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Eco-Biological, Biochemical Effect and Taxonomical Consideration on *Scytonema chiastum* Geitler (Cyanobacteria) under the Family Scytonemataceae Studied in Culture

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Abstract

The present study of filamentous heterocystous Cyanobacterial (BGA) strain *Scytonema chiastum* Geitler under the family Scytonemataceae deals with the different observations in the Cyanobacterial culture isolated from various localities of diversified habitats. Filaments are very long and broad, sheath thick with parallel lamellation, branches more lateral geminate, less opposite geminate and single branches; Hormogonia are irregular in shape, heterocytes very long cylindrical. Meristematic zone is very long, apical cells yellowish dome-shaped, Polybranches also present. The present algal growth rate was higher in the nitrogenous medium than the nitrogen-deficient medium. Chlorophyll-a was gradually increased in maximum days. Nitrogenase activity was more in a shorter period than a longer period, which was just reversed compared to the growth rate.

Keywords: Heterocyst, Growth-rate, Cyanobacteria, Taxonomy, Nitrogenase activity, Scytonemataceae.

1. Introduction

Scytonema chiastum Geitler under the family Scytonemataceae (Cyanobacteria) plays an important role in maintaining and building soil fertility, consequently increasing rice growth and yield as a natural bio-fertilizer. The paddy field ecosystem provides a favourable environment for the growth of Cyanobacteria concerning their requirement for light, water, temperature and nutrient availability. The family Scytonemataceae Rabenhorst came into existence in 1865 while the genus *Scytonema*. Agardh was described four decades ago by Agardh (1820).

Geitler (1925, 1932) maintained the same concept so far as Scytonemataceae and Microchaetaceae are concerned. Subsequent workers, including Fritsch (1945) and Desikachary (1959), also continued the same trend as followed by Geitler (1932). Komarek and Anagnostidis (1988) established genera like *Hassallia, Petalonema,* and *Camptylonemopsis*. According to them, *Scytonema* is a member of Scytonemataceae and the rest viz. Microchaete, *Hassallia, Petalonema* and *Camptylonemopsis* are the members of Microchaetaceae. Bharadwaja (1933) made a significant contribution in understanding the details of Scytonemataceae. Jeeji Bai (1977) studied *Scytonema stuposum* in cultures for morphological variations and observed that various culture media induce various changes in the morphology of the alga. Pandey (1974) observed an interesting feature in the reproduction method in *Scytonemopsis ghazipurensis*. Anand and Gunaseeli (1978b) further studied both the genera *Scytonema* and *Tolypothix* in different culture media. They found that depending on growth in different media and the same way, Scytonema strain could be identified as *S. cincinnatum* or *S. coactile*. Komarek and Anagnostidis (1988, 2005) emphasized

the mode of germination of hormogonium either isopolar or heteropolar. On this basis, *Scytonema*has been retained in the family Scytonemataceae. Komarek and Anagnostidis (1988) referred to the International Association for cyanophyte Research (IAC) and suggested the need for more intransitive study under various cultural conditions and not merely on the basis of the natural material. Continued field study, laboratory cultural and Eco-biochemical morphological studied Cyanobacteria showed diversified characters in different times.

2. Materials and Methods

2.1. Isolation and Maintaine of strains

In total 21 strains belonging to the genera *Scytonema* of the family Scytonemataceae have been taken for the present cultural study and which are isolated from their natural materials and from enrichment cultures of the soil samples collected from various fields of rice growing localities of several districts of Uttar Pradesh and some districts of West Bengal.

The present Cyanobacterial strains and soil samples were collected from different habitats. All the strains were raised in BG-11 medium (Stainer et al., 1979) in the absence and presence of Nitrate Nitrogen and amonical nitrogen in solid and liquid medium. The quantity of $NaNO_3$ and NH_4Cl for different concentrations are taken as follows: (1) Nitrogen-deficient medium (-N; without nitrogen) (2) Normal concentration of nitrate-nitrogen and ammonical nitrogen (+N; 20 mM), (3) More concentration of nitrate-nitrogen and ammonical nitrogen (+1.5N; 30 mM). (4)Rich (+2N; 40 mm) and high (+4N; 80 mM) concentrations of nitrate nitrogen and ammonical nitrogen, respectively.

For nitrogen deficient medium, either $NaNO_3$, NH_4Cl or any form of nitrogen was totally eliminated from the medium and this medium was divided into two parts: one for solidified medium (Agar slant) by adding agar (15 gm/Lit) and the other for liquid medium. Which were maintained and observed in the laboratory and natural conditions. Which are as follows (A) these strains were inoculated in 15 ml, 50 ml, 100 ml in different sterile solid and liquid medium in a taste tube, petridishes, conical flasks (different in size i.e., 100 ml ,200ml, 500ml, 1000 ml, 2000 ml), and within a polythene packets containers (i.e., generally 1000 ml and 2000 ml) and also with in sterile soils in a petridish and incubated for 20-30 days at 28 ± 2 and 4000-5000 Lux light intensity under 14/10 LD cycle. (B) Its growth was also maintained and observed in an open. 6'' x 3" x 1''within a cemented tank into natural trap water conditions. The isolation of filamentous forms was maintained by streaking methods (Kaushik, 1987). The purity of the organisms was tasted by repeated culturing and subculturing obtained bacteria-free (axenic) culture of Cyanobacteria. All the isolated strains of filamentous Cyanobacteria are being maintained in our culture room, Department of Botany.

2.2. Habitats of all the filamentous Cyanobacteria

All the 21 strains of filamentous Cyanobacteria have been isolated from various habitats from Uttar Pradesh and West Bengal in India. These strains mostly grew as a terrestrial, aquatic, epiphyte, thermal spring and sub aerial forms.

2.3. Morphological observation and identifications

Morphological observations were recorded with the help of Nikon microscope with attaching photosystem.

2.4. Identification of these filamentous cyanobacteria

The taxonomic identifications of isolated filamented strains were made by following the key given by Desikachary (1959), Komarek & Anagnostidis (1988, 2005) and our present observations.

2.5. Estimation of growth and Dry weight (mg/100 ml)

The assessment of the growth after the treatment of different conditions was based on their dry weight and by chlorophyll-a estimation. The measurement of growth was taken in term of dry weight. After the definite time of the growth, the algal material was harvested by the filtration through Whatman filter paper No.1 and washed twice with distilled water. The filtration was done by pre-weighed filter paper and these filter papers with algal material were dried at 50^0C in oven. After drying the biomass, the dry weight was determined after 24 hours up to its constant weight.

2.6. Determination of chlorophyll-a (µg/ml)

The replicates were in the test tubes containing 10 ml of liquid medium. The growth of strains was measured after a definite period i.e., 10^{th} day, 20^{th} day and 30^{th} day respectfully, of inoculation by an increase in

chlorophyll-a (µg/ml) through spectro-photometer (Sico model spec- 100). (De Marser and Hawmard, 1988). It was analysed against transmission a blank (96% methanol alone) as 100% transmission at 665 nm and readings were calculated with the following equations;

Total chlorophyll-a (µg/ml) = E 665 x 13.9

where, E is the extinction at 665 nm. All experiments were performed three replicates

2.7. Estimation of (a) Nitrogen fixation (Acetylene Reduction Assayand (b)Nitrogenase activity

Nitrogenase activity was analysed in the cultures grown in nitrogen-deficient medium .at the exponential stage of growtg. The activity was measured in terms of Acetylene Reduction Assay (ARA) (Kaushik and Venkataraman-1983)using Gas Chromatograph (Amil-Nucon model-5700) with para pack N and T columns (Stewart et al., 1967). Acetylene equivalent to 10% of the total air space was injected into a glass vial of 15 ml capacity. The vials were stopped with sub seals and incubated for 120 minutes at 28 ± 2^0C under 4000 – 5000 lux light intensity. The reaction was stopped by injecting 0.1 ml of 50 % TCA (Trichloro acetic acid) and the gas phase was analyzed for ethylene and the activity was expressed as n mole C_2H_4/ µg chl / h. Experiments were performed in three replicates.

3. Results

3.1. Habits and Habitat

It is a free-floating alga and forms big radiated patches on a surface of water or submerged into water and this alga is not recorded from India up to 1959. (Desikachary, 1959; Tiwari, 1972a, 1975, 1979). But this agla is collected from the surface of submerged watery paddy fields near Allahabad (U.P.) and some districts of West Bengal.

Table 1. Different day's on Growth (Chl.a µg/ml) of *Scytonema chiastum*.

	10^{th} Day	20^{th} Day	30^{th} Day
Cont.	0.202	0.538	0.556
(---) Growth absent.			

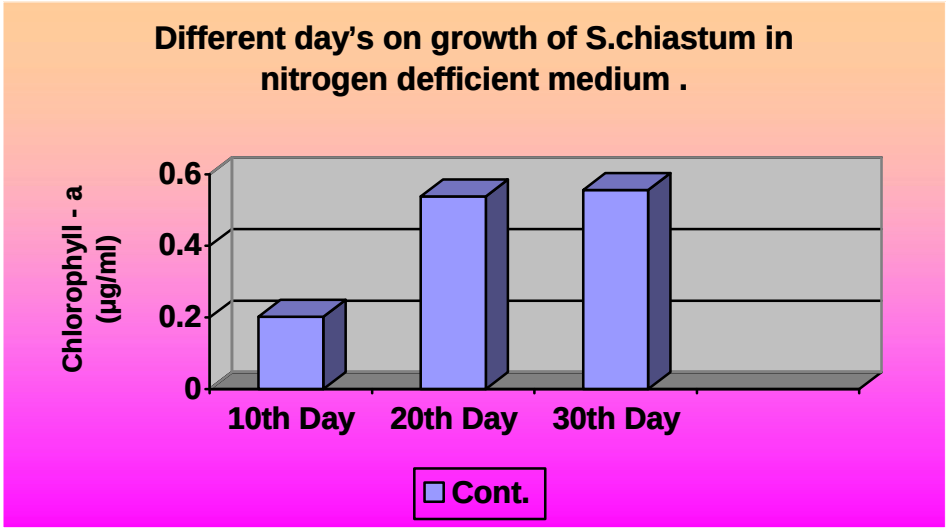

Figure-1. Different day's on Growth (Chl.a µg/ml) of *Scytonema Chiastum.*

Table 2. Different day's Nitrogenase activity (n mole C_2H_4/µg chl. a/h) of *Scytonema chiastum.*

	10^{th} Day	20^{th} Day	30^{th} Day
Cont.	1.90	1.42	0.58
(---) nitrogenase activity absent.			

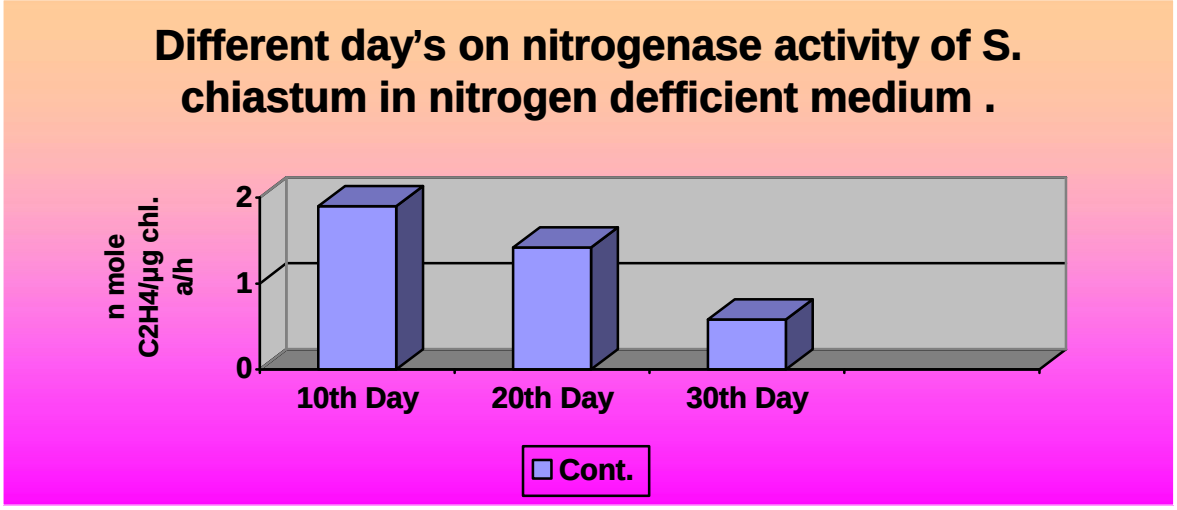

Figure- 2. Different day's Nitrogenase activity (n mole C_2H_4/µg chl. a/h) of *Scytonema chiastum*.

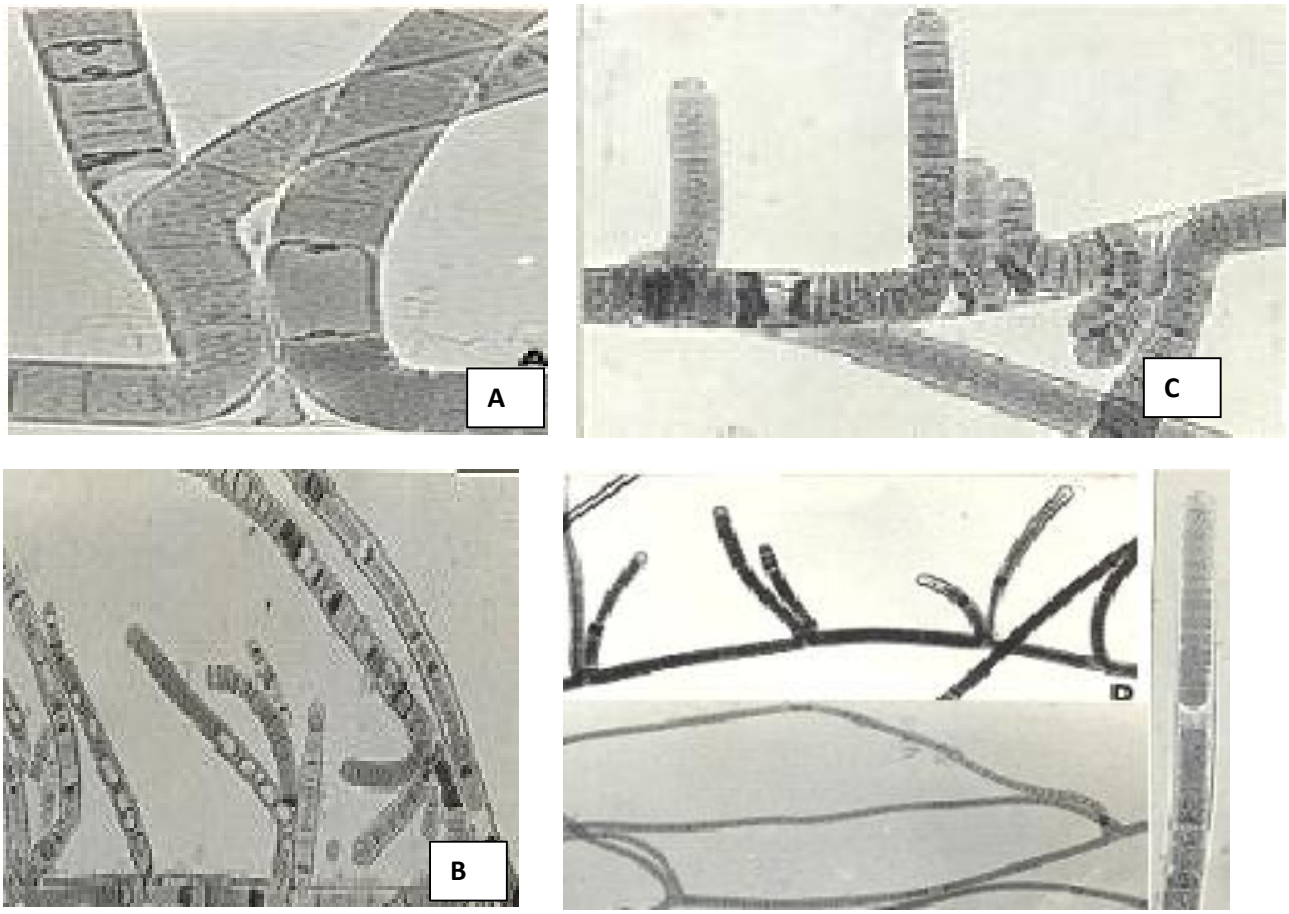

Plate-1: *Scytonema chiastum* shows different short & long branching types, heterocysts and separation for hormogones, poly branching are present and germination of perennating filaments.

3.2. Thallus and Growth (Pl-2, Figs. B & F)

The colour of young and mature thalli of the present alga appears dark blue-green by naked eyes and bright blue-green by microscope in both the conditions (liquid and solid). Old growth looks light orange colour under the microscope and dark brownish colour by the naked eyes. The present alga forms large cushion like thallus (6-20 mm in length) of radiating (prostrate and erect) filaments and normally erect filaments posses 1 to 3.5 mm height on 30th day.

The present alga grows in the form of large clusters on agar medium and its creeping filaments usually do not penetrate int the agar and grow only in upward direction. However, it grows as a free floating or on the surface of the liquid medium and branches radiate in all directions. The generation time of the alga varies in between 2 to 3 days under standard experimental conditions where it has been mentioned.

The present alga also shows 1.5 times more growth in nitrogenous medium than the nitrogen-deficient medium on 15th day. Its growth is maximum in two times nitrate nitrogen containing medium and the growth is hastically suppressed in medium containing two times ammonical nitrogen.

3.3. Filaments and Sheath

Filaments are thick, long and visible by naked eyes. The filaments' diameter varies from 14- 22 µm in different medium conditions. It is 14-18(-20) µm in nitrogen-deficient medium, 17-20 µm in normal nitrogenous medium and 18-22 µm in rich (2N) nitrogen medium. The breadth of the filaments is 7-11% more in the solid medium than liquid medium. They are also longer (upto 20mm) in the nitrogen deficient medium, which becomes short (upto 13mm) in the normal nitrogenous medium.

In the present alga, the sheath is usually thick, hyaline and lamellated in both young and mature stage. But it becomes yellowish brown colour in older growth and more yellowish from sheath appeared in the solid medium than liquid medium.

3.4. Vegetative cells and Cyanophycin granules (Pl-2, C & G)

The shape of vegetative cells is generally quadrate to discoid in nitrogen deficient medium. The present character is variable due to change in length of vegetative cells with the change of nitrogen composition in growth medium. The diameter and length of the filaments vary according to change in medium composition. In general, the length of the vegetative cells is 8-16 µm which is 12-16 µm longer in nitrogen deficient medium, 11-14 µm in normal nitrogenous medium and only 8-12 µm (shorter) in rich (2N) nitrogenous medium. However, diameter has reverse nature in such conditions. It is 11-15 µm (narrower) in nitrogen deficient medium, 13-17 µm in normal nitrogenous medium and 14-19 µm (broader) in rich (2N) nitrogen medium. Normally, mature cells are distinctly granulated, with indistinct cross walls and look dark blue-green in colour. Above characters are more prominent in nitrogenous medium than nitrogen deficient medium. However, certain older cells get vacuolisation in both conditions, whereas maximum in nitrogen-rich liquid medium.

Highly broader cyanophycin granules appear in most of the vegetative cells in mature thallus in nitrogenous and rich (2N) nitrogen medium due to this reason cross wall became incident.

3.5. Hormogonia and Heterocytes (Pl-2, Figs. A & B)

Hormogonia formation and their liberation is frequent in nitrogen deficient medium which possess mostly 7-12 celled length and shows active gliding movement on solid agar medium for some period. Their shape varies according to their mode of growth and period i.e., straight, spiral, crescent and irregular. They are straight or spiral in shape upto 60 celled stage. In hormogonia, intercalary heterocysts form when hormogonia become more than 70 celled long in normal condition on 3rd day. After that, they gradually adopt a typical crescent shape, become upto 120 celled long, and form irregular shapes from 120 to above the celled stage.

The heterocytes frequency is higher in nitrogen deficient liquid medium than solid medium and it is very poor in number in rich (2N) nitrogen medium. In the present alga, the shape of heterocytes is usually cylindrical or rectangular and occasionally they are quadratic in shape. They appear mostly single in number, but they are in series at certain places. The colour of heterocysts is blue-green tint or yellowish but in the case of a few long cylindrical heterocytes, the presence of constriction at the middle part confuse as division of heterocytes. In general, the heterocytes are present after 16 to 109 vegetative cells but their average ratio with vegetative cells is 1 :31 on 10th day in nitrogen deficient medium.

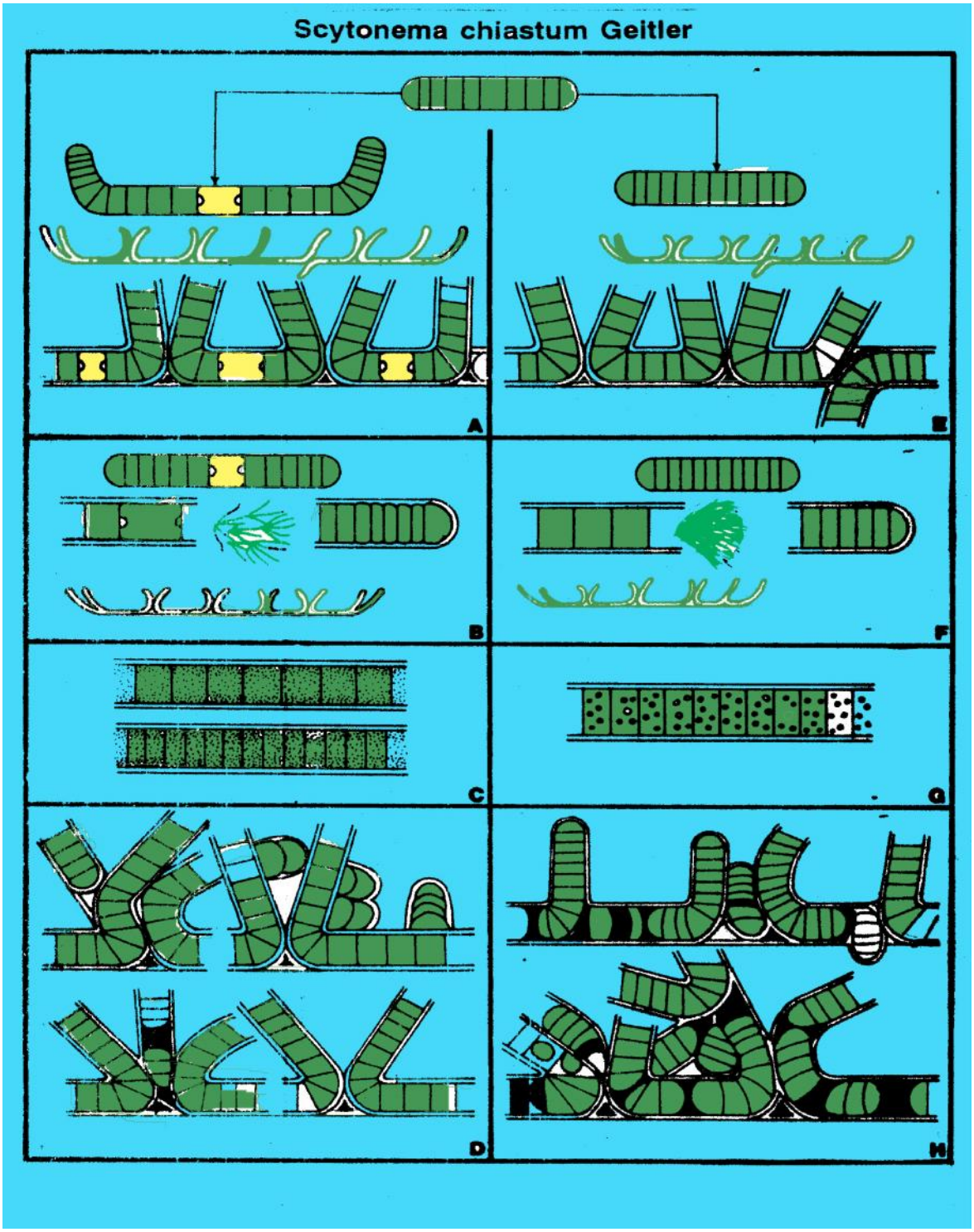

Plate-2: Figs. A-D, showing in the nitrogen-deficient medium; E-H, in the nitrogenous medium; A & E, in a liquid medium; B & F, in solid agar medium; C & G, perennating filaments; D & H, Germination of Perennating filaments.

3.6. False Branches (Pl-2, Figs. A,B,E & F)

The present alga produces 21% medium false branches in nitrogen deficient liquid medium than agar slant of the same medium and in liquid conditions, they are 14% and 27% more in nitrogenous and rich (2N) nitrogen medium respectively than their solid medium. However, when we compare the branches in solid medium only, they are 12% and 18% more in nitrogenous normal and rich (2N) nitrogen medium than nitrogen deficient medium respectively. Lateral geminate branches are in series but single branches are less in number than geminate branches. In single branches, terminal heterocytes appear occasionally only in nitrogen deficient medium. Over all 58% lateral geminate, 9% opposite geminate, 25% single and 8% single branches with terminal heterocytes are recorded in nitrogen deficient liquid medium and 63% lateral geminate, 30% single and 7% single branches with terminal heterocytes are present in solid medium. It is also observed that more longer branches were nitrogen deficient than nitrogenous medium.

3.7. Apical Part (Pl-2, Figs. A & B), Meristematic zone and Apical cell

The present alga has a distinct constricted 7-9 celled meristematic zone at apical parts of each filament in nitrogen deficient medium but indistinct in old growth and nitrogenous medium.

Apical cells of the filaments are broader than other vegetative cells and have a broadly oblong or hemispherical shape with yellowish colour in nitrogen-deficient medium, while they appear comparatively less broad and oblong in nitrogenous medium.

3.8. Perennating Bodies (Pl-2, Figs. C & G) and Germination (Pl-2, Figs. D & H)

The filaments which grow in nitrogen deficient medium have dark orange red coloured sheath around highly granulated cells as perennating central part which do not have dead cells. In nitrogenous medium, most of the filaments produce parennating bodies that have comparatively more broadness and granulation. In such filaments, cross wall constrictions are not clear.

The perennated filaments of the alga become torulose and blue-green in colour during germination. After a few days, they produce mostly lateral geminate branches in such conditions. Occasionally, these lateral branches develop more lateral geminate branches in series and several places. In addition, some spiral and irregular geminate and polybranches are observed.

3.9. Life cycle Pattern

The median intercalary heterocytous hormogones germinate and produce mostly lateral geminate and less number of single branches in nitrogen deficient medium, whereas in nitrogenous medium, number of hormogones is less and parent filaments produce maximum lateral geminate branches and a few single branches.

4. Discussion

Vasishta first reported it in 1965 from rice fields of Hoshiarpur, Punjab. Agarkar (1967) reported it from Tigra Dam, Gwaliar (M.P.) where it grew along with *Nostoc* colonies. Laloraya and Mitra (1973) and Kamat (1975) also reported from paddy field soils of Hyderabad (A.P.) and Amaravati, Vidarbh (Maharashtra), respectively.

Filaments are very long and broad; sheath thick with parallel lamellation. The range of breadth of the vegetative cells is 11-19 µm length is 6-20 mm. Branches are more lateral geminate, less opposite geminate and less single branches than geminate .Hormogonia straight, spiral, crescent and irregular in shape. Heterocytes are very long cylindrical or rectangular yellowish or blue-green and are often constricted by long cylindrical heterocytes. Meristematic zone is very long; 7-9 celled and highly constricted. Apical cells are yellowish and dome-shaped. Perennating bodies dark orange in colour sheathed. Densely granulated without dead cells. Germination spiral coiled lateral branches in series, Polybranches are also present.

The present alga has 6 mg/100 ml and 9 mg/100 ml dry weight in nitrogen-deficient and nitrogenous liquid medium, respectively. The growth rate was higher in the nitrogenous medium than the nitrogen-deficient medium. It has 0.202 µg/ml, 0.538 µg/ml and 0.556 µg/ml chlorophyll-a indifferent 10^{th} day, 20^{th} day and 30^{th} day, respectively. Chlorophyll-a was gradually increased in maximum days. Nitrogenase activity was 1.90, 1.42, and 0.58 (n mole C_2H_4/µg chl. a/h) in different 10^{th}, 20^{th} and 30^{th} days. Nitrogenase activity was more in a shorter period of time than a longer period, which was reversed compared to the growth rate.

5. Conclusion

Scytonema chiastum Geitler *(*Cyanobacteria) plays an important role in maintaining and building soil fertility, consequently increasing rice growth and yield as a natural bio fertilizer. The paddy field ecosystem provides a favourable environment for the growth of Cyanobacteria concerning their requirement for light, water, temperature and nutrient availability. It is one of the best filamentous multi-cellular heterocystous Cyanobacterial strains which details cultural and Eco-biological, biochemical different effect on morphological features in different conditions in different times and point out all characters in details, Chlorophyll-a, different day's on growth rate (Chl.a µg/ml) and Nitrogenase activity (n mole C_2H_4/µg chl. a/h) i.e., nitrogen fixing potential was characterized and all the results were very good as compare to other strains.

Conflict of Interest

The authors of this paper have no conflict of interest

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