Morphological identification, isolation, and culturing of cyanobacteria derived from hot spring of Cisolok and Galunggung Mountain based on enrichment method

N B Prihantini
Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Indonesia, Kampus UI Depok, Depok 16424, Indonesia

Corresponding author’s e-mail: nining.prihantini@gmail.com

Abstract. Research on isolating and culturing of cyanobacteria from hot springs of Cisolok and Galunggung Mountain in West Java Indonesia had been done. This research was a study to find a fast and accurate method to identify, isolate and culture the cyanobacteria originating from extreme region, especially from the hot springs. Identification based on morphological characters performed in line with the process of isolation of the founded cyanobacteria strains. In the process of isolation, enrichment methods carried out in stages to determine the viability of each strain to be easily cultured (culturable). Four (4) types of media used in this study, i.e. BBM, BG-11, CT and MA. Pipette method was used to create a single culture. At the initial observations of the fresh samples, 3 types of cyanobacteria thallus were found, i.e. the form of thread/filament (unbranched filament), branched filament, and rounded shapes (coccoid). Furthermore, from all the enrichment samples (stage-1 and stage-2), 12 genera of cyanobacteria were found, i.e. Leptolyngbya, Gloeocapsa, Chroococcus, Oscillatoria, Synechococcus, Fischerella, Scytonema, Nostoc, Microcystis, Gloeotrichia, and Stanieria. In the first stage of enrichment culture samples of Cisolok, eight (8) genera were found, i.e. Leptolyngbya, Chroococcus, Oscillatoria, Synechococcus, Scytonema, Hapalosiphon, Nostoc, and Gloeotrichia, while in the hot spring of Galunggung mountain, only four (4) genera were found (Leptolyngbya, Oscillatoria, Hapalosiphon, and Nostoc). There were several filaments and coccoid cyanobacteria that could not be identified by morphological characters. Meanwhile, in the second stage of enrichment culture samples of Cisolok, nine (9) genera were found, i.e. six (6) genera similar to those found in the first stage of enrichment (Leptolyngbya, Chroococcus, Oscillatoria, Synechococcus, Scytonema, Hapalosiphon), and added with the genus of Gloeocapsa, Microcystis, and Stanieria. From the hot spring of Mt Galunggung, nine (9) genera were found, i.e. four (4) genera similar to those found in the first stage of enrichment (Leptolyngbya, Oscillatoria, Hapalosiphon, and Nostoc), and added with the genus of Chroococcus, Gloeocapsa, Microcystis, Synechococcus, and Fischerella. Both from Cisolok and from Galunggung, the three genera (Leptolyngbya, Oscillatoria, Hapalosiphon) could be found in fresh samples and enrichment samples (stage-1 and stage-2). The research concluded that the enrichment process in stages (first and second) can be used for the isolation of strains of cyanobacteria to be adaptable, and therefore easy to be cultured (culturable). The genus Scytonema and Gloeotrichia are genera that known to be difficult to be cultured, and in this study, they grow only in stage-1, while the genus Stanieria appears in stage-2. Isolates obtained consist of 31 strains from Cisolok hot spring, and 13 strains from Galunggung mountain hot spring, then would be identified (re-identification) using molecular characters (16S rDNA).

Keywords: indigenous cyanobacteria, hot spring, culturable, isolation method
1. Introduction

Cyanobacteria are a prokaryotic alga that has an ability to photosynthesize because it has chlorophyll and other accessory pigments. Some cyanobacteria are known to live at high or thermophilic temperatures [1], in other words, cyanobacteria can live in extreme environments with very high temperatures. For example, the cyanobacteria can live in Yellowstone National Park hot springs. Jackson conducted an enrichment sample of cyanobacterium/ microalgae taken from Yellowstone National Park hot springs and grew the sample at different temperature variations. Based on the results of Jackson's research, cyanobacteria genus of Synechococcus, Synechocystis, and Mastigocladus can survive at up to 55 °C. Therefore, Jackson stated that the cyanobacteria are thermophilic (can live at high temperatures). It proves that there are certain types of microalgae that can live in extreme environments [2]. Research on cyanobacteria that can live at high temperatures has been done by Prihantini [3] on cyanobacteria from several hot spring locations in Indonesia (Ciseeng, Raw Daan Banten, Red Crater/Kawah Merah-Pancar mountain, Ciater, Maribaya and Domas Crater/Kawah Domas Tangkuban Perahu mountain). Some cyanobacteria can be isolated and cultured, but some cannot be cultured [3].

Hot spring is formed from the flow of water beneath the layer of the earth's crust that is located close to the hot stones in which the temperature can reach 148 °C. The water does not become steam because there is no contact with air. The hot water can exit to the surface of the earth through cracks or cracks in the earth's crust. Meanwhile, geyser is a gap or hole on the surface of the earth that spouted the column of hot water mixed with steam periodically. Some geysers have a volume of hot springs up to thousands of liters, with a range of bursts of up to tens of meters in the air.

Cisolok and Mt Galunggung in West Java are area that has hot springs. Cisolok hot spring and Mt Galunggung are hot water sources that are visited by many people and tourists. Cisolok geyser is located in the western part of Sukabumi Regency and bordered with Lebak District, West Java, Indonesia. The geothermal system in Cisolok is an outflow system of a high-temperature geothermal system originating from Halimun Mountain located 20 km in the northern part of Cisolok [4]. There are ± 20 hot springs at the meeting point between the Cisolok and Cikupa rivers with an area of 5 hectares. Water temperature geyser ranges in Cisolok are from 46–98 °C. The content of Cl water in Cisolok Geyser is 330 ppm with pH 7.5. Meanwhile, Mt Galunggung is located at an altitude of 2,167 meters above sea level, about 17 kilometers from downtown Tasikmalaya. Mt Galunggung erupted in 1822, 1894, 1918, and 1982. The early signs of the eruption were known in July 1822. The eruption resulted in very hot reddish sand, soft ash, hot clouds and lava. The eruption of Mt Galunggung that occurred in 1982 resulted in the formation of the Mt Galunggung Crater [5]. There are two hot spring locations in Mt Galunggung, Galunggung crater and Cipanas Galunggung. Galunggung crater has a diameter of 1000 m and a depth of 11 m with an estimated water volume of about 8 million m³. Cipanas Galunggung is a tourist attraction in the form of hot water bath. The spring in this bathhouse comes from Panoong Waterfall, which is the meeting point of hot, and cold waters source [5].

Several studies on the diversity of microorganisms in Cisolok hot springs have been conducted, one of which is research conducted by Myung-Ji et al. [6]. They analyzed the diversity of microorganisms based on 16S rRNA gene sequences in several geothermal areas in Indonesia, one of which is the Cisolok hot spring. The results show that there is an abundance of various microorganisms, one of which is the cyanobacteria of the genus Thermosynechococcus in the Cisolok hot spring region [6]. Unlike in Cisolok, research on cyanobacteria that live in the hot springs of Mt Galunggung to date has not been done. Neither location is known in regards to the culturable cyanobacteria. Therefore, research to obtain information to know members of cyanobacteria that live in the region, especially culturable cyanobacteria, is performed.

Based on previous studies [3,7], culturing cyanobacteria from extreme temperatures was often difficult to use only the pipette method. Then, the first-stage enrichment method has also been tried [3] and produced some of the cyanobacteria isolates obtained for a long time. Some of the encountered obstacles partly because of the adaptation power of some cyanobacteria are less when grown in conditions that are not exactly the same as the origin environment. This ability causes only a few cyanobacteria that grows in enrichment cultures and grows very slowly. Therefore, it is necessary to find a faster method for obtaining cultured cyanobacteria. The study aims to find a fast and accurate method for identifying, isolating, and culturing the cyanobacteria from extreme areas, especially from hot springs. The results are compared based on the morphological character of the first stage and the second stage of enrichment methods.
2. Materials and methods

2.1. Location, time, equipment, sample and medium
The research was conducted in the Laboratory of Plant Taxonomy, Department of Biology FMIPA Universitas Indonesia in Depok during the period of 2016 until early 2017. The tools used in the research were the equipment commonly used in the observation and culturing of microalgae including cyanobacteria. The identification book used was the cyanobacteria identification book [8]. The sample of cyanobacteria used was a sample of Cisolok (19 samples; sample code is CK) and samples from Mt Galunggung (9 samples from Kawah Galunggung with sample code KG and 9 samples from Cipanas Galunggung with sample code CG). Cyanobacteria samples were taken once from the above-mentioned water. The medium used in the study consisted of microalgae growth medium and cyanobacteria, medium of Blue Green 11 (BG-11), Bold Basal Medium (BBM), Cyanobacteria TAPs (CT), and Microcystis Aeruginosa (MA) [9].

2.2. Treatment of fresh cyanobacteria samples (1-stage enrichment)
The first stage of enrichment was conducted against the fresh enrichment samples in the field and laboratory [7]. Upon received in the laboratory, transfer process of cyanobacteria samples were performed into an ice cream cup, i.e. 0.5 mL of culture from freshly transferred samples into 15 mL medium (BBM, BG-11, CT, and MA) contained in ice cream cup. Furthermore, ice cream cup containing medium and cyanobacteria samples were incubated at 30 ± 5 °C and 50 ± 5 °C for 2 months.

2.3. Treatment of the stage-1 enrichment (2-stage enrichment)
The treatment of second stage enrichment was done after 2 months of the first stage enrichment. The second stage enrichment was done in aseptic transfer box. A total of 0.5 mL cultures from the first stage enrichment sample were taken using sterile Pasteur pipettes and transferred into 15 mL medium (BBM, BG-11, CT, and MA) contained in ice cream cup. Furthermore, ice cream cup containing medium and cyanobacteria samples was incubated at 30 ± 5 °C and 50 ± 5 °C for 2 months.

2.4. Identification of cyanobacteria and other microalgae
The successfully grown cyanobacteria was observed and identified under a light microscope and microscope in the laboratory of Bio Imaging, Department of Biology, FMIPA UI, Depok. The observed cyanobacteria and microalgae were photographed, and then identified by using Whitton & Brook microalgae identification book [8].

2.5. Isolation of cyanobacteria strains
Cyanobacteria strains isolation was conducted on fresh cyanobacteria samples and enrichment samples, using the pipette method (Pipette Method) in liquid medium. The genera of cyanobacteria contained in the fresh sample were immediately photographed with the Leica DM500 digital camera in the Dell brand light microscope and the Power Logic Futura Neo Xv100 CPU.

3. Results and discussion

3.1. Observation on first stage enrichment culture
Observations were initiated after cyanobacteria were seen in enrichment cultures about 1 month after the inoculation of fresh samples. Observation of cyanobacteria identification was performed for 2 months. Identification of cyanobacteria was based on morphological characters. In the first stage enrichment culture of the Cisolok samples, microalgae were identified as members of the Cyanobacteria and Bacillariophyta divisions (Diatoms). Diatoms are a group of microalgae that are easy to grow in culturing processes, and often become contaminants in the isolation process. Cyanobacteria found in Cisolok consists of 8 genera, among others are Leptolyngbya, Hapalosiphon, Oscillatoria, Nostoc, Gloeotrichia, Scytonema, Synechococcus, and Chroococcus. The most common cyanobacteria were Leptolyngbya, while the rarest was Gloeotrichia. The Leptolyngbya was discovered at almost all sample points. Leptolyngbya is cyanobacteria that can be well grown in culturing [10]. Gloeotrichia was found only at one sample point (sample code is CK1 C2 on BG-11 medium). The result of identification can be seen in table 1.

In the first stage enrichment culture from samples of Mt. Galunggung, microalgae members of the Cyanobacteria, Chlorophyta and Bacillariophyta divisions were found. Some identifiable genera of
### Table 1. Identification results of cyanobacteria on enrichment samples from Cisolok (sample code is CK) incubated at ± 35 °C

| Sample Code | Medium | Genus / thallus form in 1st Enrichment | Genus / thallus form in 2nd Enrichment | Genus / thallus form in 1st & 2nd Enrichment |
|-------------|--------|----------------------------------------|----------------------------------------|---------------------------------------------|
| CK1 A1      | BBM    | Coccoid                                 | Gloeocapsa, Coccoid like fil           | Leptolyngbya                                |
|             | CT     | Leptolyngbya                            | Gloeocapsa                             |                                             |
|             | MA     | Leptolyngbya                            |                                        |                                             |
| CK1 A2      | BBM    | Hapalosiphon, Coccoid                   | Gloeocapsa, Chroococcus                | Leptolyngbya                                |
|             | CT     | Hapalosiphon                            |                                        |                                             |
|             | MA     | Coccoid                                 |                                        |                                             |
| CK1 A3      | BBM    | Coccoid                                 |                                        |                                             |
|             | CT     | Leptolyngbya                            |                                        |                                             |
|             | MA     | Coccoid                                 |                                        |                                             |
| CK1 A4      | BBM    | Navicula                                |                                        |                                             |
|             | CT     | Hapalosiphon, Oscillatoria              |                                        |                                             |
|             | MA     | Oscillatoria, Synechococcus             |                                        |                                             |
| CK1 A5      | BBM    | Coccoid                                 |                                        |                                             |
|             | CT     | Leptolyngbya                            |                                        |                                             |
|             | MA     | Leptolyngbya                            |                                        |                                             |
| CK1 B1      | BBM    | Navicula                                |                                        |                                             |
|             | CT     | Diadesmis, Leptolyngbya                |                                        |                                             |
|             | MA     | Leptolyngbya                            |                                        |                                             |
| CK1 B2      | BBM    | Coccoid, Hapalosiphon, Coccoid like fil |                                        |                                             |
|             | CT     | Leptolyngbya                            |                                        |                                             |
|             | MA     | Leptolyngbya                            |                                        |                                             |
| CK1 C1      | BBM    | Chroococcus, Hapalosiphon              | Coccoid, Coccoid like filament,        | Leptolyngbya                                |
|             | CT     | Chroococcus                            |                                        | Leptolyngbya, Oscillatoria, Synechococcus, |
|             | MA     | Chroococcus, Oscillatoria, Synechococcus, |                                        |                                             |
|             |        |                                        |                                        |                                             |
| CK1 C2      | BBM    | Gleotrichia, Hapalosiphon, Synechococcus, | Oscillatoria                           | Leptolyngbya                                |
|             | CT     | Coccoid like filament, Hapalosiphon     |                                        |                                             |
|             | MA     | Coccoid like filament, Hapalosiphon     |                                        |                                             |
| CK2 A1      | BBM    |                                        |                                        |                                             |
|             | CT     | Hapalosiphon, Oscillatoria              |                                        | Hapalosiphon                                |
|             | MA     |                                        |                                        |                                             |
| CK2 B1      | BBM    | Leptolyngbya                            |                                        | Leptolyngbya, Oscillatoria                 |
|             | CT     |                                        |                                        | Oscilltaria                                 |
|             | MA     |                                        |                                        |                                             |
| CK3 A1      | BBM    |                                        |                                        |                                             |
|             | CT     | Coccoid gr, Leptolyngbya, Oscillatoria  |                                        | Leptolyngbya, Oscillatoria                 |
|             | MA     |                                        |                                        |                                             |
| CK3 A2      | BBM    |                                        |                                        |                                             |
|             | CT     | Hapalosiphon, Oscillatoria              |                                        |                                             |
|             | MA     |                                        |                                        |                                             |
| CK3 B1      | BBM    |                                        |                                        |                                             |
|             | CT     | Leptolyngbya                            |                                        | Leptolyngbya                                |
|             | MA     |                                        |                                        |                                             |
| CK3 B2      | BBM    |                                        |                                        |                                             |
|             | CT     | Coccoid gr, Leptolyngbya, Oscillatoria  |                                        | Leptolyngbya, Oscillatoria                 |
|             | MA     |                                        |                                        |                                             |
| CK3 C1      | BBM    |                                        |                                        |                                             |
|             | CT     | Coccoid like filament                    |                                        | Leptolyngbya                                |
|             | MA     |                                        |                                        |                                             |
| CK3 D1      | BBM    |                                        |                                        |                                             |
|             | CT     | Hapalosiphon, Oscillatoria              |                                        | Leptolyngbya                                |
|             | MA     |                                        |                                        |                                             |

**Note:** The table provides a summary of the identification results of cyanobacteria on enrichment samples from Cisolok (sample code is CK) incubated at ± 35 °C, detailing the genus/thallus form in each enrichment step and the identification results across multiple media.
cyanobacteria are Oscillatoria, Leptolyngbya, Hapalosiphon, and Nostoc. Oscillatoria was very often found in the cultured sample results, the results show that 25 of the 72 enrichment samples were able to grow Oscillatoria. Meanwhile, the Leptolyngbya genus can be found in 18 of the 36 enrichment samples in the KG code cultured at ± 30 °C. Genus of Hapalosiphon can only be found in a sample of the first stage enrichment results. Hapalosiphon was not widely found in the enrichment samples. This genus was found to be larger in samples cultured at ± 50 °C and only found in 13 of the 72 enrichment samples. In addition to Hapalosiphon, another not-so-common genus was Nostoc. Nostoc was only found in 3 samples from a total of 72 enrichment samples i.e. with the sample code of KG 2, KG 6, and KG 9.

3.2. Observation on second stage enrichment culture
As well as in the first stage enrichment culture, the identification of microalgae, especially cyanobacteria, were done after 2 months of second stage enrichment process using morphological characters. Data from the identification of second stage enrichment culture can be seen in table 2. The results of the identification of microalgae contained in the second stage enrichment culture of Cisolok samples, which found two divisions of microalgae, i.e. Cyanobacteria and Chlorophyta. The genus of cyanobacteria found in Cisolok, namely Leptolyngbya, Gloeocapsa, Chroococcus, Oscillatoria, Synechococcus, Syctyonema, Hapalosiphon, Microcystis, and Stanieria. Meanwhile, the cyanobacteria genera found in hot springs of Mt Galunggung were Leptolyngbya, Gloeocapsa, Chroococcus, Oscillatoria, Synechococcus, Fischerella, Microcystis, Nostoc. Other forms of thallus found in Cisolok and Galunggung hot springs but not yet identified were Coccoid, Coccoid-like-filament, and Filaments.

The data in the table shows that cyanobacteria are higher than other microalgae. Based on the literature, cyanobacteria are photosynthetic microorganisms that can live in extreme environments such as low pH, high temperature, and high salinity. Cyanobacteria can grow at a high temperature of about 73—74 °C [11]. In the culture of the second stage enrichment, Leptolyngbya was the most dominant genus among the other genera. According to Komárek et al. [12] species of the Leptolyngbya are often found in a variety of habitats, such as soil, rock, sea, fresh water, even hot spring. Other genera of Cyanobacteria found quite often were the genus Oscillatoria. Like
Table 2. Identification results of cyanobacteria on enrichment samples from hot springs of Kawah Galunggung (sample code is KG) and Cipanas Galunggung (sample code is CG) incubated at \( \pm 35 \) °C

| Sample Code | Genus / thallus form in 1st & 2nd Enrichment |
|-------------|---------------------------------------------|
| **1st Enrichment** | **2nd Enrichment** |
| KG 1 | Coccoid, Hapalosiphon | Oscillatoria, Leptolyngbya |
| BG-11 | Oscillatoria, Diatom | Filament | Leptolyngbya |
| CT | Coccoid, Oscillatoria, Leptolyngbya | - | - |
| MA | Coccoid, Diatom | - | Leptolyngbya, Oscillatoria |
| KG 2 | Coccoid, Leptolyngbya, Scenedesmus | Chroococcus, Gloeocapsa | Oscillatoria, Leptolyngbya |
| BG-11 | Nostoc | Coccoid, Coccoid like filament | - |
| CT | Diatom | Leptolyngbya, Chroococcus | Coccoid |
| MA | Coccoid, Oscillatoria, Diatom | - | - |
| KG 3 | Diatom | Fischerella, Synechococcus | Coccoid like filament, Oscillatoria, Leptolyngbya |
| BG-11 | Diatom | Synechococcus, Leptolyngbya, Coccoid | like filament |
| CT | Coccoid, Leptolyngbya, Diatom | - | - |
| MA | Coccoid, Diatom | Leptolyngbya, Oscillatoria, Chroococcus, Coccoid green | - |
| KG 4 | Coccoid, Diatom, Lyngbya | Chroococcus | - |
| BG-11 | Coccoid, Diatom, Oscillatoria | - | - |
| CT | Diatom | Leptolyngbya | Coccoid, Oscillatoria |
| MA | Coccoid, Diatom, Oscillatoria | - | - |
| KG 5 | Lyngbya | Microcystis, Oscillatoria | - |
| BG-11 | Coccoid, Coccoid like filament, Lyngbya, Nostoc, Scenedesmus | Bracteacoccus | - |
| CT | Diatom, Lyngbya | - | Coccoid |
| MA | Coccoid, Coccoid like filament, Lyngbya, Scenedesmus | Bracteacoccus, Leptolyngbya | Oscillatoria |
| KG 6 | Lyngbya | Bracteacoccus | - |
| BG-11 | Coccoid, Diatom, Oscillatoria, Lyngbya | - | - |
| CT | Diatom, Oscillatoria | - | Oscillatoria |
| MA | Coccoid, Oscillatoria | - | Coccoid |
| KG 7 | Diatom, Oscillatoria, Coccoid | Bracteacoccus | - |
| MA | Diatom | Coccoid, Oscillatoria, Lyngbya | - |
| KG 8 | Lyngbya | Oscillatoria, coccoid, coccoid like filament, Nostoc | - |
| BG-11 | Lyngbya, Oscillatoria, Coccoid | - | Coccoid like filament |
| CT | Diatom, Coccoid | Chroococcus, Bracteacoccus | Oscillatoria |
| MA | Diatom | Coccoid | Oscillatoria |
| KG 9 | Lyngbya, Diatom | Chroococcus, Fischerella, coccoid like filament | Coccoid, Hapalosiphon, Oscillatoria |
| BG-11 | Lyngbya, Oscillatoria, Chroococcus | - | Coccoid like filament, Hapalosiphon |
| CT | Coccoid like filament, Hapalosiphon, Nostoc, Diatom | Lyngbya | Coccoid, Oscillatoria |
| MA | Diatom | - | Oscillatoria, Lyngbya |
| CG 1 | Coccoid, Hapalosiphon | - | - |
| BG-11 | Coccoid like filament | - | - |
| CT | - | - | - |
| MA | - | - | - |
| CG 2 | Hapalosiphon | - | - |
| BG-11 | Coccoid like filament | - | - |
| CT | - | - | - |
| MA | Diatom | - | - |
| CG 3 | Coccoid | - | - |
| BG-11 | Coccoid like filament | - | - |
| CT | Coccoid like filament, Hapalosiphon | - | - |
| MA | Coccoid like filament | - | - |
Leptolyngbia, Oscillatoria was found in many environments, such as rocks, freshwater, ponds, sea, and wetlands [12]. From these two locations, cyanobacteria can be identified as filamentous and coccoid cyanobacteria, consisting of 12 genera derived from the order Chroococcales (Gloeocapsa, Chroococcus, Microcystis), Oscillatoriales (Oscillatoria), Synechococcales (Synechococcus, Leptolyngbia), Nostocales (Nostoc, Gloetrichia), and Stigonematales (Fischerella, Scyttonema, Hapalosiphon), and Pleurocapsales (Stanierea).

3.3. Another result of the first and second stage enrichment culture

During the enrichment culture process, some things can be known, namely there is a “genus that disappears” and there is a “genus that appears”. Genus lost in question is a genus that existed at the first stage enrichment but cannot be found anymore or very little at the second stage enrichment. Based on the first enrichment data, the genus Gloetrichia was obtained from the first stage enrichment sample, whereas in the second stage enrichment, it was not found. In addition, Scyttonema appeared in the first stage enrichment, but only in one of second stage enrichment culture (sample code CK1 C1). The genus is known to be quite difficult to be cultured. Like other microorganisms, cyanobacteria also known as difficult to be cultured (non-culturable or not yet culturable). This might be caused by, among other things, different species having different ecological requirements and adaptations in their natural habitat. Cyanobacteria strains may have different responses when transferred to standard cultural conditions. Cyanobacteria strains can also experience stress when transferred to a standard cultivation conditions, so that culture can change morphological or ecophysiological characters [13]. Until this paper is made, the genus Scyttonema originating from Cisolok and Mt Galunggung has not been successfully isolated. Preferably, the isolation of the Scyttonema genus is carried out directly from fresh samples of cyanobacteria and from samples at the first stage enrichment culture.

The number of isolates isolated from both sites was 34 isolates. From the 34 isolates, most were derived from the second stage enrichment. The successful isolation of several genera of cyanobacteria from a sample of second stage enrichment cultures is likely due to the genus grown in the sample of stage-2 already adapted to the media environment used. Therefore, the genera may have adapted to their environment (especially medium and incubation temperature) in order to survive and multiply. The ability to adapt of every living being is different. The enrichment process can be done by modifying the physical conditions such as regulating the temperature and pH of the growth environment, nutrition or enrichment the growth medium content with certain nutrients needed by the microalgae [14]. The isolates are then re-identified using morphological characters and will be identified with molecular characters. Figure 1 shows the enrichment cultures of samples from Cisolok and Mount Galunggung.
4. Conclusions

The conclusions that can be drawn from the research results are as follows: from Cisolok, 11 genera can be identified, i.e. Leptolyngbia, Hapalosiphon, Oscillatoria, Nostoc, Gloeotrichia, Scyttonema, Synechococcus, Chroococcus, Gloeocapsa, Microcystis, and Stanieria, while from Galunggung Mountain, 9 genera can be identified, i.e. Oscillatoria, Leptolyngbia, Hapalosiphon, Nostoc, Gloeocapsa, Chroococcus, Synechococcus, Fischereilla, Microcystis. The genus Leptolyngbya dominates the Cisolok and Galunggung hot springs samples. The enrichment methods can predict strains that are not easily isolated and cultured. For examples Scyttonema and Gloeotrichia are genera that only appear in the first stage enrichment. Moreover, the enrichment methods can grow strains that initially are not seen clearly. For example Stanieria is a genus that often appears in the second stage enrichment.

Acknowledgements

This work was funded by Hibah Penelitian Unggulan Perguruan Tinggi (PUPT) 2017 to Nining Betawi Prihantini, grant no. 2708/UN2.R3.1/HKP05.00/2017. Author thank to Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok for the facilities provided.

References

[1] Bold H C and Wynne M J 1985 Introduction to The Algae: Structure and Reproduction 2nd Edition (Englewood Cliffs: Prentice-Hall Inc)
[2] Jackson J E and Castenholz R W 1975 Limnol. Oceanogr. 20 305–22
[3] Prihantini N B 2015 Polyphasic Taxonomy of Culturable Cyanobacteria Isolated from Hot Springs in West Java Indonesia (Depok: Universitas Indonesia) Ph.D. Dissertatoin
[4] Suryantini and Hendrasto F 2009 Cisolok Excursion Guide Book (Bandung: Institute Teknologi Bandung) p 14
[5] Widodo W 2014 Biosaintifika 6 29–37
[6] Seo M J, Kim J N and Pyun Y R 2012 Microbiology and Biotechnology Letters 40 268–73
[7] Prihantini N B, Sjamsuridzal W and Yokota À 2012 Proc. 5th International Seminar of Indonesian Society for Microbiology (ISISM), ed D P M Ludong et al. (Manado: Indonesian Society for Microbiology Manado) p 318
[8] Whitton B A 2002 The Freshwater Algal Flora of the British Isles: An Identification Guide to Freshwater and Terrestrial Algae, ed John D M et al. (Cambridge: Cambridge University Press) pp 31–158
[9] National Institute for Environmental Studies (NIES) 2000 Microbial Culture Collection (MCC) NIES List of Strains 9th ed, ed M M Watanabe et al. (Tsukuba: MCC-NIES Japan) p 164
[10] Guiry M D 2018 AlgaeBase, ed M D Guiry and G M Guiry (Galway: National University of Ireland) Available at http://www.algaebase.org/
[11] Seckbach J 2007 Algae and cyanobacteria in extreme environments Cellular Origin, Life in Extreme Habitats and Astrobiology, ed J Seckbach (Dordrecht: Springer) pp 3–25
[12] Komárek J, Kling H and Komárková J 2003 Freshwater Algae of North America Ecology and Classification ( ed J D Wehr and R G Sheath R G (Academic Press Elsevier: Amsterdam) p 932
[13] Komárek J 2006 Algae 21 349–75
[14] Howland J L and Garfield A 1993 Biochem. Educ. 21 218