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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. Colchicine is one of the chemical which apply to produce polyploid organisms, such as plant. This study aimed to determine the effect of colchicine on the size and phycocyanin content of *Spirulinaplatensis*.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 highphycocyanin content obtained by treatment of 0.025 % (0.091 mg/ml).

1. Introduction

Microalgae have been developed for the purpose of research and technology. The development of microalgae has advantages in terms of fast growth and high fat and protein content [1]. *S. platensis*are microalgae with complete and high protein content [2]. *S. platensis* are small in size, and their threads consist of a series of cylindrical cells of trichomes with thin cell wall diameter of 1-12 μm [3]. The high phycocyanin content in these microalgae causes blue-green color. *S. platensis*'strichomes have a spiral structure with filaments, but they have no heterocyst [4]. High demand for *S. plantesis*greatly impacts on the need for the improvement of their quality. One of the alternative to improve *S. plantesis*'squality is changing them into polyploid. Polyploidy has been used to increase genetic variance [5]. Polyploidy can be obtained by using colchicine.

Colchicine, chemicals when administered to plants, can resulted in polyploid individuals. Common traits displayed by polyploid plants are bigger size and larger parts such as roots, stems, leaves, flower and fruit [6]. High colchicine concentration and soaking time are not sufficient for producing polyploid individuals [7]. Most studies on polyploidy have been conducted on plants, such as onion [8], garden balsam and soybean, while on microalgae, the effects of colchicine treatment are yet to be known. This study was aimed to investigate the effect of colchicine treatment on the diameter and phycocyanin content of *S. platensis*.

2. Methodology

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

2.1. Preparation of S. platensis culture

Some instruments and media were used for *S. platensis* culture prior to sterilization using autoclave [9,10]. The *S. platensis* used for culture were collected from Brackishwater Aquaculture Development Center (BADC) Situbondo. Walne fertilizer with a concentration of 0.1 % (v/v) was used to improve the growth of *S. platensis* population. The initial stock density of *S. platensis* was 1×10^4 cells/mL.Calculation of the cell according to Satyantini [11].

$$V1 = \frac{N2 \times V2}{N1} \quad (1)$$

Note:
V1 = volume of seed for initial stocking (ml)
N1 = density of plankton seeds (cells / ml)
V2 = volume of desired culture media (ml)
N2 = density of desired plankton seed (cells / ml)

2.2. Calculation of S. platensis Density

The population growth of *S. platensis* was observed every day in five days of culture by calculating the density according to Octhreeani [12].

$$\text{Phytoplankton density (cells / mL)} = \frac{na+nb+nc+nd+ne}{5 \times 4 \times 10^{-6}} \quad (2)$$

Note:
na, nb, nc, nd, ne = number of cells *of S. platensis* in box a, b, c, d, e
5 = number of boxes counted
$4x10^{-6}$ = area of small box (a, b, c, d or e)

2.3. Treatment of Colchicine Solution

The doses of colchicine used were 0.01, 0.025, 0.05, 0.075 and 0.1% (w/v), and one treatment without colchicine solution served as control. Each treatment was triplicates. The colchicine solution treatments were administered 90 minutes after the initial culture of *S. platensis*. The immersion of *S. platensis* in the colchicine solution was conducted for 12 hours.

2.4. Measurement of S. platensis's Size

The diameter of *S. platensis*was measured every day using a microscope camera completed with OpticLab and ImageRastersoftware on the computer.

2.5. Measurement of S. platensis's Phycocyanin Content

The phycocyanin extract of *S. platensis* was modified according to Lorenz [13] method. An acetic acid solution (pH 7) was added to the *S. platensis* sample at aratio of 1:5 (v/w). Then, the mixture of acetic acid solution and *S. platensis* was shaken using a vortex. The sample was stored for 24 hours. The mixture was shaken and centrifuged to separate phycocyanin from biomass. The centrifugation was conducted at a minimum speed of 3.500 rpm for 5 minutes. Afterwards, the extraction was tested on a spectrophotometer at wavelengths of 652 and 620 using equation 1 [14]:

$$\text{CPC} = \frac{(OD_{620} - 0.474 OD_{652})}{5.34} \quad (3)$$

3. Result and Discussion

3.1. Diameter Spirulina platensis

Based on the observations of the diameter of *S. platensis* from day 1 to day 5 showed that the dose of colchicine had a significant effect on the diameter of *S.platensis,* which is presented in table 1. The

data was analysed using analysis of varians and Tuckey test. The Tuckey test results showed that the largest diameter observed at a concentration of 0.1. The smallest diameters of control observed on day 1 and 2 were 7.91 and 8.23 μm, respectively. In addition, the smallest diameters observed at a concentration of 0.01 on day 1 and 2 were 8.45 and 9.34 μm, respectively. This happened because the doses administered were easily absorbed by *S. platensis.* The colchicine concentration and the dipping duration were not appropriate, thus polyploid individuals would not be produced by nature [7].

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 undergoes four phases, namely adaptation phase, exponential phase, stationary phase and death phase. Adaptation phase, was shown on day 1 in all treatments. The second phase was the exponential phase, which began on day 2 in all treatments. In this phase cell division started. The third phase is the exponential phase, which was characterized by increasing cell density.

The fourth phase was the stationary phase, which was characterized by slow increase of cells although the number of living cells remains. Every treatment in this research demonstrated stationary phase, which began on the third day. The last phase was marked with increased number of cell deaths and decreased density of *S. platensis*.

3.2. S. platensis'sphycocyanin content

Phycocyanin is a protein compound that belongs to the phycobilliprotein group like allophycocyanin and phycoeritrin. The whole phycobilliprotein group is insoluble in water and forms a compound attached to the phycobilisometilacoid membrane. Phycocyanin functions as the main photosynthetic pigment in *S. platensis*and as a store of reserves of nitrogen and amino acids.

Based on the results of this study, *S. Platensis*'s highest phycocyanin content was found after the administration of colchicine at a dose of 0.025 % and concentration of 0.1 %, which showed no significant difference from the result demonstrating the lowest phycocyanin content at a concentration of 0.1 % and colchicine highest dose of 0.1 %. This is consistent with the statement of Sofia [7] that administration of colchicine at improper concentration can cause a failure in plant breeding. The phycocyanin content 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 ($P<0.05$).

4. Conclusion

The research results showed that the administration of colchicine at different doses affected the diameter and phycocyanin content of *S. platensis.*

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