Chapter 6

Morphological and electron microscopic studies on established pure microalgal cultures
6. Morphological and electron microscopic studies on established pure microalgal cultures

6.1 Introduction

The techniques of electron microscopy have contributed valuable information to systematic studies of plants. Taxonomic problems in plants and animals frequently arise from inabilities to define certain structural relationships based on light microscopic observations, which is critical in establishing the taxonomic position of a species. The taxonomy of the microalgae is mainly based on the cell form, cell arrangement, and cell wall ornamentation. Several morphological characters of microalgae are difficult to resolve on the light microscope because of their small size. Examination of the microalgae with the electron microscope result in better resolution about their relationships. The electron microscopic studies have been very important for distinguishing the genera *Scenedesmus* and *Desmodesmus*. Smith (1916) emphasized the importance of wall ornamentation, especially the distribution of spines and ridges. In addition to spines, narrower and longer appendages called bristles are also observed in the genus *Scenedesmus* (Trainor and Burg, 1965). Bisalputra et al (1964) examined the fine structure of the wall of *S. quadricauda* and showed that the outer pectic layer consisted of the hexagonal network elevated by a system of props and spine was an aggregation of long props. The characteristic wall ornamentation pattern of Scenedesmaceae is not always visible in light microscopy, but it is well visible under the SEM, although there are also coenobia that lack any cell wall ridges or ornamentation. The genera *Desmodesmus* and *Scenedesmus* have elongate cells of different shapes that are arranged in flat coenobia of two to 32 cells in one or two rows (Komárek and Fott, 1983). Most *Desmodesmus* species have one
or several spines or dents on the cells. The specie *D. costato-granulatus* (Skuja) E. Hegewald, is a small spineless two-celled coenobia, which is abundant and dominant in the phytoplankton communities of oligo- to eutrophic waters (Vanormelingen et al., 2007). It is difficult to distinguish this taxon from other small spineless *Desmodesmus* taxa, unless investigated under the electron microscope. The coenobia belonging to *D. costato-granulatus* shows the presence of a combination of small and large warts, latter often merge into ribs (Hegewald and Krienitz, 1993; Hegewald et al., 1994). The most important differentiating criteria for the taxonomy of the genus *Desmodesmus* are three groups of structures: warts and related structures, tubes and rosettes. Large warts are visible under the light microscope as granulations, the bundles of tubes as teeth and spines or rows of tubes as ribs. However, if these structures are not visible under the light microscope, they may still be present and can be detected under the electron microscope (Hegewald, 1997).

### 6.2 Materials & Methods

#### 6.2.1 Sample preparation for Scanning Electron Microscopy

1. **Cell harvesting**

An aliquot of each culture was centrifuged at 5000 rpm for 5 minutes at 25°C. The supernatant was removed and the pelleted cells were washed once with sterile BG11 medium. The cells were again suspended in the same medium.

2. **Primary fixation**

The pellet containing the algal cells was kept for 4 hours in 3% Glutaraldehyde at 4°C. The fixative was removed and the pellet was washed in 0.1M Sodium Cacodylate buffer. Total three changes of fifteen minutes each at 4°C were given.
3. **Secondary fixation**

The secondary fixation was done with 1% Osmium Tetraoxide in 0.1 M sodium cacodylate buffer at 4°C for 1 hour. This was followed by the three washings of fifteen minutes each with 0.1 M sodium cacodylate buffer at 4°C.

4. **Dehydration**

The dehydration was performed in an acetone series (30, 50, 70, 80, 90, 95 and 100% acetone). All the steps were carried at 4°C.

5. **Drying**

The dehydrated cells were immersed in Tetra Methyl Silane for 5-10 minutes for two changes at 4°C.

6. **Mounting**

Individual samples were mounted on the brass stubs.

7. **Sputter coating**

The coating with gold was carried using a JEOL Fine Coat Ion Sputter JFC-1100 and JEOL JFC-1600 Auto Fine Coater.

8. **Sample viewing**

The stubs carrying the microalgae specimens were observed under the JEOL JSM6360 and JEOL JSM-6390 LV scanning electron microscope.
6.3 Results and Discussions

The microalgae isolates were morphologically characterized using scanning electron microscopy. The wall ornamentations of the microalgae species are characteristics morphological features that allow their taxonomic differentiation. The cells of *Ankistrodesmus angustus* DRLMA1 were curved and crescent shaped and lack mucilage (Figure 6.1). The cells dimensions along the horizontal and vertical axis were 0.25 µm and 15 µm respectively.

![Figure 6.1 (a-d) Morphological characters of *Ankistrodesmus angustus* DRLMA1. a) light microscope image showing crescent shaped cell. b-d) scanning electron micrographs showing smooth cell surface and characteristic shape of cells.](image-url)
The *Desmodesmus* sp. DRLMA7 organisms were observed as coenobia consisting of 2-4 oblong cells (3-13μm in diameter and 7-14 μm long) in one series, in which outer cells possessed long curved spines at each pole and the inner cells were without spines. Under the scanning electron microscope, the cells showed unornamented but granulated cell wall topology (Figure 6.2). The microalgal isolate *D. elegans* DRLMA13 consisted of coenobia of 2-4 oblong cells without any spines (Figure 6.3 a, b). The length and breadth of the coenobium was 7-10 μm and 6-7 μm respectively with uninucleate cells possessing two parietal chloroplasts. The wall ornamentation of
DRLMA13 under the scanning electron microscope showed the presence of dents at the two poles of the coenobia (Figure 6.3 c, d). The most striking feature was the uninterrupted pattern of ribs. Absence of large warts showed that the cell wall was non-granulated (Figure 6.3).

Figure 6.3 (a-f) Morphological characters of *Desmodesmus elegans* DRLMA13. a-b) light microscope image showing coenobia consisting of two- four cells. c) light microscope image of a two celled coenobium showing two parietal chloroplasts at the distal ends. d-f) scanning electron micrographs of two celled coenobia showing ornamented cell wall with uninterrupted rib pattern. The arrows shows the dents observed at the poles of each cell of coenobium. The asterisk shows the rupture line; no large warts were observed.

The cells of *Scenedesmus* sp. DRLMA9 were solitary, fusiform with pointed ends 16-26 μm long and 3-4.4 μm in breadth. (Figure 6.4). Colonies grown on agar were also fusiform, dense and light green in color. Cells become oval with wall thickening
observed in the cells of older cultures (Figure 6.4 b) and dividing cells make two to four daughter cells. No motile-stage and colony formation was observed in cultures. The cells of *Scenedesmus* sp. DRLMA5 were solitary crescent to elongate with pointed ends. No oval or spherical cells observed in the cultures. Long thin extensions evident at each end of crescent cells (Figure 6.5). The cells were 10-12 μm long and 2-3 μm wide. Cells uninucleate with parietal chloroplast Small protrusions were observed near poles in some cells (Fig 6.5). No motile stages or colony formation observed in liquid culture. The cell wall was unornamented as observed under scanning electron microscope and lacked any spines.
Figure 6.4 (a-f) Morphological characters of *Scenedesmus* sp. DRLMA9. a) light microscope image of a coenobia consisting of two cells each bearing spines at poles. b) light microscope image showing wall thickenings in the older cells. c) cluster of cells as observed under the scanning electron microscope. d) an individual cell highlighted to show the unornamented cell wall pattern.
Figure 6.6 (a-d) Morphological characters of *Chlorella* sp DRLMA3. a) light microscope image of a single spherical containing a single chloroplast. b) spherical isolated cells c-d) scanning electron micrographs showing that cells do not have mucilaginous envelopes or other cell wall ornamentation.
The organisms belonging to the genera *Desmodesmus* and *Scenedesmus* were morphologically differentiated by the presence of spines in the genus *Desmodesmus* (Hegewald, 1997). The genus *Scenedesmus* includes all spineless species with obtuse cell poles and without cell wall structures of the sporopolleninic cell wall (Hegewald, 1997). The genus *Desmodesmus* containing the highest number of taxa is characterized by four sporopolleninic layers as the outer cell wall layer; the fourth layer produces cell wall structures that are often visible under the light microscope as granulations or ribs (Hegewald, 1997). However, Vanormelingen et al. (2007) have reported several small spineless species of *Desmodesmus* with characteristic cell wall structures. Classical studies have showed morphological variations, unicellular organisms and the spiny isolates of *Scenedesmus*, which were classified in the genera.
Chodatella, Lagerheimia, and Franceia (Wolle, 1887; Swale 1965; Trainor and Rowland, 1968). We have characterized two organisms belonging to these genera, one is the unicellular spineless isolate of Scenedesmus and the other is spineless Desmodesmus specie. The light microscopic studies showed polymorphism in the cells of Scenedesmus and lack of spines. The cells of Desmodesmus were characterized by the presence of coenobia of 2-4 cells without spines as observed under the light microscope. The Scanning Electron Microscope examination of our specie of Desmodesmus showed no similar type of cell wall ornamentation as reported in the studies of Vanormelingen et al., 2007. Cell wall structures observed under the electron microscope has been considered as the most important differentiating criteria to distinguish the species of Desmodesmus (Hegewald et al., 1990; Hegewald and Schnepf, 1991). Hegewald (1997) has given a detailed description of warts, tubes and other related cell wall structures, which allows the identification of Scenedesmus and Desmodesmus taxa. However, often they are identifiable with difficulty or not observed under the light or electron microscope. Besides the morphological variability of these genera, the phenomenon of phenotypic plasticity has also been observed, which is defined as the ability of a single genotype to produce one or more morphiorms in response to environmental conditions (West-Eberhard, 1989).

The Desmodesmus specie, D. costa-granulatus (Skuja) E. Hegewald, is a small spineless taxon, which is quite abundant and even dominant in the phytoplankton communities of oligotrophic to eutrophic waters (Heynig, 1962; Krienitz 1984; Casper, 1985). Mainly it occurs as two-celled coenobia or as four-celled coenobia in the var. elegans (Hortob.) E. Hegewald et Krienitz. Hegewald et al (1994) have also reported single cells, which are produced in some cultures exclusively as unicells. It is difficult to distinguish this specie from other small
spineless *Desmodesmus* taxa under the light microscope. However, under the electron microscope, coenobia are easily recognized as belonging to *D. costato-granulatus* by the presence of a combination of small and large warts, the latter often merged into ribs (Hegewald and Krienitz, 1993; Hegewald et al., 1994). The old genus *Scenedesmus* Meyen included all colonial green algae having flat colonies that consisted of two to 16 fusiform or oblong cells linearly arranged along their long axes. The fusiform cells lack spines and oblong cells have various arrangements of spines or are spineless (Trainor et al., 1976). The members of the genus *Scenedesmus*, have become the equivalent of laboratory rats in many fields of limnology and are commonly used as standard organisms in numerous areas of aquatic research, biotechnology, and water management (Wiltshire et al., 2000).

Conventionally, in the genus *Chlorella*, only coccoid, solitary algae with spherical morphology that do not possess any mucilaginous envelope were included. We isolated spherical cells without mucilaginous envelope and cell wall ornamentation. These solitary cells with a single pyrenoid were identified as *Chlorella* (Figure 6.6). All *Chlorella* species reproduce asexually by autospores. In the culture of *Chlorococcum macrostigamatum* DRLMA12, we observed spherical to oblong cells of varied size. Cells were solitary as well found in irregular clumps. Each cell possessed single cup-shaped parietal chloroplast. No mucilage covering was observed around the cells (Figure 6.7).