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Cyanobacteria: Isolation, Purification and Principles

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ABSTRACT: The primary means of nutrient uptake for cyanobacteria is oxygen-producing photosynthesis. Their ecological variety is astounding; they occupy a very wide range of lighted ecological niches in terrestrial, marine, and freshwater habitats. Despite this apparent metabolic consistency, they exhibit tremendous phylogenetic diversity. The fact that cyanobacteria have certain physiological and metabolic traits that are exclusively seen in prokaryotes significantly broadens this spectrum. The capacity to fix nitrogen in an aerobic manner under light is a special characteristic. Apart from Gonotheca species, all aerobic, nitrogen-fixing cyanobacteria—a structurally diverse group—produce heterocyst, the highly specialised cells that allow them to fix nitrogen efficiently in a fully aerobic environment by preventing the oxygen-sensitive enzyme nitrogenase from being inactivated quickly in vivo (Hazelton, 1978; Stanier and Cohen-Bazire, 1978; Stewart, Haystead, and Pearson, 1969). The same quantities of cyanobacteria could be isolated from freshwater using a technique using nutrient-saturated glass fibre filters, but the quantity of accompanying heterotrophic bacteria was reduced by 2- to 15-fold. a broad-spectrum antibiotic called imipenem. In comparison to some other Plactam antibiotics, the B-lactam antibiotic that inhibits peptidoglycan biosynthesis, was more effective at lowering the levels of heterotrophic bacterial contaminants associated with newly isolated cyanobacteria to a point that made it easier to grow axenic cyanobacterial cultures.

KEYWORDS: Blue Green Algae, Cyanobacteria, Photosynthetic Bacteria, Prokaryotic.

INTRODUCTION

Cyanobacteria are a varied category of Gram-negative photosynthetic prokaryotes in terms of morphology. In practically every imaginable ecosystem on Earth, they may be found, which makes them unique (Ferris et al., 1996; Ward et al., 1997; Nubel et al., 1999; 2000; Abed and Garcia-Pichel, 2001; Garcia-Pichel and Pringault, 2001; Abed et al., 2009; Sharma et al., 2011). According to Sinha and Hader, 1996; Zehr et al., 2000; Kalib, 2002; Saha et al., 2003, they may live in harsh settings such hot springs, rocky beaches, drought, desiccation, osmotic, and UV stressors, photooxidation, heat and cold shock, anaerobiosis, and nitrogen deprivation, among others. Because of their innate ability to use nitrogenase and rubisco enzymes, respectively, to fix atmospheric CO2 and N2, cyanobacteria play a key role in the worldwide cycle of nutrients (Sinha et al., 1995; 1997). Certain cyanobacteria can fix atmospheric nitrogen by forming heterocysts (Capone et al., 2005). Cyanobacteria were previously only of academic interest and were largely disregarded as a nuisance, but recent research has shown them to be potential candidates for extensive biotechnological use (Richmond, 1990; Sundararaman and Sekar, 2001; Thajuddin and Subramanian, 2005; Govindjee and Shevela, 2011). Due to their historical placement among the algae, phycologists working under the guidelines of the Botanical Code devised the categorization of these species (Stafleu et al., 1972).

For research on physiology, genetics, and taxonomy, cyanobacteria must be grown in axenic cultures. Axenic cultures of microscopic and macroscopic cyanobacteria are typically made by single-cell isolation using a variety of techniques, such as serial dilution technique, streak plate method, UV irradiation, filtration, treatment with different antibiotics (Rippka, 1989; Choi et al., 2008), other germicidal chemicals (Kim et al., 1999), density gradient centrifugation, and rinsing (Vaara et al., 1979; Bolch and Blackburn, 1996).Given the poor success rate, dealing with cyanobacteria can be difficult and time-consuming, especially when trying to acquire axenic cultures. For several reasons, estimation and conservation of cyanobacterial biodiversity from yet unexplored habitats become very important, which need to be initiated with \systematic survey followed by collection, establishment of pure culture and their \characterization (Biswas, 1930; 1934; Bordoloi, 1980; Singh et al., 1997; Singh et al., \s1997a, 1997b; Ahmed et al., 1999; Devi et al., 1999; Rout and Dey, 1999; Singh et al., \s2011). Because of their prevalence and distinctive hues of green, blue green, and olive green in nature, cyanobacteria can be distinguished from other creatures with ease. Although there are some signs of cyanobacteria in nature, such as these, they must be inspected under a microscope and their pigment composition determined (Rippka, 1979).

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Even though cyanobacteria with a diversity of morphologies can be found in many different terrestrial and aquatic environments, research on these bacteria has only focused on a small subset of them. This appears to be partially due to issues that arose during the isolation and later purification of these microbes. The number of cyanobacterial species that can be easily grown has been hypothesised to be heavily constrained by the methods typically used to separate them. Agar is frequently used as a solidifying agent in bacterial media and is known to contain contaminants. Some of these impurities are thought to be the cause of the repeated observations that agar inhibits the development of some cyanobacteria. Various strategies have been employed to lessen or get rid of agar's growth-inhibitory impacts. Low agar amounts have been used, as have agar-washing techniques, the cleaning of agar and nutrient solutions separately, and the use of agarose or other substitute solidifying agents. The effort required to create axenic cultures is frequently laborious and time-consuming, compared to the low success rate, which is perhaps an even more aggravating aspect of working with cyanobacteria. To create more effective ways to cleanse tainted cyanobacteria, a variety of strategies have been used. As part of our strategy, we sought to completely do away with the use of agar or any other organic hardening agents in our separation media and looked for efficient ways to produce axenic cultures of cyanobacteria. Here, we reveal that glass fibre screens can replace organic solidifying agents effectively and that imipenem, in contrast to some other P-lactam antibiotics, a relatively new, broad-spectrum, -lactam antibiotic that inhibits bacterial peptidoglycan biosynthesis is more efficient at lowering the quantity of heterotrophic bacterial contaminants present with freshly isolated cultures of cyanobacteria, assisting in the development of axenic cultures.

MATERIALS AND METHODS

MEDIA AND NUTRITIONS

The components of BG-13 medium were as follows: NaNO3 (1.5 g), NaHCO3 (1.7 g), K2HPO4 (31 mg), MgSO4 7H20 (75 mg), CaCl2 H20 (36 mg), Na2CO3 (20 mg), Citric Acid (6 mg), Ferric Ammonium Citrate (6 mg), Disodium Magnesium EDTA (1 milligrams), H3BO3 (2.86 mg), Mn2Cl2 4H20 (1 (40, g). When kept in an environment containing 5% (vol/vol) CO2 in air, BG13 medium had a pH of 7.5 to 7.6. The pH 7.5 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) buffer was present in BG-12 medium, which was made up of BG-13 medium but without the sodium bicarbonate. pH 7.5. For some tests, cycloheximide and nystatin were added to the BG-12 and BG-13 media at a concentration of 100 ug/ml for each, as well as modest concentrations of organic nutrients as described in the text. A Milli-Q water system was used to purify the water used to make the BG-12 and BG13 media to an impedance of 18 Mil (Millipore). 1.5% (wt./vol) Noble agar was used to make all agar plates (Difco). All media were autoclaved for 20 minutes at 15 lb/in2 to ensure their sterility. According to Allen's technique, double-strength agar and doublestrength nutrient solution were made, sterilised individually, and then mixed for BG-12 and BG-13 agar media (3). In order to create BG-13 glass fibre plates, a Pyrex glass petri plate measuring 100 by 20 mm was filled with five Whatman 934-AH glass fibre filters with a width of 90 mm, covered, and sterilised for 30 minutes at 260°C. 20 cc of clean BG-13 medium was infused into the filters after cooling. Under an atmosphere of 5% (vol/vol) CO2 in air, inoculated dishes were kept for 25 hours. The dishes were exposed to 3 to 5 lux of irradiance from 40-W cool-white, fluorescent bulbs. The same temperature, atmosphere, and lighting circumstances as specified for the dishes were used to shake the broth cultures at 180 to 200 rpm. Other than this we also use media enriched with components like The chemicals used were of laboratory quality and included sodium nitrate, dipotassium phosphate, magnesium sulphate, ammonium chloride, calcium chloride, ferric chloride, potassium dichromate, 1, 5 diphenyl carbazide, methanol, hydrogen sulphate, glucose, and ferrous ammonium sulphate. The distilled water used to make these chemicals and media.

COLLECTIONS OF SAMPLES

WATER SAMPLES

Water samples from various ponds, lakes, and rivers in various locations from Maharashtra and Haryana India, were gathered for the current research**.**

SOIL SAMPLES

Soil samples were collected from several sites in Maharashtra and Haryana. Throughout the year 2020, cyanobacterial samples from different environments were collected at various periods (dry soil, wet soil, aquatic bodies, and rocky substratum).

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COLLECTION METHODS:

FOR SOIL SAMPLES:

The scalpels were used to gather soil samples from 7–18-centimetre depth earth layers. The scalpel was inserted into the earth and moved in a circular before being raised up with the soil. To maximise gathering, random collections were taken from various locations within a particular area. Soil samples were gathered and labelled in polythene resealable pouches measuring 5 x 6 inches. Now of gathering, the following information was recorded: location, habitat, date of capture, temperature, and soil type. In order to obtain the greatest variety, soil samples were gathered from well-watered, rarely irrigated, and dry regions. Fresh biomass was also gathered from the algae adhered to the rocky substratum and stored in airtight plastic vessels. The sample vessels were branded with a permanent marker. These labelled plastic sacks and jugs containing gathered biomass were taken to the laboratory and used for isolation and identification. The soil samples were shade dried and kept in wide mouth screw closure glass vials.

FOR WATER SAMPLES:

Freshwater and sediment samples were gathered from different locations throughout the Indian states of Maharashtra and Haryana.

PRESERVATION OF SAMPLES:

FOR SOIL SAMPLES:

All samples were stored individually in a 4% formalin solution. Each 50 ml 4% formalin solution was put into a 100 ml sterile receptacle for storage, and the sample was filled. The receptacles were stamped with the location of the sample and the date. These conserved samples were used for morphological research as well as identifying.

FOR WATER SAMPLES:

Water samples were inoculated in standard conical borosilicate flask or Erlenmeyer flasks containing algal culture medium (composition in gm/lit; sodium nitrate 0.1gm, dipotassium phosphate 0.25 gm, magnesium sulphate 0.051 gm, ammonium chloride 0.0051gm, calcium chloride 0.005gm, and ferric chloride 0.0005 gm) and incubated at room temperature under continuous dark

A) SOIL SAMPLING TECHNIQUE:

ENRICHMENT TECHNIQUE:

The percentage of cyanobacterial cells was minimal in some of the samples. Enrichment cultures were used to raise the cell population of wanted cyanobacteria in earth samples in order to enable isolation of cyanobacteria from such samples. Garden dirt was used to create the soil water substrate for this. The medium was put into culture flasks and sanitised for 30 minutes at 15 lbs and 121℃. The samples were injected with this medium and kept at 28±2 ℃ .under 20 mol m-2 s-1 light.

LIQUID ENRICHMENT TECHNIQUE:

This technique was helpful for rapidly isolating cyanobacteria. Because of multiple liquid subcultures, the cyanobacterial species that were prevalent in the inoculated sample survived in medium. All species present in inoculated samples grown evenly on medium in a streak plate or other technique used for cyanobacterial separation. BG11 medium was made and put into sterile test tubes. The civilizations that grew quickly and well were homogenised with a mixer and pestle. A few strands from these homogenised colonies were rinsed three times with sterile BG11 medium. In test tubes, 2 to 3 of these strands were seeded on sterile BG11 medium. All these efforts were made in front of laminar air movement. In the culture chamber, these test tubes were maintained at 282℃ temperature under 26.66 mol m-2 s-1 light (cool-white, fluorescent light, 8 hr light 16 hr dark cycle).

ISOLATION AND PURIFICATION OF CYANOBACTERIA FROM SOIL SAMPLES:

The isolation of individual cyanobacteria species from crude samples was critical for the creation of clean colonies. Other bacterial and fungus particles may be present in the field samples because they may contain more than one cyanobacterial species. Single species must be isolated from these samples. Mechanical separation of cyanobacterial species from other organisms is widely regarded as the most acceptable technique for getting pure cyanobacterial cultures. The following techniques were used to isolate and purify cyanobacterial strains collected from different locations.

DIRECT ISOLATION:

An outdoor sample was examined under a microscope. Aliquots of materials with uniform colonies or filament were immediately seeded in test containers on BG11 nutrient medium solidified with 1% agar. Some samples were immediately inoculated in culture flasks with 30 cc clean BG11 medium. Under these conditions, the vessels were fermented. At a temperature of 282℃ and a light intensity of 26.66 mol m-2 s-1 (Philips cool-white LED light, 8 hr light 16 hr dark cycle).

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SERIAL DILUATION TECHNIQUES:

This technique is founded on the idea that the sample mixture becomes dispersed, and subsequent dilutions result in the isolation of single species. In 9 millilitre of sterile distilled water, 1 gm of material was combined. After that, 1 mL of this combination was mixed with 9 mL of clean distilled water. This procedure was done until the material was diluted 1/10 (10-fold) (Allen and Stanier, 1967, Castenholz, 1988). Following that, 1 ml of this combination was used as an inoculum and was distributed on newly made solidified nutrient media on petri plates (agar plates).

STREAK PLATE TECHNIQUE:

The streak plate technique was used to separate specific cyanobacteria species. This aids in the separation of individual cyanobacterium clusters on agar. The streak plate technique was used to isolate cultures that were healthy and developing quickly. 1.2% Agar was used to solidify the BG11 material. The medium was put into Petri plates with a diameter of 90 millimetres (Anumbra). 20 cc BG11 medium was poured into each petri plate and left to settle and solidify. The sample culture was blended with a grinder and pestle. This pulverised sample was streaked with an inoculating (nichrome) loop onto hardened BG11 medium. The Petri plates were covered with parafilm and incubated in a culture chamber at 282℃ temperature and 26.66 mol m-2 s-1 light (Philips cool-white light, 8 hr light 16 hr dark cycle). The complete procedure was carried out aseptically in front laminar air flow.

IDENTIFICATION OF THE BACTERIA:

Slides were made and examined under the trinocular microscope to identify the cyanobacterial samples. Morphometric research was carried out using stage and eye micrometry. Prescott (1951) and Desikachary (1951) books and keys were used for identifying (1959). Desikachary's classification scheme was used to organise the found taxa (1959). Following determination, the micro-preparations were photomicrographed**.** All the identified cyanobacteria were photographed using a trinocular research microscope at 40x and 100x magnifications.

EASTABLISHMENT OF CULTURAL MEDIA:

NUTRIENT MEDIA

BG11 medium was used for culture introduction, development of laboratory culture, and pure culture. Dow Inc provided all laboratory quality chemicals. Double purified water was used to make the stock solutions. The compositions of the standard solutions were 100 times greater than those indicated in the BG11 medium. The standard remedies were all kept in the refrigerator.

PREPRATION OF STOCK SOLUTIONS:

The macronutrient and mineral stock solutions for BG11meduim were made according to the chart.

MEDIA PREPRATION

The necessary stock solutions of the BG11 medium's nutrient makeup were added as shown in table I. All macronutrient stock solutions were added with a concentration of 10ml L- and 1 ml L- of micronutrient stock solution were added first in a small amount of double distilled water, then the end volume of required quantity was raised with double distilled water. With 1N HCl, the pH was corrected to 7.5.

Table no 1 – Media composition

Composition	Concentration (mg/L)
NaNO3	1500
$K_2HPO4\ 3H_2O$	52
K_2HPO4	40
$MgSO4.7H_2O$	75
$CaCl_2\ 2H_2O$	36
$CaCl_2$	27
Na_2CO3	20
$C6H8O7\ H_2O$	6
(NH4)3FeC12H10014	6
$C10H14N2Na2O8\ 2H_2O$	1

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H3BO3	2.86
$MnCl_2$ $4H_2O$	1.86
$ZnSO4.7H_2O$	0.22
Na_2MoO4 $2H_2O$	0.39
$CuSO4.5H_2O$	0.08
Co(NO3)2 6H2O	0.05

CULTURE INNOCULATION:

A) STREAK PLATE TECHNIQUE:

A colony or filament from a streak plate culture was gathered up by an inoculum loop and placed in test containers with 10 ml of clean BG11 medium. These colonies or strands were selected from various zones in petri dishes and seeded in test tubes. In test tubes, filaments from cultures produced by direct isolation were sub cultured on newly prepared nutrient media. All these test tubes were maintained in a culture chamber at 28±2℃temperature and 26.66 mol m-2 s-1 light (cool-white, fluorescent light, 8 hr light 16 hr dark cycle).

B) BROTH CULTURE TECHNIQUE:

After growing in test tubes, the cultures were sub cultured in 400 ml culture flasks on 50 cc sterile BG11 medium. The pH of the BG11 nutrient solution was set to 7.5. Each 50 millilitre medium container was filled with medium and sanitised in an autoclave at 1.06 kg cm-1 for 20 minutes. Following inoculation, the flasks were incubated in a culture chamber at 28±2℃ temperature and 26.66 mol m-2 s-1 light (cool-white fluorescent light, 8 hr light 16 hr dark cycle).

SUBCULTURING:

All cultures were sub cultured again after a 21-day delay. Every time, the cultures were sub cultured in culture flasks with 50 mL of newly made nutrient medium. The culture from one culture bottle was sub cultured in two culture bottles to acquire biomass, and the culture was moved in three culture bottles from one culture bottle in subsequent subcultures.

B) WATER SAMPLING TECHNIQUES:

CHEMICALS:

Fresh aqueous stock solutions (0.5% [wt./vol]) of cefoxitin, ampicillin, penicillin G, imipenem, and cycloheximide (1.25% [wt./vol]) were made, sterilised by filtering, and added to chilled molten agar or broth media. Nystatin stock solutions (10% [wt./vol]) were made in N, N-dimethylformamide. Hi MEDIA provided the penicillin G (potassium salt), Sigma provided the ampicillin (sodium salt), the cycloheximide and nystatin. Merck Sharp & Dohme Research Labs provided the cefoxitin (sodium salt) and imipenem. All other chemicals were of the greatest purity possible.

ENUMERATION OF BACTERIAL COLONIES:

Freshwater and sediment samples were gathered from different locations in Maharashtra and Haryana. A sample was agitated to dislodge sediment, and then duplicate 10-pd aliquots were extracted and dissolved into 100 ml of sterile distilled water, which was vacuum filtered through a sterile 47-mm-diameter polycarbonate membrane filter (0.4-, um pore diameter; Nucleopore). The filters were aseptically moved, inoculum side up, onto plates of BG-12 or BG-13 medium containing nystatin and cycloheximide. After 14 days, the cyanobacterial colonies developing on the surface of the membrane filters were enumerated using a dissecting microscope. The mean CFU per filter was determined, and the difference between means was examined using the Student's t test (95% confidence interval).The number of heterotrophic bacteria present on the surface of a membrane filter was then determined by immersing each filter in 10 ml of sterile dilution buffer (, 1.2 g/litre; NaNO3, 1.5 g/litre; pH 7.5) and dispersing the cells for 3 minutes in an ultrasonic cleaning bath . The resulting cell suspensions were serially diluted and plated in triplicate on BG-12 medium enriched with glucose (1.0 g/litre), nutrient broth (0.8 g/litre), and cycloheximide.

After one week of incubation in the dark at 25°C in an ambient environment, the bacterial colonies on the dishes were enumerated using a colony metre . The mean CFU per filter was determined, and the difference between means was examined using the student's t test (95% CI). Similarly, the number of heterotrophic bacterial contaminants present in cyanobacteria broth cultures

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was determined by plating serial dilutions of the broth culture in duplicate on BG-12 medium containing nystatin and cycloheximide and supplemented with glucose, yeast extract, and Bacote-Peptone, all at 100 mg/litre.

PURIFICATION OF CYANOBACTERIAL STRAINS BY RUNNING ANTIBIOTIC TESTS:

A Contaminated cyanobacterial colonies were picked off the surface of membrane filters overlaid with glass fibre medium and moved into a 50-ml beaker containing 20 ml of sterile BG-12 medium. The mixed culture was incubated for 3 to 4 weeks under the previously described conditions to obtain sufficient cyanobacterial biomass, and then 400, ul of a sterile nutrient solution (SNS) consisting of 2.5% (wt/vol) sucrose, 0.5% (wt/vol) yeast extract, and 0.5% (wt/vol) Bacote-Peptone was added, along with 400 RI of sterile antibiotic solution (see below). The culture was then cultured for 18 to 24 hours in the dark at 180 to 200 rpm under a 5% (vol/vol) CO2 in air environment. After culture, the cyanobacteria were collected by centrifugation at 17,000 x g for 15 minutes at 25°C. The cells were washed twice by centrifugation in quantities of clean BG-12 medium equivalent to the original culture volume before being suspended in 1/10 of the original volume. To create a cell suspension that could be pipetted and plated onto BG-12 agar containing nystatin and cycloheximide, the cyanobacterial cells were typically lightly dispersed with a sterile tissue homogenizer. The plates were left in incubator for 2 to 4 weeks and cyanobacteria development was monitored at weekly intervals. Purified colonies or filaments of cyanobacteria were picked and moved to dishes of BG-12 agar using a dissecting microscope (x 10 to x 50 magnification). The purity of the isolates was verified using phase-contrast microscopy (x 500 to x1,250 magnification) and by growing cyanobacterial cultures in BG-12 broth enriched with 0.01% (wt/vol) glucose, yeast extract, and Bacote-Peptone and into GNB broth medium, which contained 1.0% (wt/vol) glucose and 0.8% (wt/vol) nutritional broth. Broth cultures were incubated at 25°C with stirring at 180 to 200 rpm under ambient circumstances and statically under anaerobic conditions in an anaerobic container (Gas-Pak; BBL). After a month of incubation, the cultures were deemed axenic if no growth of heterotrophic bacteria was detected under any of the circumstances. Cyanobacteria were identified using phase-contrast imaging and growth traits.

RESULTS

A) FOR SOIL SAMPLING:

STUDY OF THE FLORA RELATED TO THE CYANOBACTERIAL CULTURE:

Complete cyanobacterial samples were gathered from various locations in Maharashtra and Haryana. Among the samples gathered, 20 cyanobacterial species pertaining to 13 genera, 8 families, and 3 orders were discovered. The Nostocales order was found to be dominant with 85% frequency among the three orders of cyanobacteria, and the Nostocaceae family was found to be dominant with 40% frequency among the families. Stigonematales (10%) and Chroococcales (5%) were discovered only infrequently in soil samples taken from all three districts. After the Nostocaceae family, the Oscillatoria family (30%) was prominent. Groups such as Chroococcaceae, Scytonemataceae, Macrochaetae, and Stigonemataceae were discovered to have very few families and species. Nostoc was identified to be the most common among the 13 families, accounting for approximately 38.46% of the total, followed by Anabaena (23.08%). The greatest relative frequency was found in Nostoc calcicola Brebsson ex Born. et Flah was discovered in samples with a relative frequency of 57.71%. Nostoc punctiforme Born. Et Flah. was discovered in 119 samples with 52.42% relative abundance, followed by Nostoc entophytum Born. Et Flah. in 102 soil samples with 44.93% relative abundance.Anabaena sp. was discovered in samples with a relative frequency of 37.89%.

GROWTH OF CULTURE FOUND IN STREAK PLATE AND ISOLATION:

Initially, the samples did not exhibit signs of development. After 6 days of inoculation, the development was sluggish. After direct separation on solid medium, the Nostoc and Anabaena cultures grew well.

After 4 days, the inoculum began to develop on agar dish. Most of the samples grew well on an agar dish after repeated dilution. After 4 weeks, most cultures' cultures were completely distributed on agar plates. After 3 weeks, the filaments of Oscillatoria had grown well and filled most of the area of the petri dishes. While Phormidium and Lyngbya grew slowly. After 24 days of injection, these species grew.

IDENTIFICATION OF SAMPLES:

There are a total of 20 cyanobacterial types, including Microcystis aeruginosa. Spirullina platensis Kütz (Nordst.) Geitler, Oscillatoria sp. Vaucher Phormidium is a delicate plant (Meneghini)Gomont, Lyngbya majuscula Harvey ex. Gomont, Lyngbya sp., Microcoleus lacustris (Rabenh.) Farlow, Nostoc punctiforme Born. et Flah., Nostoc calcicola Brebsson ex Born. et Flah., Nostoc entophytum Born. et Flah., Nostoc ellipsosporum (Desm.) Rabenh. Ex Born. et Flah., Nostoc muscorum Ag. ex-Born. at Flah.

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Anabaena ambigua, Anabaena subcylindrica, Anabaena sp., Scytonema mirabile (Dillw.) Born., Microchaete uberrima N. Carter, Calothrix javanica de Wilde, Hapalosiphon welwitschii . West, and Westiellopsis prolifica Janet was recognised.

B) FOR WATER SAMPLING:

ISOLATION AND COLLECTION OF CULTURE

Water sample was inoculated in Erlenmeyer flask with algal culture medium (composition in gm/lit; sodium nitrate 0.1gm, dipotassium phosphate 0.25 gm, magnesium sulphate 0.051 gm, ammonium chloride 0.0051gm, calcium chloride 0.005gm, and ferric chloride 0.0005 gm) and incubated at room temperature under continuous dark and sunlight period of 15-20 days. Isolated colonies were examined under a microscope for physical characteristics. Forceps were used to disseminate isolated culture on a glass slide for microscopic viewing. The cultures were covered with glass cover slides and examined using a compound light microscope's low (10X) and high power (45X) objective lenses.

Table no 2– Characteristics of Isolates

Sr.No.	ISOLATES	MICROSCOPIC OBSERVATIONS	MORPHOLOGY
1	CN-1	Short, straight, parallel, fibrous, and heterocystous, soft, green viscous, membranous cells.	Anabaena species
2	CN-2	Spherical, unicellular, non-filamentous, and non-heterocystous cells.	Gloeothece species
3	CN-3	Individual, golden brown, filamentous, unevenly curled, bulbous at the base, and heterocystous.	Calothrix species
4	CN-4	Bluish green, viscous, densely packed cells that are oblong, uneven, filamentous, and heterocystous.	Nostoc species
5	CN-5	Extended, fibrous, entangled, and heterocytes.	Plectonema species

DISCUSSION AND CONCLUSION

The existence of cyanobacteria from three orders, eight families, thirteen genera, and twenty species is revealed in this research. The cyanobacterial flora is abundant in the research region. The most prevalent order, Nostocales, contains 17 species divided into 10 groups and 5 families: Oscillatoriaceae, Nostocaceae, Scytonemataceae, Microchaetaceae, and Rivulariaceae. Six species from the family Oscillatoriaceae were discovered, with Lyngbya having two species and Spirulina, Oscillatoria, Phormidium, and Microcoleus lacustris each having one. The Nostocaceae family contained eight species divided into two families. The study region contained 5 Nostoc species among the 4 genera and 21 species identified. Similarly, Venkataraman (1975) identified Nostoc sp. as the prevalent fungus in Assam, Haryana, Kerala, Tamil Nadu, and West Bengal. Nostoc species predominated in the paddy paddies of Orissa, according to Sahu et al. (1996). Auti and Pingle (2006) discovered Nostoc as the most prevalent species in Maharashtra's Northern Circle. In contrast, Madane and Shinde 25 (1993) found an unusual abundance of Nostoc sp. from salt-affected soils in the some regions of Maharashtra region.

The Scytonemataceae family is characterised by a single species and families. According to Madane and Shinde (1993), the Scytonema mirabile species is prevalent in salty soils. The families Microchaetaceae and Rivulariaceae are characterised by single species and groups. In comparison, Auti and Pingle (2006) did not find Calothrix in Northern Circle. The order Stigonematales is made up of two families (Mastigocladopsidaceae and Stigonemataceae), each with a unique genus and species.

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