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Title : **The effect of colchicine on the size and bioactive compound of microalgae
*Spirulina platensis***

We are pleased to inform you that your paper based on your full paper submission has been accepted by Scientific committee of the ASEAN-FEN, IFS 2017 to be published in IOP Conference Series (Scopus indexed).

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Sincerely,

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The Chief of Local Committee, ASEAN-FEN – IFS 2017

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The Effect of Colchicine on the Size and Bioactive Compound of Microalgae *Spirulina platensis*

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Abstract. Polyploidy is one of the techniques used to increase the genetic variant and once used as a breeding method of plant. Generally, colchicine is one of the chemicals which used to produce polyploid organisms, such as plant. This study aimed to determine the effect of colchicine on the size and phycocyanin content of *Spirulina platensis*. Research was used six treatments of colchicine concentration with three replications. *S. platensis* were immersed in the colchicine solution for 12 hours and were observed for 5 days culture. This research was showed that colchicine concentration of 0.1% were resulted highest diameter of *S. platensis* (12.57 μm) while most phycocyanin content resulted by concentration treatment of 0.025% (0.091 mg/ml).

Keywords: colchicine, diameter, phycocyanin content, *Spirulina platensis*

Introduction

Microalgae has been developed to purpose research and technology. The advantages in development of microalgae are faster growth and high fat and protein contents (Sheehan *et al.* 1998). *S. platensis* is microalgae with complete and high proteins content, so that *S. platensis* has merit as a source of protein (Amanatin and Nurhidayati 2013). *S. platensis* has a very small form and the thread is a series of cylindrical trichomes cell with thin cell wall diameter of 1-12 μm (Hariyati 2008). The high phycocyanin content in this microalgae causing the color tends to blue green. *S. platensis* trichomes have a spiral structure with filaments are mortal and do not have heterosit (Kabede and Ahlgren 1996). Highly demand of *S. plantesis* was highly impact on the need to improve their quality. One of the alternatives to improve the *S. plantesis* quality is made polyploidy. Polyploidy was used to increase the genetic variant and once used as a method of plant breeding (Dinarti *et al.* 2006). Polyploidy can be done using colchicine.

Chahal and Gosal (2002) state that colchicine is one of the chemicals which when given to plants can lead to polyploid individuals. A common trait displayed by polyploid plants are plants into larger, plant parts such as roots, stems, leaves, the flower and the fruit becomes larger. Highly colchicine concentration and soaking time are not yet result polyploid individuals (Sofia 2007). Most studies of polyploidy use colchicine treatments have been conducted in plants, such as onion (Suminah *et al*

2002), water girlfriend and soybean (Sofia 2007), while in microalgae, the effects of colchicine treatment are not yet know. This study was determined effect of colchicine treatment on diameter and phyocyanin content of *S. platensis*.

Materials and Methods

This research was conducted at Laboratory of Fisheries Education, Faculty of Fisheries and Marine, Universitas Airlangga Surabaya.

Culture Preparation of *S. platensis*

The equipments and medium were used to *S. platensis* culture prior to sterilized by autoclave according by Ekawati (2005) and Purnamawati *et al* (2013). *S. platensis* that used to culture were collected from Brackishwater Aquaculture Development Center (BADC) Situbondo. Walne fertilizer with concentration of 0.1 % (v/v) were used to improve the population growth of *S. platensis* while initial stock density of *S. platensis* are 1×10^4 cells/mL according calculation of the plankton amount by Satyantini (2008).

$$V1 = \frac{N2 \times V2}{N1}$$

Note:

V1 = volume of seed for initial stocking (ml)

N1 = density of plankton seeds cells / ml

V2 = volume of the desired culture media (ml)

N2 = density of plankton desired seed cells / ml

Calculation of Density *S. platensis*

Population growth of *S. platensis* was observed every day until five days of culture by accounting density according by Ochthreeani *et al* (2014).

$$\text{Phytoplankton density (cells / ml)} = \frac{na+nb+nc+nd+ne}{5 \times 4 \times 10^{-6}}$$

Note:

na, nb, nc, nd, ne = number of cells of *S. platensis* in box a, b, c, d, e

5 = number of boxes counted

4×10^{-6} = area of the small box (a, b, c, d or e)

Treatment of Colchicine Solution

Dose of colchicine were used 0.01, 0.025, 0.05, 0.075 and 0.1 % (w/v) and one treatment without colchicine solution as control. Each treatments were replicated three times. Start of colchicine solution treatments were done 90 minutes after the initial culture of *S. platensis*. Immersion *S. platensis* in the colchicine solution was conducted for 12 hours.

Measurement of *S. platensis* Size

Diameter of *S. platensis* was measured every day using a microscope camera completed otomatically opticlabb and imageraster application program on the computer.

Measurement of *S. platensis* Phycocyanin Content

Phycocyanin extract of *S. platensis* was modified according by Lorenz (1998) method. Acetic acid solution (pH 7) was add on the sample *S. platensis* ratio of 1:5 (v/w). Then, acetic acid and *S. platensis*

solution were shaken using a vortex to mix all. The samples were stored for 24 hours. The mixture is shaken and centrifuged to separate phycocyanin from biomass. *Spirulina* Centrifugation done with a minimum speed of 3500 rpm for 5 minutes. After the extraction is tested on a spectrophotometer with a wavelength of 652 and 620 using the equation 1 (Bennett and Bogorad 1973):

$$CPC = \frac{(OD_{620} - 0.474OD_{652})}{5.34}$$

Result and Discussion

Diameter *Spirulina platensis*

Based on observations in diameter *S. platensis* conducted during the five days of culture, the results obtained during the culture was tested using the results of analysis of variance (ANOVA) were conducted from the first day until the fifth day. Results of analysis of variance (ANOVA) on day 1 to day 5 were showed that the dose of colchicine have a real influence on the diameter difference *S. platensis*, as well as showed Table 1. The statistical test is then followed by a test using Tuckey test. Based on Tuckey test was showed that the highest diameter in 0.1 concentration on day 4 of 12,19 μm and day 5 of 12,57 μm , which significantly different compared with other concentrations and control. The smallest diameter in control on day 1 and 2 of 7.91 and 8,23 μm , respectively also the smallest diameter in 0.01 concentration on day 1 and 2 of 8,45 and 9,34 μm , respectively. This was caused the doses administered easily absorbed *S. platensis*. Sofia (2007) states that colchicine concentration and dipping duration is not yet right that would not result polyploid individuals with nature.

Table 1. Diameter of colchicine-immersed *S. platensis* in different concentrations

Day	Concentration of Colchicine (%)					
	0 (Control)	0.01	0.025	0.05	0.075	0.1
1	7.91 ^a ±0.02	8.44 ^b ±0.02	8.93 ^c ±0.01	9.34 ^d ±0.01	9.59 ^e ±0.01	11.25 ^f ±0.01
2	8.23 ^a ±0.21	9.33 ^b ±0.02	9.83 ^c ±0.01	10.02 ^d ±0.03	11.18 ^e ±0.01	11.43 ^f ±0.06
3	9.10 ^a ±0.02	9.37 ^b ±0.01	11.28 ^c ±0.02	11.67 ^d ±0.01	11.72 ^d ±0.01	12.20 ^e ±0.01
4	9.59 ^a ±0.02	10.50 ^b ±0.02	11.41 ^c ±0.02	11.98 ^d ±0.01	12.09 ^e ±0.01	12.19 ^f ±0.01
5	9.70 ^a ±0.07	10.54 ^b ±0.01	11.65 ^c ±0.01	12.17 ^d ±0.02	12.46 ^e ±0.01	12.47 ^e ±0.02

Note: Data represent as means±SD. Different superscript in the same row indicates significant differences (P<0.05).

S. platensis has four phases, namely the growth of the adaptation phase, exponential phase, stationary phase and death phase. Adaptation phase or resting phase in this study consisted of all treatments, this phase is shown on day 1 in all treatments. The second is the exponential phase begins on day 2 at all treatments, this phase is the phase of the start of cell division of *S. platensis*. The third phase is the exponential phase is characterized by increasing cell density but the rate of decline.

The fourth phase is a stationary phase characterized by increased cell numbers slowly although the number of living cells remain. Each treatment in this phase have the same stationary phase, all treatments stationary phase begins on the third day. The last phase is the phase that is marked increase the cell death number and decrease the density of *S. platensis*, this phase occurs after the stationary phase.

The content of phycocyanin *S. platensis*

Phycocyanin is a protein compound that belongs to the group phycobilliprotein like allophycocyanin and phycoeritrin. The whole phycobilliprotein group is insoluble in water and form a compound

attached to the phycobilosome thylakoid membrane. Phycocyanin is the main photosynthetic pigment in *S. platensis* in addition to its role as a store of reserves of nitrogen and amino acids (Arlyza 2005).

Based on the results of this study, the phycocyanin content was found in *S. platensis* after administration of the highest colchicine with a dose of 0.025%, the control and concentration of 0.1% were showed no difference too much, and the lowest for the phycocyanin content of 0.1% concentration with the highest dose that is 0.1%. This is consistent with the statement of Sofia (2007) that administration of colchicine with improper concentration can create a failure in plant breeding. The content of phycocyanin in *S. platensis* can be seen in Table 2.

Table 2. Average content of phycocyanin *S. platensis*

Concentration of Colchicine (%)	Content of Phycocyanin
0 (Control)	0,034 ^a ± 0,00
0.01	0,081 ^d ± 0,00
0.025	0,091 ^e ± 0,00
0.05	0,071 ^c ± 0,00
0.075	0,058 ^b ± 0,00
0.1	0,033 ^a ± 0,00

Note: Data represent as means±SD. Different superscript in the same row indicates significant differences (P<0.05).

Water Quality

Factors affecting the diameter of *S. platensis* was composed of nutrient availability and environmental. Environmental factors in the culture of *S. platensis* includes water quality parameters consisting of temperature, salinity, pH and light range. Water quality during the research was showed normal range to growth and life of *S. platensis*, i.e. temperature of 28-29°C, salinity of 30 ppt, pH of 8.4-8.7 and light of 2368-2034 lux.

Conclusion

Based on the research that the administration of colchicine with different doses were affect the diameter and content of phycocyanin *S. platensis* highest dose of 0.1% produce the highest diameter *S. platensis* 12,57 µm and phycocyanin content high value determined at a dose of 0.025% is as much as 0.91 mg/ml. Further study about the effect of colchicine on phytoplankton as aquaculture development efforts in Indonesia is necessary.

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