

***PSEUDOCHLORELLA ENCAPSULATA* SP. NOV. (CHLOROPHYCEAE)
FROM MAHAGONI BARK, DHAKA, BANGLADESH**

MAHIN MOHID AND ABDUL AZIZ*

Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh

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Aziz et Mohid sp. nov.

Abstract

Morphology and reproduction of a coccoid subaerial green alga isolated from mahagoni tree (*Swetenia mahagoni*) bark were studied in controlled batch culture using light and scanning electron microscope. Vegetative cells are predominantly spheroidal to ellipsoidal, a few are spherical, 7.0-12.0 µm in diameter. Cell wall about 1.00 µm thick surrounded by over 1.00 µm thick uneven sheath. Chloroplast single, massive cup with incised margin and a central spherical pyrenoid surrounded by two to many spherical starch granules of over 1.00 µm diameter. Cultures were initially green, turning into orange at the initiation of stationary phase. Reproduction by vegetative cell division producing 2 - 3 celled short filament, later dissociate by autospores formation. Autospores in young cultures are 4 - 16 in each autosporangium, mostly spherical to spheroidal or ellipsoidal, up to 8.00 µm long and about 4 µm broad; number of autospores in old cultures are up to 32, always spherical but smaller, about 4.00 µm diameter. Among the coccoid algae the present strain Dh97 resembles *Pseudochlorella* Lund in some characters. Presence of sheath around vegetative cell wall, incised chloroplasts and starch granules around pyrenoids led to consider the alga as a new species *Pseudochlorella encapsulata* Aziz et Mohid sp. nov.

Introduction

Much confusion still exists concerning the validity of morphological and cellular attributes of unicellular algae for identification. Taxonomic criteria of coccoid green algae include the presence or absence of vegetative cell division, cell shape and size, and cell wall thickness in log and stationary phase cultures. Other criteria used were nuclear number, presence or absence of an extracellular gelatinous sheath, number of pyrenoids, type of starch sheath, zoospore present or absent, position of the nucleus in the zoospore, presence or absence of a stigma in the zoospore, and color of the algal colonies (Hildreth and Ahmadjian 1981). Morphology and reproduction of an alga if studied in culture mimicking natural conditions, provides a detailed information on its life forms and processes making it possible to identify difficult organisms following morphometric artificial systematics. New genera and species are being described using light and electron microscopes and many of the coccoid green algae have been found to be properly identified when compared with barcoding (Krienitz and Bock 2012). The present authors while culturing subaerial algae from mahagoni tree (*Swetenia mahagoni*) barks came across an orange-red azoosporic coccoid green alga. Present communication includes an investigation of strain Dh97 having *Chlorella*-like autospore producing cells (Bock *et al.* 2011) but with vegetative cell divisions like *Pseudochlorella* (Guiry and Guiry 2017), sheathed cell wall, and chloroplast massive stellate with one spherical pyrenoid surrounded by two to many starch granules in older cells. The morphology and reproduction have been described, illustrated and discussed in the present account for identifying the alga on the basis of morphometric artificial systematics.

*Author for correspondence: <dr.aziz.botany@gmail.com>.

Materials and Methods

Unicellular green alga *Pseudochlorella encapsulata* sp. nov. Dh97 was isolated from tree barks of mahagoni (*Swetenia mahagoni*) in front of Sir Salimullah Muslim Hall, Dhaka University by inoculating in Chu 10D medium in 100 ml conical flasks (Fig. 1a). On the bark there was no sign of lichens around. Flasks were incubated in a growth room at $25\pm 1^\circ\text{C}$ with an average irradiance of about $75\ \mu\text{mol}/\text{m}^2/\text{s}$ from bottom. Culture contaminated by *Chlorella pyrenoidosa*, a fast growing green alga was made unialgal and clonal by spraying well homogenized dilute culture on 55°C liquid agar plate when only grayish-yellow discrete colonies appeared (Fig. 1b) each being derived from a single parent cell. Culture obtained from one such colony was considered as a clone, and Dh97 clone (out of nine, Dh91-Dh99) having brightest color was considered in the present study. Morphological studies were carried out subsequently using Bold's basal medium (BBM) and maintained in the National Professor AKM Nurul Islam Laboratory, University of Dhaka. Detailed surface morphology of algal cells was studied by scanning electron microscope (JEOL JSM- 6490LA) of the Center for Advanced Research in Science, Dhaka University in addition to light microscopy.



Figs 1a-c. Isolation of *Pseudochlorella encapsulata* sp. nov.: (a) Unialgal culture growing attached to the flask bottom and radiating upwardly. (b) Petri agar plate, inoculated with very dilute algal culture had small discrete colonies after seven-day-growth. (c) Three such colonies on another agar plate after 45 days.

Results and Discussion

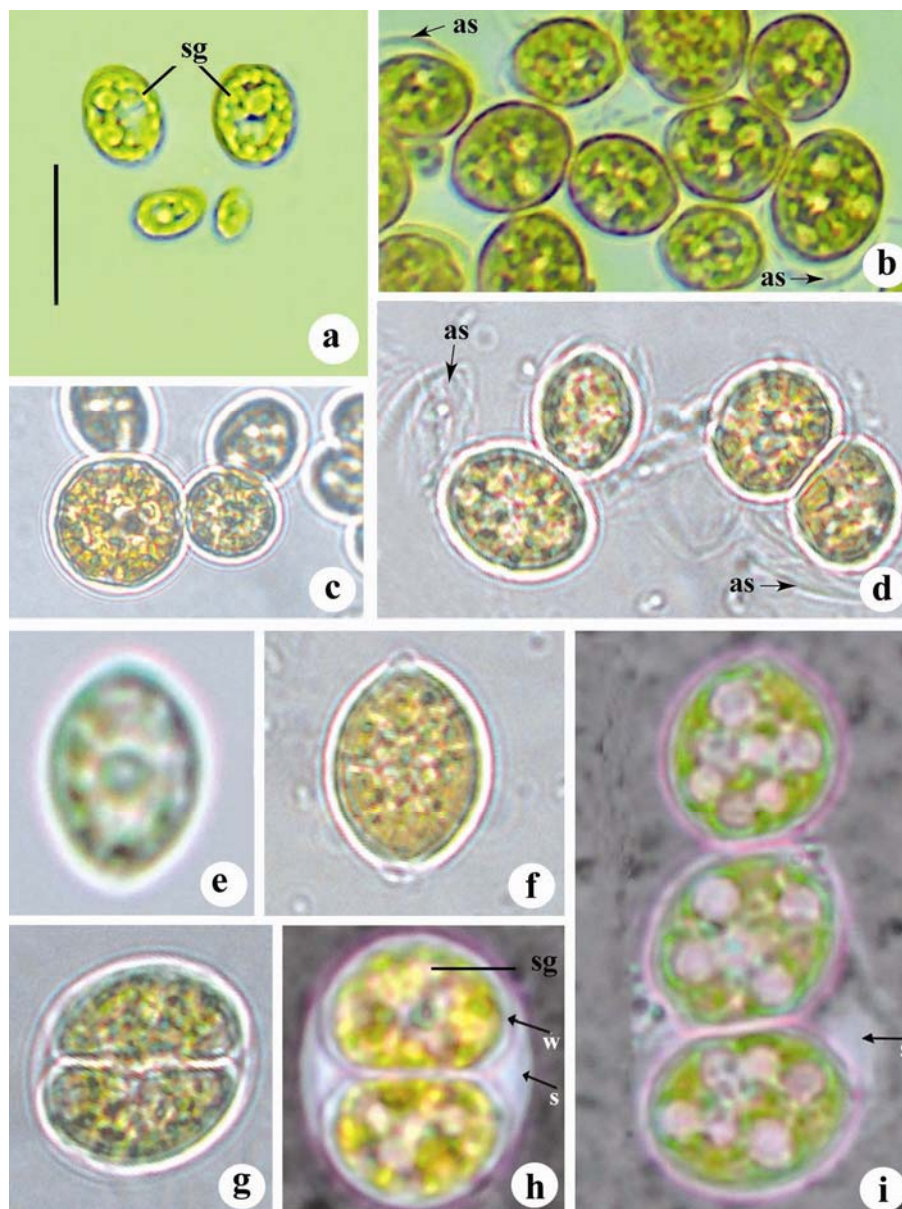
Detailed cell morphology, cell division patterns and processes of reproduction have been studied for identifying the alga based on morphometric artificial systematics.

Class: Chlorophyceae; Order: Chlorellales; Family: Chlorellaceae

***Pseudochlorella encapsulata* A. Aziz et M. Mohid sp. nov.**

(Figs 1-5)

Cells solitary, unicellular, free-living; mostly spheroidal ($7.04 - 12.03 \times 7.21 - 11.33\ \mu\text{m}$) to ellipsoidal ($8.85 - 11.87 \times 7.61 - 10.83\ \mu\text{m}$) and few are spherical ($7.00 - 11.34\ \mu\text{m}$ diameter) (Figs 2a-c). Cell wall about $1.00\ \mu\text{m}$ thick, surrounded by over $1.00\ \mu\text{m}$ thick uneven mucilaginous sheath mostly with longitudinal ridges, sometimes cord-like connection between daughter cells resulting cell clumping (Figs 3a-d). Chloroplast single, massive cup-shaped, parietal with incised margin and stellate in top view with single central spherical pyrenoid. Pyrenoid surrounded by a few to many spherical starch granules over $1.00\ \mu\text{m}$ diameters (Figs 2a, 4b, 5e-f). Reproduction by vegetative cell division and autospore formation. Vegetative cell divisions by binary fission



Figs 2a-i. *Pseudochlorella encapsulata* Aziz et Mohid sp. nov. Dh97: (a) cells of different shape and ages with spherical starch granules around pyrenoids, (b) mature vegetative cells released from autospores (some cells are with ruptured autospore sheath), (c) spherical and spheroidal vegetative cells, (d) germination of ensheathed autospores releasing vegetative cells, (e) a young vegetative cell with stellate Chloroplast. (f) mature ellipsoidal ensheathed autospore, (g, h) vegetative cell division by binary fission from young (g) and old cultures (h), (i) two successive transverse divisions producing 3-celled short filament with several pyrenoids in each with remnants of parent sheath near cross wall. Cells in Figs (h) and (i) were mounted on Indian ink. as: Autospore sheath. s: Sheath. sg: Starch granules. w: Cell wall. Bar = 10 μ m.

producing 2 - 3 celled short filaments (Figs 2g-i). Number of autospores in young cultures was 4-16 (rarely 2), mostly spherical to spheroidal or ellipsoidal up to 8.00 μm (Figs 4a-g), whereas in old cultures up to 32, always spherical and smaller, about 4.00 μm diameter (Fig. 5d). Autosporangia may attain over 20 μm diameter. Autospores are released by rupturing the parent wall at one end (Fig. 4g), and germinate by longitudinally splitting its sheath into two halves (Fig. 2d) and transform into vegetative cells of various shapes and sizes depending on the compactness in autosporangium (Figs 2a-c).

Holotype: Material of strain Dh97

Isotype: 5% formaldehyde-preserved as well as an air-dried samples of strain Dh 97, deposited at the National Prof. AKM Nurul Islam Laboratory, Department of Botany, University of Dhaka.

Type locality: Barks of mahagoni (*Swetenia mahagoni*) in front of Sir Salimullah Muslim Hall, Dhaka University, Bangladesh.

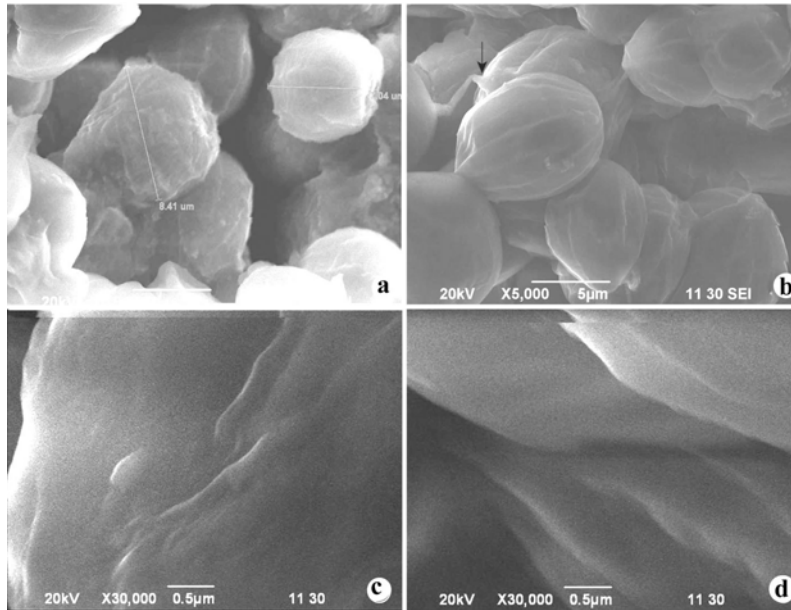
Etymology: From Latin: Encapsulata = within mucilage covering.

Authentic strain: Dh97

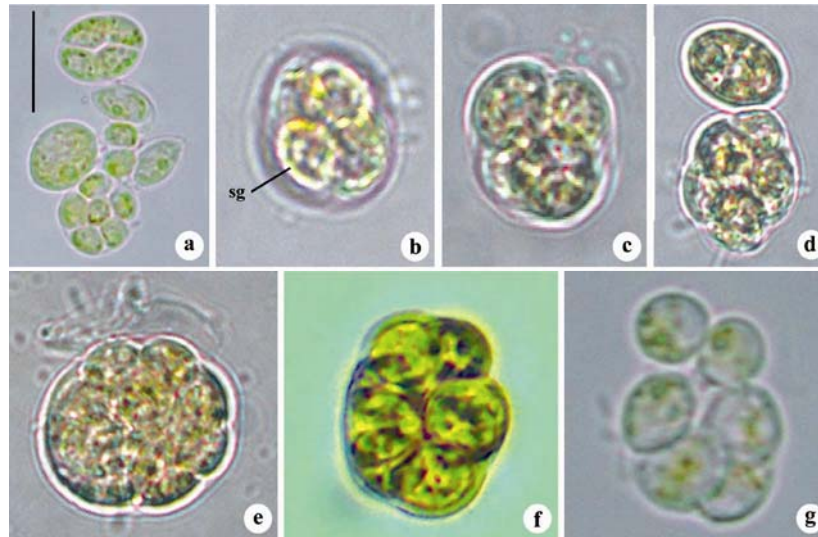
Iconotype: Figs 2-5.

To ensure proper identification of the unicellular green algal isolate, cellular characteristics were compared with that of *Trebouxia*, *Pseudotrebouxia*, *Parachlorella beijerinckii*, *Pseudochlorella*, *Spongiococcum*, *Neospongiococcum* and *Chlorella* (Table 1). *Trebouxia* Puymaly 1924 is zoosporic and Archibald (1975) separated a zoosporic organism that undergoes vegetative cell division, as *Pseudotrebouxia* Archibald. The strain Dh97 reproduces by vegetative cell division and produces ensheathed autospore (Fig. 2d) but no zoospores. Considering vegetative cell division, the strain Dh97 is close to *Pseudotrebouxia*, but a zoosporic or autosporic indicates relationship with *Chlorella* (Table 1). Vegetative cell division was also found in the genus *Spongiococcum* but absent in *Neospongiococcum*, but both are zoosporic. Therefore, the present coccoid alga Dh97 could not be placed under *Pseudotrebouxia*, *Spongiococcum* or *Neospongiococcum*. The algal isolate was also compared with some very closely related autospore forming species. Like the present organism, *Parachlorella beijerinckii* possesses mucilage sheath and produce autospores, but vegetative cell division was absent and cell shape, dimension and chloroplast structure were different. Several species of *Chlorella* resemble in terms of cell shape, chloroplast structure, mucilage in some species and production of autospore with the strain Dh97 but all differ by the absence of vegetative cell division. Moreover, *Chlorella* autospores do not produce polar thickening(s) (Fig. 2f) and are not released by longitudinal splitting of its sheath (Figs 2d, 5e) as is found in the present material. Autospore producing *Pseudochlorella* Lund was separated from *Chlorella* by the presence of vegetative cell division. The present organism also show vegetative cell division and autospore formation (Figs 2g-i, 4a-g) like *Pseudochlorella*, in addition to the cell shape, cell size, single pyrenoid and number of autospores produced per autosporangium (Table 1).

The present organism differs from *Pseudochlorella* by the presence of mucilage sheath around the cell wall, chloroplast margin incised, single pyrenoid is always spherical and surrounded by a few to many spherical starch granules. It appears that the coccoid algal strain Dh97 belongs to *Pseudochlorella* Lund and considering specially the sheath around vegetative cells, incised chloroplasts and starch granules around the pyrenoids led to consider the alga as a new species *Pseudochlorella encapsulata* Aziz et Mohid sp. nov.



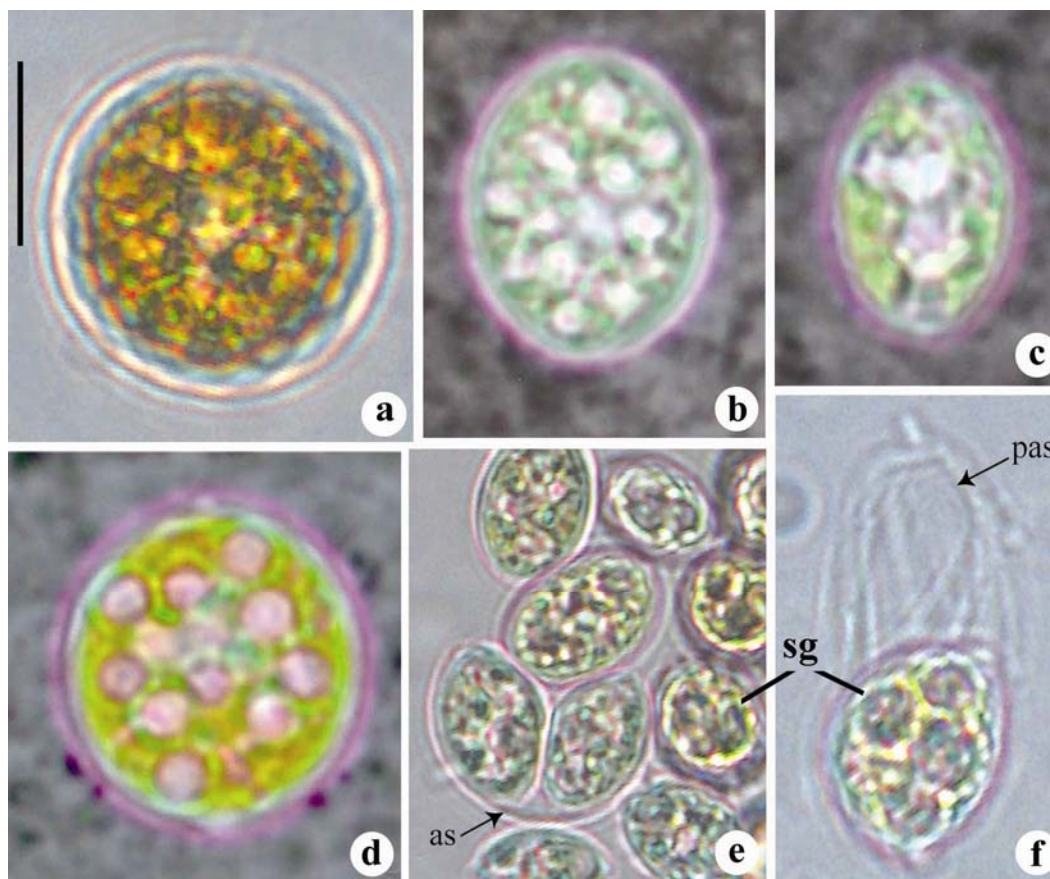
Figs 3a-d. SEM of outer morphology of *Pseudochlorella encapsulata* sp. nov. Dh97: Pictures from powdery sample, cells nearly spherical to irregular (a) and from layer forming sample, one end of cells widely pointed, while other end rounded nearly egg-shaped connected by sheath forming cord-like structure (arrow) (b); (c, d) further enlargement of cell surfaces showing detailed uneven sheath layers nearly longitudinal markings making cell surface rough.



Figs 4a-g. Autosporangia and autospore formation in *Pseudochlorella encapsulata* Aziz et Mohid sp. nov. Dh97 in young culture: (a) various type of cells and stages from young cultures: ellipsoidal (medium left), ellipsoidal dividing cell (upper), elliptical cells (mid region), octad autosporangium in top view (lower); (b) tetrad autosporangium from old culture, note many starch granules (sg) around each pyrenoid; (c) tetrad autosporangium at relatively young stage; (d) compactly arranged visible autospores in autosporangium; and upper mature ellipsoidal vegetative cell; (e) 16 compact autospores are formed within the autosporangium; (f) compactly arranged autospores ready for release; (g) spherical autospores are being released by rupturing autosporangium wall. Bar = 10 μm.

Table 1. Comparison of coccoid genera with the present alga.

Genera	Cell shape	Dimension	Mucilage sheath	Chloroplast	Veg. reproduction	Autospore/aplanospore	Reference
<i>Trebouxia</i>	Spherical, ellipsoidal, elliptical	4.4-8.8 µm	Present in some species	Massive, axial with irregularly lobed margin; single pyrenoid	Absent	Zoospore or arrested permanent aplanospore	Ahmadjian 1960
<i>Pseudotrebouxia</i>	Spherical to slightly oviform, elliptical	Maximum 30.0 µm	Absent	Axial, single pyrenoid surrounded by starch sheath	Present	Zoospores and aplanospores	Hildreth and Ahmadjian 1981
<i>Spongio-coccum</i>	Young cell ellipsoidal, spherical with age	Maximum 30.00 µm	Absent	Chromatophore spongy with single eccentric pyrenoid	Present, forming non motile diad/tetrads	Zoospores ellipsoidal	Deason 1971
<i>Neospon-giococcum</i>	Cell ellipsoidal, Spherical, sub-spherical or pyriform	60 - 180 µm	Absent	Chromatophore spongy, pyrenoids one or more	Absent	Zoospore/aplanospore	Deason 1971
<i>Parachlorella</i>	Globose/egg shaped	2.5 - 5.0 × 3.0 - 8.0 µm	Present, 2.0-4.0 µm thick	Parietal/cup shaped, pyrenoid single, ellipsoidal	Absent	2, 4, 8 autospore	Kreinitz <i>et al.</i> 2004
<i>Chlorella</i>	Spherical/globose/oval	6.7 - 9.0 µm	Present	Parietal, cup shaped, pyrenoid ellipsoidal	Absent	Autospore only 2 - 8 (16), release by rupturing parent wall into four flaps	Bock <i>et al.</i> 2011
<i>Pseudochlorella</i>	Round to ellipsoid, slightly irregular	4.4 - 6.8 µm long, 4.4 - 6.6 µm wide	Absent	Cup shaped, massive and central chloroplast, pyrenoid single, central surrounded by starch sheath	Division of Protoplast	Autospores, (2) 8 - 16 ellipsoid, release by rupturing parent wall	Guiry and Guiry 2017
<i>Pseudochlorella encapsulata</i> sp. nov.	Spheroidal to ellipsoidal, rarely spherical	7.04 - 12.03 µm long, 7.00 - 11.34 µm diam.	Sheath present, autosporangial wall surrounded by mucilage sheath of parent cell.	Parietal cup stellate, pyrenoid single, spherical covered by two to many starch granules	Present, binary fission	Autospores (2) 8 - 16 (32), spheroidal, thin walled released by dissolving parent wall at one end, or ellipsoidal with nipple-like ends released by rupturing parent wall at one end longitudinally into two halves.	Present study



Figs 5a-f. Autosporangia in old cultures: (a) spherical developing autosporangium, (b) ellipsoidal developing autosporangium, (c) elliptical developing autosporangium, (d) maturing autosporangium containing 32 pyrenoids each surrounded by many starch granules and will transform into autospores, (e) elliptical, ensheathed autosporangia. Near the bottom two celled autosporangium sheath ruptured longitudinally releasing autospores; some autospores on the right are seen in polar view, (f) a four celled autosporangium (transformed from an autospore) is being released from the parent autospore sheath. In Figs (b) to (d) cells were mounted on Indian ink. as: autosporangium sheath ruptured. pas: parent autospore sheath. sg: starch granules. Bar = 10 μ m.

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