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Growth pattern and new developmental stages in *Chroococcus* 10501 (Chroococcales, Cyanobacteria) under culture conditions

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Chroococcus 10501 was studied from the material collected from natural habitat and from growth in cultures. During exponential phase of growth, cell repeatedly divide in one plane only and the daughter cells grow to original size and get separated. Later, cells divide by two in a plane at right angle to each other and result into daughter cells that appeared hemispherical or quadrantal. In old cultures, when most of the cells die, only a few cells become enlarged, develop dark blue-green content and become perennating cells. Under favourable conditions these perennating cells divide in various planes and result into parenchymatous mass of cells and termed here as "germ colonies". Later, its individual cells get separated and form new colonies. *Chroococcus* 10501 is comparable with *C. prescottii*, *C. minutus* and *C. tenax* but differs in measurement and other characters. *Chroococcus* 10501 is unique in forming parenchymatous mass of cells from perennating cells.

Key-words—Coccoid cyanobacteria, *Chroococcus*, Growth pattern, Germ colonies.

INTRODUCTION

EVER since cyanobacteria are included in Bergey's Manual of Systematic Bacteriology (Staley *et al.*, 1989 and Boone and Castenholz, 2001), it has become necessary to study these organisms in cultures under defined conditions. Critical studies on coccoid cyanobacteria in culture were initiated by Waterbury and Stanier (1978), Rippka *et al.* (1979), and many morphological details have been given. India is known to have 33 genera and 326 species of coccoid cyanobacteria (Tiwari *et al.*, 2007). In India, Varma and Mitra (1962), Varma (1965), Padmaja and Desikachary (1967,1968), Padmaja (1972), Tiwari (1972), Sarma (1981), Kant *et al.* (2003, 2005a-d, 2006) have studied coccoid cyanobacteria in culture. Present communication deals with a detailed study of growth and developmental stages of *Chroococcus* ACC 10501 (Pl. 1, Figs 1-22).

MATERIAL AND METHOD

Chroococcus Nägeli was found growing in a cemented pond (2x4m) used as water reservoir for irrigation of garden plants in Allahabad. The pond was filled with water, twice in a month by a tube well. The growth of *Chroococcus* was monitored throughout the year (2005-2006). It was growing as a metaphyte mixed with algae and other submerged plants like *Hydrilla verticellata* (L.f.) Royle and *Valisnaria spiralis* L. etc. It was isolated in culture from a single 2 celled colony in BG 11 medium (Stanier *et al.*, 1971). It grew well in liquid and as well as solid media under 3 K lux light intensity, 28±2°C temperature, 10/14 dark and light cycles. It is maintained in germ plasm collection of Cyanobacteria, Allahabad Culture Collection, Department of Botany, University of Allahabad and its accession number is ACC 10501.

OBSERVATIONS

In Nature – During August to October, *Chroococcus* was frequent and showed one or two celled stages indicating that the cells were dividing continuously by single plane only and not in two planes at right angle to each other in sequence, often mentioned as characteristic of the genus. Cells after division got enlarged and then separated. The cells were light blue-green or grayish in colour, and finely granulated (5-9 µm in diam). Cells were covered by thin inconspicuous hyaline sheath (Figs 1-2). During November to March, cells gradually enlarged (10-20 µm), 2-4 celled colonies were formed by two-plane division at right angle to each other (Figs 3-6). However, frequency of cell division was reduced. The daughter cells appeared hemispherical and quadrantal and did not enlarge to original size for a long duration. The content of cells was of various colours and granulated. Surrounding hyaline sheath was 1-3 layered and closely appresed to cells (Fig. 6). From

April to July its appearance was occasional and mostly in the form of 2-4 celled colonies with brownish content and conspicuous laminated sheath. These colonies were found mostly adhering to aquatic plants or on bottom mud entangled in mucilaginous matrix.

In culture – During exponential growth of organism under favourable condition only single celled or 2 celled stages were frequently observed and they were the result of apparently one plane division, quick enlargement and separation of two daughter cells from each other and it continued in succession for several generations (Figs 7-8). In this duration four celled colonies were not observed.

In these growing cells and colonies, distinct surrounding sheath was not discernible. Single celled stages measured 6-9 µm in diameter. After 30 days growth most of two celled colonies were converted into 4 celled and 8 celled colonies (Figs 9-11). Mucilaginous envelopes in culture condition were not so distinct as in natural habitat. However, thin mucilage could be seen under phase contrast condition. The cells of these 4 celled and 8 celled colonies did not increase in size and remained hemispherical or quadrantal for almost one or two months. These cells were dark blue-green and subsequently converted into brownish colour. Later, when nutrients were exhausted the cells settled at bottom of the culture flasks in a mucilaginous mass. At this stage the cells remain dormant for several months (upto 24 month recorded). During this period, with passage of time most of the grayish cells turned into yellow-green or grayish colour. However, certain cells (less than 10%) became dark blue-green and got enlarged up to 20µm (Figs 12-13). These enlarged dark cells have been designated here as perennating cells. All cells (dead as well as enlarged) were surrounded by thick (1-3µm), colourless and homogeneous sheath (Figs 20-21). When this cell biomass was transferred to a fresh medium, most of the grayish cells degenerated whereas large dark coloured cells started dividing (Figs 13-14). The planes of division were highly variable (Figs 15-18). A cell may divide continuously by single plane to produce upto 4 cells in a row (Fig 14) or several planes of division at right angle to each other or occasionally in oblique planes and result into a parenchymatous multi-cellular mass of 8-16 or 32 cells of various shapes (Figs 15-18). Such parenchymatous multi-cellular 8-32 celled colonies were quite different from normal colonies and therefore they have been termed here as "germ colonies" (Figs 16-18). Following this the cells enlarge (Fig. 19), get separated and form new colony of normal morphology.

Cells–During active phase of growth, cells immediately after divisions appear hemispherical but soon enlarged and became almost rounded. During decline phase, the cells after division remain hemispherical or quadrantal for long time. In culture condition, it was clearly revealed that while certain cells survived and get enlarged and became dark blue-green in colour, many other cells become pale green to grayish in colour.

Sheath–During exponential phase single cells or 2 celled colonies did not have surrounding sheath. After active growth phase was over they do secrete a distinct, laminated and hyaline sheath around cells and colonies. In culture, only after lapse of 4-5 months growth, cells started forming thick hyaline and smooth sheath (upto 3 µm) around individual cells.

Pattern of cell division–During exponential phase, cells divided by one plane only and two daughter cells enlarged and then separated. Individual cells again divided by one plane and the cycle continued for several generations. When active phase of growth was over cells divided by two at right angle plane division and four celled colonies were formed. The daughter cells were unlike the cells of exponential phase and did not increase in size and remained hemispherical or quadrantal for a long time. During late phase of growth, under unfavourable conditions only certain cells perennated and they were much larger in size. On the advent of favourable conditions, these perennating cells divide in various planes and form multicellular and compact colonies.

Taxonomic status–The characteristics of the organism include cell division in 1-3 or more planes at different stages of growth cycle and at some stage cells are surrounded by concentric layers of hyaline envelopes. Cell envelopes may be inconspicuous at exponential phase of growth but later develop into concentric and closely appressed hyaline layers. Daughter cells at exponential phase get immediately enlarged and separated but later daughter cells do not enlarge and remain hemispherical, quadrantal or polygonal after division in various planes. All these above features relate the organisms to the genus *Chroococcus*. Its comparison with *Gloeocapsa* Kützing is superficial because in the latter, cells always grow into normal size and shape immediately after division, and envelopes are wide and loose (Fig. 22). *Gloeocapsa* is also known to show various stages including status lamellosus, coloratus, perdurans and solutus and such stages are absent in the present form.

The genus *Chroococcus* is known to have 39 species at the global level and 31 species in India. On the basis of measurement of cells of exponential growth the present strain of *Chroococcus* comes close to *C. prescottii* Drouet et Daily and *C. minutus* (Kützing) Nägeli but on the basis of size of mature and perennating cells it can be compared with *C. tenax* (Kirchner) Hieronymus. The present form has unique stages of perennating cells and forming "germ colonies" which are not known in any other species. It may indicate that this taxon is quite different and may be a new species. Since no other Indian species of *Chroococcus* are described in culture and its modern characterization are not made, at present it is designated by strain number *Chroococcus* 10501.

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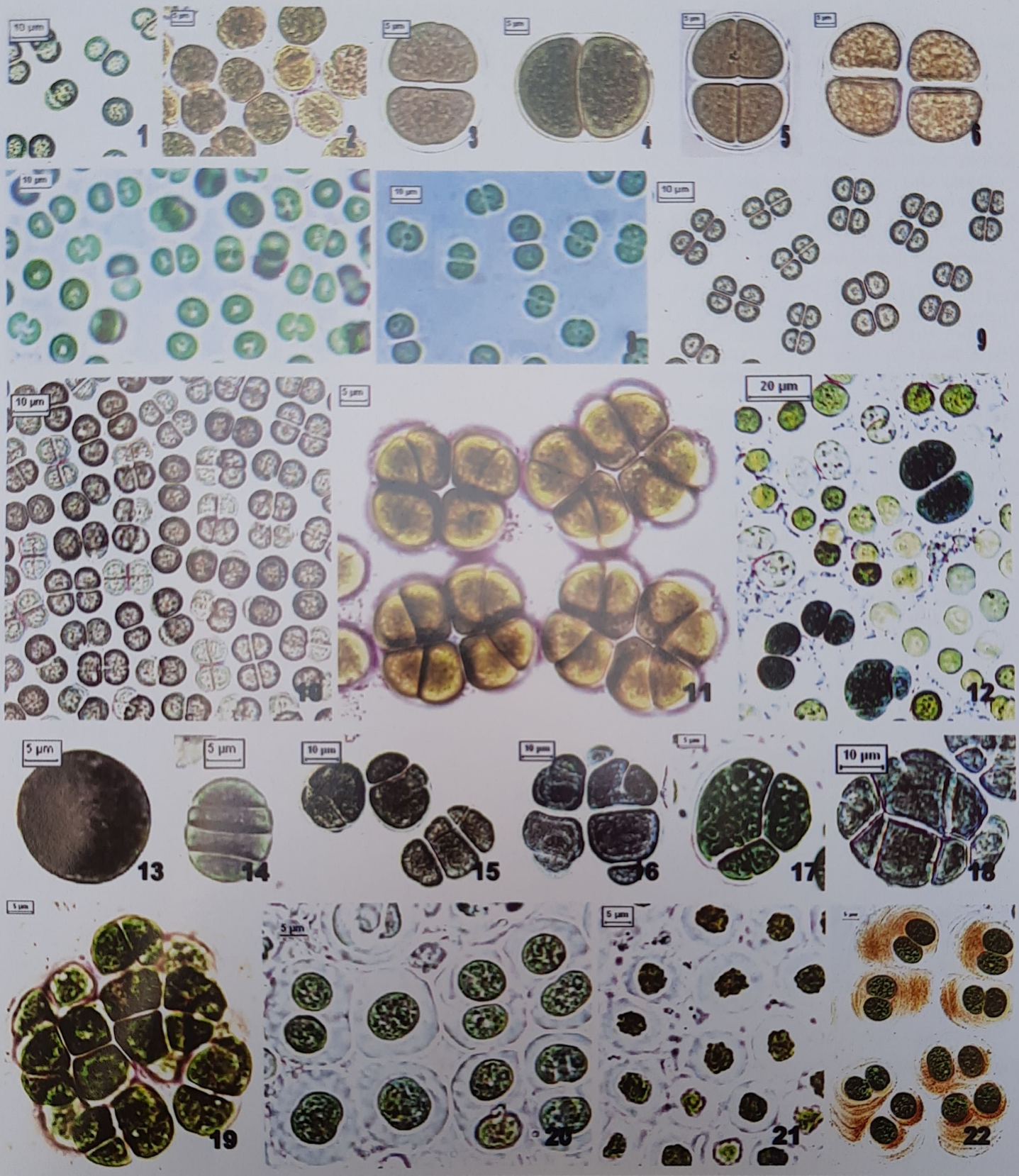

PLATE - 1

Explanation of Figures 1-22, *Chroococcus* 10501

1,2. Single to 2 celled stages in nature. 3-5. Dividing and developmental stages with 2 to 4 celled in nature. 6. Four celled stages covered by thin, 2-3 layered, hyaline sheath in nature. 7,8. Single to 2 celled stages of exponential growth in culture. 9,10. Showing 4 celled stages with two at right angle planes of division in culture. 11. Four groups of 16 celled colonies. 12,13. Degenerating cells and dark blue-green perennating cells. 14-19. Dividing stages of perennating cells and formation of germ colonies. 20,21. Cells surrounded by thick homogeneous sheath. 22. *Gloeocapsa* with concentric laminated sheath.

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