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Isolation and Cultivation of Green Alga, *Pediastrum* spp. for Nutritional Value Study

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Green microalgae *Pediostrum* spp. from Chiang Mai Moat, Chiang Mai province, northern Thailand were isolated in Jaworski's medium (JM) at 25 °C under continuous light. Dominant species in this study were selected for optimal study of media, pH and temperature. Cell density was determined spectrophotometrically at a wavelength of 665 nm, cell counts by whole counts method and biomass productivity as dry weight. It was found that *P. duplex*, *P. simplex* and *P. tetras* grew best in JM followed by the growth in Bold's Basal Medium (BBM) and Algal Medium (AM) media respectively. They grew better at pH 8.0 in JM and exhibited highest growth at room temperature. The protein content (36.45 ± 3.75 g/100 g) was highest in *P. duplex* and highest carbohydrate (43.86±1.75 g/100g) was found in *P. tetras*. Protein and carbohydrate were the major components in these algae which can be applied as food supplement in human and animal feed and pharmaceutical industries. Carbohydrate value was interesting as polysaccharide for sources of antioxidant. *Pediostrum* spp. have a large size which is easy for the cells to be harvested.

Keywords: Green microalgae, *Pediastrum*, Isolation, Cultivation, Nutritional Value.

Algae are the most important producer in the aquatic ecosystem. They are rich sources of carbohydrate, protein, enzyme and fiber. Besides, many vitamins and minerals e.g. vitamin A, C, B1, B2, B6, magnesium and calcium are abundantly found in algae [1]. Besides, it is environmentally friendly and can be effective in regulating global warming and climate change by controlling the level of pollution. One of the most promising strategies is using algae to mitigate the amount of carbon dioxide emitted into the atmosphere [2]. The coenobial freshwater algae, *Pediastrum* are members of the family Hydrodictyaceae which is placed within the order Chlorococcales of the Chlorophyta [3]. Their dominant characteristics are disc shape or stellate coenobia. Cell wall of *Pediastrum* is composed of thick inner layer of cellulose derivatives and the outer layer is composed of sporopolenin combined with silicon oxide which makes them highly resistant to decay [4]. Preliminary data indicated that *Pediastrum* spp. grow significantly faster than other algae and has a high protein content up to 46% [5]. There have been only a few previous studies on *Pediastrum* spp. in Thailand. In addition, the applications of *Pediastrum* spp. have not yet been fully studied due to lack of basic information. Study on the diversity, new and rare taxa of *Pediastrum* spp. in some freshwater resources in Thailand found 60 taxa consisting of 26 species and 22 taxa were newly recorded [6]. Three species, namely *Pediastrum duplex* var. *duplex* Meyen, *P. tetras* (Ehrenberg)

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Ralfs and *P. simplex* var. *simplex* Meyen were the dominant species. However, the conditions for culturing in laboratory have not yet been fully studied. The main objective of this research was to isolate and cultivate dominant species of *Pediastrum* spp. from Chiang Mai moat, Chiang Mai province of Thailand. Moreover, these dominant species were selected for optimal study of media, pH, temperature and nutritional analysis.

MATERIALS AND METHODS

Pediastrum spp. were collected by filtering 10 liters of water samples from sampling site in Chiang Mai moat, Chiang Mai province with 10 µm pore size plankton net. The samples were kept in a cool box for isolation and cultivation in the laboratory [7].

Isolation of *Pediastrum* spp.

Colonies of dominant species of *Pediastrum* spp. (*Pediastrum duplex*, *P. tetras* and *P. simplex*) in the water samples were studied under a microscope and single colonies were isolated with a glass micropipette. Each colony was washed at least five times with sterile medium and cultivated in the 12 multi well cell culture plate containing Jaworski's medium (JM). The alga was then purified by streak plate method on JM. Sub-culture was carried out until monoculture was obtained and transferred to JM broth for using as stock culture. The culture was incubated at 25 °C under continuous 10.8 $\mu mol.m^{-2}.s^{-1}$ illumination by florescent light[5].

Cultivation

Pediastrum spp. were cultivated in 3 media: algal broth (AM), bold basal medium (BBM) and Jaworski's medium (JM) for comparison and were washed with each sterile medium before being transferred to all media. They were cultivated at pH: 6.5, 7.0, 7.5 and 8.0 and finally incubated at 25 °C and room temperature under continuous 10.8 $\mu mol.m^{-2}.s^{-1}$ illumination with florescent light and aeration by bubbling air-line in 500 ml erlenmeyer flasks containing 300 ml of medium (Fig. 1).

Cell density was determined spectrophotometrically at 665 nm and cell counts by whole counts method [8]. When the growth reached the stationary phase, the cells were

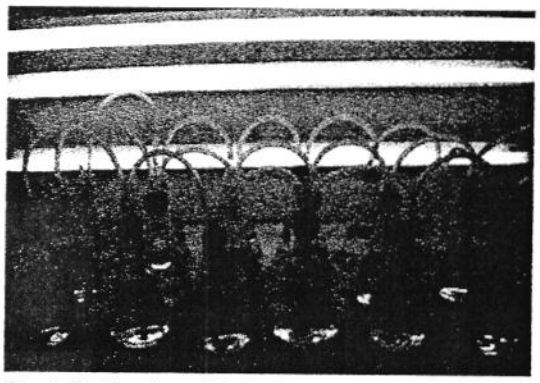

Fig. 1. Cultivation of *P. duplex*, *P. simplex* and *P. tetras* in AM, BBM and JM media

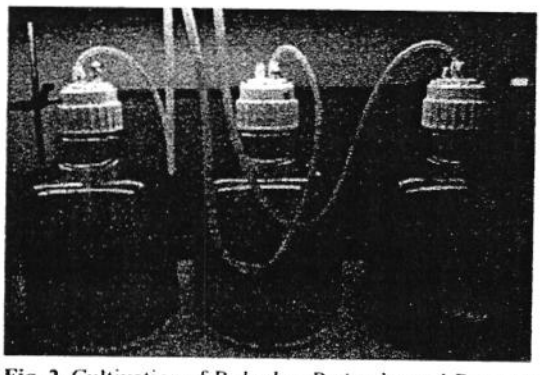

Fig. 2. Cultivation of *P. duplex*, *P. simplex* and *P. tetras* in 20 L JM medium

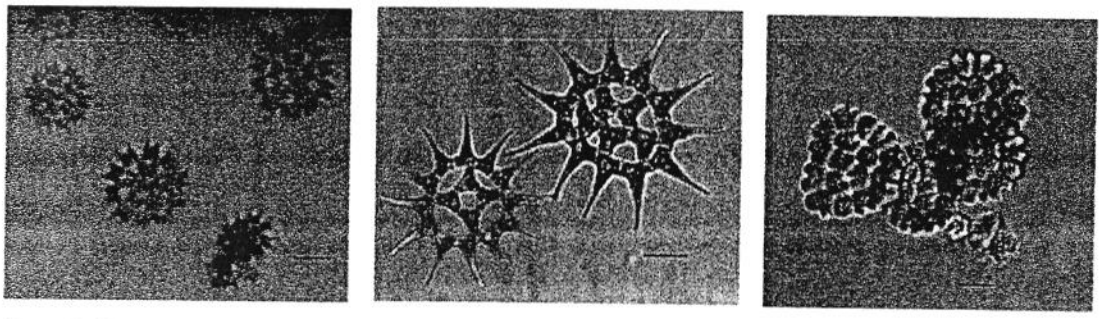

Fig. 3. *Pediastrum* spp. under compound microscope; (A) *P. duplex*, (B) *P. simplex* and (C) *P. tetras*. Scale bar = 10 µm

harvested by centrifugation and dried at 60°C for 48 hr [9], and the biomass productivity (P) was calculated as maximum productivity (mg/L/d) [10].

$$P = \frac{1000.\ (X1 - X0)}{(t1 - t0)}$$

X0 is the initial biomass (g/L) at time t0 (d)
X1 is the final biomass (g/L) at any time t1(d)

After the optimal condition for growth was identified, cultivation was scaled up to 20 L media (Fig. 2). Ten percent (V/V) of each stock culture was inoculated to 20 L of medium cultivated at room temperature with continuous illumination and aeration by bubbling air-line in plastic carboy tank. When the growth reached the stationary phase, the cells were harvested by sedimentation and siphoned supernatant away and centrifugation then dried at 60°C for 48 hr.

Nutritional analysis

At the end of cultivation, the biomass of the dominant species of *Pediastrum* were selected and subjected to nutritional analysis i.e. protein analysis by micro kjeldahl method [11], fatty acid analysis by acid hydrolysis method [11], fiber analysis by acid detergent method, ash analysis by burned at 450 °C 1 hour, carbohydrate analysis by calculating from

Carbohydrate (%) = 100 – (protein + fat + moisture + ash) [11]

Statistical analysis

Growth of *Pediastrum* in each experiment was analyzed as mean ± S.D. Statistical comparison between groups was determined by one way ANOVA followed by Tukey's post hoc test at $p<0.05$.

RESULTS AND DISCUSSIONS

Isolation and Cultivation of *Pediastrum* spp.

The dominant species of *Pediastrum* i.e. *P. duplex, P. simplex* and *P. tetras* were selected for optimal study on media, pH and temperature (Fig. 3).

Effect of media

The growth of the three dominant species of *Pediastrum* was compared in 3 media: Algal medium (AM), Bold basal medium (BBM) and Jaworski's medium (JM). Cell density was determined spectrophotometrically at a wavelength of 665 nm and cell counts by whole counts method. It was shown that *P. duplex, P. simplex* and *P. tetras* grew best in JM with OD_{665} at 0.87, 0.80 and 0.74 respectively with cell number of 94×10^6, 78×10^6 and 45×10^6 cell/mL respectively (Figs. 4 and 5). The

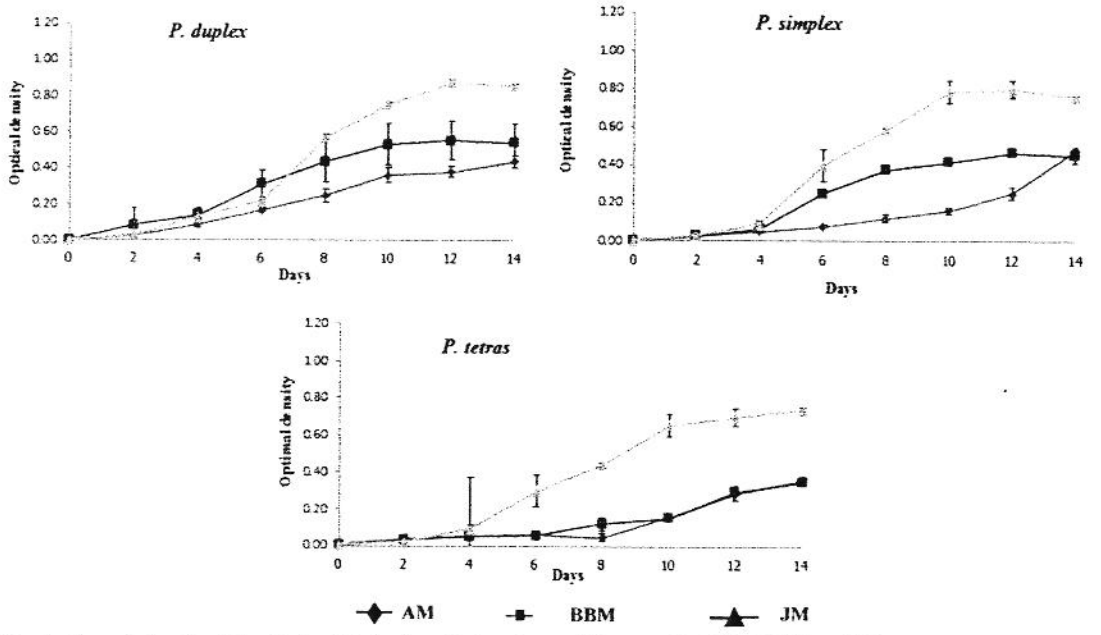

Fig. 4. Growth (optical density) of *P. duplex, P. simplex* and *P. tetras* in AM, BBM and JM