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The Growth of *Leptolyngbya* HS-16 and HS-36 on 35 °C at Different Acidity

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Cyanobacteria are prokaryotic organisms belonging to the kingdom Eubacteria. Cyanobacteria can be found in hot spring. *Leptolyngbya* is one genus of cyanobacteria that can be found in hot spring. The observation of *Leptolyngbya* growth on temperature of 35 °C with initial pH variation had been done. The study was experimental trial. The study aimed to determine the best initial growth pH for *Leptolyngbya* HS (Hot Spring)-16 and HS-36. *Leptolyngbya* HS-16 was isolated from Pancar Mountain hot spring, while *Leptolyngbya* HS-36 was isolated from Maribaya hot spring. The acidity (pH) of Pancar Mountain and Maribaya hot spring was 7. Each strain was grown in Blue Green medium number 11 with variation of initial pH (6, 7, 8 and 9) and incubated at 35 °C. Parameters was wet biomass weight of *Leptolyngbya* in each strain. The results of 15 days observation showed that the best initial pH for growing *Leptolyngbya* HS-16 is 7, while *Leptolyngbya* HS-36 is 9. From this study it could be seen that *Leptolyngbya* HS-16 and HS-36 could be cultured with alkaline condition.

Key words: hot spring, *Leptolyngbya,* pH

Cyanobacteria merupakan organisme prokariotik yang berasal dari kingdom *Eubacteria*. Cyanobacteria dapat ditemukan pada sumber air panas. Salah satu genus dari cyanobacteria yang ditemukan dalam sumber air panas, yaitu *Leptolyngbya*. Pengamatan pertumbuhan *Leptolyngbya* pada suhu 35 °C dengan variasi pH awal telah dilakukan. Penelitian ini merupakan penelitian eksperimental. Penelitian ini bertujuan untuk mengetahui pH pertumbuhan awal terbaik untuk *Leptolyngbya* HS-16 dan HS-36. *Leptolyngbya* HS-16 diisolasi dari sumber air panas di gunung Pancar, sedangkan *Leptolyngbya* HS-36 diisolasi dari sumber air panas di Maribaya. Derajat keasaman (pH) air dari sumber air panas di gunung Pancar dan Maribaya adalah 7. Masing-masing strain ditumbuhkan pada medium Blue Green nomor 11 dengan variasi pH awal (6, 7, 8 dan 9) dan diinkubasi pada suhu 35 °C. Parameter yang diteliti adalah berat basah biomassa *Leptolyngbya* pada masing-masing strain. Pengamatan dilakukan selama 15 hari dengan 11 sampling. Hasil pengamatan 15 hari menunjukkan bahwa pH awal terbaik untuk pertumbuhan *Leptolyngbya* HS-16 adalah 7, sedangkan *Leptolyngbya* HS-36 adalah 9. Berdasarkan penelitian dapat diketahui bahwa *Leptolyngbya* HS-16 dan HS-36 dapat dibiakkan dengan kondisi alkalin.

Kata kunci: *Leptolyngbya*, pH, sumber air panas

Cyanobacteria are prokaryotic organisms belonging to the kingdom Eubacteria (Van den hoek 2002). These organisms has photosynthetic apparatus that plays role in producing energy (Scholnick *et al*. 2006). Cyanobacteria has photosynthetic pigment named phycobilin. These organisms stores glycogen as its food storage (Markou *et al*. 2014).

The growth phases of cyanobacteria are same with other microorganisms. The growth curve of population in microorganisms known as exponential growth. Exponential growth in microorganisms consist of lag phase, exponential phase, stationary phase and death phase. Lag phase occur when microorganisms has been innocculated into a new medium and adaptation with new environmental with different condition of their habitat. Exponential phase happened when microorganism could adapted with their new environmental condition and their population increased twice of their first population. The population of microorganisms increased because they could used source of their new environmental. Stationary phase occurred after exponential phase. Stasioner phase is the phase that microorganisms could not do doubling cell. This phase occurred because the nutrients present in the growth medium are insufficient for doubling cell and accumulation of the metabolic waste of the microorganisms. Death phase will occured after stationer phase (Madigan *et al*. 2015).

Cyanobacteria can be found in soil, rocks and waters (Bold *et al*. 1978). Another source where cyanobacteria can be found is hot spring. Maribaya hot spring and Pancar mountain hot spring are the example of source whereas cyanobacteria can be found. Maribaya hot spring has pH 6 to 7, while Pancar Mountain hot spring has pH 7. *Leptolyngbya* is one genus of cyanobacteria that can be found in Maribaya and Pancar Mountain hot spring (Prihantini 2015).

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Leptolyngbya have filamentous as the form of the colony. Characteristic of these organisms have a thin filament with 0.5 to 3.5 μm wide with simple trichome, and some species have sheath (Komarek 2007). *Leptolyngbya* can be found in environmental condition with pH 7 to 8.5 (Olsson-francis *et al*. 2012)

Leptolyngbya have many benefit for our life. It could be seen *Leptolyngbya* could produce lipid as biofuel feedstock, produce secondary metabolites as antibiotic, and could react as bioremediator in dairy waste (Abazari *et al* 2012; Beetul, 2014; Khemka *et al* 2015). In order to beneficial of *Leptolyngbya*, it is important to cultivated *Leptolyngbya*. The acidity (pH) in the environment can affect the growth rate of cyanobacteria especially *Leptolyngbya*. Cyanobacteria can also live in environmental conditions with a wide range of pH, but some species are sensitive to acidic conditions (Gerloff-Elias *et al*, 2005). Cyanobacteria could barely find in freshwater with range of pH 4 to 5 (Bold *et al*. 1978). Variation of initial pH in growth medium will affect the growth of cyanobacteria. The *Leptolyngbya* HS-16 and HS-36's best initial growth pH have not known yet. The aim of this study is to determine the best initial growth pH for *Leptolyngbya* HS-16 and HS-36 in Blue Green number 11 medium (BG-11).

MATERIALS AND METHODS

Microorganisms and Growth Medium. The microorganisms used in this study were cyanobacteria genus *Leptolyngbya* strain HS-16 and HS-36. *Leptolyngbya* HS-16 was isolated from Pancar Mountain hot spring, while *Leptolyngbya* HS-36 was isolated from Maribaya hot spring. Those strains were grown in Blue Green number 11 medium/ BG-11 (NIES 2007) with variations of pH value 6, 7, 8 and 9. The BG-11 medium were made as reported by Prihantini (2015).

Cyanobacteria Cultivation in BG-11 Medium. The first step of cyanobacteria cultivation was inoculated 30 mg biomass of each strain into 100 mL growth medium in 250 mL Erlenmeyer flask. Before inoculation of cyanobacteria into medium, the medium had been adjusted the variation of pH value into 6, 7, 8 and 9. The treatment of variation pH value was repeated twice in each strain. Those strain were incubated at temperature 35 °C.

Measurement the weight of wet biomass Leptolyngbya HS-16 and HS-36. Measurement of *Leptolyngbya* HS-16 and HS-36 biomass were done in 15 days with 11 times of sampling. Sixteen of sterile eppendorf tube 2 mL was measured at analytical measurement tool. Biomass of those strain were taken aseptically with sterile micropipet amount of 2 mL. Eppendorf tube with biomass of those strain inside were centrifuged with Biofuge Primo R machine in room temperature for 10 minute (in 10.000 rpm). The supernatant of those strain were taken out and wet biomass weight were measured with analytical measurement tool. The growth curves were made by comparrasion between wet biomass weight as the ordinate axis Y with observed time as absisca X. The growth curves were made by Microsoft Excel.

RESULTS

The study of growth *Leptolyngbya* HS-16 and HS-36 had been done. It took 15 days with 11 times of sampling. The result produced growth curve of *Leptolyngbya* HS-16 and HS-36. The growth curve showed the growth of both strains in the adaptation stage to the pH condition of the medium. It could be seen at the growth curve of *Leptolyngbya* HS-16 and HS-36, which each strain produce the growth curve unstable. The age of inoculum that used in this study were 5 months old.

Macroscopic observation of *Leptolyngbya* were observed. Color appereance of *Leptolyngbya* based on Faber Castle standard color. Color appereance of *Leptolyngbya* HS-16 was emerald green, while *Leptolyngbya* HS-36 was brown ochre at day 0 (t0). At day-15 (t15), the color appereance of *Leptolyngbya* HS-16 in pH 9 was changed from emerald green into apple green, while *Leptolyngbya* HS-36 in pH 6, 7 & 8 were changed from brown ochre into apple green.

The average of wet weight biomass of *Leptolyngbya* HS-16 and HS-36 are shown on Table 1. The growth curve of *Leptolyngbya* HS-16 shown on Figure 3, while the growth curve of *Leptolyngbya* HS-36 shown on Figure 4. The growth curve of *Leptolyngbya* HS-16 and HS-36 were made based on their wet weight of biomass.

After 15th day of incubation, both *Leptolyngbya* were able to grow on initial pH 6 medium, with wet weight 0.0295 g L^{-1} for HS-16 and 0.02905 g L^{-1} for HS-36. The growth curve of those strain in initial pH 6 were slightly rise at 2^{th} until 10^{th} day, and drasticaly increased at 13^{th} until 14^{th} day, but decreased at 15^{th} day. On the other medium with initial pH 7, *Leptolyngbya* HS-16 were able to grow and produced maximum amount of wet weight than other initial pH, but

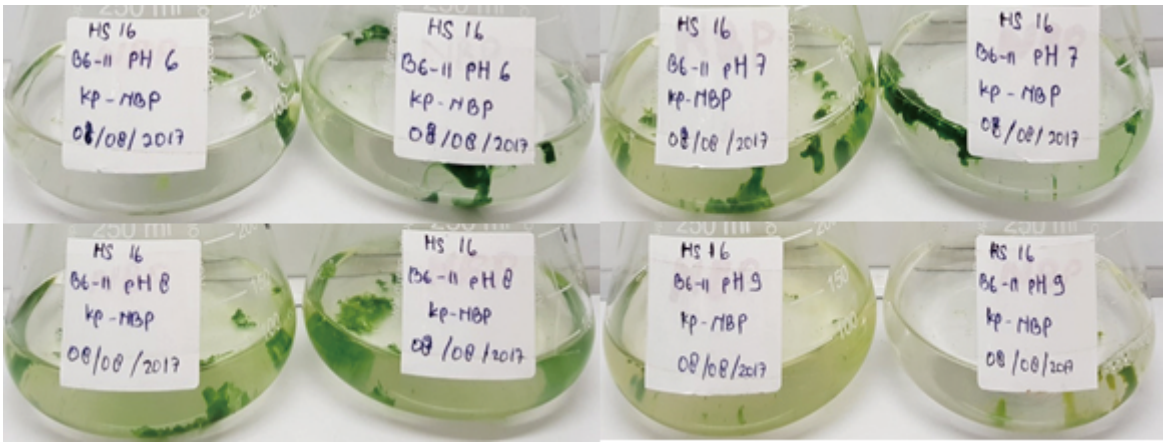

Fig 1 The color appereance of *Leptolyngbya* HS-16 at day-15 (t15).

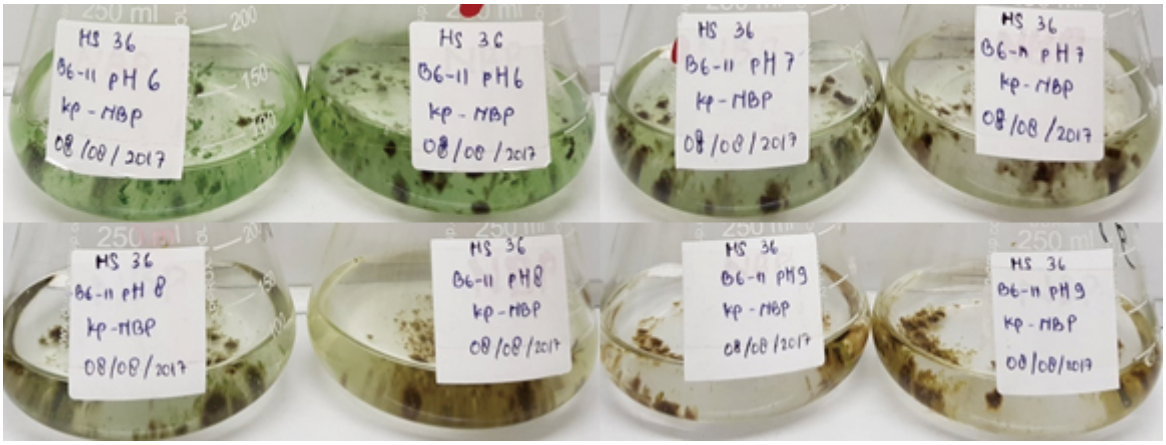

Fig 2 The color appereance of *Leptolyngbya* HS-36 at day-15 (t15).

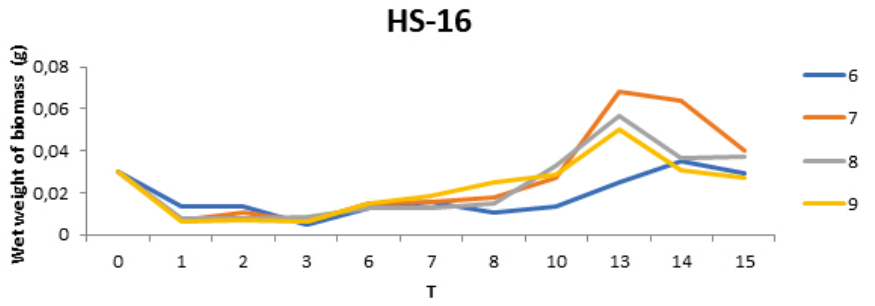

Fig 3 The growth curve of *Leptolyngbya* HS-16.

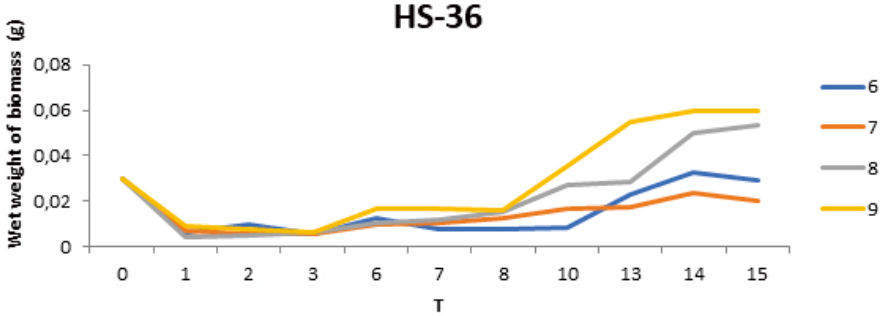

Fig 4 The growth curve of *Leptolyngbya* HS-36.

Table 1 The average of wet weight *Leptolyngbya* HS-16 and HS-36 (g L^{-1})

	Wet weight of biomass (g L^{-1})							
T	HS-16				HS-36			
	6	7	8	9	6	7	8	9
0	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
1	0.01375	0.00685	0.00755	0.00645	0.00615	0.0069	0.0046	0.00925
2	0.01345	0.01075	0.00755	0.00705	0.0101	0.00545	0.0052	0.00745
3	0.00445	0.00655	0.00835	0.0061	0.00555	0.0058	0.0064	0.00645
6	0.01305	0.0151	0.01245	0.01515	0.0129	0.01	0.01075	0.0166
7	0.01535	0.01555	0.0126	0.0188	0.0077	0.0108	0.01205	0.01705
8	0.0107	0.0179	0.01465	0.02485	0.00755	0.0127	0.01525	0.0163
10	0.0132	0.02695	0.033	0.0285	0.0086	0.01675	0.02685	0.03555
13	0.02525	0.0681	0.05635	0.05005	0.02285	0.01765	0.0284	0.0548
14	0.0348	0.06415	0.0362	0.031	0.0325	0.02345	0.04965	0.0596
15	0.0295	0.0404	0.03725	0.02735	0.02905	0.01995	0.05345	0.05995

Leptolyngbya HS-36 produced minimum amount of wet weight than other initial pH. The wet weight of *Leptolyngbya* HS-36 was 0.0404 g L^{-1} and *Leptolyngbya* HS-36 was 0.01995 g L^{-1}. The growth curve of *Leptolyngbya* HS-16 in initial pH 7 was decreased at 2th until 4th day then increased at 3th until 13th day and decreased at 14th until 15th day, while *Leptolyngbya* HS-36 was increased constantly at 1th until 14th day and decreased at 15th day. Wet weight of *Leptolyngbya* HS-16 and HS-36 in growth medium with initial pH 8 were 0.03725 g L^{-1} and 0.05345 g L^{-1}. Both of those strain well adapted in medium with initial growth pH 8, especially *Leptolyngbya* HS-36. *Leptolyngbya* HS-16 was increased at 1th until 13th day then slightly decreased at 14th until 15th day, while *Leptolyngbya* HS-36 was increased at 1th until 15th day. Wet weight of *Leptolyngbya* HS-16 and HS-36 in growth medium with initial pH 9 were 0,02735 g L^{-1} and 0,05995 g L^{-1}. *Leptolyngbya* HS-36 produced maximum amount of wet weight than other initial pH. *Leptolyngbya* HS-16 was decreased at 1th until 3th day then increased at 6th until 13th day and decreased at 14th until 15th day, while *Leptolyngbya* HS-36 was decreased at 1th until 3th day then increased at 6th day, but slightly decreased again at 7th until 8th day and increased again until 15th day.

DISCUSSION

Based on the result of this experiment, the color of *Leptolyngbya* HS-16 changed on growth medium with initial pH 9, and *Leptolyngbya* HS-36 changed on growth medium with initial pH 6, 7, and 8. It hapened because of their physiology adaptation mechanism in new environmental. The physiological adaptation caused the alteration phycobilin content (Muster *et al*. 1983). As we can see on the growth curve, both strains still in lag phase on growth medium with initial pH 6, 7, 8, and 9. It proven that the curve still in unstable stage. Lag phase sometimes could be the longest phase for some microorganisms because in this phase, microorganisms must adapt with new environmental conditions like new source of nutrient, pH and temperature (Hogg 2005).

Both of these strains were able to grow in initial growth pH 6 medium, but not as good as in alkaline condition. *Leptolyngbya* were often be found in neutral to alkaline condition (Madigan *et al*. 2015). *Leptolyngbya* HS-16 had the most average in growth medium with pH 7 than other pH. It could be *Leptolyngbya* HS-16 was already adapted in growth medium with pH 7, because *Leptolyngbya* HS-16 was isolated from Pancar mountain, which has pH 7 (Prihantini 2015). *Leptolyngbya* HS-16 was well adapted in medium growth with pH 7, which coud be grouped into neutrophile organisms (Madigan *et al*. 2015). *Leptolyngbya* HS-36 were well adapted in growth medium with initial pH 8 and 9, especially *Leptolyngbya* HS-36 had the most average in growth medium with initial pH 9. It happened because *Leptolyngbya* were often found in environmental with pH 8 (Olsson-francis *et al*. 2012). It could be also that

Leptolyngbya was alkalophile, which microorganisms that could live with pH 8 to 10 (Madigan *et al*. 2015). Those strains were decreased at 15th day of observation. It could be happened because the nutrients in the growth medium had been reduced. The nutrients in the growth medium used those strain for metabolisms to their growth (Madigan *et al*. 2015). Based on the observation of the 15th day and the discuccion that had been done, the best growth of *Leptolyngbya* HS-16 was on growth medium with initial pH 7, while the best growth of *Leptolyngbya* HS-36 was on growth medium with initial pH 9.

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