A techniques comparison of preparation for the ultrastructure of trabeculae in Caulerpa sp.

Ellen Lavoie¹, Scott Braswell²
1,2: University of Washington, Molecular Analysis Facility, MolES, Seattle, WA

Introduction:

Caulerpa is a genus of tropical marine algae within the phylum Chlorophyta morphologically are made up of three basic distinct regions: root-like rhizoids, stem-like stipes, and leaf-like blades. Within the giant coenocytic (single cell, multi-nucleated), unique structures called trabeculae appear in a network to form what appears as scaffolding. Sporatic work has been done looking at the cellular structure of the stipes, blades, and the growing apical tips of the stipes, while little has been done with the rhizoids (and will not be addressed here). In addition, cytoplasmic streaming within the single celled plant has been observed by (Janse 1890, Dawes and Barilotti, 1969) leaving curiosity as to the movement of trabeculae. Fagerberg et. Al. (2010) showed three distinct cytological regions with developing and/or mature trabeculae and Lavoie (-Hodges) and Fagerberg (unpublished studies) have quantitated the trabeculae in regions of the stipes. An obvious difference between the trabeculae ultrastructure of the mature either blades or stipes compared to those at the apical tip.

This paper presents a comparison of techniques for TEM preparation and imaging of the trabeculae in Caulerpa in order to further determine the solidity or lack of in the distinct cytological regions. The stipe and blade of Caulerpa prolifera and C. mexicana were used for both transmission electron microscopy (TEM) and scanning electron microscopy (SEM) ultrastructure comparisons utilizing the methods of traditional ultramicrotomy and focus ion beam (FIB) milling. The goal of the project is to distinguish between artifacts and either solid or hollow ultrastructure of the trabeculae structures found exclusively in all species of the tropical marine algae Caulerpa. In previous studies the trabeculae have been associated with the role of not only structural scaffolding in the giant unicellular
providing resistance to tension and compression and provide and increase in surface area enabling the structures to work as a conduit channel in and out of the cell (Fagerberg et. Al., 2010). Utilizing the FIB for both serial live cross sectioning and lift out cross sections is possibly a method to determine if the trabeculae are a conduit channel by determining the contents in the rod like structures in the distinct cytoplasmic regions. Embedding and ultramicrotoming is a traditional method to observe the ultrastructure of cells but in this paper the proposed outcome is that the trabeculae will appear in more of a natural state if milled with the ion beam from a dried state only.

**Materials and Methods:**

*Ultramicromed Sections of Fixed and Embedded:*
*Caulerpa sp* blade and stipes were taken from live cultured plants and chemically fixed with a modified Karnovsky’s fixative, post fixed with 1% OsO4, dehydrated, and embedded in Mollenhauer 2 Epon resin mix. Sectioning was done with a Leica UCT or Leica UC-6 ultramicrotome, followed by uranyl acetate and lead citrate staining. Sections on either 400 mesh grid (Electron Microscopy Sciences) or 200 mesh carbon coated grids (Ted Pella or Electron Microscopy Sciences) were imaged on an FEI Tecnai Spirit at 100 kV or an FEI Tecnai F20 at 200 kV

*Critical Point Dried followed by FIB:*
*Caulerpa sp.* were fixed and dehydrated in the same manner as the ultramicrotomed samples, followed by critical point drying with a Tousimis SamDri CPD and sputter coated with approximately 20 nm AuPd. The critical point dried samples allowed us to image the trabeculae in such a state without the use of any epoxy resin infiltrated in the hollowed areas.

**Results and Conclusions:**
FIB Milling of Trabeculae:
Embedding and ultramicrotoming is a traditional method to observe the ultrastructure of cells but in this paper the proposed outcome is that the trabeculae will appear in more of a natural state if milled with the ion beam from a dried state only.

Figure 1: a. shows a mature stipe region of *C. mexicana* (previously embedded) completely milled through and b. shows the internal mostly solid yet “porous” structure of the trabeculae in same stipe region.

The nearly solid nature of the trabeculae in the internal cytoplasm region (Fig. 1) of the mature stipe may indicate a true structural and scaffolding role. The more hollow nature of the rod like structures found (no images provided presently) near the cell wall or peripheral region of the plant indicate that possibly the role of the trabeculae here is that of a channel. Serial FIB live sectioning may show the trabeculae in “action” transporting in and out of the cell. Figure two shows a substance near an opening in the cell wall of a blade of *C. mexicana*.

Figure 2: The top surface of a C. Mexicana blade with proposed as bacteria at the entrance of opening which may be a trabeculae “pore”.

**Future Results:**
At press the ultramicrotomed followed by TEM processed samples have been found to exhibit results that are consistent with Fagerberg et. Al. (2010) showing distinct regions within the cytoplasmic structure and the trabeculae within. Lift out cross sections of the trabeculae from CPD or embedded samples are still in the results collection stage. In order to provide a more accurate depiction of the structure via FIB’d cross sections, a later paper will present full results whereas the poster presented with show preliminary data only.

This work was performed in part at the Molecular Analysis Facility (MAF) at the University of Washington, a part of the Molecular Engineering Institute and the Center for Nanoscale Systems (CNS), which was supported by the National Science Foundation. NS is part of the Faculty of Arts and Sciences at Harvard University.

References:

