

Next Generation Sequencing (NGS) for Cyanobacterial study in Agung and Sunter Barat Lakes, North Jakarta, Indonesia

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Abstract. Hendrayanti D, Prihantini NB, Ningsih F, Maulana F. 2023. Next Generation Sequencing (NGS) for Cyanobacterial study in Agung and Sunter Barat Lakes, North Jakarta, Indonesia. *Biodiversitas* 24: 1117-1124. The Next Generation Sequences (NGS) is one of the established metabarcoding methods for analyzing microbial communities. Investigation of the cyanobacterial community in eutrophic freshwater habitats is important, especially for those known as toxic-released cyanobacteria. This study aimed to analyze the structure community of cyanobacteria in Agung and Sunter Barat Lakes in North Jakarta, DKI Jakarta Province using the NGS method. The physico-chemical parameters were also measured. Sampling plots were selected by purposive sampling method. The result of the study successfully analyzed the bacterial community structure and portrayed the dominancy of the cyanobacterial population on the two lakes. Several cyanobacteria genera previously reported in 2008 research were found, including *Arthrospira* and *Planktothrix*. The diversity index in Agung Lake (4.7) was higher than in Sunter Barat Lake (3.7). Two dominant populations were found, which were *Raphidiopsis* (in Agung Lake) and *Planktothrix* (in Sunter Barat Lake). Both genera have been acknowledged as toxin-releasing cyanobacteria, which could be harmful to the water-biotic community as well as human health. The presence of potentially toxic cyanobacteria in the recreation area of Agung Lake should be taken into consideration for biomonitoring management.

Keywords: biomonitoring, cyanobacteria, metabarcoding, microbial community, Sunter Lake

INTRODUCTION

A new technology referred to as 'Next Generation Sequencing (NGS)' was introduced in 2005 that leads to the determination of millions of DNA sequences in a single process (Shokralla et al. 2012). Using this new method, community structure, the alpha and beta taxonomy of microbial communities can be analyzed. The NGS method was developed on several platforms, such as Illumina, Ion Torrent, PacBio, and Nanopore (De Corato 2020). The use of such platforms depends on the hypothesis and purpose of the research determined by the researcher. Hence, the high-output NGS approaches in biodiversity science also have the potential for lake or river biomonitoring (Hajibabaei et al. 2011), which is traditionally dependent on the measurement of physico-chemical characteristics.

Cyanobacteria are part of a bacterial community commonly found in a body of water (Pope and Patel 2008; Soltani et al. 2012; Rodriguez et al. 2016; Affe et al. 2018). Some members of Cyanobacteria, such as *Planktothrix* and *Microcystis* can secrete toxic compounds that damage animal nerves and liver (Chorus and Welker 2021). Such ability makes cyanobacterial blooming pose a dangerous risk to aquatic biota, especially humans. Cyanobacterial blooming in eutrophic water was frequently a cyclic phenomenon led by a continuous supply of nutrients, mainly nitrogen and phosphorus, coupled with optimal pH

and temperature conditions (Wilhelm et al. 2020). The effectivity of management of cyanobacterial blooming through controlling physico-chemical factors can be improved with metagenomic analysis. Metagenomic method can detect cyanobacterial populations with low relative abundance (<5% from total reads sequences) that have the potential of germinating in high numbers (Affe et al. 2018). *Microcystis* blooming monitoring using the metagenomic method was able to distinguish water areas dominated by cyanobacteria groups, such as *Microcystis*, *Aphanizomenon*, and *Planktothrix* from those dominated by non-toxic cyanobacteria and algae, such as *Chlamydomonas* and *Volvox* (Kurobe et al. 2018). This makes monitoring at-risk areas right on target.

Sunter is an administrative village located in Tanjung Priok Subdistrict, North Jakarta, Indonesia. Sunter area is geologically a river basin because Sunter River runs along the neighborhood. The river starts from eastern Jakarta and ends its 37 km journey up in Jakarta Bay, which is only 8 km far from Sunter Village. Sunter River is frequently flooded, because it cannot accommodate the surface runoff, especially during the rainy season above 100 mm (Office of Environmental Services DKI Jakarta Province 2021). The catchment area of Sunter River near Jakarta Bay is supported by the occurrence of several lakes, such as Situ Kodamar, Situ Kelapa Gading, Situ Rawa Badak, and Waduk Sunter 1 (now separated as Agung and Sunter

Barat Lakes). The water quality of those lakes is decreasing from 2015 to 2021 because of growing domestic and industrial activities around Sunter area (Hendrawan 2005). The aforementioned lakes have mesotrophic status. The mesotrophic status of body water is frequently associated with high nutrients of nitrogen and phosphor, that trigger the blooming algae phenomenon (Office of Environmental Services DKI Jakarta Province 2021).

The Jakarta Municipal Office revitalized Agung and Sunter Barat Lakes in 2014. According to the Spatial and Regional Planning Office, the two lakes have different functions. Agung Lake is designated as an ecotourism area, while Sunter Barat Lake serves as a flood controller and domestic waste shelter. As an ecotourism area, biomonitoring of lake management should be applied because physico-chemical-based monitoring cannot reflect the potential harm to the biotic population inhabiting the lakes. This study aimed to use the NGS Method to examine the structure community of Cyanobacteria in Agung and Sunter Barat Lake. Lake monitoring using the advanced method of NGS was hypothesized to be faster and more accurate than the conventional method. Information on the cyanobacterial structure community will provide a better understanding of the harmful risk to lake management.

MATERIALS AND METHODS

Study area

The sampling site was located at Agung and Sunter Barat Lakes, Sunter Village, North Jakarta, DKI Jakarta Province, Indonesia (Figure 1). The coordinate site of Agung Lake was 6°8'44.13"S, 106°52'38.08"E and Sunter Barat Lake was 6°8'48.00"S, 106°52'15.98"E.

Procedures

Water sampling

Field sampling was carried out in November 2021. Sampling plots were selected by purposive sampling

method. The environmental conditions of both lakes were quite different. As a public area, the water surface of Agung Lake was near the pedestrian walk so that easily to be disturbed. On the contrary, Sunter Barat Lake was concealed with concrete so that the water surface is far from the edge of the concrete (about 5 m below). The concrete turned out to be a protection for the lake from public activities. At the sampling plots, as much as 4 L of water was collected with a 5 L plastic bucket and poured into a plankton net (mesh 20 μ m). This was repeated 5 times so that the final volume that filtered on the plankton net was 20 L. The samples were separated and later used for isolation dan culture. For the metagenomic study, 2 L of water volume was collected and kept in a sterilized bottle (Acharya et al. 2020). All samples were labeled and immediately brought to the Culture Algae Laboratory, Department of Biology, Universitas Indonesia. Samples for the metagenomic study were kept in a refrigerator (4 °C) for a night before the filtration time.

The environmental parameters were measured on sampling sites. Parameters of temperature, pH, conductivity, dissolved oxygen, and nitrate were measured using a multiparameter meter VERNIER (GDX-FPH, ODO-BTA, GDX-NO3). Water transparency was estimated using a Secchi disc. Light intensity was measured using a light meter.

Metagenomic library preparation

Water samples were filtered through a polycarbonate membrane (Merck GS, 0.22 μ m pore, Darmstadt, Germany) with a vacuum pump. The membrane filters were stored in microtubes kept in a -20 °C freezer until DNA extraction. For metagenomic analysis, membrane-filtered samples were directly sent to Genetika Science for gDNA extraction and NGS Service using Oxford Nanopore Technologies (GridION) Platform with the full-length 16S Barcoding for Metagenomics package. The data presented in this study are accessible on EZBioclouds under the project number PKSSU4.0.

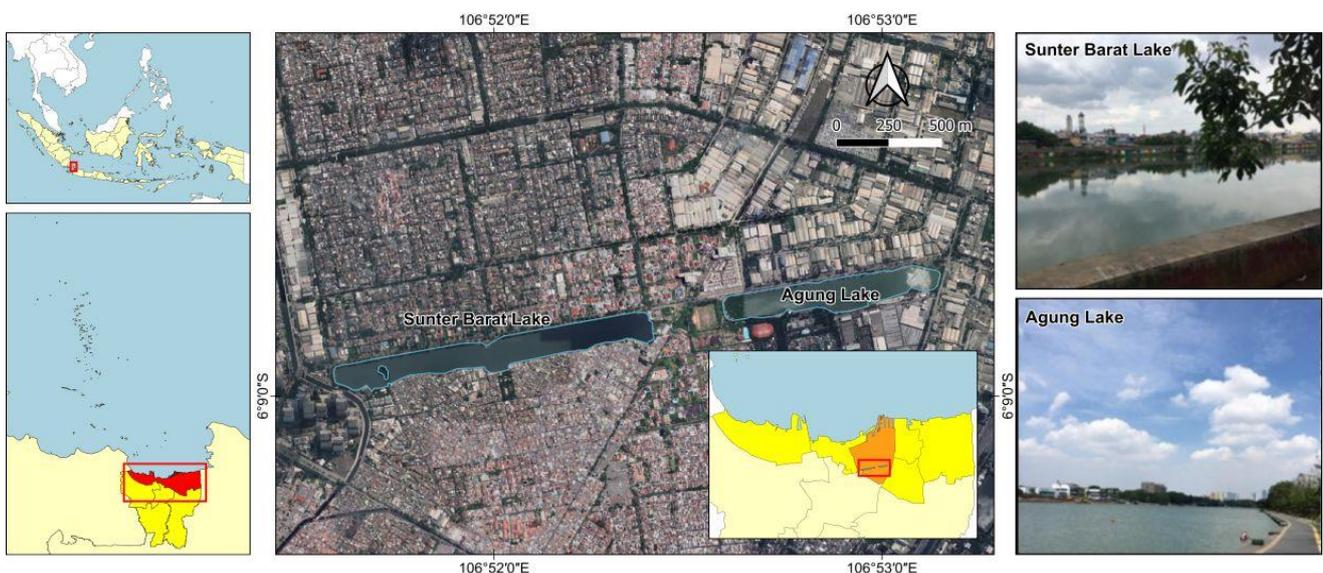


Figure 1. Sampling sites of Agung and Sunter Barat Lakes, North Jakarta, DKI Jakarta Province, Indonesia. (Source: Google Earth)

Enrichment and isolation

The fresh sample was subjected to culture enrichment using several media of Blue Green 11 (BG-11) nitrogen-free, Bold Basal Medium (BBM), and CT (Andersen and Kawachi 2005). Later, enrichment cultures were isolated without purification by serial dilution and then transferred to their original medium used in the enrichment process. Algal identification to the genus level from fresh samples was performed using morphological analysis based on Geitler (1985) and Komarek and Mares (2012). A Stereo Olympus SZX16 wide-zoom microscope and Olympus IX73 light microscope were used for morphological examination.

Data analysis

Taxonomic assignment for microbial diversity from each sample was estimated by EzBioCloud 16S-based Microbiome Taxonomic Profiling (MTP) program (Yoon et al. 2017; ChunLab, Inc., Seoul, Republic of Korea). The paired-end reads data from samples were uploaded to EzBioCloud 16S-based MTP server (<https://www.ezbiocloud.net/contents/16smtp>) to check the data quality. Trimming primer sequences, detecting, and filtering out low-quality sequences were done by EzBioCloud 16S-based MTP program. Sequences with a length of less than 100 bp or more than 2000 bp and having averaged Q value of less than 25 were filtered out. Extracting non-redundant reads was performed for the identical sequences to reduce computational time. Detection and calculation of sequence similarities of the query paired-end reads against the EzBioCloud 16S database were carried out using VSEARCH program (Rognes et al. 2016) corresponded to the 97% of 16S rRNA sequence similarity cutoff to reference species level against EzBioCloud 16S database. Chimeric sequences were removed using the UCHIME program. The 16S rRNA sequences that did not match with the reference sequences with at least 97% similarity cutoff were then clustered into Operational Taxonomic Units (OTUs) using the UCLUST tool. Alpha diversity indices were measured by EzBioCloud 16S-based MTP server in order to obtain the corresponding number of species richness and species diversity (Shannon Diversity Index).

RESULTS AND DISCUSSION

Environmental parameters

Several environmental factors showed that both lakes support the occurrence of Cyanobacteria as well as another group of algae (*Chlorophyta*, Diatom) (Table 1). The degree of acidity (pH) of the waters in both lakes tends to be alkaline conditions, Lake Agung is more alkaline than Sunter Barat Lake. The pH data corresponds to the pH of the waters needed for the optimum growth of Cyanobacteria that inhabit neutral or alkaline waters. Similarly, the water temperature (30-31 °C) is a good temperature range for the optimal growth of Cyanobacteria as thermophilic microbes with strong phototaxis (He et al. 2020).

Dissolved oxygen (DO) in Agung Lake (7.87 mg/L) was lower than Sunter Barat (12.94 mg/L), while the nitrate content of Agung Lake (62.54 mg/L) was higher than Sunter Barat (28.27 mg/L). Dissolved oxygen and nitrogen content are used to employ for determining the trophic status of a lake. Quality assessment of Sempor reservoir in Kebumen, Central Java, reported the mesotrophic status of the water with a DO value of $10^{-3} - 10^{-1}$ (mg/L) and nitrogen content of 4-8 (mg/L) (Shaleh et al. 2014). Compared to the trophic status of Sempor reservoir, Agung and Sunter Barat Lakes can be considered as eutrophic lakes.

The water brightness is a condition that reflects the ability of sunlight penetration to penetrate the water column to a certain depth. The brightness is intensified with the increasing turbidity. Nutrient content, mud, and abundance of phytoplankton can affect light penetration. The higher penetration of light to the water column in Agung Lake affected the abundance of phototrophic cyanobacteria and algae in water bodies, as shown in microscopic examination.

Bacteria taxonomic composition

The bacterial community in Sunter Barat and Agung Lakes comprised of phyla Cyanobacteria, Proteobacteria, Bacterioidetes, and Firmicutes (Figure 2). Cyanobacteria dominated the bacterial community in Sunter Barat Lake (49%) while it was found in equal proportion with Proteobacteria and Bacterioidetes in Agung Lake, about 31% of the total community. In total, 1,109 and 1,004 OTUs bacteria were identified in Agung and Sunter Barat Lakes, respectively. Shannon Diversity Index showed that Agung Lake had higher diversity (4.68) than Sunter Barat Lake (3.70). High diversity in Agung Lake was reasonable because of cyanobacterial dominance in Sunter Barat Lake. The dominance of certain populations could decrease the species diversity in one community. In Sunter Barat Lake, Cyanobacteria occupied almost 50% of the bacterial community, followed by Proteobacteria (40%) and the rest (10%) were divided among other small groups. A summary of sequences' read counts and bacterial phylum composition is provided in the supplementary material (Supplementary Figure S1, Table S1, and S2).

Table 1. Environmental parameters in Agung and Sunter Barat Lakes, Jakarta, Indonesia

Parameters	Value	
	Agung Lake	Sunter Barat Lake
Weather	Partly cloudy	Sunny
Temperature (°C)	31	34
Precipitation (%)	72	57
Light intensity (lux)	298,000-520,000	481,000-570,000
Depth (cm)	28	18
Water temperature (°C)	30.2	31.6
pH	9.02	8.81
Conductivity (µS/cm)	881	838
Dissolved oxygen (mg/L)	7.87	12.94
Nitrate (mg/L)	62.54	28.27

A previous study has reported the abundance of Cyanobacteria in freshwater small lakes, namely Cikaret, Cincin, Sunter, and Telaga Warna Lakes in the Ciliwung watershed (Sulastrri et al. 2022). The dominance of Cyanobacteria was observed in Telaga Warna and Sunter Lakes with a total abundance of 71 to 100%, respectively. Meanwhile, Cyanobacteria was detected at only 22 % in Cikaret and 24 % in Cincin Lakes. According to Sulastrri et al. (2022), Sunter Lake was considered a hypereutrophic which supported the blooming of Cyanobacteria, especially the species *Planktothrix agardhii*. Lake Sunter has faced algae bloom due to rich organic matter in the water since 2000. Sunter Barat and Agung Lakes are in the city of Jakarta as an urban area which can influence the quality of water in both lakes. Urban areas contributed to producing the city's sewage and industrial wastewater, which can cause a high concentration of phosphate and nitrate in waters and thus quicken eutrophication (Sulastrri et al. 2008).

The low dissolved oxygen concentration in Agung Lake (7.87 mg/L) might correlate with the high diversity of non-cyanobacterial groups. Most members of Proteobacteria, Bacteroidetes, and Firmicutes are anaerobes, either facultative or obligate (Madigan et al. 2012). In such cells, oxygen as an electron acceptor of cell respiration is replaced with other molecules, such as nitrate (NO_3^-) or sulfate (SO_4^{2-}). Hence, Agung Lake provided a suitable condition for non-cyanobacterial groups. On the contrary, autotrophic Cyanobacteria release oxygen during photosynthesis. This may explain the high DO concentration in Sunter Barat Lake (12.94 mg/L) where Cyanobacteria dominated the water body.

According to Cox (1991), there are two important considerations in favoring organisms as parameters for monitoring the quality of the aquatic environment. First, organisms respond to environmental change on a daily-based. Second, biotic observations in freshwater environments allow us to maintain a healthy and diverse biological community. Biomonitoring is used to carry out by calculating the relative species abundance-density and diversity index. This method takes time and needs an expert to identify species. The Next-Generation Sequencing (NGS) method used in the present study can be considered an advantage. In a matter of days, information on the cyanobacterial community in Agung and Sunter Barat Lakes can be performed. The identification of species is validated through the genome database.

Cyanobacteria taxonomic composition

The alpha taxonomy assignments of Cyanobacteria showed that Oscillatoriales, Nostocales, and Chroococcales were constituents of Agung Lake, while the last two order was not found in Sunter Barat Lake (Figure 3). Hence, Oscillatoriales predominated both lakes, by a percentage of 32% in Agung Lake and 98% in Sunter Barat Lake. Besides *Planktothrix*, other genera of Oscillatoriales were also found, such as *Oscillatoria*, *Pseudanabaena*, and *Limnothrix*. Members from Nostocales included *Nostoc*, *Raphidiopsis*, and *Cylindrospermopsis*, while Chroococcales were Cyanobacteriaceae, Chroococcaceae, and *Merismopedia*. The molecular approach used in the present study found three genera in Agung Lake that had not been recorded in previous research by Prihantini et al. (2008) using the conventional method. The three genera were *Oscillatoria*, *Chroococcus*, and *Merismopedia*.

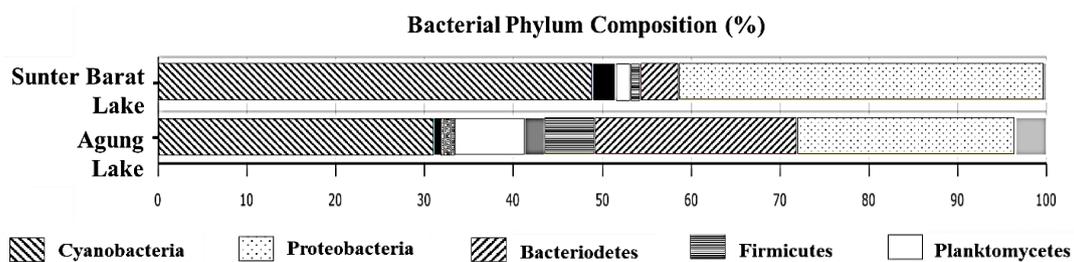


Figure 2. Major bacterial composition in Agung and Sunter Barat Lakes, Jakarta, Indonesia

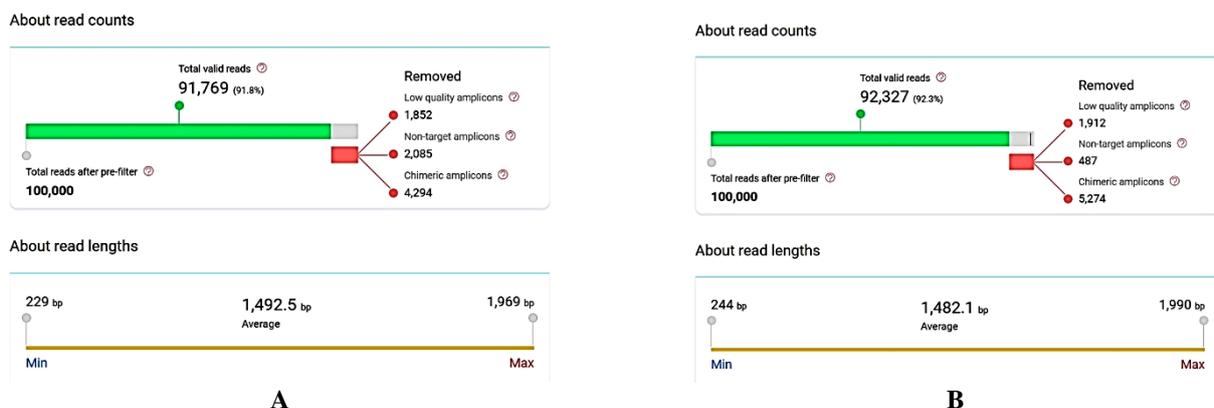


Figure S1. Percentage of Sequences' Read Counts and Lengths in Agung (A) and Sunter Barat (B) Lakes, Jakarta, Indonesia derived from EzBioCloud 16S-based MTP server.

Table S1. Bacterial composition in Agung Lake, Jakarta, Indonesia

Taxon name	Count	Proportion (%)
Cyanobacteria	28276	30.8121
Proteobacteria	22550	24.5726
Bacteroidetes	20887	22.7604
Planctomycetes	7221	7.8687
Firmicutes	5254	5.7252
Microgenomates_OP11	2021	2.2023
Verrucomicrobia	1396	1.5212
Actinobacteria	988	1.0766
Acidobacteria	581	0.6331
Hydrogenedentes_NKB19	432	0.4707
Chloroflexi	431	0.4697
Saccharibacteria_TM7	317	0.3454
Bacteria_uc	263	0.2866
Armatimonadetes	258	0.2811
Chlorobi	243	0.2648
TM6	136	0.1482
Rhodothermaeota	103	0.1122
Omnitrophica_OP3	83	0.0904
Fusobacteria	56	0.061
Gemmatimonadetes	45	0.049
Deinococcus-Thermus	41	0.0447
Spirochaetes	38	0.0414
Peregrinibacteria	17	0.0185
WS5	17	0.0185
Nitrospirae	16	0.0174
JQ072929_p	15	0.0163
Berkelbacteria	11	0.012
Chlamydiae	10	0.0109
Parcubacteria_OD1	10	0.0109
Elusimicrobia	7	0.0076
JMYB36	6	0.0065
Lentisphaerae	6	0.0065
Cloacamonas_p	6	0.0065
Fibrobacteres	5	0.0054
WS6	5	0.0054
GU454958_p	3	0.0033
Aminicenantetes_OP8	2	0.0022
WS1	2	0.0022
Caldithrix_p	2	0.0022
BRC1	1	0.0011
CPR2	1	0.0011
TDNP	1	0.0011
WM88	1	0.0011
WWE3	1	0.0011
CU922841_p	1	0.0011
EU266861_p	1	0.0011
FJ437850_p	1	0.0011
GU208502_p	1	0.0011

Although the NGS method could provide big sequence data, not all sequences could be read at the species level. For example, sequences of Nostocaceae and Cyanobacteriaceae, which highly occurred in Agung Lake, represented a bulk of sequences that did not match the sequence database library. The 16S rRNA gene is widely and well-accepted as a marker gene for bacteria species identification due to its nature as an evolutionarily conserved gene and found in large copy numbers (Garcia-Pichel 2008). However, compared to other groups of

Table S2. Bacterial composition in Sunter Barat Lake, Jakarta, Indonesia

Taxon name	Count	Proportion (%)
Cyanobacteria	45177	48.9315
Proteobacteria	37623	40.7497
Bacteroidetes	3990	4.3216
Actinobacteria	2351	2.5464
Planctomycetes	1554	1.6831
Firmicutes	1020	1.1048
Bacteria_uc	221	0.2394
Deinococcus-Thermus	113	0.1224
Verrucomicrobia	84	0.091
Chloroflexi	46	0.0498
Spirochaetes	23	0.0249
Chlorobi	21	0.0227
Microgenomates_OP11	18	0.0195
Acidobacteria	15	0.0162
Omnitrophica_OP3	9	0.0097
Peregrinibacteria	8	0.0087
TDNP	8	0.0087
WS6	7	0.0076
TM6	6	0.0065
Saccharibacteria_TM7	5	0.0054
Fusobacteria	4	0.0043
Hydrogenedentes_NKB19	4	0.0043
Synergistetes	3	0.0032
BRC1	2	0.0022
Gemmatimonadetes	2	0.0022
Latescibacteria_WS3	2	0.0022
Parcubacteria_OD1	2	0.0022
WS5	2	0.0022
Berkelbacteria	1	0.0011
Elusimicrobia	1	0.0011
GN04	1	0.0011
AY345499_p	1	0.0011
Caldithrix_p	1	0.0011
EU048619_p	1	0.0011
GU454958_p	1	0.0011

bacteria, cyanobacterial sequence databases are very limited, especially in Indonesian indigenous cyanobacteria. This makes species identification difficult to obtain. Therefore, molecular research on indigenous cyanobacteria becomes important to open a much more understanding of Indonesian cyanobacterial systematics and their ecological function.

Nostocales have been known as N₂-fixing cyanobacteria (Komarek and Mares 2012). The abundance of Nostocales in Agung Lake did not necessarily correlate with the high-level nitrate in the water body (62.54 mg/L). The process of N₂ reduction is energy-consuming because up to 16 ATP are needed to break down the triple bonding of the N₂ molecule. The formation of heterocyst, the cell that is responsible for N₂ fixation in the filamentous body of Nostocales, is prevented if there are high or adequate concentrations of nitrate and/or ammonium in the growth medium (Whitton and Mateo 2012). Therefore, in Agung Lake, instead of reducing atmospheric nitrogen the members of Nostocales consumed the existence of nitrate in the environment.

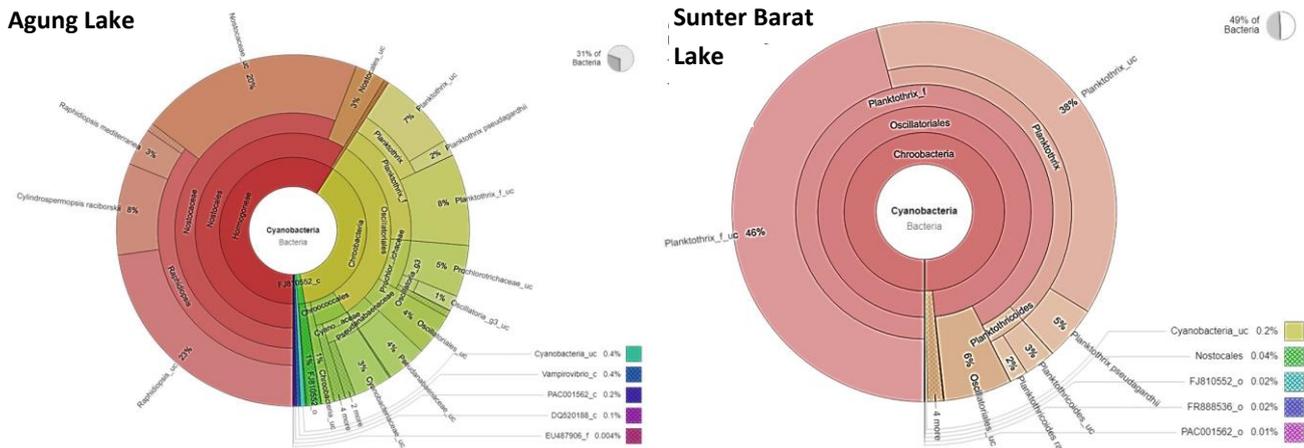


Figure 3. Krona plot of the cyanobacterial community composition in: (A) Agung Lake and (B) Sunter Barat Lake, Jakarta, Indonesia as determined by the 16S rRNA gene sequencing. *Cyanobacteria* composition in Agung Lake covered 31% of the bacterial community, while it was 49% in Sunter Barat Lake

Table 2. Composition of *Cyanobacteria* found in Agung and Sunter Barat Lakes, Jakarta, Indonesia

Taxa	Occurrence of cyanobacterial community (%)	
	Agung Lake	Sunter Barat Lake
Order Oscillatoriales	32	99.96
<i>Planktothrix</i> spp.	17	94
<i>Oscillatoria</i> sp.	1	5.7
Pseudanabaenaceae	11	-
Other	3	0.26
Order Nostocales	57	0.04
<i>Raphidiopsis</i> spp.	26	-
Nostocaceae	20	0.04
<i>Cylindrospermopsis raciborskii</i>	8	-
Other	3	-
Order Chroococcales	9	-

The krona plot obviously showed *Planktothrix* predominantly inhabited the water of both lakes. At least, three phenotypes of *Planktothrix* were found, e.g., *Planktothrix pseudagardhii*, *Planktothrix_uc*, and *Planktothrix_f_uc*. *Planktothricoides_uc* and *Plantothricoides ra*, the close relatives of *Planktothrix* within Phormidiaceae (Komárek and Komarkova 2004) were also found. The taxonomic assignment showed a large number of genera in Ordo Oscillatoriales (8 families). However, in the strain level, Ordo Nostocales showed a higher number instead of only 33 genera recorded.

Identification of dominant species found in the lakes

The dominant population in Agung Lake was different from Sunter Barat Lakes (Table 2). *Raphidiopsis* (syn. *Cylindrospermopsis*) was the major population in Agung

Lake, while *Planktothrix* dominated Sunter Barat Lake. It was obvious that non-Oscillatoriales cyanobacteria have been pressured to natural selection in Sunter Barat Lake, for example members of Nostocales covered only 0.04% of cyanobacterial populations.

Under microscope examination, *Arthrospira* was observed in freshwater samples (Figure 4G, H), along with coccoid algae and diatom (Figure 4G). However, the sequence failed to obtain this single-cell cyanobacterium. Interestingly, enrichment and isolation of water samples in several growth media (Blue Green 11, Basal Bold Medium, and CT medium) recovered the minor population instead of the dominant ones, for example, *Oscillatoria* and *Nostoc*. The present study successfully isolated and cultured *Raphidiopsis*-like and *Nostoc* sp. under laboratory conditions (Figure 4I, J). Another interesting fact was that *Microcystis* was found only in small numbers, both in sequencing data and microscopic observation. *Microcystis*, a member of *Chroococcales*, often form a large colony and are responsible for blooming phenomena in eutrophic freshwater (Affe et al. 2018).

Oscillatoria and *Raphidiopsis* (syn. *Cylindrospermopsis*) were found high in Agung Lake. These two filamentous cyanobacteria are associated with the blooming algal phenomenon and have dangerous potential because they can produce the toxic compound cyanotoxin (Aquilera et al. 2018). Morphologically, *Raphidiopsis* and *Cylindrospermopsis* are often distinguished by the presence or absence of heterocyst character (Komarek and Mares 2012). However, based on data from sequences 16S rRNA, 16S–23S ITS, and *cpcBA-IGS*, it is known that both have the same lineage (monophyletic) so that they can be included in one genus *Raphidiopsis* (Aquilera et al. 2018).

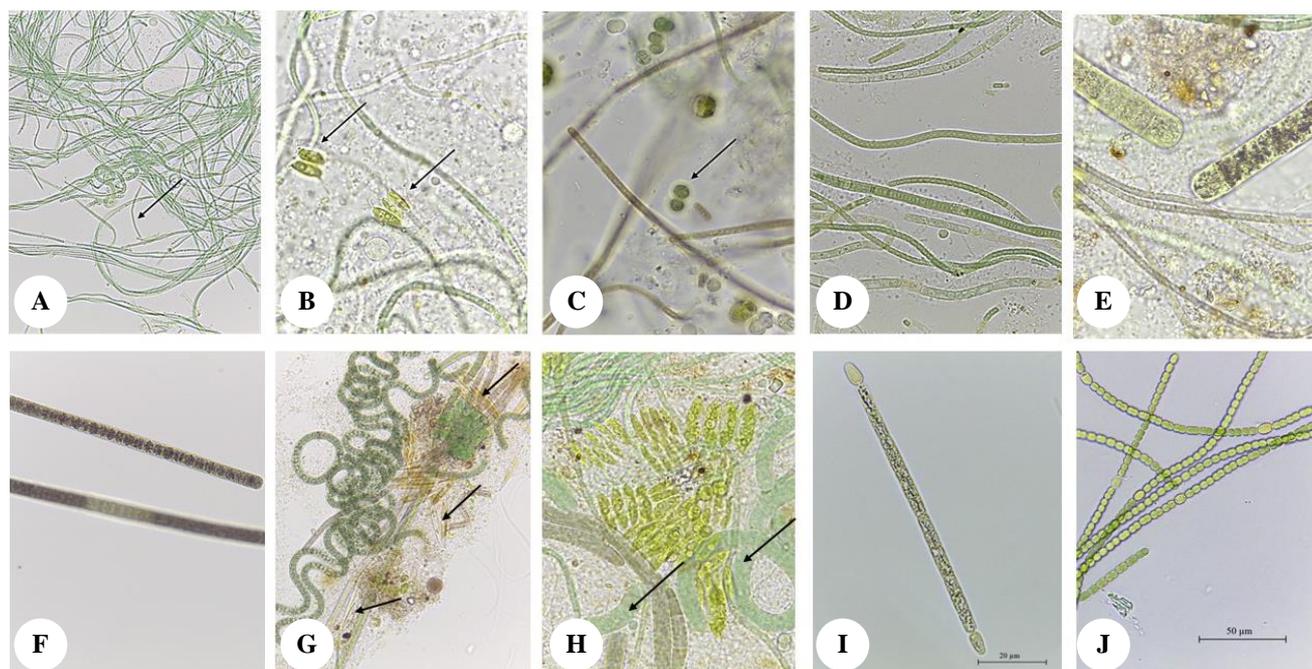


Figure 4. Diversity of cyanobacteria from the fresh samples of: (A-D) Agung Lake and (E-H) Sunter Barat Lake. A. *Oscillatoria* and *Limnothrix* (smaller filaments, arrow), B. *Scenedesmus* (colonial green algae), C. *Coscinodiscus* (colonial cyanobacteria, arrow) and filamentous cyanobacteria, D. *Oscillatoria*, E. *Planktothrix* (big filaments) and *Oscillatoria* (small filaments), F. *Planktothrix*, G. *Arthrospira* (coiled filaments), *Eudorina* group (green, packed cells), and some diatoms (arrow), H. Colony of *Scenedesmus* (center) and short fragments of *Arthrospira* (arrow), I. Culture of *Raphidiopsis* sp. isolated from Agung Lake water sample, J. Culture of *Nostoc* sp. isolated from Sunter Barat Lake water sample. Magnification: A, F, G=40x; E=10X; D, H=100X.

Research related to the toxicity of *Cylindrospermopsis* and its toxin cylindrotoxin (CYN) has been widely reported. The increase in the number of cells has a positive correlation, not only with the onset of odor and discoloration of the waters but also with the increase in the concentration of toxins released. Vehovszky et al. (2015) reported a phenomenon of dead fish in Francsika Pond (East Hungary) along with the discovery of *C. raciborskii* blooming with a cell density of 3.2×10^4 filaments per mL. Yang et al. (2017) reported a significant positive correlation between CYN production and cell growth rate. The total CYN (intra and extracellular) content of *C. raciborskii* CHAB3438 isolated from Lake Xianghu reached 45.34–63.83 fg per cell. Extracellular CYN proportion reached 11.49–20.44% at the stationary growth phase. The existence of *Raphidiopsis* and *Cylindrospermopsis* in Lake Agung needs to be monitored and researched further because of their potential that can endanger human health when it is in excessive numbers.

The present study confirmed the persistence of *Planktothrix* on both lakes must be considered seriously. Its occurrence in Agung Lake had been reported before in high numbers (Prihantini et al. 2008). Following the high-density algae ($10,030,652$ plankter/ m^3) in Sunter Lake, *Planktothrix* and *Arthrospira* were identified as dominant populations of algae in the water column, while *Oscillatoria* was also found in significant numbers. It is a common assumption that many toxic cyanobacteria cause harm to aquatic and human life when they are present in high concentrations (blooms). However, a study in

Überlingen embayment of Lake Constance revealed that even when the density of *Planktothrix* was low, the expression of the intracellular toxin microcystin gene (*mcyE*) was significantly high (Fournier et al. 2021). Therefore, the occurrence of toxic cyanobacteria cannot be underestimated and must be monitored.

In conclusion, Next Generation Sequencing Method used in the present study proved to be fruitful in projecting the cyanobacterial community structure amongst bacterial communities inhabited Agung and Sunter Barat Lakes. Dominant as well as low-density cyanobacterial populations were identified as cyanobacteria able to produce toxins. This information can be used for biomonitoring and management of the two lakes.

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