

Cyanobacterial Richness in Paddy fields of Saidabad Block, Prayagraj District, UP, India

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ABSTRACT

Cyanobacteria are the common microbial population present in paddy fields. Their presence and diversity indicate the health of the soil and also give an idea of the types of fertilizers used in the field. This study on isolation and identification of cyanobacterial strains was carried out for the first time from ten paddy fields in Saidabad block, Prayagraj district, to identify the local and dominant strains for future preparation and application of biofertilizer in different crop fields. These strains also have the prospect of being utilized as liquid trees for the purification of the environment at the study site. In the present work, a total of 37 cyanobacterial species belonging to 26 genera and ten families were recorded. Data indicates the dominance of filamentous heterocystous types like *Nostoc*, *Anabaena*, *Aulosira*, and *Gloeotrichia* in paddy fields. However, the frequency of occurrence and species diversity were the maximum of non-heterocystous form *Oscillatoria*. Nostocales was the most dominant order with representatives of all its five families, i.e., Oscillatoriaceae, Microchaetaceae, Nostocaceae, Scytonemataceae, and Rivulariaceae.

Highlights

- Cyanobacteria are ubiquitous, prokaryotic organisms that commonly inhabit paddy fields. Their occurrence in soil improves soil characteristics and adds nutrients and growth substances to the soil that enhance plant productivity.
- The study isolated and identified the region-specific cyanobacterial strains from paddy fields of Saidabad block, Prayagraj district. It is important as indigenous strains are well adapted in their local niche and give optimum benefits to plants, soil, and environment when utilized as biofertilizers.
- The study area showed the dominance of filamentous heterocystous types like *Nostoc*, *Anabaena*, *Aulosira*, and *Gloeotrichia* in paddy fields.
- Heterocystous forms were more in overall species diversity, but the frequency of occurrence and number of species was found to be the maximum of a non-heterocystous form *Oscillatoria*.
- Nostocales was the most dominant order with representatives of all its five families i.e. Oscillatoriaceae, Microchaetaceae, Nostocaceae, Scytonemataceae, and Rivulariaceae.

Keywords: Cyanobacteria, Species diversity, Nitrogen fixation, Saidabad block.

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INTRODUCTION

Cyanobacteria are photosynthetic prokaryotes that are ubiquitous and can grow at any place and in any environment where air, sunlight and moisture are available. The paddy field is one such ecosystem that supports the luxuriant growth of cyanobacteria. Their abundance and diversity in terms of number and species, particularly in paddy fields, is due to the unique ecological environment of paddy wetlands. The occurrence of cyanobacteria in paddy fields shows a type of symbiotic relationship in which paddy fields provide appropriate niches to cyanobacteria, the plant provides food and space while cyanobacteria give nitrogen, phosphorus, other minerals and growth-promoting substances such as hormones, vitamins and amino acids to the plants besides improving soil health (Song *et al.*, 2022). Cyanobacteria or BGA are multipurpose organisms that require minimal conditions for growth. They are used as biofertilizers as they enrich the soil with nutrients and growth-promoting substances, reclaim the salt-affected soil, improve the physical and chemical properties of soil and bioremediate the waste in the environment (Usharani and Naik, 2019; Kumar and Rai, 2020; Jan *et al.*, 2023). Their role in the purification of the environment in the form of liquid tree is the current field of research (Dhar *et al.*, 2023). Cyanobacteria can also boost

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agricultural practices in arid regions by overcoming challenges such as water shortage, high temperatures, high salinity, and infertile soil (Bibi *et al.*, 2024). Saidabad block of Prayagraj district is an agricultural area with unexplored ecology and diversity of cyanobacteria. The documentation on cyanobacteria helps in understanding the nutrient status of the field and might be applied for sustainable agricultural practices by controlling the application of chemical fertilizers. Furthermore the information

on dominant strains could be utilized in the preparation of area-specific biofertilizers for enhancing productivity in other crops. Experiments conducted using cyanobacterial biofertilizers or inoculants on growth performance, yield and nutritional status showed positive results in the improvement of plants like *Lupinus termis* (Haroun *et al.*, 2003), pea (Osman *et al.*, 2010), wheat (Rana *et al.*, 2012), cotton (Prasanna, 2015), maize crop (Prasanna, 2016), and *L. luteus* (Shedeed *et al.*, 2022). In the present study, an attempt has been made to isolate and identify the local cyanobacterial strains for the first time from Saidabad block (Prayagraj district, Uttar Pradesh, India). These strains will be utilized in the production of biofertilizers and as a prospect in the purification of the environment in the form of liquid trees.

MATERIALS AND METHODS

Soil Sampling Site

Soil samples were collected from paddy fields of Saidabad block, Prayagraj district (Uttar Pradesh), during the rice cultivation period at the panicle initiation stage. The block, having a geographical area of 191.42 km² is situated at latitude and longitude coordinates of 25.370766 and 82.109756, respectively. The major source of income in the block is agriculture. Here rice is grown as a kharif crop with a sowing and harvesting period between June - July to November - December.

Collection of Soil Samples

Soil samples were collected at the panicle initiation stage from paddy fields of ten villages: Amora, Bajaha Mishran, Beerpur, Binda, Dhokari Holagarh, Garaghanpur, Kaithwal, Mahuwa Diha, Sarai Mansoor and Saidabad. Five soil samples were taken from randomly selected spots of each of the ten fields by scraping the upper soil layer having green patches. These soil samples were collected in sterilized air-tight polythene bags and transported to the botany laboratory of DDU Government PG College, Saidabad, Prayagraj, for analysis. Soil samples of each field were homogenized and mixed thoroughly to make ten composite soil samples.

Taxonomic identification of Cyanobacteria

A suspension of each of the composite soil samples of ten fields was prepared by dissolution of one gram of soil in 10ml sterilized distilled water. A few drops of this suspension were observed under the research microscope Magnus MX21i Tr for identification of dominant cyanobacterial species by observing characteristics like the shape of cells, presence or absence of heterocyst, akinete and trichome sheath. The growth pattern, color and texture of the colony were also observed in culture media as an identification source. The identification of cyanobacterial species was made using standard monographs suggested by Halder (2015).

Isolation, Purification and Culture Maintenance of Cyanobacterial strains

Serial dilution of 10⁻¹ until 10⁻⁶ was carried out for each of the ten composite soil samples. Each dilution was plated on nitrogen-free BG-11 medium at 25 ± 2°C in a culture room under continuous light intensity of 1200 to 1400 lux for 10 to

12 days. After the growth of cyanobacterial colonies, a dilution factor of 10⁻⁵ was found suitable for CFU (Colony Forming Units) calculation. Individual strains were transferred to fresh medium for unialgal culture. These cultures were used for morphological identification and kept for future preparation and application of biofertilizers. Pure cultures were maintained by renewing the unialgal strains after a definite time interval to avoid contamination.

Data Analysis

Frequency of Occurrence (%) = $\frac{\text{Number of samples containing the species}}{\text{Total number of samples studied}} \times 100$

CFU (/ml) = $\frac{\text{No. of visible colonies on petriplate} \times \text{Total dilution factor}}{\text{Volume of culture plated in ml}}$

RESULTS AND DISCUSSION

About 50 soil samples collected from 10 independent fields located at a distance of about 5 to 10 km from each other showed 26 genera and 37 species of cyanobacteria belonging to 10 families. Among them, family Nostocaceae and Oscillatoriaceae showed maximum number of species (Fig. 1). On comparison of structural forms 19 species were filamentous heterocystous, 09 were filamentous non-heterocystous and 09 were unicellular (Table 1). Thus in the study area, filamentous heterocystous form was found to be more prevalent in distribution (Fig. 2). Order Nostocales was represented by all the five families, i.e., Oscillatoriaceae, Microchaetaceae, Nostocaceae, Scytonemataceae and Rivulariaceae showing better survival ability.

The maximum frequency of occurrence (90–100%) was of *Anabaena oryzae*, *Aphanocapsa bififormis*, *Gloeocapsa punctata*, *Lyngbya spiralis*, *Nostoc muscorum*, *Oscillatoria limosa*, *Oscillatoria princeps* and *Oscillatoria tenuis* while minimum frequency of occurrence (10–20%) was that of *Nodularia* sp., *Tolypothrix tenuis*, *Trichormus* sp., *Nostochopsis* sp., *Haplosiphon* sp., *Pleurocapsa microbewiki*, *Rivularia aquatica*, *Schizothrix calcicola* and *Scytonema* sp. The maximum species diversity was of *Oscillatoria* represented by four species (Table 2).

Earlier nitrogen fixation ability was considered as an attribute of heterocystous cyanobacteria. The study on variety of non-heterocystous BGA present in paddy field soil has revealed their ability to fix atmospheric nitrogen (Berrendero *et al.*, 2016). Paddy fields with stagnant water provide appropriate conditions to non-heterocystous cyanobacteria to fix atmospheric nitrogen in anoxic or micro-oxic conditions (Rajasekaran and Raja, 2021). *Oscillatoria* is a non-heterocystous alga that uses a temporal strategy in which the cells reduce nitrogen into ammonium during night with a halt in photosynthesis (Hendrayanti *et al.*, 2018). Aerobic nitrogen fixation in other non-heterocystous cyanobacteria has also been discussed by Fredriksson *et al.*, (1998) and Misra *et al.* (2003). Similar dominance of BGA has been observed in rice field surveys of other localities (Dey *et al.*, 2010; Choudhary, 2011; Srinivas and Aruna, 2016; Sebastian, 2017; Ghadage and Karande, 2019; Petkar *et al.*, 2020).

Native cyanobacterial species flourish in paddy fields as they can utilize optimum conditions of temperature, light and water needed for their growth. The isolation of region-specific strains is important as indigenous strains are well adapted in

Table 1: Structural forms of cyanobacteria observed in the study area

| S. No. | Structure | Family | Number of genus | Number of species |
|--------|-------------------------------------|--------|-----------------|-------------------|
| 1. | Filamentous heterocystous forms | 07 | 15 | 19 |
| 2. | Filamentous non-heterocystous forms | 01 | 04 | 09 |
| 3. | Unicellular forms | 02 | 07 | 09 |

Table 2: FO (%) of identified cyanobacterial sp. in studied samples of different paddy fields of Saidabaad block, Prayagraj district.

| S. No. | Family of Species | Name of Species | Structure | FO (%) |
|--------|-------------------|----------------------------------|-------------------------------|--------|
| 1. | Chroococcaceae | <i>Aphanocapsa biformis</i> | Unicellular | 90 |
| | | <i>Aphanothece stagnina</i> | Unicellular | 60 |
| | | <i>Chroococcus montanus</i> | Unicellular | 60 |
| | | <i>Chroococcus pallidus</i> | Unicellular | 70 |
| | | <i>Gloeocapsa atrata</i> | Unicellular | 80 |
| | | <i>Gloeocapsa punctata</i> | Unicellular | 90 |
| | | <i>Microcystis aeruginosa</i> | Unicellular | 70 |
| | | <i>Synechococcus aeruginosus</i> | Unicellular | 40 |
| 2. | Mastigocladaceae | <i>Mastigocladus laminosus</i> | Filamentous heterocystous | 40 |
| 3. | Microchaetaceae | <i>Microchaete aequalis</i> | Filamentous heterocystous | 30 |
| 4. | Nostocaceae | <i>Aulosira prolifica</i> | Filamentous heterocystous | 80 |
| | | <i>Anabaena cylindrica</i> | Filamentous heterocystous | 80 |
| | | <i>Anabaena oryzae</i> | Filamentous heterocystous | 90 |
| | | <i>Cylindrospermum muscicola</i> | Filamentous heterocystous | 30 |
| | | <i>Nodularia</i> sp. | Filamentous heterocystous | 10 |
| | | <i>Nostoc commune</i> | Filamentous heterocystous | 80 |
| | | <i>Nostoc muscorum</i> | Filamentous heterocystous | 90 |
| | | <i>Nostoc sphaericum</i> | Filamentous heterocystous | 70 |
| | | <i>Trichormus</i> sp. | Filamentous heterocystous | 10 |
| 5. | Nostochopsidaceae | <i>Nostochopsis</i> sp. | Filamentous heterocystous | 20 |
| 6. | Oscillatoriaceae | <i>Lyngbya rubida</i> | Filamentous non-heterocystous | 70 |
| | | <i>Lyngbya spiralis</i> | Filamentous non-heterocystous | 90 |
| | | <i>Oscillatoria tenuis</i> | Filamentous non-heterocystous | 90 |
| | | <i>Oscillatoria limosa</i> | Filamentous non-heterocystous | 100 |
| | | <i>Oscillatoria princeps</i> | Filamentous non-heterocystous | 90 |
| | | <i>Oscillatoria sancta</i> | Filamentous non-heterocystous | 80 |
| | | <i>Phormidium mucosum</i> | Filamentous non-heterocystous | 60 |
| | | <i>Schizothrix tenuis</i> | Filamentous non-heterocystous | 30 |
| | | <i>Schizothrix calcicola</i> | Filamentous non-heterocystous | 20 |
| 7. | Pleurocapsaceae | <i>Pleurocapsa microbewiki</i> | Unicellular | 20 |
| 8. | Rivulariaceae | <i>Calothrix braunii</i> | Filamentous heterocystous | 50 |
| | | <i>Gloeotrichia indica</i> | Filamentous heterocystous | 60 |
| | | <i>Gloeotrichia natans</i> | Filamentous heterocystous | 70 |
| | | <i>Rivularia aquatica</i> | Filamentous heterocystous | 20 |

| | | | | |
|-----|-----------------|---------------------------|---------------------------|----|
| 9. | Stigonemataceae | <i>Haplosiphon</i> sp. | Filamentous heterocystous | 20 |
| 10. | Scytonemataceae | <i>Scytonema</i> sp. | Filamentous heterocystous | 20 |
| | | <i>Tolypothrix tenuis</i> | Filamentous heterocystous | 10 |

Table 3: Colony Forming Units (CFU/ml) of identified Cyanobacterial strains at ten different sites of Saidabad Block, Prayagraj District, UP

| Sites | Localities | CFU/ml |
|-------|------------------|----------------------|
| 1 | Amora | 48 X 10 ⁶ |
| 2 | Bajaha Mishran | 35 X 10 ⁶ |
| 3 | Beerpur | 29 X 10 ⁶ |
| 4 | Binda | 65 X 10 ⁶ |
| 5 | Dhokari Holagarh | 68 X 10 ⁶ |
| 6 | Garaghanpur | 28 X 10 ⁶ |
| 7 | Kaithwal | 72 X 10 ⁶ |
| 8 | Mahuwa Diha | 40 X 10 ⁶ |
| 9 | Sarai Mansoor | 76 X 10 ⁶ |
| 10 | Saidabad | 36 X 10 ⁶ |

their local niche and give optimum benefits to plant, soil and environment when utilized as biofertilizers (Abdelaziz *et al.*, 2014). Their nitrogen-fixing ability makes them an important microbial component in paddy fields that also contributes to its fertilization (Song *et al.*, 2005). As compared to different growth stages of paddy cultivation, panicle initiation stage has been chosen as during this period occurrence of a broad rice canopy and high phosphorus content in soil provides more favorable environment for the growth of cyanobacteria (Hendrayanti *et al.*, 2018).

The CFU/ml of soil solution calculated from each of the study site revealed the highest CFU index at sites 7 and 9 (above 70 colonies at 10⁻⁵ diluton), while the minimum CFU index was at 3 and 6 (below 30 colonies at 10⁻⁵ diluton) (Table 3).

The innumerable microbial community of soil ecosystem maintains the natural texture and fertility of soil. Excessive use of pesticides, fungicides and insecticides disturbs the community structure and productivity of soil and has an adverse impact on growth and enzyme activity in cyanobacteria (Liu *et al.*, 2006; Rajendran *et al.*, 2007; Debnath *et al.*, 2012). They also express a negative effect on growth, production and nitrogen-fixing ability of BGA (Mazlan *et al.*, 2017). In some studies insecticides are found to inhibit the phycocyanin, chlorophyll a, and carotenoids of some strains (Prasad *et al.*, 2005). Less diversity (below 30 colonies at 10⁻⁵ diluton) indicates the indiscriminate application of chemical fertilizers and biocides in the paddy fields of III and VI while the fields that used organic fertilizers supported the growth of cyanobacteria that acted as soil conditioners, enhancing crop productivity (Saadania and Riahi, 2009; Prasanna *et al.*, 2013; Chittora *et al.*, 2020; Nascimento *et al.*, 2019; Iniesta-Pallarés *et al.*, 2021). These observations coincide with the experimental result of the ecotoxicological impact of higher doses of pesticides and chemical fertilizers on the growth structure and function of native cyanobacterial species present in the paddy field ecosystem (Singh *et al.*, 2018; Singh *et al.*, 2020).

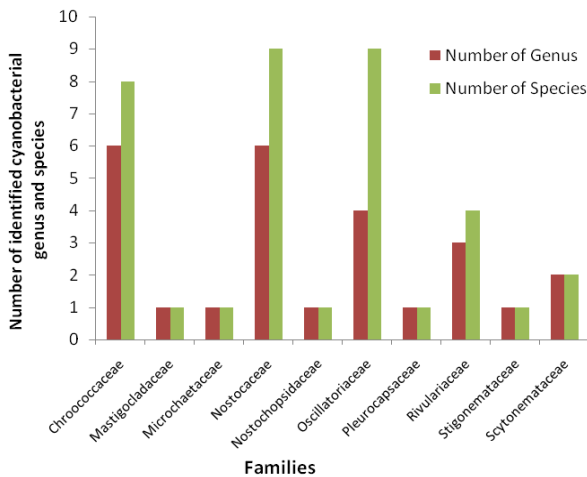


Fig. 1: Diversity of genus and species in different families indentified from saidabad block, Prayagraj district

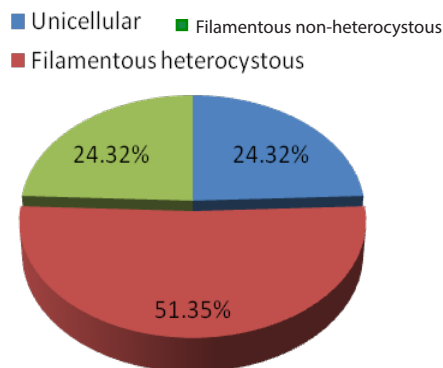


Fig. 2: Prevalence of structural forms of BGA in the study area

CONCLUSION

In an agriculturally dependent community, cyanobacterial biofertilizers will save money and provide nutrient-rich, safe and healthy food for the growth and welfare of population. Besides this, cyanobacteria are well documented as biocontrol agents and in bioremediation of pollutants. Therefore, for sustainable agriculture and balance of the soil ecosystem, there is an urgent need to focus on the use of biofertilizers. The present work pioneered the study of the distribution and diversity of cyanobacterial strains in the Saidabad block of Prayagraj district (Uttar Pradesh, India). This study documented a wide range of cyanobacterial diversity in rice fields. *Anabaena oryzae*, *Aphanocapsa biformis*, *Gloeocapsa punctata*, *Lyngbya spiralis*, *Nostoc muscorum*, *Na commune*, *O. limosa*, *O. princeps* and *O. tenuis* are the species that occurred in maximum frequency. *Oscillatoria* was the most dominant genera in paddy fields of

Saidabad.

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AUTHORS CONTRIBUTION

Dr. Swati Chaurasia has written the manuscript. Dr Rahul Soni helped in data compilation and Dr Amita Pandey contributed to the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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