The Screening of Antidiabetic Activity and The Cultivation Study of Local Marine Microalgae

S Priatni*, D Ratnaningrum and W Kosasih
Research Unit for Clean Technology, Indonesian Institute of Sciences. Jl. Sangkuriang, Bandung 40135, West Java, Indonesia.

*sripriatni@gmail.com

Abstract. Microalgae are rich source of primary and secondary metabolites which have potential bioactive compounds for application in the pharmaceutical industry. The study of antidiabetic activity from microalgae have been carried out because it can be cultivated in a small area and efficient in light capturing. This study aimed to screen the local marine microalgae which potential as the antidiabetic source. Five microalgae strains (Chlorella sp A and B, Nanochlropsis sp, Porphyridium sp, and Skeletonema sp were screened according to their growth rates, antidiabetic activity (α-glucosidase inhibition) and biomass yield. Results showed that Porphyridium sp cells were grown very active compared to other strains. After 8 days of cultivation, the cells' number of Porphyridium sp was 345x10^3 cells/ml. The highest activity in α-glucosidase inhibition was detected in Porphyridium sp biomass (12.63%). The biomass yield of Porphyridium sp (6.71 g) was obtained after cultivated at 5 L scale production. The exponential growth rate of Porphyridium sp which cultivated with aeration pump system (Rexp= 0.177, TD= 3.8 days) was higher than cultivated with shaker incubator system (Rexp= 0.045, TD= 15.4 days). We concluded that Porphyridium sp is the best microalgae compared to other microalgae both its productivity and antidiabetic activity. The cultivation of Porphyridium sp by using aeration pump system is much recommended.

1. Introduction
Diabetes mellitus is a metabolic disease due to the glucose content in the blood exceeds the allowed standard. This is caused by the insufficient production of insulin which causes increase glucose content and induces mismatches in the metabolism of fat and protein [1, 2]. According to the data in 2011, about eight million people in Indonesia were affected by diabetes and it is predicted to increase to 21.3 million sufferers in 2030 [3]. Lifestyle, obesity, food, and other medical conditions implicated in the increase of diabetes prevalence [2]. The disease is characterized also by hyperglycemia that highly contributes to type 2 diabetes development. Therapy for type 2 diabetes by various oral anti-diabetic drugs are available in the market. However, these drugs have proven side effects significantly. Alternative therapy has been explored by using natural products [4].

There are several ways for obtaining the anti-diabetic compounds from natural products, including by extraction of the metabolites from animals, plants, and microbes. Tropical marine microalgae are interesting subjects to study due to its potential as a new source of bioactive compounds. Microalgae is a rich source of primary and secondary metabolites that has potential bioactive compounds for
application in the pharmaceutical industry. Microalgae are important sources of proteins and bioactive compounds that benefit pharmaceutical and nutritional purposes [6]. The study of antidiabetic activity from microalgae has been carried out by some researchers. Biomass of *Rhodella* showed was potential in lowering the level of insulin and glucose of rodents. The other study presented the capacity of sulfated polysaccharides from *Porphyridium* was the potential in lowering the glucose level in the blood of diabetic mice [7].

Marine microalgae have the advantage because it can be cultured and cultivated in a small area, the cultivation time is between 7-10 days and very efficient in light capturing [8,9]. The growth of microalgae is influenced by cultivation conditions which have four types: photoautotrophic, heterotrophic, mixotrophic, and photoheterotrophic cultivation [10]. The culture growth of *Porphyridium purpureum* was optimized at specific conditions to increase the phycobiliproteins content, yield, and the quality of the product. The growth process of *P. purpureum* is following the exponential kinetic model which used light and sodium bicarbonate concentration as a variable experimental design [11]. In our previous study, antidiabetic activity screening has been carried out to extracellular and intracellular polysaccharide and the total biomass of marine cyanobacteria. *Pseudanabaena* sp showed the highest activity in α-glucosidase inhibition. This study aimed to screen the local marine microalgae which potential as antidiabetic sources, so that can be applied in the pharmaceutical industry. In this study was used the microalgal strain: *Chlorella*, *Nanochloropsis*, *Porphyridium*, and *Skeletonema*. The culture growth condition of selected microalgae was then evaluated in increasing the biomass production.

2. Experimental

2.1. Materials

Five marine microalgae isolates (*Chlorella* sp A, *Chlorella* sp B, *Nanochloropsis* sp, *Porphyridium* sp, and *Skeletonema*) were obtained from Microbiology Laboratory – Research Center for Oceanography, Indonesian Institute of Sciences. The modification of F2 medium was the processed seawater which enriched with the following additives (per liter): NaNO₃ 0.075 g, Na₂HPO₄ 0.05 g, Na-EDTA 0.00436 g, ZnSO₄.7H₂O 0.022 g, Na₂MoO₄.H₂O 0.0063 g, CuSO₄.5H₂O 0.0098 g, MnCl₂.4H₂O 0.18 g, FeCl₃ 0.00315 g, CoSO₄ 0.00100 g. The medium was sterilized (120°C, 20 min) before use. Cyanocobalamin 1 mg, thiamin HCl 0.2 mg and biotin 1 mg (per liter) was added to the sterile medium.

2.2. Methods

2.2.1. Microalgae cultivation. The cultivation of marine microalgae was carried by a modified method [12], as follows: (i) 1 ml stock culture was cultivated in 5 ml of F2 medium, continued to 50 ml of F2 medium. The cultivation was carried out in an incubator at 25°C for 8 days. (ii) The cultivation of marine microalgae was increased to 500 ml of medium. Microalgae in this experiment were cultivated in bottles that connected to an aeration pump (pump output: 70 L per minute) exposed to 2x10 W white lamp equal to 500-2000 lux. The growth of microalgae cells was monitored and counted the cell number for every 2 days.

2.2.2. Cell number counting. The microalgae cells in the cultures were counted with a cell counter on a light microscope at a magnificent 400x. the cell number (N) was calculated as follow:

\[
N = \frac{\left( \frac{\sum N1 + \sum N2}{2} \right) \times 1}{1 \ mm \times 0.2 \ mm \times 0.1 \ mm} \times 10^{-3} \ mL
\]
2.2.3. **Biomass extraction.** The biomass of microalgae was extracted by using methanol with a ratio of 1:20 and the extraction was carried out at 60°C, 100 rpm for 2 hours. The methanol extract was then centrifuged at 4°C, 10,000 rpm for 10 minutes and evaporated to have the concentrate of extract.

2.2.4. **Intracellular polysaccharides (IPS) extraction.** The extraction of IPS was carried out by the modified method [13]. IPS was extracted by homogenizing the biomass in distilled water (50 mL) and heated in a water bath at 95°C for 6 hours. The extracts were filtered through the Whatman No.2 filter paper to separate the rest of the biomass. The filtrate was then precipitated with 5% ethanol (ratio 1:4), stirred vigorously and keep for overnight at 4°C. The precipitated IPS was recovered by centrifugation at 8,000 rpm for 15 min and the supernatant was discarded.

2.2.5. **Determination of inhibition of α-glucosidase activity [1].** The inhibition of α- glucosidase activity was determined by using p-nitrophenyl -D-glucopyranose (pNPG) as a substrate. The sample assay contained 0.25 mL of 0.1 M phosphate buffer (pH 7.0), 0.25 mL of substrate solution (2.5 mM pNPG in 0.1 M phosphate buffer) and 0.1 mL of sample solution in DMSO. The solution was then incubated at 37°C for 5 minutes and added with 0.25 mL of enzyme solution (0.2 U/mL α-glucosidase in 0.01 M phosphate buffer containing 0.2% BSA). The solution was continued the incubation for 15 min at 37°C. The reaction was stopped by dropped 0.1 mL of 0.2 M sodium carbonate. The amount of p-nitrophenol released was measured by the absorbances at 400 nm. The % inhibition of α-glucosidase was calculated as follow:

\[
\% \text{ inhibition} = \frac{\text{Abs (Control)} - \text{Abs (Sample)}}{\text{Abs (Control)}} \times 100
\]

2.2.6. **The kinetic calculation of microalgae growth.** The growth of selected microalgae was evaluated the process kinetic by the modified method [11]. Table 1 presented the equations for kinetic calculation of microalgae.

| Parameters                                | Equation            |
|-------------------------------------------|---------------------|
| The rate of cell growth at a certain time  | \( \frac{dN}{dt} = RexpN \) |
| The total cell number at the exponential growth | \( N = N_0 \exp(Re xpT) \) |
| The exponential growth rate of microalgae  | \( \frac{N}{N_0} = Re xpT \) |
| The doubling time (TD)                    | \( TD = \frac{0.6931}{Re xp} \) |
| The doubling number (ND)                  | \( ND = \frac{Re xp}{0.6931} \) |

3. **Results and Discussion**

The screening of local marine microalgae for antidiabetic have been carried out by monitoring the growth of microalgae culture samples by using F2 medium in the processed seawater. The important parameters in microalgae growth are nutrient, light, pH, aeration, salinity, and temperature [11]. In this study, the cultivation was carried out in a laboratory-scale and monitored every two days. The results analysis of cell counting during microalgae cultivation is presented in Figure 1. The result trends of microalgae growth displayed that *Porphyridium* sp cells were grown very active compared to other
strains. After 8 days of cultivation, cell number data of *Porphyridium* sp was $345 \times 10^3$ cells/ml. The intensity of light is important for algaculture, it will depend on the cell concentrations and the culture depth that the light will penetrate through the culture, 1000 lux is suitable for Erlenmeyer scale [14]. The light absorption and the number of photosynthetic is the key factor in the light reaction [15].

![Growth curve of five microalgae strains (Chlorella sp A, Chlorella sp B, Nanochloropsis, Porphyridium sp and Skeletonema)](image)

**Figure 1.** Growth curve of five microalgae strains (Chlorella sp A, Chlorella sp B, Nanochloropsis, Porphyridium sp and Skeletonema)

The screening of antidiabetic activity has been carried out to five strains of local marine microalgae. The screening was carried out by extraction of the total biomass with methanol and extraction IPS of microalgae (Table 1). The results demonstrated that all methanol extract samples can inhibit the activity of α-glucosidase and *Porphyridium* sp showed the highest activity (12.63%). However, for IPS samples were not found positive data in α-glucosidase inhibition. Some metabolites from natural resources have been evaluated the inhibition of glucose production and absorption in the intestine or gut. α-glucosidase is located around the surface membranes of intestinal which has a role in carbohydrate digestion. This enzyme catalyzes the cleaved of α-1,4-glucosidic linkages of carbohydrates into simpler sugars such as glucose. Phlorotannins from *Ecklonia cava* have been extracted and analyzed their α-Glucosidase inhibitory effects which showed % of inhibitions were between 10.8 to 49.5% [4]. Pigments from *Spirulina* sp, such as anthocyanin and phycocyanin, have potential as antihyperglycemic agents which can inhibit the increase of glucose level in rat experiments [16]. In our study, the pigments of *Porphyridium* sp (12.63%) and *Chlorella* sp A (12.55%) which contained in methanol extract potentially showed α-glucosidase inhibition. Our previous study reported the growth of *Porphyridium* sp in media containing Fe$^{3+}$ and Co$^{2+}$ promotes the production of phycoerythrins, phycocyanins and allophycocyanins pigments.
Table 2. Antidiabetic activity of an IPS and methanol extract of local marine microalgae

| Microalgae       | Antidiabetic activity ( % inhibition) |
|------------------|--------------------------------------|
|                  | IPS        | Methanol extract |
| Control          | 91.05      | 91.05            |
| Chlorella sp A   | -4.73      | 12.55            |
| Chlorella sp B   | -4.10      | 9.38             |
| Nanochlropsis sp | -5.36      | 7.29             |
| Porphyridium sp  | -6.64      | 12.63            |
| Skeletonema sp   | -9.31      | 11.26            |

Based on the data in Table 2, the study was continued to evaluate the biomass yield of Porphyridium sp and Chlorella sp A, both in 1 L scale and 5 L scale for ten days cultivation. Figure 2 showed that at 5 L scale cultivation the biomass yield of Porphyridium sp (6.71 g) was higher than Chlorella sp A (4.40 g). However, these two strains show a similar yield in 1 L scale cultivation.

The results showed that Porphyridium sp is the best microalgae compared to other microalgae both its productivity and antidiabetic activity. *P. purpureum* produces macromolecular products such as polysaccharides, phycobiliproteins, polyunsaturated and fatty acids. The biomass composition is highly variable and that product formation is regulated in a complex way [15]. Sulfated polysaccharides (sPS) from Porphyridium sp significantly decreased the glucose levels in the blood of diabetic mice, causing no modifications in the pancreatic island cells and no fibrosis, or hemorrhagic necrosis in cells, either. The experiments suggested the potential of sulfated polysaccharides for hypolipidaemic and hypoglycaemic agents [7]. According to our study, the antidiabetic activity of intracellular polysaccharide (IPS) of microalgae samples were shown the negative result. We assumed that the polysaccharides were not in the sPS molecule structure. According to these data, Porphyridium sp was chosen for further study. To optimize the growth of Porphyridium sp, the effect of aeration systems on growth rates was evaluated. The cultivation was carried out with two aeration systems by using the shaker incubator and aeration pump. The rate of cell number growth was monitored every day until day 10 (Figure 3).
Figure 3. The exponential growth rate of *Porphyridium* sp (Rexp) cultivated by using aeration pump and shaker incubator system

Data on Figure 3 shows the exponential growth rate of *Porphyridium* sp which cultivated with aeration pump system (Rexp = 0.177, TD = 3.8 days) was higher than cultivated with shaker incubator system (Rexp = 0.045, TD = 15.4 days). Cultivation of microalgae with aeration pump system can accelerate the mixing process that is necessary for preventing the sedimentation of the algae. The mixing process will ensure all cells are equally exposed to the light and nutrients, avoid thermal stratification, and support gas exchange between the medium and the air [14]. Aeration is a technique that involves introducing dissolved oxygen into a lake. In some lakes, aeration can help increase dissolved oxygen content, control algae growth, decrease internal recycling of phosphorus and convert ammonium to nitrate [17].

4. Conclusion
The antidiabetic activity from *Chlorella* sp A, *Chlorella* sp B, *Nanochloropsis* sp, *Porphyridium* sp, and *Skeletonema* has been studied and evaluated by cultivated of these microalgae in F2 medium. The results showed that *Porphyridium* sp is the best microalgae compared to other microalgae both its productivity and antidiabetic activity. The cultivation of *Porphyridium* sp by using aeration pump system is much recommended.

Acknowledgments
This research was supported by Research Unit for Clean Technology and Research Centre for Oceanography - Indonesian Institute of Sciences (LIPI).

References
[1] Yang JP, Hsu T, Lin F, Hsu W and Chen Y 2012 *Carbohydr. Polym.* 90 174-80
[2] Islam MS, Ali S, Rahman M, Islam R, Ali A, Azad AA and Islam MR 2011 *J. Med. Plant Res.* 5 3745–50
[3] Mulyanti S, Musthapa I and Aisyah S 2010 *Jurnal Sain dan Teknologi Kimia* 1 1–9
[4] Lee SH and Jeon YJ 2013 *Fitoterapia* 86 129–36
[5] Morada NJ, Metillo EB, Uy MM and Oclarit JM 2011 *International Conference on Asia Agriculture and Animal* IPCBEE 13 197–200
[6] Priyadarshani I and Rath B. 2012 *J. Algal Biomass Util.* 3 89-100
[7] de Jesus Raposo MF, de Morais RMSC and de Morais AMMB 2013 Mar. Drugs 11 233-52
[8] Kabinawa INK 2014 Jurnal Aplikasi Teknologi Pangan 3 103–9
[9] Huang G, Chen F, Wei D, Zhang X and Chen G 2010 Appl. Energy 87 38–46
[10] Chen CY, Yeh KL, Aisyah R, Lee DJ and Chang JS 2011 Bioresour. Technol. 102 71–81
[11] Velea S, Ilie I and Filipescu L 2011 U.P.B. Sci. Bull. Series B 73 4
[12] Priatni S, Budiwati TA, Ratnaisingrum D, Kosasih W, Andryani R, Susanti H and Susilaningsih D 2016 Biodiversitas 17 642-6
[13] El-sheekh MM, Khairy HM and El-Shenody R 2012 Iranian J. Environ. Health Sci. Eng. 9 10
[14] Coutteau P 1996 FAO Fisheries Technical Paper 7 41
[15] Csőgőr Z, Kiessling B, Perner I, Fleck P, Posten C 2001 J. Appl. Phycol. 13 317–24
[16] Setyaningsih I, Bintang M and Madina N 2015 Procedia Chem. 14 211–15
[17] Priyadarshani I, Thajuddin N and Rath B 2014 Int. J. Curr. Microbiol. App. Sci. 3 173–82