

Morphological diversity of culturable cyanobacteria from habitats in Segara Anakan, Central Java, Indonesia

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Abstract. Fitri SGS, Sutarno, Sasongko H, Rosyadi H, Ratnasari M, Chairunisa S. 2021. Morphological diversity of culturable cyanobacteria from habitats in Segara Anakan, Central Java, Indonesia. *Biodiversitas* 22: 5617-5626. Cyanobacteria are Gram-negative bacteria that can undertake oxygenic photosynthesis. This ancient group of microorganisms is ubiquitous, occurs in freshwater, marine, cold, hot, and terrestrial habitats. Can survive on a minimum requirement of light, carbon dioxide (CO₂), and water, and able to fix the atmospheric N₂ are among of their unique characteristics. These have collectively offered these bio-agents as the precious bio-resource for sustainable development. A variety of cyanobacterial strains promise further advances in biotechnology, including as an alternative source of energy. Before its final use, cyanobacteria undergo several necessary bioprocesses, including strain isolation and selection. This study aims to isolate and identify culturable-cyanobacteria from several habitats in Segara Anakan, Cilacap, Indonesia. The current study is an outset step to obtain potential and suitable agents for biofuel production. Morphological identification using light microscopy showed that cyanobacteria found in samples from different habitats varied. There are 39 cyanobacterial isolates obtained from freshwater, brackish water, and rhizosphere-mangrove soil habitats. Some cyanobacteria are unicellular (Order Chroococcales, Synechococcales, Pleurocapcales), the others are filamentous cyanobacteria (Order Oscillatoriales). These cyanobacteria grow well on BG-11 media. Numerous re-streakings on fresh medium and treatments of ampicillin and nystatin as antibiotic and antifungal, respectively, were carried out to obtain pure cultures. The classification of cultured cyanobacteria has been problematic and complicated. The polyphasic approach was applied to justify the taxonomic classification of the isolates. It is a combination of different methods based on modern molecular, cytomorphological, and ecological methods.

Keywords: Cyanobacteria, estuary, freshwater, mangrove, marine, Segara Anakan

INTRODUCTION

Cyanobacteria are previously known as cyanophytes, blue-green algae. They are among the most abundant and potent primary producers on Earth, responsible for primary productivity and nitrogen fixation. This ancient group of microorganisms comprises unicellular to multicellular prokaryotes. They possess chlorophyll *a* and undertake oxygenic-photosynthesis associated with photosystems I and II. Cyanobacteria are commonly called cosmopolitan microorganisms, which means can be found in various ecological habitats. The prominent habitats of cyanobacteria are limnic and marine environments (Mur et al. 1999). However, they occur in freshwater, marine, cold, hot, and terrestrial habitats. Several studies showed that cyanobacteria live as plankton in waters (Joseph 2005; Nedumaran et al. 2008), as benthos in sediments (Kyaruzi et al. 2003; Silambarasan et al. 2012), and as an epiphyte in aerial roots of mangroves (Toledo et al. 1995; Nedumaran et al. 2008; Pérez-Estrada et al. 2012).

As an oxygenic-photosynthetic bacterium, cyanobacteria have several unique features, such as their ubiquity presence,

short generation time, and capability to fix the atmospheric N₂. Cyanobacteria can also easily survive on minimal light, carbon dioxide (CO₂), and water. They have high biomass yield, grow on non-arable lands and various water sources, generate biofuels and other valuable by-products, and reduce greenhouse gas emissions. These unique characteristics have collectively made cyanobacteria as the precious bioresource for sustainable development. Genetically engineered cyanobacteria have been devised with novel genes to produce several biofuels such as bio-diesel, bio-hydrogen, bio-methane. Therefore, it opens new avenues for the generation of biofuels in an economically sustainable manner (Singh et al. 2016). Nevertheless, to date, microalgal, including cyanobacterial biofuels production has not transited into reality primarily due to economic competitiveness. Heimann (2016) mentioned that for targeted high-value bio-product markets, the cultivation of specific strains, which have the biochemical profile, yields, and productivities to meet required productivities, is a necessary aspect yet receiving little attention.

Segara Anakan is a large lagoon with a humid tropical climate comprised of the only large estuarine-mangrove forest left in Central Java. The lagoon water fluctuates due

to water hydrodynamics and changes in the monsoon. The semidiurnal tides with seawater enter through two channels west and east of the island. Its western part is affected by the Citanduy, the fifth largest river in Java. The Segara Anakan Lagoon (SAL) consists of a shallow estuarine lagoon, fringing mangroves, adjacent rice fields, and rural settlements. Mangrove is one of the very productive coastal ecosystems. Mangrove provides a nutrient-rich habitat for many organisms, including cyanobacteria, as one of the nutrient providers in the mangrove ecosystem (Kathiresan and Bingham 2001). Unfortunately, some studies reported environmental issues regarding land conversion by various anthropogenic activities, which cause the high sedimentation process and expansion of mangrove vegetation in the lagoon area. Ardli and Wolff (2009) reported dynamic changes in land use and land cover in the SAL, mainly due to resource overexploitation, degradation and pollution through unsustainable land-use practices in the hinterland, and pollution from industry and households in the SAL ecosystem. Such conditions may influence the ecological conditions, and as a result, it can affect the biodiversity of its natural resources, such as phytoplankton, including cyanobacteria (Dewi et al. 2019).

This study aims to isolate and identify culturable-cyanobacteria from several habitats which compose the SAL ecosystem. The exploration study was conducted to obtain various native strains with unique and superior properties. It is essential as an initial step to other advanced studies in applied biotechnology, including biofuel production.

MATERIALS AND METHODS

Study area

Sampling sites comprise of different habitats (mangrove soil, brackish water, freshwater, and marine) in the Segara

Anakan area, located in the south-western part of Central Java (108°46'-109°05'E; 7°34'-7°48'S), west of Cilacap city, bordering to West Java, Indonesia (Pangandaran) (Figure 1). The sampling was carried out by random sampling method at three stations from each habitat. Soil samples were taken from the rhizosphere of mangrove vegetation areas in Places. In addition, the marine samples were taken at Teluk Penyau, freshwater samples were taken at Timong river, and brackish-water samples were taken at the lagoon area (Citanduy estuary, Pusaran Batu Kelir).

Procedures

Sampling and isolation of Cyanobacteria

Samples used in this study were collected from mangrove soil, estuary (brackish water), freshwater, and marine in Segara Anakan by random sampling method. Physiochemical parameters of samples, such as temperature, pH, salinity, and light intensity, were observed and recorded *in situ*. Water and soil samples were collected in a sterile bottle and plastic, stored in a container, taken to the laboratory for another treatment.

Cyanobacteria from the soil, freshwater, and brackish water samples were isolated by plating them in a solid BG-11 media. As the marine cyanobacteria were isolated using ASN-III media. The medium composition is listed in Table 1. The culture was incubated at $25 \pm 2^\circ\text{C}$ under continuous illumination <500 lux for 7-14 days. Numerous restreakings on fresh medium and treatments of ampicillin and nystatin, as antibiotic and antifungal, respectively, were applied to obtain a pure culture (Rippka et al. 1979). Morphological features of cyanobacteria were done by using a light microscope (Leica) and identified by several kinds of literature, including Rippka et al. (1979), Waterbury (2006), Castenholz (2001), and Komarek (2003).

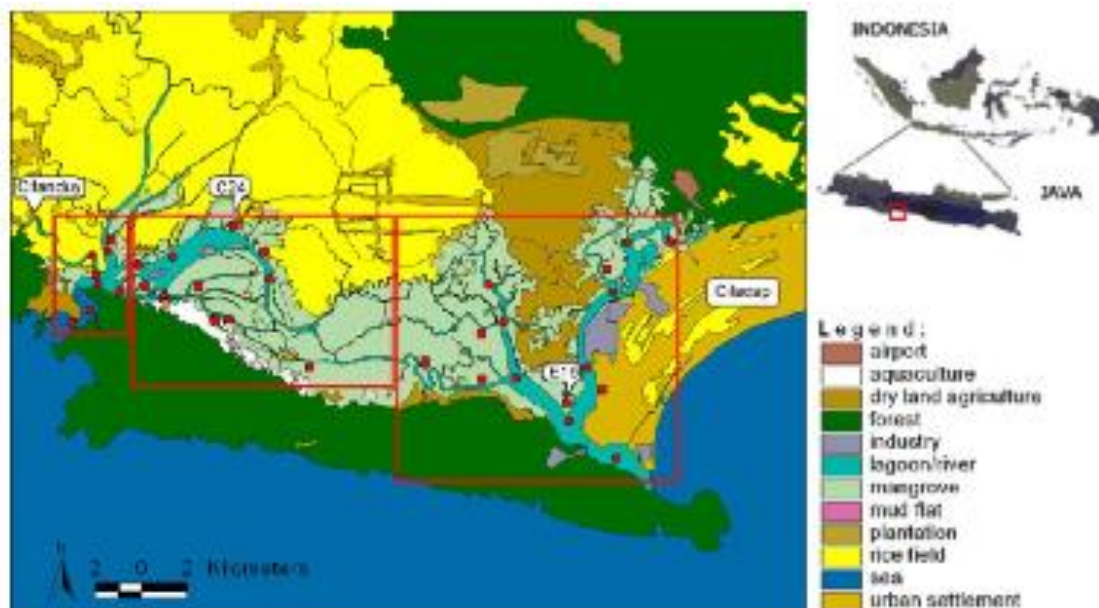


Figure 1. Location of Segara Anakan Lagoon, Cilacap District, Central Java, Indonesia (Nordhaus et al. 2018)

Table 1. The composition of culture medium

Component	Culture medium (g/L)	
	BG-11	ASN-III
NaNO ₃	1.5	0.75
K ₂ HPO ₄	0.04	0.02
MgSO ₄ · 7H ₂ O	0.075	3.5
MgCl ₂ · 6H ₂ O	-	2.0
CaCl ₂ · 2H ₂ O	0.036	0.5
NaCl	-	25.0
KCl	-	0.5
Na ₂ CO ₃	0.02	0.04
Citric acid	0.006	0.003
FeNH ₃ -citrate	0.006	0.003
Microelement solution *)	1 mL	

Morphological and molecular identification

According to manufacturer instructions, genomic DNA extractions from 50-100 mg wet of a few cyanobacteria samples were conducted using Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, D6005). Obtained genomic DNAs were amplified by 2x MyTaq HS Red Mix (BIO-25048), using universal bacterial primers for the 16s gene region for all the cyanobacteria samples. The universal bacterial primer used has sequences for forward primer as 27F: 5'-AGA GTT TGA TCC TGG CTC AG-3' and for reverse primer as 1492R: 5'- GGT TAC CTT GTT ACG ACT T-3'.

The 16s rRNA gene regions' PCR products were sequenced using ABI PRISM 3730xl Genetic Analyzer developed by Applied Biosystems, USA and BigDye® Terminator v3.1 Cycle Sequencing Kit. Additional sequences for further phylogenetic analyses were acquired from GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>).

RESULTS AND DISCUSSION

Sampling and physiochemical parameters of the sites

Segara Anakan has a tropical and humid climate. The observation of physiochemical parameters of the sites

shows the different results for each habitat (Table 2). Environmental parameters may influence cyanobacterial diversity, such as temperature, pH, salinity, and light intensity.

Morphological identification

Prior to isolation on solid media in a laboratory, the water samples were observed under a light microscope to ensure the availability of cyanobacteria. As a result, cyanobacteria from Order Chroococcales, such as *Synechococcus* dan *Synechocystis*, were observed in freshwater samples (Figure 1). Morphological identification is based on Rippka et al. (1979) and Waterbury (2006). Order Chroococcales contains all unicellular or non-filamentous aggregates of cells held together by extracellular slime or sheath layers. This group reproduces by binary fission (family Chroococcaceae) and by budding (family Chamaesiphonaceae). Both *Synechococcus* and *Synechocystis* divide by binary fission and have thylakoids. On the other hand, *Synechococcus* is in one plane and has a cell diameter of <3 µm. In contrast, *Synechocystis* divide into two or three planes and has coccoid cells, occurring singly, in pairs, or aggregates held together by amorphous capsular material.

Cyanobacteria assigned as Order Pleurocapsales are also observed in freshwater samples and, among them, identified as *Dermocarpa* and *Myxosarcina* (Figure 2). The Order Pleurocapsales comprises cyanobacteria that reproduce by forming small spherical cells (called baeocytes) through multiple fission. The unicellular genera of the Order divide exclusively by multiple fission. In other genera, cell aggregates are produced by binary fission, after which some or all of the cells in the aggregates undergo multiple fission and release baeocytes (Waterbury 2006). Cell characteristics of *Dermocarpa* are that it divides solely by multiple fission, and its baeocytes are motile. *Myxosarcina* also has motile baeocytes, but it divides by a combination of binary and multiple fission. Its baeocyte development, followed by repeated binary fission to produce a cubical aggregate of cells.

Table 2. Physiochemical parameters of several habitats in Segara Anakan Lagoon, Central Java, Indonesia

Habitat	GPS Coordinate	Abiotic parameter					
		Temp. (°C)		pH	Salinity (‰)	Water clarity (cm)	Light Intensity
		water	air				
Freshwater							
Station 1	S 07°39.113'; E 108°48.983'	28.5	34.7	7.7	2	21.7	5179
Station 2	S 07°39.138'; E 108°48.975'	29	33.6	7.8	3	23.4	1732
Station 3	S 07°39.265'; E 108°48.906'	28.5	32.4	7.8	3	26.3	1306
Brackish water							
Station 1	S 07°40.761'; E 108°48.005'	27.7	29	7.9	20	19	7255
Station 2	S 07°40.779'; E 108°47.980'	27.3	29.4	8	23.3	19	4347
Station 3	S 07°40.821'; E 108°48.164'	27.3	29.4	8.1	21	40.7	3697
Marine water							
Station 1	S 07°45.611'; E 109°01.168'	29	33	8.7	78	124	4970
Station 2	S 07°45.874'; E 109°02.068'	29	32.5	8.7	77	82	5228
Station 3	S 07°45.796'; E 109°01.533'	29	33	8.8	75	106	4403
Rhizosphere-mangrove soil							
Station 1	S 07°41.051'; E 108°48.958'	29.2	30.7	6.3	-	>100 *)	2185
Station 2	S 07°41.095'; E 108°49.614'	27.2	35	6.4	-	>100 *)	3823
Station 3	S 07°40.950'; E 108°49.742'	26.2	31.1	6.6	-	>100 *)	3199

Note: *) Humidity



Figure 1. The Order Chroococcales, A-B. *Synechococcus* (100x dan 400x magnification); C. *Synechocystis* (400x magnification). Reference. Rippka et al. (1979)



Figure 2. The Order Pleurocapcales, A-B. *Dermocarpa* (400x dan 1000x magnification); C. *Myxosarcina* (400x magnification). Reference. Rippka et al. (1979).

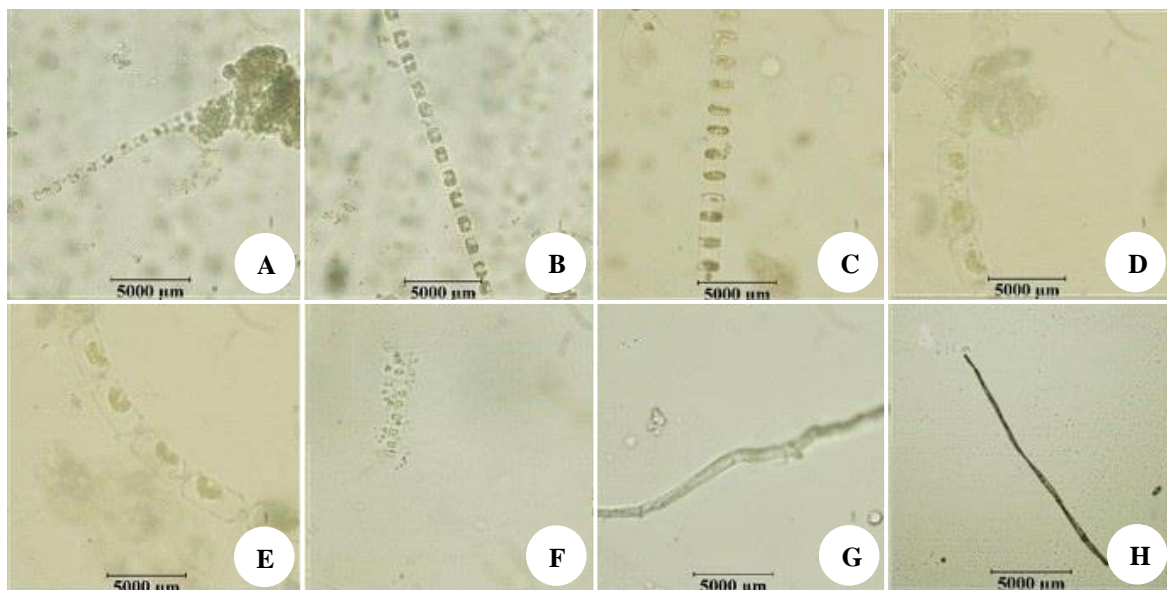


Figure 3. A-E. Filamentous Cyanobacteria found in brackish water sample; F-H. Filamentous Cyanobacteria found in the marine water sample

In samples of brackish water and seawater, various filamentous cyanobacteria cells are observed (Figure 3). They might belong either to the order Oscillatoriales or Nostocales (Rippka et al. (1979) and Waterbury (2006)). A further examination needs to be carried out to identify which genera the cell belongs to, i.e., trichomes (vegetative cells in the terminal of filament), the characteristics of extracellular sheath material, size, shape, and the arrangement of the cells within filaments. It is also essential to examine the presence or absence of heterocyst

(specific cellular site of nitrogen fixation under aerobic conditions) and akinet (thick-walled resting cells).

Isolation and cultivation of Cyanobacteria

Cyanobacterial colonies isolated from freshwater, brackish water, and rhizosphere-mangrove soil grow well on the medium BG-11. However, marine cyanobacteria fail to grow on solid medium ASN-III. Some cultures have been pure, while others are still contaminated. Figure 4

shows cyanobacterial colonies (green to yellowish color) grow on solid medium BG-11.

Observation on morphological features using light microscopy showed that the isolates are cyanobacteria. A total of 39 isolates are identified as unicellular or filamentous cyanobacteria. The unicellular cyanobacteria are assigned either as Order Chroococcales, Synechococcales, or Order Pleurocapsales. The filamentous cyanobacteria are assigned as Order Oscillatoriales. Figure 5. A and 5. B show 22 isolates from brackish water samples. Nine isolates from freshwater samples and eight isolates from rhizosphere-mangrove soil samples are shown in Figure 6 and Figure 7, respectively.

Identification of cyanobacterial isolates was carried out by using identification key from several kinds of literature, including Rippka et al. (1979), Waterbury (2006), Castenholz (2001), and Komarek (2003), as a guide. The unicellular cyanobacteria from brackish water were identified as *Synechocystis* (Figure 5. h, j, l), *Pleurocapsa* group (Figure 5. c), *Microcystis* sp.1 (Figure 5. d and i), *Aphanothece* (Figure 5. p), *Chroococcus* (Figure 5. s), *Microcystis* sp. 2 (Figure 5. m1 and m2), and *Gloeocapsa* (Figure 5. v). The filamentous cyanobacteria were identified as *LPP* group B (Figure 5. n, r, t, and u) and *Leptolyngbya* (Fig 5. a; Fig 5. o, and q).

Cyanobacteria isolated from freshwater habitats were mostly unicellular form. Based on morphological characteristics, these isolates were identified as *Chamaesiphon* sp.1 (Figure 6.b), *Chroococcus* (Figure 6.d), *Cyanobacterium* (Figure 6.e), *Cyanothece* (Figure 6g), *Chamaesiphon* sp.2 (Figure 6.h), and *Microcystis* (Figure 6f and i). The filamentous cyanobacteria were

identified as *LPP* group B (Fig 6. a) and *Planktolyngbya* (Figure 6.c).

In this study, one isolate from rhizosphere-mangrove soil (station 1), identified as *Synechococcus*, is shown in Figure 7f. It shares the morphological properties of members assignable to the botanical genus *Synechococcus* Nägeli 1849. The cells are spherical- to rod-shaped, ranging from 0.6 to 2.1 μm in diameter, containing peripheral photosynthetic thylakoids. The cells may be united into colonial aggregates by mucilage formation (Castenholz 2001). The genus is common in soil. Cells are solitary or in irregular groups or agglomeration. They do not form distinct colonies, have no slimy envelopes, or only a very fine, diffuse, and narrow gelatinous layer (Komarek 2003). Komarek (2016) assigned *Synechococcus* to family Synechococcaceae, Order Synechococcales.

Chroococcus sp. 1 (Figure 7. a), *Synechocystis* (Figure 7.b), *Aphanothece* sp. 1 (Figure 7.c), *Microcystis* (Figure 7e), *Chroococcus* sp.2 (Figure 7.g), and *Aphanothece* sp.2 (Figure 7.h) are found in rhizosphere-mangrove soil samples. The only filamentous cyanobacteria isolated from the soil habitat was identified as *Lyngbya* (Figure 7.d).

Molecular identification was carried on several isolates using the 16s rRNA gene sequence. PCR products (± 1500 bp) of the gene were analyzed by electrophoresis gel, as is shown in Figure 8. DNA sequencing analysis justifies the identification done based on morphology. Several isolates were confirmed by BLAST analysis on NCBI, as *Leptolyngbya* (accession no. MG822743.1), *Microcystis* (accession no. MT534588.1), *Cyanobacterium* (accession no. MK139949.1), and *Cyanothece* (accession no. KF157401.1).

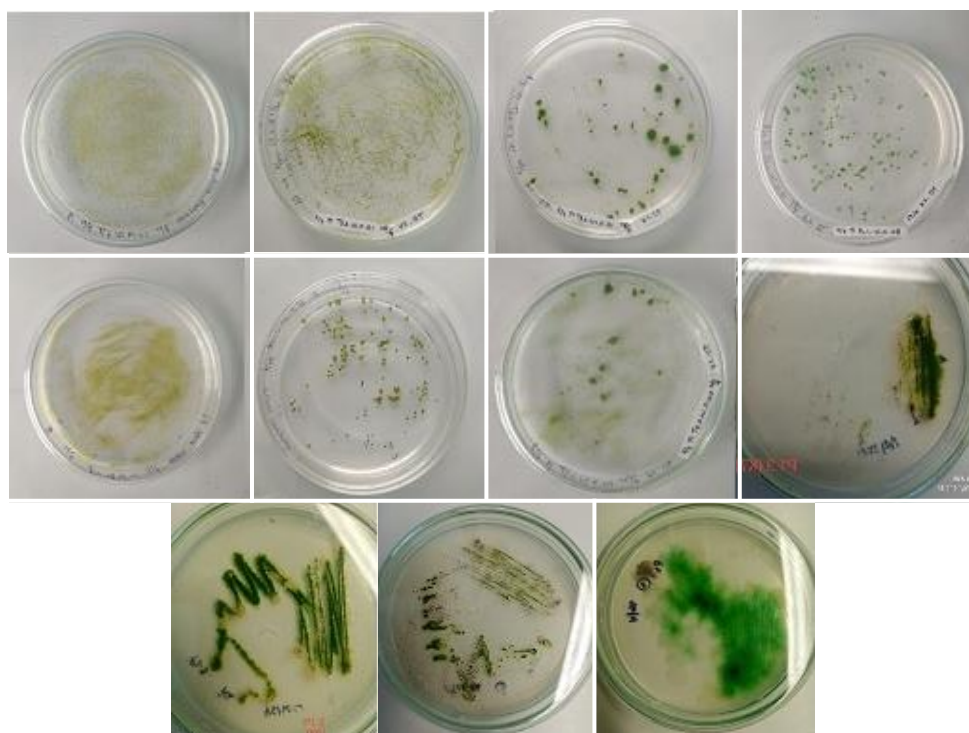


Figure 4. Cyanobacterial colonies growth on solid medium BG-11

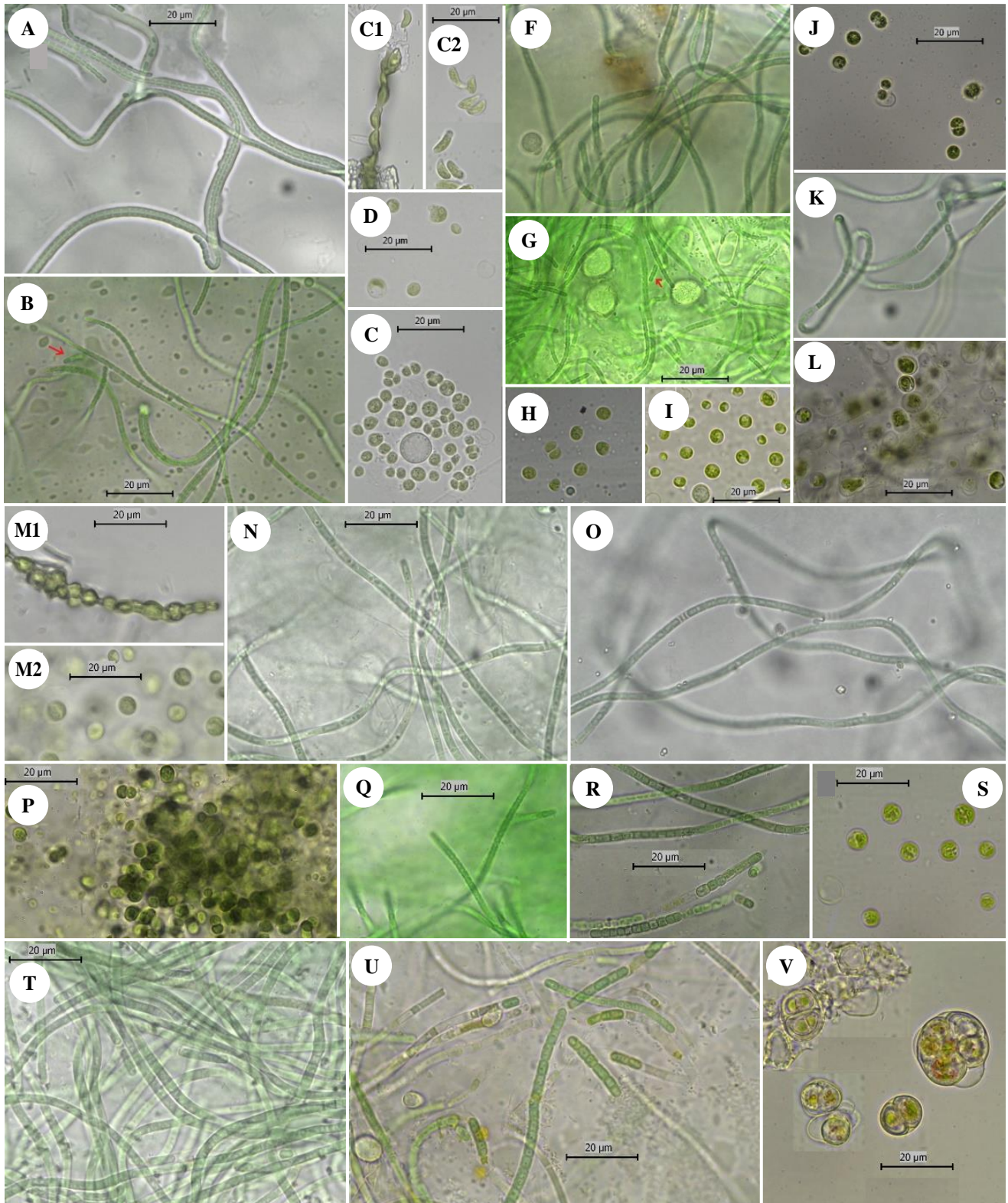


Figure 5. Isolates from brackish water habitat in Segara Anakan. A. *Leptolyngbya*; B. *LPP* group B; C. *Pleurocapsa* group; D. *Microcystis*; E. *Chroococcus*; F. *Leptolyngbya*; G-H. *Synechocystis*; I. *Microcystis*; J. *Synechocystis*; K. *Leptolyngbya*; L. *Synechocystis*; M1-M2. *Microcystis*; N. *LPP* group B; O. *Leptolyngbya*; P. *Aphanothece*; Q. *Leptolyngbya*; R. *LPP* group B; S. *Chroococcus*; T. *LPP* group B; U. *LPP* group B; V. *Gloeocapsa*

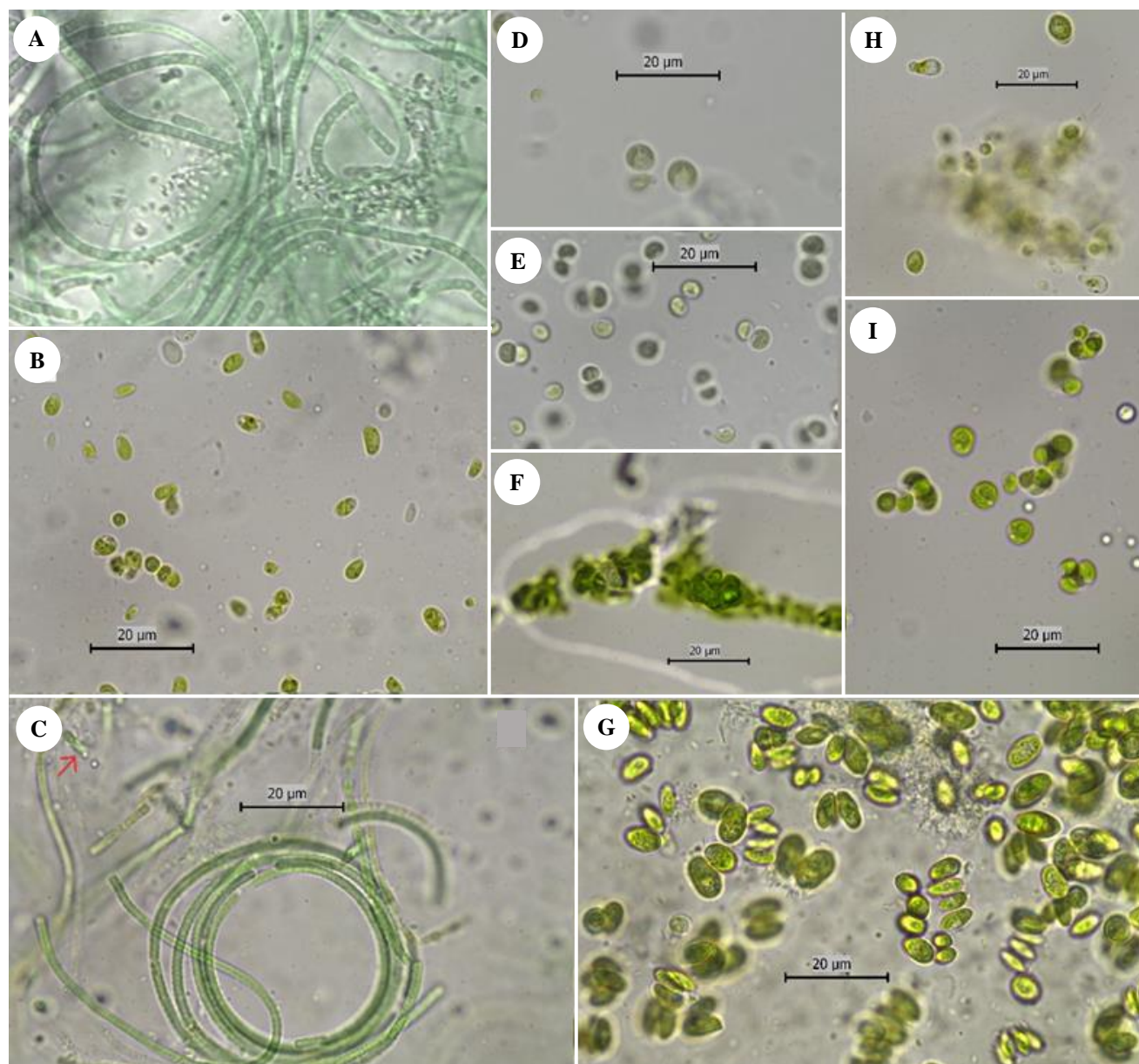


Figure 6. Isolates from freshwater habitat in Segara Anakan. A. *LPP* group B; B. *Chamaesiphon* sp.1; C. *Planktolyngbya*; D. *Chroococcus*; E. *Cyanobacterium*; F. *Microcystis*, G. *Cyanothece*; H. *Chamaesiphon* sp.2; I. *Microcystis*

Discussion

A thorough description of the sampling site is the first important step in isolating cyanobacteria. The site location and description, and parameters, such as light intensity, temperature, pH, and salinity, should be recorded *in situ*. This information helps describe the natural habitat of the cyanobacteria to be isolated and can be critically important when designing culture media and conditions of incubation (Waterbury 2006). Media should be designed to emulate the chemical composition of the environment from which cyanobacteria are to be isolated. According to Rippka et al. (1979), medium BG-11 is widely used to isolate and maintain many cyanobacteria originating from freshwater, soil, or thermal and marine source materials. However, some marine cyanobacteria isolates are halo-tolerant and

grow equally well on a medium with seawater or freshwater basis. Others have obligate sodium, magnesium, calcium, and chloride concentrations, which can only be fulfilled with seawater-based media, such as medium MN. As in this study, cyanobacteria collected from marine water failed to grow on medium ASN-III, which contains synthetic seawater. Hence, it is worth trying to grow it on a seawater-based medium.

Cyanobacteria are found in quite different temperature environments, ranging from freezing to 40°C. Its optimum growth temperature is between 20°C and 35°C. Naturally, cyanobacteria occur under a wide range of light conditions. However, they usually grow best when cultured at light intensities varying from 10-75 $\mu\text{Ein} \cdot \text{m}^{-2} \cdot \text{sec}^{-1}$ supplied by warm- or cool-white fluorescent lamps or grown under

continuous illumination Fluorescent White lamps, at relatively low intensity (<500 lux). Naturally, cyanobacteria have been cultured under constant illumination conditions. However, some cyanobacteria, such as non-heterocystous nitrogen fixers that temporally separate dinitrogen fixation and photosynthesis, require a light-dark cycle (Waterbury 2006). Higher intensities should be avoided since many strains (particularly those containing phycoerythrin) are light-sensitive (Rippka et al. 1979).

Many researchers studied the vital role of cyanobacteria in ecosystems and their potential as biological agents in various applications. Some studies explore the diversity of native and novel cyanobacterial strains from natural resources. Other studies focus on finding suitable, rapid, and effective methods for a cyanobacterial bio-products generation. For example, Rohmah et al. (2014) studied

planktonic cyanobacteria from brackish water samples of Cagar Alam Pulau Dua (CAPD), Banten, Indonesia. They found 13 genera of different orders, i.e., *Chroococcus*, *Synechococcus*, *Merismopedia*, *Coelosphaerium*, *Myxosarcina*, *Chroococcidiopsis*, *Dermocarpa*, *Lyngbya*, *Oscillatoria*, *Spirulina*, *Phormidium*, *Nostoc*, and *Calothrix*. Fatimahsari et al. (2014) collected nine genera of epiphytic-cyanobacteria from *Avicennia marina* pneumatophores in the CAPD mangrove ecosystem, namely *Chroococcus*, *Aphanothece*, *Aphanocapsa*, *Myxosarcina*, *Oscillatoria*, *Microcoleus*, *Lyngbya*, *Phormidium*, and *Calothrix*. Edwin et al. (2019) recovered 11 isolates affiliated with Chroococcales, Oscillatoriales, Pleurocapsales, and Nostocales, from Lake Magadi, an alkaline saline Lake in Kenya.

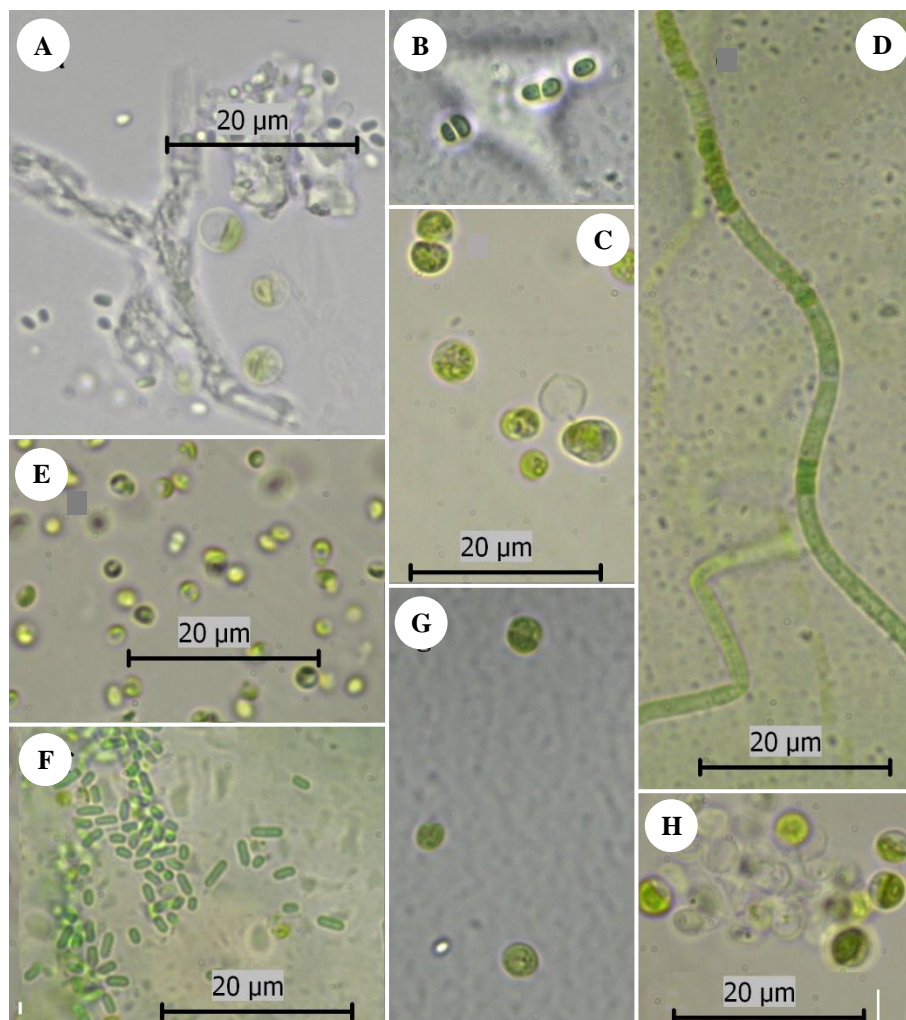


Figure 7. Isolates from mangrove rhizospheric soil in Segara Anakan. A. *Chroococcus* sp.1; B. *Synechocystis*; C. *Aphanothece*; D. *Lyngbya*; E. *Microcystis*; F. *Synechococcus*; G. *Chroococcus* sp.2; H. *Aphanothece*

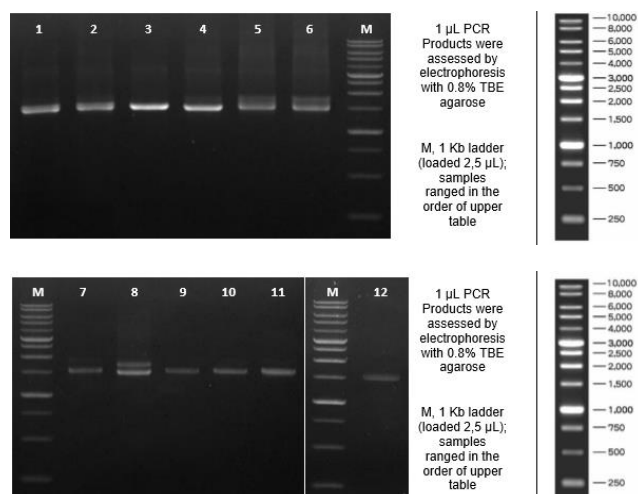


Figure 8. Gel electrophoresis result of 16s rRNA PCR product of cyanobacteria isolates

The culturable-cyanobacteria collected from habitat is usually under-estimated than the actual numbers. Moreover, many studies encounter difficulty in the isolation of axenic cultures of cyanobacteria. Some factors affect cyanobacteria cultivation, i.e., suitable medium, conditions of incubation, and isolation and purification methods. Temraleeva et al. (2016) declared that the stab-inoculation technique on a solid nutrient medium could enrich cyanobacteria growth. It is because some cyanobacterial species grow poorly on the agar surface but develop nicely inside the agar.

Many purification methods are admitted to being rapid and efficient in separating cyanobacteria from fungi or other bacteria contaminants. These methods include ordinary plating procedures, such as numerous streaking on fresh mineral medium, treatments with antifungal/antibacterial, and mechanical separations, such as micro-manipulation, filtration, and equilibrium centrifugation. For example, Vaara et al. (1979) recognized that scoring the agar plate before incubation under unidirectional light is rapid and effective in separating gliding filamentous-cyanobacteria from contaminants. In addition, Varaa proposed obligate photoautotroph-cyanobacteria selected by culturing them in tryptone-yeast extract-glucose broth with cycloserine in darkness.

Waterbury (2006) mentioned that purification of unicellular cyanobacteria could be achieved by restreaking on solid media. Furthermore, he suggested transferring isolated colonies picked from agar plates into a liquid medium and to allow them to grow up before restreaking. Contaminants that are frequently found in cyanobacterial cultures are small heterotrophic bacteria. Thus, it is not an easy and rapid process to purify cyanobacteria; it requires diligence.

According to Komárek (2016), taxonomic classification is crucial for understanding and exploring knowledge about organismal diversity. However, the classification of cultured cyanobacteria has been problematic and complicated. Cyanobacteria consist of simple unicellular

forms to multicellular types with a differentiated and diversified thallus. Their cells are cytologically relatively simple but variable in shape. Komárek proposes that combining different methods based on modern molecular, cytomorphological, and ecological methods is helpful to avoid a misleading classification. This polyphasic approach is considered a unique, modern, unambiguous, unequivocal, and entirely acceptable methodological procedure. However, it is rarely used nor possible for all known cyanobacterial populations.

However, in the traditional botanical treatments, morphological and developmental features form the basis for the description of taxa at the level of genera and above (Waterbury 2006). Recent research revealed an abundance of cryptic diversity evidenced by molecular analyses, most notably the 16S rRNA gene sequence. Many traditional genera have proven to be polyphyletic, but revisions are in process (Dvorak et al. 2017). Cyanobacteria exhibit a relatively high degree of morphological features compared to other prokaryotes. Morphology, the primary photosynthetic pigment, and molecular (phylogeny) are among of characteristics used to distinguish Cyanobacteria. Cyanobacteria were identified and categorized using morphological traits, such as cell dimensions, shape, color, type of branching, sheath characteristics, and cell contents.

Rashid et al. (2019) state that microalgae, including cyanobacteria, are convincing to be a tremendous potential biomaterial of performing environmental services and energy recovery to promote carbon-neutral bio-economy. Despite its tremendous potential, its large-scale application is facing challenges related to some technical and economic issues. Cyanobacteria undergo several necessary bioprocesses to attain their final use. The primary yet most crucial step of the bioprocesses is the isolation and selection of novel strains. Different characteristics of cyanobacteria may impact the quantity and quality of bioproduct obtained from the biomass. Therefore, according to Zahra et al. (2020), it is important to discover the right choice of material and its conversion to biofuel. Cyanobacteria are considered to have prominent potential as biofuel feedstock and may replace the majority of fossil fuel usage. They can accommodate the better biofuel production, economically and environmentally.

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