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Culture of microalgal strains isolated from natural habitats in Thailand in various enriched media

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ABSTRACT

Six freshwater microalgal strains in the class of Chlorophyceae, including *Chlorococcum humicola, Didymocystis bicellularis, Monoraphidium contortum, Oocystis parva, Sphaerocystis* sp., and *Scenedesmus acutus* were isolated from natural habitats in Thailand. The six strains were compared for their biomass yield, lipid content, and lipid productivity in four enriched culture media in batch mode. Significant differences were found across algal strains and culture media. The best strain was found to be *C. humicola,* which had the highest biomass yield of 0.113 g/l/d (in Kuhl medium), the highest lipid content of 45.94% (in BG-11 medium), and the highest lipid yield of 0.033 g/l/d (in 3NBBM medium). The 3NBBM medium, which has the lowest nitrogen concentration among the four culture media, was considered the optimal culture medium for *C. humicola* for lipid production. The fatty acid profile of *C. humicola* was also found to be affected by the culture medium. More oleic acid (C18:1) but less linolenic acid (C18:3) was accumulated in BG-11 and 3NBBM than in Kuhl and N-8 media. Lipid profiles of *C. humicola* were comparable to palm oil in the percentage of palmitic acid and the total amount of saturated fatty acids; however, *C. humicola* made more poly-unsaturated fatty acids such as linoleic (C18:2) and linolenic (C18:3) acids than oil palms. Lipids from *C. humicola* were believed to be acceptable for biodiesel production.

1. Introduction

As one of the major biofuels, biodiesel plays a significant role in diversifying the source of world transportation fuels [1,2]. Traditional oilseed crops such as soybean, rapeseed, and oil palm have been adopted extensively for biodiesel production. However, to meet the increasing demand of biodiesel, seeking lipid-rich biological materials other than traditional oilseed crops has attracted much attention [3]. Microalgae are considered a potential source of biodiesel because of a number of advantages, such as their relatively simple cellular structure, high lipid content and photosynthetic efficiency, and, consequently, their higher biomass and lipid yields when compared to traditional oilseed crops [4–6]. Moreover, some microalgae can be cultivated in non-arable lands using non-potable water or even wastewater, thus reducing or avoiding competition with food/feed crops for agricultural land and freshwater [7,8].

Choosing the right species is critical in microalgae-based biodiesel production. High lipid content, fast growth rate, ease of harvest, and the ability to adapt to local environments are some essential criteria. The climate and local land geology of different areas should also be considered [5]. Extensive studies have been conducted seeking the "best" algal species for biofuels production. Some researchers believe that indigenous microalgal species isolated from natural water bodies or wastewater treatment plant sites can adapt to local environments better and grow faster than non-native species [9,10]. Because of this, numerous species have been isolated, most of which are in the division of Chlorophyta [11–13]. In Thailand, Yeesang and Cherisilp [14] isolated *Botryococcus* sp. and found that they could achieve an oil content and yield of 25.8% and 46.9 mg/l/d, respectively. Yongmanitchai and Ward [15] screened 92 microalgal strains from various freshwater reservoirs in Thailand and found that some of them grew rapidly and accumulated lipids with various yields.

In addition to species screening, the composition of culture media is another fundamental factor that significantly affects the growth rate, product yields, and biochemical composition of microalgae [16]. Dayananda et al. reported that *Botryococcus braunii*

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cultivated in BG-11 medium had higher biomass yield and oil (hydrocarbon) content than in BBM and BBMa media [17]. Similarly, Phukan et al. cultivated *Chlorella* MP-1 in BG-11 medium and obtained higher gross biomass yield than Basal, BBM, and Modified Chu 13 media [13]. Nitrogen and phosphorus are the essential macronutrients in culture media. Their forms and concentrations are the major factors influencing biomass and lipid yields of microalgae, as well as their biochemical compounds. For example, Huerlimann et al. [18] grew four algae (*Isochrysis* sp., *Nannochloropsis* sp., *Tetraselmis* sp., and *Rhodomonas* sp.) in three different culture media (L1, f/2, and K-medium) and found that significant differences in biomass yield, lipid content and lipid productivity existed due to both species and medium differences. Huang et al. [19] believed that complex nitrogen might be superior to simple nitrogen source for lipids production in heterotrophic culture of microalgae. Lourenco et al. [20] found that macronutrients including nitrogen, phosphorus and their ratios had significant effects on the contents of protein, total amino acids, carbohydrate, lipid, fatty acids, chlorophyll-a and carotenoids of *Tetraselmis gracilis*.

Thailand is located in the tropical zone and has abundant sunlight, land, and water resources. The unique geographic location of Thailand makes year-round high-yield algal cultivation possible. Thailand also has diverse species of microalgae, implying that some of the native species could be excellent candidates for biofuel production. The objectives of this study were to (1) isolate, identify, and compare the lipid production performance of microalgae strains found in natural habits of Thailand, and (2) study the influence of four enriched culture media on biomass productivity, lipid content and yield, and lipid profile for select algae strains.

2. Materials and methods

2.1. Isolation and identification of microalgae from natural habitats

Microalgae were collected by plankton net (20-μm pore size) from three provinces of Thailand (Nakonsawan province, Songkla province, and Phetchaburi province). Microalgae samples of about 5 ml were inoculated into 5-ml autoclaved BBM medium [21] in 20-ml test tubes and cultured at room temperature (25 °C) for 2 wk with cool white fluorescent light. The light intensity was approximately 40 μmol photons/m^2/s and the diurnal cycle was 12 h dark/12 h light. The pre-cultured samples were streaked on BBM medium-enriched agar plates and cultured for another 1–2 wk with cool white fluorescent light using the same light intensity. The single colonies on agar were picked up and cultured in liquid BBM medium, and the streaking and inoculation procedure was repeated until pure cultures were obtained. The morphology of pure strains was regularly examined under an optical microscope. The strains were identified for taxonomy by Thailand Institute of Scientific and Technological Research (Pathum Thani, Thailand).

2.2. Culture conditions and culture media

Isolated and purified microalgae were inoculated in 250-ml Erlenmeyer flasks containing 125 ml culture medium. Flasks were placed on a reciprocating shaker at 120 rpm for 7 d at room temperature of 25 °C. Light was provided by cool white fluorescent lamps at an intensity of 80 μmol photons/m^2/s. The inoculums were then transferred to 500-ml Erlenmeyer flasks containing 450 ml of one of the four enriched media shown in Table 1. The different media were chosen mainly by their nitrogen and phosphorus sources and concentrations. The culture conditions were the same as in inoculation except that aeration of 1 vvm (450 ml/min) was provided. All experiments were carried out in triplicate.

2.3. Biomass measurement

Growth curves of the six strains in all media were developed by measuring the OD of samples at 680 nm using a UV–VIS spectrophotometer (Aquarius CE7200, Cecil instrument, Cambridge, UK). Cells were harvested in the stationary phase by centrifugation at 8000 rpm for 5 min and the cells were washed twice using distilled water. The cell pellets were lyophilized using a freeze-dryer (ALPHA 1-4, Christ company, Osterode, Germany) for 2 d and placed in a dessicator until constant weight. Dry weight (DW) of cells was obtained using an analytical balance with a precision of 0.1 mg.

2.4. Lipid content and fatty acid analysis

To quantify lipid contents, lipids were extracted using the solvent system of chloroform–methanol (1:2 v/v) following the method of Bligh and Dyer [25], which is briefly described as follows. After freeze-drying algal cells, algal dry powder of 0.08 g was mixed with 8 ml of chloroform–methanol (1:2) solvent and ultrasonicated with a sonicator (Bandelin SONOREX SUPER RK 156 BH, PK Elektronik, Berlin, Germany) for 30 min. Algal solid was removed by centrifugation at 8000 rpm for 5 min, and the residual solid went through the solvent extraction procedure three times to ensure that most lipid was extracted. The solvents were then evaporated to obtain lipids. For fatty acid analysis, freeze-dried algae samples containing approximately 10–50 mg fatty acids were added to 15-ml screw cap tubes. Two ml of benzene containing internal standard (methyl-C13, 2 mg/ml) and 3 ml of methanolic-HCl were added, vortexed and incubated at 70 °C for 2 h. After cooling for 15 min, 5 ml of 6% K_2CO_3 and 2 ml benzene were added to the same tube and vortexed again. The benzene layer was separated by centrifuging at 2400 rpm for 5 min and transferred to vials for analysis of fatty acid methyl esters. The fatty acid methyl esters were analyzed by using a Hewlett–Packard 5890 gas chromatograph equipped with a SP-2560 capillary column (Supelco, Inc., Bellefonte, PA). Injection port and detector temperatures were 250 °C with a flow rate of 1 ml/min helium, a split ratio of 100:1, and a 1 μl injection volume. Oven temperature began at 140 °C and increased at 2 °C/min–200 °C, then at 4 °C/min–245 °C; samples were held for 17 min.

2.5. Statistical analysis

The statistical analyses were performed using the software SPSS 16.0 (Statistical Program for Social Sciences 16.0). The means of biomass yield, lipid content and lipid productivity were compared among strains and culture media by one-way analysis of variance in conjunction with Tukey's HSD test, Samples were considered significantly different at $p < 0.05$.

3. Results and discussion

3.1. Characteristics of isolated strains

Six strains of microalgae, including *Chlorococcum humicola*, *Didymocystis bicellularis*, *Monoraphidium contortum*, *Oocystis parva*, *Sphaerocystis* sp., and *Scenedesmus acutus* were selected for further study from more than a dozen strains that were isolated from three locations in Thailand due to their fast growth rate in preliminary tests (data not shown). All six strains are belonging in the division of Chlorophyta and the class of Chlorophyceae. The name, source, and specific scientific classification of each strain are summarized in Table 2.