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The effect of temperature variation on the growth of *Leptolyngbya* (cyanobacteria) HS-16 and HS-36 to biomass weight in BG-11 medium

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ABSTRACT

Leptolyngbya is known to be used for human purposes, including for biofuel production. Therefore, it is important to study the physiology of these microorganisms. This study is expected to provide information on the temperature of growth ability of *Leptolyngbya* HS-16 and *Leptolyngbya* HS-36, so that storage conditions of culture space in subsequent research can be arranged to prevent these strains from dying or not growing well. In the utilization of cyanobacteria, a clear physiological characterization of the cyanobacteria is required. Temperature is one of the major factors affecting the growth of cyanobacteria. The growth differences between cyanobacteria strains *Leptolyngbya* HS-16 and *Leptolyngbya* HS-36 which were incubated in 20 °C, 35 °C, and 50 °C had been studied. Those strains were isolated from Gunung Pancar (*Leptolyngbya* HS-16) and Maribaya (*Leptolyngbya* HS-36) hot springs which located in West Java, Indonesia. The water temperature of habitat was 69 °C (Gunung Pancar) and 42 °C (Maribaya). Those strains were grown in batch culture methods for 21 days in BG-11 medium. This research aims to determine the best growth temperature of *Leptolyngbya* HS-16 and *Leptolyngbya* HS-36 based on the biomass weight of chlorophyll content. The result showed that the biomass weight and chlorophyll content in 35 °C of *Leptolyngbya* HS-16 and *Leptolyngbya* HS-36 were the highest. Both *Leptolyngbya* were likely thermotolerant cyanobacteria and had optimum cultured temperature 35 °C. There was no correlation between biomass weight and chlorophyll of *Leptolyngbya* HS-16 and *Leptolyngbya* HS-36. Further research should be focus on utilizing *Leptolyngbya* on various photobioreactor systems and biofuel development due their ability to growth optimally in that temperature.

1. Introduction

Cyanobacteria is a group of photosynthetic prokaryotic microorganism that can live in extreme environments (Sze, 1998). Cyanobacteria can live in extreme environments because they have ability to adapt to their environments, such as adaptation to high temperature environments. Cyanobacteria that can adapt to high temperature environments have thermostability associated with metabolic processes (Zhaoqi, 1994). Such thermostability allows some cyanobacteria to adapt in high temperature environments, such as in hot springs.

The hot springs of Gunung Pancar and Maribaya are examples of hot springs in West Java, Indonesia. Gunung Pancar hot spring has water temperature of 69 °C, while the water temperature at the Maribaya hot spring lower than Gunung Pancar Hot Spring. Water temperature of Maribaya hot spring is 42 °C. Only a few genera of Cyanobacteria can adapt to high temperature in the hot springs of Gunung Pancar and Maribaya, one of which comes from the genus *Leptolyngbya* (Prihantini, 2015). Prihantini (2015) succeeded in isolation and identifying the *Leptolyngbya* HS-16 isolated from the Gunung Pancar hot springs and *Leptolyngbya* HS-36 which were isolated from Maribaya hot springs. HS-16 and HS-36 are strain codes with HS stands for ‘Hot Spring’.

According to Komarek (2014), *Leptolyngbya* is a Cyanobacteria belonging to kingdom *Eubacteria*, phylum *Cyanobacteria*, classes *Cyanophyceae*, order *Synechococcales*, dan family *Leptolyngbyaceae* having certain characteristics. Characteristic of genus *Leptolyngbya* is thin filament with a thickness of 0.5–3.2 μm. Genus *Leptolyngbya* can generally grow optimally at 22 °C–40 °C, but there are some species that can grow at 70.1 °C, one of which is *Leptolyngbya tentaculiformis* (McGregor and Rasmussen, 2007).

Some cyanobacteria are known to be used as a source of chemicals

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that are useful for humans. *Leptolyngbya* can be used for biofuel production, because *Leptolyngbya* can produce lipids (Giddings and Newman, 2015). Beetul et al. (2014) observed the production of lipids produced by several types of microalgae, one of which was *Leptolyngbya*. In this study it was reported that *Leptolyngbya* produced lipids of 19.09% of total biomass. Meanwhile, *Leptolyngbya* HS-16 and HS-36 which are isolated *Leptolyngbya* strains from Indonesia (native to Indonesia, Indonesia Indigenous strain) are known to produce fatty acid (Prihantini et al., 2018) and pigment (Prihantini, 2015).

Temperature is one of the major factors affecting the growth of cyanobacteria (Fogg and Thake, 1987). Temperature may decrease or increase the rate of cell metabolism. So that storage conditions of culture space in subsequent research can be arranged to prevent these strains from dying or not growing well. In order to utilize *Leptolyngbya* strains, physiological characterization, especially growth temperature, is important to be known as the basis for further application research. This study will continue with the culture of cyanobacteria, especially strains of *Leptolyngbya* HS-16 and *Leptolyngbya* HS-36. The aim of the research is to know the best temperature of the growth of HS-16 and HS-36 *Leptolyngbya* grown at temperatures of 20 °C, 35 °C, and 50 °C. In addition, this study also wants to know the effect of temperature variations of 20 °C, 35 °C, and 50 °C on the weight of biomass and the chlorophyll content of *Leptolyngbya* HS-16 and HS-36.

2. Materials and methods

2.1. Microorganisms and medium

The cyanobacteria strains which used in this study are *Leptolyngbya* HS-16 and *Leptolyngbya* HS-36. *Leptolyngbya* HS-16 was isolated from Gunung Pancar Hot Spring (S06035.336′ dan E106055.453′) and *Leptolyngbya* HS-36 was isolated from Maribaya Hot Spring (S06′49.892′ dan E107′39.443′). Both hot springs are in West Java, Indonesia. Isolation of the two strains using the pipette method, and the selection of the use of both strains is based on rapid growth (fast-growing strains). Identification using morphological and molecular characters (Prihantini, 2015). Those strains were grown in BG-11 liquid culture medium. The Blue Green medium number 11 (BG-11) is medium which has a pH of 7.4. BG-11 medium contains macro nutrients and micro nutrients (NIES-collection, 2007). This medium is commonly used for cyanobacteria enrichment.

2.2. Molecular identification of Leptolyngbya strain HS-16 and HS-36

The strains used were identified using information obtained with 16S rRNA gene sequences. The alignment of sequences and phylogenetic trees revealed that the HS-16 and HS-36 strains could be identified as the genus *Leptolyngbya* (Fig. 1). The *Leptolyngbya* HS-16 strain has the same 98% (688/702) with *Leptolyngbya* sp. O-77 (AB668059). Meanwhile, the *Leptolyngbya* HS-36 strain has similarities around 97% (396/407) with *Leptolyngbya* sp. CR_35M (EF545619). *Leptolyngbya* sp. O-77 (AB668059) originates from Japan, while *Leptolyngbya* sp. CR_35M (EF545619) comes from geothermal springs from Costa Rica.

2.3. Making stock culture and working culture of Leptolyngbya strains

Culture preparation was done by preparing stock culture and working culture. Stock culture and working culture were made by inserting 180 mL of BG-11 medium and 20 mL of *Leptolyngbya* HS-16 and HS-36 culture into a 250 mL Erlenmeyer flask. Both cultures were grown in incubation cabinets at 35 °C. Making starter culture is also the same way as making stock culture and work culture. Starter cultures were done by entering each 20 mL working culture into an Erlenmeyer flask containing 180 mL BG-11 medium. Photoperiodicity of 12 h of light/12 h of dark was regulated by a timer.

2.4. Culturing of cyanobacteria starter and harvesting cultures

Culturing cyanobacteria starter is first step of the observation. The starter of biomass was grown for 28 days. Amount 60 mg of starter were inoculated into 200 mL of medium BG-11 in 250 mL Erlenmeyer flask. That step was done four times for each strain. Those cultures then incubated in three incubators (20 °C, 35 °C, and 50 °C), and with 12L/12D of photoperiodicity.

The selection of the three temperature variations based on the optimal growth temperature range of the genus *Leptolyngbya* ranged from 22 °C to 40 °C (McGregor and Rasmussen, 2007). The first variation of temperature (20 °C) was set as lower limit and the last variation of temperature (50 °C) was set as upper limit. Observations were done in 21 days and biomass weight was the parameter of the growth.

2.5. Macroscopic observation

Macroscopic observation of two strains of *Leptolyngbya* cultures were taken for 10 times at t_0, t_1, t_2, t_3, t_4, t_7, t_{10}, t_{14}, t_{17}, dan t_{21}. Photos were taken by the camera. The changing of culture color was identified based on the Faber Castell standard color.

2.6. Measuring biomass weight of Leptolyngbya

Measuring of biomass weight were done 10 times at t_0, t_1, t_2, t_3, t_4, t_7, t_{10}, t_{14}, t_{17}, dan t_{21}. Eppendorf tube 2 mL was measured at analytical measurement tool. Biomass of *Leptolyngbya* were taken into 2 mL Eppendorf tube then vortex for 10 min and centrifugated for 10 min with 10,000 rpm. The supernatant was taken out and biomass weight was measured. Growth curves were made by comparing the value of the biomass weight as the ordinate axis Y by biomass weight measuring time as absisca X. The growth curves could determine adaptation (lag) phase, exponential (log) phase, stationary phase, and death phase made by Microsoft Excel software. The normality statistic of Kolmogorov Smirnov and Friedman were used in this study to know is there any differences of between the biomass weight of those strains incubated in 20 °C, 35 °C, and 50 °C.

2.7. Measuring of chlorophyll content

Measuring of chlorophyll content were done in 10 times at t_0, t_1, t_2, t_3, t_4, t_7, t_{10}, t_{14}, t_{17}, dan t_{21}. The measurement of chlorophyll was done by extracting the pigment by spectrophotometric method using spectrophotometer UV vis Nanodrop 1000 and measured chlorophyll concentration at 645 nm and 663 nm wavelength. Based on Meeks (1974), calculating the content of chlorophyll *a* (mg. L^{-1}) is obtained by entering the absorbance value to the Arnon formula, which is the difference of 12.7 (D663nm) with 2.69 (D645nm).

2.8. Statistical analyses

Data from observations in the form of quantitative and qualitative data. Quantitative data in the form of temperature (°C), light intensity (lux), pH, cell size (μm), weight of biomass (mg. mL^{-1}) and chlorophyll content (mg. L^{-1}). Qualitative data in the form of morphological and color forms of *Leptolyngbya* HS-16 cells and *Leptolyngbya* HS-36. Heavy biomass data (mg. mL^{-1}) and chlorophyll content (mg. L-1) will be displayed in the form of tables and curves, while the morphological and color data form of *Leptolyngbya* HS-16 cells and *Leptolyngbya* HS-36 will be displayed in the form of microphotographs.

Data analysis begins with a normality test using the Kolmogorov Smirnov test. Data that is not normally distributed is tested using the Friedman test. The Friedman test was conducted to determine whether there was an effect of temperatures of 20 °C, 35 °C and 50 °C to the mean biomass of the *Leptolyngbya* HS-16 and *Leptolyngbya* HS-36 biomass.

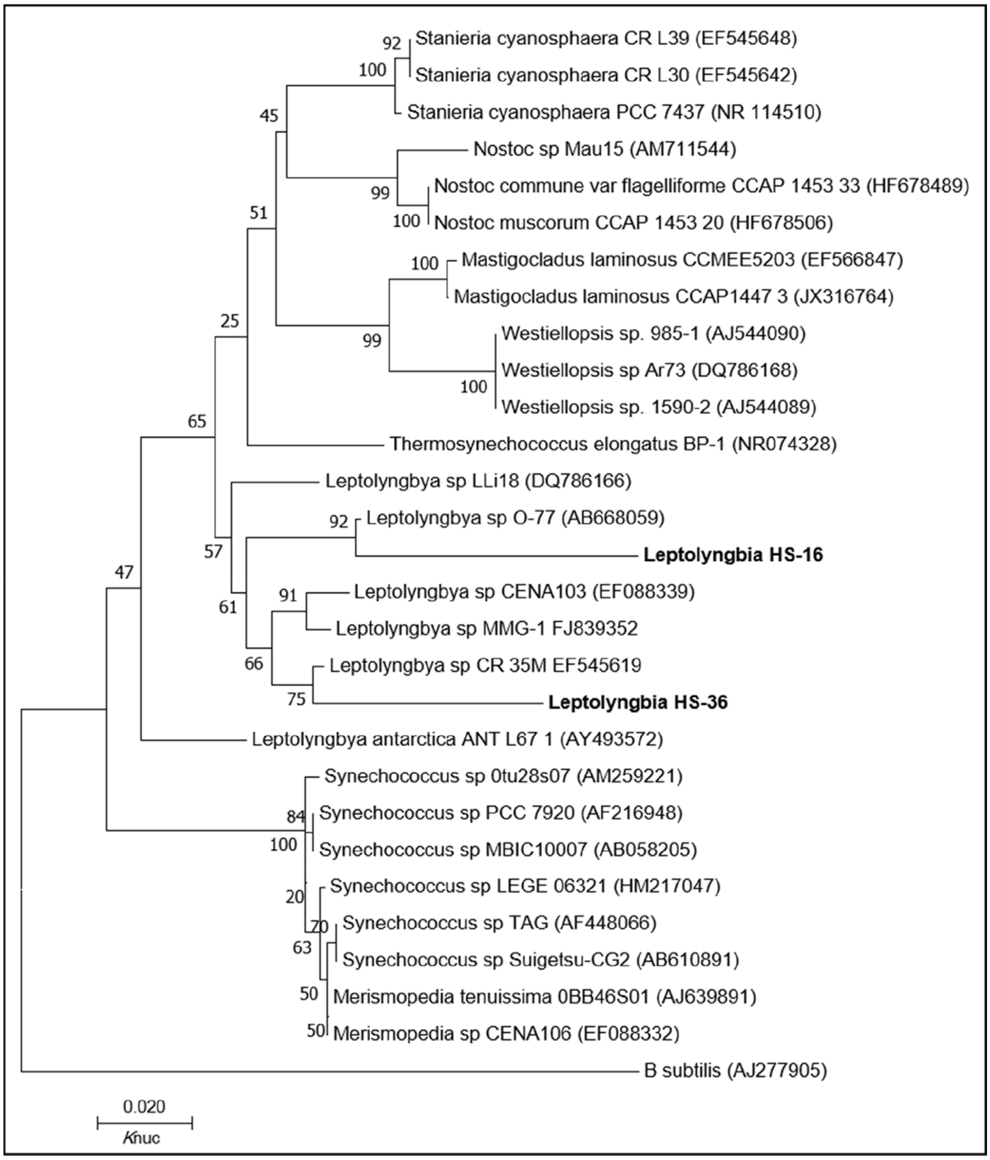

Fig. 1. The Phylogenetic tree of *Leptolyngbya* strains (HS-16 and HS-36), and other genera of cyanobacteria based on 16S rRNA gene sequences data. Tree was constructed by Neighbor-Joining method (Saitou and Nei, 1987). The GenBank accession numbers are indicated in parentheses. The numerals in the branches indicate the confidence level of bootstrap values based on 1000 replication (Felsenstein, 1985). *K*nuc, Kimura's parameter (Kimura, 1980). Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016).

The data obtained will be processed using Microsoft Excel and SPSS 16.00 computer software as a tool for making graphs and observation table results. Based on the data obtained, the analysis of the data on the growth of the growth curve, can determine the distribution of the phases of the growth of *Leptolyngbya* HS-16 and HS-36, namely the adaptation phase, log phase, stationary phase, and death phase. The growth curve is a graph of growth between the weight of the biomass (Y axis) and the time of observation (X axis). Meanwhile, the correlation between the biomass weight and chlorophyll content of the *Leptolyngbya* HS-16 and the *Leptolyngbya* HS-36 were tested using the Spearman test statistic.

3. Results and discussion

3.1. Macroscopic and microscopic of Leptolyngbya HS-16 and HS-36 cultures

The macroscopic color of starter culture of the *Leptolyngbya* HS-16 and HS-36 can be seen in Fig. 2. Based on the Faber Castell color table

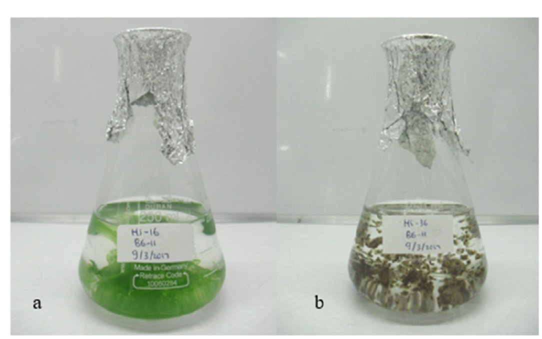

Fig. 2. The starter culture of the *Leptolyngbya* HS-16 and HS-36. a, Starter culture of *Leptolyngbya* HS-16; b, Starter culture of *Leptolyngbya* HS-36.

(Digital, 2008), there are macroscopic color differences in the starter culture of *Leptolyngbya* HS-16 and HS-36. The macroscopic color of the starter culture of the *Leptolyngbya* HS-16, namely Emerald Green. The macroscopic color of the starter culture of the *Leptolyngbya* HS-36,

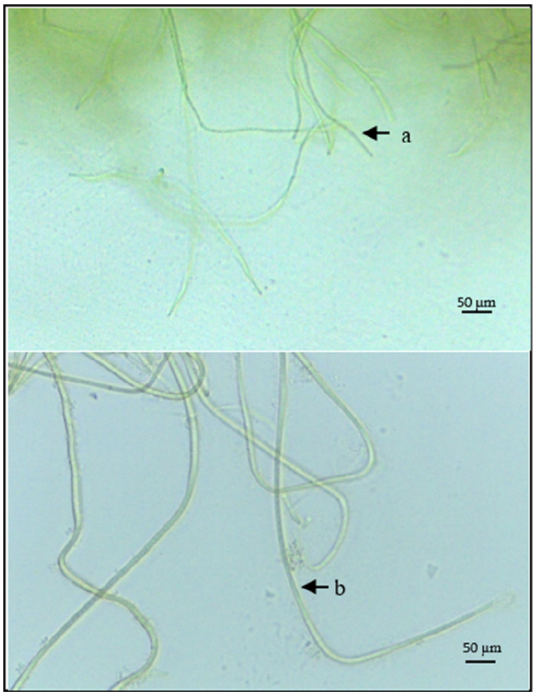

Fig. 3. Microscopic appearance of *Leptolyngbya* HS-16 (a) and *Leptolyngbya* HS-36 (b).

namely Brown Ochre.

Microscopic observations of the *Leptolyngbya* HS-16 and *Leptolyngbya* HS-36 cultures can be seen in Fig. 3. The morphological characters of *Leptolyngbya* HS-16 and HS-36 are microscopically shaped long filaments with septa that cannot be clearly seen. The *Leptolyngbya* genus is a filamentous long cyanobacteria (Whitton and Brook, 2002). The microscopic color of *Leptolyngbya* HS-16 and *Leptolyngbya* HS-36, which are green.

3.2. Macroscopic observation

The macroscopic observations of *Leptolyngbya* HS-16 and HS-36 cultures were done on t_0, t_1, t_2, t_3, t_4, t_7, t_{10}, t_{14}, t_{17}, and t_{21}. The culture color of *Leptolyngbya* HS-16 which were incubated in 20 °C at day-17 (t_{17}) changed into Juniper Green and slightly yellowish. The color of culture of *Leptolyngbya* HS-16 which were incubated in 35 °C and 50 °C were not change, which were still Emerald Green. Macroscopic colors on *Leptolyngbya* HS-36 grown at 50 °C changed to be Gold Ochre. The color of culture of *Leptolyngbya* HS-36 which grown at 20 °C and 35 °C unchanged, which were still brown ochre. Macroscopic appearance of *Leptolyngbya* HS-16 and HS-36 cultures can be seen in Fig. 4.

The color change was likely due to the chlorophyll degradation process. In the chlorophyll degradation mechanism, there is a reaction of phaeophytin formation. Phaeophytin is a form of chlorophyll that loses the Mg^{2+}ion. The Mg^{2+}ion in the center of the molecule will be released and replaced by a hydrogen ion, so the color expressed becomes yellowish. The heat stress accelerates the reaction of phaeophytin formation because heat can degrade proteins. The proteins that protect chlorophyll are denatured so that chlorophyll is in free form. Chlorophyll in free form will be easily attacked by acid, so that the Mg^{2+} ion will be replaced by hydrogen ion (Brandis et al., 2006).

3.3. Leptolyngbya biomass weight calculation

The biomass weight calculation was performed 10 times at t_0, t_1, t_2, t_3, t_4, t_7, t_{10}, t_{14}, t_{17}, and t_{21} at the same time of sampling. Biomass weight calculations were performed to support macroscopic and microscopic data of *Leptolyngbya* HS-16 and HS-36. Starter cultures were used as a 28-day-old culture of *Leptolyngbya* HS-16 starter biomass, 18.5 mg/mL and *Leptolyngbya* HS-36 starter biomass, 12.5 mg/mL. The inoculum cultures were derived from starter cultures grown at 35 °C in BG-11 medium with pH 7. The amount of inoculum put into the test culture was 60 mg in 200 mL of medium.

The mean of biomass weight between *Leptolyngbya* HS-16 and HS-36 grown at 20 °C, 35 °C, and 50 °C was statistically tested to see if there was a difference between the two strains. The statistical test began with the normality test using the Kolmogorov Smirnov test. The normality test showed that the mean biomass weight of *Leptolyngbya* HS-16 and HS-36 data were not normally distributed. The result of Friedman test was no significant difference between the mean weight of *Leptolyngbya* HS-16 and *Leptolyngbya* HS-36 biomass during peak which were grown at 20 °C, 35 °C and 50 °C respectively. However, qualitatively data showed that there were differences of mean biomass weight of *Leptolyngbya* HS-16 and HS-36 grown at 20 °C, 35 °C and 50 °C (see Table 1 and Fig. 5).

The subsequent qualitative data showed the difference of *Leptolyngbya* HS-16 biomass weight grown at 20 °C, 35 °C, and 50 °C. The weight of *Leptolyngbya* HS-16 biomass grown at 50 °C was less than *Leptolyngbya* HS-16 grown at 35 °C. The smallest biomass weight was found in *Leptolyngbya* HS-16 grown at 20 °C. The high *Leptolyngbya* HS-16 lines graph and the increased rate of biomass weight indicate that *Leptolyngbya* HS-16 could grow better at 35 °C, rather than 20 °C and 50 °C.

Fig. 5 also shows the weight differences of *Leptolyngbya* HS-36 biomass grown at 20 °C, 35 °C, and 50 °C. Based on the figure, the highest *Leptolyngbya* biomass weight was at 35 °C. The *Leptolyngbya* HS-36 biomass weight grown at 20 °C was lower than *Leptolyngbya* HS-36 grown at 35 °C. The smallest biomass weight was found in *Leptolyngbya* HS-36 grown at 50 °C.

The biomass weight of *Leptolyngbya* HS-36 grown at 20 °C, 35 °C, and 50 °C can be known. *Leptolyngbya* HS-36 grown at 20 °C began to experience a significant increase in biomass weight starting at t_7 to t_{10}. This contrasted with *Leptolyngbya* HS-36 grown at 35 °C which increase in biomass weight at t_4 to t_{14}. The subsequent differences were seen in *Leptolyngbya* HS-36 grown at 50 °C which experienced an increase in biomass weight at t_4—t_{10}, but subsequently decreased drastically to t_{21}. Fig. 1 shows that *Leptolyngbya* HS-36 had a better biomass weight when grown at 35 °C incubation temperature. This is probably due to *Leptolyngbya* HS-36 was mesophilic and it could be thermotolerant. Thermotolerant mesophilic microorganisms can grow at a temperature of 20–45 °C (Wiley et al., 2008). The original Habitat of *Leptolyngbya* HS-36, which comes from Maribaya hot spring with a temperature of 42 °C.

3.4. Observation chlorophyll content of Leptolyngbya HS-16 and HS-36

The measurement of *Leptolyngbya* HS-16 and HS-36 chlorophyll content was performed 10 times, i.e. at t_0, t_1, t_2, t_3, t_4, t_7, t_{10}, t_{14}, t_{17}, and t_{21}. Chlorophyll content measurements were performed to support macroscopic, and biomass weight data. The content of chlorophyll on day 0 (t_0) to the day one (t_1) tend to decrease in *Leptolyngbya* HS-16 and HS-36 at 20 °C, 35 °C, and 50 °C (Fig. 6 and Table 2). On the first day (t_1) to the second day (t_2), the *Leptolyngbya* HS-16 and HS-36 chlorophyll content at 20 °C, 35 °C and 50 °C temperature treatments increased again. Fig. 6 shows the highest differences in chlorophyll content in HS-16 *Leptolyngbya* at temperatures of 20 °C, 35 °C, and 50 °C. The highest chlorophyll content in *Leptolyngbya* HS-16 at temperatures of 20 °C and 35 °C is on the 14th day (t_{14}). The *Leptolyngbya* HS-16 grown at 50 °C has the highest chlorophyll content on day 17 (t_{17}). Furthermore, Fig. 6 also shows the differences in the highest *Leptolyngbya* HS-36 chlorophyll content grown at temperatures of 20 °C, 35 °C, and 50 °C. The highest chlorophyll content of *Leptolyngbya* HS-36 grown at a temperature of 20 °C was on day 0 (t_0). The difference in chlorophyll content occurs mostly in *Leptolyngbya* HS-36 at a

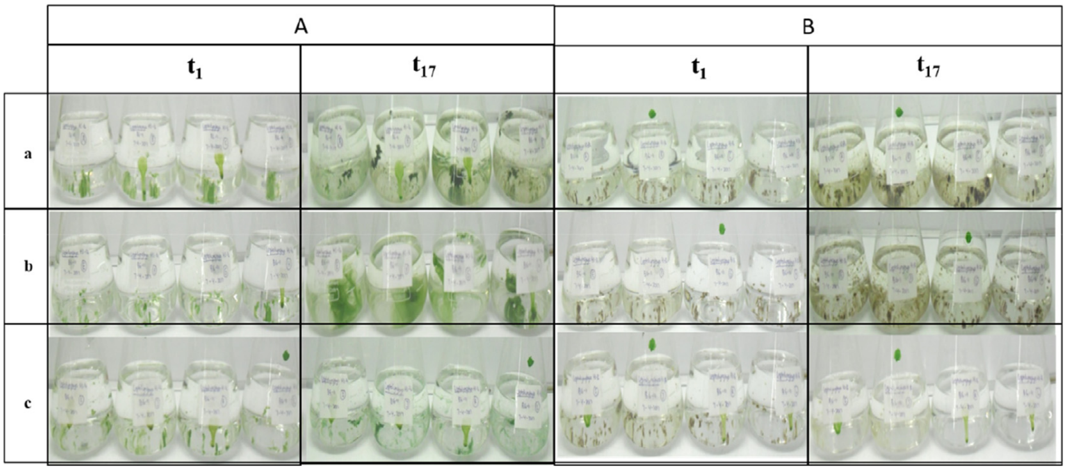

Fig. 4. Macroscopic appearance of *Leptolyngbya* HS-16 and HS-36 cultures. t1, Observation on day-1; t17, Observation on day-17; a, temperature of 20 °C; b, temperature of 35 °C; c, temperature of 50 °C.

Table 1
The average weight of biomass *Leptolyngbya* HS-16 and HS-36 with 4 replications.

Observation Time (Days)	Average of Biomass Weight (mg/mL)					
	Temperature 20 °C		Temperature 35 °C		Temperature 50 °C	
	HS-16	HS-36	HS-16	HS-36	HS-16	HS-36
t_0	0,300 ± 0,000	0,300 ± 0,000	0,300 ± 0,000	0,300 ± 0,000	0,300 ± 0,000	0,300 ± 0,000
t_1	0,125 ± 0,050	0,275 ± 0,170	0,150 ± 0,100	0,200 ± 0,141	0,175 ± 0,170	0,175 ± 0,150
t_2	0,325 ± 0,150	0,450 ± 0,191	0,425 ± 0,236	0,550 ± 0,250	0,325 ± 0,121	0,275 ± 0,287
t_3	0,575 ± 0,189	0,825 ± 0,330	0,125 ± 0,044	0,325 ± 0,030	0,175 ± 0,150	0,825 ± 0,531
t_4	0,250 ± 0,100	0,275 ± 0,121	0,725 ± 0,227	1,525 ± 0,665	0,450 ± 0,210	0,400 ± 0,244
t_7	1,550 ± 0,793	0,300 ± 0,141	2,125 ± 0,793	7,750 ± 3,413	0,275 ± 0,187	1,600 ± 0,571
t_{10}	2,450 ± 0,500	5,700 ± 0,826	4,775 ± 1,303	9,350 ± 1,693	3,700 ± 1,295	**4,825 ± 1,510**
t_{14}	**2,675 ± 1,111**	4,500 ± 2,796	**6,100 ± 2,315**	**12,625 ± 2,657**	3,250 ± 1,795	3,350 ± 1,369
t_{17}	1,950 ± 0,405	**5,925 ± 1,763**	4,825 ± 1,721	10,750 ± 4,199	**3,875 ± 1,813**	1,500 ± 0,605
t_{21}	1,600 ± 0,860	4,950 ± 1,590	4,150 ± 1,740	9,125 ± 0,928	2,925 ± 0,785	0,275 ± 0,170

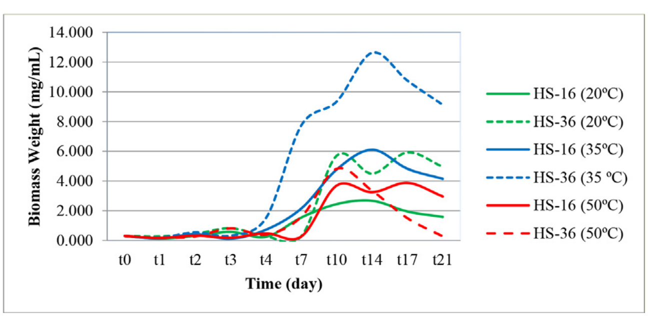

Fig. 5. Observation of biomass weight of *Leptolyngbya* HS-16 and *Leptolyngbya* HS-36.

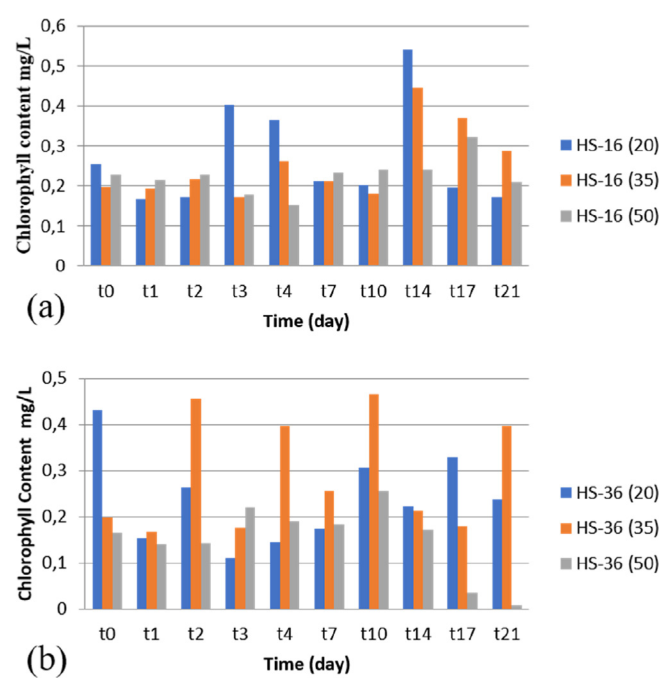

Fig. 6. Chlorophyll content of strain of *Leptolyngbya* HS-16 (a) and strain of *Leptolyngbya* HS-36 (b).

temperature of 35 °C, which is on the 10th day (t_{10}). The *Leptolyngbya* HS-36 grown at 50 °C has the highest chlorophyll content on the 10th day (t_{10}).

3.5. Correlation between the weight of biomass and the chlorophyll content of Leptolyngbya HS-16 and HS-36

Spearman test results on the content of *Leptolyngbya* HS-16 and HS-36 chlorophyll showed no correlation between biomass weight and *Leptolyngbya* HS-16 and *Leptolyngbya* HS-36 chlorophyll content grown at 20 °C, 35 °C and 50 °C. This is in accordance with the literature which states that the content of chlorophyll *a* has no relationship with the biomass weight of microalgae culture. Chlorophyll is not a reactant

Table 2
The mean chlorophyll content of *Leptolyngbya* HS-16 and HS-36.

Observation Time (Days)	Average of Biomass Weight (mg/mL)					
	Temperature 20 °C		Temperature 35 °C		Temperature 50 °C	
	HS-16	HS-36	HS-16	HS-36	HS-16	HS-36
t_0	0,254 ± 0,131	**0,430 ± 0,121**	0,197 ± 0,074	0,197 ± 0,036	0,229 ± 0,093	0,165 ± 0,052
t_1	0,166 ± 0,052	0,152 ± 0,012	0,193 ± 0,089	0,167 ± 0,021	0,213 ± 0,153	0,141 ± 0,079
t_2	0,171 ± 0,014	0,264 ± 0,018	0,216 ± 0,119	0,213 ± 0,121	0,227 ± 0,182	0,143 ± 0,032
t_3	0,203 ± 0,186	0,110 ± 0,038	0,172 ± 0,025	0,176 ± 0,054	0,178 ± 0,051	0,220 ± 0,174
t_4	0,195 ± 0,045	0,145 ± 0,026	0,179 ± 0,015	0,178 ± 0,032	0,151 ± 0,012	0,189 ± 0,063
t_7	0,211 ± 0,080	0,173 ± 0,015	0,211 ± 0,187	0,255 ± 0,083	0,234 ± 0,067	0,184 ± 0,026
t_{10}	0,402 ± 0,143	0,305 ± 0,193	0,261 ± 0,069	**0,465 ± 0,227**	0,239 ± 0,131	**0,255 ± 0,180**
t_{14}	**0,540 ± 0,212**	0,222 ± 0,117	**0,445 ± 0,213**	0,455 ± 0,294	0,240 ± 0,094	0,171 ± 0,072
t_{17}	0,366 ± 0,133	0,328 ± 0,148	0,369 ± 0,131	0,396 ± 0,165	**0,321 ± 0,118**	0,034 ± 0,008
t_{21}	0,171 ± 0,014	0,237 ± 0,098	0,286 ± 0,073	0,396 ± 0,145	0,209 ± 0,130	0,009 ± 0,000

agent in the process of photosynthesis, but as a biocatalyst. It makes chlorophyll likely to have no relationship to the weight of biomass. Chlorophyll is an important component of photosynthesis. As a biocatalyst agent, the presence of chlorophyll is essential for the process of photosynthesis, but the process is not influenced by the concentration of chlorophyll in microalgae cells (Ramaraj et al., 2013).

4. Conclusion

The results showed that *Leptolyngbya* HS-16 and *Leptolyngbya* HS-36 can grow well at 35 °C. *Leptolyngbya* HS-16 and *Leptolyngbya* HS-36 produce maximum amount of biomass 6,100 ± 2,315 mg/mL and 12,625 ± 2,657 respectively on that temperature. Both strains have the potential to be used as biological agents to multiply biofuel feedstock. This is caused by the growing temperature in accordance with the photobioreactor temperature range that is exposed to the tropical temperature in Indonesia (22–38 °C), and both strains can grow in that range of temperature. Further research can focus on utilizing *Leptolyngbya* on various photobioreactor systems and biofuel development.

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