

## TAXONOMIC DESCRIPTION OF UNICELLULAR BLUE-GREEN ALGAE OF RICE FIELDS

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### ABSTRACT

Cyanobacteria (BGA) are one of the most important microorganism in paddy fields. They plays an important role in the enhancement of soil fertility along with yield of rice. It is having unique feature of nitrogen fixation, which leads to enhancement of productivity. In the present study we have selected few areas close to Allahabad like soraon, mau-aima etc. Our sample collection area is only paddy fields and would like to collect different genera of blue green algae, along with this we like to culture these cyanobacteria in lab conditions. We have cultured them and taken pictures of blue green algae and differentiate them on the basis of genera. In the present study we concluded few most important genera with different species found in abundance in paddy fields.

**KEYWORDS:** Cyanobacteria, Paddy fields, Nitrogen fixation, Desikachary's cyanophyta, Biofertilizer.

### INTRODUCTION

A cyanobacterium (Blue-Green Algae) plays an important role in the fixation of biological nitrogen in paddy felids. The rice cultivation is directly related to the nitrogen fixed in paddy fields. This fixation of soil nitrogen is beneficial for soil as well as rice plants as nitrogen is second most important limiting factor for growth of plants (Malik et al., 2001). Cyanobacteria enhance soil fertility and yield of rice fields get increased. It is a natural biofertilizer and is free living photosynthetic nitrogen fixers (Song *et al.*, 2005). It forms mucilaginous colonies and thus they increased water holding capacity of soil (Roger PA, Reynaud PA 1982). In spite of that after its death, decay they also increase soil biomass, prevents growth of weeds

(Saadatnia and Riahi, 2009). They can utilize ammonium, nitrate, nitrite as most of the plants are not able to utilize this combined form of nitrogen. These natural biofertilizers (BGA) are fuel independent, cost effective and easily available alternative source of nitrogen. The nitrogen fixation is done in an anaerobic condition in specialized cells called heterocyst (Fleming, H. and Haselkorn, R. 1973). Blue - green algae (cyanobacteria) are world-wide distributed and play an important role in fertility of soil. The presence of blue-green algae in rice fields has been reported in many articles (Fritsch. F.E. 1907a & Fritsch.F.E. 1907b). Many reports from different countries indicates that the blue-green algal flora is often rich in species (Gupta.A.B. 1966 & Saha.K.C. and Mandal. L.N. (1979)). The study of Blue-green algae are important, as it is most serious microorganism in rice fields, helps in the maintenance of rice fields fertility. The rice fields are agriculturally managed ecosystem well known for diversity of cyanobacteria. The present work on Blue-green algae of rice fields resulted into identification of 19 taxa. They include 08 genera and 19 different species. The description of each taxa is based on the material collected from natural habitat and in certain cases observations from cultures have also been added. The genera and species are arranged on the basis of Desikachary's cyanophyta.

## MATERIAL AND METHODS

Cultural studies of selected algal strains have been done at standard laboratory condition in sterilized glassware and media. Estimation of algal strains at controlled condition has given all the informations about their structure, polymorphic behaviour, role and physiology.

Initially, strains were collected from various localities of rice fields of Allahabad and were kept in enrichment culture medium for further isolation of newly developed colonies. The medium for growing strains was BG-11 medium (Stainier *et al.*, 1971) which is modification of G-11 medium. (Hughes *et al.*, 1958).

The BG-11 medium was prepared in distilled water and had following composition.

### BG-11 MEDIUM (per litre)

K <sub>2</sub> HPO <sub>4</sub>	40 mg
Mg SO <sub>4</sub>	75 mg
CaCl <sub>2</sub>	36 mg
Citric acid	36 mg
Ferric ammonium citrate	6 mg

EDTA	1 mg
Na <sub>2</sub> CO <sub>3</sub>	20 mg
Trace metals	1 ml
For + N medium NaNO <sub>3</sub>	1.5 gm
For solid agar-agar	15 gm

**Method for preparation of trace metal mix (gm / litre)**

H <sub>3</sub> BO <sub>3</sub>	2.8600
MnCl <sub>2</sub> .4H <sub>2</sub> O	1.8100
ZnSO <sub>4</sub> .2H <sub>2</sub> O	0.222
Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O	0.3900
CuSO <sub>4</sub> .5H <sub>2</sub> O	0.0790
Ca(NO <sub>3</sub> ) <sub>2</sub> .6H <sub>2</sub> O	0.0494

All the above mentioned chemicals were dissolved in one litre of distilled water and its final pH was adjusted at 7.0, culture media used were either in solid or liquid form. Concentrations of nitrate in medium were as follows.

- Normal concentration of nitrate nitrogen (1.5 gm/ l)
- Nitrogen free medium or without nitrogen.

**IDENTIFICATION OF TAXA:** Taxa collected from nature in natural conditions. Further these cultures were studied under microscopes for their morphological features. Photographs were taken by using camera lucida and photomicrographs were prepared. Identification of taxa is based on the following literature: Minnesota algae (Tilden, 1910), Kryptogamenflora (Geitler, 1932), The algae of Illinois (Tiffany and Britton, 1952), Cyanophyta (Desikachary, 1959), Phycological studies (Baker and Bold, 1970). Some papers were also studied for giving all the strains taxonomic completeness (Srinivasan, 1963; Kamat, 1963, 1974; Khan and Rawat, 1972; Subba Raju, 1972; Pal and Yadav, 1974; Tiwari, 1972, 1975, 1979; Pandey and Pandey, 1982; Somashekhar, 1984; Tiwari and Pandey, 1976, 1985; Pal and Santra, 1984, 1985; Maity and Santra, 1985 and Khan, 1985).

**1. *Gloeocapsa* Kütz**

**General Features:** Cell is single or 2-8 in irregular colony, spherical with a number of concentric envelopes; individual sheath vesicular or broad, firm lamellated; cell division very

regularly in three direction, occasionally with nanocyte; spores with firm thick wall often formed in a number of species.

Key features the identification of different *Gloeocapsa* species.

- |                                   |                       |
|-----------------------------------|-----------------------|
| 1. Sheath indistinctly lamellated | <i>G. atrata</i>      |
| 2. Cells 6-8 $\mu$ broad          | <i>G. decorticans</i> |
| 2. Cells 3.5 – 4.5 $\mu$ broad    | <i>G. rupestris</i>   |

(a) *Gloeocapsa atrata* (Turp.) Kütz. (Identification basis Geitler, Kryptogamenflora, 188, 1932). (Plate 2; Fig 17) Sample no.: HJM 3, Locality: Mau-aima U. P.

Thallus blackish, mucilaginous; cells without sheath, 3.5 to 4.5  $\mu$  diameter, with sheath 9-14.5 $\mu$  diameter, pale blue green; 2-8 or many cell in colony, cell content dark blue-green; sheath colourless, thick unlamellated or indistinctly lamellated. In India it was first reported by Turner (1892). It is widely reported from regions of Uttar Pradesh, Bihar, Karnataka, Orissa, Tamil Nadu.

(b) *Gloeocapsa decorticans* (A. Br.) Richter (Identification basis Geitler, 184, f. 83 b, 1932.; Desikachary, 114, pl. 24, f. 9, 1959). Sample no.: HJS 51, Locality: Soraon, Phulpur.

Thallus blue green; cell spherical, 2-8 in colonies, colonies small, sometime punctiforme, seldom many with a number of concentric envelopes; sheath well stratified thick and colourless, sheath thick distinctly lamellated, hyaline diffluent in older stage; Single cell with sheath, 10-14  $\mu$  long, 9-13  $\mu$  broad single cell without sheath, 6-8  $\mu$  long, 4-6  $\mu$  broad, two cell colony with sheath, 19-36  $\mu$  long, 15-28  $\mu$  broad. In India it was first reported by Mitra (1951). It is reported from regions of Uttar Pradesh, Andhra Pradesh, Bihar, Karnataka, Kerala, Tamil Nadu, West Bengal. Previous reports of BGA from rice fields of different states have been reported (Tiwari *et al.* 2006).

(c) *Gloeocapsa rupestris* Kütz. (Identification basis Geitler, 194, f. 88 c, 89, 1932; Desikachary, 117, 1959). Plate 1; Fig 13., Sample no.: HJS 52, Locality: Soraon & Phulpur Thallus brownish; cells single or 2-4 together spherical, blue-green, cells without sheath 6-9 (-11)  $\mu$  in diameter, and with sheath 12-15  $\mu$  m in diameter; sheath yellow to brown in colour, very distinctly lamellated; colonies 15-75  $\mu$  in diameter outer daughter colonies often pale yellow to nearly colourless. In India it was first reported by Prain (1905). It is also reported

from Uttar Pradesh, Arunachal Pradesh, Karnataka, Madhya Pradesh, Tamil Nadu and West Bengal.

## 2. *Chroococcus* Näg.

**General Features:** Cells single or few together in a shapeless colony, spherical, sub spherical or hemispherical with a distinct envelope, cells after division in small groups of 2-4 individual sometime 8-16; sheath not vesicular generally lamellated, in some homogenous, persistently or irregularly broken; reproduction by cell division and fragmentation of colonies, division of cell in three direction nanocyte occasionally seen.

Key features the identification of different *Chroococcus* species.

1. Cells single or only few celled thallus	2
2. Cells forming a large thallus	3
3. Cells without sheath 16-21 $\mu$ broad	<i>C. tenax</i>
4. Cells without sheath 3-4 $\mu$ broad	<i>C. minor</i>
5. Cells with sheath 4-5 $\mu$ broad	<i>C. minimus</i>
6. Cells with sheath 7-11.5 $\mu$ broad	<i>C. pallidus</i>

**(a) *Chroococcus tenax* (Kirchn.) Hieron.** Plate 2; Fig 20 (Identification basis Geitler, Kryptogamenflora, 231, fig. 111 a, 1932). Sample no. : HJS 91, Locality : Soraon, Phulpur.

Thallus blue-green; cells mostly in groups of 2-4, sometime 8-16, cells without sheath 16-21  $\mu$ , with sheath 20-26  $\mu$  in dia.; sheath very thick, very distinctly lamellated, colourless or yellow to brown. In India it was first reported by Ganapati (1940). Its presence was also from Assam, Bihar, Gujrat, Karnataka, Kerala, Maharashtra, Rajasthan.

**(b) *Chroococcus minor* (Kütz.) Näg.** Plate 2 Fig 19 (Identification basis Geitler, Kryptogamenflora, 231, fig. 111 a, 1932). Sample no.: HJP 92, Locality : Soraon, Phulpur.

Thallus blue green, small; cells 3.5 – 4.5  $\mu$  in dia., cells usually single, sometime in groups of 2-4, spherical or hemispherical, when single with indistinct sheath, cell without sheath 4-5  $\mu$  broad, 5-7  $\mu$  long, thin colonies with sheath 10-12  $\mu$  broad, 14-15  $\mu$  long; sheath hyaline, unlamellated. In India it was first reported by Turner (1892). It is also reported from Uttar Pradesh, Assam, Bihar, Karnataka, Kerala, Orissa, Tamil Nadu.

(c) *Chroococcus minimus* (Keissler) Lemm. Plate 2 Fig 21. (Identification basis Geitler, Kryptogamenflora, 232, 1932). Sample no.: HJP 32, Locality: Phulpur Cells spherical, mucilaginous, generally in twos, without sheath 2-3  $\mu$  dia., with sheath 4-5  $\mu$  in dia. Colonies, 35-75  $\mu$  dia.; sheath unlamellated. In India it was first reported by Rao C. B. (1937), also found in Maharashtra.

(d) *Chroococcus pallidus* Näg. (Identification basis Geitler, 238, f. 116 b, 1932; Desikachary, 108, pl. 26, f. 1959). Sample no.: HJP 1, Locality: Phulpur Thallus blue green or yellow or colourless, gelatinous; cells single or in group of 2, sometime 4, single cell without sheath (5-) 6 – 8.5  $\mu$  long; (4.5-) 5-8  $\mu$  broad, single cell with sheath (6.5-) 7.5-10  $\mu$  long; (6-) 7-9.5  $\mu$  broad; sheath thin, unlamellated, colourless. In India it was first reported by De Toni, 1907. It is also found in Uttar Pradesh, Assam, Bihar, Karnataka, Kerala, Orissa, Tamil Nadu.

### 3. *Gloeothece* Näg.

**General features:** Cells single or few together in shapeless colony, cylindrical to ellipsoidal, straight or bent, not attenuated at the end but broadly rounded sheath firm, vesicular; division of cells at right angles to the longitudinal axis.

Key features the identification of different *Gloeothece* species.

- |  |                       |
|--|-----------------------|
| 1. Mucilage envelope coloured  | 2                     |
| 2. Mucilage envelope, colourless, only rarely yellowish                | 3                     |
| 3. Envelope yellow to brown  | <i>G. rupestris</i>   |
| 4. Cells without envelope 2.5-4.5 $\mu$ broad, cells ellipsoidal       | <i>G. samoensis</i>   |
| 5. Cells without envelope 4 – 6 $\mu$ broad, cells upto 10 $\mu$ broad | <i>G. membranacea</i> |

(a) *Gloeothece rupestris* (Lyngb.) Bornet Plate 2; Fig 14,15,16 (Identification basis Geitler, 221, 1932; Desikachary, 127, pl. 25, f. 4, 1959). Sample no.: HJS 77, Locality: Soraon.

Cells ellipsoidal to cylindrical, 2-4 in a spherical colony, colony 28-35  $\mu$  dia., without envelope 4-5.5 (-6)  $\mu$  broad and 10-14  $\mu$  long, with envelope 8-12  $\mu$  broad, 14-18  $\mu$ ; long, envelope colourless, hyaline distinctly lamellated contents mostly blue green, inside the colonies diffuents. In India it was first reported by Parukutty (1940). It is also reported from regions of Uttar Pradesh, Assam, Jammu & Kashmir, Karnataka, Kerala.

(b) *Gloethece samoensis* Wille Plate 1; Fig 5,10 (Identification basis Geitler, 220, 1932; Desikachary, 128, pl. 23, f. 6, 1959). Sample no.: HJP 76, Locality: Phulpur Cells usually single or in groups of 2-4 in a common mucilaginous, colourless envelope, ellipsoidal without sheath 4-5  $\mu$  broad and 8  $\mu$  long, with envelope, 8-13  $\mu$  m broad, cells yellowish or bluish green in round colonies; sheath broad, unlamellated. In India it was first reported by Desikachary (1959). It is also reported from Uttar Pradesh, Assam, Karnataka, Kerala.

(c) *Gloethece membranacea* (Rabenh.) Bornet Plate 1; Fig 11,12 (Identification basis Geitler, 220, 1932; Desikachary, 128, 1959). Sample no.: HJS 57, Locality: Soraon.

Thallus blue-green small globular or large irregularly lobed, expanded, gelatinous, leathery; cells ellipsoidal without sheath, 4.5 – 5.5  $\mu$  broad and 7.5 – 8.8  $\mu$  long, with sheath, 6-12  $\mu$  broad, 10-16  $\mu$  long; sheath colourless, distinct, upto 5  $\mu$  in diameter. In India it was first reported by Freymy (1942). It is also reported in Uttar Pradesh, Karnataka, Maharashtra, Punjab and West Bengal.

#### 4. *Synechococcus* Näg.

**General features:** Cell single or few together in a shapeless colony, elongated, cylindrical or ellipsoidal, erect without common mucilage, without a sheath, cell division transverse.

(a) *Synechococcus elongatus* Näg. Plate 2; Fig 29 (Identification basis Geitler, Kryptogamenflora, 273, fig. 133 a –c, 1932). Sample no.: HJS 78, Locality : Soraon, Phulpur. Cell cylindrical, single or 2-4 cells together, 1.4-2  $\mu$  broad 1.5 – 3 times as long as broad, contents of cell homogenous and light blue green. In India it was first reported by Parukutty (1940), also reported in Tamil Nadu.

#### 5. *Microcystis* Kütz.

**General Features:** Cell generally in single colony and in a general amorphous mucilage, very densely arranged with or without few distinct sheath around the individual cells, typically well packed in to microscopic colonies of definite shapes, mostly planktonic, spherical or elongated, ellipsoidal or irregularly overlapping or net like colony, often attached daughter colonies, cell division in all direction, generally transverse in elongate cells, nanocyte present.



Key features the identification of different *Microcystis* species.

- |                         |                      |
|-------------------------|----------------------|
| 1. Colony not clathrate | <i>M. flos-aquae</i> |
| 1. Colony clathrate     | <i>M. aeruginosa</i> |

(a) *Microcystis flos-aquae* (Wittr.) Kirchner Plate 2; Fig 24,25 (Identification basis Geitler, 138, f. 59 c, Desikachary, 94, pl. 17, f. 11 & pl. 18, f. 11, 1959). Sample no.: HJP 54, Locality: Phulpur.

Colony spherical to ellipsoidal, air spaces not present, colonial mucilage indistinct; diffluent cells spherical, blue-green, with gas vacuole 3-5  $\mu$  in diameter, nanocytes not seen. In India it was first reported by Bruhl and Biswas (1926). It is also reported in Uttar Pradesh, Kerala, Maharashtra, Orissa and West Bengal.

(b) *Microcystis aeruginosa* Kütz. Plate 2 Fig 22,23 (Identification basis Geitler, 137, f. 59 d, 1932; Desikachary, 93, pl. 17, f. 1,2,6; pl. 18, f. 10, 1959). Sample no.: HJP 93, Locality: Phulpur.

Colonies rounded, solid when young, becoming clathrate with distinct hyaline colonial mucilage; cells spherical, with gas vacuoles, 3.4 – 7.5  $\mu$  in diameter. In India it was reported by Martens (1870a). It is also reported from Uttar Pradesh, Assam, Bihar, Jammu & Kashmir, Karnataka, Orissa, Kerala, Tamil Nadu, West Bengal.

## 6. *Aphanocapsa* Näg

**General Features:** Cells generally many in single colony, spherical, without any regular or definite arrangement, cells in a general amorphous mucilage, with or without a few distinct sheath around the individual cells, loosely arranged; cell division in two direction, nanocyte present in some species, formed by repeated division.

Key features the identification of different *Aphanocapsa* species

- |                        |                   |
|------------------------|-------------------|
| 1. Planktonic species  | <i>A. pulchra</i> |
| 2. Terrestrial species | <i>A. montana</i> |

(a) *Aphanocapsa pulchra* (Kütz.) Rabenh. Plate 1; Fig 1 (Identification basis Geitler, 155, f. 69 g, 1932; Desikachary, 132, pl. 21, f. 2, 1959). Sample no.: HJS 55, Locality :Soraon, Phulpur.



Thallus blue-green, gelatinous expanded, tuberculate; cells spherical 3-5  $\mu$  in dia., loosely arranged, single or in twos, cells content not granulated; individual sheath absent. In India it was first reported Hof and Fremy (1933). It was also reported from Uttar Pradesh, Andhra Pradesh, Karnataka, Kerala, Madhya Pradesh, Orissa, Punjab, Tamil Nadu, West Bengal.

(b) *Aphanocapsa montana* Cramer (Identification basis Geitler, Kryptogamenflora, 159, 1932). Sample no.: HJM 57, Locality: Mau-aima.

Thallus light blue-green in colour, gelatinous; colonies globular or irregular shape, mucilage colourless; and diffluent cells 2.5-3.5 in diameter, single or in twos, spherical content homogenous; individual sheath absent. From India it was first reported by De Toni (1907). Previously reported from Uttar Pradesh, Assam, Bihar, Karnataka, Orissa, and West Bengal.

### 7. *Aphanothece* Näg.

**General Features:** Cells ellipsoidal to cylindrical, straight or slightly bent, generally many in single colony, without any regular or definite arrangement in a general amorphous mucilage, with or few distinct sheath around the individual cells, cells loosely arranged, division transverse nanocyte present.

Key features the identification of different *Aphanothece* species.

- |                                 |                    |
|---------------------------------|--------------------|
| 1. Cells (3-) 5 – 8 $\mu$ broad | <i>A. pallida</i>  |
| 1. Cells 3.5 – 5 $\mu$ broad    | <i>A. naegelii</i> |

(a) *Aphanothece pallida* (Kütz.) Rabenh. Plate 1; Fig 4,6-9 Geitler, 171, f. 78, 1932; Desikachary, 140, pl. 22, f. 3, 1959. Sample no.: HJM 33, Locality: Mau-aima (U.P.)

Thallus blue-green to brownish colour, gelatinous, soft; cells elliptical or oblong 3-8  $\mu$  broad and 1.5-3 times as long as broad, sometimes cells with sheath, cells without sheath 6-8 (9)  $\mu$  broad and 10-12  $\mu$  long, cells with sheath 8.5-11.5  $\mu$  broad and 10-15  $\mu$  long, cell content coarsely granular of dirty blue green colour; sheath often lamellated, sheath diffluent in the inner part. In India it was first reported by Gonzalves and Joshi (1946). It also reported from Uttar Pradesh, Andhra Pradesh and Bihar.

(b) *Aphanothece naegelii* Wartm. (Geitler, 172, 1932; Desikachary, 141, pl. 22, f. 7, 1959). Plate 1; Fig 2, 3. Sample no.: HJS 58, Locality : Soraon, Mau-aima.

Thallus pale blue-green, gelatinous; cells oval to ellipsoidal, cells 3.5-5  $\mu$  and 6.5-8.5 $\mu$  long; individual sheath absent, diffluent, easily detectable. In India it was first reported by Rao, C. B. (1936). It is also reported from Uttar Pradesh, Andhra Pradesh, Assam, Jammu & Kashmir, Kerala, Maharashtra, Punjab Tamil Nadu and West Bengal.

### 8. *Merismopedia* Meyen

**General Features:** Cells generally many in single, tubular or cubical or three dimensional colony, free floating, cell in regular transverse and longitudinal rows, multiplication by division in two direction perpendicular to the plane of the colony.

Key features the identification of different *Merismopedia* species.

- |                                |                    |
|--------------------------------|--------------------|
| 1. Cells 2.5 – 3.5 $\mu$ broad | <i>M. punctata</i> |
| 2. Cells 3 – 6 $\mu$ broad     | <i>M. glauca</i>   |

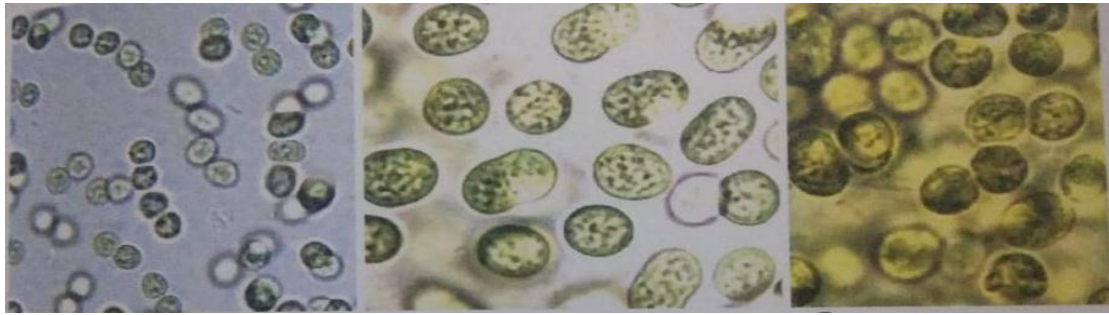
(a) *Merismopedia punctata* Meyen Plate 2; Fig 26 Geitler, Kryptogamenflora, 263, fig. 129 c, 1932. Sample no.: HJP 2, Locality: Phulpur.

Thallus pale blue-green; cell spherical or ovoid, 5 – 3.5  $\mu$  broad, colonies small, 4-64 cells about 60  $\mu$  broad. In India it was first reported by Rao C. S. (1940). It is also reported from Uttar Pradesh.

(b) *Merismopedia glauca* (Ehrenb.) Näg. Plate 2; Fig 27,28 (Geitler, Kryptogamenflora, 264, fig. 129 d, 1932). Sample no.: HJP 94, Locality: Phulpur.

Thallus blue-green colonies mostly small with 16-128 cells, free floating amongst other algae; cells oval or hemispherical, closely arranged, 3-6  $\mu$  broad. In India it was first reported by Beck and Zahlbruchner (1897). It is also reported from Bihar, Jammu & Kashmir, Karnataka, Kerala, Orissa and Tamil Nadu.

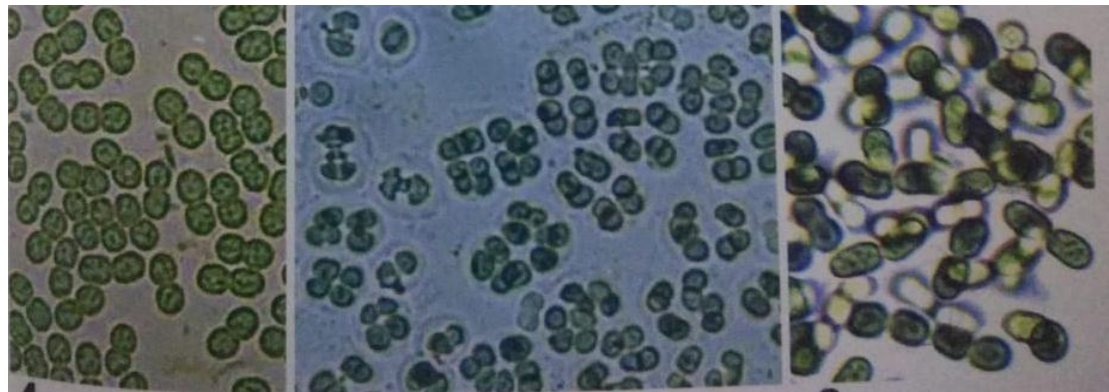
Images of algal cell culture taken in laboratory conditions



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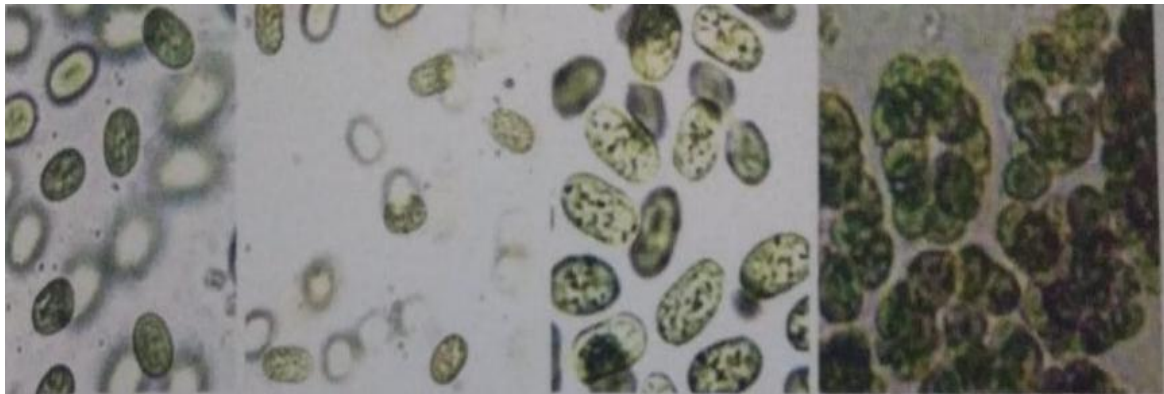
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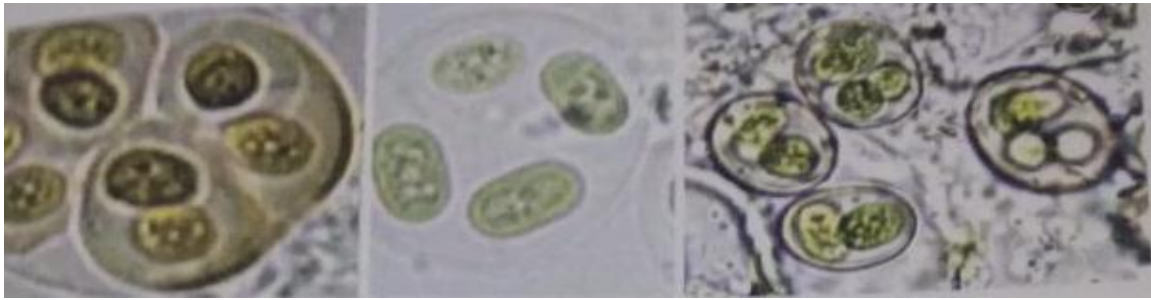


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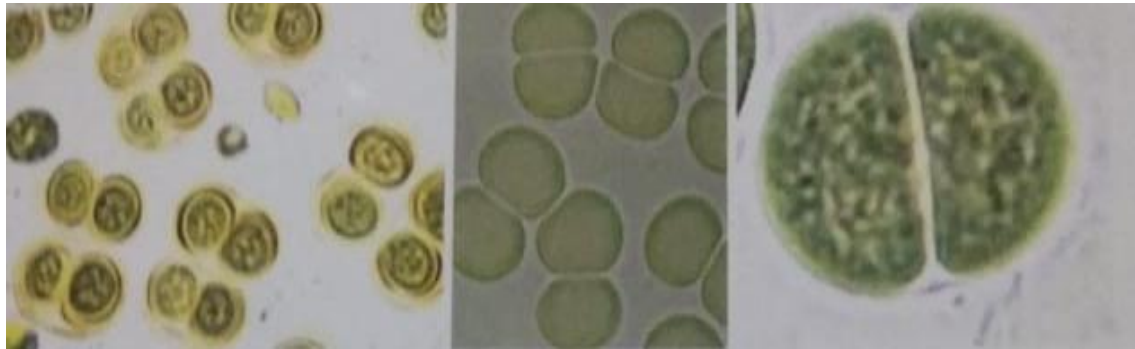




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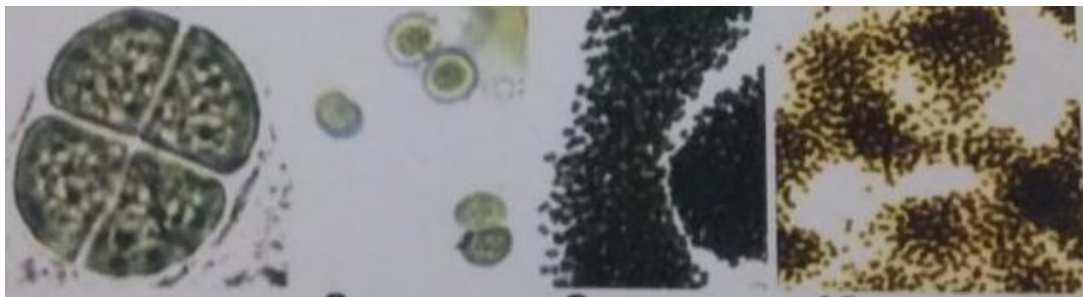
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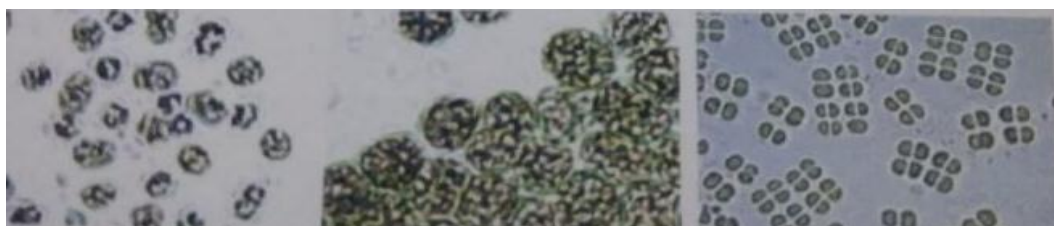


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