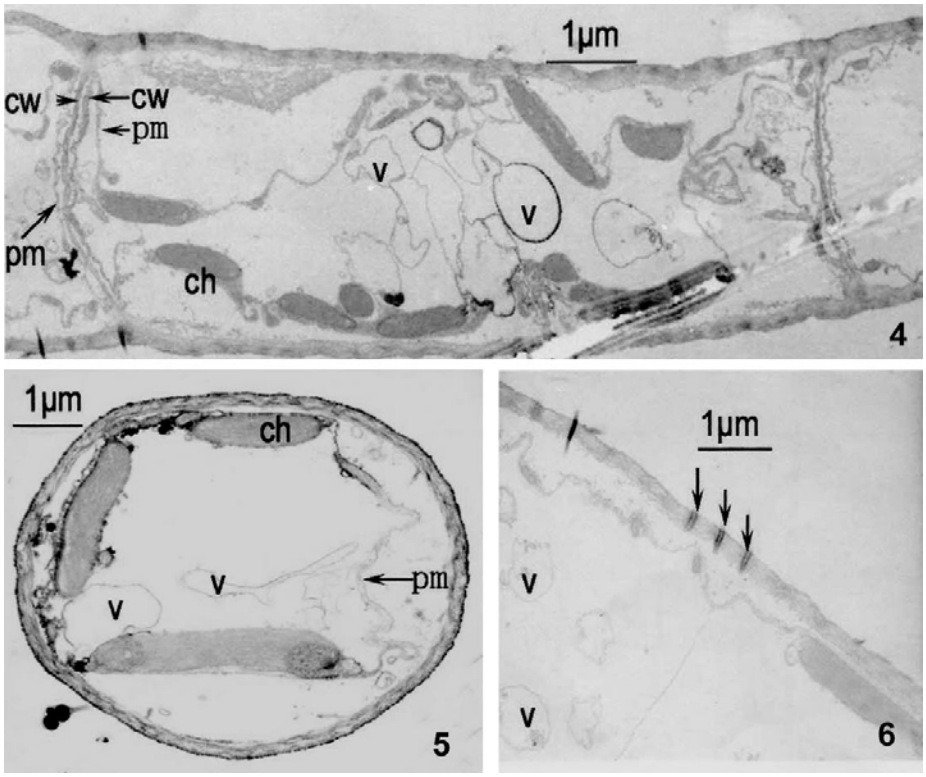
It has been reported that LBs are related to phototaxis of swarm cells. In the swarm cells of the Phaeophyceae, the posterior flagellum usually has a swelling which fits into a depression of the cell immediately above the eyespot (Kawai and Kurogi 1992). The eyespot consists of 40 to 80 LBs arranged in a single layer between the outermost band of the thylakoids and the chloroplast envelope. The eyespot acts as a concave mirror focusing light onto the flagellar swelling, which is the photoreceptor site for phototaxis in brown-algal flagellate cells.

In this study, the LBs were found in the cells of sporophyte. Compared to number of LBs as constituent (40-80 LBs) of eyespot in motile cell of the Phaeophyceae, the number in sporophyte cells was much fewer. In adition, the LBs were dispersive with two or more together among thylakoids in the cells of sporophyte, while in motile cells, LBs could be found between the outermost band of the thylakoids and the chloroplast envelope.

Do LBs exist in plurispore? Do LBs seen in Ultramicrographs originated from ones in plurisopre? If the LBs are present then it was in accordance with the fact that the attachment of

plurispores had positively correlated with illumination to a certain extent during the process of seeding.

Ultrastructures revealed that hair was also surrounded by a thick (0.3 µm) and dense side wall (Fig. 4). The hair cells are characterized by plasmolysed cells (Fig. 5), with fewer cell content including the mitochondria but highly vacuolated with clearly visible pathholes in the side wall (Fig. 6)



Figs 4-6. TEM of hair cells. 4. L.S. of hair cells showing highly vacuolated elongated cell, with plasma membrane dissociated from the cell wall. Pathholes present in the cell wall (dark areas on the cell wall at the left hand side). 5. Cross section of a hair cell with a few cellular content as in the L.S. ch, chloroplast; v, vacuole. 6. Pathholes (arrowheads) in the cell wall.

The existence of hairs in a large proportion is common in brown algae. However, as to the function of hairs in brown algae, there has had no clear conclusion until now. There is opinion that hairs act as passage through which algae absorb nutrients from the surrounding medium, and also exchange gases (Bisalputra 1974). The present ultrastructure revealed many pathholes on the side wall, which was in fact the first report of the occurrence on hair cells. Since hairs develope at the discoid stage, it was not certain that organism acquired nutrition just through pathhole. However, the discovery of pathholes, to a certain extent, supported the latter assumption. Hairs in blue-green algae were found to be developed mainly due to PO_4-P deficiency and found to produce alakaline phosphatase that makes the organic-PO_4 into assimilable from (Sinclair and Whitton 1977) and that hair is a degenerating organ (Aziz 1993). It should be looked into if the hair of *C. okamuranus* functions like that of blue-green algae.

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