Morphology, growth and perennation in *Aphanothece*, Cyanoprokaryote

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Aphanothece UACC 1001 (comparable to *A. pallida*) was studied in detail under different culture conditions and a new method of perennation is described. The young colonies are microscopic, but later due to conglomeration of numerous colonies thallus becomes large and macroscopic. As the nutrients deplete, after about six months most of the scattered cells decolorize and die, but clustered cells form characteristic packets. These packets are able to protect a few perennating cells in centre. The packets germinate *in situ* after breaking off the covering cells. The perennating cells are large and divide repeatedly to form chracteristic aphanothecoid cells.

Key-words - *Aphanothece,* growth pattern, perennation, germination.

INTRODUCTION

APHANOTHECE is a common genus and exhibits conspicuous growth on roadside pavements during rains. Certain species of the genus grow abundantly in rice-fields and contribute towards nitrogen fertility of soil. Some forms produce large size colonies (up to 15 cm) and may be found free floating in water bodies. Present survey of literature indicates that details of some 35 species of *Aphanothece* are available at the global level. In India, however, the genus in represented by 14 species only. Kovacik (1988) reported that two daughter cells produced by single plane transverse division change their position and get placed lateral to each other. He also noticed that daughter cells grow to original size before next division occurs. In India, Varma (1965) and Padmaja (1972) have made comparative study of certain unicellular and colonial genera including *Aphanothece* and recorded variations in their morphology and measurements. Since nothing is known about the pattern of growth and perennation in the genus *Aphanothece,* the present communication deals with these aspects studying *Aphanothece* UACC 1001 under culture conditions.

MATERIAL AND METHOD

The strain of *Aphanothece* UACC 1001 was collected from rice-fields and maintained in our germplasm collection of Cyanobacteria, Botany Department, University of Allahabad. A few other strains of *Aphanothece* viz., UACC # 1002., 1003, 1004, 1005, 1006 and of *Gloeothece* UACC # 1301, 1303 were also available for comparative studies. All the strains were axenic and cloned from single few celled (2-4) colonies. They were grown in BG 11 medium (Stanier *et al.* 1971) with a series of four modifications (i) BG 11 (normal medium); (ii) BG 11 without combined nitrogen source (nitrogen deficient medium); (iii) BG 11 without PO_4 source (PO_4 deficient medium); and (iv) BG 11 with double amount of combined nitrogen source (nitrogen rich medium). Cultures were grown in 150 ml or 1L flasks containing 75 ml or 500 ml liquid medium respectively with culture chamber provided with light intensity of 4 k lux (bright light) and 0.5 k lux (dim light) for 14:10 for LD region at 30 ± 2 ^{0}C temperature, cultures were maintained for one year for regular observations. Morphological observations were made under Leica DMLB microscope with digital camera having Leica Quin imaging system.

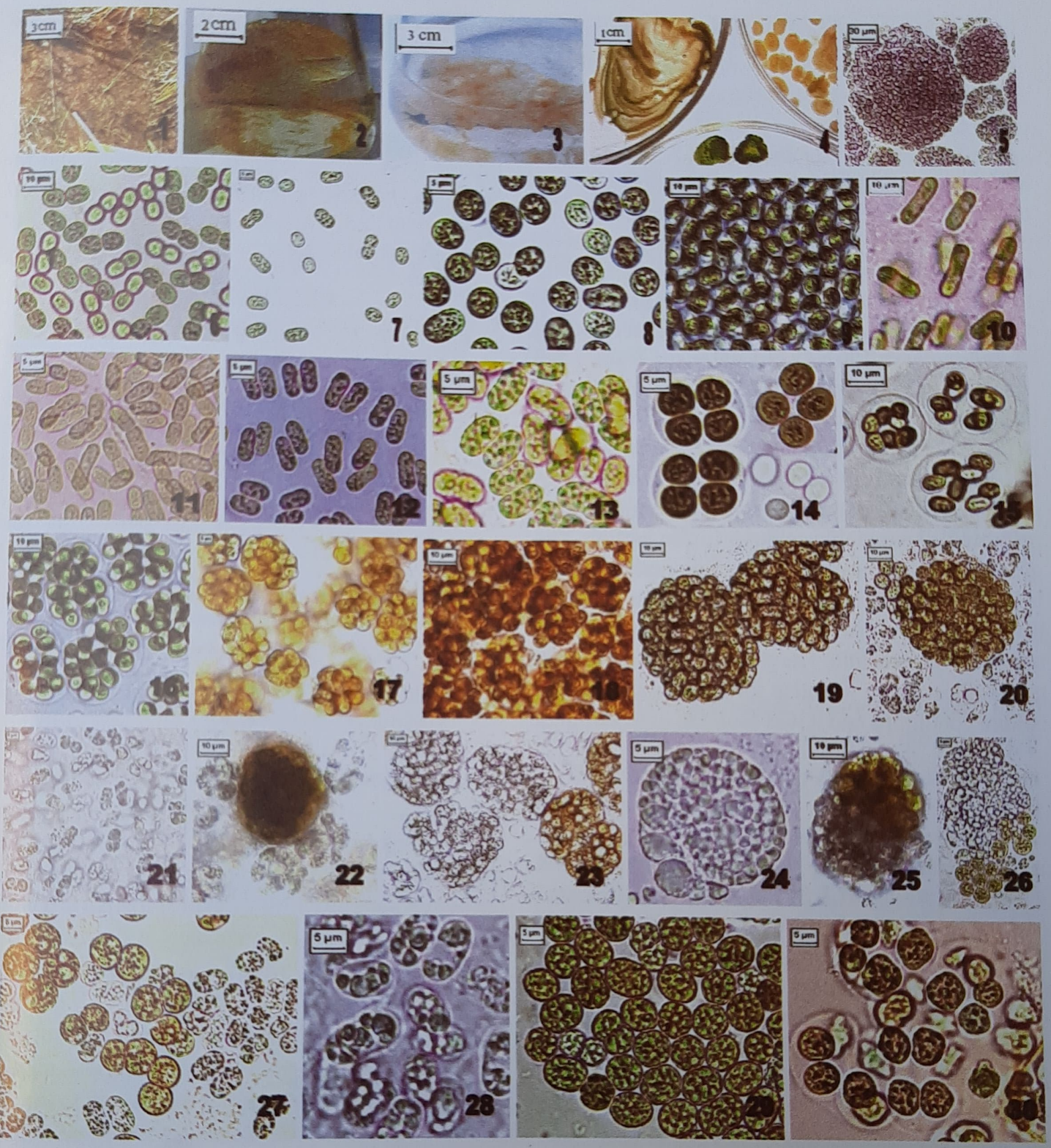
OBSERVATION

Aphanothece UACC 1001 growing in normal liquid medium under bright light condition initially forms minute spherical colonies, but later gets transformed into yellow brown and expanded thalli (Figs. 1, 2). The mature thalli keep on floating due to entrapment of air bubbles formed during photosynthesis. These mature thalli growing in cultures were quite comparable to the material collected from the natural habitat. A mature colony may have cells at different stages of development in different part of the colony. A minute colony represents typical character of *Aphanothece* where numerous elongated cells are embedded in amorphous mucilaginous matrix. The cells measure 3-5 μm in breadth and 3.5-7 μm in length. The taxonomic comparisons with known species indicate its similarity with *Aphanothece pallida* (Kutz.) Rabenh. As the colony increases in size due to secretion of mucilage, individual cells may also develop very thin inconspicuous colourless surrounding mucilaginous envelope. As the colony grows, older, peripheral cells may acquire yellowish or yellow-brown colour (scytonimin) in their envelopes to cope with bright light conditions (Figs. 14-18). All enveloped cells keep on dividing continuously at different speed depending on their position in the colony for availability of light and nutrients. As the colony increases in size a homogenous colony becomes heterogeneous where entire thallus may contain many celled packets of different size mixed with loosely arranged, senescent and elongated cells (Fig. 19-23). The packets may contain 10-50 cells, as nutrients gradually get depleted loose single cells get deformed, vacuolated and may accumulate opaque granules. At this stage, if normal medium is added to old cultures, cells of most of the packets revive and form new colonies as many packets. If the condition of culture medium is not changed for more than three months the cells of even small packets meet the same fate as the loose cells. The entire white mass of thalli still keeps on floating or remain suspended into the medium (Fig. 3). It appears that most of dying cells accumulate numeous gas vacuoles which provide buoyancy to the thallus (Fig. 21) and certain other may accumulate large granules (Fig. 28). After a lapse of six months, in floating white mass only large size packets were conspicuous structures. The surface cells of these packets also become colourless, deformed and accumulate large opaque shining granules and act as protective covering (Fig. 24). If these packets are pressed under cover slips only some of them liberate a few (5-25) yellow-green, granulated and spherical viable perennating cells (Fig. 26, 27). There were many packets, which did not have even a single perennating cell and all cells became colourless and deformed (Fig. 23).

Perennating cells are spherical and larger as compared to the vegetative cells (Figs. 25, 26). Under favourable conditions perennating cells germinate *in situ* and covering cells of the packets break open into pieces (Fig. 26). The germinating cells become blue-green, granules become homogeneous and divide by single plane (Fig. 29). It is only after 2 or 3 divisions, the cells acquire typical aphanothecoid shapes and produce a minute colony (Fig. 30).

Growth of *Aphanothece* in normal medium under dim light conditions is slow; thallus remains blue-green and packets may be surrounded by hyaline sheath even after lapse of three months. Its growth in nitrogen deficient conditions under bright light is slow and diffluent mucilage becomes yellowish within 15 days. Cells are widely spaced from each other, narrower and elongated (Fig. 7). In phosphate deficient cultures only little yellow-brown growth occurs, cells become granulated and may show parallel arrangement of paired cells (Fig. 10, 12, 13). In nitrogen rich medium growth is highly condensed, dark blue-green (Fig. 4), cells are broader; shape is oval or somewhat rounded and closely crowded (Figs. 8, 9).

It has been observed that growth of organism in normal medium gives a range of structure which could also be compared with natural growth. Observations on growth in other media indicate that size of colony is a matter of age and nutrient conditions. Young colonies appear 50-100 μm in diameter (Figs. 5, 6), whereas continued growth may result into 5-6 cm colony, which represents a composite structure of hundreds of colonies growing as a single mass. Actually each packet grows in its own way and represents a colony.



Figures 1-30. *Aphanothece* UACC 1001

1. Yellow-brown growth in rice fields, 2. Two month old growth in normal medium, 3. Four month old growth in normal medium, 4. Growth in culture with different concentrations of nitrogen in medium; growth in normal medium (left) in nitrogen deficient medium (right) and in nitrogen rich medium (below), 5. Young colonies 6. Actively dividing cells in normal medium, 7. Cells distantly arranged in nitrogen deficient medium, 8., 9 Cells oval or rounded in nitrogen rich medium, 10. Cells elongated in nitrogen and phosphorus deficient medium, 11. Two celled pseudofilamentous stage in normal medium, 12.-13 Parallely arranged cells in phosphorus deficient medium, 14-18. Developmental stages of packet formation, 19.-20. Packets with closely arranged cells for perennation, 21. Degenerating cells, 22. Viable (dark) and degenerating (light) packets, 23. Packets with and without perennating cells, 24. Covering cells of packet with large opaque granules, 25. Packet with perennating and covering cells, 26. Packet liberating perennating cells, 27. Perennating and covering cells after breaking of packet, 28. Cells of old colonies with large granules, 29. Revived blue-green perennating cells, 30. Development of mucilage surrounding the new growing cells,

DISCUSSION

Cells growing in different media give a full range of spherical, oval, elongated or cylindrical and bent cells. Cells may be loosely scattered and widely spaced or closely aggregated. Cell content is homogeneous, granulated, yellowish or blue-green. In classical literature (Geitler 1932; Desikachary 1959) different species of *Aphanothece* are identified on the basis of cell shapes and colour of cells, but culture studies indicate that one and the same species may show many variations. Formation of packets in growth of *Aphanothece* 1001is a characteristic feature, but has never been described in detail or emphasized to be of any importance (Varma 1965; Padmaja 1972). In the present study, growth patterns were followed for more than two year and it was concluded that packets help in perennation of the organism by protecting some viable cells for future growth. The growth pattern compared with three other strains UACC 1002, 1003 and 1004 isolated from different habitats revealed that colonial organization was more or less similar in nature. The growth pattern of an allied genus *Gloeothece* was also followed under similar culture conditions and it was found to have different type of packets and cells; packets were characteristically enveloped by laminated sheath.

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