



Cyanobacterial flora in “Usar” soils of Azamgarh and Varanasi districts, Uttar Pradesh

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Abstract

Cyanobacteria are microscopic organisms coming under the class Cyanophyceae. They are prokaryotic and diazo-trophic organisms that can perform both photosynthesis and nitrogen fixation. Cyanobacteria consists of about 2000 species coming under 150 genera and 5 orders. Among these species, they show variation in size and shape. For present study three sites were collected namely- Bunda (Site- 1st), Alipur (Site- 2nd) of Azamgarh district and Bhawanipur (Site-3rd) of Varanasi district. Only a few algae like *Nostoc sp.*, *Calothrix sp.* and *Anabaena sp.* could survive during highly saline condition. Cyanobacteria identified to grow on “Usar” soils were unicellular species like *Chroococcus*, *Aphanocapsa*, *Aphanothece*, *Gloeocapsa*, *Gloeothece* and filamentous species like *Microcoleus chthonoplastes*, *M. vaginatus*, *Nostoc calcicola*, *N. punctiforme*, *N. spongiaeforme*, *Calothrix brevissima*, *Anabaena ambigua*, *A. fertilissima*, *Scytonema sp.*, *Cylindrospermum licheniforme*, *Aphanothece parietina*, *Aulosira fertilissima*, *Gloeocapsa stegophila*, *Phormidium sp.*, *Oscillatoria subbrevis*, *Chroococcus macrococcus*, *spirullina sp.*, *Anabenaopsis circularis*, *Lyngbya ceylanica* and *Scytonema*, *Calothrix* and *Tolypothrix*. The major economic importance of Cyanobacteria is that they can be used as bio fertilizer in agriculture fields. The filamentous forms of Cyanobacteria we collected from the study area possess heterocyst, thus they can fix atmospheric nitrogen. This property of Cyanobacteria could be further studied and used for the preparation of bio fertilizers.

Keywords: cyanobacteria, azamgarh, varanasi, heterocyst and biofertilizers

Introduction

Cyanobacteria are a large and morphologically diverse group of phototrophic prokaryotes, which occur in almost every habitat on earth. This versatility may explain the remarkable lack of morphological change seen in 3.5-billion-year-old fossilized cyanobacteria and their modern day counterparts. Cyanobacteria have the ability to perform mutually compatible functions like nitrogen fixation and photosynthesis (Selvi and Selva kumar 2012). Cyanobacteria have applications in various fields like Microbiology, Biotechnology, Pharmacology, Agriculture, etc. The presence of wide spectrum of bioactive compounds in cyanobacteria gives antiviral, antioxidant, anticancer, antibacterial and antifungal substances. Cyanobacteria are used as bio fertilizers due to their nitrogen fixing capacity. It has the capacity to degrade the environmental pollutants and being used as a promising source 199 of alternate energy (Ananya *et al.*, 2014) [1]. Cyanobacteria can be used for phytoremediation purposes also (Telor and Forni 2011). Cyanobacteria are a photosynthetic prokaryotes and are grouped under the Gram-negative bacteria and their morphology varies from unicellular to multicellular. Some species have unique cells, called heterocysts that are capable of fixing atmospheric nitrogen. They are found in a wide variety of habitats, namely, moist soil, saline and sodic soil as well as in thermophilic and psychrophilic conditions and in symbiotic associations. Their long evolutionary history has been marked by key geochemical and biotic transition, including the creation of the development and proliferation

of metabolically complex microbial and higher eukaryotic life forms.

Materials and methods

Study area (site location)

The site selected for present study includes of two districts. The first site Bunda and second site Alipur in Azamgarh districts, has an average elevation of 64 meter (209 ft) and its geographical are coordinates 26°3'36" North 8°11'10"East. The third site Bhawanipur in Varanasi districts is located at an elevation of 80.71 meters (264.8 ft) and coordinates 25.28°N 82.96°E. The temperature between 22 and 46°C (72-115°F) in the summers. In the wenter from December to February temperature bellow 5°C (41°F) are not uncommon. The average rainfall is 1110 mm (44 inch). The temperature and rainfall like as a Varanasi districts. Soil samples was collected from ‘Usar’ fields during May 2016 to April 2018. Occurrence of Cyanobacteria in saline/alkaline soil has been described by (Singh, 1950; Singh, 1961) [5, 7]. Soil pH is an important factor in Cyanobacterial distribution in soil (Sardeshpande and Goyal, 1981). The vegetation is floristically rich and diverse and represents several unique habitats. The richness of plant diversity at Azamgarh and Varanasi plain depends on climatic, geographic, topographic and edaphic factors.

Collection of specimens

Sample collection and identification

‘Usar’ soils samples were collected from Site-1 Bunda (Azamgarh), Site-2 Alipur (Azamgarh) and Site-3 Bhawanipur (Varanasi) districts during May-2016 to April-2018. Soil samples were taken at the depth of 15cm. in zigzag pattern across the required areas.

A composite soil sampling was performed at each site. Soil samples were collected 5-10 cm below the surface after digging a pit of 5"× 5" inch and packed into polythene bag and delivered to the laboratory on the same day to avoid unpredictable changes and interference in characteristics. The collected samples were dried at shaded place at room temperature, grinded and sieved for removal of debris and stones. The sieved soil was used for analysis. Moist culture of soil algae were prepared by spreading a layer of soil (about 1 cm. thick) and moistened with sterilized distilled water periodically in Petri dish covered with a sheet of glass both previously sterilized (John, 1942). In about a fifteen night after incubation, the visible growth of algae appear in the culture. One of the replicates was disturbed for microscopic examination while other were left undisturbed for further observation. For making unialgal culture, a few drops of the culture of the algal flora were transferred to agar plate with the help of fine pipettes. After 12-15days (about 2 week), Petri dishes were observed for algal colonies. The colonies were then transferred into various nutrient medium for their isolation and identification. The identification of the cyanobacterial samples were carried out by using the taxonomic publication of Desikachary (1959) [2], and Anand (1989).

Results and discussion

Entirely 18 genus of Cyanobacteria belonging to 3 orders (Chroococcales, Nostocales and Stigonematales) 7 families (Chroococcaceae, Oscillatoriaceae, Nostocaceae, Scytonemataceae, Microchaetaceae, Stigonemataceae and Rivulariaceae) were identified. The detailed taxonomic descriptions are given below;

Taxonomic enumeration

Order: Chroococcales

Family: Chroococcaceae

Chroococcus micrococcus

Thallus mucilaginous, somewhat broad, yellowish brown, more or less dilated; cells spherical, 2-4 together, also single, 25- 50 μ diam., with sheath 30-80 diam.; sheath thick, colorless lamellated.

Microcystis aeruginosa

Colonies when young round or slightly longer than broad, soild, when old becoming clathrate, with distinct hyline colonial mucilage; cells 3-7 μ diam., spherical, generally with gas-vacuoles.

Order: Nostocales

Family: Oscillatoriaceae

Microcoleus chthnoplastes

Filaments single, or forming an expanded dirty to dark green lamellated thallus, coiled seldom branched; sheath sometimes gelatinizing, uneven, thick, not coloured violet by chlor-zinc-iodide, having many closely grouped trichomes; trichome constricted at the cross-wall, 2.5-6μ broad, not granulated at the cross-wall, cell 1-2 times as long as broad, blue-green, 3.6-10μ long; end cells not capitates; pointed conical.

Lyngbya arboricola

Thallus forming a continuous layer, when moist blue-green, when dry more or less reddish brown, velvety; filamentous nearly straight or moderately flexuous, long, 18-22μ broad; sheath colourless at first or when empty, mostly reddish brown, firm, 1.5-2μ thick, homogenous or slightly stratified, often slightly transversely wrinkled; trichome shallowly but distinctly constricted at the septa, septa not granulated, blue-green, rounded at the extremity; cells usually 5-6 sometimes 6-10μ long, contents, blue-green, densely granular; homogones (2) to 20- celled.

Lyngbya majuscula

Thallus expanded, upto 3 cm. long, dull blue-green to brown or yellowish brown; filaments very long, curved or seldom only slightly coiled; sheath colourless, lamellated upto 11μ thick, outside often rough, not coloured violet by chlor-zinc-iodine; trichome blue-green, brownish green or grey violet; not constricted at the cross walls, not attenuated at the ends, 16-60μ (or upto 80μ) broad, mostly 20-40μ broad; cells very short 1/6-1/5 times as long as broad, 2-4μ long, cross-walls not granulated; end cells rotund, calyptas absent.

Lyngbya mucicola

Filaments single in the mucilaginous thalli of other algae, variously bent, 1.5 μ broad, sheath delicate, hyaline; trichome 0.5 μ broad, not constricted at the cross-wall, cross-walls marked by a strongly refractive granule on either side; cells 1.5 μ long, pale blue-green; ends not attenuated, end cell rotund.

Oscillatoria subbrevis

Trichomes 9.5-10μ, cells 1-1.8μ long, 5-6μ broad, nearly straight, not attenuated at the apices; cells 1-2^u long, not granulated at the cross walls; end cell rounded, calyptas absent.

Oscillatoria limosa

Thallus dark blue-green to brown; trichome more or less straight, dull blue-green, brown or olive-green, not constricted at the cross-walls or only slightly constricted, 11-20 (-22) μ, commonly 13-16 μ broad; cells 1/3-1/6 as long as broad, 2-5 μ long, cross-wall frequently granulated; end cell flatly rounded with slightly thickened membrane.

Spirulina laxissima

Thallus is symbiotic, multicellular, and filamentous blue-green microalgae with symbiotic bacteria that fix nitrogen from air. It is recognizable by the arrangement of the multicellular cylindrical trichomes in an open left hand helix along the entire length. They are undifferentiated and filamentous.

Phormidium anamola

Thallus thick expanded, soft, mucilaginous, deep blue-green, 3-6 mm thick; trichome subparallel of uniform width, 8-10μ (-10.5) μ broad without constrictions at the cross-walls; sheath thin, colourless, not stained by chlor-zinc-iodine, persistent or dissolved; cells disc-shaped, much broader than long, 0.8-1.2 (-2) μ long; end cells bluntly rounded without cap or calyptas.

Phormidium purpurascens

Thallus compact, leathery, purple to brownish violet; trichome strongly bent, entangled, not constricted at the cross-wall, ends not attenuated, 1.5-2.5 μ broad, dark violet; sheath more or less diffuent, not coloured violet by chlor-zinc- iodide; cells nearly two time longer than broad, 2-4.5 μ long cross-walls marked by two granules on either side; end cell rounded, calyptra absent.

Order: Nostocales

Family: Nostocaceae

Cylindrospermum licheniforme

Thallus mucilaginous, orbicular, confluent, later more or less expanded, blackish green; trichome 2.5-4.2 μ broad, constricted at the cross walls, pale blue-green; cell quadrate to cylindrical, 4-5 μ long; heterocysts oblong, 5-6 μ broad, 7-12 μ long; spores oblong to ventricose elliptic, 12-14 μ broad, 20-30 μ long, with a smooth reddish brown episore.

Cylindrospermum musicola

Thallus expanded, mucilaginous, blackish-green; trichomes 3-4.7 μ broad, constricted at the cross-walls, light blue-green; cells 4 (-5) μ long, cylindrical, or nearly quadrate; heterocysts oblong, 4 (-5) μ broad, 5-7 μ long; spores oval, 9-12 μ broad, 10-20 μ long, episore smooth, yellowish brown.

Anabaena doliolum

Plant mass mucilaginous, blue-green; trichome single straight, curved or slightly coiled, 3.6-4.2 μ broad slightly tapering at the ends with conical apical cell, possessing almost pointed apex, cells and heterocyst barrel-shaped, 5.2-6.3 μ broad and 6.3- 9.4 μ long; spores ellipsoidal, with almost pointed apices in short and long chains, adjoining the heterocyst but developed centrifugally, episore thick, smooth and hyaline or yellow brown 4.2-6.2 μ broad and 6.3-11.5 μ long.

Nostoc calcicola

Thallus mucilaginous, slightly diffuent, expanded, olive, grey or blue green, often up to 5 cm. in diam., filament loosely entangled; sheath mostly indistinct, or distinct only at the periphery of the thallus, colourless or yellowish brown; trichome 2.5 μ broad, pale blue-green; cells barrel shaped, sub spherical, rarely longer than broad; heterocyst subspherical, 4 (-5) μ broad; spores sub spherical 4-5 μ broad, with smooth yellowish membrane.

Nostoc punctiformae

Thallus sub-globose up to 2mm diam., scattered or confluent, attached; filaments flexuous, densely entangled; sheath delicate, hyaline, mucous; trichome 3-4 μ broad, cells short barrel-shaped or sub spherical, or oblong, 5-6 μ broad and 5-8 μ long, episore thick and smooth.

Nostoc ellipsosporum

Thallus gelatinous, irregularly expanded, attached by the lower surface, reddish brown; filamentous flexuous, loosely entangled; trichome about 4 μ broad, light blue-green or olivaceous; cells cylindrical, 6-14 μ long; heterocysts subspherical, or oblong, 6-7 μ broad, 6-14 μ long; spores ellipsoidal to oblong cylindrical, 6-8 μ broad, 14-19 μ long, episore smooth, hyaline or brownish.

Nostoc linckia

Thallus varying in size, sometime punctiform, sometime tuberculate, at first globose latter irregularly expanding, torn, gelatinous, blue-green to violet, or blackish green or brown; filaments densely entangled, flexuous or highly coiled; sheath diffuent and colourless inside, distinct only in the peripheral portion; trichomes 3.5-4 μ broad pale blue-green; cells short barrel-shaped; heterocysts subspherical; spores subspherical, 6-7 μ broad, 7-8 μ long, episore smooth.

Nostoc spongiaeforme

Thallus at first globose, gelatinous, later expanding verrucose, bullose, light blue-green, violet or brownish, filaments, loosely entangled; sheath diffuent in the inside, more or less distinct along the periphery, yellowish- brown; trichome about 4 μ broad blue-green to violet; cells partly cylindrical up to 7 μ long partly short barrel-shaped; heterocysts subspherical or oblong, 7-8 μ broad, and 10-12 μ long, episore smooth, at first colorless, later yellowish.

Order: Nostocales

Family: Scytonemataceae

Scytonema coactile

Thallus radially expanded, woolly, caespitose, green or blue-green, up to 15 cm broad; filament 18-24 μ broad, 4 cm or more long; false branches long, erect; sheath firm, membranaceous hyaline or yellowish; trichome 12-18 μ broad; cells subquadrate or longer than broad; heterocysts sparse, subquadrate.

Tolypothrix byssoidea

Thallus woolly, cushion like, brownish or blackish; filaments up to 1 mm long, 10-15 μ in diam., irregularly false branched; false branches short, erect, curved; sheath thin, close to the trichome yellowish to brownish, fragile, tubular, sometime somewhat ocreaceous; trichome 9-11 μ broad, torulose; cells barrel-shaped, 1/2-1/3 times as long as broad; heterocysts basal, rarely intercalary, single or twos; spores seen ones, in series, ellipsoidal, longer than the vegetative cells, yellowish green.

Order: Nostocales

Family: Microchaetaceae

Microchaete calothrichoides

Filamentous 10-16 μ broad, seldom up to 20 μ broad, single or in tufts, forming a dirty greyish green thallus, straight or curved; sheath thick, lamellated often more or less incrustated, colourless; cells at the base of filaments 6-8 μ broad, 1/3 to as long as broad, distinctly constricted at the cross-wall, olive coloured, heterocysts mostly basal, nearly oval to long ellipsoidal, 6 μ broad, up to 8 μ long.

Calothrix marchica

Filaments in groups, irregularly bent and closely entangled, 9.6-14.4 μ broad, upto 450 μ long; sheath thin, firm, yellowish, or hyaline; trichome 8.4-12.8 μ broad, constricted at the septa, ends tapering but without a hair, end cell conical with a rounded apex, sometimes pointed cells quadratic, as well as shorter or longer than broader, 2-3.3 μ long, at the apices upto 4.8 μ long; heterocysts single, basal,

spherical or sub spherical, 8.2-12.5 μ broad and upto 5 μ long.

Order: Stigonematales

Family: Stigonemataceae

Hapalosiphon welwitschii

Filaments single among other algae, somewhat flexuous, 5.5-7.5 μ broad; sheath very close, hardly visible, colorless; cells subspherical or elongate, as long as broader or longer; lateral branches short, as broad as the main filaments or narrower, 3.5 5.7 μ broad; heterocysts rare, intercalary, quadrate rounded or long cylindrical, 6 μ broad 6-8 μ long; spores sub-spherical or oblong, 5 μ broad, 1-2 times as long as broad.

Stigonema dendroideum

Thallus expanded, brownish black, up to 1 mm high; filamentous creeping, entangled, 16-22 μ seldom up to 25 μ broad, richly branched, branches erect, when young

narrower latter as broad as the main filament, with many secondary branches, secondary branches gradually attenuated; sheath in side colourless close to the trichome, not lamellated; trichome in the main filament and primary branches with one or two seldom three rows of cells 14-20 μ broad; cells 8-14 (-20) μ broad; heterocysts sparse, intercalary or lateral; hormogones 10-14 μ broad and 40-60 μ long; conidia and planococci formed at the ends of filaments.

Order: Stigonematales

Family: Rivulariaceae

Calothrix castelli

Thallus spongy, cushion shaped, widely expanded, dull blue-green, surface pubescent by projecting ends of filaments; filaments bent, erect, densely aggregated, 12-13 μ broad, swollen at the base and prostrate, 4-8 mm long; sheath thin close to the trichome

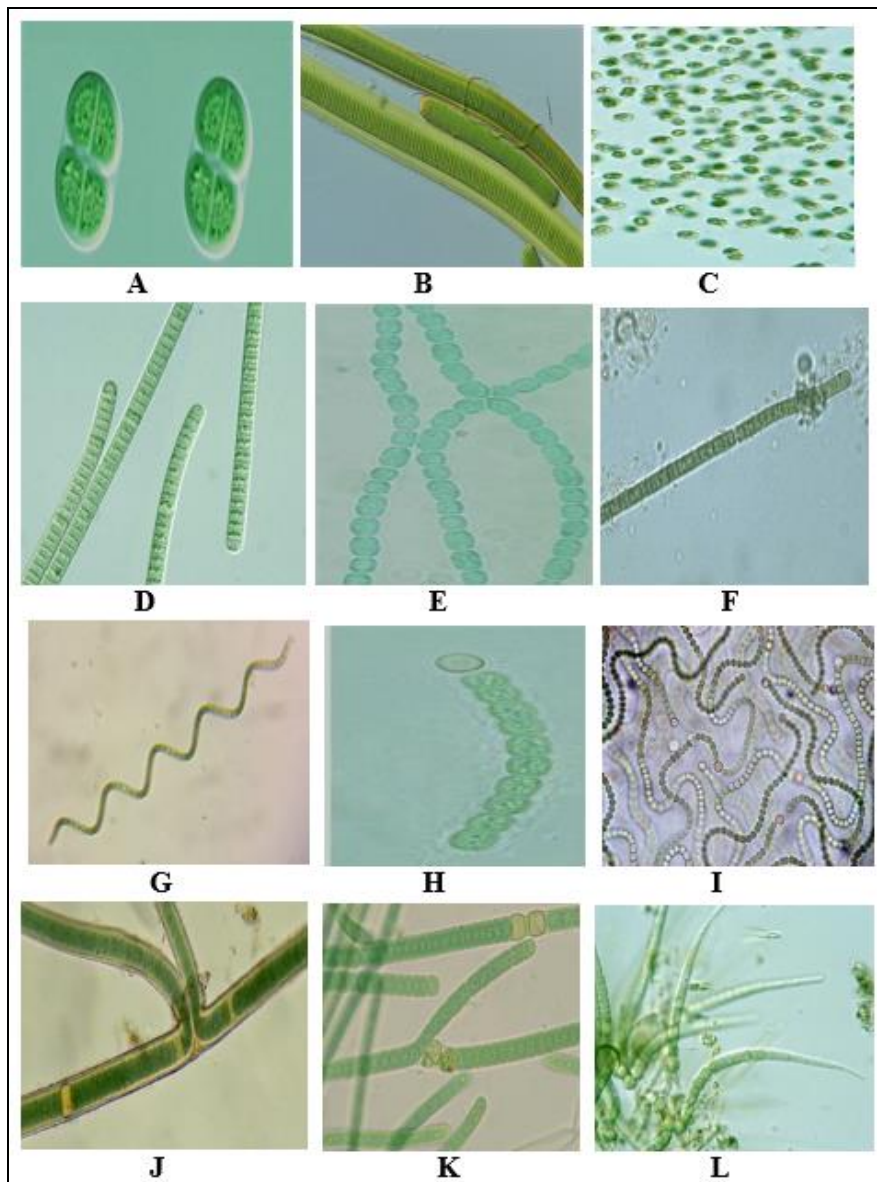


Fig 1: Some characteristic species found in the Azamgarh and Varanasi (U.P.) of "Usar" soils: (A) *Chroococcus macrococcus* (B) *Lyngbya arboricola* (C) *Microcystis aeruginosa* (D) *Oscillatoria subbrevis* (E) *Anabaena doliolum* (F) *Phormidium anomala* (G) *Spirulina laxissima* (H) *Nostoc calcicola* (I) *Nostoc linkia* (J) *Scytonema coactile* (K) *Tolypothrix byssoidea* (L) *Calothrix castelli* (M) *Microchaete calothricoides* (N) *Haplosiphon welwitschii* (O) *Stigonema dendroideum*

Conclusion

The current study deals with the species level taxonomic documentation of cyanobacteria from Varanasi and Azamgarh districts. Varanasi selected one site Bhawanipur and Azamgarh collected two sites Alipur and Bunda. The Cyanobacteria belonging to 3 orders (Chroococcales, Nostocales and Stigonematales) and 7 families (Chroococcaceae, Oscillatoriaceae, Nostocaceae, Scytonemataceae, Microchaetaceae, Stigonemataceae and Rivulariaceae) were identified. Of the 18 cyanobacteria genera collected in the present study, were identified as *Nostoc calcicola*, *Nostoc linkia*, *Scytonema coactile*, *Lyngbya arboricola*, *Anabaena doliolum*, *Tolypothrix byssoidea*, *Oscillatoria subbrevis* and *Calothrix castellii* were recorded. The study concluded that the Azamgarh and Varanasi districts rich in cyanobacterial diversity and there are heterocysts bearing cyanobacteria and unicellular types are prominent. *Nostoc* and *Anabaena* are known to be most versatile diazotrophic cyanobacterial genera, observed in all types of environment, existing both in free living and symbiotic state. Such halotolerant N₂-fixing cyanobacterial strains can be used to improve prove properties of salt affected soils due to their survival under stress condition.

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