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Dynamic population of N_2-fixing cyanobacteria in an organic rice field

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Abstract. *Hendrayanti D, Rusmana I, Santosa DA, Hamim. 2019. Dynamic population of N_2-fixing Cyanobacteria in an organic rice field. Biodiversitas 20: 2883-2890.* The existence of free living N_2-fixing cyanobacteria in rice fields has been acknowledged as an advantage for rice crops. At present, implementation of organic rice-systems has been increasing as an alternative way for keeping rice fields healthy. Therefore, investigation of N_2-fixing cyanobacteria as a part of the soil components is important. Dynamic populations of the filamentous N_2-fixing cyanobacteria assemblage in organic rice field at Ciparay, South Bandung, was investigated during the crop's growth cycle (January-March 2018). Soil samples were collected from four plots of 20 ha rice fields. At each plot, soil from three random stations with three replications was taken using a 3-cm-diameter plastic cylinder. Composite samples from each station were analyzed for colony enumeration (TPC method), relative abundance and frequency, and species identification. The results show that population reached peak on the 80 days after planting (194 x 10^6 cfu/g soil). Species number decreased following increased density of the rice canopy. Among the 23 morphospecies found along the rice growth, four species were always found during all stages of growth: *Halotia wernerae* CSO2, *Roholtiella mojavensis* CSO6, *Hapalosiphon welwitschii* CSO7, and *Desmonostoc danxiaense* CSO3. The community of N_2-fixing cyanobacteria found in the organic rice field was different to those reported from non-organic rice field.

Keywords: Nitrogen fixation, rice phase, soil cyanobacteria, structure community

INTRODUCTION

The rice field is a dynamic ecosystem considering that at the beginning of rice growth, the rice field is filled with water and drained at the flowering stage until harvest. When rice field is flooded with water, the capacity of soil to exchange gases with atmosphere decrease. Soil is less aerobic, and soil temperature is low. As rice growth and water are drained from the surface, light reach soil surface directly makes the temperature of soil increase. This situation may not long (2-3 weeks) because the rice canopy soon covers the soil, created shading effect for any kind of microorganisms' underneath, including the N_2-fixing cyanobacteria.

Nitrogen-fixing cyanobacteria is known to be the ecological balancing of rice field ecosystem through interactions among biophysical, biochemical, and biodiversity milieus. The role of N_2-fixing cyanobacteria in enhancing soil fertility by nitrogen fixation has been well documented and reviewed (Kaushik 2014; Singh et al. 2014a). The free-living, as well as symbiotic N_2-fixing cyanobacteria, act as bio-fertilizer systems for paddy soil. They release ammonia and nitrogenous polypeptide during active growth, while most fixed products are made available mainly through autolysis and decomposition (Sinha and Hader 1996). Dinitrogen-fixing cyanobacteria like *Anabaena torulosa* and *Nostoc carneum* were reported producing extracellular phosphatase and organic acids (Prasanna et al. 2013). Through these processes, N_2-fixing cyanobacteria maintain NPK balance and C:N ratio of rice field.

The occurrence and diversity of N_2-fixing cyanobacteria living on the soil surface of rice field are influenced by the wet and dry conditions as well as the growing rice. Population growth and survival are varied upon the species type. For example, *Nostoc commune* and *Scytonema* sp. had been reported to be more tolerant to sunlight radiation compared to *Anabaena* sp., due to the thickness of mucilaginous sheath on the two species (Sinha and Hader 2006). Populations which are more sensitive to sunlight took advantage with the growing of rice canopy. During rice growth, the plants increase in size and shades the soil surface. Choudhary (2011) reported the gradual intensify of cyanobacteria population from 30 days to 60 days after rice planting.

Extensive studies of N_2-fixing cyanobacteria had been carried out in many countries, particularly in India (Choudhary 2011; Syiem et al. 2011; Vijayan and Rai 2015) and a few in Philippines (Roger et al. 1992), Spain (Fernandez-Valiente and Quesada 2004), China (Song et al. 2005), and Iran (Saadatnia and Riahi 2009). Research from conventional rice fields showed that N_2-fixing cyanobacteria such as *Aulosira*, *Anabaena*, *Nostoc*, and *Scytonema* were found abundant, while *Cylindrospermum* and *Rivularia* inhabited deep-water type of rice fields (Sinha and Hader 1996). Contrast to the extensive studies on chemical-fertilized rice fields, N_2-fixing cyanobacteria occurrence in an organic rice field, especially organic rice field in Indonesia is not many reported .

Granhall et al. (1987) reported the predominance of rice field N_2-fixing cyanobacteria under low concentration of

nitrogen fertilizer in Srilanka. Over 90% of heterocystous N2-fixing cyanobacteria from a chemical-fertilized rice fields in South Korea belonged to *Anabaena* and *Nostoc* (Kim and Lee 2006). Chaudary and Bimal (2010) reported a similar distribution pattern. *Anabaena* spp. and *Nostoc* spp. were dominant populations in fertilized and unfertilized rice fields in Bihar, India. While the occurrence of *Nostoc* spp. was equal in both type of rice fields, *Anabaena* spp. were found more in unfertilized rice fields.

The present study aims to examine the diversity and occurrence of N_2-fixing cyanobacteria in an organic rice field in South Bandung, West Java, Indonesia. Species identification was based on 16s rRNA gene. The presence of dominant species during rice growth will be highlighted with the role of N_2-fixing cyanobacteria as nitrogen source in the rice field.

MATERIALS AND METHODS

Study area

This research was conducted in Sarinah Organic Rice Field area of Ciparay Subdistrict, Bandung District, West Java, Indonesia. Four plots were designated as soil sampling stations, i.e.: Plot 1 (-7.022458 S, 107.695115 E), Plot 2 (-7.020229 S, 107.693090 E), Plot 3 (-7.021429 S, 107.692446 E), and Plot 4 (-7.022559 S, 107.69364 E).

Procedures

Soil sampling

The sampling site was located at border area of three vilages, namely: Serang Mekar, Bumiwangi, and Ciheulang of Ciparay Subdistrict, Bandung District, West Java, Indonesia. The organic rice field belongs to Sarinah Organic Foundation and was established in 2004. Field sampling was carried out from January to March 2018. The crop cycle consisted of four stages: two vegetative phases (40 dap [days after planting] and 60 dap) and two generative phases (80 dap and 110 dap). Composite soil samples were collected from four plots of 20 ha rice fields. At each plot (size about 120 m^2), three random sampling sites were selected. From each sampling site, three soil samples from 0-5 cm depth were collected with a 3-cm-diameter plastic cylinder. Then, the soil samples were mixed at the laboratory as composite soil samples (Bisen 2014). A total of 48 composite samples (four stages x four plots x three sampling sites) were analyzed at the laboratory.

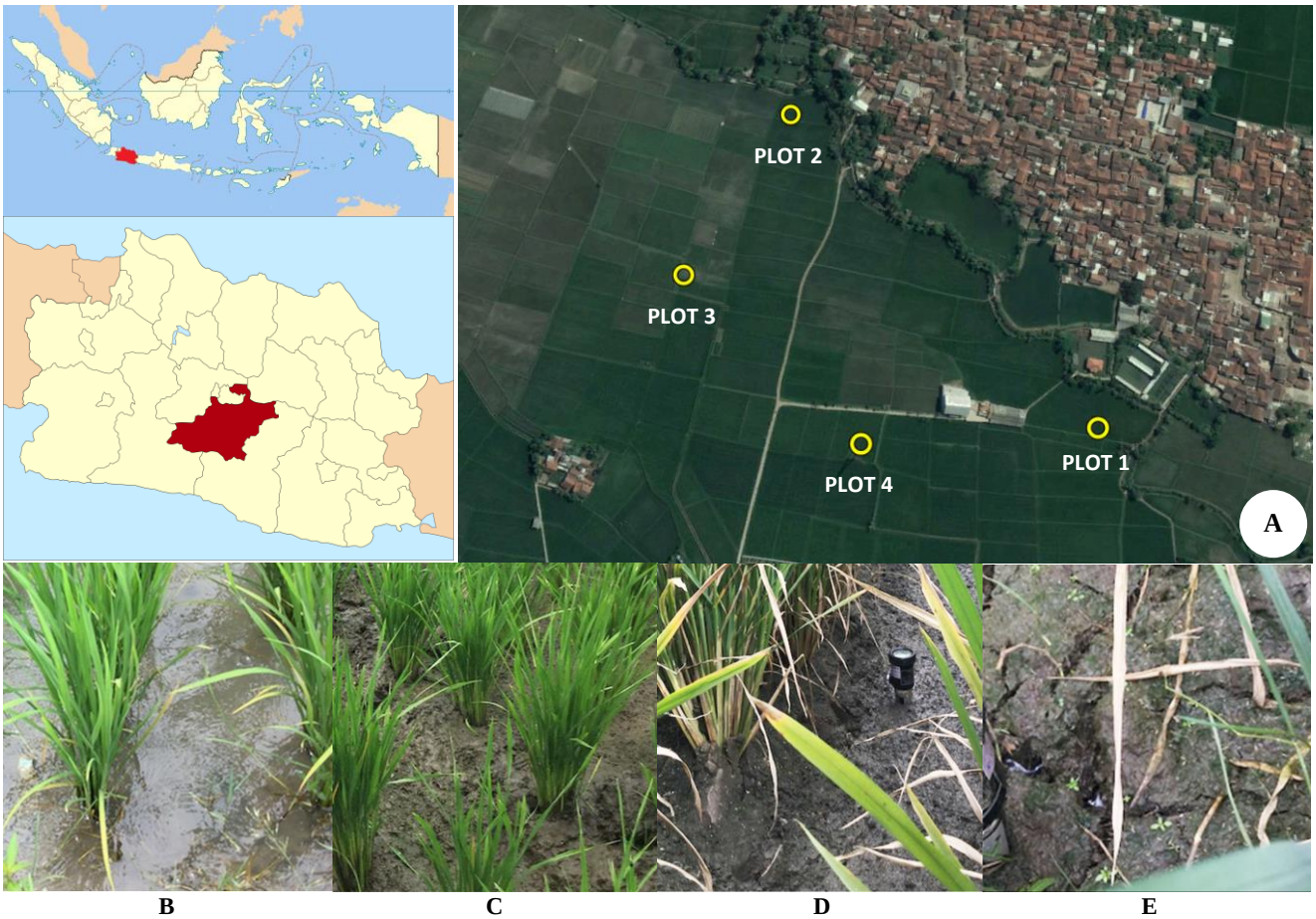

Figure 1. Rice field area at Ciparay Subdistrict, Bandung District, West Java, Indonesia and sampling plots. A. Four plots were designated as soil sampling stations: Plot 1 (-7022458S, 107695115E), Plot 2 (-7020229S, 107693090E), Plot 3 (-7021429S, 107692446E), and Plot 4 (-7022559S, 10769364E). B-E. Condition of soil surface during the rice growth phase of 40 (B), 60 (C), 80 (D), and 110 days after planting (E)

Colony enumeration

The soil samples analyzed in this study was one cm depth soil layer. To make a dilution of 10^{-1}, a certain volume of sterilized distilled water was adjusted to the volume of the sample, which was counted using the formula πr^2 x t (r [tube radial] =1.5 cm; t [depth-soil layer] = 1 cm) (Roger et al. 1992). Serial dilution of 10^{-1} until 10^{-6} was carried out, and one mL of each dilution was pour-plated on a nitrogen-free Blue Green 11 agar medium ($BG11_0$). The plates were put on a rack provided with continuous light intensity of 1,200-1,400 lux at room temperature of 28°C ± 2°C. After three weeks, the growing colonies were counted.

Isolation of cyanobacteria

The techniques of isolation and purificaton followed Andersen and Kawachi (2005) and Guillard (2005). For isolation, agar and liquid $BG11_0$ media were used. Single colonies of cyanobacteria that grew from TPC agar plate were picked and transferred into a well-plate containing liquid $BG11_0$. Growing isolates were then streaked on an agar plate and single colonies were transferred again into the well-plate. This process was repeated until a single culture of isolates was established. For culture maintenance, the plates were placed on the rack under the same conditions mentioned above.

Observation of morphological characters

The morphological characters of the culturable isolates were examined, including the nature of the filaments, the shape and size of vegetative cells, heterocysts and akinetes (if found), the location and color of heterocysts, the color, growth pattern, and surface-texture of the colony. A Stereo Olympus SZX16 wide-zoom microscope and Olympus IX73 light microscope were used for morphological examination. The description of characters followed Komarek (2016).

Molecular identification

DNA extraction was performed using DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's protocols. Three primers were used to amplify the 16S rRNA gene: CYA359F (5`-GGGGAATYTTCCGCAATGGG-3`), CYA781R (5`-GACTACTGGGGTATCTAATCCCATT-3`) (Gharaei-Fathabad et al. 2007), and 1494R (5'-GTACGGCTACCTTGTTACGAC-3') (Neilan et al. 1997). Polymerase chain reactions (PCRs) were performed in a 12.5 µl mixture using Gotaq PCR reaction (Promega, Madison, WI, USA). PCR was performed under the following conditions: five minutes at 95°C, followed by 30 cycles of 15 seconds each at 95°C, 30 seconds at 58°C, and 60 seconds at 72°C. PCR products were visualized by electrophoresis in a 1% agarose in a tris-EDTA buffer at 100 V for 20 minutes. Purified PCR products were sent to Macrogen for sequencing. The sequenced forward and reverse fragments were checked using the software package DNA Baser (Heracle BioSoft SRL, Romania). The 16S rRNA gene sequences obtained were run into the BLASTn (Basic Local Alignment Search Tool nucleotide) program from GenBank to check species identity with reference sequences.

Data analysis

Collected data were analyzed to obtain the number of colonies per species, relative abundance, and relative frequency. Relative abundance was calculated based on the following formula:

$$\text{Relative abundance} = \frac{\text{Total colony of a species}}{\text{Total colony of all species}} \times 100\%$$

Frequency is another important parameter, which reflects the spread of a species in a given area. The frequency of a species is given in percent and calculated as follows:

$$\text{Relative frequency} = \frac{\text{Number of plots in which species occurred}}{\text{Total number of plots studied during rice growth}} \times 100\%$$

RESULTS AND DISCUSSION

Sarinah Organic Foundation has been cultivating brown rice variety Aek Sibundong since 2007. Plant transplanting applied Jajar Legowo System of 25x12.5x50 cm. The field was submerged with water at the beginning of rice planting and drained at 60 dap. Application of fertilizer (solid manure) on the field was carried out first on the land tillage, followed by spraying of liquid fertilizer once every 10 days for a total of six times. The last application of fertilizer was done at the end of 60 dap. All fertilizers used were organic.

Identification of dominant species found in the rice field

Concerning heterocystous N_2-fixing cyanobacteria strains, 23 morphospecies were recognized. Among 23 morphospecies, only 13 isolates were successfully cultured. Different shapes of vegetative cells (elliptical, spherical, sub-spherical, barrel, and cylindrical) and heterocysts (elliptical, spherical, barrel, and cylindrical) were observed. Seven genera from Ordo Nostocales (straight filamentous cyanobacteria) were found: *Allinostoc*, *Calothrix*, *Cylindrospermum*, *Desmonostoc*, *Halotia*, *Nostoc*, and *Roholtiella*.Two genera from Ordo Stigonematales (branched filamentous) were observed: *Hapalosiphon* and *Pelatocladus*. *Cylindrospermum* and *Calothrix* possessed akinetes, presented intercalary or terminally. Description and documentation of 13 culturable isolates were presented in Table 1 and Figure 2, respectively.

Previous research on the diversity of N_2-fixing cyanobacteria in rice fields was based on morphological identification that portrayed *Nostoc* and *Anabaena* as the most common genera (Table 2). The DNA sequencing of cyanobacteria cultures in the current study identified some genera (*Allinostoc*, *Halotia*, and *Desmonostoc*), whose occurrences in the rice field have not been reported before.

Strain CSO2 had high sequence similarity with *Halotia wernerae* (98.5% homology), while sequences of CSO3 and CSO13 were similar to *Desmonostoc danxiaense* (99% and 89.8% homology, respectively). Actually, *Halotia* and *Desmonostoc* are synonymous of *Nostoc*. Recent phylogenetic studies on *Nostoc* concluded that many members of this genus are polyphyletic taxa. Thus, several taxa were split out of *Nostoc* and assigned into new genera: *Dolichospermum* (Ralfs, Bornet, & Flahault) Wacklin, Hoffmann, & Komárek (Wacklin et al. 2009), *Roholtiella* Bohunická, Pietrasiak, & Johansen, gen. nov. (Bohunicka et al. 2012), *Halotia* D. B. Genuario et al. gen. nov. (Genuario et al. 2015), and *Desmonostoc* Hrouzek & Ventura (Hrouzek et al. 2013). This study shows conflict between morphological and molecular identification of cyanobacteria population that occurred in rice field. Therefore, we suggest that population study of cyanobacteria should shift from a morphological approach to molecular since the systematics of many genera of cyanobacteria have been undergoing reconstruction.

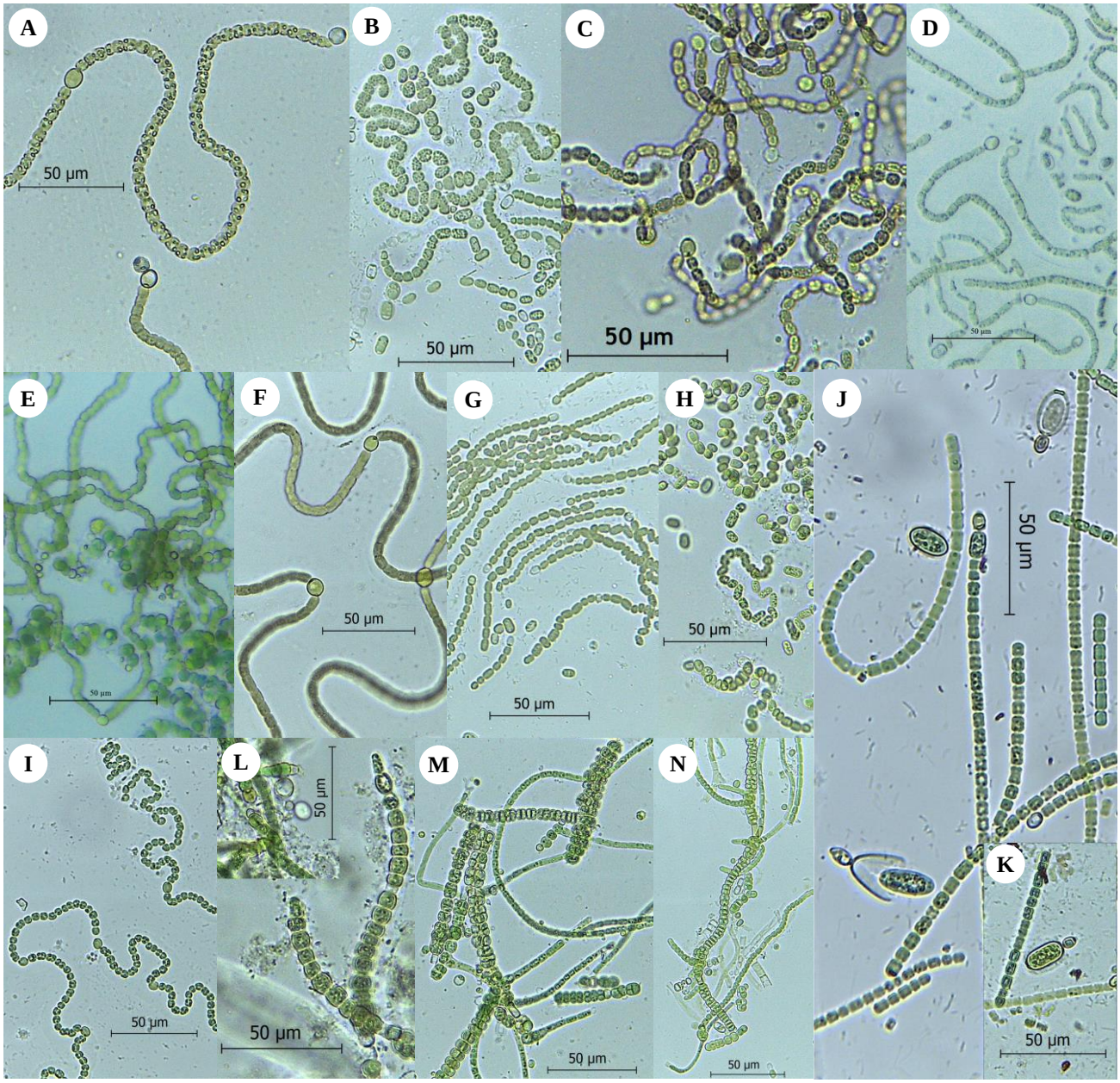

Figure 2. Thirteen culturable strains found in Sarinah Organic Rice Field of Ciparay Subdistrict, Bandung District, West Java, Indonesia. Strains belong to Ordo Nostocales (straight filamentous): A. CSO2, B. CSO3, C. CSO4, D. CSO6, E. CSO13, F. CSO15, G. CSO17, H. CSO20, I. CSO21, J. CSO23, K. Akinet of CSO23, L. CSO8. Strains belong to Ordo Stigonematales (branched-filamentous): M. CSO7, N. CSO11.

Table 1. Morphological description of 13 strains from Sarinah Organic Rice Field of Ciparay Subdistrict, Bandung District, West Java, Indonesia

Order	Family	Strains	Cell size (µm)	Morphology
Nostocales	Nostocaceae	CSO2	Vegetative cells barrel-shaped; L: 5-9, W: 4-7; Heterocysts spherical; L: 8-10; W: 7-10	Isopolar filaments, trichomes constricted at cross wall, heterocysts terminally or intercalary, cells granulated
		CSO3	Vegetative cells cylindrical; L: 3-5; W: 3-5; Heterocysts cylindrical; L: 3-6; W: 3-5	Isopolar filaments, filaments loosed (cells individually arranged in trichome), heterocysts terminally or intercalary, cells granulated
		CSO4	Vegetative cells cylindrical, L: 3-6; W: 3-4; Heterocysts ellipsoidal; L: 4-7; W: 4-6	Isopolar filaments, filaments so thickly entwined (difficult to recognized individual trichomes)
		CSO6	Vegetative cells barrel-shaped; L: 3-7; W: 3-5; Heterocysts almost spherical; L: 4-7; W: 4-6	Isopolar or heteropolar filaments; heterocysts terminally or intercalary, cells granulated
		CSO13	Vegetative cells sub-sperical; L: 3-6; W: 3-4; Heterocyst cells spherical L: 3.7-6; W: 3-4	Isopolar filaments, filaments loosed (cells individually arranged in trichome), heterocysts terminally or intercalary
		CSO15	Vegetative cells cylindrical; L: 4-8; W: 3-5; Heterocysts spherical or ellipsoidal; L: 5-8; W: 4-7	Isopolar or heteropolar filaments; heterocysts terminally or intercalary, cells having black spots
		CSO17	Vegetative cells barrel-shaped L: 3-6; W: 3-4; Heterocyst cells L: 4-6; W: 3-5	Isopolar filaments, filaments loosed (cells individually arranged in trichome), heterocyst terminally or intercalary
		CSO20	Vegetative cells cylindrical L: 3-6; W: 4-7; Heterocyst cells L: 4-6; W: 3-5	Isopolar filaments, filaments loosed (cells individually arranged in trichome), heterocyst intercalary
		CSO21	Vegetative cells barrel-shaped or cylindrical; L: 3-5; W: 3-4; Heterocysts sub-spherical; L: 4-8; W: 4-6.	Isopolar filaments, filaments loosed (cells individually arranged in trichome), heterocysts terminally or intercalary, cells granulated
	Rivulariaceae	CSO8	Vegetative cells at basal approx 8 µm long and 6 µm wide; cells at apex approx 3.8 µm long and 5.4 µm wide; heterocysts cells approx 6.7 µm long, 6.8 µm wide	Heteropolar filaments, tapering trichomes (trichomes attenuated into thin hairs), basal heterocysts, single in thick brownish yellow-pigmented sheaths, false-branching
		CSO23	Vegetative cells barrel-shaped or cylindrical; L: 3-5; W: 2-4; Heterocysts cylindrical; L: 4-6; W: 3-4; Akinet cylindrical; L: 5-8; W: 3-5	Heteropolar filaments, tapering trichomes, basal heterocyst with large akinet adjacent to heterocyst, cells having black spots
Stigonematales	Stigonemataceae	CSO7	Vegetative cells sub-sperical; L: 4-7; W: 6-9; Heterocyst cells cylindrical; L: 6-10; W: 4-7	Isopolar filaments, T-type true branching, uni- or multiseriate main filament with uniseriate branches, heterocysts intercalary
		CSO11	Vegetative cells sub-sperical; L: 4-6; W: 4-7; Heterocyst cells cylindrical; L: 5-11; W: 3-6. Akinet cell if found approx 7.6 µm long, 6.1 µm wide	T-type true branching, uni- or multiseriate filaments and branches, heterocysts intercalary

Table 2. Comparison of N_2-fixing cyanobacteria genera found in the present study to the common genera reported in some literatures

Ordo	Genus	Reference*)				Present study
		1	2	3	4	
Nostocales	*Nostoc*	+	+	+	+	+
	Anabaena	+	+	+	+	-
	Calothrix	-	+	+	+	+
	Cylindrospermum	-	+	+	-	+
	Scytonema	+	+	-	+	-
	Tolypothrix	-	+	-	+	-
	Allinostoc	-	-	-	-	+
	Desmonostoc	-	-	-	-	+
	Halotia	-	-	-	-	+
	Roholtiella	-	-	-	-	+
Stigonematales	*Hapalosiphon*	+	-	+	-	+
	Pelatocladus	-	-	-	-	+

Note: +: found; -: not found. *) Reference: 1. Vijayan et al. (2015), 2. Singh et al. (2014b), 3. Deep et al. (2013), 4. Choudhary (2011)

Table 3. The psychochemical parameters of soil in Sarinah Organic Rice Field of Ciparay Subdistrict, Bandung District, West Java, Indonesia

Parameters	Rice growth phase		
	60 dap	80 dap	110 dap
Soil pH	5.8-6.8	5.8-6.8	6.8-7
Soil temperature (°C)	30-32	26-30	20-24
Light intensity (lux)	21,800-36,200	6,500-16,200	81,600-95,200
Light intensity under canopy (lux)	10,100-30,400	3,500-5,200	3,100-4,200
Weather	sunny	cloud-rainy	cloud-sunny
Water content (%)	6.88	11.5	5.46
C organic (%)	2.78	2.77	2.29
Total N (%)	0.21	0.23	0.14
P_2O_5 (%)	4.88	8.29	1.68
K_2O (%)	0.27	0.26	0.64
C/N ratio	13.24	12.04	16.36

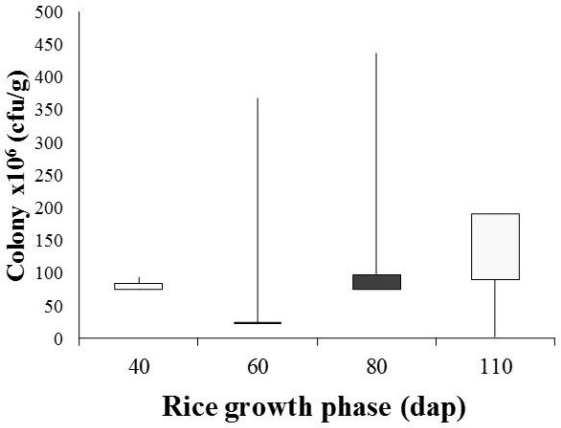

Figure 3. Total colony of N_2-fixing cyanobacteria in Sarinah Organic Rice Field of Ciparay Subdistrict, Bandung District, West Java, Indonesia during the rice growth phase

Population dynamic of N_2-fixing cyanobacteria during rice growth

During rice growth, population growth of N_2-fixing cyanobacteria in the four study plots ranged between 3 x 10^6 cfu/g and 437 x 10^6 cfu/g. There were significant blooming populations at 60 dap and 80 dap, as indicated by the high bar chart in Figure 3. At 60 dap, strain CSO11 outcompeted strain CSO2 that had been dominating in the previous rice phases. However, after outpacing CSO2, CSO11 "crashed" during the following phase, changed by CSO13. A blooming phenomenon is usually observed when environmental conditions favor a certain population. The changing of light irradiation during rice growth is one factor that can influence blooms (Quesada et al. 1998). Another factor that could influence population blooms is the changing of nutrient in soil. Table 3 showed some psychochemical parameters of rice soil at 60, 80, and 110 dap. As nitrogen fixer, cyanobacteria dependency on phosphor is higher than nitrogen. As a component for ATP, phosphor is needed in nitrogen assimilation that require a lot of energy. The raise of phosphor content (%) in soil, from 4.88 at 60 dap to 8.29 at 80 dap, give cyanobacteria an opportunity to multiply and flourish on the soil surface.

The abundance of N_2-fixing cyanobacteria populations found in this rice field study was 86 x 10^6 cfu/g at the beginning of the rice growth cycle. The number increased to 119 x 10^6 cfu/g, then reached peak at 80 dap (194 x 10^6 cfu/g). The abundance of the colony decreased as rice entered the harvest period (86 x 10^6 cfu/g). The dynamic of population abundance may be due to the growing canopy of the paddy that became denser and covered the surface of the soil. As a photosynthetic organism, N_2-fixing cyanobacteria use energy from sunlight to grow. During the vegetative phase, the paddy plants were short and the canopy was not yet formed. The soil surface was directly exposed to sunlight. Measurements of light intensity on the soil surface were 10,100-30,400 lux during the vegetative phase. Later in the generative phase, as the paddy canopy covered the light, the light intensity on the soil surface was only 3,100-5,200 lux. Only the species that have a wide spectrum to light intensity were able to survive. Hence, those species dominated their habitat and contributed to the high abundance of the next rice growth phase. Light as a decisive factor on the abundance of dominant cyanobacterial genera has been reported. Quesada et al. (1998) found that the three main heterocystous components (*Anabaena*, *Nostoc*, and *Calothrix* spp.) responded differently to the different levels of irradiation. A higher abundance of *Nostoc* coincided with a lower abundance of the other two genera. In this study, the high number of *Hapalosiphon* CSO7 at 110 dap showed adaptation to low light. Adaptation to low light involved the changing of the thylakoid membrane structure and formation of glycogen granules as a carbon energy source while metabolism activity was suppressed (Baulina 2012).

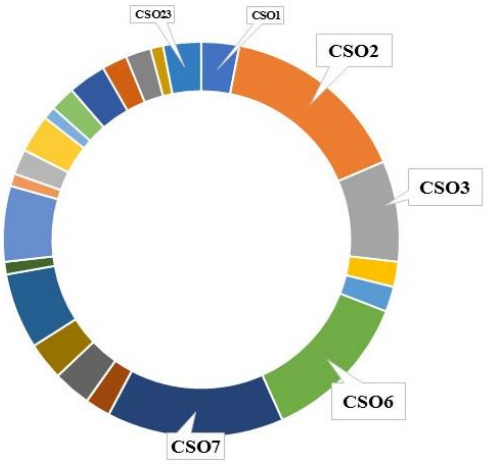

Figure 4. Relative frequency of N2-fixing cyanobacteria in Sarinah Organic Rice Field. Each color represents one strain. The sequence of strains is clockwise, starts from CSO1 and ends up with CSO23. Four strains (CSO2, CSO3, CSO6, and CSO7) are always found during the rice growth phase.

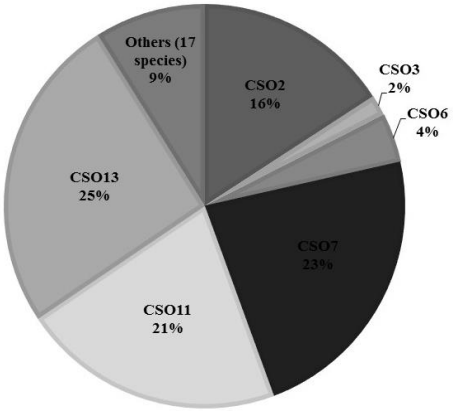

Figure 5. Relative abundance of N2-fixing cyanobacteria during the rice growth phase. Six strains had high population number compare to the rest of populations.

Twenty-three morphospecies were found in the present study. At the beginning of rice growth, there were 17 species. The number of species decreased to 15 at 60 dap and then went down to 13 at 80 dap. At the end of the rice growth, the number of species was only seven. Some species were found in all phases of rice growth, some were in two or three phases, while others were only in one phase. Species that were encountered only once during rice growth were more numerous (eight species). Relative frequency of species ranged from 6.25% to 93.75%. *Halotia wernerae* CSO2 was frequently found (93.75%), followed by *Hapalosiphon welwitschii* CSO7 (87.5%), *Desmonostoc danxiaense* CSO6 (75%), and *D. danxiaense* CSO3 (50%) (Figure 4). Relative abundance of species was shown in four species with a high percentage: *H. wernerae* CSO2, *H. welwitschii* CSO7, *Pelatocladus maniniholoensis* CSO11, and *D. danxiaense* CSO13 (Figure 5). The relative abundance of those four species was even higher than the total population of 17 other species. In this study, frequently found species were not always those with a high population number.

Eight species were found only during one phase of rice growth and failed to be cultured in the laboratory. This showed that either most populations were in a dormant or inactive state or that the populations were too small to expand in the rice microbial community. However, some populations appeared to be in a "potentially active" condition, such as CSO3, CSO11, and CSO13. According to Blagodatskaya and Kuzyakov (2013), potentially active microorganisms have the characteristic or ability to change immediately (physiologically) from an inactive condition to being active. Populations with a small number of colonies might lose the competition for life-supporting factors, such as nutrients and light. When such conditions occur, cells differentiate into akinetes (dormant cells), and sporulation is halted for the next phase. This might explain the interchanging of the colony's succession between CSO11 and CSO13 at plot 2 during 60 dap and 80 dap. Dinitrogen fixing cyanobacteria have unique filament development, not only involving parallel cell division but also heterocyst differentiation and elongation of trichomes (Mateo et al. 2011). Heterocyst differentiation in N_2-fixers is important because this transparent, thick-walled cell serves as a nitrogen-producing chamber. Short trichomes (called hormogonia) are first developed by parallel cell division, and then vegetative cells differentiate into heterocysts. Heterocysts become a pivot for the trichome's elongation. After they reach a certain size, filaments break up at the heterocyst site. Filaments turn into hormogonia, and the life cycle of new filaments starts again.

There is a common argument that living cells of cyanobacteria release fixed atmospheric nitrogen into soil. But, as the rice field is already contained nitrogen, supplied by the farmer, there is the possibility that cyanobacteria use this available nitrogen-fertilizer and stop synthesizing their own heterocyst cells. In this case, cyanobacteria do not hold a role as nitrogen supplier anymore. Under laboratory experiments, we found that 13 culturable isolates had various value of ammonium production. The ability of strains found in the Sarinah Organic rice field to fix atmospheric nitrogen is not lost, even though populations have been exposed to available nitrogen for years. Amongst 13 strains, CSO2, CSO6, and CSO7 produced ammonium (ppm/mg) higher than others (unpublished data).

In conclusion, the present study shows that the community structure of N_2-fixing cyanobacteria in a rice field is shaped by the active and dominant population of *H. wernerae* CSO2, *D. danxiaense* CSO6, and *Hapalosiphon welwitschii* CSO7, which consistently occur and are distributed over the community during rice growth. Dominant population of N_2-fixing cyanobacteria in rice fields donates a certain amount of ammonium to the rice soil. Under what conditions and how much ammonium is released into the environment are still open questions that need further research.

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REFERENCES

Andersen RA, Kawachi M. 2005. Traditional microalgae isolation techniques. In: Andersen RA (eds) Algal Culturing Techniques. Elsevier Academic Press, Burlington.

Baulina OI. 2012. Ultrastructural plasticity of cyanobacteria under dark and high light intensity conditions. In: Baulina OI. (eds) Ultrastructural Plasticity of Cyanobacteria. Springer-Verlag, Berlin.

Bisen PS. 2014. Laboratory Protocol in Applied Life Sciences. CRC Press, New York.

Blagodatskaya E, Kuzyakov Y. 2013. Active microorganisms in soil: Critical review of estimation criteria and approaches. Soil Biol Biochem 67: 192-211.

Bohunicka M, Pietrasiak N, Johansen JR, Gomez EB, Hauer T, Gaysina LA, Lukesova A. 2012. Roholtiella, gen. nov. (Nostocales, Cyanobacteria) a tapering and branching cyanobacteria of the family Nostocaceae. Phytotaxa 197 (2): 84-103.

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

Deep PR, Bhattacharyya S, Nayak B. 2013. Cyanobacteria in wetlands of the industrialized Sambalpur District of India. Aqua Biosys 9: 1-12.

Fernandez-Valiente E, Quesada A. 2004. A shallow water ecosystem: rice fields. The relevance of cyanobacteria in the ecosystem. Limnetica 23 (1-2): 95-108.

Genuario DB, Vieira Vaz MGM, Henschke GS, Sant'Anna CL, Fiore MF. 2015. Halotia gen. nov. a phylogenetically and physiologically coherent cyanobacterial genus isolated from marine coastal environments. Int J Sys Evol Microbiol 65: 663-675.

Gharaei-Fathabad E, Yazdi MT, Seyed-Naser O, Shokravi S, Sepehrizadeh Z, Faramarzi MA, Amini M. 2007. Nostoc piscinale Gt-

319, a new Cyanobacterial strain with cytotoxic activity. Biotechnology 6 (4): 505-512.
Granhall U, Kulassoriya SA, Hirimburegama WK, de Silva RSY, Lindberg T. 1987. Nitrogen fixation in some rice soils in Sri Lanka. World J Microbiol Biotech 3 (4): 67-88.
Guillard RRL. 2005. Purification method for microalgae. In: Andersen RA (eds) Algal Culturing Techniques. Elsevier Academic Press, Burlington.
Hrouzek P, Lukesova A, Mares J, Ventura S. 2013. Description of the cyanobacterial genus Desmonostoc gen. nov. including *D. muscorum* comb. nov. as a distinct, phylogenetically coherent taxon related to the genus Nostoc. Fottea 13 (2): 201-213.
Kaushik BD. 2014. Developments in Cyanobacterial biofertilizer. Proc Indian Nat Sci Acad 80 (2): 379-388.
Kim JD, Lee CG. 2006. Diversity of heterocystous filamentous Cyanobacteria (Blue-green algae) from rice paddy fields and their differential susceptibility to ten fungicides used in Korea. J Microbiol Biotech 16 (2): 240-246.
Komarek J. 2016. Taxonomic classification of cyanoprokaryotes (cyanobacterial genera), using a polyphasic approach. Preslia 86: 295-335.
Mateo P, Perona E, Berrendero E, Leganes F, Martin M, Golubic S. 2011. Life cycle as a stable trait in the evaluation of diversity of Nostoc from biofilm in rivers. FEMS Microbiol Ecol l76: 185-198.
Neilan BA, Jacobs D, Del Dot T, Blackall LL, Hawkins PR, Cox PT, Goodman AE. 1997. rRNA sequences and evolutionary relationships among toxic and nontoxic cyanobacteria of the genus Microcystis. Int J Sys Bacteriol 47: 693-697.
Prasanna R, Sharma E, Sharma P, Kumar A, Kumar R, Gupta V, Pal RK, Shivay YS, Nain L. 2013. Soil fertility and establishment potential of inoculated cyanobacteria in rice crop grown under non-flooded conditions. Paddy Water Environ 11: 175-183.
Quesada A, Nieva M, Leganes E, Ucha A, Prosperi C, Fernandez-Valiente E. 1998. Acclimation of cyanobacterial communities in rice fields and response of nitrogenase activity to light regime. FEMS Microbiol Ecol 35: 147-155.
Roger PA, Jiminez R, Santiago-Ardales S. 1992. Methods for studying blue-green algae in rice field: distributional ecology, sampling strategies, and estimation of abundance. IRRI Res Paper Series 150: 1-17.
Saadatnia H, Riahi H. 2009. Cyanobacteria from paddy fields in Iran as biofertilizer in rice plants. Plant Soil Environ 55 (5): 207-212.
Singh H, Khattar JS, Ahluwalia AS. 2014a. Cyanobacteria and agricultural crops. Vegetos 27 (1): 37-44.
Singh SS, Kunui K, Minj RA, Singh P. 2014b. Diversity and distribution pattern analysis of cyanobacteria isolated from paddy fields of Chhattisgarh, India. J Asia-Pacific Biodivers 7: 462-470.
Sinha RP, Hader DP. 1996. Photobiology and ecophysiology of rice field cyanobacteria. Photochem Photobiol 64 (6): 887-896.
Sinha RP, Hader DP. 2006. Impact of UV radiation on rice-field cyanobacteria: Role of photo protective compounds. In: Ghetti F (ed) Environmental UV Radiation: Impact on Ecosystems and Human Health and Predictive Models. Springer, Netherlands.
Song T, Martensson L, Ericsson T, zheng W, Rasmussen U. 2005. Biodiversity and seasonal variation of the cyanobacterial assemblage in a rice field in Fujian, China. FEMS Microbiol Ecol 54: 131-140.
Syiem MB, Nongrum NA, Nongbri BB, Bhattacharjee A, Biate DL, Misra AK. 2011. Molecular identification and characterization of a rice field cyanobacterium for its possible use as biofertilizer in acidic environment. Research & Reviews: J Microbiol Virol 1 (3): 1-12.
Vijayan D, Ray JG. 2015. Ecology and diversity of Cyanobacteria in Kuttanandu paddy wetlands, Kerala, India. Am J Plant Sci 6: 1-16.
Wacklin P, Hoffmann L, Komarek J. 2009. Nomenclatural validation of the genetically revised cyanobacterial genus Dolichospermum (Ralfs ex Bornet et Flahault) comb. nova. Fottea 9 (1): 59-64.