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DIVERSITY OF CYANOBACTERIA FROM SOME SELECTED TERRESTRIAL AND AQUATIC HABITATS IN HIGH ALTITUDES OF UTTARAKHAND, INDIA

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Abstract

Cyanobacteria, as most primitive life forms on earth, have minimal nutrient requirement but at present they reflect very diverse morphology by inhabiting diverse habitats, and are enable to colonize almost every illuminated habitat on Earth such as aquatic, terrestrial, arctic deserts, hydrothermal hot springs and ice-cold habitats. During the course of present investigation, a total of seventeen species of cyanobacteria representing 15 genera, and belonging to 10 families and 4 orders, were isolated from the thirteen studied freshwater and terrestrial habitats, located in high altitude region (above 1500 m *a.s.l.*) of Uttarakhand.

Key words : Cyanobacteira, high altitude, taxonomy, diversity.

Introduction

Cyanobacteria, commonly known as blue green algae, are morphologically diverse and widely distributed group of photosynthetic gram–negative prokaryotes with oxygenic photosynthesis similar to those of plants and green algae (Stanier and Cohen-Bazire, 1977; Fogg *et al.,* 1973). The diversity of cyanobacteria presently can be seen in the multitude of structural unicellular to multicellular, branched to unbranched and functional aspects of cell morphology and in variations in metabolic strategies, motility, cell division, developmental biology etc. Cyanobacteria have minimal nutrient requirement and are enable to colonize almost every illuminated habitat on Earth such as aquatic (fresh water, saline water), terrestrial (soils, rocks, buildings, tree barks, exposed rock surfaces in highly isolated and high altitude environments), arctic deserts, hydrothermal hot springs and ice-cold habitats (de los Rios *et al.,* 2007; Gorbushina, 2007). Their effective protective and tolerance mechanisms against various types of abiotic stresses, such as desiccation (Dadheech, 2010), salinity (Hagemann, 2011), ultraviolet radiation (Zeeshan and Prasad, 2009), high light intensity (Lakatos*et al.,* 2001), extremes of temperature (Singh *et al.,* 2005), oxidative (Hossain and Nakamoto, 2003), acid (Gopalaswamy *et al.,* 2007) and heavy metals (Turner and Robinson, 1995) were well documented. Many cyanobacteria form heterocyst for changes in nitrogen availability.

Cyanobacteria are characterized by the low state of cellular differentiation, simple thallus organization, copious production of mucilage (by many genera and species), absence of motile cells, and the presence of chlorophyll *a*, β-carotene, myxoxanthin, myxoxanthophyll and phycobilins or phycobiliproteins (phycocyanin, allophycocyanin and phycoerythrin) as photosynthetic pigments (Carr and Whitton, 1982). Cyanobacteria are photoautotrophs; however some of them show photoheterotrophy and chemoheterotrophy under certain conditions (Castenholz and Waterbury, 1989). Cyanobacteria play a significant role in carbon, oxygen and nitrogen cycling (Tomitani *et al.,* 2006), improving the physico–chemical properties and structure of soils, reducing soil erosion, mobilizing phosphorus, secreting plant growth promoting substances and enhancing soil microflora activity (Zulpa*et al.,* 2008). Cyanobacteria are one of the useful organisms widely used in food industries and in biotechnological applications (Rastogi and Sinha, 2009). They store reserve food materials which can be used as the source of pigments, lipids, proteins, vitamins and certain secondary metabolites. The ability of many cyanobacteria to perform photosynthesis and nitrogen fixation together with their efficient nutrient uptake mechanisms, minimal requirement of nutrients and adaptation to low light intensity makes them highly productive and efficient biological system.

Uttarakhand- a northern state of India, lies on the southern slope of the Himalaya range, offers excellent habitats for an extremely large variety of vegetation, which has been consistently providing an unparallel attraction to the entire globe and challenges to explore them from different points of views. Uttarakhand comprises a group of low lying hills with rivers, springs, streams, rivulets, pits, ponds, agricultural lands and rocks that reflect diversified natural habitats for flora and fauna. During the present investigation, the study will provide the knowledge of cyanobacterial species, especially from taxonomic point of view, inhabiting various habitats in Uttarakhand.

Material and Methods

Samples were collected randomly at once during 2012-13, from thirteen sites *i.e.*, natural freshwater (lakes, rivulets, puddle) and terrestrial (soils, rocks) habitats, situated at an altitude of 1500m and above, in Uttarakhand state table 1 by following the guidelines provided by Rippka (1988). Algal species inhabiting water bodies were collected in the sterilized polythene bags and wide mouth bottles. For collection of soil inhabitants, few drops of sterile water were added in to the sampling tubes to keep the interior moist. The collected samples were transported to the laboratory in sampling tubes for identification, cultivation and further study. The collected samples were initially examined under the light microscope to evaluate the content of cyanobacterial species. Isolation and purification of algal strain was done by repeated sub-culturing on solidified and in liquid media (BG-11, Chu-10 and Allen & Arnon) by dilution and pour plate technique and standard microbiological methods as described by Rippka (1988). The identification of cyanobacterial species was made by following the literature (Desikachary, 1959; Rippka *et al*., 1979).

Results

During the present investigation, a total of 17 species of cyanobacteria table 2 representing 15 genera, and belonging to 10 families and 4 orders, were isolated from the thirteen different studied sites (*i.e.,* nine terrestrial and four aquatic habitats) of the high altitude regions (above 1500 m *a.s.l.*) of Uttarakhand. The taxonomic description of the isolated cyanobacteiral species are described as follows-

1. ***Anabaena*** **sp. Boryex Bornet & Flahault (Or.- Nostocales, Fa.- Nostocaceae. Fig.,1.A):** Filamentous; filaments simple (unbranched); thallus soft green, gelatinous trichomes uniformly broad throughout; trichomes short, straight or curved, densely aggregated, generally parallel, a firm individual sheath absent, but a soft mucilaginous covering is often present; cells more or less barrel-shaped, 2.5-3 µm broad, 1½-2 times as long as broad; the terminal cells tapered or conical in shape; heterocysts and akinetes present; heterocysts terminal and intercalary; akinetes single or in series, formed from near the heterocysts or in between the heterocysts.

2.***Aphanocapsa montana*** **Cramer (Or.- Synechococcales, Fa.- Merismopediaceae. Fig., 1.B):** Unicellular-colonial; cells more or less spherical (2.5-4.5 µm in diameter), loosely arranged in a common mucilage envelope to form many celled colony; cells without own mucilage envelope.

Table 1: Location co-ordinates, altitude (above mean sea level) and habitats of the sampling sites.

S.N.	Site Name	District	Habitat nature	Location co-ordinates		Altitude(m)
				Latitude	Longitude	
1	Jageshwar	Almora	Rock	29°38'18"N	79°51'10"E	1570
2	Surkanda Devi	Tehri Garhwal	Rock	30°24'40"N	78°17'17"E	2674
3	Dhanoulti	Tehri Garhwal	Rock	30°25'36"N	30°25'36"E	2250
4	Joshimath	Chamoli	Rock	30°33'28"N	79°33'36"E	1890
5	Chakrata	Uttarkashi	Rock	30°42'05"N	77°52'10"E	2270
6	Mandal	Chamoli	Agri. Soil	30°27'25"N	79°16'28"E	1503
7	Mussoorie	Dehradun	Moist Rock	30°27'19"N	78°04'26"E	1971
8	Munsyari	Pithoragarh	Moist Soil	30°04'17"N	80°14'14"E	2200
9	Chamba	Tehri Garhwal	Moist Soil	30°20'43"N	78°23'40"E	1676
10	Naini Lake	Nainital	Water	29°38'18"N	79°27'48"E	1960
11	Auli	Chamoli	Puddle	30°31'44"N	79°34'13"E	3049
12	Bhulla Lake	Pauri Garhwal	Water	29°50'22"N	78°41'54"E	1706
13	Purola	Uttarkashi	Rivulet	30°55'26"N	78°03'13"E	1524

3. ***Aphanothece*** sp. **Nageli (Or.- Chroococcales, Fa.- Aphanothecaceae. Fig., 1.C):** Unicellular-colonial; cells ellipsoidal, elongated or cylindrical with rounded tips, straight, or slightly bent; yellow- brownish colored; cells 4-5 µm broad and 6-9 µm long; cells densely embedded in amorphous and tough mucilaginous or gelatinous matrix to form macroscopic colony.

4.***Calothrix*** sp. **Bornet & Flahault (Or.- Nostocales, Fa.- Rivulariaceae. Fig., 1.D):** Filamentous; filaments simple (unbranched); filaments heteropolar, differentiated in to basal and apical parts, solitary (single) or in small bundles, filaments arranged more or less parallel, mostly straight, distinctly attenuated towards the apex; sheath mostly firm, sometimes seen only at the base, trichomes with tapered morphology ranging in diameter in their basal region from 2.5 to 18 µm; cells cylindrical or barrel shaped; trichomes always with single, basal, more or less spherical or hemispherical heterocysts; akinetes not seen.

5. ***Cylindrospermum*** sp. **Kutzing ex Bornet & Flahault (Or.- Nostocales, Fa.- Nostocaceae. Fig., 1.E):** Filamentous; filaments simple (unbranched); thallus mucilaginous, mostly dull blue-green; trichome untapered (uniformly broad), short, without sheath, but in a common mostly very delicate and often imperceptible, mucilage of thin consistency; cells cylindrical, constricted at the cross-walls; heterocysts oblong, boarder than the trichomes, heterocysts terminal in position developing at both ends or at one end only; spores (akinetes) single, rarely in series, developing adjacent to the heterocyst; spores ellipsoidal, 10-15 µm broad, much bigger than the vegetative cells; epispore brownish with distinct papillae.

6. ***Gloeocapsa*** sp. **Kutzing (Or.-Chroococcales, Fa.- Microcystaceae. Fig., 1.F):** Unicellular-colonial; cells spherical (6 µm) in diameter; 2-8 cells in a colony with concentrically lamellated (layered) envelope/ sheath; sheath colourless or partly colored.

7. ***Gloeotrichia ghosei*** **Singh 1939 (Or.- Nostocales, Fa.- Gloeotrichiaceae. Fig., 1.G):** Filamentous; filaments simple (unbranched), whiplike and heteropolar with basal heterocyst and apical hair-like ends with own sheath; filaments united radially into gelatinous spherical or hemispherical colony; trichomes oriented with heterocysts in to the centre of the colony; akinetes present next to the basal heterocysts; filaments with a thin stratified and brown sheath ; cells at the base barrel – shaped, much shorter than broad, 3-7 µm long; heterocysts single, spherical (10-13 µm diameter); akinete long ellipsoidal with hyaline smooth outer wall.

8. ***Hapalosiphon*** sp. **Bornet (Or.- Nostocales, Fa.- Haplosiphonaceae):** Filamentous, filaments true branched; thallus caespitose, floccose, thin; filaments free, not coalescing laterally; filaments irregularly arcuated; cells in one or two rows; sheaths thin, colourless; continuously branched; branches irregularly lateral, true, branches erect from the primary prostrate filaments, erect branches as broad as and similar to the main filament; heterocysts intercalary, cylindrical; akinetes (spores) solitary.

9. ***Lyngbya*** sp. **Agardh ex Gomont (Or.- Oscillatoriales, Fa.- Oscillatoriaceae. Fig., 1.H-I):** Filamentous; filaments simple (unbranched); filaments thick; firm sheaths which are sometimes stratified and brownish colored; trichome isopolar, single, straight and sometimes spirally coiled; cells are cylindrical or barrel shaped; heterocysts and akinetes absent.

10. ***Nostoc muscorum*****. Agardh ex Bornet & Flahault (Or.- Nostocales, Fa.- Nostocaceae. Fig., 1.J):** Filamentous; filaments simple (unbranched); thallus mucilaginous (gelatinous); filaments within colony irregularly coiled or contorted and loosely or densely agglomerated or entangled; sheaths around trichomes present; trichome isopolar (same width along the whole length) and moniliform; apical cells morphologically similar to other cells; cells spherical or ovoid; heterocyst and akinetes present; heterocysts solitary and intercalary; akinetes spherical or oblong, formed centrifugally in series between the heterocysts; trichome 3-4 µm broad; cells twice as long as broad; heterocysts nearly spherical, 6-7 µm broad.

11. ***Oscillatoria limosa*** **Agardh ex Gomont (Or.- Oscillatoriales, Fa.- Oscillatoriaceae. Fig., 1.K):** Filamentous; filaments simple (unbranched); sheath absent; thallus dark blue- green to brown; trichome more or less straight; cells are cylindrical or barrel-shaped and shorter than wide; cells 1/3-1/6 times as long as broad; cells 2-5 µm long, cross-walls frequently granulated; end-cell flatly rounded with slightly thickened membrane; heterocysts and akinetes absent; motile under convenient environmental conditions (waving, trembling, oscillation).

12. ***Oscillatoria proboscidea*** **Gomont (Or.- Oscillatoriales, Fa.- Oscillatoriaceae. Fig., 1.L):** Filamentous; filaments simple (unbranched); sheaths absent; thallus dull green to dark blue-green; trichome more or less straight, not constricted at the cross-walls, slightly curved or sometimes spirally coiled; cells are cylindrical or barrel-shaped; cells shorter than wide; cells 1/3-1/6 times as long as broad, 2-4 µm long, not granulated at the cross-walls; end-cell flatly rounded, capitate, with slightly thickened membrane; heterocysts and akinetes absent; motile under convenient environmental conditions (waving, trembling, oscillation).

13. *Phormidium foveolarum* Gomont (Or.- Oscillatoriales, Fa.- Oscillatoriaceae. Fig., 1.M): Filamentous; filaments simple (unbranched);. Fig. 1. sheath thin, tube-like, firm, unlamellated and open at the ends containing single trichome; trichome isopolar, not attenuated at the ends, constricted at the cross walls; trichome straight or slightly bent, 1.5 µm broad; trichome composed of slightly barrel-shaped cells, cells 1-1.5 µm long, end cells of the trichome rounded; heterocysts and akinetes absent; calyptra absent.

14. *Phormidium tenue* Gomont (Or.- Oscillatoriales, Fa.- Oscillatoriaceae. Fig., 1.N-O): Filamentous; filaments simple (unbranched); sheath thin, tube like, firm and unlamellated containing single trichome; trichome straight or slightly bent, slightly constricted at the cross walls, attenuated at the ends; trichome 1-2 µm broad; cells 2.5-5 µm long, end cells acute-conical; heterocysts and akinetes absent; calyptra absent.

15.*Synechococcus* sp. Nageli (Or.- Synechococcales, Fa.- Synechococcaceae. Fig., 1.P): Unicellular; cells solitary or in colonies of 2 cells after division; cells oblong or oval in shape (2µm broad and 5 µm long); mucilage envelope absent or very thin; contents homogenous, light blue-green in colour.

16.*Synechocystis* sp. Sauvageau (Or.- Synechococcales, Fa.- Merismopediaceae. Fig., 1.Q): Unicellular; Cells solitary or in colonies of 2 cells after division; cells spherical (5 µm in diameter, mucilage envelope lacking.

17. *Scytonema* sp. Agardh ex Bornet & Flahault (Or.- Nostocales, Fa.- Scytonemataceae. Fig., 1.R-S): Filamentous, filaments false branched; trichomes isopolar, cylindrical, uniseriate, sheathed and constricted at cross walls; trichome single in each sheath; sheaths firm, lamellated, usually yellow-brown; false branches formed laterally in between two heterocysts; both branches grow parallel aside or in crossing position; heterocysts intercalary, solitary, rarely in pairs; akinetes (spores) not seen, but known only in a few species, spherical or ovate, exospores thin and smooth.

Discussion

Cyanobacteria (Blue-green algae) are an ancient, morphologically diverse, metabolically versatile and widely distributed group of oxygenic (O_2-evolving) photosynthetic prokaryotes which successfully colonize and inhabit a wide range of terrestrial and aquatic habitats, including those with extreme conditions. The ability of

Table 2: Distribution of Cyanobacteria in different study sites.

S.No.	Cyanobacteria	Jageshwar	Surkanda Devi	Dhanoulti	Joshimath	Chakrata	Mandal	Mussoorie	Munsyari	Chamba	Naini Lake	Auli	Mussoorie (Kempty fall)	Bhulla Lake	Purola
		Terrestrial habitats									Aquatic habitats				
1	*Anabaena* sp.									+					
2	*Aphanocapsa montana*										+				+
3	*Aphanothece* sp.									+					
4	*Calothrix* sp.			+		+									
5	*Cylindrospermum* sp.									+					
6	*Gloeocapsa* sp	+	+		+										
7	*Gloeotrichia ghosei*											+			
8	*Hapalosiphon* sp.											+			
9	*Lyngbya* sp.			+											
10	*Nostoc muscorum*						+								
11	*Oscillatoria limosa*										+				
12	*Oscillatoria proboscidea*													+	
13	*Phormidium foveolarum*										+			+	
14	*Phormidium tenue*												+		+
15	*Synechococcus* sp.										+			+	
16	*Synechocystis* sp.										+			+	
17	*Scytonema* sp.							+	+						

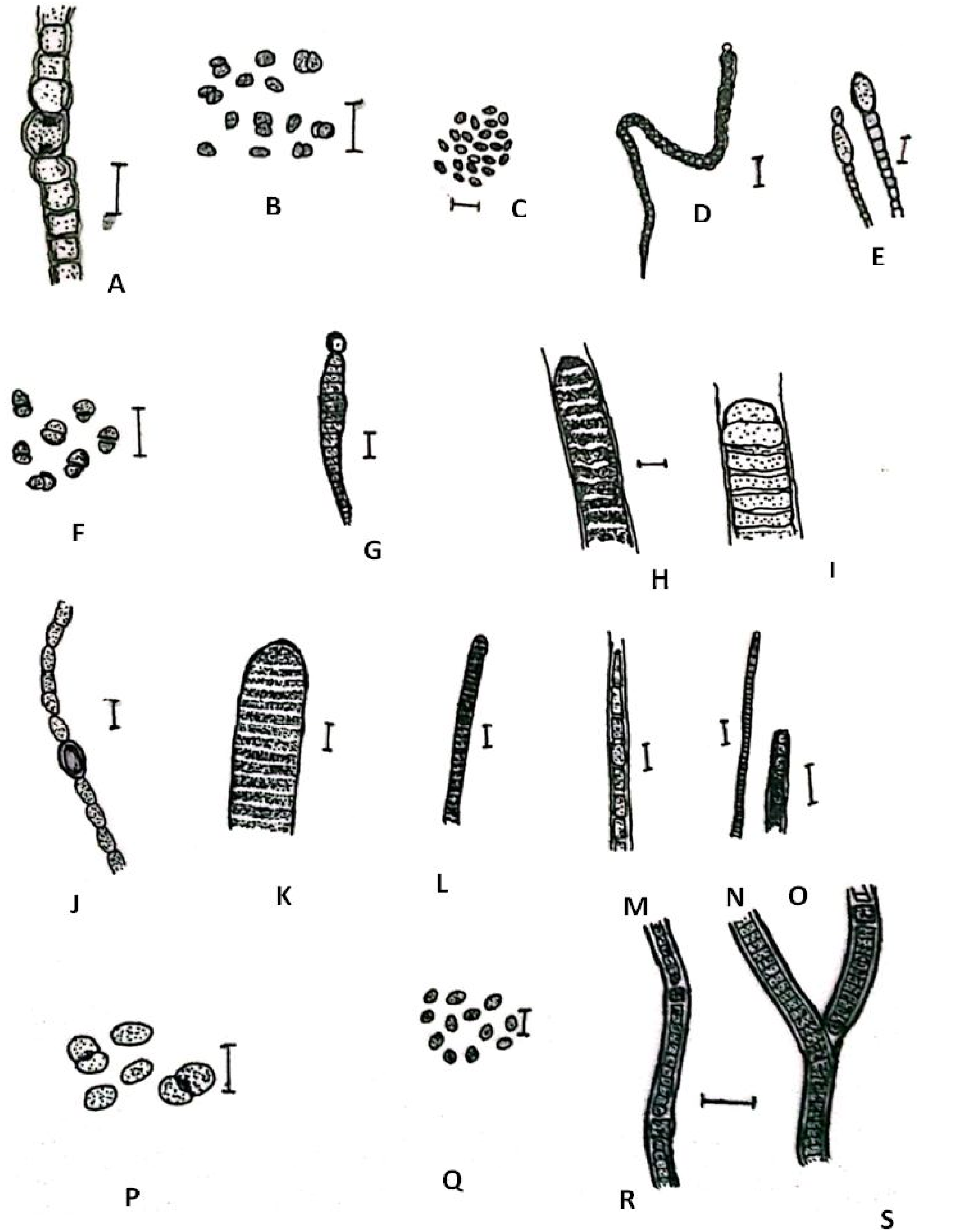

Fig. 1: (A)*Anabaena* sp. (B)*Aphanocapsa montana* (C)*Aphanothece* sp. (D)*Calothrix* sp. (E)*Cylindrospermum* sp. (F)*Gloeocapsa* sp. (G)*Gloeotrichia ghosei* (H-I)*Lyngbya* sp. (J)*Nostoc muscorum* (K)*Oscillatoria limosa* (L)*O. Proboscidea* (M)*Phormidium foveolarum* (N-O)*P. tenue* (P)*Synechococcus* sp. (Q)*Synechocystis* sp. (R-S)*Scytonema*sp. *(fig. A,B,F,O,P,R & S in 10 µm scale*; *fig. C-E, G-N & Q in 5 µm scale).*

many cyanobacteria to perform both photosynthesis and nitrogen fixation together with their efficient nutrient uptake mechanisms and adaptation to low light intensity makes them highly productive and efficient biological system.

A total of 17 cyanobacterial species representing 15 genera, and belonging to 10 families and 4 orders, were isolated from various freshwater and terrestrial habitats located in high altitude region of Uttarakhand. During present study, a total of eight species of cyanobacteria were recovered from terrestrial habitat, whereas twelve cyanobacteria were isolated from aquatic habitat (Table 2). In reference to cyanobacterial density at individual site, the maximum number (05) of Cyanobacteria was isolated from aquatic habitat in comparison to terrestrial habitat (03) sites. During present investigation, the highest number (05) of Cyanobacteria was isolated from Nainital lake, followed by Bhulla lake (04), Purola (02), Auli (02) and Kempty water fall (01) from aquatic habitats (Kumavat, 2016). On the other hand, in terrestrial habitats, maximum number (03) of cyanobacteria were isolated from the moist soil of Chamba, followed by Dhanoulti (02), while only one species of Cyanobacteria was observed from the rest of the seven study sites (Kumar *et al.,* 2011). Isolated and identified cyanobacterial species represented five morphological categories: coccoid (unicellular/colonial; *Gloeocapsa* sp., *Synechococcus* sp., *Synechocystis* sp., *Aphanocapsa momtana* and *Aphanothece* sp.), non-heterocystous simple filamentous (*Lyngbya* sp., *Phormidium foveolarum*, *Phormidium tenue*, *Oscillatoria proboscidea*, *Oscillatoria limosa*), heterocystous simple filamentous (*Nostoc muscorum*, *Calothrix* sp., *Cylindospermum* sp., *Gloeotrichia ghosei* and *Anabaena* sp.), heterocystous false branched filamentous (Scytonema sp.), and heterocystous true branched filamentous (*Hapalosiphon* sp.). Isolated cyanobacteria were grown in medium BG-11, Chu–10 and Allen and Arnon medium.

The results of the present investigation revealed the occurrence, diversity and distribution of various cyanobacterial species in various high altitude freshwater and terrestrial habitats in Uttarakhand. Furthermore, the results revealed the occurrence of promising cyanobacterial species which can be exploited as a source of phycobiliproteins, the commercially or biotechnologically important natural products.

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