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The Role of Indonesian Indigenous Cyanobacteria Culture Collection as An Ex-situ Conservation Effort and Microalgae Biodiversity Study Material

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Abstract: Exploratory research has been carried out on Indonesian indigenous Cyanobacteria in an effort to collect these isolates to be used as learning materials for microalgae biodiversity. Cyanobacteria are known as photosynthetic bacteria and prokaryotic microalgae. The members of the cyanobacteria found were isolated, cultured, identified, and collected. The location for sampling cyanobacteria is the UI Depok campus area which consists of water from small lakes (situ) and soil. The results showed that 22 strains of Cyanobacteria were isolated and cultured properly, and consisted of 12 genera/species, namely *Merismopedia* sp. (1 isolate), *Synechococcus* sp. (1 isolate), *Limnothrix redekei* (1 isolate), Oscillatoria sp.1 (1 isolate), *Oscillatoria* sp.2 (1 isolate), *Pseudanabaena* sp. (3 isolates), *Nostoc* sp. (4 isolates), Anabaena sp. (1 isolate), *Scytonema* sp. (1 isolate), *Fischerella* sp. (1 isolate), *Stigonema* sp. (1 isolate), and *Hapalosiphon* sp. (6 isolates). Twenty-two (22) strains were then collected and treated by subculture method. Of the 11 genera found, there are 3 genera that have been known as genera that have potential as sources of biobased materials, biofuels, biofertilizers, and others, namely *Synechococcus*, *Nostoc*, and *Anabaena*.

Keywords: Cyanobacteria; Ex-situ conservation; Identification; Microalgae; Taxonomy

Introduction

Conservation efforts are carried out with various objectives, including preserving the environment or a group of organisms so that they remain good so that they can be used as study material for biodiversity. Conservation is an effort to maintain and protect an organism on a regular basis from damage and destruction on a regular basis by being preserved. There are 2 types of known conservation, namely in-situ conservation and ex-situ conservation. In-situ conservation is the conservation of organisms in their natural habitats or the conservation of genetic resources in natural populations of plant or animal species, such as genetic forest resources in natural populations of tree species. Meanwhile, ex-situ conservation literally means, "off-site conservation" is the process of protecting organisms outside their natural habitat. Ex-situ conservation is usually used for microorganisms (Uzunova-Doneva et al., 2005).

Given the frequent occurrence of environmental changes that cause changes in the composition of organisms, especially Cyanobacteria in waters, it is advisable to continue ex-situ conservation efforts in line with data collection on the presence of these microalgae on an ongoing basis. Ex-situ conservation efforts include correct identification and culturing, as well as observations of all aspects and potentials of microalgae species including Cyanobacteria.

In line with knowing the benefits of microorganisms both directly, knowledge about biodiversity and conservation of the gene pool of microorganisms becomes important. Therefore, in parallel with the isolation, selection and genetic engineering processes, a way to preserve strains is needed, knowing their vitality, specificity, activity, and other properties under laboratory conditions. The support of culture collections is an important element of microbiological science, practice and development. At the beginning of the twentieth century, methods for the conservation of microorganisms were developed.

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There are various methods available for the conservation of strains, which maintain their vitality and authenticity. The main difference lies in the timing of using the target strain. Depending on the purpose, methods can be divided into two, namely short-term storage methods and long-term storage methods (Uzunova-Doneva et al., 2005). Short-term methods include sub cultivation, storage under mineral oil, in water and water buffer, by cooling to 4—8 °C or drying etc., while long-term storage methods include freezing at different temperatures, freeze-drying which causes specimens can be maintained unchanged for decades.

The conservation used for microalgae in general and Cyanobacteria in particular is the same as for microorganisms in general, i.e., ex-situ conservation. Both short-term and long-term methods have been used. However, the subculture method (sub cultivation) is still the most widely used method in countries that have a collection of microalgae cultures, such as Australia, Germany, Japan, and others.

Cyanobacteria which are also commonly referred to as Cyanophyta, blue green algae, or Myxophyta are a group of prokaryotic algae (Graham et al., 2000) that have a variety of shapes, namely unicellular, filamentous, or colony. Unicellular cyanobacteria consist of one cell that is spherical or oval in shape (Vashishta, 1999). Strands of cells that form a thread-like structure and are covered by a sheathing layer are commonly called filaments (Graham et al., 2000; Vashishta, 1999). Filaments in Cyanobacteria vary widely, some are unbranched and unbranched. There are two kinds of unbranched forms, namely straight and twisted.

Cyanobacteria can show morphology (phenotype) that is easy to change (phenotypic plasticity) and different appearance (visual) depending on the conditions in which Cyanobacteria grow (Mollenhauer, 1988; Rippka, 1988). Therefore, apart from the identification of cyanobacteria based on morphological characteristics, identification based on molecular characteristics is also better and is a popular choice in the phylogenetic analysis of cyanobacteria (Turner, 1997). There are several genetic markers used for systematic cyanobacteria, one of which is small subunit rRNA (16S) (Nelissen et al., 1995, 1996; Robertson et al., 2001; Turner, 1997; Wilmotte, 1994; Wilmotte et al., 1992, 2015).

Cyanobacteria have an important role in ecosystems and for human needs. Cyanobacteria are used as biofertilizers, single cell protein (SCP), food, biomass for grazers and in reclamation of salt and alkaline soils (Singh, 1961). Cyanobacteria play a major role in the primary productivity and nitrogen economy of various ecosystems. About 20–30% of the total fixed carbon on earth is caused only by cyanobacteria.

Cyanobacteria are recognized as a group of organisms that have the potential to produce bioactive compounds (Fish et al., 1994; Schlegel et al., 1998). Cyanobacteria are prokaryotes that can photosynthesize and can be used as food for humans. This microalga is also known to be an excellent source of vitamins and protein. In addition, it has been reported that Cyanobacteria can act as antiviral, antifungal, antibacterial, anticancer, and anti-HIV. Therefore, screening of cyanobacteria for antibiotics and other pharmacological compounds is becoming increasingly in demand and being investigated as a potential source for new drugs (Fish et al., 1994; Schlegel et al., 1998).

These microorganisms are cosmopolitan, not only found in aquatic habitats but also in terrestrial habitats (Hoek et al., 1995). Cyanobacteria species that live in aquatic habitats are mostly found in freshwater (Rheinheimen, 1980), while only a few species are found in marine waters (Vashishta, 1999). There are cyanobacteria that live as plankton and some that live as benthic. Species that are planktonic are generally species that cause a population explosion (blooming) (Vashishta, 1999). Some members of Cyanobacteria are able to grow in trees or soil in a humid environment, in rice fields, or live in damp soil or in symbiosis with mosses.

Environmental factors greatly affect the distribution of cyanobacteria, including water temperature, light intensity, acidity (pH), and nutrients. Usually, optimal growth of cyanobacteria occurs when the water temperature is warm enough, exposed to sufficient intensity of sunlight, neutral to alkaline pH, and sufficient nutrients in the water. Cyanobacteria group can grow optimally in waters that have a temperature range of 25—35 ^{0}C (Nicholas, 1980). However, some members of Cyanobacteria, such as *Synechococcus* are able to grow at high temperatures up to 73^0 C (Vidal et al., 2021), the genus Oscillatoria is an organism that can adapt to hot springs in Cipanas, Tarogang, and Garut where the temperature reaches 70 ^{0}C.

The Universitas Indonesia (UI) Depok Campus area is most likely also a suitable habitat for the Cyanobacteria group. The UI campus with an area of about 312 Ha consists of urban forest areas, open spaces, waters, and buildings. There are 6 small lakes located on the UI Depok Campus which function as water catchments and accommodate rainwater and waste water in the campus environment, especially those from buildings or buildings around the Situ (Taqyuddin et al., 1997).

The six small lakes (*SITU*) are *Situ* Kenanga, *Situ* Agathis, *Situ* Mahoni, *Situ* Puspa, *Situ* Ulin, and *Situ* Salam (K.A.M.P.U.S). *Situ* Kenanga is located in the middle of campus, while the other five lakes starting from *Situ* Agathis stretch from south to north within the UI campus. The flow (water input source) comes from the Ciliwung Cisadane watershed water system. In addition to the waters based on previous research,

various kinds of microalgae, including cyanobacteria, were found in the aquatic and soil habitats on the UI Campus. From the results of research in 2001 (Prihantini, 2002). Until 2003 (unpublished), Cyanobacteria species were found in the waters of the UI campus. The results of these studies show that there are at least 14 genera of cyanobacteria found in the waters of the UI campus. The number is not always the same every year, but varies due to the influence of environmental factors. And since 2004, cyanobacteria have started to bloom in UI waters. The dominant cyanobacteria species found in Ulin-Salam are *Merismopedia* sp., *Oscillatoria* sp. 2, *Chroococcus* sp., *Microcystis aeruginosa*, and *Synechococcus* sp., while in *Situ* Agathis is *Planktothrix agardhii*. However, apart from these species, it seems that there are still many members of Cyanobacteria that have not been identified.

Research conducted in 5 small lakes in the Jakarta-Depok-Bogor area in the period August-September 2006 found that Cyanobacteria had dominated 4 of the 5 observed small lake (Prihantini et al., 2008). Several species of potentially toxic cyanobacteria were found in some waters, and even dominated the waters, especially at Sunter 2 Lake (North Jakarta) and *Situ* Agathis (UI Campus).

The scope of research is the taxonomy and physiology of Cyanobacteria with the following research assumptions. Based on the fact that in Indonesia, Cyanobacteria are organisms that have not been explored much. This causes knowledge about Indonesian indigenous cyanobacteria, such as taxonomic studies (morphology and genetics), physiology, toxicity, and other potential materials, which are still very lacking and unclear. Therefore, cyanobacteria originating from local habitats is a great opportunity to be explored and investigated further, especially as a potential source of active substances. Therefore, research on the existence of Cyanobacteria which is then identified, isolated, and preserved is a necessity that needs attention. Ex-situ conservation by sub cultivation method is used as the initial stage.

The purpose of this research is part of a long-term research objective on Indonesian indigenous Cyanobacteria. Long-term goals include making collections of Cyanobacteria cultures that have grown optimally and stably with the best characteristics at a laboratory scale (ex-situ conservation efforts), as well as knowing their bioprospecting. The immediate goal to be achieved is to explore, collect, propagate (culture), identify (based on morphological characters) of Indonesian indigenous Cyanobacteria, especially from the UI area. Collections of these cultures can be used as learning materials for the biodiversity of cyanobacteria.

This ex-situ conservation effort was carried out considering the campus environment which was always changing. Germplasm in this case, especially cyanobacteria, may be lost. Therefore, the response to this extinction must be implemented immediately. The target to be achieved is to obtain pure isolates of Cyanobacteria from the campus area of the University of Indonesia in Depok. Isolates of pure cyanobacteria and in optimal and stable conditions.

This research is also expected to help the Indonesian microalgae collection process (ex-situ conservation) conducted by the Department of Biology, FMIPA UI. Cyanobacteria isolates that were purified, grew optimally and stably were stored and cared for in the Algae Culture Collection Room, Plant Taxonomy Laboratory, Department of Biology, FMIPA, University of Indonesia, Depok Indonesia. The name of the place for the collection of microalgae culture at UI which is in the process of being developed is the Universitas Indonesia Microalgae Culture Collection (UIMCC).

Method

Research methods include sampling in the field (sampling locations) and work in the laboratory. It can be seen on the scheme in Figure 1. The research began with the preparation of tools and the manufacture of culture medium (BBM, BG-11, CT, MA). Sampling in the field consisted of determining the sampling location, sampling, and measuring environmental parameters. The work in the laboratory consisted of identification of Cyanobacteria (morphology), isolation of Cyanobacteria, purification of isolates, and storage of isolates.

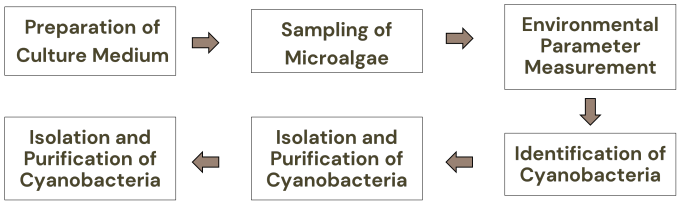

Figure 1. Research scheme

Preparation of Culture Medium

The medium used in the research consisted of four types of algae culture medium. These mediums include BBM (Bold Basal Medium) (Nichols, 1973), BG-11 Medium (Blue Green no.11), CT Medium (Cyanobacteria TAPS), and MA Medium (Microcystis Aeruginosa) (N.I.E.S.-collection, 2007). The acidity (pH) values for each medium were 8.6 for BBM; 7.4 for BG-11; 8.2 for CT; and 8.6 for MA.

Sampling Location

The research subjects were Cyanobacteria, both aquatic (originating from water) and terrestrial (originating from moist soil) in the UI Campus, Depok. Cyanobacteria sampling locations consisted of 7 sampling locations within the UI campus area. The seven sampling locations are vegetation and water areas,

namely 1) around the FMIPA UI area; 2) around *Situ* Agathis; 3) around the Politeknik Negeri Jakarta (Poltek); 4) around the Student Activity Center (Pusgiwa) and Situ Mahoni; 5) around *Situ* Puspa; 6) around *Situ* Kenanga; and 7) around *Situ* Ulin-Salam and UI student dormitories. Sampling locations can be seen in Figure 2 (Taqyuddin et al., 1997). Sampling was done by purposive sampling, which is based on environmental conditions around the sampling. The samples taken were planktonic and benthic samples (in sediments or attached to plants, etc.), as well as moist soil on the edge, under moss, urban forests, plantations, and rice fields. Soil sampling locations were to the north, namely the urban forest area (around Puspa, Ulin, and Salam), and to the south, namely the FMIPA building area and its surroundings, as well as around *Situ* Agathis. Sampling in the field was carried out 2 times.

Sampling Method

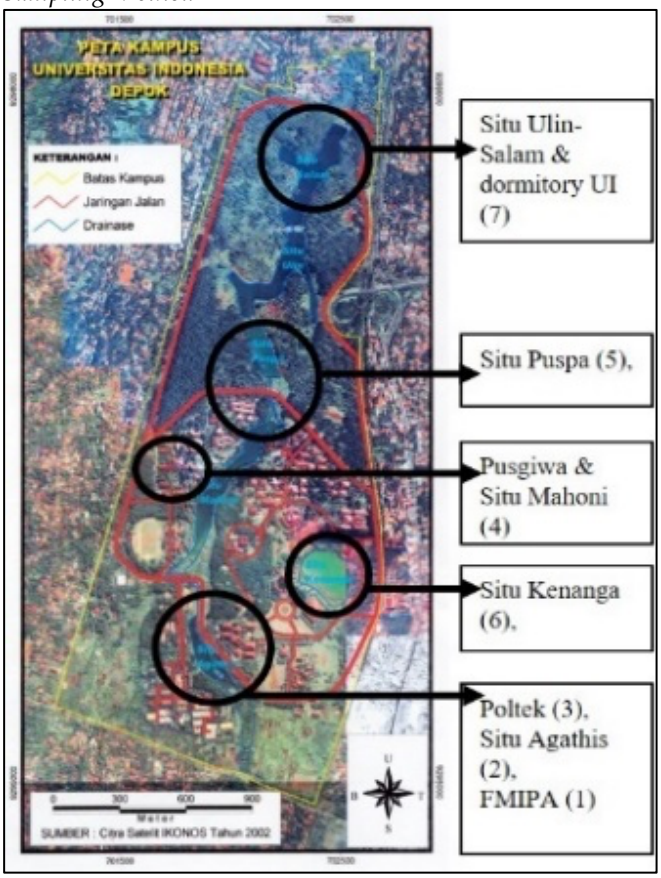

Figure 2. Location of water and soil sampling in the UI campus area

Planktonic sampling was carried out using a horizontal method using a plankton-net. Cyanobacteria samples attached to the substrate consisted of 2 types, namely Cyanobacteria samples attached to aquatic plants or litter (epiphytic), and Cyanobacteria samples found in sediments at the bottom of the water. Plants or litter in plastic squeezed (Squeeze Method). Soil samples taken were moist soil or slightly waterlogged soil. The soil sample was taken using a cloth. A little soil is put in a sealed plastic and labeled.

Environmental Parameter Measurement

Environmental parameters were measured at each location and at the time of collection. These environmental parameters are also needed to determine the approximate suitable culture medium for certain cyanobacteria. In this study, the environmental parameters measured were the degree of acidity (pH), temperature, season, and weather taken into consideration for the time of sampling.

Identification of Cyanobacteria

Cyanobacteria identification using morphological characters was carried out on fresh samples. The diagnostic morphological characters used are morphological characters with a taxonomic approach and studies according to Anagnostidis et al. (1988), Desikachary (1959), Prescott (1962), and Anand et al. (1990).

Books or literature used to identify an organism including microorganisms, in this case microalgae, must be scientifically accurate literature. Therefore, the literature for identification is often old literature because there has been no update. Identification using a Nikon SE light microscope at gradual magnifications of 10 x 4, 10 x 10, 10 x 40, with the aid of identification books (Edmondson, 1963; Geitler, 1985; Pentecost, 1984; Santra, 1993; B. A. Whitton, 2002). Identification is also carried out with the help of photographs.

Isolation and Purification of Cyanobacteria

For samples from water and soil, enrichment is carried out in the laboratory. In the laboratory, a few drops of samples from the water were put into an ice cream cup with a lid that had been filled with medium, while the soil samples were cultured in several growth media including BBM (Nichols, 1973), BG-11, CT, and MA (N.I.E.S.-collection, 2007). Each enrichment is made in duplicate. One set of enrichment samples was placed/incubated on a culture rack in an algal culture room at a temperature of 20—25 ^{0}C, while the other set was placed on a culture rack at a temperature of 28—31^0 C. Isolation of cyanobacteria species was carried out on fresh cyanobacteria samples (directly), and enriched samples. Cyanobacteria isolation was carried out by pipette (Pipette Method) and dilution (Dilution Method) in liquid and solid medium (Allen, 1973), and a combination of the two methods.

Storage of Cyanobacteria Isolate Culture

Cultivation is carried out on culture shelves in the Algae Culture Room (temperature approximately 20^0 C) Lab., Plant Taxonomy Dep. Biology, Faculty of Mathematics and Natural Sciences (FMIPA) UI. The

light intensity used is 3000 lux with white light (Fig. 2a). In this research used subculture method. The main principle in culturing is the transfer of a number of stock culture colonies (cultures) into the same medium.

Result and Discussion

Sampling in the field was carried out 2 times. This period was the rainy season. Cyanobacteria samples taken were aquatic samples, benthic samples, and samples from soil, trees, stone that were moist with moss. The number of samples of water, soil-trees-mossy stone was 119 samples (Table 1).

Table 1. Sampling Location, Type of Sample, pH Value, Number of Samples

Sampling Locations	Type of Samples	pH	Subtotal
Around the FMIPA UI area	mossy ground/stone/ trees	5—6	36
Situ Agathis & surroundings	mossy ground/stone/ trees	6—7	10
	Water	6—7	15
Around Politeknik Negeri Jakarta (Poltek)	mossy ground/trees	5—6	8
	Water	5—6	2
Around Pusgiwa &	mossy soil/stone	6—8	10
Situ Mahoni	water	6—8	8
Situ Puspa & surroundings	mossy ground	5—6	10
	water	6	2
Situ Kenanga & surroundings	mossy soil/stone	5—7	6
	water	7	3
Situ Ulin-Salam & surroundings	mossy ground/stone/ trees	4—7	7
	water	7	2
Total Sample			119

Value of Acidity (pH) of Cyanobacteria Sample Habitat in 7 Sampling Locations

In this research, only the degree of acidity (pH) was measured and took into account the season and the weather. The pH data is used for the selection of the growth medium used. The value of the degree of acidity (pH) of the samples from each habitat can be seen in Table 1. The pH values at the sampling locations ranged from 4—8. The pH data is a pH that is quite suitable for the growth of several Cyanobacteria strains. Cyanobacteria generally live in neutral or alkaline waters. Nonetheless, some can be found or grown at an acidic pH of not less than 4 (Prihantini et al., 2008). According to Brock in 1973 (Oliver et al., 2000). Cyanobacteria are generally not found in waters with a pH less than 4. Apart from pH, the season at the time of sampling was the rainy season and sunny weather after the rain. This condition causes the sampling habitat to be moist and is expected to be suitable for the growth of Cyanobacteria, especially those in the soil.

The Results of Identification and Isolation from Fresh Samples and Enrichment Cultures

The 119 samples of water and soil/trees/mossy stone were enriched using 4 types of medium, namely BBM, CT, BG-11, and MA, and incubated on a culture rack (Figure 3). Not all enrichment cultures grow well or do not grow cyanobacteria at all. Before and during the incubation process, the enrichment culture was checked/identified for the cyanobacteria present in the sample and the enrichment culture. It was used for the further isolation of cyanobacteria.

The isolation results obtained from a number of isolations for all samples at different time periods were 105 isolates of Cyanobacteria. Of the 105 isolates, there were 62 isolates that were quite stable. Cyanobacteria culture that is stable or stable enough is a culture that will be easy to breed and not easily contaminated, so it will be easy to treat.

In Table 2 it can also be seen that of the 4 mediums used for isolation, only 3 mediums that looked good for the growth of Cyanobacteria were isolated from the UI Campus samples. The three mediums were BBM (for 3 isolates), CT (for 12 isolates), and MA (for 5 isolates). There are some that grow well on BG-11 medium but are not stable. When subculture, the culture sometimes does not grow well or even dies.

Description with Morphological Characters of Cyanobacteria Derived from Fresh Samples, Enriched, and Isolated (Culture)

By using a limited microscope magnification, microalgae identification data was generated which can be seen in Table 2 and Figure 4. The data obtained are Cyanobacteria which can be observed using a microscope with 10 x 10 and 10 x 40 magnifications. Therefore, some Cyanobacteria can only be identified up to the genus, and it is likely that many Cyanobacteria cannot be seen with this limited magnification. However, the isolates obtained can be seen as one species even though the species marker has not been identified, except for *Limnothrix redekei*. Therefore, from 22 cyanobacteria isolates isolated there were 12 cyanobacteria species.

The cyanobacteria species found were grouped into four orders based on morphological diagnostic characteristics, namely differences in body shape. Those with a body shape of non-filamentous colonies are grouped in the order Chroococcales (*Synechococcus*, *Merismopedia*) and those with a body shape in the form of filamentous colonies (trichomes) are grouped in the order Oscillatoriales (*Pseudanabaena*, *Limnothrix*, *Oscillatoria*). Cyanobacteria that have unbranched filaments and have heterocysts are grouped in the order Nostocales (*Nostoc*, *Scytonema*, *Anabaena*), while those with branched filaments, both pseudo and true branches, are grouped in the order Stigonematales (*Hapalosiphon*, *Stigonema*, *Fischerella*).

Collection of Cyanobacteria Culture at the Department of Biology FMIPA UI

From the results, 62 isolates that were quite stable were re-selected by subculture and purification methods, and 22 isolates were obtained which were stable and could be stored (Table 2). The 22 isolates mostly came from samples of water and soil/trees/mossy stone and from sampling locations numbered 1 to 5 (around Politeknik Negeri Jakarta (Poltek); around *Situ* Agathis; around the Student Activity Center (Pusgiwa); around the FMIPA area. UI;around *Situ* Puspa), because the isolates from sample locations number 6 (around *Situ* Kenanga) and 7 (around *Situ* Ulin-Salam) were not stable. The 22 stable Cyanobacteria isolates were then combined with other collection isolates from previous researches.

Table 2. Cyanobacteria Isolate Data Based on the Analyzed Morphological Characters

Test Tube No.	Isolate Name/Species	Ordo	Sampling Location	Habitat	Strain Code	Medium
1	*Pseudanabaena* sp.	Oscillatoriales	Agathis	water	Ag 7 air	CT
5	*Pseudanabaena* sp.	Oscillatoriales	Agathis	water	Ag 7 air	CT
6	*Synnecococcus* sp.	Chroococcales	Agathis	water	Ag 7 air	MA
9	*Merismopedia* sp.	Chroococcales	Agathis	water	Ag 9 air	MA
10	*Pseudanabaena* sp.	Oscillatoriales	Agathis	water	Ag 9 air	CT
11	*Limnothrix redekei*	Oscillatoriales	Agathis	water	Ag 9 air	CT
25	*Nostoc* sp.	Nostocalles	Pusgiwa	soil	Pus 4 tn	BBM
29	*Nostoc* sp.	Nostocalles	Pusgiwa	soil	Pus 4 tn	BBM
30	*Nostoc* sp.	Nostocalles	Pusgiwa	soil	Pus 4 tn	BBM
31	*Hapalosiphon* sp.	Stigonematales	Pusgiwa	stone	Pus 2 bt	CT
32	*Hapalosiphon* sp.	Stigonematales	Pusgiwa	stone	Pus 2 bt	CT
33	*Nostoc* sp.	Nostocalles	Pusgiwa	stone	Pus 2 bt	CT
34	*Hapalosiphon* sp.	Stigonematales	Pusgiwa	stone	Pus 2 bt	MA
35a	*Hapalosiphon* sp.	Stigonematales	Pusgiwa	stone	Pus 2 bt	MA
35b	*Oscillatoria* sp.1	Oscillatoriales	Pusgiwa	stone	Pus 2 bt	MA
36b	*Hapalosiphon* sp.	Stigonematales	Pusgiwa	stone	Pus 2 bt	BBM
38	*Stigonema* sp.	Stigonematales	Pusgiwa	stone	Pus 2 bt	MA
39a	*Scytonema* sp.	Nostocalles	Pusgiwa	stone	Pus 2 bt	CT
39b	*Fischerella* sp.	Stigonematales	Pusgiwa	stone	Pus 2 bt	CT
43a	*Anabaena* sp.	Nostocalles	Pusgiwa	stone	Pus 2 bt	CT
50	*Oscillatoria* sp.2	Oscillatoriales	Pusgiwa	stone	Pus 2 bt	CT
51	*Hapalosiphon* sp.	Stigonematales	Pusgiwa	stone	Pus 2 bt	CT

Unlike other groups of microorganisms, microalgae cultures, especially cyanobacteria, are often difficult to isolate and grow in culture. This is probably because these organisms grow very slowly. In addition, when culturing, cyanobacteria are very sensitive to environmental factors, especially light intensity, pH, and nutrients in the medium. The low light intensity caused the Cyanobacteria isolates to not grow optimally and even not grow again after being subcultured. Some isolates grew better in Erlenmeyer than in test tubes. These constraints often make it difficult to obtain a large number of isolates. Another obstacle is the presence of members of Cyanobacteria that are difficult to culture (unculturable Cyanobacteria). In addition, it seems that these constraints still need to be studied to obtain optimal and stable isolates. Optimal and stable isolates can be used to observe genetic diversity, toxicity, and other benefits and applications.

From the research results, it is known that several mediums are suitable for the growth of Cyanobacteria. Good medium for growth of Cyanobacteria is medium MA (abbreviation: Microcystis Aeruginosa), CT (Cyanobacteria TAPS), BG-11 (Blue Green no. 11), and BBM (Bold Basal Medium). Three (3) mediums are specific medium for Cyanobacteria, while BBM is a universal medium for every freshwater microalgae. BBM is also good for use as a medium for isolation and incubation (culturing). In this study, the BG-11 medium was not very good for the growth of the isolated strains.

The selection of nutrient media for culturing a strain is very important for the application of the method. Selecting the correct nutritional compounds is the basis for further conservation of the taxonomic, morphological and biochemical properties of cultures (Uzunova-Doneva et al., 2005). The composition of the nutrient medium affects cell resistance. On nutrient rich media it is recommended in some conservation recipes, because the percentage of cells grown will be higher compared to those cultured on poor media.

Compared to other methods, the subculture method is the cheapest method, but it takes time to carry out culture transfer. In addition, in repeated subcultures, changes in culture conditions or sometimes even mutations may occur. In view of this, research is being conducted on the collection of microalgae and cyanobacteria for appropriate conservation methods for the strains that have been collected at the Department of Biology, FMIPA UI.

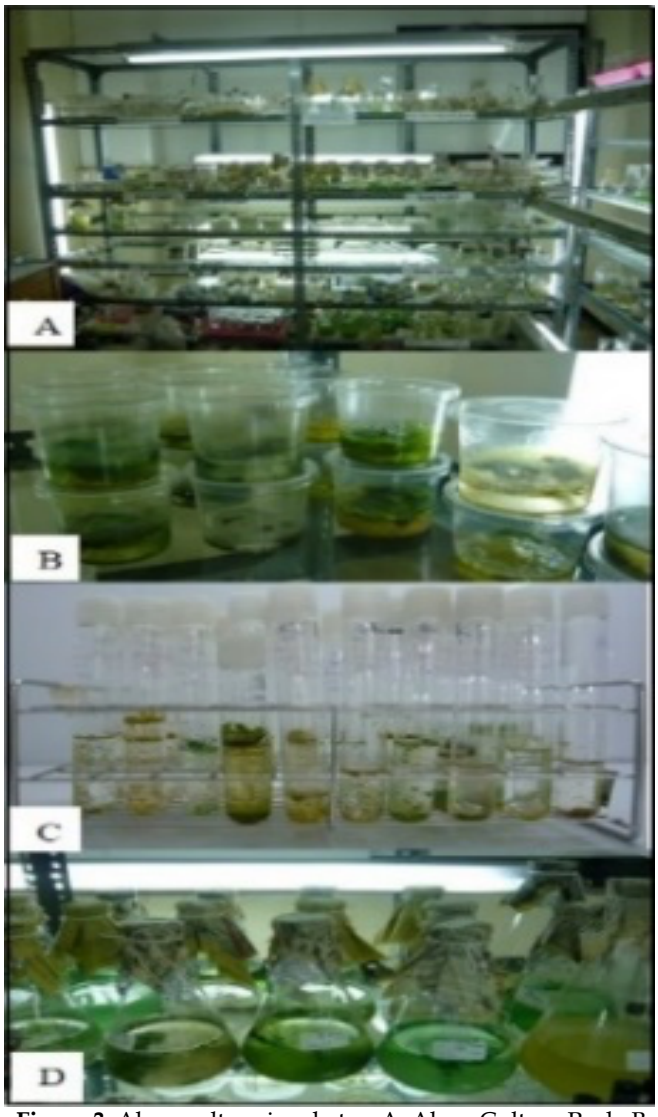

Figure 3. Algae culture incubator: A. Algae Culture Rack; B. Culture Enrichment on the shelf culture; C. Cyanobacteria isolates; D. Cyanobacteria biomass in 500 ml and 1000 ml Erlenmeyer

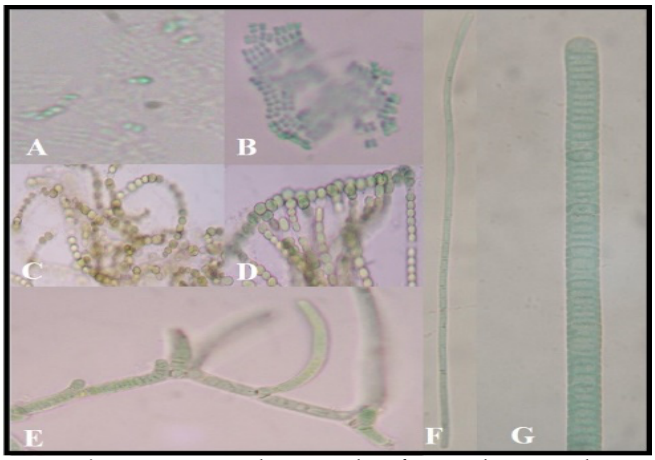

Figure 4. Microphotographs of some discovered cyanobacteria genera: A. *Synnechococcus*; B. *Merismopedia*; C. *Nostoc*; D. *Hapalosiphon*; E. *Stigonema*; F. *Limnothrix*; G. *Oscillatoria* (Photos not to scale)

Conclusion

Several conclusions can be drawn from this research, namely: 1) Several genera/species of Cyanobacteria found in fresh samples and enrichment culture results cannot be isolated and cultured (there are Unculturable Cyanobacteria); 2) Cyanobacteria strain stabilization process in culture depends on the nature of the strain, but on average it takes a long time (around 1 month); 3) Twenty-two (22) strains of Cyanobacteria were isolated and cultured properly consisting of 12 genera/species, namely *Merismopedia* sp. (1 isolate), *Synechococcus* sp. (1 isolate), *Limnothrix redekei* (1 isolate), Oscillatoria sp.1 (1 isolate), Oscillatoria sp.2 (1 isolate), *Pseudanabaena* sp. (3 isolates), *Nostoc* sp. (4 isolates), *Anabaena* sp. (1 isolate), *Scytonema* sp. (1 isolate), *Fischerella* sp. (1 isolate), *Stigonema* sp. (1 isolate), and *Hapalosiphon* sp. (6 isolates).

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