Cyanobacteria from *Sorghum bicolor*-Grown Fields of Ecopark at Cibinong Science Center-Botanic Gardens, Indonesia

7

Debora Christin Purbani

Contents

Abstract

Cyanobacteria (blue-green algae, BGA) are a group of microalgae that plays an essential role in the fixing of atmospheric nitrogen which is important for the soil fertility. BGA can be an economically and ecologically alternative solution for fertilizers in increasing the productivity of *Sorghum bicolor*. Isolation of these cyanobacteria from natural sources in pure form is an essential step for their efficient use as biofertilizer. The purpose of this study was to investigate cyanobacteria from *S. bicolor*-grown fields of Ecopark at Cibinong Science Center-Botanic Gardens, Indonesia, as a baseline data. The isolation of pure cultures was done by selecting a single colony from mixed cultures grown on selected media BG-11 and bold basal media, as different cyanobacterial strains can grow on different media. The same medium in liquid form was used for further purification and subculturing. The pure cultures were transferred to liquid media for further studies. From 20 soil sample cultures, 4 predominant isolates were identified on the basis of their morphological characteristics under light microscopy. Observations were made on heterocystous and non-heterocystous forms. The genera of *Nostoc* and *Anabaena* were found as the dominant heterocystous group, while the non-heterocystous group consisted of *Lyngbya* and *Oscillatoria* in the *S. bicolor*-grown fields.

D. C. Purbani (✉)
Research Center for Biology, Indonesian Institute of Sciences, Cibinong Science Center, Cibinong, Indonesia

R. Z. Sayyed et al. (eds.), *Plant Growth Promoting Rhizobacteria (PGPR): Prospects for Sustainable Agriculture*,
https://doi.org/10.1007/978-981-13-6790-8_7

Keywords
Cyanobacteria · *Sorghum bicolor* · Biofertilizer · Plant growth promotion

7.1 Introduction

Cyanobacteria (blue-green algae, BGA) is a photoautotrophic prokaryote organism that has incipient nucleus types, hexagonal carboxysomes, loosely arranged thylakoids, gas vesicles which help maintain certain vertical positions in water to respond to physical and chemical properties, ribosomes, phycobilisomes, and also various storage granules consisting of glycogen, cyanophycin, or polyphosphate lipids (Singh et al. 2013). The BGA group name is given based on the color of the cell seen under a microscope. The color expression of their cells is caused by existing pigments such as chlorophyll-a, phycocyanin, and phycoerythrin. Many species have a golden brown or dark color, although sometimes red in cells around individual cells or throughout filaments and sheaths (Kondo and Yasuda 2003). BGA is a major producer in natural ecosystems (Field et al. 1998; Ohkouchi et al. 2006). BGA can be found in diverse habitats (Whitton 2000). They exhibit a wide distributional spectrum; they are ubiquitous under different soil, water, and agroclimatic condition, occurring in fresh and marine water, terrestrial environment, and growing wherever little light and moisture is available (Sao and Kritika 2015).

In farmlands, BGA plays a vital role in increasing the fertility of various ecological agricultures. These organisms can produce organic materials that can increase soil fertility (Mishra and Pabbi 2004). BGA excretes substances such as hormones, vitamins, amino acids, and organic acids that can increase plant growth and affect other organisms in many ways (Wilson 2006). The presence of BGA can also reduce erosion because it can stabilize the soil surface (Hu et al. 2004). Soil porosity, aggregation, and water holding capacity can increase with the presence of polysaccharides produced by several BGAs (Choudhary et al. 2007; Kaushik 2007). BGA can carry out two biological processes such as oxygen photosynthesis and nitrogen fixation, thus becoming the preferred biological fertilizer.

Song et al. (2005) reported that inoculation of agricultural land with algae could increase grain yield by 15–25%. BGA inoculation is reported to have beneficial effects on plants such as rice, corn, wheat, soybeans, oats, tomatoes, radishes, cotton, sugar cane, chili, peanuts, muskmelon, and lettuce (Thajuddin and Subramanian 2005; Maqubela et al. 2008; Karthikeyan et al. 2007). Svircev et al. (1997) also reported that plant growth was enhanced in the presence of BGA, even without organic N fertilizer application. Some species are widespread in agricultural land and are known to contribute significantly to their fertility, such as *Nostoc*, *Anabaena*, *Tolypothrix*, *Aulosira*, *Cylindrospermum*, *Scytonema*, *Westiellopsis*, and several other genera (Rao et al. 2008; Choudhary 2011). Although some BGAs have been widely studied, information in the diversity and ecological characteristics of BGA is very limited. No previous studies have been reported about cyanobacterial diversity from *S. bicolor* fields. In this study, we conducted an isolation and characterization

of cyanobacteria from S. *bicolor*-grown fields of Ecopark at Cibinong Science Center-Botanic Gardens, Bogor, Indonesia, using morphological observations, as the first step to their efficient use as a biofertilizer.

7.2 Materials and Methods

7.2.1 Collection of Soil Sample

Collection of soil samples was carried out at a depth of 0–5 cm on 20 different plots of *Sorghum bicolor*-grown fields of Ecopark at Cibinong Science Center-Botanic Gardens (CSC-BG), Bogor, Indonesia. Ecopark is built as an extension of Bogor Botanical Gardens that has a common purpose, namely, as a means of education, research, and ecotourism. Currently, Ecopark has a garden collection grouped by bioregion, covering Sumatera Bioregion, Java and Bali, Borneo, Lesser Sunda Island, Celebes, the Moluccas, and Papua. In addition, there is a lake known as Dora Lake. However, there is also empty land dominated by grass which has not been used and developed by the manager so that not all areas can be used by visitors. In previous research, we try converting *Imperata* grassland in CSC-BG into agriculture field by planting the *S. bicolor*.

7.2.2 Enriching of Soil Sample

Soil samples were placed on sterile Petri dishes which had contained sterile BG-11 media with a pH of 7.1 and bold basal media because different cyanobacterial strains could grow on different media. Incubation is carried out for 2 weeks by placing Petri dishes in the culture chamber at 25 °C and a light 12/12 h dark cycle. After colonization, for isolation and purification, loops are used to move parts of each colony to new plates with liquid media.

7.2.3 Isolation of Cyanobacteria by Capillary Micropipetting

Equipment such as silicon slope, sliding glass, microscope, and flat bottom 24-well plates including elongated Pasteur pipette with specific size and length of holes for the capillary micropipetting procedure prepared. The base of the Pasteur pipette is connected to the silicon slant. BG-11 medium with pH 7.1 and bold basal media were poured into 24-well plates. The presence and diversity of cyanobacteria on the plate were observed under a light microscope. Cyanobacteria that have been determined as the target of isolation are sucked using a sharp-tipped Pasteur pipette. The single-celled cyanobacteria were brewed and cultivated into one of the holes of 24-well plate containing the media. The observations were made every day and recorded accordingly. Repeated subculturings were performed until pure axenic cultures were obtained. Single cells from cyanobacteria were cultivated and selected for further studies.

7.2.4 Morphological Analyses

Morphological observations were carried out using an Olympus BX5 microscope fitted with software (Cell Sens Standart) and a digital camera. Observations were made on the shape and size and vegetative cell, presence and absence of sheaths, heterocysts, akinetes (if any), and the position of the axenic culture cyanobacterial branching pattern. Magnification used to capture cyanobacterial images is 100x. The strains were identified based on their morphological features and phenotypic characters following standard keys (Prescott 1951; Desikachary 1959; Wehr et al. 2002; Komárek and Anagnostidis 2005).

7.3 Results and Discussion

7.3.1 Morphological Analyses

Morphological characteristics studied were the cell shape and size, the heterocyst, the shape of the akinetes, and the presence or absence of a sheath. Four predominant cyanobacteria (blue-green algae, BGA) were obtained from the study sites. The genera of *Nostoc* and *Anabaena* were found as the dominant heterocystous group, while the non-heterocystous group consisted of *Lyngbya* and *Oscillatoria* in the *S. bicolor*-grown fields (Fig. 7.1 and Table 7.1).

***Nostoc* (Order: Nostocales; Family: Nostocaceae)**
Nostoc has a gelatin body consisting of many internal filaments which are packaged in a sheath or skin called trichomes. Each trichome being simple free and curved or circular in shape. The population looks denser toward the outer "skin" of the body. When gelatin colonies rupture, filaments or "trichomes" are in great abundance and

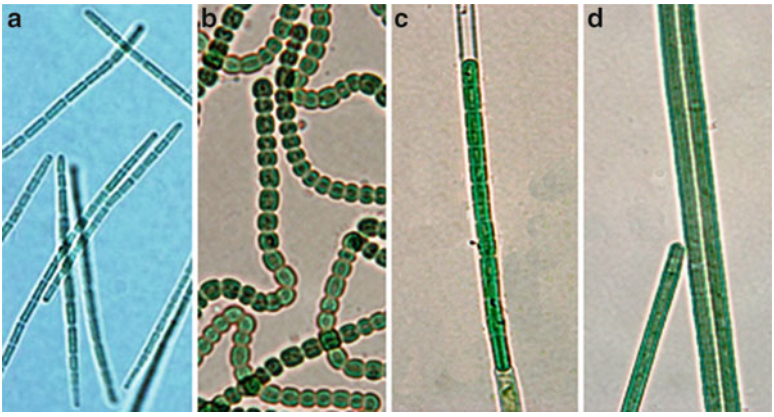

Fig. 7.1 Microscopy of cyanobacteria from *Sorghum bicolor* grown fields of Ecopark at Cibinong Science Center-Botanic Gardens: (**a**) *Nostoc*; (**b**) *Anabaena*; (**c**) *Lyngbya*; (**d**) *Oscillatoria*

Table 7.1 General morphological characteristics of the isolated cyanobacteria from *Sorghum bicolor*-grown fields of Ecopark at Cibinong Science Center-Botanic Gardens

Genus	Cell width (μm)	Cell length (μm)[a]	Sheath[a]	Motility[a]	Cellular shape	Other characteristic
Nostoc	2–8	NM	−	+	Cylindrical, spherical	Formation of thallus, heterocystous
Anabaena	2–20	NM	−	+	Cylindrical, ovoid	Filamentous, heterocystous
Lyngbya	4–15	20–80	+	−	Cylindrical, barrel shaped	Filaments occur within a firm sheath, non- heterocystous
Oscillatoria	2–15	35–65	+	+	Cylindrical	Transverse septa visible, non-heterocystous

[a]Meanings of symbols: NM, not measured; +, present; −, absent

cause a feeling of slimy. Vegetative reproduction will continue inside the colony until the moisture disappears. Then many cells produce blackish masses, forming spores. The majority of the cells seen do not have a nucleus, but a number of chromatin granules have the same size. Heterocyst cells occur in the filaments (subspherical and intercalary) which develop into larger ones with vegetative reproduction (fragmentation) and at which point they break into new colonies called hormones. Cells as a gelatinous body mature in a trichome change into akinetes, capable of forming new filaments.

***Anabaena* (Order: Nostocales; Family: Nostocaceae)**
Trichomes are untapered with conspicuous constrictions at cross-walls. Trichomes may be straight, curved, or helically (spirally) formed. The cells are cylindrical or ovoid (barrel-shaped) and not shorter than broad (or only slightly so). The terminal cells may be rounded, tapered, or conical in shape. Heterocysts are intercalary or terminal or both intercalary heterocysts are nearly spherical to cylindrical with rounded ends; terminal heterocysts are similar or sometimes conical. Akinetes are usually formed, and their position in trichomes differs with the species. A form of individual sheath is absent, but a soft mucilaginous covering is often present. Trichomes, when free of adhesive mucilage, are normally motile, and colonies are not formed. Reproduction is by fragmentation of "parental" trichomes into shorter trichomes indistinguishable in cell dimensions from the former trichome.

***Lyngbya* (Order: Oscillatoriales; Family: Oscillatoriaceae)**
Lyngbya is filamentous organisms that produce a distinct and persistent sheath. Thin sheaths can be seen with phase contrast optics when they go beyond trichome terminal cells. Trichomes of *Lyngbya* are usually non-motile within the sheath, but when placed on a new agar-solidified medium, the short section of the trichome

(hormogonia) sometimes moves slowly. Some strains produce many hormogonia which glide freely from the sheath and repr*oduc*e the new sheath. The terminal parts appear sheathless, with rapid growth extending the trichome out of the old casing, in some cases. All filament threads in liquid culture will unite because the sheaths of some strains are quite prominent and strong. In large-diameter species, laminated sheaths often occur.

***Oscillatoria* (Order: Oscillatoriales; Family: Oscillatoriaceae)**
Oscillatoria is a filamentous organism that can divide exclusively in one plane through binary fission. The trichomes are flexible or semirigid and straight to loosely to the tortuous apices. Transverse septa can be seen under a light microscope. Constructions can occur in cross-walls with total curvature never exceeding one-eighth of the diameter of a trichome. Generally, longitudinal walls are thinner than cross-wall septums. Fission of the cytoplasmic membrane that separates the new membrane of a daughter cell has a thinner peptidoglycan layer (cells may be much shorter than wide, appearing as a pile of disks), cells shorter than long. The trichome is motile and rotates from left or right in a scattered manner. The curved free end will oscillate when the trichome rotates if the terminal area does not come into contact with the substrate. When the trichome moves on a solid substrate, the sheath is barely visible. In some trichomes during periods of immobility in liquid cultures, the sheaths appear to accumulate in several trichomes.

Morphological, biochemical, and physiological properties that allow growth in a wide range of habitat can express the diversity of cyanobacteria. Its complex structure, unlike ordinary prokaryotes, can be their taxonomic differentiator based on phenotypic, mostly morphological properties. The diversity of heterotopic forms of cyanobacteria on agricultural land has been extensively studied (Choudhary 1999). The lower number of forms during the initial cultivation stage can be due to the effect of inhibiting high light intensity, while the loss of nutrients and low light intensity reaching the surface cause fewer forms in the later part.

Anabaena and *Nostoc* as nitrogen-fixing species were found in the of S. *bicolor*-grown fields of Ecopark, Cibinong Science Center-Botanic Gardens, Bogor, Indonesia, in this study. The same number was found in non-N-fixing species, namely, *Lyngbya* and *Oscillatoria*. Despite the application of periodic fertilizers, indications of N deficiency can be identified by the occurrence of N-fixing species in the soils (Nayak and Prasanna 2007). Enumeration of cyanobacteria during the mid-cultivation cycle of the farm fields can reveal the maximum diversity (Choudhary 2009). This study documented the biodiversity of cyanobacteria from S. *bicolor* in the fields of Ecopark, Cibinong Science Center-Botanic Gardens. According to Choudhary (2011), documentation regarding nitrogen-fixing cyanobacteria and their application in agricultural fields can be used for the management of nitrogen fertilizers at various stages of cultivation by making a supportive environment for nitrogen fixers for sustainable agriculture. Further studies are necessary to investigate the potential of cyanobacteria for soil fertility and productivity of crop plants such as S. *bicolor*.

Acknowledgment This research is supported by the Science and Technology Research Partnership for Sustainable Developments (SATREPS) Project 2016–2021, entitled "Revegetation of *Imperata cylindrica* field combined with sustainable production and utilization of biomass," obtained by PKT Kebun Raya-LIPI in cooperation with Satker in Dept. of Life Sciences-LIPI, Pusinov-LIPI, Ministry of Agriculture and Ministry of Forestry, Indonesia.

References

Choudhary KK (1999) Ex-situ conservation of cyanobacterial germplasm of North Bihar, India. Ph. D. thesis, B.R.A. Bihar university, Muzaffarpur, Bihar, India

Choudhary KK (2009) Ecological and biotechnological relevance of cyanobacteria. In: Gupta RK, Kumar M, Vyas D (eds) Soil microflora. Daya Publishing House, New Delhi, pp 324–339

Choudhary KK (2011) Occurrence of nitrogen-fixing cyanobacteria during different stages of paddy cultivation. Bangladesh J Plant Taxon 18(1):73–76

Choudhary KK, Singh SS, Mishra AK (2007) Nitrogen-fixing cyanobacteria and their potential applications. In: Gupta RK, Pandey VD (eds) Advances in applied phycology. Daya Publishing House, New Delhi, pp 142–154

Desikachary TV (1959) Cyanophyta. Indian Council of Agricultural Research, New Delhi, p 700

Field CB, Behrenfeld MJ, Randerson JT, Falkowski P (1998) Primary production of the biosphere: integrating terrestrial and oceanic components. Science 281:237–240

Hu CX, Zhang DL, Liu YD (2004) Research progress on algae of the microbial crusts in arid and semiarid regions. Prog Nat Sci 14:289–295

Karthikeyan N, Prasanna R, Nain L, Kaushik BD (2007) Evaluating the potential of plant growth promoting cyanobacteria as inoculants for wheat. Eur J Soil Biol 43:23–30

Kaushik BD (2007) Cyanobacterial biofertilizer technology. In: Kannaiyan S, Kumar K, Govindarajan K (eds) Biofertilizers technology. Scientific Publishers, New Delhi, pp 53–59

Komárek J, Anagnostidis K (2005) Cyano-prokaryota: *Oscillatoriales*. In: Büdel B, Gärtner LK, Schlagerl M (eds) Süßwasserflora von Mitteleuropa, vol 19/2. Elsevier GmbH, München, p 760

Kondo M, Yasuda M (2003) Seasonal changes in N_2 fixation activity and the environment in paddy soils of Japan. Jpn Agric Res Q 37:105–111

Maqubela MP, Mnkeni PNS, Malamissa O, Pardo MT Acqui LPD (2008) *Nostoc,* cyanobacterial inoculation in South African agricultural soils enhances soil structure, fertility and maize growth. Plant Soil 315:79–92

Mishra AK, Pabbi S (2004) Cyanobacteria: a potential biofertilizers for rice. Resonance 9:6

Nayak S, Prasanna R (2007) Soil pH and its role in cyanobacterial abundance and diversity in rice field soils. Appl Ecol Environ Res 5:103

Ohkouchi N, Kashiyama Y, Kuroda J (2006) The importance of diazotrophic cyanobacteria as a primary producer during cretaceous oceanic anoxic event. Biogeosciences 3:467–478

Presscott GW (1951) Algae of the Western great lakes area. Pub.Otto Koellz Science Publishers, Koenigstein

Rao DB, Srinivas D, Padmaja O, Rani K (2008) Blue-green algae of rice fields of south Telangana region, Andhra Pradesh. Indian Hydrobiol 11(1):79–83

Sao S, Kritika S (2015) Study of cyanobacteria as biofertilizer fro, the rice field. World J Pharma Res 4(3):1696–1706

Singh P, Singh SS, Elster J, Mishra AK (2013) Molecular phylogeny, population genetics and evolution of heterocystous cyanobacteria using nifH gene sequences. Protoplasma 250:751–764

Song T, Martensson L, Eriksson T, Zheng W, Rasmessen U (2005) Biodiversity and seasonal variation of the cyanobacterial assemblage in a rice paddy field in Fujian, China. FEMS Microbiol Ecol 54:131–140

Svircev Z, Tomas I, Nenin P, Drobac A (1997) Co-cultivation of N2-fixing cyanobacteria and some agriculturally important plants in liquid and sand culture. Appl Soil Ecol 6:74–81

Thajuddin N, Subramanian G (2005) Cyanobacterial biodiversity and potential application in biotechnology. Curr Sci 89:47–57

Wehr JD, Sheath RG, Thorp JH (2002) Freshwater algae of North America: ecology and classification. Aquatic Ecology Press, pp 917

Whitton BA (2000) Soils and riceefields. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria: their diversity in time and space. Kluwer Academic, Alphen aan den Rijn, pp 233–255

Wilson LT (2006) Cyanobacteria: a potential nitrogen source in the rice fields. Texas Rice 6:9–10