# Chapter 3 Rhizospheric Diversity of Cyanobacteria and Their Significance in Tropical Ecosystem



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Abstract Cyanobacteria are gram-negative diverse group of unicellular to filamentous photoautotrophs. They are found ubiquitously in nature. Ability of the cyanobacteria for nitrogen fixation and phosphorous solubilization makes them promising biofertilizers, and their plant growth-promoting potential in the rhizosphere makes them a suitable candidate for sustainable agriculture. Cyanobacteria in the rhizosphere and their importance in tropical ecosystems have been highlighted in this chapter.

Keywords Cyanobacteria · Diversity · Biofertilizer · Plant growth promotion · Tropical

# 3.1 Introduction

Cyanobacteria are gram-negative photosynthetic microorganisms involved in global oxygen supply and primary production of biomass in aquatic ecosystem. Cyanobacteria have also been reported for nitrogen fixation  $(N_2)$  and carbon dioxide  $(CO<sub>2</sub>)$  sequestration, thus contributing toward the carbon and nitrogen economy of different ecological habitats (Singh et al. [2016;](#page-10-0) Wyatt and Silvey [1969\)](#page-11-0). Cyanobacteria have a wider adaptability and are found in diverse ecological niches. Bagul et al. ([2018\)](#page-8-0) reported different types of heterocystous and non-heterocystous cyanobacteria from diverse ecological niches of India, including hot water spring of Odisha, cold regions of Leh and Uttarakhand, marine water from Odisha, and arsenic-contaminated field of Ballia, Uttar Pradesh. Cyanobacteria are also capable of tolerating biotic and abiotic stress such as salt, heavy metals, and drought and cold

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conditions. Comprising about 150 genera with more than 2000 species, they exhibit remarkable diversity in their morphology, ranging from simple unicellular and colonial to complex filamentous forms with or without branching (Van den Hoek et al. [1995\)](#page-11-1). They flourish in nitrogen-deficient environment. Cyanobacteria can grow in purely inorganic medium using light as energy and  $CO<sub>2</sub>$  and  $N<sub>2</sub>$  as sole carbon and nitrogen sources, respectively (Wyatt and Silvey [1969\)](#page-11-0). Over several years, cyanobacteria have been utilized as biofertilizer in rice crop for nitrogen fixation along with plant growth-promoting activities. Inoculation of cyanobacteria into the rice field has been practiced in many tropical countries which helps in reducing the cost of expensive chemical fertilizers. Cyanobacterial extracts have been reported to have a significant response to food crops like wheat, maize, rice, tomato, and cucumber (Priya et al. [2015;](#page-10-1) Bidyarani et al. [2016](#page-8-1); Gayathri et al. [2017\)](#page-8-2). Cyanobacteria have the ability to produce extracellular substances and modulate pH, temperature, and redox activity, besides playing a role in the volatilization of ammonia and methane generation; therefore, application of cyanobacteria in the rice field directly or indirectly has been utilized in the management and productivity of rice ecosystem (Prasanna et al. [2002\)](#page-9-0). A group of heterocyst-forming cyanobacteria such as Anabaena, Calothrix, Hapalosiphon, and Nostoc have also been reported to enhance soil microbial parameters, seed germination, and yield of rice crop (Obana et al. [2007;](#page-9-1) Prasanna et al. [2013;](#page-10-2) Hussain and Hasnain [2012;](#page-9-2) Mazhar et al. [2013;](#page-9-3) Karthikeyan et al. [2007](#page-9-4)).

# 3.2 Rhizospheric Diversity of Cyanobacteria in Tropical Ecosystem

The rhizosphere is the region of large number of microbial population and diversity. Higher metabolic activity in the rhizosphere region related to the successful production of crops and increase soil fertility. However, abundance of cyanobacteria and its diversity are meagerly explored in the rhizosphere of crop plants. The general belief that cyanobacteria are obligate phototrophs has been perhaps the major reason for the dearth of information on these organisms in this niche (Karthikeyan et al. [2009\)](#page-9-5). Soil enzymes play a vital role which regulate elements' transformation in the soil and increase the fertility of the soil. The process of elemental transformation is the result of microbial activity as microorganisms have a role in nutrient cycling; thus, both soil fertility and microbial activity are generally closely related, leading to differences in yields and changes in various soil parameters (Nain et al. [2010\)](#page-9-6). The rhizospheric diversity has been reported by many research groups from different tropical countries including India, Sri Lanka, Iraq, Saudi Arabia, Singapore, etc. The cyanobacteria that are reported to dominate in the rhizosphere are the population of non-heterocystous cyanobacteria. Adhikary and Baruah ([2015\)](#page-8-3) studied comparative diversity and composition of nitrogen-fixing cyanobacteria in three different land use systems of upper Assam, i.e., rice field, reserve forest, and coal field. Nitrogen-fixing

cyanobacteria belonging to nine genera were isolated which included six heterocystous forms, viz., Anabaena, Nostoc, Scytonema, Calothrix, Rivularia, Westiellopsis, and three non-heterocystous forms, viz., Lyngbya, Phormidium, and Oscillatoria. The dominance of Nostoc and Anabaena in the reserve forests and rice fields, whereas both were missing in the coal-contaminated sites. Oscillatoria was the dominant genus, and the species belonging to this genus were abundant in coal field areas. Jena and Adhikary ([2007\)](#page-9-7) reported 56 taxa from eastern and northeastern states of the country belonging to 21 genera: Chlorococcum  $(1)$ , Treubaria  $(1)$ , Pediastrum (9), Hydrodictyon (1), Botryococcus (1), Coenochloris (1), Radiococcus (1), Coenocystis (1), Oocystis (1), Glaucocystis (1), Chlorella (1), Kirchneria (2), Kirchneriella (1), Ankistrodesmus (10), Coelastrum (3), Actinastrum (2), Tetrastrum (1), Crucigenia (1), Crucigeniella (1), Desmodesmus (6), and Scenedesmus (9). All these species were recorded first time from this region, and out of these, 16 species were reported first from India (Singh et al. [2018\)](#page-10-3). Paddy ecosystem harbors nitrogenfixing cyanobacterial species mainly dominated by Nostoc, Anabaena, Tolypothrix, Aulosira, Cylindrospermum, Scytonema, Westiellopsis, and several other genera commonly flourishing in Indian paddy (Nayak et al. [2004;](#page-9-8) Prasanna and Nayak [2007;](#page-9-9) Saadatnia and Riahi [2009](#page-10-4)). Bora et al. ([2016\)](#page-8-4) have isolated six strains of two closely related genera—Nostoc and Cylindrospermum—Nostoc carneum, Nostoc hatei, Nostoc muscorum, Cylindrospermum muscicola (strain A), Cylindrospermum muscicola (strain B), and Cylindrospermum indicum from terraced paddy field and jhum land of biodiversity hotspot zone of Assam, Northeast India. Debnath and Bhadury ([2016\)](#page-8-5) have isolated five abundant cyanobacteria from the rice fields of arsenic-affected Bengal Delta Plains (BDP) of South Asia and maintained in vitro. The characterized isolates resembled Leptolyngbya sp. (isolate LBK), Nostoc sp. (isolates NOC and NOK), and Westiellopsis sp. (isolates WEC and WEK) based on polyphasic taxonomy. Haider and Haifaa ([2018\)](#page-8-6) have identified 96 species, including four heterocystous species represented by Anabaena, Calothrix, Cylindrospermum, and Nostoc. However, the non-heterocystous species were represented by 13 species: Aphanocapsa, Aphanothece, Arthrospira, Chroococcus, Gloeocapsa, Lyngbya, Merismopedia, Microcystis, Microcoleus, Oscillatoria, Phormidium, Schizothrix, and Spirulina. Soil samples were collected from six different agricultural sites in Al Diwaniyah City, Iraq. The dominant species of cyanobacteria was Oscillatoria, followed by Phormidium, Chroococcus, Gloeocapsa, and Lyngbya. Several arid zones like Shantiniketan (West Bengal, India) and the Thar Desert along with Achrol, Jaisalmer, Manwar, and Pokhran (Rajasthan, India) have been studied for cyanobacterial diversity in India. In Shantiniketan, a novel cluster of *Scytonema* and *Tolypothrix* cyanobacteria has been found which possessed abundant scytonemin in a sheath of cells for protection from high solar irradiance (Kumar and Adhikary [2015](#page-9-10)). In the Thar Desert, the dominance of Phormidium, Oscillatoria, and Lyngbya followed by Nostoc, Scytonema, and Calothrix has been reported (Bhatnagar et al. [2008\)](#page-8-7). Several novel strains of Oscillatoriales have been also reported from the Thar Desert by Dadheech et al. ([2012\)](#page-8-8). Silambarasan et al. ([2012\)](#page-10-5) in his study have isolated marine cyanobacteria from rhizosphere soil samples of the three mangroves, viz.,

Parangipettai, Ariyankuppam, and Mudasal Odai mangroves southeast coast of India. Jing et al. ([2015\)](#page-9-11) studied the diversity of the diazotroph communities in the rhizosphere sediment of five tropical mangrove sites with different levels of pollution along the north and south coastline of Singapore by pyrosequencing of the nifH gene and found that Scytonema sp. and Pseudanabaena sp., which belong to the Nostocales (heterocyst forming) order of cyanobacteria. Moreover, filamentous non-heterocystous cyanobacteria Microcoleus were detected at all five sampling sites. Amarawansa et al. [\(2018](#page-8-9)) found 13 different cyanobacteria genera from paddy soil crust in the intermediate and dry zones of Sri Lanka based on their morphological characteristics. Among them, six cyanobacteria genera were unicellular (Chroococcus, Aphanocapsa, Aphanothece, Synechococcus, Johannesbaptistia, Microcystis), and seven genera were filamentous types (Lyngbya, Oscillatoria, Leptolyngbya, Pseudanabaena, Anabaena, Spirulina, Nostoc).

## 3.3 Significance of Cyanobacteria in Tropical Ecosystem

#### 3.3.1 Phytohormone Production

Cyanobacteria have been reported to produce phytohormones such as IAA, IBA, gibberellins, cytokinin, abscisic acid, and jasmonic acid (Manickavelu et al. [2006\)](#page-9-12). Table [3.1](#page-4-0) shows different cyanobacterial strains reported for phytohormone production. A non-heterocyst Chroococcidiopsis sp. MMG-5 has been studied and showed significant amount of IAA (25 μg/mL) production, and when co-treated with wheat, mung beans, and pea crop, it showed significant increase in shoot and root length (Ahmed et al. [2010a\)](#page-8-10). Ahmed et al. ([2010b](#page-8-11)) reported Arthrospira platensis MMG-9 with 194.3 μg/mL IAA production and with enhanced root and shoot parameters. Anabaena vaginicola has been reported to produce IBA and IAA, 2146.9 ng/g and 9.93 ng/g fresh weight, respectively. The effects of these strains have been evaluated and are found beneficial on several vegetable and herbaceous crops (Hashtroud et al. [2013\)](#page-8-12). Prasanna et al. ([2013\)](#page-10-2) investigated the effect of Anabaena sp. (RPAN59/8) amended with compost and found enhanced growth parameters as well as enhanced quality of tomato fruit. Co-inoculation of plant growth-promoting rhizobacteria along with cyanobacteria has also been reported to increase the plant growth and grain yield significantly (Nain et al. [2010\)](#page-9-6). Karthikeyan et al. [\(2007](#page-9-4)) investigated the potential of cyanobacteria on wheat along with different dose of chemical fertilizers; interestingly. all the treatments showed enhanced plant growth and yield parameters. A study with Anabaena and Trichoderma viride biofilm showed 12–25% increase in yield of soybean as well as enhanced microbial activity. Cyanobacterial association with *Gunnera* has shown production of arabinogalactan proteins that might have played important role in plant growth and development (Bergman et al. [1996\)](#page-8-13). Kumar and Kaur [\(2014](#page-9-13)) studied the germination behavior of wheat seeds with cyanobacterial filtrate and found that germination, vigor index, and number of seedlings were higher as compared to untreated. The abovementioned studies

<span id="page-4-0"></span>

Table 3.1 Different cyanobacterial strains exhibiting phytohormone production Table 3.1 Different cyanobacterial strains exhibiting phytohormone production

indicate that cyanobacteria could be a potential component in integrated nutrient management. The world is looking for organic farming; certainly cyanobacteria are one of the important catalysts that could play a vital role.

## 3.3.2 Cyanobacteria as a Biofertilizer

Sustainable agriculture is the present trend in agriculture that has gained an attention by reducing the use of chemical pesticides and inorganic fertilizers and increasing the use of biofertilizers as an alternative to improve crop yield (Nain et al. [2010\)](#page-9-6). Cyanobacteria have the ability to fix atmospheric nitrogen. They are important component of rice ecosystem and known to fix 20–25 kg N/ha/season (Prasanna and Kaushik [2006](#page-9-15)). Cyanobacteria are categorized into heterocystous and non-heterocystous forms. Plate [3.1](#page-5-0) depicts confocal and light microscopic images of heterocystous and non-heterocystous cyanobacteria. Heterocyst is a specialized structure and nitrogen fixation site. Filamentous cells form heterocyst when inorganic nitrogen source is deprived of cultivation medium (Fig. [3.1\)](#page-6-0). These cells lack photosystem II and maintain microaerobic environment which is required for nitrogenase enzyme responsible for nitrogen fixation. PS I provides ATP for nitrogen fixation in this process which is an energy intensive process. Non-heterocystforming cyanobacteria fix atmospheric nitrogen by temporal  $(CO<sub>2</sub>$  fixation during day time and  $N_2$  fixation at night) and spatial separation. However, a new study reveals the constitutive nitrogenase activity in the presence of light and oxygen by Cyanothece sp. ATCC 3051142 (Young et al. [2019](#page-11-2)). These findings could pave the way for auto mode of nitrogen fixation in plants in the future. Cyanobacteria mimic the photosynthesis of plants; however, its metabolism has been regarded as bacterial. Studies have shown that the artificial inoculation of cyanobacteria to marine mangroves has significant effect on germination and nitrogen fixation. The researchers indicated the use of cyanobacteria in affected area to establish mangroves (Toledo

<span id="page-5-0"></span>

Plate 3.1 Confocal images of cyanobacteria (first row left to right, *Hapalosiphon* sp., *Nostoc* sp., Calothrix sp., Leptolyngbya sp.). Light microscopic images of cyanobacteria (second row left to right, Tolypothrix sp., Hapalosiphon sp., Anabaena sp., Leptolyngbya sp.)

<span id="page-6-0"></span>

Fig. 3.1 Nitrogen fixation factory (heterocyst cell in cyanobacteria)

et al. [1995\)](#page-10-9). Rice seedling treated with Anabaena sp. showed increased root and shoot length as compared to control plant as a result of nitrogen fixation by cyanobacteria (Saadatnia and Riahi [2009](#page-10-4)).

Phosphorous is another important nutrient required for plant growth after nitrogen. However, solubilization of mineral P is affected by different factors, and plant could take it easily. Reports suggest that cyanobacteria could solubilize inorganic phosphorous such as tricalcium phosphate, FePO<sub>4</sub>, AlPO<sub>4</sub>, hydroxyapatite  $(Ca_5(PO_4)_3$ <sup>-</sup>OH), and rock phosphate. Yandigeri et al. ([2011\)](#page-11-3) investigated cyanobacteria for Mussoorie rock phosphate and tricalcium phosphate solubilization and found that Westiellopsis prolifica and Anabaena variabilis were able to solubilize it. Roychoudhury and Kaushik [\(1989\)](#page-10-10) also reported P solubilization by cyanobacteria. Chinnusamy et al. [\(2006](#page-8-15)) have tested the combination of cyanobacteria, VAM fungi, Azospirillum, and PSB and found significant improvement of growth and yield in the plant. Nutritional status and fertility of the soil were also enhanced with these treatments. Stihl et al. ([2001\)](#page-10-11) reported alkaline phosphatase activity in Trichodesmium sp. Natesan and Shanmugasundaram [\(1989](#page-9-16)) studied the Anabaena ARM 310 for phosphate solubilization and found that this cyanobacterium was able to solubilize tricalcium phosphate. Aulosira fertilissima was able solubilize tricalcium phosphate (4.02 μg/mL) and showed improved seed vigor and growth of the wheat plant (Kumar and Kaur [2014\)](#page-9-13). Cyanobacteria have the dual advantages of nitrogen fixation and P solubilization which make them suitable biofertilizer for agricultural use.

# 3.3.3 Advantages of BGA Biofertilizers

Cyanobacteria play an important role in the maintenance and build-up of soil fertility, consequently increasing rice growth and yield as a natural biofertilizer (Song et al. [2005\)](#page-10-12). The other roles of cyanobacteria include:

- 1. Increasing soil pores with having filamentous structure and production of adhesive substances
- 2. Excretion of growth-promoting substances such as hormones (auxin, gibberellin), vitamins, and amino acids (Roger and Reynaud [1982](#page-10-13); Rodríguez et al. [2006](#page-10-14))
- 3. Increasing water-holding capacity through their jelly structure (Roger and Reynaud [1982\)](#page-10-13)
- 4. Increasing soil biomass after their death and decomposition (Saadatnia and Riahi [2009\)](#page-10-4)
- 5. Decreasing soil salinity (Saadatnia and Riahi [2009\)](#page-10-4)
- 6. Preventing weeds' growth (Saadatnia and Riahi [2009\)](#page-10-4)
- 7. Increasing soil phosphate by excretion of organic acids (Wilson et al. [2006](#page-11-4))

# 3.4 Plant Protection Against Diseases and Pest

Cyanobacteria could produce induced systemic resistance by producing diverse range of biologically active molecules in the rhizosphere which elicit the plant growth under different stresses (Prasanna et al. [2009a](#page-10-15), [b](#page-10-16), [2010](#page-10-7)). Cyanobacteria could protect plant by providing mechanical and physical strength of the cell wall. Physiochemical reactions are altered by producing defense-related chemicals against the phytopathogens. Major defense enzymes involved in plant growth are chitinase, phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPOs), phenolics, and phytoalexins (Kloepper et al. [1992\)](#page-9-17). Prasanna et al. [\(2013](#page-10-2)) showed enhanced production of defense enzymes 189% and 239% of PAL and PPO, respectively, resulting in increased plant growth parameters and bioprotection against Fusarium wilt of tomato. Radhakrishnan et al. [\(2009\)](#page-10-17) studied the effect of cultural filtrate of Calothrix elenkinii on fungicidal and algicidal activity which showed promising result. The strain was also evaluated for plant growth promotion, and the dual advantages of plant growth promotion and biocontrol potential make the strain more suitable candidate for agricultural use. Biondi et al. ([2004\)](#page-8-16) have reported insecticidal as well as nematicidal activity of Nostoc ATCC53789 on Helicoverpa armigera and Caenorhabditis elegans. Prasanna et al. [\(2008](#page-10-18)) showed biocidal activity against phytopathogenic fungi with Anabaena strain. Cyanobacterial extracts have been reported to reduce the infection of Botrytis cinerea in strawberries and Erysiphe polygoni in turnips and tomato seedlings, besides reducing the growth of saprophytic organisms and soilborne fungal pathogens (Kulik [1996](#page-9-18); Prasanna et al. [2013](#page-10-2)). Application of Calothrix elenkinii and augmentation with copper nanoparticles (CuNPs) exhibited 76% disease control efficacy in pathogenchallenged plants such as tomato as compared to control. Similarly, augmentation enhanced the chitosanase activity by 10% and 7%, compared to CuNPs and Calothrix elenkinii alone. Higher dehydrogenase activity and increased root and shoot length have been also recorded in the rhizosphere soil of diseased plants as compared to healthy plants. Total PLFA content in the soil also increased significantly by 1.4–3.3-fold, compared to the control (Mahawar et al. [2019](#page-9-19)).

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