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POLYPHASIC EVALUATION OF CYANOBACTERIAL COMMUNITY OF UNEXPLORED NORTH HILL REGIONS OF CHHATTISGARH, INDIA

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Abstract

Present investigation deals with the characterization, documentation and diversity analysis of the cyanobacterial community of the northern hills region of Chhattisgarh. Based on the morphological attributes (shape, size, number and position of vegetative cell, heterocyte and akinete), these regions were dominated by the isolated twenty cyanobacterial species viz. *Scytonema* sp. 1ss, *Anabaena* sp. 2ss, *Calothrix* sp. 3ss, *Aphanothece* sp. 4ss, *Aphanothece* sp. 5ss, *Scytonema* sp. 6ss, *Anabaena* sp. 7ss, *Tolypothrix* sp. 8ss, *Nostoc* sp. 9ss, *Aphanothece* sp. 10ss, *Anabaena* sp. 11ss, *Anabaena* sp. 12ss, *Calothrix* sp. 13ss, *Calothrix* sp. 14ss, *Anabaena* sp. 15+ss, *Nostoc* sp. 16ss, *Nostoc* sp. 17ss, *Scytonema* sp. 18ss, *Anabaena* sp. 19ss and *Anabaena* sp. 20ss. These cyanobacteria were also characterized on the basis of physiological activities (pigment analysis, protein, carbohydrate and enzymatic activities related to nitrogen metabolism) which suggested great diversity at the species level. Further, biochemical and molecular approaches have also been assessed for phylogenetic comparison and the result validated a high level of heterogeneity based on SDS-PAGE and 16S rRNA gene sequences respectively. The phylogenetic tree constructed on the basis of Neighbor Joining (NJ), Maximum Likelihood (ML), Maximum Parsimony (MP) showed same tree topologies with good bootstrap value at all nodes. It also showed a clear representation of monophyletic origin in Clusters I (*Aphanothece* sp. 4ss, *Aphanothece* sp. 5ss, *Aphanothece* sp.10ss) whereas cluster II, III and IV proved and confirmed polyphyletic origin of the order Nostocales. Cluster VI might be suggested that the strains found in this cluster consists of certain morphological features that might be used for the classification of the tested strains at the species level.

***Keywords*:** Cyanobacteria, Diversity, Nitrogenase activity, phylogenetic tree.

Introduction

Cyanobacteria, the prominent group of oxygenic gram negative prokaryotic photoautotrophs, bear proficient photosynthetic mechanism and their existence has been originated from the Precambrian Era. They have extraordinarily survival strategies which make their phenotypic plastic behaviour as well as ubiquitous distribution at all the realm of ecosystem and environmental extremities (Kothari *et al.*, 2013; Lau *et al.*, 2015; Coutinho *et al.*, 2016; Singh *et al.*, 2016a; Singh *et al.*, 2016b; Singh *et al.*, 2017a; Singh *et al*., 2017b; Basavaraja and Naik, 2018; Mogany *et al.*, 2018). Cyanobacteria are growing in free living condition but in an extreme case they also establish symbiotic association with other organisms such as algae, fungi, bryophytes, pteridophytes, gymnosperm and angiosperm (Anand *et al.*, 2019).

The filamentous cyanobacteria have different types of cellular organization i.e. vegetative cell, akinetes and heterocytes. The former cell type performs photosynthetic metabolic activities whereas akinetes are the resting spores and the third one the heterocyte is responsible for the nitrogen fixation and assimilation. During nitrogen deficient condition, vegetative cells are transformed into the heterocytes where special enzyme complex i.e. Nitrogenase is synthesized for reducing the molecular nitrogen to ammonia and in this way, they contribute in fulfilling the global demand of nitrogen budget. Cyanobacteria also utilize nitrate, nitrite and ammonia as combined nitrogen sources for their metabolic activity to construct amino acids, proteins, carbohydrate, lipids and specific pigment profile. Hence, they also help in carbon sequestration (Kothari *et al.*, 2013; Lau *et al.*, 2015; Prabha *et al.*, 2016; Mogany *et al.*, 2018). Their existence might have resulted time dependent alterations into some complex structural organization. Further on the basis of their structural organization, many views have been proposed (Brito *et al.*, 2016; Pfeffer *et al.*, 2016; Mogany *et al.*, 2018). Traditionally, the cyanobacteria were characterized on the basis of morphological appearances such as shape and size of vegetative cell, heterocyte, akinete; presence of sheath, constriction in cross wall, branching patternetc. Earlier, the entire cyanobacterial community was divided into five orders. Thenafter, cyanobacterial taxonomy has been focused on the molecular approaches and they are classified into 8 orders and researchers nowadays try to unravel the basic of genetic evolution of cyanobacterial community (Komarek *et al*., 2014; Singh *et al.*, 2014b; Anand *et al.*, 2019). In recent times, biochemical markers, DNA fingerprinting, 16S rRNA and other gene sequences along with phylogenetic assessment tools are widely used to study the uniqueness of cyanobacteria and their historical trends

Chhattisgarh is a torrid zone of India which is situated at 17º 46’ N to 24º 5’ N latitude and 80º 15’ E to 84º 20’E longitude where temperature varies among the climatic ambience. It is divided into three agro climatic zones Chhattisgarh plains, Bastar Plateau and Northern Hills. These regions have an average to minimum rainfall i.e. 1200-1600 mm which is the idol ecological environment for the survival of cyanobacteria (Minj *et al.*, 2017; Bhaurya *et al.*, 2018; Kunui and Singh, 2020).

The current research work has the aim to characterize the cyanobacteria isolated from north hills of Chhattisgarh and also to assess the phylogenetic comparison among them by using polyphasic approach (morphological, physiological, biochemical and molecular characterization).

Materials and Methods

Sampling and culturing of cyanobacteria

During the study, the different zones comprising of 15 sampling areas of north hills of Chhattisgarh were covered. Major area covered was belonging to district Jashpur Nagar, Sarguja and Surajpur. The cyanobacterial strains were brought in our laboratory and the collected samples were maintainedat specific culture conditions (7.2 pH, 28± 2ºC andlight intensity of 50 - 55 µE $m^{-2}s^{-1}$ with a 14:10 hour light -dark cyclephotoperiod) in the nutrient supplementation of BG 11º medium. The shaking of the cultures was done twice within 12 h (Rippka *et al.*, 1979; Singh *et al.*, 2013). Strains were subcultured many times to maintain uniform and axenic culture.

Physico-chemical properties of soil

The physicochemical properties of soil such as pH, temperature, conductivity, total dissolved solids, salinity and dissolved oxygen were analyzed with the help of Water and Soil Analysis Kit Model 161 (EI product, Panchkula, Haryana, India).

Phenotypic assessment and cluster analysis

All together the collected 20 cyanobacterial strains were phenotypically identified on the basis of preliminary examination i.e. shape and size of the vegetative cells; shape, dimension and position of the heterocytes and akinetes under Leica DM 2000 (100X resolution) microscope with the provision of digital camera. An identification of the cyanobacteria was done using the keys of Desikachary (Desikachary, 1959).

On the basis of morphological attributes a principal component analysis was performed. The main objective of this PCA was to determine the hierarchy and the elucidean distance among the species. Principal component (PCA) and hierarchial analysis were carried out using Biodiversity pro software (Version 2). The software Sigma plot 11 was used to generate the graphical representation of the values generated by principal component analysis. The data was placed in software SPSS version 12.

Direct Absorbance

An exponentially grown axenic cyanobacterial cultures were re-inoculated in a sterilized Erlenmeyer flask and the growth of the 20 cyanobacterial species were analyzed on the basis of optical density measured at 663 nm ($O.D_{663}$). The absorbance was taken at every alternate days with help of UV-Vis Spectrophotometer (Elico SL 210).

Pigment analysis

After attaining the exponential growth phase,5 ml each cyanobacterial cultures were harvested and thoroughly washed for determination of chlorophyll analysis. Thenafter cultures were centrifuged and the pellet obtained was mixed with 95 % methanol. The content was again re-centrifuged. The pellet was kept for phycobillin protein estimation. The absorbance of the supernatant was recorded at 665 and 420 for chlorophyll a and carotenoids estimation respectively (MacKinney, 1941). On the other hand, preserved pellet was suspended with 5 ml of potassium phosphate buffer (pH 6.8) for overnight. Then pellet was centrifuged at 8,000 X g for 5 min and the obtained pellet was re-extracted 4 times with 5 ml of potassium phosphate buffer (pH 6.8). The total phycobillin content was calculated as per the protocol of Bennet and Bogorad (Bennet and Bogorad, 1973).

Estimation of protein and carbohydrate

Total cellular protein content in the cyanobacteria was estimated using the protocol of Lowry *et al.* (1951). An exponentially grown culture was treated with1N NaOH (1.0 ml) and the samples were incubated at water bath for 10 minutes. Then samples were cooled at room temperature and treated with 2.5 ml of freshly prepared solution of sodium carbonate, copper sulphate with sodium potassium tartarate and the solution was mixed thoroughly. 0.5 ml Folin reagent was added and left for 15 minutes for the development of blue color in an entire solution and the absorbance was measured against blank at 650 nm. The protein content was estimated using a standard protein assay curve of lysozyme and expressed in terms of µg ml^{-1}.

The carbohydrates content was analyzed by the phenol–sulphuric acid method (Dubois *et al.,* 1956). Exponentially grown 1 ml culture was treated with 1 ml phenol (5% w/v) and 5ml of concentrated H_2SO_4 and allowed for 10 minutes at 30º C in water bath. Finally, a yellow color solution was developed and the absorbance was taken at 485 nm.

Enzymatic assay

Nitrogenase (EC 1.18.6.1) activity was estimated as per the protocol of acetylene reduction assay.5 ml of exponentially grown cyanobacterial cultures were transferred in the 10 ml sterilized stoppered vacutainer tubes. The tubes carrying cyanobacterial cells where 10% acetylene gas was injected and incubated at 29°C for6 hours. The reaction was terminated by adding 10% of Trichloroacetic acid (TCA). 100 µl ethylene gas produced was withdrawn from the tubes and injected directly into the gas chromatograph (GC, Agilent 6890N and 7890A). The nitrogenase activity was expressed as nmole C_2H_4 mg^{-1} protein h^{-1} (Kunui and Singh, 2020).

To determine nitrate reductase (EC 1.7.7.2) activity, an exponentially grown cyanobacterial cultures were centrifuged and the pellets obtained were treated with 20 µl toluene. A vigorous shake was done at 4°C. The cell suspensions were mixed with 1 ml reaction mixture containing 100 µmol $NaHCO_3$-Na_2CO_3 buffer (pH 10.5), KNO_3, methyl viologen and $Na_2S_2O_4$ in 0.3M $NaHCO_3$ and incubated for 5 minutes at 30°C (Herrero *et al.*, 1981). To estimate NO_2^-, protocol of Snell and Snell (1949) was followed. Diazotization was performed by mixing 1 ml of culture with 1 ml sulphanilamide and1 ml of NEDH reagent. A pink colour was developed and the absorbance was recorded at 540 nm. Nitrate reductase activity was calculated from the standard curve of sodium nitrite and was expressed in terms of µmol mg^{-1} protein.

For determining the glutamine synthetase (EC 6.3.1.2) activity, one ml pelleted cyanobacterial cultures were permeabilized with toluene at 4°C for 10 min. The aliquot was again centrifuged to remove the topmost toluene layer. 3 ml of reaction mixture containing 40 mM Immidazole HCl (pH 7.0), 30 mM L-glutamine, 3 mM $MnCl_2$, 0.4 mM ADP, 20 mM sodium arsenate, 60 mM hydroxylamine, was added and the solution was incubated in water bath at 30°C for 30 min. The reaction was stopped by adding 1 ml mixture of $FeCl_3.6H_2O$ prepared in 0.2 N HCl, 24% TCA and 6 N HCl in 1:1:1 ratio. Glutamine synthetase activity was expressed in

terms of nmol γ-glutamylhydroxamate μg^{-1} protein min^{-1} as quantified by the reference to standard curve obtained with γ-glutamylhydroxamate in the assay medium measured at 540 nm (Shapiro and Stadtman, 1970).

SDS-PAGE of whole cell protein

Whole cell protein was extracted as per the modified protocol of Lyra *et al.*(1997). Exponentially grown cultures (250 ml) were centrifuged at 7600 rpm for 10 minutes at 24ºC. The obtained pellets were sonicated in phosphate saline buffer (PBS) and centrifugation was done at 15,000 rpm for 45 minutes at 4ºC. The supernatant was re-suspended in denaturation sample loading buffer containing Tris HCl, SDS, glycerol, β-mercaptoethanol, Bromophenol blue and ml double distilled water) in the ratio of 1:1. The samples were heated at 100ºC for 5 minutes before loading into the wells of the gel (prepared by 12% acrylamide). Each 80 µl of samples containing100 µg ml^{-1} protein were loaded and quantification of the protein was done as per Lowry *et al.*, 1951. After electrophoresis, gel was fixed in fixative for 10-15 minutes and washed with deionized water. Coomassie Brilliant blue (R-250) staining solution was used to stain the gel and then gel was transferred indestaining solutions for 8-12 hours. Bands were visualized under gel documentation system and were compared with the molecular marker (14.3-97.4 kDa) (Bangalore Genei) run on both the sides of the gel along with the sample. The analysis was repeated thrice so as to verify the reproducibility of the patterns.

Extraction of genomic DNA

Cyanobacterial pellets were taken after centrifugation at 13,000 rpm for 10 min (Golden *et al.* 1988). Thereafter, pellets were mixed with extraction buffer (Tris HCl, pH 8.0, 20 mM EDTA, 1.4 M NaCl and 2% CTAB) and lysozyme and the samples were incubated for 60 min at 37°C with a gentle mixing. Then 3 µl Proteinase K was added and the samples were again incubated at 65°C for 1 hour. The obtained mixture was extracted twice by saturated phenol: chloroform (25:24) and once by chloroform: isoamyl alcohol (24:1) through centrifugation at 12,000 rpm for 10 min. Samples were kept overnight at -20°C for the precipitation of the DNA. Again, 1 volume of chilled isopropanol and 0.25 volume of ammonium acetate (3 M) were added to the above samples. Precipitated DNA was centrifuged and the supernatant was discarded. The precipitated DNA was washed with 70% ethanol and again centrifuged at 10,000 rpm for 2 min. The obtained DNA pellets were vacuum dried and finally dissolved in TE buffer having pH 8.0 (Tris HCl 50 mM, EDTA 10 mM).

16S rRNA analysis

The eluted DNA was used for PCR amplification of nearly 1600 bp fragments of 16S rRNA gene sequence by using Oligonucleotide primers fD1 (5'-AGAGTTTGATCCTGGCTCAG-3') and rD1 (5'-AAGGAGGTGATCCAGCC-3' (Weisburg *et al.*, 1991). An amplificatin was done in BioRad, DNA Engine, Peltier Thermal cycler according to the following program: initial denaturation at 95 °C for 3 minutes, 30 cycles of 30 seconds denaturation at 94 °C, 40 seconds annealing at 55 °C, and 1.5 minute extension at 72 °C. A final extension at 72 °C for 3 minutes was done. The amplified products were visualized on Bio-Rad Gel Documentation system after running in 1.2 % agarose gel.

The obtained DNA sequences were cross checked through the http:// blast.ncbi.nlm.nih.gov/Blast.cgi program for assessing the genetic relatednessamong the cyanobacterial strains with each other and finally submitted to the NCBI database using the online program BankIt with proper annotations in the standard format (The List of cyanobacterial strains used in the present investigation along with their accession numbers have been given in Table 3).

Phylogenetic study using the 16S rRNA Gene Sequences

Nucleotide sequences were compared with sequence information available in the NCBI database. BLAST and multiple-sequence alignment was done of all partial and complete sequences using the MEGA 6.0 (Larkin *et al.*, 2007). The Phylogenetic tree was constructed using the neighbor-joining algorithm provided in MEGA 6.0 (Tamura *et al.,* 2011).

Results and Discussion

The analysis proved that the pH of the north hills of Chhattisgarh ranged from 7.2 to 8.7. Maximum pH was recorded from the soil of paddy field of Ambikapur (8.7 ± 0.4) followed by Gholeng and Premnagar (8.6 ± 0.5). The minimum pH was recorded from the paddy field of Surajpur (7.2 ± 0.153) (Table 1). The soils of Ranidih showed maximum temperature (34.8 ± 1.0 ºC) followed by the soil of Surajpur(32.6 ± 0.569) whereas lowest was observed in the soil of Mainpat (27.43 ± 0.60 ºC). Maximum salinity was recorded from the soil of Mainpath (122.03 ± 2.57 g L^{-1}) and the lowest from Jashpur Nagar (39.47 ± 1.29 g L^{-1}). Maximum dissolved oxygen (DO) was observed in the paddy field of Gholeng (87.8±1.1 ppm) while the soil of Surajpur showed lowest DO (21.2 ± 1.021 ppm). Maximum conductivity was observed in the field of Ambikapur (152.1 ± 2.5 µ Seimen cm^{-1}) followed by Ramanujnagar (129.1 ± 1.3 µ Seimen cm^{-1}), whereas lowest conductivity was observed in the soil of Premnagar (35.2 ± 0.493). The conductivity of the rest of the soil samples collected from the different sites was ranged between 36.9 ±1.0 to 113.90 ±5.54 µ Seimen cm^{-1} (Table 1). Maximum total dissolved solid was ranged from 204.1 ± 5.9 mg L^{-1}to101.7 ± 2.0 mg L^{-1}. Apart from this, rest of the soil samples collected from different sites showed TDS range between 102.7 ± 2.722 mg L^{-1} to 146.6 ± 1.9 mg L^{-1} (Table 1).

The morphological characters such as shape and size of vegetative cells, heterocyte and akinetes along with the heterocyte frequency, Branching pattern of the cyanobacterial strains were assessed microscopically and compared with the cyanobacterial characteristics given in the keys provided by Desikachary(1959) and Rippka *et al.* (1979). (Table 2). Three unicellular species of cyanobacteria i.e. *Aphanothece* sp. 4ss, *Aphanothece* sp. 5ss and *Aphanothece*sp.10ss were reported which belongs to group Chroococcales. Maximumspecies isolated from north hills belong to order Nostocales (Section IV) that were basically heterocystous and filamentous in nature. On the basis of microphotographs of the twenty cyanobacterial strains and their important features are clearly shown in Fig. 1. Most of the cyanobacterial strains showed a linear alignment apart from *Anabaena* sp. 15ss which showed an arched orientation (Fig. 1A, 1G, 1K, 1L, 1O, 1S, 1T). *Calothrix* sp. 3ss, *Calothrix* sp. 13ss and *Calothrix* sp. 14ss always have a gradual polarity which was clearly picturized (Fig. 1C, 1M, 1N). A comprehensive marking of false branching clearly

depicts the presence of *Scytonema* sp.1ss, *Scytonema* sp. 6ss, *Tolypothrix* sp. 8ss and *Scytonema sp.* 18ss (Fig.1A, 1F, 1H, 1R).The vegetative cells were ranged from subquadrate, barrel, spherical, cylindrical, hemispherical, ovalto oblong. The dimensional orientation of vegetative cell were found to be ranged from 2.0 ±0.11 µm (L) - 3.0 ± 0.10 µm (W) (*Anabaena sp.*15ss) to 9.0 ±0.1 µm (L) – 4.5 ± 0.09 µm (W) (*Anabaena* sp. 7ss).

Simultaneously, a variation was observed in the shape of the heterocytes which ranges from subquadrate, barrel, spherical, cylindrical, hemispherical to oblong. The heterocyte were slightly larger in dimension to that of the vegetative cells (Table 2). The position of heterocyte was also varied and it was intercalary in *Scytonema sp.* 1ss, *Anabaena sp.*2ss, *Scytonema sp.* 6ss, *Anabaena sp.* 7ss, *Tolypothrix sp.*8ss, *Nostoc sp.* 9ss, *Aphanothece sp.* 10ss, *Anabaena sp.*11ss, *Nostoc sp.* 16ss, *Nostoc sp.* 17ss, *Scytonema sp.* 18ss, *Anabaena sp.*19ss, *Anabaena sp.* 20ss) and terminal in *Calothrix sp.*3ss, *Calothrix sp.*13ss, *Calothrix sp.*14ss). The largest heterocyte was observed in *Anabaena* sp. 7ss with 9.0±0.10 µm (L) - 5.0±0.10 µm (W).The highest heterocyte frequency was recorded in *Anabaena sp.* 11ss (21.05 %) whereas the lowest was recorded in *Calothrix sp.*14ss (1.75%). Akinetes was only reported in *Anabaena* sp.7ss; *Anabaena* sp.12ss and *Anabaena* sp.20ss and the largest akinetes were found in *Anabaena* sp.7ss followed by *Anabaena* sp.12ss and *Anabaena* sp.20ss (Table 2).

On the basis of the morphological analysis, a dendrogram was plotted on the basis of average linkage between groups that clearly depicted the origin of similar morphological orientation. Dendogramde picted the formation of two clusters (Cluster I and Cluster II). Cluster I comprised of solely *Calothrix* sp. 13ss and *Anabaena* sp. 20ss. Whereas, Cluster II was further bifurcated in IIA and IIB subclusters. Subcluster IIA further divided into IIA1 and IIA2 whereas cluster IIA2 again formed IIA2a and IIA2b sub-subclusters (Fig. 2). Different species of *Aphanothece* formed separate sub-subclusters.

A wide range of variation in terms of shape and size of the vegetative cell and heterocyte of cyanobacteria also proved that the distribution and richness in different area of north hills may be correlated with differences in physicochemical properties of soil. Some of the reports also supported our findings (Mishra *et al.*, 2013; Singh *et al.*, 2014b). In this context, the plastic behaviour of cyanobacteria may be the probable reason for morphological diversity so that these strains could easily adapt with the varied soil behaviour (Singh *et al.*, 2013, Silva *et al.*, 2014; Singh *et al.*, 2014b; Koch *et al.*, 2017; Singh *et al.*, 2016a; Singh *et al.*, 2016b).

The efficacy of the phenotypic and physiological characters of the cyanobacterial strains were strongly proved via principal component analysis. The component analysis showed two variant eigen values i.e., 13.981 (PC1) and 3.222 (PC2) using the software biodiversity pro software (Fig.3). *Aphanothece* sp. 4ss with *Aphanothece* sp. 5ss whereas *Anabaena* sp. 15ss with *Scytonema* sp. 18ss was found grouped together in the constructed scatter plot. Similar proximity was also observed among *Anabaena* sp. 7ss with *Calothrix* sp. 3ss and *Anabaena* 12ss with *Anabaena* sp. 19ss. *Anabaena* sp. 1ss occurred at one side, while rest of cyanobacterial strains was placed distinctly appeared in scatter plot.

The direct absorbance of different cyanobacteria, isolated from North hills region of Chhattisgarh, was taken at different time interval (0, 2, 4, 6, 8, 10 and 12 days). The result clearly depicted that the rapid growth of different strains was initiated from 2nd day and achieved exponential between 4th to 6thday of incubation (Fig 4a). In order to assess the physiological attributes, pigment content (chlorophyll a, carotenoid and phycocyanin) along with protein and carbohydrates were also investigated. The chlorophyll a content was maximally observed in case of *Aphanothece*sp.10ss (4.84 ± 0.02 µg ml^{-1}) followed by *Scytonema* sp. 6ss (3.00 ± 0.12 µg ml^{-1}) while minimum amount of chlorophyll a was observed in *Anabaena* sp. 2ss (0.650 ± 0.09 µg ml^{-1}) (Fig. 4b). Similarly, carotenoid content was recorded at the range of 19.35 ± 0.63 - 48.31 ± 0.52 µg ml^{-1} in the different cyanobacteria. The maximum carotenoid was observed in *Anabaena* sp. 11ss (48.31 ± 0.52 µg ml^{-1}) followed by *Tolypothrix* sp. 8ss (45.02 ± 1.04 µg ml^{-1}) whereas minimum was reported in *Nostoc* sp. 16ss (19.35 ± 0.63 µg ml^{-1}) (Fig. 4c).The total phycobilin protein content was recorded at a range of 0.378 ± 0.019 - 2.614 ± 0.09 µg ml^{-1}. *Anabaena* sp. 2ss (2.614 ± 0.09 µg ml^{-1}) showed maximum phycobilin protein followed by *Scytonema* sp.1ss (1.72 ± 0.022 µg ml^{-1}) whereas *Tolypothrix* sp. 8ss showed minimum content (0.378 ± 0.019 µg ml^{-1}) (Fig. 4d).

The protein content was recorded in the range of 34.7 ± 0.735 to 79.92 ± 0.49 µg ml^{-1}. The maximum protein content was recorded in *Anabaena* sp. 19ss (79.92 ± 0.49 µg ml^{-1}) whereas was found to be 34.7 ± 0.73 µg ml^{-1} in *Scytonema* sp.1ss (Fig. 5a).The Carbohydrate content was recorded between 18.715 ± 0.62 to 6.557 ± 0.327 µg ml^{-1}, maximum was in *Anabaena* sp. 15ss and minimum was in *Calothrix* sp. 3ss (Fig. 5b).

A remarkable variation was observed in biomolecules of different cyanobacteria that suggested the great heterogeneity among the cyanobacterial species. Probably, different levels of synthesis of biomolecules may be needed to different cyanobacteria for performing optimum survival and growth at their ecological niches as a primary producer (Anand *et al.*, 2019). Nitrogen fixing ability was observed among the different cyanobacterial strains and they showed great variation (Fig. 6a). Result also suggested that the maximum nitrogenase activity was found in *Anabaena* sp.7ss (211.85±10.5 nmole C_2H_4 mg^{-1} protein h^{-1}) followed by *Calothrix* sp. 13ss (211.28±10.2 nmole C_2H_4 mg^{-1} protein h^{-1}), *Scytonema* sp. 6ss (207.55±10.0 nmole C_2H_4 mg^{-1} protein h^{-1})and the minimum was reported in *Aphanothece* sp. 4ss (16.73±0.83 nmole C_2H_4 mg^{-1} protein h^{-1}). Heterocyte is the soul cellular compartment where unavailable form of nitrogen is converted into the utilizable form (Singh *et al.*, 2014b; Singh *et al.*, 2016a; Singh *et al.*, 2017b; Chakraborty *et al.*, 2017; Horváth *et al.*, 2019). The heterocytous cyanobacteria have a proficiency in nitrogen fixation criteria but some unicellular cyanobacteria such as *Aphanothece* sp. 4ss, *Aphanothece* sp. 5ss and *Aphanothece* sp. 10ss also showed efficient nitrogen fixing ability (Fig. 6a).

NR activity (mmole NO_2^- mg^{-1} protein) was observed in cells grown in nitrate supplemented medium. The level of activity was found to be highest in *Scytonema* sp. 1ss (85.0 ±1.25 mmol mg^{-1}protein) followed by *Nostoc* sp. 17ss (79.8

± 3.9 mmol mg^{-1} protein), *Anabaena* sp.19ss (79.8 ± 3.9 mmol mg^{-1} protein). Minimum NR activity was reported in *Tolypothrix* sp. 8ss (21.7 ±1.08 mmol mg^{-1} protein) followed by *Aphanothece* sp. 4ss (21.0 ±1.08) (Fig. 6b).

The level of glutamine synthetase activity among different cyanobacterial species was observed in terms of nmol γ-glutamyl hydroxamate μg^{-1} protein min^{-1}. *Scytonema* sp. 1ss showed highest level of GS activity (99.77 ± 4.9 nmol γ-glutamyl hydroxamate μg^{-1} protein min^{-1}) followed by*Nostoc* sp.9ss (83.10 ± 4.1 nmol γ-glutamyl hydroxamate μg^{-1} protein min^{-1}), *Scytonema* sp.18ss (83.10 ± 4.0 nmol γ-glutamyl hydroxamate μg^{-1} protein min^{-1}). Minimum GS activity was observed in *Aphanothece* sp. 5ss (13.43 ± 0.67) followed by *Aphanothece* sp. 4ss (14.6 ± 0.73 nanomol γ-glutamyl hydroxamate μg^{-1} protein min^{-1} (Fig. 6c).

Protein profiling of all the twenty cyanobacterial strains, isolated from North hills of Chhattisgarh, was compared by using one dimensional polyacrylamide gel electrophoresis (SDS-PAGE) and the different protein banding pattern was visualized which proved that each and every cyanobacterial strains showed specific protein profiling. Results also showed each species has different number and position of protein bands. The bands of high molecular weight were very dissimilar while considerable similarity was found among the bands of lower molecular weight protein among the cyanobacterial species (Fig.7). It is clearly observed that the common protein band of 21.0 kDawas common in all cyanobacterial strains. Band of 67.4 kDa was only found in *Calothrix* sp. 3ss while other band of 48.0 kDa was appeared in *Calothrix* sp. 3ss. On the basis of bands similarity (66.2 kDa and 48.8kDa), *Aphanothece* sp. 4ss and *Aphanothece* sp. 5ss were closely related to each other. The band of 66.2 kDa and 45.0 kDa were found in almost all cyanobacterial strains. On the basis of protein profiling, the dendogram was constructed by using Jaccard's similarity coefficient 0.11-1.0 (Fig 8). The dendogram clearly formed two major clusters I and II. Whereas cluster II comprised of the two cyanobacterial strains *viz. Aphanothece* sp. 4ss and *Scytonema* sp. 8ss. Cluster I further separated two sub clusters IA1 and IA2. Whereas sub cluster II A2 comprised of *Anabaena* sp. 9ss and sub cluster IA1 was further separated into two sub cluster IA1a and IA2b whereas IA2b has single cyanobacterial species. Dendogram was clearly showed that the sub cluster IA1a included 14 cyanobacterial species i.e. *Anabaena* sp. 1ss, *Scytonema* sp. 6ss, *Calothrix* sp. 14ss, *Aphanothece* sp. 5ss, *Anabaena* sp. 15ss, *Anabaena* sp. 2ss, *Anabaena* sp. 20ss, *Calothrix* sp. 13ss, *Calothrix* sp. 3ss, *Anabaena* sp. 12ss and *Tolypothrix* sp. 18ss.

To investigate the molecular diversity and relatedness among the cyanobacterial strains isolated from north hills region of Chhattisgarh, 16S rRNA gene was amplified using universal primers. The isolated twenty cyanobacterial strains were successfully submitted to National Centre for biotechnological Information (NCBI) database and after submission the accession number have been received (Table 2).

In the present study, the constructed phylogenetic tree based on 16S rRNA gene sequences using the algorithm where phylogenetic tree showed robust topology (Fig. 9).Results revealed that phylogenetic tree consisted of many small clusters but overall five clusters were consistently formed. Cluster I represented separate and distinct cluster and comprised the unicellular group of *Aphanothece* sp. 4ss, *Aphanothece* sp. 5ss, *Aphanothece* sp. 10ss which confirmed the monophyletic origin and shared the sequences similarity together (93%, 92%). The grouping of these results conclave with the earlier findings (Fewer *et al.*, 2002; Litvaitis, 2002). The cluster II and III (Family- Scytonemataceace) consist of *Scytonema* sp. 6ss, S*cytonema* sp. 18ss, *Scytonema* sp. 1ss and S*cytonema* sp. 8ss.

Similarly cluster IV (Family- Nostacaceae) represented the strains *Anabaena* sp. and *Nostoc* sp.; *Nostoc* sp. 17ss followed by *Anabaena* sp. 19ss, *Anabaena* sp. 15ss, *Anabaena* sp. 12ss, Anabaena sp. 12ss, *Anabaena* sp. 11ss, *Anabaena* sp. 20ss, *Anabaena* sp. 7ss, *Anabaena* sp. 2ss *and Nostoc* sp. 16ss. The Cluster V (Family- Rivulariaceae) comprised of the strains *Calothrix* sp. 14ss, *Calothrix* sp. 13ss and *Calothrix* sp. 3ss and only one strain of *Nostoc* sp. 9ss in cluster VI which shared 98.5% similarities with *Aliinostoc morphoplasticum*.

In contrast to morphological, physiological, and biochemical analysis, 16S rRNA gene sequences divided the twenty cyanobacterial strains into six clusters and it was strongly supported by bootstrap value in the constructed phylogenetic tree *viz.* NJ/ML/MP (Fig 9). It also confirmed the monophyletic origin of Clusters I whereas cluster II, III and IV proved and confirmed the polyphyletic origin of the order Nostocales.

Our report also depicted the member of genus *Scytonema* (*Scytonema* sp. 6ss, *Scytonema* sp. 18ss, *Scytonema* sp. 1ss and *Scytonema* sp. 8ss) showed the highest sequence similarity with the earlier reports of new strains of *Scytonem*a sp. from Chhattisgarh *viz. Scytonema singhii* and *Scytonema bilaspurensis.* In the present study, cluster II included four strains of *Scytonema* sp. even though three representatives viz., *Scytonema* sp. 1ss, *Scytonema*sp.18ss *and Scytonema* sp. 6ss also showed divergence even within them and were separated to fourth one. This divergence of three *Scytonema* strains was found to be incoherent with the other studies and it might be due to the possible effect of environmental conditions (Komarek *et al.*, 2013; Hentschke and Komarek, 2013; Singh *et al.*, 2016a; Singh *et al.*, 2017b; Minj *et al.*, 2017).

Cluster III represented the organization of the strain *Nostoc* sp. 16ss with the close affinities with *Anabaena* and *Nostoc* and in this way phylogenetic clustering of *Nostoc*sp.16ss in cluster III proved that the *Nostoc* species exhibited the maximum genetic heterogeneity. On the other hand *Anabaena* strains showed conflicting affiliations in both intra-generic and inter-generic perspectives (Andreja *et al.* 2015; Singh *et al.*, 2016b).

The Cluster IV include *Calothrix* sp. 3ss, *Calothrix* sp. 13ss and *Calothrix* sp. 14ss which showed the high sequence similarities with high bootstrap value between their relatives obtained from NCBI database.

Nostoc sp. 9ss is a heterocytous filamentous genera showed a high sequence similarity with that of *Aliinostoc morphoplasticum*. Our result was also supported by the cited literatures related to cyanobacterial diversity (Singh *et al.*, 2016a; Minj *et al.*, 2017).

We can conclude our findings as a first attempt made as exploration of the cyanobacterial diversity of Northern hill of

Chhattisgarh using polyphasic approach. The proficiency of cyanobacteria in terms of primary producer has been proven on the basis of pigment analysis (chlorophyll a, carotenoids and phycobillin protein content) along with the nitrogen metabolism related enzymymes (nitrogenase, nitrate reductase and glutamine synthetase). The adaptive capability was determined by the specific protein profiling and carbohydrate. Further, protein profiling based on SDS-PAGE has paved the way to understand the heirarchial orientation of the members of cyanobacterial species. The phylogenetic clustering of *Nostoc* sp.16ss cluster III showed that *Nostoc* species exhibited the maximum genetic heterogeneity.

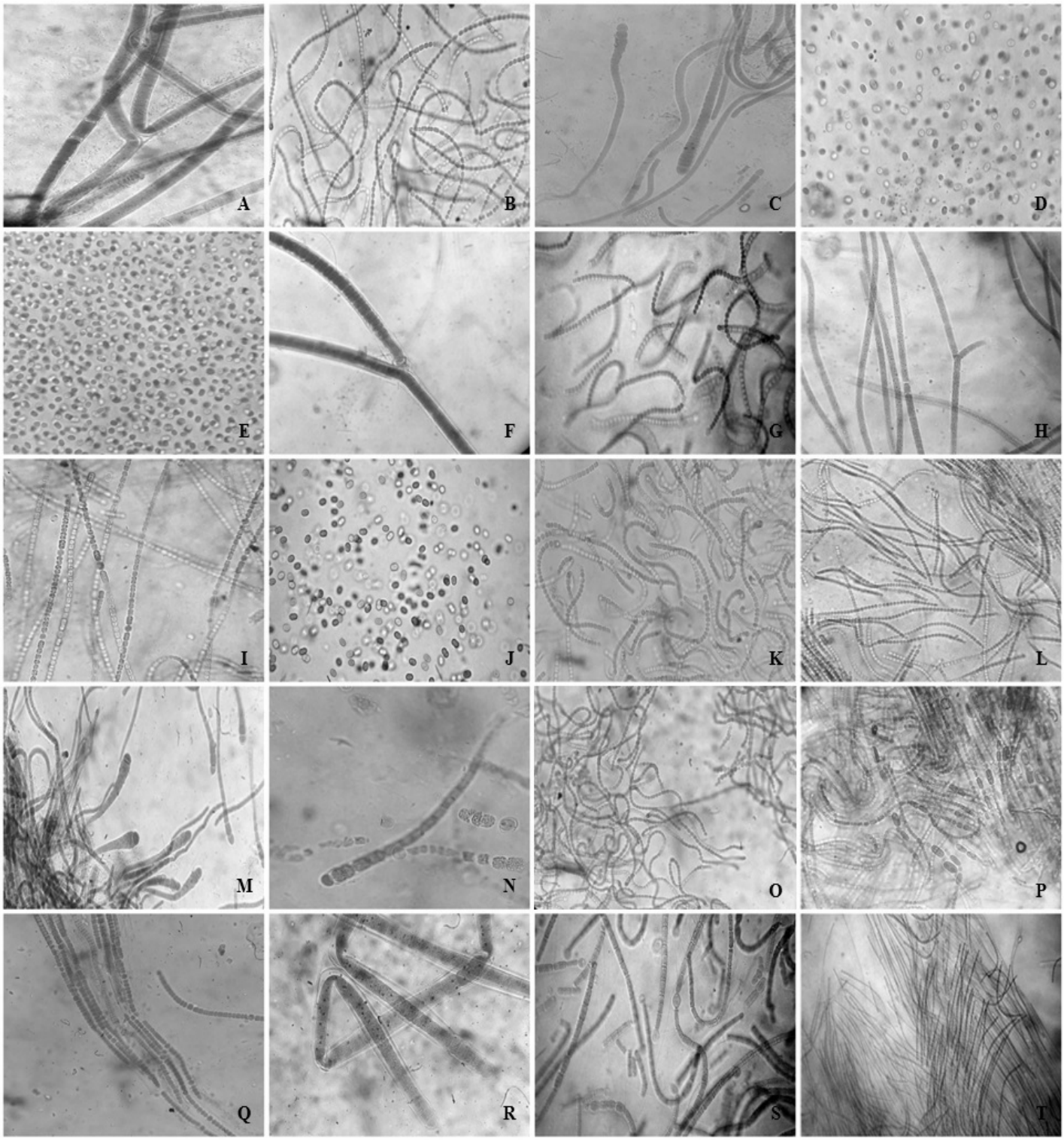

Figure 1

Fig. 1: Microphotographs of the twenty cyanobacterial strains **(A)** *Scytonema* sp. 1ss, **(B)** *Anabaena* sp. 2ss, **(C)** *Calothrix* sp. 3ss, **(D)** *Aphanothece* sp. 4ss **(E)** *Aphanothece* sp. 5ss, **(F)** *Scytonema*sp. 6ss, **(G)** *Anabaena* sp. 7ss, **(H)** *Tolypothrix*sp. 8ss, **(I)** *Nostoc*sp. 9ss, **(J)** *Aphanothece* sp. 10ss, **(K)** *Anabaena* sp. 11ss, **(L)** *Anabaena* sp. 12ss, **(M)** *Calothrix*sp. 13ss, **(N)** *Calothrix* sp. 14ss, **(O)** *Anabaena* sp. 15ss, **(P)** *Nostoc*sp. 16ss **(Q)** *Nostoc* sp. 17ss, **(R)** *Scytonema* sp. 18ss, **(S)** *Anabaena* sp. 19ss, **(T)** *Anabaena* sp. 20ss.

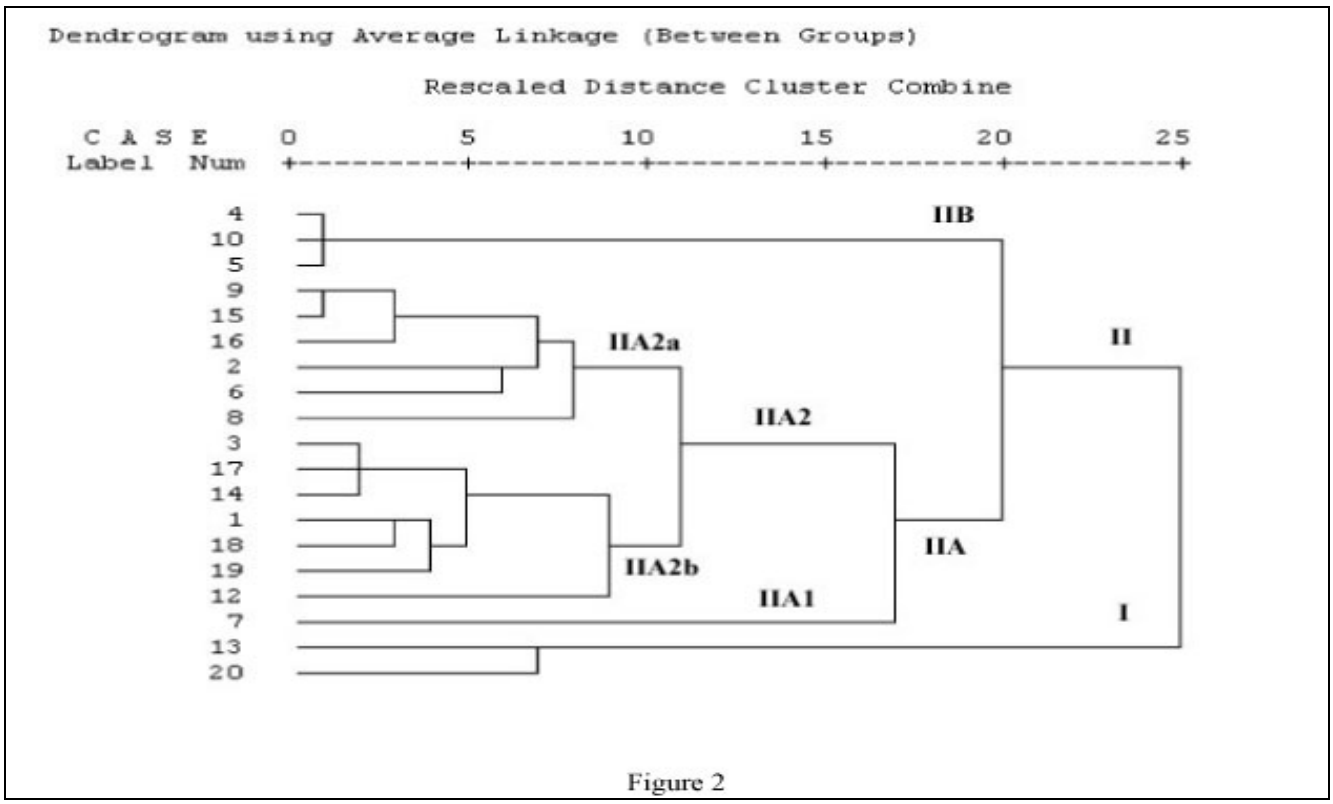

Figure 2

Fig. 2: Cluster analysis of the twenty cyanobacterial species isolated from North hills region based on the morphological attributes **(1)** *Scytonema* sp.1ss **(2)** *Anabaena* sp. 2ss **(3)** *Calothrix* sp. 3ss **(4)** *Aphanothece* sp. 4ss **(5)** *Aphanothece* sp. 5ss **(6)** *Scytonema* sp. 6ss **(7)** *Anabaena* sp. 7ss **(8)** *Tolypothrix* sp. 8ss **(9)** *Nostoc*sp. 9ss **(10)** *Aphanothece* sp. 10ss **(11)** *Anabaena* sp. 11ss **(12)** *Anabaena* sp. 12ss **(13)** *Calothrix* sp. 13ss **(14)** *Calothrix*sp. 14ss **(15)** *Anabaena* sp. 15ss **(16)** *Nostoc* sp. 16ss **(17)** *Nostoc* sp. 17ss **(18)** *Scytonema* sp.18ss **(19)** *Anabaena* sp. 19ss **(20)** *Anabaena* sp. 20ss.

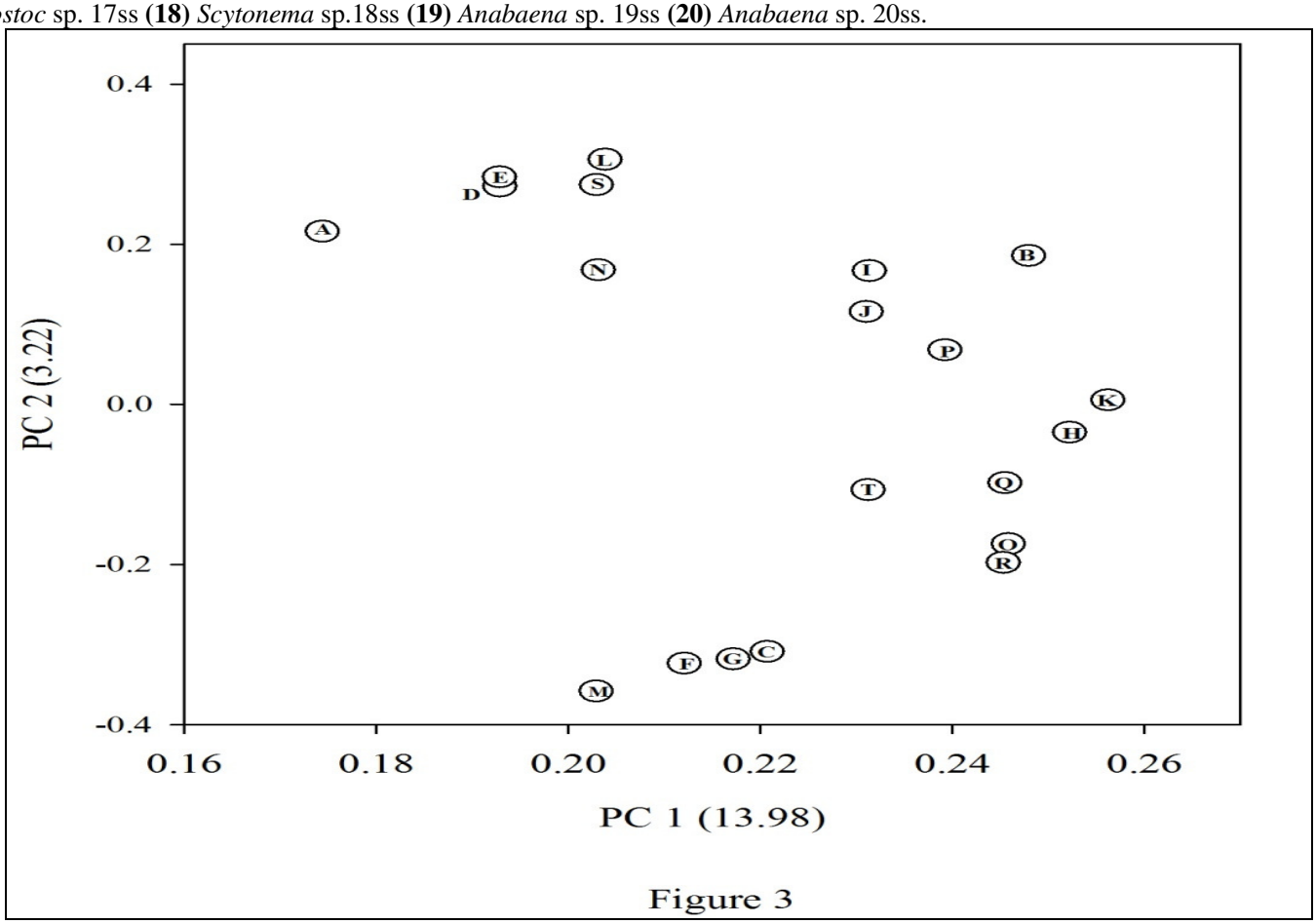

Figure 3

Fig. 3: Scatter plot based on principal component analysis considering morphological attributes. Value in parantheses account for the Eigen values for each principal component. The signs in graph plot indicate individual cyanobacterium. **(A)** *Scytonema* sp. 1ss, **(B)** *Anabaena* sp. 2ss, **(C)** *Calothrix* sp. 3ss, **(D)** *Aphanothece* sp. 4ss **(E)** *Aphanothece* sp. 5ss**, (F)** *Scytonema* sp. 6ss, **(G)** *Anabaena* sp. 7ss, **(H)** *Tolypothrix* sp. 8ss, **(I)** *Nostoc* sp. 9ss, **(J)** *Aphanothece* sp. 10ss, **(K)** *Anabaena* sp. 11ss, **(L)**

Anabaena sp. 12ss **(M)** *Calothrix* sp.13ss **(N)** *Calothrix* sp. 14ss, **(O)** *Anabaena* sp.15ss, **(P)** *Nostoc* sp. 16ss, **(Q)** *Nostoc* sp. 17ss **(R)** *Scytonema* sp. 18ss, **(S)** *Anabaena* sp. 19ss **(T)** *Anabaena* sp. 20ss.

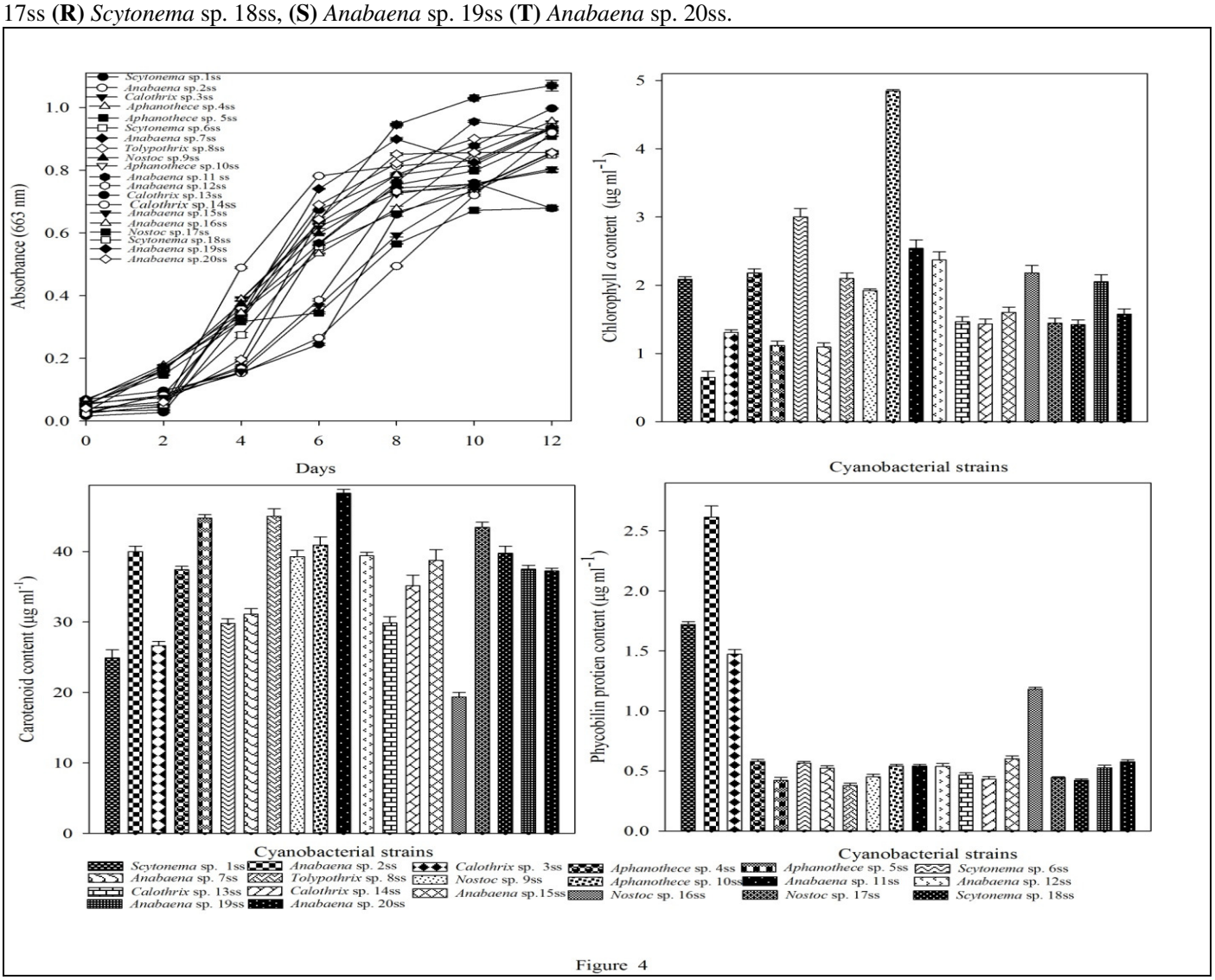

Fig. 4: Growth curve (in terms of OD), chlorophyll a content, carotenoid content and phycobilin protein content of twenty cyanobacterial strains isolated from North Hills region of Chhattisgarh.

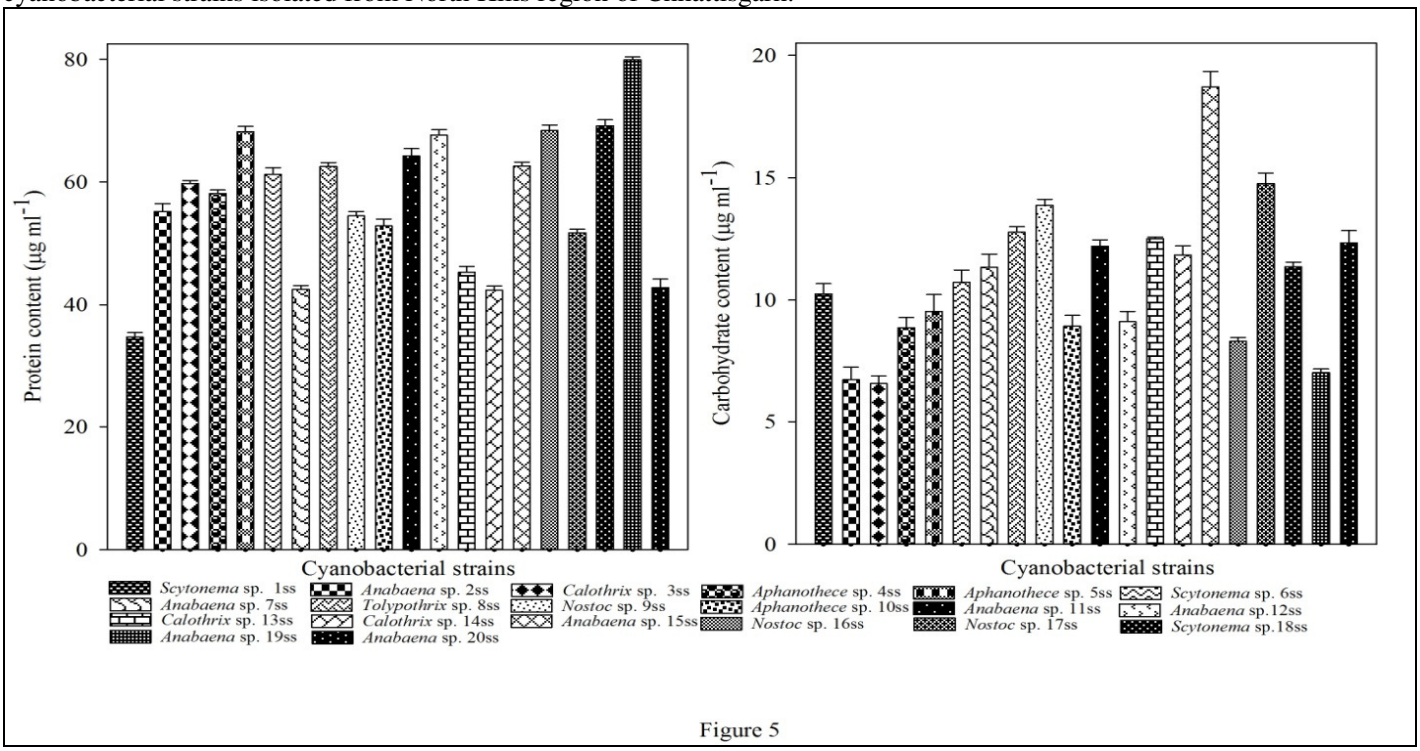

Fig. 5: Protein content and carbohydrate content of twenty cyanobacterial strains isolated from North Hills region of Chhattisgarh.

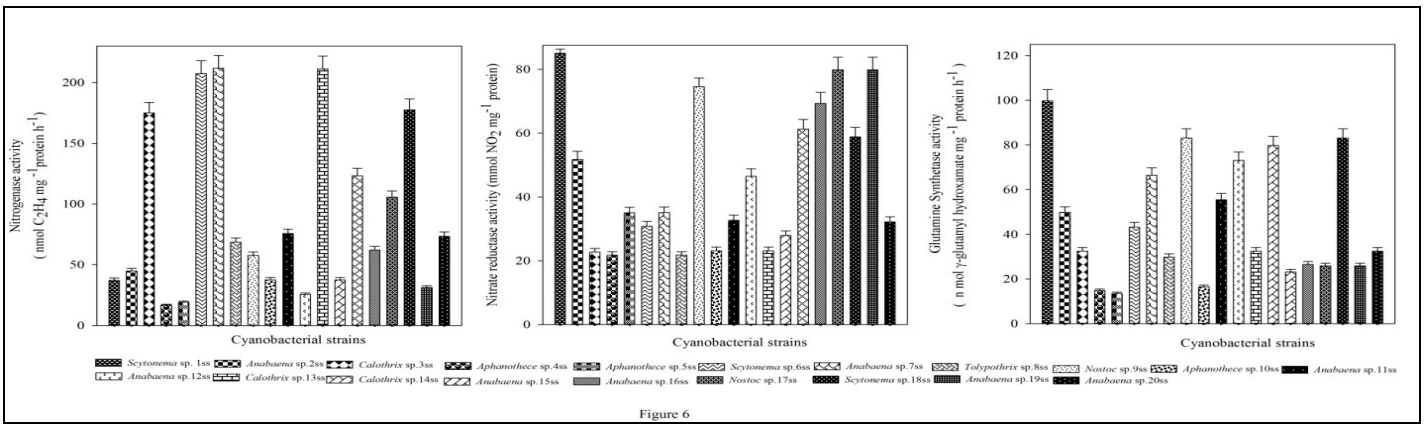

Fig. 6: Nitrogenase enzyme, Nitrate Reductase and Glutamine Synthetase activities in twenty cyanobacterial strains isolated from North hills region of Chhattisgarh.

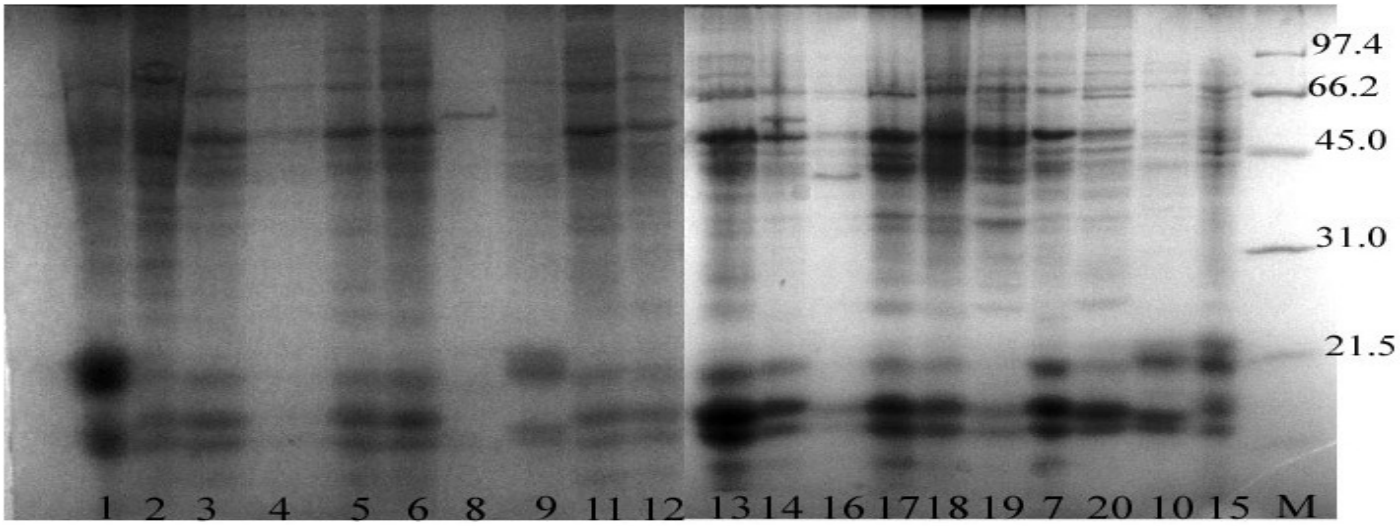

Figure 7

Fig. 7: Whole cell protein profiling pattern of the twenty cyanobacterial strains obtained by using SDS-PAGE analysis. M represents standard molecular weight marker (kDa).

(1) *Scytonema* sp.1ss **(2)** *Anabaena* sp. 2ss **(3)** *Calothrix* sp. 3ss **(4)** *Aphanothece* sp. 4ss **(5)** *Aphanothece* sp. 5ss **(6)** *Scytonema* sp. 6ss **(7)** *Anabaena* sp. 7ss **(8)** *Tolypothrix* sp. 8ss **(9)** *Nostoc* sp. 9ss **(10)** *Aphanothece* sp. 10ss **(11)** *Anabaena* sp. 11ss **(12)** *Anabaena* sp. 12ss **(13)** *Calothrix* sp. 13ss **(14)** *Calothrix* sp. 14ss **(15)** *Anabaena* sp. 15ss **(16)** *Nostoc* sp. 16ss **(17)** *Nostoc* sp. 17ss **(18)** *Scytonema* sp.18ss **(19)** *Anabaena* sp. 19ss **(20)** *Anabaena* sp. 20ss.

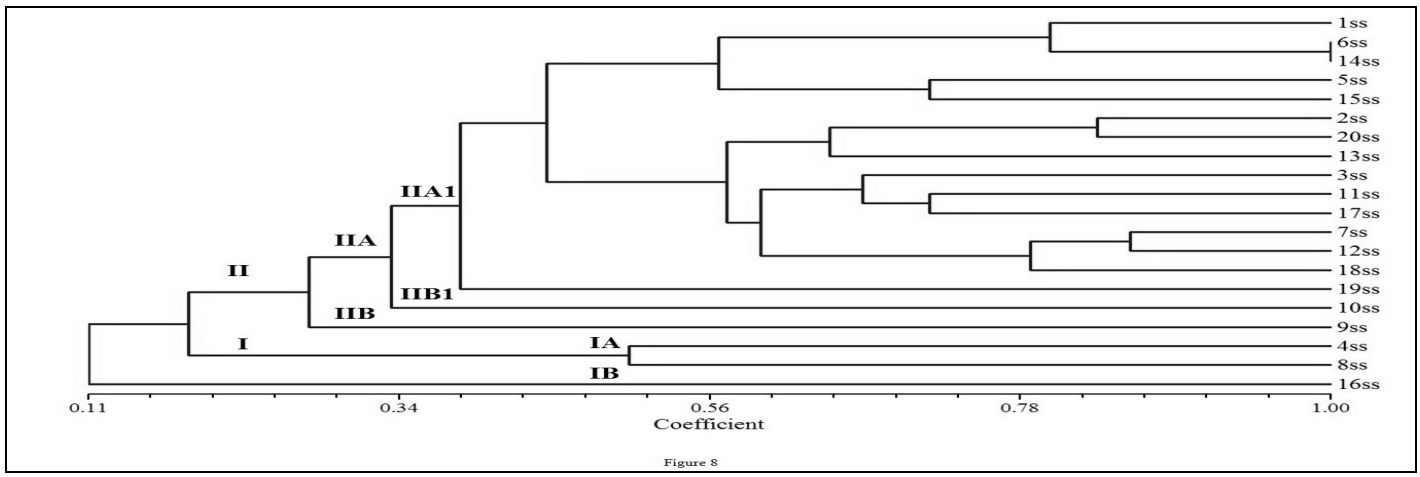

Fig. 8: Dendogram generated with UPGMA clustering algorithm by using NTYSYS software (version 2) based on SDS-PAGE of whole cell protein profiling of the twenty cyanobacterial strains. **(1)** *Scytonema* sp.1ss **(2)** *Anabaena* sp. 2ss **(3)** *Calothrix* sp. 3ss **(4)** *Aphanothece* sp. 4ss **(5)** *Aphanothece* sp. 5ss **(6)** *Scytonema* sp. 6ss **(7)** *Anabaena* sp. 7ss **(8)** *Tolypothrix* sp. 8ss **(9)** *Nostoc* sp. 9ss **(10)** *Aphanothece* sp. 10ss **(11)** *Anabaena* sp. 11ss **(12)** *Anabaena* sp. 12ss **(13)** *Calothrix* sp. 13ss **(14)** *Calothrix* sp. 14ss **(15)** *Anabaena* sp. 15ss **(16)** *Nostoc* sp. 16ss **(17)** *Nostoc* sp. 17ss **(18)** *Scytonema* sp. 18ss **(19)** *Anabaena* sp. 19ss **(20)** *Anabaena* sp. 20ss.

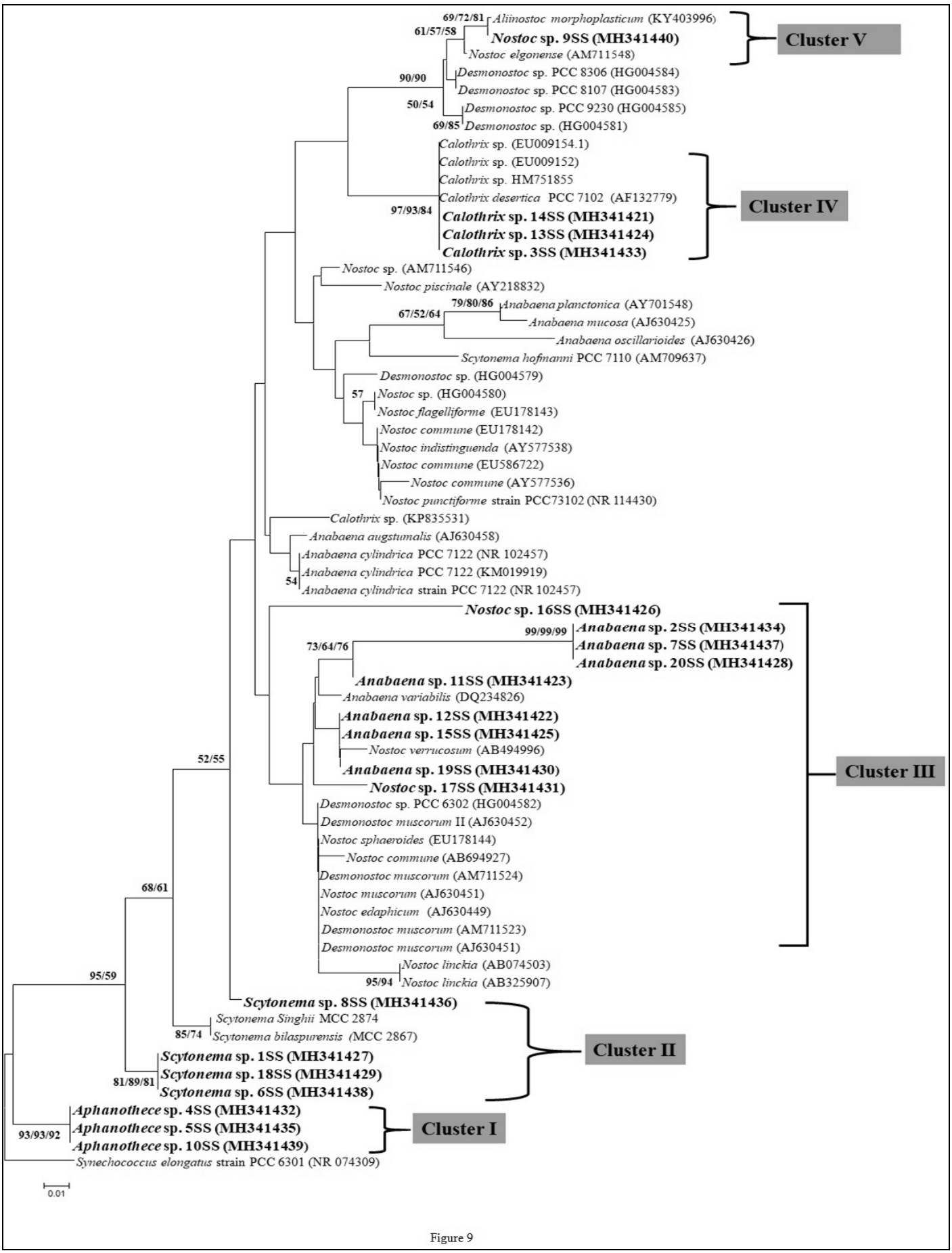

Figure 9

Fig. 9: Neighbor joining/Maximum Likelihood/Maximum parsimony tree showing Phylogenetic relationship of the cyanobacterial strains of the north hills regions based 16S rRNA gene sequences. Total 1000 bootstraps were performed and only more than 50% bootstrap support values are mentioned. Phylogenetic analysis was performed using MEGA 6.0 software. Numbers designate the clade.

Table 1: Analysis of physicochemical properties of soil samples of divergent area of Northern hills of Chhattisgarh

District	Area	pH	Temperature (˚C)	Salinity (g L^{-1})	Dissolved Oxygen (ppm)	Conductivity (µ Seimen cm^{-1})	Total Dissolved Solid (mg L^{-1})
Jashpur Nagar	Jashpur Nagar	8.30 ± 0.17	30.27 ± 1.52	39.47 ± 1.29	32.17±1.44	65.77 ±1.12	121.53 ± 2.16
	Jashpur	7.5 ± 0.5	29.7 ± 0.6	86.7 ± 1.1	45.4± 0.5	95.9 ±1.8	101.7 ± 2.0
	Deepatoli	8.33 ± 0.2	31.33 ± 0.29	40.33 ± 0.58	67.47±1.24	77.33 ±1.38	157.83 ± 1.70
	Gholeng	8.6 ± 0.5	30.9 ± 0.9	42.8 ± 1.0	87.8±1.1	110.9 ±2.1	146.6 ± 1.9
	Ranidah	7.9 ± 0.1	34.8 ±1.0	81.4 ± 1.4	57.0 ±1.0	94.4 ±1.4	204.1 ± 5.9
Sarguja	Ambikapur	8.7 ± 0.4	28.7 ±1.1	41.5 ± 0.4	58.2 ±1.3	152.1 ±2.5	106.1 ± 2.4
	Mainpaath	7.40 ± 0.35	29.73 ± 0.47	122.03 ± 2.57	30.43 ±1.36	113.90 ±5.54	122.63 ± 3.52
	Mainpaath	7.57 ± 0.29	27.43 ± 0.60	81.93 ± 2.57	68.40 ±1.32	108.57 ±1.24	142.27 ± 2.97
	Ramgarh	7.3 ± 0.3	30.2 ± 1.0	70.3 ± 0.7	57.1 ±0.9	36.9 ±1.0	150.5 ±3.1
	Sitapur	8.4 ± 0.45	30.633 ± 0.4	73.64±1.1	64.733 ±1.3	77.223 ±1.1	122.1 ± 2.38
Surajpur	Surajpur	8.43 ± 0.50	28.50 ± 0.46	61.57±2.11	36.57 ±1.59	56.83 ±1.03	110.20 ±1.59
	Surajpur	7.2 ± 0.15	32.6 ± 0.56	61.7±1.91	21.2 ±1.021	54.3 ±0.621	152.9 ±2.05
	Premnagar	8.6 ± 0.265	28.5 ±1.380	42.3±1.270	46.1 ±0.757	35.2 ±0.493	102.7 ±2.72
	Ramanujnagar	7.5 ± 0.231	32.3 ± 0.513	110.3±1.53	68.0 ±0.89	129.1±1.30	135.2 ±1.51
	Pratapur	7.77 ± 0.23	31.27 ± 0.35	57.00±0.89	68.20 ±1.47	96.47±2.20	121.93 ±2.32

Table 2: Morphological characterization of twenty cyanobacterial strains isolated from North Hills region of Chhattisgarh.

S. N.	Cyanobacterial strains	Average filament length	Vegetative Cell			Heterocyte				Akinites			
		Cell per Fillament	Shape	Dimension (µm)		Shape	Dimension (µm)		Position	Fre-quency (%)	Shape	Dimension (µM)		Position
				L	W		L	W				L	W	
1	*Scytonema sp.* 1ss	30	Subquadrate	4.5±0.15	3.5±0.11	Subquadrate	6.5±1.125	5.0±0.1	Intercalary	9.09	-	-	-	-
2	*Anabaena sp.*2ss	15	Barrel	5.5±0.09	5.5±0.09	Barrel	-	-	Intercalary	11.76	-	-	-	-
3	*Calothrix sp.* 3ss	28.5	Spherical	3.0±0.10	3.0±0.10	Spherical	4.0±0.10	4.0±0.10	Terminal	3.17	-	-	-	-
4	*Aphanothece sp.* 4ss	1	Spherical	3.0±0.10	-	-	-	-	-	-	-	-	-	-
5	*Aphanothece sp.* 5ss	1	Cylindrical	3.5±0.13	-	-	-	-	-	-	-	-	-	-
6	*Scytonema sp.* 6ss	15	Hemispherical	3±0.10	2.5±0.10	Cylindrical	8.0 ± 0.35	6.0 ± 0.35	Intercalary	11.76	-	-	-	-
7	*Anabaena sp.* 7ss	17	Cylindrical	9.0 ±0.1	4.5 ±0.09	Barrel	9.0±0.10	5.0±0.10	Intercalary	15.00	Spherical	20 ±4.0	20 ±4.0	Intercalary
8	*Tolypothrix sp.*8ss	20	Quadrate	4.0±1.15	4.5 ±1.4	Cylindrical	8.0 ± 0.35	6.0 ± 0.3	Intercalary	4.76	-	-	-	-
9	*Nostoc sp.* 9ss	23	Spherical	3.0±0.10	3.0±0.10	Spherical	4.0±0.10	4.0±0.10	Intercalary	14.81				
10	*Aphanothece sp.* 10ss	1	Oval	3.7±0.10	-	-	-	-	-	-	-	-	-	-
11	*Anabaena sp.*11ss	15	Hemispherical	3±0.10	2.5±0.10	Spherical	6.5±0.10	6.5±0.10	Intercalary	21.05	-	-	-	-
12	*Anabaena sp.* 12ss	36	Barrel	3.0±0.10	4.5±0.10	Barrel	5.5±0.10	5.0±0.10	Intercalary	4.00	Barrel	10.5 ±0.1	5.0 ±0.1	Intercalary
13	*Calothrix sp.*13ss	53	Quadrate	5.0±1.15	5.0±1.11	Round	7.0±1.0	5.0±1.0	Terminal	1.79	-	-	-	-
14	*Calothrix sp.*14ss	28	Spherical	3.0±0.10	3.0±0.10	Oblong	5.5±0.10	5.0±0.10	Terminal	1.75	-	-	-	-
15	*Anabaena sp.*15ss	23	Barrel	2.0±0.11	3.0±0.10	Ellipsoidal	5.0±0.10	5.5±0.10	Intercalary	14.81	-	-	-	-
16	*Nostoc sp.* 16ss	22	Spherical	4.0±0.10	4.0±0.10	Spherical	7.0±0.10	7.0±0.10	Intercalary	16.67	-	-	-	-
17	*Nostoc sp.* 17ss	30	Spherical	3.0±0.10	3.0±0.10	Hemispherical	5.5±0.10	5.5±0.10	Intercalary	3.23	-	-	-	-
18	*Scytonema sp.* 18ss	30	Cylindrical	5±0.10	4.5±0.10	Cylindrical	8.0±1.89	7.5±1.0	Intercalary	6.06	-	-	-	-
19	*Anabaena sp.*19ss	35	Spherical	5.0±0.10	5.0±0.10	Hemispherical	6.5±0.10	5.8±0.10	Intercalary	8.11	-	-	-	-
20	*Anabaena sp.* 20ss	58	Oblong	4±0.10	3±0.10	Spherical	5±0.10	5±0.10	Intercalary	3.33	Barrel	7.5	9.0	Intercalary

Table 3: List of accession number of cyanobacterial strains isolated from Northern Hills of Chhattisgarh.

S. N.	Cyanobacterial strains	Accession no.	S.N.	Cyanobacterial strains	Accession no.	S.N.	Cyanobacterial strains	Accession no.	S.N.	Cyanobacterial strains	Accession no.
1	*Scytonema* sp. 1ss	MH341427	6	*Scytonema* sp. 6ss	MH341438	11	*Anabaena*sp. 11ss	MH341423	16	*Nostoc* sp. 16ss	MH341426
2	*Anabaena* sp. 2ss	MH341434	7	*Anabaena* sp. 7ss	MH341437	12	*Anabaena*sp. 12ss	MH341422	17	*Nostoc* sp. 17ss	MH341431
3	*Calothrix* sp. 3ss	MH341433	8	*Tolypothrix*sp. 8ss	MH341436	13	*Calothrix* sp. 13ss	MH341424	18	*Scytonema* sp. 18ss	MH341429
4	*Aphanothece*sp. 4ss	MH341432	9	*Nostoc* sp. 9ss	MH341440	14	*Calothrix* sp. 14ss	MH341421	19	*Anabaena* sp. 19ss	MH341430
5	*Aphanothece* sp. 5ss	MH341435	10	*Aphanothece* sp. 10ss	MH341439	15	*Anabaena* sp. 15ss	MH341425	20	*Anabaena* sp. 20ss	MH341428

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